Pests and predators on genetically altered cotton (Bt-cotton) and associated host plants in South Africa

by

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DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Philosophiae Doctor in the Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, RSA.
It has not been submitted before for any degree or examination in any other University.

_____ day of _____ 2007

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ABSTRACT

The efficacy of the Bt-genes (the *cry1Ac* and *cry2Ab2 genes)* were evaluated for bollworms (i.e. the american or “african” bollworm, Helicoverpa armigera, the red bollworm Diparopsis castanea and the spiny bollworm species, Earias biplaga and E. insulana) on cotton under normal spraying conditions in different field trials in South Africa. Differences that were found in bollworm efficacy and yields are explained by comparison in various field trials. Bt-cottons (Genetically Modified Cotton) exhibiting either only bollworm resistance (NuOPAL), or cotton exhibiting both bollworm resistance and herbicide tolerance (NuOpal RR)-, or cotton exhibiting only herbicide tolerance (DeltaOpal RR) were compared with non-Bt-cotton (DeltaOPAL). At the same time the effect of the Bt-gene on non-target organisms, such as secondary pests and predator numbers was monitored. Since most of the small-scale farmers in South Africa cultivate Bt-cotton and they are required to plant a refuge as part of a Resistance Management Programme, the abundance of alternative host plants for bollworms was evaluated in the largest small-scale production region, the Makhathini Flats (KwaZulu-Natal). Finally, the acceptance of Bt-technology amongst growers are discussed. This study was undertaken over a number of years and the repetition of a number of the trials at different localities has showed that Bt-technology has proved to be not only effective against the target pests, which are the african bollworms on cotton, but it is also beneficial to farmers in the form of a higher yield production and improved crop protection. The effect of the Bt-gene on non-target organisms is minimal if present and the Bt-gene has no detrimental effect on predator numbers, especially in the presence of an increase in insect host numbers, irrelative of the cotton type planted. The increase in predator numbers and secondary pests is a result of the decrease in the number of bollworm sprays applied on Bt-cottons to control bollworm, as bollworms are effectively controlled by the Bt-gene. In some instances when additional sprays for secondary pests were applied, the benefit for the grower to plant Bt-cottons, is reflected in the higher yields and lower input costs as a result of the absence or fewer bollworm sprays. The host plant study showed that alternative host plant abundance in an area where cotton is cultivated, can provided evidence for a possible alternative refuge to conventional cotton. The fact that very little, of the cotton planted by South African farmers are conventional cotton (non-Bt-cotton) varieties, confirms the acceptance of Bt-technology amongst cotton growers, with full acceptance of the requirements of planting this cotton, while obtaining higher yields per surface area than in the case of non-Bt-cottons. The benefits of planting Bt-cotton that have been illustrated in this study, clearly demonstrates the acceptance of Bt-cotton in South Africa, especially amongst, the commercial and the small-scale farmer, by enabling cotton growers to farm more cost-effectively.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

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Background and justification of study

This study forms part of research work that has been carried out over a number of years on behalf of the industry. Some of the work completed during this time, was carried out under the auspices of the Agricultural Research Council, while the latter part of the study, mainly from 2000 onwards, was completed by the writer while doing consultancy work for Agri-Biotech Research Consultancies cc. The importance of this study must be seen in relation to the introduction of new technologies in South Africa, and in specific the introduction of genetically altered cotton varieties. Transgenic cotton, or so-called Genetically Modified (GM) Cotton, or Bt-cotton (cotton with the Bacillus thuringiensis gene) became registered in 1998 in the Republic of South Africa and some of the results here will refer to data gathered during the registration trials of Bollgard™. Cotton has a market share for the 2002 season of 39.4 % of the textile industry (Cotton SA, 2002). A large amount approximately 70% of all cotton grown in SA, is at present Bt-cotton. The numbers of hectares planted in total under cotton were 71 608 ha for 2001/02, and it has decreased to 22 551 ha at present (Cotton SA, 2006). The total of 22 551 ha includes cotton planted under irrigation and rain-fed cotton. The dissemination of the benefits of Bt-cotton is expected to motivate farmers to expand cotton production in the Republic of South Africa. In addition, besides the environmental benefit that reduced spraying cause on Bt-crops, it lowers the cost of production, which in turn raises farmers' incomes (Wambugu, 2001). Although cotton production has decreased considerably to make out only 22 000 ha in 2006/2007, about 90% of cotton planted in South Africa at present is genetically modified cotton (pers comm. H.W. Bruwer[1]) The decrease in cotton production is related to the poor price of SA cotton on the international market, with the result that commercial and small-scale farmers in South Africa get very little income from cotton in relation to other crops which are subsequently preferred. However, cotton is a cash-crop, and for many small-scale farmer and dryland commercial farmers, cotton is a preferred choice, since it can be cultivated under dryland conditions, when few other crops can be planted. In order to sustain the cultivation of Bt-cotton in South Africa, ongoing research is necessary to evaluate Bt-cotton lines with better-manifested gene expression, and which are least susceptible under variable conditions to the major pests, the bollworm complex, while the population dynamics of secondary pest species are kept in mind. In addition to bollworm numbers, other secondary pest species like leafhoppers (Jacobiella fasciata Jac.), whitefly (Bemiscia tabaci Gennadius), aphids (Aphis gossypii Glover), spider mites (Tetranychus cinnabarinus (Boisduval)), thrips (Thrips tabacci Lind.), the cotton stem weevil (Apion soleatum Wagner), and the black cotton beetle (Syagrus rugifrons Baly) were also monitored where possible on different trials. Since transgenic crops are often said to influence beneficial insect populations negatively, the numbers of spiders and various stages of lacewings and ladybirds, were also noted where possible. Some of these genetically resistant lines are still being evaluated further and no other transgenic cotton variety that shows resistance against lepidopteran pests has been registered up to date in the Republic of South Africa. The question

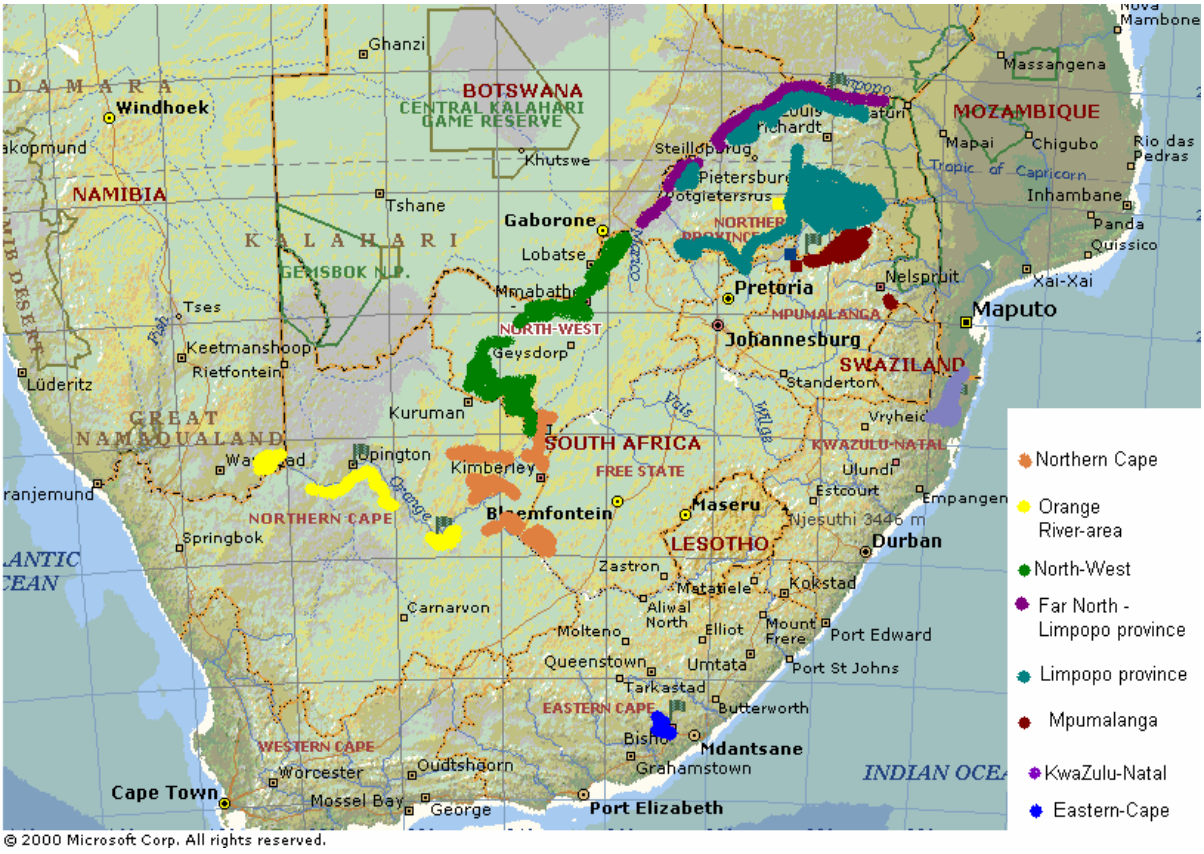
[1] Mr. H. Bruwer is the Chief Executive Officer of Cotton SA, Pretoria, S.A.

of possible resistance to the Bt-gene is probably the most common question when transgenic crops are discussed. In the Republic of South Africa, a resistance management strategy is implemented by introducing the planting of a refuge system, which is obligatory for growers who plant Bt-cotton. A part of this study was thus dedicated to determine possible alternatives to conventional cotton for use as a bollworm refuge, i.e. the investigations into alternative host plants in addition to the conventional cotton refuge that the cotton grower is obliged to plant as stated in the license agreement of Bollgard™ cotton. Scouting is used as part of an Integrated Pest Management system. The benefits of applying such an integrated system, gives the farmer the opportunity to make informed decisions about spraying and at the same time leads to cleaner production by reduced spraying. With the introduction of the Bt-cottons in South Africa, scouting has become even more important, since much emphasis is laid on secondary pests, which appears more prominent due to the lower frequency of spraying for bollworms. The occurrence of secondary pests is discussed in Chapter 3.

The word "cotton" as we know it today originates from the Arabic word "qutun". In Middle Dutch it was also known as "cotton" and with the development of Afrikaans as a spoken language it became "catoen" and eventually "katoen" (www.cottonsa.org.za). Cultivated cotton belongs to the genus Gossypium L. in the family Malvaceae, and commercial varieties belong to four species, namely G. herbaceum, G. arboreum, G. barbadense (Sea Island cotton) and G. hirsutum (Upland cotton) (Pearson and Darling 1958). Most cultivated cotton types take the form of a perennial shrub with a vigorous tap root system and a single ascending main stem that bears at each node a leaf and usually one branch. The main pests on cotton are the bollworm complex, consists of the american or “african” bollworm Helicoverpa armigera, red bollworm Diparopsis castanea and the spiny bollworm species Earias biplaga and E. insulana and are able to cause serious economic damage to the crop (Pearson and Darling 1958). The benefits of genetically modified cotton ranges from an increase in yield to the self-fulfillment of establishing a cash crop. The yields obtained from Bt-cotton will be discussed in Chapter 5. The first cotton seed was planted in 1690 in the Western Cape, more or less 40 years after the arrival of Jan van Riebeeck. Cotton, however, prefers a warm climate and requires a substantial amount of moisture for the seed to germinate (www.cottonsa.org.za). In 1846 a certain Dr Adams brought seed from America and started growing cotton in the Amanzimtoti district in the KwaZulu-Natal Province. Between 1860 and 1870 cotton was planted on a relatively large scale in both Natal and the Cape Colony due to the demand for this fibre which had arisen as a result of the American Civil War. In 1904 about 12 to 14 hectares were planted in the Tzaneen area and in 1905 a cotton gin was erected in the area where cotton could be ginned and baled mechanically. In 1913 an experimental station, which was to provide farmers with advice, was established at Rustenburg under the direction of a Mr. Taylor. Between 1913 and 1922 cotton was cultivated mainly in the Transvaal Lowveld and the Eastern Transvaal. The co-operative movement with regard to cotton had its origin in 1922 when a co-operative and a ginnery were established at Barberton (www.cottonsa.org.za).

In the Republic of South Africa cotton production takes place in the different provinces, whereof only the Limpopo Province, Mpumalanga, the Northern Cape, and KwaZulu-Natal, and to a lesser extent the North West Province is known as cotton production regions. Some initiatives are undertaken during the last years to implement cotton as a small-scale farmer crop in the Eastern Cape region, but up to now, only some demonstration trials and research projects have been undertaken in this region. The regions are illustrated in Plate I. Most of the cotton produced within the Republic of South Africa originates from the Mpumalanga and Limpopo provinces, where most of the commercial farmers are situated, while there are a few large commercial growers in the Pongola region of KwaZulu-Natal, and most of the small-scale farmers originate from the Makhathini Flats (KwaZulu-Natal).

Plate I. Cotton production areas of the Republic of South Africa.



According to Bennett et al. (2005), Bollgard™ cotton was initially available to growers in Delta and Pinelands varieties, namely NuCOTN 35^{B} and NuCOTN 37^{B}. These have been replaced with the variety NuOpal. All contained the event line 531, which produces an endotoxin or protein, Cry1Ac (Akehurst 1993). They also mentioned that research with a second event (Mon 15985), which produces the Cry2Ab2 protein for bollworm resistance, has been ongoing and the field trials on cotton have already been undertaken. Current effort is being directed to commercial approval of combinations of genes, the so-called stacked gene varieties, where more than one event is present within a single plant. These stacked gene varieties, or double gene varieties, would express more than one trait, rendering it to be both herbicide-tolerant and insect-resistant

at the same time. At present the NuOPAL RR cotton type is available in Stackgene formation, providing both bollworm resistance and glyphosate tolerance.

In order to understand bollworm resistance as expressed by Bt-cotton, it is important to recognize the particular protein in the cotton type planted, as well as the mode of action of the Bt-protein in the pest insect. Chilcott and Wigley (In: Akehurst 1993) give a review of the many Bt-crystalline proteins identified. The mode of action of the Bt-protein (delta-endotoxin) is described by many authors, including Knowles (1993), as cited by Akehurst (1993).The Bt-protein is often referred as the Bt-gene, or in specific the *cry1Ac* gene or *cry2Ab2* gene[2]. The delta-endotoxin (also referred to as a protoxin) is synthesized as an insoluble crystal protoxin, which is ingested by a bollworm. The protoxin dissolves in the alkaline environment of the insect gut and becomes proteolytically activated in the gut. It then binds on specific high-affinity receptors on the brush border of gut epithelial cells (Akehurst 1993). After binding to a receptor, the toxins diffuse into the plasma membrane forming toxic lesions, which in turn leads to cell lyses, breaking down the permeability of the gut. Intracellular contents leak into the gut lumen and the gut becomes paralyzed because of changes in the electrolyte and pH balance. The insects then stop eating and die of starvation or septicemia, normally within about 72 hours (*cf*. Bennett et al. 2005).

In this study, two proteins are dealt with. The Cry1Ac protein (Part I of chapters 2, 3, 4 & 5) as well as the Cry2Ab2 protein (Part II of chapters 2, 3, 4, 5). It is stated (Anon, 2002) that the Cry2Ab protein has a high degree of amino acid sequence similarity (97 percent) to the Cry2A protein, which is another component of various *B.t.* microbial products, and has been evaluated in numerous animal and human studies. The company concerned (Monsanto, USA), with the initial research on molecular level has also provided data and information on the allergenic and toxic potential of the Cry2Ab protein (Anon 2002). The Cry2Ab protein, and in particular the Cry2Ab2 (sic!) protein which is mentioned in this study, rather refers to the same protein (Cry2Ab) with a specific amino-acid structure which differs minimally from example, the Cry2Ab1 protein, with similar efficacy on the target organisms. The last number of the protein represents a measure of amino-acid structural difference between proteins (pers comm. W.M. Green, formerly from Monsanto SA).

The nomenclature of the different proteins is a study in itself, and for the purpose of this study, the Bt-protein concerned will be referred to as the Cry2Ab2 protein, and this protein is subsequently referred to in the P2B study of each chapter (Part II) (see Chapters 2, 3, 4 & 5).

The economic control threshold for the three-bollworm species is regarded as more than five bollworms per 24 plants scouted, after Basson 1987. For Bt-cotton, the threshold has been adapted to more than five plants found with one or more bollworms out of the 24 plants scouted (Anon, 2006). Basson (1987) also gives recommendations concerning which pesticides to apply at various cotton growth stages. Of importance is the fact that no pyrethroids should be used between eight and eleventh weeks after plant emergence. Pyrethroids should be avoided where

[2] In this study, the terms "*cry1Ac* gene" and "c*ry2Ab2* gene" respectively refer to the genome or "gene" which is related to the DNA structure in the nucleus of a cell. The terms Cry1Ac and Cry2Ab2 refer to the protein or endotoxin, as discussed above.

possible after this period as well. Pyrethroids are generally highly toxic to insects and are not selective (i.e. they are very effective against predators and parasitoïds) and when abused, give rise to outbreaks of secondary pests such as mites and aphids. Basson (1987) authored the guidelines for integrated control of various cotton pests. In it he states that it is generally not necessary to apply any form of control within the first eight weeks after plant emergence, except in exceptional cases. He recommended the intensification of scouting from approximately six weeks until 20 weeks after emergence, with scouting to be undertaken at least once a week. A cotton field should be divided into blocks of approximately 15 ha in extent, with the observations made in each block determining the control times in that block. Blocks should contain cotton of the same age, should not be geographically divided (e.g. by a vlei, hillock) and should not contain irrigated and rain-fed cotton. According to Basson (1987), 24 plants in each block should be inspected by thoroughly examining the entire plant including young bolls and squares for eggs and larvae of bollworms. This inspection should take an average of five minutes per plant. The pests found on the plant are then recorded, and should they equal or exceed the economic control threshold, control should be applied in that block as soon as possible. The management of Bt-cotton should include a strict scouting procedure to monitor bollworm levels, and the efficacy of the gene under the farmers' specific environmental conditions. Secondary pest levels should also be monitored.

In order to understand the movement (dispersal) of bollworms between cotton plants and other host plants, it is important to explain their biology and life cycles. A full detailed description of the appearance of all the bollworms, and their stages will be given in this study. References used include: Broodryk et al. 1974, Pearson and Darling 1958. The build-up of the infestation in the crop is essentially a gradual one, with no sudden influx of egg-laying moths at a particular stage during the season, as with H. armigera. A broad range of crops is hosts for H. armigera, and various wild plants complement them. This enables the pest to develop on different hosts, to possibly continue development on successive hosts and to persist in small populations in seemingly unsuitable areas (Vaissayre, 1994). In the literature, it has been found that bollworm larvae occur on a number of different host plants that occur naturally in the veldt.

Resistant management strategy for Bt-cotton

Bennett-Nel et al. (2005) mentioned that for insect-tolerant crops, the question of possible resistance to the Bt-gene is a critical one, since insects are capable of quickly developing resistance to chemicals. Research has also shown that under specific conditions, bollworms have developed resistance to the insecticidal protein produced by B. thuringiensis. It is thus essential that an Insect Resistance Management (IRM) strategy be put in place with GM crops to prevent the development of resistance for as long as possible and so preserve the benefits of this technology. A resistance strategy was chosen by the company concerned, by selecting different Bt-proteins for introduction into different cotton events, i.e. Cry1Ac and Cry2Ab2 proteins, originating from the *cry1Ac* and *cry2Ab2* genes, with similar efficacy but functioning at different receptor sites in the bollworm larvae. Insects develop resistance against a particular

substance by decreasing the number of receptor sites available for functioning for example in the stomach lining, by changing the structure of the receptor sites so that they do not match the amino-acid structure of the protein. Gould (1994) mentioned that there are four basic tactics for resistance management when using transgenic plants, which includes very high doses of one or more Bt toxins, mixtures of plants with high doses of toxin(s) and plants with no toxin expression, low doses of toxin interacting with natural enemies, and targeted toxin expression which would be tissue-specific for example, or time-specific, or variation in binding properties of specific proteins etc. In the case of introducing two different proteins in stacked gene combination (Cry1Ac & Cry2Ab2) as is illustrated in this study or as a single second protein (Cry2Ab2) with different target sites in the target organisms, the possibility of bollworms to develop genetic structural changes to combat the affect of the protein, is minimized or counteracted. The specific mechanism of working that these proteins have within the target insect is beyond the scope of this study, but both proteins will be shown to be similar in efficacy to control bollworm populations.

Another resistance management strategy was introduced by complying with the requirements of the GMO Act. In the Republic of South Africa, as part of an IRM strategy, the GMO Act, Act 15 of 1997, requires a refuge area to be planted where biotech crops are grown. According to Mallet and Porter (1992), by planting refugia, or toxin-free, non-transgenic cotton plants on the same cultivated land, the susceptible genes in the bollworm population can be conserved. If bollworms were to reach maturity in a field of Bollgard™ cotton, the specific population could give rise to resistant individuals due to the presence of a resistant gene. If the offspring of these potentially resistant larvae were to mate with one another, the survival of the resistant/tolerant gene in the population will be ensured. If, however, a susceptible population has been maintained in the toxin-free refugia, the probability is higher that potentially resistant moths (from the Bt-crop) will mate with moths from the larger susceptible population (from the refugia), resulting in dilution of resistant genes and production of susceptible offspring, ensuring susceptibility to the Bt-toxin for a longer period of time (Anon 1999).

Monsanto SA Ltd (the company commercializing biotech products in South Africa) offers a choice of two refuge options to be planted with Bollgard™ cotton. For each 100 ha of Bollgard™ planted, the farmer can plant a refuge of either 20 ha sprayed non-transgenic cotton, or 5 ha unsprayed non-transgenic cotton (Anon 1998 and Anon 1999). Upon purchase of seed, the buyer signs a license agreement with the company stating that one of the two choices will be followed. Refugia must be planted as close as possible to the Bollgard™ field and must be planted as a separate block, not mixed with the Bollgard™. The refugia should preferably not be further than 500 meters away from Bollgard™ fields and must be planted at the same time, and managed in the same way, so as to provide a suitable habitat for Bt-susceptible individuals.

Some problems can occur with the planting of refuges in the field, especially in a small-scale farmer set-up, where farmers only plant a few hectares of cotton and so may neglect to plant the refuge areas. In order to find some alternative to compliment this resistant management strategy, a host plant study was undertaken to determine the significance of alternative bollworm

host plants and the implications thereof on the refuge system in a small-scale farmer setup (Bennett-Nel et al. 2003a and 2003b). According to Monsanto SA, the monitoring of the recommended refuge system is an ongoing process, and to date, there have been no documented cases in South Africa of resistance to bollworm under normal growing practices.

Alternative resistance strategies

No resistance has been documented at this stage of bollworms against any of the registered insecticides. Bt-sprays have been available for many years in the Republic of South Africa and no cases of resistance have been reported. In cotton growing countries such as the USA, and in Australia, resistance has, however, developed. One of the reasons why South Africa escaped this build-up of resistance to insecticides may lie in the presence of natural bush and other crops surrounding many cotton fields, which act as natural refuge areas where the susceptible genes could be maintained. The naturally occurring surrounding vegetation was therefore investigated for the presence of bollworms, as well as other crops planted in the vicinity of Bollgard™ cotton. The study (Chapter 6) was extended to include the monitoring of bollworms on alternative host plants, with the aim, to get approval from the authorities to void the planting of refuge sites, with the acceptance of the hypothesis that there is enough movement of bollworms from alternative natural host plants towards cotton, so that potentially resistant populations can interbreed with "wild" populations to dilute to possible resistant effect.

The american bollworm ("african bollworm" sic!) has been associated with a number of crops (Pearson and Darling 1958; Parsons 1939, 1940), such as barley, beans (various), Cape gooseberry, chick-pea, citrus, cucurbits, cotton, flax, groundnuts, hemp, lucerne, maize, oats, peas, pigeon-peas (Cajanus cajan), sunflowers, sorghum, tobacco and tomatoes. The african bollworm has also been found on various indigenous plant species such as Acalypha segetalis (Euphorbiaceae), Amaranthus thunberghii (Amarantaceae), Ipomoea cordofana (Convulvulaceae), Malvastrum tricuspidatum (Malvaceae), Nicandra physaloides (Solanaceae), Portulaca oleracea (Portulacaceae), Sonchus oleraceus (Compositae), Tridax procumbens (Compositae) and Xanthium pungens (Compositae). Other plant families on which the african bollworm has been found include Chenopodiaceae, Leguminoseae, Gramineae, Linaceae, Rutaceae, Cucurbitaceae, Capparidaceae and Labiatae. H. armigera is widely distributed in Africa, southern Europe, the Near and Middle East, India, Central and Southeast Asia, Japan, the Philippines, Indonesia, New Guinea, eastern Australia, New Zealand and Fiji, and in these areas it constitutes a serious pest of many crops and in ornamental gardens. In the Republic of South Africa it is a primary pest of peas, various beans including soya, maize, sunflower, wheat, grain sorghum, oats, barley, tobacco, citrus, cucurbits, potato, tomato, lucerne, sun hennup and groundnuts, in addition to cotton (Annecke and Moran 1982). It has been shown that H. armigera visits many of the above crops when they are in flower, or about to flower, for the purposes of oviposition (Parsons 1940). The occurrence of bollworms on alternative host plants will be described in Chapter 5. Helicoverpa armigera (Hübner), also known as the Old World bollworm, the cotton bollworm, and locally, the "african" bollworm, is a pest of many American crops

(Pearson and Darling 1958). This insect is also generally known by its synonym, Heliothis armigera, and much of the relevant literature still refers to it under this binomial. In South African cotton, H. armigera is arguably the most destructive insect, and consequently insect control in cotton is directed to eliminating this pest. This study will present an overview of alternative host plants for the "african" bollworm, as found in the Makhathini.

Unlike D. castanea, Earias spp. does not originate as a result of a sudden moth flight derived from long-term pupae (Pearson and Darling 1958). Cotton is not a first choice of host plant for Earias spp., Abutilon and some Hibiscus species seem to be preferred. Spiny bollworms may carry over from one cotton season to the next on alternative host plants, or may multiply early in the season before the cotton is available (Pearson and Darling 1958). A number of plant species have been found to be hosts for the spiny bollworm species including Abutilon indicum, A. sonneratianum, Althaea rosea, Cienfuegosia gerrardii, C. hildebrandtii, Corchorus olitorius, C. tridens, Hibiscus calycinus, H. cannabinus, H. esculentus, H. rosa-sinensis, H. sabdariffa, Gossypium spp., Malva parviflora and Sida spp. (Broodryk et al. 1974, Pearson and Darling 1958, Williams 1933). Red bollworm, D. castanea, has been recorded to have a very confined host range (Pearson and Darling 1958), limited to the genera Gossypium (G. hirsutum, G. herbaceum and G. barbadense), Gossypioides (G. kirkii) and Cienfuegosia (C. hildebrandtii). Gossypium herbaceum subsp. africanum indigenous to the eastern lowveld areas of the Republic of South Africa (Hudchinson et al. 1947). Single records have also been found on Thespesia sp. and Abutilon spp.

No discussion of bollworm control in southern African cotton would be complete without mentioning the work done by G.A. Matthews and J.P. Tunstall (1968). They were the first to realise that the same yields could be produced by fewer pesticide applications, if the timing of these applications was correct. With Bt-cotton, management strategies should follow scouting procedures to determine if threshold values are met, whatever the circumstances under which the varieties or cotton types[3] are grown. Biotechnology presents the South African farmers with the opportunity to spray considerably less on Bt-cotton, and at the same time achieve higher yields.

Quality of Bt-cotton

Jenkins et al. (1991) have noted that foreign genes and tissue culture did not have a major adverse effect on fibre properties in transgenic strains. Independent transformation events may however, lead to consistent differences. Quality of cotton is directly link to price, which is indirectly linked to yield. By comparing fibre quality parameters (strength, length, length uniformity and micronaire) across different technology types, i.e. single gene introduction (Bt-cotton or RR-cotton), Jordan and Wakelyn (2002) found that fibre quality is just as likely to have been improved as not. They found no evidence that genetically engineering cotton has had a practical effect on fibre quality. In South Africa, Joubert et al. (2001) found no significant

[3] Cotton types is the preferred citation used by the company, Monsanto SA, who holds the patent rights for NuOPAL and NuOPAL RR, since the original variety was not altered by introducing an additional gene, and the original variety's characteristics is still expressed after the gene modification.

differences in length and strength between Bt- and non-Bt-cotton under irrigated and rain-fed conditions, although the micronaire was found to be lower in both Bt- and non-Bt-cotton under rain-fed conditions. Gin out Turn (GOT) was found to be higher in the case of Bt-cotton when compared to non-Bt-cotton (Joubert et al. 2001). They mentioned that the fibre properties of Bt-cotton were found to be more acceptable than those of non-Bt-cotton during studies in South Africa performed in the 1999/2000 and 2000/2001 seasons (Joubert et al. 2001).

The primary pest complex: Bollworm complex

The primary pest complex occurs on conventional as well as genetically modified cotton. These bollworms are the main pests on cotton and include the american or “african” bollworm Helicoverpa armigera (Hübner), the red bollworm Diparopsis castanea Hampson and the spiny bollworm species Earias biplaga Walker and E. insulana Boisduval. They are all able to cause serious economic damage to the crop (Pearson and Darling 1958). Bt-cotton is more prominent in the commercially irrigated fields, whereas the red bollworm is more common in stand-over or ratoon cotton, and in the dry land regions of the Makhathini Flats (Northern KwaZulu-Natal). Ratoon cotton is not regarded as the most suitable farming system to follow, but in the Roedtan area near Loskop that has a below average annual rainfall (below 600 mm), stand-over cotton is the preferred choice and yields obtained is financially viable, when cotton is compared to other commercial crops. A study by Bennett-Nel et al. (2003a) has shown that red bollworm and spiny bollworm is more prevalent than african bollworm. This is probably due to the availability of more red and spiny bollworm in the northern KwaZulu-Natal region. On Bt-cotton, bollworm numbers if present are very low (Bennett-Nel et al. (2006), due to the inherent resistance of the variety with the added Bt-gene. However, to understand the influence of the genetically altered variety and its impact on the pest complex, it is important to have a clear indication of the life-cycle of the bollworms and the significance thereof in relation to the availability of an artificial refuge or its potential to occur on natural host plants in the vicinity of Bt- cotton.

African bollworm (Plate II)

The african bollworm is known to occur in high population densities during three peaks or moth flights during the season. The cotton season in South Africa starts at the beginning of October and ends at about April-May the following year. The basic life-cycle is followed where the female moth lays the egg. Egg hatching occurs three to four days after egg laying, depending on temperature. The egg is a shiny oval shaped egg, 0.4-0.5 mm in diameter (Pearson and Darling, 1958), with longitudinal ridges which can often be observed with a magnifying glass.

Eggs are usually laid on the adaxial side of the leaf or on squares, singularly, or it may vary from two to five. Eggs can also occur on the abaxial side, or on the stem of the leaf, but this occurs to a lesser extent. Larvae go through seven to eight larval stages. The neonate larvae are very small and difficult to see. The larvae feed on young buds or squares, and an entrance hole is often visible at the basis of a square. Older larvae can be found in flowers, and when feeding here, they often are greenish to yellow in colour. The older larvae are often found within a green

cotton boll, where most of the unripe fibre is eaten. These bolls will eventually rot, become wilted and drop off. The lateral white stripe, to cotton growers, is still the most diagnostic feature of the larvae. Three pairs of parapodia, and a final pair of legs at the end of the abdomen characterizes the abdomen. These three pairs of parapodia, distinguishes bollworm larvae from all other leaf-eaters, with which bollworm larvae are often mistaken for by cotton growers. Larvae can be aggressive when in competition with other larvae, and younger larvae have seen to be cannibalistic in artificial surroundings when food is scarce.

Plate II. Graphical illustration of the life cycle of the american or "african" bollworm.



African bollworm larvae can be bred successfully under laboratory conditions. The final instar larva will drop to the ground to pupate in cracks in the soil underneath the plant. The pupa is brown with concentric rings, and somewhat longer than that of the red bollworm. The female pupa is slightly shorter than that of the male. Pupation is regulated by ambient temperature, and normally takes two to three weeks. In warmer seasons, these pupae can hatch and larger hatchings can result in additional unexpected moth flights. African bollworm feeds on a number of

host plants and a large number of crops (see Chapter 5). Female moths are attracted to pheromone traps and counts by means of pheromone traps are a reliable method to monitor population densities. The number of generations varies between three to five per season, but it may be as high as seven generations. On cotton, however, more than three generations are very seldom seen in South Africa.

Red bollworm (Plate III)

The red bollworm is prominent in dry land cotton most probably because african bollworm is adapted to other host plants, while red bollworm feeds only on cotton as a commercial crop. Additional host plants have been identified for red bollworm, and these will be discussed in Chapter 5. The female moth lays her eggs on the leaf petioles, the abaxial side of leaves, the smaller branches and sometimes on the main stem. The eggs appear to be somewhat bigger (0.5-0.7mm in diameter) (Pearson and Darling 1958) and flatter than that of the african bollworm, and appears to be concave when viewed from above, with an indentation on the upper side of the egg. The egg walls appear to be spiky, in comparison with the longitudinally smooth margined african bollworm egg. The colour varies from yellow to white-blue, while becoming slightly darker close to the time of hatching. Larvae hatches within two to three days, and the neonate larvae are very difficult to distinguish from the african bollworm neonate. The red bollworm neonate larvae appear more hairy when observed through a magnifying glass. Similar to african bollworm, the first instar before feeding is characterized by a prominent pronotum with a sclerotized pre-scutellum, while in the feeding stage the pronotum is more definite, reaching the lateral margins of the thorax. This characteristic is often used in scouting on cotton, to distinguish neonate larvae from 1st instar larvae that have already initiated feeding. Larval instars vary from four to five, and from the second instar the larvae are distinguishable by red markings on the dorsal side of the pronotum. Larvae feed in squares, seldom in flowers, but more commonly in cotton bolls. Most of the bolls that are damaged by bollworm, are damaged by red bollworm larvae. Red bollworm often damages all the loculi within a cotton boll. Larvae enter young bolls already in the 2nd instar, and feeds within the boll, for most of its larval cycle.

Larvae can reach up to seven instars and they leave the boll when nearing pupation to drop to the ground and enter the ground through cracks in the soil. Pupation takes place in a soil cell or mud cell that is made from their excrement. Pupation can vary from two to three weeks in summer (personal observation) or it may take up to five years depending on temperature and moisture conditions (Pearson 1958). Pupation normally takes place from about May to September during the winter months in South Africa, where moths emerge to lay eggs on alternative host plants (see Chapter 5). Red bollworm goes through five to seven generations within one season. This explains the high population pressure in some cotton growing regions. It is in these regions where ratoon cotton is still cultivated, although it is not the preferred practice. Due to the availability of cotton very early in the season, red bollworm seems to have a shorter cycle and they are more common in these regions due to the availability of a food source. Moths

are brownish in appearance with red markings on the front wing. They are attracted to light traps. No pheromone is commercially available for red bollworm.

Plate III. Graphical illustration of the life cycle of the red bollworm.



Spiny bollworm (Plate IV)

Spiny bollworm is common in all cotton growing regions, and the impact that spiny bollworm have on cotton is often under estimated. In the Makhathini region, spiny bollworm is more common than african – and/or red bollworm, probably due to the many host plants that exists in the area (see Chapter 5). Two species of spiny bollworm is present in South Africa, but a taxonomic revision of the different species that occur on cotton is long overdue. Many times, different moths and larvae are found, and few people can distinguish between the species on cotton based on the larval appearance. Larvae that seem to be orange-brown in appearance seem to be E. biplaga, while the more yellowier-greener larvae seems to represent E. insulana (Pearson and Darling 1958). Other characters given by Pearson and Darling (1958). Other characters given by

Pearson to distinguish between larvae are difficult to follow as an identification key in the field. The larvae of both species occur commonly in the flowers, and to a lesser extent in young bolls.

Plate IV. Graphical illustration of the life cycle of the spiny bollworm.



Larger larvae are seldom found in more mature or bigger bolls. The dorsal surface of the egg, is characterized by a corona by means of about 30 longitudinal ridges, of which alternate ones project upwards to form a crown or corona (Pearson and Darling, 1958). When viewed from above (dorsal view) the corona appears like “spines” that are arranged in a circular fashion. The moths lay an oval shaped light blue-green egg, which is slightly less than 0.5mm in diameter (Pearson and Darling, 1958). Eggs hatch after 2-3 days, and the neonate larvae appear blackish and have a very hairy appearance at this stage. They are easily distinguishable from african– or red larvae. They go through about seven larval instars and form a cocoon made from silk. The cocoon with the pupa inside can be found on the plant parts, usually in a fold between small leaves, or in the fold between the square (flower bud) and the outer calyx leaves. The cocoon hatches after a few months of hibernation. Pheromones are available for monitoring of both

species, but the moths are also attracted to light traps, but to a lesser extent than the red bollworm. Spiny bollworm occurs on many alternative host plants, some of which are present when no cotton is available. This might explain the increase of populations in some areas (see Chapter 5).

Scouting: The pegboard system

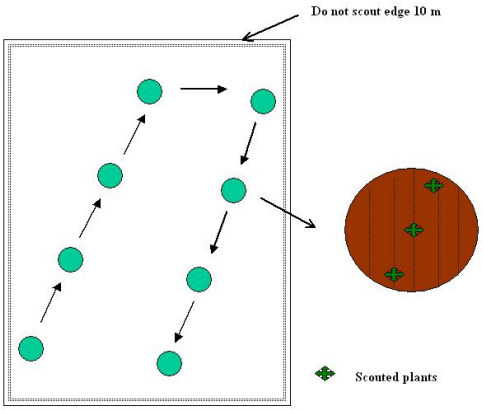
A wooden block with 6 coloured lines as indicated on Plate V is used as a scouting tool[4] (Anon, 2006). Each line has a number of holes in which a match can be fitted after noticing the required number of insects. The green line represents 24 plants. Plants are marked consecutively by moving the match further along the coloured line, on approaching more plants. The yellow line represents aphids, and three leaves (top, middle and bottom leaves) are scouted for the presence of aphids. If more than 30 aphids are found on three leaves, the match is moved to the next entrance hole in the yellow line. The yellow and blue line each has twelve holes, and spraying is recommended when the match reaches the bottom of the block in the last hole. The blue line represents leafhoppers or jassids, the name originating from the scientific family name, Jassidae. Three leaves per plant (top, middle and bottom leaves) are scouted and if more than three jassids are found in total on three leaves scouted, the match is moved to the next hole. With other words, more than three jassids per plant would indicate to move the match downwards. The threshold for leafhoppers is reached when the match reaches the 12th hole. The pink line represents six holes for bollworms. On Bt-cotton the presence of one or more larvae per plant are marked. In the case of Bt-cotton, it is rather the number of plants with bollworm, which is recorded, than the presence of larvae. The presence of all three species is marked on a particular plant and when the 6th hole is reached, it indicates spraying. The threshold for bollworm is reached when more than five plants out of 24 plants have been found with one or more larvae.

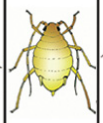
The red line represents stainers, each of the 6 holes represents a family group or focal group of stainers found. At least two adults and a number of nymphs indicate a family group. Family groups refer to common cotton stainers. The green stinkbug can be marked individually. One bug per plant would indicate to move the match. The black line represents the beneficial insects-, spiders or other beneficial invertebrates. Presence of beneficial insects counted per plant is indicated by each move on the block. In instances where aphid numbers are nearing the threshold and when beneficials are numerous, one would resume from spraying to give the predators a chance to control the pests. When the scouting per field has been completed, one would record the results on the recording sheet (Plate V).

[4] The pegboard system used in South Africa is adapted by the late Dr. SW Broodryk (pers comm.), from the system used in Kadoma Training Centre (Zimbabwe), during 2002. Adaptations for Bt-cotton was made accordingly by Broodryk and Bennett-Nel, since research gained from 1997-2002 indicated that in different environmental conditions the expression of the Bt-gene in cotton plants may vary, leading to particular plants to be less bollworm resistant and more larvae occurring on these. Immediate weed control in the surrounding areas of particular plants may also influence bollworm numbers. Three larvae on a particular plant scouted may not necessarily indicate a high population density in the field.

Plate V. Illustration of the scouting layout, pegboard system and recording booklet used for scouting pests on cotton.





	No. plants Total 24	Aphids per 3 leaves	Jassids per 3 leaves	Plants with one or more bollworms	Stainers (family groups)	Natural enemies (present per plant)
Date: _____						
	green	yellow	blue	pink	red	black
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						

The system introduced has yet to be proved scientifically and in the absence of any other monitoring system on Bt-cotton, it has been used successfully for the past four years. Scouting thresholds has been discussed by Basson (1987). However, threshold for bollworms is recorded as a minimum of more than five bollworms per twenty four plants indicates spraying. However, in the case of stress conditions such as drought, flooding, excessive weeds in a field or other host plants, some Bt-cotton plants may exhibit a lower expression of the Bt-gene, resulting in the survival of some larger bollworm larvae on some plants, or larger larvae which may originate from weeds or other host plants. Small larvae may survive in the flowers of these plants and reach larger instars. It has been found that the Bt-gene is not as well expressed in the flowers than in the squares and bolls and some larvae may survive in the flowers. This phenomenon may result in an incidence where the threshold for bollworms is reached when a few plants (two to three, or four to five plants) are scouted and the minimum larvae indicating spraying is reached. On Bt-cotton the threshold is therefore determined as a minimum of five plants with one or more larvae out of twenty four plants scouted.

Bollworm larvae on Bt-cotton have been found to disperse quicker to the lower fruiting bodies of the plant than bollworm larvae on non-Bt-cotton by Gore et al. (2002), and they mentioned that more work is needed concerning a scouting system on Bt-cotton. In South Africa, all fruiting bodies are scouted for bollworm, and the absence of bollworms in the terminal plant parts are more a result of the efficacy of the gene expression within the plant, and the system as described above to use plant infestation rather than bollworm numbers have been found to be effective.

All stages or larval instars of bollworm should be counted during scouting. Bennett (-Nel) et al. (1999) mentioned that In NuCOTN 37^{B} (containing the Bollgard^{TM} gene) all neonates and larvae upto the second instar of the bollworm complex (L1-L3) were effectively controlled throughout the season. This phenomenon was true for 1st, 2nd, and 3rd instar larvae from Rustenburg. The number of small larvae was significantly lower on the Bollgard treatments than on the conventional cultivar, DpAC 90 treatments.

The efficacies of the Bt-gene to control bollworm populations cause highest mortality rates against neonate larvae and first instar larvae or L1 larvae. Under stress conditions, the expression of the gene in different plant parts may be affected, either as a result of an environmental stress factor, or because of a high number of secondary pest insects present. During scouting it is important to record first instar larvae that have not yet feed at the time of scouting (Matlou et al., 2000). These neonate larvae that have not fed yet, show a distinct short or narrow pronotal shield and the body appears short. Neonate larvae that have ingested plant tissue, show a longer and wider pronotal shield and the larvae appear longer (pers comm. Dr. Kater Hake[5]).

Neonate larvae that has fed, will soon succumb as a result of the endotoxin. These larvae should be distinguished from L1 larvae or larvae which has off course been feeding for a time and have survived. First instar larvae may survive when they occur in flowers where the

[5] Dr. Kater Hake is a specialist on biotech cotton and plant breeder for Delta and Pinelands, USA.

Bt-gene expression is not as high as in other plant parts. The larvae counted and recorded on Bt-cotton (excluding neonate larvae) can appear to be lower than the larvae on conventional cotton at the same time of scouting, and appear to be below the threshold line. When taking the neonate larvae into account, larval numbers may increase and indicate that the plants experience “some kind of stress” and that the scout should be repeated in 2-3 days to re-examine the effect of the Bt-gene on all larvae (Matlou et al., 2000). Distinguishing between these larvae is important in taking a decision when to spray in order to limit input costs. Neonate larvae cannot be omitted from the total number of larvae on Bt-cotton, and the same is true for conventional cotton, since larvae on conventional cotton will probably survive, if left unsprayed or in case of a low natural mortality rate.

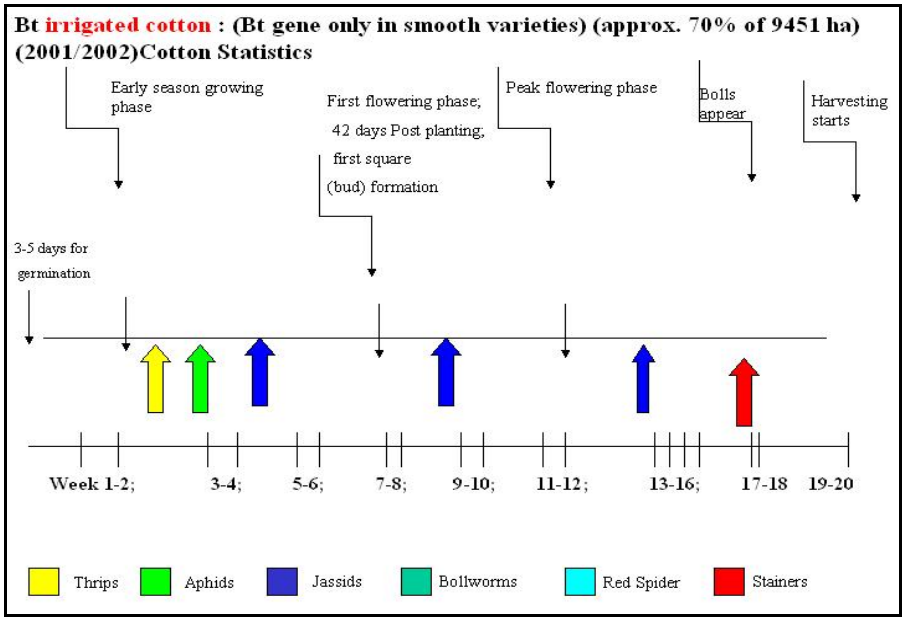
Spraying programmes used at present on Bt-cotton

Pest control in the Republic of South Africa is carried out by following and Integrated Pest Management (IPM) approach. However, many small-scale farmers are only now at the stage where they are becoming more aware of the concept of IPM through extensive training activities that are launched as part of a new National Strategy Plan for cotton production. In most instances, commercial growers and some small-scale farmers follow a calendar-spraying programme. The window period for pyrethroids are practiced in some instances, but often farmers will spray what is sold and recommended to them by pesticide agents. Some of these pesticides are not always the most appropriate, most cost-effective pesticide with the least effect on predators and the environment. Up to March 2003, organophosphate, i.e. Monocrotophos® has been commonly used. This chemical has fortunately been taken off the market due to its harsh effect on the environment and on beneficial insects. Some industry representatives still believes bollworm and secondary pests like aphids and leafhoppers should be controlled by such drastic measures, and it will still be a year or two before old stock on farms is depleted. The approach now is to train farmers in the use of the peg-board system. This system is very practical and commercial farm scouts, small-scale farmers that are illiterate can use it. The pegboard system follows a similar approached used in Zimbabwe. It is practical and one can move about within a cotton field without carrying paper and pencil. The results can be recorded on a recording sheet in the form of a coloured-in diagram, so that records can be followed throughout the season.

In order to understand the benefits associated with planting Bt-cotton, it is important to know what the spraying regime is on Bt-cotton under dryland and irrigated conditions. Bollworm control, based on scouting, is still an important aspect in the production of Bt-cotton. Preliminary research of pest insects on Bt-cotton resulted in the suggested control programmes as shown in Plates VI and VII under irrigation and rain-fed conditions respectively (Anon 2006). These spraying regimes for 2006 and 2007 form part of the learning material of the Cotton SA skills development programme at present. Mainly aphids and leafhoppers appear to be a problem early in the season. Spraying is usually recommended for these secondary insects.

With the introduction of Bt-cotton in South Africa, the pest complex has shifted from bollworms being the important pests to the secondary pest complex being more prominent. About 80% of all commercial farmers have accepted this new technology, while the figure under small-scale farmers is close to 100%. More emphasis is placed on scouting on secondary pests. On irrigated cotton, spraying is aimed at controlling secondary pests early in the season (thrips, aphids and jassids) with a single or more bollworm sprays at peak fruit formation phase if scouting results justify spraying for bollworm (Plate VI).

Plate VI. Insect occurrence and spraying programme as suggested for irrigated Bt-cotton in South Africa.



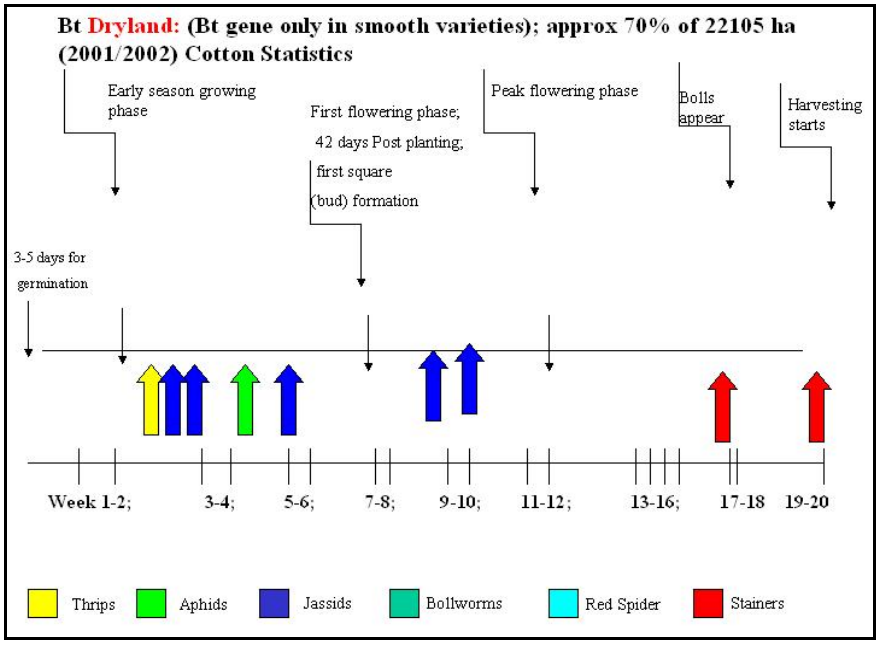
On rainfed cotton, jassid infestations are often more prevalent and this requires a number of additional sprays to be applied aimed at jassid control, since predators for jassids are scarce and they do not seem to have a significant effect on the jassid population build-up (Plate VII).

Main objectives of the study were to determine the efficacy of the Bt-gene in different gene combinations to control bollworms on cotton and demonstrate the influence of bollworm suppression and fewer bollworm sprays on secondary pest populations, yields obtained, and arthropod biodiversity within a Bt-cotton field. Furthermore to determine the influence of alternative host plants on bollworm density and diversity in a small-scale farmer set up. The acceptance of modified cotton by cotton-producers was also determined.

The trial design and experimental layout of the different experiments are reflected within the material and methods of the following chapters. Detailed information of each trial is provided in Chapter 2 and afterwards referred to. Important dates of activities are given in each chapter. Most of the outcomes are based on scouting assessments. Scouting is the consecutive counting

of insects on a number of plants, over a specific consistent period, in order to determine population fluctuations. Scouting was performed on genetically modified cotton types as well as conventional cotton.

Plate VII. Insect occurrence and spraying programme as suggested on rain-fed Bt-cotton in South Africa.



In each instance the following parameters were noted:

1. The number of bollworms of all species present. The instars of these larvae were noted, to determine if the first and final instars of the species are present.
2. The identity and numbers of bollworm eggs.
3. The presence of aphids, whitefly, spider mites, thrips and leafhoppers.
4. The numbers and identities of beneficial arthropods.

Spraying or chemical control with a registered chemical product was applied when pest numbers reached threshold values. Where appropriate, the yields of the different treatments were compared in order to evaluate the influence of bollworm resistance of the cotton types on yield.

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CHAPTER 2

THE EFFICACY OF GMO-COTTON TYPES (TRANSGENIC COTTON "VARIETIES") TO SUPPRESS BOLLWORM POPULATIONS UNDER SOUTH AFRICAN CONDITIONS

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Abstract

The Bt-gene effectively controlled the total number of bollworms observed on the Bt-cotton treatments at all localities during 1997/1998, when evaluating the *cry1Ac* gene for bollworm susceptibility. Bollworm numbers appeared to be significantly lower on the Bollgard™ treatments than on the conventional cotton.

The evaluation of the *Cry2Ab2* treatments included a transgenic 15813 Insect Protected Cotton line (IPC line); a 15985 IPC line, the transgenic control variety DP 50B (Deltapine 50 with Bollgard™), and a non-transgenic reference variety, DP 50 (Deltapine 50). The trial size was 0.507 ha (1.07acre), laid out as a randomized block design of four cotton treatments with 4 replicates of each treatment. Each plot comprised of three rows, of 4.5m (15ft) in length and 2m (6.6ft) apart, containing 30 plants per row and 90 plants per plot. The distance between each plant within a row was 15 cm (6 inches). Each plot was separated by four rows of non-transgenic cotton (DP 90). The ends of each plot were separated by a 3m (10 ft) alley. All three species of the bollworm complex, e.g. Helicoverpa armigera, Diparopsis castanea and Earias spp. on the four localities tested in South Africa, were effectively controlled by all the transgenic cotton treatments, 15813, 15985 and DP 50B, and significantly different from the DP 50 treatment. The transgenic cotton treatments have similar efficacy in controlling the bollworm complex irrespective of the bollworm species concerned. The aim of this study was to compare bollworm resistance to the Bt-gene, expressed as the *cry1Ac* gene, as well as the effects of transgenic cotton on other pest and predatory populations, at 4 localities.

In determining the efficacy of an insect resistant cotton line, 15985 (in stacked gene combination (*cry1Ac* and *cry2Ab2*) for resistance against the bollworm complex (2000) it was found that all three species of the bollworm complex, i.e., Helicoverpa armigera, Diparopsis castanea and Earias spp. were monitored on the three localities within South Africa. With regard to the different bollworm species, the transgenic cotton treatments are equally effective in controlling the bollworm complex, irrespective of the bollworm species concerned. With regard to larval instar, the transgenic cotton treatments are equally effective in controlling all bollworm larvae, irrespective of the size or instar of the bollworm larvae concerned. As expected, the sprayed control treatment (DP50s) gave significantly better bollworm control than the unsprayed control treatment (DP50u). On at least one locality, there were no significant differences in bollworm control between the transgenic 15985 treatments containing the Bollgard™ gene for the total number of all bollworms over all localities. On the other two localities the same tendency was observed. The Bollgard gene in DP50Bu provided effective control of bollworms, by giving a low number of bollworms (< 20) in the same range as other transgenic treatments. The Bollgard gene proved to be just as effective as the *cryX* gene in suppressing the total number of bollworms. It seemed that on some localities, the Bollgard gene could be better expressed in one variety than in another. At Nelspruit, it was better expressed in the DP50 variety than in the 15985 line, most probably because the amount of endotoxin synthesized and expressed is influenced by external factors which cause plant stress and which affect the two varieties differently. When a very high density of bollworm larvae occurs, as for red bollworm at Nelspruit, the sprayed transgenic

treatments (sprayed according to threshold) prove to have more potential to suppress the higher numbers. This emphasizes the importance of scouting as part of an Integrated Pest Management (IPM) system, for the control of bollworm on genetically modified cotton types. No difference in the total amount of plant damage (squares, flowers and bolls) at all the localities was observed amongst treatments with the *cryX* gene. Less damage was observed in the 15985BXs treatment than in all the other treatments, except those with the *cryX* gene, with regard to boll damage. Spraying of a transgenic variety with the *cryX* gene might give better protection against plant damage caused by bollworm. The *cryX* gene could be better expressed in the bolls than in other plant parts, in respect of both genetically modified and conventional cotton, no conclusion could be drawn with regard to the female moth's oviposition preferences.

Introduction

This study forms part of research work that has been carried out over a number of years on behalf of the industry. Some of the work completed during this time, was carried out under the auspices of the Agricultural Research Council, while the latter part of the study, mainly from 2001 to present, the research was completed as part of contract work completed by Agri-Biotech Research Consultancies cc.

The importance of this study on bollworm efficacy must be seen in relation to the introduction of biotechnology crops in South Africa, and in specific the introduction of genetically modified cotton types. Bt-cotton became registered in 1998 in South Africa and some of the results here will refer to data gathered during the registration trials of Bollgard™. Ongoing research was necessary to evaluate more resistant cotton types or events; with better manifested gene expression and which were least susceptible under variable conditions to the major pests, the bollworm complex. Some of these "potentially genetically resistant" cotton types are still being evaluated further and no other transgenic cotton variety that expresses the Cry1Ac protein has been registered and commercially launched up to date. The Cry2Ab2 protein has been registered in South Africa, but has not been launched commercially in cotton (pers comm. W.M. Green). According to Adamczyk et al. (2004), the amount of *cry1Ac* in transgenic Bacillus thuringiensis (Bt-) or Bollgard® cotton varies among commercial cultivars. These expression differences have been correlated to survival levels in Lepidoptera, indicating that all Bollgard® cultivars do not provide the same level of control. They provided data that showed that genetic background has a major effect on *cry1Ac* expression. Because backcrossing is the primary method used by commercial cotton breeders, the selection and use of donor and/or recurrent parents that will result in a high level of *cry1Ac* expression is crucial. In South Africa, the DeltaOPAL variety with the *cry1Ac* gene (Bollgard™) has proved to be efficient (see Part I of this study, see also Annexure I), but some extensive work was done to evaluate the *cry1Ac* and c*ry2Ab2* genes (Part II of this study, see also Annexure II). A cotton line containing the cry1Ac gene (Bollgard) to also contain the cry2Ab2 gene was re-engineered and the combination of both genes in one line was called MON15985. Bollgard II, i.e. MON15985 (Cry2Ab2) was registered in South Africa in 2003, but it was not released until its acceptance with Roundup Ready Flex (Mon 15985 x Mon 88913) which happened at the end of 2007 (pers comm. W.M. Green). Mellet et al. (2004) also commented on the efficacy of the Cry1Ac protein, and mentioned that the number of bollworm larvae and damaged cotton bolls are kept below threshold levels by the presence of the Bt-gene in the Bt-cotton fields and by insecticide applications in non-Bt-cotton fields. In order to demonstrate the different genes and associated proteins used in this study, they are listed below to indicate the introduction of a second genotype in order to possibly minimize bollworm resistance.

Gene	Protein	Cotton line/event	Phenotype	Study
cry1Ac	Cry1Ac1	DP50B	Bollworm resistance	Part I; Part IIb
cry1Ac	Cry1Ac1	NuOPAL (Bollgard®)	Bollworm resistance	Part I; Part IIb
cry2Ab2	Cry2Ab2	MON 15985 (Bollgard II) and 32 events	Bollworm resistance	Part IIa
cry1Ac & cry2Ab2	Cry2Ab2 & Cry1Ac	MON 15985 & MON15813	Bollworm resistance	Part IIb
None	None	DP50	None	Part IIb
cry1Ac	Cry1Ac	15985x	Bollworm resistance	Part III
cry2Ab2	Cry2Ab2	15985B	Bollworm resistance	Part III
cry1Ac & cry2Ab2	Cry1Ac & Cry2Ab2	15985BX	Bollworm resistance	Part III
cry1Ac	Cry1Ac	NuOPAL RR	Bollworm resistance & Glyphosate tolerance	Part IV
None	None	DeltaOPAL RR (Roundup Ready®)	Glyphosate tolerance	Part IV
None	None	DeltaOPAL	None	Part IV

The question of possible resistance to the Bt-gene is probably the most common question when transgenic crops are discussed. In South Africa, resistance to the gene is implemented by means of introducing a refuge system as discussed before. The efficacy of different genetically modified cotton types representing different genotypes to suppress bollworm populations is discussed in this paper. Little work has been done within South Africa to generate efficacy data, other than this study. Tabashnik et al. (2003) mentioned that despite expectations that pests would rapidly evolve resistance to such Bt-crops, increases in the frequency of resistance caused by exposure to Bt-crops in the field have not yet been documented. Literature which is applicable but not directly related to bollworm efficacy within South Africa is listed in Annexure III.

The aim of this study on bollworm efficacy was:

a) Determination of the efficacy of the Bt-gene (*cry1Ac*) against Helicoverpa armigera (Hübner) (Part I)

b) To select for the most effective bollworm resistant cotton event containing the P2B protein or the Cry2Ab2 protein, through a process of elimination by looking at the gene expression in relation to efficacy of different events, in order to reduce variability to a minimum (Part II)

c) To compare the second protein (Cry2Ab2) to the first (Cry1Ac) for efficacy, and provide data for an alternative choice, in a stack combination of the two proteins (Cry2Ab2 and Cry1Ac - 15985BX) which can be introduced as a resistant management strategy if necessary (Part III).

Part I: Determination of the efficacy of the Bt-gene (*cry1Ac*) against Helicoverpa armigera (Hübner) and other bollworms, during 1997/1998 in three field trials at Rustenburg, Potgietersrus and Groblersdal

The aim of this study was to compare bollworm resistance at different localities expressed by the cry1Ac gene (a Bt-gene).

EXPERIMENTAL PROCEDURES

Three field trials were carried out on the experimental farms of the Institute for Industrial Crops (ARC-IIC) in Rustenburg, Potgietersrus and Loskop (Groblersdal). The field trial at Loskop was repeated during the season of 1998/99 to confirm results obtained during the 1997/98 season. Reliable data collected during 1997/1998 at the Loskop locality, was recorded for further interpretation at this locality. The soil composition varied from a heavy clay soil, (turf) with a clay content of 58%, 14% silt and 28% sand content in Rustenburg, while in Potgietersrus the soil was a sandy-clay soil with a clay composition of 29%, 6% silt and 65% sand. In Loskop, the soil consisted of a sandy soil, with a clay content of 17%, 2% silt and 81% sand. All trials consisted of four treatments with six replicates laid out as a randomised block design, with each block representing a single replication. Each plot comprised 10 rows of 10 m each. Each plot was separated from adjacent plots by a 2 m pathway. The rows in the trial were planted 1.0 m apart and plant spacing within a row was 0.2 m. The treatments comprised of transgenic and non-transgenic varieties, either sprayed with insecticide for bollworm control or left unsprayed as indicated below. Total surface area = 74 m x 98 m. Treatments included NuCtn 37B with Bollgard™ – unsprayed; NuCtn 37B with Bollgard™ – sprayed according to threshold; Deltapine Acala 90 (DpAC 90) – unsprayed and Deltapine Acala 90 (DpAC 90) - sprayed according to threshold. Similar planting procedures were followed for all the trials. Important dates of applications and observations, and other actions are noted in Tables 2.1-2.6, for Rustenburg, Potgietersrus and Groblersdal (1997/1998) respectively. The soil was mechanically prepared by deep, thorough ploughing, followed by tilling. Directly thereafter, Dual® and Cotogard® were applied at the registered dosages, followed by a light irrigation of about 20 mm. The cotton was planted by hand at all the localities. One week after emergence, plants were thinned out to a density of 50 000 plants/ha, representing the conventional plant population density for irrigated cotton. When necessary, the cotton was irrigated with approximately 20-25 mm by means of an overhead sprinkler system. Thioflo® (endosulfan) was applied to the specified treatments above, when the egg threshold (12 eggs/24 plants) or larval threshold (5 larvae/24 plants) was reached or exceeded, whichever was attained first. The equivalent of endosulfan 200 l / ha spray mixture was applied at a pressure of 2.5 bar to all plants in the treatments sprayed according to threshold, by means of a CO_2 -driven knapsack sprayer fitted with a drop arm and six full-cone nozzles.

Assessments

Assessments included nine consecutive scouts starting at first square formation. Ten plants/plot were scouted for H. armigera, D. castanea and Earias spp. before the first application (approximately eight weeks after emergence) and thereafter, each week, for 8 counts, up to approximately 16 weeks after emergence. A distinction was made between small (L1-L2), medium (L3) and large (L4-L5) bollworm larvae of each species. The number of damaged fruiting bodies was noted. Insecticide application dates and weather conditions are given in Table 2.1 for Rustenburg and in Table 2.3 for Potgietersrus. Similarly, for Groblersdal insecticide application dates and weather conditions are given in Table 2.5. Larval counts were compared for the respective localities by means of an ANOVA with a regression approach. The different larval instars, as well as the bollworm egg data were compared only for the Rustenburg and Potgietersrus localities.

The statistical package GENSTAT 5.3 was used to analyze most of the data collected during 1997/1998 (Genstat 5 Committee, Rothampstead, UK, Clarendon Press, Oxford). For the 1998/1998 data from Groblersdal, a one-way chi-square analysis of variance for treatment effects, and for differences between scouting times, was performed on the data for the scouting dates. This analysis was performed for the scouts performed during February and March, in order to decrease the number of zero values and to see if there was an indication of significant differences between treatments. The chi-square test indicates whether the treatment with the highest mean differs from that of the lowest mean. Line plots of the data of the relevant counts are given. The data was also analyzed by means of a two-directional chi-square test, but the results seemed unreliable, since too many zero values occurred in the data, due to the transgenic effect of the cotton types on bollworm numbers. For further significant differences between treatments, an analysis of variance was performed on the total number of bollworms of the bollworm complex. An attempt was made to analyze the total number of bollworms found on the Bt-cottons. However, the data in the latter case was skew and a weighted log transformation was subsequently performed on the data, but the results were unreliable and were therefore omitted.

Statistical analyses

Statistical analyses on the bollworm data of Rustenburg and Potgietersrus during 1997/1998, included an analyses of variance using the regression approach, were performed on the logarithm of the counts of the following variables from the Rustenburg, Potgietersrus and where appropriate, the Loskop locality. The variables included the total number of bollworm larvae; african-, red- and spiny bollworm larvae respectively; 1st, 2nd and 3rd instar larvae and the total number of bollworm eggs. The log transformation was decided on in order to satisfy the ANOVA-assumption of homogeneous treatment variances. (This assumption could not be met for the untransformed variables; resulting in invalid ANOVA-results.) Observations were made at different time intervals. The regression approach, aided in studying the trend over time, resulted in different linear regression lines being fitted for each treatment. Regression lines that differ

significantly from one another imply that the associated treatments differ significantly. Applicable t-statistics were calculated to compare the different slopes or intercepts of the various regression lines. (The variance/covariance matrix obtained from the analyses is used in these tests.) Calculation of t-statistics and their interpretation is demonstrated for the Rustenburg locality on the total number of larvae.

An accumulated analysis of variance table (log transformation), table of treatment means, relevant regression equations; their graphical presentation, calculated t-statistics, line plots of the original, relevant counts and interpretation for each variable follow below. For each variable the logarithm of the relevant counts versus time is displayed in the graphical representation of the regression line. The line plots depict relevant counts versus time. Although the general trend will be in the same direction for both types of graphs, the regression lines are linear and the line plots often tend to be curvi-linear. The results or conclusions derived at for the transformed variables (log transformations) also apply to he untransformed variables**.**

For the data collected at Groblersdal (Loskop), during (1998/1999), a Chi-square test was used. From the one-directional chi-square test, the comparison of the total number of african bollworm larvae per scout seemed to be unreliable, since too many zero values occurred in the analysis, although significant differences between the treatments were indicated at the 5% significance level ($p = 0.005$). Differences between treatments are not discussed for the 1998/1999 season. The total number of bollworm larvae at each scouting interval is given in Table 2.7. Deductions from the statistical analyses of the respective variants from Rustenburg and Potgietersrus (1997/1998) and Groblersdal (1998/1999) are presented in Tables 2.22 and 2.23, and 2.24 respectively.

RESULTS

(See Annexure I; Tables A.2.1 - A.2.16)

Analysis of the total number of larvae in Rustenburg and Potgietersrus (1997/1998) and Groblersdal (1998/1999)

The total number of bollworm refers to the so-called bollworm complex, which in this case includes african-, red- and spiny bollworm.

The mean number of bollworms for Rustenburg (Fig. 2.1) is given in Table 2.6. The incidences of larvae over time ($p < 0.027$) and between treatments ($p < 0.013$) are significant (see Annexure I, Table A.2.7.). The regression analyses are explained in Annexure I. From the regression analyses, Treatments three (DpAC 90 unsprayed) and four (DpAC 90 sprayed) did not differ significantly ($t_{3;\,4} = 0.0789 < 1.697$). In reality, there was no difference between Treatments one (Bollgard unsprayed) and two (Bollgard sprayed), as explained above. Treatments three and four also did not differ significantly. It can therefore, be deduced that Treatment two also differed from Treatments three and four. The Bollgard cotton (Treatments one and two (sic!)) was resistant to the attack of bollworm larvae (Fig. 2.1).

The treatment means for Potgietersrus (Fig. 2.2) are given in Table 2.7. From the accumulated analysis of variance table (see Annexure I) time ($p < 0.001$) and the effects

between treatments ($p < 0.05$) were significant (Table 2.10). The non-significant interaction effect implies that the different treatment responses are represented by parallel regression lines. The regression equations were calculated ($y = mx + c$), where x =days after planting); from the estimates of the regression coefficients and for the 4 treatments (see Annexure I). By methods already discussed under the Rustenburg locality, it was established that Treatment one (Bollgard unsprayed) differed significantly from Treatments three and four (DpAC 90 unsprayed and sprayed) with regard to the y-intercepts. There was a decrease in the number of bollworm larvae over time for all treatments (Fig. 2.2). In addition, the least number of bollworm larvae was found on the Bollgard treatments (Fig. 2.2).

When the total number of bollworms was compared between treatments over the scouting period from February to March in Groblersdal (Fig. 2.3), significant differences were found between the DpAC 90 unsprayed control treatment and the other treatments ($p < 0.001$) (Table 2.8). The DpAC 90 unsprayed treatment had the highest number of bollworms. The number of bollworm larvae found on both the Bt-unsprayed, as well as the DpAC 90 sprayed plots was similar (Table 2.7).

A significant increase ($p < 0.001$) in the total number of bollworm larvae can be observed over the time period from February to March. A peak in bollworm numbers was reached at the end of January and again in the beginning of March.

An analysis of variance on the total number of all species of bollworm larvae, showed significant differences between treatments ($p < 0.001$). It was found that the DpAC 90 unsprayed treatment differed significantly from the Bt-sprayed and Bt-unsprayed treatments, but not from the DpAC 90 sprayed treatment. Similarly, the DpAC 90 sprayed treatment differed significantly from both Bt-treatments. There were no significant differences between the two Bt-treatments. The total number of bollworm larvae of the entire observation period for each treatment is given in Table 2.18.

Analysis of african bollworm larvae

For Rustenburg and Potgietersrus, (1997/1998) a similar approach was followed to the analysis of the total number of bollworm larvae, to compare african bollworm densities.

A weighted analysis of variance was done to distinguish between the treatment means of the scouting intervals, but the data was skew. In addition, an attempt was made to perform a log transformation (LY=LOG(Y+1)) on all the counts of african bollworm larvae over the whole observation period, in order to minimize the number of zero values in the Bt-treatments. Significant differences between treatments were still unreliable. For Groblersdal, the total number of african bollworm larvae over the season is shown in Fig. 2.3. From the line graph it can be seen that the african bollworm numbers were high in the beginning of the season on both DpAC 90 treatments, but decreased on the DpAC 90 sprayed treatment towards the end of the season.

The treatment means for the Rustenburg locality are given in Table 2.8. The treatment means differed significantly ($p < 0.001$). The significant effect implies that the different treatment responses do not each follow a significant different trend over time and separate parallel regression lines represent this. By showing the parallel lines of the respective treatments, the

increase african bollworm larvae were prevalent. There was an increase in the bollworm population density on all treatments.

The regression equations were calculated (Annexure I) and by studying the t-values and t-probability associated with the estimates of the regression coefficients for the y-intercept, it was found that Treatment one (Bollgard unsprayed) differed significantly from all the other treatments with regard to the y-intercept[1]. Treatments one and two differed significantly from Treatments three and four with respect to african larvae. This finding is similar to that obtained from the analysis of the total number of bollworm larvae. Lower infestation levels of Bt-cotton larvae were observed on the Bollgard treatments, than in comparison with the DpAC 90 treatments (Fig. 2.4), indicating that Bollgard was more resistant to african larvae than conventional cotton variety, DpAC 90.

For the Potgietersrus locality, treatment means are given in Table 2.12. The effect over time ($p < 0.001$) was significant. The trend over time indicated that there was a decrease in the total number of Bt-cotton larvae throughout the season on all treatments (Fig. 2.5). No significant differences were found between treatments (Table 2.12). No african larvae were found in either of the Bollgard treatments from the 4th week onwards (Fig. 2.5). Similarly african bollworm numbers dropped to zero during the 6th week on the DpAC 90 treatments (Fig. 2.5).

The total number of african bollworm larvae for Groblersdal (Fig. 2.6) was compared between treatments using the Chi square test and an analysis of variance. The comparison of the total number of african bollworms per scouting time interval was unreliable, since too many zero values occurred in the analysis. There were no significant differences between the treatments ($p = 0.005$). When the total number of african bollworms was compared over the scouting time period between treatments, the DpAC 90 unsprayed control treatment had significantly more african bollworms than the Bt-sprayed treatment ($p < 0001$) (Table 2.8). By comparing the total number of african bollworm larvae over all the treatments for each scouting period, a significant increase ($p < 0.001$) in the number of bollworm larvae was observed over time, to reach a peak at the beginning of March on the DpAC 90 treatment (Table 2.9).

Analysis of red bollworm larvae

A similar approach was followed as that in the analysis of red bollworm larvae for Rustenburg and Potgietersrus.

The means for red bollworm larvae for Rustenburg (Fig. 2.7) are given in Table 2.12. The effect of time ($p < 0.002$) and treatments ($p < 0.014$) were significant (Table 2.18). The different treatment responses each followed a separate parallel regression line. Treatment one and two were found to be significantly different from 3 and 4, although the pairs 1, 2 and 3, 4 did not differ significantly. The red bollworm population increased over time. This was in agreement with the increase in red bollworm population densities found on this locality over the past two seasons. Red bollworm numbers exceeded the threshold on the DpAC 90 treatments (Fig. 2.7).

[1] The t-values and t-probabilities associated with the estimates of the regression coefficients as well as t-tests for the comparison of all other treatments are given in the Annexure for all other bollworm larval comparisons, and will not be discussed in detail in the text.

For the Potgietersrus locality (Fig. 2.8), treatment means are given in Table 2.12. The effect of treatments ($p < 0.024$) and the effect over time ($p < 0.003$) were significant (Table 2.17). The significant effect between treatments implies that separate parallel regression lines whereof the equations are given in the Annexure I represent the different treatment responses. The negative trends over time indicate that there was a decrease in the total number of red bollworm larvae throughout the season on all treatments. Significant differences were found between treatments (Table 2.17). In summary it can be concluded that Treatments one and two differed significantly from the DpAC 90 unsprayed and from the DpAC 90 sprayed treatment; although the pairs one; two and three; four did not differ significantly. In contrast with Rustenburg low numbers of red bollworm larvae were found on both Bollgard treatments. Red bollworm numbers equaled null from the 4th week onwards (Fig. 2.8).

The analyses of red bollworm larvae from Groblersdal (1998/1999) (Fig. 2.9) (Table 2.12) was done by means of a chi-square test red bollworm larvae from each scouting period were compared between treatments. The comparison of red bollworm larvae per scouting time period was unreliable, since too many zero values occurred in the analysis. There were no significant differences between the treatments ($p = 0.026$).

The comparison of the total number of red bollworms between treatments over the whole scouting time period, showed that there were significantly more red bollworms on the DpAC 90 unsprayed control treatment than on the Bt-sprayed treatment ($p < 0001$) (Table 2.12). By comparing the total number of red bollworm larvae over all the treatments for each scouting period, there was not a significant increase ($p = 0.035$) in the number of red bollworm larvae over time. Red bollworm larval numbers appeared to vary throughout the season (Table 2.12). In addition, an analysis of variance was performed on the total number of red bollworm larvae over the entire observation period. There were significant differences between treatments at the 5% significance level ($p < 0.006$). Treatment means are indicated in Table 2.6. The DpAC 90 unsprayed treatment had significantly more bollworms than both the Bt-treatments, but did not differ significantly from the DpAC 90 sprayed treatment. There were no significant differences found between the Bt-unsprayed and Bt-sprayed treatments. The DpAC 90 sprayed treatment did not differ from either of the Bt-treatments. The number of red bollworm larvae found throughout the season is illustrated in Fig. 2.9. It seems as if the number of red bollworm larvae found on both of the DpAC 90 treatments varied at different time intervals, while the Bt-treatments had very few red bollworms.

Analysis of spiny bollworm larvae

Spiny bollworm larvae from Rustenburg and Potgietersrus (1997/1998) were compared between treatments by following the regression approach, similar to that of african – and red bollworm larvae.

For Rustenburg (Fig. 2.10), the mean number of spiny bollworms is given in Table 2.13. The effect of time ($p < 0.004$), treatments ($p < 0.001$) and the interaction between treatments and time ($p < 0.03$) were significant (Table 2.16). The significant interaction effect implies that the different treatment responses each follow a different trend over time, which is represented by

non-parallel regression lines. Treatments one and two (Bollgard™ sprayed and unsprayed) differed significantly from Treatments three and four (DpAC 90 unsprayed and sprayed). The DpAC 90 unsprayed treatment (Fig. 2.10) showed most spiny bollworm larvae throughout the season. Spiny bollworm larvae were also found on the DpAC 90 sprayed treatment, but were absent from any of the Bollgard treatments (Fig. 2.10).

For Potgietersrus (Fig. 2.11), treatment means of spiny bollworm larvae are given in Table 2.13. The effect of time ($p < 0.001$) was significant (Table 2.23), implying that the treatment responses each followed the same trend over time. The negative trend over time indicated that there was a decrease in the total number of spiny bollworm larvae throughout the season on all treatments. Spiny bollworm larvae were only found during the first 4 counts on any of the treatments (Fig. 2.11).

Spiny bollworm larvae were compared in a similar way to that of african- and red bollworms in Groblersdal (1998/1999) (Fig. 2.12). The comparison of spiny bollworm numbers by means of a Chi-square test for each scouting time period was unreliable, since too many zero values occurred in the analysis. There were no significant differences between the treatments ($p = 0.101$) (Table 2.9). When the total number of spiny bollworms was compared over the scouting time period between treatments, there were significantly more spiny bollworms on the DpAC 90 unsprayed control treatment than on any of the other treatments ($p < 0.001$) (Table 2.18), similar to what was found with african and red bollworm larvae. When the total number of spiny bollworm larvae over all the treatments for each scouting period was compared, no significant trend with regards to bollworm numbers could be established ($p = 0.247$). Spiny bollworm numbers varied during the observation period (Table 2.10).

The results obtained from the weighted analysis of variance as well as the log transformation of the 9ta proved to be unreliable. The population densities of the total number of spiny bollworm larvae between treatments are given in Fig. 2.12. Most spiny bollworm larvae were observed on the DpAC 90 unsprayed treatment, except for the 3rd, 4th and 5th week. Very few spiny bollworms were noted on either of the Bt-treatments.

Comparison of different larval instars between treatment means of each locality

For the Rustenburg locality (Fig. 2.13), a regression analysis was performed on the data of L1-L2 larvae from Rustenburg. Treatment means of first and second instar larvae (L1-L2) are given in Fig. 2.13. Treatment means are given in Tables 2.18 and 2.19. The accumulated analysis of variance table indicated that the effect of time ($p < 0.036$) and treatments ($p < 0.029$) were significant (Table 2.18). The significant treatment effect implies that the different treatment responses each follow a different trend over time. Treatment one and two (Bollgard unsprayed and sprayed) differed significantly from treatments three and four (DpAC 90 unsprayed and sprayed); though the pairs one; two and three; four did not differ significantly. Low numbers of 1st and 2nd (L1 and L2) instar larvae were found for both of the Bollgard treatments in comparison to the DpAC 90 treatments (Fig. 2.13). Treatment means of third instar larvae (L3) are given in Fig. 2.14 and treatment means are given in Table 2.14. For second instar larvae from Rustenburg, the effect of time ($p < 0.003$) and treatments ($p < 0.001$) were significant

(Table 2.16). For the total number of larvae from Rustenburg, it was established that Treatments one and two (Bollgard unsprayed and sprayed) differed significantly from Treatments three and four (DpAC 90 unsprayed and sprayed). Treatments three and four did not differ significantly. The same applied to treatment one and two. No third instar larvae were found on any of the Bollgard treatments (Fig. 2.14) except for the 8th week on the sprayed treatment.

For the Potgietersrus locality, comparison of the L1-L2 larvae and the L3 larvae revealed no significant treatment effects (Table 2.17) (Fig. 2.16) and Table 2.19, Fig. 2.17.). No L1 and L2 instar larvae were found on any of the treatments from the 4th count onwards (Fig. 2.16). However, a significant effect was found for both over time. A comparison of the total no. of L1-L2 larvae from Potgietersrus (see Annexure I) showed that the effect of time ($p < 0.001$) was significant (Table 2.17). The negative trend found indicated that the number of smaller larvae (L1 and L2) of all treatments decreased as the season progressed (Fig. 2.16). This is expected for the normal life cycle of the bollworms, indicating that peak bollworm activity is during the beginning of the season when most damage is done. The trend over time is also indicated in Fig. 2.16, in that there was a decrease in the total number of second instars larvae near the end of the season on all treatments. Very few L3 larvae (Table 2.15) were found on the Bollgard treatments in comparison with other treatments.

For the comparison of larger larvae (L4-L5 instars) the treatments means in Rustenburg showed that the effect of time ($p < 0.001$), treatments ($p < 0.001$) and the interaction between treatments and time ($p < 0.006$) were significant (Table 2.30.) (Fig. 2.15). The significant interaction effect implies that the different treatment responses each follow a different trend over time and separate non-parallel regression lines can represent this. (The equations are given in Annexure I -Table A.2.11). The total number of larvae from Rustenburg from Treatments one and two (not significantly different from one another) differed from treatment three and four (which again did not differ). Thus the Bollgard treatments were more resistant to third instar bollworm larvae. No third instar larvae were noted on the Bollgard unsprayed treatment, while the sprayed treatment had a few during the 7th count (Fig. 2.14).

In Potgietersrus, for L4-L5 larvae, the accumulated analysis of variance table for L4-L5 instar larvae from Potgietersrus indicated that treatment effects ($p < 0.041$) and time effects ($p < 0.068$) were significant. Similarly, the significant effect between treatments can be represented by separate parallel regression lines (Annexure I). Thus, it was found that for L4-L5 instar larvae, Bollgard™ was significantly more resistant than DpAC 90 (Fig. 2.18). Spraying showed no significant effect. Similar to the Rustenburg locality, very few 3rd instar larvae were found on the Bollgard treatments (Fig. 2.15).

For the Groblersdal locality (Figs 2.19, 2.20 and 2.21) (Table 2.18), instars between larvae were not compared statistically. First to second instar larvae (L1-L2) were under the threshold on the Bollgard sprayed and unsprayed treatments (Fig. 2.19), while it varied on the DpAC 90 treatments. L3 larvae exceeded the sprayed DpAC treatment on count 6, 7 and 8 (Fig. 2.20) and an L5 larva exceeded the DpAC larvae only on the 6th count, but was still below the threshold (Fig. 2.21).

Analysis of the total number of bollworm eggs (only for Rustenburg and Potgietersrus)

In Rustenburg, ttreatment means are given in Table 2.19. There was no significant difference between treatments, but that there was a significant general trend over time ($p < 0.001$). The positive slope of the line indicates that there was an increase in the total number of bollworm eggs over time of all treatments. Considerably fewer eggs were found on the Bollgard unsprayed treatment than on either of the DpAC 90 treatments (Fig. 2.22).

In Potgietersrus, treatment means are given in Table 2.19. There was no significant difference between treatments, but a general trend over time existed ($p < 0.001$). The negative slope (Fig. 2.23) obtained from the bollworm eggs in Potgietersrus, indicates that there was a decrease in the total number of bollworm eggs over time on all treatments. It seems as though the Bollgard unsprayed treatment had more eggs up to the 3rd count than any of the other treatments (Fig. 2.23). The Bollgard sprayed treatment had fewer eggs from the second count and the 3rd count than all other treatments (Fig. 2.23), while all treatments showed fewer eggs at the 5th count than at the beginning of the observation period.

Prevalence of Bollworm species

Overall, the most prevalent species that occurred on Treatment three (DpAC 90 unsprayed) was red bollworm, at the Rustenburg locality (Fig. 2.7) as well as in Potgietersrus (Fig. 2.8). A similar tendency was observed for the DpAC 90 sprayed treatment in Rustenburg (Fig. 2.7) and in Potgietersrus (Fig. 2.8). This data emphasizes the importance of red bollworm, as part of the bollworm complex in an IMP (Integrated Pest Management) approach. In Groblersdal (1998/1999), he so-called “african bollworm” was found to be the most abundant species on all four treatments (Table 2.9). Slightly more red bollworms were found than spiny bollworms on the DpAC 90 and B-unsprayed treatments.

Analysis of squares, flowers and bolls

During 1997/1998, in Rustenburg, a higher number of damaged squares were found on the DpAC 90 treatments than on the Bollgard™ treatments throughout the season, while the DpAC 90 unsprayed treatment had most damaged squares than in comparison with the sprayed treatments.

In Potgietersrus the Bollgard™ sprayed treatment had more squares throughout the season than the Bollgard unsprayed treatment and the number of undamaged flowers seemed to follow the same pattern. No trend in any of the other treatments could be seen. No damaged squares were found from the 4th week onwards of the observation period.

In the trial done in Groblersdal, during 1998/1999, very few damaged squares were found at the beginning of the observation period on both of the Bt-treatments, while most damaged squares were found on the DpAC 90 unsprayed treatment from the 3rd count onwards. The DpAC 90 treatments showed most damaged flowers during the observation period. No damaged flowers were found in either of the Bt-treatments. The DpAC 90 unsprayed treatment had most damaged flowers at the end of the observation period.

In Rustenburg, the number of damaged bolls was much lower on the Bollgard™ cotton throughout the season in comparison with the DpAC 90 cotton. The Bollgard™ sprayed treatment had the least number of damaged bolls during the 4th and 5th week of scouting, while this exceeded the number of bolls on the Bollgard unsprayed treatment at the end of the observation period. The number of damaged bolls on the DpAC 90 cotton exceeded the number of damaged bolls on both of the Bollgard treatments throughout the season.

In Potgietersrus, the differences between treatments were not as clear as at the Rustenburg locality, with respect to boll damage. However, between 6 and 7 out of the nine observations obtained, the boll damage was lower on the Bollgard unsprayed and Bollgard sprayed treatment than on the DpAC 90 cotton.

In Groblersdal (1998/1999), the total number of damaged bolls was the highest for the DpAC 90 unsprayed treatment at the 6th, 8th-10th counts. No damaged bolls were found on the Bt-sprayed treatment.

It seemed that the number of damaged squares; flowers and bolls could be related to the number of insects found on a specific variety, which depended on the inherent characteristics of the variety and /or the number of insecticide applications when sprayed.

CONCLUSION

For the Rustenburg and Potgietersrus localities (1997/1998) (Tables 2.16 and 2.17), it can be deduced that transgenic cotton has without doubt, resistance to bollworm larvae. The total number of larvae found on Treatments one and two (Bollgard unsprayed and sprayed) differed significantly from the DpAC 90 cotton (sprayed and unsprayed). The two transgenic treatments did not differ, since the threshold for bollworm larvae (> 12,5 larvae/60 plants) were never reached, explaining the similar effect obtained by Treatments one and two. Furthermore, the spray treatment for DpAC 90 consistently showed no significant effect for the variables discussed.

For the Groblersdal Locality (1998/1999) (Table 2.18), it can be concluded that the transgenic cotton has shown without doubt, resistance to bollworm larvae. The Bt-sprayed treatment seemed to give lower numbers of bollworm larvae in comparison with the Bt-unsprayed treatment, but not significantly so. In Groblersdal, the Bt-sprayed treatment and Bt-unsprayed differed significantly from both DpAC 90 treatments for red bollworm larvae. It is important to note that spraying was carried out on the egg threshold (30 eggs/60 plants). The results indicate that there could be a tendency that sprayed red bollworm is more effective than unsprayed red bollworm, based on the egg threshold.

The Bollgard™ treatments at the Rustenburg locality (1997/1998) significantly prevented bollworm infestation when compared to the DpAC 90 treatments. Significant differences in the total number of larvae, red bollworm larvae and spiny bollworm larvae were found between the Bollgard™ and DpAC 90 treatments for the Rustenburg locality. The same phenomenon was true for 1st, 2nd and 3rd instar larvae from Rustenburg. The number of small larvae was significantly lower on the Bollgard™ treatments than on the DpAC 90 treatments. The Bollgard™ unsprayed treatment differed significantly from all other treatments regarding red bollworm

larvae. A significantly lower number of larvae were found on the Bollgard™ unsprayed treatment. The natural enemies possibly had a greater effect on the red bollworm population on this treatment, than on the other treatments, where spraying or the absence of the Bt-gene resulted in high red bollworm numbers.

For the Potgietersrus locality (1997/1998), the same was true with respect to the total number of larvae. However, no significant differences were found between treatments or between the various instars, for spiny bollworm and red bollworm. The Bollgard unsprayed treatment had significantly fewer red bollworm numbers than the DpAC 90 sprayed treatment. Similarly, the natural enemies of red bollworm probably kept numbers low on the control (DpAC 90 unsprayed), while the Bt-gene kept them low on the Bollgard sprayed and unsprayed treatments. Insecticide application on the DpAC 90 treatment possibly kept natural enemy numbers low, with a subsequent increase in red bollworm numbers. No significant differences in the number of bollworm eggs were found for any of the treatments at the Rustenburg and the Potgietersrus localities.

For Groblersdal (1998/1999), the Bt-treatments controlled bollworm infestation significantly in comparison with the DpAC 90 unsprayed and DpAC sprayed treatments. The DpAC 90 unsprayed treatment differed significantly from both the Bt-sprayed and Bt-unsprayed treatment with regards to the total number of bollworm numbers similarly, the DpAC 90 sprayed treatment differed significantly from both the Bt-unsprayed and Bt-sprayed treatments. The Bt-unsprayed treatment gave significantly less red bollworm when compared to the DpAC 90 unsprayed treatment, indicating that on the Groblersdal locality, there were significantly less red bollworm on unsprayed transgenic cotton, than in comparison with unsprayed non-transgenic cotton. The most prevalent species on all of the treatments was red bollworm.

From the data collected in Rustenburg and Potgietersrus (1997/1998), one can conclude that very little damage was done to squares, flowers and bolls on the Bollgard treatments in comparison with both the DpAC 90 treatments. Bollgard has a tendency to prevent bollworm damage effectively by controlling bollworm numbers. The most prevalent species on both of the treatments on all localities was red bollworm.

In Groblersdal (1998/1999), it seemed that very little damage was done to squares on the Bollgard treatments in comparison with both the DpAC 90 treatments. Very little damage to flowers and bolls occurred in any of the treatments.

Part II (a): Efficacy of the P2B protein, containing the Cry2Ab2 protein, in transgenic cotton events against bollworm

The aim of this study was to determine the efficacy of insect resistant cotton lines containing a gene expressing the "P2B" Bt-protein (Cry2Ab2 protein) at Rustenburg, The different events or transgenic lines, all expressed the Cry2Ab2 protein, but differed in the particular circumstances when the gene was introduced into the plant for example by the "molecular gun - technique" or so-called ballistic bombardment (Madden, 1995), distinguishing them from one another as events.

EXPERIMENTAL PROCEDURES

A field trial was planted at the Institute for Industrial Crops (IIC-ARC) in Rustenburg. The area planted was 0.1184 ha. A single transgenic P2B event represented one plot. Each plot was one row of 15 m in length. Thirty three transgenic P2B events were interspersed throughout the field with two border rows of conventional cotton of the DpAC 90[2] variety. Border rows (DpAC 90) of 12 m surrounded the perimeter of the trial. Two DpAC 90 border rows were planted between each transgenic plot. All border rows were destroyed at the end of the season. A single seed was planted per plant station and plant stations were 15 cm apart. Plants of individual events were each numbered with a white tag. Treatments included 33 transgenic P2B events. The plots corresponded with the following events:

Plot 1 – Event 6149; Plot 2 – Event 6151; Plot 3 – Event 6152; Plot 4 – Event 6223; Plot 5 – Event 6227; Plot 6 – Event 6232; Plot 7 – Event 6234; Plot 8 – Event 6239; Plot 9 – Event 6241; Plot 10 – Event 6351; Plot 11 – Event 6352; Plot 12 – Event 6456; Plot 13 – Event 6460; Plot 14 – Event 6483; Plot 15 – Event 6484; Plot 16 – Event 6489; Plot 17 – Event 6491; Plot 18 – Event 6526; Plot 19 – Event 6532; Plot 20 – Event 6563; Plot 21– Event 6542; Plot 22 – Event 6718; Plot 23 – Event 6725; Plot 24– Event 6733; Plot 25– Event 6734; Plot 26 – Event 16221; Plot 27 – Event 16960; Plot 28 – Event 17079; Plot 29– Event 17133; Plot 30– Event 17180; Plot 31– Event 17206; Plot 32– Event 6233 and Plot 33– Event 15830-5. Six control plots were used (C1-C6) to include 4 transgenic varieties: DP50B (C2 = Control 2) – referring to Delta Pine 50 with the Bt-gene, C312-531 (C4 = Control 4) – Coker variety 15813 (C5 = Control 5) – a transgenic cotton type with the Bt-gene, and 15985 (C6) – a transgenic event and 2 non-transgenic varieties: DP50 – referring to Delta Pine 50 without the Bt-gene; (C1 = Control 1) and C312 (C3 = Control 3). The trial was planted on a heavy turf soil with 50 % clay, 42 % sand and 8 % silt. The pH of the soil was between 7.33 and 7.54 at a depth of 30 - 60 cm. A single insecticide application was carried out to control leafhoppers and cotton stainers on all plots at the end of the season. No spraying was performed at any stage for lepidopteran pests. The insecticides monocrotophos (Monostem®[3], 500 ml/ha) and deltamethrin (Decis®, 170 ml/ha)

[2] DpAC 90 refers to Deltapine Acala 90 non-transgenic cotton, from Delta and Pinelands, SA.
[3] At the time of performing the trial, Monostem (monocrotophos) was commercially available, but has since been withdrawn from the market, from April 2004.

were applied by means of a CO_2 -driven knapsack sprayer fitted with a drop arm and six nozzles. All plots were managed like conventional cotton with respect to fertilization, water, and control of insect pests, with the exception of lepidopteran species. No weed control was carried out before planting or at any time during the season. Fertilizer was applied on 02/11/98 (150 kg/ha $K_2SO_{4)}$; on 12/01/99 (150 kg/ha LAN); and on 22/02/99 (150kg /ha LAN) per ha. Irrigation was applied as 50 ml overhead sprinkler irrigation during the week of the 22/02/99, and another 50 ml approximately one month later.

Assessments

Stand counts were done by counting the number of plants that emerged for each plot at seven and 14 days after planting. Cotyledons that had completely cleared the soil were considered "emerged". Individual plants were tested for the gene of interest via lateral flow strips at the four-leaf stage. Leaf samples of each individual plant per event were shipped back to Monsanto for gene analysis, on two occasions during the season. The number of fully cracked bolls per transgenic plant/event was calculated over five time periods. Insect efficacy testing was done by relying on natural infestations of lepidopteran pests. Ten randomly selected plants per plot, of which the plant numbers were noted, were evaluated weekly, for eight consecutive weeks, until approximately 16 weeks after emergence. The following insects were noted: bollworm eggs, bollworm larvae which were classified as either small (L1-L2), medium (L3) or large (L4-L5) larvae, as well as other lepidopteran insects and non-target species. The number of damaged squares, white blooms, and bolls were also noted. In the case of Coccinellidae, the number of adults, larvae and pupae were combined to compare events and control plots. The number of lacewing eggs (Chrysopidae) was counted individually on scouted plants, as well as the spiders. For aphid and leafhopper comparisons, the number of plants per event on which they occurred was noted, and not the actual numbers. In the case of leaf miners, the number of plants with leaf damage per event was compared. All other insects were individually noted and are represented in a table for comparisons between events.

No statistical analyses were performed on the data since no plots (treatments) were repeated throughout the trial. The amount of seed available was just enough to plant a single row of each event. Graphical representation of the data is, however, given.

Percentage control, as indicated by gene expression, was calculated whenever the data fitted the formula described by Abbot (1925).

The following formula was applied: % Control = $X - Y/X$ x 100

Where X = the percentage of living larvae from control plots; Y = the percentage of living larvae from the treated plots. For the purpose of this study, it was assumed that the treated plots are represented by the transgenic events that vary by gene expression. This percentage is expressed as: the difference in larval counts on the non-transgenic DP50 line and the transgenic events, divided by the percentage of larvae found on the control.

The following assumptions were made with reference to the above-mentioned formula:

High increase in Y so that Y>X; results in a negative control ("-"), indicating an increase in larval density on the transgenic event.

High increase in X (control) so that X remains > Y; results in a poorer control, but, however, in a positive poorer control, indicating that the larval density on the corresponding transgenic event did not increase to a great or "potentially harmful" extent.

Planting date was on 17/11/1998 and the subsoil temperature was 19°C at the time of planting and ambient or air temperature was 22°C; Scouting commenced on 06/01/1999; 14/01/1999; 21/01/1999; 27/01/1999; 08/02/1999; 11/02/1999; 14/02/1999 and on 25/02/1999. Insecticide application was carried out on 08/04/1999, and the first gene-check sampling was on 11/12/1998, with the second to follow on the 25/01/1999. A 50 boll sample was taken on 19/04/1999, while boll counts took place on 29/03/1999; 06/04/1999; 13/04/1999; 20/04/1999 and 28/04/1999. The trial was harvested from the 17th of May to the 31 of May 1999.

RESULTS

Plant Growth data

A base-line count (plant-stand count) was done to determine the number of plants 14 days after emergence of each event. The counts are given in Table 2.19. The average stand count of each check plot was calculated and compared to the stand count of each other event (Fig. 2.24). The fewest number of plants germinated in the DP50 check plot (C1), while the C312-531 (C4) line had the highest germination potential. The two Coker lines (C3 and C4) gave the highest stand count overall, and were closely followed by the two new transgenic varieties, 15813 (C6) and 15985 (C5). The events that showed a higher germination potential than both of the Coker lines were events 6227 (plot 5), 6351 (plot 10), 6352 (plot 11), 6456 (plot 12), 6460 (plot 13), 6489 (plot 16), 6563 (plot 20), 17079 (plot 28), 17133 (plot 29), 17180 (plot 30), 17206 (plot 31) and 6233 (plot 32). Events that had a higher germination potential than C312 (C3), but lower than C312-531 (C4) were events 6234 (plot 7), 6718 (plot 22) and 6734 (plot 25). The events that produced a germination count of more than 80 plants per 15 m row included 6456 (plot 12), 6460 (plot 13), 17079 (plot 28), 17180 (plot 30), 17206 (plot 31) and 6233 (plot 32) (Fig. 2.24). After removing the plants that did not give a positive gene expression, the stand count was recalculated (Table 2.21). Event 17079 (plot 28) was the only row that had more than 80 plants after the non-transgenic plants were removed. The stand counts of the positively identified genetically transgenic plants were used to compare scout and yield data.

The events that showed a boll burst earlier in the season (above 3/plant) were 6151 (plot 2), 6232 (plot 6), 6456 (plot 12), 6484 (plot 15) and 6542 (plot 21) (Fig. 2.25). The Coker 531 check (C4) also showed an earlier boll burst. The transgenic event 15813 (C5) gave a similar number of bolls being cracked by the 3rd and 4th count. The events 16960 (plot 27), 17079 (plot 28) and 17206 (plot 31), as well as the DP50 (C1) and C312 (C3) check plots, had a later boll bursting than the other events, with event 17206 (plot 31) having the latest boll bursting through the season, where few bolls cracked in comparison with other events and at later stages (the 4th and 5th counts). These events were therefore harvested later in the season. These events seem to have a longer growth or ripening period of the bolls.

The number of cracked bolls was compared over the five time intervals (Fig. 2.26). The highest mean number of cracked bolls was found during the 4th count (20/04/1999) for most of

the events. The event 6542 (plot 21) had the highest mean number of cracked bolls per plant at the 3rd count (3.86). This event was compared to the two new transgenic check plots (C5 and C6), which gave an earlier boll burst during the 3rd count, with means of 1.78 for check 15813 (C5) and 1.43 for check 15985 (C6) respectively. Events that performed well at the 4th count were 6526 (plot 18), 6483 (plot 14) and 6460 (plot 13). The number of bolls per plant had decreased by the 5th count in all events, except event 6232 (plot 6), which showed a slight increase in boll burst.

Insect data

Comparison of bollworm eggs without removal of the data on plants showing "negative" (non transgenic plants) in the gene-check

The mean number of bollworm eggs (on 10 plants per event) was compared to those of the check plots over time (Fig. 2.27). The number of eggs found over the spectrum of events is an indication of the infestation level at the specific given time. This illustrates the probability of the population density on these plants, should they not have been of a transgenic variety or should the gene expression in the individual plant and/or event not be sufficient to kill L1-L2 bollworm larvae. Oviposition is possibly determined by physical and physiological factors of the plant itself, i.e. alkaloids, terpenoids etc., by individual fertility factors of the female moth and by external factors, such as wind, temperature and rainfall. No evidence has been found to link transgenic probabilities of a variety to the degree of oviposition that takes place on the plant. The number of eggs on most events started to increase by mid-January, or the 3rd week after scouting commenced. The mean number of eggs on 10 plants per event over the time period of eight weeks is illustrated in Fig. 2.28. Event 6460 (plot 13) and the DP50B (C2) check plot gave the highest mean number of eggs. The two transgenic check plots, 15813 (C5) and 15985 (C6), also gave a large number of bollworm eggs, indicating that the Bt-gene does not influence oviposition of the female moth.

Comparison of bollworm eggs on positively tested transgenic plants

All plants that showed a negative expression of the Bt-gene were removed from the field and the data subsequently removed from the data tables. The results are presented in Fig. 2.29. Oviposition reached a peak during the 4th week of scouting or by mid-January. All events were recognized as attractive oviposition sites since eggs were found on all events and check plots. This emphasizes that the Bt-gene does not influence oviposition.

When comparing the mean number of bollworm eggs per plant on positively identified plants (Fig. 2.30), similar results were obtained as to when the plants which tested negatively for the Bt-gene, were included. Event 6460 (plot 13) and the DP50B (C2) check plot had the highest number of eggs when compared to the other events. The results showed that there was no difference between events with regards to suitability as oviposition sites for female moths. Transgenic cotton does therefore not seem to have any effect on the female moth's choice in oviposition site. Egg counts increased during week three and reached a peak during week four

on all events. The rate of oviposition decreased over time and was the lowest during the 8th week.

The lowest mean number of bollworm eggs found over eight weeks on transgenic plants was found on events 6232 (plot 6), 6489 (plot 16), 6733 (plot 24) and 6734 (plot 25). The non-transgenic check plot C312 (C3) also had few bollworm eggs. Event 6483 (plot 14), that had no bollworm larvae throughout the season, had a mean number of 8.75 eggs per 10 plants over the eight weeks. The check plots 15813 (C5) and 15985 (C6) had a greater mean number of 10 eggs per 10 plants over the 8 weeks. The fact that the least number of larvae out of all the check plots were found on these two treatments indicates that the expression of the Bt-gene in these transgenic check plots is sufficient to kill off all larvae that hatched from these eggs and fed on the cotton.

Comparison of bollworm numbers without removal of the data on plants showing "negative" (non-transgenic plants) in the gene-check

The number of bollworm larvae was first compared without the plants that tested negative for gene expression being removed. This data (Figs 2.31 and 2.32) is given to illustrate the importance of removing negatively tested plants from the field, and noting the number of plants scouted at a specific time to get a true representation of the infestation of bollworms on so-called transgenic lines or events. The mean number of bollworm larvae (african-, red- and spiny bollworm) found each week is given in Fig. 2.14. The check plots that seemed most resistant to bollworms were the transgenic lines 15813 (C5) and 15985 (C6). A mean of one larva was found during the second week on 15985 (C6), while 15813 (C5) gave a mean of two larvae during the 5th week. A high mean number of bollworm larvae was found during the 7th week in events 6234 (plot 7), 6460 (plot 13), 6718 (plot 22), 16960 (plot 27) and in the non-transgenic C312 check plot (C3). This phenomenon might correlate with the large number of bollworm eggs found during week 4 (Fig. 2.10), which would indicate that eggs had 3-5 days to hatch and just over a week to develop further. The C312-531 check plot (C4) gave a similar high number of bollworms just a week after the egg peak (week 5).

Event 6532 (plot 19) had the least bollworm larvae, per week, (Fig. 2.31) with a mean of one larva, found during week 8. Event 6491 (plot 17) had only two larvae, found during the 3rd week of the observation period. Other promising events that did not have more than a mean number of two living larvae at any one/more weekly time period through the observation period are listed in Table 2.21.

Comparison of bollworm numbers with removal of the data on plants showing "negative" (non-transgenic plants) in the gene-check

The data from the plants that showed a negative expression of the Bt-gene were removed from the data tables, and a histogram was drawn to include bollworm numbers from only plants that expressed the Bt-gene in sufficient quantities to control bollworms (Fig. 2.33). The results showed that there was only one event, 6483 (plot 14), on which no larvae were found throughout

the season. All checks had larvae at some stage during the season, except for the transgenic line, 15813 (C5). Less than 0.2 larvae per plant per event were found at any given time throughout the season on events 6151 (plot 2), 6223 (plot 4), 6227 (plot 5), 6232 (plot 6), 6456 (plot 12), 6483 (plot 14), 6526 (plot 18), 6532 (plot19) and 6734 (plot 25). It seems as if the population pressure was much higher during the 5th week than in any of the other weeks. The plots that showed the least resistance to bollworms were the non-transgenic check plot DP50 (C1) and the events 6241 (plot 9), 16960 (plot 27), 17133 (plot 29) and 17206 (plot 31).

The total mean number of bollworms over the observation period (eight weeks) was calculated (Fig. 2.34). The event that gave the highest mean number of bollworms at any given time during the observation period was 16960 (plot 27). A mean of 0.38 living larvae per plant was found on this event over the observation period. Events 17133 (plot 29) and 17206 (plot 31) also had high bollworm infestations, with means of 0.27 and 0.29 living larvae respectively. The non-transgenic check plots, DP50 (C1) and C312 (C3), both had a high mean number of 0.26 living larvae. The events that showed the most promise in controlling bollworms are listed in Table 2.30. Throughout the season, these events, as well as the check plots 15813 (C5) and 15985 (C6), had less than or equal to a total mean number of 0.05 larvae/plant.

Comparison of the cumulative number of bollworms over time, on transgenic plants

The cumulative number of bollworms was compared by calculating the sum of the african, red and spiny bollworm larvae (Fig. 2.35). No bollworm larvae were found to occur on either of the check plots, 15813 (C5) and 15985 (C6), except for the 2nd week when 0.1 larvae were found per plant on the 15985 transgenic line (C6). Event 6241 (plot 9) gave the most larvae during the 6th count. This event exhibited the most larvae over the time period in comparison with the other events. In comparison with the other controls, the DP50 check plot (C1) showed the most larvae during the 5th time period, while the C312 check (C3) had the highest number of larvae throughout the observation time (except for the 5th count). The check plot that had the least larvae over the time period was the transgenic variety 15813 (C5). The transgenic line 15985 showed similar resistance to bollworm larvae, except for the 2nd count. Events that were not effective (< 0.5 larvae/plant/event) were 6233 (plot 32), 6718 (plot 22), 16960 (plot 27), 6241 (plot 9), 6152 (plot 3) and 17133 (plot 29).

The following events had less than 0.2 larvae per plant at any given time during the observation period: 6733 (plot 24), 17180 (plot 30), 6734 (plot 25), 6460 (plot 13), 6227 (plot 5) and 15830-5 (plot 33).

Percentage control relative to that obtained with the non-transgenic variety DP50 (C1) (Fig. 2.36)

The percentage control expressed by most events throughout the season was 10%. Where the percentage control did not increase throughout the season, it implied that larval counts did not increase on the transgenic events overall. Where control stays the same (10%) at all time periods throughout the season, it implies total control. This was true for event 6483 (plot 14). Since the infestation increases on the non-transgenic check plot DP50 (C1) for week 6, and the

corresponding transgenic event, e.g. event 6149 (plot 1), has no increase in larval counts (week 6), the percentage control would decrease slightly. However, when the larval counts on a particular event, e.g. 6241 (plot 9), increased at any given time (e.g. week 6) and the larval counts on the corresponding check plot increased during the same time period, the factors "X" and "Y" increased at the same time and the percentage control expressed by the transgenic variety would decrease. Similarly, if the increase in larvae on the transgenic event ("Y") exceeded that of the check ("X"), the numerator was found to be a smaller number and this factor was subsequently divided by a smaller denominator, resulting in a lower percentage control, often indicating a negative ("-") control. This therefore only indicated that the transgenic event did not kill off neonate larvae effectively.

When all the transgenic events were compared with the non-transgenic variety DP50 (Table 2.24), only events 6223 (plot 4) and 6483 (plot 14) gave good control. This comparison was however, not as strict since the check was non-transgenic. Events that did not give any control whatsoever (< -10%), are 6152 (plot 3), 6241 (plot 9), 6718 (plot 22), 16960 (plot 27), 17133 (plot 29) and 6233 (plot 32).

Percentage control relative to that obtained with the transgenic variety DP50B (C2) (Fig. 2.37)

When the transgenic events were compared with the commercially available transgenic variety, NuCtn 37B, with a DP50B background (Bollgard™ Monsanto SA), the selection of events was stricter than when the transgenic events were compared with a non-transgenic variety (Table 2.25). Good bollworm control was expressed by events 6223 (plot 4) and 6483 (plot 14). Moderate control implied that only a small percentage of the larvae survived when compared with the survival percentage on Bollgard. Events which gave moderate control included 6227 (plot 5), 6489 (plot 16), 6526 (plot 18) and 6532 (plot 19).

Percentage control relative to that obtained with the non-transgenic varieties C312 (C3) and C531 (C4) (Figs 2.21 and 2.22)

As with the comparison of the transgenic events with the non-transgenic variety, DP50, the comparison of events with the Coker varieties (Tables 2.24-2.27) was not as strict as with the transgenic check, Bollgard. Similar results were obtained. In a comparison of the events with Coker 312 (Fig. 2.38, Table 2.26), events 6223 (plot 4) and 6483 (plot 14) gave good control. Event 6223 (plot 4) did, however, not perform that well during week seven, when compared to Coker 531 (Fig. 2.39 Table 2.27). Events that gave reasonable control relative to Coker 312 were 6151 (plot 2), 6227 (plot 5), 6232 (plot 6), 6239 (plot 8), 6351 (plot 10), 6352 (plot 11), 6456 (plot 12), 6460 (plot 13), 6491 (plot 17), 6526 (plot 18), 6532 (plot 19), 6563 (plot 20), 6542 (plot 21), 6733 (plot 24), 6743 (plot25), 16221 (plot 26), 17180 (plot 30) and 15830-5 (plot 33) (Table 2.26).

Fewer events gave reasonable control when compared with Coker 531 (Fig. 2.39, Table 2.26), i.e. 6151 (plot 2), 6223 (plot 4), 6526 (plot 18) and 6532 (plot 19).

Percentage control relative to that obtained with the transgenic line 15813 (C5) (Fig. 2.40)

By substituting zero larvae in the formula (X = 0), the formula is undefined. A comparison of the transgenic events with the transgenic check line 15813 (C5) could not be performed since no larvae were found throughout the season on this check line. This check line seemed to have a stronger expression of the Bt-gene than the events. Event 6483 (plot 14) was the only event on which no larvae were found throughout the season, similar to the C5 check line.

Percentage control relative to that obtained with the transgenic line 15985 (C6) (Fig. 2.41)

Comparison of the transgenic events with the transgenic line 15985 (Table 2.28), showed that only event 6483 (plot 14), which had no larvae throughout the season, gave good control. By comparing the events with this transgenic line (15985), the test is stricter than with the Bollgard variety, since more larvae were obtained on the Bollgard than on this line. None of the events gave reasonable control while all the events other than the event of plot 14 (6483), were considered to have far weaker in their expression of the Bt-gene than the transgenic line 15985.

Comparison of the number of damaged squares, flowers and bolls by bollworm on transgenic events

No transgenic plants were found to have any damaged flowers or bolls. Differences in damage between events were only observed on the squares. The highest mean number of damaged squares was found in events 16960 (plot 27), followed by event 17206 (plot 31) (Fig. 2.24). Amongst the check plots, DP50 (C1) had the highest mean number of damaged squares, followed by C312 (C3). This corresponded with the high number of larvae found on these check plots (Fig. 2.34). The transgenic lines, 15813 (C5) and 15985 (C6), had less damaged squares than the other check plots and events. Very little or no damage on squares corresponded with the low larval counts found on those check lines. Events 6532 (plot 19) had the fewest damaged squares out of all the events, with a mean of 0.08 per plant. Although no larvae were found on event 6483 (plot 14), there was a mean of 0.18 damaged squares per plant. This shows that neonate larvae were present on the plants, but were killed after feeding had started. This probably supports strong expression of the Bt-gene in these plants. The positive characteristics of each line observed throughout the study are presented in a score chart (Tables 2.29 and 2.30).

CONCLUSIONS

The event 6483 (plot 14) scored 8/13 for beneficial characteristics of the event during the performance of the trial (Table 2.36). The check plot that gained the highest beneficial score was the transgenic line, 15813 (C5). Other events that gave good bollworm control were events 6227 (plot 5), 6532 (plot 19) and 6733 (plot 24).

Events that gave reasonably good bollworm control and a high yield per plant were 6239 (plot 8) and 6483 (plot 14). The highest yield obtained per plant was obtained with 15985 (C6), while DP50B (C2) gave the second highest yield amongst the check plots. The transgenic check line, 15813 (C5), achieved excellent bollworm control.

Part II (b): Evaluation of two IPC (Insect Protected Cotton) lines (15813, 15985), containing the Cry1Ac and the Cry2Ab2 proteins, a control (DP 50B) and a reference line (DP 50) for bollworm susceptibility in South Africa

This study on bollworm efficacy forms part of a wider study that was done on the same experimental plots to determine disease symptoms, and agronomic variance between the IPC lines. Only the bollworm efficacy data will be reported on in this chapter. The aim was to determine the efficacy of the Cry2Ab2 protein against bollworms, while monitoring the occurrence of secondary pests, and natural enemies.

EXPERIMENTAL PROCEDURES

Four field trials were carried out during the 1998/99 season in South Africa, to include Rustenburg (IIC-ARC), North West Province, 25° 43'S ; 27° 18'E; 1157m altitude); Brits (IIC Experimental Farm, North West Province, 25° 31 'S; 27° 49'E, 1109m altitude); Potgietersrus (IIC-ARC Experimental Farm, Northern Province; 24° 11'S 29° 01'E, 1116m altitude) and Friedenheim (Nelspruit) (IIC-ARC Experimental Farm, Mpumalanga Province; 25° 27'S; 30° 58'E) Treatments included a 15813 Insect Protec ted Cotton line (IPC line); 15985 IPC line; DP 50B (Deltapine 50 with Bollgard$^{TM)}$, DP 50 (Deltapine 50) (non-transgenic control). The trial size was 0.507 ha, laid out as a randomized block design of four cotton treatments with four replicates of each treatment. Each plot comprised of three rows, of 4.5m in length and 2m apart, containing 30 plants per row and 90 plants per plot. The distance between each plant within a row was 15 cm. Each plot was separated by four rows of non-transgenic cotton (DP 90). A 3m alley separated each plot. Planting took place with an in-furrow treatment of aldicarb (Temik®, 10kg/ha), for control of early season secondary pests. Endosulfan (Thioflo®, 1l/ha), was applied at egg threshold (40 eggs/80 plants) or larval threshold (16.6 larvae/80 plants). The threshold was applied to the number of plants scouted per plot (20) for four repetitions. The equivalent of endosulfan (Thioflo®, 1l / ha) spray mixture (2.5 bar) -CO_2 -driven knapsack sprayer fitted with a drop arm and six full-cone nozzles was applied. Endosulfan (Thioflo®) was applied to the specified cotton treatments above when the egg threshold (40 eggs/80 plants) or larval threshold (16.6 larvae/80 plants) was reached or exceeded. The threshold was applied to the number of plants scouted per plot (20) for four repetitions. Spraying was applied to all transgenic and non-transgenic cotton after consultation with the project leader[4]. After planting, plots were scouted at least once a week to assess insect, weed and disease infestation, with subsequent insect, weed and disease control. All chemical applications were documented in the field notebook (date, rate, and reason for application). All control, reference and IPC (Insect Protected Cotton lines) plots were sprayed for lepidopteran insect control, when necessary. Dual® and Cotogard® were applied, with irrigation 20 mm, followed by thinning one week later. All plots were managed the

[4] K. Hamilton, Monsanto St. Louis, who funded the project.

same way as cotton that is commercially produced, with respect to fertilization, irrigation and weed control. Similar planting procedures were followed for all three trials as explained in the field notebooks. The soil was mechanically prepared by deep, thorough ploughing, followed by tilling. The cotton was planted by hand at all the localities. Dual® and Cotogard® were applied at the registered dosages, followed by a light irrigation of about 20 mm, before germination of the seed. One week after emergence, plants were thinned out to a density of 33 000 plants/ha. When necessary, the cotton was irrigated with approximately 20-25 mm by means of an overhead sprinkler system. Plant tissue samples were collected from the field for gene expression. The dates and the names of the persons collecting the samples were recorded. The first, fully expanded true leaves (3 leaves) from 6 plants per plot were collected, frozen on dry ice in the field and shipped to Monsanto, St. Louis. Plant tissue sample kits were used to sample plant tissue. All seed were acid de-linted and shipped to Monsanto, St. Louis at ambient temperature. Total seed send included total seed cotton yield and seed from the 50 boll samples taken for fibre analyses. For the Brits locality, the seed from branches that broke off from the plants earlier in the season before harvesting due to heavy boll formation were included in the seed returned.

Several statistical tests or analyses were used for "fit" and effectiveness of the insect data. In order to understand the analyses followed for data collected from transgenic material to achieve the results explained in this report, it is necessary to state the type of analyses tested and their suitability, (pers comm. H. Muller[5]). Firstly, non-parametric chi-square tests were done on main effect of locality, site, treatment, time, locality-treatment interaction and time-treatment interaction effects. These tests were not included in the final analyses, since the tests on the interaction effects were not reliable. Unreliability of the interaction effects were decided on the presence of some entries in the contingency tables which contained frequencies of less than five, which weakens the discriminatory power of the test. The tests for the main effects confirmed the results of the ANOVA, which proved to be more informative and more suitable at the end. Furthermore, analysis of variance incorporating time was investigated as well. An attempt was made to omit those time intervals containing less than 25% infected plants. This too, seemed to prove ineffective. Since the data was collected over several time periods, the effect of time, using the regression approach was investigated. In the initial analysis, indications were that a significant time trend for Treatment two (DP 50) might exist, while no significant time trends were indicated for the other cotton treatments. According to Muller (pers comm.) the regression analysis restricted to this treatment (DP 50) indicated a poor linear fit, and it was subsequently omitted from the final analysis after collaboration with the project leader.

Finally, an analysis of variance for a randomized block design, nested over localities was performed, since the importance of comparing the effect of transgenic material on bollworms across localities (pers comm. K. Hamilton, Monsanto St. Louis) was chosen to be the important factor in comparing the data. Since sites were taken into account and replications are required to

[5] Ms. Heleen Muller is a statistician, and formerly worked for the Department of Datametrics, Pretoria, SA, at the Agricultural Research Council, but she is now working at the University of South Africa (UNISA).

perform an analysis of variance, the effect of time had to be ignored. The fact that the data is sparse by containing many zero values contributed to this. The analyses were done on transformed bollworm counts (power 0.25 transformation) in order to satisfy the ANOVA-assumptions of homogeneous variances and normally distributed residuals. Bonferroni LSD-tests were conducted on the transformed means, but conclusions reached concerning the differences between means apply directly to the untransformed mean counts.

Assessments

Planting data was noted during the performance of this trial, according to Good Experimental Practice. All production requirements were followed to be in agreement with the normal planting conditions for cotton under South African conditions. Aspects that were considered included the following: Receipt of seed and seed storage conditions; dates of planting; method of planting; equipment used; weather conditions at planting (specific information at the exact time of planting); tillage and seed bed preparation; soil temperature and moisture; seed depth; seeding density; method for cleaning equipment between plots to avoid contamination; insecticide treatment (see below); initial stand count, date of thinning (if necessary), final stand count. Maximum and minimum temperatures, as well as rainfall, associated with insect counts and insecticide applications for the separate localities are given in the tables. Assessments included a total number of seven consecutive scouts at Rustenburg, Brits and Potgietersrus, starting at two weeks after planting. No insects were recorded for the first count in Rustenburg; therefore only six datasheets are included in the Rustenburg field notebook. In Friedenheim, scouting started at six weeks after planting. Scouting commenced at first square formation on each locality. Twenty plants/plot were scouted for Helicoverpa armigera, Diparopsis castanea and Earias spp. A distinction was made between small (instar 1 and instar 2; L1-L2), medium (L3) and large (L4-L5) bollworm larvae of each species. However, since very few larvae were observed on the transgenic entries, the distinction between instars was ignored, and all larvae were grouped together across the localities. The number of damaged fruiting bodies was noted. The statistical package GENSTAT V. version 3.2 was used to analyze the bollworm data (Genstat 5 Committee, Rothampsted, UK. Clarendon Press, Oxford). Planting date in Rustenburg was on the 1998/11/09, while harvesting took place on the 1999/04/20-23. In Brits, planting date took place on 1998/11/10 and harvesting on1999/06/04 (total yield). Broken branches were harvested on the 1999/07/01. In Nelspruit, planting took place on 1998/10/28 and harvesting on 1999/03/23 (first picking), and the second picking on 1999/04/06. In Potgietersrus, planting took place on the 1998/11/11, and harvesting dates on the 1999/06/15.

In addition, the number of plants with other pest species was noted from two weeks after emergence. These secondary pests included aphids, spider mites, thrips, whiteflies, leafhoppers, cotton beetles (Nisotra spp., Chrysomelidae) and cotton stainer bugs. The number of beneficial insects, e.g. ladybirds (adults, larvae and eggs), lacewings (larvae, eggs) and spiders was also calculated. The number of cotton stainer bugs was only counted in the Rustenburg locality. For the Loskop locality, only the data concerning aphids, thrips, spider mites as well as leafhoppers were used in this report. For the comparison of damage to squares, flowers and bolls, line plots

were used for all relevant variables. No statistical analyses were done on insects other than bollworms, since this data is affected by insecticide applications. Comparison of other insects on transgenic cotton should be determined in trials where the spraying programme is similar on all cotton treatments and on all localities, or when no spraying is applied. By comparing this data statistically it might create a wrong interpretation of trends of other insects on transgenic cotton. However, the data presented do give an indication of the secondary pests present.

RESULTS

Weather data applicable to scouting dates and spraying dates is given in Tables 2.23-2.34.

Bollworm larvae

The efficacy of the transgenic cotton treatments on the different bollworm species were compared and statistically analyzed across localities.

The total number of red bollworm larvae of all 4 localities

There was a significant difference between cotton treatments over all ($p < 0.001$) with regards to red bollworms (Table A.2.18). From the table of means (Table 2.37), it is clear that there is a significant difference between the DP 50 treatment and all other cotton treatments at the 5% significance level (see Annexure II for LSD values applicable to transformed means). There were no significant differences between any of the three transgenic cotton treatments with regards to the mean total number of red bollworm numbers over all the localities. From the bar charts (Fig. 2.43), the DP 50 treatment showed the highest number of red bollworm larvae for each locality. No red bollworm larvae were observed on any of the three transgenic cotton treatments in Brits, while red bollworm larvae were present only on the 15985 line in Rustenburg and on the DP 50B variety in Nelspruit. In Potgietersrus, all cotton treatments showed a presence of red bollworm larvae. The high population of red bollworm in Potgietersrus was probably due to the presence of ratoon cotton on this locality.

The total number of spiny bollworm larvae of all 4 localities

There is a significant difference between cotton treatments over all ($p < 0.001$) with regards to spiny bollworms (Table A.2.10, Annexure II). From the table of means (Table 2.38), it is clear that there is a significant difference between the DP 50 treatment and all other cotton treatments at the 5% significance level (see Annexure II). There were no significant differences between any of the three transgenic cotton treatments with regards to the mean total number of spiny bollworm numbers over all the localities. From the bar charts (Fig. 2.44), it can be seen that spiny bollworms were present at all localities on the DP 50 treatment in larger numbers than on any of the other cotton treatments. No spiny bollworms were present on any of the other cotton treatments in Nelspruit, while some were present on the 15985 line in Rustenburg, on the DP 50B treatment in Brits, and on both the 15813 and DP 50B cotton treatments in Potgietersrus.

The total number of all species of bollworm larvae (african, red and spiny bollworms)

From the analysis of variance table (Annexure II -Table A.2.12), it can be deduced that there is a significant difference between cotton treatments over all ($p < 0.001$) with regards to the total number of bollworm larvae. From the table of means (Table 2.40), it is clear that there is a significant difference between the DP 50 treatment and all other cotton treatments at the 5% significance level (see Annexure II). There was no significant difference between any of the three transgenic cotton treatments with regards to the mean total number of bollworms in the bollworm complex, over all the localities (Table 2.41). Overall, there were no significant differences between bollworm numbers (with regards to all species) on the three transgenic lines on all localities, as well as in the interaction between treatment and locality for all larval species. This indicates that the transgenic varieties control bollworm numbers similarly on all localities, and that the main effect of locality with regards to temperature, soil, etc. does not have an effect in the expression of the Bt-gene. From the bar charts (Fig. 2.45), it can be deduced that the 15985 line showed most potential to suppress bollworm numbers, followed by the 15813 line and the DP 50 B variety. The DP 50 treatment had most larvae across all localities. When the total number of larvae from the Rustenburg locality on each treatment was compared (Fig. 2.46), the DP 50 treatment was found to have the most larvae. The 15813 line had the most larvae of the transgenic cotton treatments only on one occasion (count 5), while the DP 50B variety showed more larvae during the 4th and the 7th count in comparison with the other transgenic cotton treatments. In Brits (Fig. 2.47), the most larvae were observed in the DP 50 treatment, except for the first count, while the DP 50 B treatment gave most larvae at the end of the season in comparison with the other two transgenic cotton treatments. In Nelspruit (Fig. 2.48), a similar trend was also observed at the end of the season with regards to bollworm larvae, with the DP 50 treatment having the most larvae, and the DP 50B treatment having more than the other two transgenic lines. In Potgietersrus, however (Fig. 2.49), bollworm larvae varied during the beginning of the season on the DP 50 treatment and increased during the 4th, 6th and 7th count. At the end of the observation period, the DP 50B treatment again had more larvae than the other two transgenic cotton treatments.

A summary of the analyses of variance for bollworms from Brits, Potgietersrus, Nelspruit and Rustenburg is presented in Table 2.40. In all cases the DP 50 treatment had significantly more bollworm larvae than the other three transgenic cotton treatments (see also Fig. 2.49.).

Squares, flowers and bolls

The number of damaged squares was the highest on the DP 50 treatment in all localities (Fig. 2.50). This corresponds with the highest number of bollworm larvae found on this treatment on all localities. Rustenburg had the highest number of damaged squares on all cotton treatments except for the 15985 line, where there were more damaged squares in Potgietersrus. In Nelspruit, no damaged squares were observed in the 15813 and the 15985 lines, and only a few in the DP 50B treatment.

Most damaged flowers were found on the DP 50 treatment (Fig. 2.51), with the highest levels of damaged flowers observed at Brits. On this locality, there were no damaged flowers on the 15985 line, while no damaged flowers were found in Rustenburg and Nelspruit on the DP 50B treatment. In Potgietersrus, more damaged flowers were found on the DP 50B treatment than on the other two transgenic cotton treatments.

Most damaged bolls were found on the DP 50 treatment (Fig. 2.52), with Potgietersrus having the highest number. At Potgietersrus and Brits, there were no damaged bolls on the 15985 line. No damaged bolls occurred in Brits in the 15813 line. At Rustenburg, all three transgenic cotton treatments showed a similar number of damaged bolls.

Bollworm egg data

In Rustenburg (Fig. 2.53), DP 50 had the highest total number of bollworm eggs per treatment, except for the 3rd count when the egg counts were higher on the 15985 treatment. Bollworm population pressure seemed to be higher in Rustenburg, than in comparison with other localities. The total number of eggs laid in Rustenburg reached levels of up to 100 eggs per 20 plants during specific counts, and the peak period for egg laying seems to be somewhat earlier in the season than at the other localities.

In Brits (Fig. 2.54), the highest number of eggs was found during the 5th count (week 10) on the DP 50B treatment. This treatment had the highest number of eggs of all cotton treatments for the 4th, 5th and 6th counts during the observation period. The transgenic line 15813 had the lowest number of eggs for most of the season.

In Nelspruit (Fig. 2.55), the transgenic line 15813 had the highest number of eggs during the 3rd and the 4th count, while egg laying on the other cotton treatments varied.

In Potgietersrus (Fig. 2.56), the peak time for egg laying was during the 4th and 5th count, while a high number of eggs were also laid on the DP 50 treatment during the 7th count (14 weeks after planting). The number of eggs laid on the various cotton treatments varied throughout the season.

The peak egg laying period seems to be earlier in Rustenburg and Nelspruit (during the 3rd and 4th counts), and slightly later in Brits and Potgietersrus (during the 4th and 5th counts). Generally, higher numbers of eggs were found at Rustenburg and Potgietersrus (Figs 2.53 and 2.56) than at Brits and Nelspruit (Figs 2.54 and 2.55). Numbers of eggs found on the different cotton treatments at the different assessment periods vary and no clear preference for a particular cultivator or cotton line is apparent.

CONCLUSION

The three transgenic cotton treatments did not differ in efficacy with regards to bollworm larvae. All three transgenic cotton treatments controlled bollworm larvae significantly in comparison with the non-transgenic DP 50 treatment. All 3 species of bollworms were effectively controlled by all 3 transgenic treatments, and significantly different from the DP 50 treatment. There were no differences between the localities in the control of bollworm larvae by the transgenic cotton treatments. The DP 50 treatment had more damaged squares, flowers and bolls than any of the

transgenic cotton treatments, while damaged fruiting bodies varied between the transgenic cotton treatments. Oviposition by female bollworm moths varies between cotton treatments, and there is no trend between any of the transgenic and/or non-transgenic cotton treatments. The Bt-gene does not seem to have any effect on the selection of oviposition sites.

Part III: The evaluation of the *cry1Ac* gene and the *cry2Ab2* gene for bollworm efficacy in a segregant study

The aim of this study was to determine the level of bollworm resistance expressed by insect resistant genes in cotton in one transformed event containing the *cry1Ac* and the *cry2Ab2* genes, which was selected out for segregates.

EXPERIMENTAL PROCEDURES

Three field trials were conducted at the IIC- ARC in Rustenburg (North-West Province 25° 43'S; 27° 18'E; 1157m altitude); in Brits (IIC Experimental Farm) (North-West Province 25° 31'S; 27° 49'E, 1109 m altitude) and at Friedenheim (Nelspruit) (Mpumalanga Province 25° 27'S; 30° 58'E). The material used included the 15985BX line – containing Bollgard and *cryX*[6], 15985X – segregant containing only *cryX*, 15985B – segregant containing only Bollgard; DP 50B – original transformant of 15985 with Bollgard and DP 50 - non-transgenic recurrent parent of DP 50B. The material was used in treatments to include an unsprayed Treatment one of 15985BX; an unsprayed Treatment two of 15985X; an unsprayed Treatment three of 15985B, unsprayed Treatment four of DP50B, and an unsprayed Treatment five DP50; and sprayed Treatment six of 15985BX, which was sprayed according to threshold. Treatment seven consisted of sprayed DP50B, according to threshold and Treatment eight of DP 50, which was also sprayed according to threshold.

Trial size was 0.507 ha and the trial layout followed a randomized block design with eight treatments and eight replicates of each treatment. Double-row plots, each row 7.5 m in length, of the above-mentioned lines, with DP 90 interspersed throughout the plots in single rows. The distance between each plant within a row was 15 cm (6 inches). Each plot was separated by two rows of non-transgenic cotton (DP 90). A 3m alley separated the ends of each plot. Border rows (DP 90) of 12m surrounded the perimeter of the trial. All border rows were destroyed at the end of the season. The soil laboratory of the ARC-IIC determined the soil composition of soil samples from each locality. Methods for analysis of soil samples were those listed by Anon (1990). The pH was determined by saturated water paste and soil textures were determined by using a hydrometer. In Rustenburg, the trial was planted on a so-called turf soil with 50 % clay, 42 % sand and 8 % silt. The pH of the soil was between 6.98 and 7.68 at a depth of 30 - 90 cm. In Brits, the soil was a sandy clay loam soil of 62% sand, 33% clay and 5 % silt. The pH of the soil was between 6.89 and 7.68 at a depth of 30 - 90 cm. At Friedenheim, the soil was a sandy soil (85% sand) with 9.5% clay and a silt content of 5.3%. The pH of the soil was between 6.04 and 5.92 at a depth of 30 - 90 cm. Planting was performed by hand, by putting a single cottonseed with 3 soya bean seeds into each plant station.

[6] According to W.M. Green (formerly from Monsanto SA) (pers comm.), the term *cryX* represents the *cry1Ac* and *cry2Ab2* genes, used to for experimental purposes, where the actual event or cotton line had not been selected from multiple events or lines.

Assessments

A total weight of 2.970 kg seed was received for three trials at three localities. This excludes DP90 seed for the perimeter and the border rows. Seed was kept locked up under dry storage conditions. The following amounts of seed were used for the planting of each cotton type: 15985BX (456.0g); 15985X (264.0g); 15985B (259.2g); DP50B (494.4g); DP50 (480.0g). Planting dates are listed in Tables 2.49-2.51. Planting was performed by hand, by putting a single seed with three soy bean seeds into each planting station. This was done to enhance the germination of cotton seedlings, because little seed was available for planting. Soya bean seedlings were later removed by hand. Weather conditions at all three localities on the planting date are listed in Tables 2.4-2.51. Other trial documents that were noted included tillage and seedbed preparation, soil temperature and moisture (the seed was planted in dry soil at a temperature varying between 23-26 $^{\circ}$C at the various localities); seed depth in Brits was approx. 2 cm; in Rustenburg: approx. 2 cm and in Nelspruit: approx. 4 cm. Since the seed was planted by hand, no equipment was used, except for a planting rope to determine plant spacing. The packets of seed were kept sealed until the seeds were placed in the soil. The project manager controlled the distribution of seed over the separate plots. Maximum and minimum temperatures as well as rainfall associated with insect counts and insecticide applications are given on the separate localities in Tables 2.48, 2.49, 2.50. Herbicides (Dual and Cotogard 600 ml/ha and 4 l/ha mixed) were applied in Rustenburg on 17 November 1999, and in Brits (Dual® and Cotogard®, 600 ml/ha and 4 l /ha mixed) on 18 November 1999. In Nelspruit, MSMA 3l/ha was applied on 1 November 1999. Fertilizer was applied as 200kg LAN/ha on the 28 January 2000; 28 February 2000; and in Brits on 28 January 2000 and on 28 February 2000. In Nelspruit, 500 kg super phosphate was applied on 6 October 1999, as well as 200kg LAN/ha 23 November 1999 and on 24 January 2000 respectively. Overhead sprinkler irrigation was given in Rustenburg on 18/11/ 1999(20 mm); on 22/11/1999 (50 mm); in Brits on 23/11/1999 (20 mm and in Nelspruit during October 1999 (20 mm) and December 1999 (50 mm). No defoliant was used in any of these trials. Thioflo® (endosulfan) was applied to the specified sprayed treatments above when the larval threshold (16.6 larvae/80 plants) was reached or exceeded. The threshold of five larvae/24 plants was adapted to the number of plants scouted per plot (10) for eight repetitions. After planting, the plots were scouted at least once a week to assess insect infestation and the insects were subsequently controlled. All chemical applications are documented in Tables 2.49-2.51. A spray mixture, equivalent to endosulfan (1 l / ha), was applied at a pressure of 2.5 bar to all plants in the cotton treatments and was sprayed according to the threshold, by means of a CO_2 -driven knapsack sprayer fitted with a drop arm and six full-cone nozzles. With respect to fertilization, irrigation and weed control, all plots were managed the same way as cotton that is commercially produced. Similar planting procedures were followed for all three trials. The soil was mechanically prepared by deep, thorough ploughing, followed by tilling. The cotton was planted by hand at all the localities. Before germination of the seed, Dual and Cotogard were applied at the registered dosages and the applications were followed by a light irrigation of about 20 mm. Assessments included a total number of 10 consecutive scouts at

Rustenburg, Brits and Nelspruit, starting approximately six weeks after planting. Scouting commenced at first square formation on each locality. Ten plants/plot were scouted for african bollworm, red bollworm, and spiny bollworm. A distinction was made between small (instar 1 and instar 2; L1-L2), medium (L3) and large (L4-L5) bollworm larvae of each species. The number of damaged fruiting bodies was noted.

An attempt was made to do a simple analysis of the bollworm data. Previous experience with bollworm data collected from genetically engineered cotton indicated that complicated transformations, regressions and log regressions do in the end give differences between treatments, but these analyses are often hard for the reader to follow. In the case of bollworm data collected from transgenic cotton, it seems unnecessary to do the ultimate to prove the obvious, as can be seen from the line plots. The efficacy of the transgenic cotton treatments in controlling the total number of bollworms of the three different species was compared and statistically analyzed across localities, for Rustenburg and Nelspruit, by means of the X^2 square test. In addition, the Kolmogorov Smirnoff-test (non-parametric X^2 test) was used to test if the frequency distributions differed between treatments. Results obtained with this test supported those obtained with the Genstat non-parametric chi-square test and these results are, therefore not further discussed in this report. The analysis of spiny bollworm was omitted, since these bollworm numbers were too low or equal to zero. An attempt was made to compare the individual instar of each species of the bollworm complex, but the number of values available for comparison was far below the norm, and the many zero numbers are seen as missing values by the Genstat programme. Instead of statistical analyses between instars of the species, the graphs are represented in the Annexure A.2.1-A.2.9. In order to understand the analyses followed for data collected from transgenic material to achieve the results explained in this report, it is necessary to state the type of analyses tested and their suitability, as given by the Dept. of Datametrics (ARC – pers comm. H. Muller). Non-parametric chi-square tests were done on the total number of bollworms over all localities. Firstly, the chi-square test performed, tested if the frequency distributions of all 8 treatments were similar. From this test, it was seen that the frequency distributions of some treatments differed ($p < 0.001$). Secondly, a chi-square test was used to test if a proposed different expected frequency distribution for treatments correlated with the observed frequency distribution. With regard to the latter X^2 test, for all 3 bollworm species, the null hypothesis (H^o) that observed and expected frequencies are the same was not rejected. This proposed expected frequency then indicated which treatments differed. The data was sparse because it contained many zero values and, since the main objective of the project was to compare the effects of transgenic material on bollworm, the effect of time was ignored and the total number of bollworms observed was compared over all localities and between localities. For the Brits locality, the total number of bollworm analyzed did not include the L1-L2 Bt-cotton data, since there was uncertainty if this data did contain neonate larvae that have not fed. These hatchlings were thus not affected by the endotoxin by the time of scouting. However, this does not seem to make any difference to the results obtained in the statistical comparison of

treatments. The data was included in Table 2.45, and in the graphical representation of the total number of bollworms at all localities.

For the analyses of plant damage, a non-parametric chi-square test was performed to test if the distribution or homogeneity (ratio of increase/decrease) of damaged squares, flowers and bolls between treatments showed a similar trend over certain time periods.

RESULTS

Analyses of the total number of bollworms of all 3 localities

The chi-square test rejected the hypothesis that equal frequencies existed between treatments ($p < 0.001$; $X^2=1799.01$) (Table 2.45). When the different expected frequency distributions were compared between treatments, there was no reason to omit the hypothesis that the observed frequencies and corresponding expected frequencies differed between treatments ($p > 0.05$; insignificant at 5% level). With regard to the analysis of the total number of all bollworms over all localities (Table 2.45), there were no significant differences in bollworm control between the unsprayed transgenic 15985 treatments containing the Bt-gene. The unsprayed 15985 transgenic treatments with both the Bt-gene and the *cryX* gene (15985BXu) showed a significantly higher number of bollworms than the sprayed treatment. The unsprayed 15985 treatment with the *cryX* gene (15985Xu) showed a significantly lower number of bollworms than the unsprayed 15985 treatment with the Bt-gene. This could be as a result of the higher number of red bollworms observed at the Nelspruit locality that contributed to reach the total (Table 2.46). On at least one locality, there was no difference in the efficacy of the *cryX* gene, the Bt-gene, or the combination thereof in the 15985 variety in controlling the total number of larvae. When the total number of bollworms at Brits was compared between treatments (Table 2.45), the unsprayed transgenic 15985 variety with the *cryX* gene gave the lowest number of bollworms in comparison with the same unsprayed variety with only the Bt-gene or the combination of both genes. The sprayed 15985BXs treatment gave a correspondingly low number of bollworms in comparison with the 15985Xu treatment at all 3 localities (Table 2.46). For the 15985BXu treatment, the total number of bollworms from all 3 localities is reflected as a high expected value in comparison with the other transgenic treatments (Table 2.45). Unusually high numbers of bollworms in Nelspruit were probably the result of the poor expression of the gene, which in turn is related to environmental circumstances. If the high number of red bollworms at the Nelspruit locality is taken into account (Table 2.46), the total number of bollworms will be much lower and within the same range as numbers on the other 2 localities. This implies that numbers counted on the transgenic treatments would all be within the same range.

As expected, a sprayed control treatment gave significantly better bollworm control than an unsprayed control treatment (Table 2.45). The sprayed treatment containing Bollgard and the *cryX* segregant and the unsprayed 15985Xu treatment gave the lowest number of bollworms over the season. The 15985BXs treatment did not differ significantly from the unsprayed 15985Xu and DP50Bu treatments (Table 2.45). The total number of bollworms over all three localities is illustrated in Fig. 2.57. From the histogram, it is clear that the unsprayed control

treatment (DP50u) had most larvae throughout the season, followed by the sprayed control treatment (DP50s).

The total number of bollworm larvae from Rustenburg (Genstat -X^2)

As in the analyses of the total number of bollworm larvae across all localities, the chi-square test rejected the null-hypothesis that treatment frequencies are equal ($p < 0.001$; X^2=527.51). When the observed and expected frequencies were compared between treatments, the chi-square test indicated that there was no reason to omit the hypothesis that the treatment frequencies were different ($p = 1$). There were significant differences between the DP50u control treatment (Treatment five) and all other treatments (Table 2.45). In addition, the DP50s treatment differed significantly from all other treatments (Table 2.45). In Rustenburg, there were no differences between the treatments containing the *cryX* segregant and Bollgard, although the unsprayed Bollgard treatment (DP50Bu, Treatment four) differed significantly from the sprayed Bollgard treatment (DP50Bs, Treatment seven). Significantly fewer larvae were found on the unsprayed Bollgard treatment than on the sprayed Bollgard treatment (Table 2.45). The higher number of larvae found on the sprayed control treatment in comparison with the DP50u treatment corresponds with the higher number of eggs laid on the sprayed control treatment in general during the season (Fig. 2.70). From the line plot (Fig. 2.58), it is clear that most larvae occurred on the two DP50 control treatments. A varied mean number of larvae occurred on all the other treatments at certain weeks during the observation period (Fig. 2.58), but these treatments showed no specific trend, except for a slight increase in bollworm numbers on the DP50Bs treatment from week 7 onwards.

The total number of bollworm larvae from Brits (Genstat -X^2)

The total number of bollworm larvae from Brits, were not compared with the Genstat chi-square test, since there appeared to be no differences between the observations and the values were too low. The total number of bollworms observed at the Brits locality is given in Table 2.46. From the number of bollworms counted, it appears that there were not any significant differences between the transgenic 15985 unsprayed treatments. From the line plot (Fig. 2.59), it is evident that most larvae were found on the DP50u treatment, while the DP50Bu treatment gave a few larvae from the 5th week onwards (Fig. 2.59). The presence of larvae on the unsprayed Bollgard treatment was probably due to plant stress, which can be ascribed to less of the Bt-protein being expressed in the plant.

The total number of bollworm larvae from Nelspruit (Genstat -X^2)

As in the analyses of the total number of bollworm larvae across all localities, the chi-square test rejected the null-hypothesis that treatment frequencies are equal ($p < 0.001$; X^2=662.37). The chi-square test, then indicated that the treatment frequencies were significantly different and that there was no reason to omit the null-hypothesis that these frequencies differed between treatments (Table 2.45) ($p = 0.961$, thus $p > 0.05$). All treatments differed significantly from each other, except the 15985Xu treatment (Treatment two) and the 15985BXs treatment

(Treatment six). Only two larvae were observed in both of these treatments. These results indicate that the *cryX* gene has the potential to suppress bollworm numbers significantly. The same effect could probably have been noticed for the 15985BXu treatment, had a large number of eggs not been laid on this treatment in general during the season (Fig. 2.72). This increase in the number of eggs laid on the 15985BXu treatment was observed during counts 6, 8 and 9 and probably caused the high number of larvae found in Nelspruit on this treatment during the season (Table 2.46). The Bollgard unsprayed treatment (DP50Bu, Treatment four) had a significantly lower number of bollworm larvae than the 15985Bu treatment (Treatment three).

Thus, when only the *cryX* gene was present, the unsprayed treatment gave similar results to those obtained with the sprayed Bollgard and *cryX* gene treatment. This indicates that, at Nelspruit, the *cryX* gene on its own, or in combination with Bollgard, performed better than the Bollgard gene on its own (15985Bu), since a significantly lower number of bollworms were observed on this treatment. However, the opposite was true for the 15985BXu treatment. This might indicate that the Bollgard gene expression could be influenced by other external factors such as plant stress caused by extreme leafhopper infestation at Nelspruit or unnatural plant growth caused by the above-average rainfall in the area. From the line plot (Fig. 2.60), it can be seen that, in general, most larvae occurred on the DP50u treatment. The 15985Bu treatment, the DP50Bu treatment and the 15985BXs treatment showed a slight increase in larvae at different counts during the observation period (Fig. 2.60)

A comparison of the total number of african and red bollworms over localities (Van Ark's r x c X^2-test)

A comparison of the total number of african and red bollworm larvae of all the localities revealed significant differences between treatments ($p < 0.00001$; $X^2 = 36.857$) in rows. The minimum allowable expected frequency is 3, and therefore the observed value for red bollworm for the 15985Xu treatment and the 15985BXs treatment was increased by 1. The 15985BXu treatment (Treatment one) differed significantly from the 15985Bu treatment (Treatment three). The frequency distribution of african and red bollworm larvae was different for Treatment one in comparison with Treatment three. Similarly, there were significant differences between the 15985BXu treatment (Treatment one), the DP50u treatment (Treatment five), the DP50Bs treatment (Treatment seven) and the sprayed control, DP50s (Treatment eight) (Table 2.49).

A comparison of the total number of bollworms over localities (Van Ark's r x c X^2-test)

The total number of bollworms over all three localities was also compared between treatments (Table 2.47). The chi-square test for rows tests whether the trend in the frequency distributions over time/weeks differs for the different treatments. These frequency distributions differed significantly per treatment at the 5 % level ($p=0.00001$ and $X^2 = 45.345$). The 15985BXu treatment (Treatment one) differed significantly from the control treatment, DP50u. This significance indicated that the frequency distributions of the number of larvae observed on all localities differed for the 15985BXu treatment and the unsprayed control treatment, DP50u. This can be seen in the sharp increase in the number of larvae present on the Nelspruit locality, when

compared with the increase in larvae on the other two localities. The presence of these larvae can possibly be due to the less efficient expression of the gene due to plant stress, as described previously. The mean number of african bollworms was not statistically compared by means of the r x c X^2 test of Van Ark, for reasons mentioned before. Distinguishing between the species of the bollworm complex at the 3 localities by using graphical representation. From the line plots (Figs 2.61-2.63), it can be seen that the mean number of african bollworms, of all five instars, was the highest on the DP50u treatment (Treatment five) and on the DP50s treatment (Treatment eight).

At Rustenburg, the transgenic treatments did not differ much in their control of african bollworm (Fig. 2.61). The 15985Xu treatment had african bollworm larvae only on three occasions, during weeks 4, 9 and 10 of the observation period. Similarly, the 15985BXs treatment had african bollworms on 4 occasions, during weeks 7-10 (Fig. 2.61).

At Brits (Fig. 2.62), the 15985BXu treatment and the 15985Bu treatment were more effective in suppressing african bollworm numbers than the other treatments. The DP50Bu treatment did not suppress bollworm numbers, as well as had been expected (Fig. 2.62), probably due to the fact that the Bollgard gene was not as well expressed as under normal weather and plant growth conditions.

At Nelspruit, no african bollworm larvae were found on the 15985Bxu treatment (Fig. 2.63). The sprayed 15985BXs treatment showed larvae only at the 10th count and the 15985Bu treatment showed larvae only at the 5th count. The unsprayed Bollgard treatment (DP50Bu) had larvae only at the 5th and 7th counts, while the sprayed Bollgard treatment (DP50Bs) had a number of larvae similar to that of the DP50u treatment at the 3rd and 5th counts (Fig. 2.63).

Distinguishing between the red bollworm at the 3 localities

A few more red bollworm larvae occurred in some of the treatments at Rustenburg, namely in the 15985Bxu treatment and in the 15985Bu treatment (see also figures in Annexure V.). Red bollworm numbers were still much lower than those of the untreated control plot, DP50u (Fig. 2.64).

At Brits, the 15985Bu treatment seemed to be less efficient during weeks 5-8 and during the 9th and 10th weeks (Fig. 2.65).

At Nelspruit (Fig. 2.66), the red bollworm population peaked during the 3rd and 4th weeks, and the DP50Bs and 15985BXs treatments showed fewer larvae than the other transgenic treatments during this time period.

Distinguishing between the spiny bollworm at the 3 localities

It was obvious that the transgenic treatments suppressed the spiny bollworms effectively (Figs 2.67-2.69), except for some treatments at Rustenburg (Fig. 2.67), namely the DP50Bs treatment during week 5, the 15985Xu treatment during weeks 5 and 6, and the 15985BXs treatment during week 6. At the other localities, no spiny bollworms were noticed amongst the

transgenic treatments (Figs 2.68 and 2.69). The sprayed DP50 treatment was more effective than the unsprayed DP50 treatment in suppressing spiny bollworm numbers on all three localities.

Bollworm egg data

The bollworm egg counts were not statistically analyzed. A comparison of the number of bollworm eggs on transgenic and non-transgenic cotton over the past three seasons in trials performed at the ARC-IIC, have shown that there are no statistical differences between the number of eggs laid on transgenic cotton and the number of eggs laid on non- transgenic cotton. However, the trend in egg laying on all three localities will be discussed with reference to the line plots (Figs 2.70-2.72).

At Rustenburg (Fig. 2.70), egg counts varied on all the treatments and were moderately low up to the 4th count. Egg numbers started to increase from the 4th count onwards, up to the 9th count, where after a drop in egg numbers was observed for most treatments. Except at the 1st and 3rd counts, the egg numbers of the DP50u treatment (unsprayed control) generally tended to exceed those of most of the other treatments, except for the DP50s treatment on two occasions. The 15985Bu treatment and the DP50Bu treatment had fewer eggs than most of the other treatments throughout the season (Fig. 2.70).

At Brits (Fig. 2.71), an increase in the number of eggs was observed at the 6th and 8th counts, with a slight increase at the 10th count. It seemed that two peaks in egg laying could be observed for the Brits locality.

At Nelspruit (Fig. 2.72), a varied number of eggs were observed in all the treatments. Eggs started to increase from the 3rd count onwards, to reach a peak at the 9th count. A slight decrease in the number of eggs was observed at the 10th counts for most of the treatments. In general, it seemed that the sprayed control, DP50s, had most eggs, except before the 3rd count, and at the 6th and 7th counts. This is probably due to the effect of the insecticide on predators and parasites of bollworm eggs that results in a low degree of predation or parasitism and a subsequent increase in the total number of eggs.

Egg counts on all treatments were high at Rustenburg and Brits (Figs 2.70 and 2.71) in comparison with egg counts at Nelspruit (Fig. 2.72). On all three localities (Fig. 2.73), there seemed to be a peak in oviposition by the 9th week, where after egg numbers declined. At Rustenburg and Nelspruit egg counts seemed to increase quite steadily, while two peaks were observed at Brits, a smaller peak at the 2nd count and a higher peak at the 6th count.

Squares, flowers and bolls (Van Ark's non-parametric r x c X^2 test)

Firstly, the non-parametric r x c X^2 test of Van Ark was used to test whether the total number of damaged squares, flowers and bolls tended to be similar for all three localities over time. Thus, the chi-square test for rows is used to test whether the trend in the frequency distributions of squares, flowers and bolls differs for the different treatments over time/weeks (Table 2.49). The frequency distributions (increase/decrease) of damaged plant parts ($p = 0.0150$; $X^2 = 27.817$)

differed significantly within the treatments (Table 2.50). The 15985BXs treatment (Treatment six) differed significantly from the 15985Bu (Treatment three), DP50Bu (Treatment four), DP50u (Treatment five), DP50Bs (Treatment seven) and DP50s (Treatment eight) treatments (Table 2.50). Plant damage, specifically boll damage (Table 2.50), on Treatment six was considerably lower when compared with the other treatments. Treatment six was a sprayed treatment and fewer bolls were damaged on this treatment, possibly indicating that the combination gene (*cryX* gene and the Bollgard gene) provides better protection against boll damage when sprayed on threshold. This could have some implications in the commercial registration of the product. From the line plots (Figs 2.80 and 2.81), it can be seen that most plant damage was observed on the two control plots (DP50u and DP50s). From the line plots of the total number of squares, bolls and flowers (Fig. 2.74), it is clear that the two non-transgenic treatments (DP50u and DP50s have most damaged in comparisons with the other treatments, while the DP50 unsprayed treatment had more damaged from the 6th count onwards than the DP50 sprayed treatment. A logarithmic graph (Fig. 2.75), comparing the total number of damaged squares, flowers and bolls, gives a clearer picture of the difference in plant damage between the transgenic treatments. From Fig. 2.75, it is apparent that the 15985BXu treatment and the 15985Xu treatment proved to be more stable than the other transgenic treatments.

Comparison of the combined total number of squares, flowers and damaged bolls at all the localities (Van Ark's non-parametric r x c X^2 test)

The chi-square test was used to determine whether the distribution pattern of the total number of squares, flowers and bolls was similar for all the treatments over the observation period. The distribution of plant damage between the treatments over time is given in Table 2.50. Significant differences were found in the distribution of plant damage between treatments ($p = 0.00001$; $X^2 = 263.450$) (Table 2.53). The 15985BXs treatment (Treatment six) differed significantly from the 15985BXu (Treatment one), 15985Xu (Treatment two) and from Treatment three, 15985Bu, and DP50Bu (Treatment four) treatments and the sprayed and unsprayed DP50 treatments. All other treatments apart from the controls (Treatments one, two, three and four) (Table 2.51) showed a slight increase in the number of damaged plant fruiting bodies up to weeks 8 and 9. The number of damaged fruiting bodies stayed relatively low. The DP50Bs treatment showed a peak in damaged fruiting bodies at the 6th count, where after the number of damaged fruiting bodies declined before increasing again from the 8th count onwards (Table 2.52). The trend in Treatment six seemed to be somewhat different from that in the other treatments.

Comparison of square damage between treatments over all the localities (Van Ark's non-parametric r x c X^2 test)

In the comparison of bollworm damage to squares (Fig. 2.76), it was found that the frequency distributions differed significantly for the different treatments over time (($p = 0.00001$); $X^2 = 239.186$) (Tables 2.50 and 2.53). The 15985Xu treatment (Treatment two) differed significantly from the DP50u (Treatment five) and 15985BXs (Treatment six) treatments and the

DP50s (Treatment eight) treatments. From Figs 2.76 and 2.77, it is evident that the DP50u treatment and the DP50s treatment differed from the 15985Xu treatment, since the 15985Xu treatment generally gave the lowest number of damaged squares over time. The 15985BXs treatment (Treatment six) followed a similar trend to that of the 15985Xu treatment (Treatment two), except that bollworm damage on the two treatments peaked at different times during the observation period. Furthermore, the DP50Bs Treatment (seven) differed significantly from the unsprayed control, DP50u (Treatment five), and the sprayed control, DP50s (Treatment eight). Over time, less bollworm damage was observed on the sprayed Bollgard treatment at Rustenburg (Table 2.51) than on any of the non-transgenic DP50 treatments, whether sprayed or unsprayed. From the line plots, it can be seen that fewer damaged squared were observed at Rustenburg on the DP50s treatment than on the DP50u treatment (Fig. 2.78) while at Nelspruit (Fig. 2.80), the DP50s treatment had more damaged squares in the beginning of the observation period than the DP50u treatment. At Brits (Fig. 2.79), the DP50u treatment showed the highest number of damaged squares over the season. At Nelspruit, (Fig. 2.80) all transgenic treatments tended to have the same potential to avoid damage to squares.

Comparison of the total number of damaged squares at the Rustenburg and Brits localities, respectively (Van Ark's non-parametric r x c X^2 test)

Significant differences were observed in the frequency distributions of damaged squares at Rustenburg ($p = 0.00001$; $X^2 = 57.277$) and Brits ($p = 0.00001$; $X^2 = 114.707$) (Table 2.50).

For the Rustenburg locality, the 15985BXs Treatment (six) differed significantly from the control treatments, namely DP50u and DP50s from the 3rd week onwards. The number of damaged squares increased at a different tempo on the 15985BXs treatment in comparison with the other treatments (Table 2.50).

For the Brits locality, the 15985BXs treatment (Treatment six) differed significantly from the 15985BXu (Treatment one), 15985Xu (Treatment two), DP50u (Treatment five), DP50Bs (Treatment seven) and DP50s (Treatment eight) treatments (Table 2.57). The number of damaged squares in the 15985BXs treatment (Treatment six) decreased over time, probably as a result of the application of insecticides (Table 2.50), while the number of damaged squares in the 15985BXu treatment (Treatment one) increased slightly over time. The DP50u Treatment (five) also differed significantly from the DP50Bs Treatment (seven) and the sprayed control, DP50s (8).

Comparison of the number of damaged flowers as deduced from the line plots

At Rustenburg, the two control treatments, DP50u and DP50s, had the most damaged flowers in comparison with the other treatments (Fig. 2.81). A slight increase in the number of damaged flowers was observed in all treatments during the 9th count. No trend in respect of the number of damaged flowers could be observed between the transgenic treatments for the Rustenburg locality (Fig. 2.81).

When the transgenic treatments were compared, the 15985BXs treatment had the highest number of damaged flowers at all the counts taken at Brits (Fig. 2.82). In general, the two control treatments had the most damaged flowers at Brits.

At Nelspruit, very little damage was done to flowers overall (Fig. 2.83). However, as at Rustenburg and Brits, the most damaged flowers were observed on the two control treatments.

Comparison of the total number of damaged bolls over all the localities (Van Ark's non-parametric r x c X^2 test)

In a comparison of the total number of damaged bolls found at the respective localities during weeks 5-10, significant differences were observed in the distribution of boll damage between treatments (p=0.00001; Chi-square =85.269). When pairs of rows of the observed frequencies were compared, it was found that the DP50u control (Treatment five) differed significantly from the sprayed DP50s control (Treatment eight) as well as from the treatments with the Bollgard gene (Treatments one, three and seven), with the exception of the DP50Bu Treatment (four) (Table 2.60). The 15985BXs treatment could not be included in this analysis, because it had fewer damaged bolls than the minimum allowable frequency of 3 damaged bolls. This treatment was considered different from all the other treatments. The new variety, 15985 with the Bollgard gene, as well as the sprayed Bollgard™ variety, effectively prevented bollworm damage to the bolls over the season.

Comparison of the total number of damaged bolls at the Nelspruit locality

The number of damaged bolls at the Nelspruit locality exceeded the number at the other two localities (Table 2.53; Figs 2.84-2.86). The 15985BXs treatment was omitted from the analysis, since very few bolls were noticed on this treatment (Fig. 2.86). This indicated that this treatment differed significantly from all the other treatments (pers comm. H. Muller, Datametrics, ARC). The chi-square test for the period 5-10 weeks indicated that the DP50u and the DP50s treatments differed significantly. The new variety, 15985, with the *cryX* gene and the Bollgard gene, as a sprayed treatment on larval threshold, was effective in preventing boll damage by bollworm larvae**.** It is apparent that the two control treatments showed most boll damage at Rustenburg, Brits and Nelspruit respectively (Figs 2.84-2.86). Very few damaged bolls were noticed on any of the transgenic treatments at Rustenburg and Brits, while some damaged bolls seemed to be present on the sprayed Bollgard treatment (DP50Bs) at Nelspruit, as well as on the 15985Bu treatment (Fig. 2.86). This might suggest that the expression of the Bollgard gene is not as stable as that of the *cryX* gene under extreme weather conditions or environmental circumstances.

Analyses of the effects of the treatments on the various bollworm instars

The effects of the transgenic material on the various bollworm instars were not statistically compared. Line plots indicating the effects of the treatments on the different instars are given in Annexure V (Figs A.2.1-A.2.9) In general, the transgenic treatments were equally effective in suppressing the different bollworm instars. At Nelspruit, however, the DP50Bu treatment and the

DP50Bs treatment had a lesser effect on L3 african bollworm larvae (Fig. A.2.7 (b)). This could have been due to other stress factors on the plant. The leafhopper infestations on this trial and on the experimental farms were considered extremely high and they reached economically damaging levels. It might be that the Bollgard gene was not as well expressed on this locality, due to stress caused by other secondary insects

CONCLUSION

As expected the sprayed control treatment (DP50s) gave significantly better bollworm control, than an unsprayed control treatment (DP50u). With regard to the analysis of the total number of all bollworms over all localities, there were no significant differences in bollworm control between the unsprayed transgenic 15985 treatments containing the Bollgard gene (15985BXu and 15985Bu). The unsprayed 15985 transgenic treatment with both the Bollgard gene and the *cryX* gene (15985BXu) showed a significantly higher number of bollworms than the sprayed treatment, 15985BXs. The unsprayed 15985 treatment with the *cryX* gene (15985Xu) showed a significantly lower number of bollworms than the unsprayed 15985 treatment with the Bollgard gene. On at least one locality, there was no significant difference in the efficacy of the *cryX* gene, the Bollgard gene, or the combination thereof in the 15985 variety, to control the total number of larvae. Bollworm numbers on other localities reflected the same tendency amongst the transgenic 15985 treatments. The Bollgard gene in DP50Bu proved to be just as effective as the *cryX* gene in the 15985 unsprayed variety in suppressing the total number of bollworms. At Rustenburg, the unsprayed Bollgard treatment (DP50Bu) differed significantly from the sprayed Bollgard treatment (DP50Bs). At Nelspruit, the unsprayed Bollgard treatment (DP50u) had significantly fewer larvae than the 15985Bu treatment. On some localities, the Bollgard gene could be better expressed in one variety than in another. At Nelspruit, it was better expressed in the DP50 variety than in the 15985 line, most probably because the amount of endotoxin produced, and thus the expression of the gene is influenced by external factors which cause plant stress and which affect the two varieties differently. From the line plots, it is evident that the transgenic treatments (Treatments one, two, three, four, five and six) were more or less equally effective in controlling bollworm. It can also be seen from the line plots that the 15985BXu treatment showed no larvae throughout the season. When a very high density of bollworm larvae occurs, as for red bollworm at Nelspruit, the sprayed transgenic treatments prove to have more potential to suppress the higher numbers. Spiny bollworm numbers appeared to be low in comparison with african and red bollworm numbers. Except at Rustenburg, no spiny bollworm was noticed amongst the transgenic treatments. The transgenic treatments did not differ in their control of the various bollworm instars. The total amount of plant damage was significantly smaller on the 15985BXs treatment than on the other treatments, except the 15985BXu and 15985Xu treatments. Boll damage was also less on the 15985Xs treatment. This might suggest that the *cryX* gene is better expressed in the bolls than in other plant parts. Spraying insecticides on a combination transgenic variety might give better protection against plant damage caused by bollworm. The number of damaged squares on the DP50Bs treatment was significantly lower

than on the two control treatments, DP50u and DP50s. The 15985 variety with the Bollgard gene, the DP50Bs sprayed treatment and the DP50u treatment showed significant differences in the number of bolls with bollworm damage at Rustenburg and Brits. The new 15985 variety with Bollgard or a sprayed DP50s treatment can effectively prevent bollworm damage on these localities. In respect of both transgenic and non-transgenic cotton, no conclusion could be drawn with regard to the female moth's oviposition preferences. All transgenic treatments differed significantly from the non-transgenic control treatments. There were no significant differences between any of the transgenic treatments.

Final conclusion on bollworm efficacy data on Bt-cotton (Cry1Ac and Cry2Ab2)

Bt-cotton suppressed the bollworm complex present in South Africa efficiently in comparison with non-Bt-cotton. The Cry1Ac protein gives similar control of bollworms than the Cry2Ab2 protein, and either or both of these proteins can be introduced in an appropriate variety that gives good yield and quality in South Africa. Both proteins expressed efficient control to suppress all species of bollworms on cotton in South Africa under field conditions.

Smaller instars of bollworm (L1-L2) are more sensitive to the effect of the Bt-gene, than larger instars (L3-L5).

Bollworm egg appearance' are similar on non-Bt-cotton, and on Bt-cotton on all events, or on any other transgenic lines or non-transgenic cultivars evaluated in this study. No claim with regard to the detection of the "new genetic material and specific selection of Bt-cotton or not so by the female moth of any bollworm species can be deducted from the results of this study, and no literature has been cited where female moths can in fact distinguish between transgenic or non-transgenic cotton.

Damage to cotton, with regard to squares, flowers and bolls, is dependent on the bollworm infestation present at a particular time, which is affected by the presence of either of the proteins in the plant. Less bollworm present on the plant at a particular time, is reflected in the plants ability to maintain the number of squares, flowers and bolls, unaffected or damaged by bollworms.

Either of the proteins evaluated appear to be stable throughout the season and in all cultivars and lines evaluated, and could be used for introduction in a further resistance management strategy. Due to the protein expression not being affected by any chemical used during these trials, and throughout the season, either or both of these proteins have potential to be effective against bollworm. Either of these proteins can be used in a stackgene combination expressing bollworm resistance together with a possible herbicide tolerant gene.

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Tables

Table 2.1. Insecticide application dates, larval determination dates and climatic conditions for Rustenburg (1997/1998) in the Cry1Ac trial.

Planting Date	Insecticide Application date	Larval Determination date	MAX T (℃)	MIN T (℃)	RAIN (mm)	SUNS (Hr)	WIND (Km)
23/10/97			33.3	14.0	0.0	158.4	23.7
	27/01/98	27/01/98	31.9	18.0	0.0	193.7	25.0
	10/02/98	10/02/98	33.1	17.2	0.0	199.1	25.2
	24/02/98	24/02/98	29.7	17.8	0.0	130.2	23.8
	03/03/98	03/03/98	30.8	12.5	0.0	143.0	21.7
	10/03/98	10/03/98	33.2	16.1	0.0	176.8	24.7
	16/03/98	16/03/98	29.9	15.0	0.0	136.4	22.5
	24/03/98	24/03/98	31.0	14.3	0.0	163.5	22.7
	07/04/98	07/04/98	25.5	7.0	0.0	*	16.3

** missing value*

Table 2.2. Activities (scouting and spraying) carried out and pests monitored per treatment in Rustenburg (1997/1998) in the Cry1Ac trial.

Activity	Date	Chemicals sprayed	Pest	Treatment
Planting	26/11/97			
First scout	27/01/98			
1st spray	10/2/98	Thioflo® (1 l / ha)	Bollworms	Bollgard™ spray[E]
2nd spray	24/2/98	Thioflo® (1 l /ha)	Bollworms	Bollgard™ spray[E] DpAC 90 spray[E, L]
3rd spray	3/3/98	Thioflo® (1 l /ha)	Bollworms	DpAC 90 spray[L]
4th spray	10/3/98	Thioflo® (1 l / ha)	Bollworms	Bollgard™ spray[E] DpAC 90 spray[E, L]
5th spray	16/3/98	Thioflo® (1 l / ha)	Bollworms	Bollgard™ spray[E] DpAC 90 spray[E, L]
6th spray	24/3/98	Thioflo® (1 l / ha)	Bollworms	Bollgard™ spray[E] DpAC 90 spray[E, L]
7th spray	7/4/98	Thioflo® (1 l / ha)	Bollworms	Bollgard™ spray[E] DpAC 90 spray[E, L]
Last scout	7/4/98			

E = spray based on bollworm egg threshold of 30 eggs/60 plants

L = spray based on bollworm larval threshold of 12.5 larvae/60 plants

Table 2.3. Insecticide application dates, larval determination dates and weather conditions for Potgietersrus (1997/1998) in the Cry1Ac trial.

Planting Date	Insecticide Application date	Larval Determination date	MAX T (℃)	MIN T (℃)	RAIN (mm)	SUNS (Hr)	WIND (Km)	AVE T (℃)
23/10/97			34.4	16.5	0.0	11.7	97.0	25.5
		29/01/98	29.5	22.3	1.0	1.8	97.0	25.9
	02/02/98		34.5	18.0	0.0	10.2	53.0	26.3
		05/02/98	34.8	24.0	0.0	*	153.0	29.4
	10/02/98		33.5	19.2	0.0	*	171.0	26.4
		12/02/98	34.0	21.4	0.0	*	118.0	27.7
	16/02/98		29.9	21.5	1.5	0.8	94.0	25.7
		25/02/98	32.0	19.9	0.0	*	121.0	26.0
	26/02/98		34.4	19.5	0.0	*	60.0	26.5
		05/03/98	34.6	23.0	0.0	*	116.0	28.8
	12/03/98	12/03/98	28.0	18.0	10.0	*	82.0	23.0
	14/03/98		26.5	16.9	0.0	*	*	21.7
		19/03/98	31.0	20.0	0.0	*	112.0	25.5
		03/04/98	30.0	19.5	0.0	4.6	63.0	24.8

** missing value*

Table 2.4. Activities (scouting and spraying) carried out and pests monitored per treatment in Potgietersrus (1997/1998) in the Cry1Ac trial.

Activity	Date	Chemicals sprayed	Pest	Treatment
Planting date	23/10/97			
First scout	29/01/98			
1st spray	2/2/98	Thioflo® (1 l / ha)	Bollworms	Bollgard™ spray[E] DpAC 90 spray[E, L]
2nd spray	10/2/98	Thioflo® (1 l /ha)	Bollworms	DpAC 90 spray[E, L]
3rd spray	16/2/98	Thioflo® (1 l /ha)	Bollworms	DpAC 90 spray[L]
4th spray	25/2/98	Applaud® 125g/ha Wuxall® , 4 l /ha	Leafhoppers	Bollgard™ spray[E] DpAC 90 spray[E, L]
Last scout	3/4/98			

E = spray based on egg threshold of 30 eggs/60 plants

L = spray based on larval threshold of 12.5 larvae/60 plants

Table 2.5. Insecticide application dates, scouting dates and weather conditions at Groblersdal (1998/1999) in the Cry1Ac trial.

Insecticide Application Dates	Scouting Dates	Insecticides applied	MIN T (℃)	MIN T (℃)	RAIN (mm)
	99/01/07		31.2	17.7	1
		Thioflo® (endosulfan) 1 l /ha[E]			
99/01/11		Chess® (pymetrozine) 1kg/ha (sprayed after rain)	29.3	17.6	16
	99/1/14		33.2	15.2	0
99/1/15		Thioflo® (endosulfan) 1 l /ha[E]	31.7	14.8	0
	99/01/21		26.7	17.6	10.5
99/01/25		Thioflo® (endosulfan) 1 l /ha[E]	30.6	16.4	0
	99/01/28		28.3	15.3	0
	99/02/04		30.3	19.4	3
	99/02/11		32.3	14.5	4
99/02/12		Azodrin® (monocrotophos) 750 m l /ha	31.4	17.6	0
99/02/16	99/02/16	Thioflo® (endosulfan) 1 l /ha[E]	32.3	18.8	1
	99/02/26		33.3	15.6	0
99/03/03	99/03/03	Thioflo® (endosulfan) 1 l /ha[E]	36.4	15.3	0
99/03/11	99/03/11	Thioflo® (endosulfan) 1 l /ha[E]	32.0	15.0	0

E = spray based on egg threshold of 30 eggs/60 plants (derived from 12eggs/24 plants)

Table 2.6. Total number of larvae from Rustenburg (1997/1998) and Potgietersrus (1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Rustenburg	1.56	2.67	32.78	28.56
Potgietersrus	2.33	2.78	7.78	8.78

Table 2.7. The total number of bollworm larvae of the bollworm complex found during each count at Groblersdal (1998/1999) in the Cry1Ac trial.

Treatment Time	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
4/2/99	1	3	4	3
11/2/99	3	0	12	0
16/2/99	1	1	1	2
26/2/99	6	0	22	3
3/3/99	4	1	27	3
11/3/99	2	0	15	4
Totals	**17**	**5**	**81**	**15**

Table 2.8. The total number of each species of bollworm larvae at Groblersdal (February-March) (1998/1999) in the Cry1Ac trial.

Treatments	african bollworms	red bollworms	spiny bollworms
4/2/99	7	3	1
11/2/99	7	6	2
16/2/99	2	1	2
26/2/99	22	4	5
3/3/99	24	9	2
11/3/99	14	1	6
Totals	**66**	**25**	**18**

Table 2.9. The total number of each of the three species of bollworm larvae of all treatments per scouting time (February-March) at Groblersdal (1998/1999) in the Cry1Ac trial.

Treatments	african bollworms	red bollworms	spiny bollworms
Bollgard™ unsprayed	14	3	0
Bollgard™ sprayed	3	1	1
DpAC 90 unsprayed	52*	16*	13*
DpAC 90 sprayed	7	4	4
Totals	**78**	**24**	**18**

** differs significantly from at least the Bollgard™ sprayed treatment.*

Table 2.10. Total number of Bt-cotton larvae from Rustenburg (1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Means	0.00[a]	0.67[a]	7.11[b]	2.78[b]

* means with different letters, differ significantly ($p < 0.05$)

Table 2.11. Number of Bt-cotton larvae from Potgietersrus (1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Means	0.89	1.33	2.00	2.44

Table 2.12. Total number of red bollworm larvae from Rustenburg (1997/1998), Potgietersrus (1997/1998) and Groblersdal (1998/1999) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Rustenburg	1.56	2.00	14.33	21.89
Potgietersrus	1.11	0.78	4.78	5.56
Groblersdal	0.67[b]	0.5[b]	4.83[a]	3.17[ab]

$LSD_{Bonf(p < 0.05)} = 3.615$ for Groblersdal only

Table 2.13. Total number of spiny bollworm larvae from Rustenburg (1997/1998) and Potgietersrus (1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Rustenburg	0.00	0.00	11.33	3.89
Potgietersrus	0.33	0.67	1.00	0.78

Table 2.14. The mean number of different larval instars from Rustenburg (1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
First instar larvae	1.56	2.33	18.67	14.33
Second instar larvae	0.00	0.11	9.89	9.22
Third instar larvae	0.00	0.22	4.22	5.00

Table 2.15. The mean number of third instar larvae from Potgietersrus (1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Means	0.33	0.67	2.56	2.56

Table 2.16. Summary accumulated analysis of variance table for the Rustenburg locality (1997/1998) in the Cry1Ac trial.

Variable	Time (days)	Treatment	R^2a	Treatment Time	Deductions (using t values and probabilities from the estimates of the regression coefficients)
Total no. larvae	0.027	0.013	30.4	n.s	Treatments one and two differ significantly from three and four (Treatments one and two = not significant; Treatments three and four = not significant (n.s))
Bt-cotton	0.065	0.001	59.5	n.s.	Same as variable 1, but no trend indicated over time
red bollworm	0.002	0.014	36.2	n.s.	Same as variable 1
spiny bollworm	0.004	0.001	79.2	0.03	Same as variable 1 (trend over time non-parallel lines)
1st instar	0.036	0.029	25.8	n.s.	Same as variable 1
2nd instar	0.003	<0.001	76.2	n.s.	Same as variable 1
3rd instar	<0.001	<0.001	72.0	0.006	Same as variable 1 (trend over time, non-parallel lines)
Total no. eggs	<0.001	0.376	68.6	0.650	No differences between treatments Strong trend over time

Table 2.17. Summary accumulated analysis of variance table for the Potgietersrus locality (1997/1998) in the Cry1Ac trial.

Variable	Time (days)	Treatment	R^2a	Treatment Time	Deductions (using t values and probabilities from the estimates of the regression coefficients)
Total no. larvae	0.001	0.023	39.8	n.s	Treatments one and two differ significantly from three and four (Treatments one and two = not significant; Treatments three and four = not significant (n.s.))
Bt-cotton	0.001	n.s.		n.s.	No significant treatment differences. Strong general trend over time.
Red bollworm	0.003	0.021	33.9		Same as variable 1
spiny bollworm	0.001	n.s.		n.s.	No significant differences between treatments; General trend over time
1st instar	0.001	n.s.		n.s.	No significant differences between treatments; General trend over time
2nd instar	0.001	n.s.		n.s.	No significant differences between treatments; General trend over time.
3rd instar	0.058	0.031	22.2	n.s.	Same as variable 1
Total no. eggs	0.001	n.s.		n.s.	No differences between treatments Strong general trend over time

Table 2.18. The number of all bollworm larvae of the bollworm complex found during the entire observation period at Groblersdal (1998/1999) in the Cry1Ac trial.

Treatments	Total no. bollworm (February-March)	Total no. bollworms (February-March) (Chi-square test)	Total no. bollworms (January-March)	Mean no. bollworms (January-March) (ANOVA)
Bollgard™ unsprayed	17	14	21	3.5[a]
Bollgard™ sprayed	5	3	11	1.8[a]
DpAC 90 unsprayed	81	52*	138	23.0[b]
DpAC 90 sprayed	15	7	96	16.0[b]
		* differs significantly from at least the Bt-sprayed treatment		LSD Bonf ($p < 0.05$) = 12.467 Means sharing the same symbol are not significantly different.

Table 2.19. Bollworm eggs counted in Rustenburg (1997/1998) and Potgietersrus (1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Rustenburg	24.00	34.11	27.78	33.78
Potgietersrus	12.33	10.67	11.44	10.67

Table 2.20. Stand count for each plot before the non-transgenic plants were removed in the P2B trial.

Plot no.	Events	Event stand count	Check plot no.	Corresponding Check	Check Stand count
1	6149	60	C1	DP50	42
2	6151	71	C2	DP50B	49
3	6152	66	C3	C312	69
4	6223	70	C4	531	62
5	6227	77	C5	15813	63
6	6232	66	C6	15985	70
7	6234	73	C1	DP50	18*
8	6239	69	C2	DP50B	62
9	6241	60	C3	C312	72
10	6351	78	C4	531	71
11	6352	77	C5	15813	75
12	6456	83	C6	15985	70
13	6460	82	C1	DP50	37
14	6483	70	C2	DP50B	55
15	6484	70	C3	C312	68
16	6489	77	C4	531	83
17	6491	72	C5	15813	76
18	6526	71	C6	15985	59
19	6532	57	C1	DP50	30
20	6563	78	C2	DP50B	62
21	6542	72	C3	C312	70
22	6718	74	C4	531	81
23	6725	69	C5	15813	64
24	6733	63	C6	15985	66
25	6734	72	C1	DP50	28
26	16221	66	C2	DP50B	47
27	16960	69	C3	C312	70
28	17079	85	C4	531	82
29	17133	78	C5	15813	82
30	17180	80	C6	15985	81
31	17206	85	C1	DP50	31
32	6233	81	C2	DP50B	56
33	15830-5	69	C3	C312	83

Table 2.21. Stand count for each plot after the non-transgenic plants were removed in the P2B trial.

Plot no.	Events	Event stand count	Check plot no.	Corresponding Check	Check Stand count
1	6149	42	C1	DP50	42
2	6151	49	C2	DP50B	49
3	6152	46	C3	C312	69
4	6223	53	C4	531	62
5	6227	49	C5	15813	63
6	6232	52	C6	15985	70
7	6234	52	C1	DP50	18*
8	6239	58	C2	DP50B	62
9	6241	50	C3	C312	72
10	6351	52	C4	531	71
11	6352	73	C5	15813	75
12	6456	50	C6	15985	70
13	6460	43	C1	DP50	37
14	6483	48	C2	DP50B	55
15	6484	52	C3	C312	68
16	6489	41	C4	531	83
17	6491	54	C5	15813	76
18	6526	53	C6	15985	59
19	6532	38	C1	DP50	30
20	6563	56	C2	DP50B	62
21	6542	42	C3	C312	70
22	6718	57	C4	531	81
23	6725	55	C5	15813	64
24	6733	58	C6	15985	66
25	6734	41	C1	DP50	28
26	16221	57	C2	DP50B	47
27	16960	47	C3	C312	70
28	17079	82	C4	531	82
29	17133	60	C5	15813	82
30	17180	69	C6	15985	81
31	17206	56	C1	DP50	31
32	6233	36	C2	DP50B	56
33	15830-5	55	C3	C312	83

Table 2.22. Events with transgenic and non-transgenic plants that expressed less than or equal to two living larvae at any given time in the P2B trial.

Plot no.	Event	Time (week/s)
6	6232	3,8
8	6239	3,5
14	6483	6,8
17	6491	3
19	6532	8
24	6733	3,6
25	6734	2,3,4,5,6,7
33	15830-5	1,3,5,7,8

Table 2.23. Events that gave a total mean number of bollworm larvae smaller/ equal to 0.05 on positively checked transgenic plants in the P2B trial.

Plot no.	Event no.	Mean no of larvae/plant/ event/8 weeks)
2	6151	< 0.05
4	6223	< 0.05
5	6227	< 0.05
6	6232	< 0.05
8	6239	< 0.05
12	6456	<0.05
13	6460	= 0.05
14	6483	= 0
15	6484	<0.05
17	6491	< 0.05
19	6532	< 0.05
24	6733	< 0.05
26	16221	= 0.05

Table 2.24. Potential of transgenic events relative to that of DP50 (C1) in the P2B trial.

Plot-Event	Good control (10%) = 100%	Reasonable control (0 – 10 %)	Poor control (< 0%); negative control/high increase in larval population on event
1- 6149			
2- 6151		*	
3- 6152			*
4- 6223	*	*	
5- 6227		*	
6- 6232		*	
7- 6234			
8- 6239			
9- 6241			*
10- 6351			
11- 6352		*	
12- 6456		*	
13- 6460		*	
14- 6483	*	*	
15- 6484			
16- 6489		*	
17- 6491			
18- 6526		*	
19- 6532		*	
20-6563		*	
21- 6542		*	
22- 6718			*
23- 6725			
24- 6733		*	
25- 6734		*	
26- 16221		*	
27- 16960			*
28- 17079			
29- 17133			*
30- 17180		*	
31- 17206			
32- 6233		*	*
33- 15830-5		*	

Table 2.25. Potential of transgenic events relative to that of DP50B (C2) in the P2B trial.

Plot-Event	Good control (10%) = 100%	Reasonable control (0 – 10 %)	Poor control (< 0%); negative control/ high increase in larval population on event
1- 6149			*
2- 6151			*
3- 6152			*
4- 6223	*	*	
5- 6227		*	
6- 6232			*
7- 6234			*
8- 6239			*
9- 6241			*
10- 6351			*
11- 6352			*
12- 6456			
13- 6460			*
14- 6483	*	*	
15- 6484			
16- 6489		*	*
17- 6491			*
18- 6526		*	
19- 6532		*	
20-6563			*
21- 6542			*
22- 6718			*
23- 6725			*
24- 6733			*
25- 6734			*
26- 16221			*
27- 16960			*
28- 17079			*
29- 17133			*
30- 17180			*
31- 17206			*
32- 6233			*
33- 15830-5			*

Table 2.26. Potential of transgenic events relative to that of Coker 312 (C3) in the P2B trial.

Plot-Event	Good control (10%) = 100%	Reasonable control (0 – 10 %)	Poor control (< 0%); negative control/ high increase in larval population on event
1- 6149			*
2- 6151		*	
3- 6152			*
4- 6223	*		
5- 6227		*	
6- 6232		*	
7- 6234			*
8- 6239		*	
9- 6241			*
10- 6351		*	
11- 6352		*	
12- 6456		*	
13- 6460		*	
14- 6483	*	*	
15- 6484			
16- 6489			*
17- 6491		*	
18- 6526		*	
19- 6532		*	
20-6563		*	
21- 6542		*	
22- 6718			*
23- 6725			*
24- 6733		*	
25- 6734		*	
26- 16221		*	
27- 16960			*
28- 17079			*
29- 17133			*
30- 17180		*	
31- 17206			*
32- 6233			*
33- 15830-5		*	

Table 2.27. Potential of transgenic events relative to that of Coker 531 (C4) in the P2B trial.

Plot-Event	Good control (10%) = 100%	Reasonable control (0 – 10 %)	Poor control (< 0%); negative control/ high increase in larval population on event
1- 6149			*
2- 6151		*	
3- 6152			*
4- 6223		*	
5- 6227			*
6- 6232			*
7- 6234			*
8- 6239			*
9- 6241			*
10- 6351			*
11- 6352			*
12- 6456			*
13- 6460			*
14- 6483	*		
15- 6484			*
16- 6489			*
17- 6491			*
18- 6526		*	
19- 6532		*	
20-6563			*
21- 6542			*
22- 6718			*
23- 6725			*
24- 6733			*
25- 6734			*
26- 16221			*
27- 16960			*
28- 17079			*
29- 17133			*
30- 17180			*
31- 17206			*
32- 6233			*
33- 15830-5			*

Table 2.28. Potential of transgenic events relative to that of the transgenic line 15985 (C6) in the P2B trial.

Plot-Event	Good control (0%) = 100% (since the larval control no = 0)	Poor control (< 0%); negative control/ high increase in larval population on event
1- 6149		*
2- 6151		*
3- 6152		*
4- 6223		*
5- 6227		*
6- 6232		*
7- 6234		*
8- 6239		*
9- 6241		*
10- 6351		*
11- 6352		*
12- 6456		*
13- 6460		*
14- 6483	*	
15- 6484		*
16- 6489		*
17- 6491		*
18- 6526		*
19- 6532		*
20-6563		*
21- 6542		*
22- 6718		*
23- 6725		*
24- 6733		*
25- 6734		*
26- 16221		*
27- 16960		*
28- 17079		*
29- 17133		*
30- 17180		*
31- 17206		*
32- 6233		*
33- 15830-5		*

Table 2.29. Score sheet of beneficial characteristics observed amongst the events in the P2B trial.

Parameters Plot	Events	No. Bollworms </= 0.05	% Control	Cumulative number of Bollworms </= 0.2	< damage squares	Low freq. aphids	High no. lacewings	Low freq. Leafhoppers	Ladybirds	Spiders	> no. maize beetles	Yield (g/plant)	% GOT	Early boll burst
1	6149											*		
2	6151	*									*		*	*
3	6152												*	
4	6223	*											*	
5	6227	*		*							*		*	
6	6232	*									*		*	
7	6234								*			*		*
8	6239	*							*			*	*	
9	6241													
10	6351											*	*	
11	6352									*			*	
12	6456	*												
13	6460			*	*								*	
14	6483	*	*	*					*	*		*	*	
15	6484												*	
16	6489												*	
17	6491	*				*		*					*	
18	6526						*		*				*	
19	6532	*			*								*	
20	6563												*	
21	6542										*		*	
22	6718						*			*			*	
23	6725							*					*	
24	6733	*		*				*		*			*	
25	6734			*									*	
26	16221								*					
27	16960													
28	17079													
29	17133													
30	17180													
31	17206													
32	6233													
33	158305													

Table 2.30. Score sheet of beneficial characteristics observed amongst the control plots in the P2B trial.

Parameters Plot	Events	No. Bollworms < 0.5	% Control	Cumulative number of Bollworms < 0.2	< damage squares	Low freq. aphids	High no. lacewings	Low freq. Leafhoppers	Ladybirds	Spiders	⮝ no. maize beetles	Yield (g/plant)	% GOT	Early boll burst
C1	DP50													
C2	DP50B													
C3	C312												*	
C4	C531												*	*
C5	15813	*							*					
C6	15985	*												

Table 2.31. Insecticide application dates, scouting dates and weather conditions at Rustenburg in the Cry2Ab2 trial.

Insecticide application dates	Insecticides applied*	Scouting dates	MAX T (°C)	MIN T (°C)	RAIN (mm)
		98/11/24	31.5	16.8	0.5
		98/12/11	28.7	16.7	0.0
		99/01/06	29.1	17.2	0.0
		99/01/18	33.4	18.4	0.0
99/02/01	Thioflo® (endosulfan) 1 l /ha	99/02/01	29.0	16.6	0.2
99/02/04	Thioflo® (endosulfan) 1 l /ha		29.5	19.4	0.0
99/02/17	Thioflo® (endosulfan) 1 l /ha	99/02/17	30.5	18.4	0.0
		99/03/01	36.3	15.6	0.0

** applied when the number of larvae exceeded larval threshold of 16.6 larvae/80 plants*

Table 2.32. Weather conditions on insecticide application dates and scouting dates at Brits in the Cry2Ab2 trial.

Insecticide application date	Insecticide applied*	Scouting dates	MAX T (°C)	MIN T (°C)	RAIN (mm)
		98/11/24	31.0	17.2	0.5
		98/12/08	26.0	16.7	1.0
		99/01/05	29.0	18.2	0.0
		99/01/19	34.6	18.2	0.0
		99/02/02	30.2	18.0	0.0
		99/02/16	33.3	15.2	29.5
99/02/17	Thioflo® (endosulfan) 1 l / ha		32.0	15.0	0.0
		99/03/02	37.5	16.3	0.0

** applied when the number of larvae exceeded larval threshold of 16.6 larvae/80 plants*

Table 2.33. Insecticide application dates, scouting dates and Weather conditions at Nelspruit in the Cry2Ab2 trial.

Insecticide application dates	Insecticides applied*	Scouting dates	MAX T (°C)	MIN T (°C)	RAIN (mm)
		98/12/07	30.9	14.5	0.5
		98/12/21	25.9	19.2	0.1
		99/01/06	29.7	20.6	11.1
99/01/08	Chess® (pymetrozine) 800g/ha		27.6	18.4	0.0
99/01/14	Chess® (pymetrozine) 800g/ha Thioflo® (endosulfan) 1/ /ha		30.0	20.1	0.0
		99/01/20	32.1	19.1	0.0
		99/02/02	30.0	20.3	4.0
99/02/16	Dimethoate® (dimethoate) 800 l /ha Decis® (deltamethrin) 250 l /ha Thioflo® (endosulfan) 1 l /ha		27.1	18.3	0.0
		99/02/17	29.4	17.7	0.0
		99/03/08	29.1	17.9	0.0

** applied when the number of larvae exceeded larval threshold of 16.6 larvae/80 plants*

Table 2.34. Insecticide application dates, scouting dates and weather conditions at Potgietersrus in the Cry2Ab2 trial.

Insecticide application dates	Insecticides applied*	Scouting dates	MAX T (°C)	MIN T (°C)	RAIN (mm)
		98/11/25	29.0	16.0	10.0
		98/12/09	26.5	16.5	0.0
		99/01/06	30.5	18.5	0.0
		99/01/18	33.5	20.5	3.5
99/01/29	Thioflo® (endosulfan) 1 l/ha		30.7	20.0	0.0
		99/02/01	30.0	19.0	0.0
99/02/12	Chess® (pymetrozine) 800g/ha		34.0	17.5	0.0
		99/02/15	32.5	17.5	0.0
		99/03/01	36.5	17.0	0.0
99/03/07	Chess® (pymetrozine) 800g/ha		31.5	12.0	0.0
99/03/12	Agrimec® (abamectin) 500 ml/ha		31.0	20.0	0.0

** applied when the number of larvae exceeded the larval threshold of 16.6 larvae/80 plants*

Table 2.35. Transformed ($x^{0.25}$) mean number of african larvae of four localities in the Cry2Ab2 trial.

Treatments	No. of replicates	Means
15813	16	0.487[a]
DP 50	16	1.652[b]
15985	16	0.344[a]
DP 50B	16	0.734[a]

$LSD_{(Bonferroni\ p< 0.05)}$ = 0.3999

** means with the same symbol do not differ significantly*

Table 2.36. The untransformed mean number of african larvae of four localities in the Cry2Ab2 trial.

Treatments	No. of replicates	Total number of larvae	Means
15813	16	20	0.1786[a]
DP 50	16	157	1.4018[b]
15985	16	8	0.0714[a]
DP 50B	16	31	0.2768[a]

** means with the same symbol do not differ significantly*

Table 2.37. The untransformed mean number of red bollworm larvae of four localities in the Cry2Ab2 trial.

Treatments	No. of replicates	Total	Means[*]
15813	16	3.00	0.0268[a]
DP 50	16	44.00	0.3929[b]
15985	16	7.00	0.0625[a]
DP 50B	16	7.00	0.0625[a]

** means with the same symbol do not differ significantly*

Table 2.38. The untransformed mean number of spiny bollworm larvae of four localities in the Cry2Ab2 trial.

Treatments	No. of replicates	Total no. larvae	Means
15813	16	1.00	0.0089[a]
DP 50	16	40.00	0.3571[b]
15985	16	2.00	0.0179[a]
DP 50B	16	2.00	0.0179[a]

** means with the same symbol do not differ significantly*

Table 2.39. Summary of analysis of variance for bollworms at four localities in the Cry2Ab2 trial.

Variable	Treatments F pr	Cv% (coefficient of variance)	Treatment Locality	Deductions (using LSD values for transformed $x^{0.25}$ means)
african bollworm	0.001	69.4	n.s.	Treatment two differs from all other cotton treatments. No significant difference between transgenic cotton treatments.
red bollworm	0.001	85.7	n.s.	Treatment two differs from all other cotton treatments. No significant difference between transgenic cotton treatments.
spiny bollworm	0.001	118.6	n.s	Treatment two differs from all other cotton treatments. No significant difference between transgenic cotton treatments.
Total no. larvae	0.001	59.5	n.s	Treatment two differs from all other cotton treatments. No significant difference between transgenic cotton treatments.

n.s. indicates no significant difference between cotton treatments

Table 2.40. The untransformed mean number of bollworm larvae (african, red and spiny bollworms) of four localities in the Cry2Ab2 trial.

Treatments	No. of replicates	Total no. larvae	Means across localities
15813	16	24.00	0.2143[a]
DP 50	16	241.00	2.1518[b]
15985	16	17.00	0.1518[a]
DP 50B	16	40.00	0.3571[a]

means with the same symbol do not differ significantly

Table 2.41. Summary of analysis of variance for bollworms at four localities in the Cry2Ab2 trial.

Variable	Treatments F pr	Cv% (coefficient of variance)	Treatment Locality	Deductions (using LSD values for transformed $x^{0.25}$ means)
Bt-cotton	0.001	69.4	n.s.	Treatment two differs from all other cotton treatments. No significant difference between transgenic cotton treatments.
red bollworm	0.001	85.7	n.s.	Treatment two differs from all other cotton treatments. No significant difference between transgenic cotton treatments.
spiny bollworm	0.001	118.6	n.s	Treatment two differs from all other cotton treatments. No significant difference between transgenic cotton treatments.
Total no. larvae	0.001	59.5	n.s	Treatment two differs from all other cotton treatments. No significant difference between transgenic cotton treatments.

n.s. indicates no significant difference between cotton treatments

Table 2.42. Insecticide application dates and dosages at Rustenburg in the Cry1Ac and Cry2Ab2 stacked gene trail.

Activity	Date	Chemicals sprayed	Pest	Treatment
Planting				
First scout	10/01/2000			
1st spray	24/02/2000	Thioflo® 1 l / ha	Bollworms	DP50s*
2nd spray	16/03/2000	Thioflo® 1 l /ha	Bollworms	DP50s
3rd spray	10/04/2000	Thioflo® 1 l /ha	Bollworms	DP50s

L = spray based on larval threshold of 16.6 larvae/80 plants

**s = sprayed*

Table 2.43. Insecticide application dates and dosages at Brits in the *Cry1Ac* and Cry2Ab2 stacked gene trail.

Activity	Date	Chemicals sprayed	Pest	Treatment
Planting date	22/11/1999			
First scout	11/01/2000			
1st spray	8/02/2000	Thioflo® 1 l / ha	Bollworms[L]	DP50s*
2nd spray	22/03/2000	Thioflo® 1 l /ha	Bollworms[L]	DP50s
Last scout	28/03/2000			

L = spray based on larval threshold of 16.6 larvae/80 plants

**s = sprayed*

Table 2.44. Insecticide application dates and dosages at Nelspruit in the C*ry1Ac* and *Cry2Ab2* stacked gene trail.

Activity	Date	Chemicals sprayed	Pest	Treatment
Planting	10/11/1999			
First scout	11/01/2000			
1st spray	19/01/2000	Metasystox® 500 ml / ha	Leafhoppers	All treatments
2nd spray	25/01/2000	Thioflo® 1 l / ha	Bollworms L	DP50s
3rd spray	31/01/2000	Thioflo® 1 l / ha	Bollworms L	DP50s
4th spray	24/02/2000	Metasystox® 500 ml / ha	Leafhoppers	All treatments
Last scout	20/03/2000			

L = spray based on larval threshold of 16.6 larvae/80 plants

Table 2.45. Comparison of the total number of all bollworm larvae between localities (excluding L1-L2 african larvae from Brits) in the *Cry1Ac* and *Cry2Ab2* stacked gene trail.

Treatments	Total number of larvae Rustenburg, Brits and Nelspruit		Total number of larvae Rustenburg		Total number of larvae Nelspruit	
	Observed frequencies	Expected frequencies	Observed frequencies	Expected frequencies	Observed frequencies	Expected frequencies
15985Bxu	39	38	12	13	25	23
15985Xu	17	19	12	13	2 (3)*	4
15985Bu	37	38	15	13	12	11
DP50Bu	19	19	9	10	8	8
DP50u	482	486	159	157	174	174
15985BXs	16	19	13	13	2(3)*	4
DP50Bs	43	48	14	13	23	22
DP50s	299	286	94	95	133	133
	$X^2 = 1799.01$		$X^2 = 527.51$		$X^2 = 662.37$	
	$p < 0.001$		$p < 0.001$		$p < 0.001$	

Values adapted to the minimum critical value

Treatments with different expected frequencies differ from one another

Table 2.46. Comparison of the total number of all bollworm larvae at each locality (including L1-L2 african larvae from Brits) in the *Cry1Ac* and *Cry2Ab2* stacked gene trail.

Localities Treatments	Rustenburg african, red and spiny bollworms	Brits[a] african, red and spiny bollworms	Nelspruit african, red and spiny bollworms	Nelspruit (total number of red bollworms)
15985Bxu	12	11	25	24
15985Xu	12	8	2	0
15985Bu	15	13	12	2
DP50Bu	9	15	8	5
DP50u	159	209	174	86
15985BXs	13[b]	8[b]	2[b]	0
DP50Bs	14	18	23	4
DP50s	94	119	133	74

[a] *includes L1-L2 african bollworm larvae*
[b] *numbers did not reach the threshold, transgenic treatment was never sprayed*

Table 2.47. Significant differences in frequency distributions of the total number of bollworm larvae at Rustenburg, Brits and Nelspruit in the *Cry1Ac* and *Cry2Ab2* stacked gene trail.

Treatments	Genstat -X^2 Total no. of all 3 spp. Of bollworms (all loc.)	Genstat -X^2 Test for equal freq.: all 3 spp. of bollworms, Rustenburg	Genstat -X^2 Test for equal freq.: all 3 spp. of bollworms, Nelspruit	Van Ark's r x c X^2 test african and red bollworm (all loc.)	Van Ark's r x c X^2 test all 3 spp. of bollworms (all loc.)
15985Bxu	2,4,5,6,7,8	4,5,8	2,3,4,5,6,7,8	3,5,7,8	5
15985Xu	1,3,5,7,8	4,5,8	1,3,4,5,7,8	n.s	n.s
15985Bu	2,4,5,6,7,8	4,5,8	1,2,4,5,6,7,8	1	n.s
DP50Bu	1,3,5,7,8	1,2,3,5,6,7,8	1,2,3,5,6,7,8	n.s	n.s
DP50u	1,2,3,4,6,7,8	1,2,3,4,6,7,8	12,3,4,6,7,8	1	1
15985BXs	1,3,5,7,8	4,5,8	1,3,4,5,6,7,8	n.s	n.s
DP50Bs	1,2,3,4,5,6,8	4,5,8	1,2,3,4,5,6,8	1	n.s
DP50s	1,2,3,4,5,6,7	1,2,3,4,5,6,7	1,2,3,4,5,6,7	1	n.s
X^2	= 1799.01 (p < 0.001)	= 527.51 (p < 0.001)	= 662.37 (p < 0.001)	= 36.857 (p < 0.001)	= 45.345 (p < 0.001)

n. s = treatment not significantly different from other treatments

Table 2.48. The total number of bollworm larvae at each locality (Van Ark's non-parametric r x c X^2 test) in the *Cry1Ac* and *Cry2Ab2* stacked gene trail.

Treatments	Rustenburg	Brits	Nelspruit	Total
15985Bxu	12	3	25	40
15985Xu	12	3	3	18
15985Bu	15	10	12	37
DP50Bu	9	3	8	20
DP50u	159	149	174	482
15985BXs	13	3	3	19
DP50Bs	14	6	23	43
DP50s	94	72	133	299

Table 2.49. The total number of african and red bollworm larvae over all 3 localities (Van Ark's non parametric r x c X^2 test) in the C*ry1Ac* and *Cry2Ab2* stacked gene trail.

Treatments	Total number of african bollworm larvae	Total number of red bollworm larvae
Bxu15985	13	26
Xu15985	12	3
Bu15985	28	9
DP50Bu	14	5
DP50u	273	119
BSx15985	13	3
BsDP50	36	5
DP50s	163	101

X^2 = 36.85

p = 0.00001

** pairwise comparison tests indicate that Treatment one differs significantly from Treatments three, five, seven and eight*

Table 2.50. The total number of damaged squares, flowers and bolls of all 3 localities in the C*ry1Ac* and *Cry2Ab2* stacked gene trail.

Treatments	Total number of squares	Total number of flowers	Total number of bolls
15985Bxu	82	11	31
15985Xu	83	14	32
15985Bu	126	18	60*
DP50Bu	126	18	718
DP50u	1296	181	558*
15985BXs	114	22	20*
DP50Bs	129	13	60*
DP50s	963	118	391

$X^2 = 27.817_{(p < 0.05)}$ *for significant distribution of plant damage*

** Treatment six differs significantly from the other treatments marked with an asterisk*

Table 2.51. The total number of damaged squares at Rustenburg and Brits (Van Ark's non parametric r x c X^2 test) in the *Cry1Ac* and *Cry2Ab2* stacked gene trail.

Treatments	Rustenburg Weeks				Brits Weeks		
	3 and 4	5 and 6	7 and 8	9 and 10	5 and 6	7 and 8	9 and 10
15985BXu	3	12	14	15	6	8	14
15985Xu	5	5	11	13	3	20	8
15985B	7	9	14	26	14	20	10
DP50Bu	5	16	22	17	15	18	5
DP50u	29	156	145	129	128	314	177
15985BXs	11	3	25	21	27	7	8
DP50Bs	10	11	12	15	18	9	20
DP50s	39	128	98	104	149	108	82
	X^2 = 57.277 ; p =0.00001				X^2 = 114.707 ; p=0.00001		

Treatment six differs significantly from Treatments five and eight; Rustenburg

Treatment two differs significantly from six, seven, eight; Treatment one from six;

Treatment five from six, seven and eight: Brits

Table 2.52. The total number of damaged bolls (Van Ark's non-parametric r x c X^2 tests) in the C*ry1Ac* and *Cry2Ab2* stacked gene trail.

Treatments	Rustenburg, Brits and Nelspruit Weeks						Nelspruit Weeks		
	5	6	7	8	9	10	5 and 6	7 and 8	9 and 10
15985BXu	9	4	5	3	6	3	12	3	4
15985Xu	8	3	4	3	6	6	11	3	3
15985Bu	15	12	9	6	8	9	25	13	10
DP50Bu	14	12	3	10	18	12	24	11	14
DP50u	41	84	55	141	148	76	104	87	116
15985BXs	3	7	3	3	5	5	*	*	*
DP50Bs	17	11	3	13	10	5	27	11	10
DP50s	52	73	29	69	91	61	116	40	87
	X^2 = 85.269 ; p = 0.00001						X^2=411; p= 0.001		

** Treatment 15985BXs omitted from the analysis*

Treatment five differs significantly from Treatments one, three, seven and eight; all localities;

Treatment five differs significantly from eight; Nelspruit

Table 2.53. The total number of damaged squares, flowers and bolls of all three localities in the *Cry1Ac* and *Cry2Ab2* stacked gene trail.

Treatments	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Total
15985Bxu	3	8	5	17	16	16	17	24	19	**125**
15985Xu	7	8	8	13	11	19	20	13	28	**127**
15985Bu	7	13	9	26	29	26	29	38	25	**202**
DP50Bu	9	11	8	36	30	26	32	30	30	**212**
DP50u	59	82	47	194	263	265	451	372	274	**2007**
15985BXs	7	7	6	5	52	25	11	18	24	**155**
DP50Bs	9	15	11	31	35	16	22	28	31	**198**
DP50s	93	80	76	259	196	108	239	221	183	**1455**
Total	**194**	**224**	**170**	**581**	**632**	**501**	**821**	**744**	**614**	**4481**

Table 2.54. Significant differences in frequency distributions of damaged squares, flowers and bolls of Rustenburg, Brits and Nelspruit (Van Ark's non-parametric r x c X^2 tests) in the *Cry1Ac* and *Cry2Ab2* stacked gene trail.

Treatments	Total no. of squares, flowers, bolls (all loc.)	Test for equal freq.: squares (all loc.) (weeks 2-10)	Test for equal freq.: squares, flowers, bolls (all loc.) (weeks 2-10)	Test for equal freq.: squares (Brits) (weeks 5 10; 2-weekly)	Test for equal freq.: squares (Rustenburg) (weeks 5-10, 2-weekly)	Test for equal freq.: bolls (all loc.) (weeks 6-7, weeks 8-9)	Test for equal freq.: bolls (Nelspruit) (weeks 6-7, weeks 8-9)
15985Bxu			6	6		5	
15985Xu		5,6,8	5,6	6,7,8			
15985Bu			6			5	
DP50Bu	6		6				
DP50u	6	2,6,7,8	2,6,7,8	6,7,8	6	1,3,7,8	8
15985BXs	4,5,7,8	2,5,8	1,2,3,4,5,7,8	1,2,5	5,8		
DP50Bs	6	5,8	5,6	2,5		5	
DP50s	6	2,5,6,7	5,6	2,5	6	5	5
X^2	**= 27.817** (p = 0.0150)	**= 239.186** (p =0.00001)	**= 263.450** (p = 0.00001)	**= 114.707** (p = 0.00001)	**= 57.277** (p =.0.00001)	**= 85.269** (p = 0.00001)	**= 32.411** (p = 0.0010)

Figures

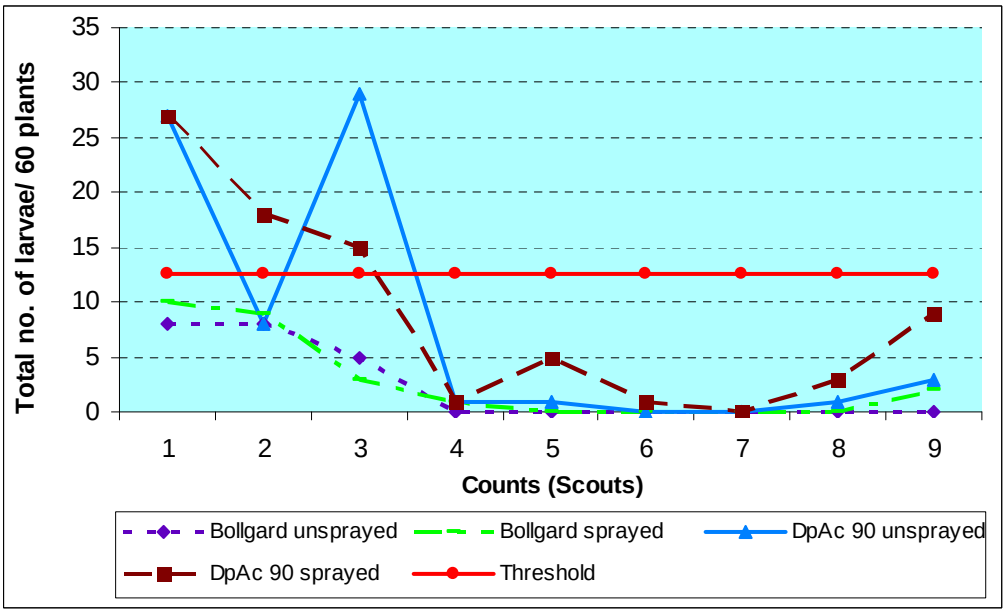


Fig. 2.1. Total number of larvae counted in Rustenburg (1997/1998) in the Cry1Ac trial.

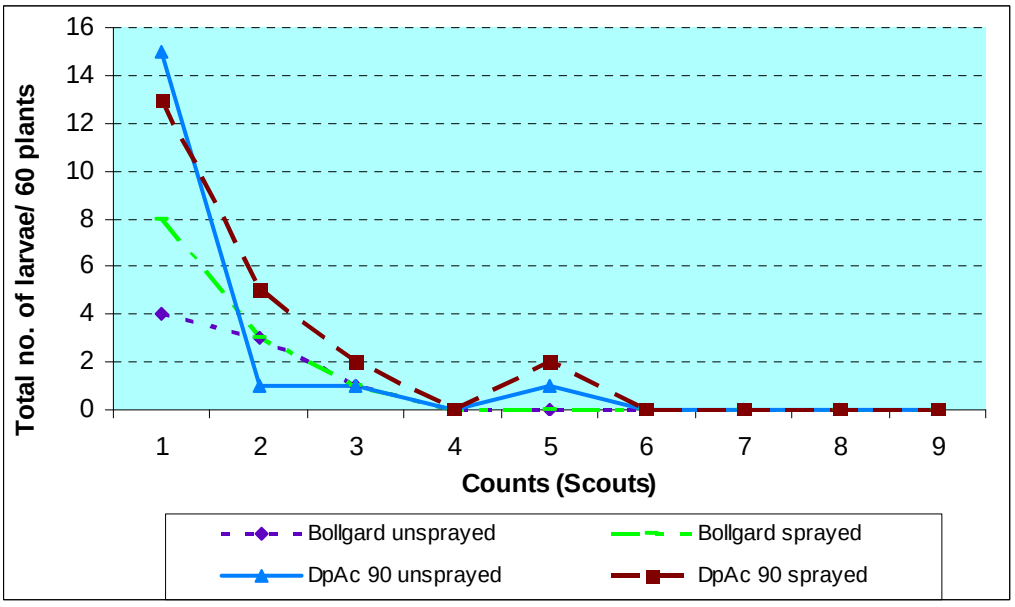


Fig. 2.2. Total number of larvae counted in Potgietersrus (1997/1998) in the Cry1Ac trial.

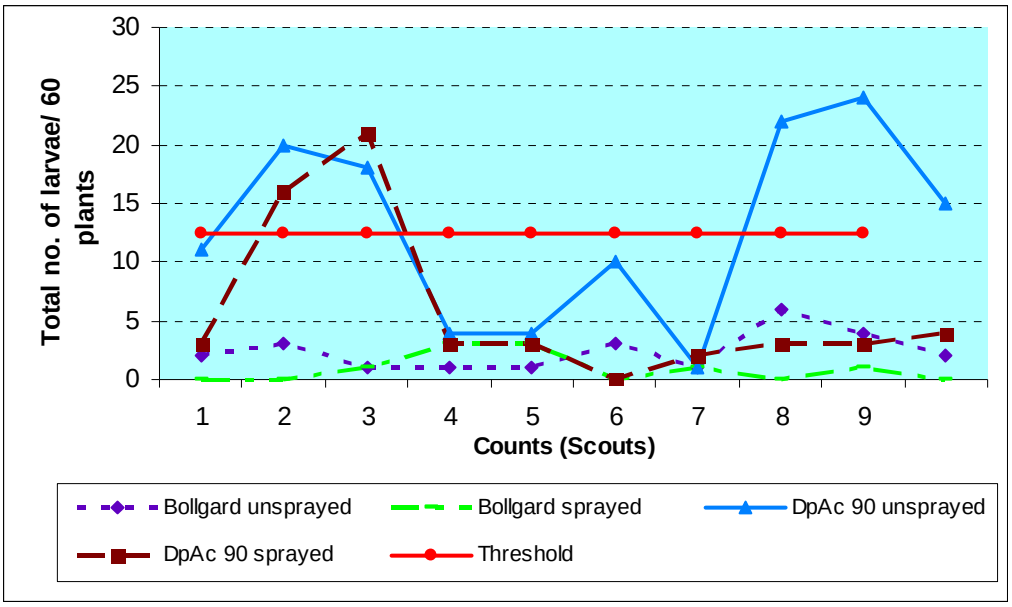


Fig. 2.3. Total number of larvae counted in Groblersdal (Loskop) (1998/1999) in the Cry1Ac trial.

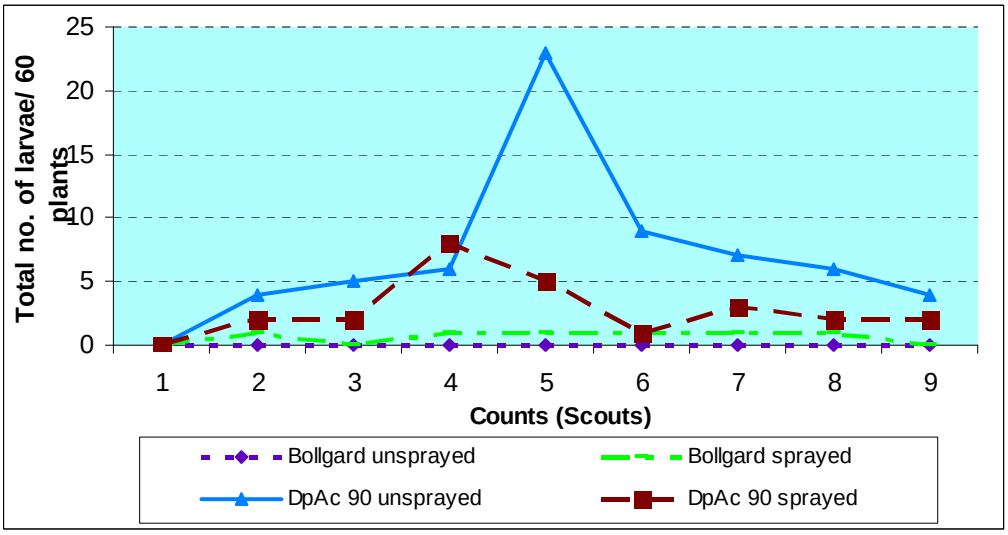


Fig. 2.4. number of african bollworm larvae in Rustenburg (1997/1998) in the Cry1Ac trial.

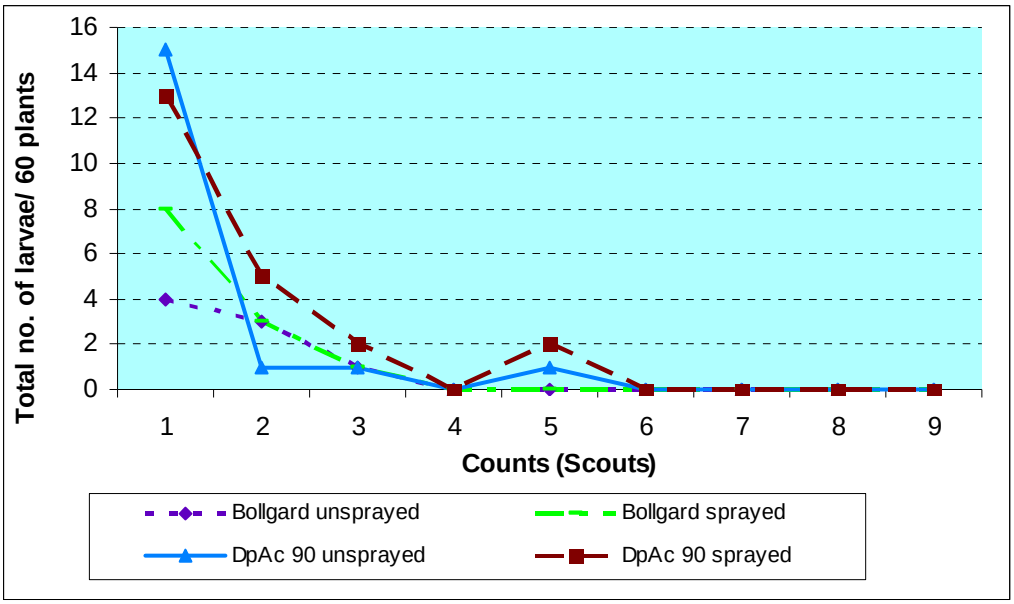


Fig. 2.5. Total number of african bollworm larvae in Potgietersrus (1997/1998) in the Cry1Ac trial.

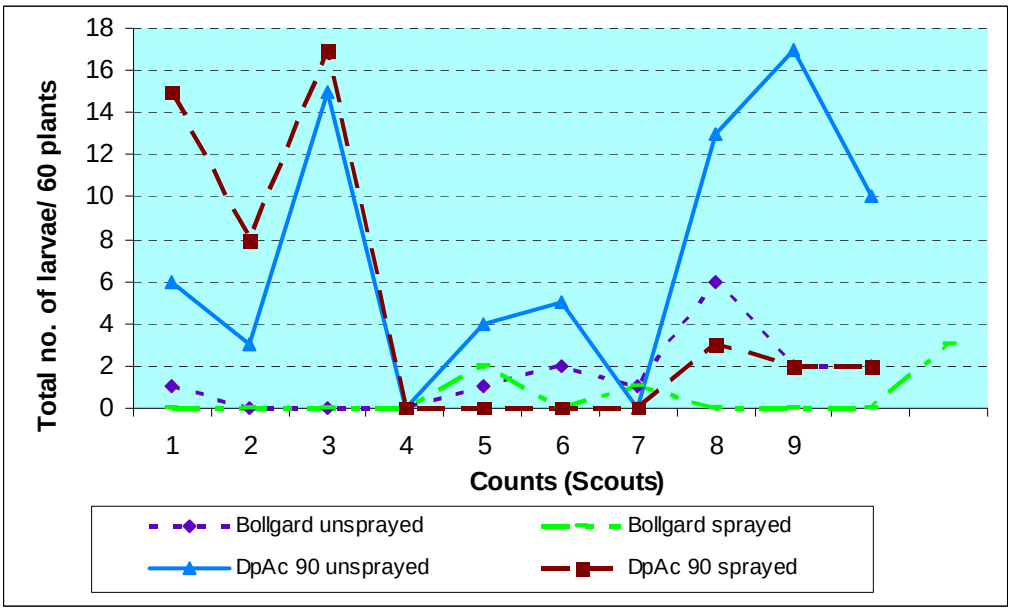


Fig. 2.6. Total number of african bollworm larvae in Groblersdal (Loskop) (1998/1999) in the Cry1Ac trial.

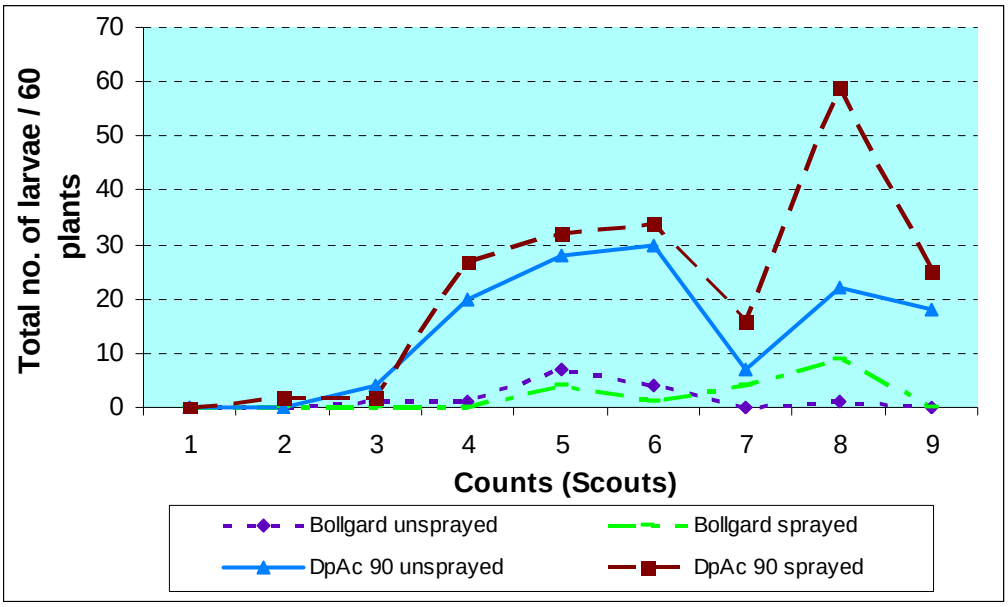


Fig. 2.7. Total number of red bollworm larvae counted in Rustenburg (1997/1998) in the Cry1Ac trial.

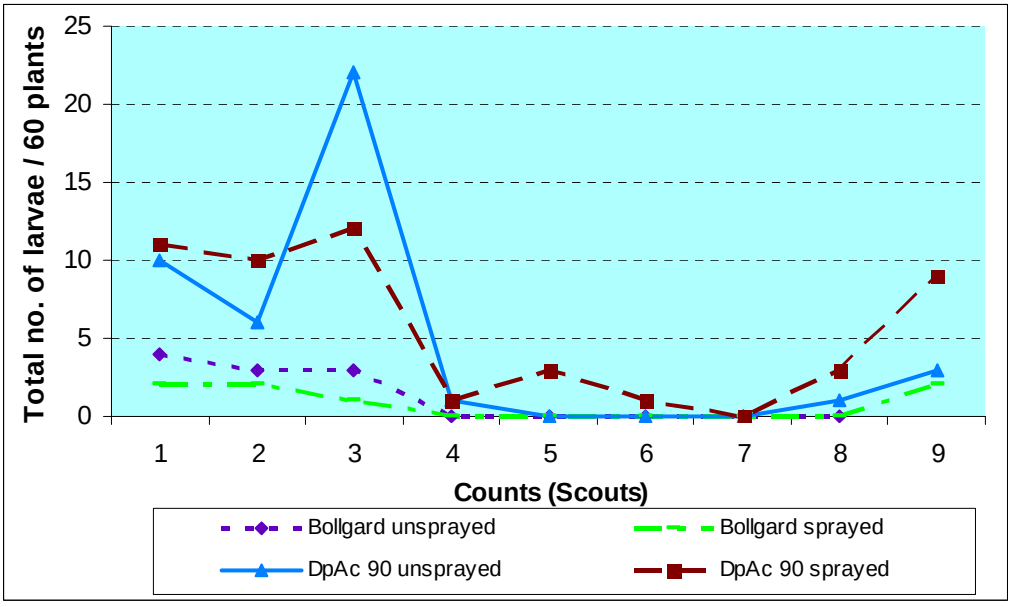


Fig. 2.8. Total number of red bollworm larvae counted in Potgietersrus (1997/1998) in the Cry1Ac trial.

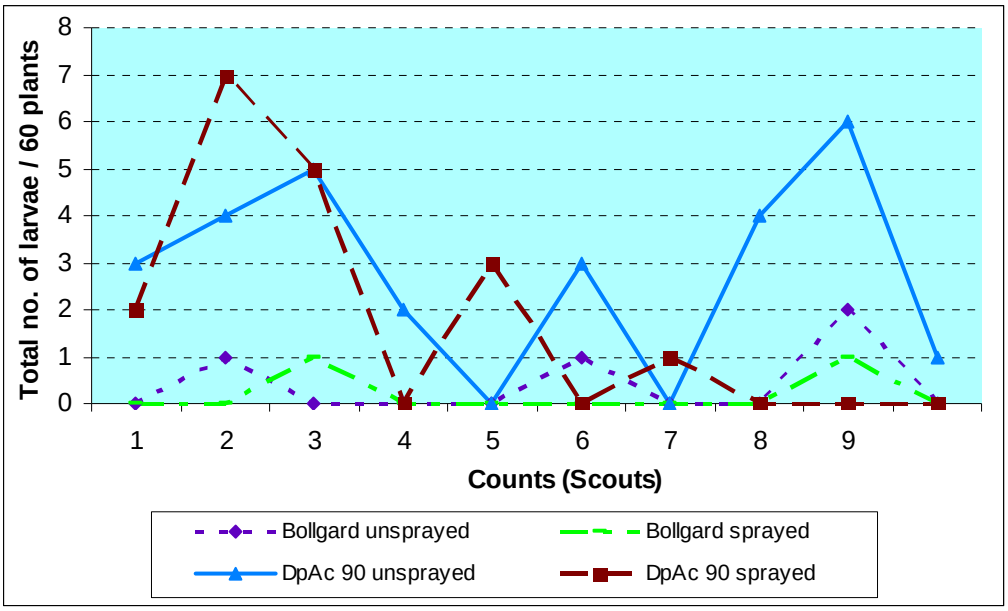


Fig. 2.9. Total number of red bollworm larvae counted in Loskop (Groblersdal) (1998/1999) in the Cry1Ac trial.

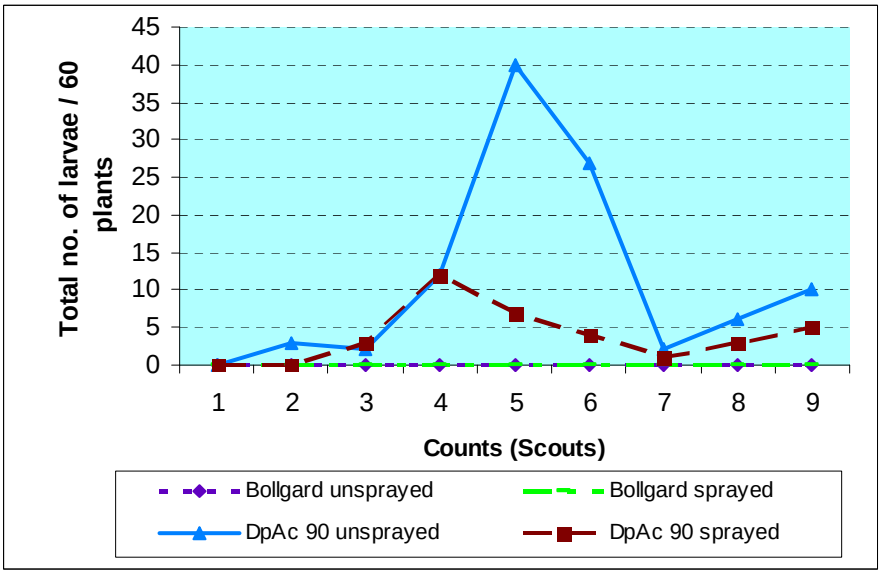


Fig. 2.10. Total number of spiny bollworm larvae in Rustenburg (1997/1998) in the Cry1Ac trial.

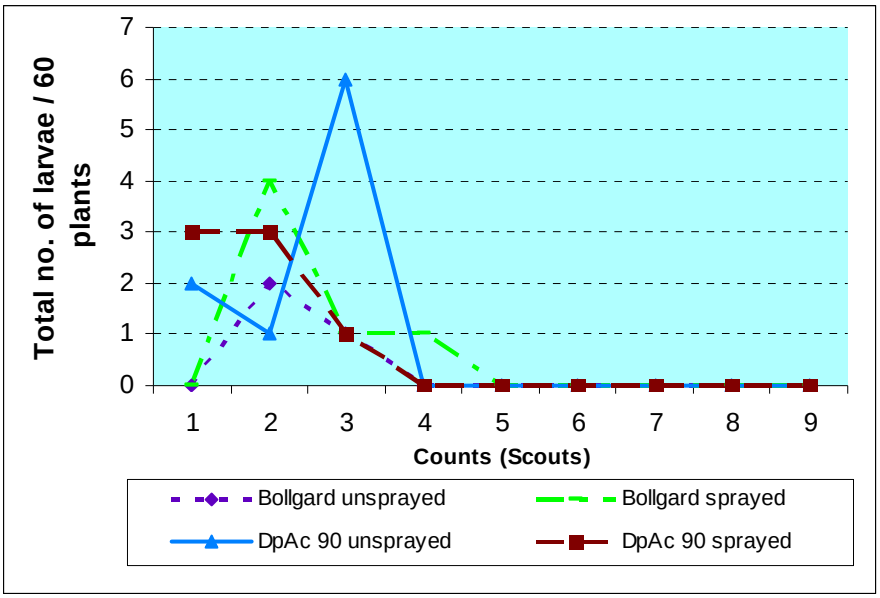


Fig. 2.11. Total number of Spiny bollworm larvae in Potgietersrus (1997/1998) in the Cry1Ac trial.

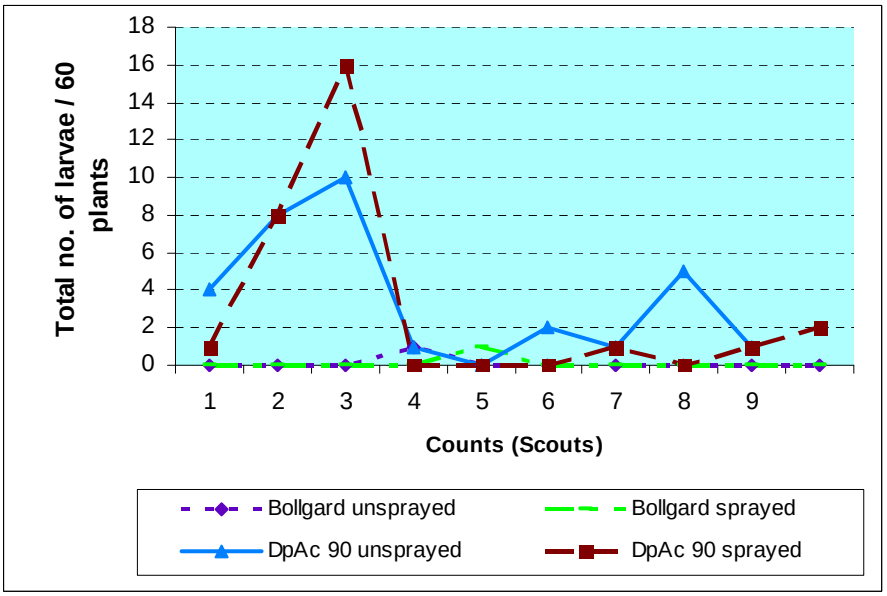


Fig. 2.12. Total number of spiny bollworm larvae in Groblersdal (Loskop) (1998/1999) in the Cry1Ac trial.

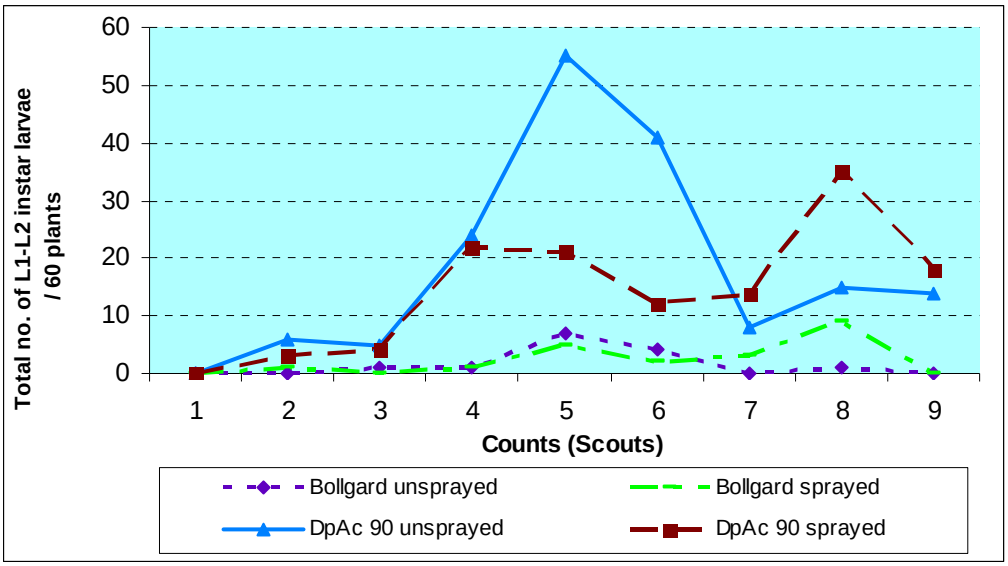


Fig. 2.13. Total number of L1-L2 instar larvae counted in Rustenburg (1997/1998) in the Cry1Ac trial.

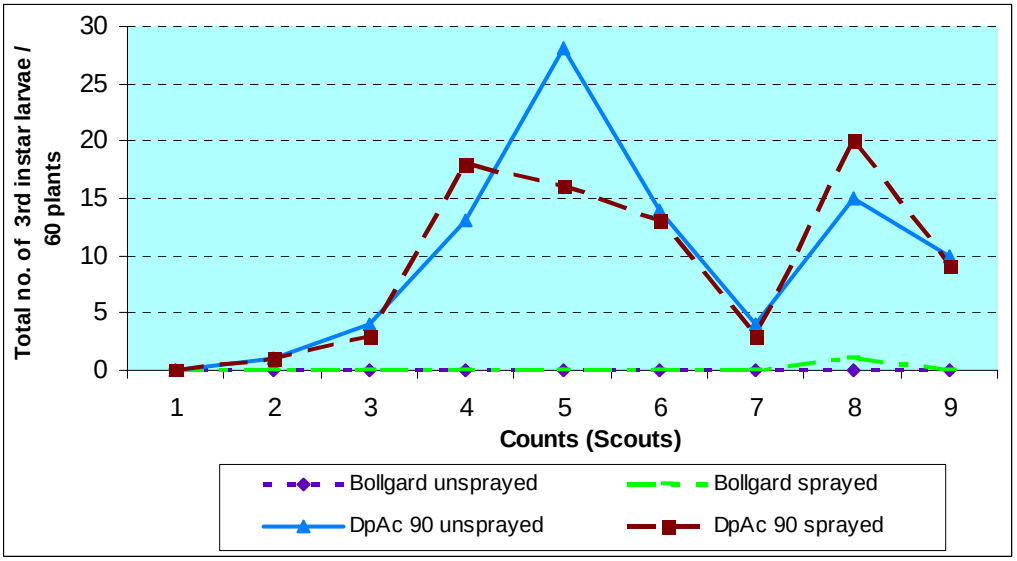


Fig. 2.14. Total number of L3 instar bollworm larvae counted in Rustenburg (1997/1998) in the Cry1Ac trial.

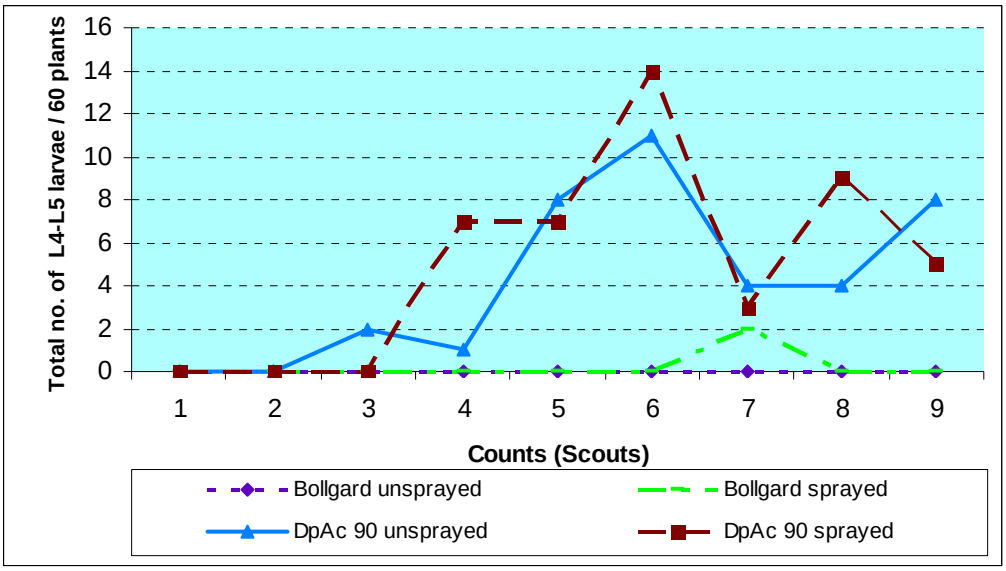


Fig. 2.15. Total number of L4-L5 instar bollworm larvae counted in Rustenburg (1997/1998).

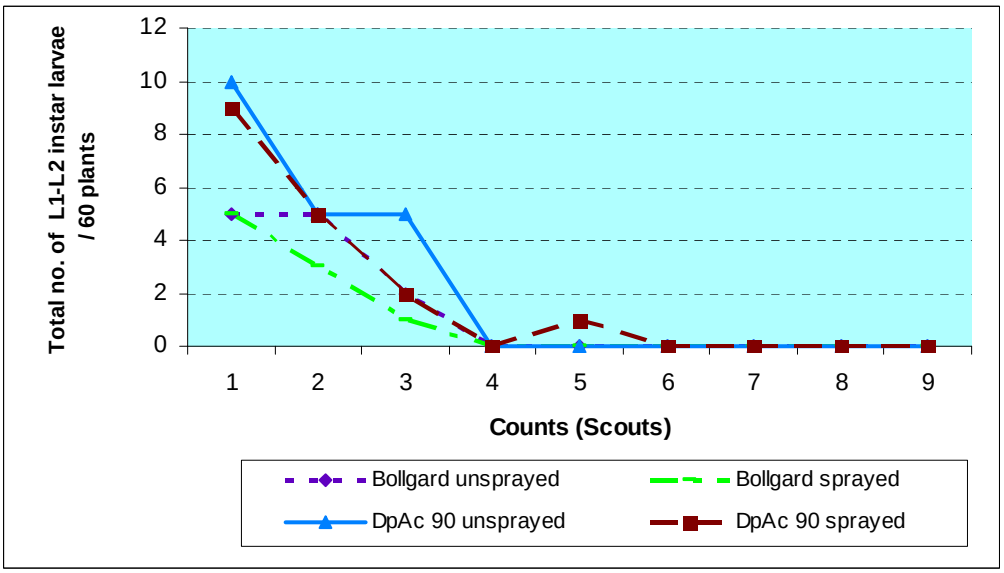


Fig. 2.16. Total number of L1-L2 instar larvae counted in Potgietersrus (1997/1998).

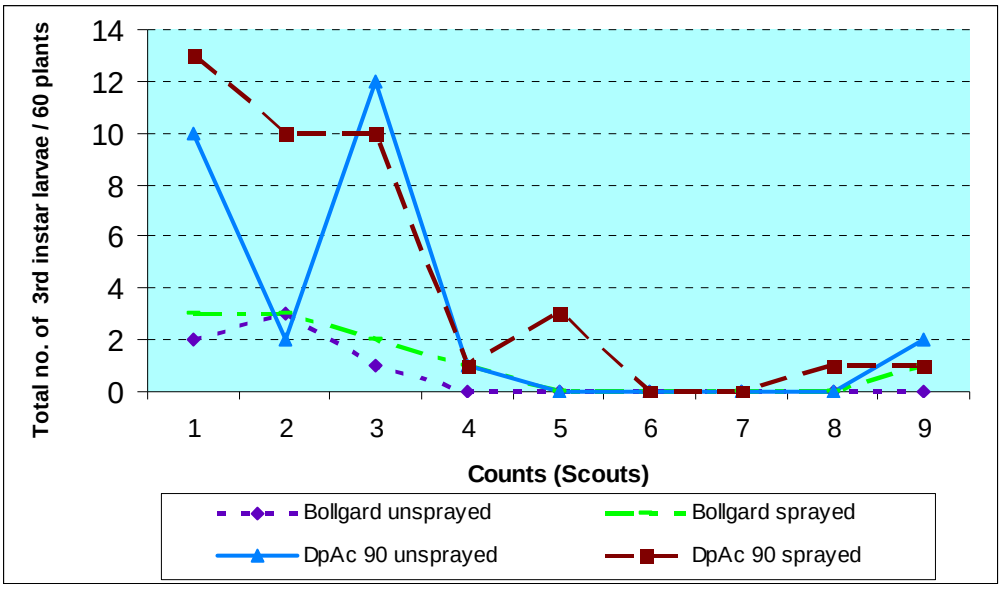


Fig. 2.17. Total number of 3rd instar larvae in Potgietersrus (1997/1998).

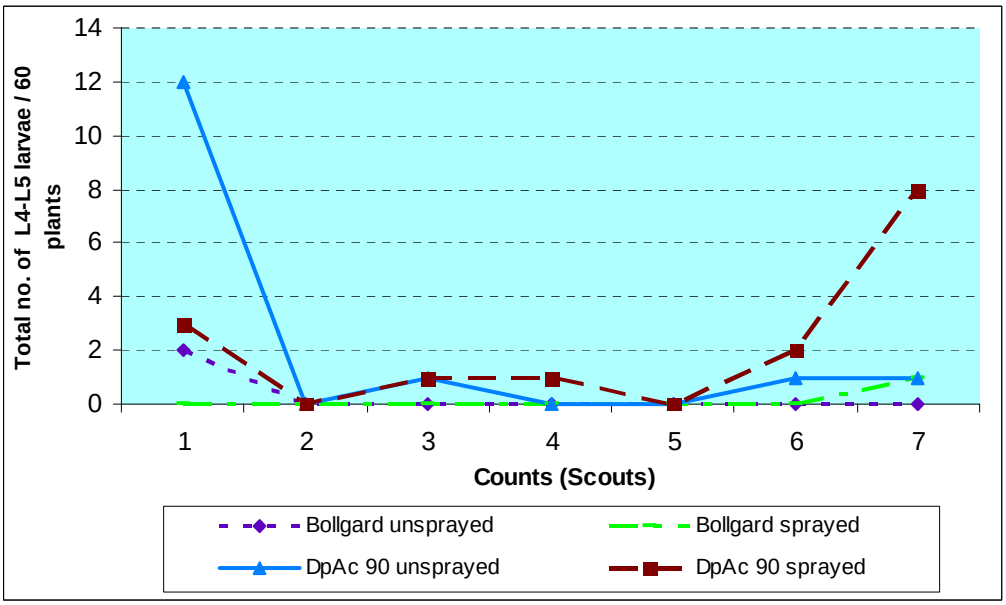


Fig. 2.18. Total number of L4-L5 instar larvae in Potgietersrus (1997/1998) in the Cry1Ac trial.

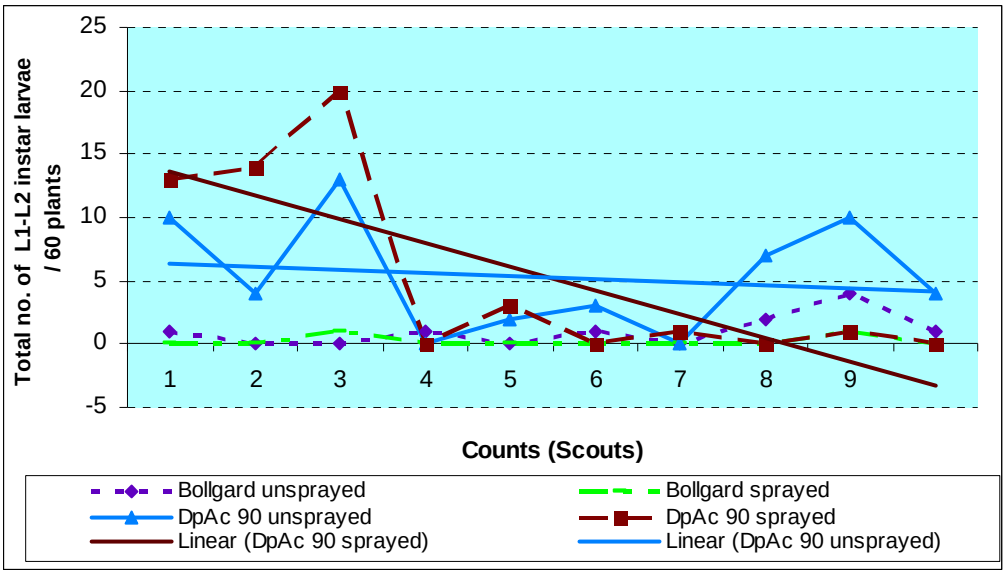


Fig. 2.19. Total number of L1-L2 instar larvae counted in Groblersdal (Loskop) (1998/1999) in the Cry1Ac trial.

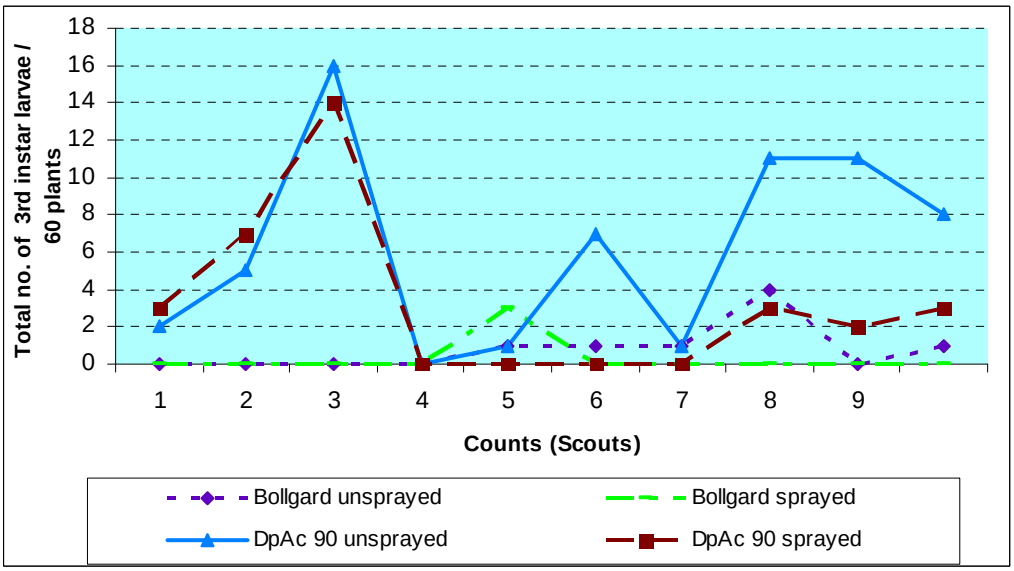


Fig. 2.20. Total number of 3rd instar larvae in Groblersdal (Loskop) (1998/1999) in the Cry1Ac trial.

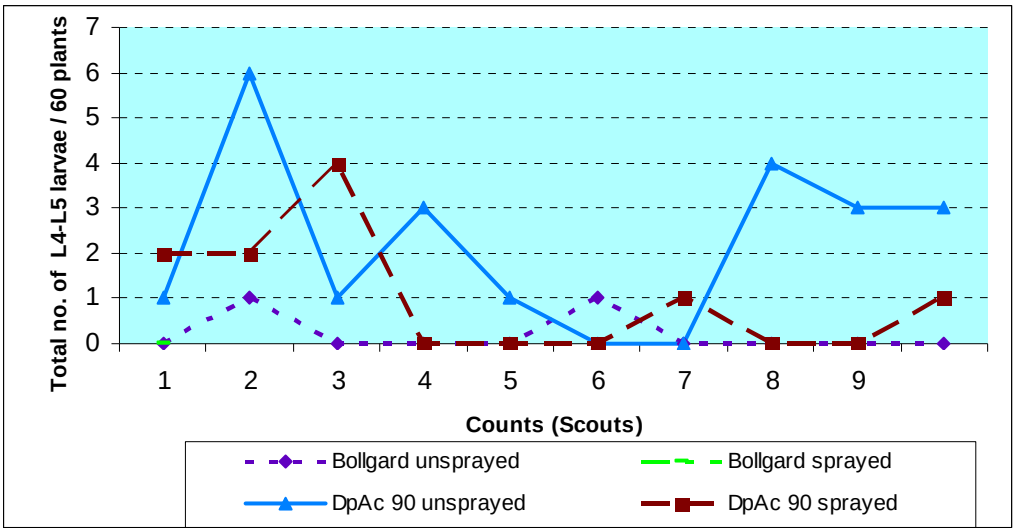


Fig. 2.21. Total number of L4-L5 instar larvae in Groblersdal (Loskop) (1998/1999) in the Cry1Ac trial.

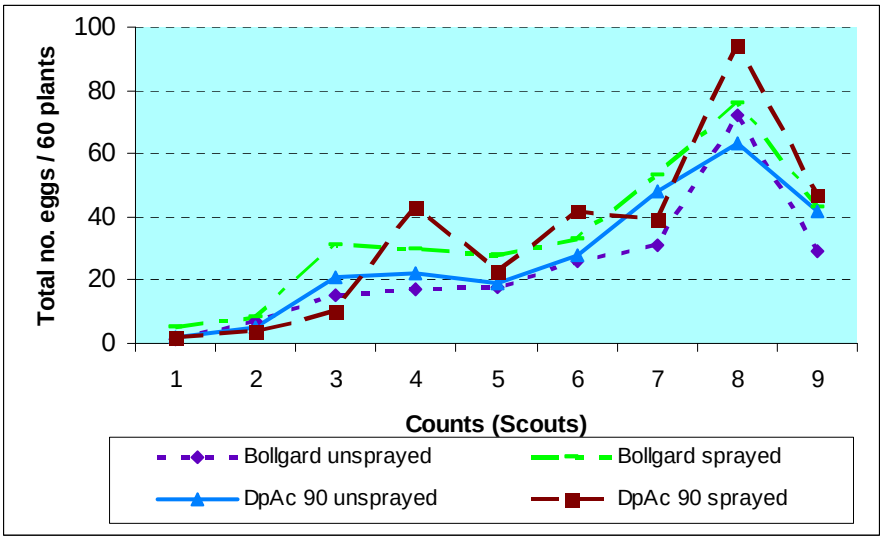


Fig. 2.22. Total number of eggs found in Rustenburg (1997/1998) in the Cry1Ac trial.

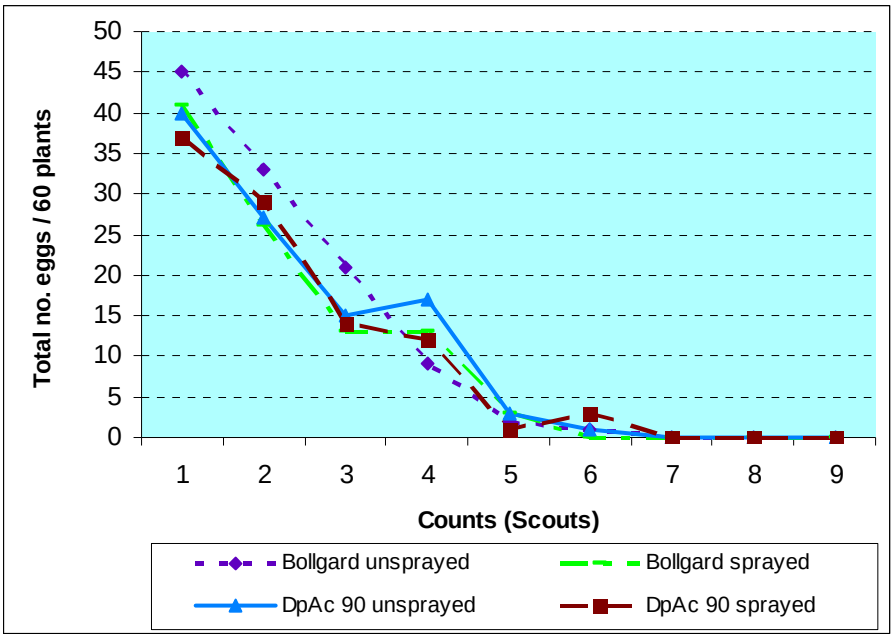


Fig. 2.23. Total number of eggs found in Potgietersrus (1997/1998) in the Cry1Ac trial.

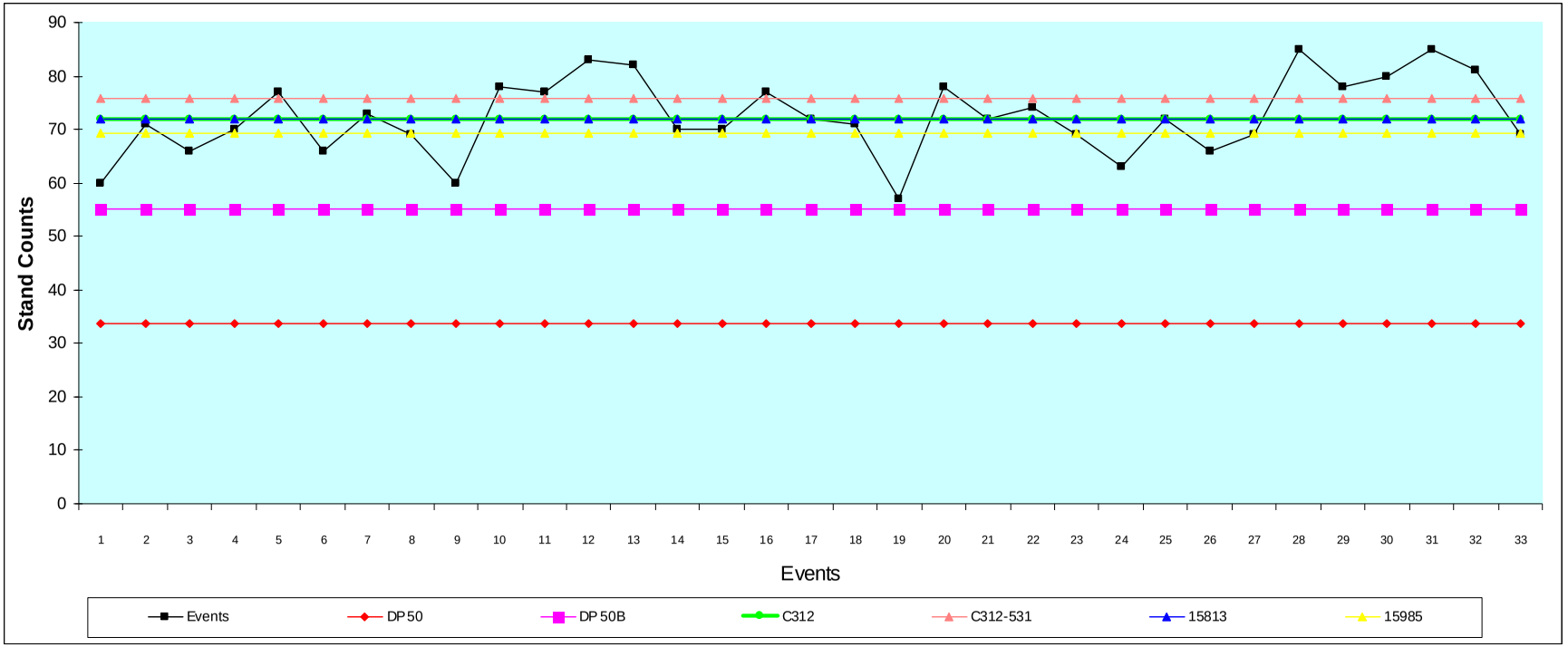


Fig. 2.24. Germination potential of each event in comparison with the control plots in the P2B event trial.

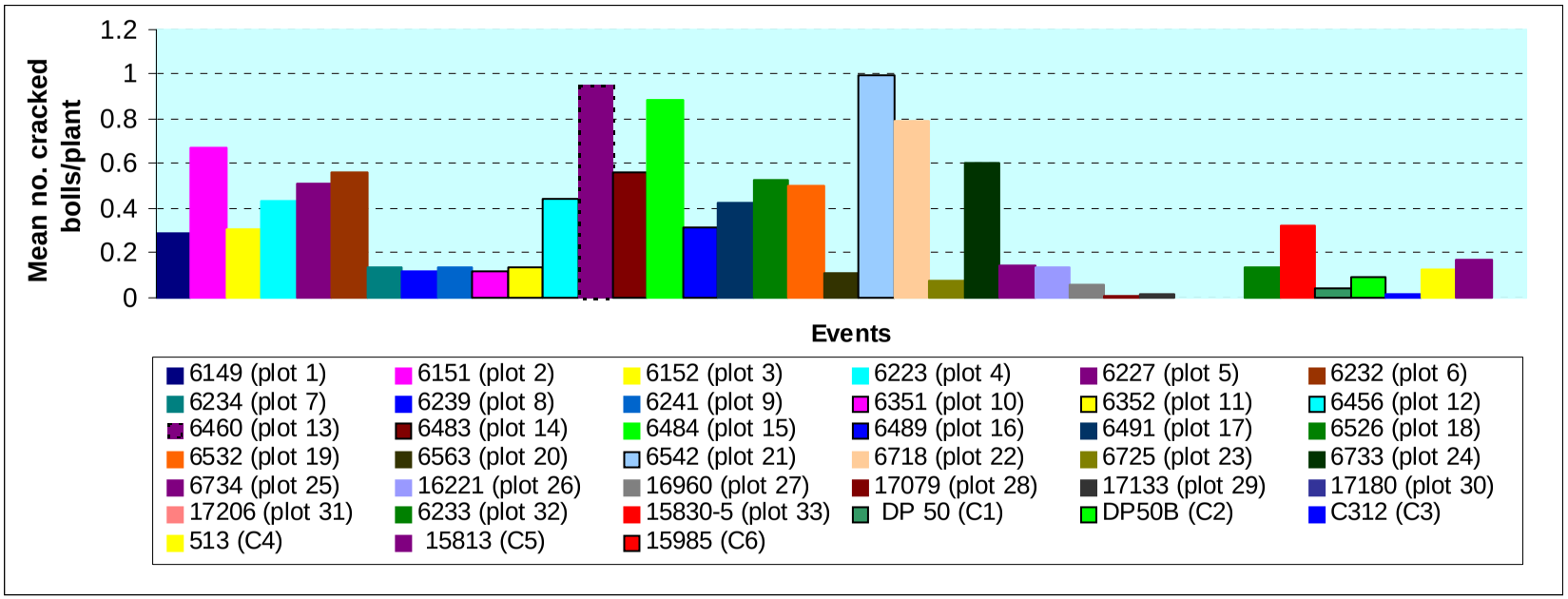


Fig. 2.25. The mean number of cracked bolls at different time intervals in the P2B event trial.

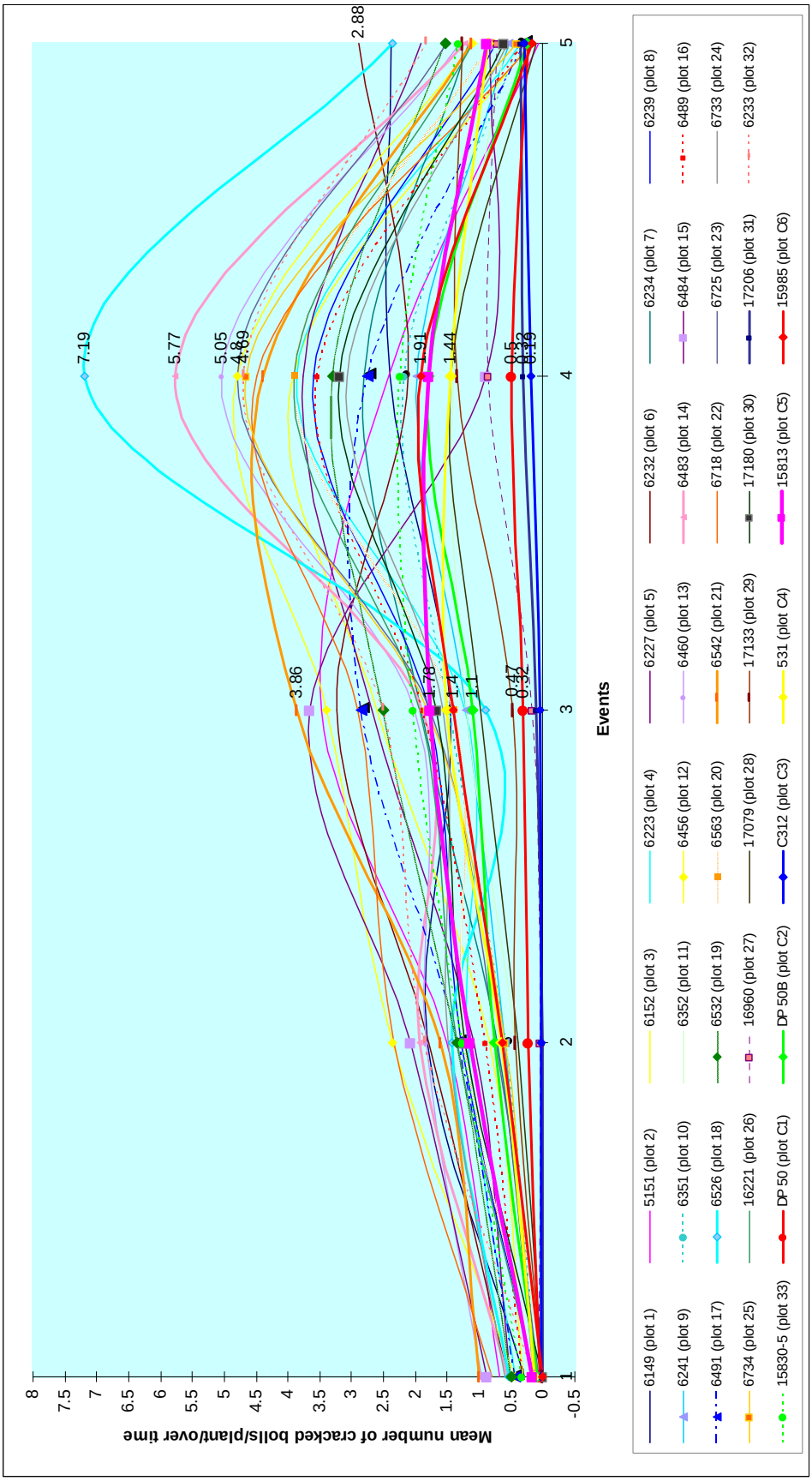


Fig. 2.26. The mean number of cracked bolls over time in the P2B event trial.

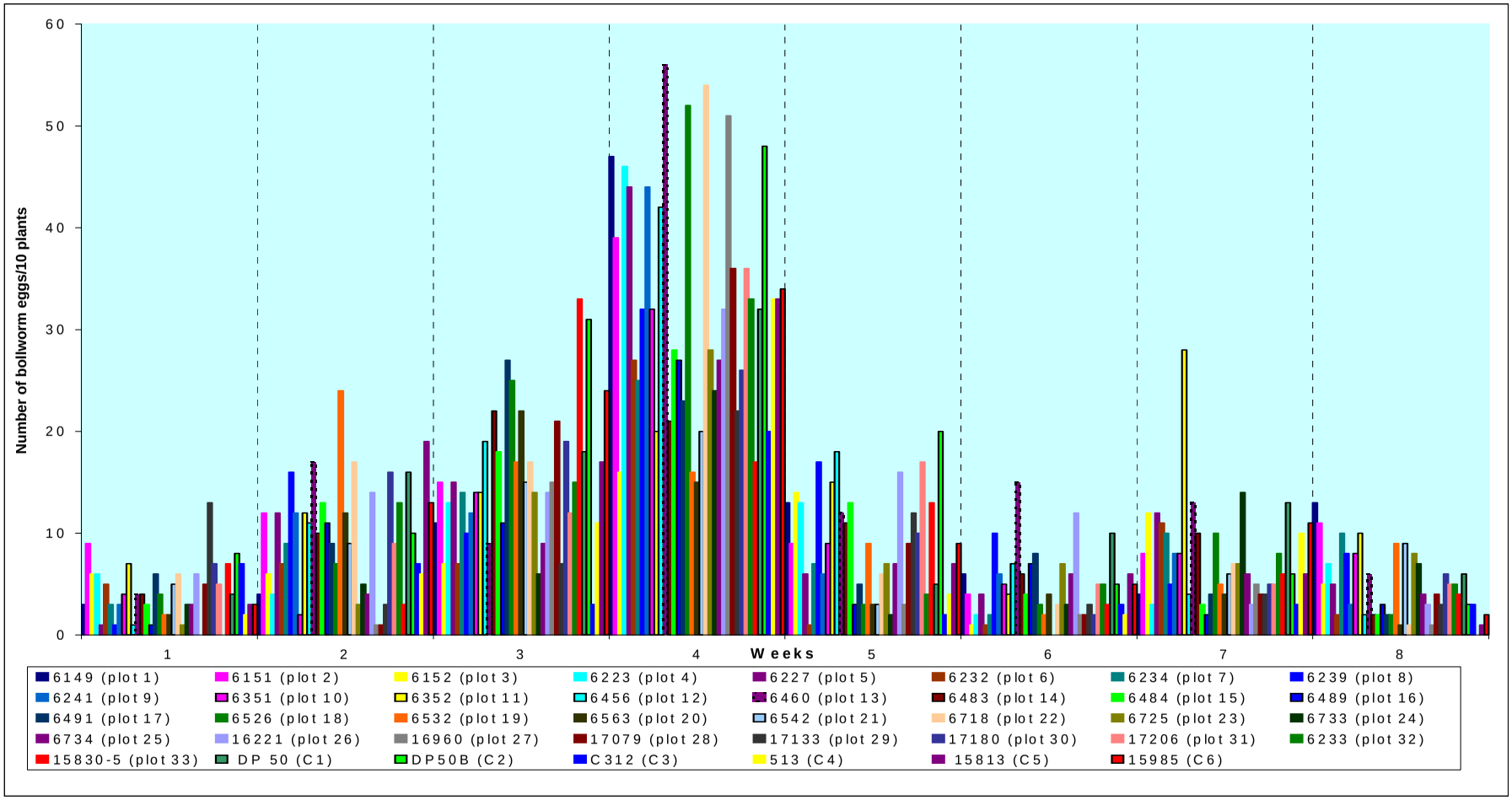


Fig. 2.27. Comparison of bollworm eggs between events at different time intervals, without the plants tested negative for gene expression removed in the P2B event trial.

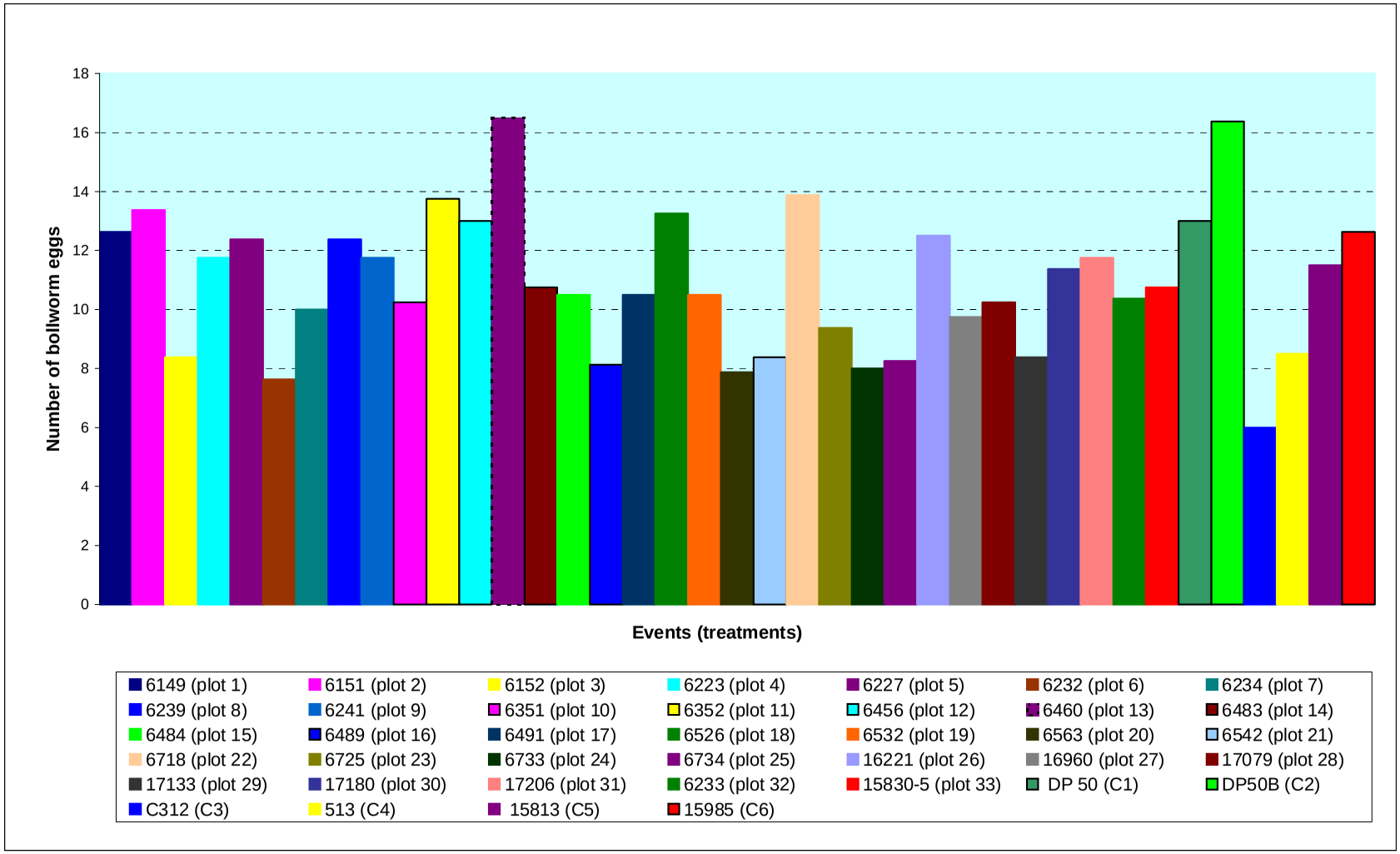


Fig. 2.28. The mean number of bollworm eggs on 10 plants between events, without the plants tested negative for gene expression removed in the P2B event trial.

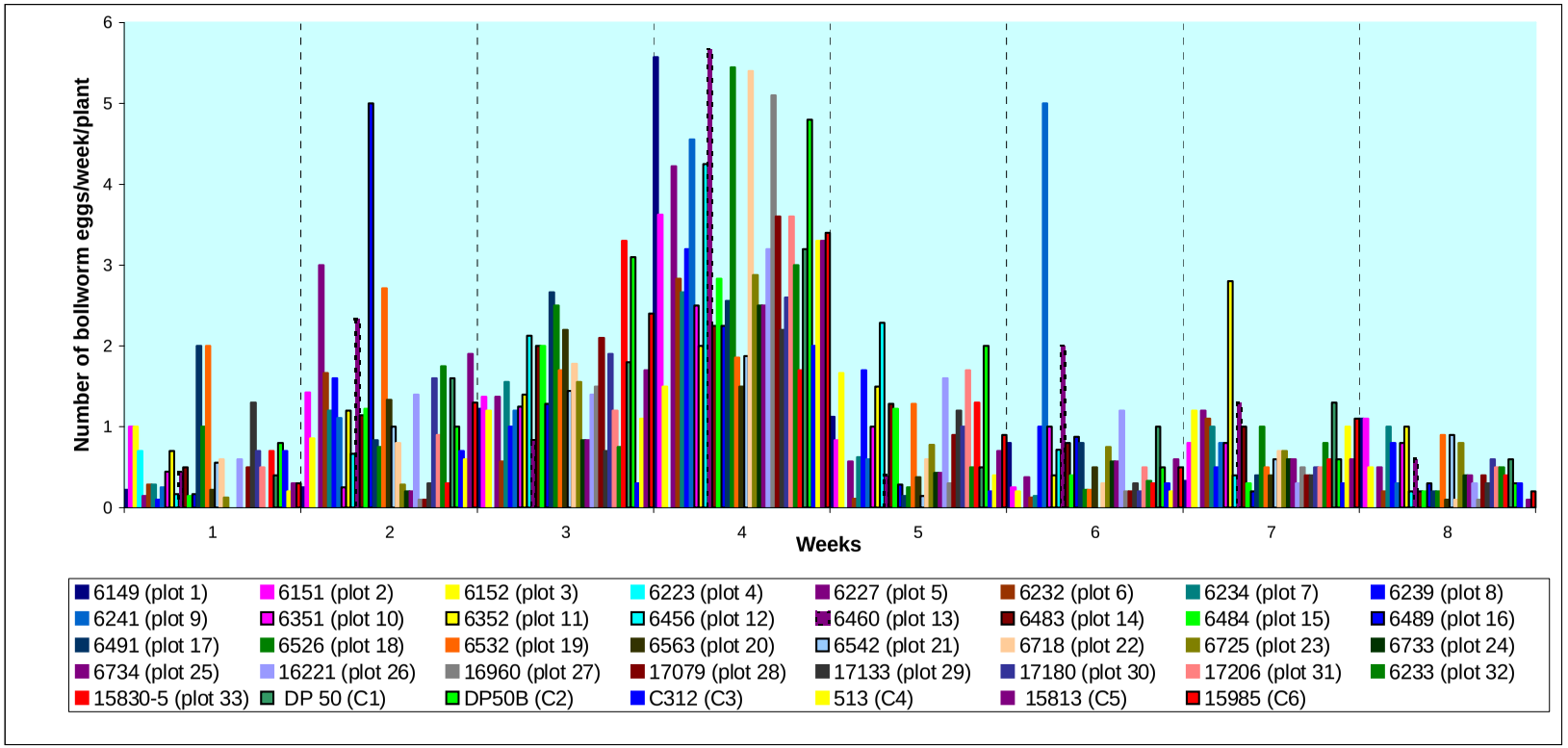


Fig. 2.29. The number of bollworm eggs at the different time periods on positively tested transgenic plants in the P2B event trial.

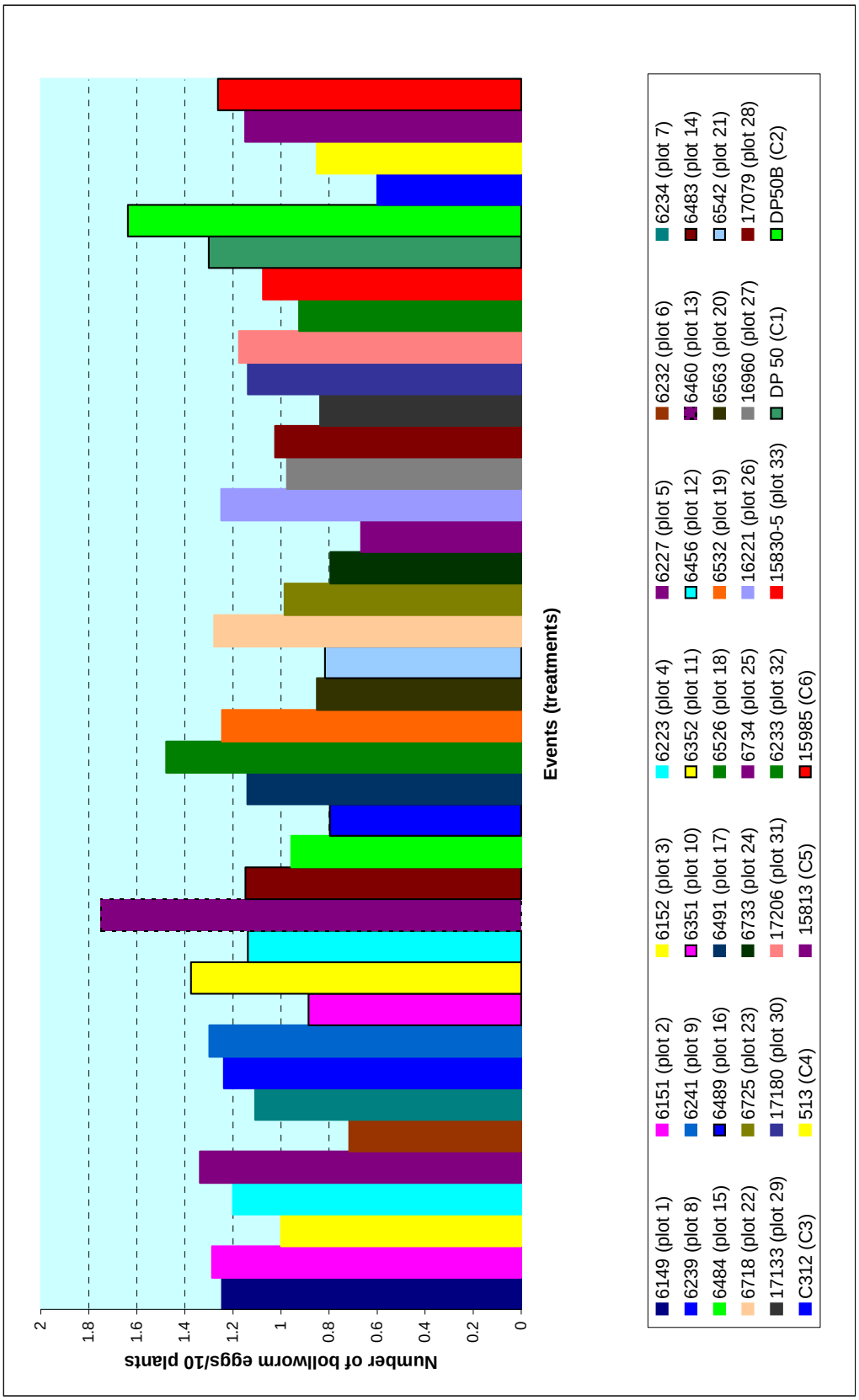


Fig. 2.30. The mean number of bollworm eggs on positively tested transgenic plants in the P2B event trial.

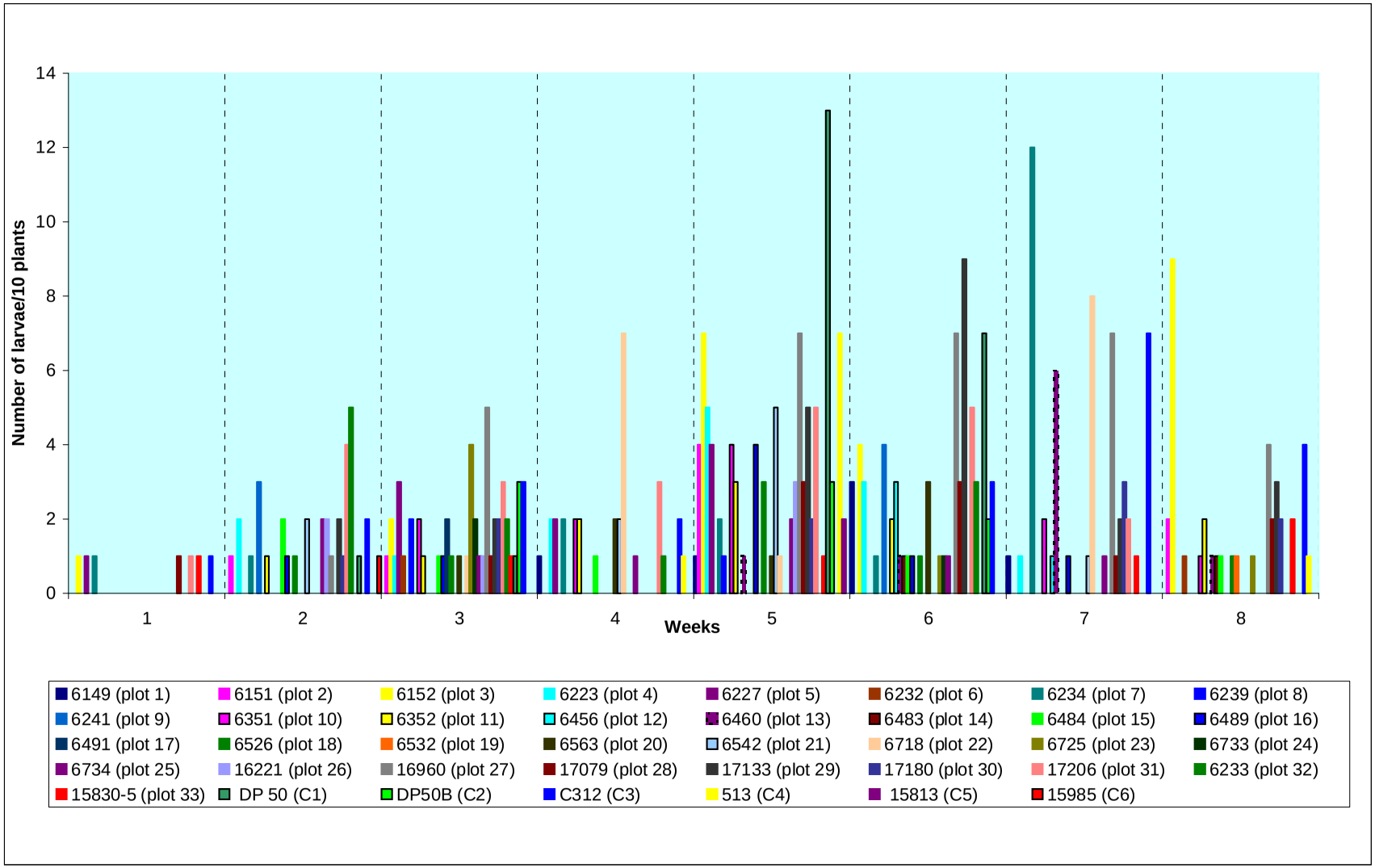


Fig. 2.31. The number of larvae between events at different time intervals, without the plants tested negative for gene expression removed in the P2B event trial.

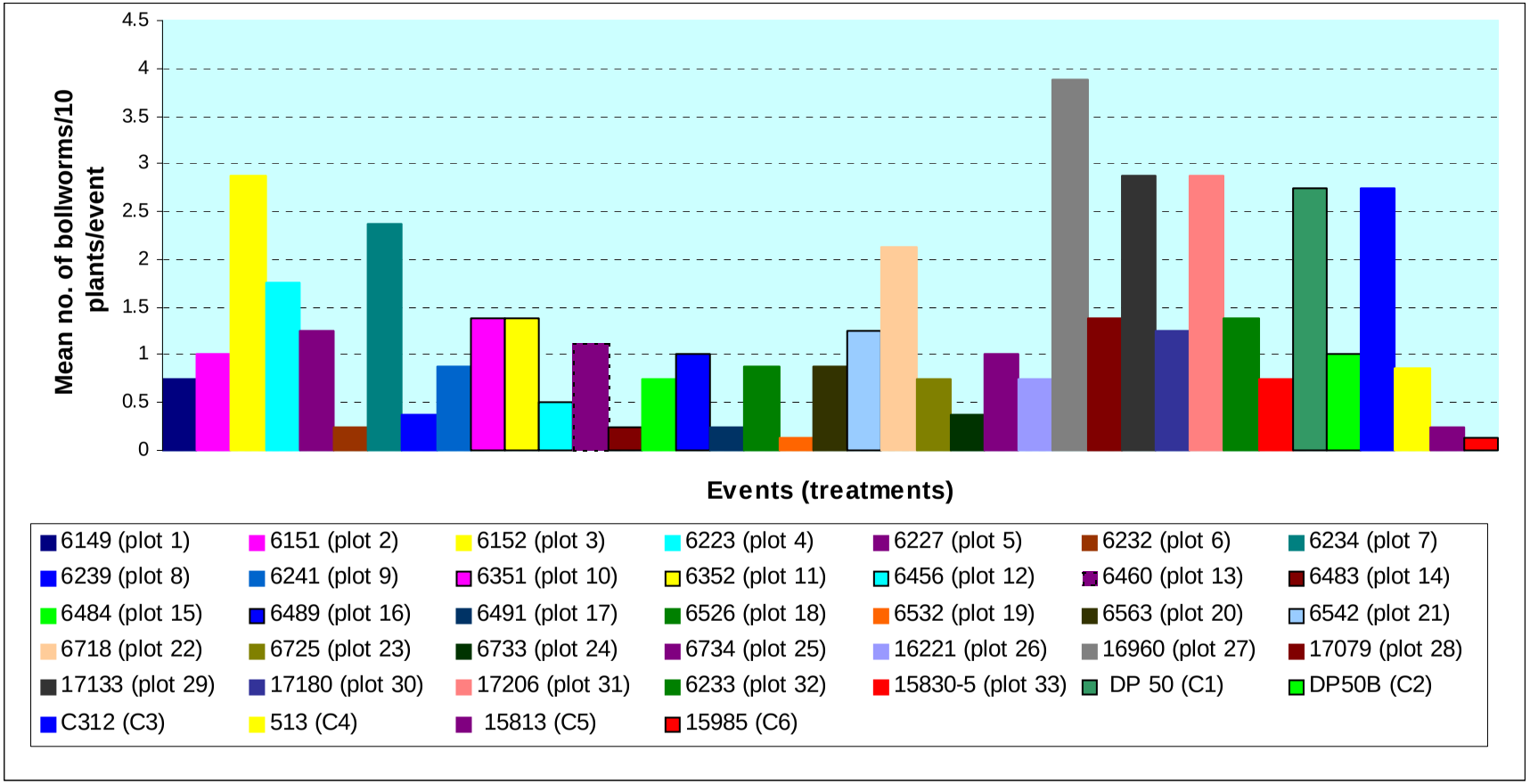


Fig. 2.32. The mean number of bollworm larvae on 10 plants between events, without the plants tested negative for gene expression removed in the P2B event trial.

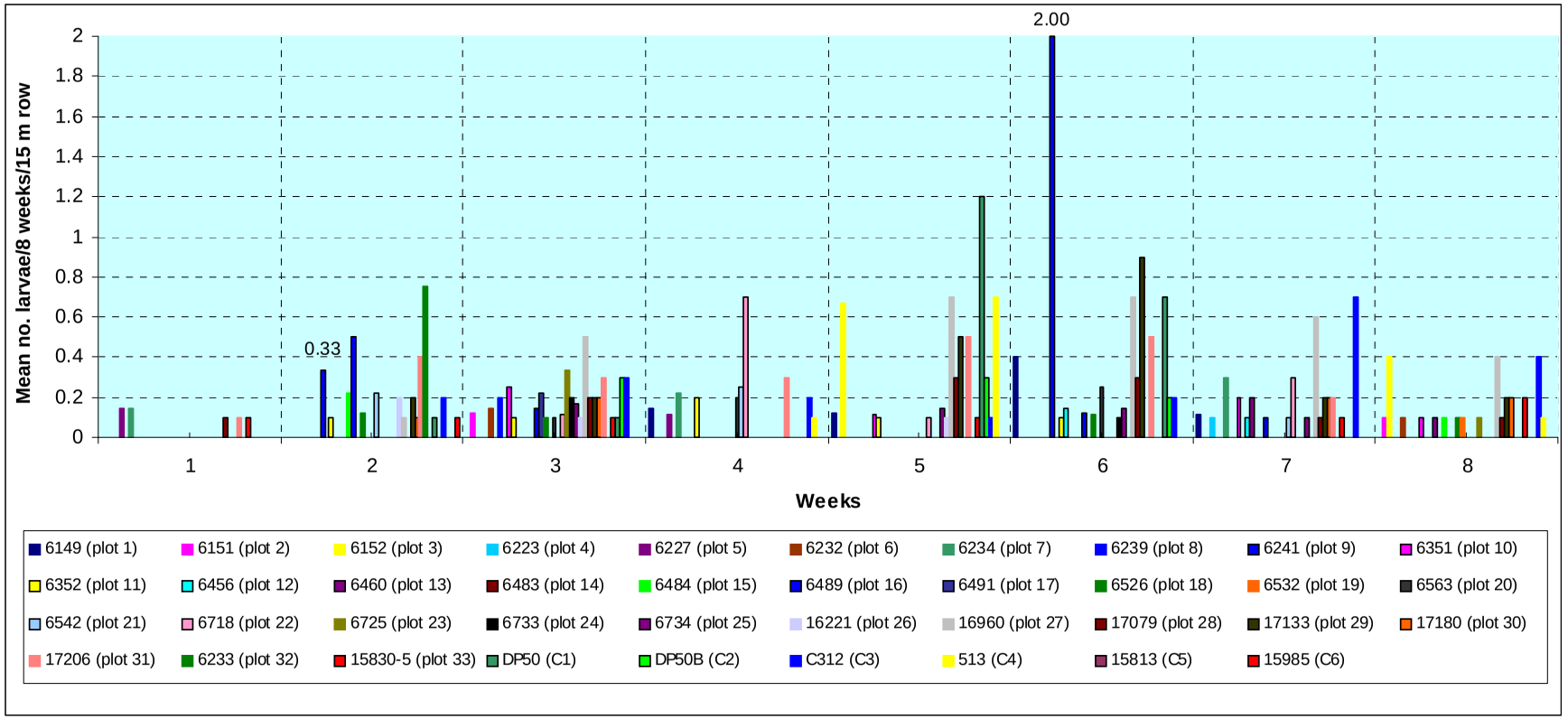


Fig. 2.33. No of bollworm larvae on positively identified transgenic plants over the time period of 8 weeks in the P2B event trial.

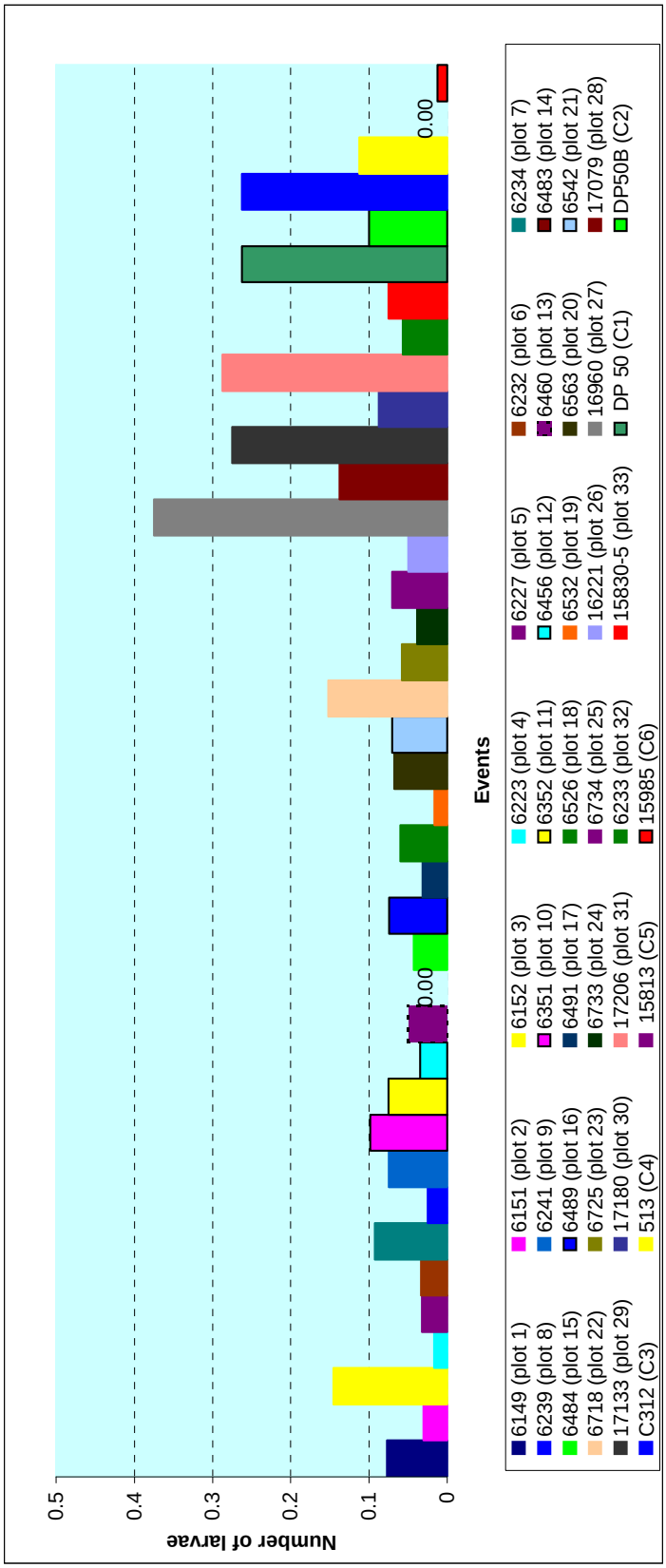


Fig. 2.34. Mean number of bollworm larvae on positively identified transgenic plants in the P2B event trial.

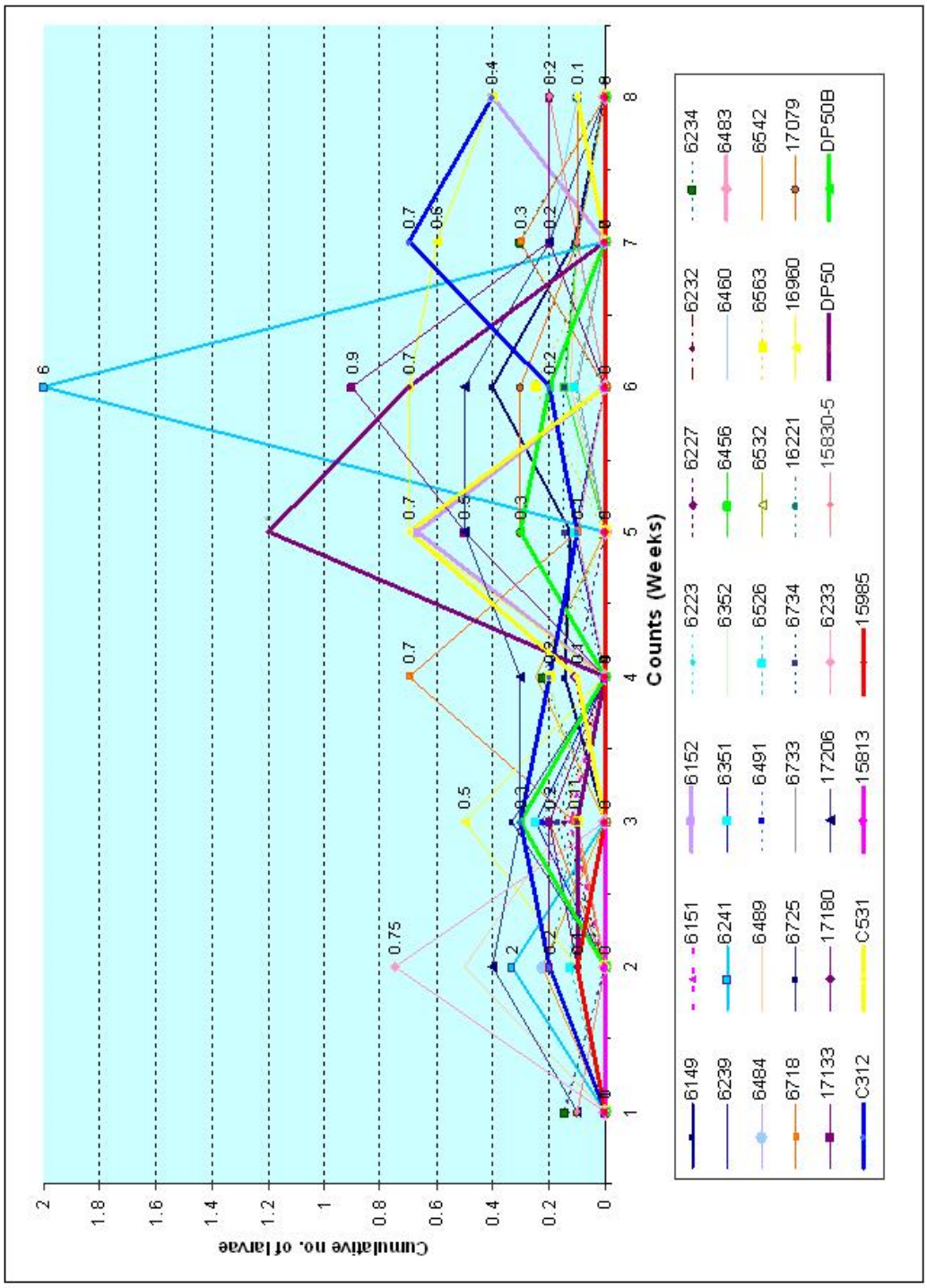


Fig. 2.35. The cumulative number of bollworm larvae over 8 weeks in the P2B event trial.

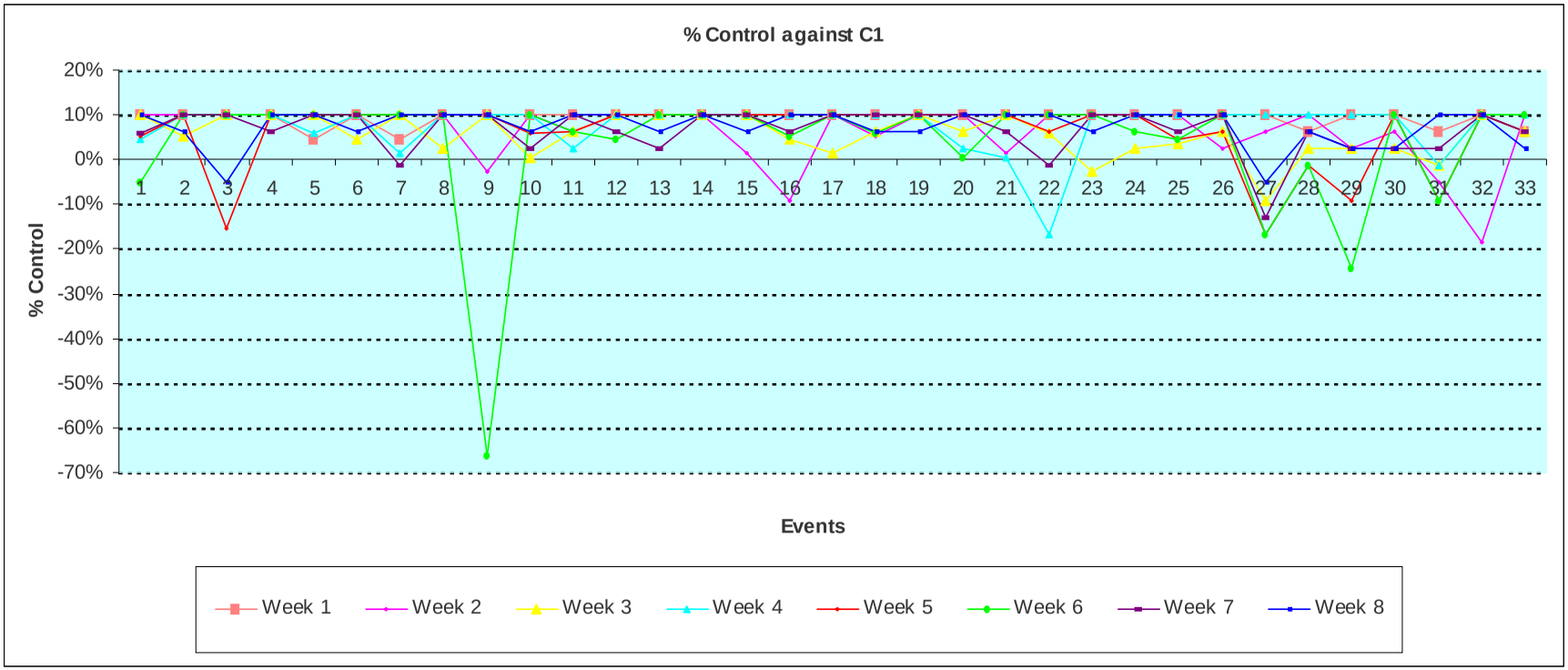


Fig. 2.36. Percentage control of bollworm larvae, measured against DP 50 (C1) in the P2B event trial

(Numbering of events: 1- 6149; 2- 6151;3- 6152; 4- 6223; 5- 6227; 6- 6232; 7- 6234; 8- 6239; 9- 6241; 10- 6351; 11- 6352; 12- 6456; 13- 6460; 14- 6483; 15- 6484; 16- 6489; 17- 6491; 18- 6526; 19- 6532; 20-6563; 21- 6542; 22- 6718; 23- 6725; 24- 6733; 25- 6734; 26- 16221; 27- 16960; 28- 17079; 29- 17133; 30- 17180; 31- 17206; 32- 6233; 33- 15830-5).

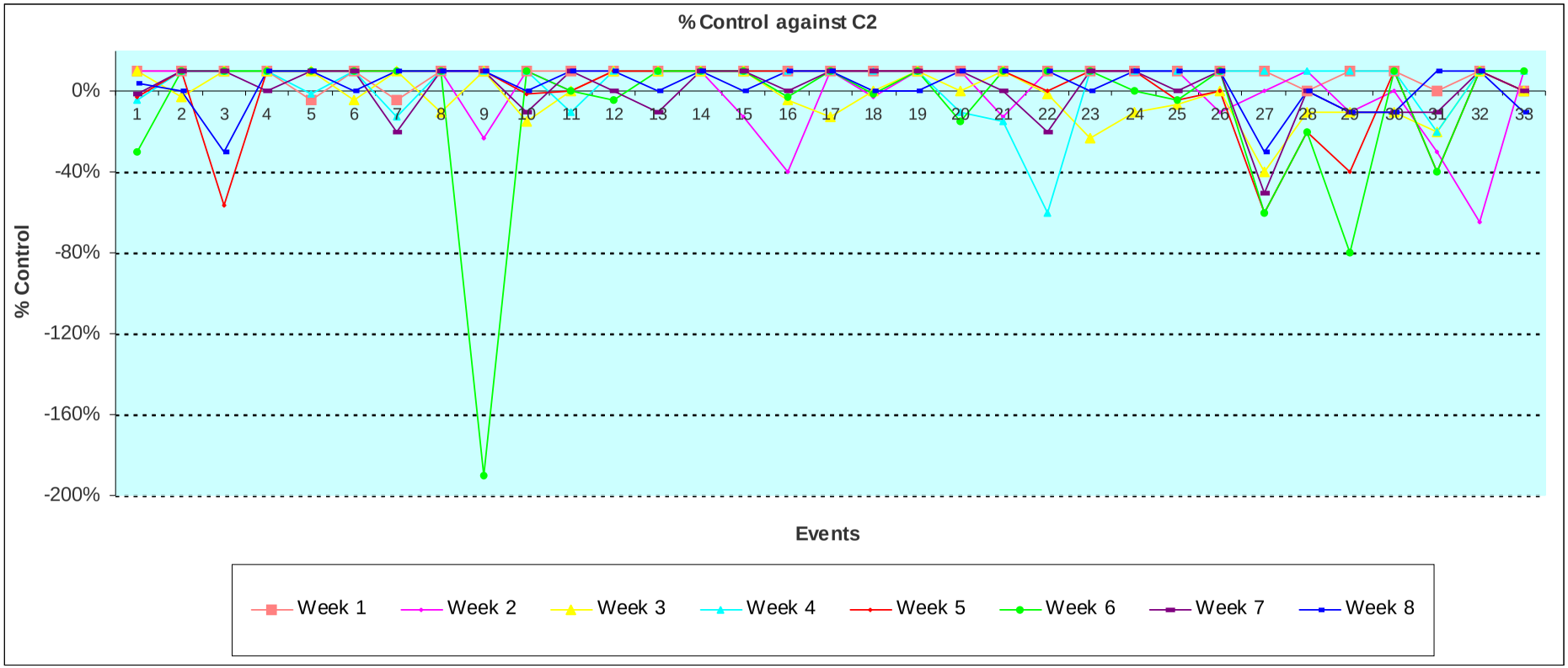


Fig. 2.37. Percentage control of bollworm larvae, measured against DP 50B (C2) in the P2B event trial

(Numbering of events: 1- 6149; 2- 6151;3- 6152; 4- 6223; 5- 6227; 6- 6232; 7- 6234; 8- 6239; 9- 6241; 10- 6351; 11- 6352; 12- 6456; 13- 6460; 14- 6483; 15- 6484; 16- 6489; 17- 6491; 18- 6526; 19- 6532; 20-6563; 21- 6542; 22- 6718; 23- 6725; 24- 6733; 25- 6734; 26- 16221; 27- 16960; 28- 17079; 29- 17133; 30- 17180; 31- 17206; 32- 6233; 33- 15830-5).

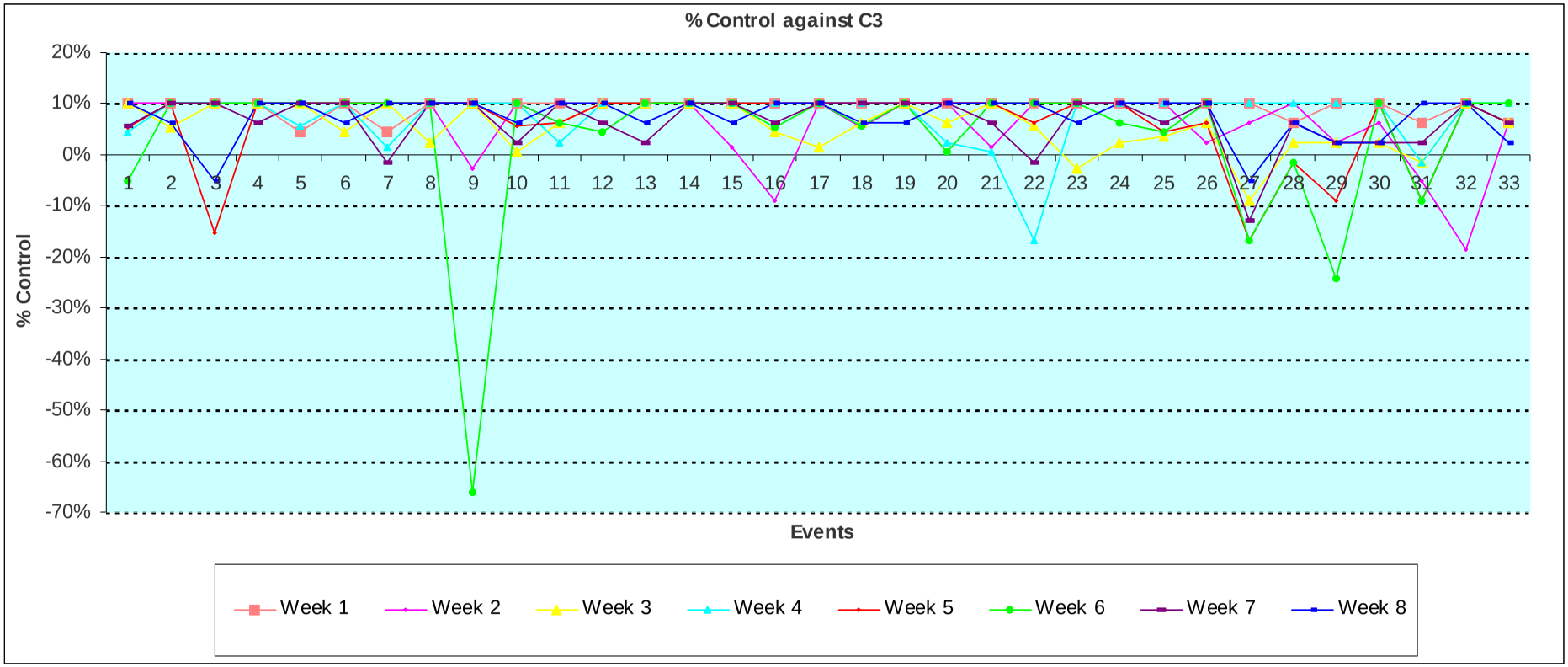


Fig. 2.38. Percentage control of bollworm larvae, measured against C312 (C3) in the P2B event trial

(Numbering of events: 1- 6149; 2- 6151;3- 6152; 4- 6223; 5- 6227; 6- 6232; 7- 6234; 8- 6239; 9- 6241; 10- 6351; 11- 6352; 12- 6456; 13- 6460; 14- 6483; 15- 6484; 16- 6489; 17- 6491; 18- 6526; 19- 6532; 20-6563; 21- 6542; 22- 6718; 23- 6725; 24- 6733; 25- 6734; 26- 16221; 27- 16960; 28- 17079; 29- 17133; 30- 17180; 31- 17206; 32- 6233; 33- 15830-5).

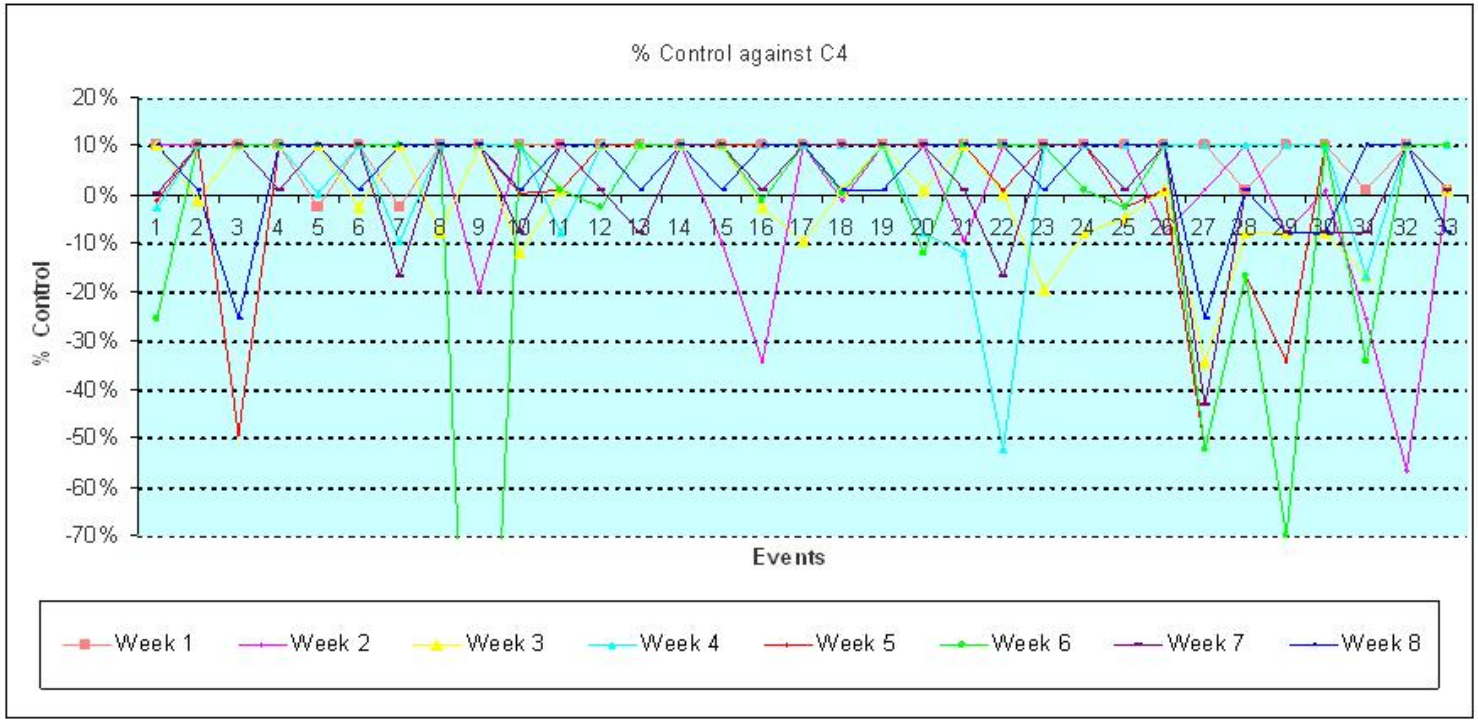


Fig. 2.39. Percentage control of bollworm larvae, measured against C531 (C4) in the P2B event trial (Numbering of events: 1- 6149; 2- 6151;3- 6152; 4- 6223; 5- 6227; 6- 6232; 7- 6234; 8- 6239; 9- 6241; 10- 6351; 11- 6352; 12- 6456; 13- 6460; 14- 6483; 15- 6484; 16- 6489; 17- 6491; 18- 6526; 19- 6532; 20-6563; 21- 6542; 22- 6718; 23- 6725; 24- 6733; 25- 6734; 26- 16221; 27- 16960; 28- 17079; 29- 17133; 30- 17180; 31- 17206; 32- 6233; 33- 15830-5).

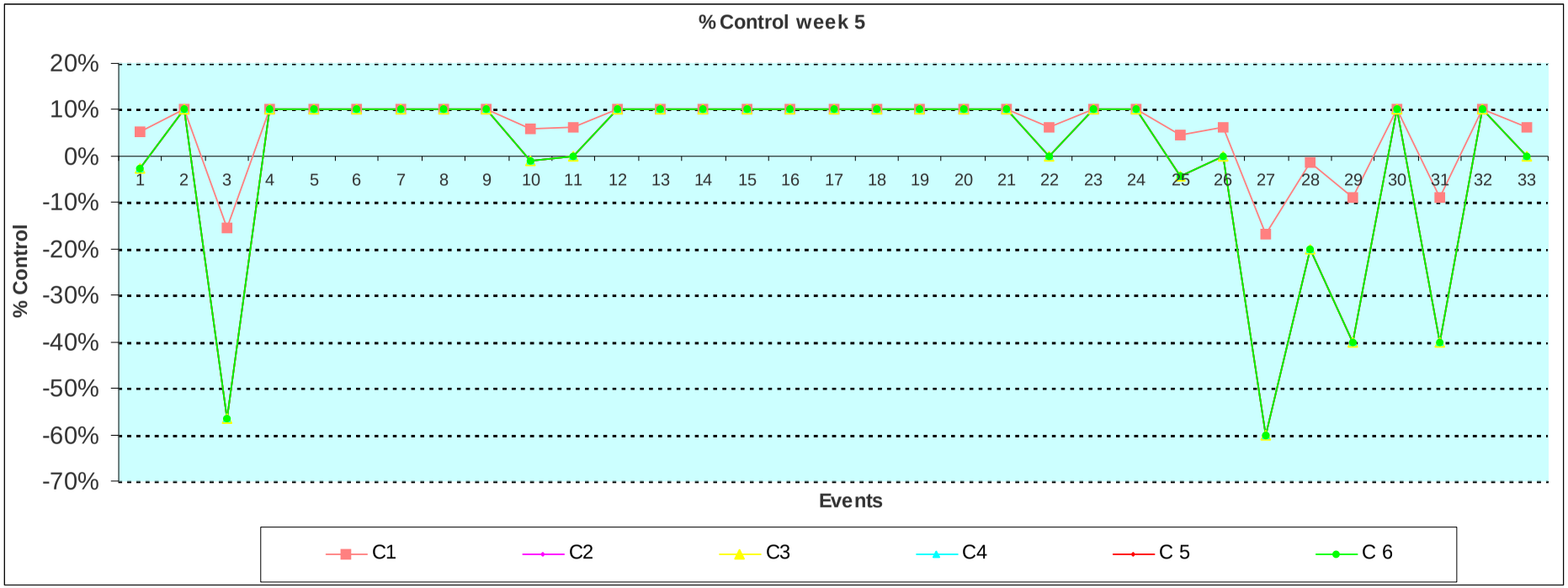


Fig. 2.40. Percentage control of bollworm larvae, measured against 15813 (C5) in the P2B event trial

(Numbering of events: 1- 6149; 2- 6151;3- 6152; 4- 6223; 5- 6227; 6- 6232; 7- 6234; 8- 6239; 9- 6241; 10- 6351; 11- 6352; 12- 6456; 13- 6460; 14- 6483; 15- 6484; 16- 6489; 17- 6491; 18- 6526; 19- 6532; 20-6563; 21- 6542; 22- 6718; 23- 6725; 24- 6733; 25- 6734; 26- 16221; 27- 16960; 28- 17079; 29- 17133; 30- 17180; 31- 17206; 32- 6233; 33- 15830-5).

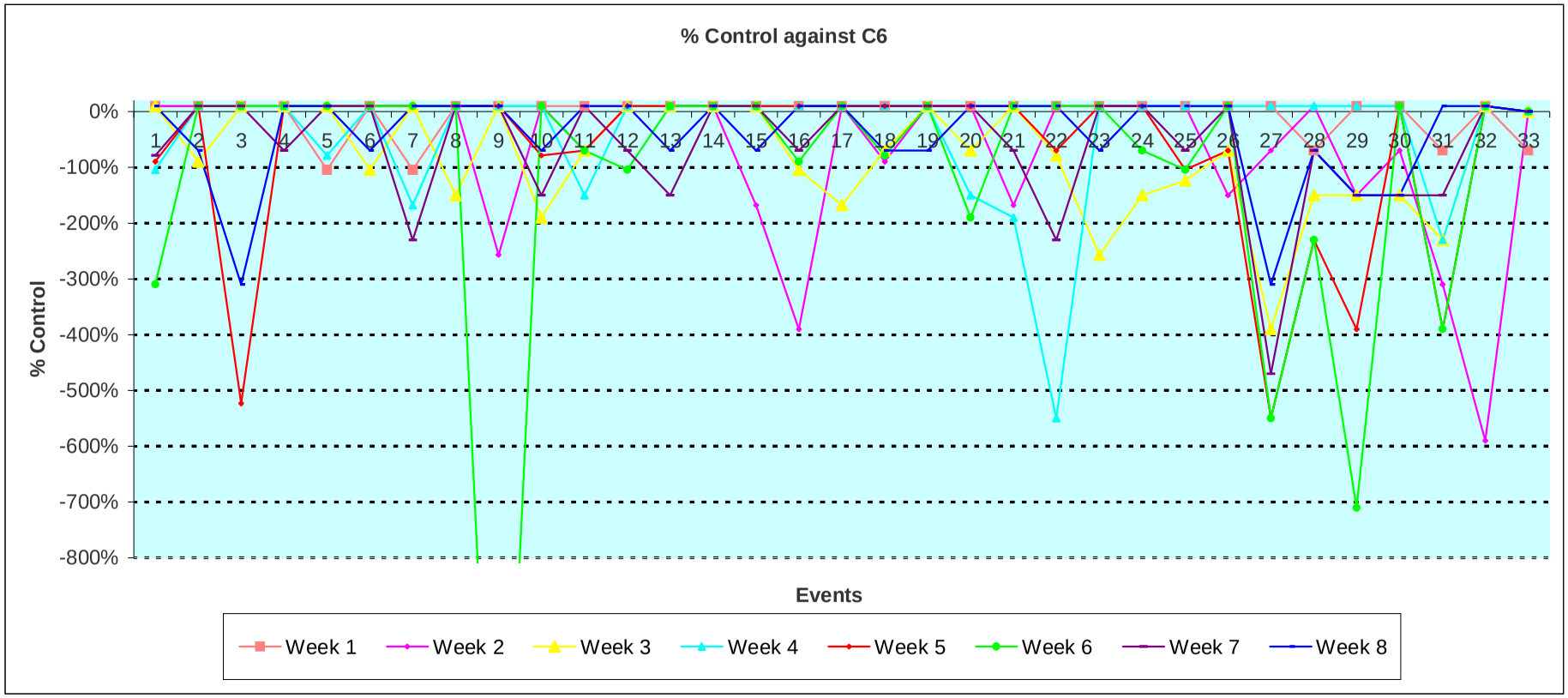


Fig. 2.41. Percentage control of bollworm larvae, measured against 15985 (C6) in the P2B event trial

(Numbering of events: 1- 6149; 2- 6151;3- 6152; 4- 6223; 5- 6227; 6- 6232; 7- 6234; 8- 6239; 9- 6241; 10- 6351; 11- 6352; 12- 6456; 13- 6460; 14- 6483; 15- 6484; 16- 6489; 17- 6491; 18- 6526; 19- 6532; 20-6563; 21- 6542; 22- 6718; 23- 6725; 24- 6733; 25- 6734; 26- 16221; 27- 16960; 28- 17079; 29- 17133; 30- 17180; 31- 17206; 32- 6233; 33- 15830-5).

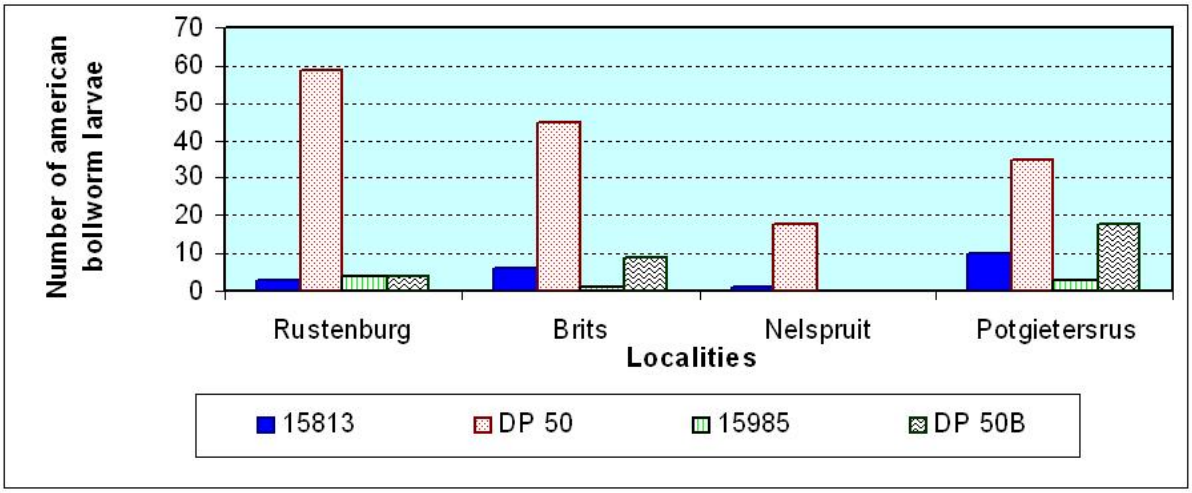


Fig. 2.42. The total number of african bollworm larvae (on 20 plants per plot) for each of the treatments and for each of the localities in the Cry2Ab2 trial.

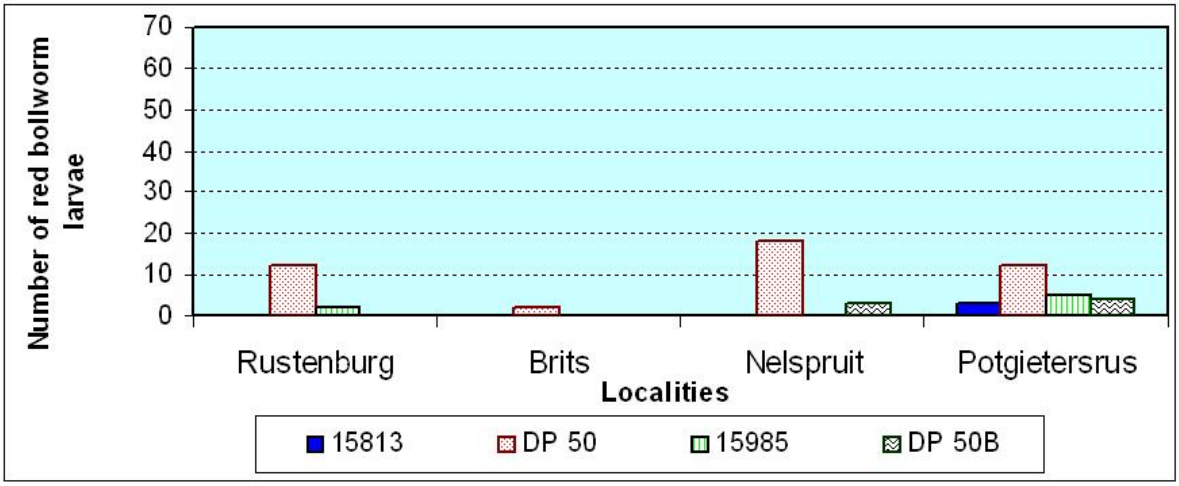


Fig. 2.43. The total number of red bollworm larvae (on 20 plants per plot) for each of the treatments and for each of the localities in the Cry2Ab2 trial.

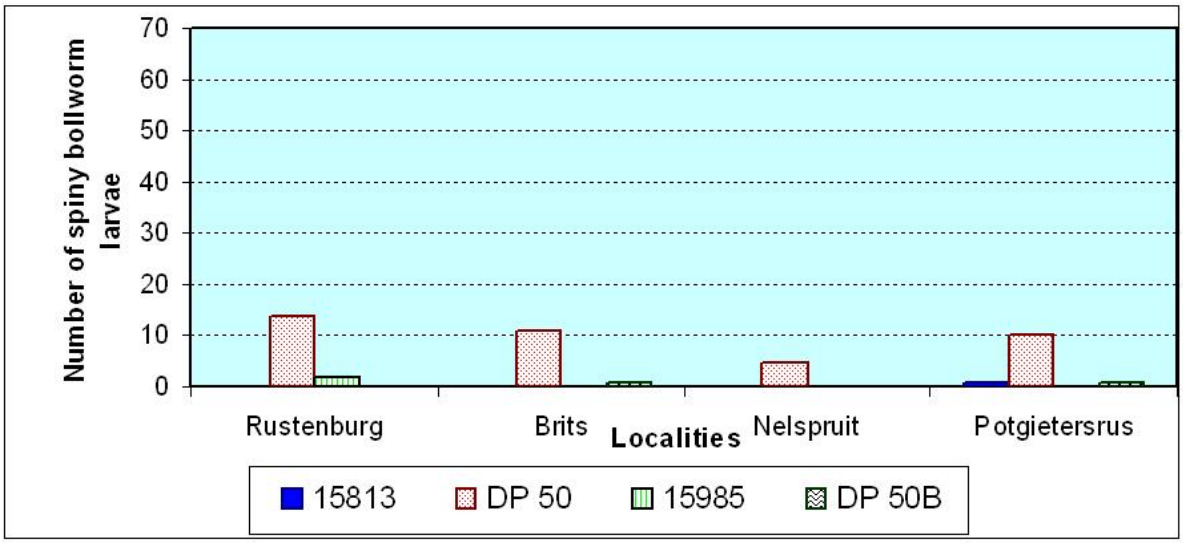


Fig. 2.44. The total number of spiny bollworm larvae (on 20 plants per plot) for each of the treatments and for each of the localities in the Cry2Ab2 trial.

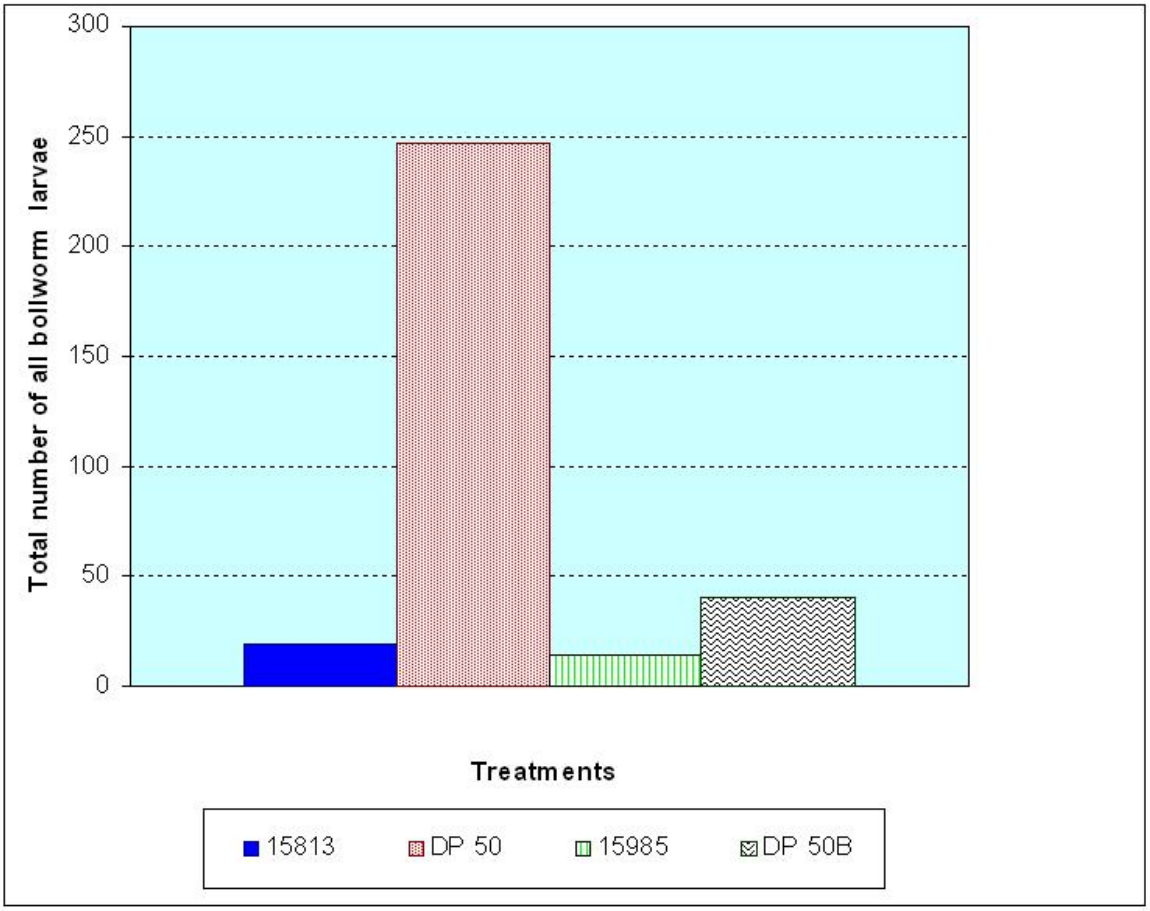


Fig. 2.45. The total number of all bollworm larvae on 20 plants per plot for each treatment for four localities in the Cry2Ab2 trial.

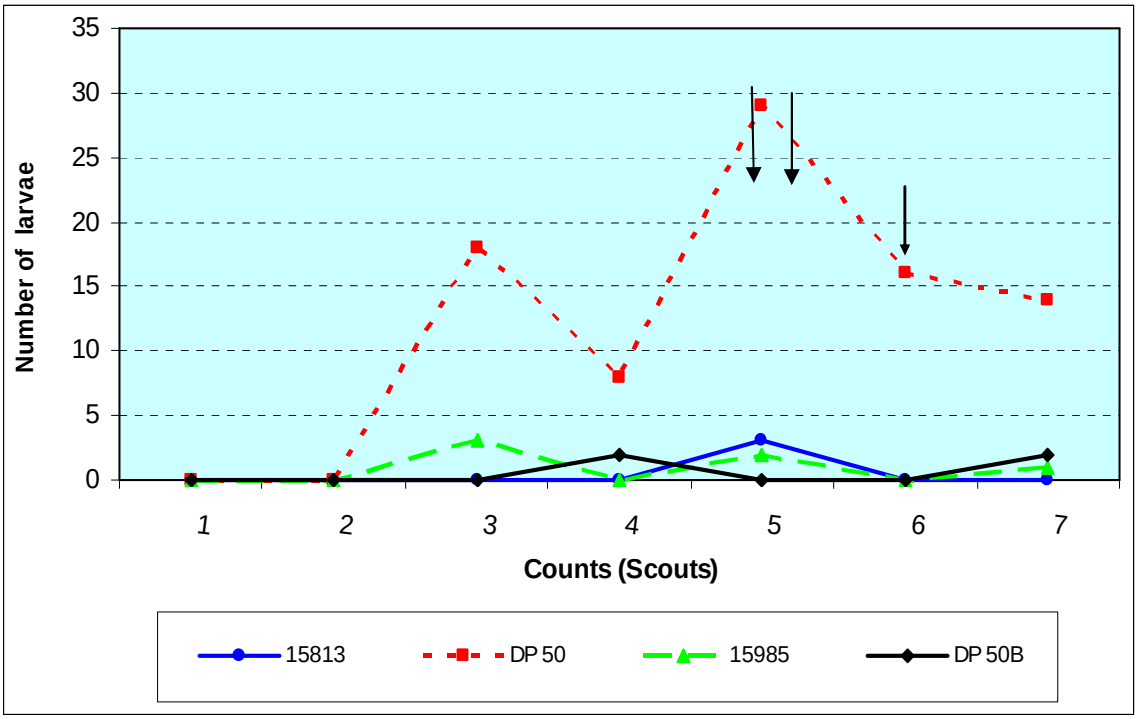


Fig. 2.46. The total number of larvae counted on 20 plants per plot for each treatment at Rustenburg (arrows - insecticide applications) in the Cry2Ab2 trial.

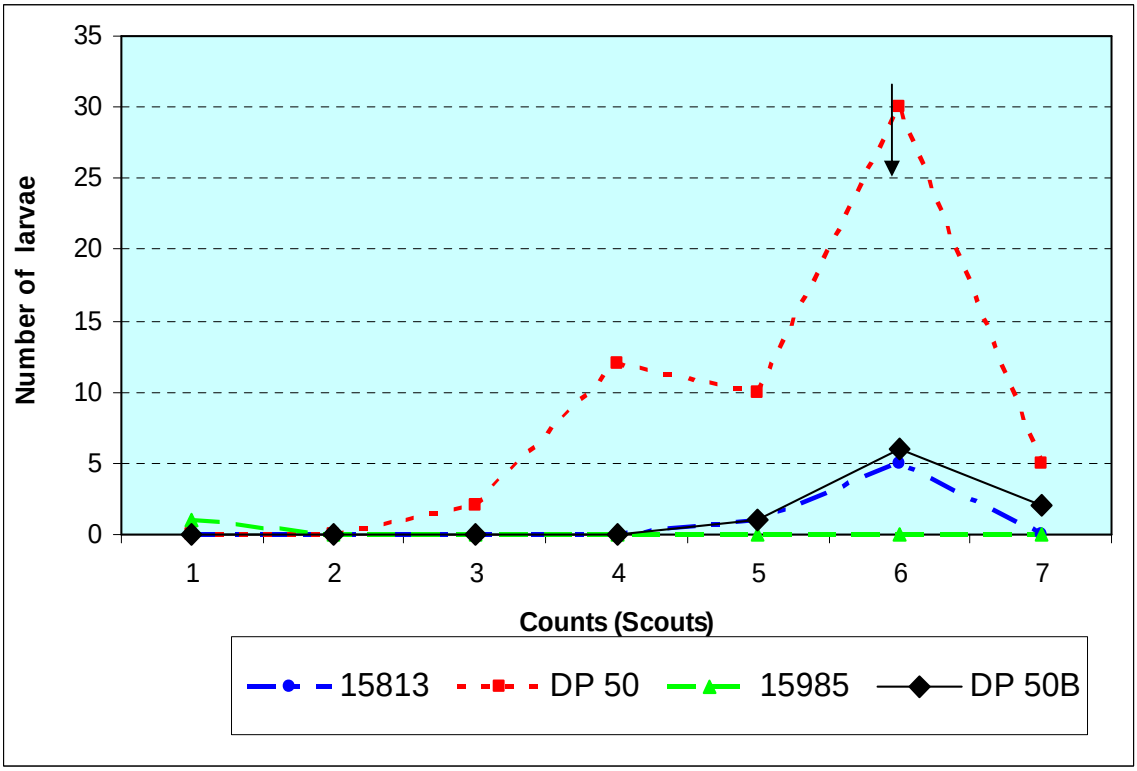


Fig. 2.47. The total number of larvae counted on 20 plants per plot for each treatment at Brits (arrows - insecticide applications) in the Cry2Ab2 trial.

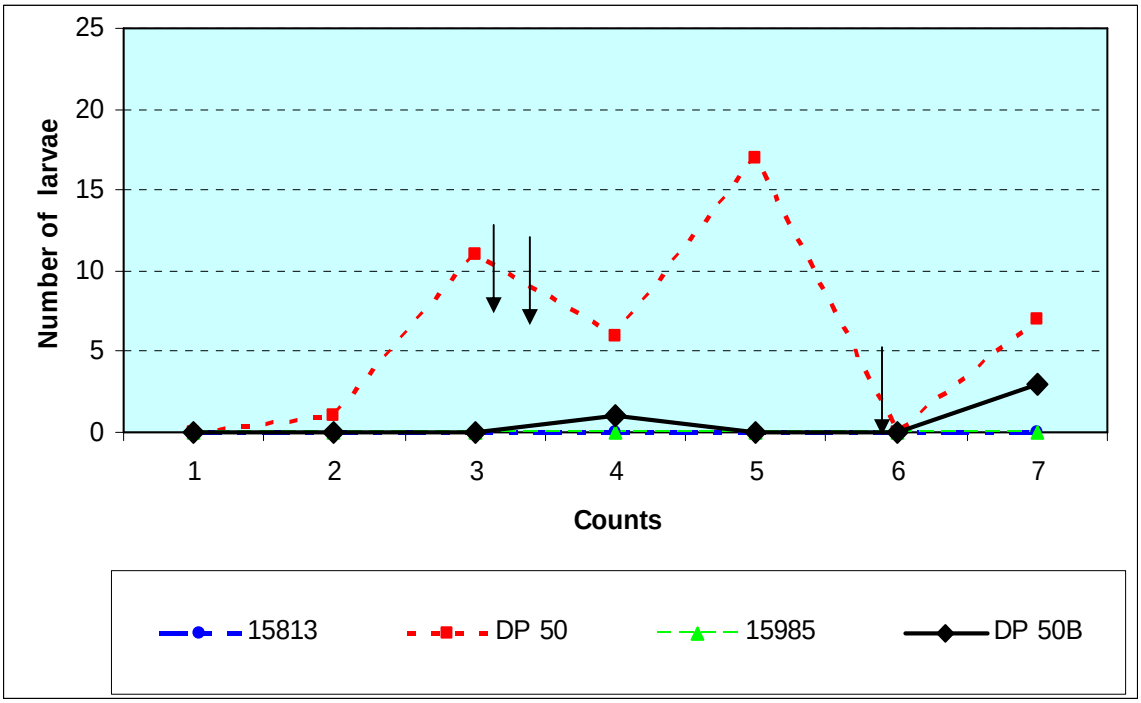


Fig. 2.48. The total number of larvae counted on 20 plants per plot for each treatment at Nelspruit (arrows - insecticide applications) in the Cry2Ab2 trial.

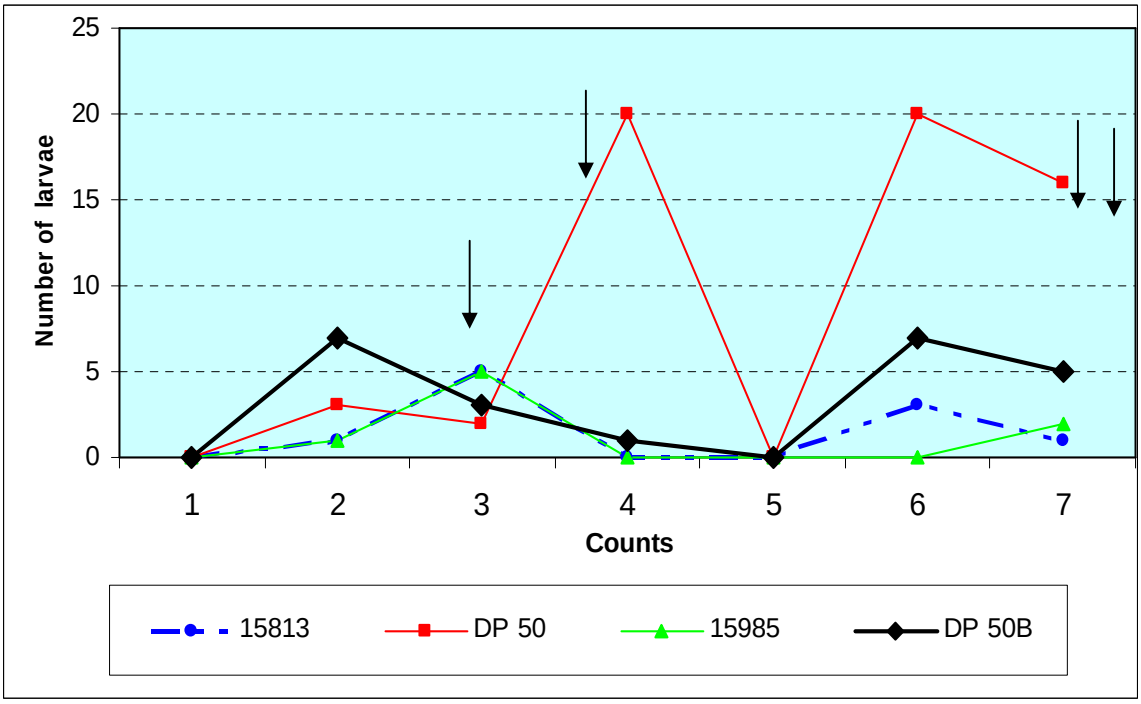


Fig. 2.49. The total number of larvae counted on 20 plants per plot for each treatment at Potgietersrus (arrows - insecticide applications) in the Cry2Ab2 trial.

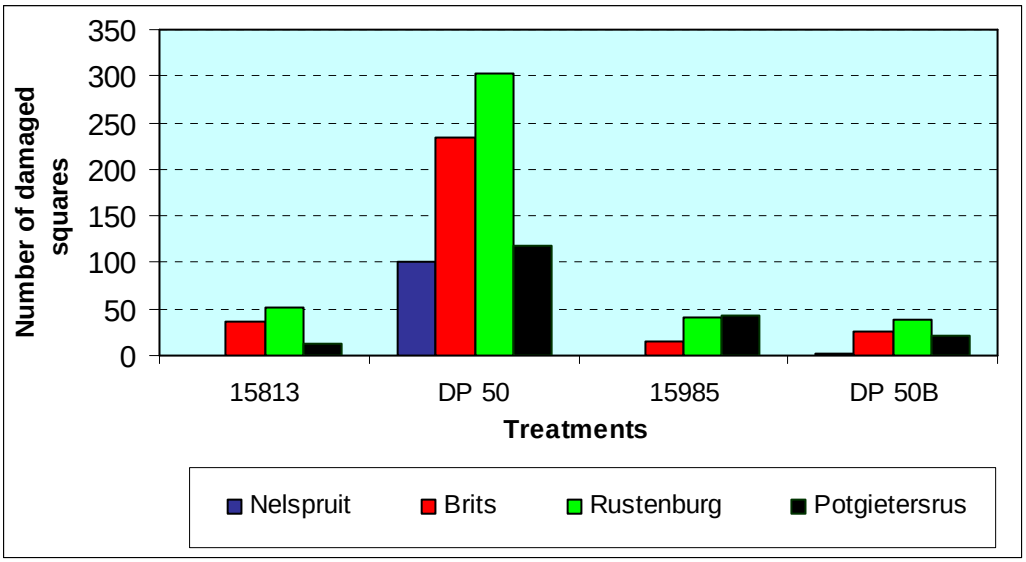


Fig. 2.50. The total number of damaged squares counted on 20 plants per plot of each treatment at the four localities in the Cry2Ab2 trial.

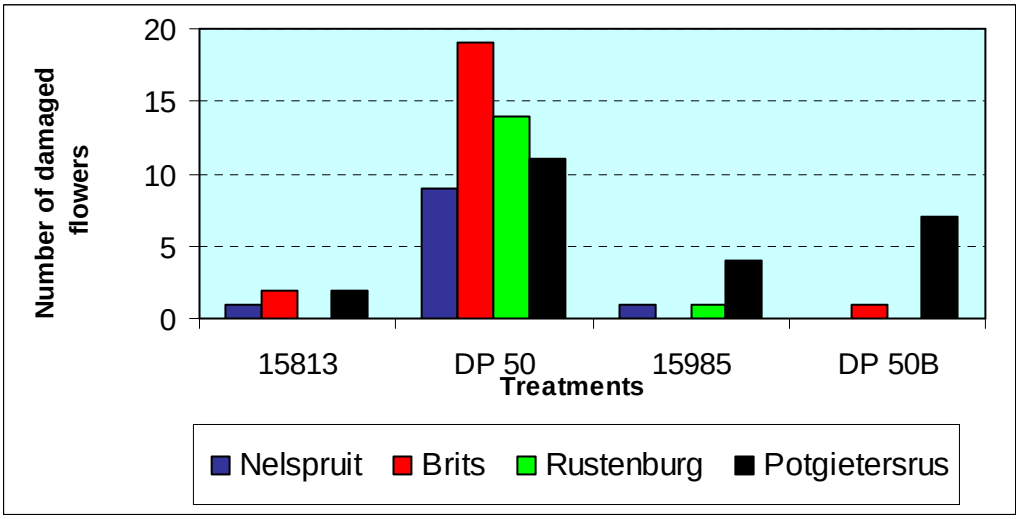


Fig. 2.51. The total number of damaged flowers counted on 20 plants per plot of each treatment at the four localities in the Cry2Ab2 trial.

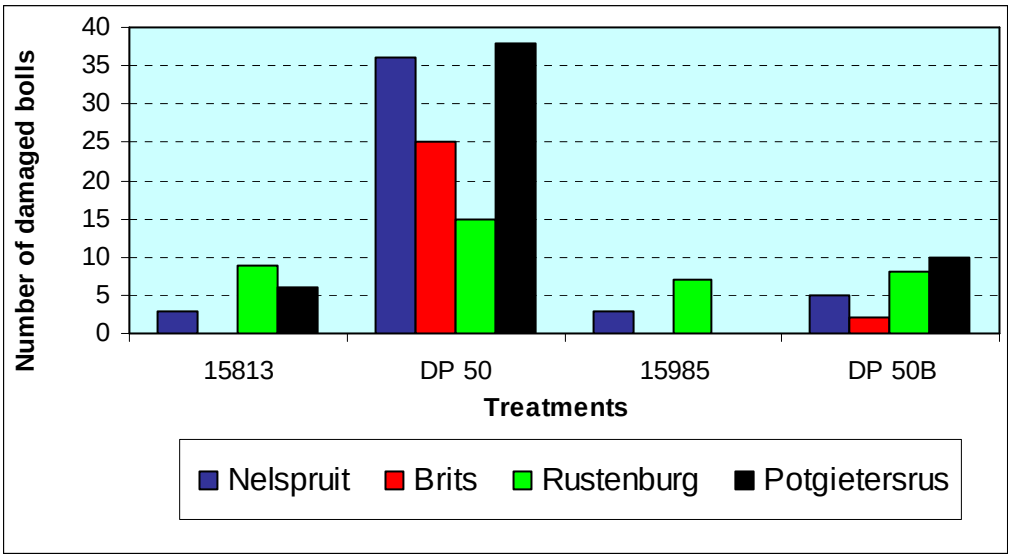


Fig. 2.52. The total number of damaged bolls counted on 20 plants per plot of each treatment at the four localities in the Cry2Ab2 trial.

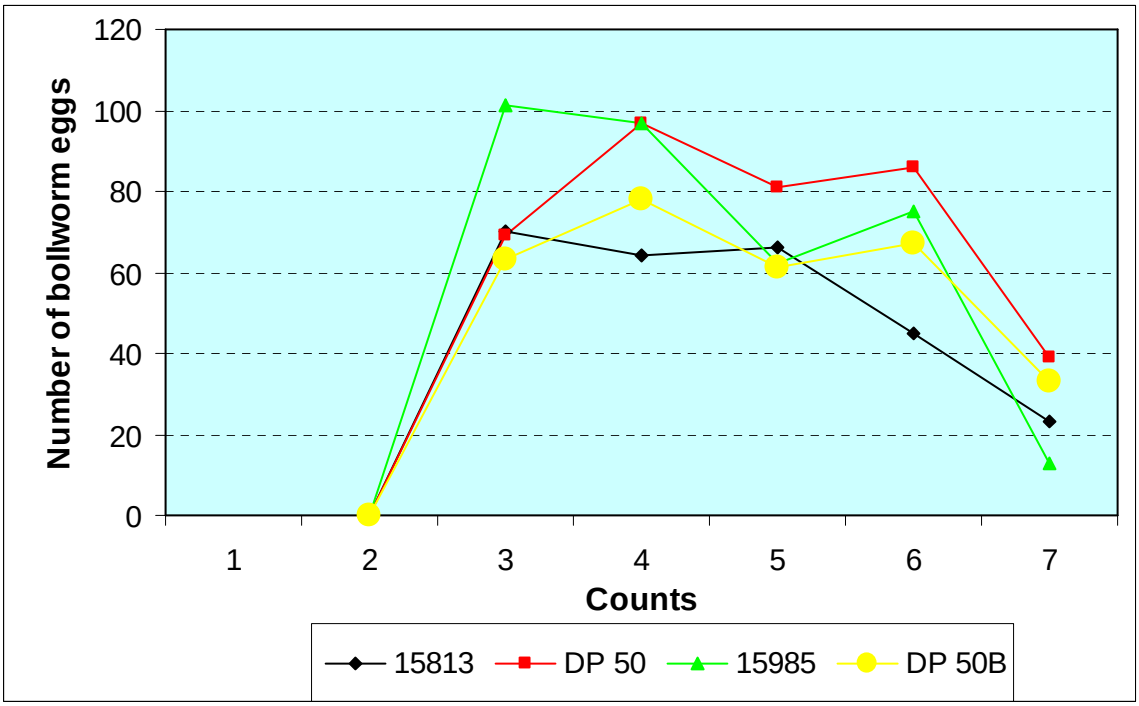


Fig. 2.53. The total number of bollworm eggs counted on 20 plants per plot for each treatment at Rustenburg in the Cry2Ab2 trial.

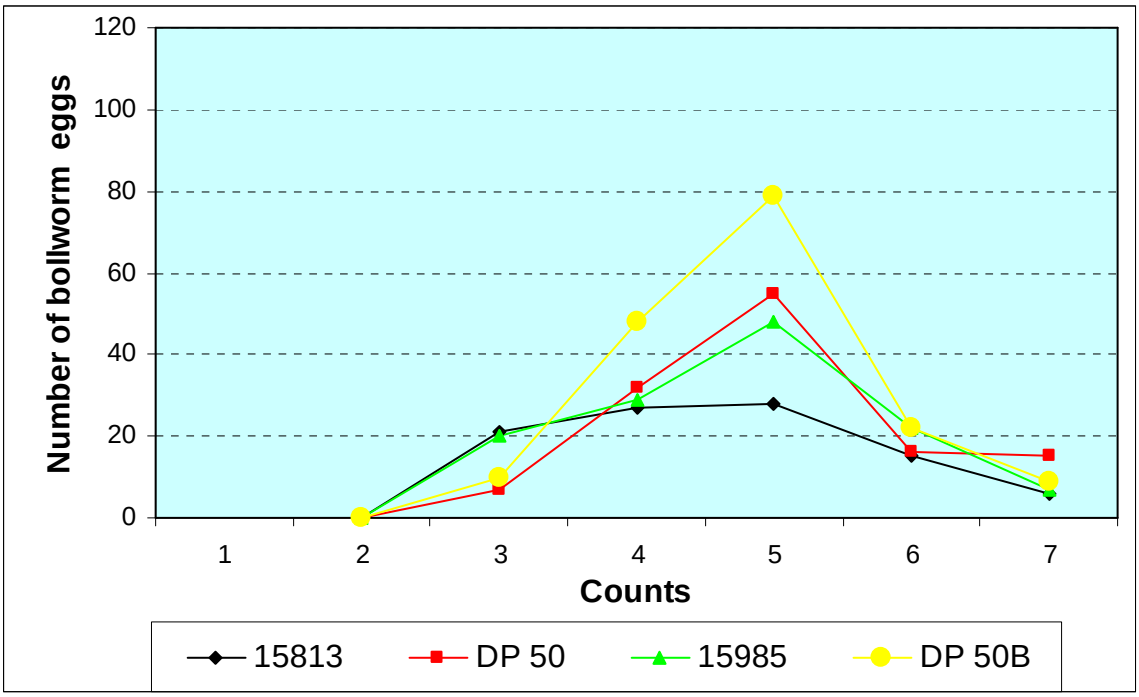


Fig. 2.54. The total number of bollworm eggs counted on 20 plants per plot for each treatment at Brits in the Cry2Ab2 trial.

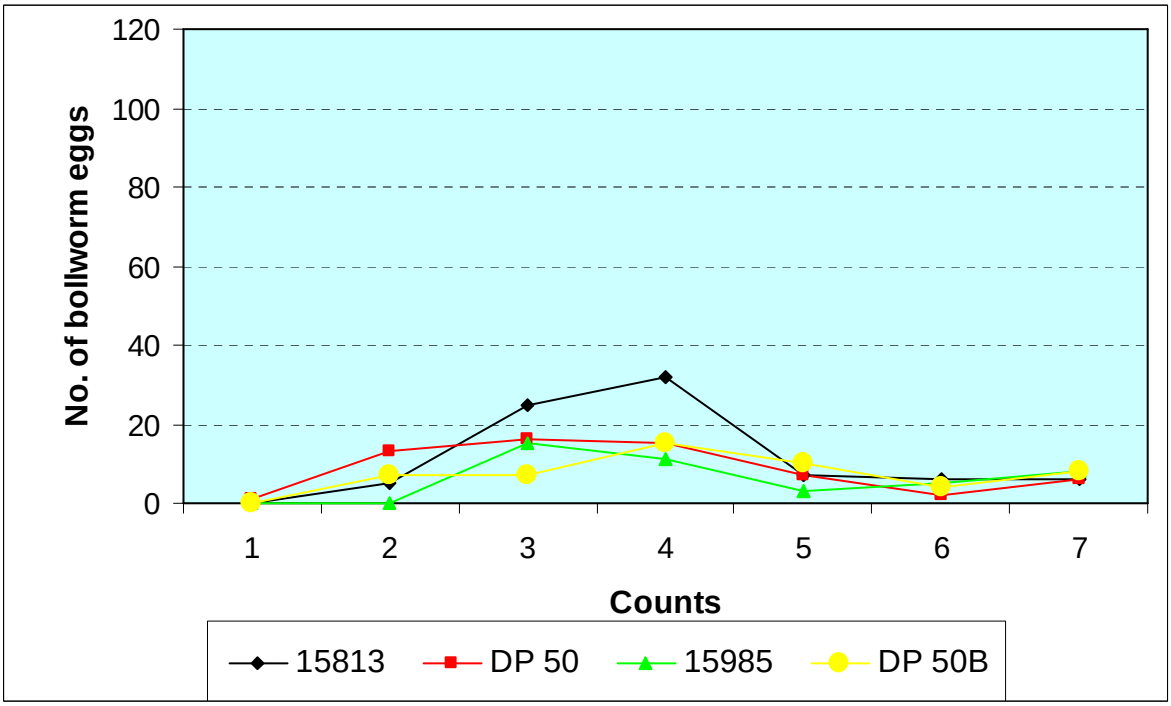


Fig. 2.55. The total number of bollworm eggs counted on 20 plants per plot for each treatment at Nelspruit in the Cry2Ab2 trial.

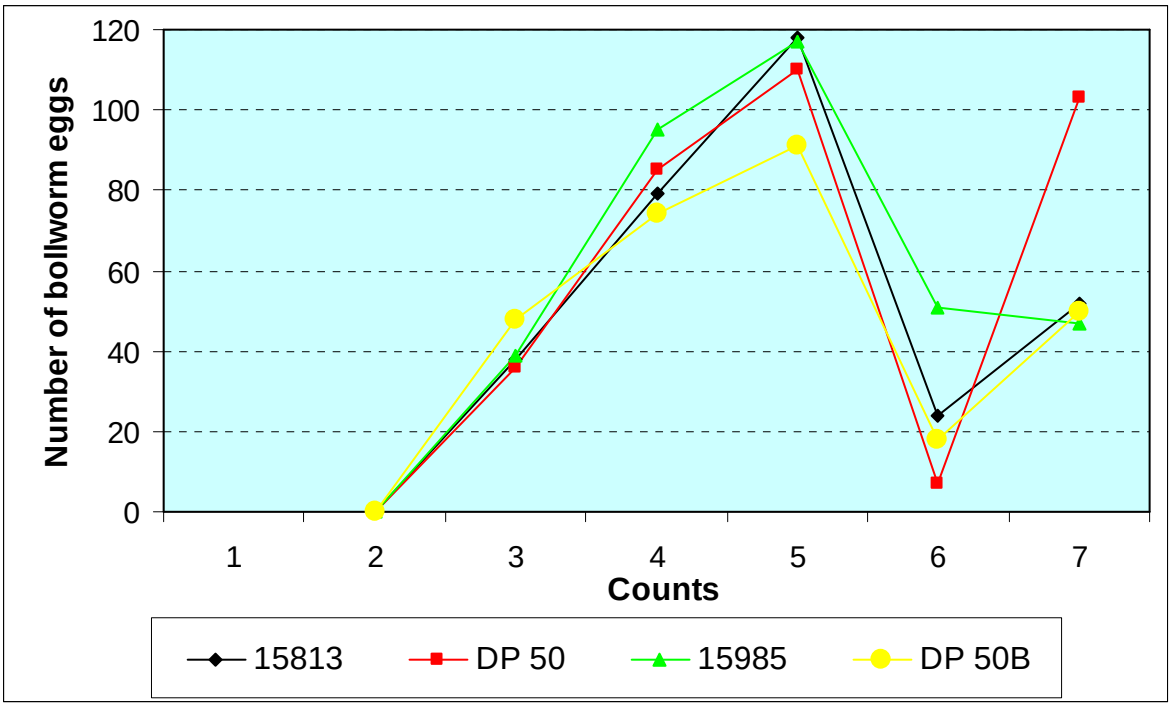


Fig. 2.56. The total number of bollworm eggs counted on 20 plants per plot for each treatment at Potgietersrus in the Cry2Ab2 trial.

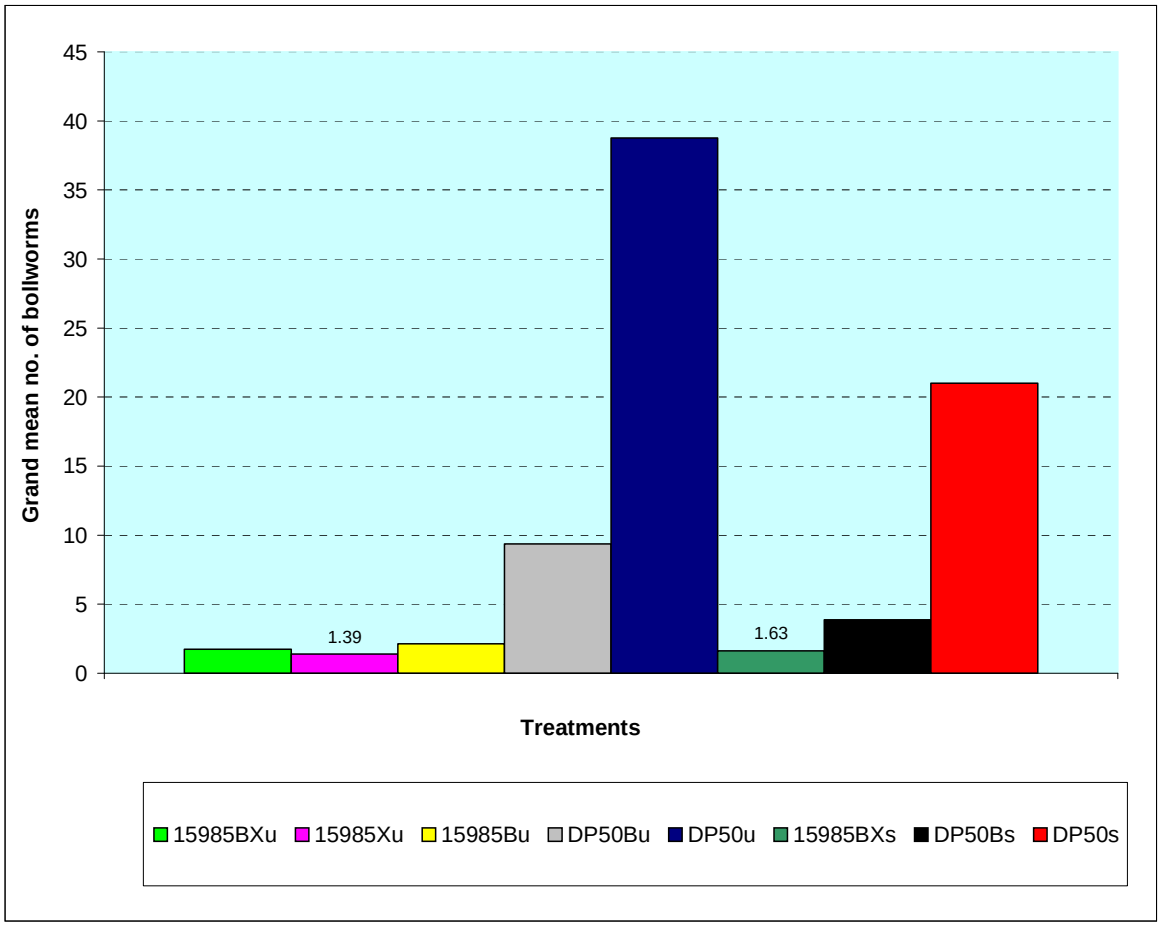


Fig. 2.57. Total number of bollworm over all the localities in the stacked Cry1Ac and Cry2Ab2 trial.

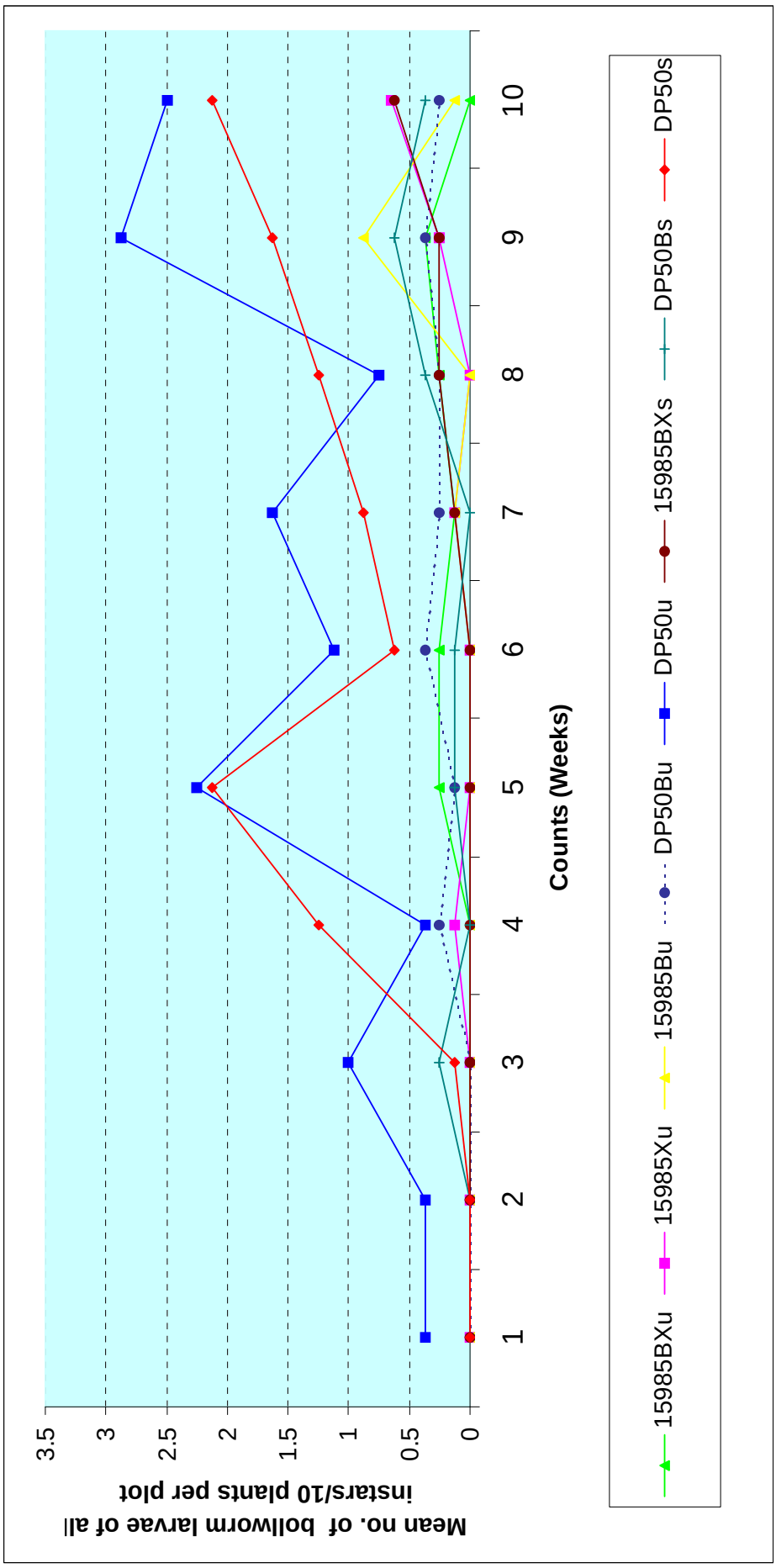


Fig. 2.58. Total number of bollworm larvae from Rustenburg in the stacked Cry1Ac and Cry2Ab2 trial.

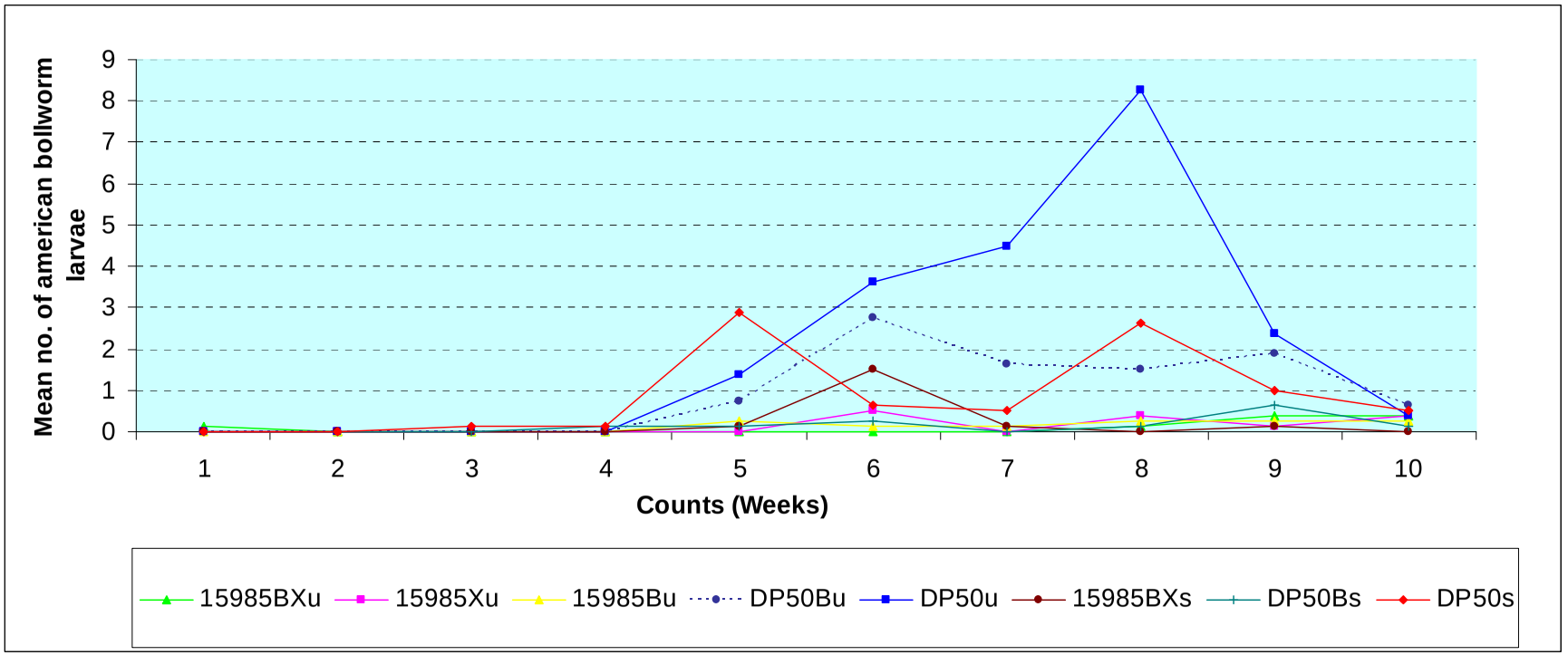


Fig. 2.59. Total number of bollworm larvae from Brits in the stacked Cry1Ac and Cry2Ab2 trial.

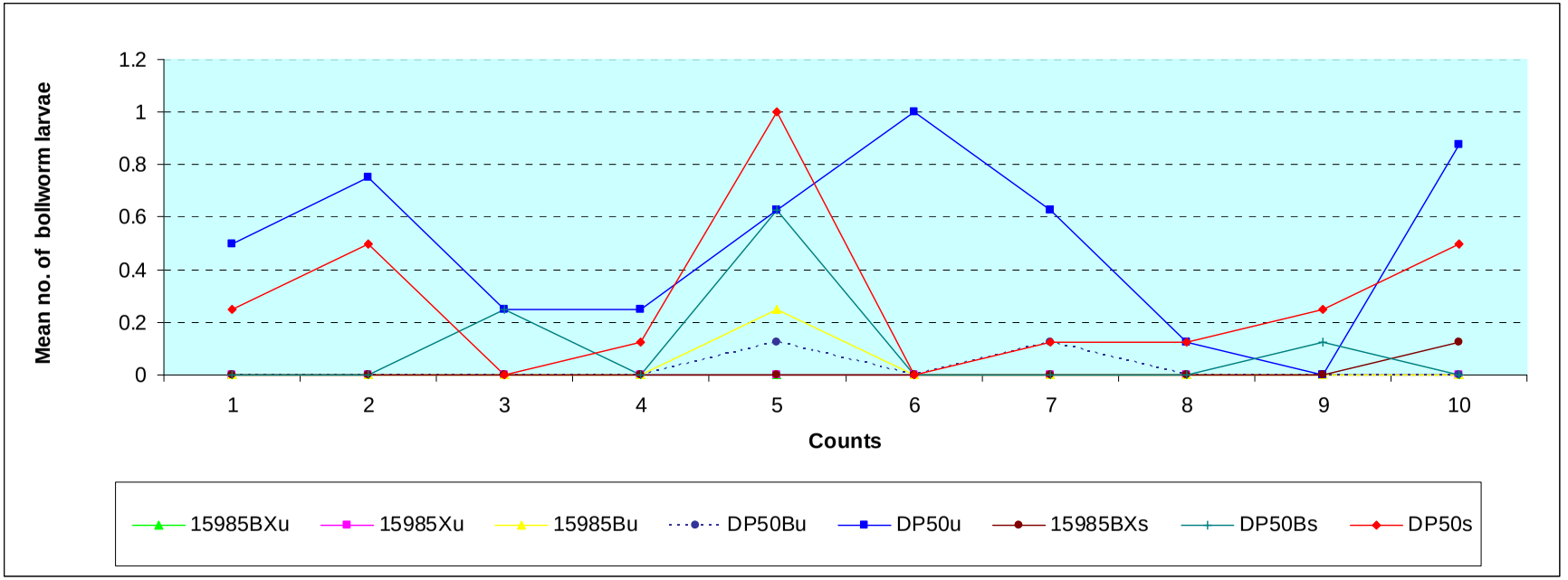


Fig. 2.60. Total number of bollworm larvae from Nelspruit in the stacked Cry1Ac and Cry2Ab2 trial.

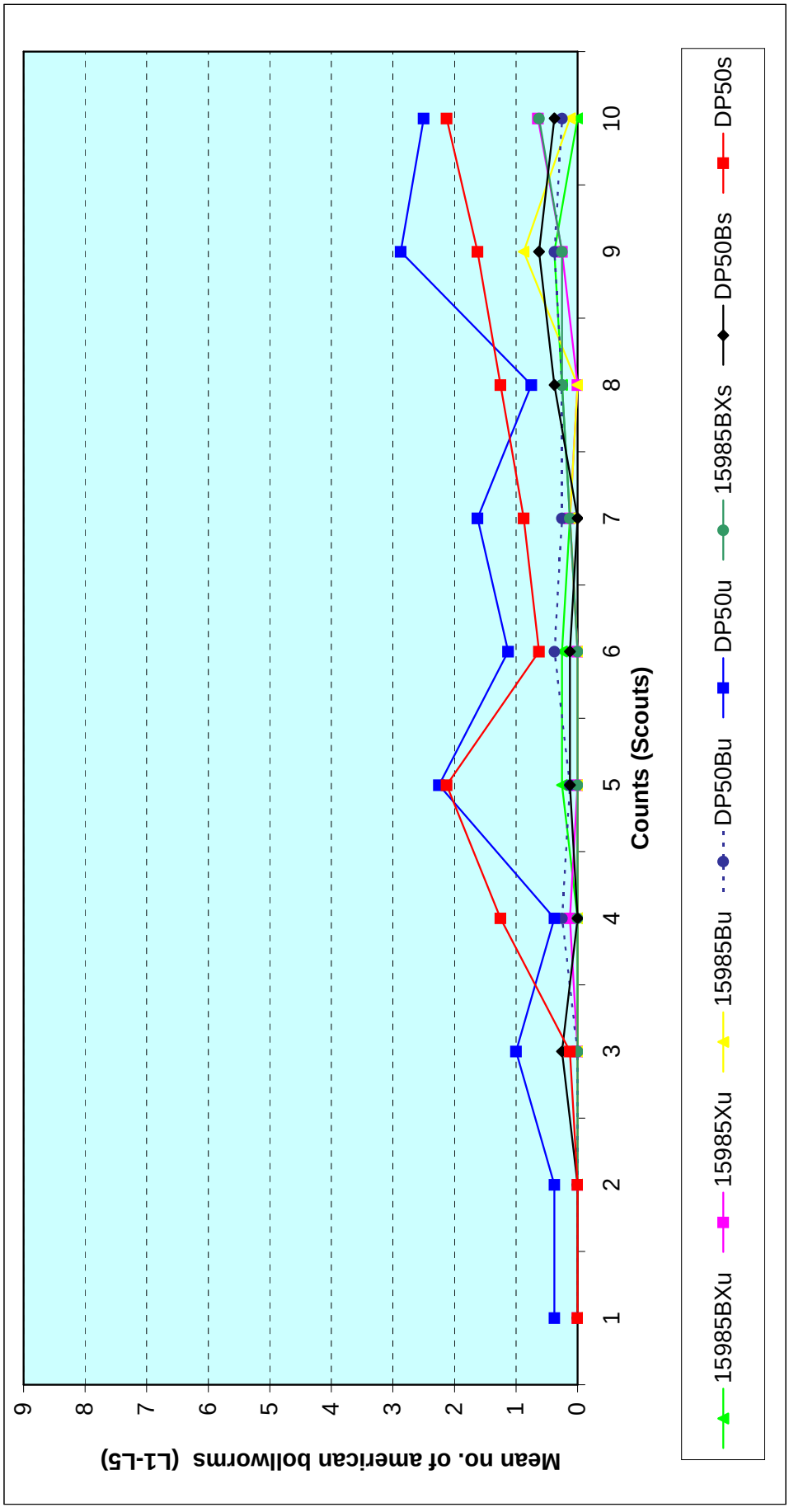


Fig. 2.61. Mean number of african bollworm larvae (L1-L5) on 10 plants per plot at Rustenburg in the stacked Cry1Ac and Cry2Ab2 trial.

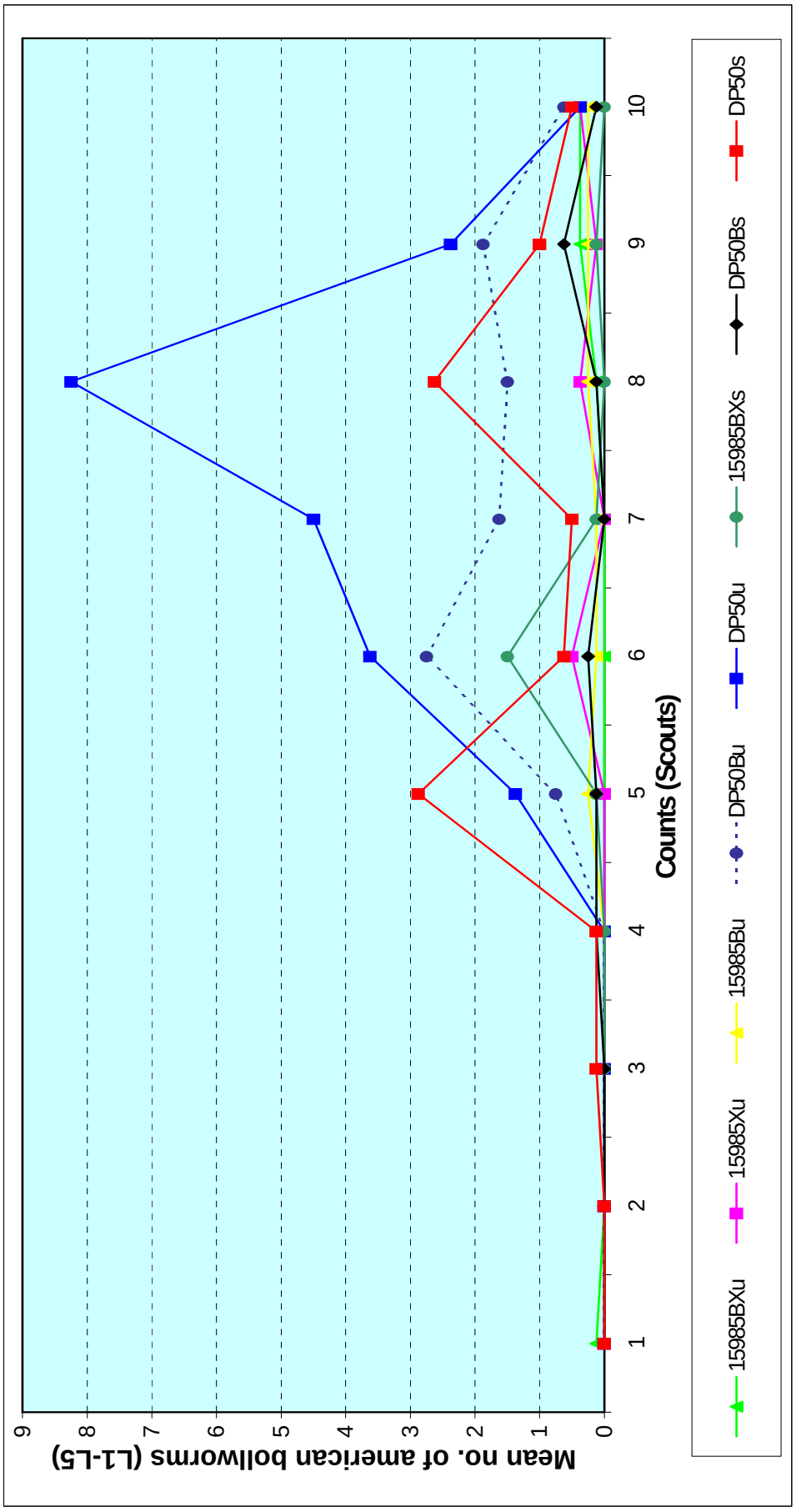


Fig. 2.62. Mean number of african bollworm larvae (L1-L5) on 10 plants per plot at Brits in the stacked Cry1Ac and Cry2Ab2 trial.

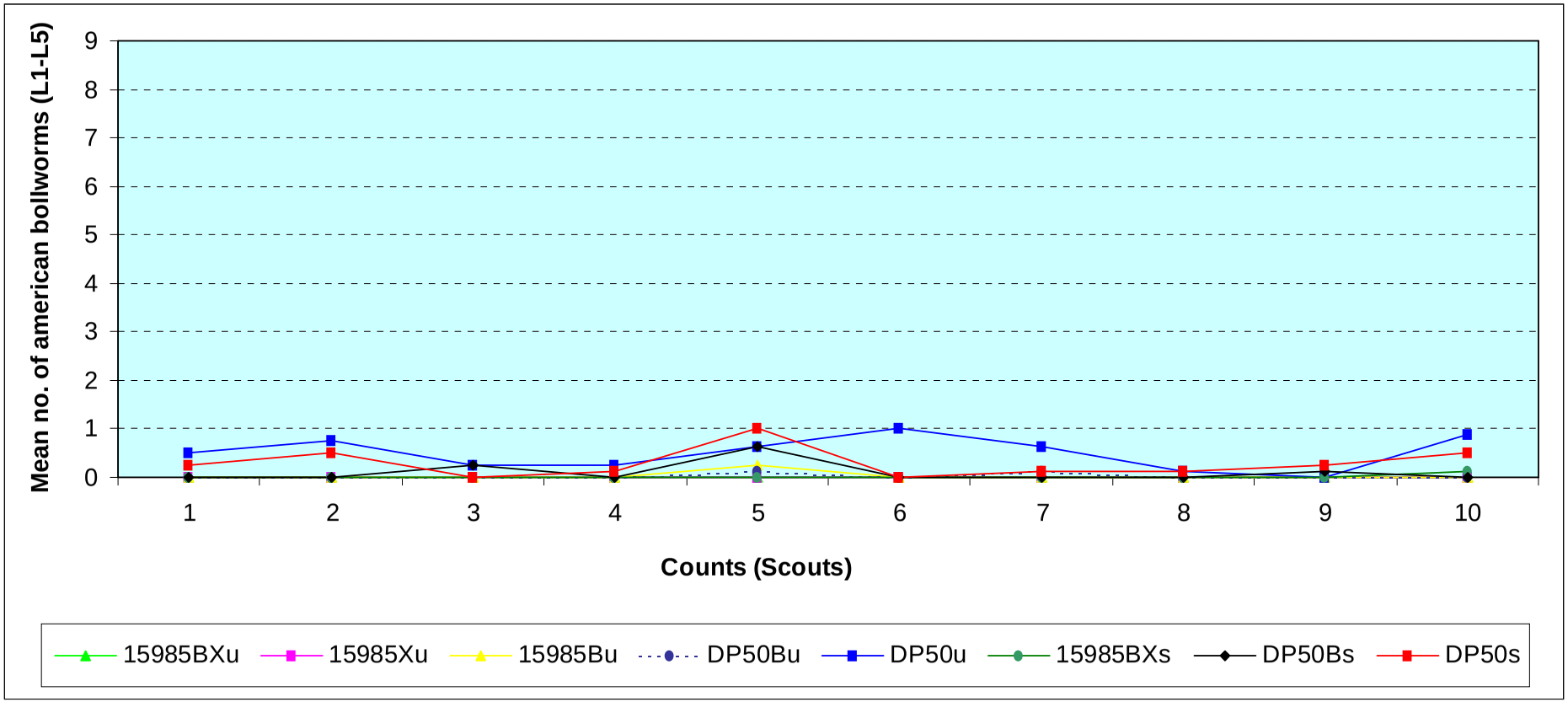


Fig. 2.63. Mean number of african bollworm larvae (L1-L5) on 10 plants per plot at Nelspruit in the stacked Cry1Ac and Cry2Ab2 trial.

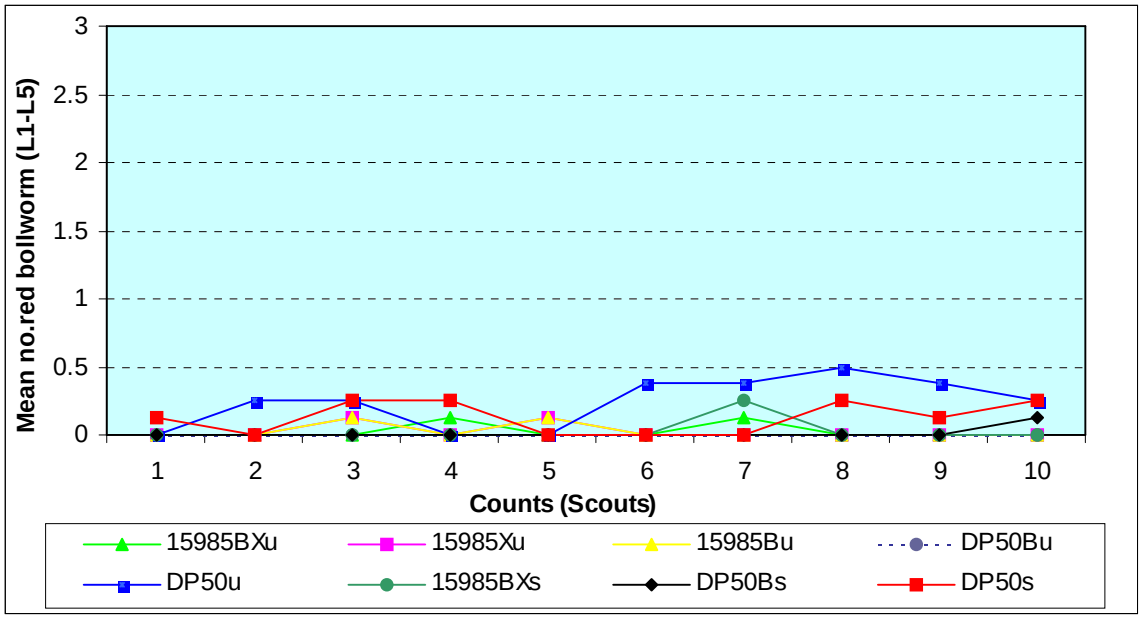


Fig. 2.64. Mean number of red bollworm larvae (L1-L5) on 10 plants per plot at Rustenburg in the stacked Cry1Ac and Cry2Ab2 trial.

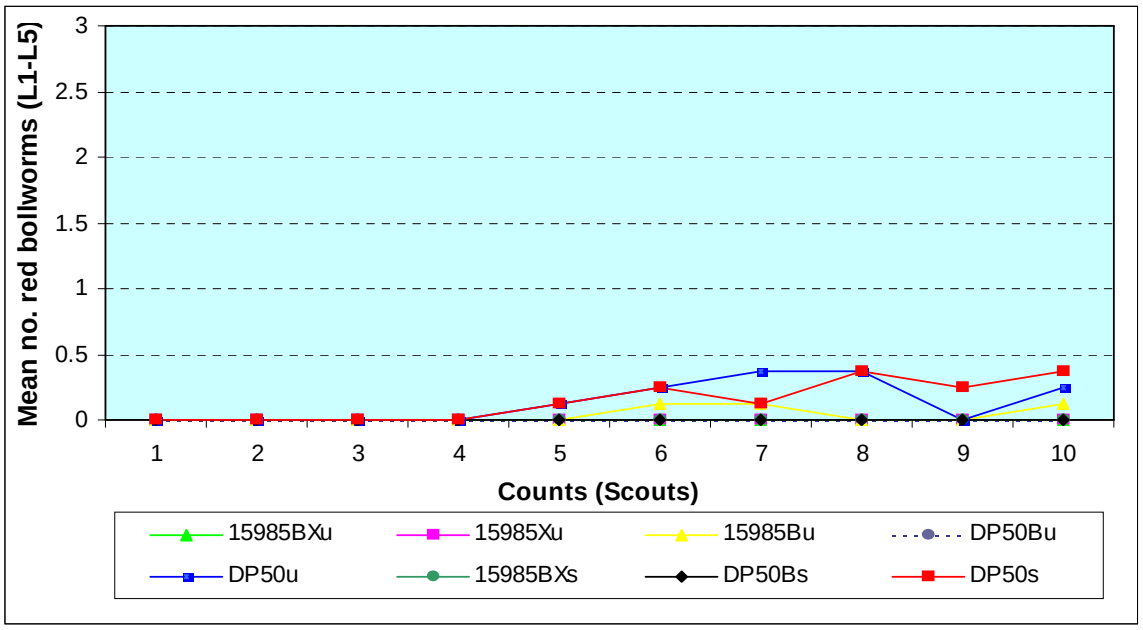


Fig. 2.65. Mean number of red bollworm larvae (L1-L5) on 10 plants per plot at Brits in the stacked Cry1Ac and Cry2Ab2 trial.

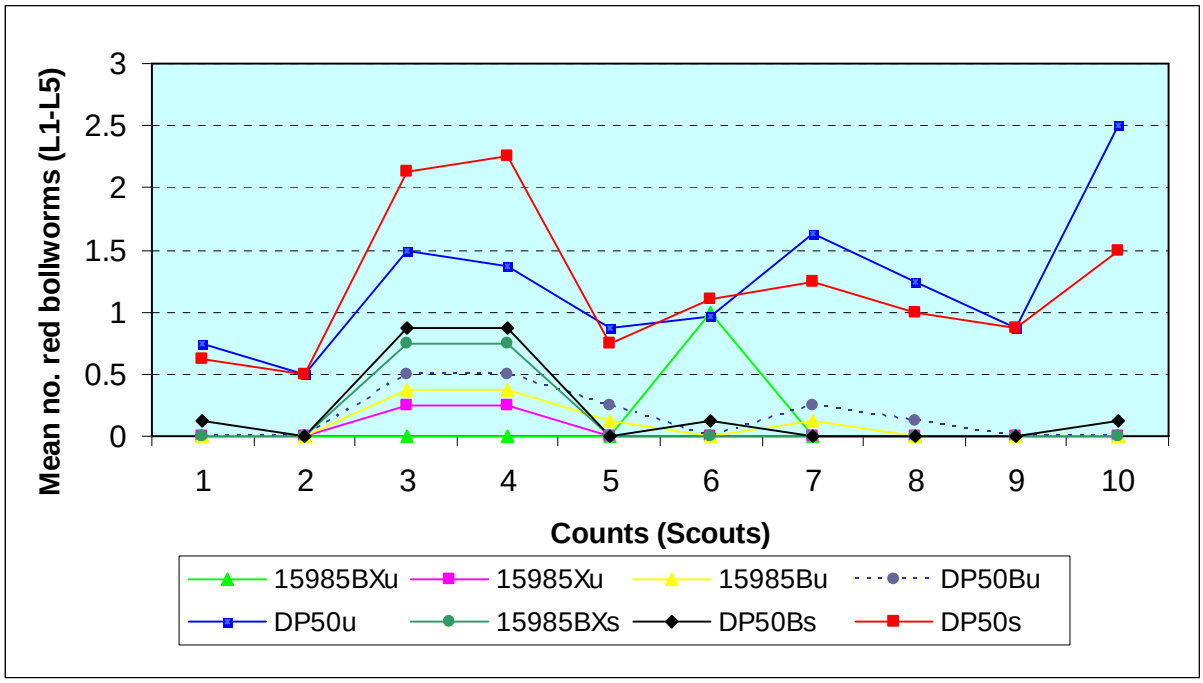


Fig. 2.66. Mean number of red bollworm larvae (L1-L5) on 10 plants per plot at Nelspruit in the stacked Cry1Ac and Cry2Ab2 trial.

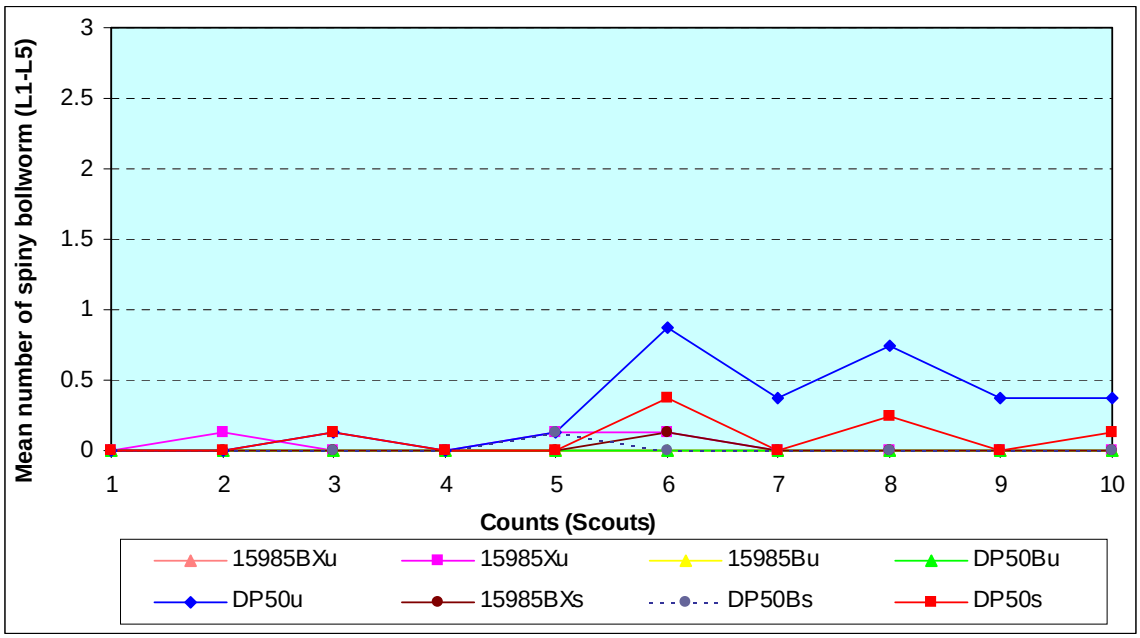


Fig. 2.67. Mean number of spiny bollworm larvae (L1-L5) on 10 plants per plot at Rustenburg in the stacked Cry1Ac and Cry2Ab2 trial.

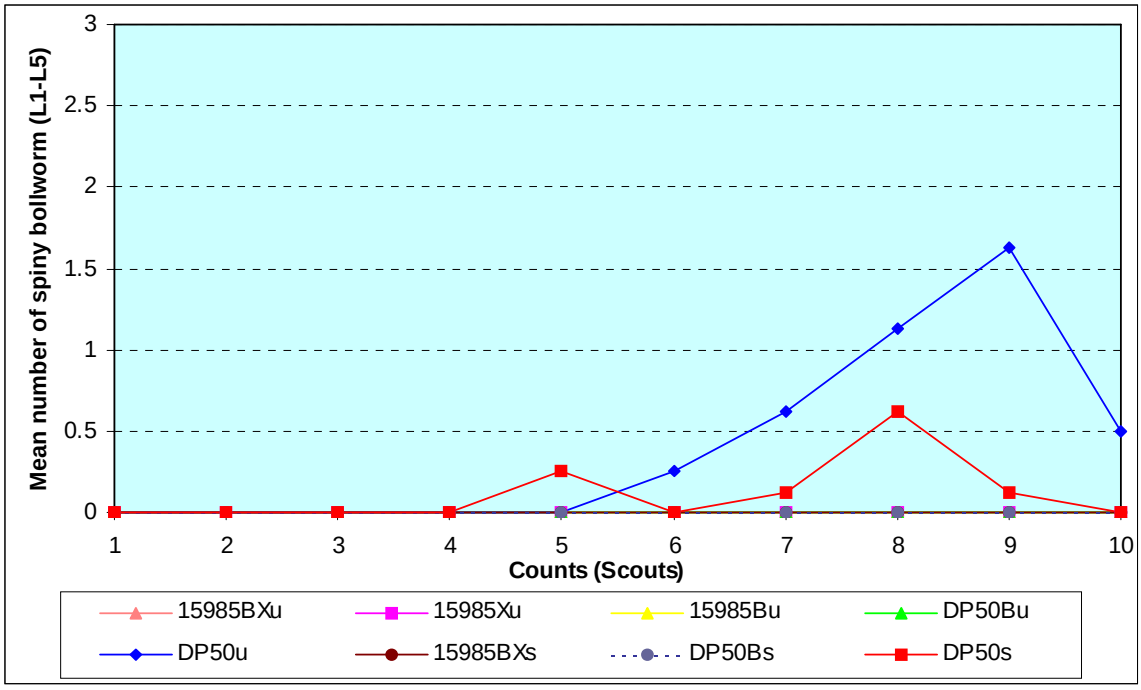


Fig. 2.68. Mean number of spiny bollworm larvae (L1-L5) on 10 plants per plot at Brits in the stacked Cry1Ac and Cry2Ab2 trial.

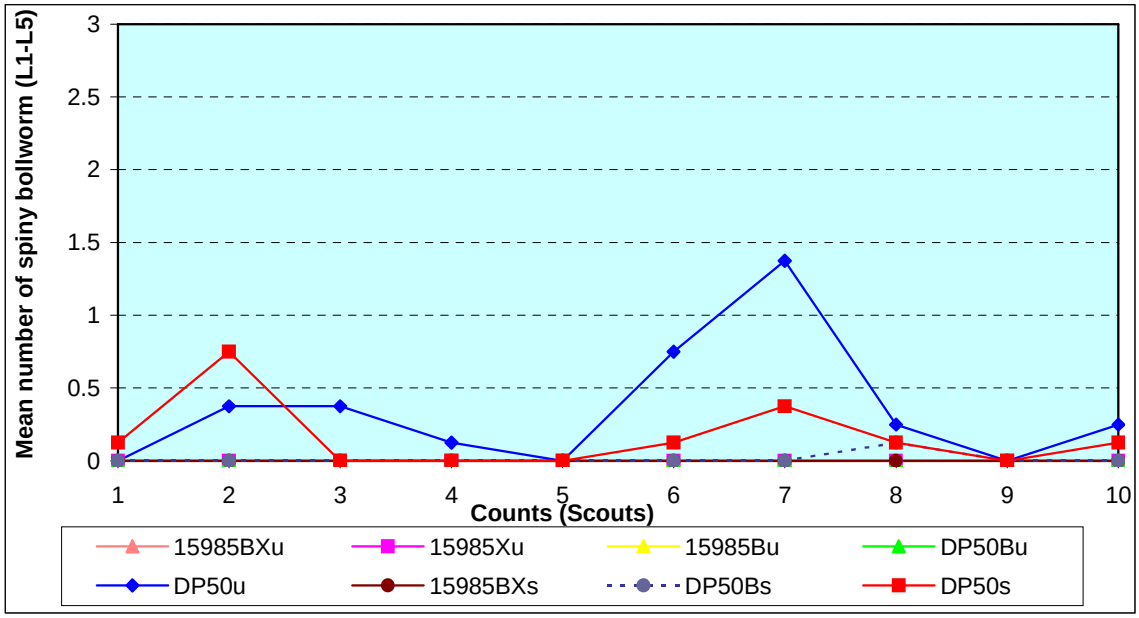


Fig. 2.69. Mean number of spiny bollworm larvae (L1-L5) on 10 plants per plot at Nelspruit in the stacked Cry1Ac and Cry2Ab2 trial.

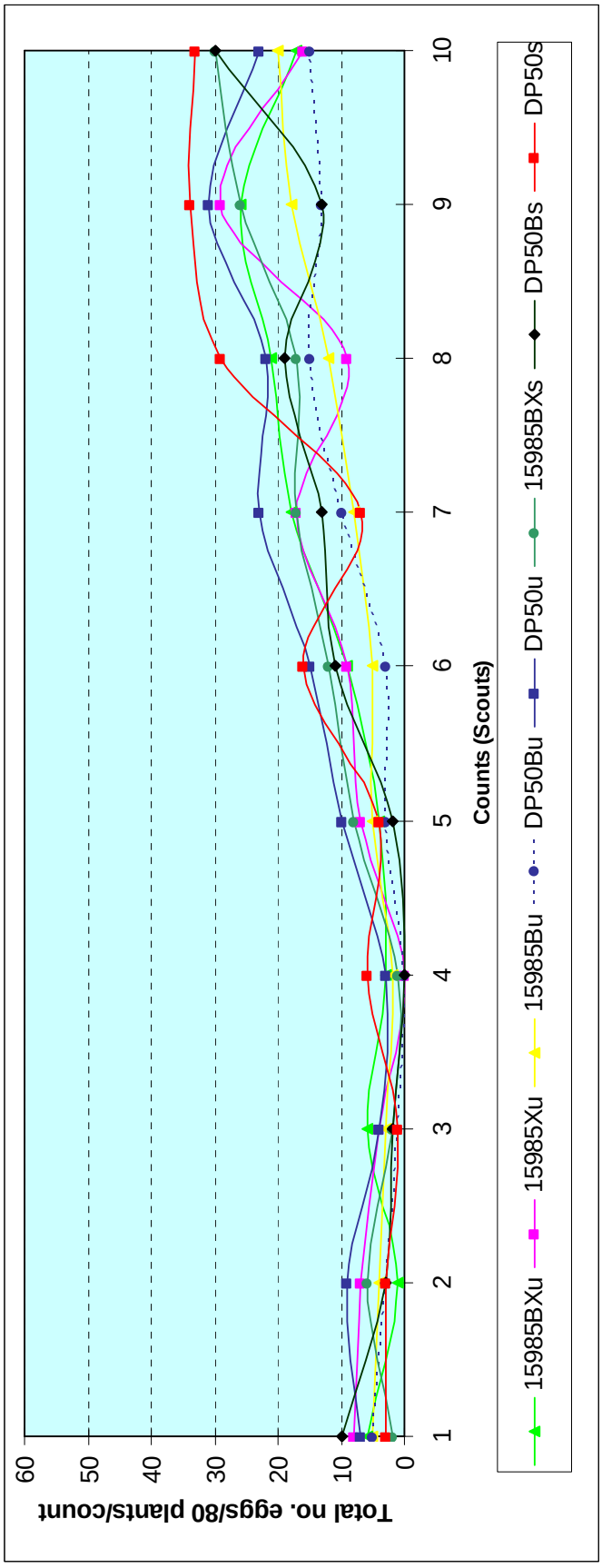


Fig. 2.70. The total number of bollworm eggs on 80 plants per treatment at Rustenburg in the stacked Cry1Ac and Cry2Ab2 trial.

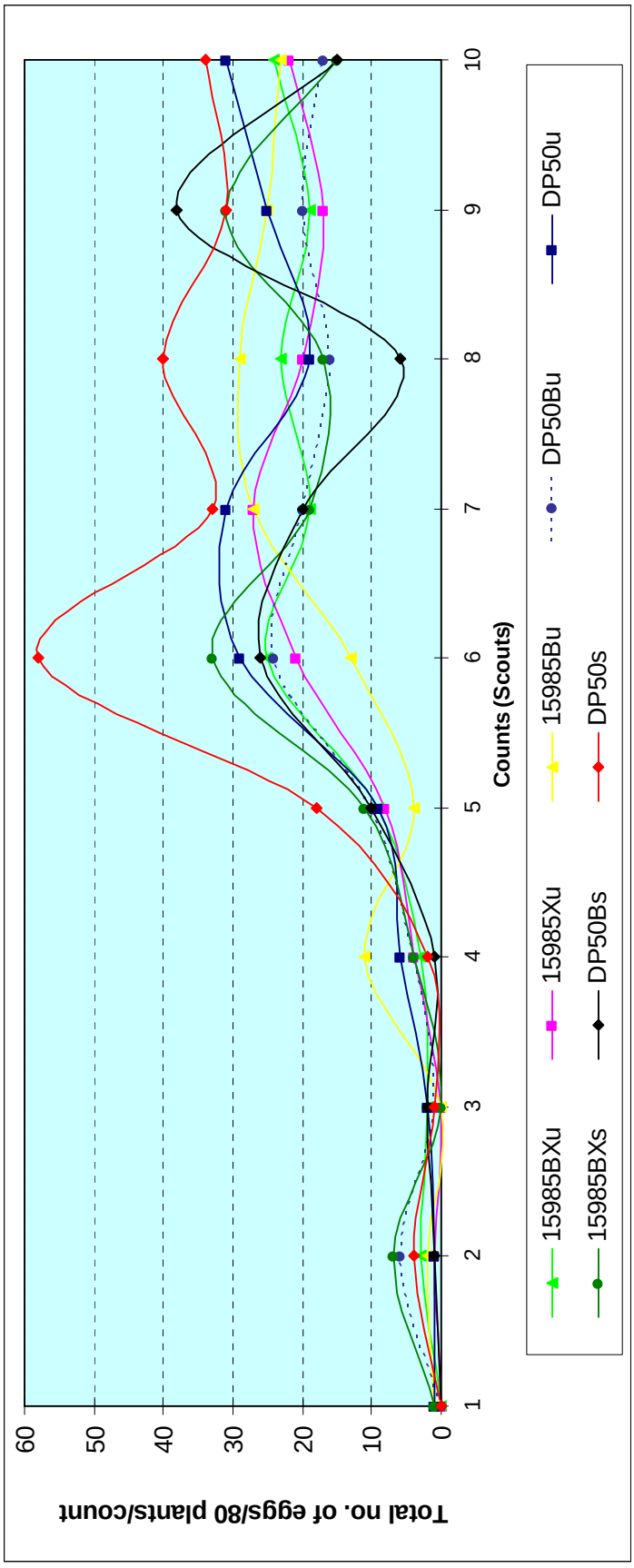


Fig. 2.71. The total number of bollworm eggs on 80 plants per treatment at Brits in the stacked Cry1Ac and Cry2Ab2 trial.

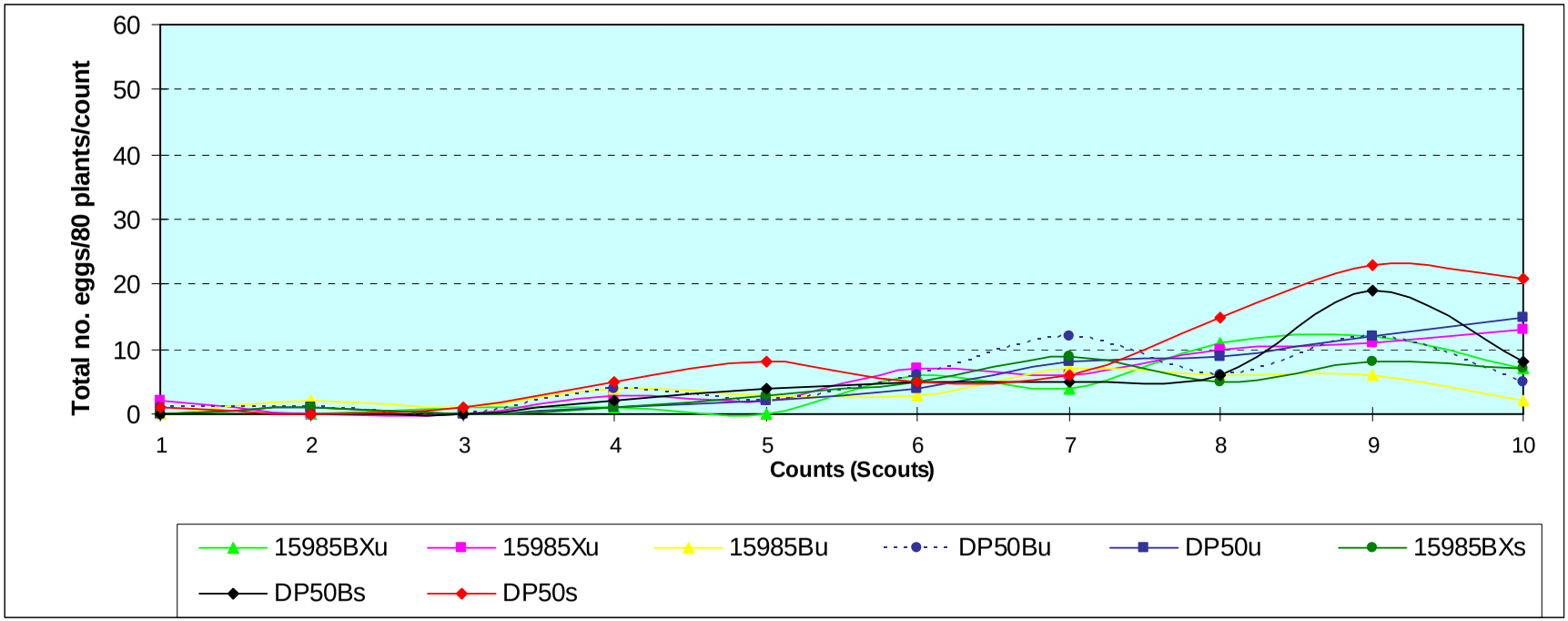


Fig. 2.72. The total number of bollworm eggs on 80 plants per treatment at Nelspruit in the stacked Cry1Ac and Cry2Ab2 trial.

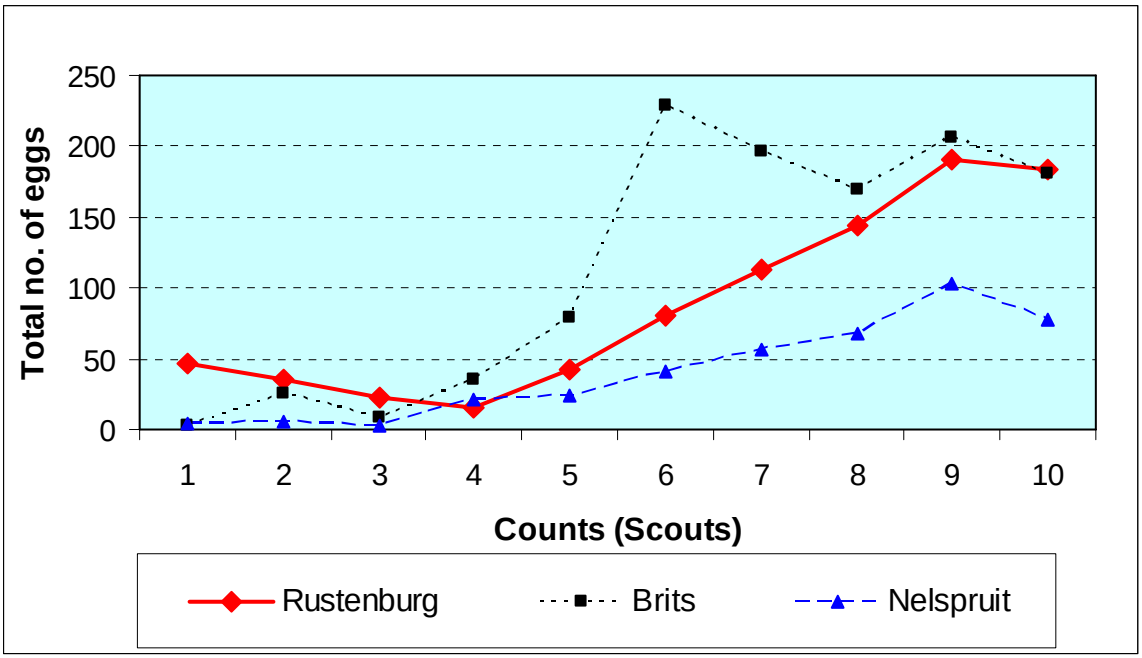


Fig. 2.73. The total number of eggs on all treatments at Rustenburg, Brits and Nelspruit (1999) in the stacked Cry1Ac and Cry2Ab2 trial.

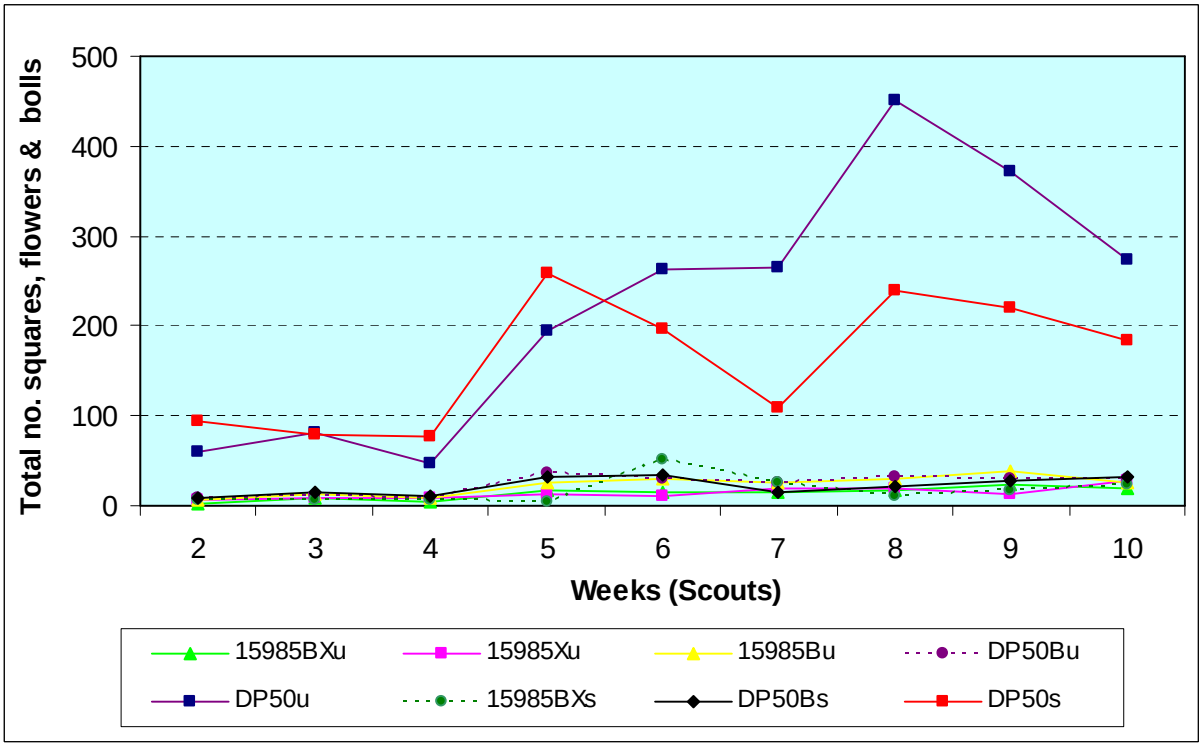


Fig. 2.74. The total number of all squares, flowers and bolls at all 3 localities (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

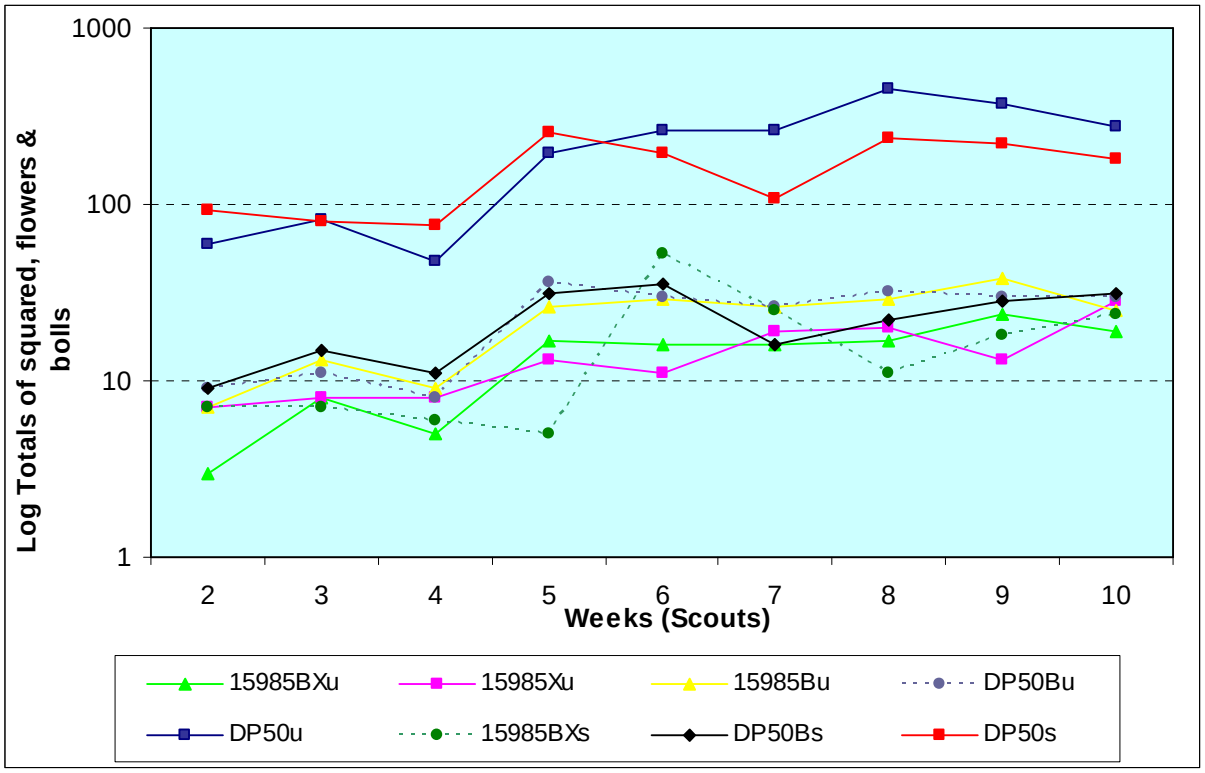


Fig. 2.75. The logarithmic of the total number of all squares, flowers and bolls at all three localities (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

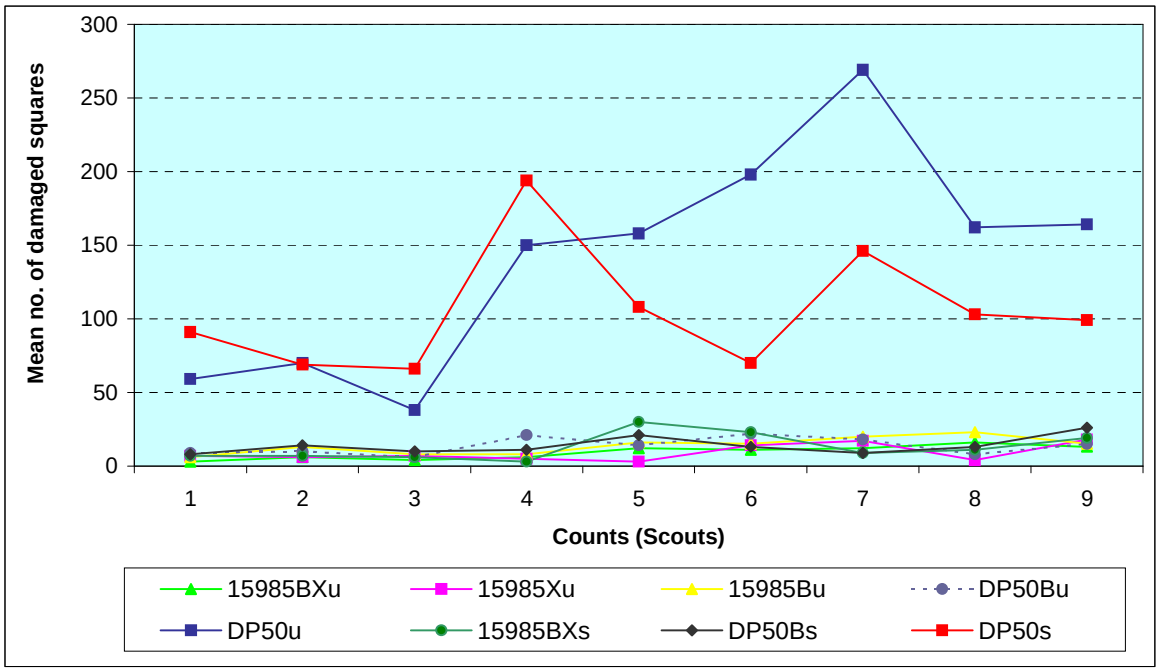


Fig. 2.76. The total number of squares at all 3 localities (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

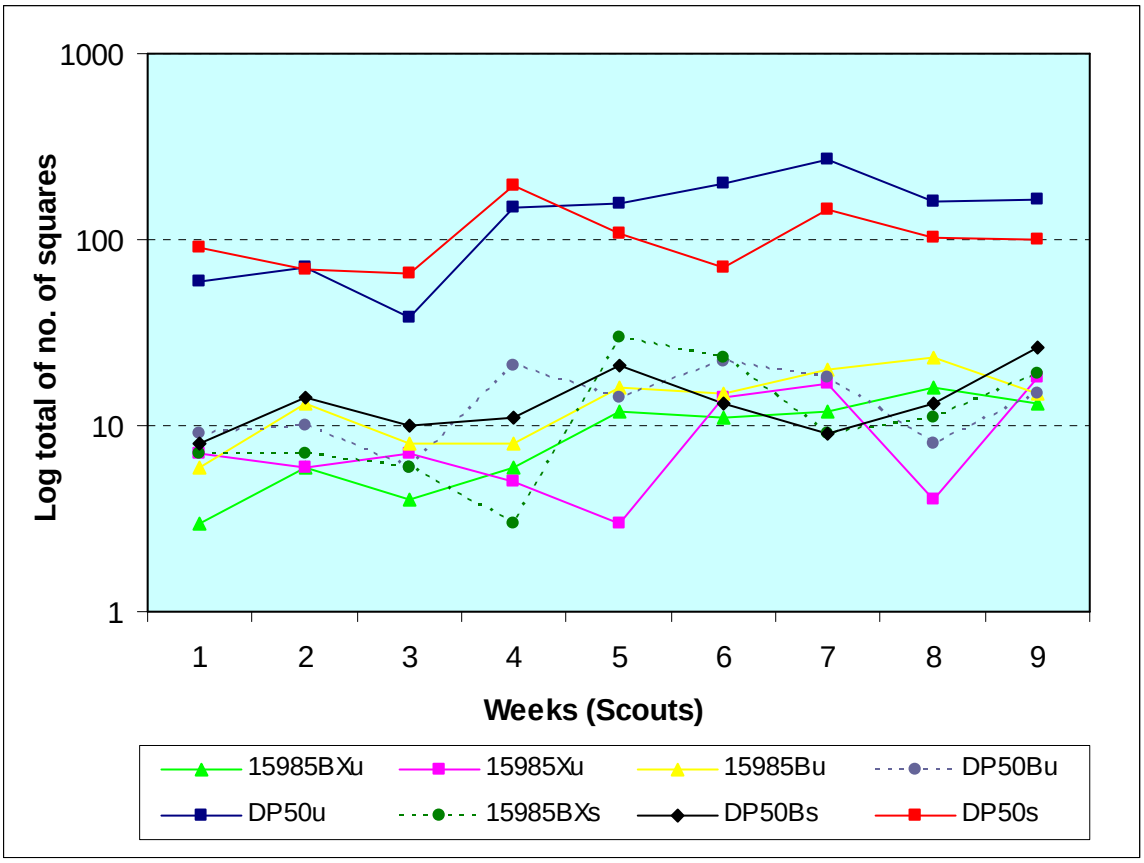


Fig. 2.77. The logarithmic of the total number of squares at all 3 localities (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

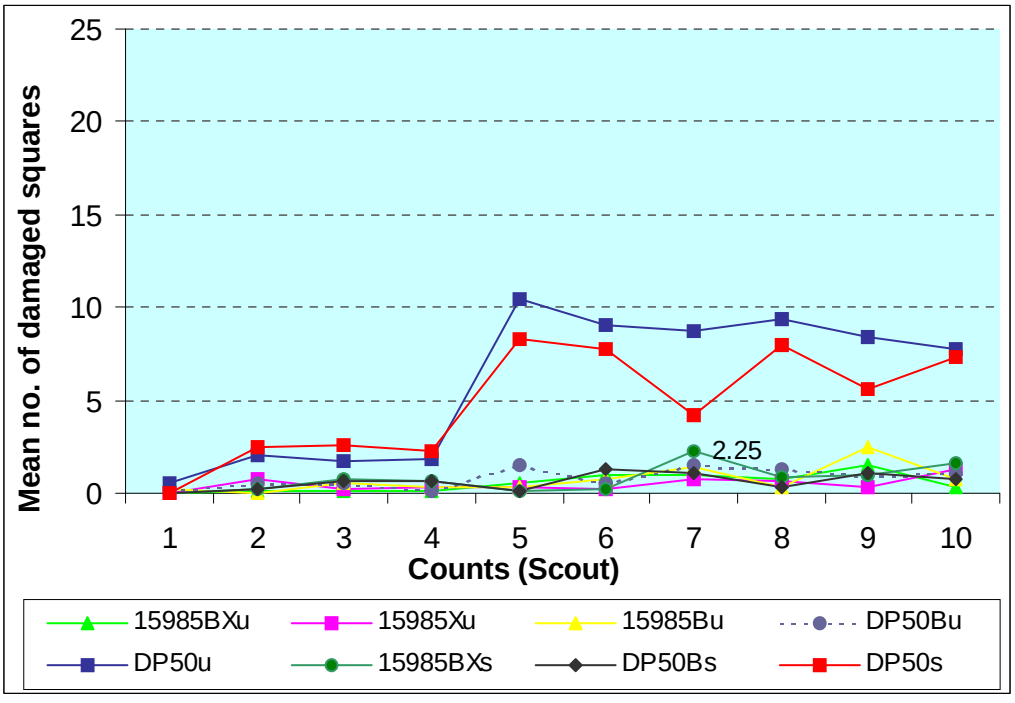


Fig. 2.78. The mean number of damaged squares on 80 plants per treatment at Rustenburg (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

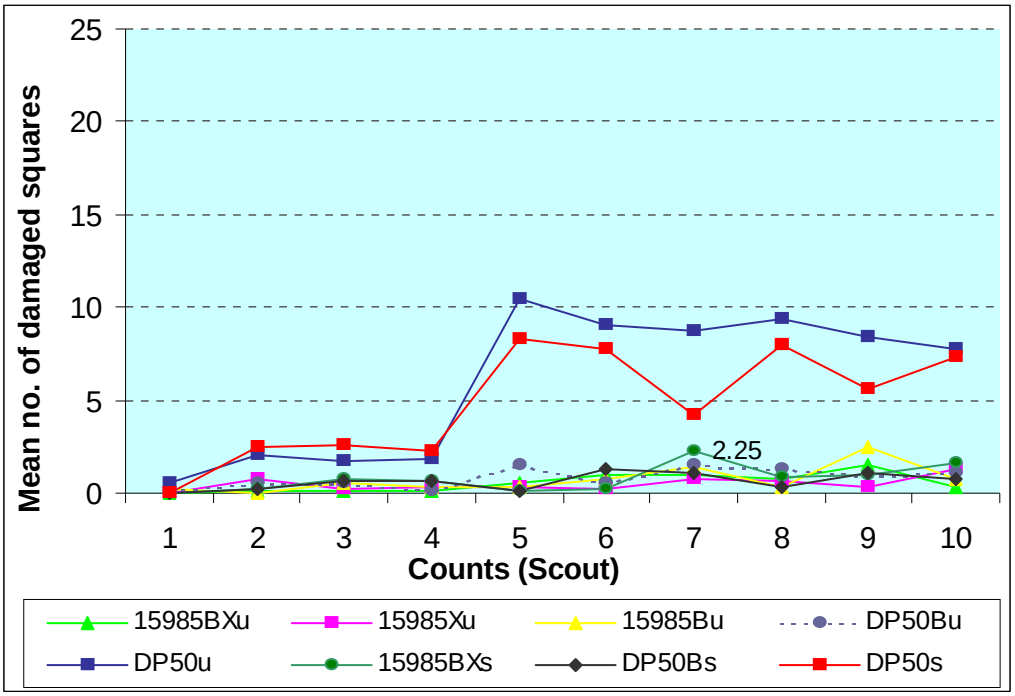


Fig. 2.79. The mean number of damaged squares on 80 plants per treatment at Brits (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

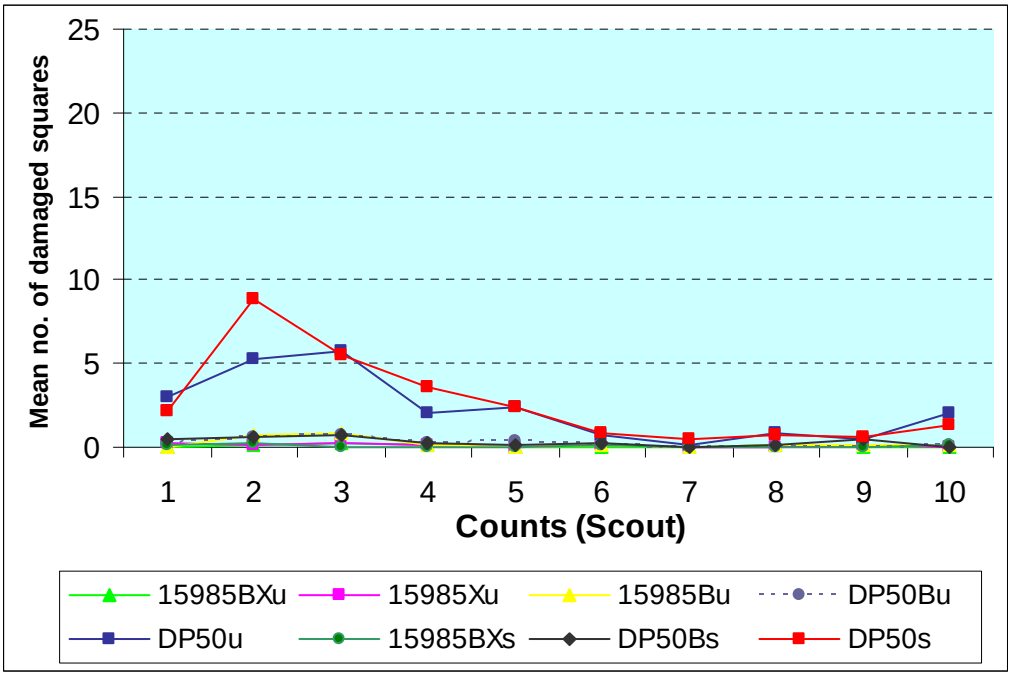


Fig. 2.80. The mean number of damaged squares on 80 plants per treatment at Nelspruit (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

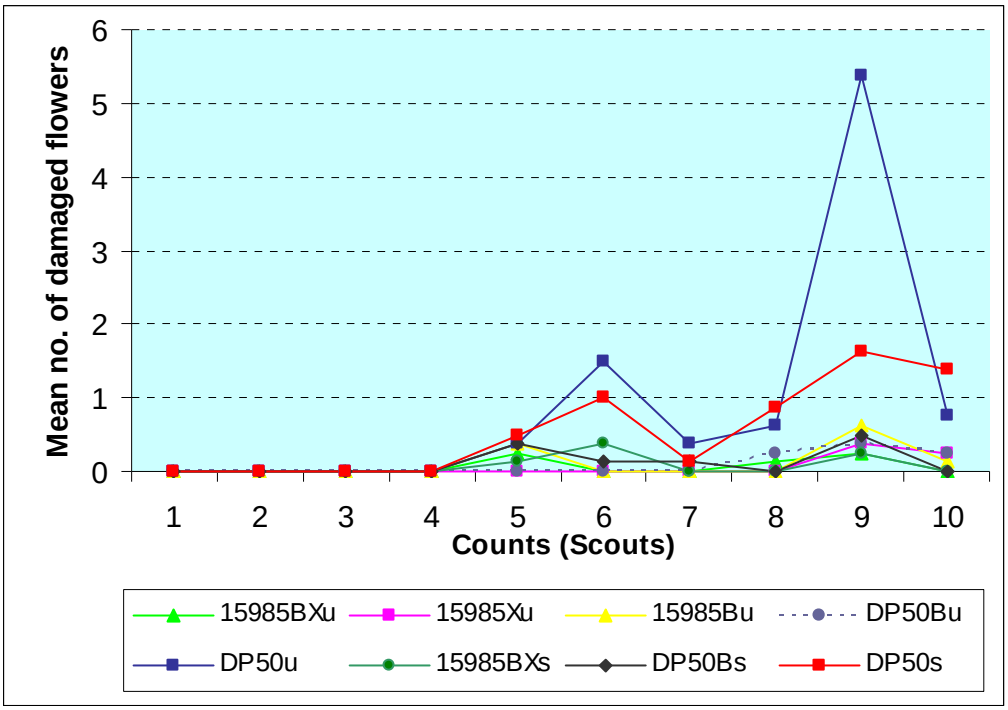


Fig. 2.81. The mean number of damaged flowers on 80 plants per treatment at Rustenburg (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

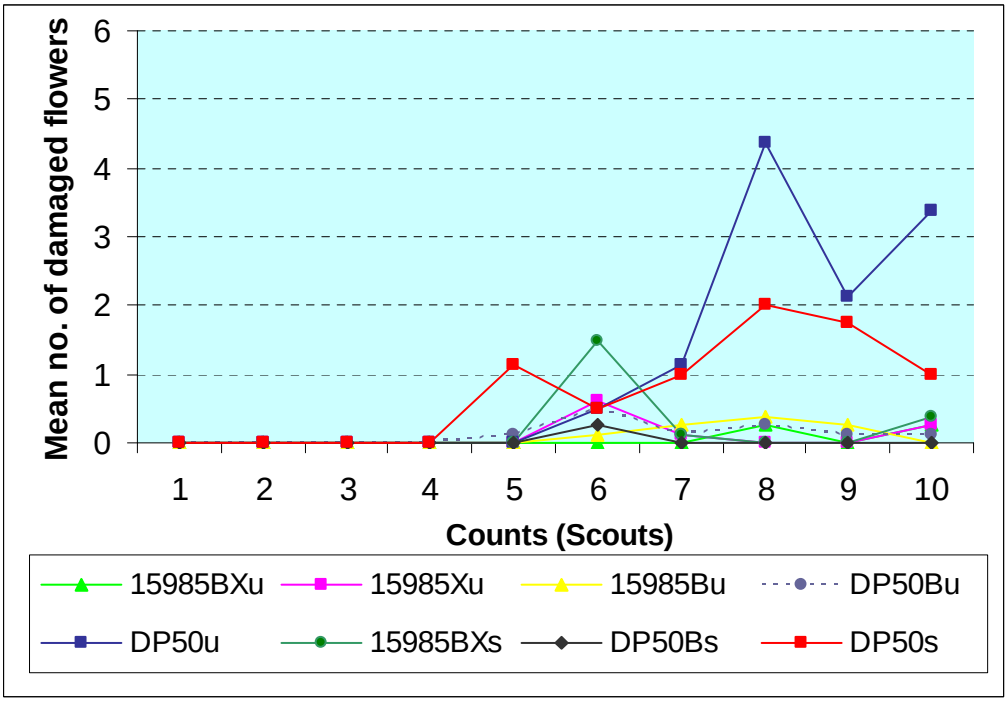


Fig. 2.82. The mean number of damaged flowers on 80 plants per treatment at Brits (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

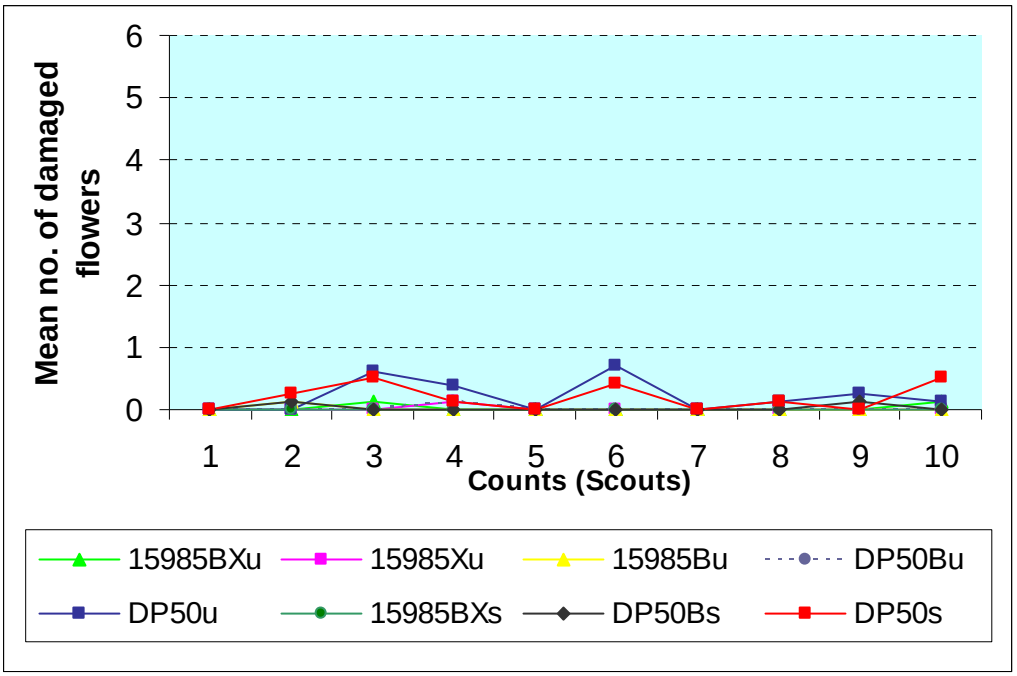


Fig. 2.83. The mean number of damaged flowers on 80 plants per treatment at Nelspruit in the stacked Cry1Ac and Cry2Ab2 trial.

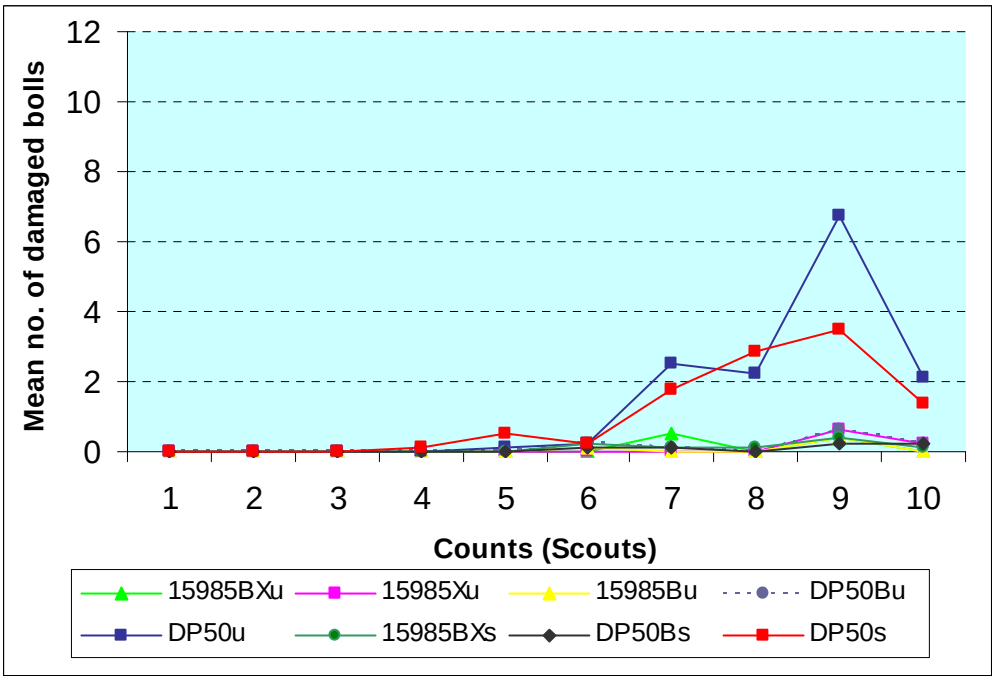


Fig. 2.84. Mean number of damaged bolls on 80 plants per treatment at Rustenburg (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

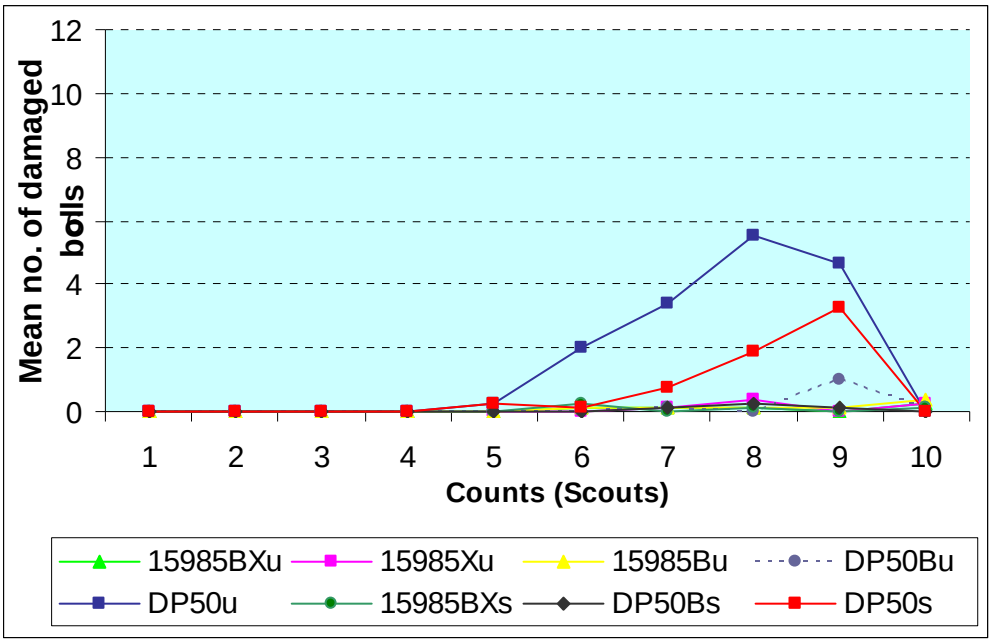


Fig. 2.85. Mean number of damaged bolls on 80 plants per treatment at Brits (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

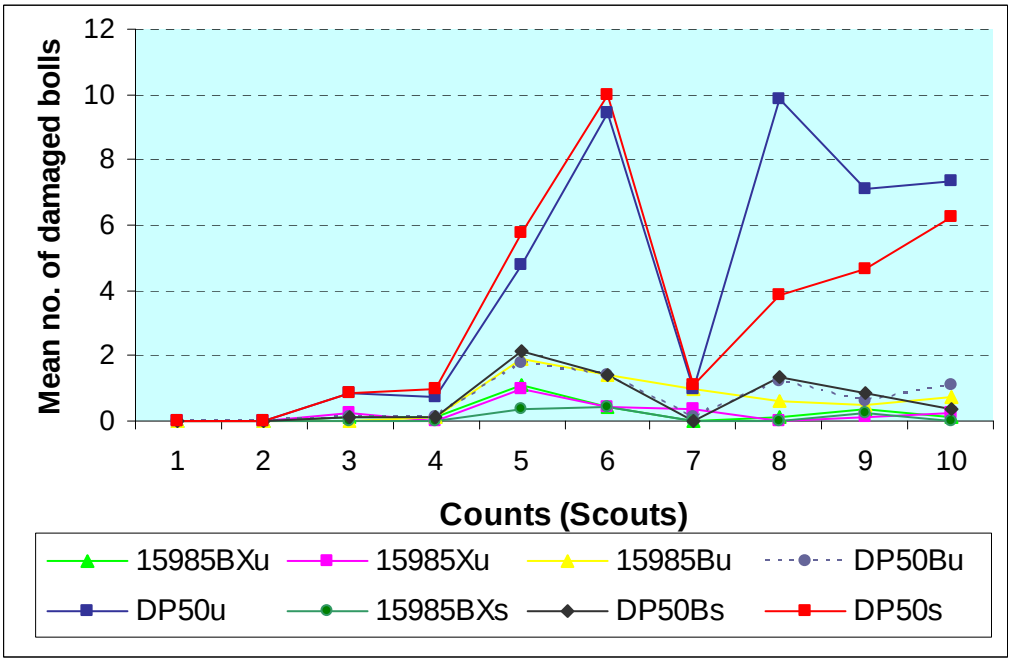


Fig. 2.86. Mean number of damaged bolls on 80 plants per treatment at Nelspruit (2000) in the stacked Cry1Ac and Cry2Ab2 trial.

CHAPTER 3

THE INCIDENCE OF SECONDARY PESTS ON GENETICALLY MODIFIED COTTON EXPRESSING BOLLWORM RESISTANCE BY MEANS OF AN INDEPENDENT GENE TRANSFER (*cry1Ac* and the *cry2Ab2* gene) AS WELL AS IN STACKED-GENE COMBINATION (*cry1Ac* with the Roundup Ready gene), WHILE TAKING INTO ACCOUNT THE GENERAL BIODIVERSITY THAT EXISTS ON A COTTON PLANT

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Abstract

No trend could be established on transgenic cotton (genetically modified) and non-transgenic cotton with regards to secondary pests and other arthropods observed. The occurrence of secondary pests on Bt-cotton seems to correlate with the spraying programme on a particular cotton type, and the particular population density of the pest or arthropod present during that particular time, when the observation was made. The *cry1Ac* gene did not have any direct effect in decreasing or attracting large numbers of secondary pests or other insect visitors.

The cotton treatments included a transgenic 15813 Insect Protected Cotton line (IPC line); a 15985 IPC line, the transgenic control variety DP 50B (Deltapine 50 with Bollgard™), and a non-transgenic reference variety, DP 50 (Deltapine 50). It seems that the transgenic treatment DP 50B appeared to have more plants with aphids in some instances in comparison with the other cotton treatments. There was no clear trend with regards to susceptibility of the cotton treatments to aphid infestation. The two transgenic cotton treatments 15985 and DP50B appeared to have more leafhoppers at certain time periods during the season on some of the localities, but no definite trend could be established. More whitefly infested plants appeared to be present in the 15985 line, followed by the DP 50B variety and the 15813 transgenic line, in comparison with the DP 50 treatment. The transgenic line 15985, followed by the DP50B treatment, appeared to have more thrips infested plants at certain time periods at some of the localities, but no continuous trend of thrips infested plants were observed on any of the cotton treatments throughout the season.

In the case where cotton which expresses both bollworm resistance and herbicide tolerance were compared during 2003/2004, scouting data showed that species diversity and richness occurring on GM cotton was similar to that found on conventional cotton, while a higher species diversity appeared to be present on Stack gene cotton when monitored by means of sweeping. The incidence of cotton pests coincided with the plant development stage of each variety. Pest management strategies, as performed by the grower, differed for each of the genetically modified (GM) cotton varieties (Stack gene, Bollgard™ and Roundup Ready™) and this had an effect on the pest and predator populations found during each count. The GM cotton varieties had no obvious negative environmental effect, when comparing the arthropod diversity within each field with uncontrolled conventional cotton. During the season of 2004/2005, the results of the previous year were statistically confirmed. Arthropod numbers (secondary insects and predators (see Chapter 3 and Chapter 4 respectively) were at least similar or in many instances higher in numbers than that which was found on conventional cotton and on Roundup Ready cotton. In the case of Roundup Ready cotton (DeltaOPAL RR), any lower numbers of arthropods at any specific time were the result of chemical application. During this study, bollworm resistant varieties required less spraying which enhanced arthropod numbers, or at least kept them approximately the same as DeltaOPAL (sprayed) and DeltaOPAL RR (sprayed). A large variety of arthropods was present on all the cotton types evaluated and no trend could be established that these arthropods are more common on a particular cotton type.

Results from the surveys on single gene and double gene traits are presented regarding species richness in Genetically Modified (GM) and non-GM (conventional) cotton in commercial cotton plantings in South Africa over two seasons. The genetically modified cultivar varieties used were NuOPAL (a single gene transformation expressing insect resistance), DeltaOPAL RR (single gene transformation expressing herbicide resistance) and NuOPAL RR (a double gene expressing both insecticidal resistance and herbicide tolerance). DeltaOPAL was used as the conventional non-GM cultivar. The studies were undertaken in the Groblersdal region in Mpumalanga Province and provided biodiversity data for GM crops which can be used as a reference tool by biosafety organizations and as a source of information for parties interested in the application of biotech crops in South Africa The first study was undertaken during 2003/04 where arthropod diversity was measured using both scouting and sweeping. The scouting results showed that species diversity and species richness on GM cotton was similar to that on conventional DeltaOPAL (non-GM) cotton. Data obtained through sweeping showed the highest species diversity in the NuOPAL RR cotton. Predators occurring on NuOPAL, DeltaOPAL RR (Roundup Ready™) and conventional DeltaOPAL cotton also occurred in NuOPAL RR cotton. The incidence of cotton pests coincided with the plant development stage of each variety. Pest management strategies performed by the grower differed for each of the cotton varieties (DeltaOPAL, NuOPAL RR, NuOPAL and DeltaOPAL RR) and this had an effect on the pest populations found during each count. The GM cotton varieties had no obvious negative environmental effect when comparing the arthropod diversity within each field with uncontrolled conventional cotton.

The second trial done the following season appeared to confirm these results. Arthropod numbers (excluding bollworms) were at least similar to, or, in many instances, higher than that which was found on conventional (DpAc 90) and DeltaOPAL RR cotton. The low numbers of arthropods found on DeltaOPAL RR is attributed to insecticide applications as the bollworm-resistant varieties required less corrective insecticidal spraying, which resulted in higher arthropod numbers, or at least similar to that on DeltaOPAL (sprayed) and DeltaOPAL RR (sprayed). A large variety of arthropods were present on all the cotton types evaluated and there was no evidence that the genetically modified cotton influenced biodiversity in any way. “Normal” pest management practices appear to play a bigger role in affecting biodiversity.

Introduction

It is generally known that a wide range of secondary pests occur on conventional cotton. The most important of these are aphids, leafhoppers, spider mites, stainers and to a lesser extent other sporadic pests like thrips, leaf miners etc. With the introduction of genetically altered cotton, and in specific Bt-cotton, the spraying regime has changed from applying several insecticide applications aimed at bollworms to one or two sprays, but more often than not, to no sprays at all. Secondary pests has generally not proved to be a main factor affecting yield on cotton in South Africa, for the past 5-10 years. However, with the changes that the introduction of Bt-cotton has brought about in the management of the pest complex, these secondary pests can appear to be more common in some years in some specific regions. The aim of this study was to demonstrate the occurrence of these pests during trial evaluations for bollworm efficacy. Thus, when a potential optimum bollworm control was achieved, by planting various genetically altered cotton types and spraying the treatments according to specific guidelines as stipulated in each trial. In each trial, the occurrence of these pests are compared to conventional cotton as a control, or to Bollgard™ cotton as a registered GM cotton type in South Africa. The occurrence of these pests on conventional cotton forms no specific pattern, although some authors have found hairy cotton, i.e. cotton leaves with an abundant number of trichomes, to be more resistant to leafhoppers, and smooth leaves to be less resistant to leafhoppers. The opposite was found to be true for the occurrence of whitefly on conventional cotton. During this study, results are presented as in incidence of these pests on the specific cotton types, and since no trend could often be established during the analyses of these results, statistical analysis has been found to be non-expressionistic to demonstrate differences between treatments. The fact of the matter is that the incidence of secondary pests seems to be not related to cotton type, but rather influenced by the specific spraying programme at any given time, the degree of predation by natural enemies, and the timing of the season, weather conditions and the presence of other alternative hosts for these pests.

The occurrence of natural pests will be demonstrated as they occur on genetically altered cotton with the *cry1Ac* gene, the *cry2Ab2* gene, and on cotton where the *cry1Ac* gene is used in stacked combination with a herbicide tolerant gene, the Roundup Ready gene. Three transgenic cotton types are currently registered for use in South Africa. Two contain single gene traits while the third is a double gene variety (Bennett et al. 2005). Cotton with single gene traits has been transformed through the introduction of genes expressing *in plant* proteins for either insect-resistance (Cotton line 531 – Cry1Ac protein) (Bollgard™ or NuOPAL) or herbicide-tolerance (Cotton line 1445 – CP4 EPSPS enzyme) (Roundup Ready™ or DeltaOPAL RR) (cf. Bennett et al. 2005). The expression of the insect-resistant protein has been shown to have specific activity on bollworm in the Order Lepidoptera. The herbicide-tolerant variety has no insecticidal activity but affords the plant tolerance to the herbicide, glyphosate. Cotton growers have benefited considerably from planting these genetically modified types. The herbicide-

tolerant "variety"[1] contributes to lowering production costs in combating weeds, particularly where cotton is produced under dryland conditions, whereas the bollworm resistant variety has reduced losses attributed to this pest, with a subsequent increase in yield. The double gene variety ("Stack Gene" or NuOPAL RR), produced by conventional cross breeding of the two single gene varieties, expresses both traits within the same plant, thus giving benefit of both insect-resistance and herbicide-tolerance to the farmer. The new Strategic Plan for South African agriculture, as set out in the Draft Vision, Mission and Strategy Declaration[2] of the South African Cotton Industry (Anon 2003), includes the training of farmers to make use of new technologies to increase cotton production (the National Cotton Strategy aims at increasing production to a total of 300 000 bales of lint by 2007). With the establishment of these genetically modified cotton types, one of the questions that arose was whether any of the genetically modified cotton has any impact on the biodiversity, with specific reference to secondary pests. In order to determine this, information regarding species diversity and richness within a commercial cotton field was a need to understanding species interaction is these crops. This data could then be compared to those obtained from GM modified cotton fields. The implications of Bt-cotton on non-target pests and beneficial insects have previously been documented (*cf*. Fitt and Wilson, 2000; Fitt and Wilson, 2002; Fitt, 2003). Transgenic cotton varieties have the potential to increase cotton production in South Africa but there is a need to demonstrate that these varieties are environmentally benign. Research is required in order to obtain a better understanding regarding the effects of GM technologies on the environment.

[1] "Variety" refers to the DeltaOPAL variety to which a single gene trait has been introduced, and not to a new variety as such.

[2] The Mission and Strategy Declaration of the SA Cotton Industry was formulated by a working-group/committee consisting of representatives of the industry and the Dept. of Agriculture, and it has been approved and supported by the government in principle.

Part I : The incidence of secondary pests on genetically modified cotton (Bt-gene), containing the Cry1Ac protein at three localities during the cotton season of 1997/1998

The aim was to compare the incidence of secondary pests on genetically modified cotton with the *cry1Ac* gene, under normal spraying regime.

EXPERIMENTAL PROCEDURES

Three field trials were planted at the Institute for Industrial Crops (IIC) (ARC) in Rustenburg, another at the IIC's Experimental Farm, Potgietersrus, while the third was planted at the Loskop Experimental Farm, Groblersdal. The field trial at Loskop was repeated during the season of 1998/99 to make sure of field data obtained during the 1997/98 season. Where data was reliable, it was included in this report for the Loskop locality. Soil composition ranged from a heavy clay soil, (turf) with a clay content of 58%, 14% silt and 28% sand in Rustenburg. In Potgietersrus the soil consisted of a sandy-clay soil with a clay content of 29%, 6% silt and 65% sand, while in Loskop, a sandy soil, with a clay content of 17%, 2% silt and 81% sand was prevalent. Four treatments with six replicates were laid out as a randomized block design, with each block representing a single replication for all localities. Each plot comprised 10 rows of 10 m each. Each plot was separated from adjacent plots by a 2 m pathway. The rows in the trial were planted 1.0 m apart and plant spacing within a row was 0.2 m. The treatments comprised of transgenic and non-transgenic varieties, either sprayed with insecticide for bollworm control or left unsprayed as indicated below. Total surface area = 74 m × 98 m. Treatments included a NuCtn 37B variety with Bollgard™ which was left unsprayed; a NuCtn 37B with Bollgard™ which was sprayed according to threshold; Deltapine Acala 90 variety (DpAC 90) which was left unsprayed, and a Deltapine Acala 90 (DpAC 90) variety, which was sprayed according to threshold. Similar planting procedures as described in Chapter 2, Part I, were followed for all three trials. Thioflo™ (endosulfan) was applied to the specified treatments above, when the egg threshold (12 eggs/24 plants) or larval threshold (5 larvae/24 plants) was reached or exceeded, whichever was attained first. The equivalent of Thioflo™ 2 l / ha spray mixture was applied at a pressure of 2.5 bar to all plants in the treatments sprayed according to threshold, by means of a CO_2 -driven knapsack sprayer fitted with a drop arm and six full-cone nozzles.

Assessments

Assessments included nine consecutive scouts starting at first square formation. Ten plants/plot were scouted for secondary pests (approximately 8 weeks after emergence) and thereafter, each week, for eight counts, up to approximately 16 weeks after emergence. Insecticide application dates and weather conditions are given in Tables 2.1 and 2.2 for Rustenburg and in Tables 2.3 and 2.4 for Potgietersrus (both listed in Chapter 2). Similarly, for Loskop, insecticide application dates and weather conditions are given in Table 2.5 (Chapter 2). In addition, the number of plants with other pest species was noted. These secondary pests included aphids, spider mites,

thrips, white fly, leafhoppers, cotton stainer bugs and spiders. For the Loskop locality, no statistical analyses were done on these variables as this particular data was regarded as an observation study. A summary of possible trends as observed from the line plots is given in the text.

The repeat of the field trial at the Loskop Experimental Farm, Groblersdal, followed the same design and management procedure as described in Chapter 2, Part II. The soil consisted of a sandy soil, with clay content of 17%, 2% silt and 81% sand. The same treatments were planted. Assessments were similar and the secondary pests included aphids, spider mites, thrips, white fly, leafhoppers and cotton stainer bugs. The number of cotton stainer bugs was also counted. Insecticide application dates and weather conditions are given in Table 3.1. Similarly, no statistical analyses were done on these variables as this particular data was regarded as an observation study. A summary of possible trends, as observed from the line plots, is given in the text.

RESULTS

Aphids

In Rustenburg, the number of plants with aphids was higher on the Bollgard™ (NuOPAL) sprayed treatment than on any of the other treatments, for 5 out of the 9 observations made. On 6 occasions, the Bollgard™ unsprayed treatment had more aphids than either of the DpAC 90 treatments during the observation period. The number of plants with aphids on the Bollgard unsprayed treatment was generally lower than the number of plants with aphids on the Bollgard sprayed treatment (Fig. 3.1), if taken into account that spraying commenced only on 10/02/98.

In Potgietersrus, on 5 occasions, the Bollgard™ unsprayed treatment had more plants with aphids than on the DpAC 90 treatments. Spraying commenced on 2/02/98. The Bollgard™ sprayed treatment had on 4 occasions after spraying commenced, more plants with aphids than the DpAC 90 sprayed treatment. The differences between treatments were not as clear as on the Rustenburg locality, and the DpAC 90 unsprayed treatment seemed to perform better than the DpAC 90 sprayed treatment, except for the first two counts (Fig. 3.2).

In Loskop 1998, only three reliable counts were made during mid-season. The number of plants with aphids at the second count on the Bollgard™ treatments was lower than at the other two localities. The number of plants with aphids was highest after 3 sprays on the Bollgard unsprayed treatment, but decreased after the following insecticide application, in comparison with the other treatments. The DpAC 90 sprayed treatment had least number of plants with aphids after 4 sprays, but had similar number of plants with aphids than the control treatment thereafter (Fig. 3.3). All the DpAC 90 treatments had a similar number of plants with aphids at the other localities (Figs 3.1 and 3.2).

In the Loskop 1999 trial, the number of plants infested with aphids declined sharply between the 1st and 2nd count on all the treatments. Spraying for bollworms during the first 3 counts did not seem to have a suppressing effect on the sprayed treatments, when compared to the unsprayed treatments. A decline in the number of aphid infested plants after the

pymetrozine spray was observed on all treatments but not for the monocrotophos spray (Fig. 3.7). All plots were sprayed with these two insecticides. The spraying of endosulfan for bollworms did not seem to affect the aphid population. After the application of endosulfan, the number of aphid-infested plants either increased or stayed more or less the same in the sprayed treatments. No trend could be observed for any of the treatments, but the DpAC 90 sprayed treatment seemed to have less infested plants for the 5th, 6th and 8th count.

Leafhoppers ("jassids")

In Rustenburg, the Bollgard™ treatments generally had a lower number of leafhoppers throughout the observation period than the DpAC 90 unsprayed treatment. However, the Bollgard™ unsprayed treatment had more leafhoppers than the sprayed Bollgard™ treatment and the DpAC 90 sprayed treatment in at least 6 of the 9 counts made (Fig. 3.4). Fewer plants with leafhoppers were noticed at the Rustenburg locality than at any of the other two localities.

In Potgietersrus, the number of plants in general, with leafhoppers was higher on the DpAC 90 sprayed plots, except for the second and the fourth count (Fig. 3.5).

The Bollgard™ sprayed treatment had most plants with leafhoppers during the first two counts, but this decreased at the end of the observation period (Fig. 3.5).

In Loskop, the population density of leafhoppers in Loskop was much higher than at the other two localities throughout the season. This could also have been a reason for the lower yields obtained at this locality (see Chapter 5). A lower numbers of plants were observed during 24/02/98 in the Bollgard™ unsprayed treatment, but they had increased markedly by the following week in all the other treatments, leafhopper numbers were somewhat higher on the Bollgard™ sprayed treatment (24/02/98) (Fig. 3.5). Plant numbers with leafhoppers were somewhat higher in Loskop than in Potgietersrus, for this time period.

The number of leafhopper infested plants in the 1999 Loskop trial declined after the spraying of pymetrozine (Fig. 3.8). By the third count, the number of leafhopper infested plants increased on the unsprayed treatments up to the 6th count. On the sprayed treatments, leafhopper numbers declined after the 3rd endosulfan spray, to increase again after the 5th count. A sharp decline in infested plants was observed after the application of monocrotophos by the 6th week, up to the 8th week, where after leafhopper infested plants increased again from the 9th count onwards. No differences could be observed between the Bollgard™ and the DpAC 90 treatments with regards to the number of leafhopper-infested plants.

Mites

In Rustenburg, the number of plants with mites on the Bollgard™ unsprayed treatment reached similar levels on a few occasions than the DpAC 90 unsprayed treatment (Fig. 3.9). The Bollgard™ sprayed treatment seems to be the most affected by spider mites.

In Potgietersrus, the DpAC 90 sprayed treatment had most plants with mites at the beginning of the observation period. This decreased, however, towards the end of the observation period (Fig. 3.10). The Bollgard™ sprayed treatment had more or a similar number of plants with mites on most occasions than the Bollgard unsprayed treatment. At the end of the

observation period, the Bollgard treatments seemed to be most affected by spider mites. However, this somewhat corresponded to the susceptibility of the DpAC 90 sprayed treatment throughout the season, in that the DpAC 90 sprayed treatment were least susceptible to spider mite infestation, and on the same occasions, either or both of the Bollgard™ treatments showed a higher number of plants with mites.

The data from Loskop was incomplete. Treatments had very similar numbers of plants with mites during the 3 weeks that counts were made (Fig. 3.11). However, a higher frequency of plants with mites occurred at this locality. This is often observed when a high leafhopper density is present. The attack on cotton plants by leafhoppers seems to make them more vulnerable to spider mite attack.

Whitefly

In Rustenburg, no real trend could be established between treatments by counting the number of plants with whitefly. On some occasions, the Bollgard™ treatments had a higher number of plants with whitefly, while on other occasions the DpAC 90 treatments had higher numbers of plants with whitefly (Fig. 3.12).

At Potgietersrus, the DpAC 90 unsprayed treatments had the least number of plants with whitefly, while the Bollgard™ treatments and the DpAC 90 sprayed treatment showed similar results (Fig. 3.13).

No significant presence of whitefly or spidermite was noted during the performance of the trial in Loskop 1999.

Thrips

In Rustenburg, most plants with thrips were found amongst the Bollgard™ unsprayed treatment throughout the season in Rustenburg (Fig. 3.14)).

In Potgietersrus, all treatments in Potgietersrus gave varied control of resistance to thrips (Fig. 3.15). No trend could be established amongst any of the treatments.

All treatments in Loskop, showed varied susceptibility to thrip infestation (Fig. 3.16). At the 3rd count however, the Bollgard™ unsprayed treatment had most plants with thrips. The occurrence of thrips were monitored by Eulitz (2003), on different cultivars, amongst others the genetically modified variety, NuCOTN 37B. Eulitz (2003) found that thrips in South Africa has no preference for any cotton cultivar and differences found between cultivars, whereof NuCOTN 37B was one of the cultivars; the differences were not consistent for any cultivar. No other reference to secondary plant infestation by thrips on GM cotton in South Africa has been dated yet.

Cotton leafbeetles (<u>Nisotra</u> spp.)

In Rustenburg, no significant trend could be established between treatments from Rustenburg. The Bollgard unsprayed treatment had the highest numbers of beetles at the end of the observation period (Fig. 3.17).

Cotton stainer bugs (Dysdercus spp.)

No cotton stainer bugs were observed in Rustenburg in any of the treatments until about 8 weeks after square formation (Fig. 3.18). The Bollgard™ unsprayed cotton showed the highest number of cotton stainer bugs at the end of the observation period in Rustenburg.

Similarly, in Potgietersrus, the cotton stainer bugs were only found at about the 7th count. The DpAC 90 sprayed treatment showed the highest number of cotton stainer bugs (Fig. 3.19). Cotton stainer bugs varied between treatments, and no tendency for the presence of these bugs could be observed on any of the treatments in Loskop in 1999 (Fig. 3.20).

CONCLUSION

Aphid populations may increase on unsprayed Bollgard™ cotton. This was noted for the Rustenburg locality and seemed a possibility for the Potgietersrus locality. Less leafhopper was found on Bollgard cotton in Rustenburg and Potgietersrus than on DPAC 90 cotton. Leafhopper infestation levels were higher in Loskop than in Rustenburg or Potgietersrus. For the Rustenburg locality, a high number of plants with thrips were observed on the Bollgard™ unsprayed treatment in comparison with other treatments. No trend was observed for spider mites between treatments. Spider mites tend to increase on sprayed Bollgard™ cotton. In some instances, it was obvious that spraying increased spider mite numbers on either DpAC 90 or Bollgard™ cotton. No trend was observed for aphid, or leafhopper infested plants between treatments in the Loskop 1999 trial.

Part II : The incidence of secondary pests on transgenic cotton with the P2B protein, namely P2B events, expressing an indeterminate degree of bollworm resistance

The aim of this study was to determine the incidence of secondary pests on insect resistant cotton lines containing a gene expressing the “P2B” Bt-protein at Rustenburg, SA.

EXPERIMENTAL LAYOUT AND DESIGN

The surface area of the trial consisted of 0.1184 ha (0.25 acre). A field trial was planted at the Institute for Industrial Crops (IIC-ARC) in Rustenburg, South Africa. The trial was planted on a heavy turf soil with 50 % clay, 42 % sand and 8 % silt. The pH of the soil was between 7.33 and 7.54 at a depth of 30 - 60 cm. A single transgenic P2B event represented one plot. Plot layout was similar to that described in Chapter 2, Part II. They included 33 transgenic P2B events, as listed in Chapter 2, Part II (under treatments). The 6 check plots (C1-C6) included 4 transgenic varieties: DP50B (C2), C312-531 (C4), 15813 (C5) and 15985 (C6), and 2 non-transgenic varieties: DP50 (C1) and C312 (C3). A single insecticide application was carried out to control leafhoppers and cotton stainers on all plots at the end of the season. Spraying was carried out after discussion with the Project Coordinator. No spraying was performed at any stage for lepidopteran pests. The insecticides monocrotophos (Monostem®, 500 ml/ha) and deltamethrin (Decis®, 170 ml/ha) were applied by means of a CO_2 -driven knapsack sprayer fitted with a drop arm and six nozzles. All plots were managed like conventional cotton with respect to fertilization (see Chapter 2, Part II) water, and control of insect pests, with the exception of lepidopteran species. A 50 ml overhead irrigation was applied to the trial during the week of the 22/02/99, and another 50 ml approximately one month later. No weed control was carried out before planting or at any time during the season. Fertilization was applied on 02/11/98 150 kg/ha K_2SO_4; 12/01/99 150 kg/ha LAN and on 22/02/99 150kg /ha LAN.

Assessments

Emergence stand counts for each plot were taken at seven and 14 days after planting. Cotyledons that had completely cleared the soil were considered “emerged”. Individual plants were tested for the gene of interest via lateral flow strips at the four-leaf stage. Monsanto provided the kit and assisted with the procedures. Leaf samples of each individual plant per event were shipped back to Monsanto for gene analysis, on two occasions during the season. Results of the gene-detected positive plants are listed in the Annexure. The number of fully cracked bolls per transgenic plant/event was calculated over five time periods. Natural infestations of pests were relied upon. Ten randomly selected plants per plot, of which the plant numbers were noted, were evaluated weekly, for eight consecutive weeks, until approximately 16 weeks after emergence. The following insects were noted: aphid and leafhoppers and the number of plants per event on which they occurred was noted, and not the actual numbers. In

the case of leaf miners, the number of plants with leaf damage per event was compared. All other insects were individually noted and are represented in a table for comparisons between events. No statistical analyses were performed on the data since no plots (treatments) were repeated throughout the trial. Seed was obtained from Monsanto was just enough to plant a single row of each event. Graphical representation of the data is, however, given, using Excel, Microsoft for Windows. Important dates are listed in Chapter 2, Part II.

RESULTS

Leafhoppers (Cicadellidae)

Leafhoppers occurred on most events throughout the season. No leafhoppers were, however, noted on events 6491 (plot 17), 6725 (plot 23), 6733 (plot 24) and the check plot 16985 (C6) (Fig. 3.21). Unlike localities such as Groblersdal (Mpumalanga Province) and the Makhathini experimental farm on the Makhathini Flats (KwaZulu-Natal), the Rustenburg locality does not represent a high infestation of leafhoppers. To determine resistance to leafhoppers, the counts should be taken on localities where the pest reaches high population densities.

Aphids (Aphididae)

Aphids are a large problem in some cotton regions, such as KwaZulu-Natal and the Northern Province. When comparing the number of plants with aphids per event (Fig. 3.22), the highest mean (n=8, where n = no. of plants with aphids) was observed during the 3rd week for event 6241 (plot 9). Other events that had high numbers of aphids were 6489 (plot 16) during week 2, and 6532 (plot 19) during the first week. No real trend could be established throughout the season. All events and check plots had aphids present at some or all of the stages during the observation period. Event 6491 (plot 17) had the lowest mean number of plants with aphids during the observation period (Fig. 3.23).

Leaf miners (Agromyzidae)

Leaf miners are only a sporadic pest in the cotton regions of South Africa. However, in some seasons, some regions experience a higher pest population density than other regions. Normally, no chemical control is necessary to control this pest. The past season had a particularly high population of leaf miners. Events 6239 (plot 8) and 6460 (plot 13), had the highest mean number of plants with leaf miner damage (Fig. 3.24). Events 6151 (plot 2), 6483 (plot 14) and 17079 (plot 28) also had means of higher than 0.2 plants with leaf miner damage. Of all the checks, 15813 (C5) had the highest damage, whereas 15985 (C6) had the least leaf miner damage. Events 6351 (plot 10), 17180 (plot 30) and 15830-5 (plot 33) had a low number of plants with leaf miner damage.

Other insects

Other insects that occurred on the transgenic events and check plots are listed in Tables 3.1 and 3.2. The factor/s that influence the occurrence of these insects on specific cotton varieties or

breeding lines is unknown. In the Rustenburg area, the cotton leaf beetles (Nisotra spp., Chrysomelidae), have been observed during the last few seasons. These insects feed on the outer leaves of the squares and on the leaves. When they occur in large numbers, they can affect the development of squares, but they rarely infest the cotton to such an extent that chemical control is necessary. High numbers of Nisotra beetles were found on most events (Table 3.1). Nisotra beetles have a patchy distribution in the field. The positive characteristics of each line observed throughout the study are presented in a score chart (Tables 3.3 and 3.4).

CONCLUSION

The appearance of secondary pests on conventional and genetically modified cotton types does not seem to follow any particular pattern, but do append on the population density of the pest at one particular time at one particular place. No differences between cotton types and conventional cotton varieties were observed for secondary insects and other occasional arthropod visitors. When expression of the Bt-gene is compared with the occurrence of these pests on genetically modified cotton, it does not seem to influence their acceptance as a host plant.

Part III : Evaluation of two IPC (Insect Protected Cotton) lines (15813, 15985), a control (DP 50B) and a reference line (DP 50), potentially bollworm resistant lines, for the occurrence of secondary pests in South Africa

The aim was to investigate the occurrence of secondary pests while determining the efficacy of the Cry2Ab2 protein against bollworms.

EXPERIMENTAL PROCEDURES

Four field trials were carried out during the 1998/99 season in South Africa, at the following sites: Rustenburg (Tobacco and Cotton Research Institute (TCRI), North West Province, 25° 43'S; 27°18'E; 1157m altitude); Brits (TCRI Experimental Farm, North West Province, 25° 31'S; 27°49'E, 1109m altitude); Potgietersrus (TCRI Expe rimental Farm, Northern Province; 24° 11'S 29° 01'E, 1116m altitude); Friedenheim (Nelspruit) (TCRI Experimental Farm, Mpumalanga Province; 25° 27'S; 30° 58'E). Treatments included a 15813 Insect Protected Cotton line (IPC line); 15985 IPC line; DP 50B (Deltapine 50 with Bollgard$^{TM)}$, DP 50 (Deltapine 50) (non transgenic control). The trial design was similar to that described in Chapter 2, Part IV.

Assessments

The conventional method of land preparation, trial management with regards to planting, irrigation and fertilize, thinning etc. was followed as described in Chapter 2, Part IV. Weather conditions applicable to insect data collection and insecticide applications are given for Rustenburg (Table 2.31, Chapter 2), Brits (Table 2.32, Chapter 2), Nelspruit (Table 2.33, Chapter 2) and for Potgietersrus (Table 2.34, Chapter 2) respectively. Thioflo® (endosulfan) was applied at egg threshold (40 eggs/80 plants) or larval threshold (16.6 larvae/80 plants). The threshold was applied to the number of plants scouted per plot (20) for four repetitions. The equivalent of endosulfan (Thioflo®) 1 l / ha spray mixture (2.5 bar) -CO_2 -driven knapsack sprayer fitted with a drop arm and six full-cone nozzles. Assessments included a total number of seven consecutive scouts at Rustenburg, Brits and Potgietersrus, starting at two weeks after planting. No insects were recorded for the first count in Rustenburg, therefore only six datasheets are included in the Rustenburg field notebook. In Friedenheim, scouting started at six weeks after planting. Scouting commenced at first square formation on each locality. Twenty plants/plot were scouted for the various bollworm species, and in addition to that the number of plants with other pest species was noted from two weeks after emergence. These secondary pests included aphids, spider mites, thrips, whiteflies, leafhoppers, cotton beetles (Nisotra spp., Chrysomelidae) and cotton stainer bugs. The number of beneficial insects, e.g. ladybirds (adults, larvae and eggs), lacewings (larvae, eggs) and spiders was also calculated. The number of cotton stainer bugs was only counted in the Rustenburg locality. For the Loskop locality, only the data concerning aphids, thrips, spider mites as well as leafhoppers were used in this report. For the comparison of damage to squares, flowers and bolls, line plots were used for all relevant

variables. No statistical analyses were done on insects other than bollworms, since this data is affected by insecticide applications. Comparison of other insects on transgenic cotton should be determined in trials where the spraying programme is similar on all cotton treatments and on all localities, or when no spraying is applied. By comparing this data statistically it might create a wrong interpretation of trends of other insects on transgenic cotton. However, the data presented do give an indication of the secondary pests present.

Dual® and Cotogard® were applied, with irrigation 20 mm, followed by thinning one week later. Important dates are given in Chapter 2, Part IV. Normal insect control was carried out as stipulated in Chapter 2, Part I (rate, and reason for application). All control, reference and IPC (Insect Protected Cotton lines) plots were sprayed for lepidopteran insect control, when necessary. The equivalent of Thioflo®, 1 l / ha spray mixture was applied at a pressure of 2.5 bar to all plants in the cotton treatments sprayed according to threshold, by means of a CO_2 -driven knapsack sprayer fitted with a drop arm and six full-cone nozzles. All plots were managed the same way as cotton that is commercially produced, with respect to fertilization, irrigation and weed control. Similar planting procedures as described in Chapter 2, Part I, were followed. After planting, all plots were monitored throughout the season by recording plant growth characteristics, as well as insects and diseases every two weeks. Insects scouted for included pests, e.g. bollworm, aphids, leaf-miners, leafhoppers, thrips, red spider mite, whiteflies and Nisotra beetles. Several statistical tests or analyses were tested for "fit" and effectiveness for the insect data.

An analysis of variance for a randomized block design, nested over localities was performed, since the importance of comparing the effect of transgenic material on bollworms across localities (pers comm. K. Hamilton[3]) was chosen to be the important factor in comparing the data. Since sites were taken into account and replications are required to perform an analysis of variance, the effect of time had to be ignored. An assumption of homogeneous variances and normally distributed residuals was made. Bonferroni LSD-tests were conducted on the transformed means, but conclusions reached concerning the differences between means apply directly to the untransformed mean counts.

RESULTS

Aphids

At Rustenburg, the number of plants with aphids varied at the time intervals. A varying number of aphid-infested plants were observed from the 3rd to the 7th count on the 15985 transgenic line (Fig. 3.25). The number of infested plants varied more on the DP 50B treatment and the 15985 line, than on the other two cotton treatments over the observation period (Fig. 3.25). However, there was no clear trend as to any preferences for a particular line or cultivar. The 15813 transgenic line and the DP 50 non-transgenic treatment had a more or less similar number of plants with aphids throughout the season (Fig. 3.26). The number of plants infested with aphids

[3] K. Hamilton, was the project leader, and a researcher for Monsanto St. Louis, USA.

was the lowest on the DP 50B treatment, except for the last two counts, while the transgenic line 15985 gave the highest number of plants with aphids during the 5th count. Overall, it seems as if the DP 50B treatment was less infested with aphids.

The number of plants with aphids was slightly lower at Nelspruit than in the other localities (Fig. 3.27). The 15813 line had a slightly higher number of aphids than the other three cotton treatments during the 2nd count. All four cotton treatments had, however, a similar number of aphids present throughout the season (Fig. 3.27), with the aphid population decreasing during mid-season, and increasing again at the end of the season. Only at the 7th count, did DP 50 and 15985 have somewhat more aphids than the other cotton treatments.

In Potgietersrus, the non-transgenic variety, DP 50, had a similar number or fewer numbers of plants infested with aphids, except for the 4th count (Fig. 3.28). The DP 50B treatment had more plants with aphids, or a similar number of infested plants than other cotton treatments at all time except the 3rd and 4th counts. All four cotton treatments did, however, show very similar trends with regards to aphid numbers through out the season.

Overall, it seems as if the aphid populations reached higher levels in Potgietersrus than at the other three localities. It seems that the transgenic treatment DP 50B appeared to have more plants with aphids on at least one locality in comparison with the other cotton treatments. There was no clear trend with regards to susceptibility of the cotton treatments to aphid infestation.

Leafhoppers ("jassids")

At Rustenburg, the transgenic line 15985 and the DP 50B variety had more plants with leafhoppers during the first part of the season, while DP 50 had more infested plants than the other cotton treatments later in the season (from the 5th count onwards) (Fig. 3.29). Although spraying with endosulfan was carried out on 3 occasions during the season, the tendency in all cotton treatments were the same, except for the DP 50B treatment that showed a higher increase in the number of plants infested with leafhoppers after the 6th count (Fig. 3.29).

In Brits, the transgenic line 15985 had an equal, or a higher number of plants infested with leafhoppers than the other cotton treatments throughout the season, except for the 7th count (Fig. 3.30). The leafhopper infestation declined after the 5th count on all cotton treatments, but increased again on the 15813 line and on the DP 50 variety at the 7th count. An application of endosulfan was carried out just after the 6th count, which possibly contributed to the decline in infested plants on the 15985 and DP 50B cotton treatments. However, the increase in infested plants on the DP 50 and 15813 cotton treatments after the 6th count was unexpected. DP 50B had fewer plants infested with leafhoppers than in comparison with the 15985 line throughout the season. Both the 15985 and the DP 50B cotton treatments showed a high number of infested plants at the 5th and 6th count, before the application of any insecticides. DP 50B seems to be less susceptible than 15985 to leafhopper infestation on this locality. The DP 50 treatment had fewer plants with leafhoppers than DP 50B at the 5th and 6th counts. The variation in dispersal of a leafhopper population on sprayed cotton treatments should be studied further.

In Nelspruit, a higher number of plants infested with leafhoppers were noted on the DP 50B treatment than on the 15985 line, except for the 1st count. All cotton treatments followed a similar trend concerning the variation in the number of infested plants, after the application of insecticides (Fig. 3.31). However, the 15985 line, had the least number of infested plants from the 3rd count onwards, opposite to that what was found at the Brits locality. Although leafhoppers were sprayed on the Nelspruit locality during the season, and at a later stage during the season at Brits, the number of infested plants on the 15985 line was lowest in comparison with the other cotton treatments (Figs 3.31 and 3.32;). It seems that a cultivar effect on a specific locality could contribute to the dispersal of the pest species in a field.

In Potgietersrus, the DP 50 treatment had fewer plants with leafhoppers than the 15985 line and the DP 50B treatment, at the 5th and 6th count. However, a similar number of infested plants were noted on the DP 50 treatment and the 15985 line at the 7th count, to reach high levels at the end of the season (Fig. 3.32). Fewer numbers of plants was infested for all cotton treatments in Nelspruit during the 7th count, than in comparison with Potgietersrus. The higher number of infested plants on all cotton treatments in Potgietersrus could be explained by the fact that insecticide applications of pymetrozine and abamectin was applied only after the 7th count was made, while insecticides in Nelspruit were applied before the 6th and 7th. (see Tables 3.5, 3.6, 3.7 and 3.8). The 3 transgenic cotton treatments had more leafhopper infested plants during the 6th count in Brits before any insecticide application, while the same was true for the Potgietersrus locality at the 6th count. Before spraying, the DP 50B treatment, as well as the 15985 line appeared to have more leafhopper infested plants during the 5th count at Brits and Potgietersrus than in comparison with the other two cotton treatments. These results might suggest higher susceptibility in DP 50B and 15985 than in the other cotton treatments at Potgietersrus and Brits, but further research is necessary to determine which factors influences dispersal of the pest and why the opposite happened on the Nelspruit locality.

Whitefly

At Rustenburg, the transgenic variety DP 50B had most whitefly at the 3rd count, while the 15985 line had most whitefly at the 4th, 5th and 6th counts. The number of plants infested with whitefly increased at the end of the season (7th count) on the DP 50 treatment (Fig. 3.33).

In Brits, the DP 50 treatment had fewer plants with whitefly from the 4th count onwards, in comparison with the 15985 line and with DP 50B (Fig. 3.34). The DP 50B variety had more whitefly-infested plants than the DP 50 non transgenic treatment, on the 5th, 6th and 7th count. The number of plants infested with whitefly increase only from the 3rd count onwards on all cotton treatments.

In Nelspruit, the number of plants with whitefly was similar on most cotton treatments for most of the counts made during the observation period, except for the 3rd count where the 3 transgenic cotton treatments showed a higher number of infested plants than the DP 50 treatment (Fig. 3.35).

In Potgietersrus, very few whiteflies were observed during the first five counts (Fig. 3.36), while at the end of the season, the 15985 line had the highest number of plants with whitefly at

the 6th count. The numbers of infested plants were higher on the 15985 and 15813 lines at count 7 than in comparison with the other cotton treatments. The least number of infested plants was observed on the DP 50 treatment at the last two counts. Overall, more whitefly infested plants were observed on the 15985 line, followed by the DP 50B variety and the 15813 transgenic line, in comparison with the DP 50 treatment.

Thrips

In Rustenburg (Fig. 3.37), the 15985 line had a higher number of thrips-infested plants only during the 5th count, while the DP 50B treatment had more thrips-infested plants at the 7th count.

No thrips were observed on any of the plants in Nelspruit. In Brits, the predominantly susceptible treatment seemed to be the transgenic line 15985, followed by the DP 50B treatment (Fig. 3.38), from the 4th to the 7th count.

At Potgietersrus, most plants with thrips were found on the 15985 line at the 6th count, while the number of infested plants at the 7th count were similar on the 15985 and 15813 lines and higher than on the other two cotton treatments (Fig. 3.39).

CONCLUSION

Results with aphids varied and there is no clear distinction between cotton treatments for aphid numbers, but it seemed as if the DP 50B and the transgenic line 15985 tended to have more aphid infested plants than the other transgenic line 15813. Results with leafhopper infested plants varied and there is not clear distinction between cotton treatments for leafhopper presence. The 15985 line may be more susceptible to whitefly infestation, than the other transgenic cotton treatments. Whenever thrips occurred, the highest number of plants infested with thrips was found amongst the transgenic line 15985, followed by the DP 50B cultivar, than in comparison with the other cotton treatments. El-Wakeil et al. (2003) found flea beetles to be significantly higher between Bt- and non-Bt-cotton before jasmonate applications and in overall densities of pests. Pyke (2003) mentioned that minor pests such as jassids and green vegetable stink bug required more sprays on GM-cotton (INGARD™) in Australia than on conventional cotton, particularly in areas where IPM practices had reduced spraying for other pests to very low levels. Very few references are made to the occurrence of secondary pests on Bt-cotton, and when such a reference is made, it fails to confirm the increase of these pests on Bt-cotton, or else the reasons why differences are found at certain plant stages during the season. The effect of spraying and the particular IPM programme followed on GM- cotton can however, appear to have an increasing effect of secondary pests on Bt-cotton in the lower incidence of spraying, probably by the lesser effect of the pesticides that would have been applied for bollworm control under non-GM cotton, since many of these pesticides in South Africa, e.g. endosulfan is also registered for some of the secondary pests.

Part IV : Arthropod biodiversity on transgenic cotton in single gene traits, expressing either herbicide tolerance or bollworm resistance (*cry1Ac*), and in a double gene trait, expressing both herbicide tolerance and bollworm resistance in South Africa

A recent South African study was carried out on the presence of bollworms in Bt (NuOPAL) and non-Bt-cotton (conventional) by Mellett et al. (2004), but did not include herbicide-tolerant nor double gene varieties. The aim of this study was to evaluate arthropod biodiversity within GM and non-GM cotton fields, containing single and double gene traits, over two cotton-growing seasons (2003/04 and 2004/05). The occurrence of secondary pests and beneficial insects was evaluated after normal spray programmes aimed at controlling bollworm populations and were compared to those populations found in unsprayed fields. In the case of insect-resistant cotton, the absence of spraying could lead to an increase in secondary pest numbers as these were often incidentally controlled. Insect populations (whether primary pests, secondary pests or non-target insects) may be affected by the repetitive spraying of an insecticide.

This study was aimed at establishing the baseline for insect biodiversity in genetically altered cotton fields in South Africa. The purpose was to collect data on the population levels of arthropod species during the fruiting phase of cotton on a commercial cotton farm, under common management practices for each cotton type (conventional (cultivar DeltaOPAL), Bollgard™ (cultivar NuOPAL), Roundup Ready (cultivar DeltaOPAL RR) and Stack gene (cultivar NuOPAL RR)). This data could be used to determine the possible impact of the cultivation of single gene and double gene traits on arthropod diversity, and to provide a set of data for decision makers interested in developing biosafety regulations for transgenic cotton.

INTRODUCTION

Three transgenic cotton types are currently registered for use in South Africa. Two contain single gene traits while the third is a double gene variety (Bennett-Nel et al. 2005). Cotton with single gene traits has been transformed through the introduction of genes expressing *in plant* proteins for either insect-resistance (Cotton line 531-Cry1Ac protein; Bollgard® or NuOPAL) or herbicide-tolerance (Cotton line 1445-CP4 EPSPS enzyme; Roundup Ready® or DeltaOPAL RR) (Bennett-Nel et al. 2005). The expression of the insect-resistant protein has been shown to have specific activity on bollworms in the Order Lepidoptera. The herbicide-tolerant variety has no insecticidal activity but affords the plant tolerance to the herbicide, glyphosate. Cotton growers have benefited considerably from planting these genetically modified types. The herbicide-tolerant 'variety' (refers to the DeltaOPAL variety to which a single gene trait has been introduced, and not to a new variety as such) contributes to lowering production costs in combating weeds, particularly where cotton is produced under dryland conditions, whereas the bollworm resistant variety has reduced losses attributed to this pest, with a subsequent increase in yield. The double gene variety (Stack Gene or NuOPAL RR), produced by conventional cross

breeding of the two single gene varieties, expresses both traits within the same plant, thus giving benefit of both insect-resistance and herbicide-tolerance to the farmer.

The new Strategic Plan for South African agriculture, as set out in the Draft Vision, Mission and Strategy Declaration of the South African Cotton Industry (Anon 2003), includes the training of farmers to make use of new technologies to increase cotton production. The National Cotton Strategy aims at increasing production to a total of 300'000 bales of lint by 2007. Transgenic cotton varieties have the potential to increase cotton production in South Africa but there is a need to demonstrate that these varieties are environmentally benign. Research is required in order to obtain a better understanding of the effects of GM technologies on the environment.

One of the questions that arose was whether any of the genetically modified varieties have any impact on the biodiversity. In order to determine this, information regarding species diversity and richness within a commercial cotton field was needed to understand species interactions within these crops. This data could then be compared to those obtained from transgenic (also referred to as genetically altered or modified) cotton fields. The implications of Bt-cotton on non-target pests and beneficial insects have previously been documented (Fitt and Wilson, 2000; Fitt and Wilson, 2002; Fitt, 2003). A recent South African study was carried out on the presence of bollworms in Bt (NuOPAL) and non-Bt-cotton (conventional) by Mellett et al. (2004), but did not include herbicide-tolerant nor double gene varieties.

Trials were undertaken to obtain data on arthropod biodiversity within GM and non-GM cotton fields over two cotton-growing seasons (2003/04 and 2004/05). The occurrence of secondary pests and beneficial insects was evaluated after normal spray programmes aimed at controlling bollworm populations and were compared to those populations found in unsprayed fields. In the case of insect-resistant cotton, the absence of spraying could lead to an increase in secondary pest numbers as these were often incidentally controlled. Insect populations (whether primary pests, secondary pests or non-target insects) may be affected by the repetitive spraying of an insecticide.

These studies aimed to establish the baseline for insect biodiversity in genetically altered cotton fields in South Africa. The purpose was to collect data on the population levels of arthropod species during the fruiting phase of cotton on a commercial cotton farm, under common management practices for each cotton type, conventional cotton (cultivar, DeltaOPAL), single traits e.g. Bollgard® (cultivar, NuOPAL) and Roundup Ready® (cultivar, DeltaOPAL RR) and in combined traits, e.g. cultivar, NuOPAL RR. This data could be used to determine the possible impact of the cultivation of single gene and double gene traits on arthropod diversity, and to provide a set of data for decision makers interested in developing biosafety regulations for transgenic cotton.

EXPERIMENTAL PROCEDURES

The 2003/04 trial was planted in heavy black clay soil on a commercial cotton farm approximately 15 km SE of Groblersdal (Mpumalanga, S 25º 21.157'; E 29º 21.609'), an accepted cotton growing region in South Africa. NuOPAL was planted on 40 ha, DeltaOPAL RR on 50 ha and

NuOPAL RR on 50 ha. DeltaOPAL was planted on 0.3 ha adjacent to NuOPAL. A further 0.3 ha was planted adjacent to the NuOPAL RR plantings.

Planting details are illustrated in Fig. 3.1. There was a two-week interval between the plantings of the different cultivars. The Delta and Pine Land Co. (D and PL S.A. Inc.) seed multiplication blocks were used and the study was limited to the available GM and non-GM cotton blocks and their management practices. An area of approximately 1 ha in the centre of the blocks of GM cotton was selected for assessments while the whole of the unsprayed conventional cotton blocks were assessed. The DeltaOPAL RR cotton was sprayed six times for bollworms; four endosulfan sprays (Thioflo® 1.5l/ha) and two cypermethrin sprays (Karate® 150 ml/ha), while it was sprayed only once for sucking pests with acetamiprid (Mospilan®). The NuOPAL cotton was sprayed twice for sucking pests, once with carbosulfan (Marshal® 300 ml/ha), and once with acetamiprid (Mospilan® 75g/ha). The NuOPAL RR cotton was sprayed twice for sucking pests with acetamiprid (75g/ha). The conventional DeltaOPAL cotton received no insecticide treatments since this was managed by the farmer as an unsprayed refuge area for insect-resistant cotton, conforming with the license agreement with Monsanto SA (Anon 1998).

The 2004/05 trial was planted in Groblersdal at the Agricultural Research Council - Institute for Industrial Crops (ARC-IIC) Experimental Farm, on a red clay (20-25%)-loam soil. The trial was planted on 13 December 2004 in a complete randomized block design, with eight treatments and four replicates. Treatments were DeltaOPAL, DeltaOPAL RR, NuOPAL and NuOPAL RR, all with and without (control) insecticidal spraying for bollworms. Treatments were initiated once scouting had determined that the bollworm threshold level of 5 larvae/24 plants was exceeded (Table 3.1). Plots consisted of 10 cotton rows, each 10 m in length. Data were collected from the middle 6 m x 6 m (the inner 6 rows and inner 6 m of each row) i.e. there were 2 m of guard plants surrounding the data plants. Twelve border rows of unsprayed conventional cotton (DeltaOPAL) surrounded the trial. Control plots were never sprayed and considerable care was taken to ensure that no spray drifted into these plots.

Assessments

During 2003/04, insects and other invertebrate species were monitored by both sweep netting and scouting. The scouting was done every two weeks (Fig. 3.40). Thirty-six plants in each transgenic cotton block were assessed. This is 1.5 times the number of plants recommended by Basson (1987), who advocates that 24 plants within each field and the entire plant, including young bolls and squares, should be examined thoroughly. The number of any pest found on the plant is recorded and if this is equal to or more than the predetermined economic control threshold, control measures should be applied. Even when planting Bt-cotton, a scouting programme monitoring bollworm levels should be conducted to monitor bollworm levels, as this will determine the efficacy of the gene expression to resist pests. The monitoring for secondary pests must be undertaken, as spraying must be done to control these pests. In each of the conventional cotton blocks, 24 plants were assessed and data were generated on a total of 48 plants. All invertebrates observed (including eggs, pest species, predator species, pollinators, soil insects and spiders) were recorded and identified to species where possible. In addition to

scouting, sweeping of 50 random sweeps was done on each transgenic cotton variety (5 repetitions of 10 sweeps), while 25 sweeps were done in the two conventional cotton blocks (5 repetitions of 5 sweeps). Each insect was identified to species level where possible and counted. For this preliminary data set, it was decided to omit any statistical analyses of the data, large variation existed amongst all parameters compared, mainly due to the inherent characteristics of the cotton types and seasonal occurrences of the insects, the planting dates (Fig. 3.1) and surface areas planted with each cotton type. Management procedures followed on each cotton variety also differed according to how the commercial cotton grower would typically manage each cotton type and the farmer's spraying programme was monitored for interpretation of the results (Fig. 3.2).

Scouting was the only method used to monitor the invertebrate populations in 2004/05. Assessments were carried out on a 7 to 10 day cycle starting at the 2nd node stage of cotton development. The observation schedule was adjusted following the application of an insecticide by performing an assessment on the day following the application. The 7 to 10 day cycle of inspection was then resumed using the last assessment date as day 1 of the new cycle. Assessments were continued until the first boll burst stage was reached. Six plants per plot, each randomly selected within each data row were assessment. All arthropods were identified to at least family/subfamily level, and where possible to generic and species. Insecticide applications were based on predetermined economic threshold levels (bollworm control sprays were applied according to a larval threshold of five larvae per 24 plants scouted on conventional cotton, whereas on Bt-cotton, a threshold of more than 5 plants with bollworm larvae out of 24 plants scouted, was used. Secondary pest sprays were applied according to the following thresholds: Aphids: more than 30 aphids per 12 plants out of 24 plants inspected; Jassids: more than three jassids per plant on 12 plants out of 24 plants; Stainers: more than six family groups of between three or more insects present on 24 plants scouted[4]. In order to limit the variation of insect populations between treatments, when the threshold of one of the non-Bt-sprayed treatments was exceeded, spraying was conducted on all replicates of the non-Bt-sprayed treatments (DeltaOPAL and DeltaOPAL RR). If bollworm threshold for Bt-cotton were to be reached, insecticides would have been sprayed on all replicates of the Bt-sprayed treatments (NuOPAL and NuOPAL RR). The purpose was to be able to register the impact of the spray on insect pests and beneficial non-target insects. Three sprays of endosulfan were applied for bollworms. One spray of acetamiprid was applied to all the cotton to control sucking pests. These insecticides are considered to be "soft" option and are recommended for an Integrated Pest Management Strategy. The first herbicide application was done between the 3rd and 4th node stage of the cotton and the second after the 4th node stage. Care was taken to prevent contact with the cotton plant leaves, as stipulated on the Roundup Ready™ herbicide label.

[4] Thresholds used for secondary pests are those currently used by the cotton industry, according to the Cotton Production Skills Training Manual of S.A. (Cotton SA 2005). These thresholds are adapted from those used at the Kadoma Training Centre (Zimbabwe).

Statistical analyses

For this preliminary data set in the 2003/04 trial, it was decided to omit any statistical analyses of the data, due to the large variation that existed in the parameters measured and amongst cotton types (mainly due to the inherent characteristics of the cotton types) and seasonal occurrences of the insects, planting dates (Fig. 3.1) and surface areas planted with each cotton type. Management procedures followed on each cotton variety also differed according to how the commercial cotton grower would typically manage each cotton type and the farmer's spraying programme was monitored for interpretation of the results (Fig. 3.2).

To facilitate interpretation of the 2004/05 data, arthropods were classified into four categories: (i) primary pests (bollworms), (ii) secondary pests, (iii) predators and (iv) other arthropods. Analyses were conducted using SAS (University of Pretoria), following the GENMOD procedure for bollworms. Chi-square tests were carried out. The data followed a Poisson distribution and log-transformations were done on the treatment means, since many zero values were observed, due to the inherent characteristics of the NuOPAL and NuOPAL RR. Significant values indicating differences between log-means are applicable to 'true' means recorded or calculated.

The GLM (General Linear Model) procedure was used to analyze secondary pest data. A comparison of means between each sprayed and unsprayed mean of the same treatment was then made (for example, secondary pest means of DeltaOPAL sprayed compared with secondary pest means of DeltaOPAL unsprayed). A comparison between each mean with all other treatment means was done (for example, comparison of secondary pest means across all treatments). The data followed a normal distribution and treatment means were compared by t-tests (Link-Unity), tested at a significance level of $p<0.05$ (5%) for a General Linear Model.

For other arthropod data, no statistical analyses were performed, since many of the parameters gave zero values. The total of this data group was tabulated. These occasional visitors to cotton may include pollinators, leaf feeders or sap-sucking insects and their presence varies due to environmental factors and chance visits to cotton plants.

RESULTS

The spraying regime is given in Fig. 3.40.

Secondary Pests (2003/04 season)

Secondary pests were assessed in 2004/05. Leafminers, Aphids, leafhoppers, whiteflies, thrips and red spider mites were grouped as 'secondary pests' and analyzed as a group. Fig. 3.41 shows the secondary pests found calculated as the percentage of plants infested.

Leafminers

Leafminers are considered as minor pests of cotton in South Africa They are not considered, as having any economically damaging effect and it has not been necessary to carry out any control measures for the past 10-15 years. They do, contribute to the overall biodiversity on a cotton

plant, and were found to be similar on all cotton types, except for DeltaOPAL, on which no leaf miners were found**.**

Aphids

Aphids were found to have infested a higher percentage of plants in the three GM varieties than in the conventional cotton (Fig. 3.41). On the NuOPAL cotton, fewer aphids were present than in the other two GM varieties, due to the carbosulfan spray. Dead aphids were observed on most plants that were inspected at this time. Aphid predators also declined considerably on NuOPAL cotton due to the carbosulfan spray. Natural enemies were probably responsible for the low number of aphids found on the conventional cotton. This cotton was unsprayed, allowing for the buildup of natural enemies and manifesting their ability to control the pest naturally.

Leafhoppers

During 2003/04, the conventional cotton had the highest percentage of plants infested with leafhoppers (Fig. 3.41). Leafhoppers have few known natural enemies and populations of these pests often increase in unsprayed cotton fields. The heavy spraying regime undertaken on the DeltaOPAL RR cotton apparently suppressed leafhopper numbers. This was particularly evident where the grower sprayed the contact pesticide, cypermethrin. Acetamiprid was sprayed twice on the NuOPAL RR cotton. The result of using a less harsh chemical on the NuOPAL RR cotton type was that more plants were infested with leafhoppers than on the DeltaOPAL RR. NuOPAL was sprayed with carbosulfan, a more effective, systemic control agent, and this single spray resulted in fewer plants being colonized with leafhoppers than in the other three cotton types. The number of leafhoppers found during sweeping (Fig. 3.9) was highest on the NuOPAL RR, with the exception of counts 3 and 6 where DeltaOPAL RR had more leafhoppers. Sweeping was not seen as the most appropriate technique for monitoring leafhoppers, due to the fact that many leafhoppers escape during collection. Many more were monitored during scouting.

Whiteflies

During 2003/04, the conventional cotton had higher whitefly populations than the three GM varieties (Fig. 3.8). For the three GM cotton types, NuOPAL RR had slightly more whiteflies than NuOPAL, followed by DeltaOPAL RR. These differences are thought to be a consequence of insecticidal applications. The higher number of sprays and “harsher” chemicals sprayed on the NuOPAL and DeltaOPAL RR cotton seemed to reduce whitefly populations when compared with the acetamiprid sprayed on the NuOPAL RR cotton.

Thrips

During 2003/04, the percentage of thrips was similar on all four cotton varieties (Fig. 3.8). Thrips usually occur early in the season, during the first three weeks after planting. Earlier observations may indicate varietal differences. Mid-season thrips inhabit the flowers, and since spraying was not directed at this part of the plant, these pests remained unaffected by these applications, hence the similar numbers.

Red spider mites

During 2003/04, the DeltaOPAL RR cotton had the highest percentage of plants infested with red spider mites (Fig. 3.8). The heavy spraying regime, which suppressed predator populations within the field, is probably responsible for the phenomenon. The unsprayed conventional cotton had the fewest plants infested by red spider mites. Since the conventional cotton was left unsprayed, predator populations could build up and keep the red spider mite populations in control.

Secondary pests (2004/05 season)

Comparison of the means of sprayed treatments

During 2004/05, secondary pests, such as aphids, leafhoppers and whitefly, were pooled to form one group (Table 3.4). The mean number of secondary pests found per six plants on sprayed DeltaOPAL, DeltaOPAL RR, NuOPAL and NuOPAL RR are shown in Figs 3.10, 3.11, 3.12 and 3.13 respectively. Table 3.10 shows that there were statistically significant differences between the mean number of secondary pests found in each sprayed treatment. The probability values are given in Table 3.11.

During count 1, the number of secondary pests did not differ between any of the sprayed treatments. Since the bollworm numbers had, however, reached threshold, Thioflo® (endosulfan) was applied directly after count 1 to the DeltaOPAL and DeltaOPAL RR treatments. The second count was then made two days later to determine the effect of the spray on the arthropod numbers. It was found during count 2 that the DeltaOPAL RR sprayed treatment had significantly fewer secondary pests than the NuOPAL RR treatment and this is attributed to the endosulfan spray that was applied to the DeltaOPAL RR treatment for bollworm control. Bollworm sprays exert a significant level of secondary pest control. In this case it was clear that on the NuOPAL RR treatment, secondary pests increased in the absence of bollworm spraying, while the opposite was true for the DeltaOPAL RR sprayed treatment. A slight decrease in secondary pest numbers was also observed on the DeltaOPAL sprayed treatment after the endosulfan application but not significantly so. On the NuOPAL treatment there appeared to be a slight decrease in secondary pest numbers too, but there was a higher number of predators found on this treatment at this time (possibly due to the absence of the bollworm spray, allowing predator numbers to increase), which could account for this. During count 3, no significant differences in secondary pest numbers were found. By count 4, however, significantly more secondary pests were found in the DeltaOPAL sprayed treatment than in the NuOPAL sprayed treatment. This could be due to fewer predators in the sprayed treatments, allowing pest numbers to build up. Thioflo® (endosulfan) was once again applied after count 4, to the DeltaOPAL and DeltaOPAL RR treatments due to bollworm numbers reaching threshold, and by count 5, the number of secondary pests had decreased in these two sprayed treatments. By counts 5, 6 and 7, no significant differences were found between the treatments regarding secondary pest numbers. Acetamiprid was applied directly after count 6 to control sucking pests and a sharp decrease was

found in the number of secondary pests on all treatments after the acetamiprid application (count 7).

Comparison of the means of unsprayed treatments

Table 3.12 shows the mean number of secondary pests found in each unsprayed treatment, indicating any possible effect of the inherent differences between cotton types on secondary pests. The probability values are given in Table 3.13. In the absence of spraying, there were no significant differences between treatments with regards to secondary pest numbers from counts 1 to 6. During count 7, however, the NuOPAL RR treatment had significantly more secondary pests than DeltaOPAL RR. It was interesting that the DeltaOPAL and DeltaOPAL RR treatments tended to have a similar but somewhat lower total number of secondary pests over the season than the NuOPAL and NuOPAL RR treatments (Table 3.12). In general, data collected from unsprayed conditions, to minimize the possible effect of insecticides, indicated that the abundance of secondary pests is independent of the cotton type evaluated.

Comparison of treatment means across all treatments

T-tests were used to compare each mean of a particular treatment (sprayed and unsprayed means) to all other sprayed and unsprayed treatment means (pairwise comparison of $means_{secondary\ pests}$) (Table 3.14). The probability values are given in Table 3.15. For count one, there were no significant differences found between treatment means. The same was true for counts 3, 4, 5 and 6. During count 2, the DeltaOPAL RR sprayed treatment had significantly fewer secondary pests than the NuOPAL unsprayed treatment ($t_{0.05}$=0.0173) ($p<0.05$)) and the NuOPAL RR sprayed treatments ($t_{0.05}$=0.0263 ($p<0.05$) (Tables 3.14 and 15). During count 7, significantly more secondary pests were found on the DeltaOPAL unsprayed treatment than on the DeltaOPAL sprayed ($t_{0.05}$=0.0220 ($p<0.05$) and the DeltaOPAL RR sprayed treatments ($t_{0.05}$=0.0271) (Table 3.15). The DeltaOPAL RR sprayed treatment also had significantly fewer secondary pests than the NuOPAL unsprayed ($t_{0.05}$=0.0430) and NuOPAL RR unsprayed treatments ($t_{0.05}$=0.0003), probably due to the effect of spraying on DeltaOPAL RR. The DeltaOPAL RR unsprayed treatment had significantly fewer secondary pests than the NuOPAL RR unsprayed treatment. The unsprayed NuOPAL RR treatment also had significantly more secondary pests then the sprayed NuOPAL treatment ($t_{0.05}$=0.0017), as one might expect, due to the effect of spraying on secondary insect populations. The DeltaOPAL sprayed treatment had significantly fewer secondary pests than the NuOPAL unsprayed treatment, once again emphasizing the effect of chemicals on secondary pests ($t_{0.05}$=0.0351) (Table 3.14). The NuOPAL unsprayed treatment had significantly fewer secondary pests than the unsprayed NuOPAL RR ($t_{0.05}$=0.0511). The NuOPAL RR sprayed treatment had significantly fewer secondary pests than the NuOPAL RR unsprayed treatment ($t_{0.05}$=0.0016), probably due to the Mospilan® (acetamiprid) spray applied after count 6.

The total number of secondary pests found on each treatment over the season is shown in Fig. 3.14. When the sprayed treatments were compared, there were no differences found during counts 1, 3, 5, 6 and 7. During the 2nd count, the NuOPAL RR sprayed treatment had

significantly more secondary pests than the DeltaOPAL RR sprayed treatment (Table 3.10). During count 4, the DeltaOPAL sprayed treatment had significantly more secondary pests than the NuOPAL sprayed treatment. The number of secondary pests found over the season tended to be higher in the unsprayed treatments (Table 3.12) than in the sprayed treatments (Table 3.10) (Fig. 3.14). The DeltaOPAL RR unsprayed treatment had significantly fewer secondary pests than the NuOPAL RR unsprayed treatment during only count 7, possibly indicating that there is no trend in the occurrence of secondary pests on cotton with a Bt-gene left unsprayed compared to non-Bt-cotton left unsprayed. In general, where the Bt-gene was present in an unsprayed treatment, the number of secondary pests was higher than in the corresponding unsprayed, non-Bt-treatment (Table 3.14). The results emphasised that spraying did have an influence on the occurrence of secondary pests during the trial.

Other Arthropods (2004/05 season)

The total number of other arthropods found per treatment during the 2004/05 season is shown in Table 3.20. When specific arthropods were compared between treatments, no trends could be observed, for example, Spotted maize beetle varied between 175 (DeltaOPAL sprayed) and 313 individuals (NuOPAL sprayed). In total, the NuOPAL RR sprayed treatment had the most arthropods (718), whereas the least (492) was found in the DeltaOPAL sprayed treatment.

To summarize the findings, a matrix was set up of the incidence of significance obtained during the statistical analyses performed (Table 3.21) by scoring the number of times the Bt-cotton showed a significantly higher diversity for secondary pests and predators. The NuOPAL RR treatment differed significantly from other treatments more than any other treatment did from another, indicating a higher level of biodiversity on this cotton type.

CONCLUSIONS

The 2003/04 study on arthropod diversity provided data on the arthropod species richness occurring within GM and non-GM cotton fields during the peak fruiting stages of cotton. The number and species composition varied on the different cotton types that were subjected to general farm weed and pest management practices. It was hypothesized that the number of arthropods would be similar on GM and non-GM (conventional) cotton. This, however, could not be established due to the high within-trial procedural variability, such as differences in the spraying regime, the differing ages of the cotton varieties and the absence of good weed control in the conventional blocks. This preliminary data did, however, demonstrate that species diversity varied according to the insecticides applied. Arthropod diversity monitored by sweeping tended to be higher in the cotton with a double trait (NuOPAL RR) than in the other cotton types, especially regarding spiders (both in number and in species). Roundup Ready cotton (DeltaOPAL RR) tended to have the lowest diversity and this was attributed to the many insecticide applications applied within this field and possibly the fewer number of weed species observed.. The results indicated that spraying affected pest numbers. Since NuOPAL RR cotton is bollworm-resistant and has the added benefit of herbicide-tolerance, the management of this variety is easier for the farmer. This variety also seems to be of benefit to the environment, since the number of

predators (discussed in Chapter 4) and other arthropods were higher on this variety than on the other varieties. This data on species diversity also indicates that a farmer is likely to conserve species diversity by improving the management practices on his farm.

Results obtained in bollworm comparisons by Bennett-Nel et al (2006), showed that NuOPAL and NuOPAL RR express efficient bollworm control in the absence of bollworm sprays and this reduction in sprays can result in an increase in numbers of predators and other arthropods. The highest total number of predators (Chapter 4) and other arthropods was found on NuOPAL RR at the end of the observation period.

Final conclusion with regards to secondary pests on transgenic cotton

Secondary pests occur commonly on all cotton types, be they conventional cotton varieties or genetically modified cotton types. The main secondary pests on South African cotton which have an economic effect are aphids, leafhoppers and common cotton stainers. Other secondary pests include whitefly, thrips, red spider mite, cotton stem weevil in some areas, the black cotton beetle in some areas, and occasional visitors like the green vegetable stinkbug, dusky cotton stainers, mirids and others. In general, on the Bt-cottons, secondary pests appear to be present in higher densities as a result of fewer insecticides applied which are aimed at bollworms, due to the inherent resistance to bollworms of these cotton types. In the absence of spraying, secondary pests appear to be similar on all cotton types, be they conventional or genetically modified. The Bt-protein does not have an influence on secondary pest population by affecting them negatively. There appear to be a shift towards the up build of secondary pests on Bt-cottons due to a change in the spraying regime on these cotton types. Cotton that possesses the Roundup Ready gene expresses similar secondary pest infestations due to a similar bollworm control programme to be followed on this cotton type. Biodiversity with regards to secondary pests were similar on all cotton types, which included conventional varieties, Bt-cottons and cotton containing the Roundup Ready gene.

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Tables

Table 3.1. Total number of insects found on transgenic events at Rustenburg in the P2B trial.

Plots	Events	Dusky stainers	Common Cotton stainers	Whitefly	Nisotra beetles	Stink-bugs	Cotton leaf Worms	Plants with thrips
1	6149	0	0	1	7	1	1	0
2	6151	0	0	1	3	0	1	0
3	6152	3	0	0	43	0	1	0
4	6223	0	0	0	12	0	0	2
5	6227	0	0	1	12	0	0	0
6	6232	0	0	1	99	0	1	0
7	6234	2	0	1	19	1	0	0
8	6239	0	5	2	0	0	1	0
9	6241	0	0	0	10	0	0	0
10	6351	0	10	0	1	4	0	0
11	6352	0	0	0	3	2	0	0
12	6456	0	6	0	68	0	0	0
13	6460	0	0	3	18	0	1	0
14	6483	0	0	2	0	2	0	0
15	6484	5	1	0	16	0	0	0
16	6489	0	0	0	4	0	0	0
17	6491	0	0	0	0	0	2	0
18	6526	1	0	0	35	1	1	0
19	6532	0	21	0	0	0	0	0
20	6563	8	4	0	48	0	0	0
21	6542	1	9	2	13	0	0	0
22	6718	2	0	0	1	3	0	0
23	6725	0	0	0	99	1	0	0
24	6733	2	28	1	25	0	0	0
25	6734	15	0	2	19	1	1	0
26	16221	0	0	1	0	2	0	0
27	16960	0	0	1	4	0	0	0
28	17079	4	0	0	0	0	2	0
29	17133	0	2	0	2	0	2	0
30	17180	0	0	0	0	0	1	0
31	17206	0	0	0	0	0	1	0
32	6233	0	4	1	1	0	1	0
33	15830-5	5	0	12	12	0	1	0

Table 3.2. Total number of insects found on control plots at Rustenburg in the P2B trial.

Plots	Events (Controls)	Dusky Cotton stainers	Common Cotton stainers	Nisotra beetles	Fly pupae	Miridae	Assassin bugs	Stink-bugs	Cotton leaf worms	Plant with thrips
C1	DP50	0	1	0	0	0	0	4	0	0
C2	DP50B	2	0	1	0	0	0	1	0	0
C3	C312	0	0	0	0	0	0	0	0	0
C4	C531	4	0	0	0	2	1	1	1	0
C5	15813	0	3	2	0	0	0	0	0	0
C6	15985	2	1	0	0	0	182	8	0	0

Table 3.3. Score sheet of beneficial characteristics observed amongst the events at Rustenburg in the P2B trial.

Parameters Plot	Events	Low frequence Aphids	Low frequence Leafhoppers	> no. Maize beetles	Early boll burst
1	6149				
2	6151			*	*
3	6152				
4	6223				
5	6227			*	
6	6232			*	
7	6234				*
8	6239				
9	6241				
10	6351				
11	6352				
12	6456				
13	6460				
14	6483				
15	6484				
16	6489				
17	6491	*	*		
18	6526				
19	6532				
20	6563				
21	6542			*	
22	6718				
23	6725		*		
24	6733		*		
25	6734				
26	16221				
27	16960				
28	17079				
29	17133				
30	17180				
31	17206				
32	6233				
33	158305				

Presence of beneficial characteristic found per event.

Table 3.4. Score sheet of beneficial characteristics observed amongst the control plots at Rustenburg in the P2B trial.

Parameters Plot	Events	Low frequence Aphids	Low frequence Leafhoppers	no. Maize beetles	Early boll burst
C1	DP50				
C2	DP50B				
C3	C312				
C4	C531				*
C5	15813				
C6	15985				

Table 3.5. Weather conditions on insecticide application dates and scouting dates at Rustenburg in the Cry2Ab2 trail.

Insecticide application dates	Insecticides applied*	Scouting Dates	MAX T (℃)	MIN T (℃)	RAIN (mm)
		98/11/24	31.5	16.8	0.5
		98/12/11	28.7	16.7	0.0
		99/01/06	29.1	17.2	0.0
		99/01/18	33.4	18.4	0.0
99/02/01	Thioflo® (endosulfan) (1 l / ha)	99/02/01	29.0	16.6	0.2
99/02/04	Thioflo® (endosulfan) (1 l / ha)		29.5	19.4	0.0
99/02/17	Thioflo® (endosulfan) (1 l / ha)	99/02/17	30.5	18.4	0.0
		99/03/01	36.3	15.6	0.0

** applied when the number of larvae exceeded larval threshold of 16.6 larvae/80 plants*

Table 3.6. Weather conditions on insecticide application dates and scouting dates at Brits in the Cry2Ab2 trail.

Insecticide application date	Insecticides applied*	Scouting dates	MAX T (°C)	MIN T (°C)	RAIN (mm)
		98/11/24	31.0	17.2	0.5
		98/12/08	26.0	16.7	1.0
		99/01/05	29.0	18.2	0.0
		99/01/19	34.6	18.2	0.0
		99/02/02	30.2	18.0	0.0
		99/02/16	33.3	15.2	29.5
99/02/17	Thioflo® (endosulfan) (1 l / ha)		32.0	15.0	0.0
		99/03/02	37.5	16.3	0.0

** applied when the number of larvae exceeded larval threshold of 16.6 larvae/80 plants*

Table 3.7. Weather conditions on insecticide application dates and scouting dates at Nelspruit in the Cry2Ab2 trail.

Insecticide application dates	Insecticides applied	Scouting dates	MAX T (°C)	MIN T (°C)	RAIN (mm)
		98/12/07	30.9	14.5	0.5
		98/12/21	25.9	19.2	0.1
		99/01/06	29.7	20.6	11.1
99/01/08	Chess® (pymetrozine) 800g/ha		27.6	18.4	0.0
99/01/14	Chess® (pymetrozine) 800g/ha * Thioflo® (endosulfan) 1/ /ha		30.0	20.1	0.0
		99/01/20	32.1	19.1	0.0
		99/02/02	30.0	20.3	4.0
99/02/16	Dimethoate® (dimethoate) 800 l /ha Decis® (deltamethrin) 250 l /ha * Thioflo® (endosulfan) 1 l /ha		27.1	18.3	0.0
		99/02/17	29.4	17.7	0.0
		99/03/08	29.1	17.9	0.0

** applied when the number of larvae exceeded larval threshold of 16.6 larvae/80 plants*

Table 3.8. Weather conditions on insecticide application dates and scouting dates at Potgietersrus in the Cry2Ab2 trail.

Insecticide application dates	Insecticides applied*	Scouting Dates	MAX T (°C)	MIN T (°C)	RAIN (mm)
		98/11/25	29.0	16.0	10.0
		98/12/09	26.5	16.5	0.0
		99/01/06	30.5	18.5	0.0
		99/01/18	33.5	20.5	3.5
99/01/29	Thioflo® (endosulfan) (1 l/ha)		30.7	20.0	0.0
		99/02/01	30.0	19.0	0.0
99/02/12	Chess® (pymetrozine) (800g/ha)		34.0	17.5	0.0
		99/02/15	32.5	17.5	0.0
		99/03/01	36.5	17.0	0.0
99/03/07	Chess® (pymetrozine) (800g/ha)		31.5	12.0	0.0
99/03/12	Agrimec® (abamectin) (500 ml/ha)		31.0	20.0	0.0

** applied when the number of larvae exceeded the larval threshold of 16.6 larvae/80 plants*

Table 3.9. Arthropods found on the different varieties during scouting (x) and sweeping (&) during the 2003/04 biodiversity trial.

Common name	Order / Class	Family	Genus / Species name	NuOPAL RR (x = scouting; & = sweeping)		DeltaOPAL RR (x = scouting; & = sweeping)		NuOPAL (x = scouting; & = sweeping)		DeltaOPAL (x = scouting; & = sweeping)	
African (African) bollworm	Lepidoptera	Noctuidae (Noctuinae)	Helicoverpa armigera (Hübner)	x	&	x	&	x	&	x	&
Red bollworm	Lepidoptera	Noctuidae (Acronyctinae)	Diparopsis castanea Hampson	x	&	x	&	x	&	x	&
Spiny bollworm	Lepidoptera	Noctuidae (Westermaniinae)	Earias biplaga Walker	x	&	x	&	x	&	x	&
Spiny bollworm	Lepidoptera	Noctuidae (Westermaniinae)	Earias insulana Boisduval	x	&	x	&	x	&	x	&
Cotton leaf-miner damage	Lepidoptera	Gracillariidae	probably Acrocercops bifasciata Wals	x		x		x		x	
Leafeaters	Lepidoptera	Noctuidae (Acronyctinae)	probably Spodoptera littoralis (Boisduval)	x		x		x		x	
Leaf worm	Lepidoptera	Noctuidae	Xanthodes graellsi Feisthamel	x		x		x		x	
Semi-looper	Lepidoptera	Noctuidae	Anomis spp.					x	&		&
Ephemeroptera**	Ephemeroptera	?	-								
Cotton aphid	Hemiptera (Homoptera)	Aphididae	Aphis gossypii Glover	x	&	x	&	x	&	x	&
Tobacco whitefly	Hemiptera (Homoptera)	Aleyrodidae	Bemisia tabaci (Gennadius)	x	&	x	&	x	&	x	&
Snout bug	Hemiptera (Homoptera)	Fulgoridae	?		&						
Spittle bugs	Hemiptera (Homoptera)	Cercopidae	?	x	&				&		&
Wax scale **	Hemiptera (Homoptera)	Coccidae	?		&						
Stem sucking bugs	Hemiptera (Heteroptera)	Miridae	? probably Helopeltis spp.		&		&				
Dusky cotton stainer	Hemiptera (Heteroptera)	Lygaeidae	Oxycarenus hyalinipennis (Costa).	x	&	x		x		x	
Common cotton stainers	Hemiptera (Heteroptera)	Pyrrhocoridae	Dysdercus fasciatus Signoret D. nigrofasciatus Stăl D. intermedius Distant	x		x	&	x		x	
Green vegetable stinkbug	Hemiptera (Heteroptera)	Pentatomidae	Nezara viridula (L.)	x				x		x	
Blue bug**	Hemiptera (Heteroptera)	Scutelleridae	probably Calidea dregii Ger.					x			
Small Hemipterans	Hemiptera (Heteroptera)	Lygaeidae?	probably Lygus spp.	x	&		&	x		x	&
Pirate Bugs	Hemiptera (Heteroptera)	Anthocoridae	Orius spp.		&		&		&		&
Leafhoppers	Hemiptera (Homoptera)	Cicadellidae	Jacobiella facialis Jacoby J. libyca	x		x		x		x	
Western flower thrips**	Thysanoptera	Thripidae	Frankliniella occidentalis (Pergande)	x	&	x	&	x	&	x	&
Onion thrips**	Thysanoptera	Thripidae	Thrips tabaci Lindeman	x	&	x	&	x	&	x	&

(table continued)

Cotton bud thrips	Thysanoptera	Thripidae	Frankliniella schultzei (Trybom)	x	&	x	&	x	&	x	&
Spotted maize beetles	Coleoptera	Melyridae	Astylus atromaculatus Blanchard	x	&	x	&	x	&	x	&
Ladybirds	Coleoptera	Coccinellidae	Coccinella septempunctata L.	x	&	x	&	x	&	x	&
Ladybirds	Coleoptera	Coccinellidae	Scymnus spp.	x	&	x	&	x	&	x	&
Flea beetles	Coleoptera	Chrysomelidae	Nisotra spp.	x	&	x	&	x	&	x	&
Ant beetle	Coleoptera	Anthicidae	Noxos sp.	x	&	x	&	x	&	x	&
Rove beetle	Coleoptera	Staphylynidae			&				&		
Sap-feeding beetles	Coleoptera	Nitidulidae	Probably Carpophilus spp. (?)		&		&		&		&
Leaf beetle - Black beetle, brown spots	Coleoptera	Chrysomelidae	?	x							
Black cotton beetle**	Coleoptera	Chrysomelidae	Syagrus rugifrons Baly	x	&			x	&	x	&
Cotton snout beetle	Coleoptera	Curculionidae	Protostrophus sp. (probably P. amplicollis (Fab.)).						&		
Tumbling flower beetle	Coleoptera	Mordellidae	?		&						&
Green lacewing	Neuroptera	Chrysopidae	Chrysopa spp.	x	&	x	&	x	&	x	&
Green lacewings	Neuroptera	Chrysopidae	Chrysoperla spp.	x				x			
Hoverfly	Diptera	Syrphidae	Syrphus spp.?	x	&	x	&	x	&	x	&
Mosquitoes	Diptera	Culicidae	?		&		&				&
Midges	Diptera	Chironomidae	?	x		x			&		&
Mosquitoes	Diptera	Tipulidae	?						&		
Booklice	Psocoptera	?	?					x			
Earwigs	Dermaptera	Forficulidae			&						
Dragonfly	Odonata	Aeshnidae	Aeshna spp.?		&						
Praying mantis	Mantodea	Mantidae	?	x		x					
Grasshopper	Orthoptera (Caelifera)	Pamphagidae	?		&						
Common garden cricket	Orthoptera (Ensifera)	Gryllidae	Gryllus bimaculatus de Geer		&				&		
Honeybee	Hymenoptera	Apidae	Apis mellifera L.	x		x		x		x	
Ants	Hymenoptera	Formicidae	Crematogaster sp.		&						
Parasitoid	Hymenoptera	Braconidae	Bracon spp.? Apanteles spp?						&		&
Parasitoid	Hymenoptera	Ichneumonidae	?		&		&				&
Parasitoid	Hymenoptera	Trichogrammatidae	Trichogramma spp.?		&		&		&		&
Red spider mites	Acari (Class)	Tetranychidae	Tetranychus cinnabarinus Boisduval			x	&		&		&
Predatory mites	Acari (Class)	Phytoseidae?	?				&				
Spiders	Arachnidae	Thomisidae	Misumenops rubrodecoratus Millot		&		&		&		&
Spiders		Thomisidae	?		&		&				
Spiders		Linyphiidae	Ostearius melanopygius (Cambridge)								&
Spiders		Linyphiidae	?						&		
Spiders		Philodromidae	Tibellus minor Lessert								&
Spiders		Salticidae	Heliophanus sp.						&		
Spiders		Salticidae	Aelurillus sp.						&		
Jumping spider		Salticidae	Pardosa sp.		&						
Jumping spider		Salticidae	Heliophanus sp.		&						
Spiders		Theridiidae	Euryopis sp.						&		

(table continued)

Spiders	Theridiidae	Afropisaura sp.	&	
Spiders	Lycosidae	Pardosa sp.		&
Spiders	Lycosidae	Theridion sp.	&	
Spiders	Miturgidae	Cheiracanthium sp.		&
Spiders	Araneidae	Neoscona subfusca (Koch)		&
Fishing spiders	Pisauridae	Philodromus sp.	&	
Spiders	Lycosidae	Pardosa sp.	&	
Spiders	Salticidae	Heliophanus sp.	&	

** Species unconfirmed;*
*** Species noticed during scouting and sweeping, and not necessarily collected and recorded*

Table 3.10. Arthropods, classified per category, found during the 2004/05 biodiversity trial.

Category 1: Bollworms	Category 2: Secondary pests	Category 3: Predators	Category 4: Other arthropods		
African Bollworm (Noctuidae) Helicoverpa armigera (Hűbner)	Aphis gossypii Glover	Ladybird eggs Coccinellidae (Coleoptera)	Spodoptera littoralis Boisduval (Lepidoptera	Staphylinidae (Coleoptera)	Primitive bug (Hemiptera)
Red bollworm Diparopsis castanea Hampson (Acronyctinae)	Leafhoppers (Jacobiella fasciata Jacobi)	Ladybird larvae Coccinellidae (Coleoptera)	Anomis spp. (Lepidoptera)	Click beetle (Elateridae Coleoptera)	Tip wilter (Hemiptera)
Spiny bollworm Earias biplaga Walker; Earias insulana (Boisduval)	Whitefly (Bemisia tabaci (Genn))	Ladybird pupae Coccinellidae (Coleoptera)	Looper eggs (Lepidoptera)	'Velvet beetle' (Lagriidae Coleoptera)	Lygus bug (Hemiptera)
Neonate larvae (all spp.)	Thrips (Thrips tabaci Lind.)	Ladybird adult Coccinellidae (Coleoptera)	Unid. Noctuidae (Lepidoptera)	Fruit chafer (Cetoniinae; Scarabaeidae Coleoptera)	Stinkbug egg packet (Pentatomidae; Hemiptera)
	Dusky cotton stainer Oxycareneus hyalinipennis (Costa)	Lacewing eggs, larvae, pupae and adults (Neuroptera)	Yellow butterfly (Pieridae; Lepidoptera)	Black small beetle unid. Coleoptera)	Assassin bugs (Reduviidae; Hemiptera)
Common cotton stainer Dysdercus fasciatus Sign, D. nigrofasciatus Stäl, D. intermedius Dist.	Hover fly larvae Syrphidae	Acrocerops spp. (Lepidoptera)	Anthicidae (Coleoptera)	Assassin bug eggs (Reduviidae; Hemiptera)	Common cotton stainer Dysdercus fasciatus Sign, D. nigrofasciatus Stäl, D. intermedius Dist.
Green stinkbug Nezara viridula (L.)	Hoverfly adults Syrphidae	Spodoptera (Lepidoptera)	Eggs?	Midget (Chironomidae; Diptera)	Green stinkbug Nezara viridula (L.)
	Spiders (Arachnida)	Unid. Noctuidae (Lepidoptera)	Beetle unid. (Coleoptera)	(Calliphoridae; Diptera)	Spiders (Arachnida)
		Spider egg package Arachnida	Unid. Pieridae (Lepidoptera)	Protostrophus spp. (Coleoptera)	Long-legged mosquito (Tipulidae; Diptera)
		Earwigs Dermaptera	Acrocerops spp. (Lepidoptera)	Chrysomelidae (Coleoptera)	Lyriomyza spp; (Diptera)

(table continued)

	Predatory mites	Aphis mellifera (Hymenoptera)	CMR beetle (Meloidae; Coleoptera)	Small unid. Black fly? (Diptera)
	Predatory thrips	Wasp (Hymenoptera)	Black beetle, unid. (Coleoptera)	Zonocerus elegans; (Orthoptera)
	Predatory Hemiptera	Formicidae (Hymenoptera)	Nisotra spp. (Coleoptera)	Brown leafhopper
		Minute pirate bug	Astylus spp. (Melyridae, Coleoptera)	Cricket (Gryllidae; Orthoptera)
		Parasitic wasps Hymenoptera?	Flea beetle (Coleoptera)	Red spider mites (Tetranychus cinnabarinus (Boisduval))
		Parasitized Aphididae (Homoptera)	Mordeliidae (Coleoptera)	Parasitized Aphididae (Homoptera)

Table 3.11. Scout dates and insecticide applications during the 2004/05 biodiversity trial.

Date	Assessments	Insecticide application rate/dosage	Treatments (Cotton types)
09/02/2005	1	Endosulfan (1litre/ha)	DeltaOPAL and DeltaOPAL RR
11/02/2005	2	No spray	-
23/02/2005	3	No spray	-
07/03/2005	4	Endosulfan (1litre/ha)	DeltaOPAL and DeltaOPAL RR
09/03/2005	5	No spray	-
17/03/2005	6	Endosulfan (1litre/ha)	DeltaOPAL and DeltaOPAL RR
		Acetamiprid 75g/150 liter water	DeltaOPAL, DeltaOPAL RR, NuOPAL and NuOPAL RR
19/03/2005	7	No spray	-

Table 3.12. The mean numbers of secondary pests of 6 plants per repetition of treatments in the 2004/05 biodiversity trial*.

Treatments	Count 1	Count 2 Df=15 (P>/t/=0.0247)	Count 3	Count 4 Df=15 (P>/t/=0.0179)	Count 5	Count 6	Count 7	Totals
DeltaOPAL sprayed	22.63[a]	18.75[ab]	30.00[a]	52.70[a]	45.00[a]	62.75[a]	15.00[a]	246.88
DeltaOPAL RR sprayed	19.25[a]	12.50[b]	40.00[a]	35.00[ab]	33.00[a]	73.00[a]	17.00[a]	229.75
NuOPAL sprayed	24.63[a]	21.25[ab]	56.50[a]	28.00[b]	44.50[a]	66.25[a]	30.50[a]	271.63
NuOPAL RR sprayed	21.50[a]	31.25[a]	51.50[a]	42.00[ab]	49.75[a]	70.25[a]	30.00[a]	296.25

[*] *Means followed by the same symbol/s within a column are not significantly different (p<0.05)*

Table 3.13. Significant differences $_{(p<0.05)}$ in secondary pest numbers between sprayed treatments in the 2004/05 biodiversity trial*.

Treatment	Significance (Pr >/t/) (Df = 15)						
	Count 1	Count 2	Count 3	Count 4	Count 5	Count 6	Count 7
DeltaOPAL Sprayed	< 0.0001	0.0035	0.0094	<0.0001	0.0001	<0.0001	0.0149
DeltaOPAL RR sprayed	< 0.0001	0.0324 *(0.0247)	0.0014	0.0001	0.0012	<0.0001	0.0074
NuOPAL sprayed	< 0.0001	0.0014	<0.0001	0.0009 *(0.0179)	0.0001	<0.0001	<0.0001
NuOPAL RR sprayed	< 0.0001	< 0.0001	0.002	<0.0001	<0.0001	<0.0001	0.0001

Probability values for the Least Square Means (Pr>/t/ for Ho)

Table 3.14. Significant differences $_{(p<0.05)}$ in secondary pest numbers between unsprayed treatments in the 2004/05 biodiversity* trial.

Treatments	(Df= 15) P>/t/=0.0464							
	Count 1	Count 2	Count 3	Count 4	Count 5	Count 6	Count 7	Totals
DeltaOPAL Unsprayed	18.75[a]	16.50[a]	48.25[a]	43.75[a]	37.75[a]	60.50[a]	66.00[ab]	291.50
DeltaOPAL RR Unsprayed	21.25[a]	20.00[a]	45.50[a]	48.50[a]	44.50[a]	58.75[a]	41.00[a]	279.50
NuOPAL Unsprayed	17.88[a]	32.75[a]	50.00[a]	44.75[a]	39.75[a]	73.25[a]	61.50[ab]	319.88
NuOPAL RR Unsprayed	20.25[a]	24.00[a]	44.50[a]	44.25[a]	50.25[a]	41.50[a]	104.25[b]	329.00

[*] *Means followed by the same symbol/s within a column are not significantly different (p<0.05)*

Table 3.15. Significant differences $_{(p<0.05)}$ between unsprayed treatments for secondary pests in the 2004/05 biodiversity trial*.

Treatments	Significance (1%) (Pr > /t/) (DF = 15)						
	Count 1	Count 2	Count 3	Count 4	Count 5	Count 6	Count 7
DeltaOPAL Unsprayed	< 0.0001	0.0176	0.0004	0.0002	0.0014	0.0007	0.0066
DeltaOPAL RR Unsprayed	< 0.0001	0.0060	0.0007	<0.0001	0.0004	0.0009	0.0645
NuOPAL unsprayed	< 0.0001	0.0001	0.0003	0.0002	0.0010	0.0001	0.0100
NuOPAL RR unsprayed	< 0.0001	0.0018	0.0008	0.0002	0.0001	0.0093	0.0002 *(0.0464)

** Probability values for the Least Square Means (Pr>/t/ for Ho)*

Table 3.16. Comparison of treatment means for secondary pests across all treatments in the 2004/05 biodiversity trial*.

Treatments	Count 1	Count 2	Count 3	Count 4	Count 5	Count 6	Count 7	Totals
DeltaOPAL sprayed	22.63[a]	18.75[a]	30.00[a]	52.75[a]	45.00[a]	62.75[a]	15.00[c]	246.88
DeltaOPAL unsprayed	18.75[a]	16.50[a]	48.25[a]	43.75[ab]	37.75[a]	60.50[a]	66.00[ab]	291.50
DeltaOPAL RR sprayed	19.25[a]	12.50[a]	40.00[a]	35.00[ab]	33.00[a]	73.00[a]	17.00[c]	229.75
DeltaOPAL RR unsprayed	21.25[a]	20.00[a]	45.50[a]	48.50[ab]	44.50[a]	58.75[a]	41.0[bc]	279.50
NuOPAL sprayed	24.625[a]	21.25[a]	56.50[a]	28.00[b]	44.50[a]	66.25[a]	30.50[bc]	271.63
NuOPAL unsprayed	17.88[a]	32.75[a]	50.00[a]	44.75[ab]	39.75[a]	73.25[a]	61.50[ab]	319.88
NuOPAL RR sprayed	21.50[a]	31.25[a]	51.50[a]	42.00[ab]	49.75[a]	70.25[a]	30.00[bc]	296.25
NuOPAL RR unsprayed	20.25[a]	24.00[a]	44.50[a]	44.25[ab]	50.25[a]	41.50[a]	104.25[a]	329.00

[*] *Means followed by the same symbol/s within a column are not significantly different (p<0.05)*

Table 3.17. Significant differences $_{(p<0.05)}$ of secondary pests treatment mean comparisons across all treatments in the 2004/05 biodiversity trial.

Treatments	Significance (1%) (Pr > /t/) (DF = 24)						
	Count 1[$]	Count 2	Count 3	Count 4	Count 5	Count 6	Count 7
DeltaOPAL (sprayed)	<0.0001	0.0027	0.0056	<0.0001	<0.0001	<0.0001	0.3184
DeltaOPAL (unsprayed)	<0.0001	0.0070	<0.0001	<0.0001	0.0002	<0.0001	0.0002 *(0.0220) *(0.0271)
DeltaOPAL RR (sprayed)	<0.0001	0.0352 *(0.0173) *(0.0263)	0.005	<0.0001	0.0008	<0.0001	0.2596 *(0.0430) *(0.0003)
DeltaOPAL RR (unsprayed)	<0.0001	0.0015	0.0001	<0.0001	<0.0001	<0.0001	0.0103 *(0.0057)
NuOPAL (sprayed)	<0.0001	0.0009	<0.0001	0.0001	<0.0001	<0.0001	0.0492
NuOPAL (unsprayed)	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	0.0003 *(0.0351) *(0.0511)
NuOPAL RR (sprayed)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0528 *(0.0016)
NuOPAL RR (unsprayed)	<0.0001	0.0003	0.0001	<0.0001	<0.0001	0.0013	<0.0001

* *Probability values for the Least Square Means (Pr>/t/ for Ho)*

Table 3.18. Numbers of arthropods found on the different varieties by means of sweeping (excluding Diptera and Hymenoptera) in the 2003/04 biodiversity trial.

Summary data (Common or family names)	NuOPAL RR	NuOPAL	DeltaOPAL RR	DeltaOPAL
Dragonflies	1	0	0	0
African (“american”) bollworm eggs	1	0	0	0
African (“american”) bollworms	5	0	1	11
Red bollworm eggs	0	0	0	1
Red bollworms	0	0	0	0
Spiny bollworm eggs	0	0	0	0
Spiny bollworms	0	0	1	0
African (“american” bollworm moths	0	0	0	0
Acraeidae (unid. moth).	0	0	1	0
Leaf eaters (loopers)	0	2	0	4
Aphids	12	8	0	15
Leafhoppers	19	3	13	26
Whitefly	14	9	7	23
Thrips	9	3	2	6
Dusky cotton stainers	3	0	0	0
Common cotton stainers	0	0	1	0
Green vegetable stinkbug	0	4	1	2
Spittle bugs	1	8	0	1
Scuttelleridae (Shield backed bug)	0	9	9	2
Small black hemipterans (unid.)	11	2	0	11
Minute pirate bug	1	0	0	0
Predatory bug	0	0	3	0
Small brown hemipterans (unid.)	17	0	0	8
Unid. Bugs (Lygus spp.?)	0	0	0	2
Stinkbugs	0	0	0	2
Fulgoridae	0	0	0	1
Predatory thrips	5	0	0	1
Wax lice	0	0	21	1
Grasshoppers	0	17	5	7
Cricket (Gryllidae)	0	1	0	0
Pamphagidae	0	0	1	0
Ladybird eggs	2	0	9	0
Ladybird larvae	12	3	1	14
Ladybird pupae	0	0	0	0
Ladybird adult	32	16	0	24
Lacewing eggs	0	10	31	2
Lacewing larvae	2	4	2	2
Lacewing pupae	0	0	0	1
Lacewing adults	9	1	0	3
Spotted maize beetles	42	0	1	18
Flea beetles (Nisotra spp.)	18	14	14	58
Coleoptera (unidentified)	4	0	2	7
Click-beetles	0	0	0	1

(table continued)				
Lagriidae	0	0	0	1
Black cotton beetles	0	1	0	0
Mordellidae (unid. Coleoptera)	1	0	0	1
Anthicidae (unid. Coleoptera)	25	47	29	24
Nitidulidae (unid. Coleoptera)	6	13	4	7
Flea beetles (unid. Chrysomelidae)	15	17	5	1
Braconidae (unid. Hymenoptera)	20	57	19	8
Egg parasitic wasps (unid. Hymenoptera)	5	0	22	1
Braconidae (unid. Hymentoptera)	0	8	0	1
Ichneumonidae (unid. Hymenoptera)	1	0	1	6
Ants (unid. Formicidae)	1	0	0	0
Earwigs (unid. Dermaptera)	2	0	0	0
Flies (unid. Diptera)	0		8	10
Long-legged mosquitoes (unid.Tipulidae)	0	0	0	1
Mosquitoes (Culex spp.?)	4	0	2	53
Midges (unid. Chironomidae)	0	3	0	64
Hoverfly larvae (Syrphidae)	1	2	5	2
Hoverfly adults (Syrphidae)	0	0	0	0
Red spider mites	0	3	8	2
Spiders	40	1	8	12
Spider egg packages	0	7	8	5
Predatory mites	0	0	3	0
Total	366	319	254	478

Table 3.19. Total number of arthropods found by sweeping per variety (excluding Diptera and Hymenoptera) in the 2003/04 biodiversity trial.

Important arthropod orders	NuOPAL RR	NuOPAL	DeltaOPAL RR	DeltaOPAL
Lepidoptera	7	0	2	12
Hemiptera* (Includes aphids, leafhoppers, whitefly)	111	66	48	122
Coleoptera	168	125	98	165
Thysanoptera	9	3	2	6
Arachnids (spiders)	40	1	8	12

Table 3.20. Total number of other arthropods over the observation period under sprayed and unsprayed conditions in the 2004/05 biodiversity trial.

Totals	Delta OPAL (3 endosulfan sprays; 1 acetamiprid spray)	Delta OPAL (Unsprayed)	DeltaOPAL RR (3 endosulfan sprays; 1 acetamiprid spray)	DeltaOPAL RR (Unsprayed)	NuOPAL (1 acetamiprid spray)	NuOPAL (Unsprayed)	NuOPAL RR (1 acetamiprid spray)	NuOPAL RR (Unsprayed)
Red spider mite	98	105	84	95	93	84	85	85
Spodoptera	15	0	2	2	2	3	0	2
Spotted maize beetle	175	201	217	205	313	284	239	257
Nisotra beetle	1	0	1	0	0	0	0	0
Anthicidae	3	6	7	1	2	4	4	3
Honeybee	3	9	27	13	10	9	10	14
Looper	2	6	2	5	0	1	0	0
Looper eggs	3	0	0	0	0	0	0	0
Staphylinidae	13	12	14	19	10	7	13	8
Nitidulidae	2	1	0	1	5	1	6	0
Flea beetles	0	3	2	3	5	2	3	2
Ants	2	7	17	4	5	8	9	7
Miridae	71	98	107	107	95	114	147	115
Lagriidae	2	2	3	1	1	0	5	11
Click beetle	1	0	0	0	1	0	0	0
Fruit chafer	0	1	9	0	0	3	0	0
Tip wilter	2	0	5	3	3	2	0	0
Lygus bug	14	50	20	66	37	59	47	42
Ephemeroptera	1	0	5	0	0	1	0	3
Lyriomyza	1	5	2	0	4	4	5	0
Nezara egg packet	1	0	18	0	0	0	64	0
Brown leafhopper	30	0	0	0	0	4	0	0
Hemipteran eggs?	30	69	63	0	15	16	25	25
Chironomidae	2	1	11	0	0	10	2	0
Chrysomelidae	5	10	6	5	7	6	2	7
Gryllidae	0	0	0	0	0	0	3	0
Assassin bug	0	1	1	0	1	0	1	0
Coccinellidae?	1	0	0	0	0	0	1	2
Mordellidae	0	1	1	1	0	3	0	0
Calliphoridae (Diptera)	1	0	0	0	0	0	0	0
Pyrghomorphidae	3	0	2	0	1	0	1	1
Tipulidae	3	0	0	0	0	0	2	1
Curculionidae	2	1	1	1	0	0	3	2
Chrysomelidae	0	2	0	2	1	2	4	3
Sphecidae	0	3	1	4	3	0	5	6
Chironomidae	5	6	4	12	6	3	8	8
Moth unid.(?)	0	5	2	8	7	1	7	7
Pieridae?	0	6	3	10	9	1	8	9
Meloidae	0	7	4	12	11	3	9	11
Totals	492	618	641	580	647	635	718	631

Table 3.21. Matrix indicating highest treatments with statistical significance in all three statistical comparisons in the biodiversity of the 2004/05 trial.

Arthropod category	Treatments	Significant highest number present							Score Total
		Count 1[$]	Count 2	Count 3	Count 4[$]	Count 5	Count 6	Count 7	
Bollworms	DeltaOPAL sprayed				X	X	X	X	4
	DeltaOPAL unsprayed					X	X	X	3
	DeltaOPAL RR sprayed								
	DeltaOPAL RR unsprayed				X	X		X	3
	NuOPAL sprayed								
	NuOPAL unsprayed								
	NuOPAL RR sprayed								
	NuOPAL RR unsprayed								
Secondary Pests	DeltaOPAL sprayed				X				1
	DeltaOPAL unsprayed		X					XX	3
	DeltaOPAL RR sprayed								
	DeltaOPAL RR unsprayed							X	1
	NuOPAL sprayed								
	NuOPAL unsprayed		X					XXX	4
	NuOPAL RR sprayed		X					X	2
	NuOPAL RR unsprayed							XXXX	4
Predators	DeltaOPAL sprayed					X	XXXXXXXX		9
	DeltaOPAL unsprayed		XX	X			X		4
	DeltaOPAL RR sprayed			XX					2
	DeltaOPAL RR unsprayed		XX	X			X	X	5
	NuOPAL sprayed		XXXXX	X		XXXX	X		10
	NuOPAL unsprayed			X			X	X	3
	NuOPAL RR sprayed	X	XX	XXX		X	X		8
	NuOPAL RR unsprayed	XX	XX			XXXXX			9

(1) Significant incidence of secondary pests and predators on all Genetically Modified cotton types (excluding bollworm incidences): DeltaOPAL RR sprayed – 10; unsprayed -8; NuOPAL sprayed –10; unsprayed -7; NuOPAL RR sprayed -8; unsprayed -9;
(2) Significant incidence of secondary pests and predators on all DeltaOPAL (excluding bollworms): DeltaOPAL sprayed –9; unsprayed –9.

Figures

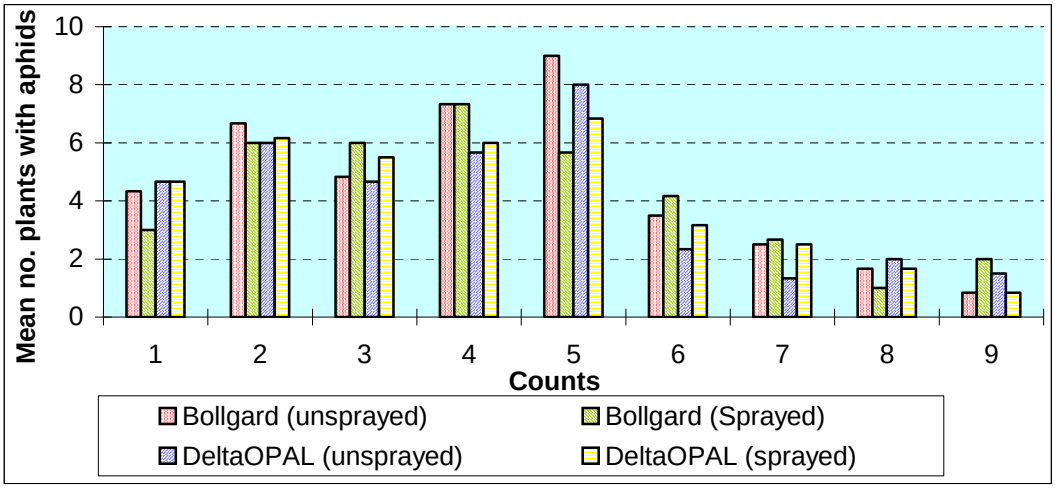


Fig. 3.1. The mean number of plants with aphids in Rustenburg (1997/1998) in the Cry1Ac trial.

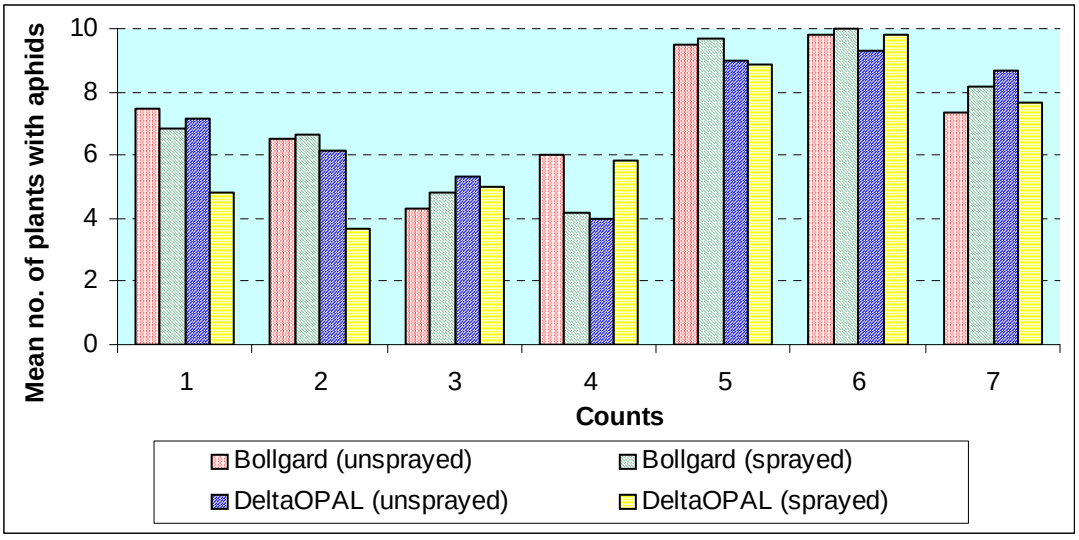


Fig. 3.2. The mean number of plants with aphids in Potgietersrus (1997/1998) in the Cry1Ac trial. (1998).

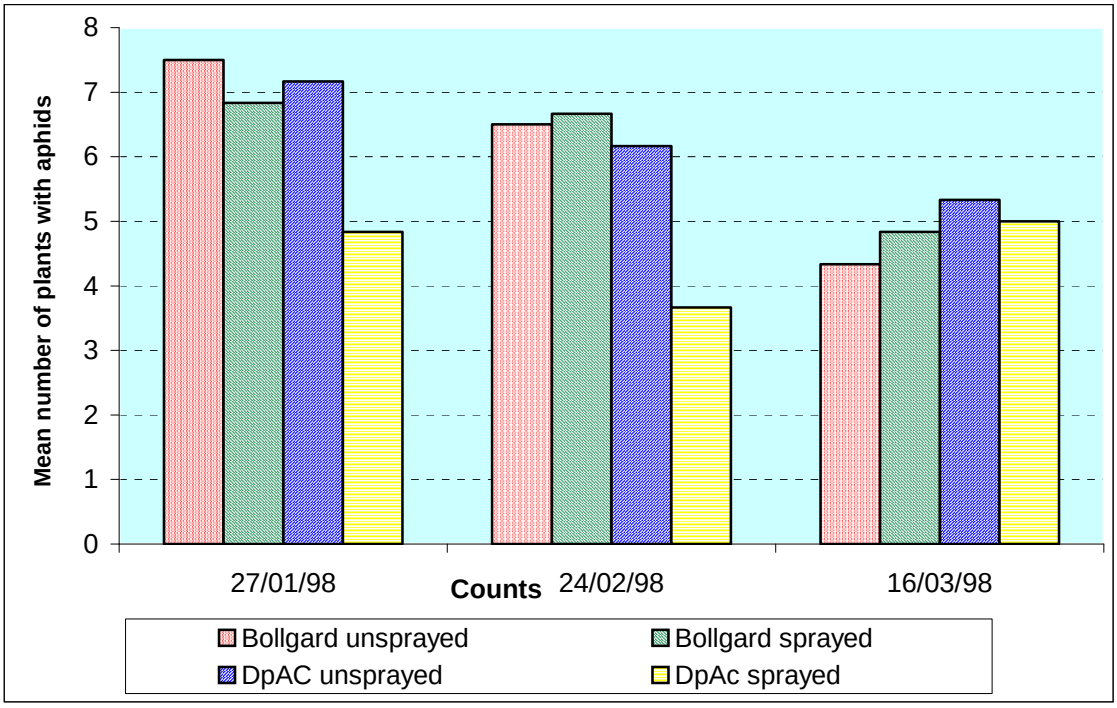


Fig. 3.3. The mean number of plants with aphids in Groblersdal (Loskop) (1998/1999) in the Cry1Ac trial.

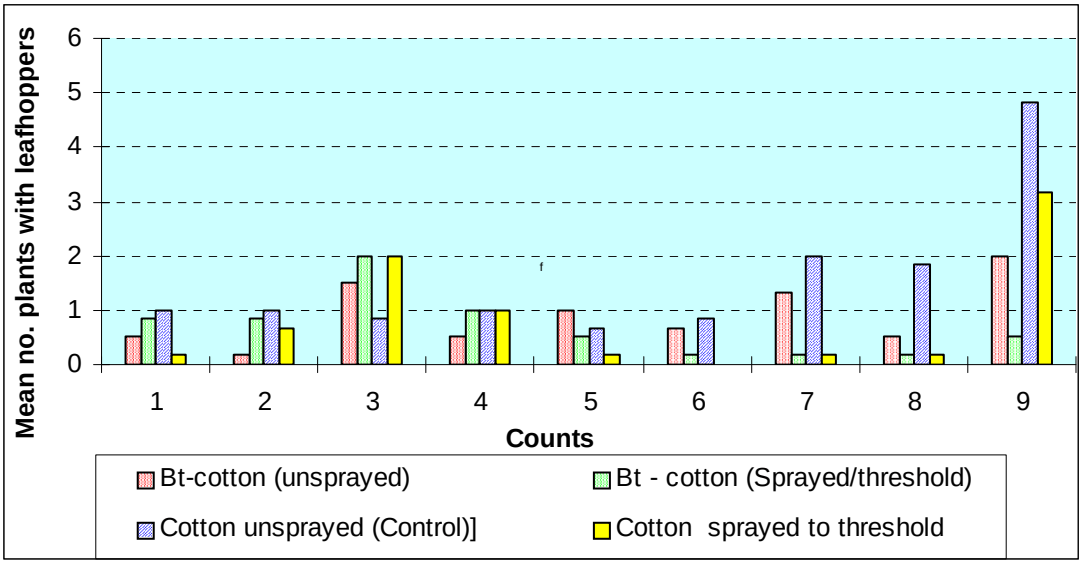


Fig. 3.4. The mean number of plants with leafhoppers in Rustenburg (1997/1998) in the Cry1Ac trial.

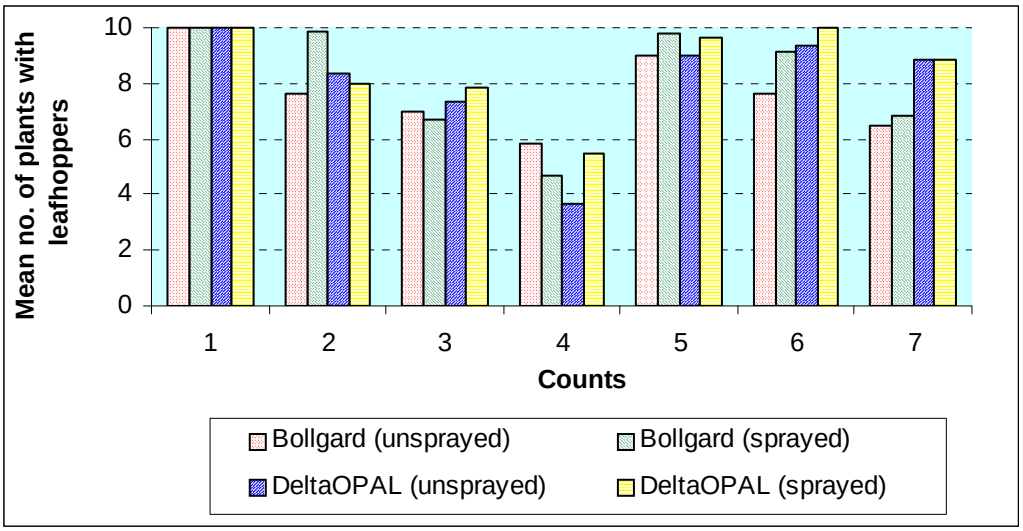


Fig. 3.5. The mean number of plants with leafhoppers in Potgietersrus (1997/1998) in the Cry1Ac trial.

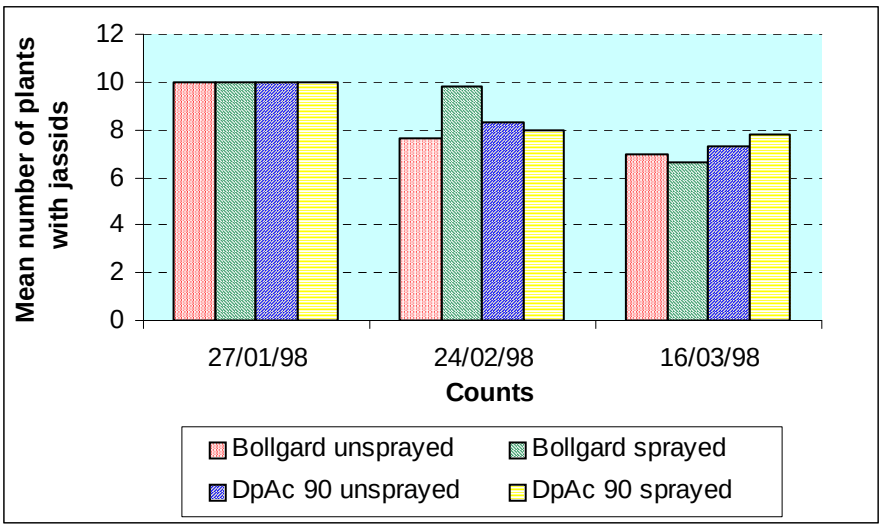


Fig. 3.6. The mean number of plants with leafhoppers in Loskop (Groblersdal) (1998/1999) in the Cry1Ac trial.

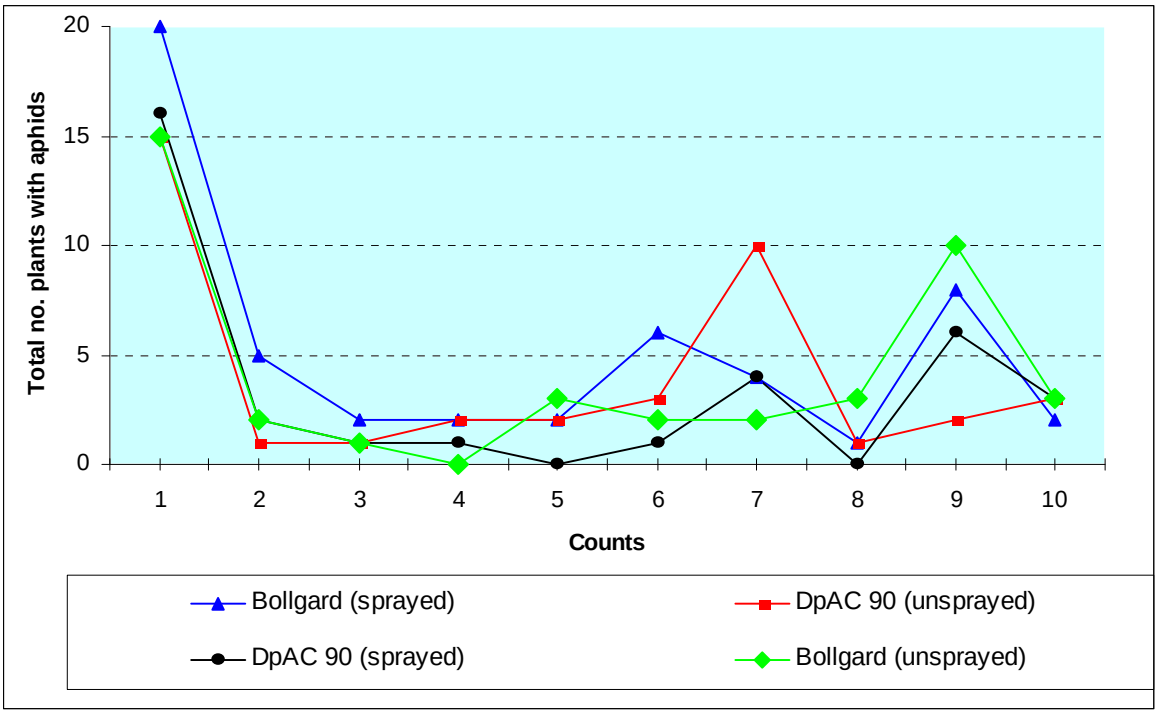


Fig. 3.7. The total number of plants with aphids in (Loskop) (1998/1999) in the Cry1Ac trial.

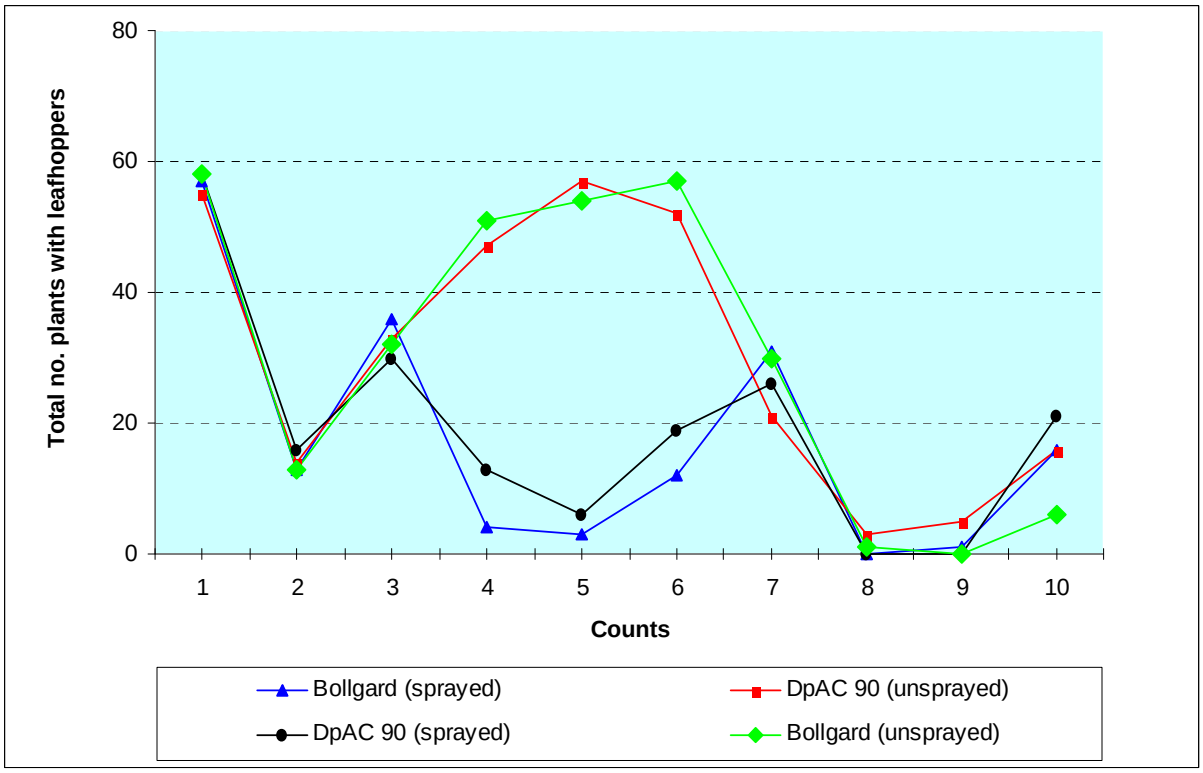


Fig. 3.8. The total number of plants with leafhoppers in (Loskop) (1998/1999) in the Cry1Ac trial.

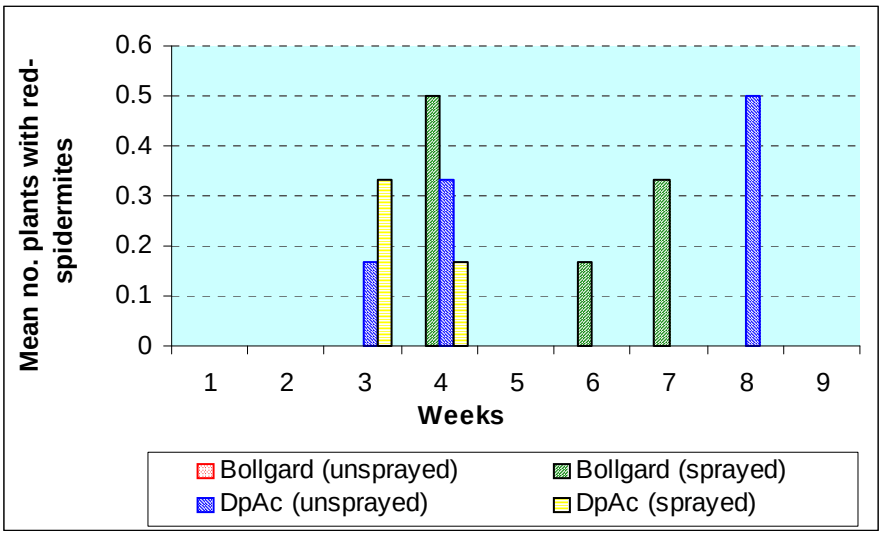


Fig. 3.9. Spider mites on the different treatments in Rustenburg (1998).

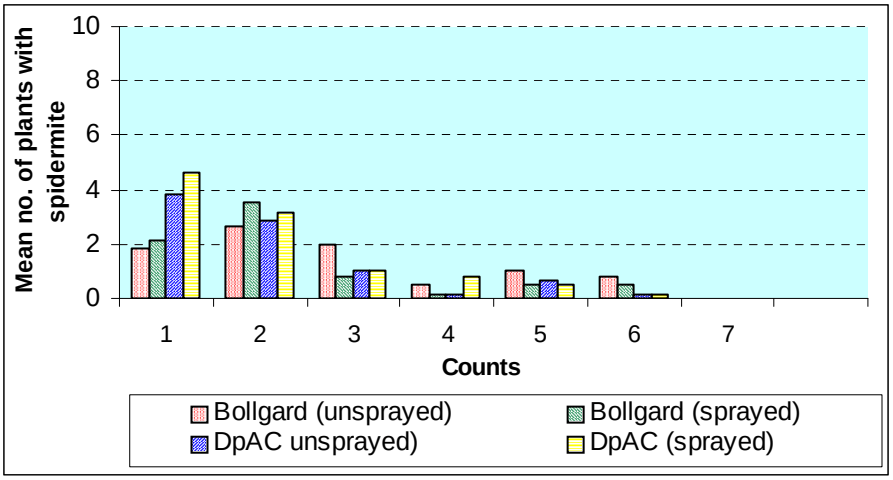


Fig. 3.10. Spider mites on the different treatments in Potgietersrus (1997/1998) in the Cry1Ac trial.

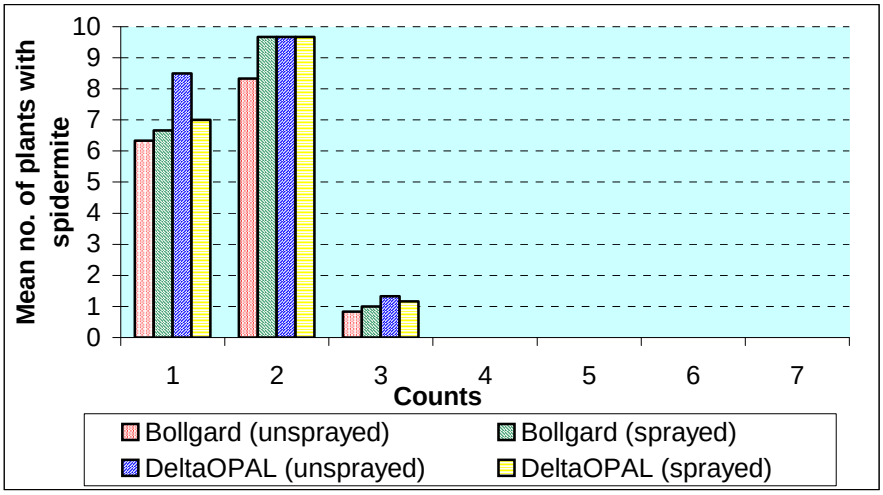


Fig. 3.11. Spider mites on the different treatments in Loskop (Groblersdal) (1998/1999) in the Cry1Ac trial.

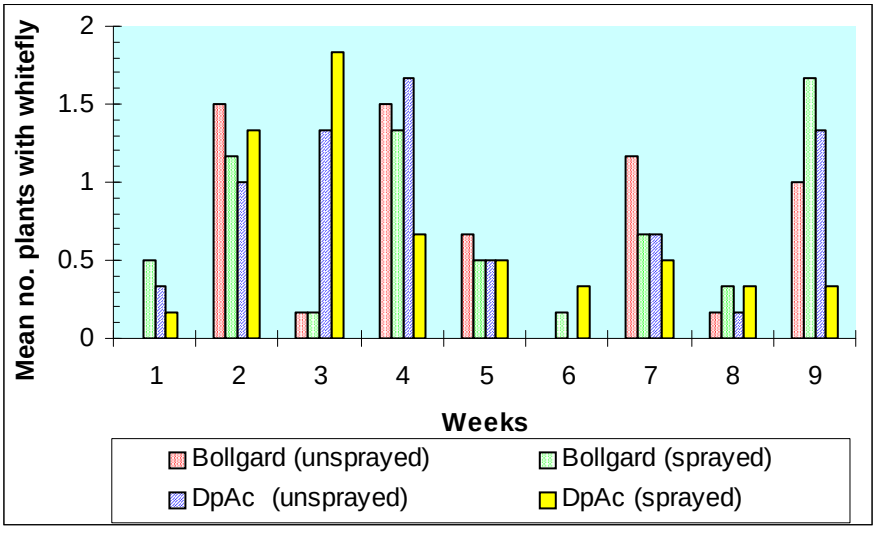


Fig. 3.12. Whitefly in Rustenburg (1997/1998) in the Cry1Ac trial.

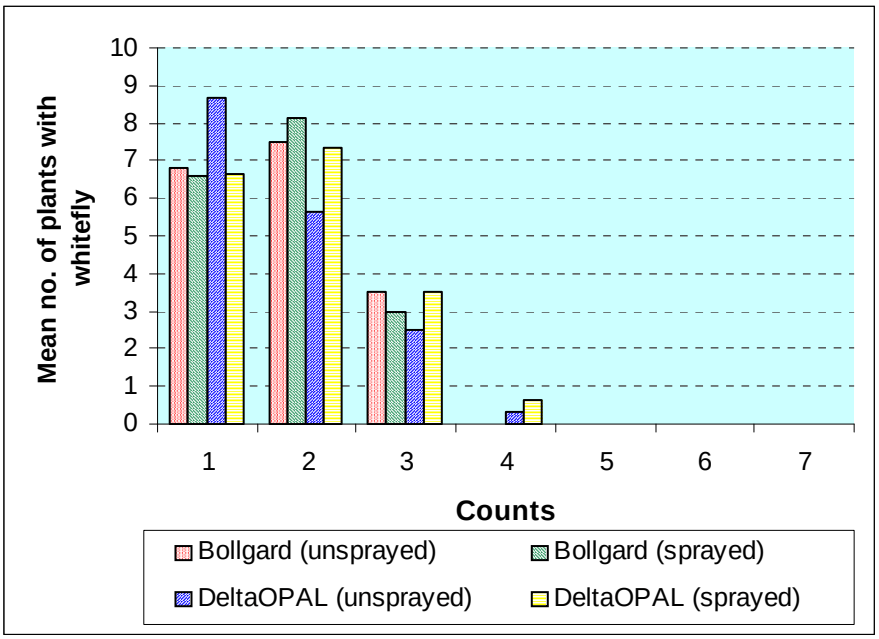


Fig. 3.13. Whitefly in Potgietersrus (1998).

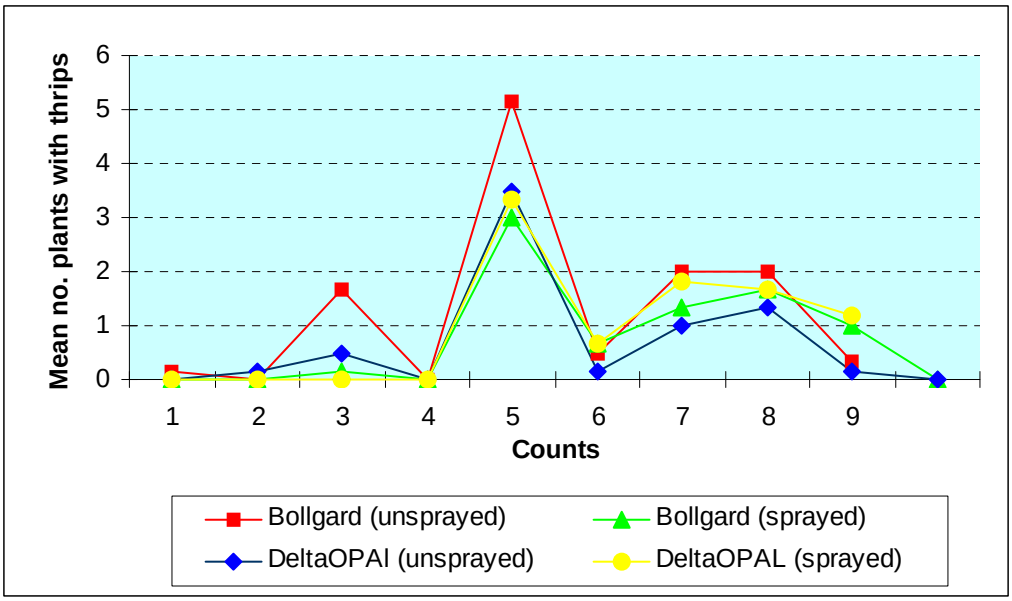


Fig. 3.14. Thrips in Rustenburg (1998).

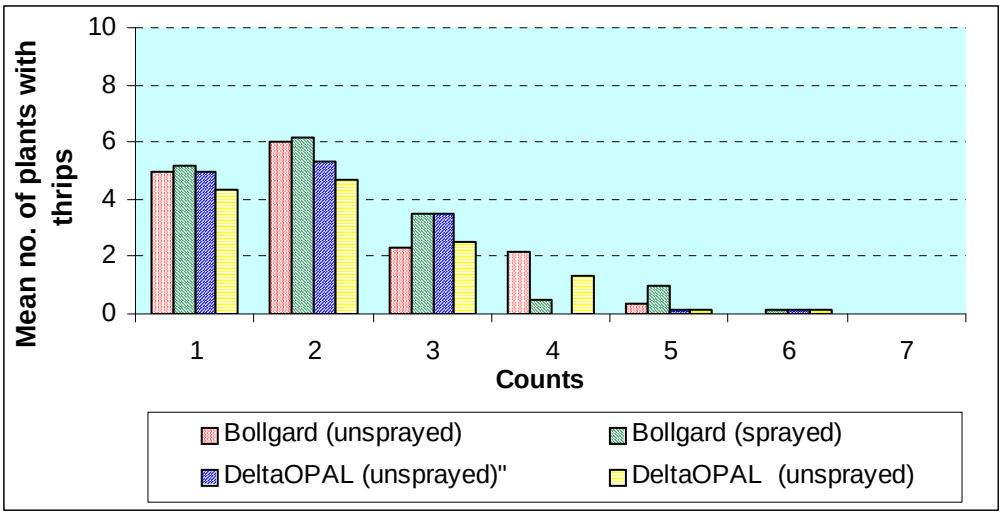


Fig. 3.15. Thrips in Potgietersrus (1998) in the Cry1Ac trial.

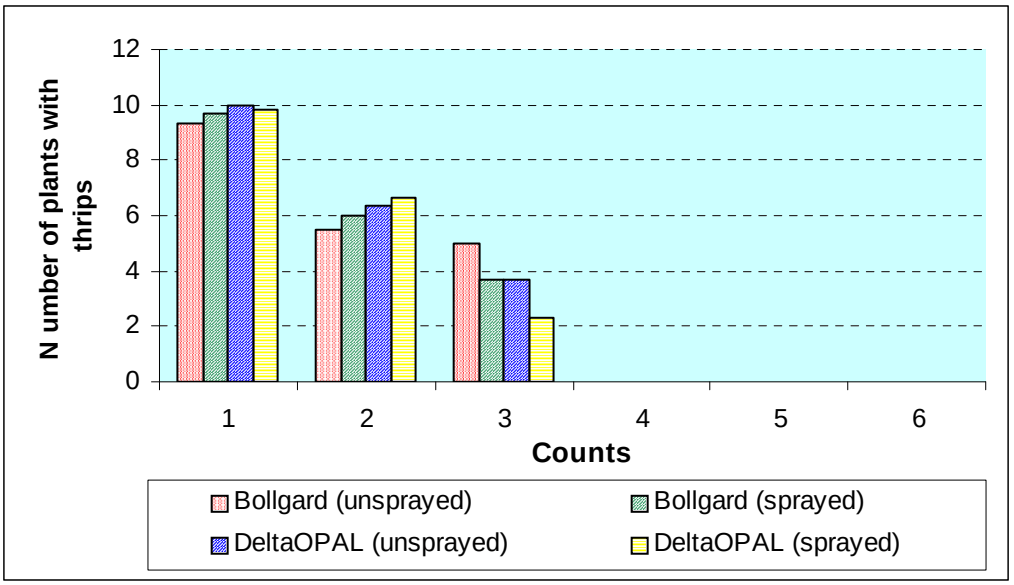


Fig. 3.16. Thrips in Loskop (Groblersdal) (1998/1999) in the Cry1Ac trail.

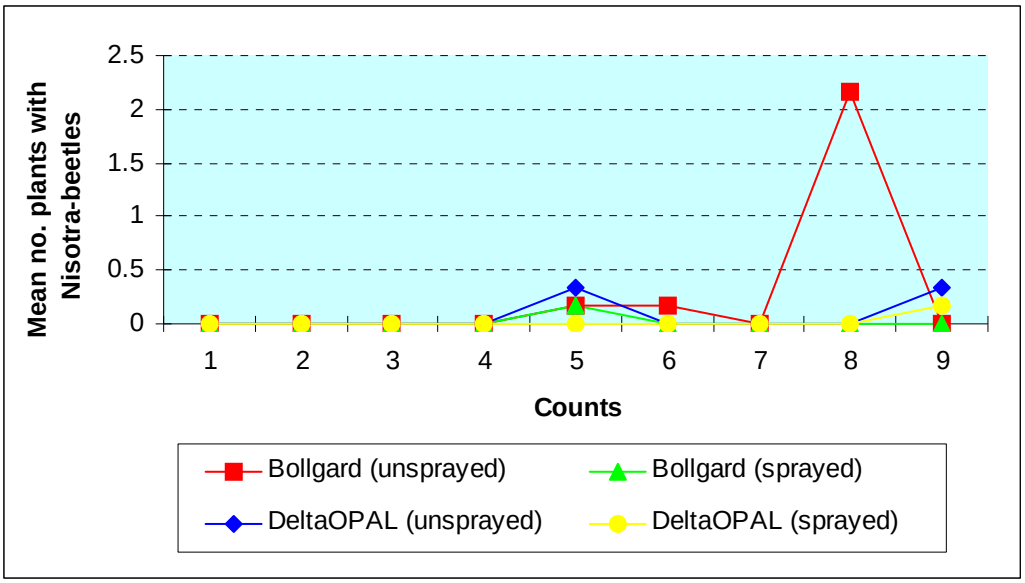


Fig. 3.17. Nisotra spp. in Rustenburg (1997/1998) in the Cry1Ac trial.

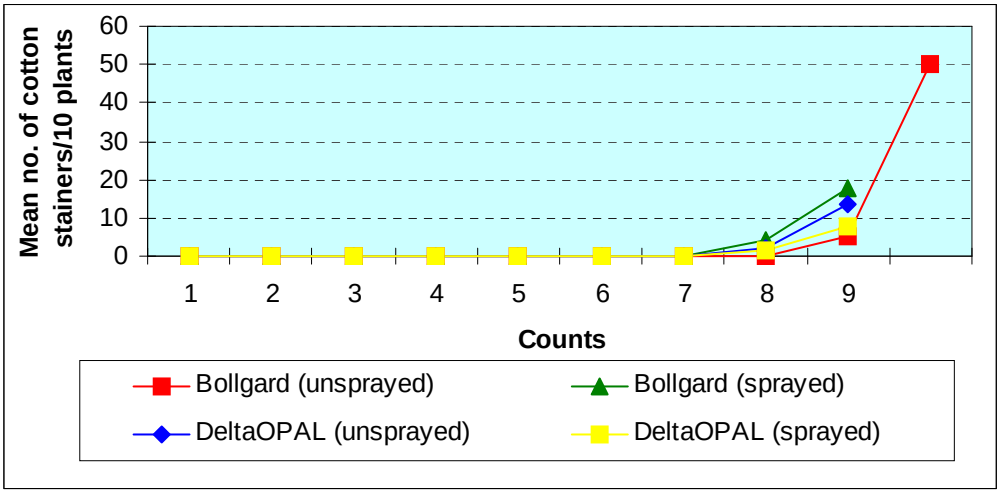


Fig. 3.18. Cotton stainers in Rustenburg (1997/1998) in the Cry1Ac trial.

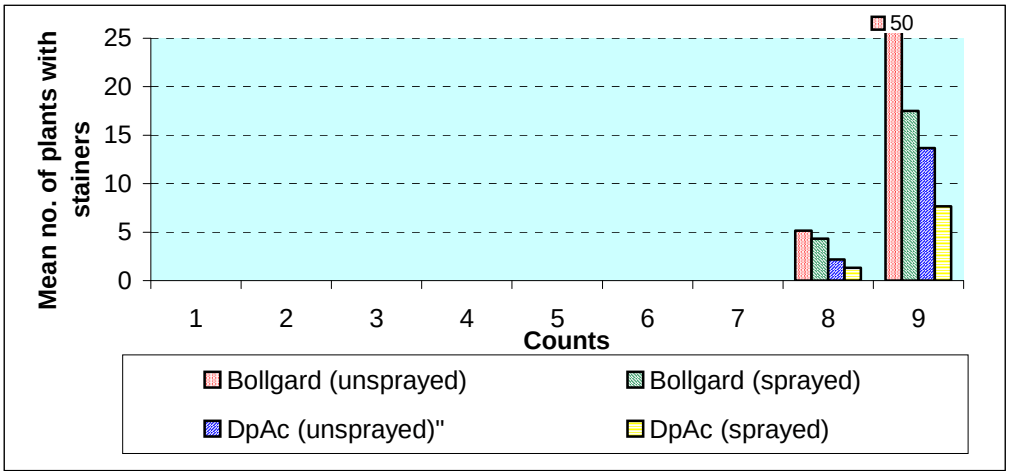


Fig. 3.19. Cotton stainers in Potgietersrus (1997/1998) in the Cry1Ac trail.

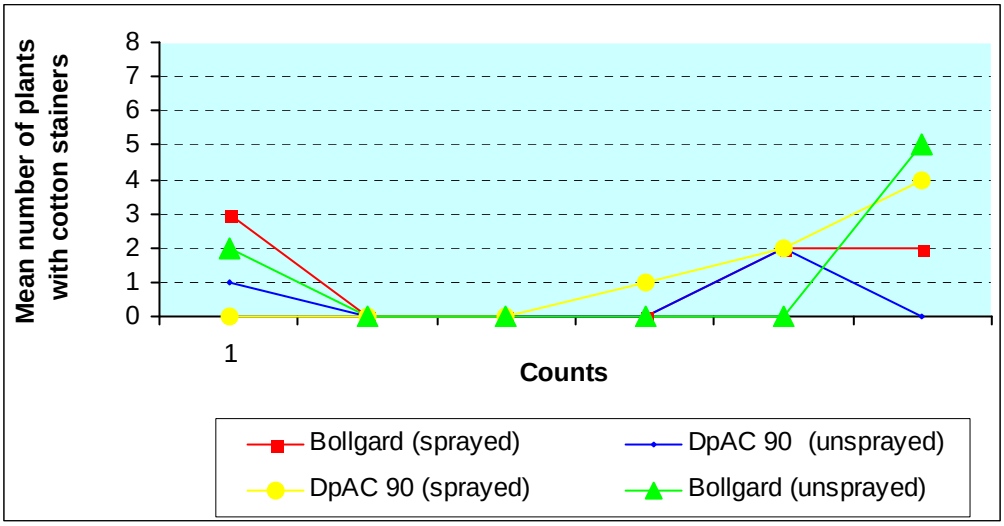


Fig. 3.20. Cotton Stainers in Loskop (Groblersdal) (1998/1999) in the Cry1Ac trial.

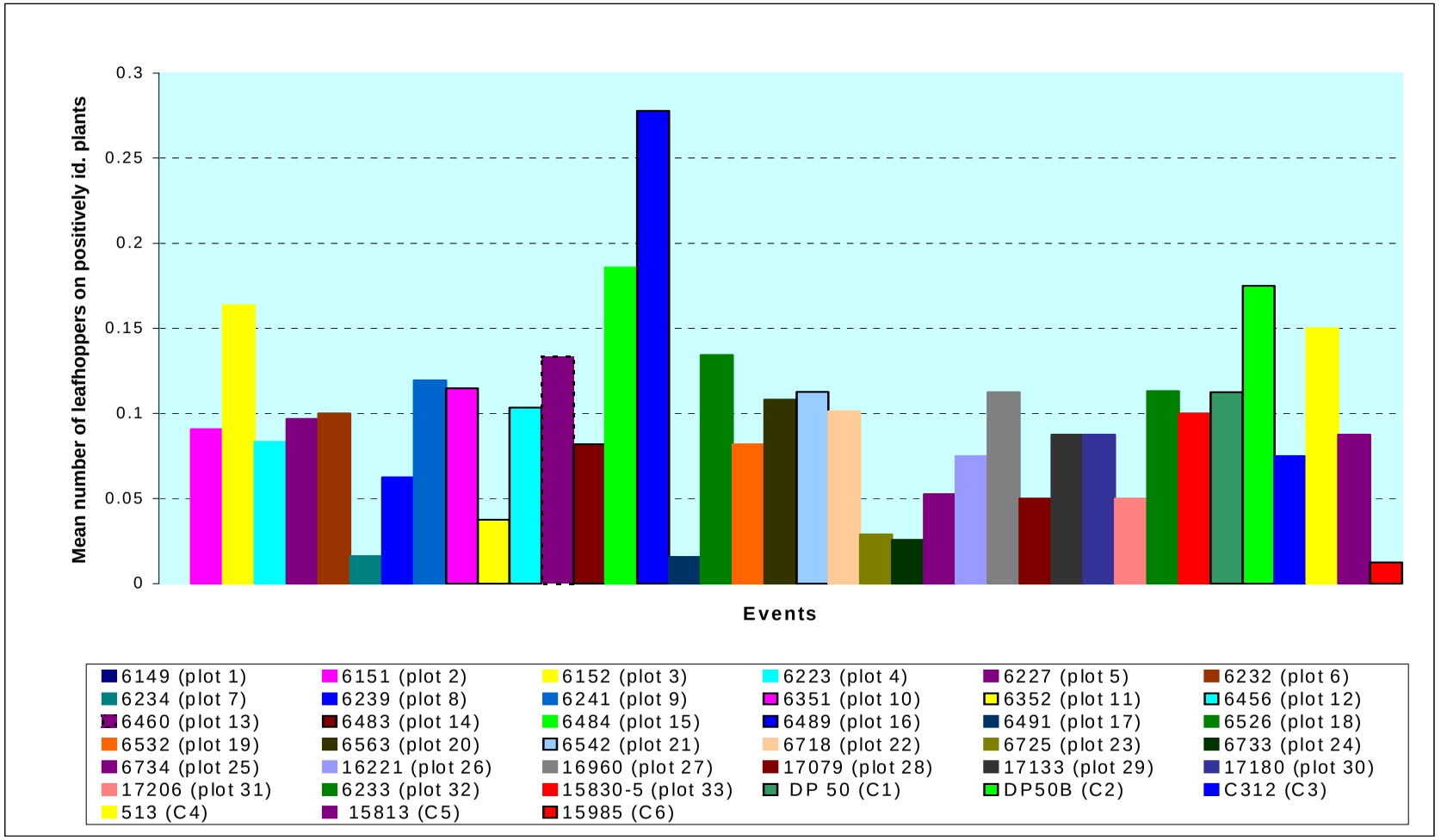


Fig. 3.21. Mean number of plants with leafhoppers between events, on positively identified transgenic plants in the P2B event trial.

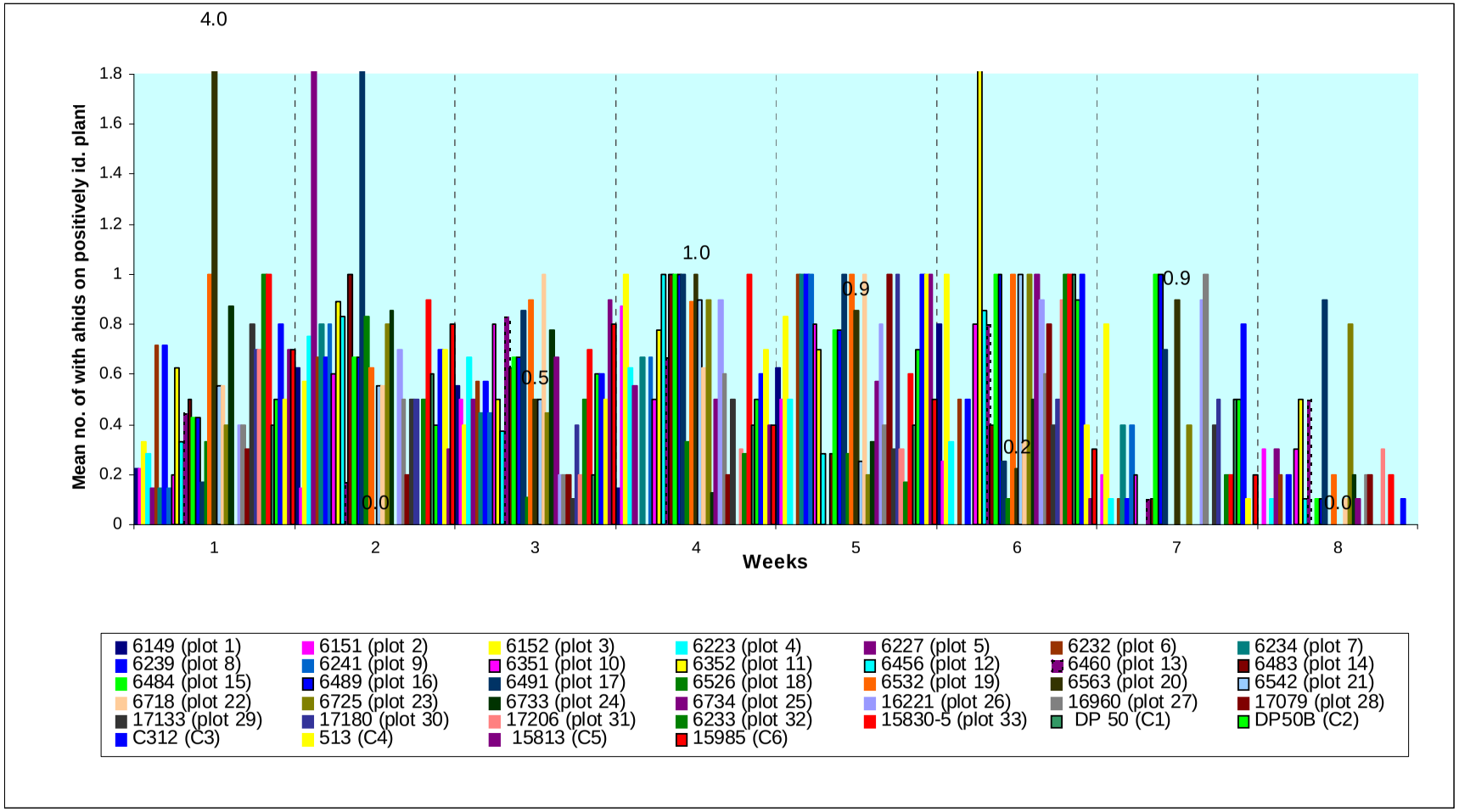


Fig. 3.22. Total number of plants with aphids, on positively identified transgenic plants at different time intervals in the P2B event trial.

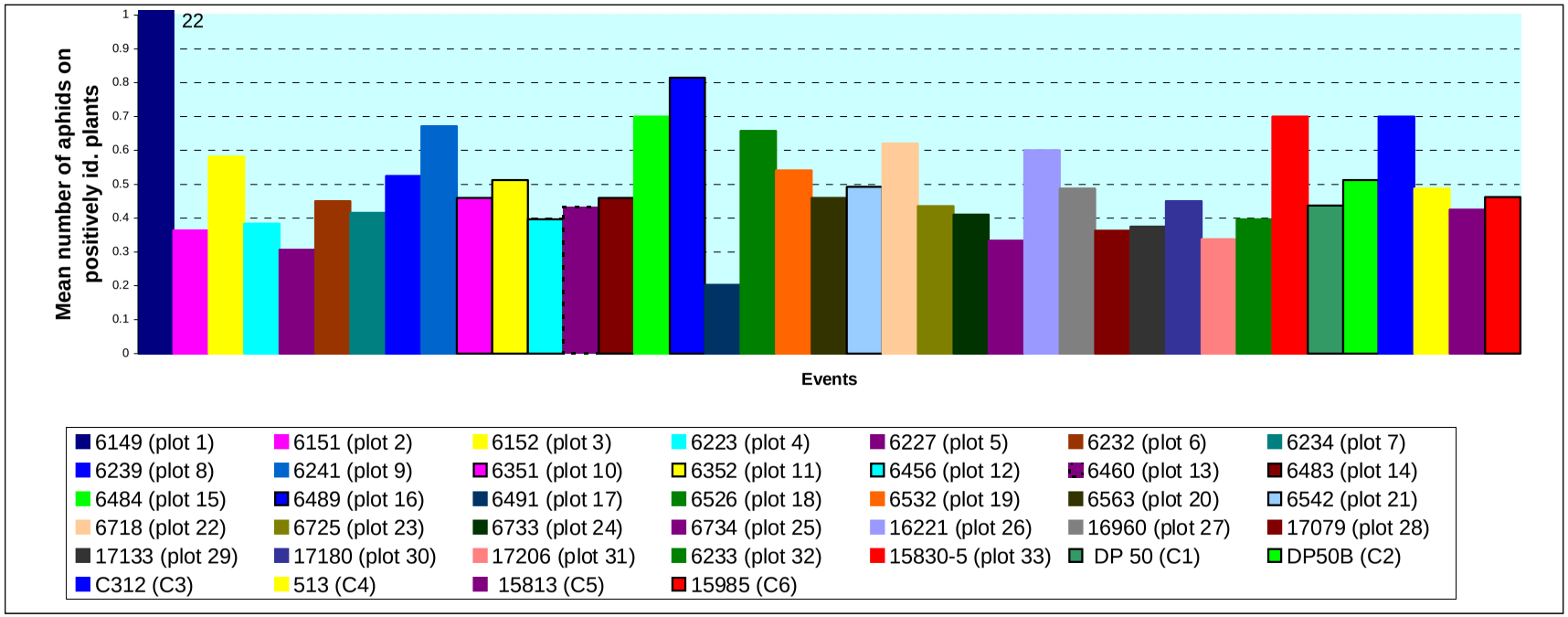


Fig. 3.23. Mean number of plants with aphids, on positively identified transgenic plants in the P2B event trial.

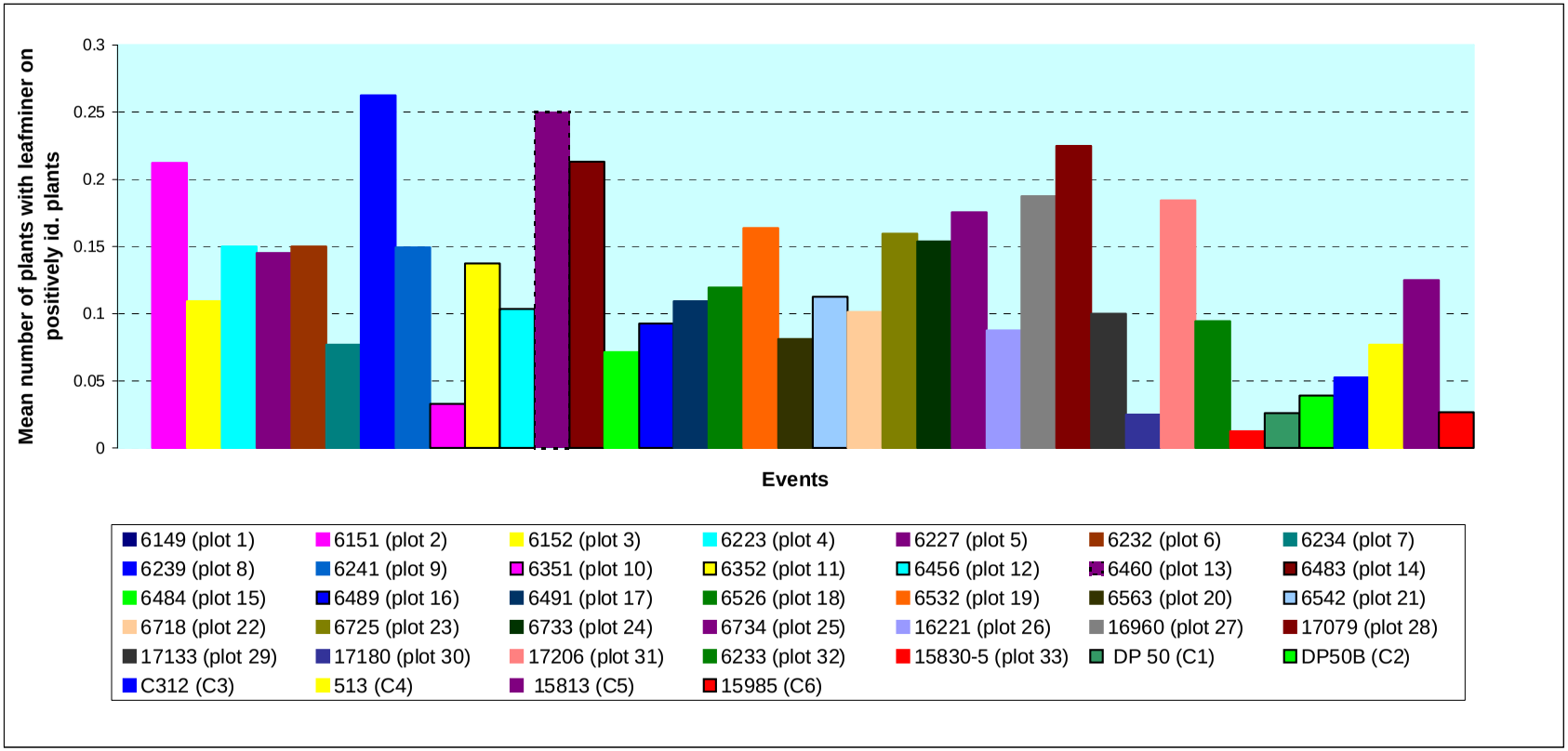


Fig. 3.24. Mean number of plants with leaf miner, on positively identified transgenic plants in the P2B event trial.

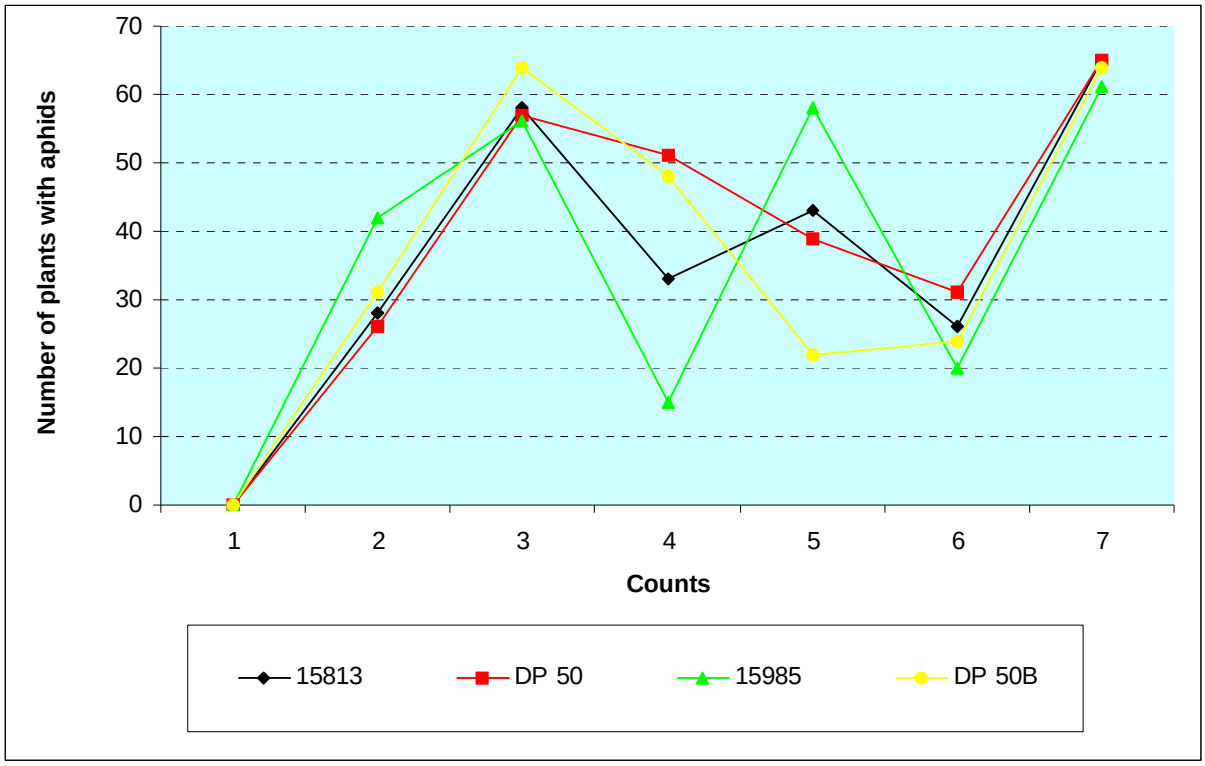


Fig. 3.25 The number of plants with aphids counted on 20 plants per treatment in the Cry2Ab2 trial.

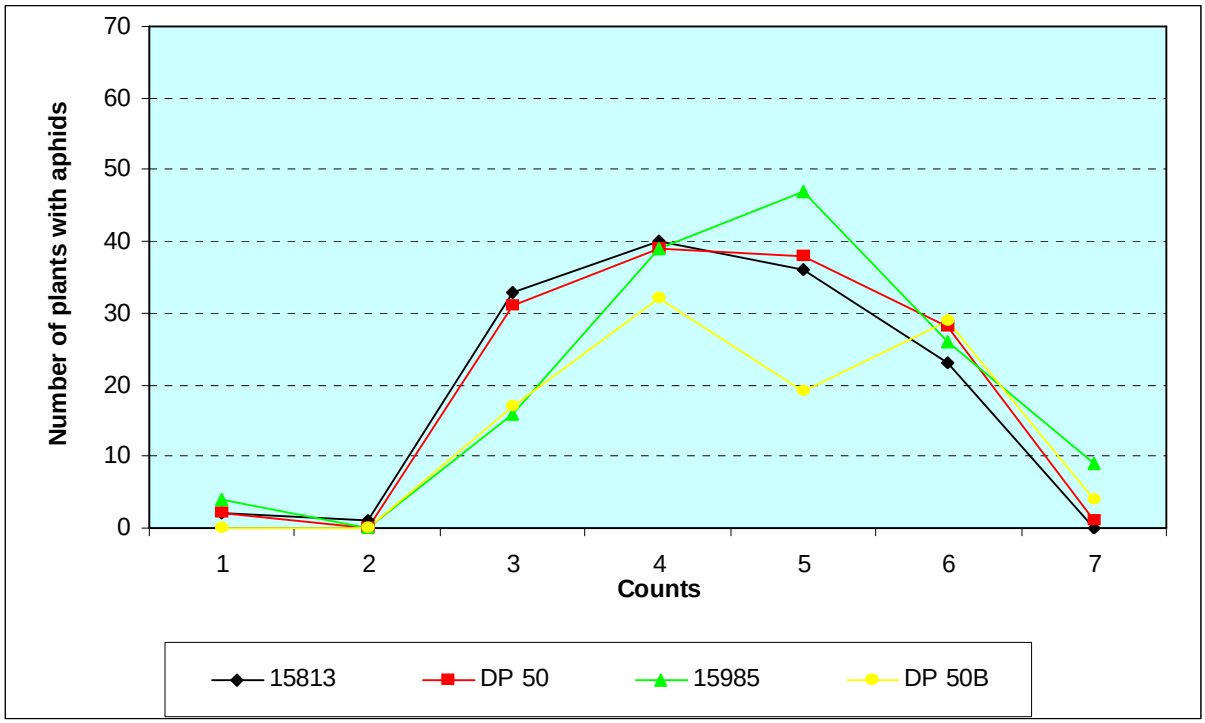


Fig. 3.26. The number of plants with aphids counted on 20 plants per plot in each treatment at Brits in the Cry2Ab2 trial.

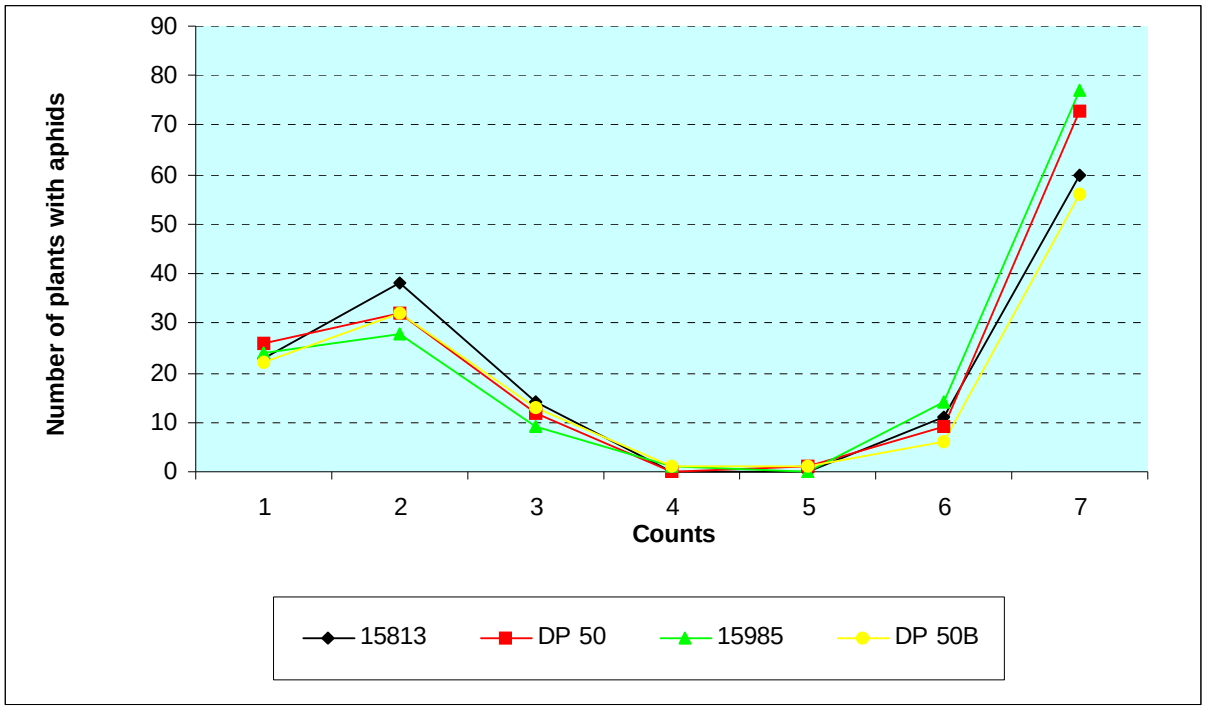


Fig. 3.27. The number of plants with aphids counted on 20 plants per plot in each treatment at Nelspruit in the Cry2Ab2 trial.

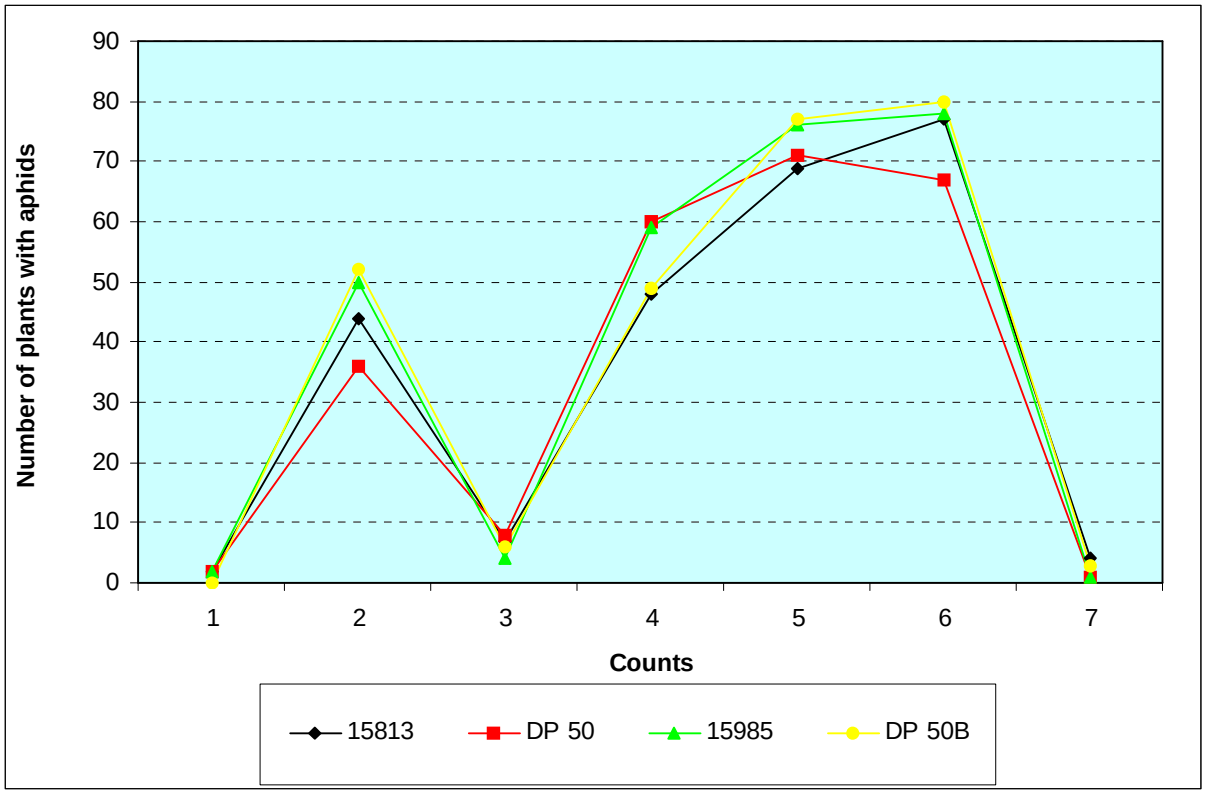


Fig. 3.28. The number of plants with aphids counted on 20 plants per plot in each treatment at Potgietersrus in the Cry2Ab2 trial.

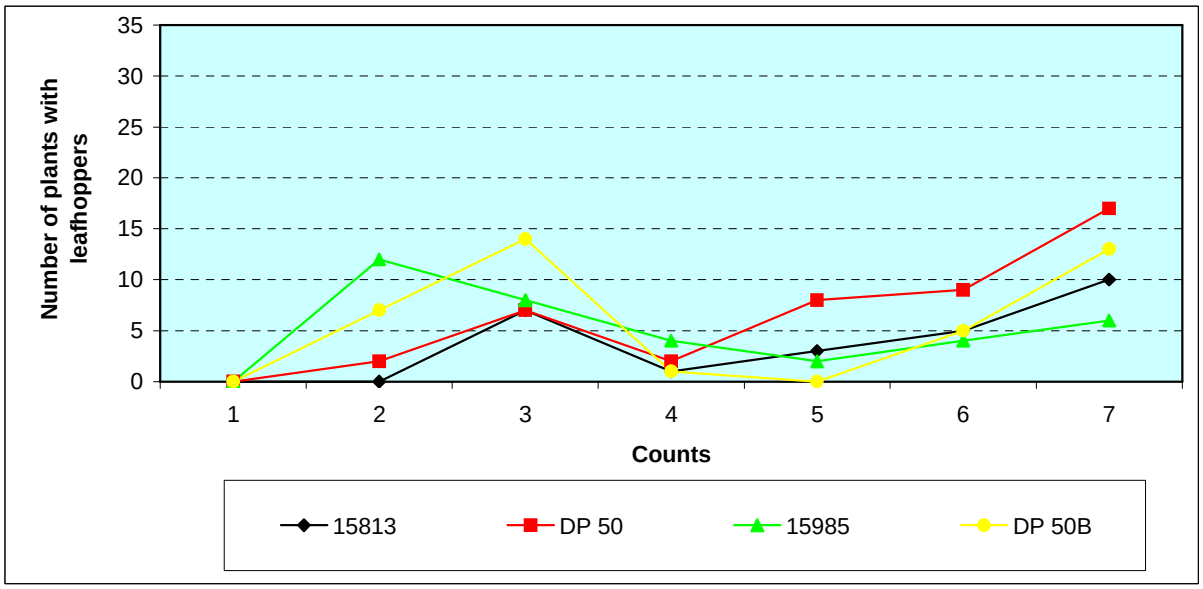


Fig. 3.29. The number of plants with leafhoppers counted on 20 per plot in each treatment at Rustenburg (arrows - insecticide applications) in the Cry2Ab2 trial.

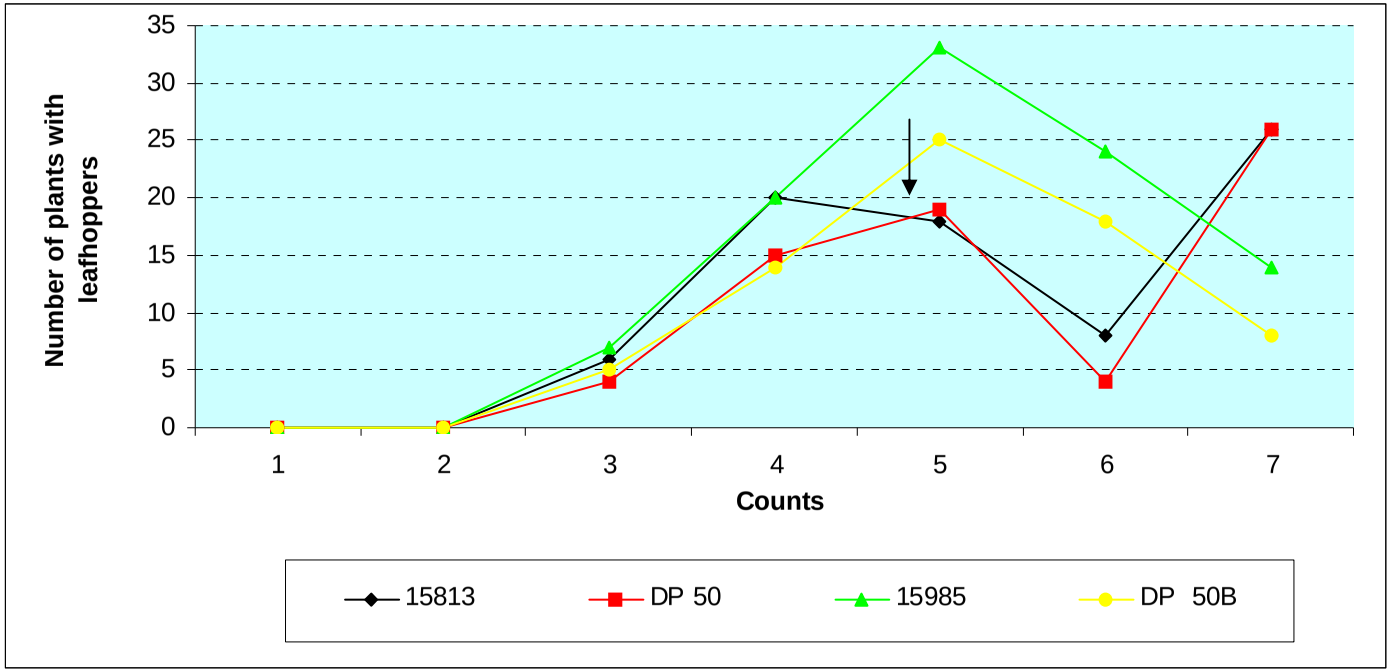


Fig. 3.30. The number of plants with leafhoppers counted on 20 plants per plot in each treatment at Brits (arrow - insecticide application) in the Cry2Ab2 trial.

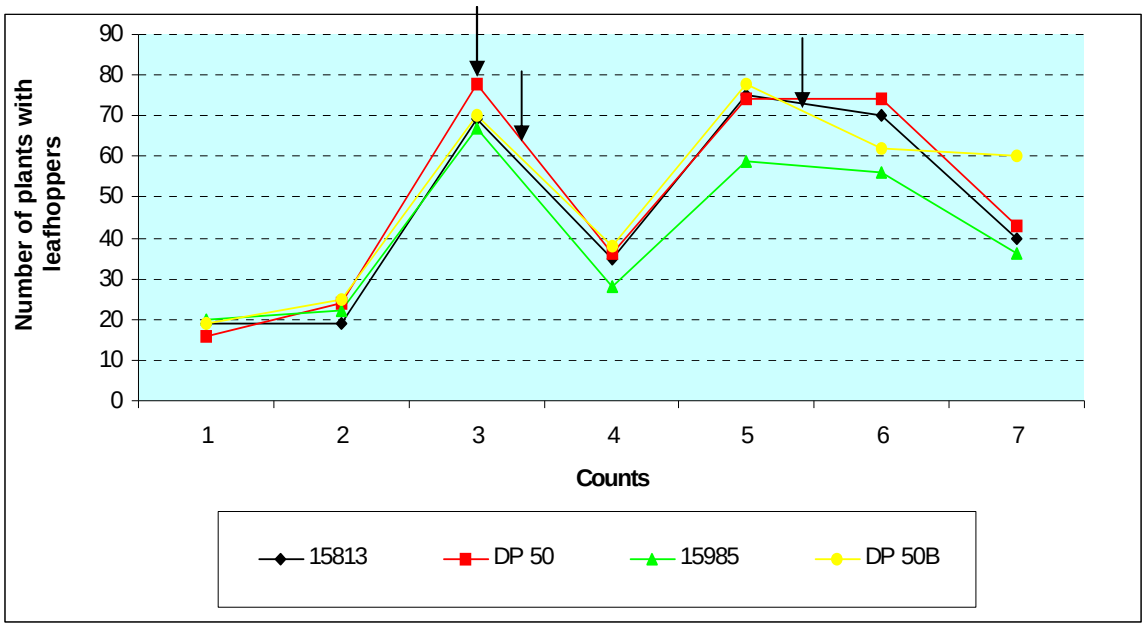


Fig. 3.31. The number of plants with leafhoppers counted on 20 plants per plot in each treatment at Nelspruit (arrows - insecticide applications) in the Cry2Ab2 trial.

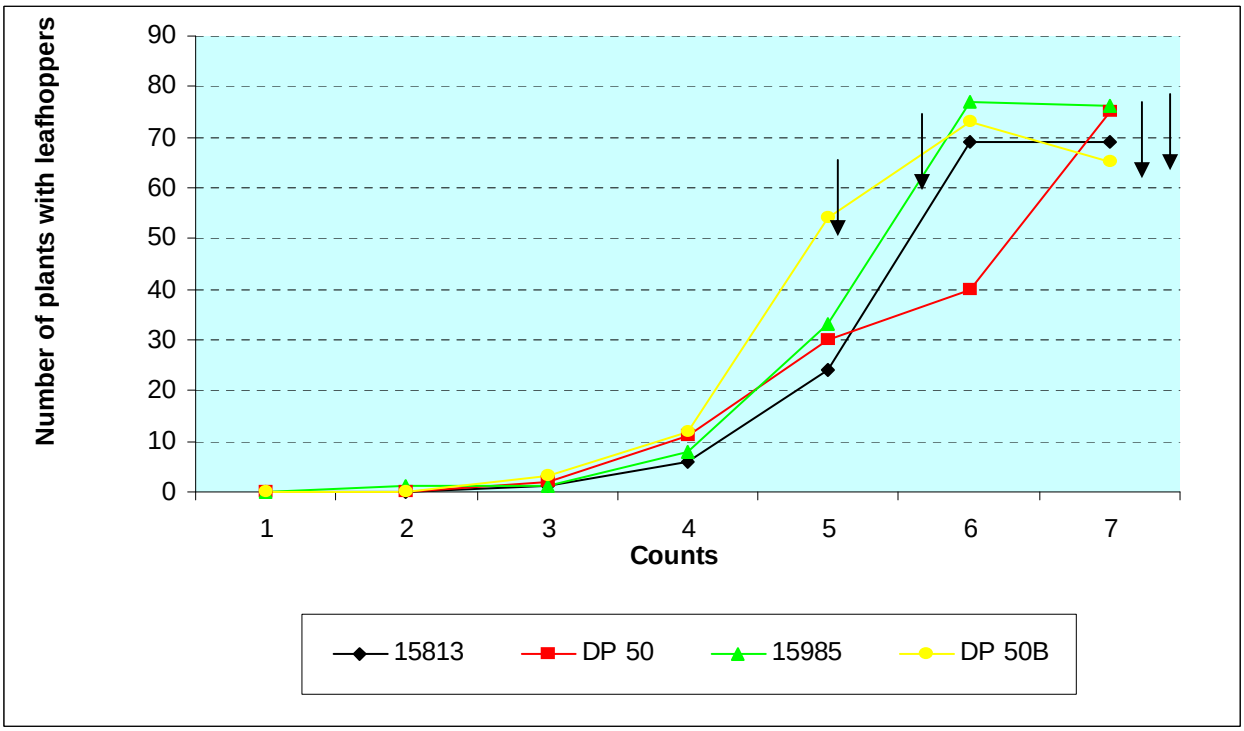


Fig. 3.32. The number of plants with leafhoppers counted on 20 plants per plot in each treatment at Potgietersrus (arrows - insecticide applications) in the Cry2Ab2 trial.

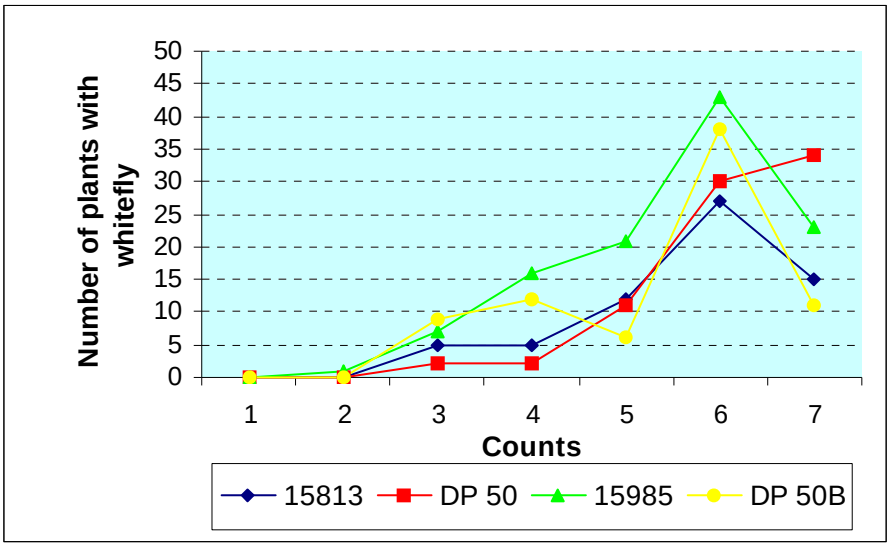


Fig. 3.33. Mean number of plants with whitefly from Rustenburg (1999) in the Cry2Ab2 trial.

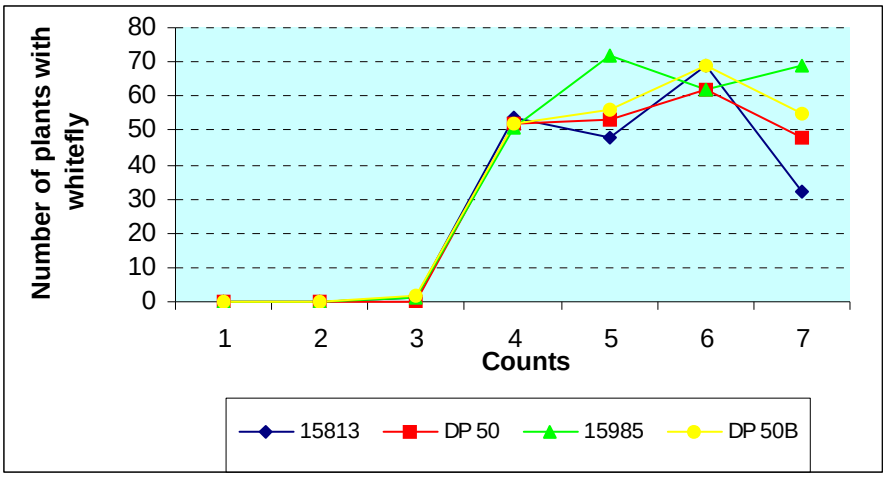


Fig. 3.34. Mean number of plants with whitefly from Brits (1999) in the Cry2Ab2 trial.

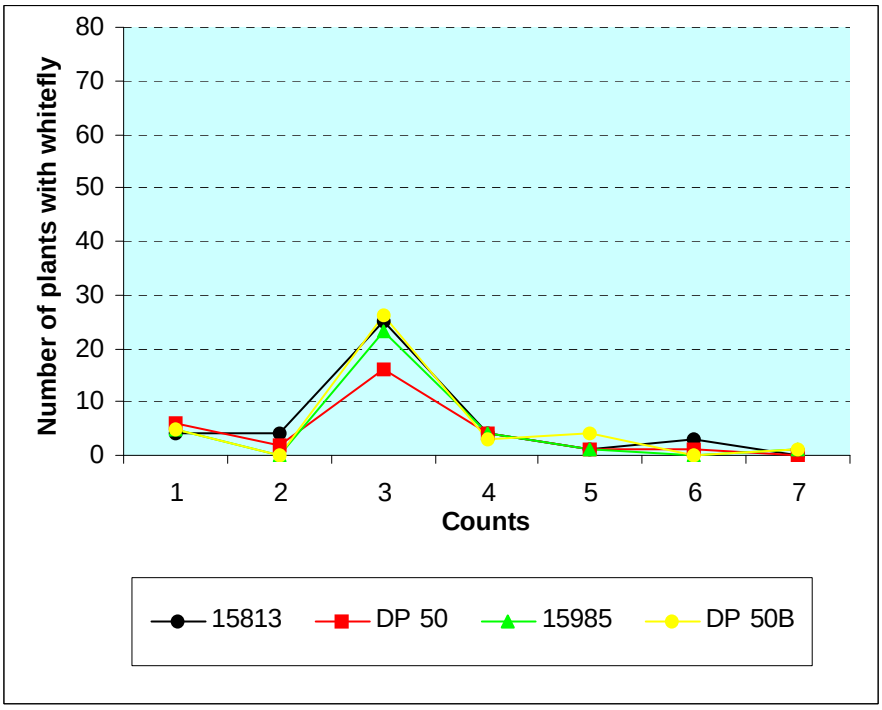


Fig. 3.35. Mean number of whitefly on 20 plants per plot in Nelspruit in the Cry2Ab2 trial.

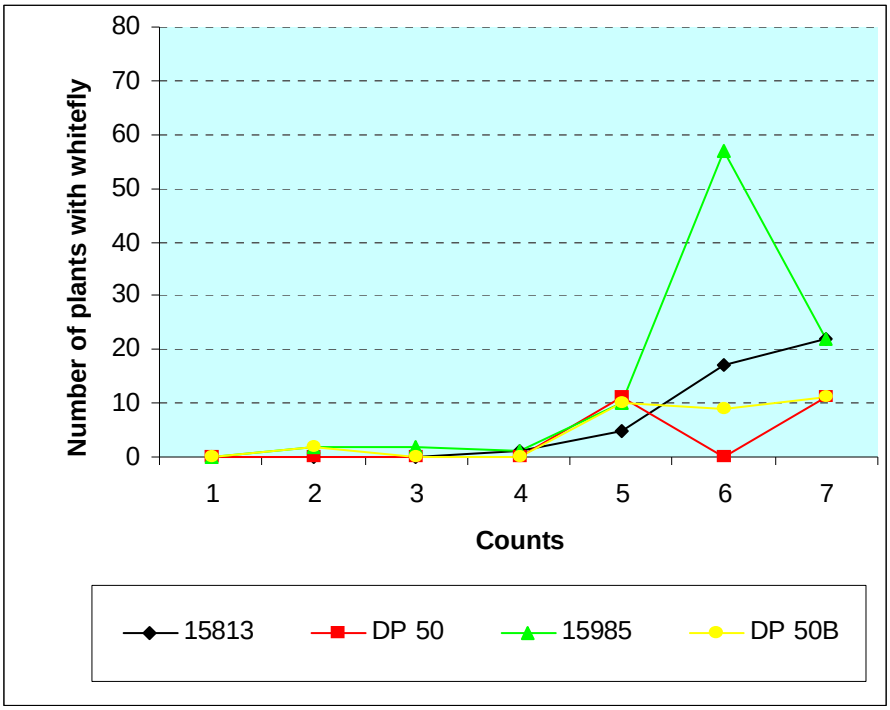


Fig. 3.36. Mean number of whitefly on 20 plants per plot in Potgietersrus in the Cry2Ab2 trial.

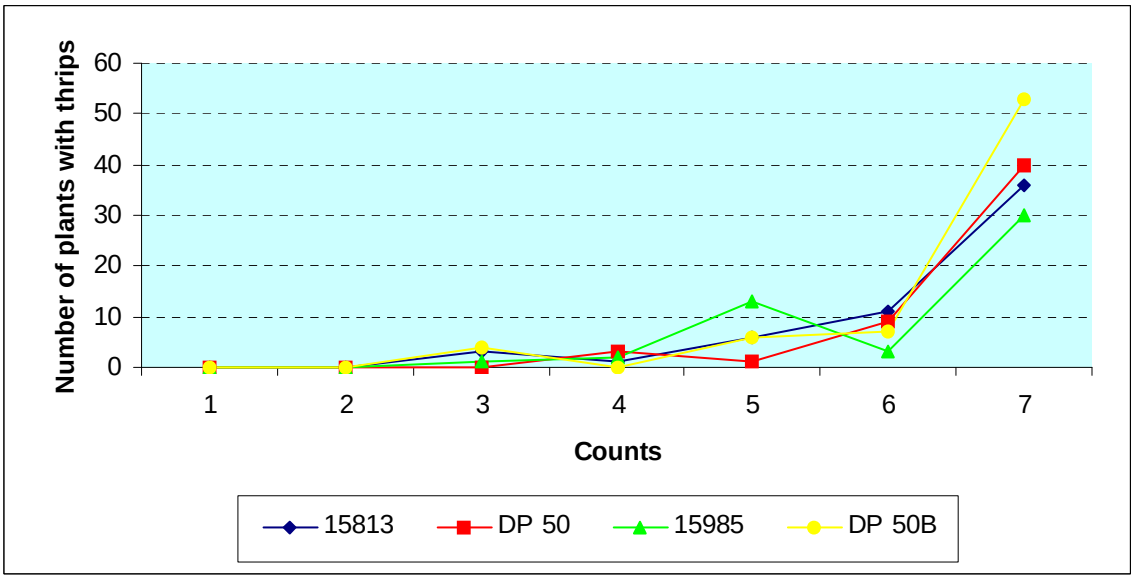


Fig. 3.37. Mean number of plants with thrips from Rustenburg (1999) in the Cry2Ab2 trial.

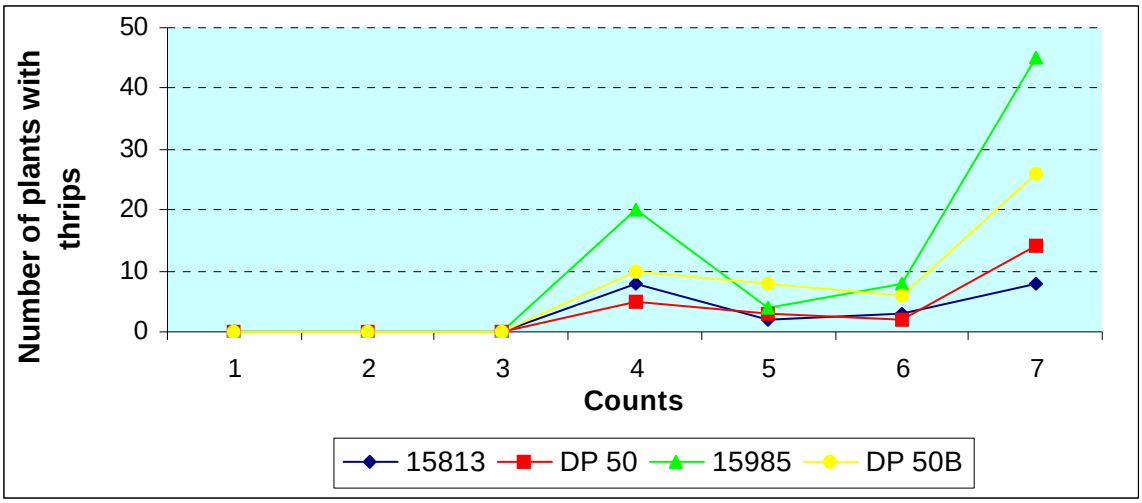


Fig. 3.38. Mean number of thrips on 20 plants in Brits (1999) in the Cry2Ab2 trial.

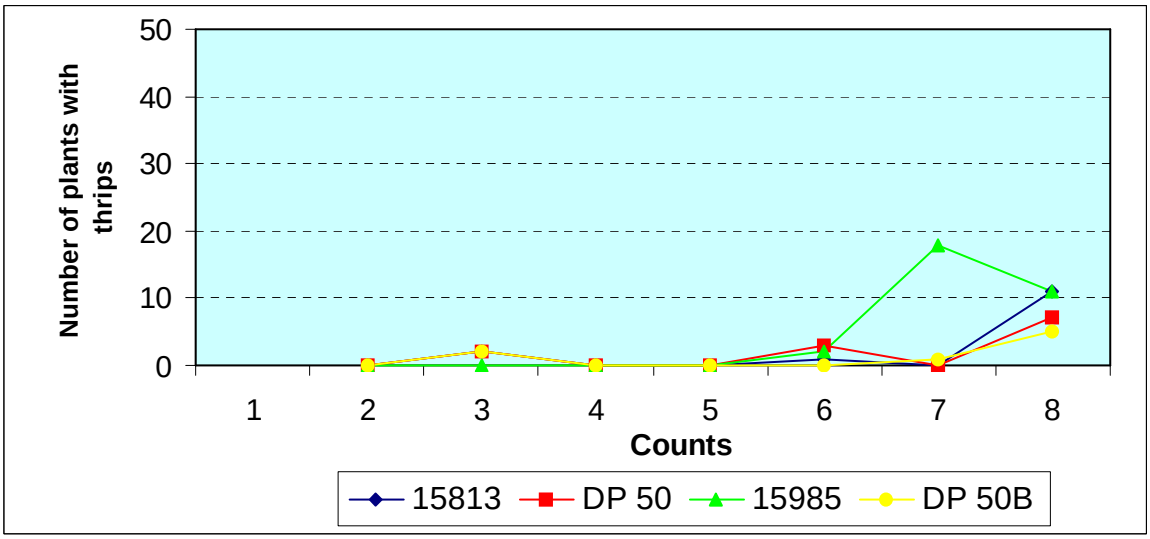


Fig. 3.39. Mean number of thrips in Potgietersrus in the Cry2Ab2 trial.

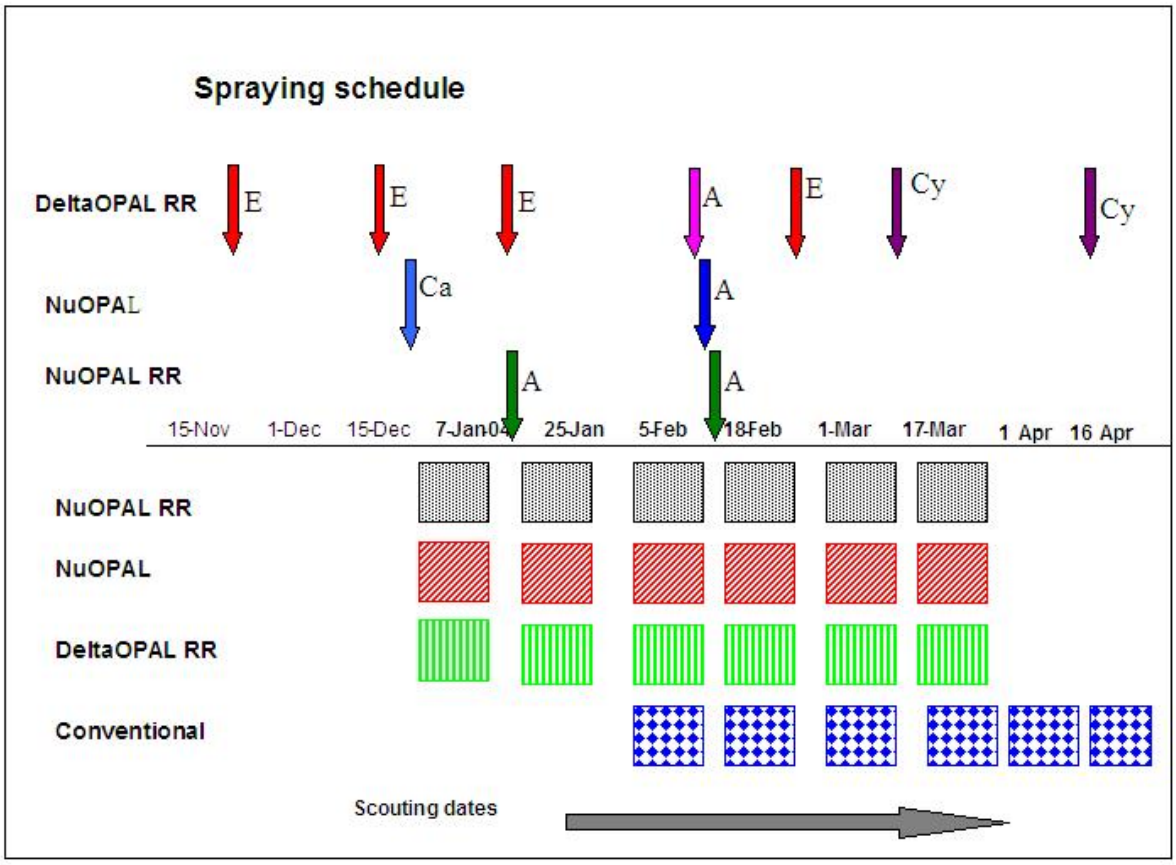


Fig. 3.40. Spraying schedule and scouting dates for the different cotton types in the 2003/2004 biodiversity trial (E = endosulfan; Cy = cypermethrin; Ca = carbosulfan; A = acetamiprid)

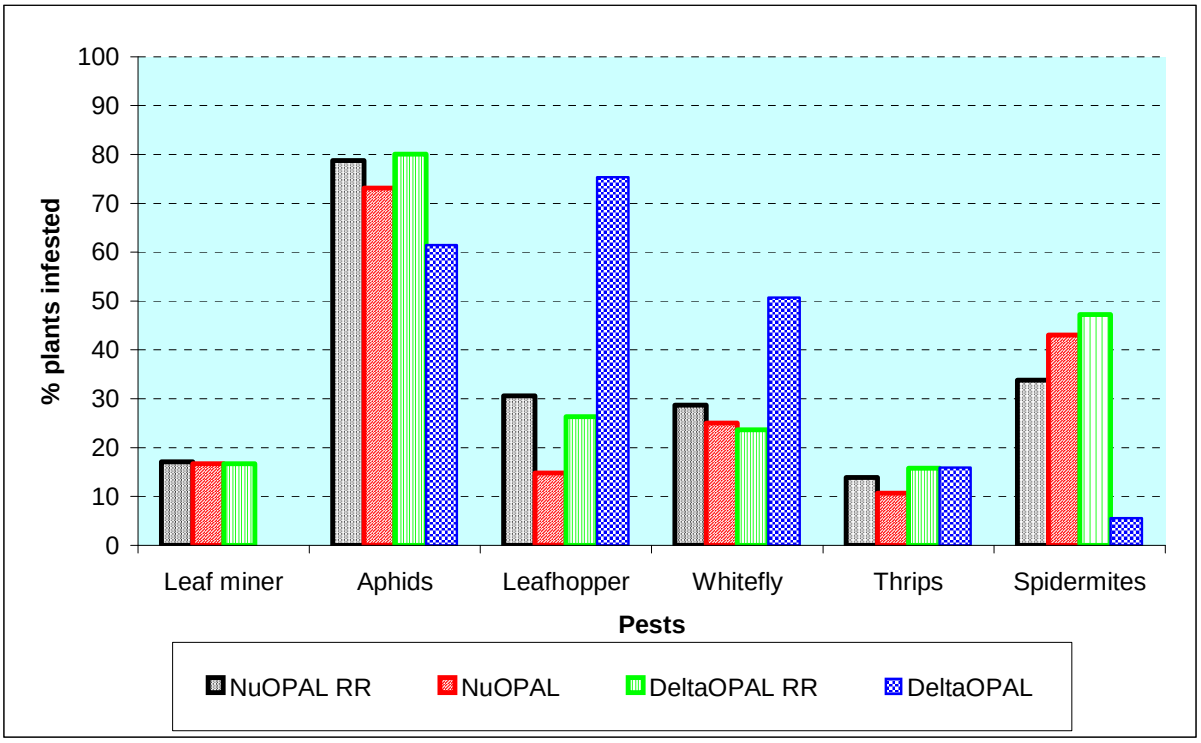


Fig. 3.41. The mean percentage plants infested with sucking pests for each count in the 2003/04 biodiversity trial.

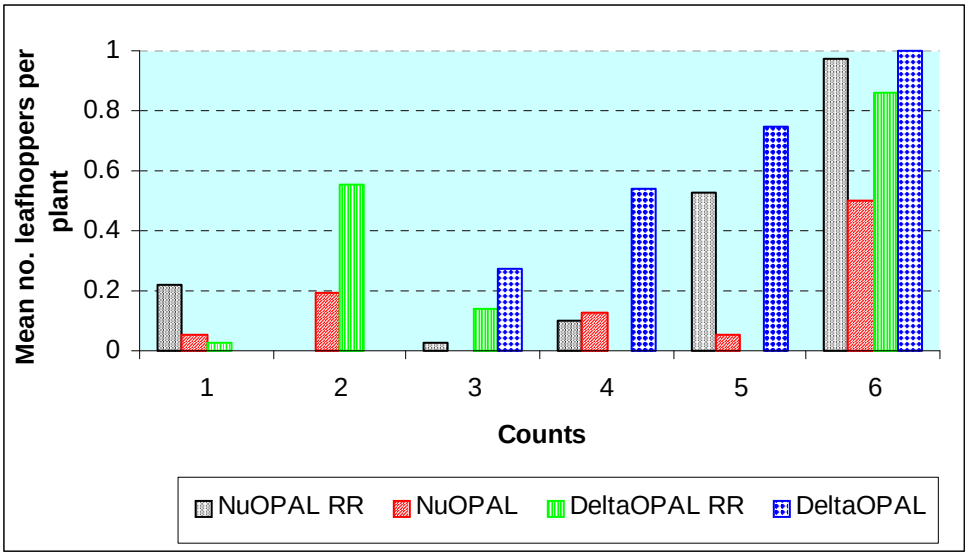


Fig. 3.42. Mean number of leafhoppers found during sweeping in the 2003/2004 biodiversity trial.

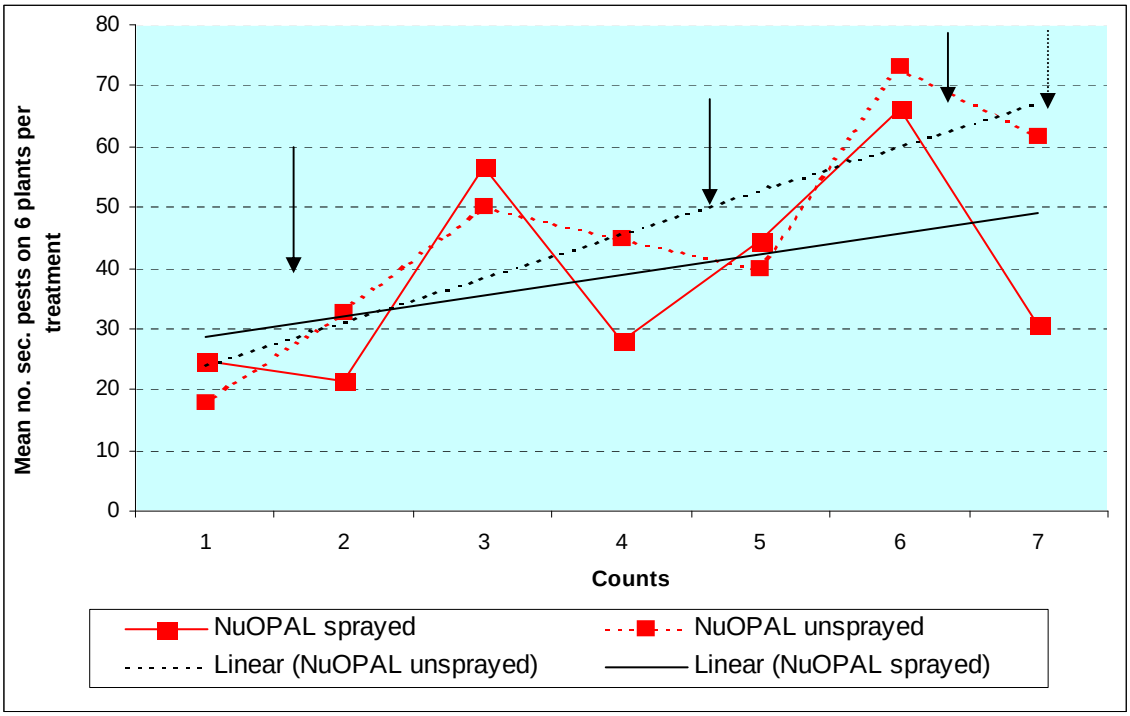


Fig. 3.43. Mean number of secondary pests on 6 plants per DeltaOPAL treatment in the 2004/05 biodiversity trial.

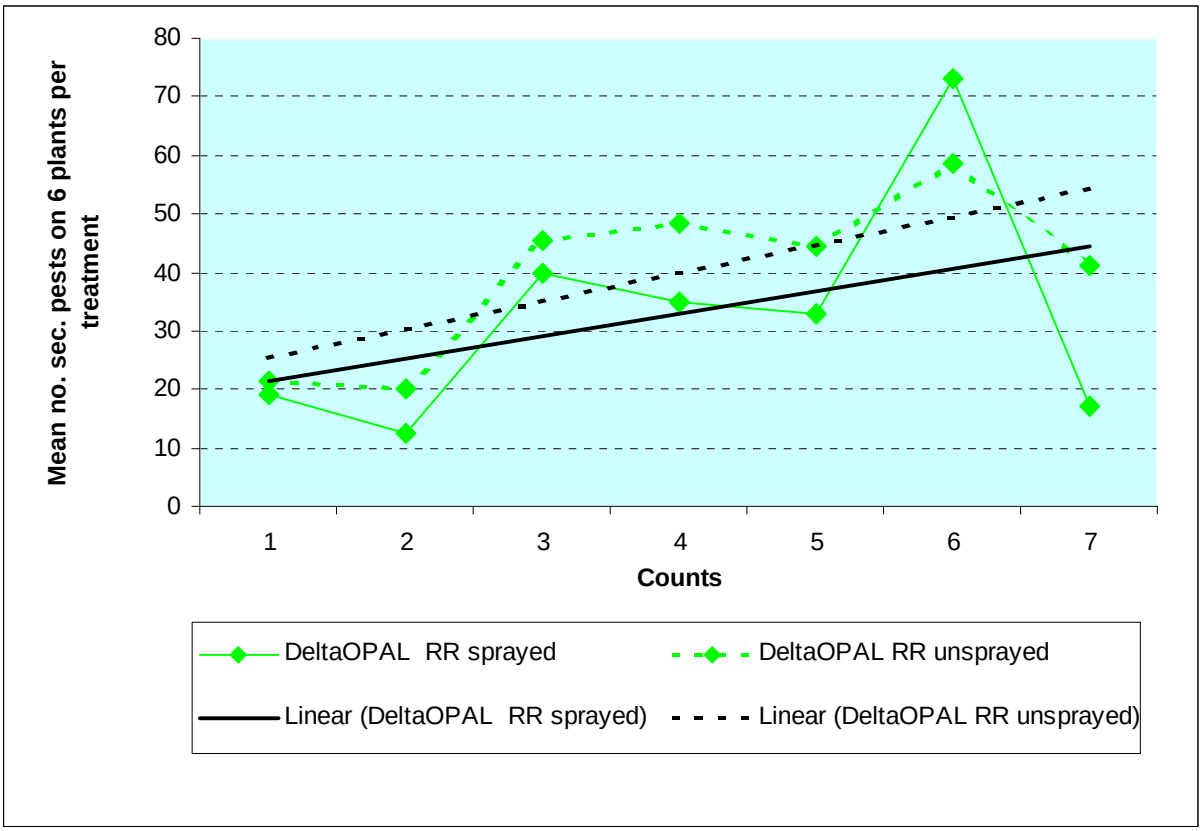


Fig. 3.44. Mean number of secondary pests on 6 plants per DeltaOPAL RR treatment in the 2004/05 biodiversity trial.

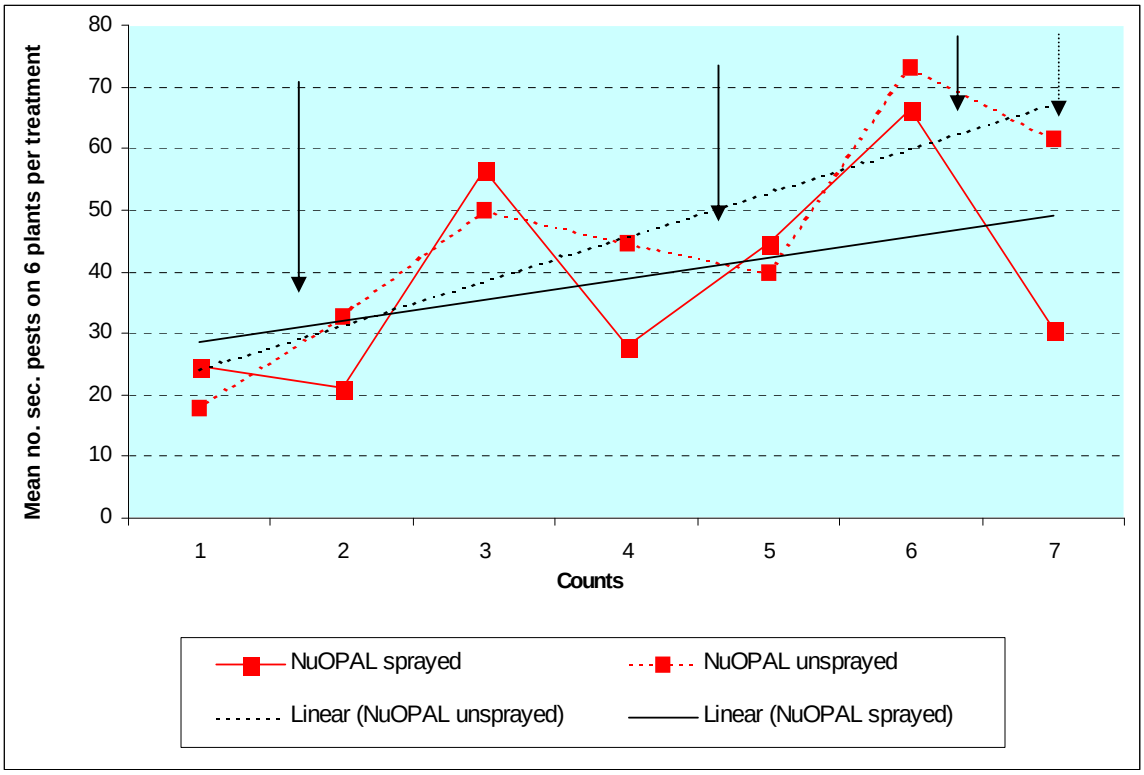


Fig. 3.45. Mean number of secondary pests on 6 plants per NUOPAL treatment in the 2004/05 biodiversity trial.

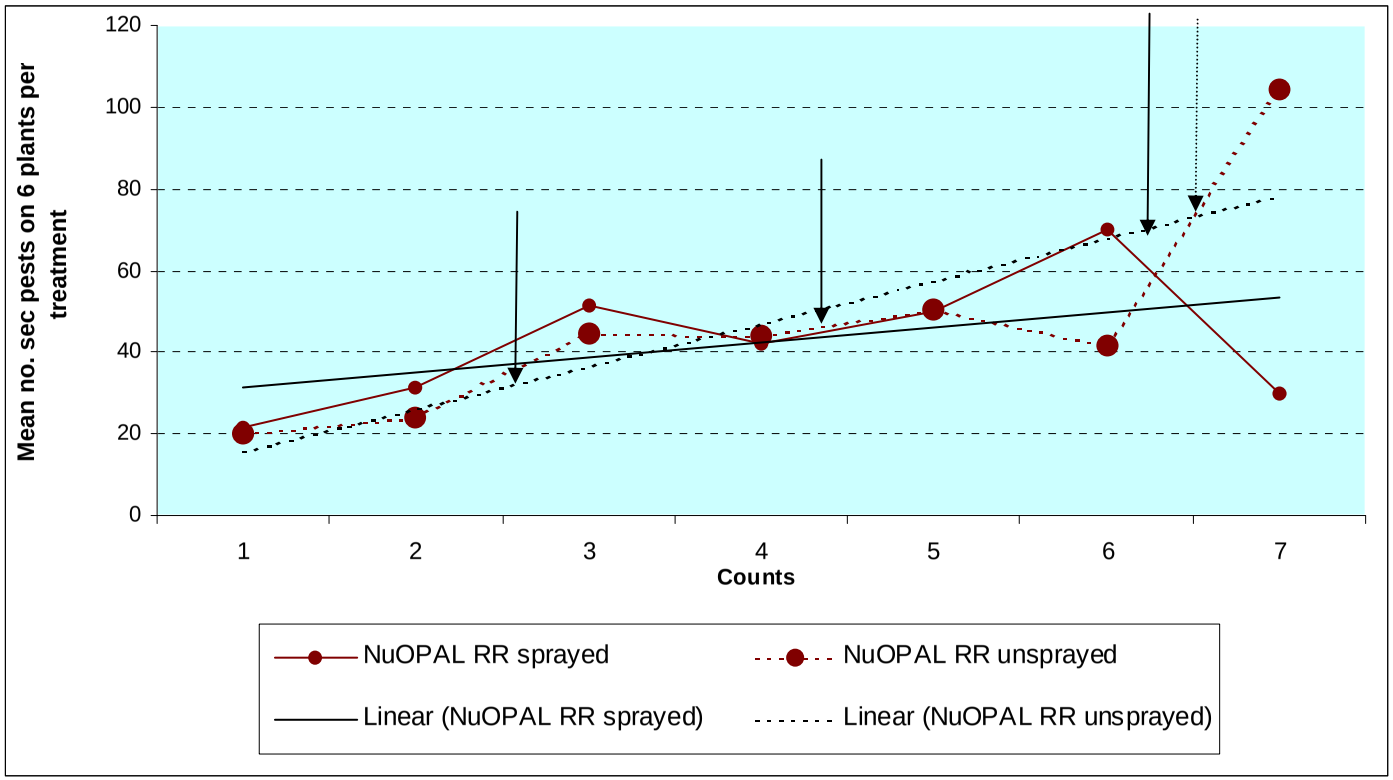


Fig. 3.46. Mean number of secondary pests on 6 plants per NuOPAL RR treatment in the 2004/05 biodiversity trial.

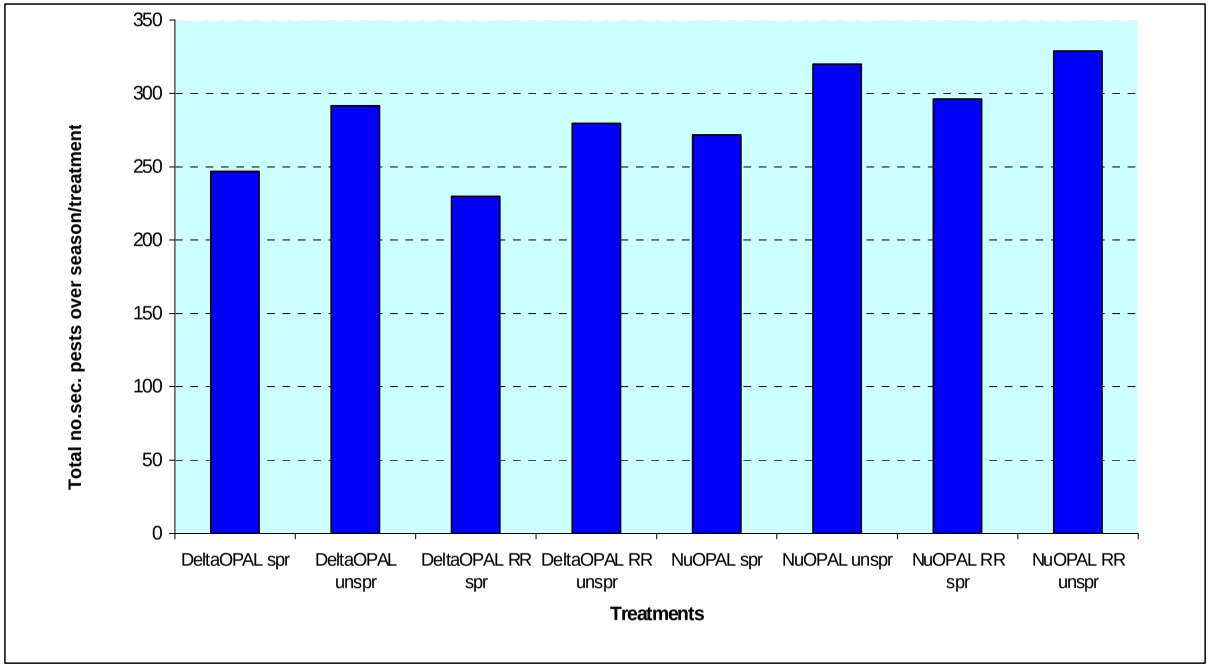


Fig. 3.47. The total number of secondary pests per treatment over 7 counts in the 2004/05 biodiversity trial.

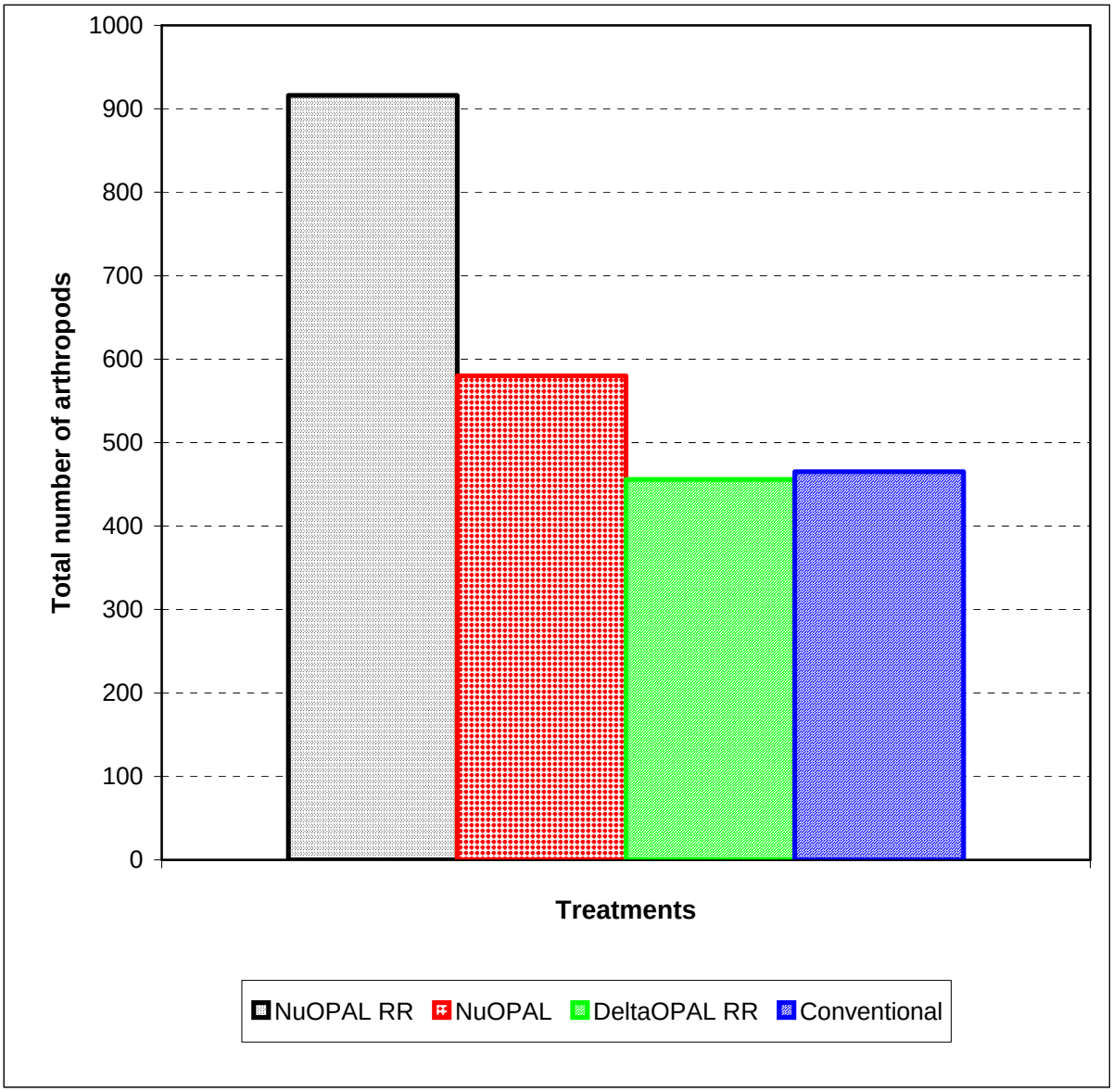


Fig. 3.48. The total number of arthropods found per variety by means of sweeping in the 2003/2004 biodiversity trial.

CHAPTER 4

THE OCCURRENCE OF BENEFICIAL INSECTS ON TRANSGENIC COTTON, IN SINGLE GENE TRAITS AND IN DOUBLE GENE TRAITS

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Abstract

Predator numbers were found to be high on Bollgard cotton. Under unsprayed conditions, there was a tendency for conventional cotton to also show a high number in predator numbers. Spraying seems to affect predator numbers on sprayed Bt-cotton and sprayed conventional cotton.

When comparing the Insect Protected Cotton lines (Part III), there did not seem to be a specific preference for oviposition by female ladybird beetles on any of the cotton treatments. Adult ladybird numbers varied on all cotton treatments, though the 15985 line and the DP 50B treatment tended to have more ladybirds and egg packets at certain counts, while high numbers were also found on the DP50 treatment in some instances. It seemed that higher numbers of ladybird pupae occurred on the transgenic line 15985. The number of lacewing eggs increased towards the end of the season at all four localities, and the 15985 line tended to have more lacewing eggs than the other cotton treatments at certain time periods. Spider numbers did not seem to show any specific trend over the season. Spiders did, however, seem to be more prevalent on the 15813 treatment except for one locality.

The predator biodiversity study where biotech cotton types were compared in single gene traits and in stacked gene combination with the Roundup Ready gene showed that spraying affected pest and predatory numbers. During 2003/2004, the NuOPAL RR variety did not have any negative effect on predator numbers within the cotton field but rather showed either a higher or similar species diversity or number of sensitive predator species, especially spiders, than the other varieties. Since NuOPAL RR cotton is bollworm-resistant and has the added benefit of herbicide-tolerance, the choice of this cotton type will provide added benefit to the grower when an Integrated Pest Management programme is used. This variety also seems to be of benefit to the environment, since the number of predators and other arthropods were higher on this variety than on the other varieties. For the 2004/2005 season, the highest total number of predators and other arthropods was found on NuOPAL RR overall. NuOPAL and NuOPAL RR tended to have higher numbers of predators than the sprayed DeltaOPAL and DeltaOPAL RR varieties, indicating that a higher biodiversity or species richness is possible in the GM types than on conventional varieties sprayed with insecticides.

Introduction

Predators play an important role in Integrated Pest Management on cotton. Several predators occur which of some are more important, because they predate on primary cotton pests, while other are less important mainly because their impact on cotton pests is not that well understood. The primary pests are mostly affected by some Hymenoptera species, while the wide range of secondary pests e.g. aphids, leafhoppers, spider mites, stainers and to a lesser extent other sporadic pests like thrips, leaf miners etc., are affected by spiders, hemipterans and some predatory coleopterans. With the changes that the introduction of Bt-cotton has brought about in the management of the pest complex, these secondary pests can appear to be more common in some years in some specific regions, resulting in the situation where the cotton grower should pay more attention to the conservation of predators. The objective of this study was to demonstrate the occurrence of beneficial arthropods on both conventional and Bt-cotton under specific spraying programmes. Thus, when a potential optimum bollworm control was achieved, by planting various genetically altered cotton types and spraying the treatments according to specific guidelines as stipulated in each trial. In each trial or combination of trails, the occurrence of the predators is compared to conventional cotton as a control. At present, three cotton types are registered in South Africa. Two of these types, contain single gene traits while the third is a double gene variety (Bennett-Nel et al. 2005). Cotton with single gene traits has been transformed through the introduction of genes expressing *in plant* proteins for either insect-resistance (Cotton line 531-Cry 1Ac protein; Bollgard® or NuOPAL) or herbicide-tolerance (Cotton line 1445-CP4 EPSPS enzyme; Roundup Ready® or DeltaOPAL RR; Bennett-Nel et al. 2005). The expression of the insect-resistant protein has been shown to have specific activity on bollworm in the Order Lepidoptera. The herbicide-tolerant variety has no insecticidal activity but affords the plant tolerance to the herbicide, Roundup Ready® with active ingredient glyphosate. Cotton growers have benefited considerably from planting these genetically modified types.

The herbicide-tolerant 'variety' (refers to the DeltaOPAL variety to which a single gene trait has been introduced, and not to a new variety as such) contributes to lowering production costs in combatting weeds, particularly where cotton is produced under dryland conditions, whereas the bollworm resistant variety has reduced losses attributed to this pest, with a subsequent increase in yield. The double gene variety (Stack Gene or NuOPAL RR), produced by conventional cross breeding of the two single gene varieties, expresses both traits within the same plant, thus giving benefit of both insect-resistance and herbicide-tolerance to the farmer. During this study, results are presented as in incidence of these predators on the specific cotton types, and statistical analysis was only carried out for the Stackgene (double trait cotton type). Graphical presentation between treatments has proved to be efficient to demonstrate variation that occurs between treatments. The occurrence of predators will be demonstrated as they occur on genetically altered cotton with the *cry1Ac* gene, the *cry2Ab2* gene, and on cotton where the *cry1Ac* gene is used in stacked combination with the *cry2Ab2* gene and with a herbicide tolerant gene, the Roundup Ready gene. In addition, the occurrence of beneficial arthropods on cotton containing the P2B protein will be evaluated. Three transgenic cotton types are currently

registered for use in South Africa. One of the questions that arose was whether any of the genetically modified varieties have any impact on predators or the number of predators. In order to determine this, information regarding predator species diversity and richness within a commercial cotton field was needed to understand species interaction in these crops. This data could then be compared to those obtained from transgenic (also referred to as genetically altered or modified) cotton fields.

The occurrence of natural pests will be demonstrated as they occur on genetically altered cotton with the *cry1Ac* gene, the *Cry2Ab2* gene, and on cotton where the *cry1Ac* gene is used in stacked combination with a herbicide tolerant gene, the Roundup Ready gene. With the establishment of these genetically modified cotton types, one of the questions that arose was whether any of the genetically modified cotton have any impact on the biodiversity and in specific on predator numbers.

In order to determine this, information regarding species diversity and richness within a commercial cotton field was a need to understanding species interaction in these crops. This data could then be compared to those obtained from GM modified cotton fields. The implications of Bt cotton on non-target pests and beneficial insects have previously been documented (*cf*. Fitt and Wilson, 2000; Fitt and Wilson, 2002; Fitt, 2003). A comparison was made between different conventional treatments and genetically modified cotton types in different gene introduction, e.g. in the presence of *cry1Ac* gene, or *cry2Ab2* gene in various events. In this case the incidence of beneficials or predators on potentially bollworm resistant cotton line, 15985 in a stacked gene combination (*cry1Ac* and *cry2Ab2*) is not presented here due to limitations in the time-span when the project was carried out for bollworm efficacy. In addition the effect of the Bt-gene in combination with the Roundup Ready gene on beneficial insects is given**.**

Part I: The incidence of beneficial arthropods on the genetically modified cotton (Bt-gene), expressing bollworm resistance with the *cry1Ac* gene

The aim was to compare the incidence of beneficial arthropods on genetically modified cotton with the *cry1Ac* gene, under a normal spraying regime.

EXPERIMENTAL PROCEDURES

Three field trails were planted at the Institute for Industrial Crops (IIC) (ARC) in Rustenburg, another at the IIC's Experimental Farm, Potgietersrus, while the third was planted at the Loskop Experimental Farm, Groblersdal. The data collected was done in addition to bollworm data and secondary pest data collected as described in Chapter 2 and Chapter 3 respectively on the same field trials, on Bt-cotton containing the *cry1Ac* gene. The field trial at Loskop was repeated during the season of 1998/99 to make sure of field data obtained during the 1997/98 season. Weather conditions and insecticide application dates are listed in Tables 2.1 and 2.2 (Rustenburg); Tables 2.3 and 2.4 (Potgietersrus) and Table 2.5 (Potgietersrus) in Chapter 2. Soil composition ranged from a heavy clay soil, (turf) with a clay content of 58%, 14% silt and 28% sand in Rustenburg. In Potgietersrus the soil consisted of a sandy-clay soil with clay content of 29%, 6% silt and 65% sand, while in Loskop, a sandy soil, with a clay content of 17%, 2% silt and 81% sand was prevalent. Four treatments with six replicates were laid out as a randomized block design, with each block representing a single replication for all localities. Each plot comprised 10 rows of 10 m each. Each plot was separated from adjacent plots by a 2 m pathway. The rows in the trial were planted 1.0 m apart and plant spacing within a row was 0.2 m. The treatments comprised of transgenic and non-transgenic varieties, either sprayed with insecticide for bollworm control or left unsprayed as indicated below. Total surface area = 74 m x 98 m. Treatments included a NuCtn 37B variety with Bollgard™ which was left unsprayed; a NuCtn 37B with Bollgard™ which was sprayed according to threshold; Deltapine Acala 90 variety (DpAC 90) which was left unsprayed, and a Deltapine Acala 90 (DpAC 90) variety, which was sprayed according to threshold. Similar planting procedures as described in Chapter 2, Part I, were followed for all three trials. Thioflo™ (endosulfan) was applied to the specified treatments above, when the egg threshold (12 eggs/24 plants) or larval threshold (5 larvae/24 plants) was reached or exceeded, whichever was attained first. The equivalent of Thioflo™ 2 l / ha spray mixture was applied at a pressure of 2.5 bar to all plants in the treatments sprayed according to threshold, by means of a CO_2 -driven knapsack sprayer fitted with a drop arm and six full-cone nozzles.

Assessments

Assessments included nine consecutive scouts starting at first square formation. Ten plants/plot were scouted for secondary pests (approximately 8 weeks after emergence) and thereafter, each week, for eight counts, up to approximately 16 weeks after emergence. Insecticide application dates and weather conditions are given in Tables 2.1 and 2.2 for Rustenburg and in Tables 2.3 and 2.4 for Potgietersrus (both listed in Chapter 2). Similarly, for Loskop, insecticide application

dates and weather conditions are given in Table 2.5 (Chapter 2). The repeat of the field trial at the Loskop Experimental Farm, Groblersdal, followed the same design and management procedure as described in Chapter 2, Part II. The soil consisted of a sandy soil, with clay content of 17%, 2% silt and 81% sand. The same treatments were planted). No statistical analyses were done on the predator data, since the data gathered was at the time considered as an observational study. A summary of possible trends as observed from the line plots is given in the text. Counts were done on the number of ladybirds, lacewing larvae, spiders.

RESULTS

Ladybird larvae (several spp.)

In Rustenburg, the number of ladybird larvae observed on 10 plants per treatment was higher on either or both of the Bollgard treatments, in comparison with the DpAC 90 sprayed treatment. Similarly, a high number of ladybird larvae were found on the DpAC 90 unsprayed treatment as expected. The DpAC 90 sprayed treatment had the least number of ladybird larvae over the observation period (Fig. 4.1).

In Potgietersrus, the Bollgard unsprayed treatment had more ladybird larvae than all other treatments only on one occasion. A similar increase in ladybird larval numbers were observed for the Bollgard unsprayed treatment at the Rustenburg locality during the same time period (Fig. 4.2). There was a decrease in the numbers of ladybirds found in all treatments over time.

Adult ladybirds and pupae

In Rustenburg, more ladybird adults and pupae were observed than ladybird larvae. Higher numbers of adults and pupae were counted on at least 6 of the 9 counts on either or both of the Bollgard treatments, except for one count where the DpAC 90 unsprayed treatment exceeded that of the Bollgard cotton. The latter was expected to occur more often, since it is the control treatment. The Bollgard unsprayed treatment outperformed the control treatment in general. The DpAC 90 sprayed treatment had the least number of ladybird adults and pupae than the Bollgard unsprayed treatment and the DpAC 90 unsprayed treatment throughout most of the observation period, except for the first 2-3 counts (Fig. 4.3). The Bollgard cotton in this case, proved to be more “environmentally friendly” towards adult ladybirds. More of the larvae reached maturity on the Bollgard cotton than on the DpAC 90 cotton.

Similarly, in Potgietersrus, higher numbers of adults and pupae were found on at least 3 occasions on the Bollgard cotton than on the DpAC 90 sprayed treatment. The DpAC 90 treatments had more ladybird numbers on the 12/02/98. This could be considered as an unreliable data point, since this observation did not correspond with the general trend of larval and adults numbers (Fig. 4.4).

The total number of adult ladybirds found during the Loskop 1998/1999 trial (Fig 4.5), seemed to be similar on all treatments. However, the sprayed treatments showed a somewhat

lower number of adult ladybirds during the first 3 counts. No trend with regards to the presence of adult ladybirds could be established between any of the treatments.

Ladybird eggs

In Rustenburg, the highest number of ladybird egg packets was found in the Bollgard treatments (Fig. 4.6). Very few egg packets were found in any of the DpAC 90 treatments in Rustenburg.

While in Potgietersrus, a varied number of ladybird egg packets were found in all treatments at Potgietersrus (Fig. 4.7). However, the highest number of egg packets was found in the Bollgard sprayed treatment.

Lacewing larvae

Most lacewing larvae in Rustenburg, were found on either of the Bollgard treatments or the control treatment (DpAC 90 unsprayed), in comparison with the DpAC 90 sprayed treatment. As one can expect, many lacewing larvae occurred on these treatments, since they were not sprayed during the observation period (Fig. 4.8). The number of lacewing larvae on the DpAC 90 unsprayed treatment mostly exceeded any of the numbers found in any of the other treatments.

In Potgietersrus, the highest number of lacewing larvae was found in the Bollgard unsprayed treatment (except for once, when the DpAC 90 unsprayed treatment exceeded it), while high numbers were found on the Bollgard sprayed treatment for the 4th count (Fig.4.4). Overall, the Bollgard treatments and the control had higher numbers of lacewing larvae than the DpAC 90 sprayed treatment.

Lacewing eggs

All treatments in Rustenburg, showed large variation in the number of lacewing eggs. The Bollgard sprayed treatment, showed on 6 occasions a higher number of lacewing eggs than both of the DpAC 90 treatments. The Bollgard unsprayed treatment had on only 2 occasion a higher number of eggs than on the DpAC 90 treatments (Fig. 4.10).

All treatments from the Potgietersrus locality gave varied number of lacewing eggs (Fig. 4.11). However, most eggs were found on either of the Bollgard treatments at the beginning of the observation period. At the 3rd count, the control treatment also showed a high number of lacewing eggs as expected, but the Bollgard sprayed treatment exceeded it.

For the 1998/199 Loskop trial, the number of lacewing eggs (Fig. 4.12) was highest on the Bt-unsprayed treatment for 6 counts of the counts during the observation period. Lacewing eggs tended to be lower on either of the DpAC 90 treatments on most occasions, except for the 2nd, 6th and to some extent the 7th count, but not significantly so.

Spiders

All treatments in Rustenburg gave a varied number of spiders during the observation period. The DpAC 90 sprayed treatment had the lowest number of spiders throughout the season, while the Bollgard treatments gave the highest number of spiders at the end of the season (Fig. 4.13).

The number of spiders found in Loskop during 1998/1999 (Fig. 4.14) varied between treatments, but tended to be lower on the DpAC 90 sprayed treatment, except for the 2nd, 6th and 7th count. There was no difference between the DpAC 90 unsprayed treatment and any of the Bt-treatments.

CONCLUSION

A high number of ladybird larvae, adults, pupae and eggs were found on the Bollgard treatments at the Rustenburg and Potgietersrus localities. Similarly, high numbers of lacewing larvae and eggs, as well as high number of spiders were observed on the Bollgard treatments at Rustenburg. In some instances, the DpAC 90 unsprayed treatment showed similar high numbers of predatory insects or eggs.

At Loskop during 1998/1999, a high number of adult ladybirds were found on the Bollgard treatments. The Bollgard unsprayed treatment had a significantly lower yield than the Bollgard sprayed treatment. This might suggest that spraying on egg threshold could produce a significantly higher yield in transgenic cotton.

Part II: The incidence of predators on different transgenic cotton events, expressing the P2B protein, at different levels

The aim of this study was to evaluate transgenic lines for the presence of beneficials when expressing some degree of bollworm resistance.

EXPERIMENTAL PROCEDURES

The same field trial which was planted at the Institute for Industrial Crops (IIC-ARC) in Rustenburg, South Africa was used for the assessments on predators and beneficial insects. Experimental procedures followed the same trend as in Chapter 2, Part II. Observations were done on the same ttreatments which included 33 transgenic P2B events, as listed in Chapter 2, Part II (under treatments). The six check plots (C1-C6) included four transgenic varieties: DP50B (C2), C312-531 (C4), 15813 (C5) and 15985 (C6) and two non-transgenic varieties: DP50 (C1) and C312 (C3). Soil type and soil pH was similar to that described in Chapter 2, Part II. A single insecticide application was carried out to control leafhoppers and cotton stainers on all plots at the end of the season. No spraying was performed at any stage for lepidopteran pests. The insecticides monocrotophos (Monostem®, 500 ml/ha) and deltamethrin (Decis®, 170 ml/ha) were applied by means of a CO_2 -driven knapsack sprayer fitted with a drop arm and six nozzles, when the threshold for secondary pests was reached. All plots were managed like conventional cotton with respect to fertilization (see Chapter 2, Part II) water, and control of insect pests, with the exception of lepidopteran species. No weed control was carried out before planting or at any time during the season.

Assessments

Assessments included stand counts for each plot counted at seven and 14 days after planting. Cotyledons that had completely cleared the soil were considered “emerged”. Individual plants were tested for the gene of interest via lateral flow strips at the four-leaf stage. Leaf samples of each individual plant per event were shipped back to Monsanto for gene analysis, on two occasions during the season. The number of fully cracked bolls per transgenic plant/event was calculated over five time periods. Natural infestations of lepidopteran pests were relied upon. Ten randomly selected plants per plot, of which the plant numbers were noted, were evaluated weekly, for eight consecutive weeks, until approximately 16 weeks after emergence. All beneficials observed were noted. In the case of Coccinellidae, the number of adults, larvae and pupae were combined to compare events and control plots. The number of lacewing eggs (Chrysopidae) was counted individually on scouted plants, as well as the spiders. No statistical analyses were performed on the data since no plots (treatments) were repeated throughout the trial. Seed was obtained from Monsanto was just enough to plant a single row of each event. Graphical representation of the data is, however, given, using Excel, Microsoft for Windows. Important dates are listed in Chapter 3, Part II (see Table 3.1).

RESULTS

The total number of predators found on the different transgenic events is given in Table 4.1. Of all the events spiders and lacewings were most numerous. Table 4.2, showed that amongst the control plots, the spiders varied between 15 and 36 spiders per plot. Some events scored higher than others (Table 4.3), varying from having spiders and/or lacewings, or spiders and/or ladybirds, or the absence of all three parameters. Amongst the control plots (Table 4.4) it was only the transgenic control, which showed the presence of some ladybirds.

Ladybird adults, larvae and pupae (Coccinellidae)

The total number of Coccinellids was compared between events (Fig. 4.15 and 4.16). The highest number of coccinellids at any given time throughout the observation period was found on event 6460 (plot 13) during the 5th week, and on event 6532 (plot 19) during the first week. Coccinellids were found on all the events during the observation period, and it seemed as if during the 1st and the 5th week, there was an increase in the number of coccinellids in some events. These insects are predators on bollworm eggs and on the immature stages of other pests such as whiteflies, spider mites and leafhoppers. Bollworm egg numbers were high on most events during the 4th week, which might explain the increase in the number of coccinellids during the following week. A comparison of the mean number of coccinellids over the eight week observation period (Fig. 4.16) revealed that the highest means (> 0.2) were found in events 6234 (plot 7), 6239 (plot 8), 6460 (plot 13), 6483 (plot 14), 6526 (plot 18), 16221 (plot 26) and the check plot 15813 (C5).

Lacewing eggs (Chrysopidae)

No trend could be established when comparing the number of lacewing eggs between events (Figs 4.17 and 4.18). However, event 6532 (plot 19) showed the highest individual count (n = 6, where n = number of lacewing eggs) for lacewing eggs during the first week (Fig. 4.17). In a comparison of the mean number of lacewing eggs per plant over the observation period (Fig. 4.18), events 6526 (plot 18), 6718 (plot 22) and 15830-5 (plot 33) had the most lacewing eggs. The check plots DP50B (C2) and 15813 (C5) had more lacewing larvae than the other checks. Many of the events did, however, have more lacewing eggs than the checks.

Spiders

Throughout the observation period, all events had spiders present at some stage. The highest mean number of spiders (Fig. 29) was found on the DP50 check plot (C1) (mean = 36), while events 6352 (plot 11), 6483 (plot 14), 6718 (plot 22), 6733 (plot 24), 17079 (plot 28) and the check 15813 (C5) all had a mean of more than 20 spiders over the observation period.

CONCLUSION

The event 6483 (plot 14) scored 8/13 for beneficial characteristics of the event during the performance of the trial. The check plot that gained the highest beneficial score is the transgenic line, 15813 (C5).

Could you conclude that the cry2ab2 protein had any clear effect on beneficiaries? The cry2ab2 protein did not affect the numbers or occurrence of predators at any one time on any of the events during this study. Predators were not repelled by the presence of the *cry2Ab2* gene. A wide variety of predators were present which include spiders, possibly as a result of the variety of food available in the form of bollworm eggs, secondary pests etc.

The protein is specific in controlling only bollworms in the larval stage. No distinct trend could be observed with regards to the occurrence of beneficial insects in addition to that of secondary pests (discussed in Chapter 3) over a number of weeks for any of the events. Some of the predator species can also prey on bollworm eggs. The event 6483 with most beneficial characteristics had an average number of aphids for example over the weeks which the event was monitored. The presence of aphid predators can be attributed to food availability and the absence of competition by other particular pest species. A high number of bollworm eggs were found on this event, but not as high as event 6460 (see chapter 2).

With regards to the abundance of beneficials, a high number of spiders was observed on the 6483 event, although bollworm numbers appeared to be low on this event (chapter 2). When one considers that an average number of bollworm eggs were found on this event, it might explains that predation on bollworm eggs by spiders might have managed to keep bollworm numbers very low or minimum, as explained in chapter 2. In contrast, the event positioned next to it, (event 6460), had a considerable high number of bollworm eggs, predation was not so high on this event, and bollworm numbers were a bit higher than on 6483. It is not thought that the position of the event affected the abundance of beneficiaries, since the events were interspersed by two rows on conventional cotton. The abundance of beneficiaries is probably an indication of food availability, which is in this case bollworm eggs. The presence of beneficial insects could be a factor attributed to the availability of prey insects who are present at any one time.

Part III: The evaluation of two potentially bollworm resistant lines, called IPC (Insect Protected Cotton) lines (15813, 15985), a control (DP 50B) and a reference line (DP 50) for the occurrence of beneficial arthropods in South Africa

The aim was to investigate the occurrence of predators and other beneficial insects on potentially bollworm resistant lines with the Cry2Ab2 protein.

EXPERIMENTAL PROCEDURES

Four field trials were carried out during the 1998/99 season in South Africa, at Rustenburg (Tobacco and Cotton Research Institute (TCRI), North West Province, 25° 43'S; 27° 18'E; 1157m altitude); Brits (TCRI Experimental Farm, North West Province, 25° 31'S; 27° 49'E, 1109m altitude); Potgietersrus (TCRI Experimental Farm, Northern Province; 24° 11'S 29° 01'E, 1116m altitude) and Friedenheim (Nelspruit) (TCRI Experimental Farm, Mpumalanga Province; 25° 27'S; 30° 58'E). The cotton treatments included a transgenic 15813 Insect Protected Cotton line (IPC line); a 15985 IPC line, the transgenic control variety DP 50B (Deltapine 50 with Bollgard$^{TM)}$, and a non-transgenic reference variety, DP 50 (Deltapine 50). The trial size and trial layout was similar to that described in Chapter 3, Part III. Planting took place with an in-furrow treatment of aldicarb (Temik®, 10kg/ha), for early season secondary pests.

The conventional method of land preparation, trial management with regards to planting, irrigation and fertilize, thinning etc. was followed as described in Chapter 2. Maximum and minimum temperatures, as well as rainfall, associated with insect counts and insecticide applications for the separate localities are given in Chapter 2, Part III. Endosulfan (Thioflo®) was applied at egg threshold (40 eggs/80 plants) or larval-threshold (16.6 larvae/80 plants). The threshold was applied to the number of plants scouted per plot (20) for four repetitions. The equivalent of endosulfan (1 l / ha spray mixture) was applied with a CO_2 -driven knapsack sprayer fitted with a drop arm and six full-cone nozzles (2.5 bar). Dual® and Cotogard® were applied, with irrigation 20 mm, followed by thinning one week later. Important dates are given in Chapter 3, Part III. Normal insect control was carried out as stipulated in Chapter 2, Part III. All control, reference and IPC (Insect Protected Cotton lines) plots were sprayed for lepidopteran insect control, when necessary. All plots were managed the same way as cotton that is commercially produced, with respect to fertilization, irrigation and weed control. Similar planting procedures were followed for all three trials.

Assessments

After planting, all plots were monitored throughout the season by recording the predators every two weeks to include spiders, ladybirds, lacewings (predators), as well as spotted black maize beetles (pollinator of cotton). Twenty plants per plot were screened for beneficial insects. All stages of the predators and beneficial insects were scouted, recorded and compared between treatments. Assessments included a total number of seven consecutive scouts at Rustenburg,

Brits and Potgietersrus, starting at two weeks after planting. No insects were recorded for the first count in Rustenburg; therefore only six datasheets are included in the Rustenburg field notebook. In Friedenheim, scouting started at six weeks after planting. Scouting commenced at first square formation on each locality. Twenty plants/plot were scouted for the various bollworm species, secondary pest species and other predators. The number of beneficial insects, e.g. ladybirds (adults, larvae and eggs), lacewings (larvae, eggs) and spiders was also calculated. No statistical analyses were done on insects other than bollworms, since this data is affected by insecticide applications. By comparing this data statistically it might create a wrong interpretation of trends of other insects on transgenic cotton.

Statistical analyses

All statistical analyses followed are explained in Chapter 2, Part III. Several statistical tests or analyses were tested for fitness for the insect data. In order to understand the analyses followed for data collected from transgenic material to achieve the results explained in this report, it is necessary to state the type of analyses tested and their suitability (pers comm.[1] H. Muller). Firstly, non-parametric chi-square tests were done on main effect of locality, site, treatment, time, locality and treatment interaction; and time and treatment interaction effects. These tests were not included in the final analyses, since the tests on the interaction effects were not reliable. Unreliability of the interaction effects were decided on the presence of some entries in the contingency tables which contained frequencies of less than five, which weakens the discriminatory power of the test. The tests for the main effects confirmed the results of the ANOVA, which proved to be more informative and more suitable at the end. Furthermore, analysis of variance incorporating time was investigated as well. The suggestion (pers comm. K. Hamilton[2]) was that those time intervals containing less than 25% infected plants should be omitted. This too, seemed to prove ineffective. Since the data was collected over several time periods, the effect of time, using the regression approach was investigated. In the initial analysis, indications were that a significant time trend for Treatment two (DP 50) might exist, while no significant time trends were indicated for the other cotton treatments. According to Muller (pers comm. Dept. Datametrics, ARC) the regression analysis restricted to this treatment (DP 50) indicated a poor linear fit, and it was subsequently omitted from the final analysis after collaboration with the project leader.

Finally, an analysis of variance for a randomized block design, nested over localities was performed, since the importance of comparing the effect of transgenic material on bollworms across localities (pers comm. K. Hamilton) was chosen to be the important factor in comparing the data. Since sites were taken into account and replications are required to perform an analysis of variance, the effect of time had to be ignored. The fact that the data is sparse by containing many zero values contributed to this. The analyses were done on transformed bollworm counts (power 0.25 transformation) in order to satisfy the ANOVA-assumptions of homogeneous

[1] Ms. Heleen Muller is a statistician, and worked formerly for the Department of Datametrics, Pretoria, SA, at the Agricultural Research Council, but she is now working at the University of South Africa (UNISA).
[2] K. Hamilton, was the project leader, and a researcher for Monsanto St. Louis, USA.

variances and normally distributed residuals. Bonferroni LSD-tests were conducted on the transformed means, but conclusions reached concerning the differences between means apply directly to the untransformed mean counts.

RESULTS

For the comparison of ladybird pupae (several species), the transgenic line 15985 had the highest number of ladybird pupae during the 3rd and 4th counts (Fig. 4.19) in Rustenburg, and few ladybirds were observed from the 5th count onwards. In Brits, most ladybird pupae were observed on the transgenic lines 15813 (count 3) followed by the 15985 line, while the 15985 line had the most ladybird pupae at the 4th count (Fig. 4.20). In Nelspruit, the most ladybird pupae were present in the beginning of the season (count 1) on the DP 50B treatment, while the DP 50 treatment had more ladybird pupae at count 3, 4 and 7 than the other cotton treatments (Fig. 4.21). The 15813 line had a similar number of ladybird pupae than the DP 50 treatment at the 3rd count. In Potgietersrus, the transgenic line 15985 seemed to have more ladybird pupae than the other two cotton treatments at the 6th and 7th counts, while the15813 line had slightly more pupae at the 5th count (Fig. 4.22). However, the number of ladybird pupae on the DP 50 treatment exceeded that of 15813 and DP 50B at the 6th count. Overall it seems that higher numbers of ladybird pupae occurred on the transgenic line 15985. This is probably because this line tended to be more susceptible to leafhoppers, aphids and whitefly at certain observation periods on some of the localities. More research is however necessary, to determine pest and predatory relationships on transgenic varieties.

For the comparison of ladybird adults and egg packets, the DP 50B line had most ladybird adults and eggs during the 3rd and 7th count, followed by the DP 50 variety during the 4th, 6th and 7th count (Fig 4.23) in Rustenburg. In Brits, *t*he highest number of eggs and adults were found on the non-transgenic line, DP 50 (Fig. 4.24). In Nelspruit, the DP 50 treatment had most ladybirds and egg packets at the 2nd and 3rd count, while at the end of the season; the 3 transgenic cotton treatments had more ladybirds and egg packets (Fig. 4.25). In Potgietersrus, the 15985 line had the highest number of adults and eggs during the 6th count followed by the DP 50 treatment at the same count (Fig. 4.26). There does not seem to be a specific preference for oviposition on any of the cotton treatments, though the 15985 line and the DP 50B treatment tended to have more of ladybirds and egg packets at certain counts. However, high numbers of these beneficial insects were also found on the DP50 treatment in some instances. Factors that influence the presence of these insects should be taken into account e.g. Insecticides applied and time of application, population densities of other secondary pests and the inherent chemical and physical nature of the variety, with regards to the selection of an oviposition site. As with the pupal counts, the number of available prey insects on these plants probably influences the adult and egg numbers. The availability of food for these predators is probably the factor influencing their dispersal within a given area. An increase in the number of aphids and whitefly infested plants at e.g. Nelspruit during count 2 to 3, before spraying, probably explains the higher number of beneficial insects found during this period on all cotton treatments.

For lacewing eggs the DP 50B and 15813 cotton treatments reached a peak in Rustenburg during the 7th count (Fig. 4.27). Lowest numbers were observed on the DP 50 treatment during counts 3 and 4. Line 15813 had more lacewing eggs during most counts (except for count 6) in comparison with the other cotton treatments at this locality. On the Brits locality, most lacewing eggs were found on the DP 50 treatment at count 4 and 5, while at count 6 and 7, the transgenic cotton treatments DP 50B and the 15985 line had most lacewing eggs respectively (Fig. 4.28). In Nelspruit, the number of lacewing eggs was lowest on the DP 50B treatment throughout the season, except for the 7th count (Fig. 4.29). Lacewing eggs were the highest on the DP 50 treatment during the 5th and the 7th count, while the 15985 line had most lacewing eggs during count 6. In Potgietersrus the number of lacewing eggs reached a peak during the 5th and 6th counts on the two transgenic lines, 15813 and 15985 respectively, and was lower during the same period on the DP 50B treatment, followed by the DP 50 treatment (Fig. 4.30). The number of lacewing eggs seemed to increase towards the end of the season at all four localities. The line 15985 tended to have more lacewing eggs than the other cotton treatments at the 6th count at Nelspruit and Potgietersrus, and at Brits on the 7th count. As with ladybirds, the dispersal of lacewings will be determined by the availability of prey.

When spiders were compared, the number of spiders was the highest on the 15813 transgenic line from the 5th count onwards (Fig. 4.31) in Rustenburg and spider numbers between the other cotton treatments varied over the observation period. In Brits, spider numbers started to increase from the 2nd count onwards on all cotton treatments (Fig. 4.32). The transgenic line 15813 had least number of spiders throughout the season, while the numbers varied on the other 3 cotton treatments. In Nelspruit, there did not seem to be any trend with regards to spider numbers on any of the cotton treatments (Fig. 4.33). There were spiders on all cotton treatments throughout the observation period. In Potgietersrus, spider numbers varied on all cotton treatments (Fig. 4.34). The transgenic line 15813 tended to have more spiders than the other cotton treatments, while DP 50B had most during the 6th count. Spider numbers did not seem to show any specific trend over the season. Spiders did, however, seem to be more prevalent on the 15813, except for the Brits locality, where they were the least.

CONCLUSION

The transgenic line 15985 seemed to have more ladybird pupae, adults and egg packets, probably because it appears to have more whitefly (nymphs) and, in some cases, leafhoppers (nymphs) during certain periods of the season. Leafhopper eggs and nymphs were found on cotton leaf surfaces. It has not been proved that ladybird adults and/or larvae do not feed on leafhopper nymphs/eggs. It is known that they are predators of aphid nymphs and whitefly nymphs. The transgenic line 15985 seemed to have more lacewing eggs in some cases during the season. The transgenic line 15813 had more spiders than the other cotton treatments overall, whenever spiders were present.

Part IV: The incidence of predator biodiversity on transgenic cotton in single gene traits, expressing either herbicide tolerance or bollworm resistance (*cry1Ac*), and in a double gene trait, expressing both herbicide tolerance and bollworm resistance in South Africa

In broader terms, this study was done in order to obtain a better understanding of the effect of spraying regimes of Bt-cotton on predators, in order to formulate recommendations for and Integrated Pest Management strategy on Bt-cotton. The aim of this study was to compare the incidence of beneficial insects and other predatory arthropods on genetically modified cotton under a normal spraying regime. The data presented here, was collected during the evaluation of the expression of the Bt-gene for bollworm efficacy mentioned in Chapter 2.

Trials were undertaken to obtain data within GM and non-GM cotton fields over two cotton-growing seasons (2003/04 and 2004/05). The occurrence of beneficial insects was evaluated under normal spray programmes aimed at controlling bollworm populations and were compared to those found in unsprayed fields. In the case of insect-resistant cotton, the absence of spraying could lead to an increase in secondary pest numbers with a subsequent increase in predators. Insect populations whether pests of predators, may be affected by the repetitive spraying of an insecticide.

EXPERIMENTAL PROCEDURES

The 2003/04 trial was planted in heavy black clay soil on a commercial cotton farm approximately 15 km SE of Groblersdal (Mpumalanga, S 25º 21.157'; E 29º 21.609'), an accepted cotton growing region. NuOPAL was planted on 40 ha, DeltaOPAL RR on 50 ha and NuOPAL RR on 50 ha. DeltaOPAL was planted on 0.3 ha adjacent to NuOPAL. A further 0.3 ha was planted adjacent to the NuOPAL RR plantings. Planting details are illustrated in Fig 4.1. There was a two-week interval between the plantings of the different cultivars. The Delta and Pine Land Co. (D & PL S.A. Inc.) seed multiplication blocks were used and the study was limited to the available GM and non-GM cotton blocks and their management practices. An area of approximately one hectare in the centre of the blocks of GM cotton was selected for assessments while, the whole of the unsprayed conventional cotton blocks were assessed. The DeltaOPAL RR cotton was sprayed six times for bollworms; four endosulfan sprays (Thioflo® 1.5l/ha) and two cypermethrin sprays (Karate®, 150 ml/ha), while it was sprayed only once for sucking pests with acetamiprid (Mospilan®). The NuOPAL cotton was sprayed twice for sucking pests, once with carbosulfan (Marshal®, 300 ml/ha), and once with acetamiprid (Mospilan®, 75g/ha). The NuOPAL RR cotton was sprayed twice for sucking pests with acetamiprid (Mospilan®, 75g/ha). The conventional DeltaOPAL cotton received no insecticide treatments since this was managed by the farmer as an unsprayed refuge area for insect-resistant cotton, conforming to the license agreement with Monsanto SA (Anon 1998).

The 2004/05 trial was planted in Groblersdal at the Agricultural Research Council - Institute for Industrial Crops (ARC-IIC) Experimental Farm, on red clay (20-25%)-loam soil. The trial was planted on 13 December 2004 in a complete randomized block design, with eight treatments and four replicates. Treatments were DeltaOPAL, DeltaOPAL RR, NuOPAL and NuOPAL RR, all with and without (control) insecticidal spraying for bollworm, which served as the control plot for each variety. Treatments were initiated once scouting had determined that the bollworm level of 5 larvae/24 plants was exceeded. Plots consisted of 10 cotton rows, each 10 m in length. Data were collected from the middle 6 m x 6 m (the inner six rows and inner six m of each row) i.e. there were 2 m of guard plants surrounding the data plants. Twelve border rows of unsprayed conventional cotton (DeltaOPAL) surrounded the trial. Control plots were never sprayed and considerable care was taken to ensure that no spray drifted into these plots. In order to limit the variation of insect populations between treatments, when the threshold of one of the non-Bt-sprayed treatments was exceeded, spraying was conducted on all replicates of the non-Bt-sprayed treatments (DeltaOPAL and DeltaOPAL RR). If the bollworm threshold for Bt-cotton were to be reached, insecticides would have been sprayed on all replicates of the Bt-sprayed treatments (NuOPAL and NuOPAL RR). The purpose was to be able to register the impact of the spray on beneficial non-target insects. Three sprays of endosulfan were applied for bollworms. One spray of acetamiprid was applied to all the cotton to control sucking pests (Table 4.2). These insecticides are considered to be 'soft' options and are recommended for an Integrated Pest Management Strategy. The first herbicide application was done between the 3rd and 4th node stage of the cotton and the second after the 4th node stage. Care was taken to prevent contact with the cotton plant leaves, as stipulated on the Roundup Ready® herbicide label.

Assessments

During 2003/04, insects and other invertebrate species were monitored by both sweep netting and scouting, similar to that described in Chapter III, Part IV. The scouting was done every two weeks (Fig.4.36). Thirty-six plants in each transgenic cotton block were assessed. This is 1.5 times the number of plants recommended by Basson (1987) who advocated that 24 plants within each field and the entire plant, including young bolls and squares, should be thoroughly examined. The numbers of any pest found on the plant is recorded and if this is equal to or more than the predetermined *economic control threshold*, control measures should be applied. Monitoring for predators was undertaken while scouting for secondary pests, as described in Chapter III, Part IV. Twenty- four plants were assessed and data were generated on a total of 48 plants. All predator - invertebrates were observed and recorded and identified to species where possible. In addition to scouting, sweeping of 50 random sweeps was done on each transgenic cotton variety (five repetitions of 10 sweeps); while 25 sweeps were done in the two conventional cotton blocks (five repetitions of five sweeps). Each insect was identified to species level where possible and counted.

Scouting was the only method used to monitor the predator invertebrate populations in 2004/05. Assessments were carried out on a seven to 10 day cycle starting at the 2nd node stage of cotton development. The observation schedule was adjusted following the application of an

insecticide by performing an assessment on the day following the application. The seven to 10 day cycle of inspection was then resumed using the last assessment date as day one of the new cycle. Assessments were continued until the first boll burst stage was reached. Six plants per plot, each randomly selected within each data row were assessed during each scout. All arthropod predators were identified to at least family/subfamily level, and where possible to genus and species level. Predator numbers are influenced by iinsecticide applications. These were applied similarly to that described in Chapter III, Part IV, where they were based on predetermined economic threshold levels for bollworm and secondary pest sprays were applied according to the thresholds mentioned in Chapter III, Part IV.

Statistical analyses

Similarly as for secondary pests, described in Chapter III, Part IV, it was decided to omit any statistical analyses of the predator data for this preliminary data set (2003/2004), due to large variation in all parameters. To facilitate interpretation of the 2004/05 data, arthropods were classified into four categories: primary pests (bollworms) (not discussed here for the purpose of this study), secondary pests and other arthropods (see Chapter 3) and predators. Analyses were conducted using SAS (University of Pretoria), following the GENMOD procedure for bollworms. Chi-square tests were carried out. The data followed a Poisson distribution and log-transformations were done on the treatment means, since many zero values were observed, due to the inherent characteristics of the NuOPAL and NuOPAL RR. Significant values indicating differences between log-means are applicable to 'true' means recorded or calculated. The GLM (General Linear Model) procedure was used to analyze predator data. A comparison of means between each sprayed and unsprayed mean of the same treatment was then made (for example, predator mean of DeltaOPAL sprayed, compared with predator mean of DeltaOPAL unsprayed). A comparison between each mean with all other treatment means was done (for example, comparison of predator means across all treatments). The data followed a normal distribution and treatment means were compared by t-tests (Link-Unity), tested at a significance level of $p<0.05$ (5%) for a General Linear Model.

RESULTS

2004/2005 season

Predator arthropods found during 2003/04 are listed in Table 4.8 and those from 2004/05 in Table 4.8. The mean number of predators found during 2003/04 per plant during each assessment is illustrated in Fig. 4.35a, b, c and 4.36. The number of predators per plant/assessment is shown in Table 4.15, while the number of predators per sprayed and unsprayed cotton type is given in figures 4.42-4.45 respectively. Most predators were collected from the NuOPAL cotton, followed by the NuOPAL RR, while it seemed that the predators varied on the DeltaOPAL RR, and on the conventional blocks. The high numbers of predators that were recorded on the NuOPAL is associated with the high number of ladybird eggs found during the first count (Table 4.5). During this count most other insects were found to be dead on the

NuOPAL as a result of the carbosulfan spray. Predators observed to have died as a result of this spray included spiders and ladybirds (both larvae and adults), while dipterans and hemipterans were also affected. The effect of the spraying can be seen in the decrease in predator numbers on the NuOPAL after the first count (Fig. 4.35b). The possibility that these ladybird eggs (71 egg packets) would not hatch was therefore high and the presence of these egg packets could not give a true reflection of predator numbers on the NuOPAL during that count and were thus excluded in the final mean per plant in Table 4.15. When the ladybird eggs were included, NuOPAL had the highest number of predators per plant (1.25). Conventional (DeltaOPAL) and NuOPAL RR had a similar number (0.69 and 0.72 respectively) and DeltaOPAL RR the least (0.54) due to the spraying programme. If the number of ladybird eggs is excluded, the mean of NuOPAL (0.66) drops to that similar to the means on conventional and NuOPAL RR cotton varieties (0.67 and 0.72 respectively), with DeltaOPAL RR still the least (0.45). Lacewings of all stages were most common on the NuOPAL RR variety, while many predatory thrips were also more frequently recorded on this variety compared to the other varieties. The spider numbers on the GM cotton were more or less similar, while more spiders were noticed on the conventional cotton. Parasitized aphids occurred on all varieties, indicating that parasitism occurs on GM cotton. High numbers of spider mites and aphids were also observed later in the season. Predators increased on the Roundup Ready cotton after the acetamiprid application following the 3rd count, to reach a maximum during the 4th count. Acetamiprid appears to conserve natural enemies, and have little, if any, affect on them. The DeltaOPAL RR cotton had a low number of predators per plant at count 1, possibly due to the two endosulfan applications during the month of November-December, and since the plants were nearing their mid-season plant stage (approximately nine weeks after planting) when aphid populations tend to decline. The NuOPAL and NuOPAL RR were entering the fruiting stage at this period. The few predators that were found on the conventional cotton were probably due to the mismanagement of the conventional cotton. Weed control was exceptionally poor in this plot on several occasions, and it is possible that predators preferred other food sources on the weeds. The mean number of predators per plant and the total number of predators associated with each variety are shown in Figs 4.16 and 4.17 respectively. The number of predators tended to vary on all the cotton types. In general, NuOPAL tended to have the most predators initially (counts 1 to 3) but numbers dropped drastically due to the carbosulfan spray. The low number of predators on the Roundup Ready could probably be attributed to the endosulfan and cypermethrin sprays. Table 4.7 shows the total number of predators found by sweeping during 2003/04. NuOPAL RR had the most predators, followed by NuOPAL. This is probably due to the lack of bollworm sprays on these two varieties. The conventional cotton had many weeds, possibly influencing the numbers caught, and a heavy spraying regime was followed on DeltaOPAL RR, which reduced predator numbers. Both scouting and sweeping data showed the most predators in the NuOPAL RR and NuOPAL cotton types. Since bollworm sprays are seldom required on the NuOPAL and NuOPAL RR varieties, the reduced use of insecticides could have a positive effect on the environment as indicated by measured predator densities.

2004/05 season

Comparison of the means of sprayed treatments

During 2004/05, the predators were pooled into one group (Table 4.8). The mean number of predators found per six plants on DeltaOPAL, DeltaOPAL RR, NuOPAL and NuOPAL RR are shown in Figs 4.37, 4.38 and 4.39 respectively. The latter three figures explain the different spraying regimes followed on each cotton type, as listed in the legend of each figure. Figure 40 illustrates the number of predators over six counts. Table 4.18 shows the number of predators found in each sprayed treatment. The probability values are given in Table 4.18.

During count 1, DeltaOPAL and NuOPAL RR sprayed treatments had significantly fewer predators than the NuOPAL sprayed treatment (Table 4.11). Bollworm numbers reached threshold during count 1 in both DeltaOPAL and DeltaOPAL RR and these two treatments were sprayed directly after the count with Thioflo. During count 2, a decrease in the number of predators was observed in the DeltaOPAL sprayed treatment and in the DeltaOPAL RR sprayed treatment due to endosulfan spray. The number of predators in the NuOPAL and NuOPAL RR treatments increased as no endosulfan was sprayed in these two treatments. During count 2, the number of predators in the NuOPAL treatment was significantly higher than those in the DeltaOPAL treatment. The NuOPAL RR treatment also had significantly more predators than DeltaOPAL (Table 4.12; count 2). Also, the NuOPAL sprayed treatment had significantly more predators than the DeltaOPAL RR sprayed treatment. During count 3, all three GM cotton types (DeltaOPAL RR, NuOPAL and NuOPAL RR) showed significantly higher numbers of predators than DeltaOPAL, but did not differ significantly from one another. During count 4, no significant differences were found between treatments $_{(p< 0.05)}$. By count 5, the DeltaOPAL RR treatment had significantly fewer predators than the NuOPAL treatment at the 5% significance level, while the same was true for NuOPAL RR $_{(p<0.1)}$ This is probably due to the fact that endosulfan was sprayed on DeltaOPAL and DeltaOPAL RR directly after count 4, resulting in a decrease in the number of predators on both of these treatments.

There were no significant differences between treatments during counts 6 and 7. Acetamiprid was applied directly after count 6 to control sucking pests and it seems to have caused a slight decrease in predator numbers by count 7 (Table 4.12).

Comparison of the means of unsprayed treatments

Table 4.10 shows the number of predators found in each unsprayed treatment. The probability values are given in Table 4.14. For the first four counts, no significant differences in predator numbers were found between treatments. During Count 5 and count 6, the probability values (Table 4.14) showed that the NuOPAL RR treatment had significantly more predators $_{(p<0.05)}$ than the DeltaOPAL, DeltaOPAL RR and NuOPAL treatments (Table 4.13). No significant differences were found between treatments by count 7. The total mean number of predators found over the season was similar in all unsprayed treatments and no significant differences were found during most counts. Since the only factor affecting predator numbers in the unsprayed treatments would

be cotton type, it thus seems that cotton type does not have a significant effect on predator numbers.

Comparison of treatment means across all treatments

When all predators means were compared across all treatments (Tables 4.15 and 4.16), the differences observed followed the same tendency as for the previous two analyses. Significantly fewer predators were found on the conventional sprayed cotton (DeltaOPAL), than the NuOPAL sprayed and unsprayed cotton, as well as the NuOPAL RR unsprayed cotton respectively. The same results were achieved when the sprayed treatments were compared for count 1 (Table 4.13). The NuOPAL sprayed and unsprayed treatment had significantly more predators than the NuOPAL RR sprayed treatment. The NuOPAL RR unsprayed treatment had significantly more predators than the NuOPAL RR sprayed treatment. For count 2, treatment means differed between all the unsprayed and sprayed treatment means, except between the means of the Bt-cotton types. The DeltaOPAL sprayed treatment had significantly less predators than all other treatments, except the DeltaOPAL RR sprayed treatment, indicating the possible effect of spraying endosulfan on predators. Also, the DeltaOPAL RR sprayed treatment had significantly less predators than that of the NuOPAL sprayed treatment, and the NuOPAL unsprayed treatment. During count 3, the DeltaOPAL sprayed treatment had significantly fewer predators than all other treatments, except for the NuOPAL RR unsprayed treatment. Also, during this count the latter treatment differed significantly from the DeltaOPAL RR unsprayed, NuOPAL sprayed and NuOPAL RR sprayed treatment (Table 4.15). The low number of predators on the NuOPAL RR unsprayed treatment could not be explained, except for possibilities like hyper parasitism that could exist, or the fact that fewer prey (bollworm) were available. During count 4, no significant differences at $p<0.05$ were found. For count 5, the DeltaOPAL RR unsprayed treatment had significantly fewer predators than the NuOPAL RR sprayed treatment, though this comparison was not important for the purpose of the trial. The NuOPAL sprayed treatment differed from the DeltaOPAL sprayed and unsprayed treatments and the NuOPAL unsprayed treatment. The NuOPAL unsprayed treatment had significantly fewer predators than the DeltaOPAL sprayed treatment and both the NuOPAL RR sprayed and unsprayed treatments, indicating that the NuOPAL RR has more predators. The NuOPAL RR unsprayed treatment has significantly more predators than the sprayed treatment, as well as the DeltaOPAL RR sprayed treatment and the DeltaOPAL unsprayed treatment. In general, significantly more predators seem to occur on the unsprayed NuOPAL RR treatment in comparison with most of the other sprayed and/or unsprayed treatments. For count 6, only the NuOPAL unsprayed treatment had significantly fewer predators than all other treatments. The reason for this is unclear, but it could be seasonal, or some other non-observed form of predation on beneficial insects. By Count 7, the DeltaOPAL sprayed treatment had significantly fewer predators than the DeltaOPAL RR unsprayed and NuOPAL unsprayed treatments. The total number of predators on all treatments over seven counts is given in Fig. 4.46.

To summarize the findings, a matrix was set up of the incidence of significance obtained during the statistical analyses performed (Table 4.19) by scoring the number of times the GM cotton showed a significantly higher diversity for secondary pests and predators.

The NuOPAL RR treatment differed significantly from other treatments more than any other treatment did from another, indicating a higher level of biodiversity on this cotton type.

Other arthropod beneficials

During 2003/2004, a few unidentified predatory thrips species and most spiders were collected on the NuOPAL RR cotton. The presence of spiders is indicative of a stable ecological system. The large number of predators found on the NuOPAL RR cotton could be due to the combination of 'softer' insecticide (acetamiprid) sprayed on the NuOPAL RR as well as the presence of more prey items, such as aphids and bollworm eggs, serving as food for predators (pers comm. Broodryk, 2005)[3]. The total number of predator arthropods found per treatment during the 2004/05 varied between treatments and no trends could be observed.

CONCLUSIONS

The 2003/04 study on arthropod diversity provided data on the arthropod species richness occurring within GM and non-GM cotton fields during the peak fruiting stages of cotton. The number and species composition varied on the different cotton types that were subjected to general farm weed and pest management practices. It was hypothesized that the number of arthropods would be similar on GM and non-GM (conventional) cotton. This, however, could not be established due to the high within-trial procedural variability, such as differences in the spraying regime, the differing ages of the cotton varieties and the absence of good weed control in the conventional blocks. This preliminary data did, however, demonstrate that species diversity, and in specific predator diversity, varied according to the insecticides applied. Arthropod diversity monitored by sweeping tended to be higher in the Stack gene cotton than in the other cotton types, especially regarding spiders (both in number and in species). Roundup Ready cotton tended to have the lowest diversity and this was attributed to the many insecticide applications applied within this field and possibly the fewer number of weed species observed. The number of predators (spiders, Hemiptera, ladybirds and lacewings) that were found by sweeping the Stack gene variety was higher than on the other varieties. The mean number of predators found through scouting was similar on the NuOPAL, NOPAL RR and conventional (DeltaOPAL) cotton and the lowest in the DeltaOPAL RR. It was determined by scouting that the NuOPAL RR cotton had a higher number of lacewings than the other cotton types. The results indicated that spraying affected pest and predatory numbers, even with the application of an insecticide (endosulfan) generally accepted as 'soft' towards predators. The NuOPAL RR variety did not have any negative effect on arthropod populations within the cotton field but rather showed higher or similar species diversity and a number of sensitive predator species, especially

[3] The late Dr. SW Broodryk, served as the Integrated Pest Management Advisor to Cotton SA, as part of the DANIDA (Danish International Development Assistance) funded Cleaner Textile Production Project, Cotton SA, 2002-2005).

spiders, than the other varieties. Since NuOPAL RR cotton is bollworm-resistant and has the added benefit of herbicide-tolerance, the management of this variety is easier for the farmer. This variety also seems to be of benefit to the environment, since the number of predators and other arthropods were higher on this variety than on the other varieties.

For the 2004/2005 season, the highest total number of predators and other arthropods was found on NuOPAL RR overall. NuOPAL and NuOPAL RR tended to have higher numbers of predators than the sprayed DeltaOPAL and DeltaOPAL RR varieties, indicating that a higher biodiversity or species richness is possible in the GM types than on conventional varieties sprayed with insecticides.

Overall conclusion

Neither the Cry1Ac or Cry2Ab2 protein affected beneficial insects or spiders in any way. The presence of predators seemed to be as a result of the availability of a food source on all cotton types/events evaluated. These results confirm that the Bt-proteins have no affect on non-target organisms such as insect predators and spiders present on Bt-cotton.

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Tables

Table 4.1. Total number of predators found on transgenic events in the P2B trial.

Plots	Events (Control treatments)	Spiders	Lacewings	Fly pupae	Hover fly Larvae	Mirid bugs	Assassin bugs	Parasitic wasps
1	6149	12	1	0	0	0	0	0
2	6151	20	1	0	0	0	0	0
3	6152	9	0	0	0	0	0	0
4	6223	14	0	0	0	0	0	0
5	6227	17	1	0	0	0	0	0
6	6232	13	1	0	0	0	0	0
7	6234	18	1	1	0	0	0	0
8	6239	15	2	1	0	0	0	0
9	6241	19	0	5	0	0	1	0
10	6351	17	0	0	0	0	0	0
11	6352	24	0	0	0	0	0	0
12	6456	17	0	0	0	0	0	0
13	6460	18	3	0	0	0	0	0
14	6483	29	2	0	0	0	0	0
15	6484	15	0	0	0	0	0	0
16	6489	8	0	1	0	0	0	0
17	6491	9	0	1	0	0	0	1
18	6526	11	0	0	0	0	0	0
19	6532	14	0	0	0	0	0	0
20	6563	15	0	0	0	0	0	1
21	6542	8	2	0	0	0	0	0
22	6718	22	0	0	0	0	0	0
23	6725	15	0	0	0	0	0	0
24	6733	22	1	0	0	1	1	0
25	6734	16	2	0	0	0	0	0
26	16221	20	1	0	0	0	0	0
27	16960	13	1	0	0	0	0	0
28	17079	21	0	0	0	0	0	0
29	17133	17	0	0	0	3	0	0
30	17180	13	0	0	0	0	0	1
31	17206	13	0	2	0	0	2	0
32	6233	12	1	1	0	0	0	0
33	158305	17	12	0	0	1	1	0

Table 4.2. Total number of predators found on control plots in the P2B trial.

Plots	Events (Controls treatments)	Spiders	Lacewings	Parasitic wasps
C1	DP50	36	0	0
C2	DP50B	15	1	0
C3	C312	16	0	0
C4	C531	15	0	1
C5	15813	22	2	0
C6	15985	13	0	0

Table 4.3. Score sheet of beneficial characteristics observed amongst the events in the P2B trial.

Parameters Plot	Events	High no. lacewings	Ladybirds	Spiders
1	6149			
2	6151			
3	6152			
4	6223			
5	6227			
6	6232			
7	6234		*	
8	6239		*	
9	6241			
10	6351			
11	6352			*
12	6456			
13	6460			
14	6483		*	*
15	6484			
16	6489			
17	6491			
18	6526	*	*	
19	6532			
20	6563			
21	6542			
22	6718	*		*
23	6725			
24	6733			*
25	6734			
26	16221		*	
27	16960			
28	17079			
29	17133			
30	17180			
31	17206			
32	6233			
33	158305			

Table 4.4. Score sheet of beneficial characteristics observed amongst the control plots in the P2B trial.

Plots	Events (Control treatments)	High no. lacewings	Ladybirds	Spiders
C1	DP50			
C2	DP50B			
C3	C312			
C4	C531			
C5	15813		*	
C6	15985			

Table 4.5. Cotton treatments in the 2004/05 biodiversity trial.

	Treatments	Action	Herbicide applications
1	Delta OPAL	Scout and spray programme[*]	Pre emerge (Dual/Cotogard)
2	Delta OPAL	None (Control)	Pre emerge (Dual/Cotogard)
3	DeltaOPAL RR	Scout and spray programme	RRH (2 x)[¥] – 1 OT[§] – 1D[$]
4	DeltaOPAL RR	None (Control)	RRH (2 x) – 1 OT – 1D
5	NuOPAL	Scout and spray programme	Pre emerge (Dual/Cotogard)
6	NuOPAL	None (Control)	Pre emerge (Dual/Cotogard)
7	NuOPAL RR	Scout and spray programme	RRH (2 x) – 1 OT – 1D
8	NuOPAL RR	None (Control)	RRH (2 x) – 1 OT – 1D

[*] *Scout and spray programme = Spray according to bollworm threshold (5 larvae/24 plants)*
[¥] *RRH (2 x) = Two Roundup Ready Herbicide applications*
[§] *OT = Over the top of the cotton canopy*
[$] *D = as a directed spray*

Table 4.6. Scout dates and insecticide applications in the 2004/05 biodiversity trial.

Date	Assessments	Insecticidal applied (rate)	Treatments (Cotton types)
9/02/2005	1	Endosulfan (1l/ha)	DeltaOPAL and DeltaOPAL RR
11/02/2005	2	No spray	-
23/02/2005	3	No spray	-
7/03/2005	4	Endosulfan (1l/ha)	DeltaOPAL and DeltaOPAL RR
9/03/2005	5	No spray	-
17/03/2005	6	Endosulfan (1l/ha); Acetamiprid 6g/15 l water	DeltaOPAL and DeltaOPAL RR; Delta OPAL, Delta OPAL RR, NuOPAL, and NuOPAL RR
19/03/2005	7	No spray	-

Table 4.7. Predators found on the different varieties during scouting (x) and after sweeping (&) during 2003/04.

Common name	Order / Class	Family	Genus / Species name	NuOPAL RR		DeltaOPAL RR		NuOPAL		DeltaOPAL	
Pirate Bugs	Hemiptera (Heteroptera)	Anthocoridae	Orius spp.		&		&		&		&
Leafhoppers	Hemiptera (Homoptera)	Cicadellidae	Jacobiella facialis Jacoby J. libyca	x		x		x		x	
Ladybirds	Coleoptera	Coccinellidae	Coccinella septempunctata L.	x	&	x	&	x	&	x	&
Ladybirds	Coleoptera	Coccinellidae	Scymnus spp.	x	&	x	&	x	&	x	&
Ant beetle	Coleoptera	Anthicidae	Noxos sp.	x	&	x	&	x	&	x	&
Rove beetle	Coleoptera	Staphylynidae			&				&		
Green lacewing	Neuroptera	Chrysopidae	Chrysopa spp.	x	&	x	&	x	&	x	&
Green lacewings	Neuroptera	Chrysopidae	Chrysoperla spp.	x				x			
Hoverfly	Diptera	Syrphidae	Syrphus spp.?	x	&	x	&	x	&	x	&
Earwigs	Dermaptera	Forficulidae			&						
Dragonfly	Odonata	Aeshnidae	Aeshna spp.?		&						
Praying mantis	Mantodea	Mantidae	?	x		x					
Ants	Hymenoptera	Formicidae	Crematogaster sp.		&						
Parasitoid	Hymenoptera	Braconidae	Bracon spp.? Apanteles spp?						&		&
Parasitoid	Hymenoptera	Ichneumonidae	?		&		&				&
Parasitoid	Hymenoptera	Trichogrammatidae	Trichogramma spp.?		&		&		&		&
Spiders	Arachnidae	Thomisidae	Misumenops rubrodecoratus Millot,		&		&		&		&
Spiders		Thomisidae	?		&		&				
Spiders		Linyphiidae	Ostearius melanopygius (Cambridge)								&
Spiders		Linyphiidae	?						&		
Spiders		Philodromidae	Tibellus minor Lessert								&
Spiders		Salticidae	Heliophanus sp.						&		
Spiders		Salticidae	Aelurillus sp.						&		
Jumping spider		Salticidae	Pardosa sp.		&						
Jumping spider		Salticidae	Heliophanus sp.		&						
Spiders		Theridiidae	Euryopis sp.						&		
Spiders		Theridiidae	Afropisaura sp.		&						
Spiders		Theridiidae	Enoplognatha sp.		&						
Spiders		Lycosidae	Pardosa sp.				&				
Spiders		Lycosidae	Theridion sp.		&						
Spiders		Miturgidae	Cheiracanthium sp.				&				
Spiders		Araneidae	Neoscona subfusca (Koch)				&				
Fishing spiders		Pisauridae	Philodromus sp.		&						
Spiders		Lycosidae	Pardosa sp.		&						
Spiders		Salticidae	Heliophanus sp.		&						

* *Species unconfirmed*

** *Species noticed during scouting and sweeping, and not necessarily collected and recorded*

Table 4.8. Predators found, classified according to categories in the 2004/05 biodiversity trial.

Category 3: Predators		Category 4: Other arthropods	
Spiders (Arachnida)	Unid. Noctuidae (Lepidoptera)	Beetle unid. (Coleoptera)	(Calliphoridae; Diptera)
Spider egg package Arachnida	Unid. Pieridae (Lepidoptera)	Protostrophus spp; (Coleoptera)	Long-legged mosquito (Tipulidae; Diptera)
Ear-wigs Dermaptera	Acrocerops spp. (Lepidoptera)	Chrysomelidae (Coleoptera)	Lyriomyza spp; (Diptera).
Predatory mites	Aphis mellifera (Hymenoptera)	CMR beetle (Meloidae; Coleoptera)	Small unid. Black fly? (Diptera)
Predatory thrips	Wasp (Hymenoptera)	Black beetle, unid. (Coleoptera)	Zonocerus elegans; (Orthoptera)
Predatory Hemiptera	Formicidae (Hymenoptera)	Nisotra spp. (Coleoptera)	Brown leafhopper
Minute pirate bug	Ephemeroptera	Astylus spp. (Melyridae, Coleoptera)	Cricket (Gryllidae; Orthoptera)
Parasitic wasps Hymenoptera?		Flea beetle (Coleoptera)	Red Spider mites (Tetanychus cinnabarinus)
Parasitized Aphididae (Homoptera		Mordeliidae (Coleoptera)	

Table 4.9. Numbers of predators found on the different varieties by means of scouting (2003/04) in the biodiversity trial.

Predators	NuOPAL RR	NuOPAL	DeltaOPAL RR	DeltaOPAL
	(36 plants x 6 counts)	(36 plants x 6 counts)	(36 plants x 6 counts)	(48 plants x 6 counts)
Ladybird eggs	1	129	21	8
Ladybird larvae	25	21	8	31
Ladybird pupae	8	12	2	4
Ladybird adult	16	23	9	25
Lacewing eggs	42	31	32	35
Lacewing larvae	11	1	2	2
Lacewing pupae	5	2	0	2
Lacewing adults	5	1	1	2
Hover fly larvae	2	0	0	0
Hover fly adults	0	1	0	0
Spider	6	5	6	22
Spider egg package	3	6	0	0
Praying mantis	1	1	0	0
Dragonfly	0	1	0	0
Dermaptera	0	1	1	0
Predatory mite	3	18	8	5
Predatory thrips	9	0	3	1
Predatory bug	8	5	3	37
Minute pirate bug	5	0	0	7
Parasitic wasps	0	0	0	0
Parasitized aphids	6	13	22	19
Totals	156	271	118	200
Mean per plant/assessment	0.72	1.25	0.54	0.69
Mean per plant/assessment (excluding ladybird eggs)	0.72	0.66	0.45	0.67

Table 4.10. The total number of predators found by sweeping during the 2003/04 biodiversity trial.

Predators (all life stages)	Varieties			
	NuOPAL RR	NuOPAL	DeltaOPAL RR	DeltaOPAL
Ladybirds	46	19	10	38
Lacewings	11	15	33	8
Spiders	40	1	8	12
Hoverfly	1	2	5	2
Earwigs	2	0	0	0
Parasitic wasps (Braconidae)	20	57	19	8
Predatory thrips	5	0	0	1
Minute pirate bug	1	0	0	0
Total	**126**	**94**	**75**	**69**

Table 4.11. The mean number of predators per sprayed treatment per count in the 2004/05 biodiversity trial.

	Treatments	Count 1[$]	Count 2	Count 3	Count 4[$]	Count 5	Count 6[$¥]	Count 7	Totals
1	DeltaOPAL sprayed	7.25 a	4.00 ac	4.50 a	16.25 a	14.50 a	18.75 a	12.00 a	77.25
3	DeltaOPAL RR sprayed	10.00 ab	6.25 a	12.25 b	18.25 a	10.25 a	14.75 a	13.00 a	84.75
5	NuOPAL sprayed	13.13 b	14.50 b	15.25 b	14.25 a	16.75 b	19.00 a	18.25 a	111.13
7	NuOPAL RR sprayed	4.88 a	12.00 ab	14.75 b	16.75 a	17.00 a	15.50 a	12.25 a	93.13

Means followed by the same symbol/s within a column are not statistically significant ($p<0.05$).
[$] *sprayed with endosulfan*
[¥] *sprayed with acetamiprid*

Table 4.12. Significant differences $_{(p<0.05)}$ between sprayed treatments comparing predator numbers (2003/04).

Treatment number	Treatments	Significance (Pr > /t/) (Df = 15)						
		Count 1[$]	Count 2	Count 3	Count 4[$]	Count 5	Count 6[$¥]	Count 7
1	DeltaOPAL sprayed	0.0498	0.0844	0.0018	<0.0001	0.0001	<0.0001	0.0058
3	DeltaOPAL RR sprayed	0.0009	0.0124 *(0.0112)	<0.0001 *(0.0004)	<0.0001	0.0022 *(0.0963)	<0.0001	0.0035
5	NuOPAL sprayed	< 0.0001 *(0.0276)	<0.0001 *(0.0028)	<0.0001 *(<0.0001)	<0.0001	<0.0001 *(0.0374)	<0.0001	0.0010
7	NuOPAL RR sprayed	0.0549 *(0.0257)	<0.0001 *(0.0208)	<0.0001 *(<0.0001)	<0.0001	<0.0001	<0.0001	0.0051

** Probability values for the Least Square Means (Pr>/t/ for Ho)*
[$] sprayed with endosulfan
[¥] sprayed with acetamiprid

Table 4.13. The mean number of predators per unsprayed treatment per count in the 2004/05 biodiversity trial.

	Treatments	Count 1[$]	Count 2	Count 3	Count 4	Count 5	Count 6	Count 7	Totals
2	DeltaOPAL unsprayed	10.13 a	15.50 a	12.75 a	13.25 a	11.25 a	15.25 a	17.50 a	95.63
4	DeltaOPAL RR unsprayed	10.00 a	14.25 a	14.50 a	15.25 a	8.75 a	18.50 a	21.25 a	102.50
6	NuOPAL unsprayed	11.38 a	12.00 a	13.00 a	14.50 a	7.25 a	16.00 a	21.75 a	95.88
8	NuOPAL RR unsprayed	11.00 a	13.50 a	8.75 a	18.50 a	19.75 b	7.00 b	19.25 a	97.75

** Means followed by the same symbol/s within a column are not statistically significant (p<0.05).*

Table 4.14. Significant differences $_{(p<0.05)}$ for predator means between unsprayed treatments in the 2004/05 biodiversity trial.

Treatment number	Treatments	Significance (1%) (Pr > /t/) (DF = 15)						
		Count 1[$]	Count 2	Count 3	Count 4	Count 5	Count 6	Count 7
2	DeltaOPAL unsprayed	<0.0001	<0.0001	0.0002	0.0002	<0.0001	<0.0001	<0.0001
4	DeltaOPAL RR unsprayed	<0.0001	<0.0001	<0.0001	<0.0001	0.0008 *(0.0102)	<0.0001	<0.0001
6	NuOPAL unsprayed	<0.0001	0.0001	0.0002	0.0001	0.0032 *(0.0020)	<0.0001 *(0.0096)	<0.0001
8	NuOPAL RR unsprayed	<0.0001	<0.0001	0.0034	<0.0001	<0.0001 *(0.0008)	0.0213 *(0.0476) *(0.0331)	<0.0001

**Probability values for the Least Square Means (Pr>/t/ for Ho)*

Table 4.15. Comparison of predator treatment means across all treatments in the 2004/05 biodiversity trial.

Treatments	Count 1[$]	Count 2	Count 3	Count 4[$]	Count 5	Count 6[$¥]	Count 7	Totals
DeltaOPAL sprayed	7.25[c]	4.00[c]	4.50[a]	16.25[a]	14.50[a]	18.75[a]	12.00[a]	66.00
DeltaOPAL unsprayed	10.13[b]	15.50[a]	12.75[bc]	13.25[a]	11.25[a]	15.25[a]	17.50[ab]	85.50
Pr>F	0.0792	0.0010	0.0049	0.3740	0.3345	0.3528	0.2262	
Delta OPAL RR sprayed	10.00[b]	6.25[c]	12.25[bc]	18.25[a]	10.25[a]	14.75[a]	13.00[ab]	74.75
DeltaOPAL RR unsprayed	10.00[b]	14.25[a]	14.50[b]	15.25[a]	8.75[abc]	18.50[a]	21.25[b]	92.50
Pr>F	1.0000	0.0152	0.4061	0.3740	0.6535	0.3202	0.0747	
NuOPAL sprayed	13.13[a]	14.50[a]	15.25[b]	14.25[a]	16.75[b]	19.00[a]	18.25[ab]	98.00
NuOPAL unsprayed	11.38[ab]	12.00[ab]	13.00[bc]	14.50[a]	7.25[be]	16.00[a]	21.75[b]	84.50
Pr>F	0.5373	0.2989	0.6427	0.5034	0.0016	0.5482	0.1709	
NuOPAL RR sprayed	4.88[c]	12.00[ab]	14.75[b]	16.75[a]	17.00[ad]	15.50[a]	12.25[ab]	88.25
NuOPAL RR unsprayed	11.00[b]	13.50[a]	8.75[ab]	18.50[a]	19.75[bc]	7.00[b]	19.25[ab]	86.75
Pr>F	0.0384	0.6286	0.0335	0.6021	0.4129	0.0304	0.1270	

** Probability values for the Least Square Means (Pr>/t/ for Ho)*
[$] sprayed with endosulfan
[¥] sprayed with acetamiprid

Table 4.16. Significant differences $_{(p<0.05)}$ in predators means across all treatments in the 2004/05 biodiversity trial.

Treatment number	Treatments	Significance (1%) (Pr > /t/) (DF = 15)						
		Count 1[$]	Count 2	Count 3	Count 4[$]	Count 5	Count 6[$¥]	Count 7
1	DeltaOPAL sprayed	0.0184	0.0770 *(0.0010) *(0.0027) *(0.0012) *(0.0152) *(0.0152) *(0.0048)	0.0249 *(0.0049) *(0.0076) *(0.0010) *(0.0012) *(0.0039) *(0.008)	<0.0001	<0.0001 *(0.0379)	<0.0001	0.0008 *(0.0475) *(0.0375)
2	DeltaOPAL unsprayed	<0.0001	<0.0001 *(0.0010) *(0.0059)	<0.0001 *(0.0049)	<0.0001	<0.0001 *(0.0274) *(0.0166)	<0.0001	<0.0001
3	DeltaOPAL RR sprayed	<0.0001	0.0081 *(0.0152) *(0.0071) *(0.0263)	<0.0001 *(0.0010)	<0.0001	0.0002 *(0.0083)	<0.0001	0.0004
4	DeltaOPAL RR unsprayed	<0.0001	<0.0001 *(0.0152) *(0.0027)	<0.0001 *(0.0010)	<0.0001	0.0010 *(0.0197)	<0.0001	<0.0001
5	NuOPAL sprayed	<0.0001 *(0.0078) *(0.0070)	<0.0001 *(0.0012) *(0.0071)	<0.0001 *(0.0012)	<0.0001	<0.0001 *(0.0140) *(0.0048)	<0.0001	<0.0001
6	NuOPAL unsprayed	<0.0001 *(0.0318) *(0.0289)	<0.0001 *(0.0152)	<0.0001 *(0.0039)	<0.0001	0.0048 *(0.0379) *(0.0069) *(0.0009)	<0.0001	<0.0001
7	NuOPAL RR sprayed	0.0212 *(0.0384)	<0.0001 *(0.0152)	<0.0001 *(0.0008) *(0.0335)	<0.0001	<0.0001	<0.0001	0.0007
8	NuOPAL RR unsprayed	<0.0001 *(0.0422)	<0.0001 *(0.0048) *(0.0263)	0.0001 *(0.0409) *(0.0497) *(0.0335)	<0.0001	<0.0001	0.0131 *(0.004) *(0.0351) *(0.0466) *(0.0047) *(0.0056) *(0.0226) *(0.0304)	<0.0001

** Probability values for the Least Square Means (Pr>/t/ for Ho)*
[$] sprayed with endosulfan
[¥] sprayed with acetamiprid

Table 4.17. Numbers of predators found on the different varieties by means of sweeping (excluding Diptera and Hymenoptera) for 2003/04.

Summary data (Common or family names)	NuOPAL RR	NuOPAL	DeltaOPAL RR	DeltaOPAL
Dragonflies	1	0	0	0
Minute pirate bug	1	0	0	0
Predatory bug	0	0	3	0
Predatory thrips	5	0	0	1
Ladybird eggs	2	0	9	0
Ladybird larvae	12	3	1	14
Ladybird pupae	0	0	0	0
Ladybird adult	32	16	0	24
Lacewing eggs	0	10	31	2
Lacewing larvae	2	4	2	2
Lacewing pupae	0	0	0	1
Lacewing adults	9	1	0	3
Braconidae	20	57	19	8
Egg parasitic wasps	5	0	22	1
Braconidae (unidentified)	0	8	0	1
Ichneumonidae (unidentified)	1	0	1	6
Ants	1	0	0	0
Earwigs	2	0	0	0
Hoverfly larvae	1	2	5	2
Hoverfly adults	0	0	0	0
Red spider mites	0	3	8	2
Spiders	40	1	8	12
Spider egg packages	0	7	8	5
Predatory mites	0	0	3	0
Total	?			

Table 4.18. Total number of spiders found by sweeping per variety (excluding Diptera and Hymenoptera) during 2003/04.

Important arthropod orders	NuOPAL RR	NuOPAL	DeltaOPAL RR	DeltaOPAL
Arachnids (spiders)	40	1	8	12

Table 4.19. Matrix indicating highest treatments with statistical significance in all three statistical comparisons in the 2004/05 biodiversity trial.

Arthropod category	Treatments	Significant highest number present							
		Count 1[$]	Count 2	Count 3	Count 4[$]	Count 5	Count 6[$¥]	Count 7	Score Total
Predators	DeltaOPAL sprayed					X	XXXXXXX		8
	DeltaOPAL unsprayed		XX	X			X		4
	DeltaOPAL RR sprayed			XX					2
	DeltaOPAL RR unsprayed		XX	X			X	X	5
	NuOPAL sprayed		XXXXX	X		XXXX			10
	NuOPAL unsprayed			X			X	X	3
	NuOPAL RR sprayed	X	XX	XXX		X			7
	NuOPAL RR unsprayed	XX	XX			XXXXX			9

[$] sprayed with endosulfan; [¥] sprayed with acetamiprid

Significant incidence of predators on all Genetically Modified cotton types (excluding bollworm incidences):
DeltaOPAL RR sprayed: 10 unsprayed 8
NuOPAL: sprayed: 10 unsprayed 7
NuOPAL RR sprayed: 9 unsprayed 13

Significant incidence of predators on all DeltaOPAL (excluding bollworm incidences):
DeltaOPAL sprayed: 9 unsprayed: 7

Figures

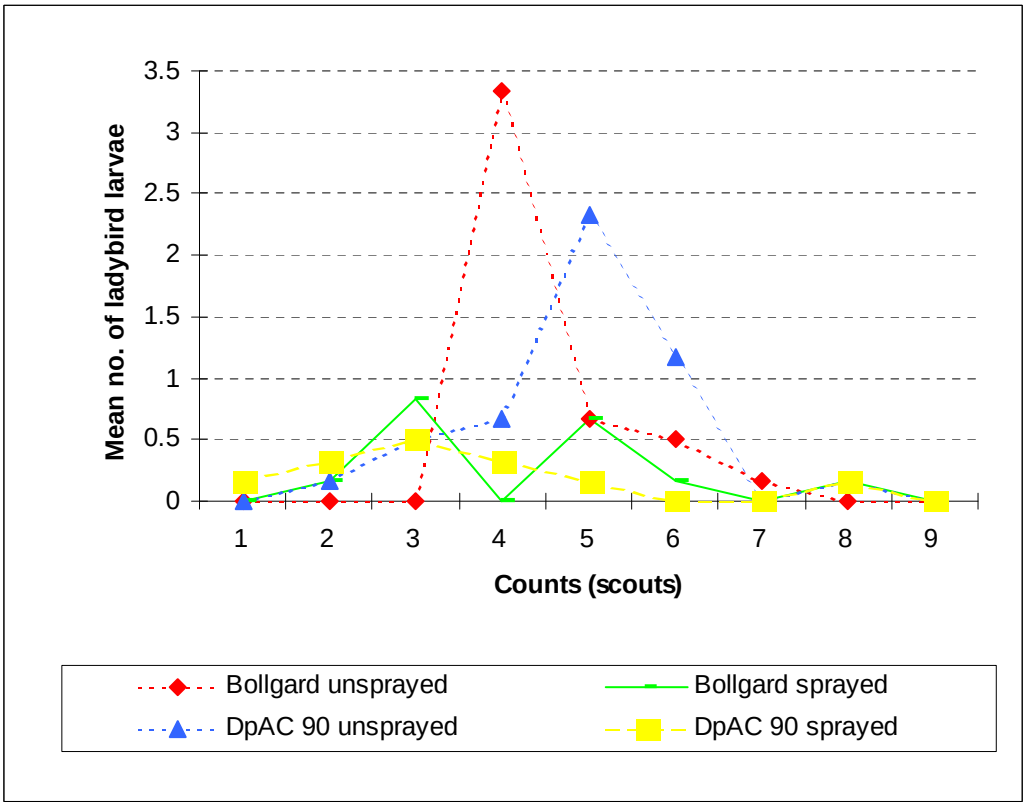


Fig. 4.1. Mean number of ladybird larvae found in Rustenburg (1997/1998) in the Cry1Ac trial.

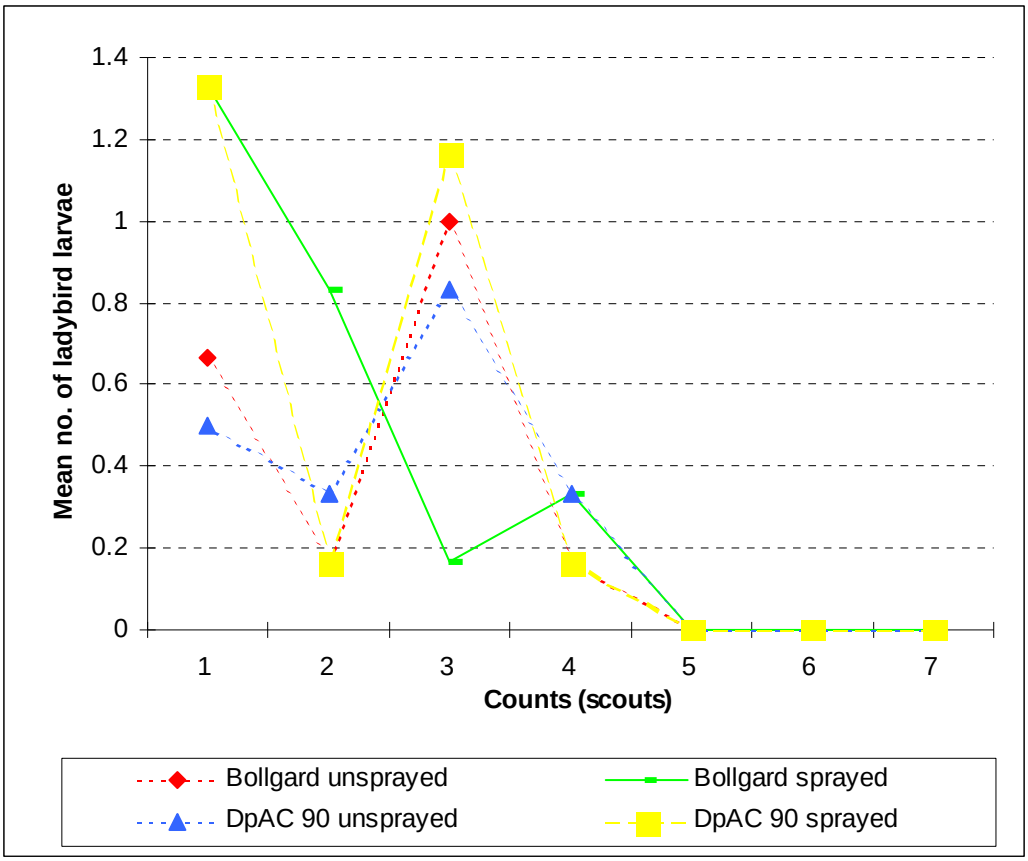


Fig. 4.2. Mean number of ladybird larvae found in Potgietersrus(1997/1998) in the Cry1Ac trial.

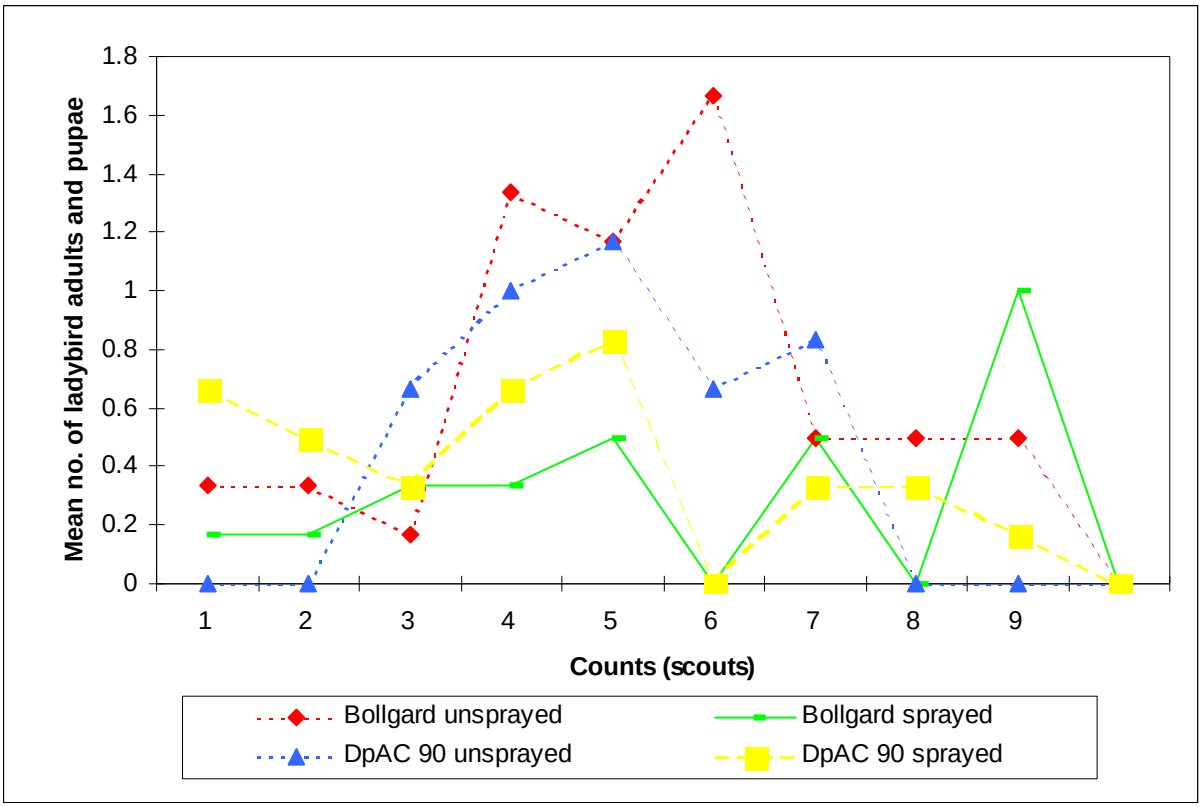


Fig. 4.3. Mean number of ladybird adults and pupae from Rustenburg(1997/1998) in the Cry1Ac trial.

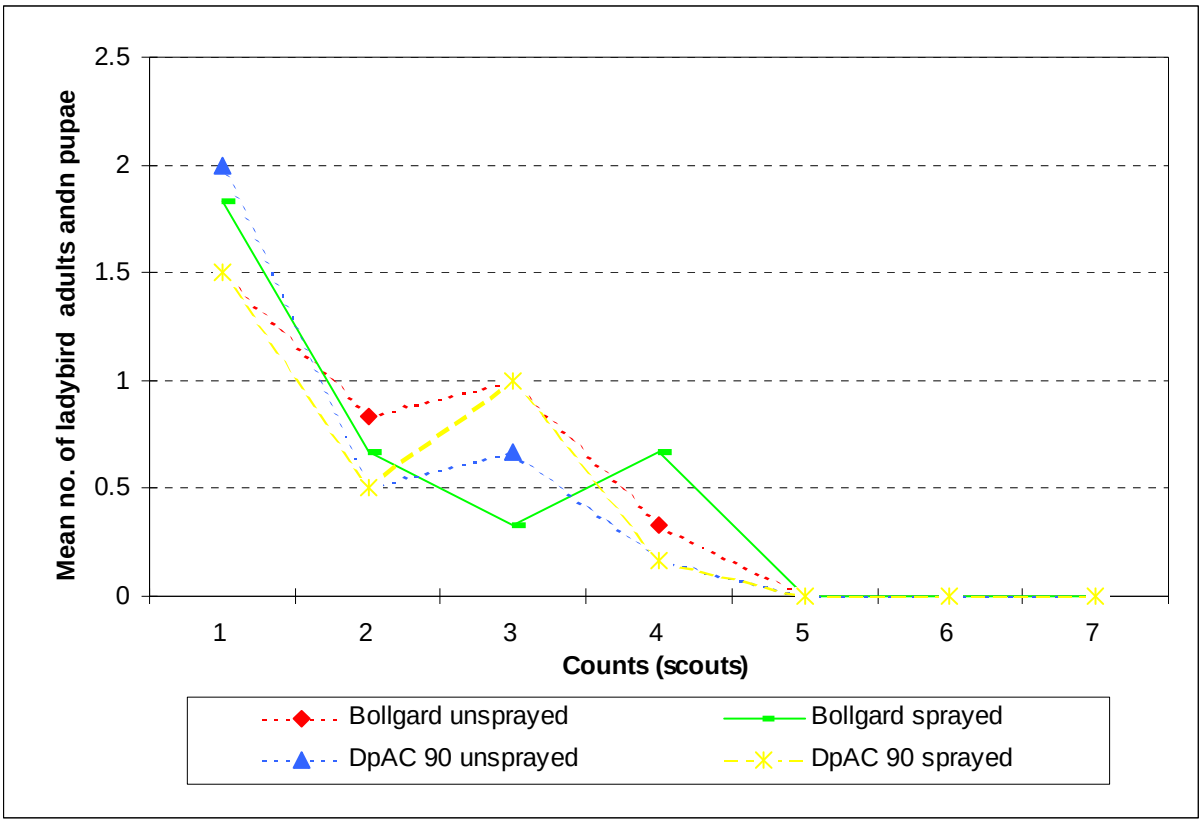


Fig. 4.4. Mean number of ladybird adults and pupae from Potgietersrus (1997/1998) in the Cry1Ac trial.

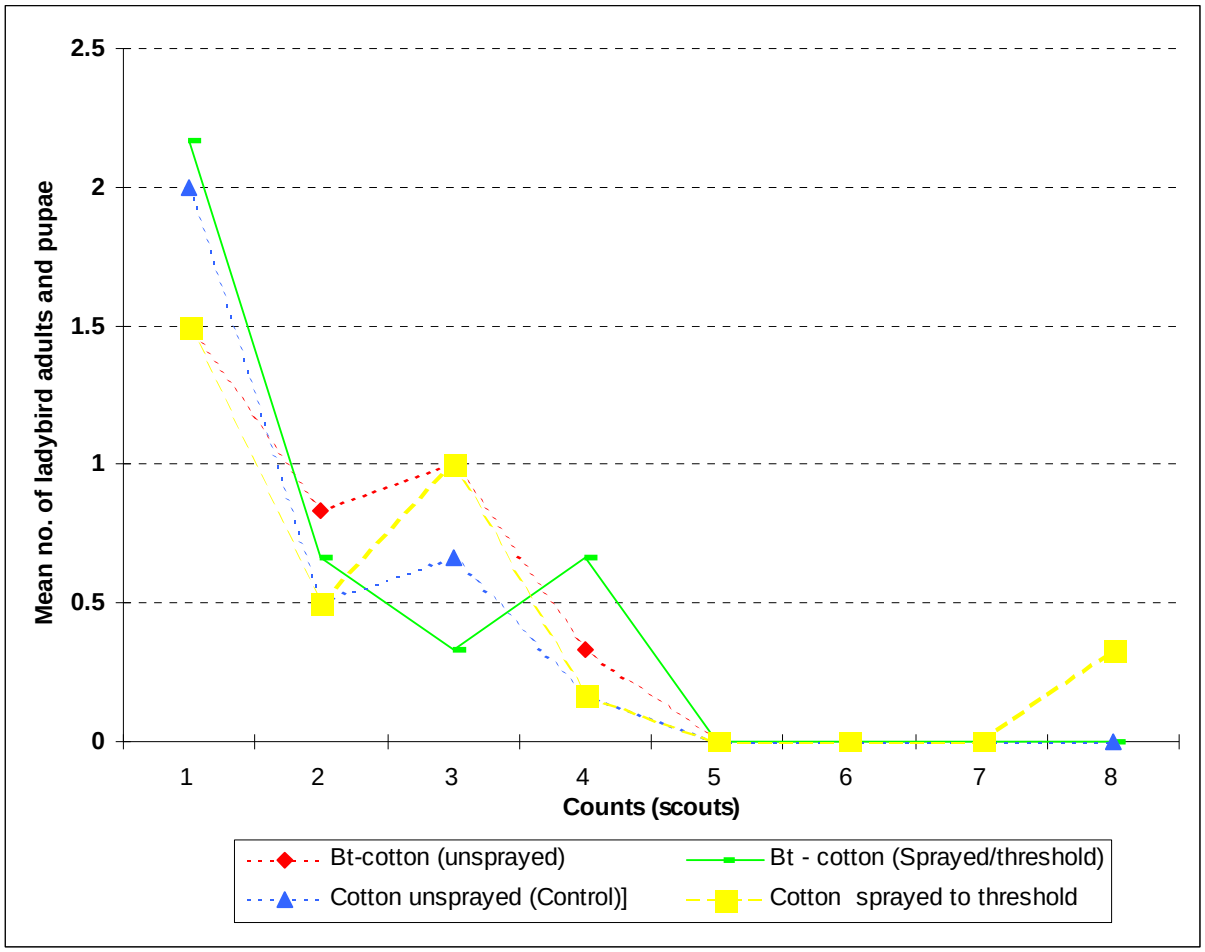


Fig. 4.5. Mean number of ladybird adults and larvae from Loskop (Groblersdal) (1998/1999) in the Cry1Ac trial.

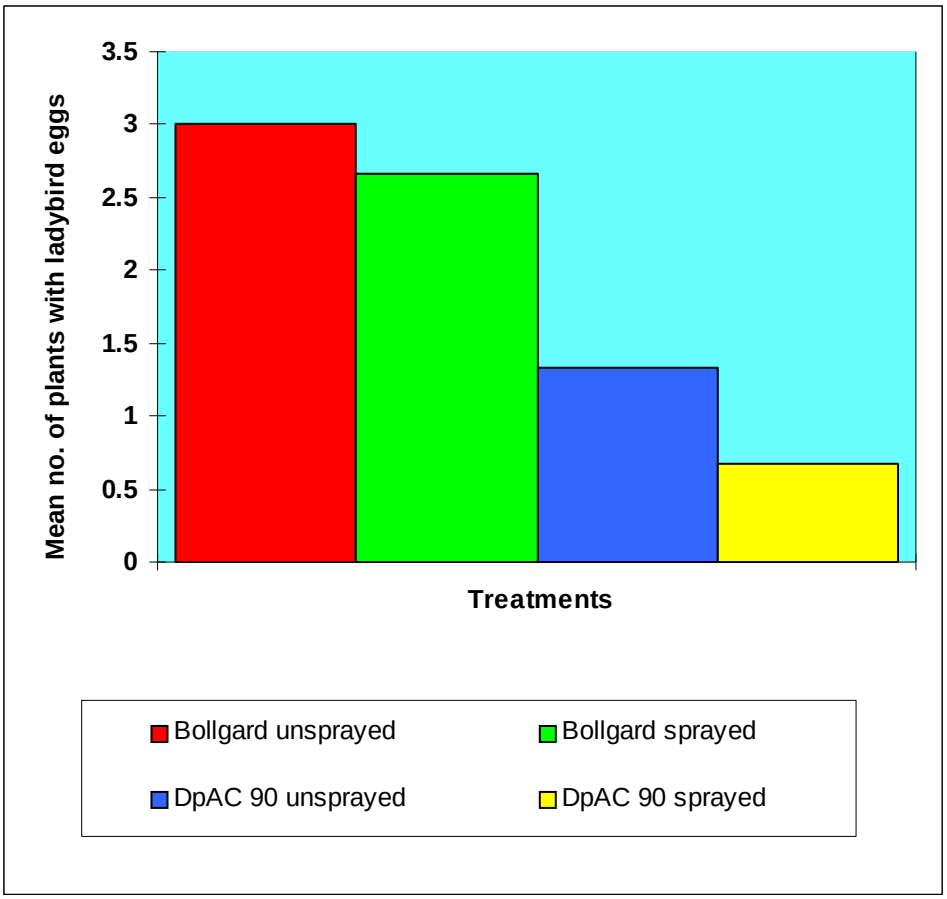


Fig. 4.6. Mean number of ladybird egg packets in Rustenburg (1997/1998) in the Cry1Ac trial.

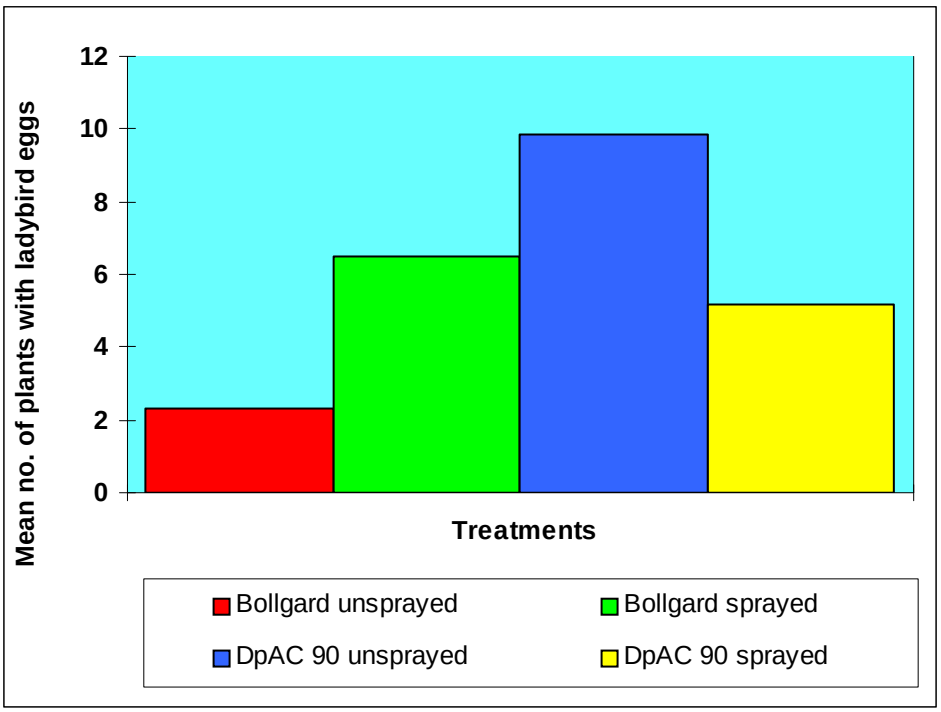


Fig. 4.7. Mean number of ladybird egg packets in Potgietersrus (1997/1998) in the Cry1Ac trial.

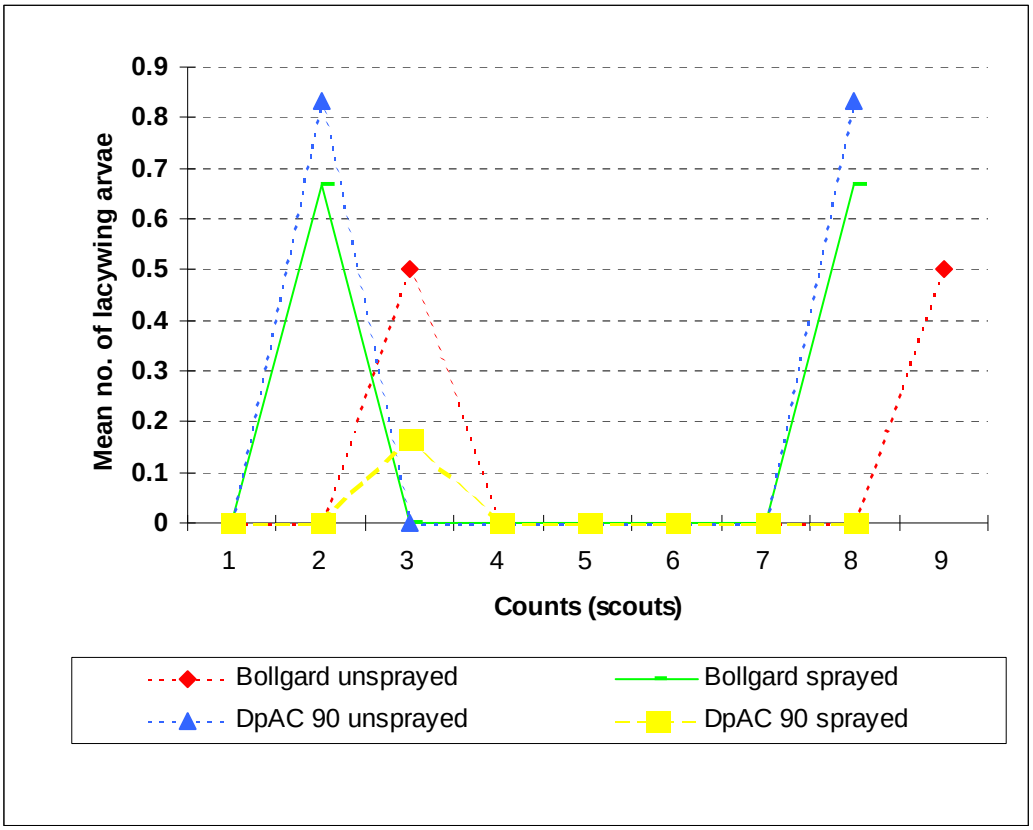


Fig. 4.8. Mean number of lacewing larvae in Rustenburg (1997/1998) in the Cry1Ac trial.

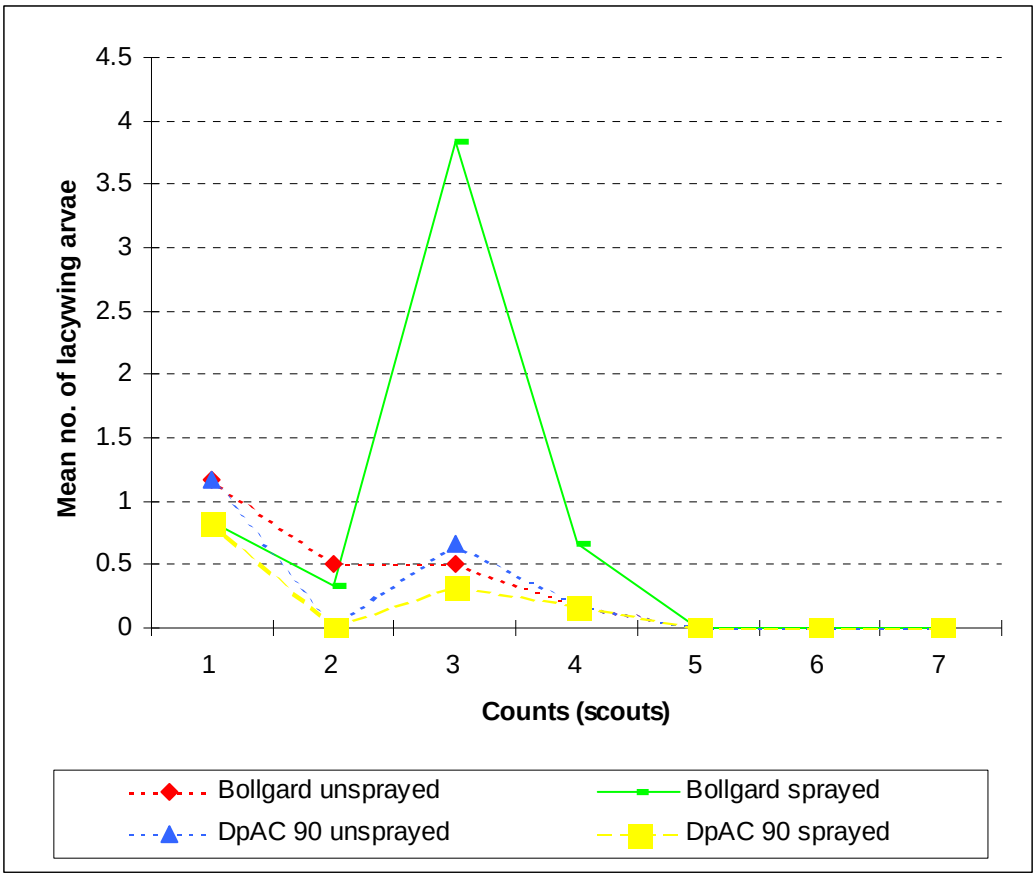


Fig. 4.9. Mean number of lacewing larvae found in Potgietersrus (1997/1998) in the Cry1Ac trial.

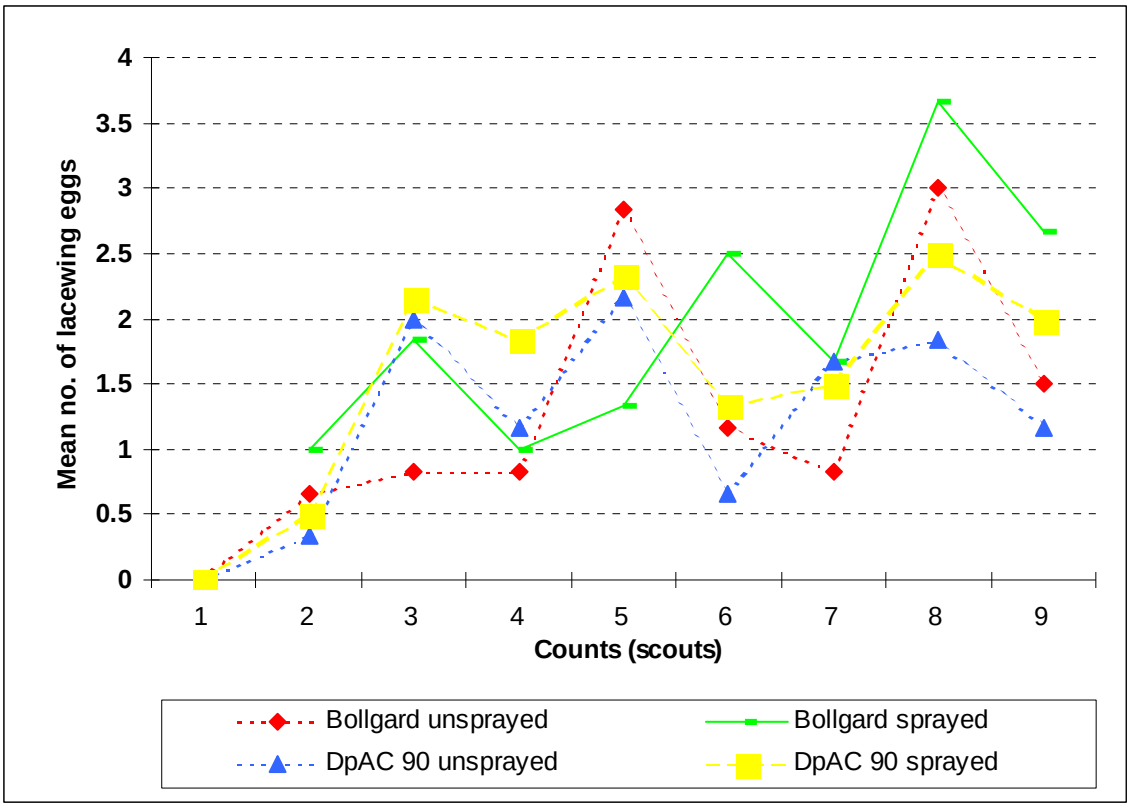


Fig. 4.10. Mean number of lacewing eggs found in Rustenburg (1997/1998) in the Cry1Ac trial.

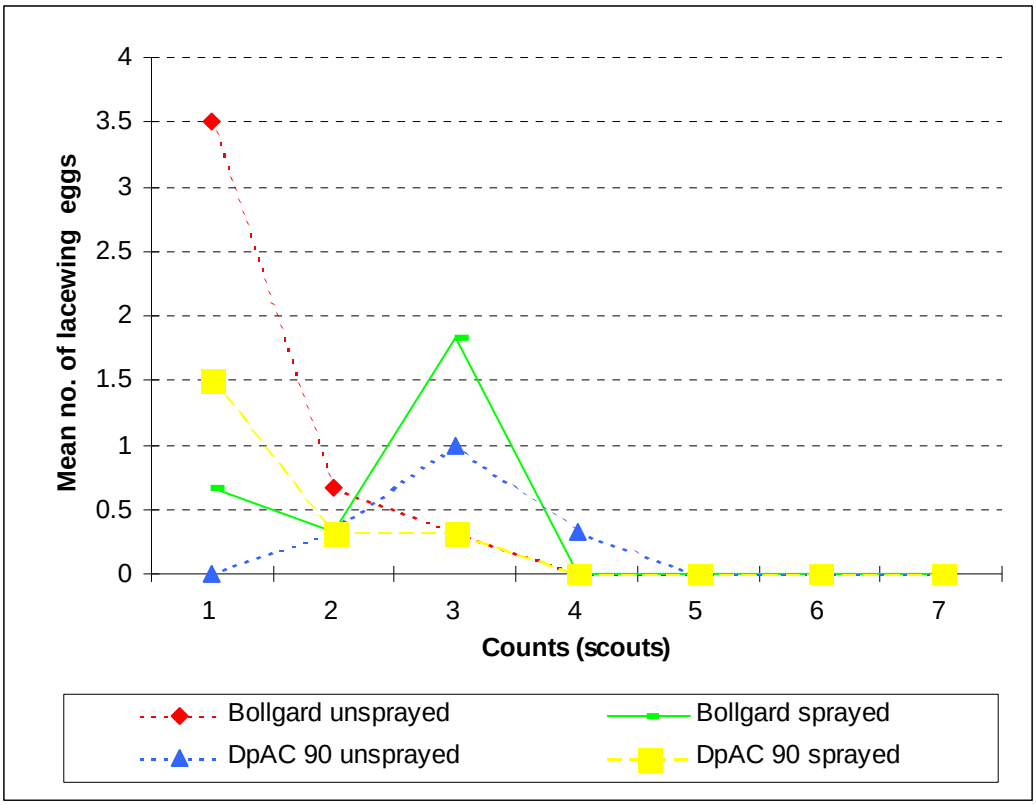


Fig. 4.11. Mean number of lacewing eggs found in Potgietersrus (1997/1998) in the Cry1Ac trial.

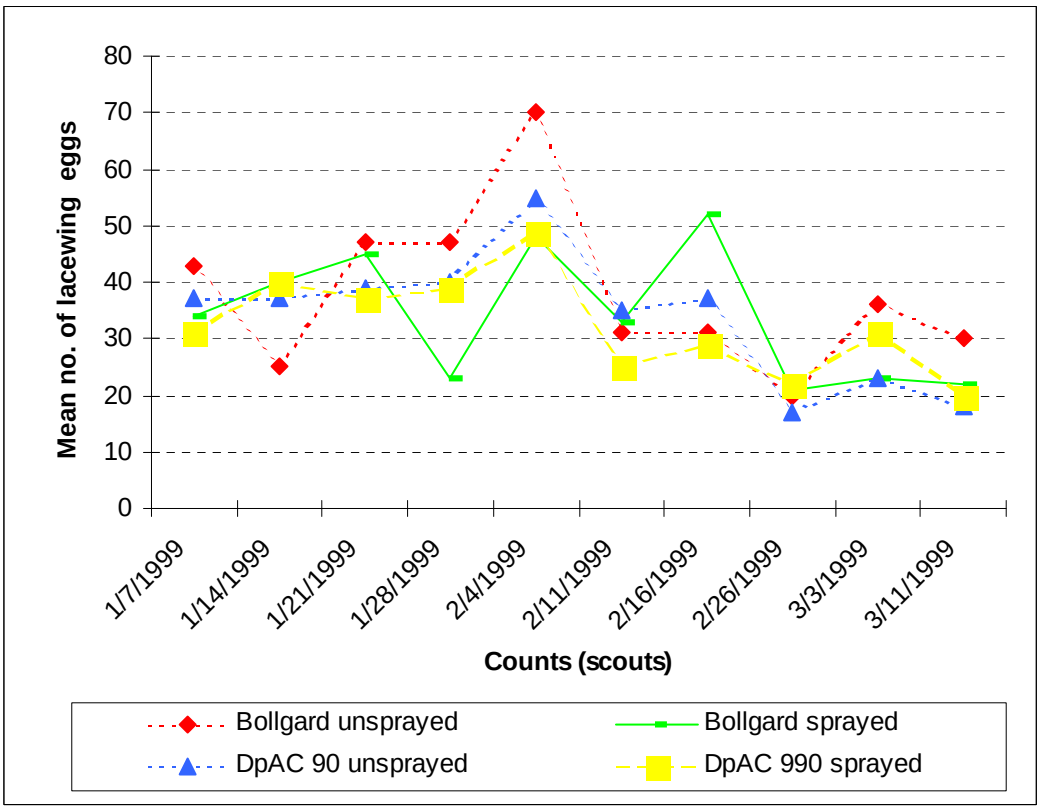


Fig. 4.12. Mean number of lacewing eggs found in Loskop (1998/1999) in the Cry1Ac trial.

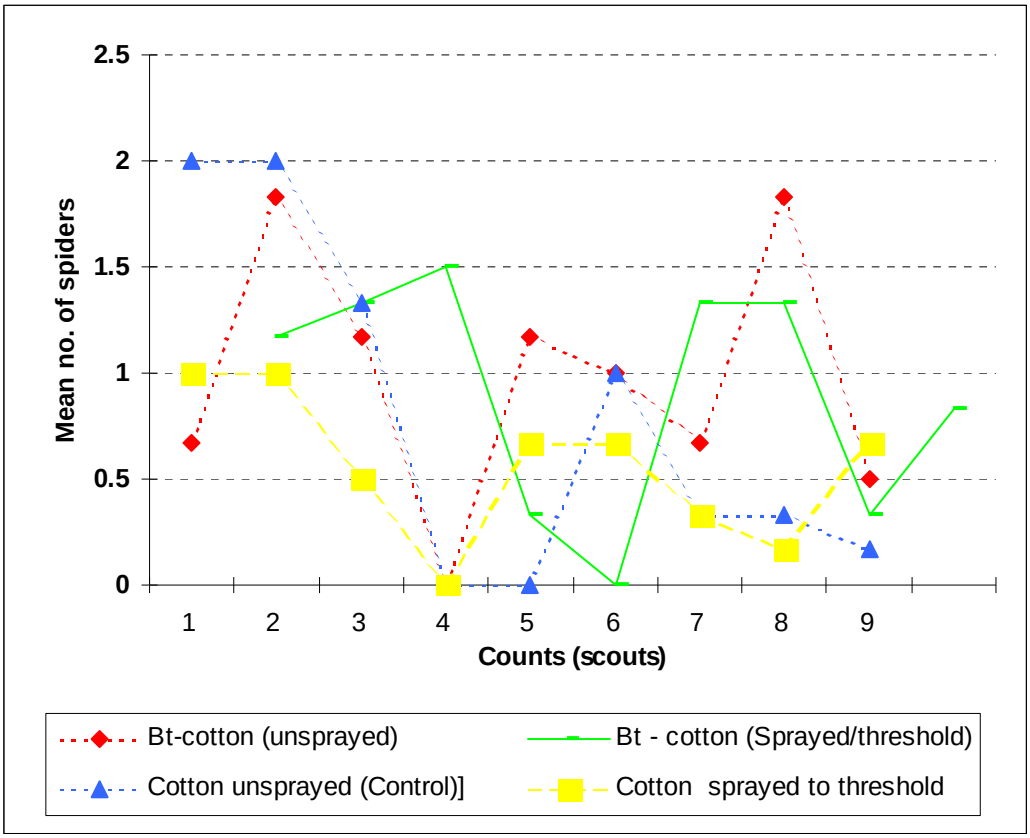


Fig. 4.13. Mean number of spiders in Rustenburg (1997/1998) in the Cry1Ac trial.

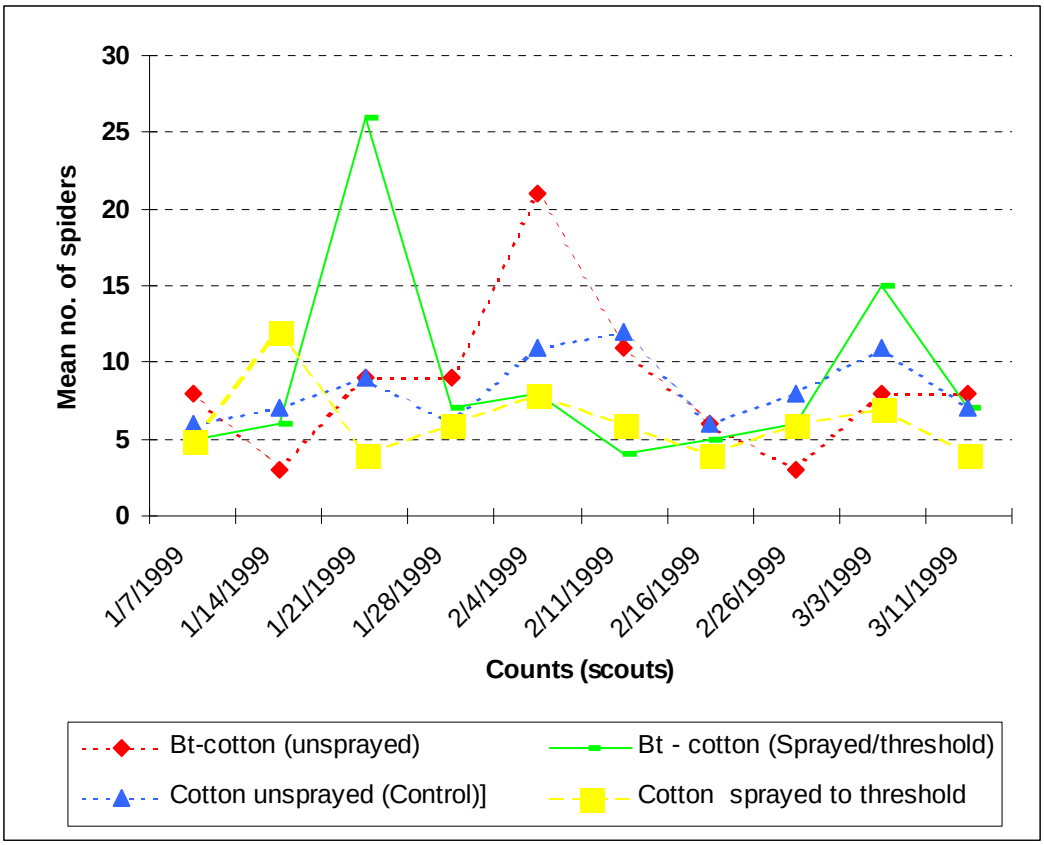


Fig. 4.14. Mean number of spiders found in Loskop (Groblersdal) (1998/1999) in the Cry1Ac trial.

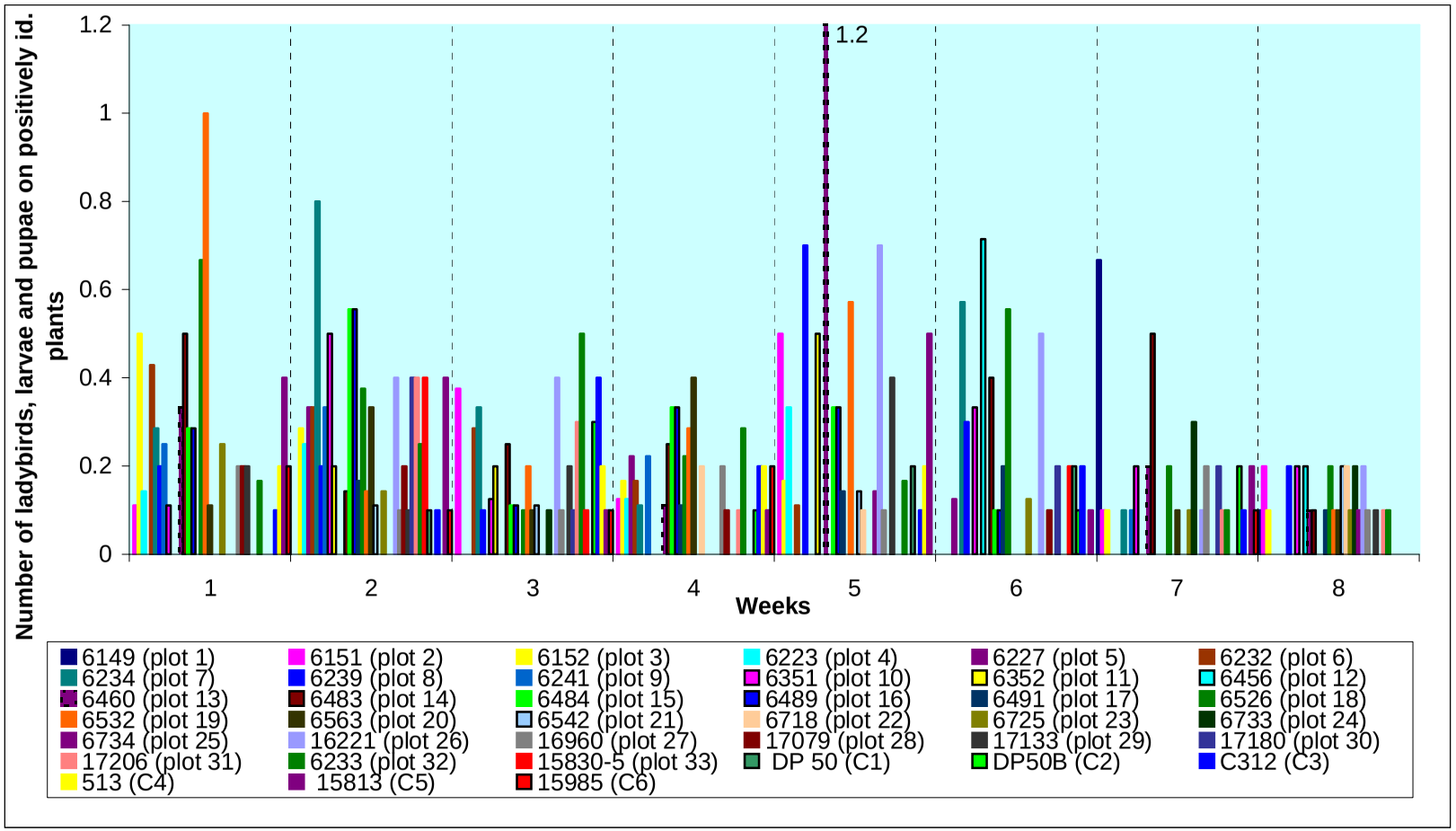


Fig. 4.15. Number of ladybird adults, larvae and pupae at different time intervals, on positively identified transgenic plants in the P2B event trial.

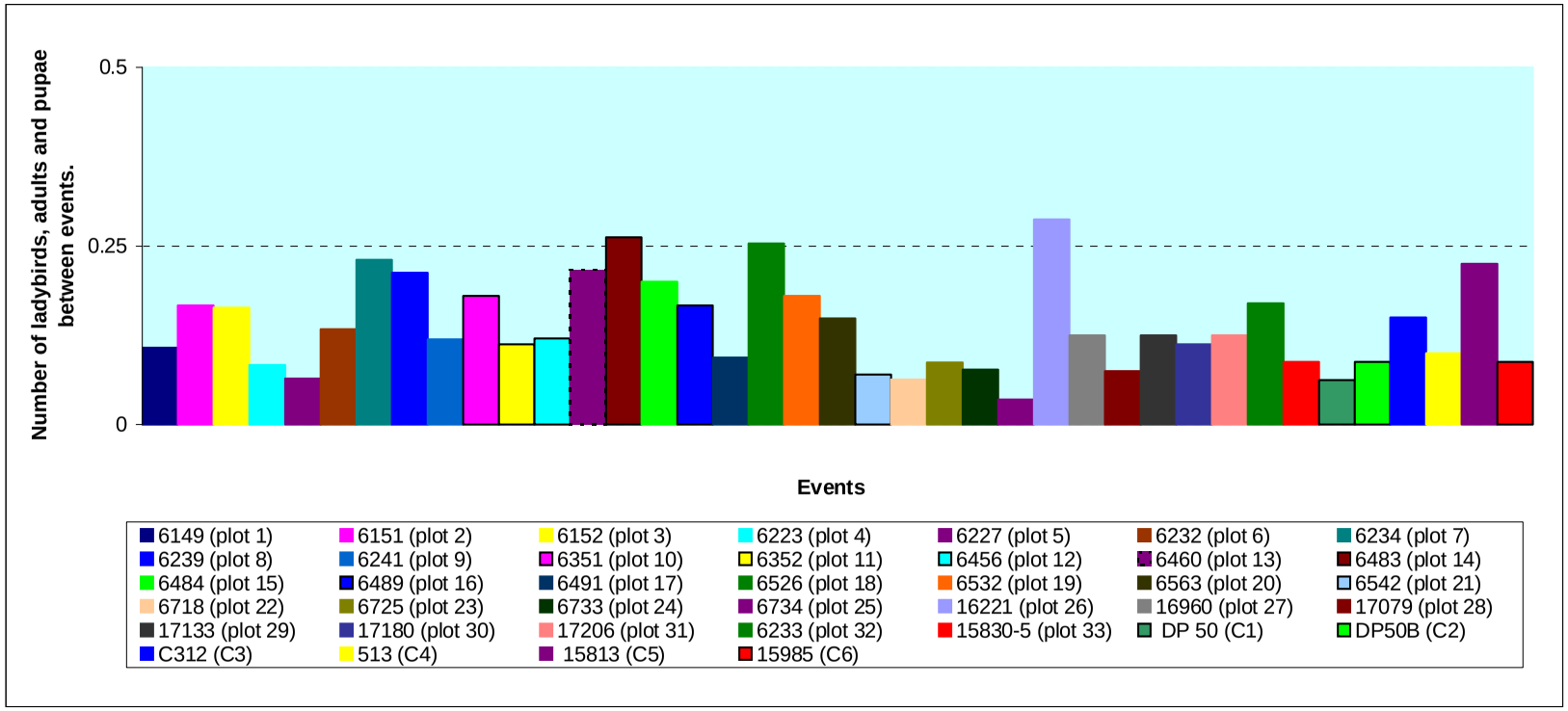


Fig. 4.16. Mean number of ladybird adults, larvae and pupae in the P2B event trial.

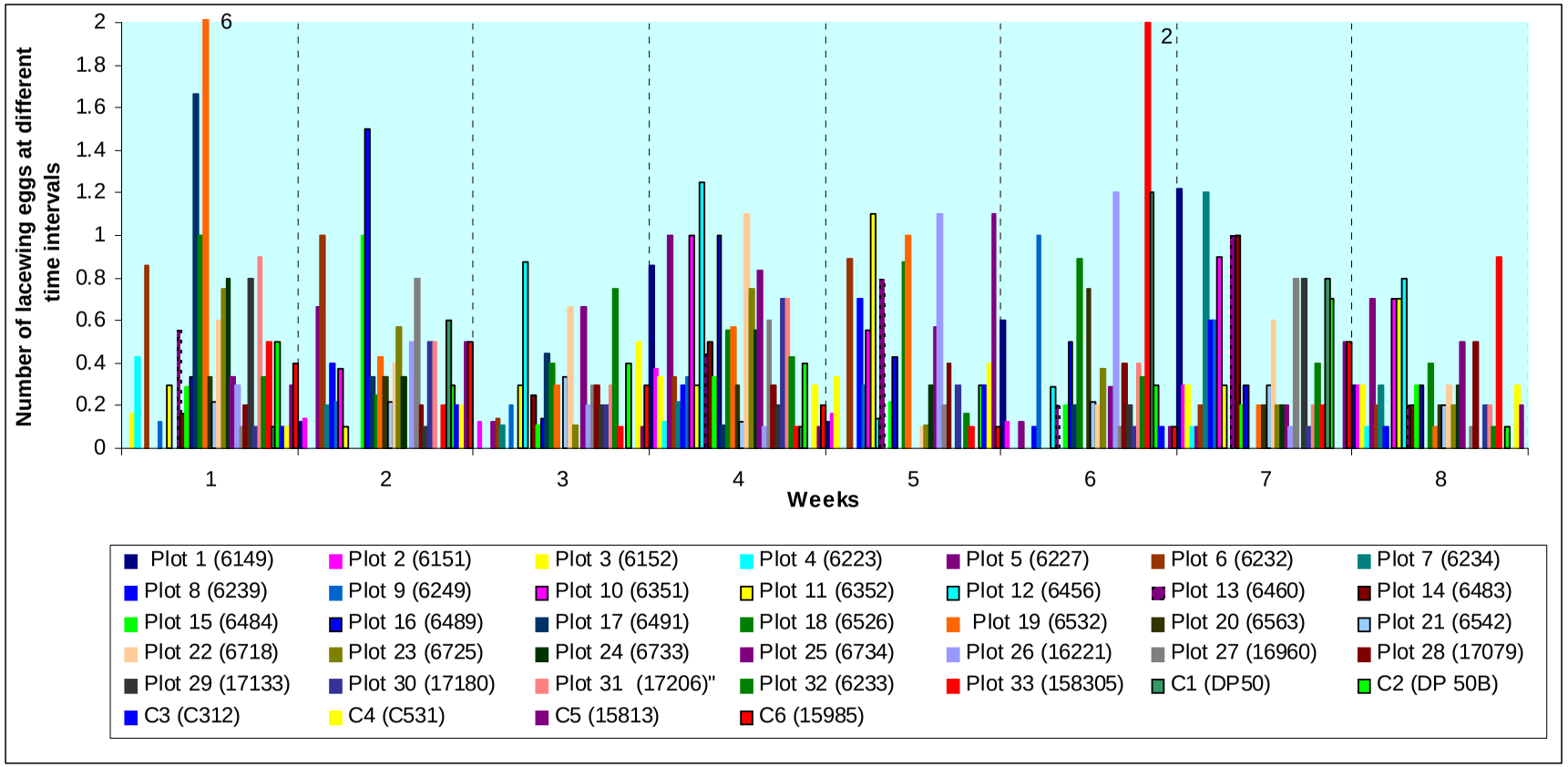


Fig. 4.17. Number of lacewing eggs at different time intervals, on positively identified transgenic plants in the P2B event trial.

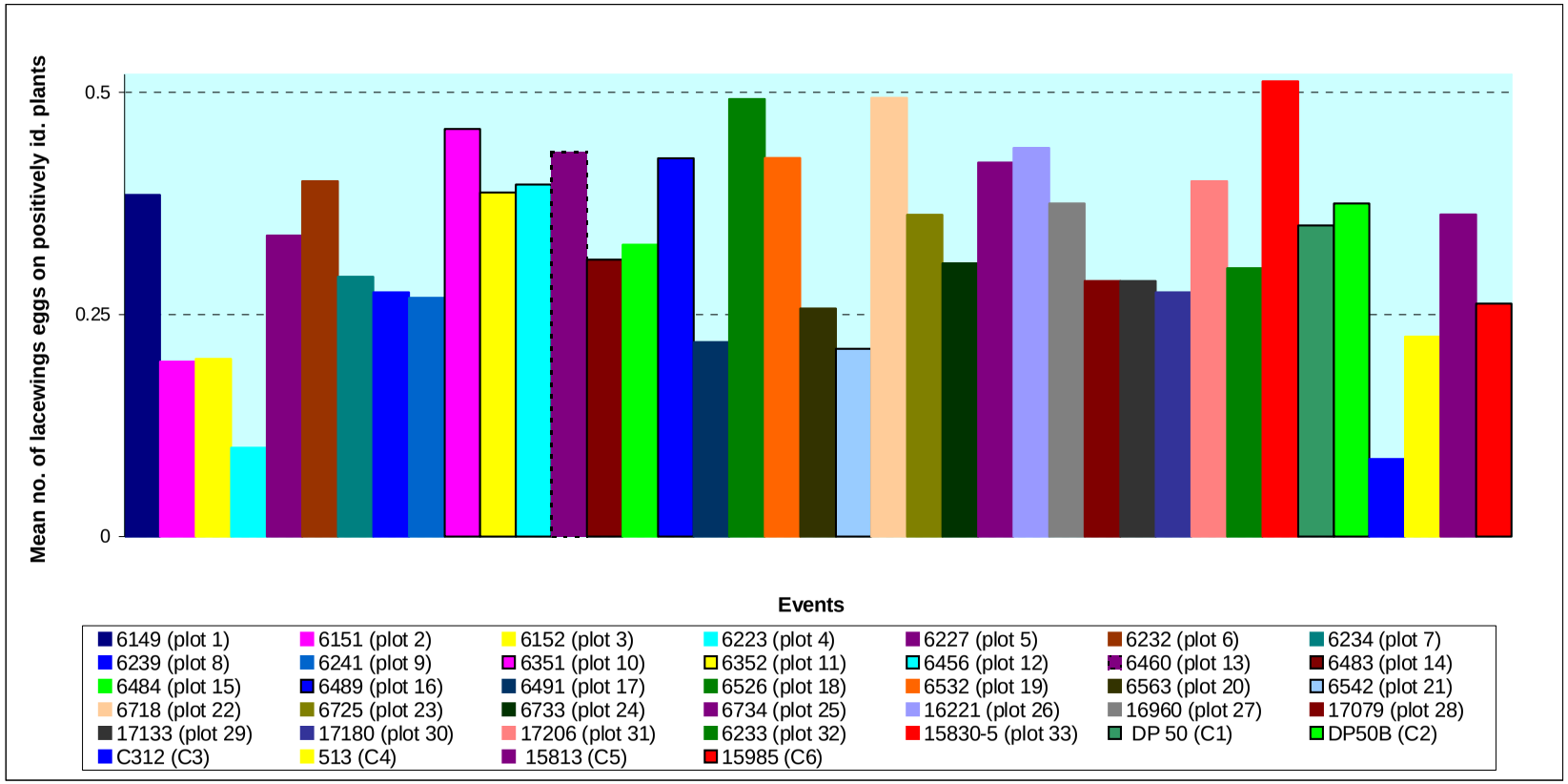


Fig. 4.18. Mean number of lacewing eggs between events, on positively identified transgenic plants in the P2B event trial.

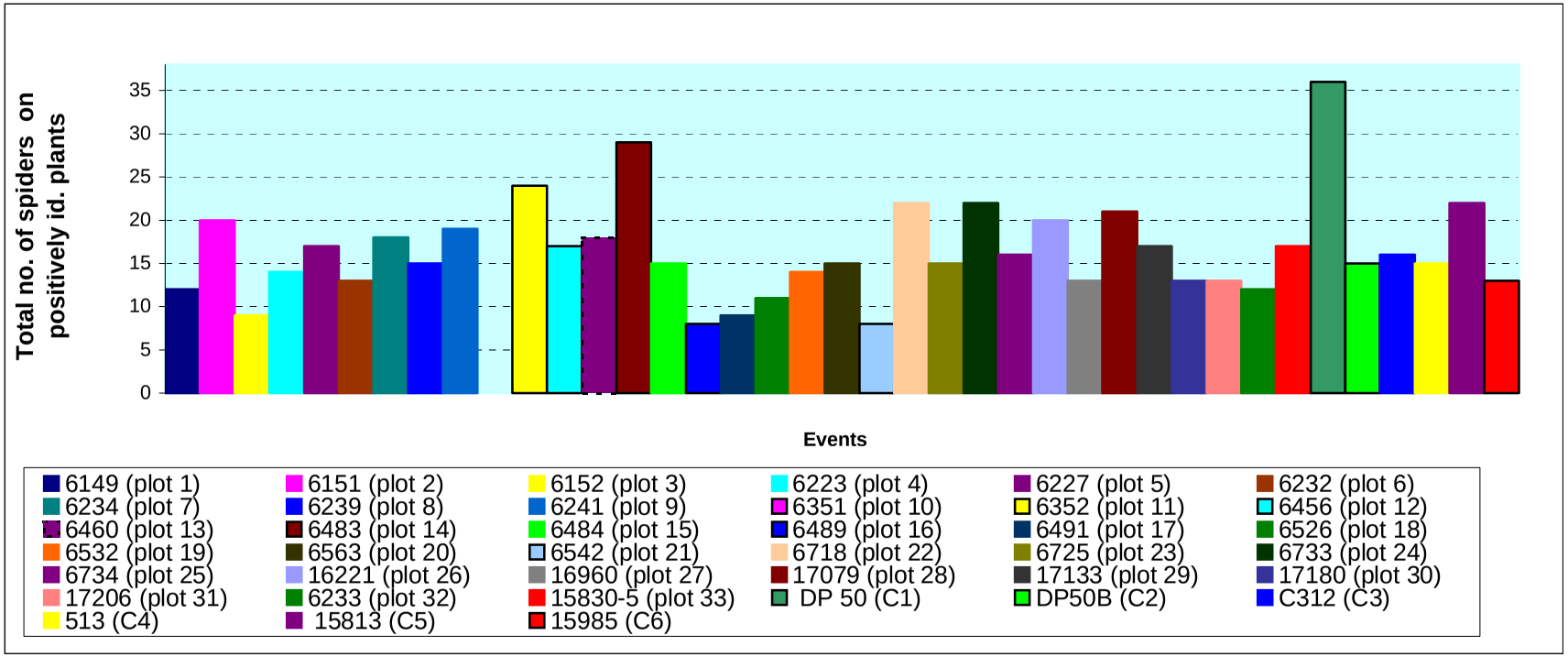


Fig. 4.19. Total number of spiders between events, on positively identified transgenic plants in the P2B event trial.

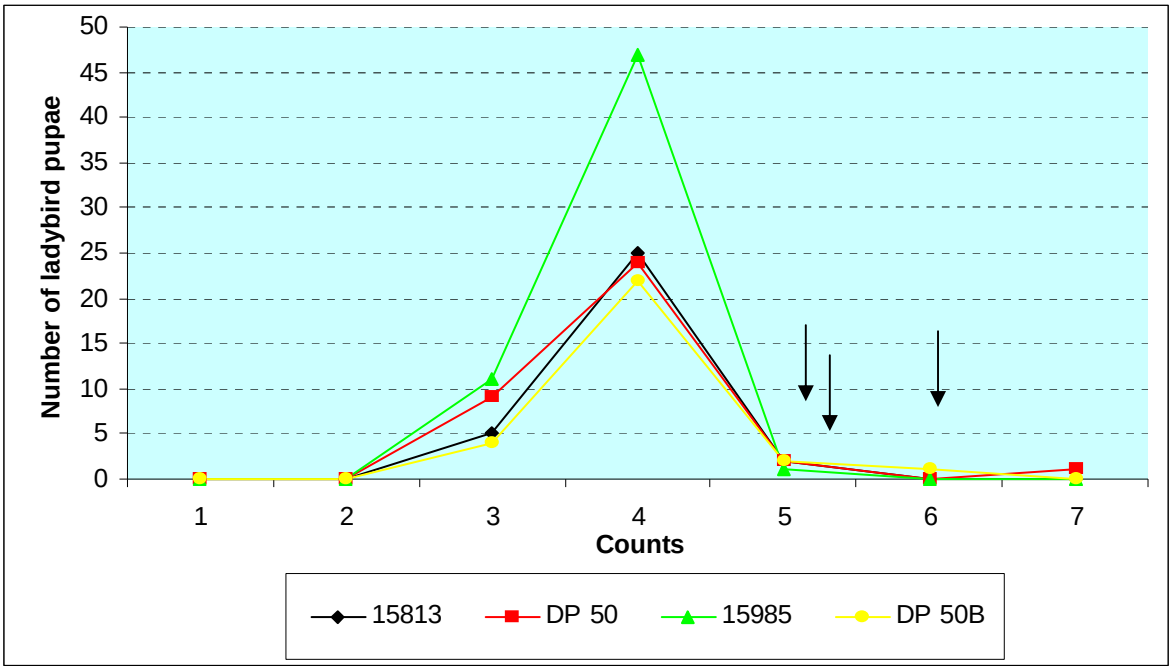


Fig. 4.20. The number of ladybird pupae counted on 20 plants per plot in each treatment at Rustenburg in the Cry2Ab2 trial. (arrows - insecticide applications).

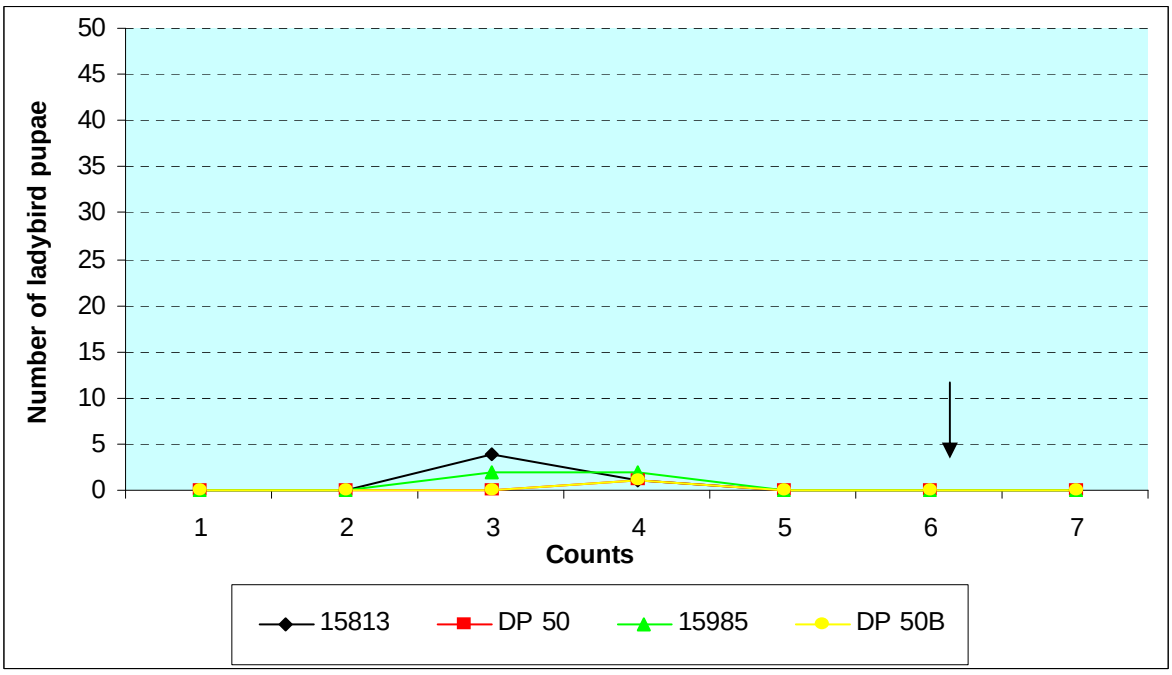


Fig. 4.21. The number of ladybird pupae counted on 20 plants per plot in each treatment at Brits in the Cry2Ab2 trial (arrow - insecticide application).

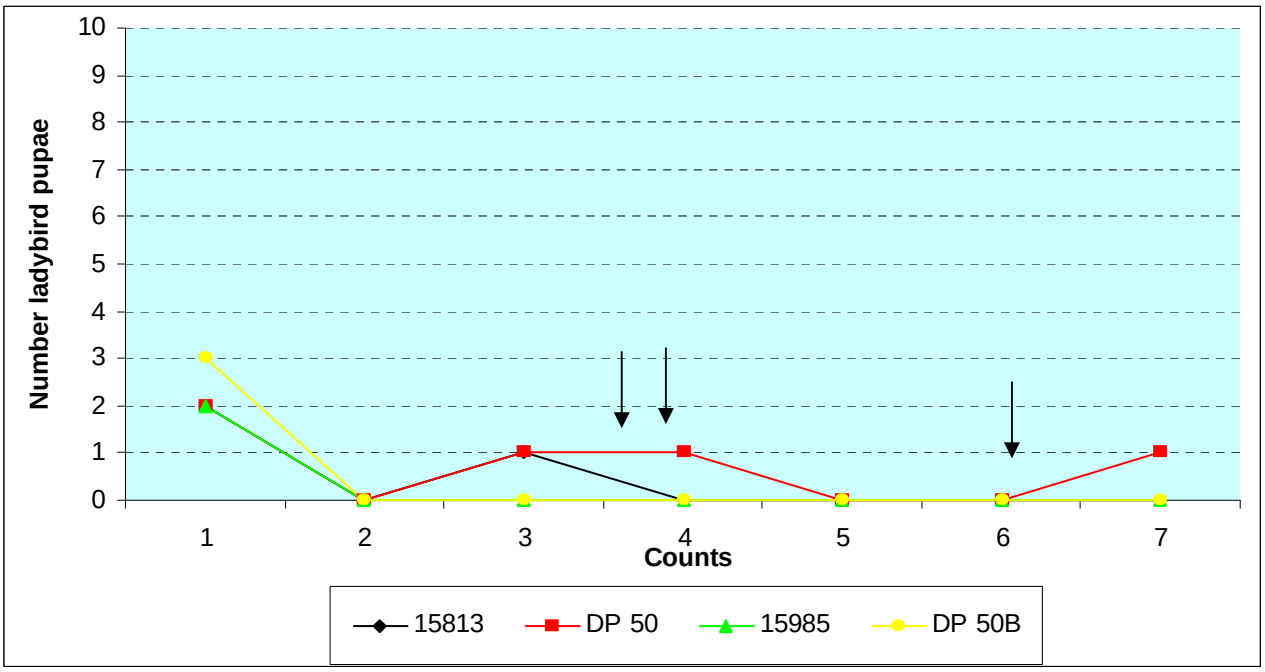


Fig. 4.22. The number of ladybird pupae counted on 20 plants per plot in each treatment at Nelspruit in the Cry2Ab2 trial (arrows - insecticide applications).

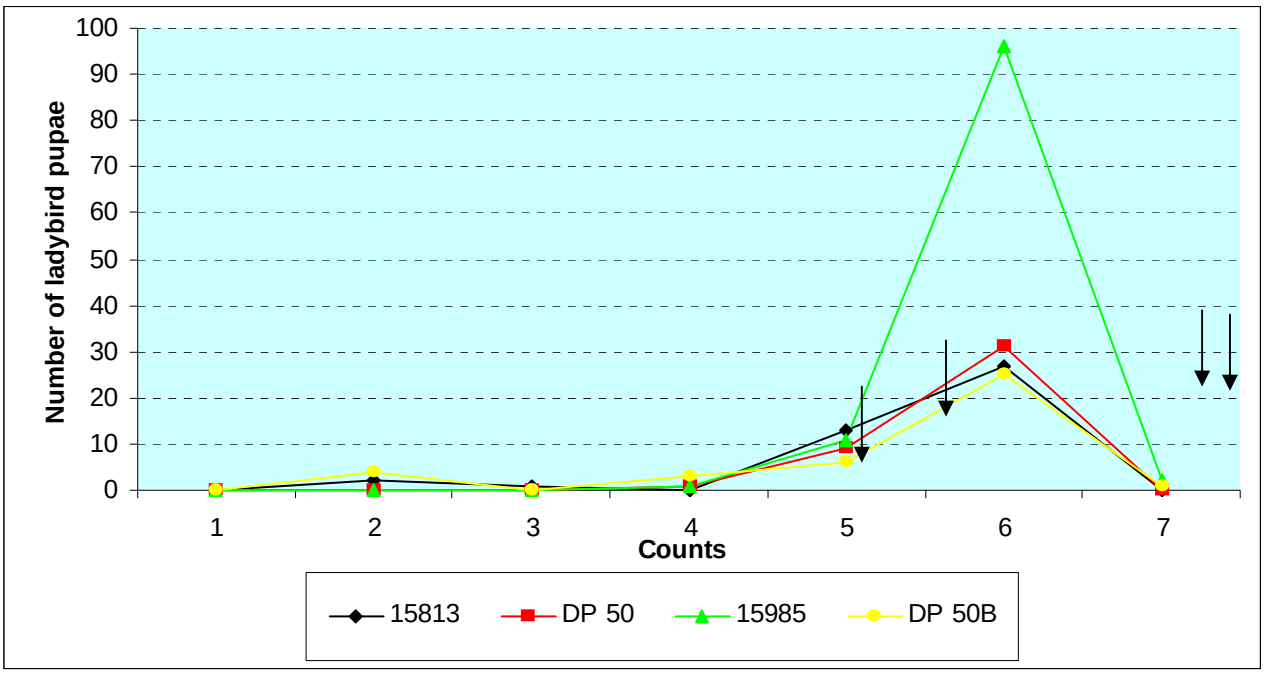


Fig. 4.23. The number of ladybird pupae counted on 20 plants per plot in each treatment at Potgietersrus in the Cry2Ab2 trial (arrows - insecticide applications).

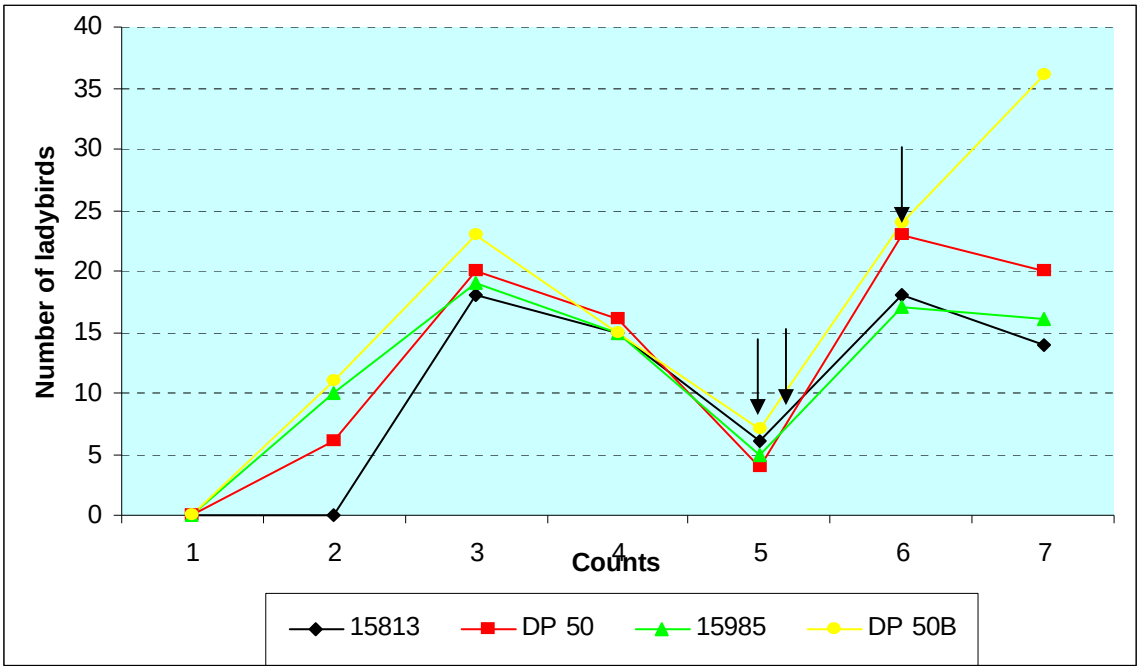


Fig. 4.24. The number of ladybird adults and egg packets counted on 20 plants per plot in each treatment at Rustenburg in the Cry2Ab2 trial (arrows - insecticide applications).

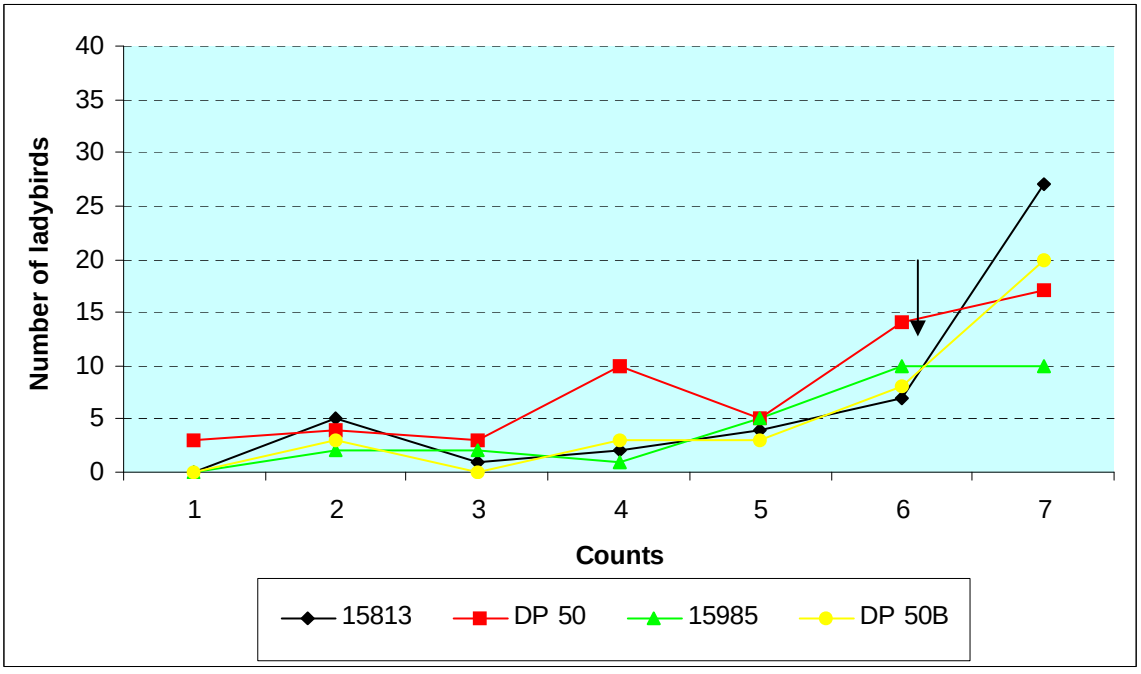


Fig. 4.25. The number of ladybird adults and egg packets counted on 20 plants per plot in each treatment at Brits in the Cry2Ab2 trial (arrows - insecticide applications).

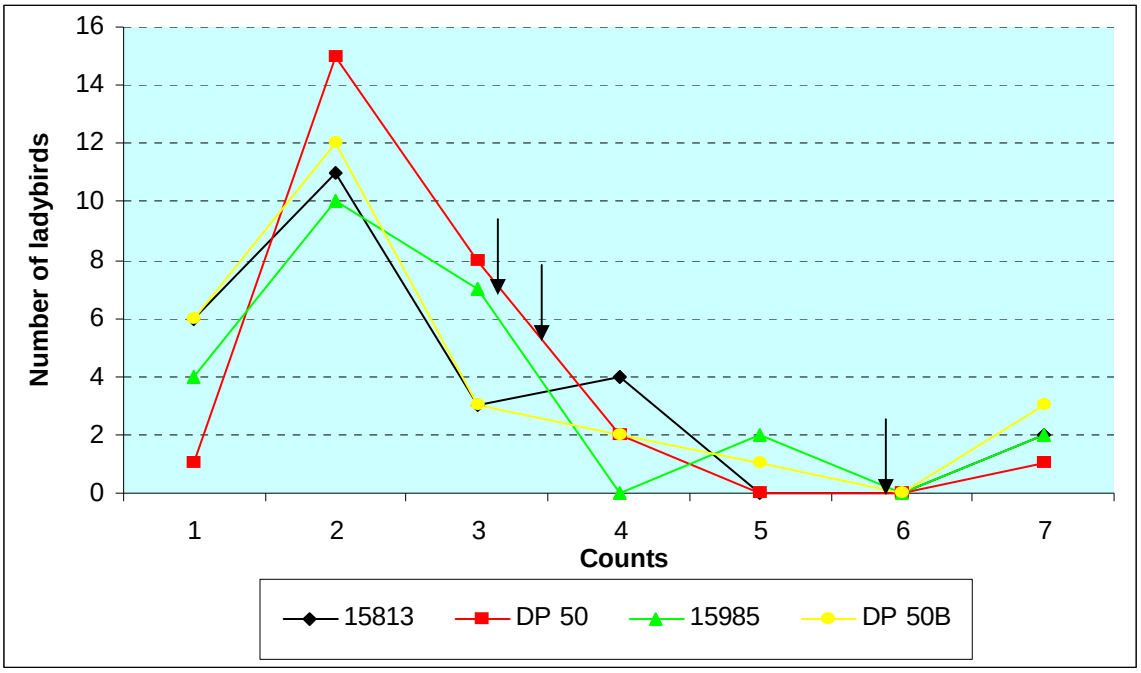


Fig. 4.26. The number of ladybird adults and egg packets counted on 20 plants per plot in each treatment at Nelspruit in the Cry2Ab2 trial (arrows - insecticide applications).

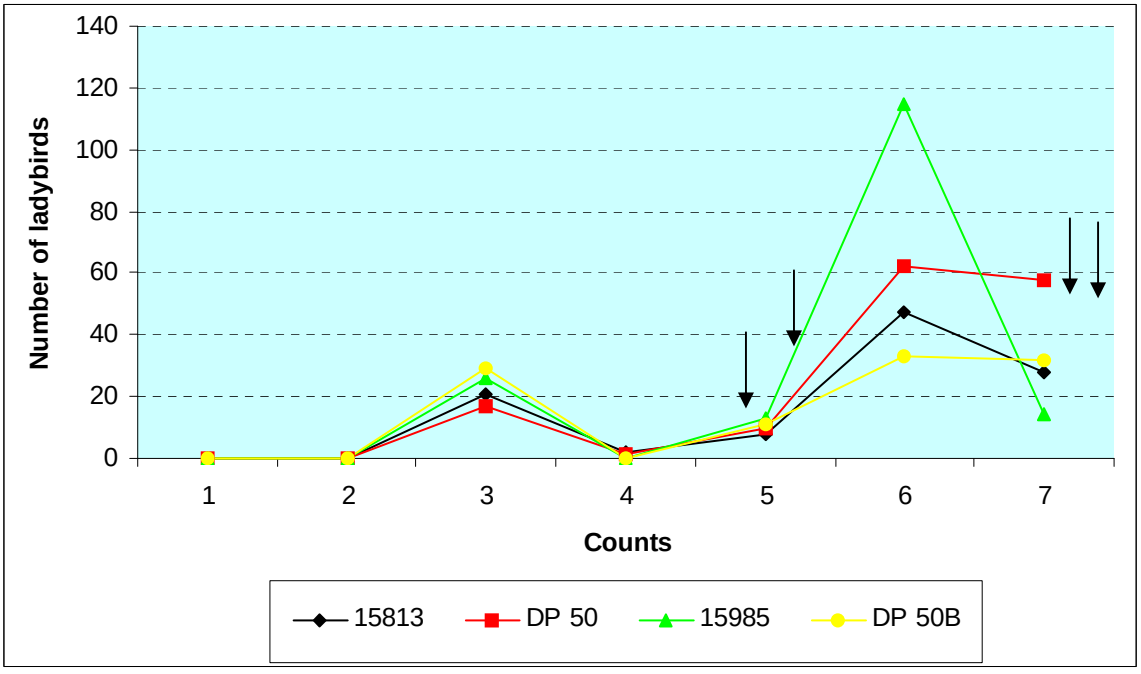


Fig. 4.27. The number of ladybird adults and egg packets counted on 20 plants per plot in each treatment at Potgietersrus in the Cry2Ab2 trial (arrows - insecticide applications).

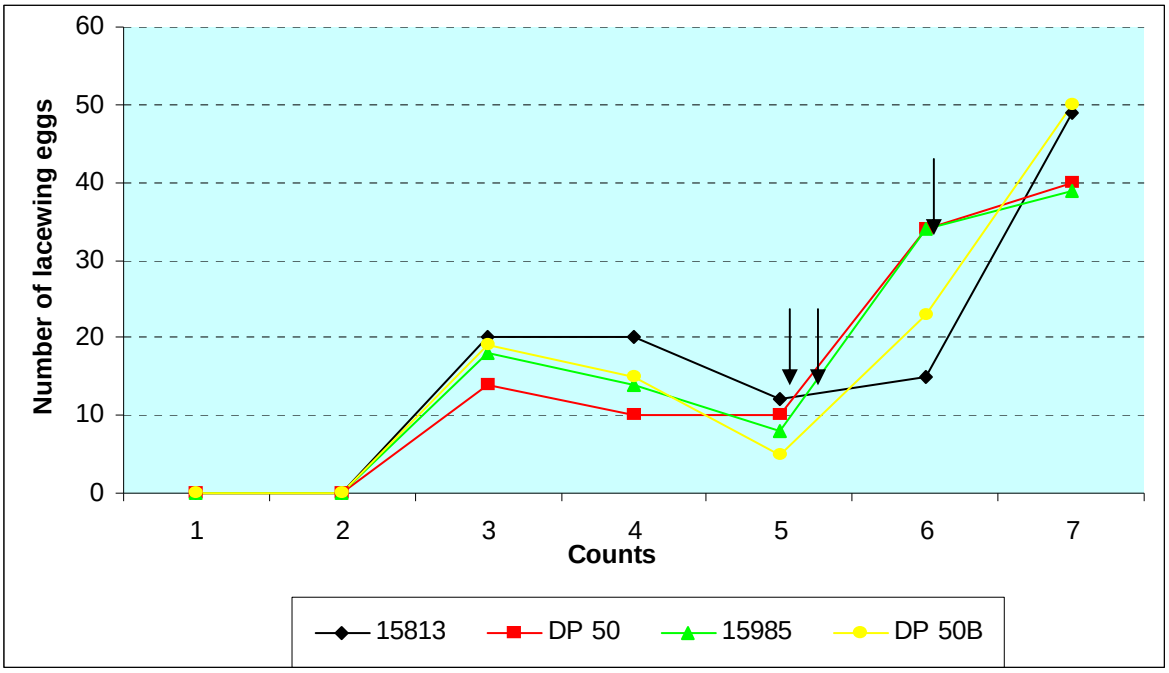


Fig. 4.28. The number of lacewing eggs counted on 20 plants per plot in each treatment at Rustenburg in the Cry2Ab2 trial (arrows - insecticide applications).

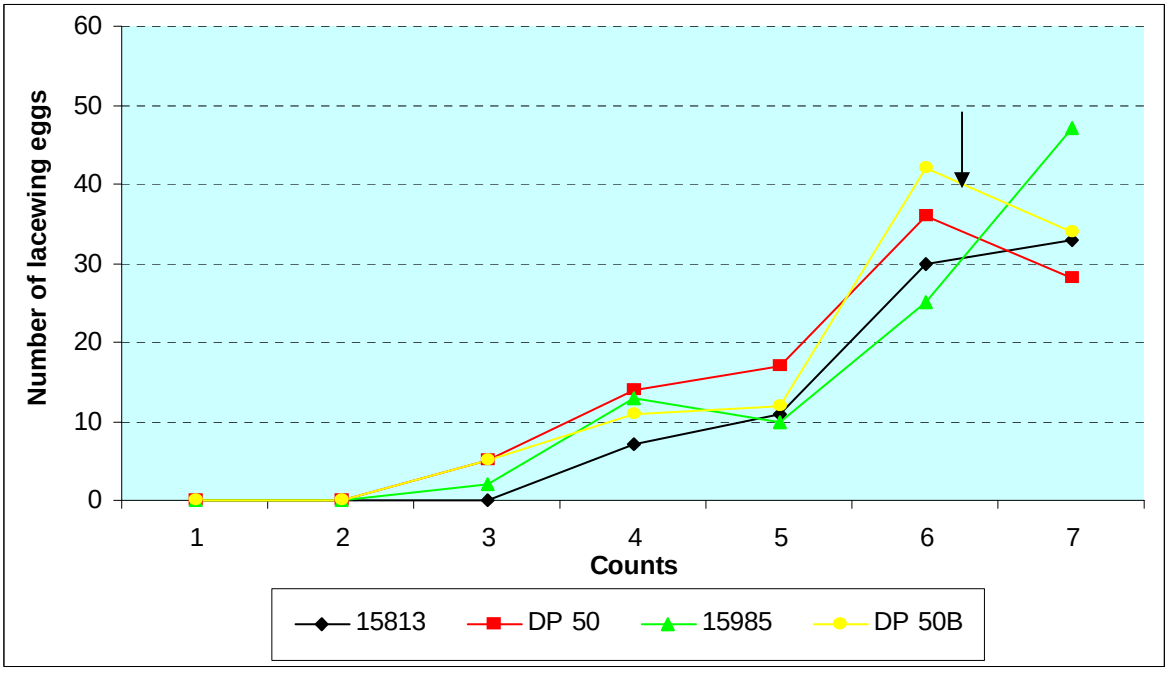


Fig. 4.29. The number of lacewing eggs counted on 20 plants per plot in each treatment at Brits in the Cry2Ab2 trial (arrow - insecticide application).

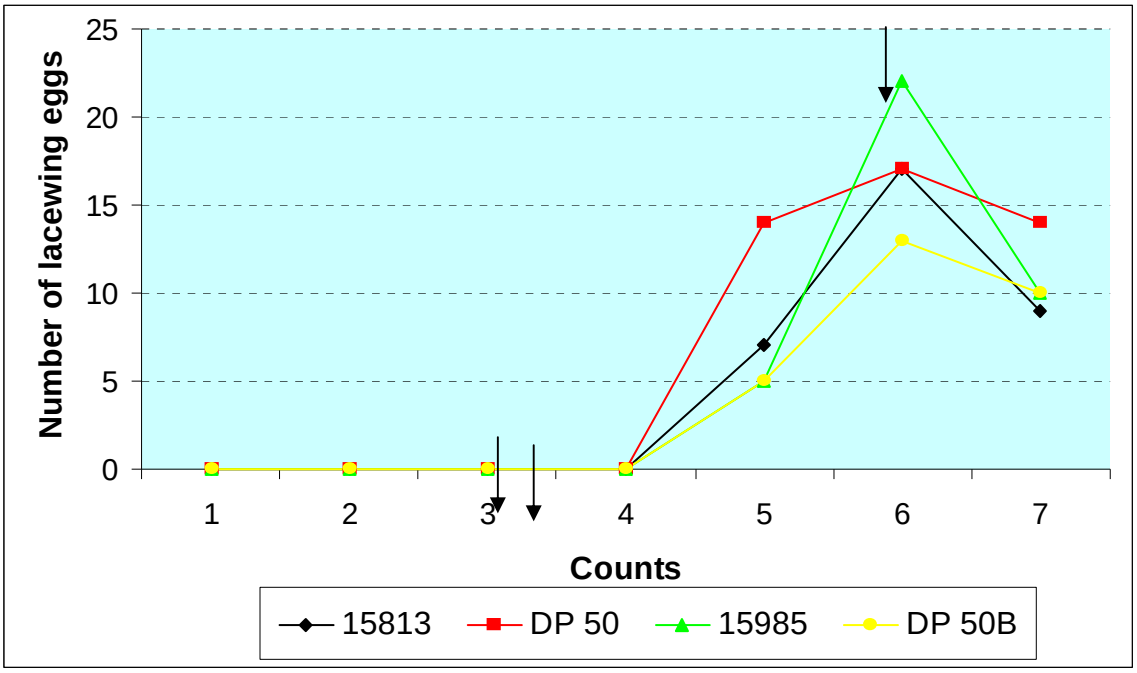


Fig. 4.30. The number of lacewing eggs counted on 20 plants per plot in each treatment at Nelspruit in the Cry2Ab2 trial (arrows - insecticide applications).

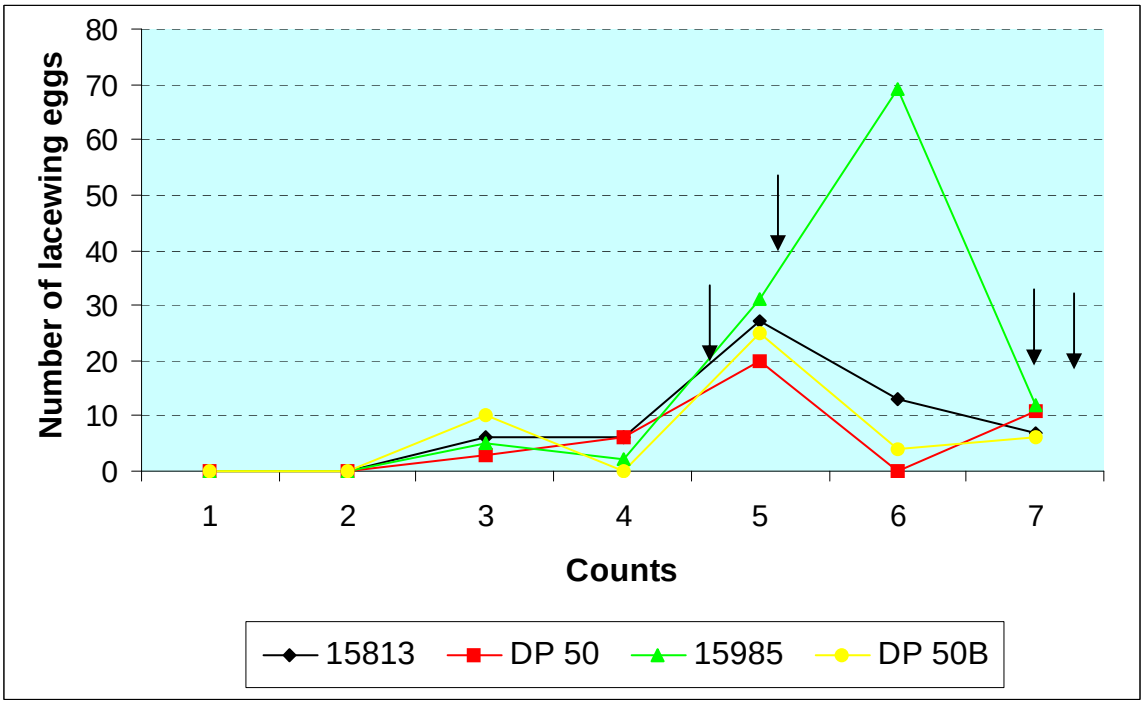


Fig. 4.31. The number of lacewing eggs counted on 20 plants per plot in each treatment at Potgietersrus in the Cry2Ab2 trial (arrows - insecticide applications).

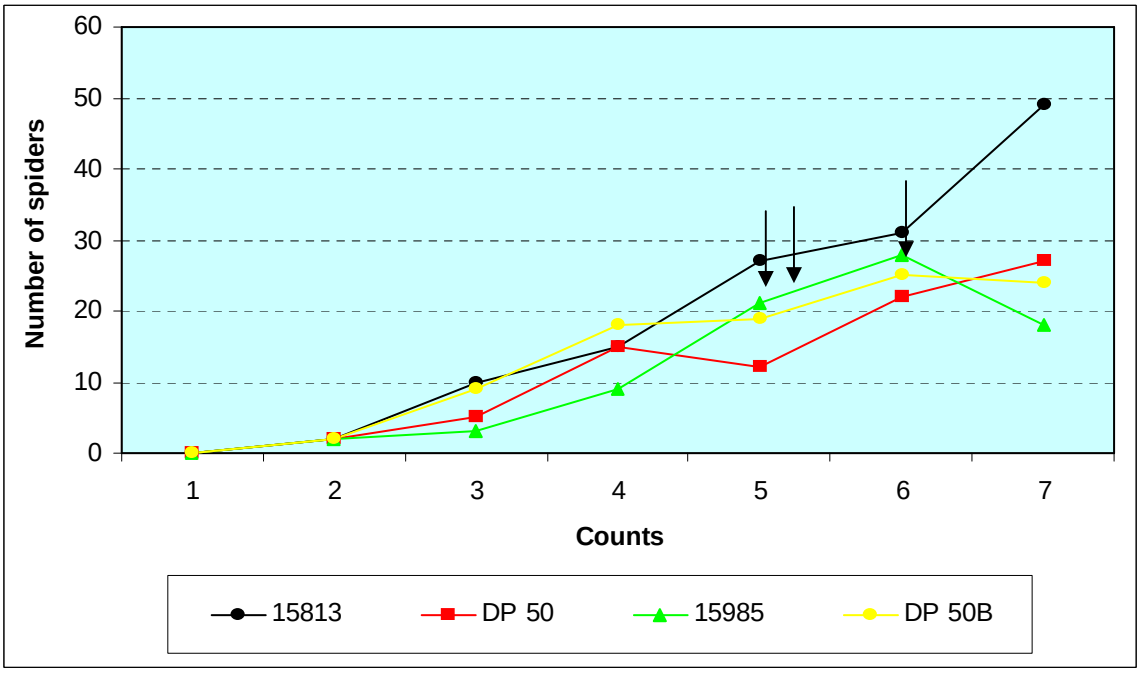


Fig. 4.32. The number of spiders counted on 20 plants per plot in each treatment at Rustenburg in the Cry2Ab2 trial (arrows - insecticide applications).

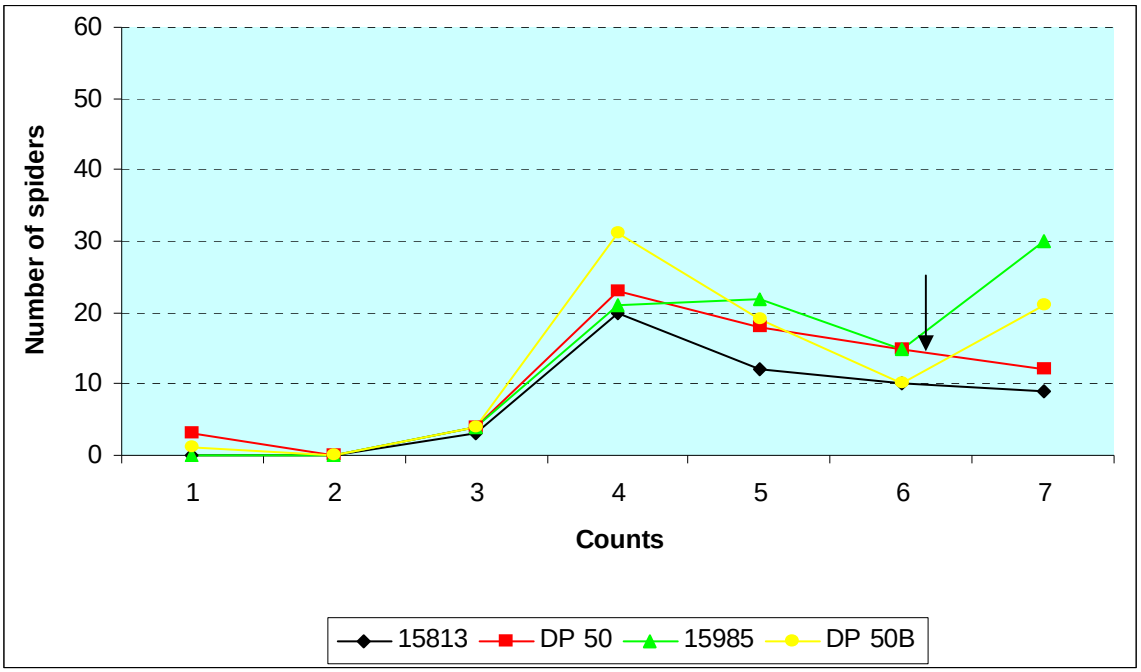


Fig. 4.33. The number of spiders counted on 20 plants per plot in each treatment at Brits in the Cry2Ab2 trial (arrow - insecticide application).

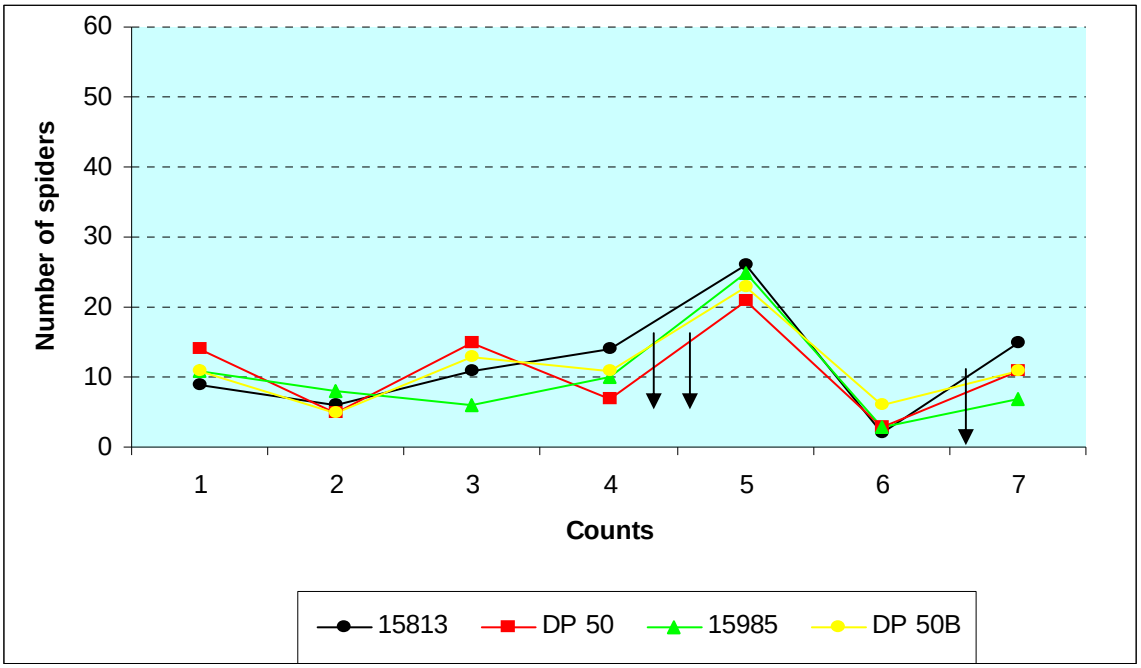


Fig. 4.34. The number of spiders counted on 20 plants per plot in each treatment at Nelspruit in the Cry2Ab2 trial (arrows - insecticide applications).

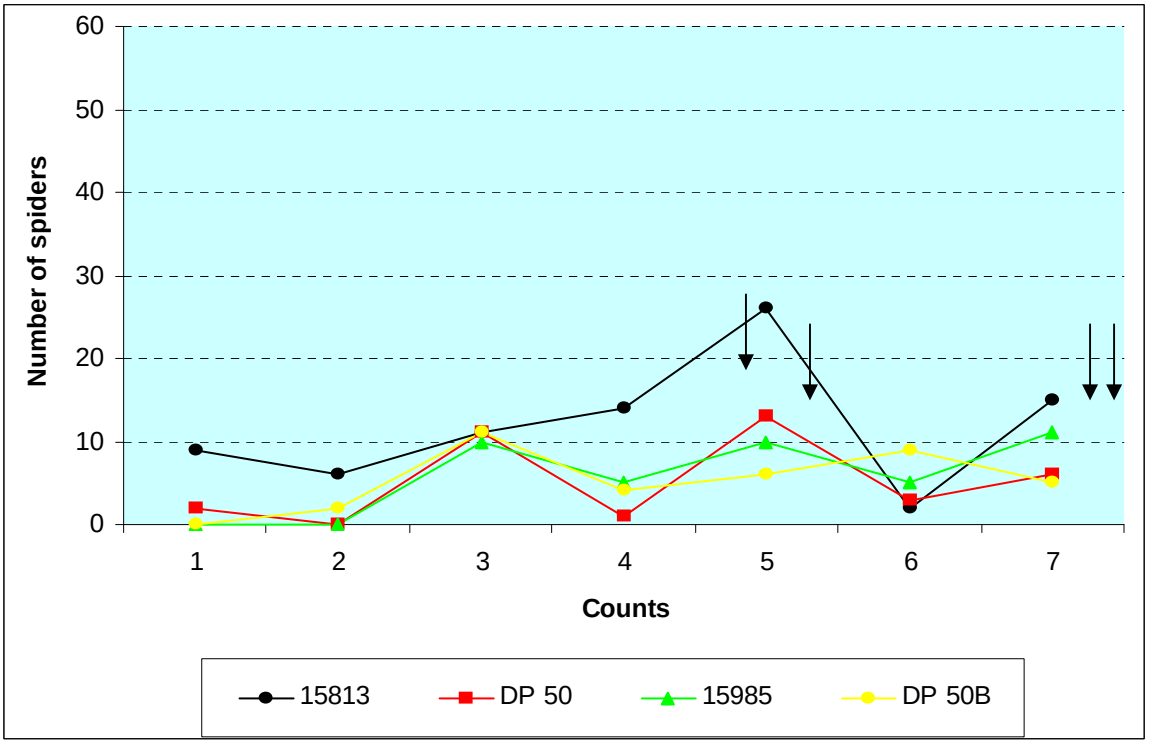


Fig. 4.35. The number of spiders counted on 20 plants per plot in each treatment at Potgietersrus in the Cry2Ab2 trial (arrows - insecticide applications).

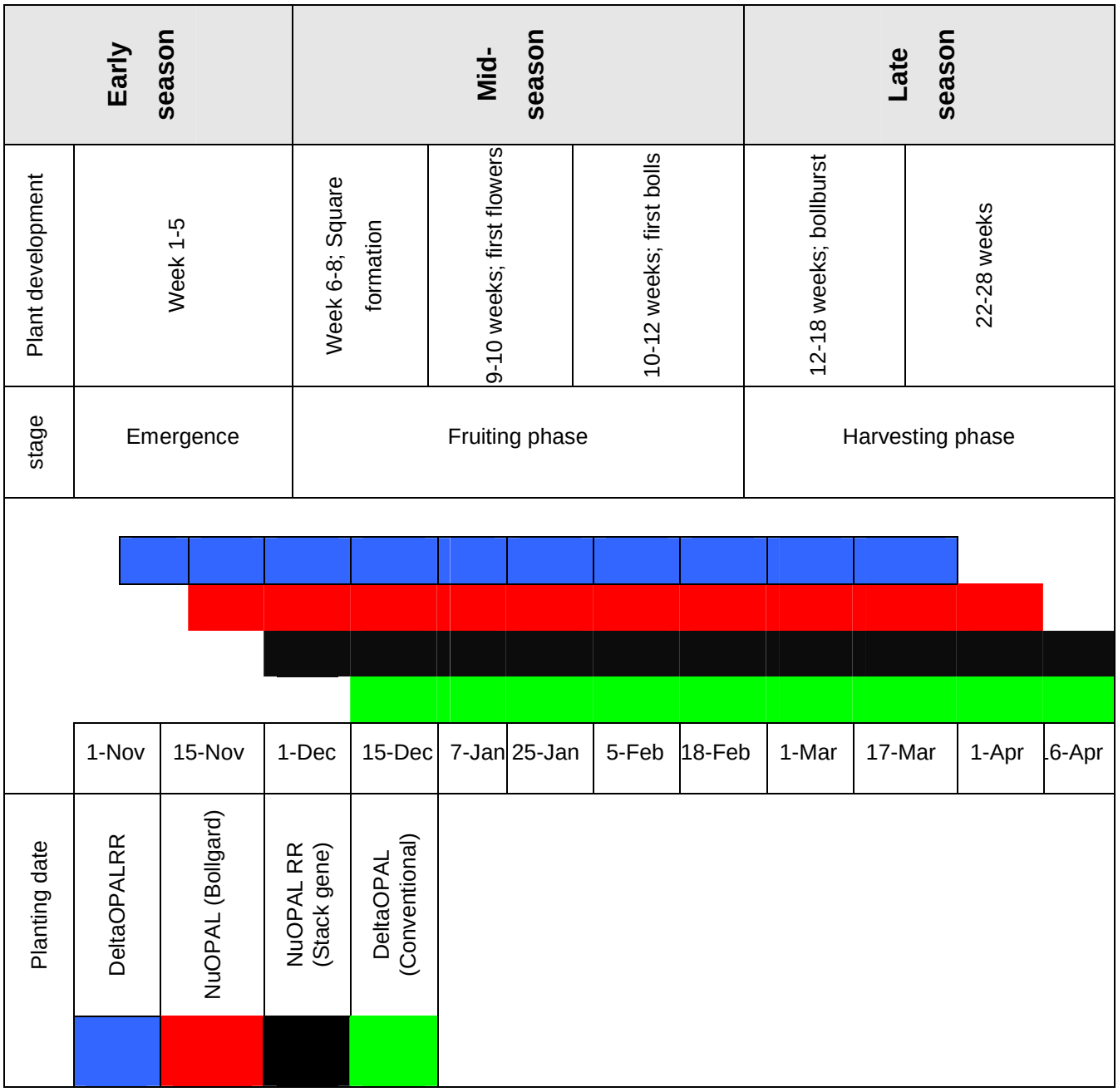


Fig. 4.36. Plant development and planting dates of the different varieties in the 2003/2004 biodiversity trial.

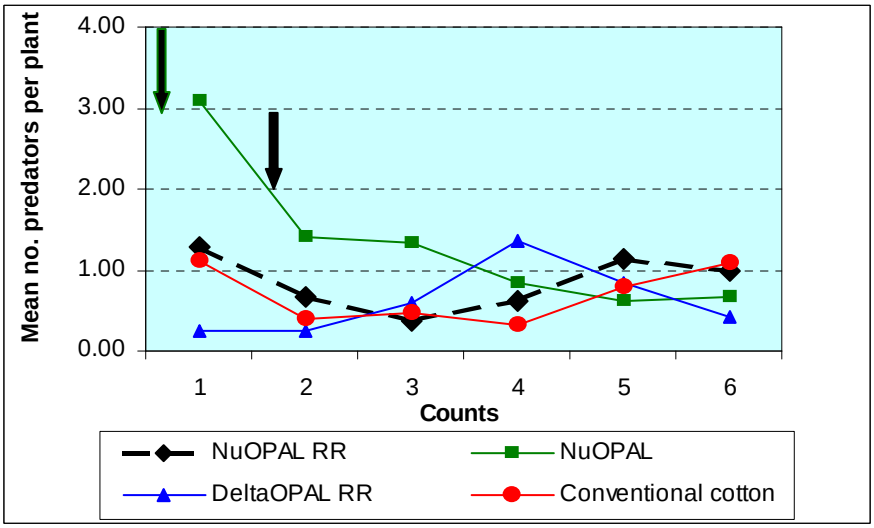


Fig. 4.37. Mean number of predators per plant on Bollgard (2003/2004): left arrow - acetamiprid; right arrow – carbosulfan in the 2003/2004 biodiversity trial.

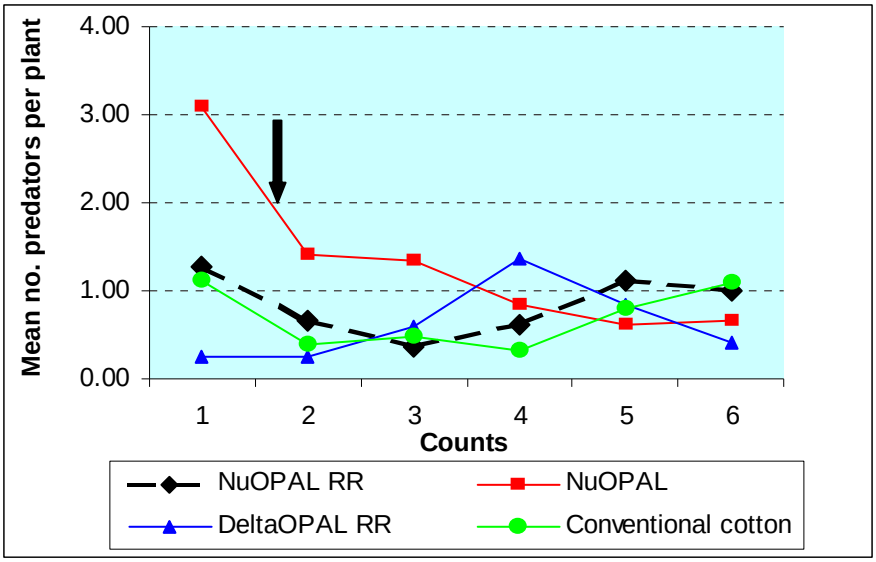


Fig. 4.38. Mean number of predators per plant on Stackgene in the 2003/2004 biodiversity trial: arrows – acetamiprid.

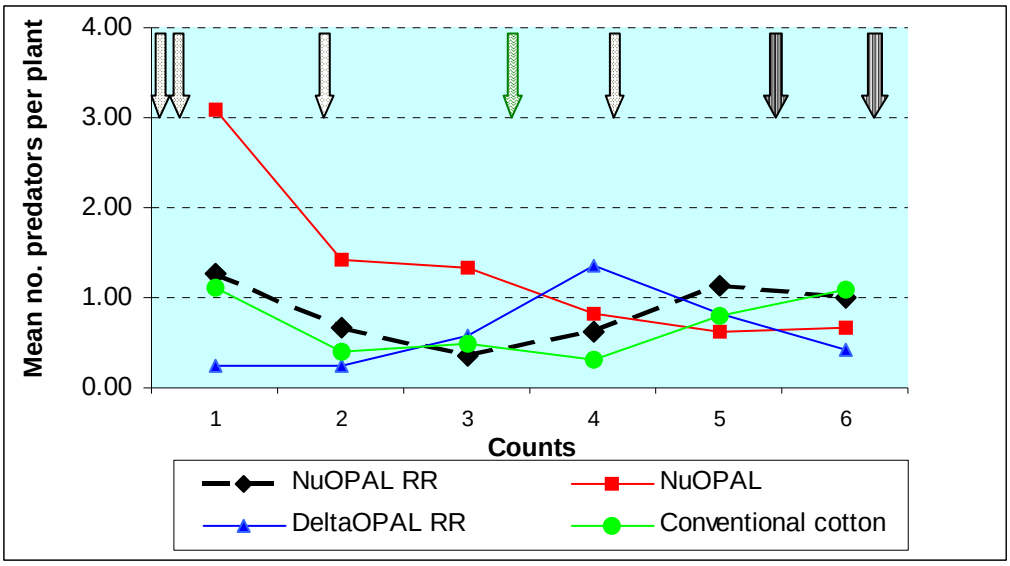
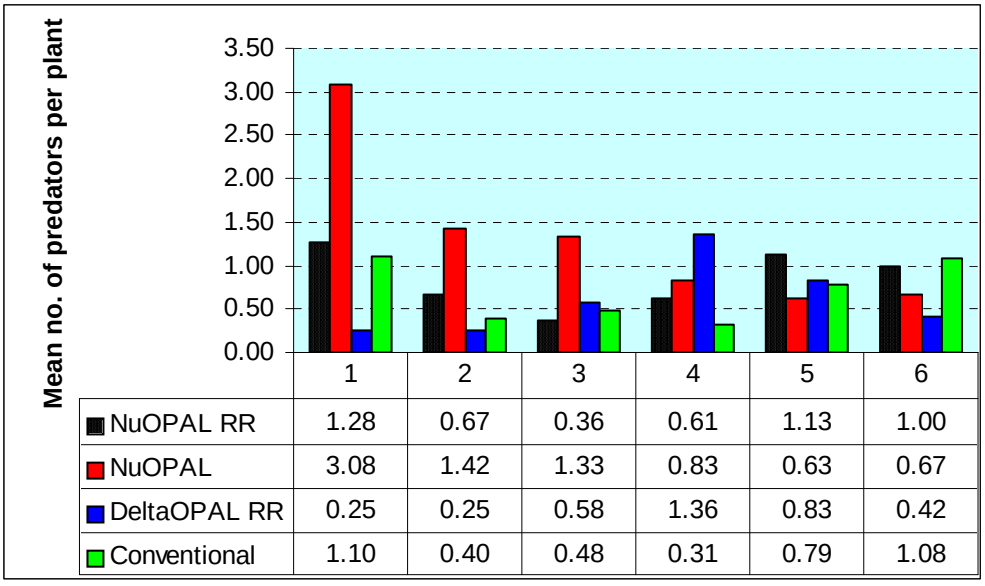


Fig. 4.39. Mean number of predators per plant on Roundup Ready in the 2003/2004 biodiversity trial: stippled arrows - endosulfan; diagonal lined arrows - acetamiprid; vertical lined arrows – cypermethrin.



	1	2	3	4	5	6
NuOPAL RR	1.28	0.67	0.36	0.61	1.13	1.00
NuOPAL	3.08	1.42	1.33	0.83	0.63	0.67
DeltaOPAL RR	0.25	0.25	0.58	1.36	0.83	0.42
Conventional	1.10	0.40	0.48	0.31	0.79	1.08

Fig. 4.40. The mean number of predators per plant observed during 6 counts in the 2003/2004 biodiversity trial (includes dead and alive predators and egg packets on the first count).

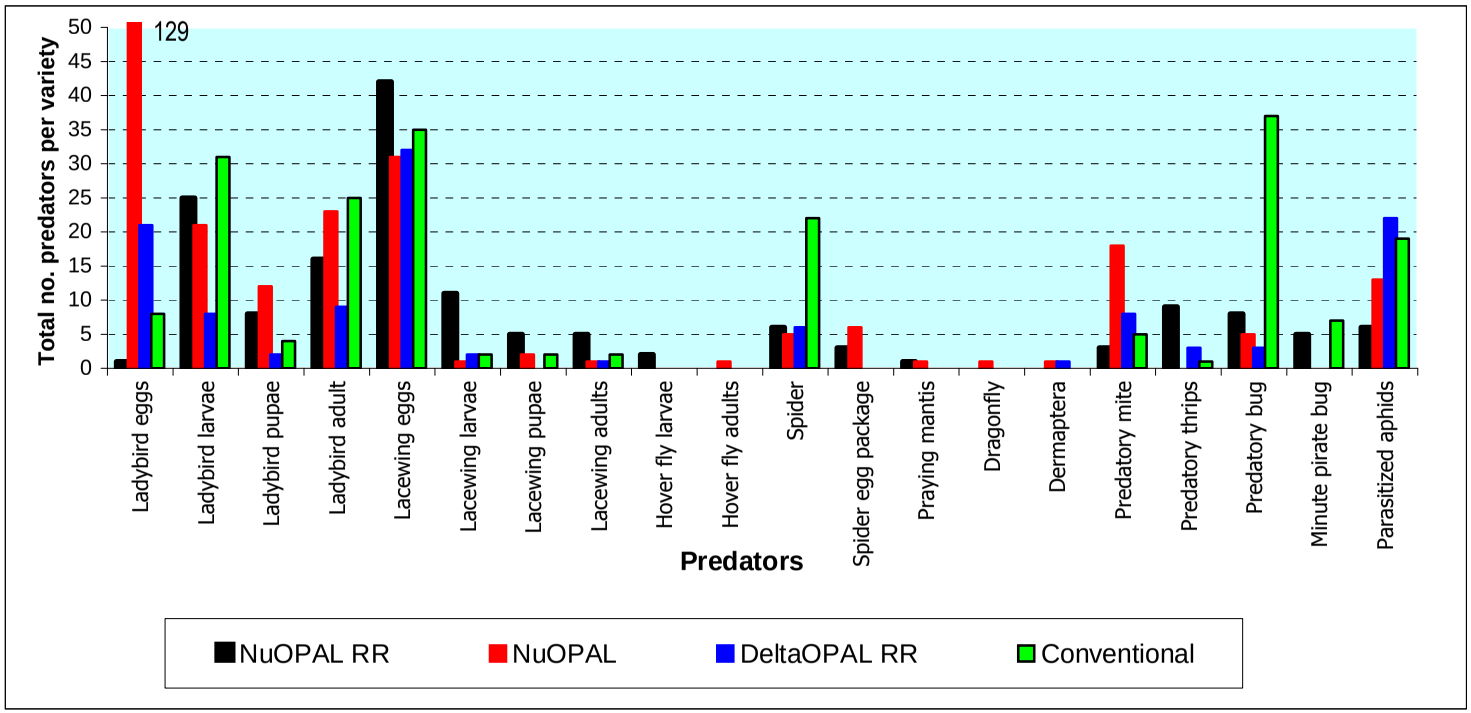


Fig. 4.41. The total number of predators found by scouting in the 2003/2004 biodiversity trial.

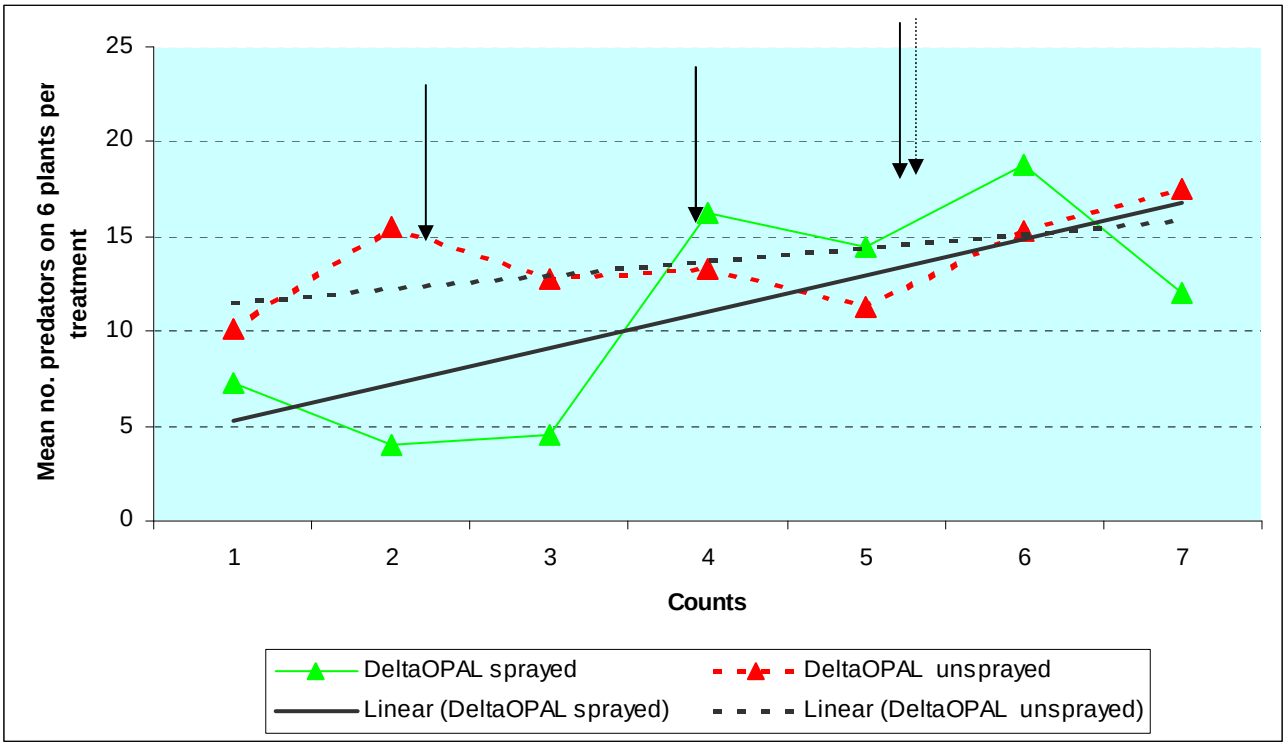


Fig. 4.42. Mean number of predators on 6 plants of the DeltaOPAL treatments in the 2004/2005 biodiversity trial: solid arrow indicates Thioflo® sprays, stippled arrow indicates Mospilan® sprays.

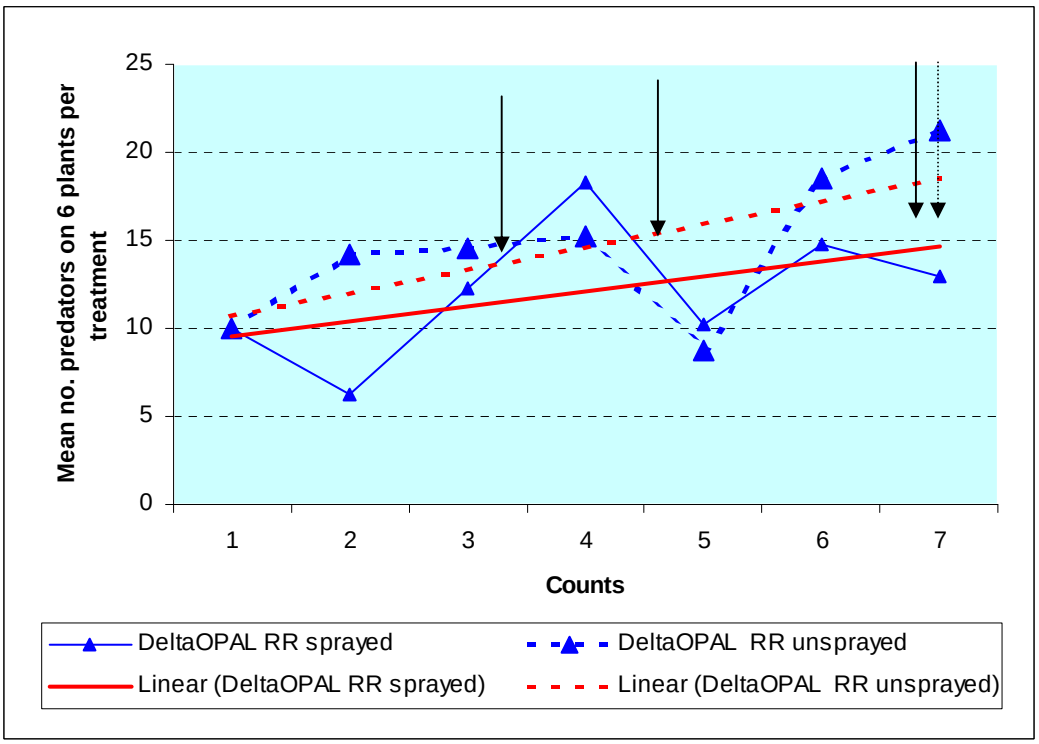


Fig. 4.43. Mean number of predators on 6 plants of the DeltaOPAL RR treatments in the 2004/2005 biodiversity trial: solid arrow indicates Thioflo® sprays, stippled arrow indicates Mospilan® sprays.

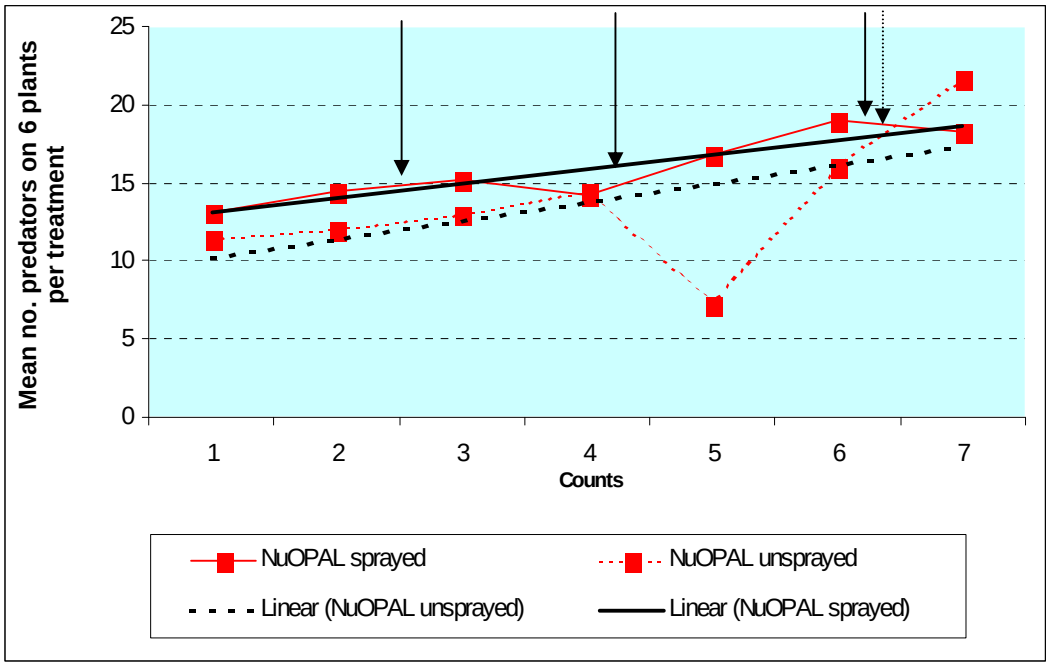


Fig. 4.44. Mean number of predators on 6 plants of the NuOPAL treatments in the 2004/2005 biodiversity trial: solid arrow indicates Thioflo® sprays, stippled arrow indicates Mospilan® sprays.

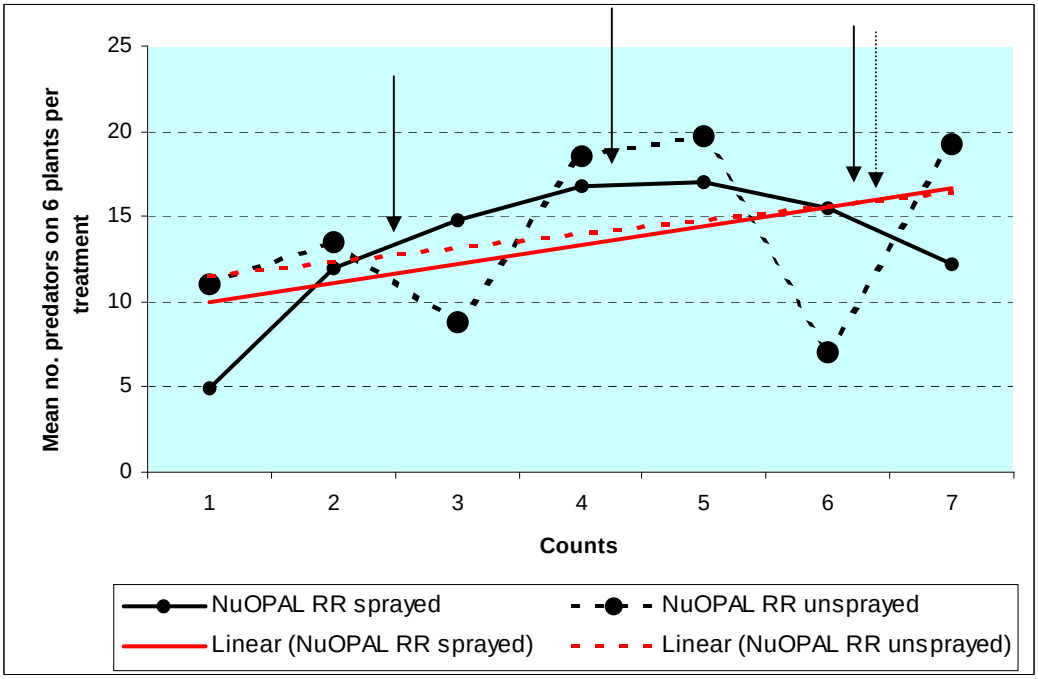


Fig. 4.45. Mean number of predators on 6 plants of the NuOPAL RR treatments in the 2004/2005 biodiversity trial: solid arrow indicates Thioflo® sprays, stippled arrow indicates Mospilan® sprays.

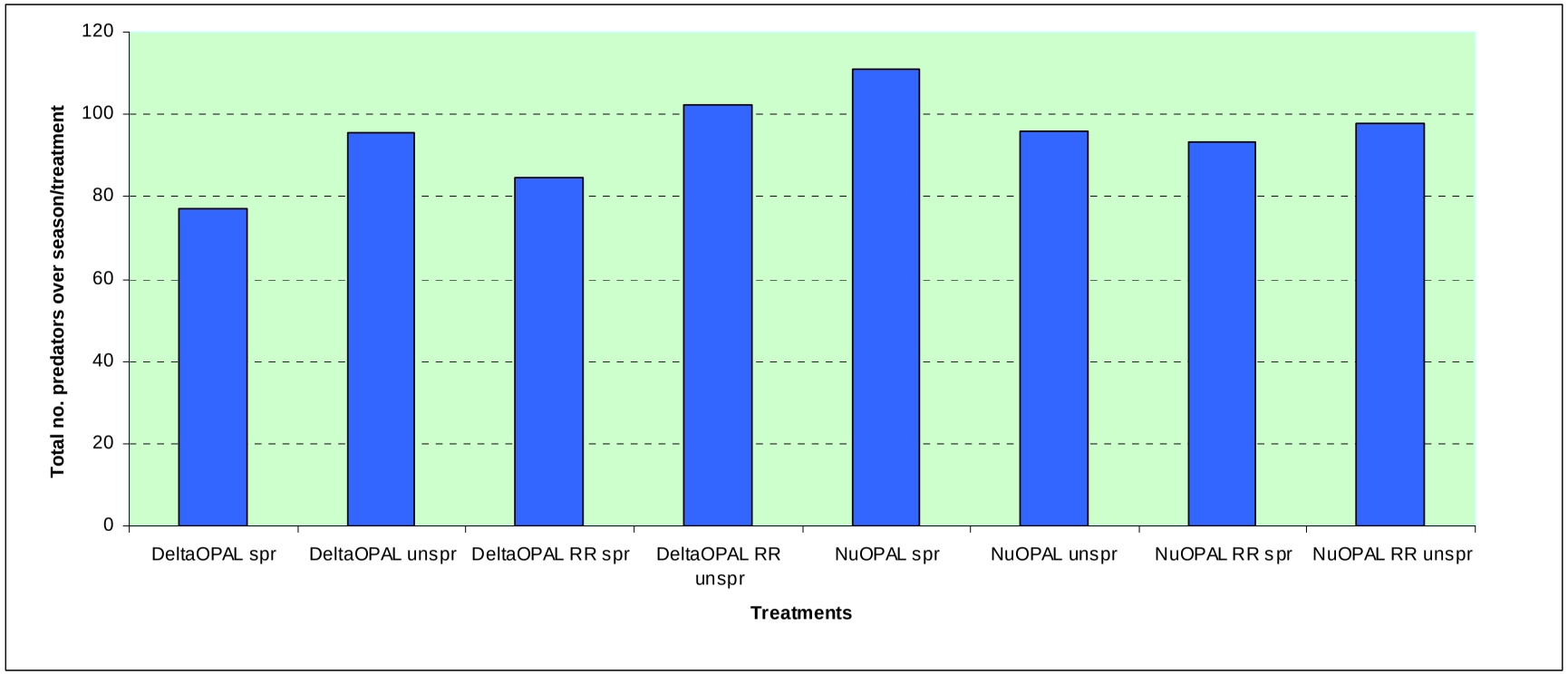


Fig. 4.46. The total number of predators on all treatments over seven counts in the 2004/2005 biodiversity trial.

CHAPTER 5

THE EFFECT OF Bt-COTTON TYPES ON YIELD

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Abstract

From the results presented here, it can be concluded that bollworm numbers affect yield. Bt-cotton or Bollgard, with the *cry1Ac* gene, gives significant yield differences when compared to conventional cotton. The *cry2Ab2* gene also provides significant yield increases when compared to conventional cotton. The best results in yield can be achieved when Bt-cotton is scouted and sprayed for the bollworm complex and secondary pests on threshold values. The sprayed or unsprayed Bollgard™ treatments, with the *cry1Ac* gene, produced a significant higher yield than the conventional sprayed and unsprayed treatments on all localities. Fibre qualities tended to be at least similar or to even better that of conventional cotton.

Different events containing the *cry2Ab2* gene indicated different results in yield. Transgenic events that show increases in yield compared to other events or non-transgenic lines are used in further selection and screening field tests where stability of the gene is monitored. The 15813 event gave the most promising results in yield and related bollworm numbers and were further compared to a 15985 line. In a further study, the *Cry2Ab2* cotton treatments included a transgenic 15813 Insect Protected Cotton line (IPC line); a 15985 IPC line, the transgenic control variety DP 50B (Deltapine 50 with Bollgard$^{TM)}$, and a non-transgenic reference variety, DP 50 (Deltapine 50). The transgenic line 15985 gave a higher yield than the other two transgenic lines. The yield obtained by the 15985 line was significantly different from the DP 50B variety, but not from that obtained by the 15813 transgenic line. The total mean seed cotton yield from all three the transgenic cotton treatments differed significantly from the DP 50 variety on averaged across all the sites. The lower yield obtained by the DP 50 variety across all sites, was expected since more bollworm larvae were found on this treatment at all sites due to the absence of the Bt-protein which affects only lepidopteran larvae. In determining the efficacy of an insect resistant cotton line, 15985 (in stacked gene combination (*cry1Ac* and *cry2Ab2*) for resistance against the bollworm complex (2000), all Bt-cotton treatments differed significantly from the conventional control treatments with regard to yield. No significant differences in yield were found between any of the transgenic treatments. The yield observed in the 15985BXs treatment was significantly higher than the 15985Bu treatment. Fibre quality and the gin out turn percentage were most probably heavily influenced by weather conditions and the trials should be repeated to obtain more accurate results.

Bt-cotton (*cry1Ac* gene) sprayed according to specific thresholds with a Micron Ulva tends to give higher yields in a small-scale farmer setup, than with in comparison with a knapsack under irrigated conditions, indicating that good management practices of Bt-cotton can improve yields.

Introduction

This chapter is a continuation of Chapters 3 and 4, where the effect of genetically altered cotton types on insect numbers were evaluated. In this chapter, the impact of Bt-cotton on yield will be demonstrated and as in the previous chapters, this work forms part of the research work that has been carried out over a number of years on behalf of the industry. The benefits in yield is further discussed in relation to net profit as expressed in Rands (RSA) in the final chapter of this work, together with the acceptance of Bt-cotton amongst commercial and small-scale farmers.

The effect of bollworm numbers on yield is the most important aspect of cotton production, especially after the introduction of genetically altered cotton varieties. Net Profit, which is directly related, to yield is the tool that would enable a cotton producer to continue farming. One of the advantages of crop biotechnology lies in the ability of these crops to increase crop productivity, and thus contribute to global food, feed and fiber security (James, 2003 and 2004). Joubert et al. (2001) mentioned that Bt-cultivars produced significantly higher seed cotton yields than the non-Bt-cultivars in trials done over three seasons in South Africa. In addition, they mentioned that the fiber properties of Bt-cotton were more acceptable than those of non-Bt-cotton under irrigated conditions in South Africa. Farmers in South Africa were of the opinion that the higher yields and reduced costs meant more money in their pockets (Joubert et al. 2001). Bennett (2001) also found that when yields were compared between the Bt-variety and its non-transgenic isoline, a mean yield increase of 27.3%, which equals an average of 388kg seed cotton/ha was observed. Bennett (2001) further mentioned, that Bt-cotton delivered a direct incremental benefit of R943.00/ha over the non-transgenic cotton. Yield increases when Bt-cotton is measured against its conventional counterparts in South Africa, according to Ismael et al. (2001), where he mentioned that Bt-variety generally resulted in a per hectare increase in yields, value of output and reduction of pesticide costs, which outweighs seed costs to give a substantial increase in gross margin during a study done in the 1999-2000 season amongst 100 smallholders. Yield increases has also been mentioned in other countries, like those mentioned by the Pesticide Action Network UK (Mayer, 2002), where they discuss that overall Bt-cotton does lead to increased yields. In Australia, significant yield increases with the introduction of the *cry1Ac* gene have been reported by Fitt (2003). In Australia, fibre quality has not been affected, while in some cases yields have increased. Hake (2003) also reported that biotechnology has been introduced as a tool to enhance yield in cotton in Australia. A dual Bt-gene product has been launched in 2002 in Australia (Hake, 2003) containing the *cry1Ac* and the *cry2Ab2*[1] gene (Bollgard II™) by Monsanto. At present (2006) no dual Bt-gene cotton product has been marketed in South Africa. The *cry2Ab2* gene has proved to be as effective as the *cry1Ac* gene, as a single gene trait or as a double gene trait (see Chapter 2), but the market value cotton has in South Africa in comparison with other crops such as maize, does not

[1] A dual Bt-gene product has been launched in 2002 in Australia (Hake, 2003) containing the *cry1Ac* and the *cry2Ab* gene (Bollgard II™) by Monsanto." The term "*cry2Ab2* gene" mentioned in this study, corresponds with the term, *cry2Ab* gene (pers comm. W.M. Green, Monsanto, SA) used in the Bollgard II™ study in Australia. Bollgard II was approved in South Africa in 2003, but the product has not been launched yet (pers comm. W.M. Green, Monsanto, SA).

encourage the introduction of a second Bt-gene for cotton. One should keep in mind however, that the pest complex on cotton differs from country to country and region to region, within one country (Anon 2006), as well as stress factors for plant populations and insect populations. Yield increases of up to 25% has been documented on the Makhathini Flats in KwaZulu Natal (cf. Anon 2005), and amongst other benefits like an decrease in production costs and a reduction in the number of pesticide applications of at least 50% yield increases has also been documented in case studies by an ISAAA report in 2002 (Anon 2005). Each scenario is different for the variety planted and managed under specific conditions. In South Africa only the DeltaOPAL variety has been alternated or modified with the Bt-gene, and now is referred to as NuOPAL. Coker 312 as a gene recipient (parent variety) (pers comm. W. M Green[2]). Coker 312 is the cultivar used to introduce "new genes" into. Coker, as a gene recipient was used in a breeding programme and crossed with DeltaOPAL in order to introduce the Bt- gene (sic) into the conventional variety. The variety, NuOPAL, was obtained through a process of conventional backcrossing breeding techniques to obtain the most desired plant characteristics and sufficient gene-expression. For the purpose of this study, further comparisons with Bt-cotton and their integration into other cotton varieties will not be discussed. This study presents an overview of research trials, which were done before and directly after the initial registration of Bollgard cotton, to determine the impact of bollworms on yield under South African conditions. The impact of NuOPAL RR (Stackgene cotton type, expressing bollworm resistance and herbicide tolerance) on yield can not be explained fully and can be attributed to a number of factors, depends on gene introduction, weed management, a changed in cultivar characteristics (NuOPAL is back crossed and introduced with Bt-gene by conventional breeding, and does not necessarily exhibit the same characteristics as NuOPAL) (pers comm. F. Friss[3]).

The objective of this chapter of this study was to determine yield between cotton lines where the *cry1Ac* genotype was introduced, so-called Bollgard™ (Part I); to compare yield when a second protein (Cry2Ab2) was introduced into different events (Part II); to compare the effect of the second protein (Cry2Ab2) to the first (Cry1Ac) for efficacy, and provide data for an alternative choice, in a stackgene combination of the two proteins (Cry2Ab2 and Cry1Ac - 15985BX) (Part III); to determine the incidence of pests on yield of a potential insect resistant cotton line 15985 in stacked gene combination (*cry1Ac* and *cry2Ab2*) and to evaluate the difference in yields obtained under different spraying regimes in a small-scale farmer setup (Part V).

[2] Wally Green was the Regulatory Manager at Monsanto SA until Dec, 2007, but now acts as a private consultant for Crop and Plant Biotechnology Services cc.
[3] Mr. Fanie Friss is a plant breeder for Delta and Pinelands, SA

Part I: Determination of the effect on yield of the Bt-gene (*cry1Ac*) which provides bollworm resistance, during 1997/1998 in three field trials at Rustenburg, Potgietersrus and Groblersdal

The aim of this study was to compare the effect of yield of bollworm resistant cotton, expressed as the *cry1Ac* gene in 3 localities.

EXPERIMENTAL PROCEDURES

Three field trials on the experimental farms of the Institute for Industrial Crops (IIC-ARC) in Rustenburg, Potgietersrus and Loskop (Groblersdal). The experimental design, and layout is summarized in this chapter, for it is similar to that describe in Chapter 2. The soil composition varied from a heavy clay soil, (turf) content in Rustenburg, while in Potgietersrus the soil was a sandy-clay soil and in Loskop, the soil varied from being a sandy soil to a sandy-clayish soil. All trials consisted of four treatments with six replicates laid out as a randomised block design (see Chapter 2). Treatments included NuCtn 37B with the Bt-protein (referred to as Bollgard) – unsprayed; NuCtn 37B with the Bt-protein (referred to as Bollgard) – sprayed according to threshold; Deltapine Acala 90 (DpAC 90) – unsprayed and Deltapine Acala 90 (DpAC 90) - sprayed according to threshold.

Assessments

Assessments included nine consecutive scouts starting at first square formation. Ten plants / plot were scouted for H. armigera*,* D. castanea *and* Earias spp*.* before the first application (approximately eight weeks after emergence) and thereafter, each week, for eight counts, up to approximately 16 weeks after emergence. The effect of yield was determined at the end of the season. All plots were harvested and the weights recorded. Samples of 50 bolls per plot were taken for fibre analyses. Determination of yield in relation to bollworm numbers on genetically modified cotton was repeated at Loskop Experimental Farm, Groblersdal during 1998/1999. In addition to bollworm numbers assessed as described in Chapter 2 (Part II), yield differences between treatments were calculated. Differences in fibre quality between treatments were analyzed. Fibre quality (strength; length and micronaire) was measured by means of High Volume Instrumentation (HVI) from Spinlab (USA).

Statistical analyses

The statistical package GENSTAT 5.3 was used to analyze most of the data collected during 1997/1998 (Genstat 5 Committee, Rothampstead, UK. Clarendon Press, Oxford). For the 1998/1999 data from Groblersdal, an attempt was made to keep the statistical analyses simple, a one-way chi-square analysis of variance for treatment effects, and for differences between scouting times, was performed on the data for the scouting dates. This analysis was performed for the February and March scouts only, in order to decrease the number of zero values and to see if there was an indication of significant differences between treatments. Deductions from the

statistical analyses of the respective variants from Rustenburg and Potgietersrus (1997/1998) and Groblersdal (1997/1998 and 1998/1999) are presented in Tables 5.1, 5.2, 5.3 and 5.4 respectively.

RESULTS

In Rustenburg, Treatment two (Bollgard sprayed treatment) had significant less bollworm than the sprayed and unsprayed conventional cotton as discussed in Chapter 2. This is reflected in the yield. Yield between treatments was compared by means of an analysis of variance. Significant treatment effects were found between treatments ($p < 0.0001$). The Bollgard™ treatments differed significantly from the DpAC 90 treatments (Table 5.1), but not from each other. The DpAC 90 sprayed treatment gave a significantly higher yield than the DpAC 90 unsprayed treatment.

For the Potgietersrus locality, the Bollgard sprayed treatment differed significantly from the DpAC 90 unsprayed treatment (Table 5.2).

For the Loskop locality (1997/1998 – Table 5.3), the yields obtained from the Bollgard treatments were significantly higher than those obtained from the DpAC 90 treatments. In the repeat of the Loskop trial, during 1998/1999 (Table 5.4), no significant differences were found between the Bollgard sprayed and Bollgard unsprayed treatments or between the DpAC 90 sprayed and unsprayed treatments. During the 1998/1999 season, the yield obtained from the Bollgard sprayed treatment was significantly higher than those obtained from the two unsprayed treatments. Significant differences were found between the Bollgard sprayed and Bollgard unsprayed treatments ($p < 0.001$), as well as between the DpAC 90 sprayed and unsprayed treatments (Table 5.4). The yield obtained on the Bt-sprayed treatment differed significantly from the Bt-unsprayed treatment, as well as from the DpAC 90 unsprayed treatment. The DpAC 90 unsprayed treatment differed significantly from the DpAC 90 sprayed treatment. These results indicate that transgenic cotton sprayed according to egg threshold, seems to give a higher yield than non-transgenic sprayed cotton but not significantly so, but indeed significantly higher when compared to transgenic unsprayed cotton. The lowest yield was obtained in the treatments with a corresponding high number of bollworm larvae, e.g. DpAC 90 unsprayed (see Chapter 2).

The analysis of variance of the comparison of yield between the localities (Rustenburg, Potgietersrus and Loskop (1997/1998)) (Table 5.5a), showed that there was a significant difference between treatments and the interaction between treatment and locality was also significant ($p < 0.001$). Significantly higher yields were obtained in the Bollgard sprayed treatments in comparison with all the other treatments (Table 5.5b), except the DpAC 90 sprayed treatment. The Rustenburg DpAC 90 sprayed treatment gave a significantly higher yield than the Potgietersrus and Loskop DpAC 90 sprayed treatments as well as all the DpAC 90 unsprayed treatments (Table 5.5c). The Bollgard sprayed treatments at each locality did not give significantly higher yields than any of the Bollgard unsprayed treatments at a specific locality.

Analysis of fibre quality

No significant differences were found between treatments with regards to fibre strength at the 5% significance level for any of the localities (Table 5.6). Fibre strength was slightly lower than the normal range of about 22.5-23.5 g/tex. Fibre strength tended to be lower on the Bollgard treatments than on the DpAC 90 treatments. With regards to fibre length, no significant differences were found in fibre length between treatments at $p < 0.05$ (Table 5.7). The Bollgard treatments from Rustenburg showed slightly lower fibre lengths than the DpAC 90 treatments. Fibre lengths between Bollgard treatments and DpAC 90 treatments were similar for the Potgietersrus and Loskop localities respectively. The minimum fibre length for A1 fibre = 1.09 (27.8mm/25.4 inches =1.09 inches). The fibre lengths of treatments at the Potgietersrus locality were below the minimum requirement. This could be due to a localized effect like the planting date, plant nutrition, soil composition differences or ripening of the cotton fibre. According to Dippenaar[4] (pers comm.) ripening of the cotton fibre could be influenced by a higher humidity throughout the season. For the Loskop and the Potgietersrus locality the rainfall was higher than for the Rustenburg locality. This could influence ripening of the fibre. The DpAC 90 sprayed treatment in Loskop produced a lower fibre length than the Bollgard treatments. This treatment differed on the 6% significance level from the Bollgard treatments. This could be due to total boll damage, or to damage to some of the locks in the boll caused by bollworm feeding (pers comm. Dippenaar). However, the DpAC 90 treatment (unsprayed control) had most larvae than the DpAC 90 sprayed treatment and than any of the Bollgard treatments. Larval numbers on the DpAC 90 sprayed treatment exceeded those of the Bollgard treatments. Thus, in this case, the difference in fibre length between treatments could not be explained by bollworm feeding damage. Treatment fibre lengths of the other two localities were above the minimum.

Significant differences were found for micronaire between treatments for the Rustenburg locality. The DpAC 90 unsprayed treatment had the smallest micronaire and differed significantly from the Bollgard sprayed treatment. There were no significant differences between the Bollgard unsprayed treatment and any of the DpAC 90 treatments for the Rustenburg localities. No other differences between treatments were found to be significant for either the Potgietersrus or the Loskop localities. The normal micronaire range for A1 cotton is between 3.5 and 4.5. The micronaire of all treatments on all three localities fitted this range. The lower micronaire given by the DpAC 90 unsprayed treatment is probably due to unripening of the cotton fibre indicating a possible cultivar effect in this case (pers comm. Dippenaar). Overall, the Bollgard™ treatments produce a fibre with a similar micronaire than DpAC 90 treatments.

The Gin Out Turn percentage (% GOT) for the Rustenburg locality did not differ significantly between treatments at the 5% significance level (Table 5.9). The % GOT, depends on the moisture content of the seed and how cleanly the cotton is picked, without any plant matter remaining in the seed cotton (pers comm. Dippenaar). Lower GOT's in the Loskop samples could be due to picking of "wet" or unripe seed cotton. Similarly, no significant

[4] Dr. MC Dippenaar was an experienced cotton agronomist, formerly working for the Institute for Industrial Crops, Agricultural Research Council - at the time of completing this field work.

differences were found between treatments of other localities (Table 5.13). The treatments for the Loskop localities did not perform as well as other localities with respect to GOT % (Table 5.13). Cotton role players consider a GOT% of above 39-40% as acceptable. Bollgard cotton did not give a significant different GOT% than DpAC 90 cotton. Cotton of all treatments from Rustenburg could be classified within the Deal, Dirk, Dolly and Duns' grades (for explanation of grades, see Anon 2006). Two samples from the Bollgard treatments represented the Deal grades. All the Potgietersrus samples, irrespective of the treatments, belonged to the Dolly grade, while all samples from Loskop belonged to the Duns grade. Lower GOT percentages of Loskop were reflected in the lower grades (Duns) achieved. Bollgard™ cotton could achieve a high grade of cotton when cultivated under optimal circumstances, as was seen for the Rustenburg locality.

CONCLUSION

Significantly higher yields were obtained with the Bollgard™ treatments than with the DpAC 90 treatments in Rustenburg and in Loskop (1997/1998). Overall, no significant differences in yield were found between the Bollgard™ unsprayed and Bollgard™ sprayed treatments.

For the Loskop locality during 1998/1999, the Bollgard™ unsprayed treatment had a significantly lower yield than the Bollgard™ sprayed treatment. This might suggest that spraying on egg threshold could produce a significantly higher yield in transgenic cotton

No significant differences in fibre strength were found between any of the treatments. Slightly lower fibre strengths were found amongst the Bollgard™ treatments than amongst the DpAC 90 treatments. Fibre lengths were similar for the Potgietersrus and Loskop (1997/1998) localities. However, on a single locality, fibre lengths did not differ between the Bollgard™ and DpAC 90 treatments, except at Loskop (1997/1998). Overall, the Bollgard™ treatments produced fibre with a similar micronaire to that of the DpAC 90 treatments. The micronaire of all treatments fell within the normal accepted range for A1 cotton. Bollgard™ cotton does not give a significantly better GOT % than DpAC 90 cotton.

Part II: The yield obtained in relation to the incidence of bollworms found on transgenic P2B cotton events

The aim of this study was to determine associated yield of potential insect resistant cotton lines containing a gene expressing the “P2B” Bt-protein at Rustenburg, SA.

This study involved mainly the evaluation of transgenic lines or events for bollworm susceptibility. In addition, the yield of the various events was determined. An event in this case, refers to the method of introducing the gene into the plant; although the gene is similar, varied methods with the gene “gun” are used to introduce foreign genetic matter into the plant.

EXPERIMENTAL PROCEDURE

A field trial was planted at the Institute for Industrial Crops (IIC-ARC) in Rustenburg, South Africa. An area of 0.1184 ha (0.25 acre) was planted under cotton existing of a single transgenic P2B event represented one plot. The design and trial layout was similar to that described in Chapter 3. For the purpose of the yield results, it would be summarized below. Treatments included 33 transgenic P2B events (numbered events are listed in Chapter 3, Part III) and 6 check plots (C1-C6) consisting of: i) 4 transgenic varieties: DP50B (C2), C312-531 (C4), 15813 (C5) and 15985 (C6); ii) 2 non-transgenic varieties: DP50 (C1) and C312 (C3) Soil composition, insect control, insecticide application and agronomic management and irrigation as described in Chapter 2, Part II. No statistical analyses were performed on the data since no plots (treatments) were repeated throughout the trial. Seed obtained from Monsanto was just enough to plant a single row of each event. Graphical representation of the data is, however, given, using Excel, Microsoft for Windows. Important dates are listed in Chapter 2, Part II.

Assessments

Emergence stand counts for each plot were taken at seven and 14 days after planting. Cotyledons that had completely cleared the soil were considered “emerged”. Individual plants were tested for the gene of interest via lateral flow strips at the four-leaf stage. Monsanto provided the kit and assisted with the procedures. Leaf samples of each individual plant per event were shipped back to Monsanto for gene analysis, on 2 occasions during the season. Results of the gene-detected positive plants are listed in the Annexure. The number of fully cracked bolls per transgenic plant/event was calculated over five time periods. All plots were harvested and the weights recorded. Samples of 50 bolls per plot were taken for fibre analyses. Yield was compared per plant per square meter, since all events did not represent an equal number of plants at the end of the season. This is because plants that gave a negative Elisa result were removed from the field, and so were not included in the comparison of yield. The total yield per event was also calculated. Total seed cotton of each P2B line was shipped back to the client for whom this work was completed. No statistical analyses were performed on the data since no plots (treatments) were repeated throughout the trial, since the trial was designed to select the event with the most desired characteristics to control bollworm and produce an

acceptable yield. The seed which was obtained from Monsanto was just enough to plant a single row of each event. Graphical representation of the data is, however, given, using Excel, Microsoft for Windows. Important dates are listed in Chapter 2 part II.

RESULTS

The spacing of the plants in each individual row influenced the yield of each event. Plant spacing varied because different numbers of non-transgenic plants were found in each event and were consequently removed. Stand counts are given in Tables 5.10 and 5.11. This enabled some of the plants in each event to spread out and so possibly produce more bolls, increasing the yield in that specific event. Yield results should be used to support entomological data collected from the field and comparison of events should not solely rely on the yields achieved in this trial. It does, however, give some indication as to which events showed potential to produce a higher yield. Yield of seed cotton per square meter per plant is given in Table 5.12. The highest yields were obtained during the first picking phase of the cotton. The yield per plant per square meter is illustrated in Fig. 5.1. Event 6483 (plot 14) showed the highest yield during the first picking. Other events that showed potential for producing a reasonably high yield early in the season were 6149 (plot 1), 6151 (plot 2), 6526 (plot 18), 6718 (plot 22), 6233 (plot 32) and 1580-5 (plot 33). Event 16960 (plot 27) and 17206 (plot 31) produced no yield early in the season. These events could possibly show more promising early yield results in a cotton producing area, which remains warmer for a longer period before winter sets in. The check plot C312 (C3) also produced no yield during the first picking. Events 6234 (plot 7), 6239 (plot 8), 6241 (plot 9) and 6351 (plot 10) showed highest yield during the second picking of cotton.

By comparing the total yield per plant per square meter over the three picking periods (Fig. 5.2), it can be deduced that event 6149 (plot 1) produced the highest yield over all. Other events that produced more than 100 g/plant/square meter included 6234 (plot 7), 6239 (plot 8), 6351 (plot 10), 6483 (plot 14), plot 16221 (plot 26), 6233 (plot 32) and 15830-5 (plot 33). Yield from the above-mentioned events proved to be almost double that produced by the check plots. Of all the check plots, the transgenic line 15985 (C6) gave the highest yield per plant. When the total yield between events (not between individual plants) were compared (Fig. 5.3), the transgenic line 15985 (C6) gave the highest yield overall. High yields were also obtained by 6239 (plot 8), 6351 (plot 10), 16221 (plot 26), 17180 (plot 30), 15830-5 (plot 33) and DP50B (C2).

Analyses of fibre quality

The minimum acceptable fibre length for A1 fibre is 1.09 inches (27.8mm/25.4 inches = 1.09 inches). The fibre lengths for each event are illustrated in Fig. 5.4. Localized effects, such as planting date, plant nutrition; soil composition differences or ripening of the cotton fibre can influence fibre length. Short fibre lengths were obtained by events 6489 (plot 16) and 6532 (plot 19).

Fibre strength for each event is illustrated in Fig. 5.5. The range obtained was slightly lower than the normal range of about 22.5-23.5 g/tex. Unacceptable fibre strength of less than

22.5 g/tex was obtained by events 6234 (plot 7), 6456 (plot 12), 16221 (plot 26), 16960 (plot 27), 17180 (plot 30), as well as by the C312 check (C3) and the transgenic 15985 check (C6).

The normal micronaire range for A1 cotton is between 3.5 and 4.5. The micronaire of all events and check plots fitted this range (Fig. 5.6). The lower micronaire given by event 17206 (plot 31) is probably due to unripening of the cotton fibre indicating a possible “cultivar” effect in this case. A high micronaire of above 5 mic was obtained by events 6456 (plot 12), 6460 (plot 13), 6489 (plot 16), 6491 (plot 17) and 6725 (plot 23).

The percentage gin out turn for each event and check plot is given in Table 5.13. Cotton growers consider a GOT of above 39 % as acceptable. The events with a GOT lower than 39 % were 6149 (plot 1), 6234 (plot 7), 6241 (plot 9), 6456 (plot 12), 6542 (plot 21), 16221 (plot 26), 16960 (plot 27), 17079 (plot 28), 17133 (plot 29), 17180 (plot 30), 17206 (plot 31), 6233 (plot 32) and 15830-5 (plot 33). All check plots gave poor % GOT, except the Coker varieties; C312 (C3) and C531 (C4) (see Tables 5.12 and 5.13). Event 6456 (plot 12) was the only event that had an acceptable level of efficacy for bollworm control, but did not have an acceptable GOT. Event 6483 (plot 14), that had no larvae throughout the trial, had a GOT of 41 %.

CONCLUSIONS

The event 6483 (plot 14) scored 8/13 for beneficial characteristics of the event during the performance of the trial (see Chapter 3). The check plot that gained the highest beneficial score is the transgenic line, 15813 (C5). Other events that gave good bollworm control were events 6227 (plot 5), 6532 (plot 19) and 6733 (plot 24). Events that gave reasonably good bollworm control and a high yield per plant were 6239 (plot 8) and 6483 (plot 14). The highest yield obtained per plant was obtained with 15985 (C6), while DP50B (C2) gave the second highest yield amongst the check plots. In addition, the transgenic check line, 15813 (C5), achieved excellent bollworm control as discussed in Chapter 2. This phenomenon can be directly responsible for the increase in yield.

Part III: Evaluation of two IPC (Insect Protected Cotton) lines (15813, 15985), a control (DP 50B) and a reference line (DP 50) for the impact of bollworm numbers on yield.

The aim was to investigate the effect of the appearance of bollworm numbers on yield, while determining the efficacy of the Cry2Ab2 protein against bollworms.

EXPERIMENTAL PROCEDURES

Four field trials were planted during the 1998/99 season in South Africa. The sites included Rustenburg (IIC-ARC), North West Province, 25° 43'S ; 27° 18'E; 1157m altitude); Brits (IIC Experimental Farm, North West Province, 25° 31 'S; 27° 49'E, 1109m altitude); Potgietersrus (IIC-ARC Experimental Farm, Northern Province; 24° 11'S 29° 01'E, 1116m altitude) and Friedenheim (Nelspruit) (IIC-ARC Experimental Farm, Mpumalanga Province; 25° 27'S; 30° 58'E). The experimental layout was similar to that described in Chapter 3, Part III. Treatments included a 15813 Insect Protected Cotton line (IPC line); 15985 IPC line; DP 50B (Deltapine 50 with Bollgard$^{TM)}$ and a DP 50 (Deltapine 50, non-transgenic control. Soil composition of each locality is described in Chapter 2, Part III. Planting took place with an in-furrow treatment of aldicarb (Temik® ,10kg/ha), for early season secondary pests. The conventional method of land preparation, trial management with regards to planting, irrigation and fertilize, thinning etc. was followed as described in Chapter 2. Maximum and minimum temperatures, as well as rainfall, associated with insect counts and insecticide applications for the separate localities are given in Chapter 2. Normal insect control was carried out by applying endosulfan (Thioflo, 1 l /ha) spray mixture and a CO_2 -driven knapsack sprayer (2.5 bar) fitted with a drop arm and six full-cone nozzles at egg threshold (40 eggs/80 plants) or larval threshold (16.6 larvae/80 plants). Normal agronomic management was performed and the herbicides Dual® and Cotogard® were applied, followed by irrigation 20 mm, and the thinning of the seedlings one week later. All control, reference and IPC (Insect Protected Cotton lines) plots were sprayed for lepidopteran insect control, when necessary (see Chapter 2).

Assessments

Yield differences between cotton treatments were calculated and differences in fibre quality between cotton treatments were analyzed. An analysis of variance was done on the untransformed yield and fibre quality data. An ordinary analysis of variance was done on the total yield data over the four sites. Thus, both the effect of sites (localities) and cultivar (cotton treatments) and their interaction (site.cult/locality.treatment) was tested (in the Genstat printouts, the locality was often referred to as "site", see Annexure). Fibre quality (strength, length and micronaire) was measured by means of High Volume Instrumentation (HVI) from Spinlab (USA), at the Cotton Board, Pretoria (South Africa). An analysis of variance was done on the cotton fibre length, strength and micronaire over the four sites or localities.

Statistical analyses

The statistical package GENSTAT V. version 3.2 was used to analyze the yield data (Genstat 5 Committee, Rothampsted, UK. Clarendon Press, Oxford). Bonferroni pairwise comparison of means for yield and fibre were done using the programme COMPMEAN, part of a biometrics package (Van Ark 1985). Harvesting data was done according to Good Experimental Field practices. This included recording of the dates of harvest, weather conditions at the time of harvest, cleaning of the equipment between plots to avoid contamination, Storage of seed cotton prior to shipping and the weight of samples. All plots were harvested, weights taken and recorded.

RESULTS

The analysis of variance showed that there was a significant difference between yield obtained on the different localities, as well as between cotton treatments on each locality, and between the interaction between locality and treatment ($p < 0.001$) (Table 5.14). Only 5.5% of the total variance accounted for (sum of squares for locality. treatment = 65.723), can be ascribed to the interaction between locality and treatment and this indicated that significant differences found between cotton treatments and localities were negligibly small. Differences between cotton treatments across localities were not discussed in detail, since it appeared to be of little importance to discuss, for example differences in yield obtained by one treatment (15813) on a given locality e.g. Rustenburg in comparison with another treatment (DP 50) on another locality e.g. Potgietersrus.

For the comparison of the main effect of total yield over all localities (Table 5.14), it was found that the highest yield over all was obtained at the Rustenburg locality, followed by Brits. The total yield obtained in Rustenburg differed significantly from that obtained in Nelspruit and Potgietersrus, while a slightly higher yield was obtained in Nelspruit than in Potgietersrus. The yield obtained in Brits, differed significantly from yields obtained in Nelspruit and Potgietersrus. The lower yield obtained in Nelspruit was probably due to flooding in the beginning of the season. On this locality only, “plant filling in” was performed to obtain a uniform plant stand. In Potgietersrus, there was very little rain throughout the season and high temperatures prevailed. Although the trial was irrigated, many squares (approximately 60%) were lost due to drought circumstances. This explains the very low yields obtained at this locality. The effect of treatment on mean seed cotton yield showed that significant differences were found between cotton treatments over all sites or localities. The transgenic line, 15985 gave the highest yield, but it did not differ significantly from the other transgenic line 15813. However, significant differences were found between the new transgenic line 15985 and the commercially available variety DP 50B. All three transgenic cotton treatments (15985, 15813 and DP 50B) differed significantly from the non-transgenic variety DP 50, which gave the lowest yield. The yield in ton/ha is illustrated in Fig. 5.7. Treatment 15985 gave the highest yield on the Rustenburg and Brits localities, while the 15813 transgenic line gave the highest yield on the Nelspruit and Potgietersrus localities (Table 5.14). The DP 50 treatment gave the lowest yield on at least 3 of the four localities. Yields

obtained in Potgietersrus and Nelspruit are probably not as a result of the inherent characteristics of the cultivars/lines, but probably due to drought and extreme heat in the case of Potgietersrus, and flooding in the beginning of the season in Nelspruit.

Analyses of fibre quality

There was a significant difference in fibre length obtained on the different localities, as well as between cotton treatments on each locality (Table 5.15). However, there were no significant differences in fibre length between the interaction of locality and treatment at $p < 0.001$, although significant differences were found at $p < 0.03$. The percentage variance accounted for by determining the interaction effect of treatment and locality, made out 13.5 % of the total variance accounted for in the analysis. Due to the fact that fibre length can be influenced by environmental circumstances, this interaction effect was not discussed further. The mean fibre length from cotton from Rustenburg and Nelspruit differed significantly from the mean fibre length from cotton from Brits and Potgietersrus respectively, but not from each other (Table 5.15). The mean fibre length of the transgenic line 15813 differed significantly from all other cotton treatments, so does the DP 50 treatment. However, there were no significant differences between the two transgenic cotton treatments 15985 and DP 50B. Table 5.22 shows a comparison of the mean fibre length between cotton treatments per locality. At Rustenburg, the transgenic line, 15813 gave the longest fibre length in comparison with that of the other cotton treatments. Similarly, at Nelspruit, the fibre length of the transgenic line 15813 tended to be longer than that of the other cotton treatments. At Potgietersrus, the fibre of 15813 and DP 50B seemed to be longer than DP 50, while the fibre length of the 15985 line fell in between. DP 50 gave the shortest fibre length of all cotton treatments on all localities except for the Nelspruit locality, where the fibre of the 15985 line was the shortest in length. The average fibre length for each treatment and for each locality is shown in Fig. 5.9. The fibre length obtained in Potgietersrus in the DP 50 treatment was below the required minimum for cotton A1 fibre. The minimum fibre length for A1 fibre = 1.09 inches (27.8mm/25.4 inches = 1.09 inches). Differences in fibre length could be due to a localized effect like the planting date, plant nutrition, soil composition differences or ripening of the cotton fibre. External factors like temperatures, rainfall and humidity could influence the ripening of the fibre. Some of these factors could have played a role determining the fibre length in Potgietersrus. This could be due to drought during the season. However, the other 3 transgenic cotton treatments performed well on this locality, by obtaining the minimum length for cotton fibre. This could indicate that transgenic cotton might not be influenced so much by stress circumstances such as drought, with regards to fibre length. In general, fibre length of the 3 transgenic cotton treatments does not seem to differ from each other. With regards to fibre strength the analysis of variance showed that fibre strength was significantly affected by the localities at $p < 0.001$ (Table 5.16). However, there were no significant differences in fibre strength between cotton treatments and in the interaction of locality and treatment. The normal acceptable range for fibre strength is 22.5-23.5 g/tex. The fibre strength at all localities was lower than the lowest acceptable level of 22.5g/tex, except for Potgietersrus, where the highest fibre strength was obtained (Table 5.16). Fibre strength measured at Brits, were 20.44, while the strength measured at Nelspruit was

slightly lower, about 19 g/tex (Fig. 5.10). Rustenburg gave the second highest fibre strength of 21.62 g/tex. The very low fibre strength achieved in Nelspruit is possibly as a result of the fact that fewer bolls were formed at the first boll count in comparison with other localities, which might have resulted in the poor yield on this locality. This poor boll setting might have resulted in the picking of cotton at a too early stage, which could have caused cotton with a lower strength than in comparison with the other localities. From the bar charts (Fig. 5.10) it seemed as if the fibre strength of all cotton treatments were slightly lower in Nelspruit, while slightly higher in Potgietersrus in comparison with other cotton treatments.

The micronaire was significantly affected by the localities ($p < 0.001$). No significant differences were found between cotton treatments or in the interaction between cotton treatments and localities. The Potgietersrus locality gave the lowest mean micronaire, which differed from the micronaire at Rustenburg, Brits and Nelspruit (Table 5.17, Fig. 5.11). These were below the normal range of 3.5 mic. The micronaire of fibre from Rustenburg and Brits was not significantly different, but that of Nelspruit differed significantly from all other localities. Each treatment gave similar micronaire's (Fig. 5.11) at each locality. The variation in fibre quality of each locality is affected by environmental circumstances as well as cultivation practices (pers comm. Dippenaar), and cannot easily be explained.

The % GOT of cotton treatments at the Potgietersrus locality was significantly lower than the GOT% found in all cotton treatments at the other three localities (Table 5.18). The highest % GOT was obtained on the Brits locality, and this differed significantly from the % GOT's of Nelspruit and Rustenburg. No differences with regards to % GOT were observed on the Nelspruit and Rustenburg localities (Table 5.18). The percentage gin out turn depends on the moisture content of the seed and how cleanly the cotton is picked, the time of harvesting, external environmental factors like drought etc. The very low % GOT obtained in Potgietersrus could have been caused by drought during the season, and this is also reflected by the low micronaire achieved on this locality (Table 5.18). By comparing the mean % GOT across all localities, it was found that the 15813 transgenic line was significantly lower than that of the non transgenic control, DP 50. No differences were observed between the GOT % of the 15985 line, DP 50B or the DP 50 treatment (Table 5.21). All cotton treatments achieved a GOT % of less than 40%, what is normally considered as acceptable. From the bar charts (Fig. 5.8), it can be seen that no real differences are apparent between the cotton treatments per locality. The Brits locality gave the highest GOT % of all cotton treatments, while the lowest %GOT was obtained at Potgietersrus. The DP 50 treatment seemed to give a slightly higher %GOT at Rustenburg, Brits and Nelspruit than any of the other cotton treatments.

CONCLUSION

The transgenic line 15985 gave the highest yield, though not significantly different from the 15813 line, but significantly higher than the DP 50B treatment and the DP 50 treatment. Over all localities, the 3 transgenic cotton treatments gave significantly better yields than the DP 50 treatment. The DP 50 treatment gave a slightly higher GOT % than the 2 transgenic lines, 15813 and 15985, but not significantly different from that of DP 50B. The 15813 line had significantly

longer fibre length than the other cotton treatments, and DP 50 gave the shortest fibre length. The 15985 line and the DP 50B variety fell between these extremes. There were no significant differences between cotton treatments with regards to fibre strength. Fibre strength was significantly higher in Potgietersrus than the other localities. There were no significant differences between cotton treatments with regards to micronaire. Micronaire was significantly lower at Potgietersrus, in comparison with the other localities.

Part IV : To determine the incidence of bollworms on yield of a potential insect resistant cotton line, 15985 containing the *cry1Ac* and *Cry2Ab2* genes, in stackgene combination, during a segregant study

The aim was to determine the effect on yield during a bollworm efficacy study of the transgenic line, 15985, by comparing different segregants from the line, containing single or double genes.

EXPERIMENTAL PROCEDURES

Three field trials were conducted at the IIC, Rustenburg (North-West Province 25° 43'S; 27° 18'E; 1157m altitude); in Brits (IIC Experimental Farm) (North-West Province 25° 31'S; 27° 49'E, 1109 m altitude) and at Friedenheim (Nelspruit) (Mpumalanga Province 25° 27'S; 30° 58'E). The treatments included unsprayed 15985BX treatments, 15985X – unsprayed; 15985B – unsprayed; DP50B – unsprayed; DP50 – unsprayed; 15985BX - sprayed according to threshold; DP50B - sprayed according to threshold and DP 50 - sprayed according to threshold. The cotton type 15985BX – contains Bollgard and the *cryX* gene, while 15985X is a segregant containing only *cryX*. The cotton type 15985B is a segregant containing only Bollgard, and DP 50B is the original transformant or parent variety of 15985 with Bollgard™. The DP 50 variety is a non-transgenic recurrent parent of DP 50B.The trial layout and design followed that which is described in Chapter 2, Part V. A summary of the trial layout is given below: Soil composition is as described in Chapter 2; Part V. Planting was performed by hand, by putting a single cotton seed with 3 soya bean seeds into each plant station. Seed depth varied from approximately 2 cm in Brits and Rustenburg, while seed depth was approximately 4 cm in Nelspruit. Insecticide application dates for all three localities are listed in Tables 2.42, 2.43 and 2.44 (Chapter 2). Seedbeds were prepared by means of ploughing and conventional tilling and the seed was planted in dry soil at a temperature varying between 23-26°C at the various localities. Herbicides were given on the 17th of November 1999 (Dual® and Cotogard®; 600 ml/ha and 4 l/ha mixed) in Rustenburg, on the 18th of November 1999 (Dual and Cotogard; 600 ml/ha and 4 l /ha mixed) in Brits, and on the 1st of November 1999 (MSMA®; 3l/ha) in Nelspruit. Fertilization and irrigation were applied according to standard procedures. Normal insect control was carried out by applying Thioflo (endosulfan) as a spray mixture, equivalent to Thioflo® (endosulfan; 1 l / ha) applied at a pressure of 2.5 bar to all plants in the cotton treatments and sprayed according to the threshold, by means of a CO_2 -driven knapsack sprayer fitted with a drop arm and six full-cone nozzles. This was done when the larval threshold (16.6 larvae/80 plants) was reached or exceeded on the specified sprayed treatments. The threshold of 5 larvae/24 plants was adapted to the number of plants scouted per plot (10) for 8 repetitions. After planting, the plots were scouted at least once a week to assess insect infestation and the insects were subsequently controlled. All chemical applications are documented in Table 2.2 (Chapter 2). No defoliant was used in any of these trials.

Assessments

Harvesting was done by hand. From the Nelspruit locality, 50 boll samples were taken, weighed and ginned for fibre analyses. The same was done at the Rustenburg locality. The cotton in Brits was of a poor quality, probably due to adverse weather conditions. It was decided not to perform fibre analyses on cotton from Brits. Total yield per plot was compared between treatments for all three localities. In addition, 8 plants per plot that seemed similar in plant growth were selected and harvested separately to compare yield per plant per plot. This was done in order to minimise the effect of very large plants in the control plots. These plants grew vigorously because of poor stand and which had an irregular number of bolls. The larger number of bolls per plant on the control plots, could lead to an incorrect interpretation of yield results per plot when compared with the number of bolls per plant on the transgenic treatments. Yield samples were weighed and the weights were recorded. Total seed included the total seed cotton yield and seed from the 50 boll samples taken for fibre analyses. Yields between treatments at each of the localities were compared by means of an analysis of variance. The effects of the different localities on the treatments were not compared because extreme climatic conditions, unusual for the specific cotton-producing regions, had prevailed at all the localities during the trials. Since fibre quality can be affected by external factors, the effects of locality on the treatments were not compared statistically. Differences in yield between cotton treatments were calculated and differences in fibre quality between cotton treatments were analyzed.

Statistical analyses

An analysis of variance was done on the untransformed yield and fibre quality data. Fibre quality (strength, length and micronaire) was measured by means of High Volume Instrumentation (HVI) from Spinlab (USA) at the Cotton Board, Pretoria (South Africa). An analysis of variance was done on the cotton fibre length, strength and micronaire over the four sites or localities. The statistical package, GENSTAT V. version 3.2, was used to analyze the yield data (Genstat 5 Committee, Rothampstead, UK. Clarendon Press, Oxford). A Bonferroni pairwise comparison of means for yield and fibre was done using the programme, COMPMEAN, part of a biometrics package (Van Ark 1985).

RESULTS

An ordinary analysis of variance was done on the total yield data at Rustenburg and Nelspruit as explained in the experimental methods. As a result of climatic conditions, which caused boll rot most probably secondary to insect damage, the yield was very poor at Brits and only a comparative sample of seed cotton was taken from this locality, to get some measure of yield between treatments. The effect of cultivar (cotton treatments) on the yield was evaluated. The effect of the interaction between yield, treatments and locality was not compared, since each locality experienced very different climatic conditions this season (see weather conditions Chapter 2).

For the effect of treatment on mean seed cotton yield in Rustenburg and Nelspruit, the analysis of variance showed that a significant difference in yield was obtained between

treatments at Rustenburg. All transgenic treatments differed significantly from the non-transgenic DP50 unsprayed and sprayed treatments, but not from each other. No differences in yield were obtained between the two DP50 unsprayed and sprayed control treatments (Table 5.19). Similarly, in Nelspruit, all the transgenic treatments differed significantly from the two DP50 control treatments, but not from each other, while the DP50u treatment differed significantly from the DP50s treatment (Table 5.19).

For the effect of treatment on mean seed cotton yield of 60 plants/treatment at Rustenburg and Brits, the number of plants harvested for total seed cotton yield was compared between treatments, significant differences were observed for Rustenburg ($p< 0.001$) and Brits ($p<0.05$). At Rustenburg, the 15985Xs treatment gave a significantly higher yield than the 15985Bu treatment the DP50 unsprayed and sprayed control treatments (Table 5.20). The latter two control treatments did not differ significantly from each other. There was no difference in the yield obtained from 60 plants between the transgenic 15985 unsprayed treatments, although the 15985 line tended to produce a higher yield with the *cryX* gene than with the Bollgard gene. This difference was not significant. At the Brits locality, there were no differences between any of the transgenic treatments, with or without the *cryX* and the Bollgard genes, and irrespective of whether they were sprayed or unsprayed. All the transgenic treatments differed significantly from the DP50u treatment. The DP50s treatment gave an unexpectedly high yield. This could be due to an incorrect number of plants harvested in this control plot and statistically this was considered as an outlier. The harvested weight for the DP50s treatment in Brits was considered as an incorrect value due to experimental error.

Analyses of fibre quality

Fibre length did not differ significantly between treatments either at Rustenburg or Nelspruit. The mean fibre length of cotton from Rustenburg varied from 1.0588 inches (15985BXu) to 1.08 inches (15985Xu). Except for the 15985BXu treatment, which seemed to give a slightly lower fibre length (< 0.01 inches), as the two controls, the mean fibre length of the transgenic lines tended to be slightly higher (> 0.01 inches) than that of the two DP50 control treatment. In Nelspruit, fibre length between treatments followed no trend and fibre length varied from 1.0963 inches (DP50s) to 1.1188 inches in the DP50Bu treatment (see Annexure). The minimum fibre length for A1 fibre = 1.09 inches (27.8mm/25.4 inches = 1.09 inches). The fibre length obtained in all the treatments at Nelspruit was above the minimum for cotton A1 fibre, while those in Rustenburg were below the minimum. Since differences in fibre length could be due to a localized effect like the planting date, plant nutrition, and differences in soil composition or ripening of the cotton fibre, the values are not tabulated here. External factors like temperature, rainfall and humidity could influence the ripening of the fibre. Some of the factors, like drought during the season, could have caused, or been partly responsible for the varying fibre length at Nelspruit.

The normal acceptable range for fibre strength is 22.5-23.5 g/tex. The fibre strength at both localities was lower than the lowest acceptable level of 22.5g/tex. The DP50s treatment gave the highest fibre strength of 22.088 g/tex at Rustenburg (Table 5.21). At Rustenburg,

significant differences in fibre strength were found between the 15985BXu treatment (Treatment one) and both the 15985BXs treatment (Treatment six) and the DP50Bs treatment (Treatment seven). The DP50s treatment differed significantly from the 15985Xu (Treatment two) and 15985Bu (Treatment three) treatments (Table 5.30). There were no significant differences in fibre strength between cotton treatments at the Nelspruit locality (5.21). Fibre strength between treatments at Nelspruit was below 20.0 g/tex for all the treatments. The very low fibre strength achieved at Nelspruit possibly resulted from the early picking of unripe cotton, necessitated by extremely unusual weather conditions.

The micronaire differed significantly between treatments at Nelspruit ($p < 0.05$), while no differences between treatments were observed at Rustenburg. At Rustenburg, all treatments gave a micronaire below the normal range of 3.5 mic. The 15985Xu treatment and the 15985Bu treatment were the only treatments with a micronaire above 3.0 mic. The latter two treatments were similar, and seemed to have a slightly higher micronaire than all the other treatments (Table 5.22). At Nelspruit, an exceptionally high micronaire was observed in all the treatments (> 5.0 mic) (Table 5.22). The three transgenic treatments containing the *cryX* gene did not differ significantly from each other, but did differ from the DP50 treatments with or without the Bollgard gene. The variation in fibre quality, i.e. micronaire, at each locality was caused by environmental circumstances as well as cultivation practices (pers comm. Dippenaar) and cannot easily be explained. However, it is possible that the large amount of rain that fell with a subsequent small number of sunshine days (number of heat units) at Nelspruit, could have influenced the fibre quality. In Rustenburg, similar wet weather conditions prevailed (see Chapter 2). Cotton bolls took longer than usual to burst and a large number of bolls had boll rot, probably caused by fungus infection secondary to insect damage.

There was a significant difference in the GOT % obtained between treatments at both localities ($p < 0.001$). The highest GOT % was obtained at the Nelspruit locality, while very low GOT's were obtained at the Rustenburg locality. The percentage gin out-turn depends on the moisture content of the seed, the time of harvesting, external environmental factors like drought, and on how clearly the cotton is picked. The very low GOT % obtained at Rustenburg, as well as the low micronaire achieved could have been caused by the unusual amount of rain received during the season (Table 5.23). All the cotton treatments achieved a GOT % of less than 40%. Normally a GOT % of 38-40% is acceptable. At Rustenburg, however, all the GOT percentages were below 34%. It seems irrelevant to discuss significant differences between treatments, since no trend could be observed between the transgenic treatments themselves, or between transgenic and non-transgenic treatments, or between unsprayed and sprayed transgenic and non-transgenic treatments. At Nelspruit, however, the 15985 transgenic variety, with or without the *cryX* gene and Bollgard gene differed significantly from the unsprayed and sprayed DP50 control treatments. Similarly, this 15985 transgenic variety, with or without the *cryX* gene and the Bollgard gene, differed significantly from the unsprayed and sprayed DP50 treatments containing the Bollgard gene (Table 5.23). In general, the 15985 transgenic variety expressed a slightly lower GOT % than the DP50 variety.

CONCLUSION

At one locality, when the transgenic varieties were compared, the yield per number of plants was significantly higher for the 15985BXs treatment than for the 15985Bu treatment, but the spraying costs should be taken into account when the economic viability of producing this variety is considered. GOT percentages were extremely low, due to unusual weather conditions that prevented the cotton from ripening. Fibre quality was suspected to be heavily influenced by weather conditions and the trials should be repeated for a correct assessment of fibre quality. All transgenic varieties increased yield whether they contained cry1Ac or cry1Ac/cry2Ab2 or a combination of both and at all location. Both genes were equally effective at controlling bollworms and therefore protecting yield.

Part V: A comparison of yields obtained with Bt-cotton in a small-scale farmer situation under different management programmes

The aim of this study was to compare different insecticide application techniques and associated yields in a small-scale farmer setup, on transgenic cotton.

INTRODUCTION

Three trials were carried out during the cotton production season of 2003/2004 in the Makhathini (KwaZulu-Natal) and Moutse West district (Mpumalanga) to compare and demonstrate the principle of Integrated Pest Management to small-scale farmers. These trials were carried out under the auspices of Cotton SA, and under management of the Cleaner Cotton Production Unit. These trials were aimed at illustrating an approach to farmers, which aims to develop a combination of control measures which are cost-effective and safe for farmers, consumers and the environment, with other words an integrated farming approach that are ecologically sustainable. The significance of discussing these results is to confirm those achieved in the above mentioned trials in Part I-III of this study. The emphasis of the demonstration trials in IPM, was on the reduction – or when possible elimination of pesticide use.

The success of demonstration trials depends on the availability of irrigation, since cotton production under dry land conditions, are often not feasible. One trial was planted in the Makhathini region under rainfed conditions and two trials in Moutse East and Moutse West respectively, under irrigation. Weekly visits were carried out and the growers performed scouting on the trials. Two field assistants in the area, under supervision, managed these trials. The scouting results and yield of the demonstration trials will be analyzed to use for further training and advise to farmers.

EXPERIMENTAL PROCEDURES

A single trial was planted in Moutse West (Marble Hall, Mpumalanga) and the cotton type demonstrated as the only treatment, was a herbicide tolerant variety – Roundup Ready®. The cotton was sprayed with the herbicide Roundup Ready® before and after planting and when plants reached the 4th leaf stage. A knapsack sprayer was used for spraying.

The trial in Moutse East was planted with Bollgard™ cotton containing the Bt-gene, and sprayed with a knapsack and Micron Ulva in two blocks, placed adjacent of each. In the first treatment the application technique was aimed at insect control with a knapsack sprayer, while in the additional treatment spraying was done with the use of a Micron Ulva. In both these two treatments, spraying was carried out on scouting results done on 24 plants per treatment, which is the acceptable number of plants to be scouted per field on cotton.

In the Makhathini, a cotton trial was planted on the farm of Mr. Ntshangase (L11, Fig 6.1, Chapter 6) and Ms. Bongani Mbatha (L12, Fig. 6.1, Chapter 6). In both of these two trials, two blocks of Bollgard were planted and sprayed with a knapsack sprayer and a Micron Ulva respectively.

Important dates are listed in Table 5.24. Trial areas were seven rows of 25 m in length, cotton rows spaced 1 m apart for each block sprayed with a particular method. The Roundup Ready was a single block of 14 rows of cotton.

For sucking pests, acetamiprid (Mospilan®, 500ml/ha), was sprayed, while chlorpirifos (Dursban®[5], 1l/ha) was used for stainers, and endosulfan (Thioflo®, 1l/ha) was sprayed for bollworms. Spraying commenced when threshold values were reached on all the trials. Threshold values were used as described in Chapter 2 (for bollworms) and 3 (for stainers) of this study. Land preparation, thinning and planting was done according to the standard procedures for cotton production.

Assessments

Yields were compared by picking the inside rows of each block, and not the two outer rows. Seed cotton was weighed and yield per ha was calculated. No statistical comparisons were made, since the trials were demonstration trials.

RESULTS

Irrigated trails were carried out only in Moutse. Yields obtained in the Bollgard™, knapsack sprayed trial in Moutse West gave the highest yield of 3 142 kg/ha seed cotton. The yield obtained on the Micron Ulva treatment of 3 028 kg/ha did not differ very much of the treatment sprayed with a knapsack. The Roundup Ready™ treatment gave the lowest yield, possibly as a result of poor bollworm control, since on average; bollworm control normally requires 6-8 insecticide applications, in comparison with the four applications, which were applied in this instance. The yield obtained at Moutse East (Table 5.25) could be attributed to the somewhat late planting time.

Rainfed trials were carried out in the Makhathini. The yields obtained under rain-fed conditions varied between 800 and 900 kg/ha between the two application methods, which are in the range normally achieved for this region. A cost analysis was performed on the small-scale farmer demonstration trials of the input items that differed between the trials (Table 5.26). The cost for land preparation, weed control and other management procedures that was similar in each instance where a comparison is made was omitted from the table, to emphasize chemical input. The net-profit for the commercial demonstration trial can only be compared between the two treatments, while the irrigated trials and the dryland trials are compared respectively. Table 5.25, indicates that the trial under irrigation in Moutse East gave a lower income, probably due to the high number of pesticides sprayed on this plot and the cost of scouting. Under dryland conditions, a low income was achieved, due to low yields obtained. This emphasizes the importance of scouting for the dryland farmer, to be able to apply pesticides only when there is no other choice. The costs of chemicals including monocrotophos which was applied by the grower early in the season, was high and it indicated that hazardous chemicals like this are still used by some growers.

[5] Dursban® (chlorpirifos) is not registered on cotton. Was used to simulate the same conditions of small-scale farmers who are using it on cotton and maize. It is registered on maize.

CONCLUSION

The small-scale farmer trials indicated that a reasonable yield could be obtained under irrigated conditions with an Integrated Pest Management programme. These trials would give better results if they are done on a larger scale, but it is difficult to get small-scale growers to co-operate under the conditions that they experience. They often have only 1ha to farm, and they have their own priorities regarding farm management. Under dryland conditions, the yields were very low, and indicated that cotton should really be grown under irrigation, even in the case of bollworm resistant varieties. The Micron Ulva treatment demonstrated the highest yield on both the Moutse Localities and in the Makhathini. The Roundup Ready demonstration trial in Moutse West appeared to give the highest yield of all the trials, if scouting is done properly and when genetically modified cotton is sprayed on threshold values.

Final conclusion of the effect of yield

The sprayed or unsprayed Bt-treatments, with the *cry1Ac* gene, produced a significant higher yield than the conventional sprayed and unsprayed treatments on all localities. Bt-cotton with the *cry1Ac* gene and the *cry2Ab2* gene, give significant yield differences when compared to conventional cotton, due to the protection the gene provides against bollworm damage. Fibre qualities tended to be at least similar or to even better that of conventional cotton.

Different events containing the *cry2Ab2* gene indicated different results in yield, depending on the inherent genetic characteristics of each event. The 15813 event gave the most promising results in yield and related bollworm numbers and were further compared to a 15985 line. In this comparison, the *Cry2Ab2* cotton treatments which included the 15813 Insect Protected Cotton line event, the 15985 event, and the transgenic control variety DP 50B (Deltapine 50 with Bollgard$^{TM)}$, and a non-transgenic reference variety, DP 50 (Deltapine 50). The yield obtained by the 15985 line was significantly different from the DP 50B variety, but not from that obtained by the 15813 transgenic line. The total mean seed cotton yield from all three the transgenic cotton treatments differed significantly from the DP 50 variety on averaged across all the sites. Bollworm numbers are higher in the absence of the Bt-protein, resulting in a lower yield. The lower yield obtained by the DP 50 variety across all sites, was expected since more bollworm larvae were found on this treatment at all sites due to the absence of the Bt-protein which affects only lepidopteran larvae.

In a stacked combination of the *cry1Ac* gene and the *cry2Ab2* gene, and in single gene format, all transgenic treatments differed significantly from the non-transgenic control treatments with regard to yield. No significant differences in yield were found between any of the transgenic treatments.

Bt-cotton (*cry1Ac* gene) sprayed according to specific thresholds with a Micron Ulva tends to give higher yields in a small-scale farmer setup, than with in comparison with a knapsack under irrigated conditions, indicating that good management practices of Bt-cotton can improve yields. The biggest benefit for the cotton grower from planting Bt-cotton types is the achievement of a substantial higher yield than in conventional cotton. With the low cotton price of about R2.20 per kg, seedcotton (pers comm. Schröder[6]), the key element in producing cotton to replace high input costs, is the achievement of higher yields, resulting in a higher net-profit per hectare.

[6] Mr. H. Schröder is the Cotton Quality Manager, at Cotton SA, Pretoria, S.A, and he is an expert on cotton quality and market trends.

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Tables

Table 5.1. Yield between treatments at Rustenburg (1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Means kg/ha)	2051.00[a]	2276.00[a]	842.00[c]	1395.00[b]

** Means sharing the same symbol do not differ significantly at p < 0.001.*

$LSD_{(Bonf.\ p < 0.001)} = 418.77$

Table 5.2. Yield between treatments at Potgietersrus (1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Means kg/ha)	1053.00[ab]	1201.00[a]	738.00[b]	895.00[ab]

** Means sharing the same symbol do not differ significantly at p < 0.001.*

$LSD_{(Bonf.\ p < 0.001)} = 437.576$

Table 5.3. Yield between treatments at Loskop (1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Means kg/ha)	1101.00[a]	1131.00[a]	625.00[b]	629.00[ab]

** Means sharing the same symbol do not differ significantly at p < 0.001.*

$LSD_{(Bonf.\ p < 0.001)} = 396.972$

Table 5.4. Mean yield between treatments at Groblersdal (1998/1999) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Means kg/ha)	1101.00[a]	1131.00[a]	625.00[b]	629.00[b]

** Means sharing the same symbol do not differ significantly at p < 0.001.*

$LSD_{(Bonf.\ p < 0.001)}$ = 396.972 (d.f. = 23)

Table 5.5.a. Analysis of variance for treatment and locality effect on yield for Rustenburg, Potgietersrus and Loskop (1997/1998) in the Cry1Ac trial.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Locality	2	8399295	4199647	54.96	< 0.001
Residual	15	1146227	76415	1.34	
Locality. Rep. Treatment	3	7483784	2494595	43.85	<0.001
Locality. Treatment	6	2296990	382832	6.73	<0.001
Residual	45	2559855	56886		
Total	71	21886150			

Table 5.5.b. Total treatment means compared across localities (Rustenburg, Potgietersrus and Loskop, 1997/1998) in the Cry1Ac trial.

Treatments	Bollgard™ unsprayed	Bollgard™ sprayed	DpAC 90 unsprayed	DpAC 90 sprayed
Means kg/ha)	20.82[bc]	32.62[a]	16.12[c]	27.02[ab]

** Means sharing the same symbol do not differ significantly*

$LSD_{(Bonf.\ p < 0.01)}$ = 6.87

Table 5.5.c. Comparison of yield between the various localities (1997/1998) in the Cry1Ac trial.

Treatment	MEAN
Loskop –DpAC 90 unsprayed	625.00[a]
Loskop –DpAC 90 sprayed	629.00[a]
Potgietersrus – DpAC 90 unsprayed	738.00[ab]
Rustenburg -DpAC 90 unsprayed	842.00[ab]
Potgietersrus –DpAC 90 sprayed	895.00[ab]
Potgietersrus – Bollgard™ unsprayed	1053.00[abc]
Loskop - Bollgard™ unsprayed	1101.00[abc]
Loskop - Bollgard™ sprayed	1131.00[bc]
Potgietersrus – Bollgard™ sprayed	1201.00[bc]
Rustenburg - DpAC 90 sprayed	1395.00[c]
Rustenburg – Bollgard™ unsprayed	2051.00[d]
Rustenburg – Bollgard™ sprayed	2276.00[d]

** Means sharing the same symbol do not differ significantly at $p < 0.05$.*

$LSD_{(Bonf.\ p < 0.05)} = 497.631$

Table 5.6. Fibre strength (g/tex) between localities (1997/1998) in the Cry1Ac trial.

Treatments	Rustenburg (p= 0.137)	Potgietersrus (p = 0.098)	Loskop (p = 0.187)
Bollgard™ unsprayed	21.30	20.68	21.61
Bollgard™ sprayed	21.72	21.30	21.94
DpAC 90 Unsprayed	22.50	22.27	22.49
DpAC 90 sprayed	22.00	21.62	22.67

Table 5.7. Fibre length (mm) at localities (1997/1998) in the Cry1Ac trial.

Treatments	Rustenburg (p = 0.331)	Potgietersrus (p = 0.857)	Loskop (p = 0.06)
Bollgard™ unsprayed	27.86	27.22	28.49
Bollgard™ sprayed	28.11	27.31	28.49
DpAC 90 unsprayed	28.32	27.52	28.38
DpAC 90 sprayed	28.36	27.26	23.53

Table 5.8. Micronaire (mic) between localities (1997/1998) in the Cry1Ac trial.

Treatments	Rustenburg (p = 0.02)	Potgietersrus (p = 0.797)	Loskop (p = 0.252)
Bollgard™ unsprayed	4.417[ab]	4.267	3.770
Bollgard™ sprayed	4.433[a]	4.383	3.745
DpAC 90 unsprayed	3.900[b]	4.217	3.790
DpAC 90 sprayed	4.083[ab]	4.283	4.050

LSD $_{Bonf\ (p< 0.05)}$ 0.533

** Means sharing the same symbol in the same column are not statistically significant.*

Table 5.9. Comparison of the Gin out Turn (%) at localities (1997/1998) in the Cry1Ac trial.

Treatments	Rustenburg (p = 0.045)	Potgietersrus (p = 0.09)	Loskop (p = 0.318)
Bollgard™ unsprayed	39.65 [n.s.]	41.44	34.40
Bollgard™ sprayed	39.27 [n.s.]	40.79	33.93
DpAC 90 unsprayed	38.22 [n.s.]	40.91	34.71
DpAC 90 sprayed	38.38 [n.s.]	42.01	34.64

LSD $_{Bonf\ (p< 0.05)}$ 1.6059

[n.s.] Treatments do not differ significantly.

Table 5.10. Stand count for each plot before the non-transgenic plants were removed in the P2b trial.

Plot no.	Events	Event stand count	Check plot no.	Corresponding Check	Check Stand count
1	6149	60	C1	DP50	42
2	6151	71	C2	DP50B	49
3	6152	66	C3	C312	69
4	6223	70	C4	531	62
5	6227	77	C5	15813	63
6	6232	66	C6	15985	70
7	6234	73	C1	DP50	18*
8	6239	69	C2	DP50B	62
9	6241	60	C3	C312	72
10	6351	78	C4	531	71
11	6352	77	C5	15813	75
12	6456	83	C6	15985	70
13	6460	82	C1	DP50	37
14	6483	70	C2	DP50B	55
15	6484	70	C3	C312	68
16	6489	77	C4	531	83
17	6491	72	C5	15813	76
18	6526	71	C6	15985	59
19	6532	57	C1	DP50	30
20	6563	78	C2	DP50B	62
21	6542	72	C3	C312	70
22	6718	74	C4	531	81
23	6725	69	C5	15813	64
24	6733	63	C6	15985	66
25	6734	72	C1	DP50	28
26	16221	66	C2	DP50B	47
27	16960	69	C3	C312	70
28	17079	85	C4	531	82
29	17133	78	C5	15813	82
30	17180	80	C6	15985	81
31	17206	85	C1	DP50	31
32	6233	81	C2	DP50B	56
33	15830-5	69	C3	C312	83

Table 5.11. Stand count for each plot after the non-transgenic plants were removed in the P2b trial.

Plot no.	Events	Event stand count	Check plot no.	Corresponding Check	Check Stand count
1	6149	42	C1	DP50	42
2	6151		C2	DP50B	49
3	6152	46	C3	C312	69
4	6223	53	C4	531	62
5	6227	49	C5	15813	63
6	6232	52	C6	15985	70
7	6234	52	C1	DP50	18*
8	6239	58	C2	DP50B	62
9	6241	50	C3	C312	72
10	6351	52	C4	531	71
11	6352	73	C5	15813	75
12	6456	50	C6	15985	70
13	6460	43	C1	DP50	37
14	6483	48	C2	DP50B	55
15	6484	52	C3	C312	68
16	6489	41	C4	531	83
17	6491	54	C5	15813	76
18	6526	53	C6	15985	59
19	6532	38	C1	DP50	30
20	6563	56	C2	DP50B	62
21	6542	42	C3	C312	70
22	6718	57	C4	531	81
23	6725	55	C5	15813	64
24	6733	58	C6	15985	66
25	6734	41	C1	DP50	28
26	16221	57	C2	DP50B	47
27	16960	47	C3	C312	70
28	17079	82	C4	531	82
29	17133	60	C5	15813	82
30	17180	69	C6	15985	81
31	17206	56	C1	DP50	31
32	6233	36	C2	DP50B	56
33	15830-5	55	C3	C312	83

Table 5.12. Seed cotton yield (g) per square meter in the P2b trial.

Plot	Pick 1 (g/15m row)	(g/ square meter)	Pick 2 (g/15m row)	(g/ square meter)	Pick 3 (g/15m row)	(g/ square meter)	Total yield (g)/15 meter row	No. of plants/ row	Total yield (g)/plant/ square meter)
1	2949.2	70.22	1124.1	26.76	684.4	16.30	4854.683	42	115.59
2	3251.1	66.35	1121.8	22.89	360.6	7.36	4068.093	49	83.02
3	2470.6	53.71	1167.7	25.38	350.7	7.62	4068.093	46	88.44
4	2703.0	51.00	1247.4	23.54	406.0	7.66	4430.936	53	83.60
5	2350.1	47.96	1614.5	32.95	299.9	6.12	4345.410	49	88.68
6	3085.0	59.33	1665.8	32.03	233.3	4.49	5075.462	52	97.61
7	2303.3	44.29	2678.7	51.51	466.2	8.97	5544.008	52	106.62
8	3068.2	52.90	2637.7	45.48	626.3	10.80	6430.578	58	110.87
9	1249.1	24.98	2343.9	46.88	605.1	12.10	4269.960	50	85.40
10	1910.4	36.74	2814.3	54.12	723.3	13.91	5538.860	52	106.52
11	1827.7	25.04	2675.9	36.66	581.6	7.97	5146.893	73	70.51
12	2640.9	52.82	474.9	9.50	0.0	0.00	3178.116	50	63.56
13	2446.5	56.90	698.8	16.25	203.4	4.73	3421.847	43	79.58
14	4017.3	83.69	826.9	17.23	269.4	5.61	5214.521	48	108.64
15	3026.6	58.20	230.1	4.43	0.0	0.00	3319.329	52	63.83
16	1903.7	46.43	755.5	18.43	246.3	6.01	2970.359	41	72.45
17	2591.6	47.99	539.2	9.99	246.2	4.56	3434.978	54	63.61
18	3604.7	68.01	725.8	13.69	232.2	4.38	4644.408	53	87.63
19	1672.9	44.02	831.2	21.87	182.7	4.81	2752.697	38	72.44
20	1797.7	32.10	769.0	13.73	270.2	4.83	2882.734	56	51.48
21	2268.9	54.02	434.8	10.35	112.6	2.68	2880.674	42	68.59
22	4024.6	70.61	533.5	9.36	211.8	3.72	4849.867	57	85.09
23	2428.5	44.16	509.5	9.26	269.5	4.90	3260.918	55	59.29
24	1752.1	30.21	287.9	4.96	0.0	0.00	2075.172	58	35.78
25	1429.9	34.88	818.0	19.95	299.7	7.31	2602.427	41	63.47
26	3177.0	55.74	1645.2	28.86	852.8	14.96	5759.600	57	101.05
27	0.0	0.00	1414.7	30.10	993.6	21.14	2438.400	47	51.88
28	2062.0	25.15	1479.8	18.05	729.0	8.89	4313.993	82	52.61
29	877.5	14.63	983.2	16.39	533.6	8.89	2425.312	60	40.42
30	3454.0	50.06	1073.1	15.55	709.9	10.29	5302.610	69	76.85
31	0.0	0.00	401.6	7.17	876.9	15.66	1285.671	56	22.96
32	2696.1	74.89	564.6	15.68	215.2	5.98	3566.475	36	99.07
33	3607.4	65.59	1347.6	24.50	551.6	10.03	5596.691	55	101.76
C1	773.9	7.74	833.2	8.33	629.7	6.30	2252.871	100	22.53
C2	3844.4	38.44	1298.1	12.98	375.6	3.76	5569.525	100	55.70
C3	0.0	0.00	1230.6	12.31	960.5	9.61	2203.406	100	22.03
C4	3095.9	30.96	1420.6	14.21	450.1	4.50	5011.765	100	50.12
C5	2664.4	26.64	1658.8	16.59	656.8	6.57	5023.232	100	50.23
C6	3239.7	32.40	2274.1	22.74	1189.2	11.89	6758.138	100	67.58

Table 5.13. Percentage Gin Out-turn of 50-boll samples for each event in the P2b trial.

Event no.	Seed mass	Fibre mass	Total seed cotton mass	Fibre/seed Fibre	% GOT
1(6149)	209.5	130.4	339.9	0.383642	38%
2(6151)	188.9	128.8	317.7	0.405414	41%
3(6152)	178.9	114.9	293.8	0.391082	39%
4(6223)	180.9	115.9	296.8	0.390499	39%
5(6227)	169.8	114.5	284.3	0.402744	40%
6(6232)	175.5	120.8	296.3	0.407695	41%
7(6234)	197.6	118.5	316.1	0.374881	37%
8(6239)	201.5	132.3	333.8	0.396345	40%
9(6241)	192.7	108.3	301	0.359801	36%
10(6351)	185.6	122.7	308.3	0.397989	40%
11(6352)	174.2	120.7	294.9	0.409291	41%
12(6456)	172	96.4	268.4	0.359165	36%
13(6460)	167.4	119.2	286.6	0.415911	42%
14(6483)	192.5	133.7	326.2	0.409871	41%
15(6484)	181.4	121.7	303.1	0.401518	40%
16(6489)	184.4	123.3	307.7	0.400715	40%
17(6491)	179.8	122.4	302.2	0.40503	41%
18(6526)	182.7	119.7	302.4	0.395833	40%
19(6532)	173.5	116.3	289.8	0.401311	40%
20(6563)	174.7	113.7	288.4	0.394244	39%
21(6542)	168.9	96.8	265.7	0.364321	36%
22(6718)	203.8	132	335.8	0.393091	39%
23(6725)	178.8	123.4	302.2	0.408339	41%
24(6733)	141.8	93.2	235	0.396596	40%
25(6734)	148.5	96	244.5	0.392638	39%
26(16221)	195.9	94.4	290.3	0.325181	33%
27(16960)	171	91.5	262.5	0.348571	35%
28(17079)	181.9	98.3	280.2	0.350821	35%
29(17133)	140.3	68.7	209	0.328708	33%
30(17180)	179.5	102.3	281.8	0.363023	36%
31(17206)	152	82.4	234.4	0.351536	35%
32(6233)	183.3	112.8	296.1	0.380952	38%
33(158305)	188	97.9	285.9	0.342427	34%
C1 (DP50)	164	90.3	254.3	0.355092	36%
C2 (DP0B)	190.9	100.4	291.3	0.344662	34%
C3 (C312)	175.7	123.5	299.2	0.412767	41%
C4 (C531)	200	128.5	328.5	0.391172	39%
C5 (15813)	192.8	98.2	291	0.337457	34%
C6 (15985)	189.5	97.1	286.6	0.3388	34%

Table 5.14. Means of total seed cotton yield per plot (kg) of four cotton treatments, containing the *cry2Ab2* gene at four localities (1998/1999)

Treatments Localities	15813	DP 50	15985	DP 50B	Means* (Localities)
Rustenburg	10.83	8.04	13.15	11.44	10.87[a]
Brits	10.59	7.13	13.22	10.30	10.31[a]
Nelspruit	4.83	3.97	3.34	3.44	3.89[b]
Potgietersrus	2.87	1.21	2.38	1.92	2.09[c]
Means* (Treatments)	7.28[ab]	5.09[c]	8.02[a]	6.77[b]	

$LSD_{(Bonf.\ p < 0.05)}$ = 1.21012 for localities (Means in the same column with the same symbol do not differ significantly at p < 0.05)

$LSD_{Bonf.\ p < 0.05)}$ = 1.21012 for cotton treatments (Means in the same row with the same symbol do not differ significantly at p < 0.05)

** Means of 16 repetitions.*

Table 5.15. Mean fibre length (inches) between cotton treatments at four localities in the *cry2Ab2* gene trial.

Treatments Localities	15813	DP 50	15985	DP 50B	Means* (Localities)
Rustenburg	1.1950	1.1000	1.1500	1.1450	1.1475[a]
Brits	1.1150	1.0950	1.0975	1.0975	1.1012[b]
Nelspruit	1.1950	1.1350	1.1150	1.1425	1.1469[a]
Potgietersrus	1.1325	1.0725	1.1000	1.1375	1.1106[b]
Means* (treatments)	1.1597[a]	1.1006[d]	1.1156[bc]	1.1306[b]	

$LSD_{(Bonf.\ p < 0.001)}$ = 0.02451 for localities (Means in a single column with the same symbol do not differ significantly at p < 0.001)

$LSD_{(Bonf.\ p < 0.001}$= 0.02451 for cotton treatments (Means in the last row with the same symbol do not differ significantly at p < 0.001)

- *Means of 16 repetitions.*

Table 5.16. Mean fibre strength (g/tex) of four cotton treatments at four localities in the *cry2Ab2* gene trial.

Localities	Rustenburg	Brits	Nelspruit	Potgietersrus
Means	21.62[b]	20.44[c]	19.27[d]	23.66[a]

$LSD_{(Bonf.\ p < 0.001)}$ =0.8130

** Means with the same symbol do not differ significantly at p < 0.001.*

Table 5.17. Mean micronaire (mic) of four cotton treatments at four localities in the *cry2Ab2* gene trial.

Localities	Rustenburg	Brits	Nelspruit	Potgietersrus
Means	4.531[b]	4.550[b]	4.850[a]	2.906[c]

$LSD_{(Bonf.\ p < 0.001)}$ =0.2941

** Means with the same symbol do not differ significantly at p < 0.001.*

Table 5.18. Mean percentage Gin out turn (% GOT) at four localities (1998/1999) in the *cry2Ab2* gene trial.

Treatments Localities	15813	DP 50	15985	DP 50B	Means* (localities)
Rustenburg	34.68	36.53	35.44	36.48	35.780[b]
Brits	36.80	37.95	38.12	37.18	37.510[c]
Nelspruit	33.14	36.21	34.44	35.08	34.78[b]
Potgietersrus	32.74	32.40	31.57	33.63	32.590[a]
Means* (treatments)	34.4[a]	35.770[b]	34.890[ab]	35.590[ab]	

$LSD_{(Bonf.\ p < 0.05}$= 1.28942 for localities (Means in a single column with the same symbol do not differ significantly at p < 0.05)

$LSD_{(Bonf.\ p < 0.05}$= 1.28942 for cotton treatments (Means in a single row with the same symbol do not differ significantly at p < 0.05)

** Means of 16 repetitions.*

Table 5.19. Mean yield (g/treatment) between treatments at Rustenburg and Nelspruit in the *cry1Ac* and *cry2Ab2* genes in stacked combinations.

Localities Treatments	Rustenburg* (g/treatment)	Nelspruit (g/treatment)
15985Bxu	1124[a]	2592[a]
15985Xu	1262[a]	2361[a]
15985Bu	1423[a]	2552[a]
DP50Bu	1324[a]	2502[a]
DP50u	467[b]	1199[c]
15985BXs	1193[a]	2389[a]
DP50Bs	1306[a]	2247[a]
DP50s	592[b]	1706[b]
$LSD_{(Bonf.\ p < 0.05)}$=	317.1	478.3
CV%	24.9	21.7

Means in a single column with the same symbol do not differ significantly at p < 0.05

** Means of 6 repetitions.*

Table 5.20. Mean yield of 10 plants per treatment at Rustenburg and Brits in the *cry1Ac* and *cry2Ab2* genes in stacked combinations.

Localities Treatments	Rustenburg (g/treatment)	Brits* (g/treatment)
15985Bxu	235.7[abc]	92.9[a]
15985Xu	255.4[ab]	81.2[a]
15985Bu	183.9[bcd]	105.0[a]
DP50Bu	273.0[a]	107.8[a]
DP50u	140.1[d]	50.0[b]
15985BXs	307.5[a]	91.6[a]
DP50Bs	277.1[a]	108.2[a]
DP50s	179.1[cd]	107.9[a]
$LSD_{(Bonf.\ p < 0.05)}$ =	76.18	29.33
CV%	28.1	26.9

Means in a single column with the same symbol do not differ significantly at p < 0.05

* Means of 6 repetitions

Table 5.21. Mean fibre strength (g/tex) of the cotton treatments at Rustenburg and Nelspruit in the *cry1Ac* and *cry2Ab2* genes in stacked combinations.

Localities Treatments	Rustenburg (F pr = 0.006)	Nelspruit (F pr =0.272)
15985Bxu	21.925[a]	17.775[a]
15985Xu	21.050[b]	17.875[a]
15985Bu	21.113[b]	18.325[a]
DP50Bu	21.450[ab]	18.425[a]
DP50u	21.463[ab]	18.588[a]
15985BXs	20.963[b]	17.813[a]
DP50Bs	20.800[b]	18.125[a]
DP50s	22.088[a]	18.138[a]
$LSD_{(Bonf.\ p < 0.05)}$ =	0.7242	0.7464
CV %	3.4	4.1

** Means with the same symbol in a column do not differ significantly at p < 0.05.*

Table 5.22. Mean micronaire (mic) between cotton treatments at Rustenburg and Nelspruit in the *cry1Ac* and *cry2Ab2* genes in stacked combinations.

Localities Treatments	Rustenburg (F pr = 0.279)	Nelspruit (F pr = 0.018)
15985Bxu	2.725[a]	5.450[a]
15985Xu	3.050[a]	5.450[a]
15985Bu	3.013[a]	5.262[ab]
DP50Bu	2.850[a]	5.100[b]
DP50u	2.750[a]	5.150[b]
15985BXs	2.963[a]	5.487[a]
DP50Bs	2.950[a]	5.150[b]
DP50s	2.838[a]	5.162[b]
$LSD_{(Bonf.\ p < 0.05)}$	0.3010	0.2765
%CV	10.4	5.2

** Means with the same symbol in a column do not differ significantly at p < 0.05.*

Table 5.23. Mean % Gin out-turn (GOT) between cotton treatments at Rustenburg and Nelspruit in the *cry1Ac* and *cry2Ab2* genes in stacked combinations.

Localities Treatments	Rustenburg	Nelspruit
15985Bxu	27.52[d]	36.70[c]
15985Xu	29.80[c]	37.168[c]
15985Bu	31.86[b]	37.238[c]
DP50Bu	33.59[a]	38.262[b]
DP50u	31.63[b]	39.136[a]
15985BXs	28.96[c]	36.919[c]
DP50Bs	33.30[a]	38.228[b]
DP50s	33.27[a]	39.780[a]
$LSD_{(Bonf.\ p < 0.05)}$ =	1.293	0.6965
CV %	4.1	1.8

** Means in a single column with the same symbol do not differ significantly at p < 0.05)*

Table 5.24. Important dates and activities for the small-scale farmer Bt-cotton trials.

Trial	Treatments	Cotton type	Scout dates	Dates of sprays	Chemicals used	Main pests	Reason why sprayed
Moutse West (0.03ha)	Knapsack	Roundup Ready™	13-Feb	17-Jan	Mospilan® (500ml/ha)	Aphids	Threshold
Plant date			28-Jan	14-Feb	Roundup (1/ha)	weeds	CT treatment
9 Nov. 2003			13-Jan	30-Jan	Thioflo® (1l/ha)	Bollworms	Threshold
			28-Feb	13-Jan	Thioflo® (1l/ha)	Bollworms	Threshold
			4-Mar	2-Mar	Thioflo® (1l/ha)	Bollworms	Threshold
Moutse East (0.0175ha)	Knapsack and Micron Ulva	Bollgard™	23-Nov	30-Dec	Dursban® (1l/ha)	grasshoppers and aphids	Threshold
Plant date			18-Dec				
24 Nov. 2003			8-Jan				
			28-Jan	9-Jan	Mospilan® (500ml/ha)	aphids	Threshold
			5-Feb	19-Jan	Mospilan® (500ml/ha)	Aphids	calendar spray
			12-Feb	27-Jan	Mospilan® (500ml/ha)	Aphids	calendar spray
			19-Feb	19-Feb		Aphids	calendar spray
			26-Feb				
Makhathini (0.03ha)	Knapsack and Micron Ulva	Bollgard™	10-Feb				
Mr. Nshangasi			28-Jan	23-Jan	Thioflo® (1l/ha)	Bollworms	Threshold
Plant date			25-Feb	27-Jan	Thioflo® (1l/ha)	Bollworms	Threshold
15-Nov-03			3-Mar	3-Mar	Thioflo® (1l/ha)	Stainers	Threshold
			15-Mar				

* Dates for knapsack and Micron Ulva treatments were the same.

Table 5.25. Yields obtained in small-scale farmer demonstration trials.

Localities	Moutse East (irrigation)	Moutse East (irrigation)	Moutse West (irrigation)	Makhathini (dryland)	Makhathini (dryland)
Spraying method	Knapsack	Micron Ulva	Knapsack	Knapsack	Micron Ulva
Hectare	0.0175	0.0175	0.0975	1	1
No. of kg sampled	55	3	200		
Yield (seed cotton (kg/ha))	962.5	927,5	2051	800	970

Table 5.26. Cost analysis of small-scale farmer trials.

Localities	Moutse East	Moutse East	Moutse West	Makhathini	Makhathini
Activities	Knapsack	Micron Ulva	Knapsack	Knapsack	Micron Ulva
	0.0175 ha	0.0175 ha	0.0975 ha	1 ha	
Insecticide sprays	4	4	5	3	3
Roundup® sprays					
Mospilan® costs (R30 / ha)	1.7775	1.575	2.925		
Dursban® (chlorpirifos) (cost; R50 /ha)	0.875	0.875			
Thioflo® (cost; R100 / ha)			100	300	300
Roundup® (spays; 5l/ha R140/ha)			40.74		
Seed costs (Price/ha and Tech fee)	3.4125	3.4125	62.4	195	195
Labour: harvesting (50c per kg)	529.1	534.6	1128.05	400	450
Costs (Expenditure)	535.165	540.4625	1334.115	895	945
Yield (seed cotton)	962.5	972.5	2051	800	970
Income per ha (R2.70/kg)	2598.75	2625.75	5537.7	2160	2619
Income – costs (net profit) (rands)	2063.585	2085.2875	4203.585	1265	1674

Costs which were similar on all trials were omitted from the analyses, i.e. land-preparation, weed control, scouting.

Figures

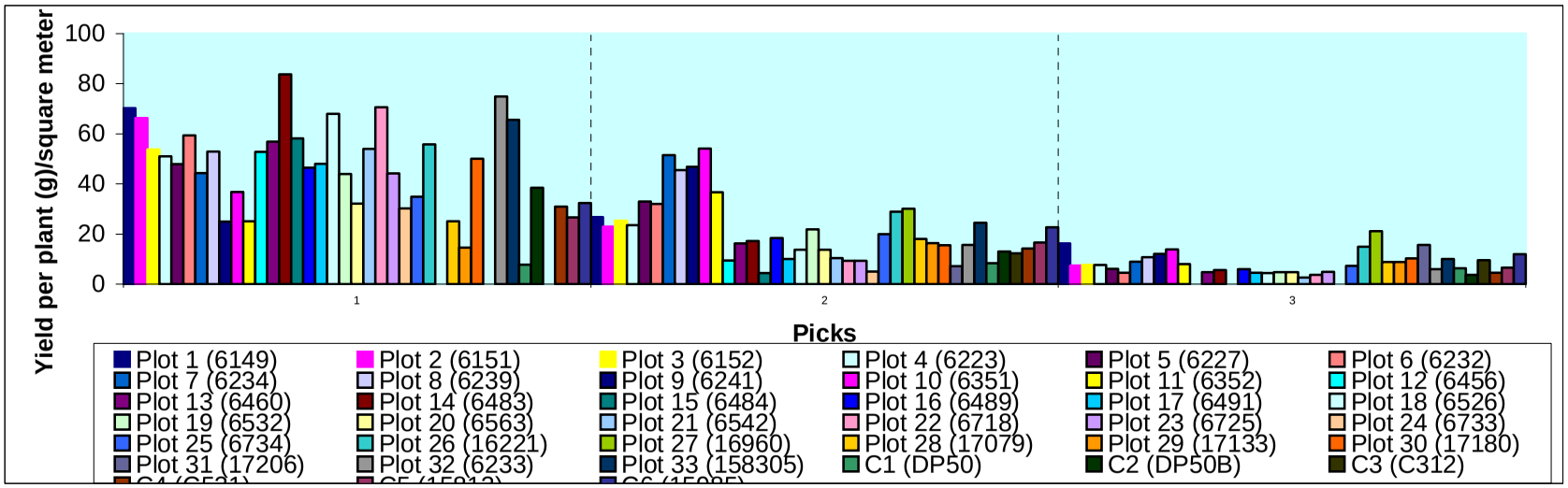


Fig. 5.1. Yield per plant per square meter for the different events at 3 picking periods in the P2B event trial.

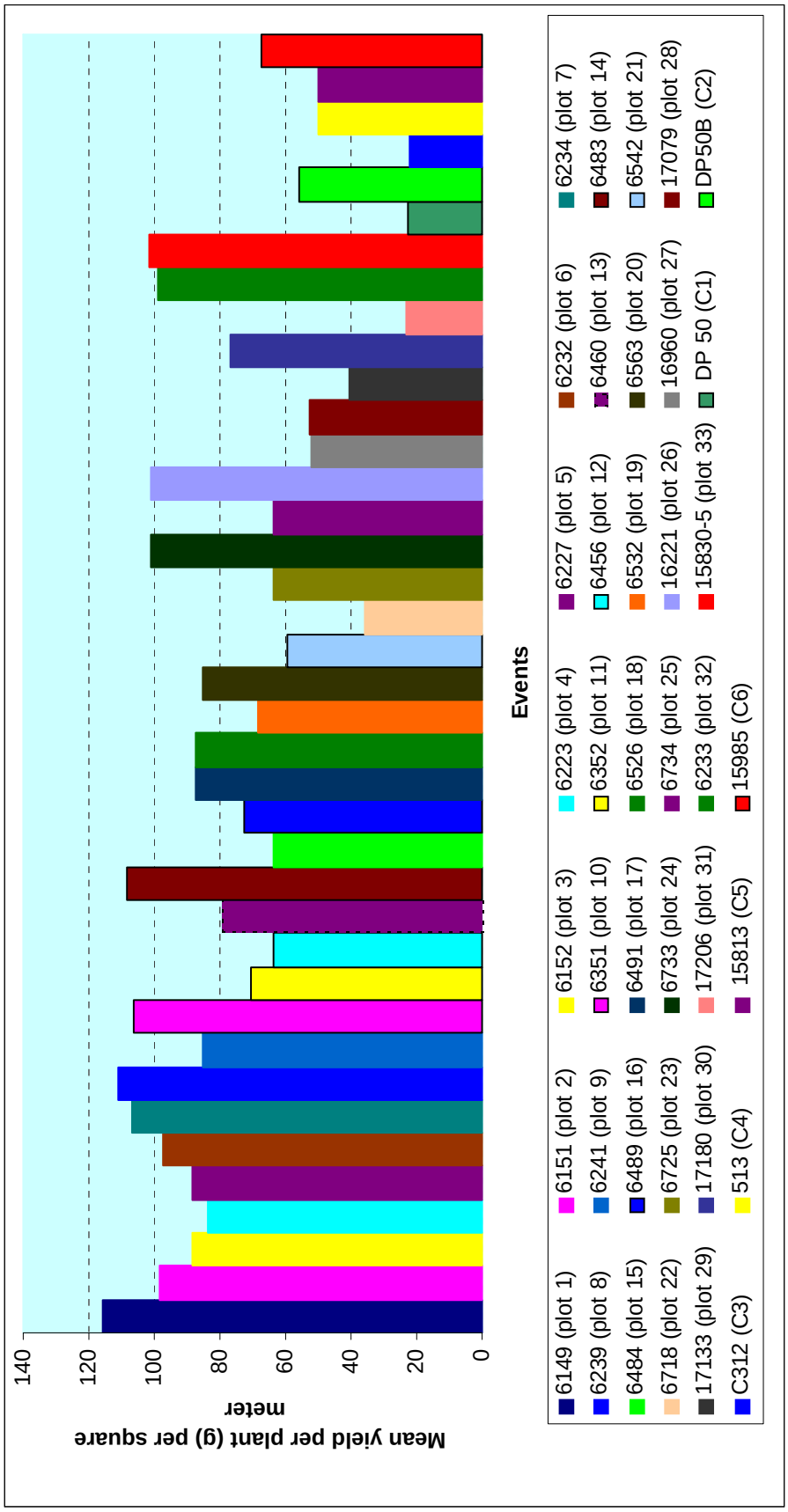


Fig. 5.2. Mean yield per plant/square meter between events, over three harvesting periods in the P2B event trial.

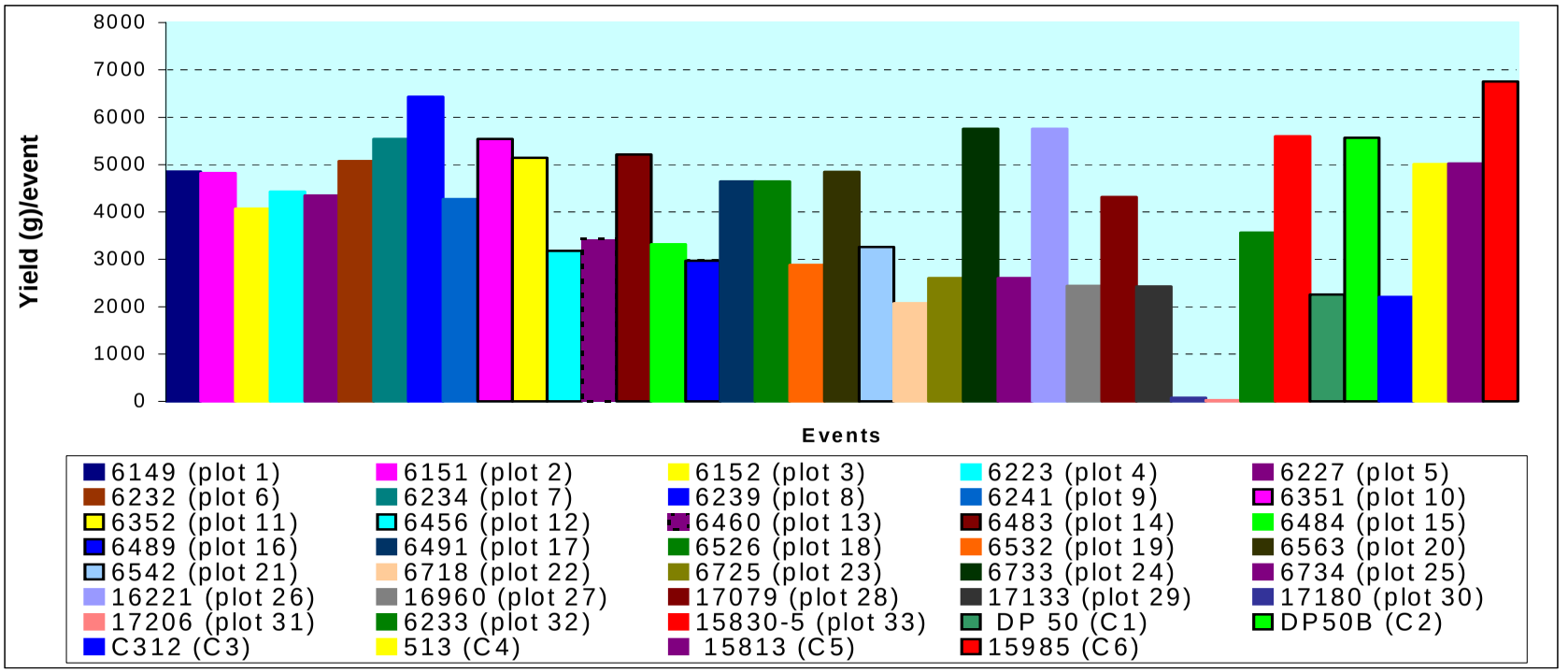


Fig. 5.3. Total yield comparison between events and control plots in the P2B event trial.

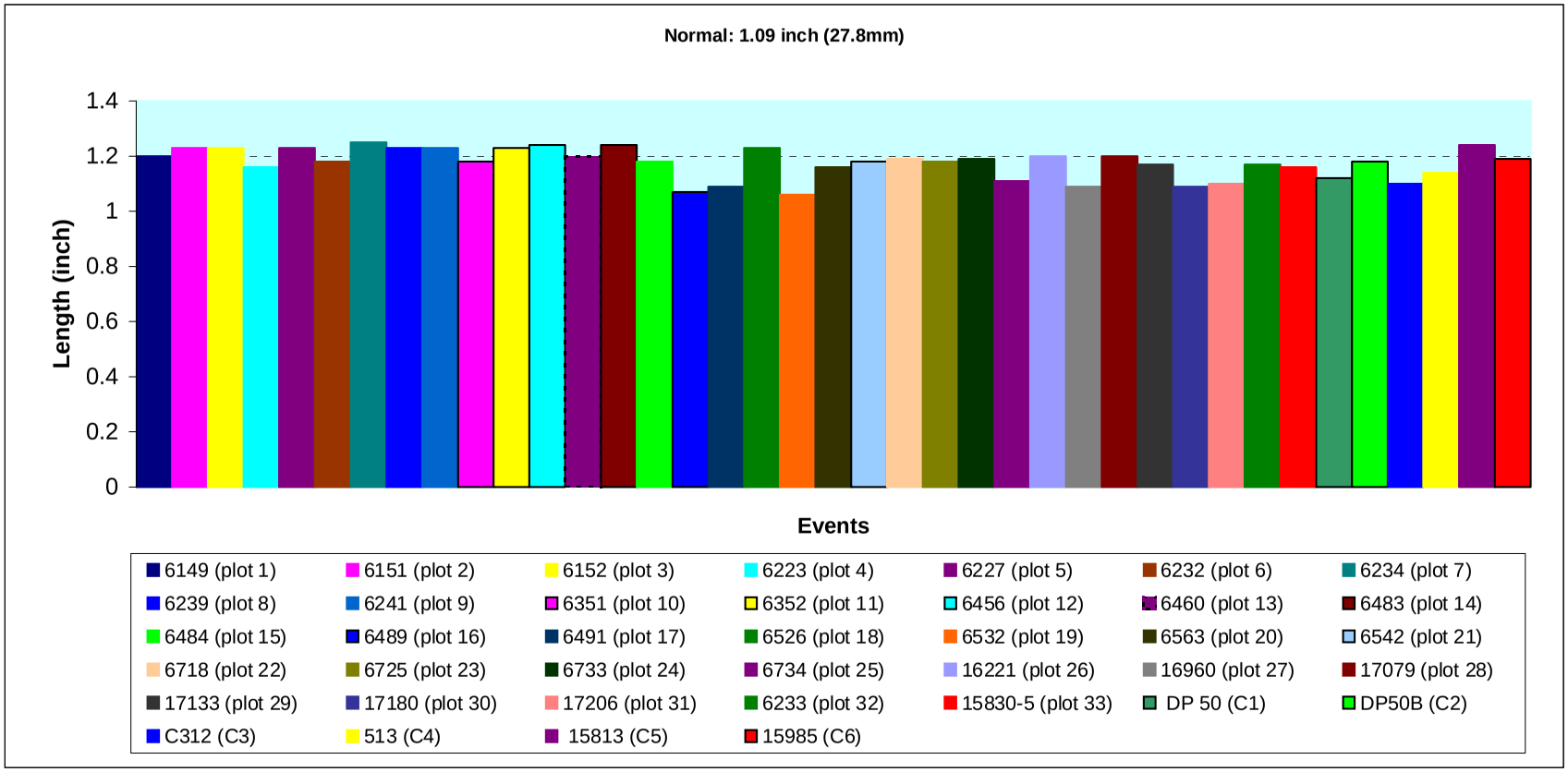


Fig. 5.4. Fibre length of the different events in comparison with the control plots in the P2B event trial.

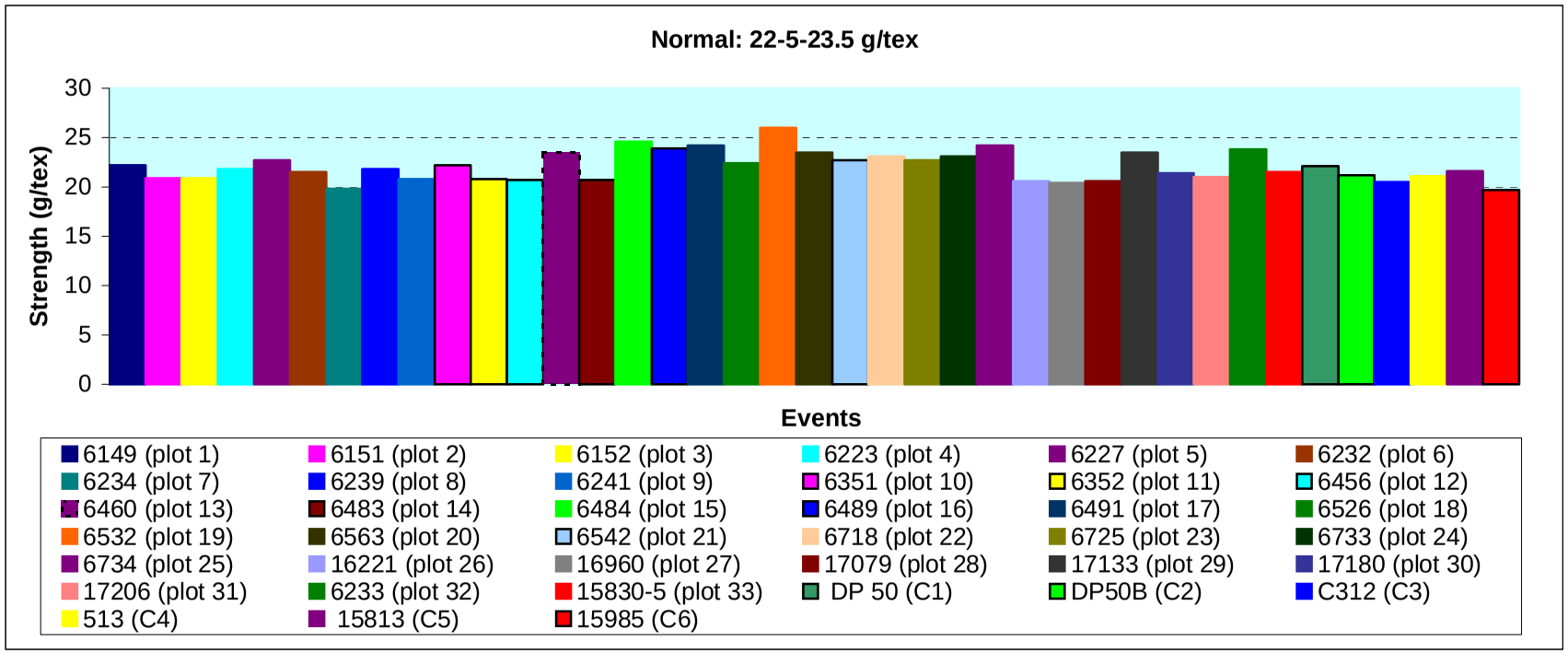


Fig. 5.5. Fibre strength of the different events in comparison with the control plots in the P2B event trial.

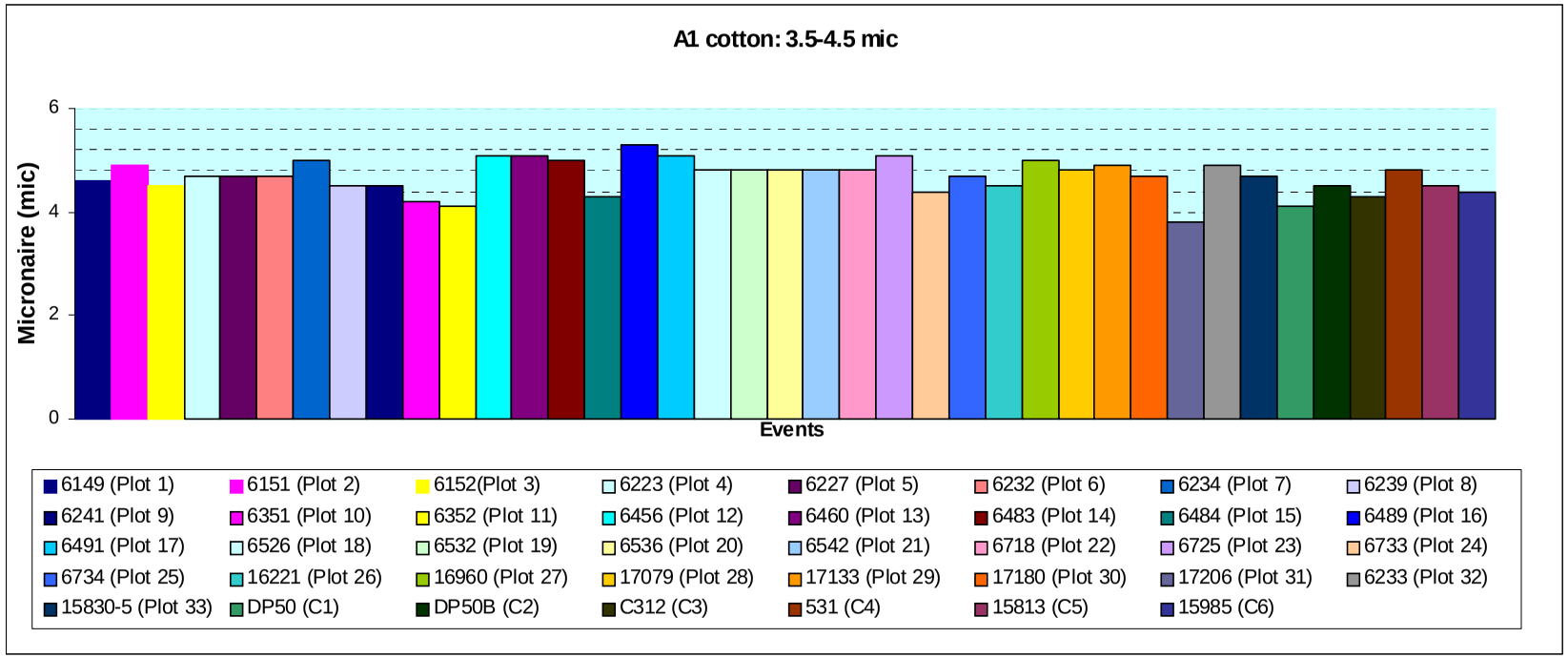


Fig. 5.6. Micronaire of the different events in comparison with the control plots in the P2B event trial.

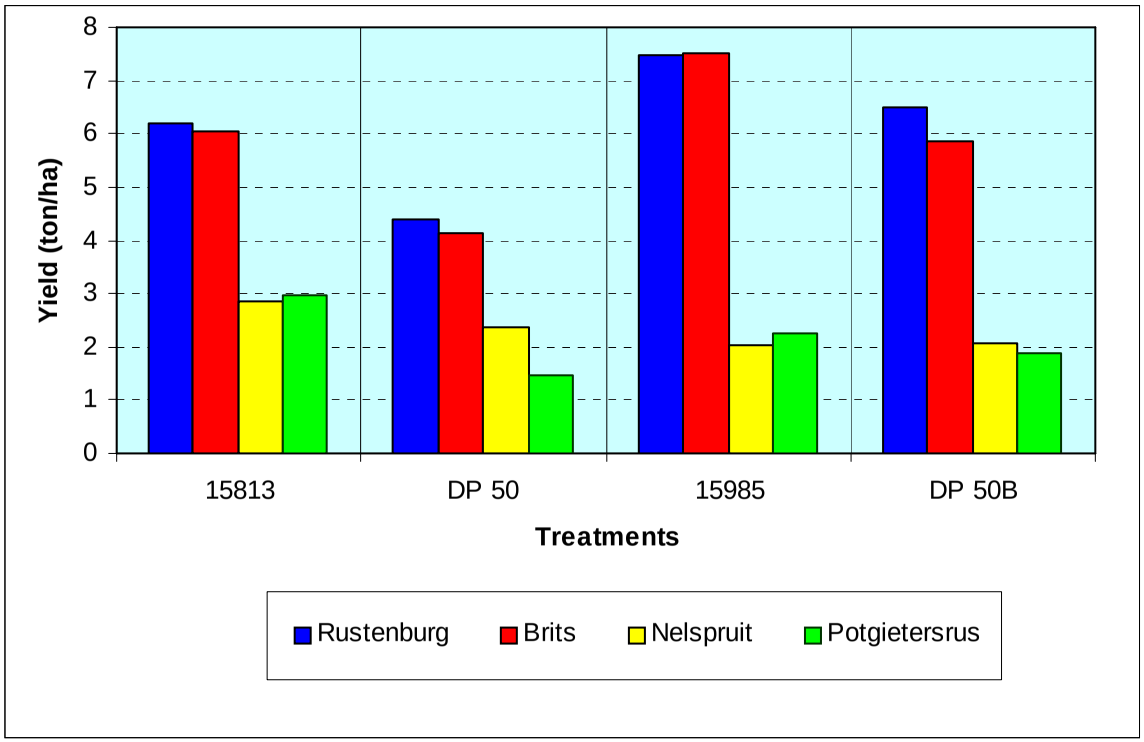


Fig. 5.7. The yield for each treatment at Rustenburg, Brits, Nelspruit and Potgietersrus (1999) in the Cry2Ab2 trial.

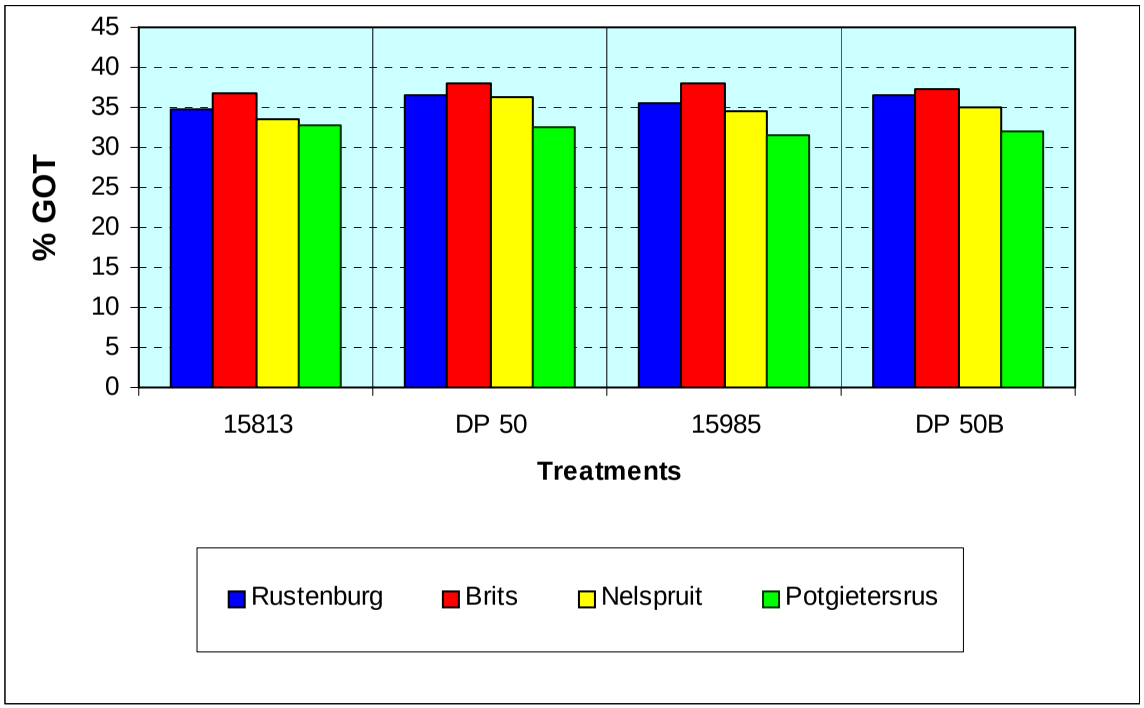


Fig. 5.8. The percentage Gin out Turn (GOT) for each treatment at Rustenburg, Brits, Nelspruit and Potgietersrus (1999) in the Cry2Ab2 trial.

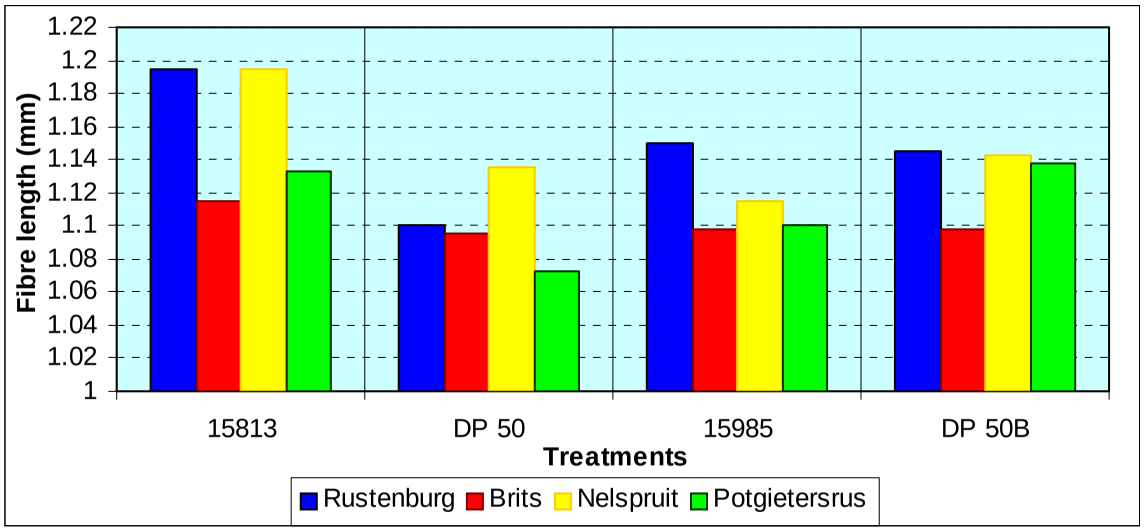


Fig. 5.9. The average fibre length for each treatment at Rustenburg, Brits, Nelspruit and Potgietersrus (1999) in the Cry2Ab2 trial.

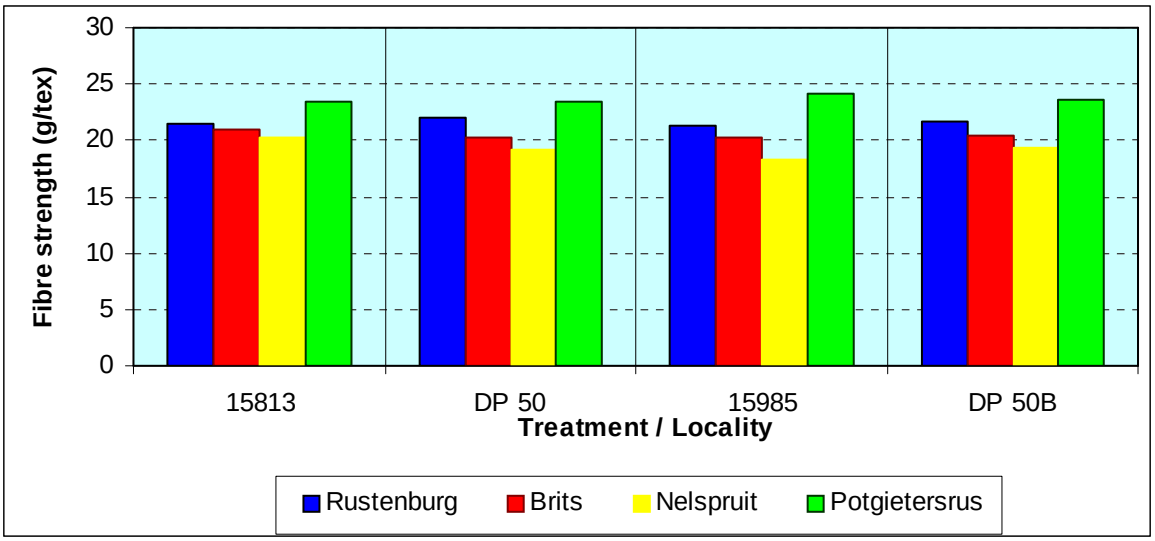


Fig. 5.10. The average fibre strength for each treatment at Rustenburg, Brits, Nelspruit and Potgietersrus (1999) in the Cry2Ab2 trial.

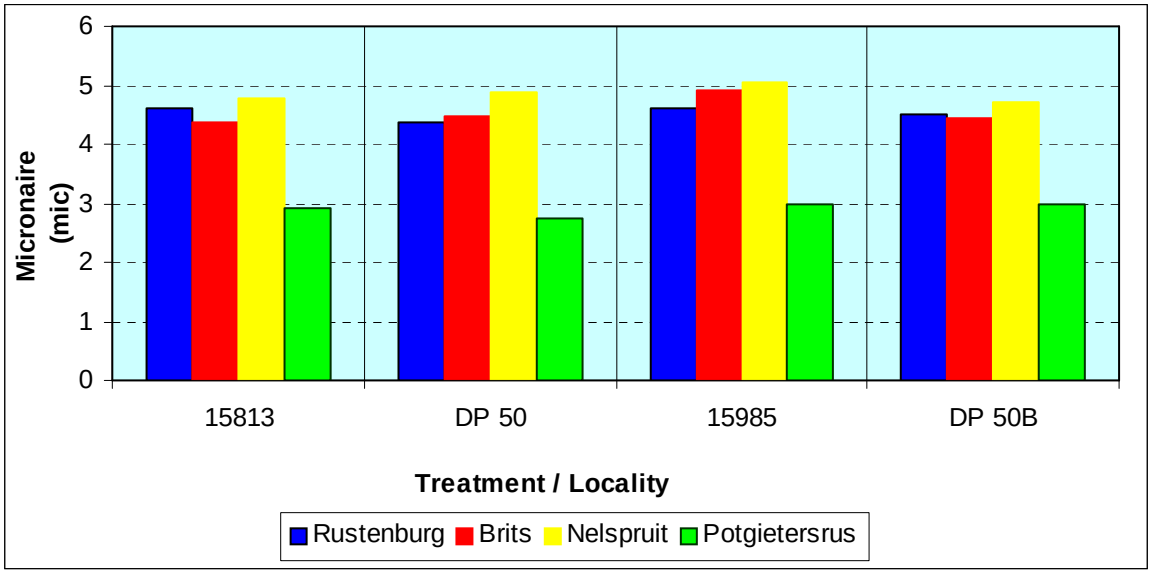


Fig. 5.11. The average micronaire for each treatment at four localities (1999) in the Cry2Ab2 trial.

CHAPTER 6

THE ROLE THAT INDIGENOUS HOST PLANT SPECIES PLAY IN BOLLWORM POPULATIONS IN AN AREA WHERE TRANSGENIC COTTON IS CULTIVATED

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Abstract

The abundance of the bollworm complex, american or african bollworm Helicoverpa armigera (Hübner), spiny bollworms Earias biplaga Walker and Earias insulana Boisduval and the red bollworm Diparopsis castanea Hampson on cotton and alternative host plants on the Makhathini flats (KwaZulu-Natal) is discussed. African bollworms were found on eight plant species, namely, Abutilon austro-africanum Hochr., Abutilon guineense (K. Schum.) Baker f. and Exell, Acanthospermum hispidum DC, Cienfuegosia hildebrandtii Gärcke, Corchorus trilocularis L., Hibiscus vitifolius L., Justicia flava (Vahl) Vahl and Pavonia burchellii (DC.) R. A. Dyer. Red bollworms were found on A. guineense, C. hildebrandtii, and Gossypium herbaceum L. Spiny bollworms were found on 15 host plants, namely, A. austro-africanum, A. guineense, Abutilon sonneratianum (Cav.) Sweet, Abutilon sp. ('leathery'), C. trilocularis, Hibiscus calyphyllus Cav., H. cannabinus L., H. praeteritus R.A.Dyer, H. vitifolius, Malvastrum coromandelianum (L.) Gärcke, Melhania forbesii Planch. Ex Mast., P. burchellii, Sida cordifolia, S. dregei and S. rhombifolia L. In the previous season, they were also found on C. hildebrandtii. More african and red bollworm eggs were found on the Bt-cotton than on the non-transgenic cotton. On the non-transgenic cotton, however, more African bollworm larvae and more damaged squares and bolls were found. No spiny bollworm eggs or larvae were found on either Bt- or non-transgenic cotton this season, although they were detected in the previous season. The mean number of bollworms on the sum of all the weed species found in the fallow field areas, the maize field and the natural veld did not differ statistically from the mean number of bollworms found on the non-transgenic cotton. This indicates that the weeds in these areas (fallow field, maize and natural veld) could be considered as an additional refuge to non-transgenic cotton.

Based on the plant growth stage data, plant species could be divided into the following groups: (1) Pre-cotton alternative host plants: C. hildebrandtii, H. calyphyllus, H. vitifolius, J. flava, S. dregei and S. cordifolia. (2) Alternative host plants during the cotton-flowering phase: A. austro-africanum, A. guineense, S. rhombifolia, M. coromandelianum and M. forbesii. (3) Post-cotton alternative host plants: A. hispidum, H. cannabinus, H. vitifolius, H. praeteritus and S. dregei. Most of the plant species identified as bollworm hosts seem to be widespread across the Makhathini flats, especially Abutilon spp., Hibiscus spp., Sida spp. and C. trilocularis. C. hildebrandtii, is abundant in small areas or patches of natural veld. Population densities of both C. trilocularis and Sida spp. did not seem to be affected by the position of the locality in relation to the distance from the Pongola River. On average, the plant population densities were highest during December. The tendency existed that the more numerous the host plants and the bigger the surface area, the more bollworm larvae were produced. The number of bollworm larvae is expected to be the highest in localities where the biggest area of natural veld occurs. Number of host plants found in a 5% potential refuge area. The mean number of bollworm larvae found on 0.05 ha fallow field on the A. guineense and Sida groups were higher than the number of larvae on the non-transgenic cotton refuge. These two plant groups therefore seem to be preferred above non-transgenic cotton. It seemed that more bollworms occurred in the fallow field and

natural veld areas on the same proportion of refuge as that of the non-transgenic cotton, which suggests that the conventional cotton as a refuge may, in this case, not be necessary.

The main objective of this study was to do a search for indigenous plant and weed species on the Makhathini flats that are host plants for bollworms and can serve as a refuge to maintain susceptibility of the bollworms to the Cry1Ac protein in Bt-cotton.

Introduction

The presence and numbers of bollworms on indigenous plants and weeds on the Makhathini flats was investigated, to determine whether alternative host plants can sustain natural bollworm populations to such an extent that these bollworms will influx transgenic cotton and that these bollworms will interbreed with possible "Bt-resistant" bollworms that survived on the transgenic cotton. Cross-breeding of susceptible and possibly "Bt-resistant" populations is necessary in order to minimize the chance of resistance of surviving bollworms on the transgenic cotton. In addition the plant densities per unit area of naturally occurring plant species and weeds that will support a similar or higher number of bollworms than the recommended non-transgenic cotton refuge was determined. The first part of this study was performed during the 2000/2001 cotton-growing season. The occurrence of the bollworm complex on cotton was monitored on transgenic cotton, non-transgenic cotton and indigenous plant and weed species. Nine plant species, out of the 33 species scouted, were identified as alternative host plants that could serve as natural hosts for the bollworm complex. All bollworm species were found to be present on some indigenous plant and weed species, while some eggs and all instars of bollworms were found on some species of indigenous plants and weeds, and moths were found in light traps placed in natural veld areas. The results of the 2000/01 season showed that the bollworm complex does occur on naturally occurring host plants. African bollworm was found on Abutilon guineense (K.Schum.) Baker f. and Exell, Abutilon sp. ('leathery')[1] and Sida rhombifolia L. Red bollworm host plants were Cienfuegosia hildebrandtii Gärcke and Abutilon austro-africanum Hochr. The spiny bollworm occurred on nine species, including A. austro-africanum, Abutilon sonneratianum (Cav.) Sweet, C. hildebrandtii and Hibiscus calyphyllus Cav. These results showed that the numbers of larvae found on these indigenous host plants and weeds suggest that alternative host plants do have the potential to serve as a refuge in the Makhathini Flats. These findings had to be confirmed during the 2001/02 season. The different plant species known to serve as bollworm hosts as well as additional new species that could sustain bollworm populations had to be monitored more extensively to determine their distribution, population density and the occurrence of bollworms on these species throughout the season. For the second season the identification of additional plant species that can sustain some or all species of bollworm was done, and the presence of bollworms on alternative host plants over a larger surface area that will be representative of the Makhathini Flats.

The Makhathini Flats, situated in the north-eastern corner of KwaZulu-Natal (KZN) (South Africa) is an area ranging from the Mozambique border to St. Lucia Lake and from the Lebombo mountains to the Indian Ocean. Situated within the Makhathini Flats is a small-scale farmer cotton production area of approximately 80 km x 30 km, ranging North of Jozini up to Ndumo and a smaller area south of Jozini in the direction of Ubombo. Most cotton cultivated here by small-scale farmers is cultivated under dryland conditions. During the 2000/01 cotton-growing

[1] The plant species Abutilon sp. ('leathery') has been identified to be A. guineense from several plant samples, but the authors of this report feel that Abutilon sp. ('leathery') differed from A. guineense, and it will, therefore, for the purpose of this study be referred to as Abutilon sp. ('leathery').

season, a total of 50 768 ha cotton was planted in South Africa, of which, 3200 ha was planted under dryland cotton and 10 ha under irrigation, in KZN, by approximately 3000 small-scale farmers. This made KZN the second largest producer of dryland cotton in South Africa. All the cotton produced in this area was handpicked. The total surface area planted under dryland conditions in the Limpopo Province (was Northern Province) was 12 515 ha, but only 25% of this dryland cotton was handpicked, and most of it was planted by commercial farmers on a large scale. This emphasizes the importance of the contribution of the Makhathini dryland farmers to the annual cotton crop. For most of the small-scale farmers, cotton is also their only source of cash income. The importance of sustainable cotton production for this area is supported by the initiatives of 'Noordelike Sentrale Katoen' (NSK), who erected a new ginnery on the Makhathini Flats. The introduction of Bollgard™ cotton, or cotton containing the Bt-gene or the *cryIAc* gene (Federici, 1993), to the South African cotton industry has been well and widely accepted. For the 2001/02 season, cotton obtained a market share of 39.4 % of the textile industry (Cotton SA 2002). In South Africa, a total of 71 608 ha cotton was planted during the 2001/02 season, of which about 5000 ha was planted by small-scale farmers in the Makhathini area (pers comm. AL Bennett[2]). A large proportion of the total number of ha planted in SA (approximately 70%) was Bt-cotton. The number of ha planted in the Makhathini is deducted from seed sales for the area. The benefits of this technology is expected to motivate farmers to expand cotton production in SA, but as a result of the low cotton price, the amount of cotton to be planted in the 2002/03 season is estimated at only 44 560 ha (Cotton SA 2002). The reduced spraying on Bt-crops has both benefits to the environment as well as to the small-scale farmer, by lowering the cost of production (Wambugu 2001). If the farmers are to obtain high yields, it is essential that they apply effective management practices. The importance of extensive training in how to apply this new technology in cotton production, not only for cotton production but also for pest management is now more prominent than ever, since It is assumed that cotton production will escalate within this area in the nearby future due to the planting of transgenic cotton, or Bollgard™, which is generating an income for many farmers in the Makhathini flats.

Important pests on cotton include the bollworm complex, (In the Makhathini, the red bollworm (D. castanea) seems to be the most important), aphids (Aphis gossypii Glover), leafhoppers (Jacobiella fascialis (Jacoby)), the cotton stem weevil (Apion soleatum Wagner) and the black cotton beetle (Syagrus rugifrons Baly). In the Makhathini Flats, insecticides are costly and difficult to come by, and those that are obtained are usually very harsh on the beneficial insects and on the environment. Bollgard™ cotton has thus become an ideal crop for small-scale farmers, due to its ability of effectively controlling the bollworm complex, which is capable of causing serious economic damage in non-transgenic cotton (Gossypium hirsutum L.) (Pearson and Darling 1958), as well as the reduced need of insecticides for the production of the crop. If the technology of Bollgard™ cotton is to be preserved for the future, it is vital that resistance to the Bt-toxin (protein or endotoxin) be prevented. According to Mallett and Porter (1992),

[2] Mr. A.L. Bennett is the Biotech Marketing Lead for Monsanto for Europe-Africa in Switzerland, and formerly from South Africa.

susceptible insects could be conserved by planting “refugia”, or toxin-free plants on the same cultivated land. Moths maturing on the Bollgard cotton are potentially more resistant or tolerant towards the gene. If a refuge area is planted and movement of moths occurs from transgenic cotton, these more resistant/tolerant moths have a higher probability of mating with a susceptible individual from the bigger population of moths originating from the refuge area. The probability of establishing the resistant/tolerant genetic pool thus becomes much smaller since this interaction could cause sufficient genetic dilution to counteract selection for resistance against the effect of the toxin expressed by transgenic cotton plants. It is for this reason that a purchaser of Bollgard™ cotton in South Africa is under obligation to sign a license agreement, stating that for each 100 ha of Bollgard™ cotton planted, a refuge of either 20 ha sprayed non-transgenic cotton, or 5 ha unsprayed non-transgenic cotton will be planted (Anon 1998). If moths occurred within the natural veld surrounding the cotton, the same dilution effect would apply and this could therefore act as a natural refuge for bollworms.

In South Africa, the bollworm complex has been sprayed since the fifties with chlorinated carbons, as well as various other insecticides, and, during the past decade, with pyrethroids. No resistance has, however, developed against any of these insecticides. Bt-sprays have been available for many years in South Africa and no cases of resistance have been reported. In cotton growing countries such as the USA (cotton belt) and in Australia, resistance has, however, developed. One of the reasons why South Africa escaped this buildup of resistance to insecticides may lie in the presence of natural bush and other crops surrounding many cotton fields, which act as natural refuge areas where the susceptible genes could be maintained. It is often not practical for small-scale farmers, planting approximately 2 ha of Bt-cotton, to plant this additional refuge area of non-transgenic cotton and this is therefore sometimes neglected. The aim of this study was therefore to investigate and determine the possibility of alternative refuge sites for the bollworm complex. Variables that play an important role in the investigation of alternative refuge sites include the distribution and plant abundance of the alternative host plants as well as the occurrence of the bollworm complex on these plants, and on cotton.

Bollworm larvae occur on a number of different host plants occurring naturally in the veld (*cf*. Broodryk et al. 1974, Kroon 1999, Pearson and Darling 1958, Williams 1933). Helicoverpa armigera (Hübner), also known as the Old World bollworm, the cotton bollworm, and locally, the African bollworm, is also still generally known by its synonym, Heliothis armigera, and much of the relevant literature still refers to it under this binomial. The african bollworm is a pest of many crops (Pearson and Darling 1958) and also occurs on various naturally occurring plants. This enables the pest to develop on different hosts, to possibly continue with development on successive hosts and to persist in small populations in areas that appear unsuitable (Vaissayre 1994). Crops on which african bollworms are known to occur include barley, beans (various), Cape gooseberry, chick-pea, citrus, cucurbits, cotton, flax, grain, groundnuts, hemp, lucerne, maize, oats, peas, pigeon-peas (Cajanus cajan), potato sunflowers, sorghum, soya, sun hemp, tobacco, tomatoes and wheat (Annecke and Moran 1982, Pearson and Darling 1958). The african bollworm has also been found on various indigenous plant species such as Acalypha

segetalis (Euphorbiaceae), Amaranthus thunberghii (Amarantaceae), Ipomoea cordofana (Convulvulaceae), Malvastrum tricuspidatum (Malvaceae), Nicandra physaloides (Solanaceae), Portulaca oleracea (Portulacaceae), Sonchus oleraceus (Compositae), Tridax procumbens (Compositae) and Xanthium pungens (Compositae). Other plant families on which the African bollworm has been found include Chenopodiaceae, Leguminoseae, Gramineae, Linaceae, Rutaceae, Cucurbitaceae, Capparidaceae and Labiatae. H. armigera is widely distributed in Africa, southern Europe, the Near and Middle East, India, Central and Southeast Asia, Japan, the Philippines, Indonesia, New Guinea, eastern Australia, New Zealand and Fiji, and in these areas it constitutes a serious pest of many crops and in ornamental gardens (Annecke and Moran 1982). H. armigera visits many of the above-mentioned crops when they are in flower, or about to flower, for the purposes of oviposition (Parsons 1940). Periodicity in oviposition activity of H. armigera with respect to crop production made it possible to distinguish five periods in a year (Parsons 1939). May, June and July represent a period of least activity, restricted by food availability and climatic conditions. A large proportion of pupae enter a resting period, rainfed crops are harvested and irrigated crops are in the seedling stage. August, September and part of October sees the first emergence of H. armigera. Larvae are supported largely on irrigated crops. October and November represents peak activity on irrigated crops. According to Parsons (1939), oviposition declines markedly in December, although he did not consider oviposition on natural hosts. January to April sees the highest oviposition on rain fed crops such as maize and cotton, and represents the major bollworm attack. Parsons (1939) found two major moth flights during the course of a year, which occurred in early November and late February and corresponded to moths bred from winter crops and summer crops respectively. In addition he found three other minor flights in September, December and March/April, which originated from over wintering pupae, intermediate crops and weeds. Although the November moth flight represented a higher number of moths than that of late February, the number of eggs laid from the second major flight of moths was greater. In South African cotton, H. armigera is arguably the most destructive insect, and consequently insect control in cotton is directed at eliminating this pest. At present, 18 chemicals under a multitude of formulations and trade names are registered against H. armigera in cotton (Vermeulen et al. 1992).

The build-up of spiny bollworm populations in the crop is essentially a gradual one, with no sudden influx of egg-laying moths at a particular stage during the season, unlike both D. castanea and H. armigera. Earias spp. does not originate as a result of a sudden moth flight derived from long-term pupae (Pearson and Darling 1958). Cotton is not a first choice of host plant for Earias spp., Abutilon and some Hibiscus species seem to be preferred. Spiny bollworms may carry over from one cotton season to the next on alternative host plants, or may multiply early in the season before the cotton is available (Pearson and Darling 1958). The main importance of alternative host plants must be sought in the extent to which their fruiting does not coincide with that of the cotton crop. Where plant growth is regulated by a strongly seasonal moisture supply, as in areas characterized by a single rainy season or by seasonal flooding, a proportion of the weed population of annual hosts may survive as biennials or perennials.

Alternative host plants may therefore provide an early breeding ground for Earias spp, which can then transfer readily to the annual crop when this becomes attractive. Alternative host plants are not usually uprooted, as is the cotton crop and they may survive in a variety of situations such as pastures, roadsides, hedges, river and canal banks, and so continue to provide food for Earias spp. when cotton is not available (Pearson and Darling 1958). A number of plant species have been found to be hosts for the spiny bollworm species including Abutilon indicum, A. sonneratianum, Althaea rosea, Cienfuegosia gerrardii, C. hildebrandtii, Corchorus olitorius, C. tridens, Hibiscus calycinus, H. cannabinus, H. esculentus, H. rosa-sinensis, H. sabdariffa, Gossypium spp., Malva parviflora and Sida spp. (Broodryk et al. 1974, Pearson and Darling 1958, Williams 1933).

Red bollworm, D. castanea, has been recorded on a very confined host range (Pearson and Darling 1958), limited to the genera Gossypium (G. hirsutum, G. herbaceum and G. barbadense), Gossypioides (G. kirkii) and Cienfuegosia (C. hildebrandtii). Gossypium herbaceum subsp. africanum is indigenous to the Eastern lowveld areas of South Africa (Hudchinson et al. 1947). Single records have also been found on Thespesia sp. and Abutilon sp. Eggs and larvae have been raised on Hibiscus calycinus and Cienfuegosia gerrardii, but these failed to mature, and were therefore not considered to be host plants. The red bollworm, D. castanea, has not been found naturally on G. kirkii, but larvae have been raised to maturity on it. It is, however, a natural host plant for another red bollworm species, D. gossypioides (Pearson and Darling 1958).

The hypothesis was formulated that there is a certain amount of movement, or dispersal, from the natural host plants of the bollworm complex, to cotton, and more specifically, transgenic cotton. After cotton has been harvested, cattle and goats feed on the stalks of cotton. Cotton is therefore not available for the bollworms for a certain time period, and the re-infestation of bollworms on the next cotton crop has to come from the surrounding vegetation. In the presence of numerous weeds or naturally occurring host plants, it is vital to determine whether bollworms will have a preference for cotton above these natural host plants.

The hypothesis being tested is therefore that bollworm larvae infest natural host plants throughout the season even in the presence of cotton.

EXPERIMENTAL PROCEDURES

Twelve study areas were identified within the area proposed by the industry for cotton cultivation in the Makhathini Flats (Fig. 6.1). Most cotton in the Makhathini is planted close to the Pongola River, which is a very fertile area due to silt deposits, thus added soil nutrients, laid down by centuries of flooding of the river. In selecting the study areas, soil composition was noted, since certain plant species prefer certain soil types. Soil types varied from clay-loamy soil near the river, to clay-sandy soil further away from the river, followed by red sandy soil even more east and west respectively.

The cotton growing areas are under supervision of small-scale farmer study groups, each with a representative society leader. These cotton-association areas were plotted on a

hand-drawn map of the Makhathini (cf. C. Matlou[3]), and this map was used as the basis for the selection of study areas.

The importance of the distance from the Pongola River on the occurrence of the bollworm complex and the plant densities of host plants was evaluated by selecting study areas in three different zones, namely: Riverside fields: fields situated in close proximity to the Pongola river and its floodplain pans (Zone 1); Midway fields: fields that were situated a few (±3) kilometres from the river (Zone 2); Distant fields: fields that were further away from the Pongola River than those in Zone 2 (Zone 3). Within each of these zones, situated east and west of the Pongola River, study areas were identified and at each study area, study points were selected, which included Bt-cotton, non-transgenic cotton (where available), fallow field, other cultivated cash crops next to the cotton field (if present) and natural veld, or vegetation growing along the fences, usually built from thorn tree branches, surrounding the cultivated fields. The selected study areas varied according to cotton varieties planted and management procedures followed (Table 6.1). For data collection, which started in October 2001, a set of six of the twelve study areas was investigated and three weeks later the other six were examined. Each study area was therefore visited every six weeks up until July 2002.

The twelve study areas were investigated for the presence of the bollworm complex by using scouting techniques (for eggs and larval stages). Within each study point, plant densities were also recorded. Light traps (for monitoring mature stages/moths) were placed at four localities and pheromone traps (for monitoring mature stages/male moths) at two localities. The study areas are discussed below.

Riverside fields

The first locality (L2) was that of Mr. T.J. Buthelezi (Grid reference: 27.19527° S 32.25455° Elevation: 51m) which was situated about four km east of the Pongola River, near to the floodplains, in natural bush, with small patches of cultivated crops and marshy areas (vlei land) in between. An area of 4.6 ha Bt-cotton was planted under dryland conditions. Minimum tillage was applied to part of this area. The soil was a red to brown sandy loam soil. Plant species scouted included C. hildebrandtii, H. calyphyllus and A. guineense. It was obvious in this area that C. hildebrandtii occurred mostly in the fallow fields next to cotton fields. These plants became more obvious when fields were neglected and not weeded properly.

The second locality (L4) was that of Mr. Qwabe Mandla (Grid reference: 27.31913° S 32.21528° E; Elevation: 65m), and this area was situated next to the riverbed on the eastern side of the Pongola River. Bt-cotton was planted on about 0.7 ha, under dryland conditions. The fields were characterized by alluvial sand mixed with partially decomposed plant material (a possible deposit from the river when in flood). Weed species grew very high and vigorously, possibly as a result of the soil composition. Plant species scouted included A. guineense, C. trilocularis and Sida spp.

[3] Mr. C. Matlou was formerly the small-scale farmer representative for Monsanto SA, residing in the Makhathini (KwaZulu-Natal) area.

Thirdly, (L6) the farm of Mr. Jobe (Grid reference: 27.26968° S 32.21830° Elevation: 68m) was about 2 km west of the Pongola River, and situated about one km north-west of the new dirt road crossing the Pongola River midway between Jozini and Ndumu. The area was characterized by thorny bushveld, with dry grass and vlei-land zones. One of these vlei-zones bordered the cotton field. An area of 4 ha non-transgenic cotton was planted under dryland conditions, on a red clay soil. The cotton was well managed with insecticides and hand hoeing. Plants scouted here included A. austro-africanum, A. guineense and C. hildebrandtii.

The farm (L10) of Mr. Mhlongo and Mrs. Zodwa (Grid reference: 27.04011° S 32.26975° E, Elevation: 41m) was selected to represent the far northern point of the study area, and was situated about 1 km from the Pongola River, while the fallow field was less than 500 m from the river. Bt-cotton was planted and neatly managed on a small piece of land (about 0.69 ha) under dryland conditions, on a brown silt loam soil. Cultivation was by hand. Plants scouted here included A. austro-africanum, A. guineense and Gossypium herbaceum L. subsp. africanum (Watt) Vollesen. The farm (L12) of Mr. Nhlenyama and Mr. A. Buthelezi Grid reference: 27.38432° S 32.139133° E, Elevation: 71m) bordered a huge pool, possibly the remains of the previous bed of the Pongola River. Both Bt- and non-transgenic cotton was planted, on 2.7 and 2.4 ha respectively, under dryland conditions, on red sandy soil of possible alluvial origin. The non-transgenic cotton was covered by morning glory Ipomoea purpurea (L.) Roth during January and February. Plants scouted in this area included A. guineense, C. trilocularis, Hibiscus vitifolius L. and S. rhombifolia.

Midway fields

The farm (l1) of Mr. N'dmande (Grid reference: 27.37190° S 32.21587° E; Elevation: 83m), is situated about 2 km west of the dirt road to Ndumu, at the turn off to Mjindi High School and about 6 km from the Pongola River. Cattle grazing disturbed the natural vald and most natural host plants were overgrown with, or misplaced by, creeper-like weeds and other thorny shrubs. An area of about 2.5 ha Bt-cotton was planted under dryland conditions, on a red sandy soil, which seemed nutrient-deficient. The cotton fields were well weeded. Very few natural host plants occurred here but some of the species scouted included A. austro-africanum, Acanthospermum hispidum DC and C. hildebrandtii.

The second midway field visited (L3) was that of Mr. Qwabe Mandla (Grid reference: 27.32282° S 32.24213° E; Elevation: 71m), which was an area of about 7.6 ha Bt-cotton planted under dryland conditions, on a red sandy soil. Planting continued over an extended period, every time after rain had fallen. The cotton was infested by common quick grass (Cynodon dactylon (L.)). A. austro-africanum, A. hispidum and C. trilocularis were amongst the plants scouted here.

The farm of Mr. Ngwenya, owned by Mr. Thakani (L8) (Grid reference: 27.24166° S 32.16167° E; Elevation: 117m), was an area situated next to a gravel road and 6.9 km from the Pongola River. An area of 4.4 ha Bt-cotton was planted under dryland conditions, on a red sandy soil. Plants scouted included A. hispidum, G. herbaceum and Hibiscus cannabinus L.

Distant fields

The next locality (L5) as referred to by Mr. C. Matlou (Monsanto SA), (Grid reference: 27.50254° S 32.11206° E; Elevation: 123m) was in the natural veld on a black clay soil with high water-holding capacity. About 1 ha of Bt-cotton and 0.5 ha of maize[4] was planted, intercropped with pumpkins, squash and beans, under dryland conditions. Plants scouted in this area included A. guineense, C. hildebrandtii and H. calyphyllus.

The farm of Mr. Xulu (l7) (Grid reference: 27.51611° S 32. 15298° E; Elevation: 93m), was situated next to the irrigation scheme of the Pongola dam. On the irrigation scheme, cotton, maize, vegetables, sugar cane and sorghum were planted. An area of about 8.03 ha Bt-cotton was planted under dryland conditions, on a heavy grayish clay soil with a high water-holding capacity. The cotton bordered a fallow field with weeds, surrounded by natural veld and vlei or wetland area. Indigenous plant and weed species scouted at this site included A. guineense, C. hildebrandtii and C. trilocularis.

Mr. Mgomezulu's farm (L10), (Grid reference: 27.23671° S 32.10933° E; Elevation: 159 m) was planted with Bt-cotton on an area of about 1.52 ha under dryland conditions, on a dark brown to black clayish soil. This cotton was, however, grazed by goats and cattle. Plants scouted at this area included A. guineense, G. herbaceum and Sida spp. Plants were also scouted amongst an area of about 0,5 ha maize.

Those of Mr. Ntshangase (L11) (Grid reference: 27.41336° S 32.21135° E; Elevation: 76 m) was situated within an inhabited area, bordering natural bush. An area of 2.5 ha Bt-cotton was planted under dryland conditions, on a brown loam to sandy soil. The cotton was overgrown with weeds. Plants scouted in this area included A. guineense, Abutilon sonneratianum (Cav.) Sweet and A. hispidum.

Scouting procedures

Cotton (both transgenic and non-transgenic), plant species known to be bollworm hosts and species that could possibly sustain bollworm populations were monitored extensively throughout the season by scouting, to determine bollworm population densities. In the cotton fields, 29 species of weeds/natural plants were scouted, 28 in the fallow field, and 30 species in the veld/indigenous bush. For each plant species, 10 whole plants were scouted within each study point (where present) and for cotton (both Bt-and non-transgenic), 24 whole plants were scouted. Scouting commenced on 9th and 10th October 2001 and continued until July 2002. The following parameters were noted during scouting: the identification and number of bollworm eggs and their position on the plant; the number and instars of bollworm larvae of all species present; bollworm damage to the plant on the squares/buds, flowers, bolls/seed capsules; plant growth stages (pre-flowering, budding, first flowers, flowering general and post-flowering).

For statistical purposes the parameter, bollworm activity, was used to analyze the data. The parameter bollworm activity included the mean number of bollworms of all stages per plant

[4] The term "maize" used here is synonymous with "corn" used in the Americas, while the term "mealie-fields" refers to a field of maize (corn) and is traditionally referred to as such in South Africa. Legends in figures labeled mealie-fields refer to corn fields in the strict sense of the word.

as well as the mean number of eggs per plant. Plant damage was excluded from the analyses because it was seen as too variable between species, since often the damage on the pods and seed capsules of the weed species could not without doubt be ascribed to bollworm damage. However, in this case, for the purpose of statistical analyses, the number of eggs found on Bt-cotton too, was considered as an inconsistent parameter, since it is not known how many eggs eventually lead to larvae that survive on the Bt-cotton. Bollworm larvae were therefore considered as a more reliable parameter. The standard error for the comparison of means was too high and it was subsequently decided to discard a statistical comparison of bollworm activity. For the analysis of bollworm activity, three analyses were completed using a Wilcoxon non-parametric t-test. For the first analysis, the bollworm activity on all the weed species in the fallow field, maize and veld areas was compared with the bollworm activity on non-transgenic cotton. In the second analysis, the bollworm activity on all the weed species in the fallow field, maize and veld areas, on the weeds in the non-transgenic cotton land and on the non-transgenic cotton, was compared with the bollworm activity on the weeds found in the Bt-cotton land and on the Bt-cotton itself. The third analysis dealt with the number of bollworm larvae on all the weed species in the non-transgenic cotton land was compared with the number of bollworm larvae on the weeds found in the Bt-cotton land.

Bollworm eggs collected in the field were placed in containers and placed in a cooler. These were taken back and allowed to hatch. Eggs from bollworm species were also identified based on results and observations made the previous season through Scanning Electron Microscopy, and experience of finding eggs again on the same host plants. To accurately identify the bollworm species, larvae were collected in the field, kept on their host plant, and raised to moths, especially in the case of spiny bollworm larvae. For the identification of plant species scouted, a representative specimen of each plant was collected from the appropriate locality, pressed in a plant press and labeled in the field. These specimens were sent to the herbarium at the National Botanical Institute in Pretoria (SA) for identification.

The growth stage of each weed species (pre-flowering, budding, first flowers, flowering general and post-flowering) was noted during each observation, since this may influence the number of bollworms on a specific plant scouted. The percentage of plants in each growth stage on a particular date (out of the total number scouted on that date) was calculated and plotted on graphs together with the number of bollworms found over the season to determine whether any trends were present.

At each locality (study area), plant densities were determined within each study point (cotton, fallow field, natural veld or the hedges surrounding the fields) for plant species on which bollworm larvae were found (the data collection sheet for plant density is added in Annexure III). Four subplots of 1 m x 10 m were selected at random and the numbers of host plants occurring within that area were counted. When plant abundances were very high, an area of 1 m^2 was surveyed. At the end of the season, when plant densities were low, the area surveyed was increased to 60 and even 100 m^2. A Global Positioning System (GPS) (Model: Vista e-Trex) was used to determine the position (latitude and longitude) of each subplot surveyed.

All host plants were divided into three groups according to the bollworm species that occurred on them, to illustrate the distribution of african bollworm host plants, red bollworm host plants and spiny bollworm host plants. Plant population densities were measured in the cotton, fallow-field and natural bush areas at each locality, by means of counting the number of each species that occurred within a grid of 1 m^2. The grid was randomly thrown four times within each area (cotton, fallow field and natural veld), and rolled to cover a distance of 10 m at each locality. During the season, when very few weed species occurred, a distance was measured, for example, 6 m x 10 m, and the species within this area was then assessed and the number expressed in plants per 10 m^2. The species variation within the grids was extreme. The variability of the parameter measured was considerable, and after deliberation with Dr. Mike van der Linde[5] it was decided to omit any statistical analyses. It was decided that the data obtained illustrates the point.

For the purpose of illustrating the distribution of these plant species, the host plants were divided into groups. The Sida species were combined to form the Sida species group, which included S. rhombifolia, S. cordifolia, S. dregei, J. flava and Malvastrum coromandelianum. Similarly, all Hibiscus spp. were combined to form the Hibiscus group, and two Abutilon spp. (A. guineense and Abutilon sp. ('leathery') were combined to form a guineense group: C. trilocularis, C. hildebrandtii and A. austro-africanum were determined individually. Plant densities were compared by calculating the number of host plants per 10 m^2, as well as the number of cotton plants per 10 m^2, keeping in mind that both Bt-cotton and non-transgenic cotton were cultivated under dryland conditions. An average of 35000 plants per ha was accepted as the general stand for cotton plants in a cotton field. Management practices varied to a large extent between the study points (Table 6.1). The number of species present in a cotton field is influenced by factors such as the natural occurrence of the species, the amount of hoeing, the use of herbicides, land preparation methods followed, the use of minimum tillage and the cultivation and management practices followed during the winter months. For the purpose of this study, the data collected from the non-transgenic cotton, fallow field and natural veld areas was added together. These three regions represent the possible refuge area that could provide a source of moths for influx into the Bt-cotton. These refuge areas were compared to those of the host plants counted in the Bt-cotton. The total number of host plants measured over the whole season per 10 m^2/locality was compared for all plants that hosted the african bollworm, spiny bollworm and red bollworm respectively. The number of weed species per locality is illustrated by making use of pie charts in order to show that most weed species, or species groups did occur on most localities.

Plant dry mass

Bollworm host plants and cotton differ in size during the growing season and thus support a different number of bollworm larvae. To compare the effectiveness of the different plant species

[5] Dr. Mike van der Linde, is an applied statistician from the Department of Statistics, University of Pretoria, Pretoria.

as host plants for the bollworms, the biomass of the plants was calculated (the biomass of a host plant is the function of the dry mass and the abundance of the plant) so that the bollworm activity on an equivalent biomass of the different host plant species could be compared. At each study point, one or more specimens of each of two plant species that serve as host plants for mainly spiny bollworm and/or cotton plants (Bt- or non-transgenic) were removed to determine the dry mass. The dry masses of the plants were measured after drying them at 80℃ for 48-72 hours. The size of the cotton land as well as the number of plants per hectare was used to calculate the total available biomass of cotton plants. The mean number of bollworm larvae per plant was correlated to the plant biomass for cotton and weeds. The biomass of the weed species found in the cotton fields and in the veld was divided by the mean biomass of a cotton plant. This gave an indication of the number of host plants for each species that represent the biomass of a cotton plant. The mean number of bollworm larvae per plant was then used to calculate a projected number of bollworms that could be found on the number of plants that were similar in biomass to that of a cotton plant. This was mainly done for spiny bollworm since these host plant species were more common (some of the host plants were weeds). One does not want to demolish less common, naturally occurring host plants of the other bollworm species that occur within the study areas.

Number of bollworms produced per locality

A GPS was used to determine the coordinates of the corners of each cotton field of the 12 localities. The same was done where definite fallow fields could be identified. Areas in the natural veld surrounding the cotton fields were also measured at certain localities. The surface area of the cotton lands, fallow fields and natural veld were then calculated. The average number of the different host plants per 10 m^2 found in the cotton lands, fallow fields and natural veld was determined by counting the plants per m^2 in several sampling grids. The total estimated number of a specific host plant that could occur within a specific cotton land, fallow field or natural veld could then be calculated by multiplying the average number of plants per m^2 by the surface area. The total number of bollworm larvae found over the season on each plant was then divided by the total number of that plant species scouted during the study period to determine the average number of bollworms per plant. This was multiplied by the approximate number of host plants found within the study point to calculate the approximate number of bollworm larvae that could theoretically be found per host plant within that specific cotton land, fallow field or veld.

A comparison of the number of bollworms on weeds in a 5 % unsprayed non-transgenic cotton refuge to that found in a 95 % Bt-cotton field on weeds was done by making use of the number of weed species in their particular groupings per 10 m^2. This was calculated to a number of plants per ha, of which 5 % was then used to represent a specific plant density. Bollworm numbers were extrapolated to determine the number of bollworms on weed species groups for this area, as well as for Bt-cotton. Plants were handed in for identification at the National Botanical Institute (NBI) in Pretoria.

RESULTS

Plants scouted on which no bollworm eggs or larvae were found, are listed in Table 6.2. A list of plants on which bollworms were found, is shown in Table 6.3. Additional species supporting the bollworm complex, not identified in the previous season were Acanthospermum hispidum DC, Gossypium herbaceum L. subsp. africanum (Watt) Vollesen, Hibiscus praeteritus R.A.Dyer, Hibiscus vitifolius L, Justicia flava, Malvastrum coromandelianum (L.) Garcke, Melhania forbesii Planch. Ex Mast., Pavonia burchellii (DC.) R.A.Dyer, Sida cordifolia L. and Sida dregei Burtt Davy.

The total bollworm activity (sum of the bollworm neonates, larvae, eggs and damage to fruiting bodies caused by the bollworm complex) on each host plant is shown in Fig. 6.2. The plants could be divided into four basic groups according to the amount of bollworm activity present: the first group consisted of plants with a relatively high activity. The plant with the highest bollworm activity was H. vitifolius, which did not have that many bollworm larvae but had many bollworm eggs present. G. herbaceum and H. calyphyllus also had high bollworm activity. The second group, were plants which showed slightly less bollworm activity and included A. austro-africanum, A. guineense, C. hildebrandtii, H. praeteritus and S. dregei. The third group included plants like Abutilon sp. ('leathery'), A. sonneratianum, G. hirsutum, M. forbesii, P. burchellii, S. rhombifolia and S. cordifolia. The fourth group consisted of plants with very low bollworm activity, namely A. hispidum, C. trilocularis, H. cannabinus, J. flava and M. coromandelianum.

If the bollworm activity on the host plants in the different study zones is compared (Fig. 6.3), it can be seen that no real differences occurred between the number of plants showing bollworm activity in the riverside, midway and distant zones. H. vitifolius had highest bollworm activity in the riverside zones, G. herbaceum had high bollworm activity in the midway zone and H. praeteritus had most activity in the most distant zone from the river.

The different study points showed that bollworm activity was far more prevalent in the natural veld, fallow fields and Bt-cotton lands (Fig. 6.4) than in non-transgenic cotton lands and the maize fields (labeled Mieliefield in figures). In the natural veld, the most bollworm activity was seen on H. vitifolius, G. herbaceum and H. calyphyllus. In the fallow field, highest activity was on H. vitifolius and H. praeteritus. In the Bt-cotton lands, most activity occurred on H. vitifolius, A. guineense, C. hildebrandtii and A. austro-africanum. Most of the activity in the non-transgenic cotton and maize lands occurred on A. guineense. Most eggs from the bollworm complex were found on G. herbaceum and H. vitifolius (Fig. 6.5). The most African bollworm eggs occurred on H. vitifolius. The most red bollworm eggs were laid on G. herbaceum and C. hildebrandtii. The most spiny bollworm eggs were found on H. vitifolius H. praeteritus and A. guineense.

African, red and spiny bollworm eggs found within each study zone are shown in Figs 6.6, 6.7 and 6.8 respectively. The most african bollworm eggs were found on H. vitifolius in all three the study zones, riverside, midway and distant (Fig. 6.6). Red bollworm eggs occurred mostly on G. herbaceum in the midway zone and on C. hildebrandtii in the distant zone. Some red bollworm eggs occurred on G. herbaceum in the riverside zone (Fig. 6.7). The most spiny

bollworm eggs (Fig. 6.8) occurred on H. vitifolius in the riverside and midway zones. The most spiny bollworm eggs in the distant zone were found on H. praeteritus.

African, red and spiny bollworm eggs found within each study point are shown in Figs 6.9, 6.10 and 6.11 respectively. The most African bollworm larvae (Fig. 6.9) were found on H. vitifolius in the natural veld, fallow field, weeds/indigenous plants in the Bt-cotton and maize field. No african bollworm eggs were found in the weeds among the non-transgenic cotton. The most red bollworm eggs (Fig. 6.10) were found on G. herbaceum in the veld. Most spiny bollworm eggs (Fig. 6.11) were found in the veld on A. guineense and in the fallow field on H. vitifolius and H. praeteritus. In the Bt-cotton, the most bollworm eggs were found on H. vitifolius and in the non-transgenic cotton, the most were found on A. guineense.

Bollworm larvae

The number of bollworm larvae found per plant is illustrated in Figs 6.12-6.20. The average number of African bollworm larvae found per plant is shown in Fig. 6.12. African bollworm larvae were found on eight host plants. The most larvae were found per plant on P. burchellii, followed by A. hispidum.

The number of african bollworm larvae per plant in the different zones is shown in Fig. 6.13. In the riverside zone, the most african bollworm larvae were found on C. hildebrandtii, followed by H. vitifolius. In the midway zone, the most african bollworm larvae were found on P. burchellii and in the distant study zone, the most were found on A. austro-africanum and A. hispidum.

The number of african bollworm larvae per plant in the different study points is shown in Fig. 6.14. African bollworm was only found on J. flava in the veld. The most african bollworm larvae were found on C. hildebrandtii in the fallow field and on P. burchellii in the Bt-cotton.

Red bollworm larvae were found on three different host plant species (Fig. 6.15). The most were found on G. herbaceum, followed by C. hildebrandtii. On A. guineense, very few larvae were found.

The number of red bollworm larvae per plant in the different zones is shown in Fig. 6.16. In the riverside zone, red bollworm larvae were found on all three of the recorded host plants, with the most on G. herbaceum. In the midway zone, most red bollworm larvae were found on G. herbaceum while in the distant zone, more were found on C. hildebrandtii.

The number of red bollworm larvae per plant in the different study points is shown in Fig. 6.17. Most of the red bollworms larvae were found in the natural veld, followed by fallow field and then the Bt-cotton fields.

The average number of spiny bollworm larvae found per plant is illustrated in Fig. 6.18. Of the four bollworm species found in the Makhathini, spiny bollworms were the most numerous and occurred on 15 of the plant species scouted. High numbers of spiny bollworm were found on some of their host plants, including H. calyphyllus, S. dregei, A. guineense, A. austro-africanum and H. vitifolius.

The number of spiny bollworm larvae per plant in the different zones is shown in Fig. 6.19. In the riverside zone, spiny bollworm larvae were found on 11 of the 15 host plant

species, of which, the most occurred on H. calyphyllus. In the midway zone, spiny bollworm larvae were found on eight of the 15 host plant species, with A. austro-africanum and A. guineense having the most larvae. In the distant zone, spiny bollworm larvae were collected on nine of the 15 host plant species scouted, of which, S. dregei and H. praeteritus had the most larvae.

The number of spiny bollworm larvae per plant in the different study points is shown in Fig. 6.20. In the natural veld, larvae were found on 12 different host plants. Most of the larvae were found on H. calyphyllus, S. dregei and H. vitifolius. In the fallow field, larvae were found on 11 different host plants, of which, H. praeteritus had the most larvae. Spiny larvae were collected on nine different host plant species in the Bt-cotton lands. Fewer Hibiscus species grew in the Bt-cotton lands than in the previous two study areas. The most larvae in the Bt-cotton fields were found on A. guineense, A. austro-africanum, S. dregei and H. vitifolius. In the non-transgenic cotton lands, a few larvae were found on three different host plants, A. guineense, A. austro-africanum and H. vitifolius. In the maize field, because of better weeding, fewer natural plants and weeds were present than in the natural veld and fallow fields. Within the maize field, larvae were, however, found in high numbers on A. guineense.

The distribution of bollworm species on the host plants

In KwaZulu-Natal, on the Makhathini flats, the four bollworm species making up the bollworm complex on cotton, african bollworm, Helicoverpa armigera (Hübner) (also called American bollworm), spiny bollworms Earias biplaga Walker and Earias insulana Boisduval and the red bollworm Diparopsis castanea Hampson, maintain natural populations on alternative host plants.

The only plant found on which African, red and spiny bollworms occurred was A. guineense. On five of the other host plants, two of the bollworm species were found. African bollworms were found together with red bollworm on C. hildebrandtii although spiny bollworms were also collected on C. hildebrandtii during the previous season. The african and spiny bollworm occurred together on A. austro-africanum, H. vitifolius, P. burchellii and C. trilocularis. On A. hispidum and J. flava, only african bollworm larvae were found. Only spiny bollworm was collected on the remaining ten host plant species.

Damage to fruiting bodies

The number of damaged buds, flowers and seed capsules is shown in Fig. 6.21. The most damaged buds were found on S. dregei and H. vitifolius. H. calyphyllus had the most damaged flowers. A. guineense and A. austro-africanum had the most damaged seed capsules.

The damage to buds, flowers and seed capsules within each study zone is shown in Fig. 6.22, 6.23 and 6.24 respectively. In the riverside and midway zones, the most damaged buds were found on H. vitifolius (Fig. 6.22). In the distant zone, the most damaged buds were found on S. dregei. The most damaged flowers were found on H. calyphyllus in the riverside zone, on H. vitifolius in the midway zone and on S. dregei in the distant zone (Fig. 6.23). A. guineense had the most damaged seed capsules (Fig. 6.24) in the riverside zone, G. herbaceum in the midway zone and H. praeteritus in the distant zone.

The damage to buds, flowers and seed capsules within each study point is shown in Figs 6.25, 6.26 and 6.27 respectively. S. dregei, A. sonneratianum and H. vitifolius had the most damaged buds in the veld. In the fallow field, H. vitifolius had the most damaged buds. S. dregei had the most damaged buds in the Bt-cotton fields, H. vitifolius in the non-transgenic cotton lands and A. guineense had the most damaged buds in the maize fields (Fig. 6.25). The most damaged flowers (Fig. 6.26) were found on H. calyphyllus in the veld, on H. vitifolius in the fallow field and on A. sonneratianum in the Bt-cotton fields. The most damaged seed capsules (Fig. 6.27) were found on H. vitifolius in the veld, on H. praeteritus in the fallow field, A. guineense and A. austro-africanum in the Bt-cotton fields and on A. guineense in the non-transgenic cotton and maize fields.

Other bollworm stages

A spiny bollworm pupa was found on A. austro-africanum in the natural veld. E. biplaga moths were observed on Xanthium strumerium L. (cockleburr) and on Sida cordifolia, both within the Bt-cotton field. An E. insulana moth was observed on Abutilon sp. ('leathery')' in a Bt-cotton field.

Scout data on cotton

The bollworm activity found on the Bt-cotton is shown in Fig. 6.28. No neonate larvae were detected. The average number of bollworms per plant scouted was very low. African bollworm larvae were only found in L1, L2 and L8. Red bollworm larvae were only found in locality L5. No spiny bollworm larvae were found. That can imply that spiny bollworm preferred their natural host plants to cotton and that there are enough natural host plants in the study area for the spiny bollworm population. Both african and red bollworm eggs were found on five localities but no spiny bollworm eggs were found, that could further demonstrate that spiny bollworms prefer their natural host plants to cotton. No damaged flowers were found. Damaged squares were found at three localities (L1, L7 and L12) and damaged bolls at six of the 11 localities (L1, L4, L5, L7, L8 and L12).

The bollworm activity found on the non-transgenic cotton (totals/plant/locality) is shown in Fig. 6.29. Since very little bollworm activity was found on the non-transgenic cotton this season, the data from the previous season's scouting on non-transgenic cotton was added. During this season, only african bollworm larvae were found, but both african and red bollworm eggs were found. No spiny bollworm eggs or larvae were found. No damaged flowers were found. During the previous year, all three bollworm species were found in all stages on the non-transgenic cotton.

The scout data for both Bt-cotton and non-transgenic cotton over the season is shown in Fig. 6.30. During this season, more african bollworm larvae were found on the non-transgenic cotton, as well as more damaged squares and bolls although more african and red bollworm eggs were found on the Bt-cotton.

Statistical analysis of bollworm data

A summary of the statistical analyses is given in Table 6.4. During the first analysis, no significant differences were found between the bollworm activity on the weed species found in the fallow field, maize and veld areas and the bollworm activity found on the non-transgenic cotton (P=0.0619 Standard error for bollworms on weeds = 0.1991, standard error for means of bollworms on non-transgenic cotton = 0.1123). This confirms that a more or less similar number of bollworms can occur on the complex of host plants during the season, that that found on the non-transgenic cotton. Weed species therefore can possibly serve as a source of bollworms or a possible refuge site.

During the second analysis, by comparing the bollworm activity for all the weed species in the fallow field, maize, non-transgenic cotton lands, veld and on non-transgenic cotton itself, with the bollworm activity on all the weed species in the Bt-cotton lands plus on the Bt-cotton itself, significantly more bollworms were found in the Bt-cotton lands and on the Bt-cotton (P=0.0130). During this study very little bollworm larvae were found on the Bt-cotton, but most larvae were found on weed species within the Bt-cotton lands. This indicates that the bigger larvae do not move to infest Bt-cotton plants, and the presence of these weed species within the Bt-land is dependent on management practices like hoeing or herbicide applications. The number of weed species within the Bt-cotton field will definitely be affected by the introduction of new cotton varieties possessing both bollworm resistance and herbicide tolerance, when the use of herbicides become more common. The larvae on these weed species within the Bt-cotton land should not be considered as the important source for bollworms that are potentially less exposed to the Bollgard™ gene, but rather the larvae found on weed species in the natural veld and fallow field areas.

In the third analysis, the total number of larvae on all the weed species within the non-transgenic cotton lands was compared with the total number of larvae on all the weeds species within the Bt-cotton lands, significantly more larvae were found on weeds in the Bt-cotton lands than in the non-transgenic cotton lands (P=0.0088). The number of non-transgenic cotton lands that were scouted may influence the mean that was compared to that of the Bt-cotton lands. The standard error for means was much greater for the larvae found in the Bt-cotton land than that of the non-transgenic cotton lands.

Host plant growth stages in relation to bollworm numbers

The number of bollworms collected on each species was plotted together with the percentage of plants in each plant stage (pre-flowering, budding, first flowers appear, flowers general and post flowering). This data is represented in Fig. 6.48. In general, the bollworm numbers tended to correspond to the budding stages of the different weed species. Most bollworms were found in the buds and seed capsules of the plants. A. austro-africanum (Fig. 6.31) indicates high bollworm numbers when the plants were in the budding stages during November, February and in May. A. guineense (Fig. 6.32) and Abutilon sp. ('leathery') (Fig. 6.33) seem to bud during November and March and again at the end of April to May. High bollworm numbers correspond with these

budding phases. Bollworms tended to occur in February and March on A. hispidum (Fig. 6.34) together with a slight peak in budding and flowering. The budding stages of A. hispidum seemed to be slightly later than that of the plant species previously mentioned. In the case of C. hildebrandtii (Fig. 6.35), an increase in bollworm numbers occurred when the plants were in the budding stage. Bollworms tended to occur on C. trilocularis (Fig. 6.36) during January and again in March to April, after which, the numbers decreased, with no bollworms occurring on this plant after May. The cotton season ends with the start of boll burst from end of March onwards in the Makhathini, and bollworm numbers are expected to decline during this period. On G. herbaceum (Fig. 6.37), budding increased from January to February, with a corresponding increase in the number of bollworms. A peak in bollworm numbers was found in March to April, when the bolls were well developed. On H. calyphyllus (Fig. 6.38), a high number of bollworms were found in middle December to January, before the cotton budding period, but numbers declined throughout the season on this plant species. This latter species may serve as an early alternative host plant for bollworms. On H. cannabinus (Fig. 6.39), bollworm numbers were low during the post-flowering period in December and in May. Bollworm numbers tended to correspond with the budding stages, which increased after February to April. In the case of H. praeteritus (Fig. 6.40), a high number of bollworms were found at the end of May. H. vitifolius showed a high number of bollworms when budding took place, in October, May and some larvae in June (Fig. 6.41). This tendency shows that it might serve as a host plant before the cotton season, and in the interim-period following boll bursting of cotton. Some bollworms were found on J. flava during November, in the pre-budding phase of cotton, which may be significant (Fig. 6.42), however, no bollworms were found for the rest of the season on this species. On M. coromandelianum (Fig. 6.43), bollworm activity was found from October to January and again in February. After February, no bollworms were found on this species, which might be an indication that bollworm moths prefer cotton to this species when it is in flower. On M. forbesii (Fig. 6.44), bollworms were found during February, corresponding with the budding period of the species. In the case of P. burchellii (Fig. 6.45), the budding, flowering and post-flowering stages form an irregular pattern, although some bollworms were noticed at stages when the plants tended to have more buds, in January and again in April and May. High bollworm numbers were found on S. cordifolia during May (Fig. 6.46). Some bollworms were found on S. dregei during December, and a few in June (Fig. 6.47), but none during February to March, indicating that this species might also serve as a host plant before and after the peak flowering period of cotton. Some larvae were found in early December and in February during the budding stage on S. rhombifolia (Fig. 6.48).

Based on the plant growth stage data, plant species could be divided into the following groups: (1) Pre-cotton alternative host plants: C. hildebrandtii, H. calyphyllus, H. vitifolius, J. flava, S. dregei and S. cordifolia. (2) Alternative host plants during the cotton-flowering phase: A. austro-africanum, A. guineense, S. rhombifolia, M. coromandelianum and M. forbesii. (3) Post-cotton alternative host plants: A. hispidum, H. cannabinus, H. vitifolius, H. praeteritus and S. dregei.

The budding stages of many of the alternative host plant species started during the middle of November, just before the cotton budding stage. This could indicate that the bollworms already present on the weed species fed on these host plants until cotton became attractive to them for feeding.

Host plant density in relation to bollworm numbers

A large amount of variation in plant densities occurred between grids with regard to localities and time in the season when they were counted. Plant densities between areas and study points are shown in Table 6.5. African bollworm host plants were found were found to be present on all localities (Table 6.5, Fig. 6.49a). The highest density of african bollworm host plants (34.357 plants / $10m^2$) was found in locality L8, and the lowest in Locality L1 (0.031 plants / $10m^2$). Most red bollworm host plants were found at locality L2 (6.688 plants / $10m^2$), while localities L4 and L8 had none of these host plants (Table 6.5, Fig. 6.49b). Spiny bollworm host plants were present at all localities (Table 6.5, Fig. 6.49c). The highest density of plants was found at Locality L2 (45.656 plants / $10m^2$), followed by localities L8 (33.500 plants / $10m^2$), then L7 and L9 with 31.553 and 31.100 plants / $10m^2$ respectively. From Figs 6.49a, b, c; it can be seen that the red bollworm host plants tended to be more localized, whereas the african and spiny bollworm host plants had more even distributions.

Figs 6.50a,b,c-Figs 6.51a,b,c illustrate the distribution of the host plants between the localities, measured as the total number of host plants per $10m^2$ over the season. The A. guineense group was most common at locality L5 (11.009 plants / $10m^2$) (Table 6.5, Fig. 6.50a). The Hibiscus group was most common in locality L3 (7.250 plants / $10m^2$) and L8 (3.214 plants / $10m^2$) (Table 6.5, Fig 6.50b). The Sida species were more evenly distributed (Table 6.5, Fig. 6.50c), with the most occurring in L2 (16 plants / $10m^2$). C. trilocularis was most common at locality L7 (25.25 plants / $10m^2$), followed by L9 (15.893 plants / $10m^2$) and L8 (13.464 plants / $10m^2$) (Table 6.5, Fig. 6.51a). A. austro-africanum was the most common at locality L1 (5.569 plants / $10m^2$), followed by L10 (3.411 plants per 10 m^2) and L5 (1.643 plants per 10 m^2) (Table 6.5, Fig. 6.51b). C. hildebrandtii was most common at locality L2 (6.656 plants per 10 m^2) followed by L6 (2.964 plants per 10 m^2) (Table 6.5, Fig. 6.51c). The A. guineense and Sida groups are important spiny bollworm host plants. C. hildebrandtii is an important red bollworm host plant. When the different species or species groups were compared between the hypothetical refuge area (fallow field, veld and non-transgenic cotton) and the host plants found in the Bt-cotton over the season (Table 6.5), only one locality, L10, showed a slightly lower number of host plants (33.107 plants per $10m^2$) in the refuge area than in the Bt-cotton (35.789 plants per $10m^2$). When the total number of cotton plants per $10m^2$ (35 cotton plants per $10m^2$) was compared at each locality with the host plants found in the non-transgenic cotton, veld and fallow field areas (proposed refuge area) of each locality, a considerably higher number of host plants were found at all localities in the “refuge area” than the number of cotton plants per $10m^2$ (except locality L5 (29.996 plants per $10m^2$)) (Table 6.5). The total number of host plants per $10m^2$ in the non-transgenic cotton fields at locality L6 (22.500 plants per $10m^2$) was close to that found in the non-transgenic cotton at Locality L12 (20.857 plants per $10m^2$) (Table 6.5).

Plant dry mass

The dry mass (g) of some of the weed species is illustrated in Table 6.6. The number of plants of the various weed species that was equivalent to the dry mass of one cotton plant varied between species. The plants in the cotton fields were generally smaller than the plants in the natural veld, probably as a result of management practices carried out by farmers in the cotton fields, so more plants were needed to make up the same dry mass as one cotton plant than in the natural veld. For example, 2.621 A. guineense plants make up the same dry mass as one cotton plant in the cotton lands, and 1.765 A. guineense plants make up the same dry mass as one cotton plant in the veld. The calculated number of bollworms on weed plants found in the veld with the same biomass as a single cotton plant was less for A. guineense, A. sonneratianum, H. cannabinus and H. vitifolius than the calculated number of bollworms found on weed plants in a cotton field with same biomass of a single cotton plant. The number of bollworms found on the same biomass (equivalent of one cotton plant) of, for example, A. guineense found in cotton, is considerably higher (1.463) than that found per cotton plant (0.953). This varied between plant species and indicated that bollworm numbers per each plant species separately could not be compared to that found on cotton. The potential refuge area should be made out of a complex of host plant species.

Number of bollworms produced per locality

The surface area of the cotton lands, fallow fields and natural veld are given in Table 6.7. In Table 6.8, the calculated number of bollworm larvae produced on the host plants in the cotton lands, fallow fields and natural veld in the different study localities are given. The tendency existed that the more numerous the host plants and the bigger the surface area, the more bollworm larvae were produced. A summary of the calculated number of bollworm larvae found per study area is given in Table 6.9. The expected number of bollworm larvae was the highest in the localities where the biggest area of natural veld was studied. In locality L2, lots of C. hildebrandtii occurred in the natural veld and an area of 13 ha was studied. Huge numbers of african, red and spiny bollworm larvae can therefore be expected from this area. In locality L3, high numbers of african and spiny bollworm can be expected. As a result of the bigger study area, more african and spiny bollworm larvae can be expected from locality L7. Over the total study area, more red bollworms can be expected than african bollworms. Spiny bollworm will be the most numerous.

Bollworm numbers on the refuge area on cotton in comparison with bollworm numbers on a possible weed host plant refuge. The number of bollworms found in the refuge area on cotton and on weeds is given in Table 6.10. The mean number of bollworms found in 0.05 ha of fallow field areas, as well as in 0.05 ha of veld, on the different weed species was higher than the mean number of bollworms found on the weed species within 0.05 ha non-transgenic cotton. The mean number of bollworm larvae found on 0.05 ha fallow field on the A. guineense and Sida groups were both higher (152.2 and 141.1 respectively) than the number of larvae on the non-transgenic cotton refuge (79.0). These two plant groups therefore seem to be preferred above

non-transgenic cotton. From this evidence, it seems that more bollworms occur in the fallow field areas and in the natural veld on the same proportion of refuge as that of the non-transgenic cotton, which suggests that the conventional cotton as a refuge is, in this case, not necessary.

CONCLUSIONS

The findings for this report support the findings of the previous years work, in that on all plant species previously identified as alternative hosts for bollworms, bollworms of various stages were again found. Additional plants hosting the bollworm complex were also identified. African bollworms were found on Abutilon austro-africanum, Abutilon guineense, Acanthospermum hispidum, Cienfuegosia hildebrandtii, Corchorus trilocularis, Hibiscus vitifolius, Justicia flava and Pavonia burchellii. Red bollworms were found on A. guineense, C. hildebrandtii, and Gossypium herbaceum. Spiny bollworms were found on A. austro-africanum, A. guineense, Abutilon sonneratianum, Abutilon sp. ('leathery'), C. trilocularis, Hibiscus calyphyllus, H. cannabinus, H. praeteritus, H. vitifolius, Malvastrum coromandelianum, Melhania forbesii, P. burchellii, Sida cordifolia, S. dregei and S. rhombifolia. Bollworm eggs were laid on most species where larvae were found. It is assumed that a high level of predation exists on the weeds since there is no chemical control that can affect these natural enemies. In the case of african bollworm, very few larvae were found in relation to the number of eggs found. Bollworms found on alternative host plants indicate that these plants could serve as an alternative to cotton plants later in the season. Cotton in the Makhathini is planted once there has been enough rain for germination of cotton seeds in the soil, and to help the plant through its early stages of development. Rains can fall at any time from October onwards but varies from year to year. For the 2001/02 season, rain fell in December and most cotton was planted only late in December and early in January. The number of heat units is then still enough to see to normal lint development in such a short season. Harvesting prevails in April to the beginning of May to June. The late planting practise in the Makhathini makes the season when cotton is available for bollworms as host plant very short, and this window in change of host plants is without doubt supported by a shift from and to alternative host plants. Based on the plant growth stage data, plant species could be divided into the following groups: (1) Pre-cotton alternative host plants: C. hildebrandtii, H. calyphyllus, H. vitifolius, J. flava, S. dregei and S. cordifolia. (2) Alternative host plants during the cotton-flowering phase: A. austro-africanum, A. guineense, S. rhombifolia, M. coromandelianum and M. forbesii. (3) Post-cotton alternative host plants: A. hispidum, H. cannabinus, H. vitifolius, H. praeteritus and S. dregei. The budding stages of many of the alternative host plant species started during the middle of November, just before the cotton budding stage. This could indicate that the bollworms already present on the weed species fed on these host plants until cotton became attractive to them for feeding. The host plants for the bollworm complex varied in their population densities. Abutilon spp. and Hibiscus spp. were the most widespread genera. C. hildebrandtii tends to grow naturally in more clayish soils and wet conditions, such as wetlands. The refuge areas had more alternative host plants than within the cotton fields, which should serve as a source of food for bollworms that can interbreed with bollworms exposed to the

Bt-gene on Bollgard™ cotton. The number of bollworms in the potential refuge areas, which include the fallow field, veld and maize areas did not differ significantly from the bollworms found on the non-transgenic cotton plants, therefore these areas may be suggested as a possible source or refuge areas where bollworms can originate from. The extrapolation of bollworm numbers found on weed species that are representative of the biomass of a single cotton plant indicates that a lot of variation exists in the biomass of these host plant species, and should a refuge be suggested, the refuge should contain most of these host plant species and not a single one or two species. The tendency existed that the more numerous the host plants and the bigger the surface area, the more bollworm larvae were produced. The number of bollworm larvae is expected to be the highest in localities where the biggest area of natural veld occurs. Weed control on the farms was generally very disappointing. Weed control was mainly based on hand hoeing and this was not sufficient for preventing the weeds from forming seeds and thus multiplying. No animal drawn tillers were used on any of the 12 localities in this study. Glyphosate was sprayed on weeds on three of the cotton fields just before minimum soil cultivation took place. Glyphosate and a defoliant were sprayed, by mistake, as a lay-by over the top of part of a cotton field at one locality. Weed species were restricted within these fields. The constant presence of weeds and indigenous plants that act as hosts for the bollworm complex provide a year round source of food for these insects. They can also provide an ideal over wintering habitat for the cotton stem weevil, as well as for possibly more Bt-tolerant strains of bollworm that may have survived on Bollgard™ cotton. No resistant bollworm populations have, however, been reported up to now. The practice of cutting and burning cotton stalks was applied too late in the season to provide a cotton-free period. The bollworms are thus able to breed on the re-growth and then invade new or existing alternative host species. Management practices of small-scale farmers in the area indirectly influence bollworm populations. Soil cultivation and the preparation for planting cotton is started too late in the season to make the best use of the rainfall occurring during late November and December. Farmers need to be trained to farm all year around and to make use of better farming practices, such as no-till farming, since these can improve yields and thus the livelihood of the farmers of the Makhathini.

Final conclusion on alternative host plants for bollworms

Cotton is the most preferred host plant for the bollworm complex. In the absence of cotton, most cotton pests take to alternative host plants commonly referred to as weeds. The occurrence of weeds within a Bt-cotton field may influence pest numbers on Bt-cotton that would otherwise not have been expected. A wide range of alternative host plants exists in the study area (Makhathini Flats) for bollworms on cotton. The density of these plants varies from region to region, depending on the management procedures applied by the cotton growers in each area. In the absence of any particular type of cotton, bollworm occurs more commonly on these plants and they could serve as a refuge. Bollworms found on conventional cotton did not differ significantly from those found on weeds. Weed species complement the conventional refuge area in terms of

bollworm numbers. Conventional cotton is required to be planted according to the license agreement of the patent holders, as a refuge area for transgenic cotton. In the absence of cotton, these host plant species tend to fill the gap as a 'potential refuge" and possibly ensures the interbreeding with individuals which have fed on the alternative host plants, and thereby providing a mixed gene pool of "wild bollworm populations" which has at least partly not been exposed to the Bt-gene, and by doing so preventing resistance against Bt. Weed control is important in a Bt-cotton field in order to avoid alternative nesting sites for pests.

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Tables

Table 6.1. Soil cultivation status and crop condition of the surveyed fields during the 2001/2002 season in the Makhathini Flats.

Locality	10 Oct 2001	30 Oct	21 Nov	4 Dec	3 Jan 2002	23 Jan	12 Feb	5 March	25 March	16 Apr	7 May and 28 May	17 June and 7 July
1	Not cultivated; Residues burned; Weeds short		Ploughed; Weed regrown	Disked; hand planted (2 Dec 2001)		8-11 nodes squares and buds. Partly hoed		13-17 nodes, flowers and bolls – opened and ripe		Harvested, open bolls	Few leaves on cotton	Cotton slashed
2			Minimum tillage; Cotton emerging		Field tilled. Weeds emerging		13-17 nodes, flowers, bolls		12-16 nodes, bolls opened, ripe			
3	Not cultivated		Not cultivated; Ratooned Cotton weeds present		Ploughed; Cotton 3rd leaf stage		10-14 nodes		14-17 nodes, flowers and bolls,		Harvest completed	
4		Partly cultivated; Cotton slashed		Overgrown with weeds	Find other cotton field	12 –19 nodes, flowers and bolls. Hoed.		16-20 nodes, bolls, opened bolls, dry		Harvested	Browsed by cattle	Stalks defoliated
5				Ploughed, cotton at 3rd leaf stage		8-15 nodes flowers and bolls. Hoed, young weeds		11-15 nodes, bolls, opened bolls		Harvested, open bolls	Browsed; Being slashed	Being slashed
6	Ploughed		Cultivated cotton emerged		Cotton 6 to 9 nodes; Looks good. Few weeds		13-17 nodes, flowers, bolls		13-19 nodes, wilted		Harvest completed	

(table continued)

Locality	10 Oct 2001	30 Oct	21 Nov	4 Dec	3 Jan 2002	23 Jan	12 Feb	5 March	25 March	16 Apr	7 May and 28 May	17 June and 7 July
7		Ploughed	Fallow weeds start to grow		Cotton 4 to 5 leaves. Stand uneven; Few weeds.		13-14 nodes, flowers, bolls		14-16 nodes, bolls opened, dry		Harvested, few bolls left	
8		Cotton slashed; Ploughed ¾		Rough seed-bed, cotton emerged		8-11 nodes. Large weeds present		10-17 nodes, flowers, bolls, open and ripen		Open bolls	Some leaves	Field bare, cotton is dead
9		Not cultivated; Crop browsed		Veld ploughed		Planted in furrows. Poor stand. Maize field hoed.		6-7 nodes, poor growth		5-6 cm, browsed	Browsed by goats and cattle	Field lay bare
10	Not cultivated; Residues burned		Ploughed and rough		Not planted. Overgrown.	Find other cotton field nearby	12-15 nodes, budding 1st flowers,		16 nodes, bolls, crop good		Harvest completed	
11		Not cultivated; Weeds grazed			4 nodes. Partly hoed						Harvest completed	
12	Not cultivated; Residues burned			Minimum tillage. Cotton at two leaf stage		12 nodes, in flower. Big weeds hoed		13-16 nodes, bolls, bolls opened, dry		Harvested, open bolls	Plants browsed; Little re-growth	Soil is bare, and tramped to dust

Table 6.2. Plants scouted on which no larvae or eggs were found during the host plant study 2001/2002.

	Plant species*	English name	Afrikaans name
1.	Amaranthus hybridus L. subsp. cruentus (L.) Thell.	Pigweed	Misbredie
2.	Amaranthus hybridus L. supsp. hybridus var. hybridus	Pigweed	Misbredie
3.	Barleria elegans S.Moore ex C.B.Clarke	-	-
4.	Bonamia velutina Verdc.	-	-
5.	Ceratotheca triloba	-	-
6.	Corchorus junodii (Schinz) N.E.Br.	-	-
7.	Ecbolium glabratum Vollesen	-	-
8.	Hibiscus meeusei Exell (Old name: Hibiscus cannabinus sensu Ulbr. and Fr., non L.)	-	-
9.	Hibiscus pusillus Thunb.	-	-
10.	Ipomoea purpurea (L.) Roth	Common morning glory	Purperwinde
11.	Lantana camara L.	Common lantana, Tickberry	Gewone lantana, Gomdagga
12.	Lantana rugosa Thunb.	-	-
13.	Leucas glabrata (Vahl) Sm. Var. glabrata	-	-
14.	Sesamum alatum Thonn.	-	-
15.	Solanum delagoense Dunal	Poison/Bitter apple	Gifappel
16.	Solanum nigrum L.	Black nightshade, Nightshade	Nastergal, Galbessie
17.	Sonchus oleraceus L.	Sowthistle	Sydissel
18.	Tagetes minuta L.	Khaki weed	Kakiebos
19.	Triumfetta pentandra	-	-
20.	Waltheria indica L.	-	-
21.	Withania somnifera (L.) Dunal	-	-

** Plants identified by National Botanical Institute (NBI)*

Table 6.3. Plants scouted on which moths, larvae and/or eggs were found during the host plant study 2001/2002.

	Plant species*	English name	Afrikaans name
1.	Abutilon austro-africanum Hochr.	-	-
2.	Abutilon guineense (K.Schum.) Baker f. and Exell	-	-
3.	Abutilon sp. ('leathery')	-	-
4.	Abutilon sonneratianum (Cav.) Sweet	Wild hibiscus	Wildemalva
5.	Acanthospermum hispidum DC	Starburr	Sterklits
6.	Cienfuegosia hildebrandtii Gärcke	-	-
7.	Corchorus trilocularis L.	Wild jute	Wildejute
8.	Gossypium herbaceum L. subsp. africanum (Watt) Vollesen	Wild cotton	Wilde katoen
9.	Hibiscus calyphyllus Cav.	-	-
10.	Hibiscus cannabinus L. var. cannabinus	Kenaf/Wild stockrose	Wildestokroos
11.	Hibiscus praeteritus R.A.Dyer		
12.	Hibiscus vitifolius L.		
13.	Justicia flava (Vahl) Vahl		
14.	Malvastrum coromandelianum (L.) Garcke		
15.	Melhania forbesii Planch. Ex Mast.		
16.	Pavonia burchellii (DC.) R.A.Dyer		
17.	Sida cordifolia L.	Flannel weed, Heart-leaf sida	Hartblaartaaiman, Verdompsterk
18.	Sida dregei Burtt Davy	-	-
19.	Sida rhombifolia L. subsp. rhombifolia	Arrow-leaf sida, Pretoria sida	Smalblaartaaiman, Taaiman
20.	Xanthium strumerium L.	Cocklebur	

- *Plants identified by NBI*

Table 6.4. Summary of the statistical analyses.

	Analysis 1		Analysis 2		Analysis 3	
	Bollworm activity on fallow field, veld and maize	**Bollworm activity on non-Bt-cotton**	**Bollworm activity on fallow field, maize, veld, weeds in non-Bt-cotton land and on non-Bt-cotton**	**Bollworm activity on weeds in Bt-cotton land and on Bt-cotton**	**No. of bollworms on weeds in non-Bt land**	**No. of bollworms on weeds in Bt land**
N	438	24	539	211	77	187
Means	1.6644	0.2917	1.436	2.872	0.3506	2.5668
Std .Dev.	4.4622	0.7717	3.8146	7.1579	0.8548	7.3871
Std. Error	0.1991	0.1123	0.1643	0.4928	0.0974	0.5402
Pr > F	< 0.0619		<0.0130		<0.0088	

Table 6.5. The mean number of plants per 10 m^2 per locality over the season of the host plant study 2001/2002.

		no. plants/10m^2												
Plant species/ group	**Localities**	**L1 Bt**	**L2 Bt**	**L3 Bt**	**L4 Bt**	**L5 Bt**	**L6 non-Bt**	**L7 Bt**	**L8 Bt**	**L9 Bt**	**L10 Bt**	**L11 Bt**	**L12 Bt**	**L12 non-Bt**
	No. times surveyed	**8**	**8**	**7**	**7**	**7**	**7**	**6**	**7**	**7**	**7**	**6**	**7**	**7**
A. guineense group	Cotton	1.164	0.719	0.286	0.679	9.675	1.000	6.398	0.143	2.286	2.571	0.375	1.357	1.714
	Fallow field	0.063	0.125	1.281	0.564	1.313	0.250	0.958	0.000	2.688	0.625	0.219	0.031	0.000
	Veld	0.000	1.250	0.036	0.500	0.021	0.116	0.049	0.000	0.129	0.007	0.028	0.000	0.000
	Total	1.227	2.094	1.603	1.743	11.009	1.366	7.404	0.143	5.102	3.203	0.622	1.388	1.714
Hibiscus group	Cotton	0.250	1.500	6.321	0.573	0.036	0.179	0.083	0.821	0.000	1.214	0.000	0.250	0.607
	Fallow field	0.000	0.031	0.571	0.000	0.000	0.000	0.000	1.429	0.000	0.000	0.000	0.000	0.000
	Veld	0.125	0.625	0.357	0.000	0.143	0.464	0.000	0.964	0.321	0.286	0.708	0.000	0.000
	Total	0.375	2.156	7.250	0.573	0.179	0.643	0.083	3.214	0.321	1.500	0.708	0.250	0.607
Sida group	Cotton	3.011	9.219	3.821	3.500	0.932	1.893	5.185	9.143	8.286	10.500	1.375	3.071	0.464
	Fallow field	0.156	6.781	0.857	0.357	0.607	0.107	0.458	2.000	1.143	0.536	1.875	0.000	0.000
	Veld	0.656	0.000	0.036	1.071	0.000	0.036	2.917	0.000	0.107	0.799	1.083	0.000	0.000
	Total	3.823	16.000	4.714	4.929	1.539	2.036	8.560	11.143	9.536	11.835	4.333	3.071	0.464
C. trilocularis	Cotton	1.375	6.094	3.321	1.714	6.107	1.036	23.792	6.286	14.500	5.071	0.417	3.821	7.536
	Fallow field	0.000	0.656	1.500	0.679	1.821	0.036	1.458	7.179	0.571	0.036	0.000	0.000	0.000
	Veld	0.000	0.000	0.000	1.964	0.107	0.000	0.000	0.000	0.821	0.107	0.083	0.000	0.000
	Total	1.375	6.750	4.821	4.357	8.036	1.071	25.250	13.464	15.893	5.214	0.500	3.821	7.536
A. austro-africanum	Cotton	4.788	0.531	0.000	0.071	0.821	0.107	0.055	0.000	0.000	2.732	0.167	0.036	0.036
	Fallow field	0.000	0.094	0.000	0.000	0.321	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000
	Veld	0.781	0.000	0.000	0.286	0.500	0.000	0.000	0.000	0.000	0.179	0.000	0.000	0.000
	Total	5.569	0.625	0.000	0.357	1.643	0.107	0.055	0.000	0.000	3.411	0.167	0.036	0.036
C. hildebrandtii	Cotton	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Fallow field	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000
	Veld	0.518	6.656	0.000	0.000	0.786	2.964	0.000	0.000	0.393	0.000	0.000	0.000	0.000
	Total	0.518	6.656	0.000	0.000	0.786	2.964	0.000	0.250	0.393	0.000	0.000	0.000	0.000

(table continued)

Plant species/ group	Localities	no. plants/10m^2												
		L1 Bt	**L2 Bt**	**L3 Bt**	**L4 Bt**	**L5 Bt**	**L6 non-Bt**	**L7 Bt**	**L8 Bt**	**L9 Bt**	**L10 Bt**	**L11 Bt**	**L12 Bt**	**L12 non-Bt**
	No. times surveyed	8	8	7	7	7	7	6	7	7	7	6	7	7
African bollworm hosts	Cotton	0.000	10.594	5.500	9.893	2.500	16.643	5.542	25.357	2.714	17.718	14.667	5.429	10.071
	Fallow field	0.000	0.000	2.179	5.321	2.000	5.143	5.833	4.893	7.500	4.179	6.208	0.000	0.000
	Veld	0.031	0.000	8.214	9.536	1.071	1.607	10.375	4.107	0.000	2.393	0.542	0.000	0.000
	Total	**0.031**	**10.594**	**15.893**	**24.750**	**5.571**	**23.393**	**21.750**	**34.357**	**10.214**	**24.289**	**21.417**	**5.429**	**10.071**
Spiny bollworm hosts	Cotton	11.098	24.250	14.000	6.039	16.786	4.893	16.693	17.286	24.607	15.250	2.604	8.536	10.750
	Fallow field	0.219	8.938	4.571	1.429	3.821	0.786	5.527	15.536	2.964	2.571	2.292	0.000	0.000
	Veld	12.514	12.469	1.500	3.679	2.246	5.786	9.333	0.679	3.529	2.393	1.875	0.000	0.000
	Total	**23.831**	**45.656**	**20.071**	**11.146**	**22.854**	**11.464**	**31.553**	**33.500**	**31.100**	**20.214**	**6.771**	**8.536**	**10.750**
Red bollworm hosts	Cotton	0.288	0.000	0.000	0.000	0.179	0.964	0.042	0.000	0.036	2.821	0.208	0.036	0.036
	Fallow field	0.000	0.000	0.000	0.000	0.000	0.357	0.292	0.000	0.000	0.500	0.125	0.000	0.000
	Veld	1.518	6.688	0.143	0.000	0.000	1.290	0.000	0.000	0.929	0.214	0.000	0.000	0.000
	Total	**1.806**	**6.688**	**0.143**	**0.000**	**0.179**	**2.611**	**0.333**	**0.000**	**0.964**	**3.536**	**0.333**	**0.036**	**0.036**
Total no. host plants in cotton fields		11.386	34.844	19.500	15.932	19.464	22.500	22.276	42.643	27.357	35.789	17.479	14.000	20.857
Total no. of host plants in fallow field, veld and non-Bt /locality (proposed refuge area)		35.139	48.951	37.464	40.821	29.996	35.826	52.218	46.071	35.779	33.107	31.899		
Total no. of host plants in fallow field and veld		14.318	28.129	16.643	20.000	9.175	15.004	31.396	25.250	14.957	12.286	11.077		
Total no. cotton plants /10m^2		35	35	35	35	35	35	35	35	35	35	35		

Table 6.6. The calculated numbers of bollworms that can occur on a number of weed plants with the same biomass (measured in grams dry mass) either in a cotton field or in the veld.

Plant species	Mean biomass of weed species in a cotton field Dry mass (g)	No. of weed plants equivalent to one cotton plant in a cotton field (biomass/biomass)	Mean biomass of weed species in the veld Dry mass (g)	No. of weed plants equivalent to one cotton plant in the veld (biomass/ biomass)	Mean no. bollworms / plant	Calculated no. of bollworms found on weed plants in a cotton field with same biomass of a single cotton plant	Calculated no. of bollworms on weed plants found in the veld with the same biomass as a single cotton plant
Non-Bt-cotton	56.795	1.000	*	*	0.953	0.953	*
A. guineense	21.667	2.621	32.176	1.765	0.558	1.463	0.985
A. sonneratianum	5.000	11.359	11.500	4.939	0.244	2.777	1.207
A. hispidum	7.750	7.328	4.750	11.957	0.020	0.146	0.238
C. hildebrandtii	0.000	*	17.000	3.341	0.517	*	1.726
C. trilocularis	6.159	9.222	3.500	16.227	0.009	0.080	0.141
H. calyphyllus	24.000	2.366	15.750	3.606	0.727	1.721	2.623
H. cannabinus	6.250	9.087	171.000	0.332	0.024	0.219	0.008
H. praeteritus	12.000	4.733	11.833	4.800	0.362	1.711	1.735
H. vitifolius	17.000	3.341	28.500	1.993	0.976	3.259	1.944
M. forbesii	*	*	19.000	2.989	0.091	*	0.272
S. cordifolia	23.333	2.434	8.983	6.322	0.063	0.154	0.399
S. rhombifolia	10.500	5.409	*	*	0.111	0.603	*

Table 6.7. The total surface area (m^2) of the study points at each locality during the host plant study 2001/2002.

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12 Bt	L12 non-Bt
Cotton land	25176	46130	76627	7405	7489	4064	80310	44971		6902	25176	26909	24002
Fallow field				8560	3092		25538		2557	16949	9503		
Veld	40550	137323	70380	25630	3270		92287	51611	77961	9726	6427		

Table 6.8. The number of bollworms calculated for each species/species group in each study area at each study point during the host plant study 2001/2002.

Plant group		A. guineense group			Hibiscus group			Sida group			C. trilocularis			A. austro-africanum			C. hildebrandtii			Total
Localities		cotton land	fallow field	veld	cotton land	fallow field	veld	cotton land	fallow field	veld	cotton land	fallow field	veld	cotton land	fallow field	veld	cotton land	fallow field	veld	
L1	African	2			1		1				7								6	17
L1	Red	2																	147	149
L1	Spiny	503			110		84	250		88	12			2169		570				3786
L2	African	3		14	14		17				56			10			274			388
L2	Red	3		14													6398			6415
L2	Spiny	570		2949	1218		1433	1403			98			441						8112
L3	African	2			96	5					51									154
L3	Red	2																		2
L3	Spiny	376		43	8087	424		966		8	89									9993
L4	African	1	0	1	1						3	1	10	0	3					20
L4	Red	1	0	1																2
L4	Spiny	87	83	220	75			86	10	91	4	2	18	9	132					817
L5	African	6	0	0	0		0				9	1	0	3	0			1		20
L5	Red	6	0	0														18		24
L5	Spiny	1245	70	1	5		8	23	6		16	2	0	110	18					1504
L6	African	0			0						1			0						1
L6	Red	0																		0
L6	Spiny	70			13			25			1			8						117
L7	African	41			1						382			2						426
L7	Red	41																		41
L7	Spiny	8830			113			1374			669			80						11066
L8	African	1			7		10				57									75
L8	Red	1																		1
L8	Spiny	110			650		876	1356			99									3091
L9	African		1	1			5					0	13						9	29
L9	Red		1	1															214	216
L9	Spiny		118	174			441		10	28		1	22							794

(table continued)

Plant group		A. guineense group			Hibiscus group			Sida group			C. trilocularis			A. austro-africanum			C. hildebrandtii			Total
Localities		cotton land	fallow field	veld	cotton land	fallow field	veld	cotton land	fallow field	veld	cotton land	fallow field	veld	cotton land	fallow field	veld	cotton land	fallow field	veld	
L10	African	1	1	0	2		1				7	0	0	8	3	1				**24**
L10	Red	1	1	0																**2**
L10	Spiny	305	182	1	147		49	239	30	26	12	0	0	339	153	31				**1514**
L11	African	1	0	0			1				2		0	2						**6**
L11	Red	1	0	0																**1**
L11	Spiny	162	36	3			80	114	59	23	4		0	76						**557**
L12 Bt	African	3			1						21			0						**25**
L12 Bt	Red	3																		**3**
L12 Bt	Spiny	627			118			273			36			17						**1071**
L12 non-Bt	African	3			3						36			0						**42**
L12 non-Bt	Red	3																		**3**
L12 non-Bt	Spiny	707			256			37			63			15						**1078**

Note: Bollworm larvae numbers were only calculated if the surface area, plant density and average bollworm larvae per plant were available.

Table 6.9. The total calculated number of bollworm larvae that could be found over the season at each locality during the host plant study 2001/2002.

Locality	African bollworm	Red bollworm	Spiny bollworm
L1	17	149	3786
L2	388	6415	8112
L3	154	2	9993
L4	20	2	817
L5	20	24	1504
L6	1	0	117
L7	426	41	11066
L8	75	1	3091
L9	29	216	794
L10	24	2	1514
L11	6	1	557
L12 bt	25	3	1071
L12 non	42	3	1078
Total	**1227**	**6859**	**43500**

Table 6.10. The mean number of bollworm larvae calculated for the non-transgenic cotton refuge area in comparison with a representative refuge area of some of the weed species during the host plant study 2001/2002.

Mean no. of larvae per 0.05 ha non-Bt-cotton (= 5% refuge)	Mean no. of larvae in 0.95 ha Bt-cotton (= 95% non refuge)	Weed species	Mean no. of larvae per 0.05 ha veld	Mean no. of larvae per 0.05 ha fallow field	Mean no. of larvae per 0.05 ha plant species within non-Bt-cotton
79.0	21.2	A. guineense group	16.8	152.2	14.6
79.0	21.2	Hibiscus group	0.0	0.2	0.0
79.0	21.2	Sida group	63.8	141.1	1.4
79.0	21.2	C. trilocularis	7.1	12.3	0.0
79.0	21.2	A. austro-africanum	8.9	5.9	0.1
79.0	21.2	C. hildebrandtii	26.0	0.2	0.0

Figures

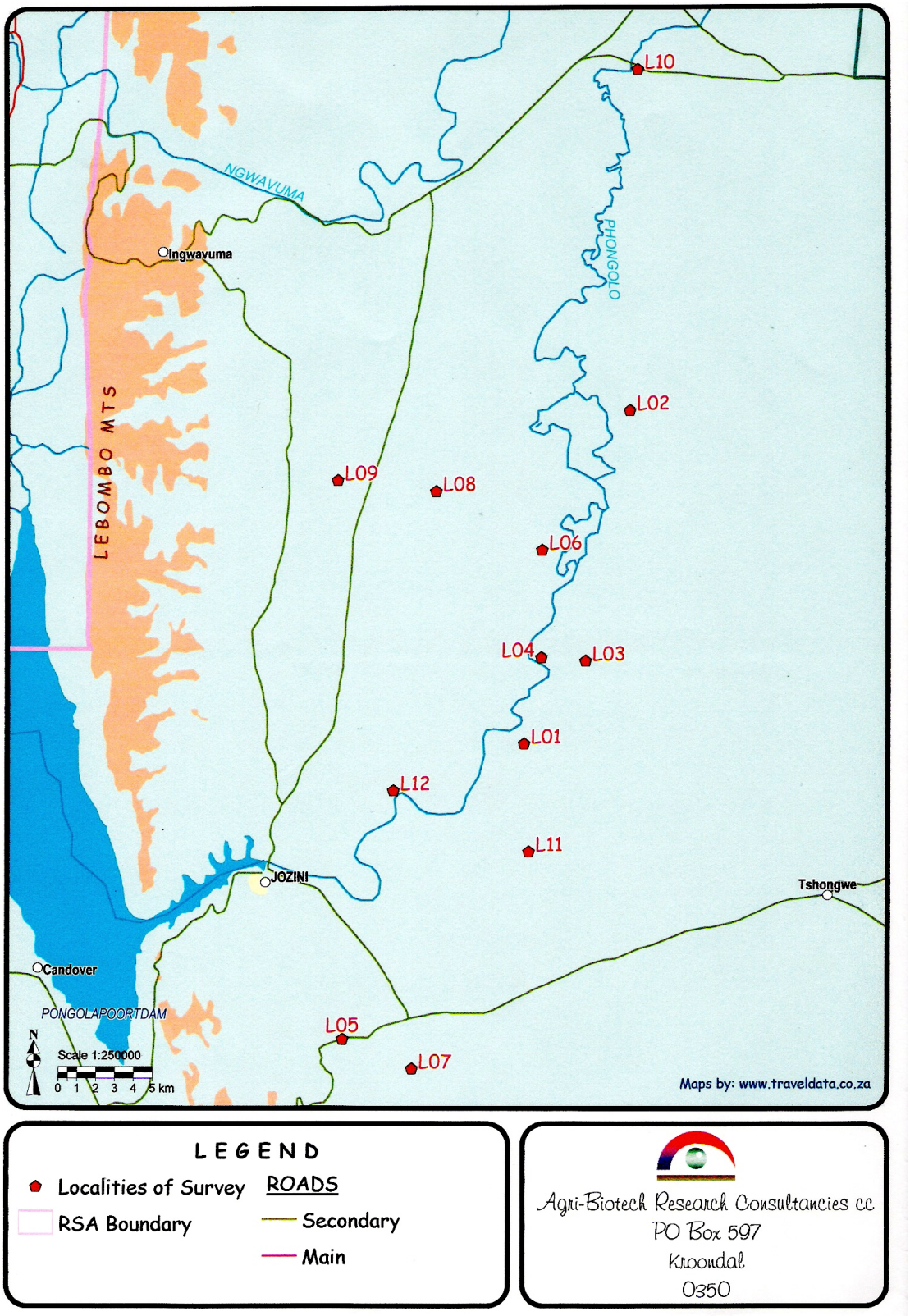


Fig. 6.1. Study areas on the Makhathini Flats – KwaZulu Natal, RSA.

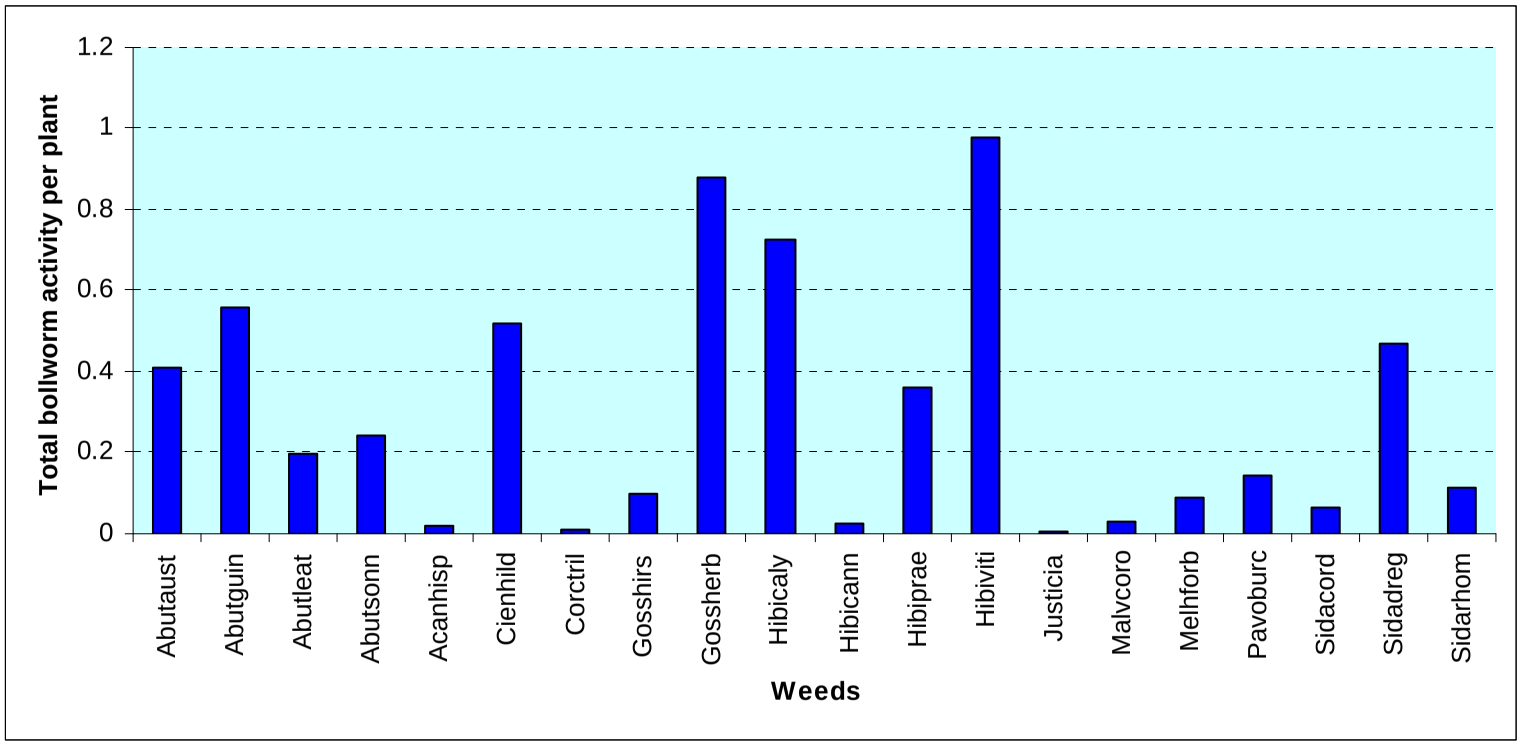


Fig. 6.2. Total bollworm activity per plant over the season during the host plant study 2001/2002.

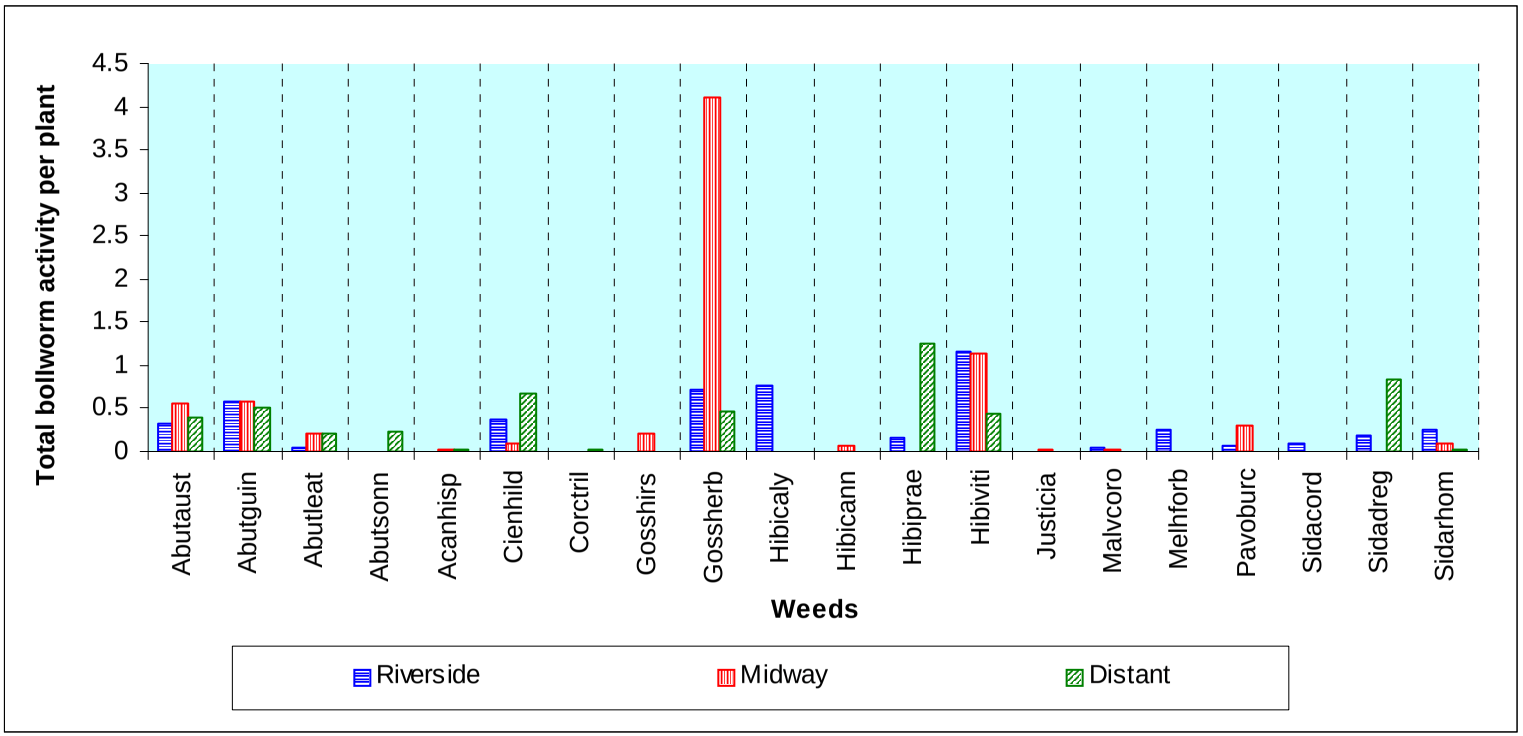


Fig. 6.3. Total bollworm activity per plant in the three study zones during the host plant study 2001/2002.

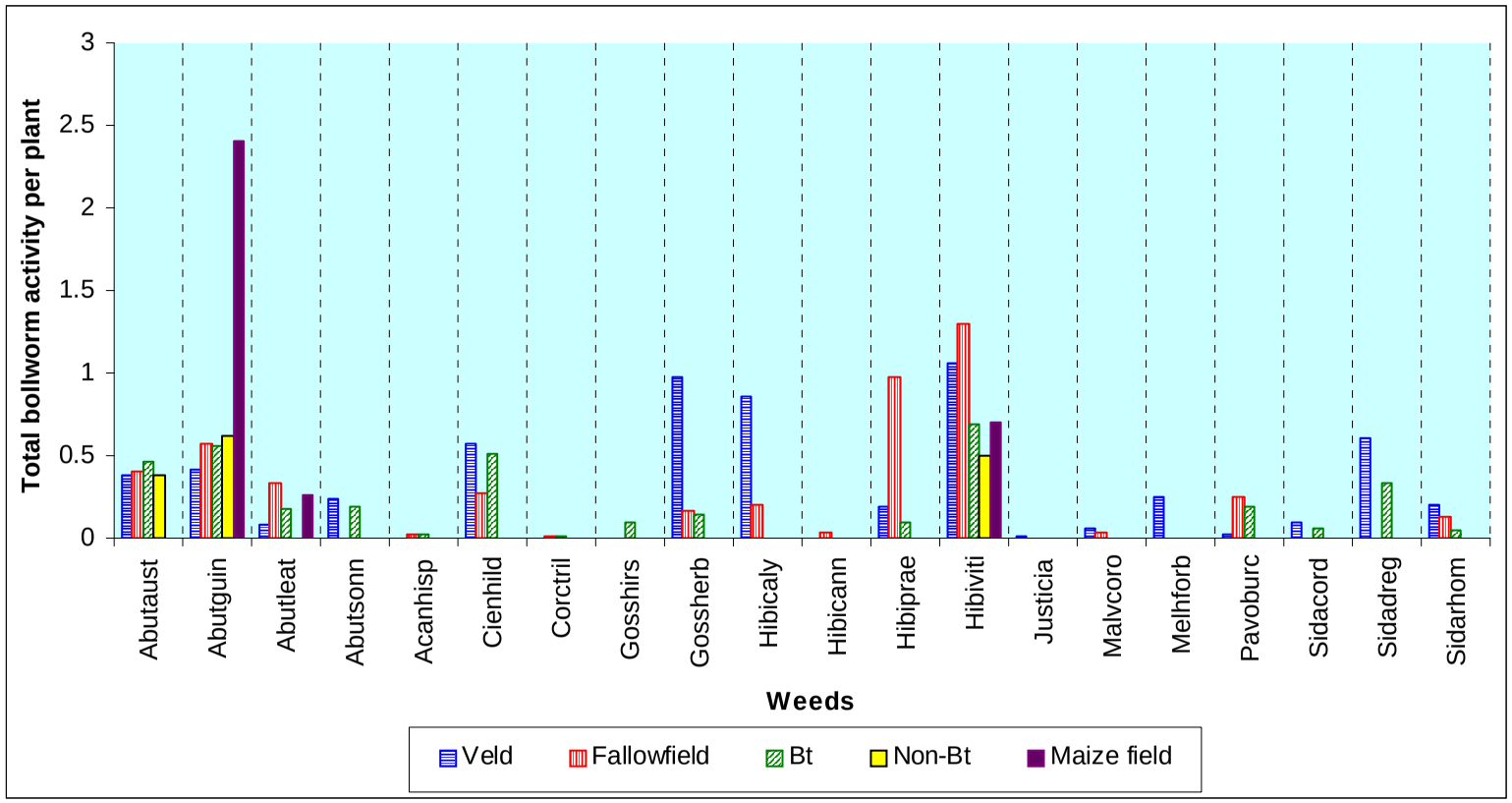


Fig. 6.4. Total bollworm activity per plant in the natural veld, fallow field, cotton fields and maize fields during the host plant study 2001/2002.

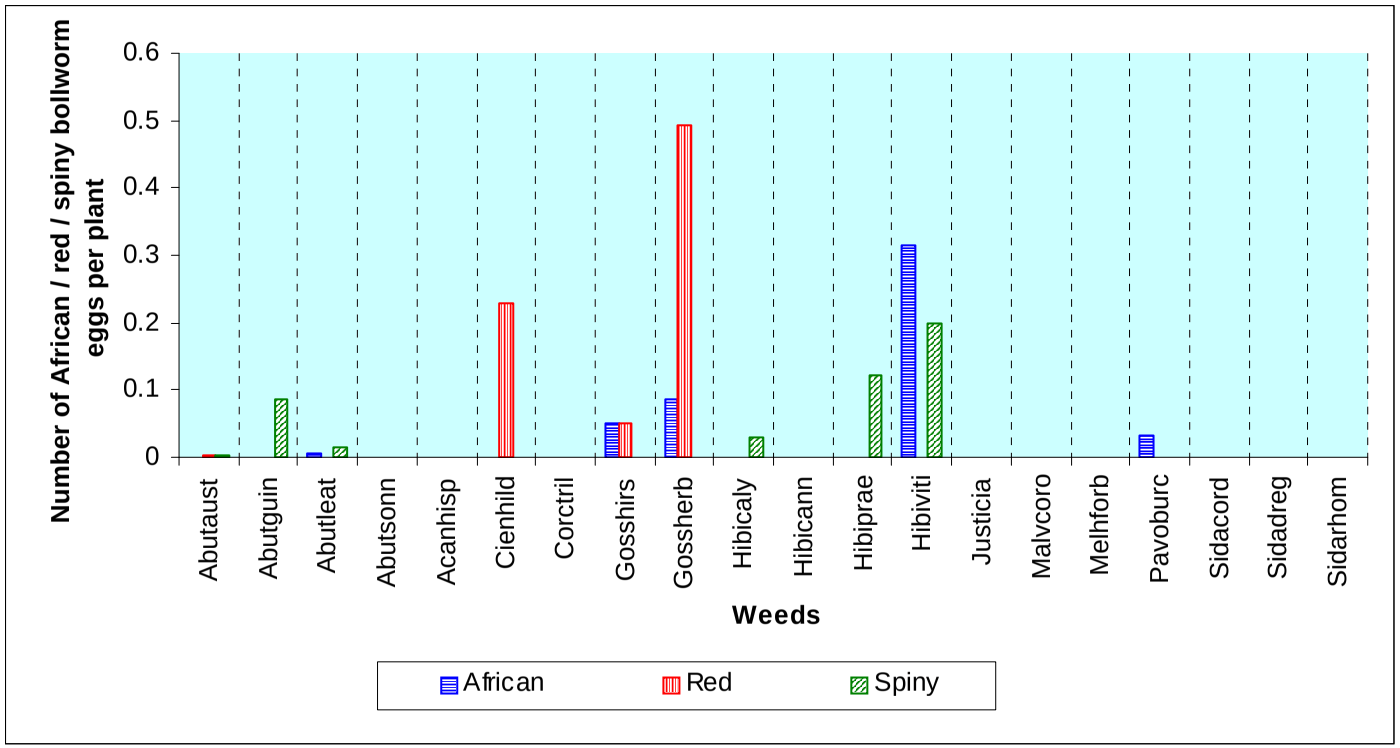


Fig. 6.5. The mean number of african, red and spiny bollworm eggs per plant over the season during the host plant study 2001/2002.

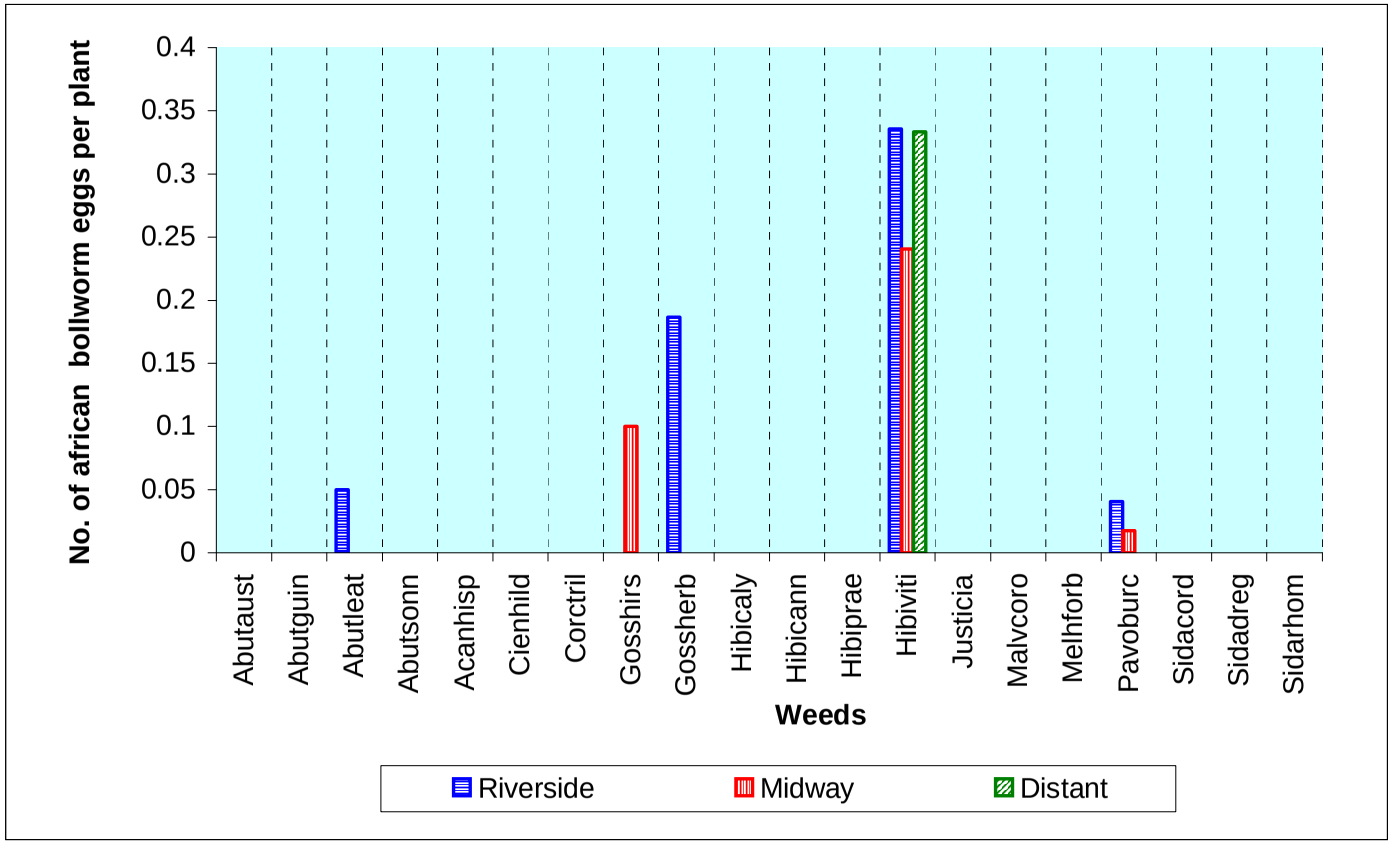


Fig. 6.6. The mean number of african bollworm eggs per plant in the three study zones during the host plant study 2001/2002.

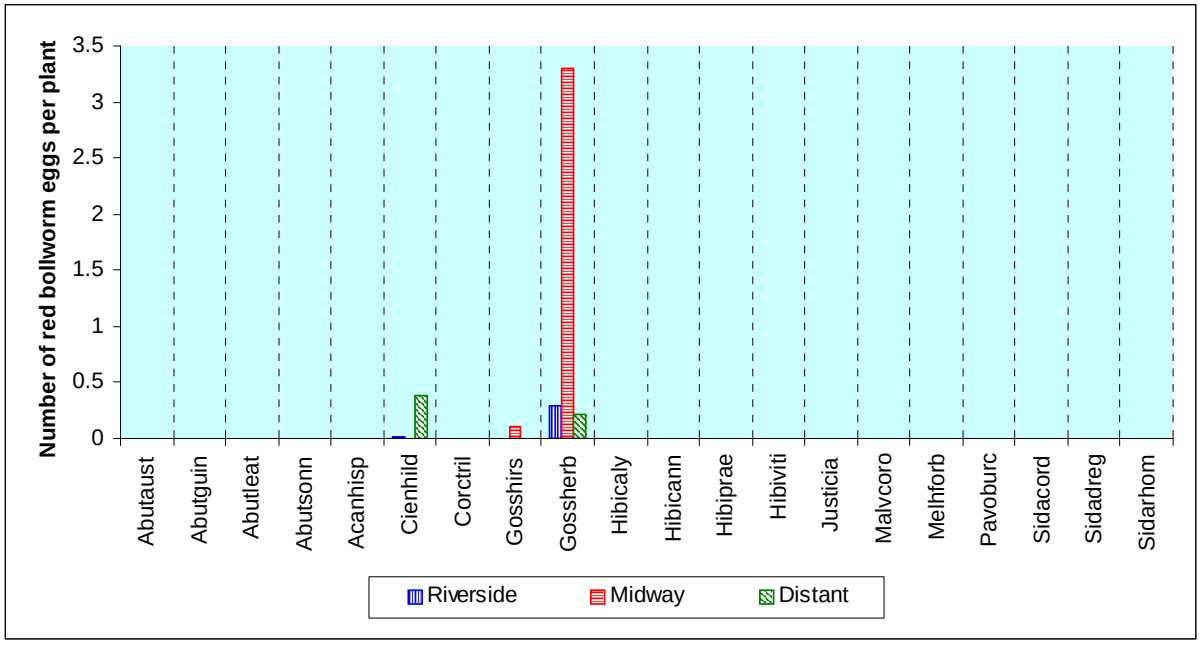


Fig. 6.7. The mean number of red bollworm eggs per plant in the three study zones during the host plant study 2001/2002.

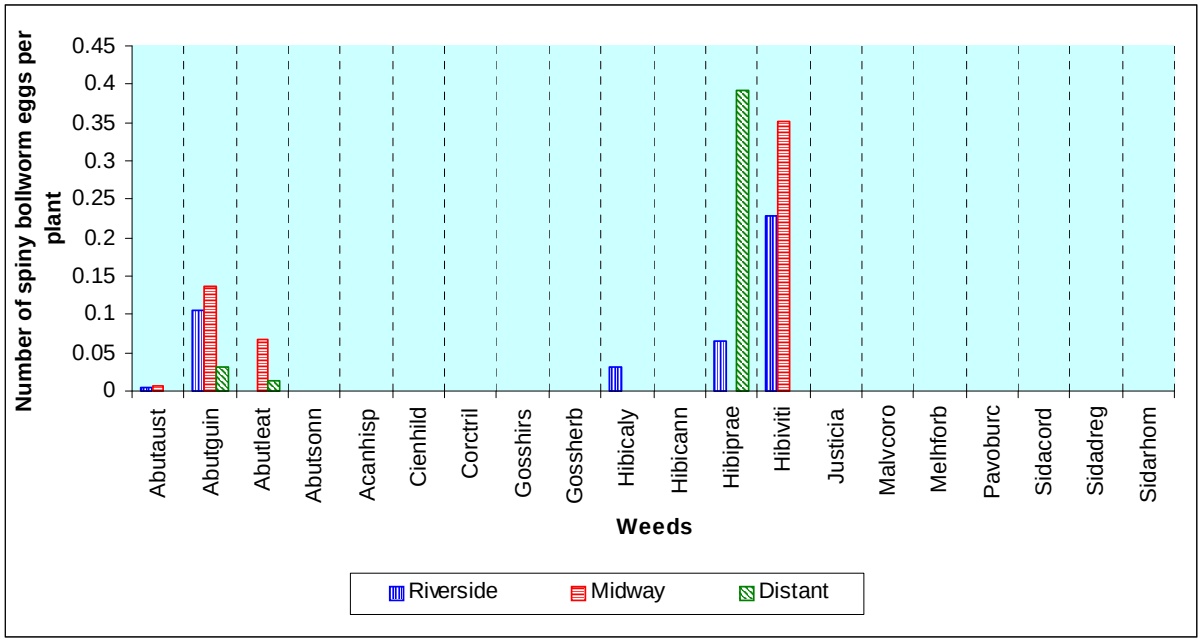


Fig. 6.8. The mean number of spiny bollworm eggs per plant in the three study zones during the host plant study 2001/2002.

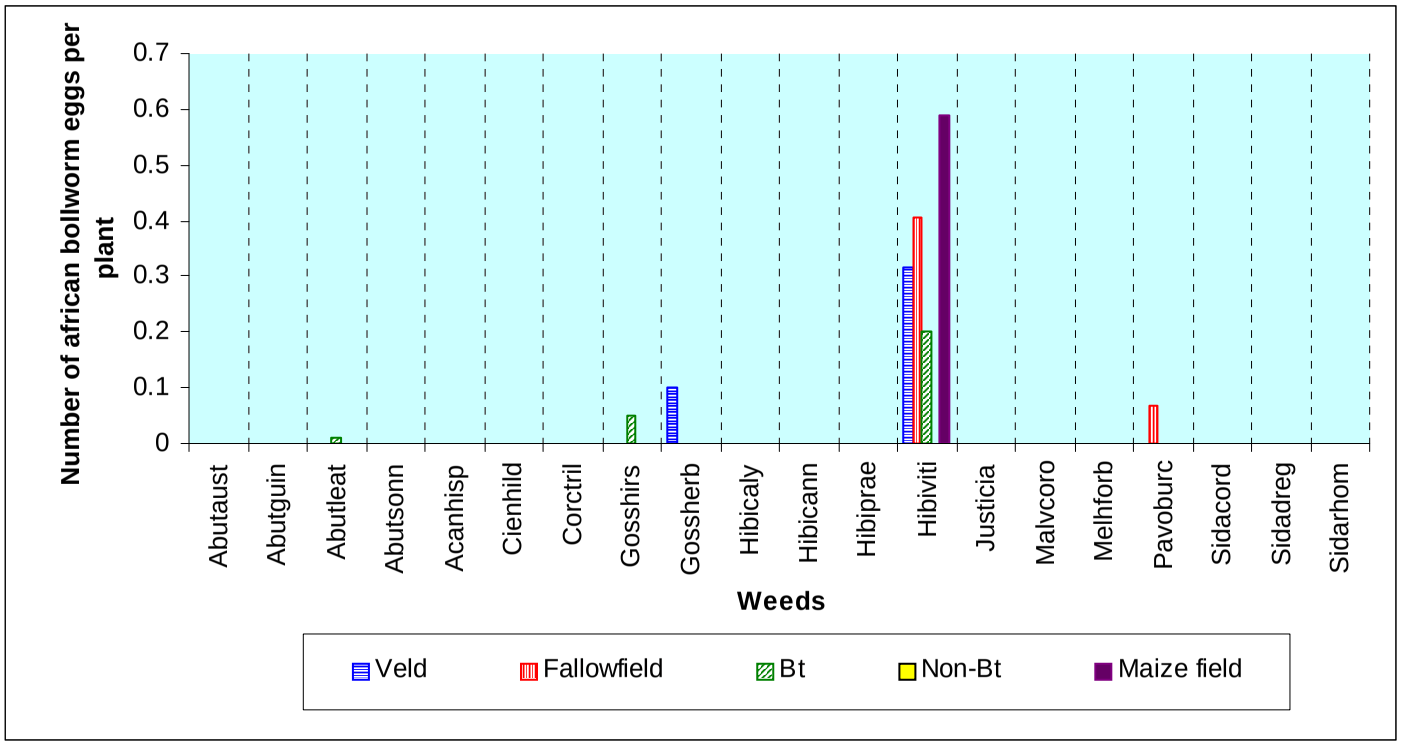


Fig. 6.9. The mean number of african bollworm eggs per plant in the natural veld fallow field, cotton fields and maize fields during the host plant study 2001/2002.

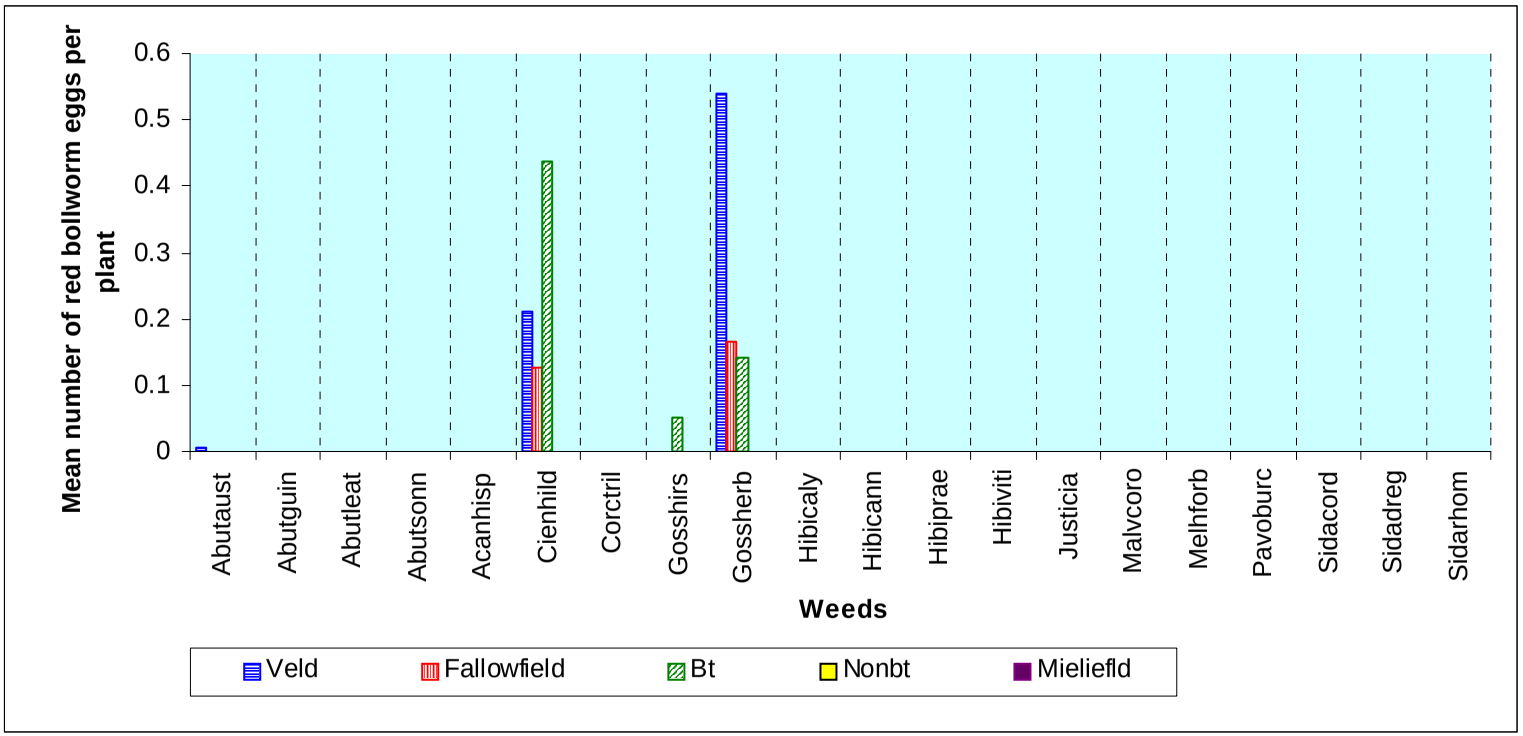


Fig. 6.10. The mean number of red bollworm eggs per plant in the natural veld, fallow field, cotton fields and maize fields during the host plant study 2001/2002.

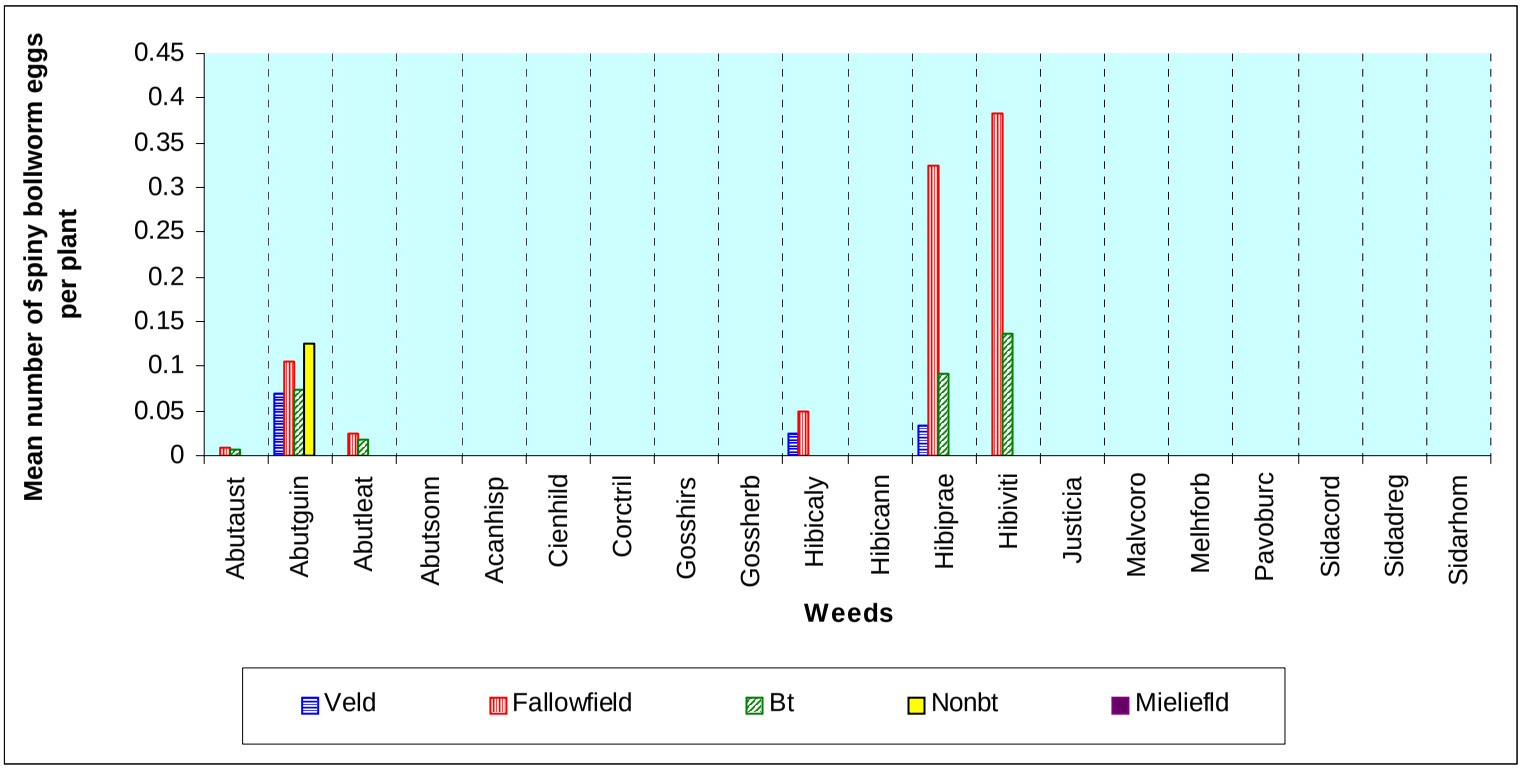


Fig. 6.11. The mean number of spiny bollworm eggs per plant in the natural veld, fallow field, cotton fields and maize fields during the host plant study 2001/2002.

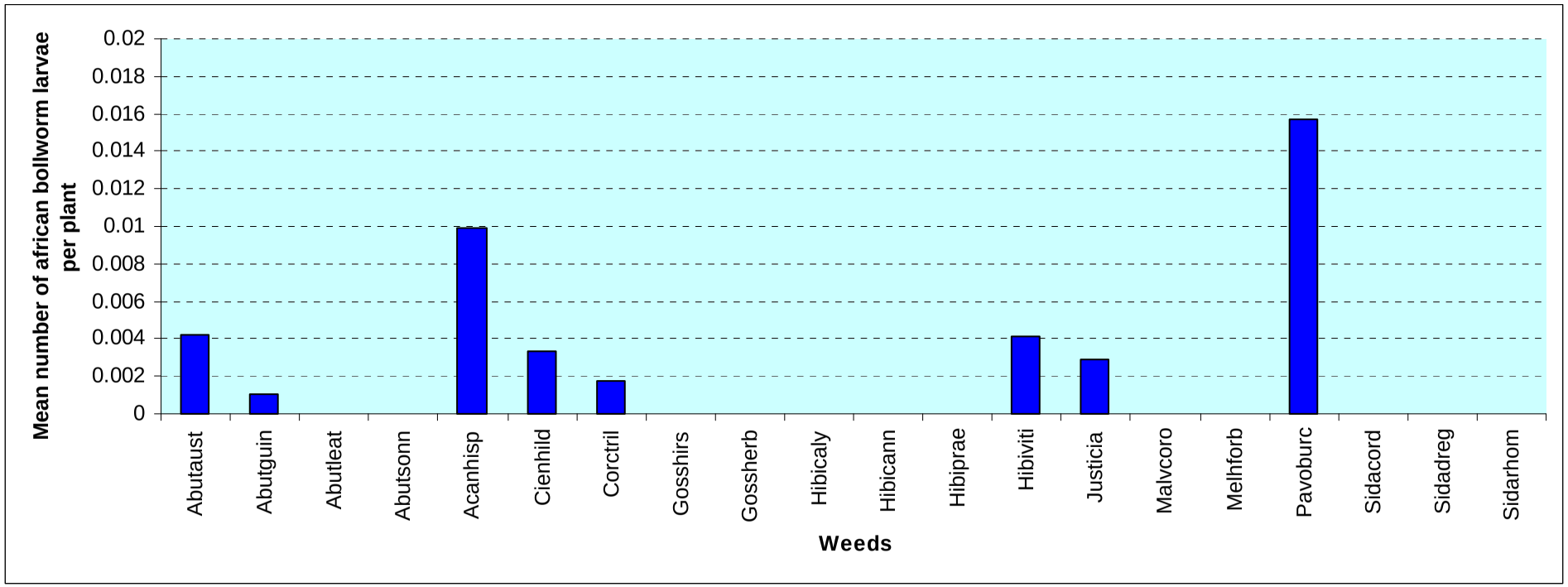


Fig. 6.12. The mean number of african bollworm larvae per plant over the season during the host plant study 2001/2002.

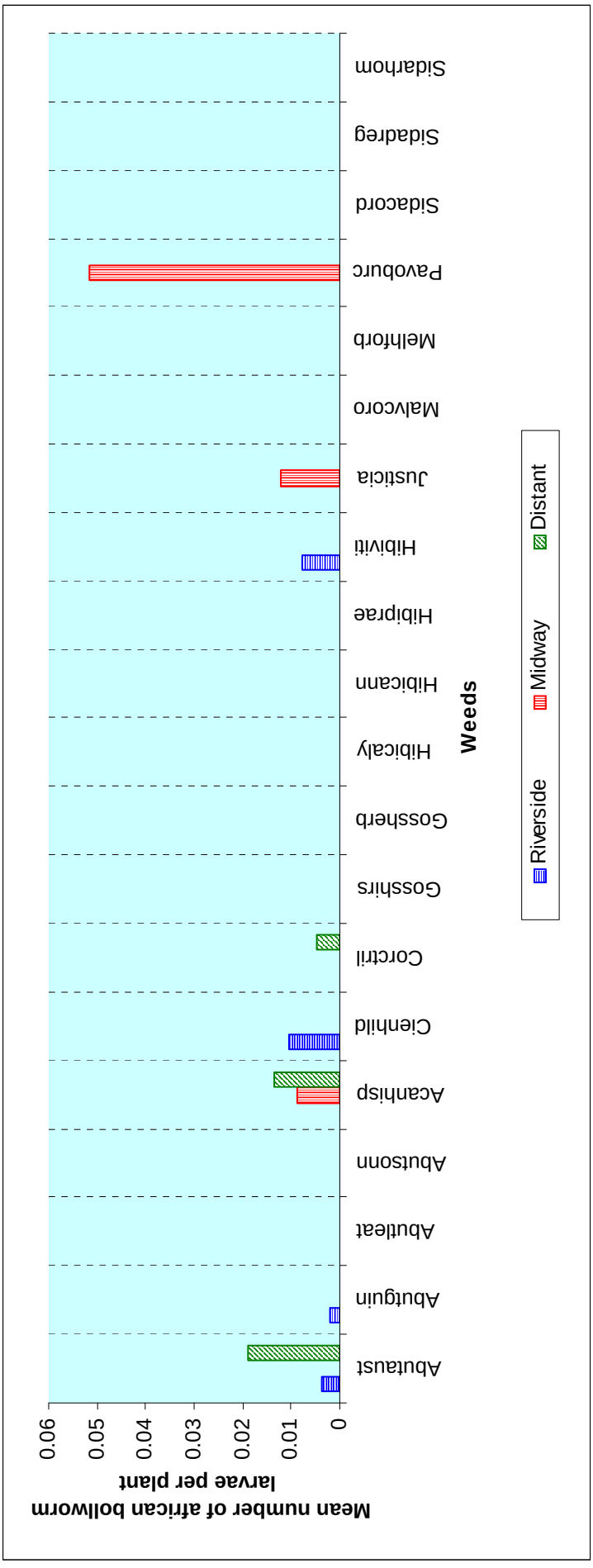


Fig. 6.13. The mean number of african bollworm larvae per plant in the three study zones during the host plant study 2001/2002.

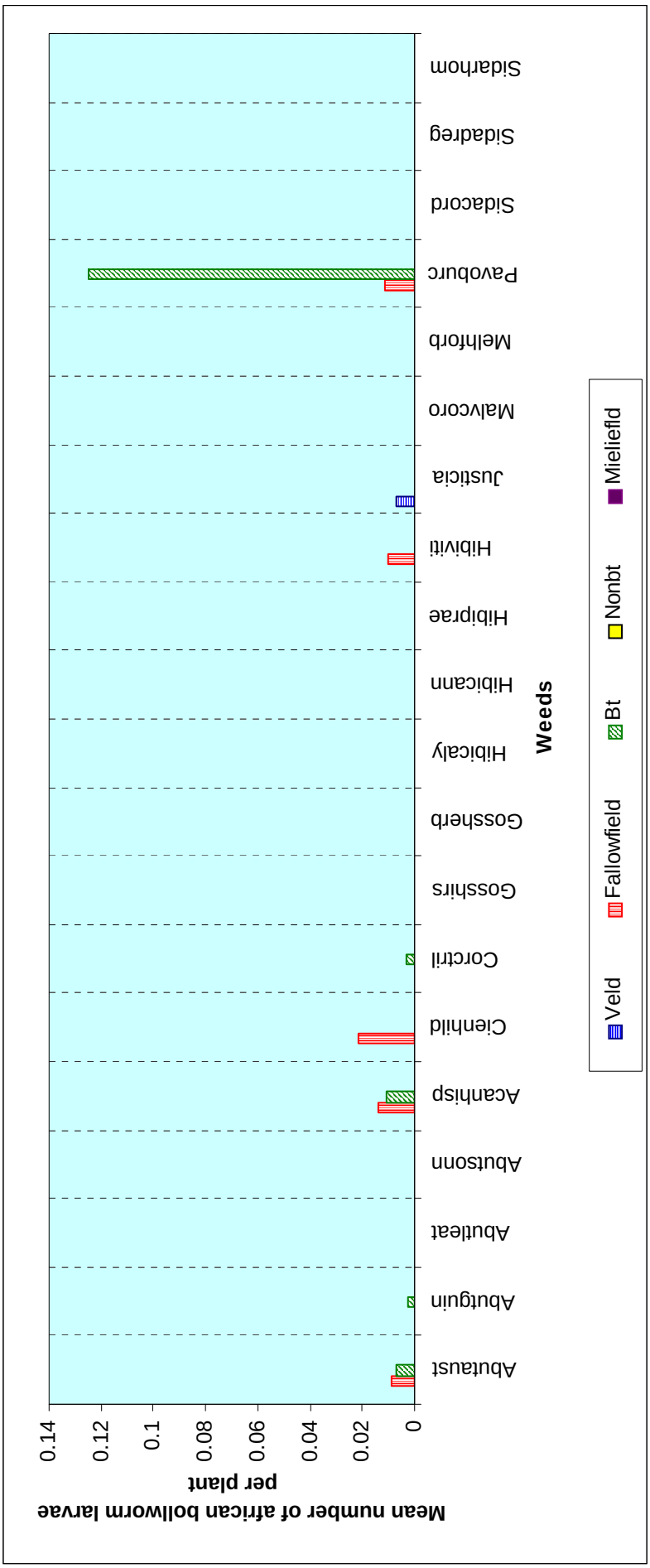


Fig. 6.14. The mean number of african bollworm larvae per plant in the natural veld, fallow field, cotton fields and maize fields during the host plant study 2001/2002.

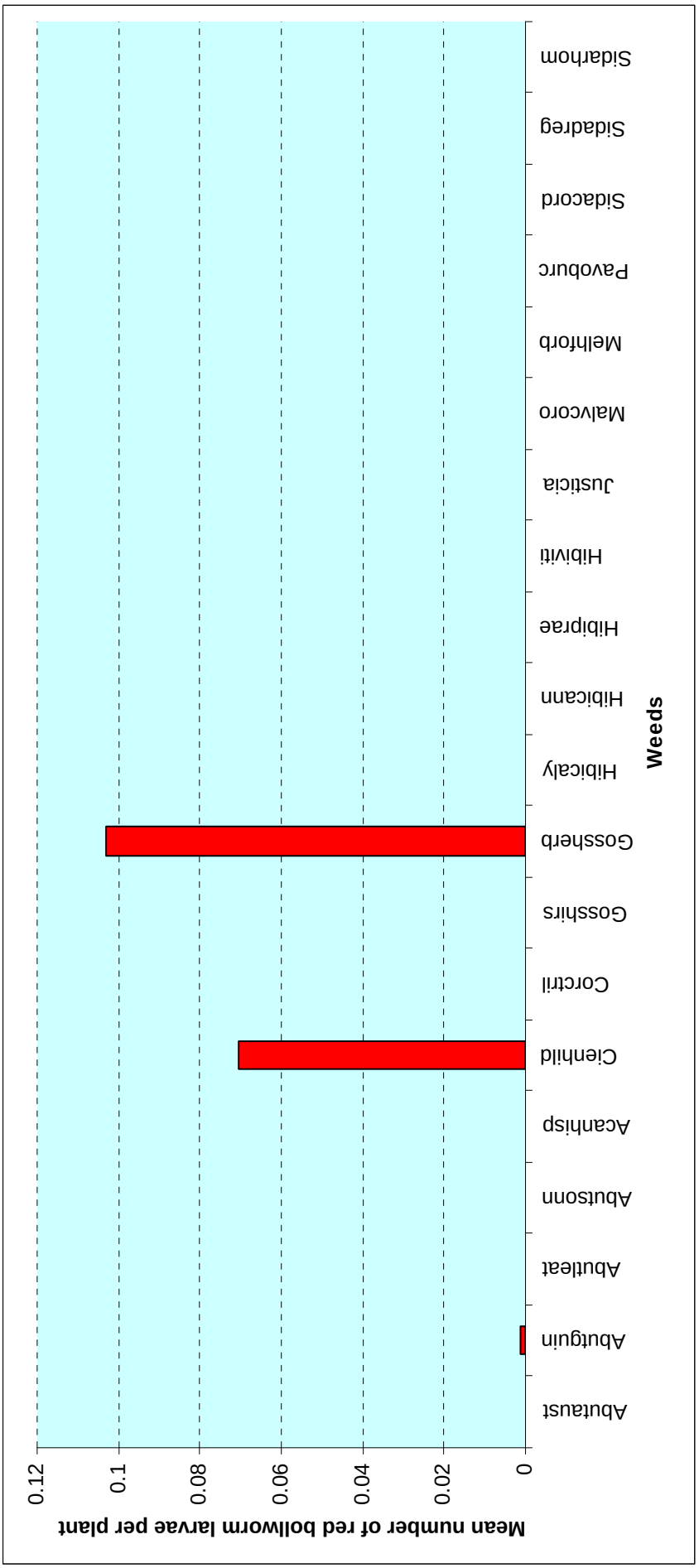


Fig. 6.15. The mean number of red bollworm larvae per plant over the season during the host plant study 2001/2002.

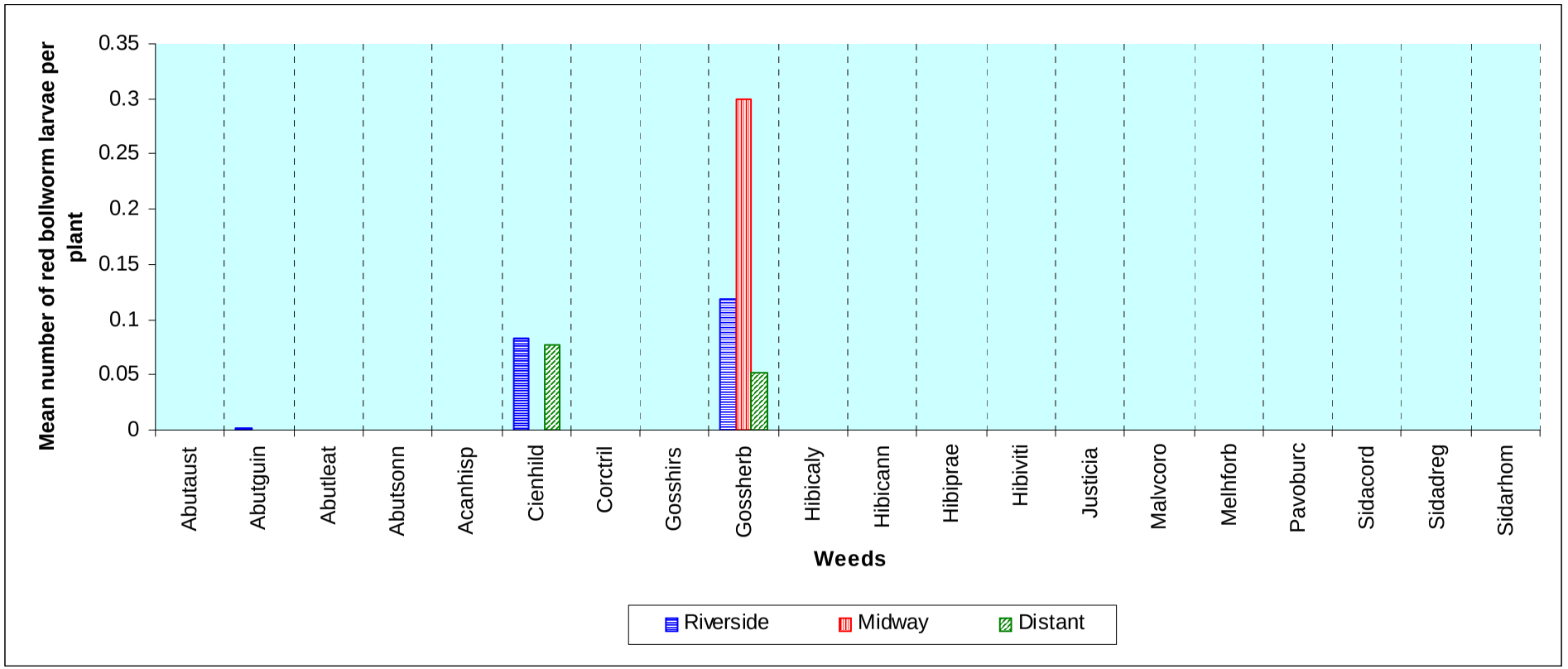


Fig. 6.16. The mean number of red bollworm larvae per plant in the three study zones during the host plant study 2001/2002.

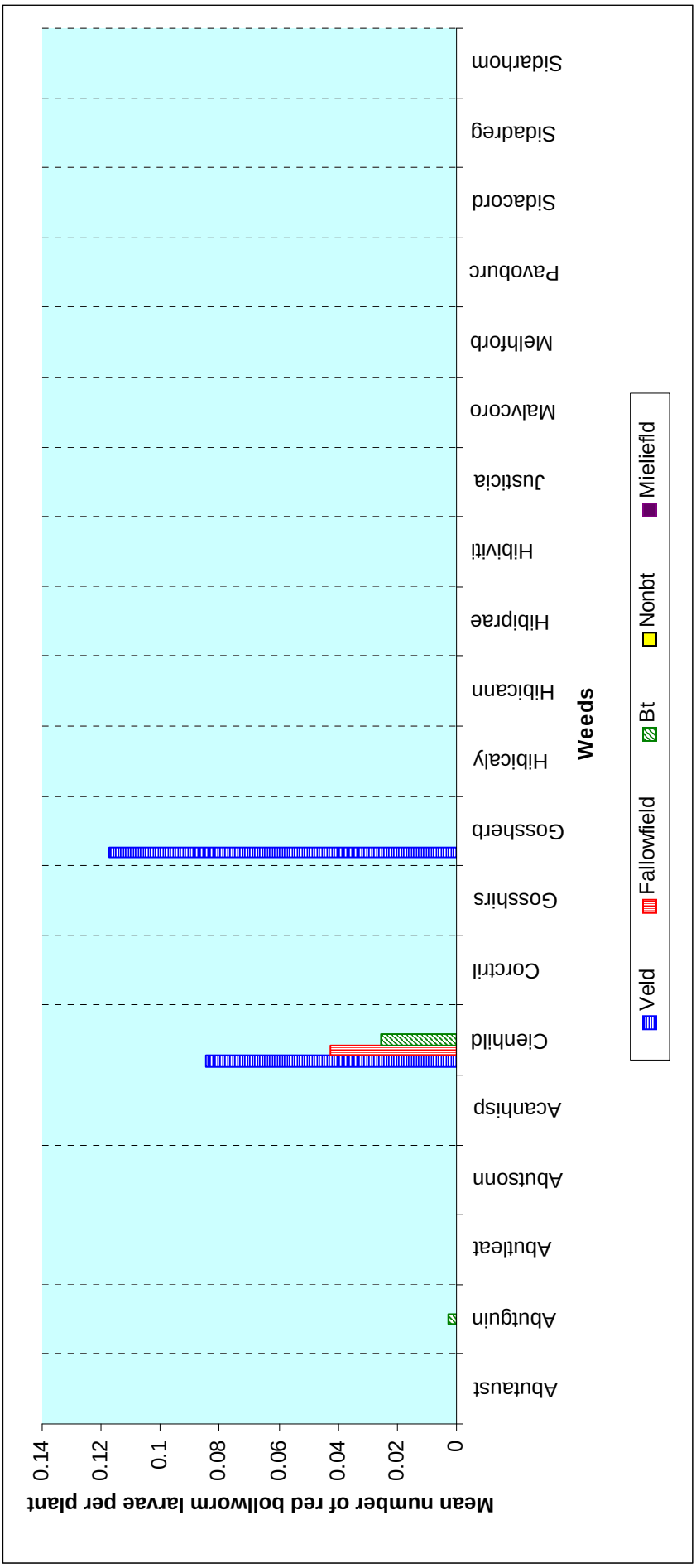


Fig. 6.17. The mean number of red bollworm larvae per plant in the natural veld, fallow field, cotton fields and maize fields during the host plant study 2001/2002.

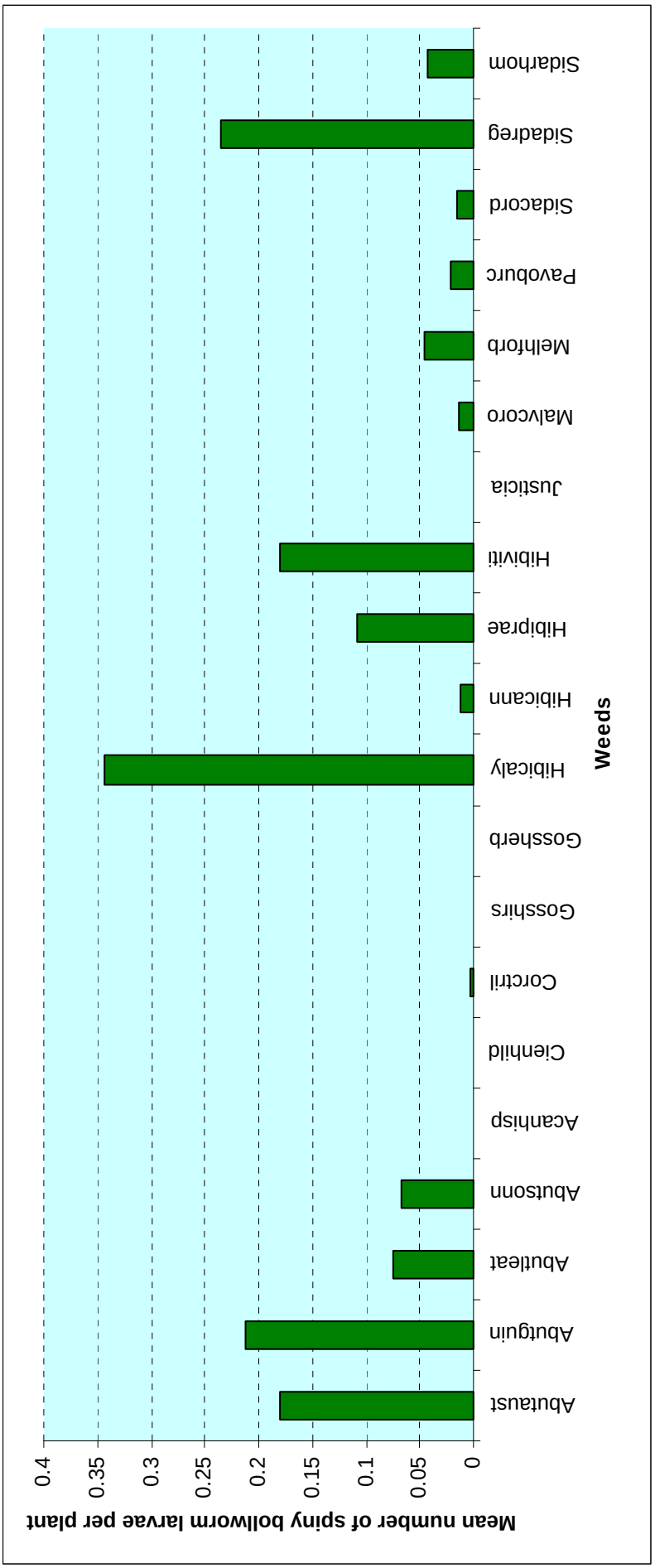


Fig. 6.18. The mean number of spiny bollworm larvae per plant over the season during the host plant study 2001/2002.

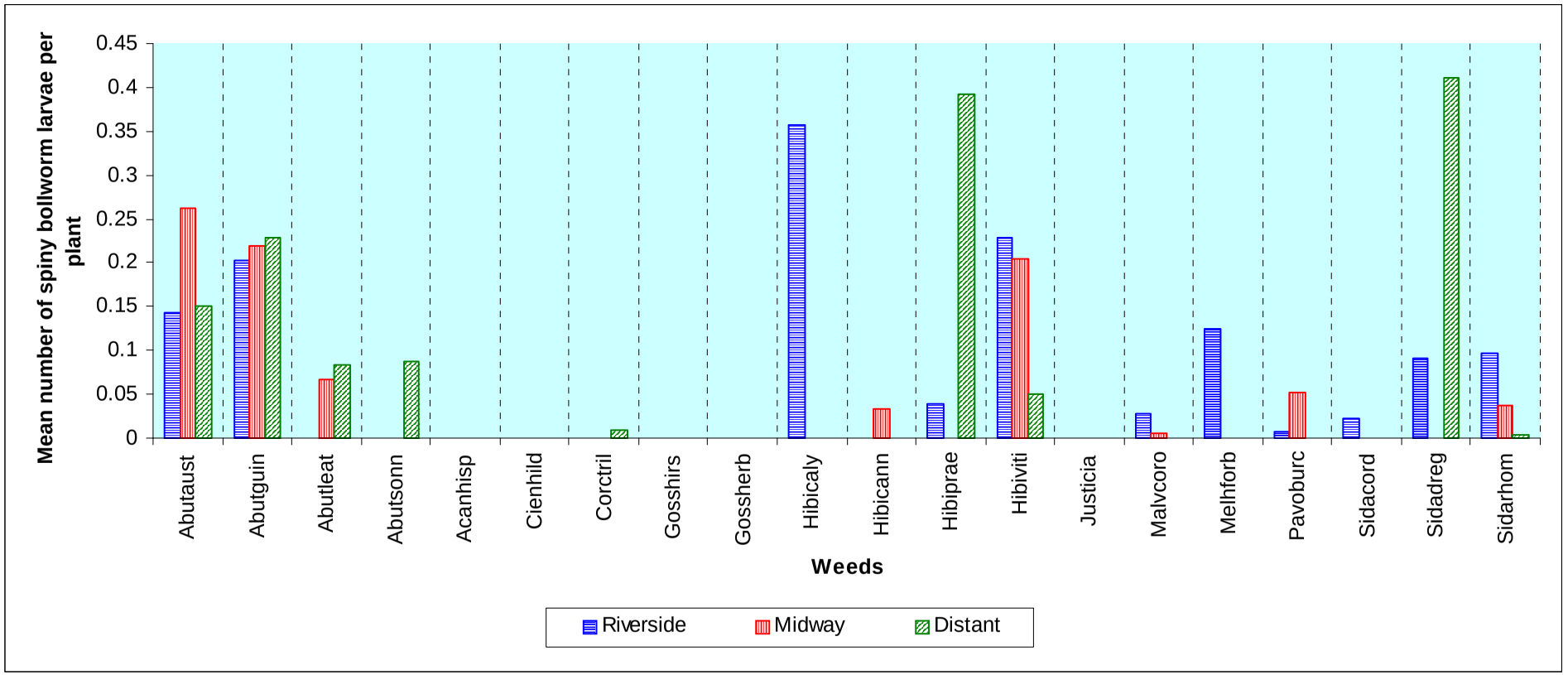


Fig. 6.19. The mean number of spiny bollworm larvae per plant in the three study zones during the host plant study 2001/2002.

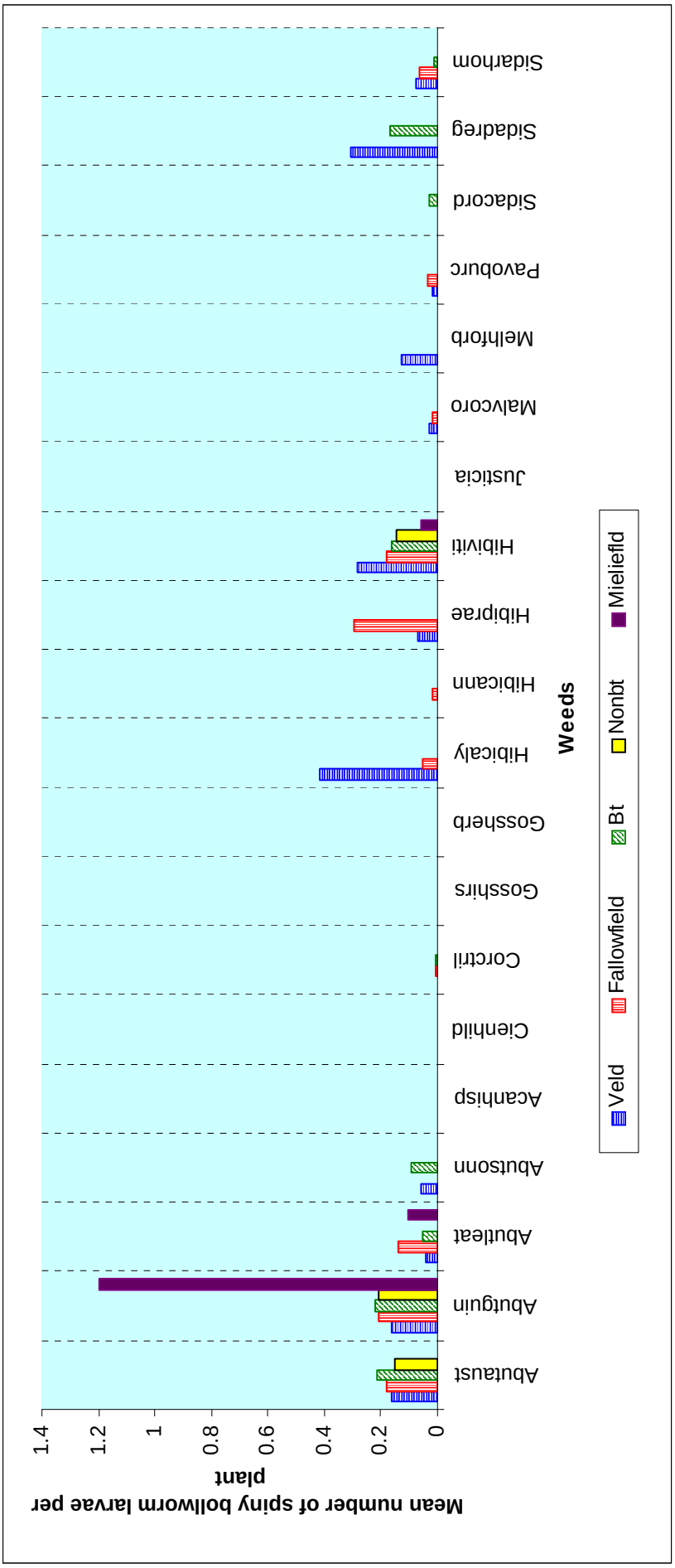


Fig. 6.20. The mean number of spiny bollworm larvae per plant in the natural veld, fallow field, cotton fields and maize fields during the host plant study 2001/2002.

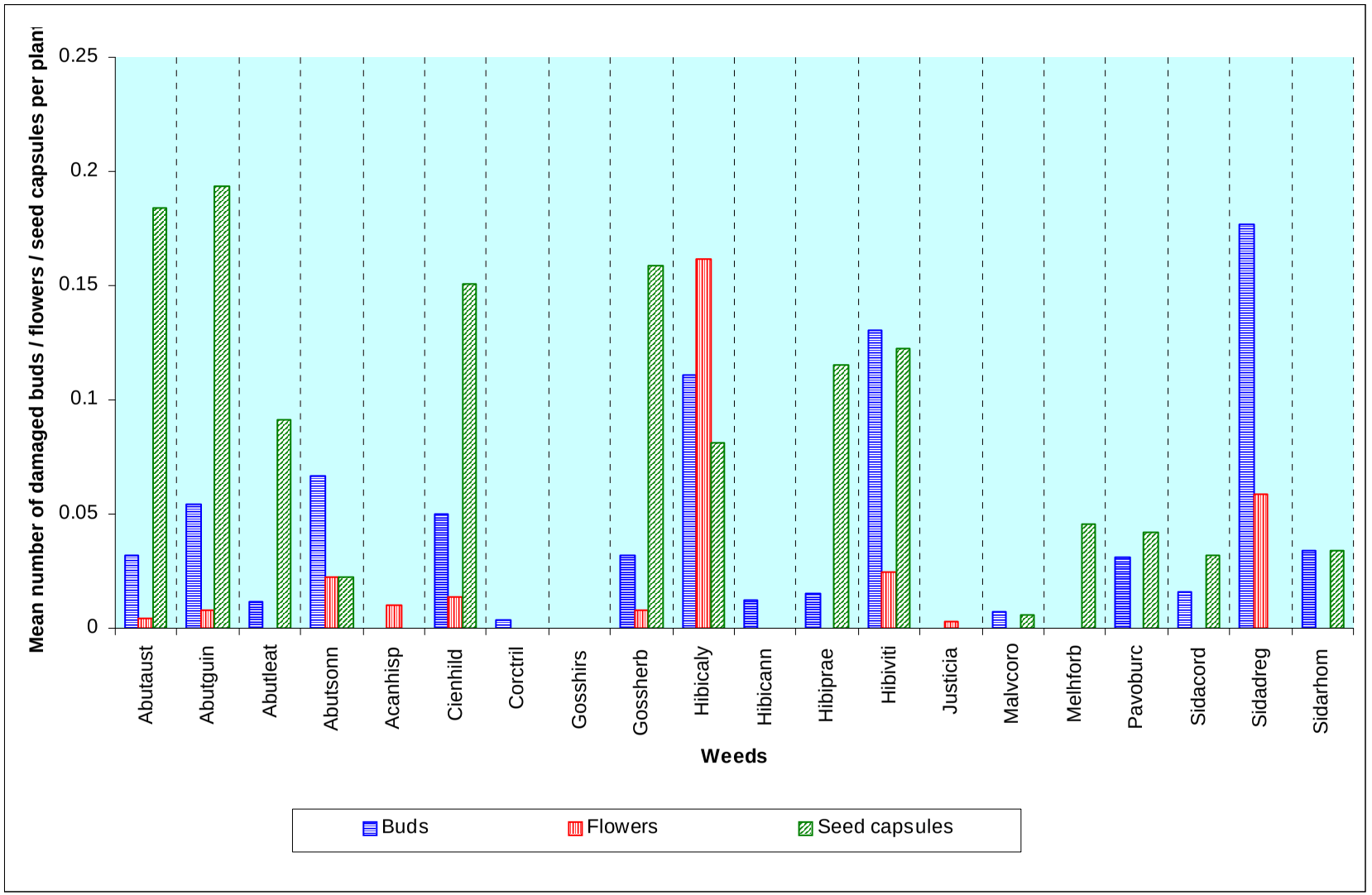


Fig. 6.21. The mean number of damaged buds, flowers and seed capsules per plant over the season during the host plant study 2001/2002.

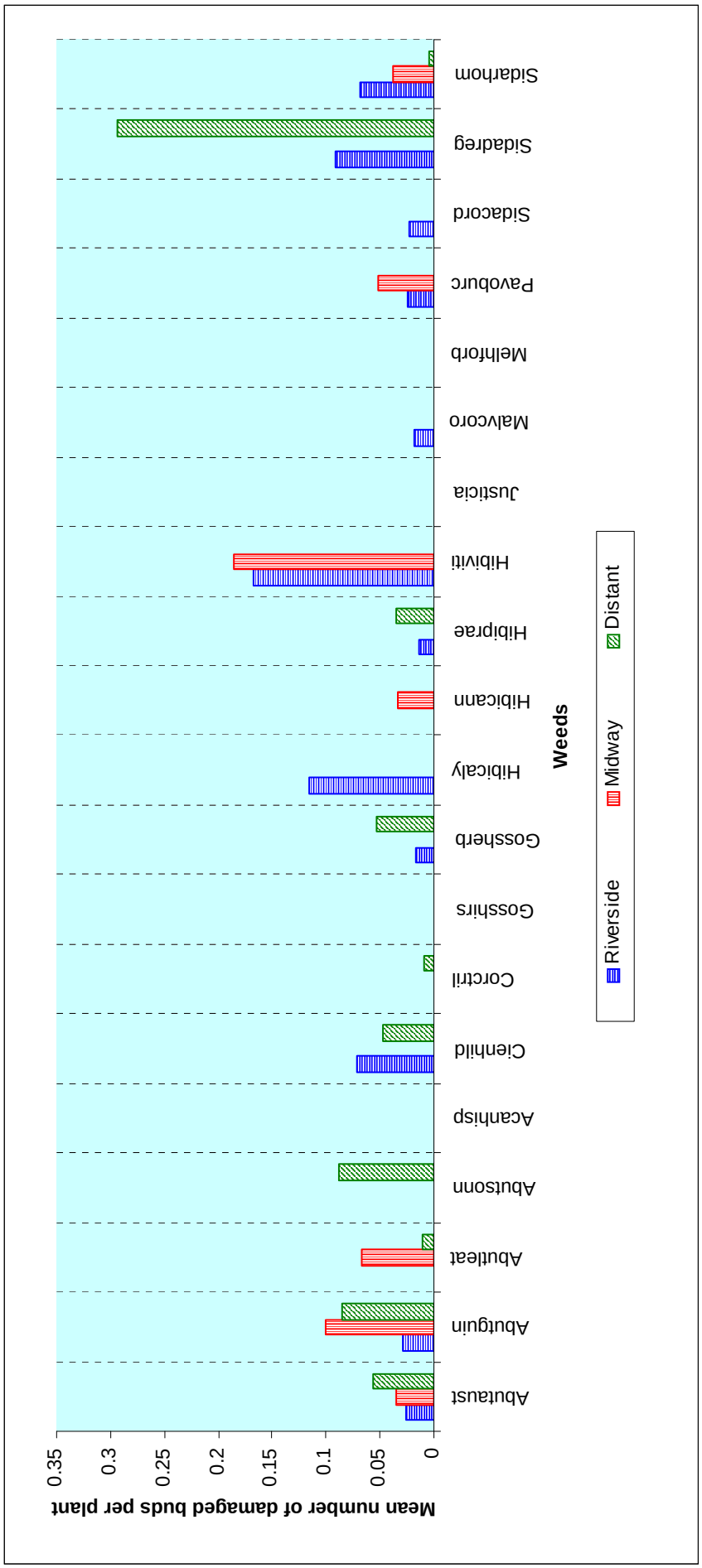


Fig. 6.22. The mean number of damaged buds per plant in the three study zones during the host plant study 2001/2002.

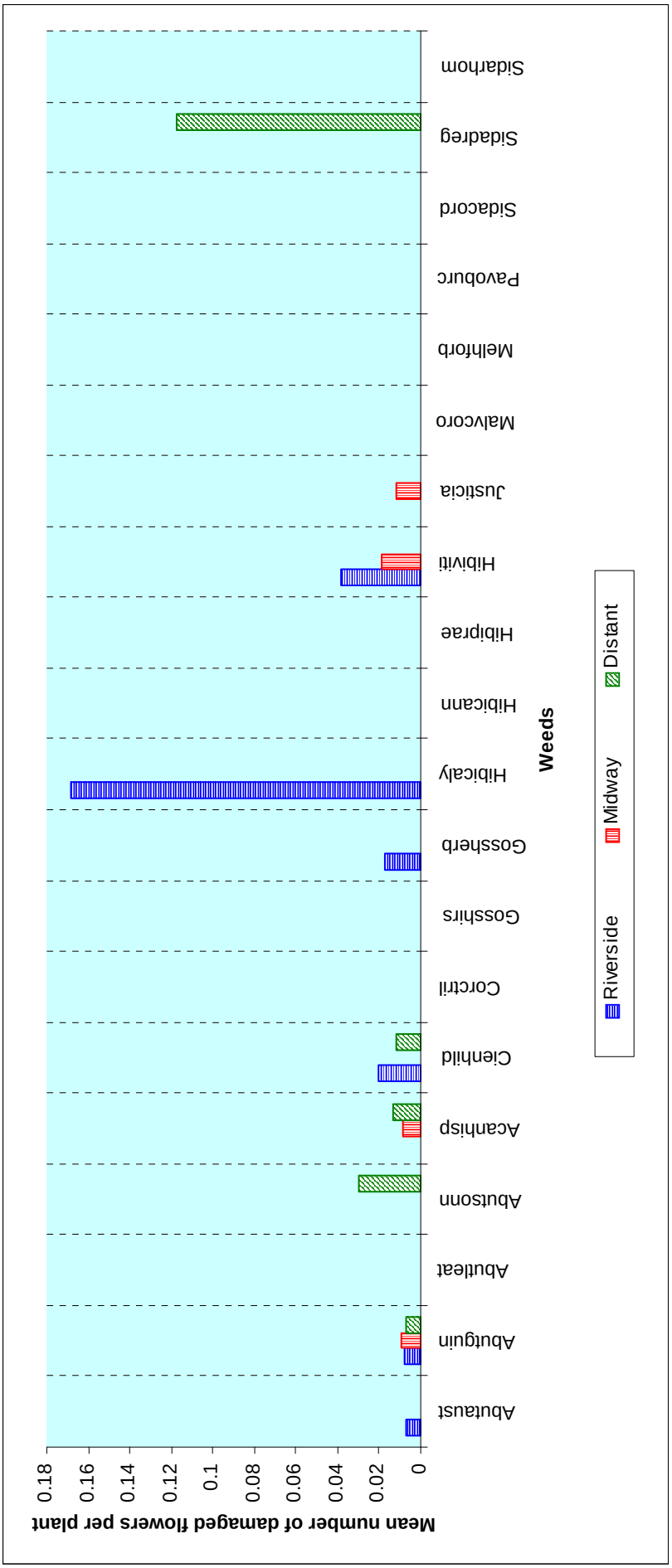


Fig. 6.23. The mean number of damaged flowers per plant in the three study zones during the host plant study 2001/2002.

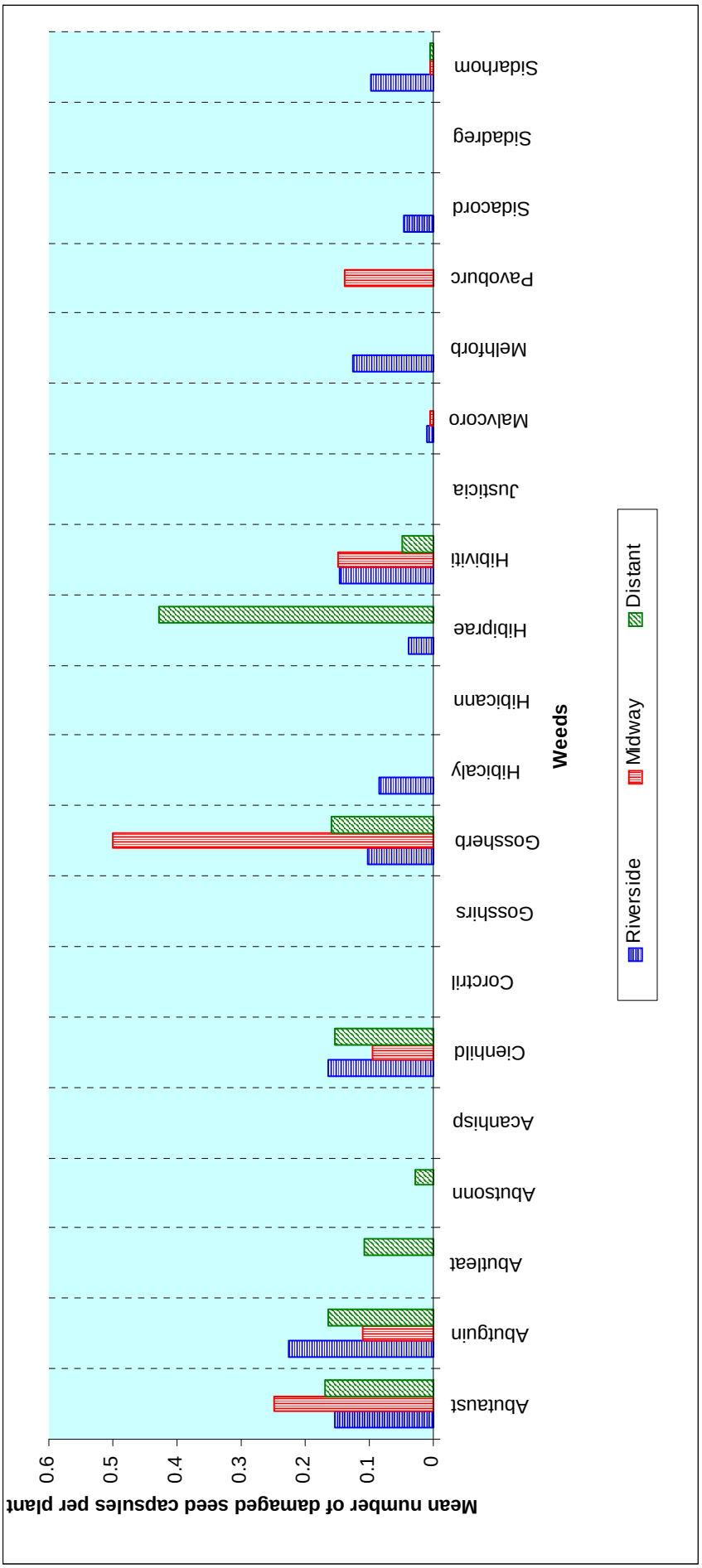


Fig. 6.24. The mean number of damaged seed capsules per plant in the three study zones during the host plant study 2001/2002.

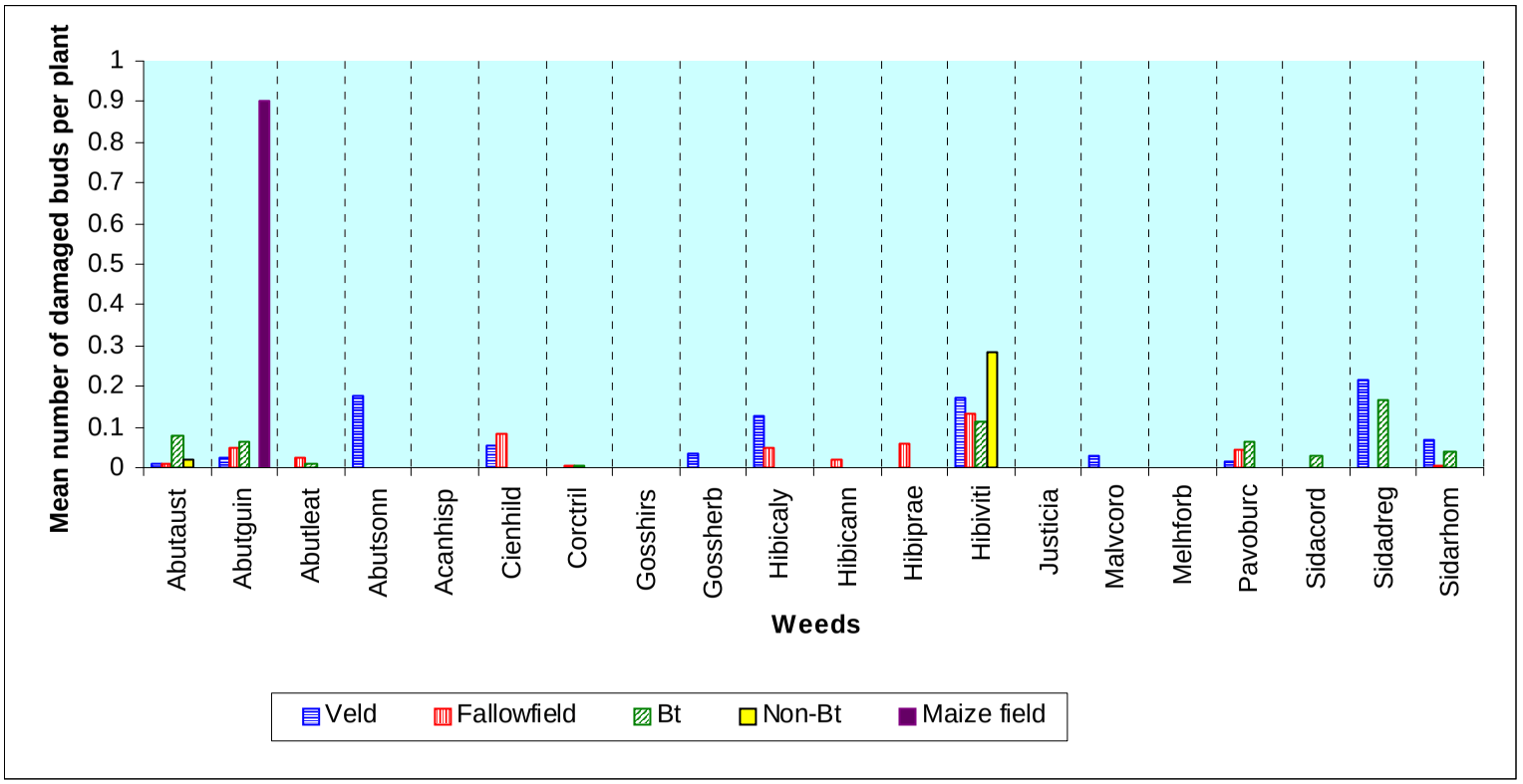


Fig. 6.25. The mean number of damaged buds per plant in the natural veld, fallow field, cotton fields and maize fields during the host plant study 2001/2002.

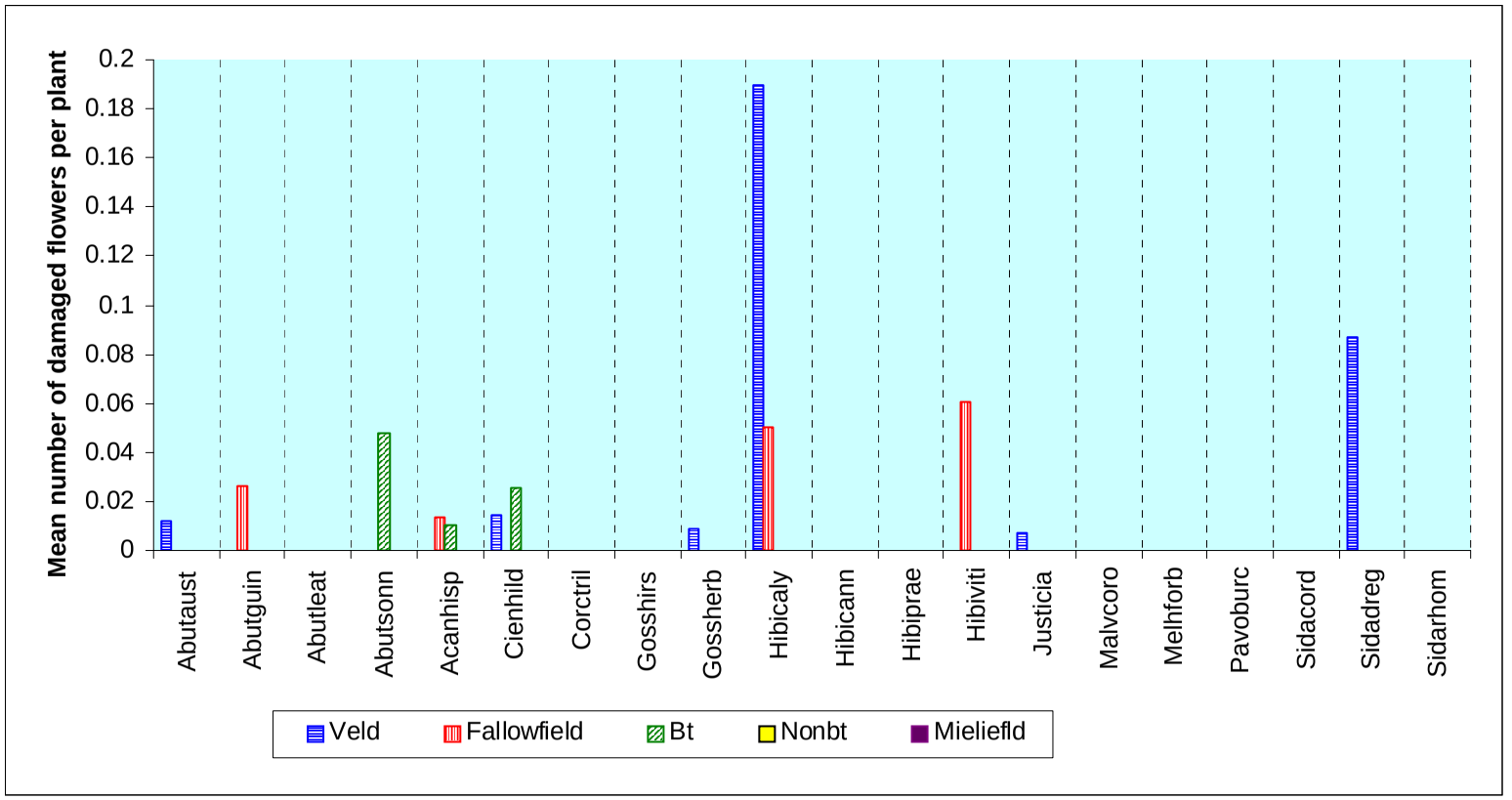


Fig. 6.26. The mean number of damaged flowers per plant in the natural veld, fallow field, cotton fields and maize fields.

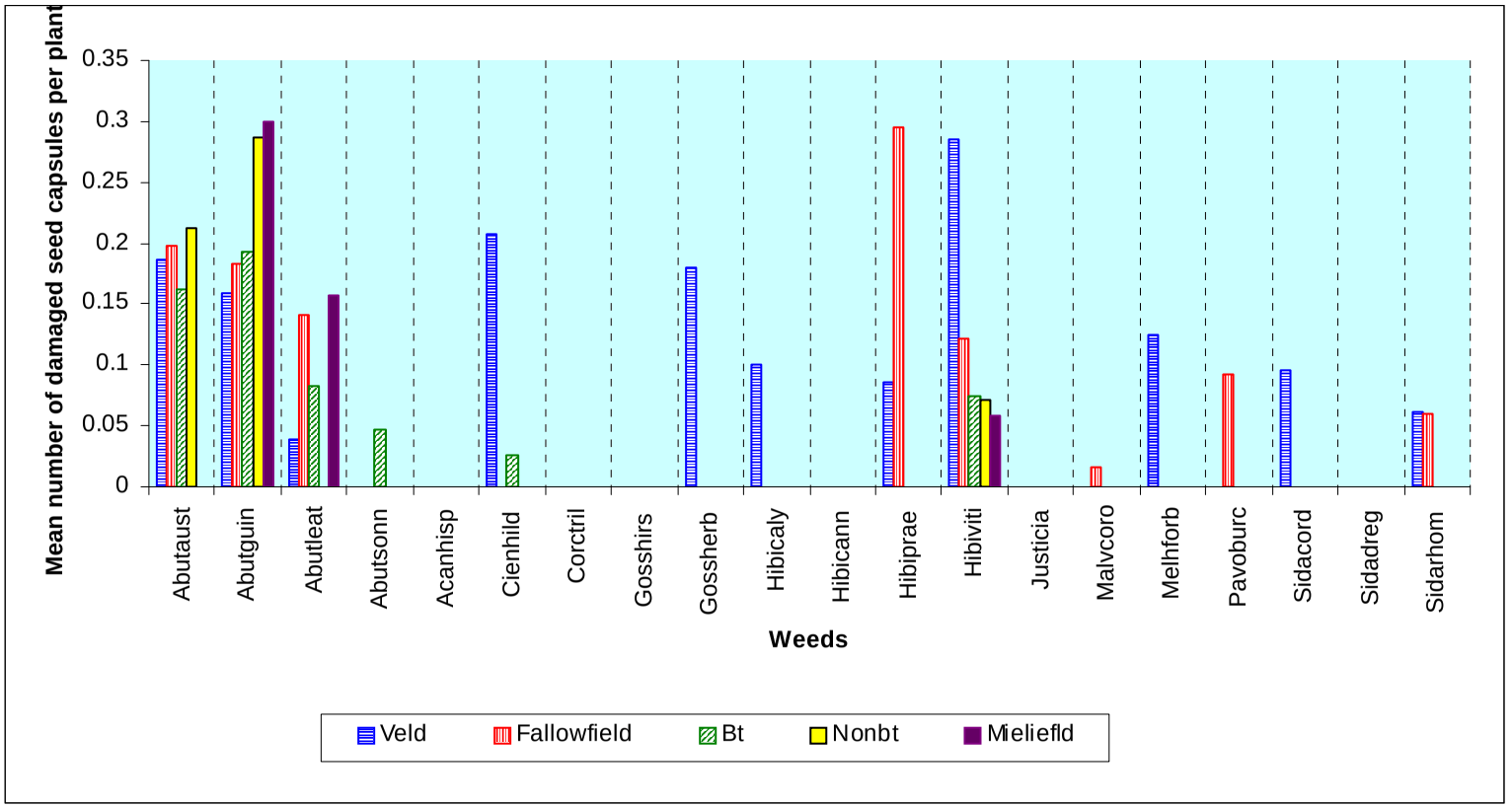


Fig. 6.27. The mean number of damaged seed capsules per plant in the natural veld, fallow field, cotton fields and maize fields during the host plant study 2001/2002.

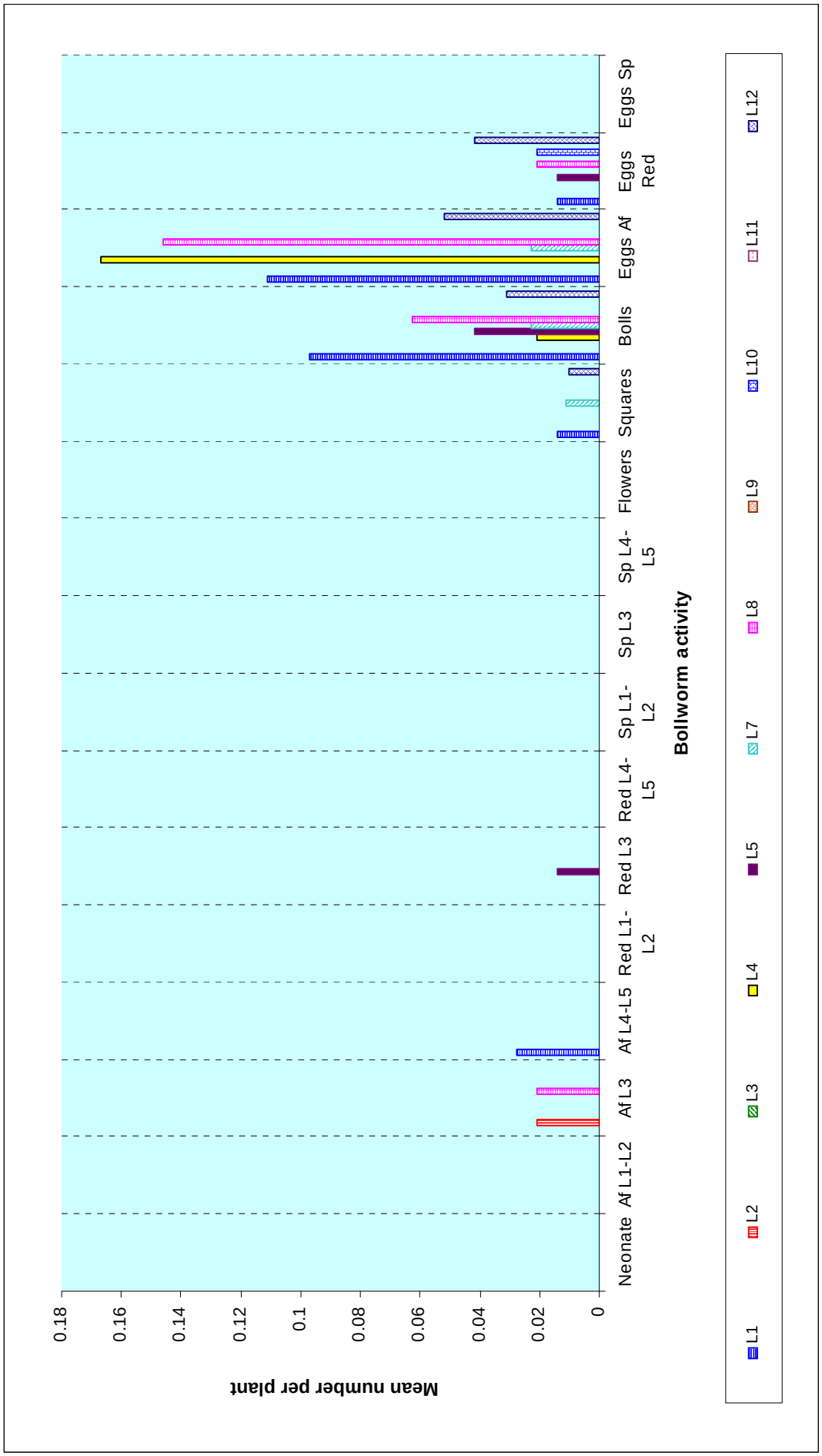


Fig. 6.28. Scout data for Bt-cotton per locality over the season during the host plant study 2001/2002.



Fig. 6.29. Scout data for non-transgenic cotton per locality over the season during the host plant study (2000/01; 2001/2002).

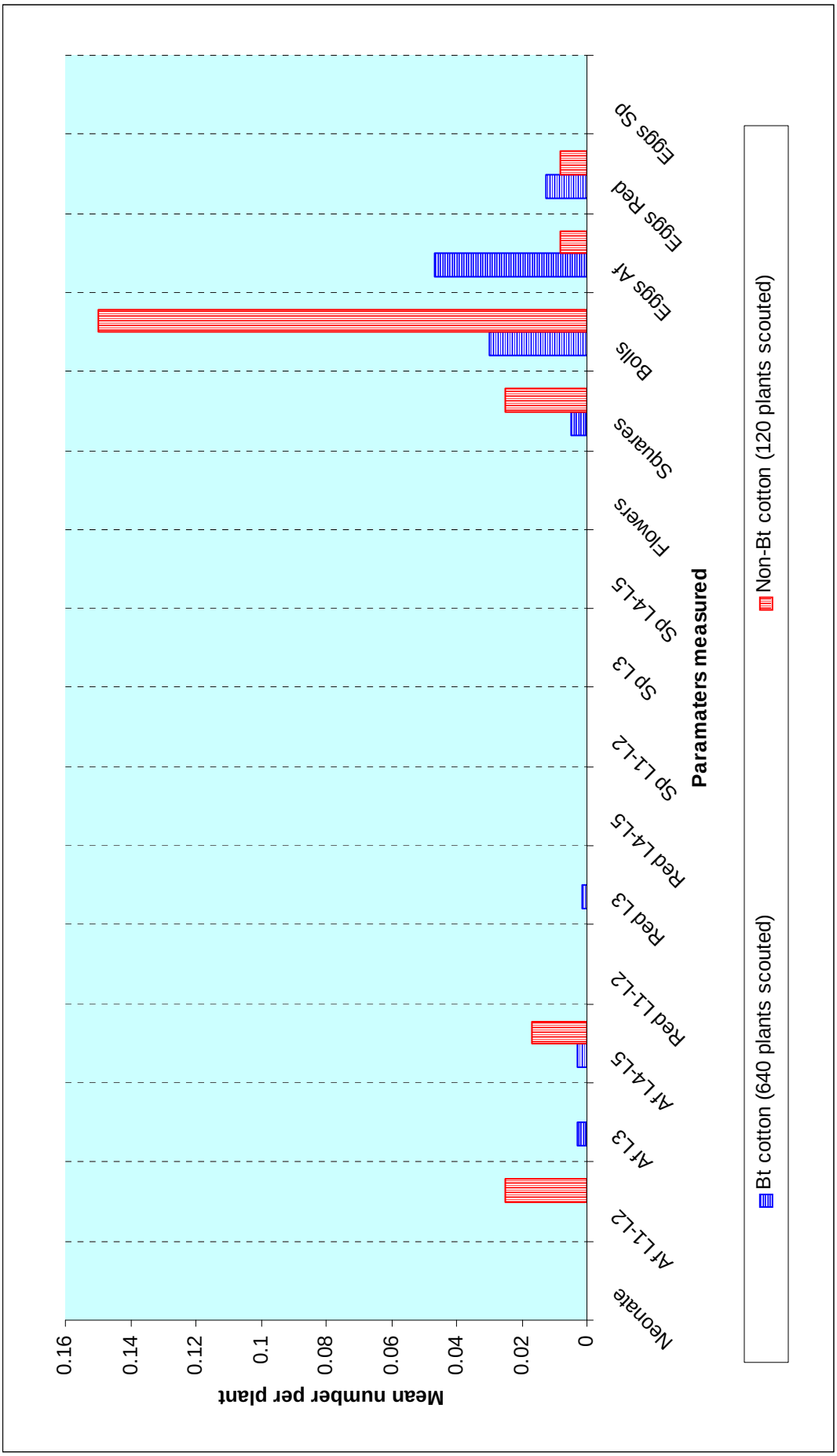


Fig. 6.30. Scout data for both Bt-cotton and non-transgenic cotton over the season during the host plant study 2001/2002.

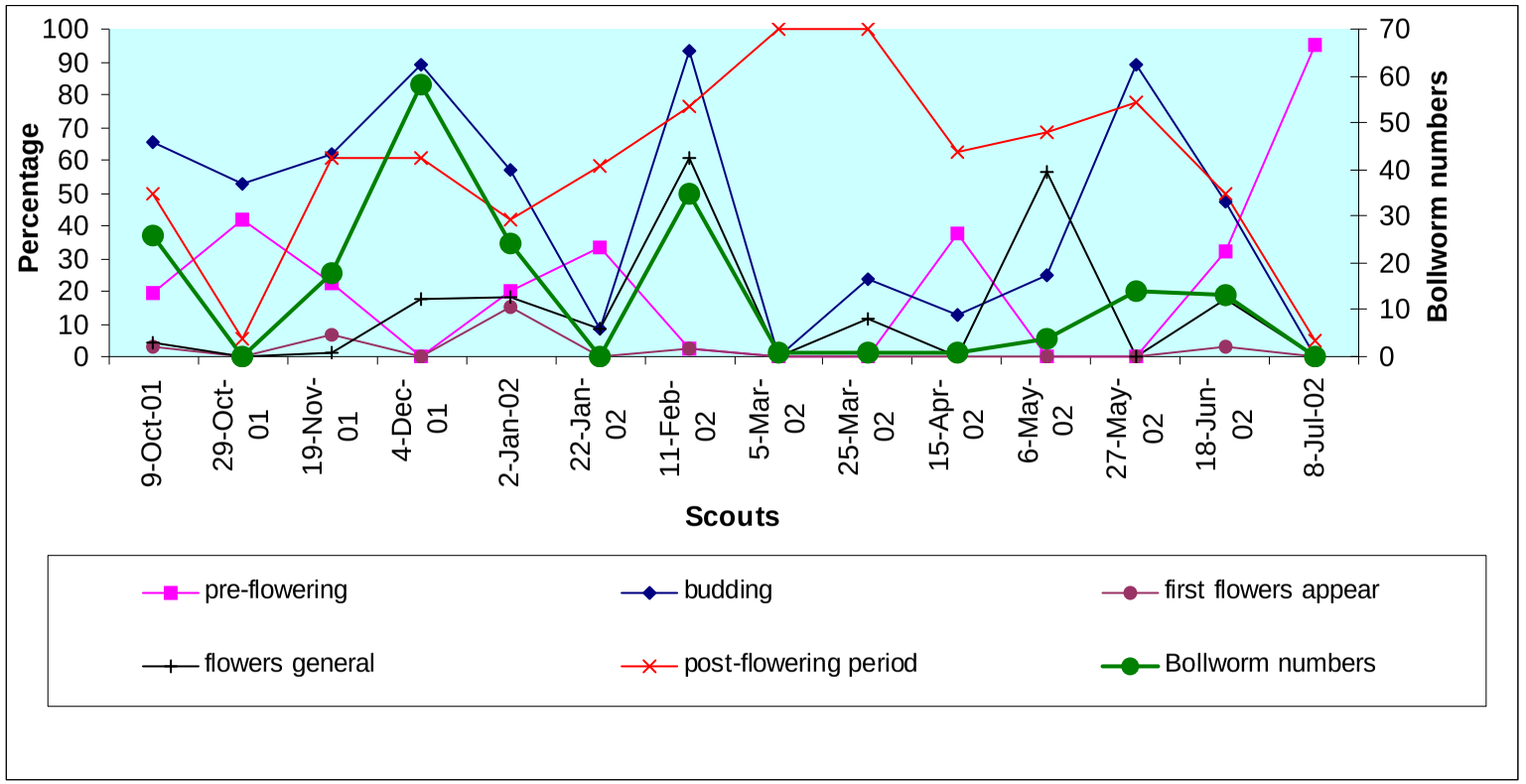


Fig. 6.31. Flowering stages of Abutilon austro-africanum over the season, together with the number of bollworms found during the host plant study 2001/2002.

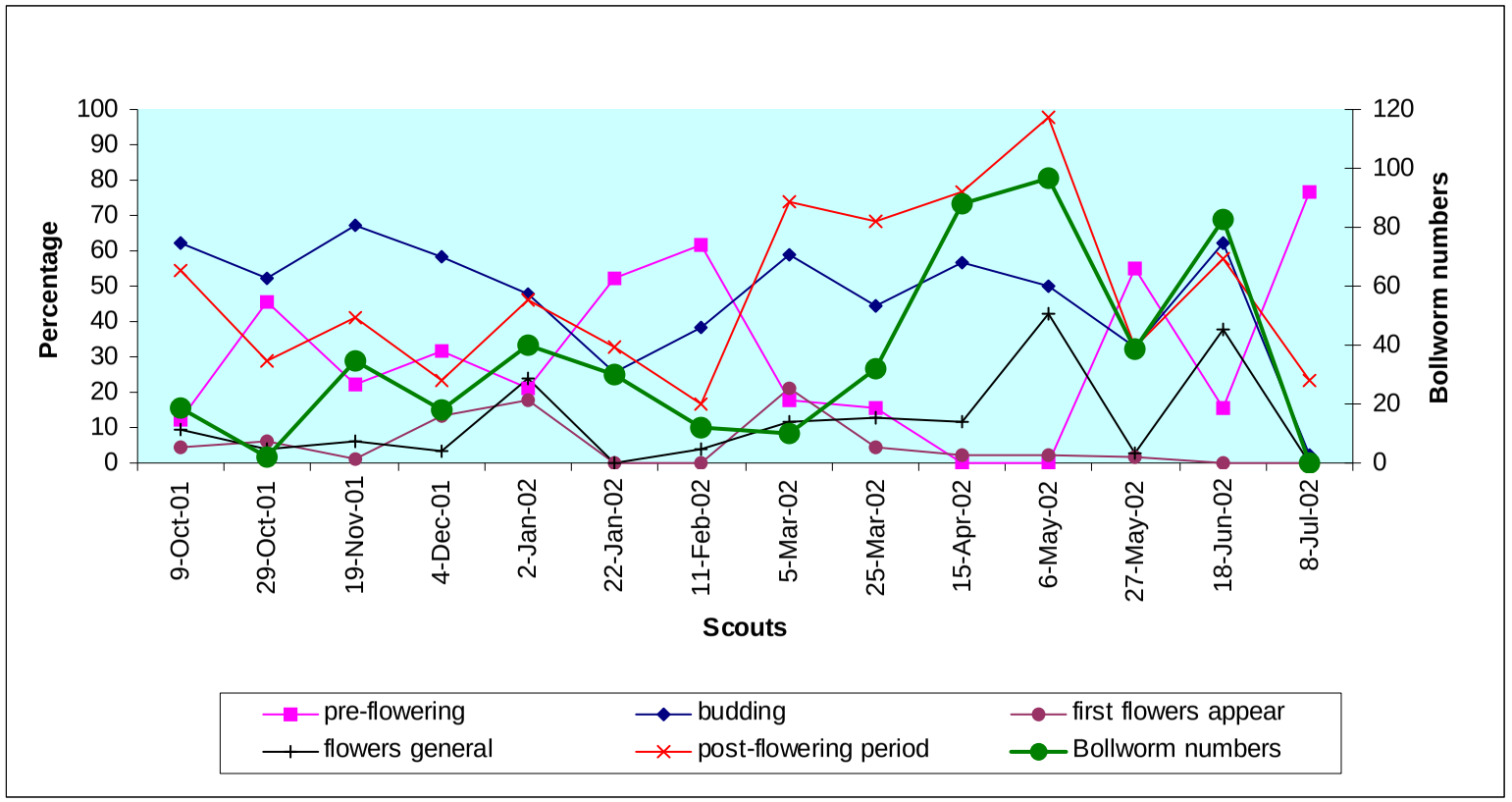


Fig. 6.32. Flowering stages of Abutilon guineense over the season, together with the number of bollworms found during the host plant study 2001/2002.

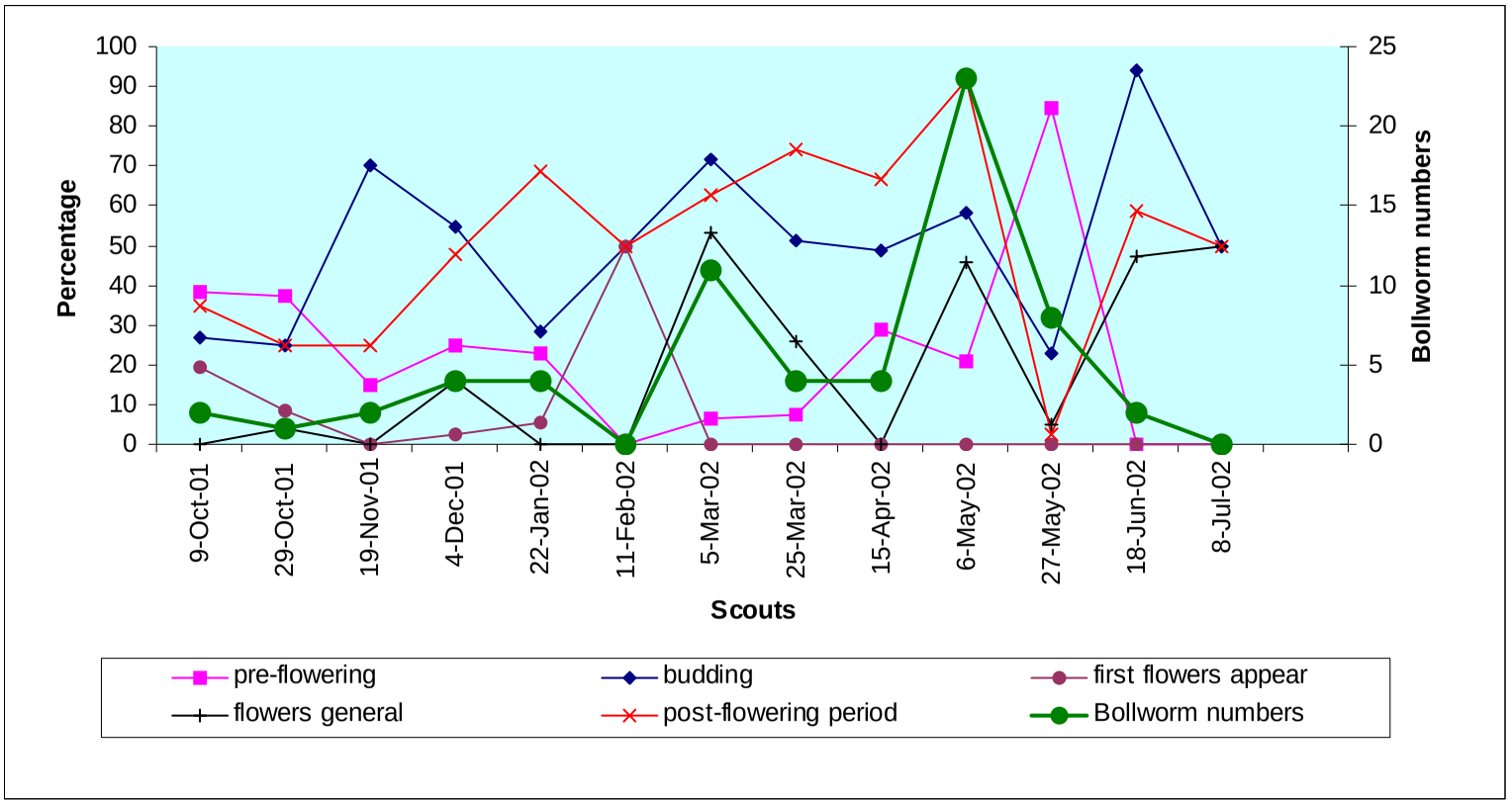


Fig. 6.33. Flowering stages of <u>Abutilon</u> sp. ('leathery') over the season, together with the number of bollworms found during the host plant study 2001/2002.

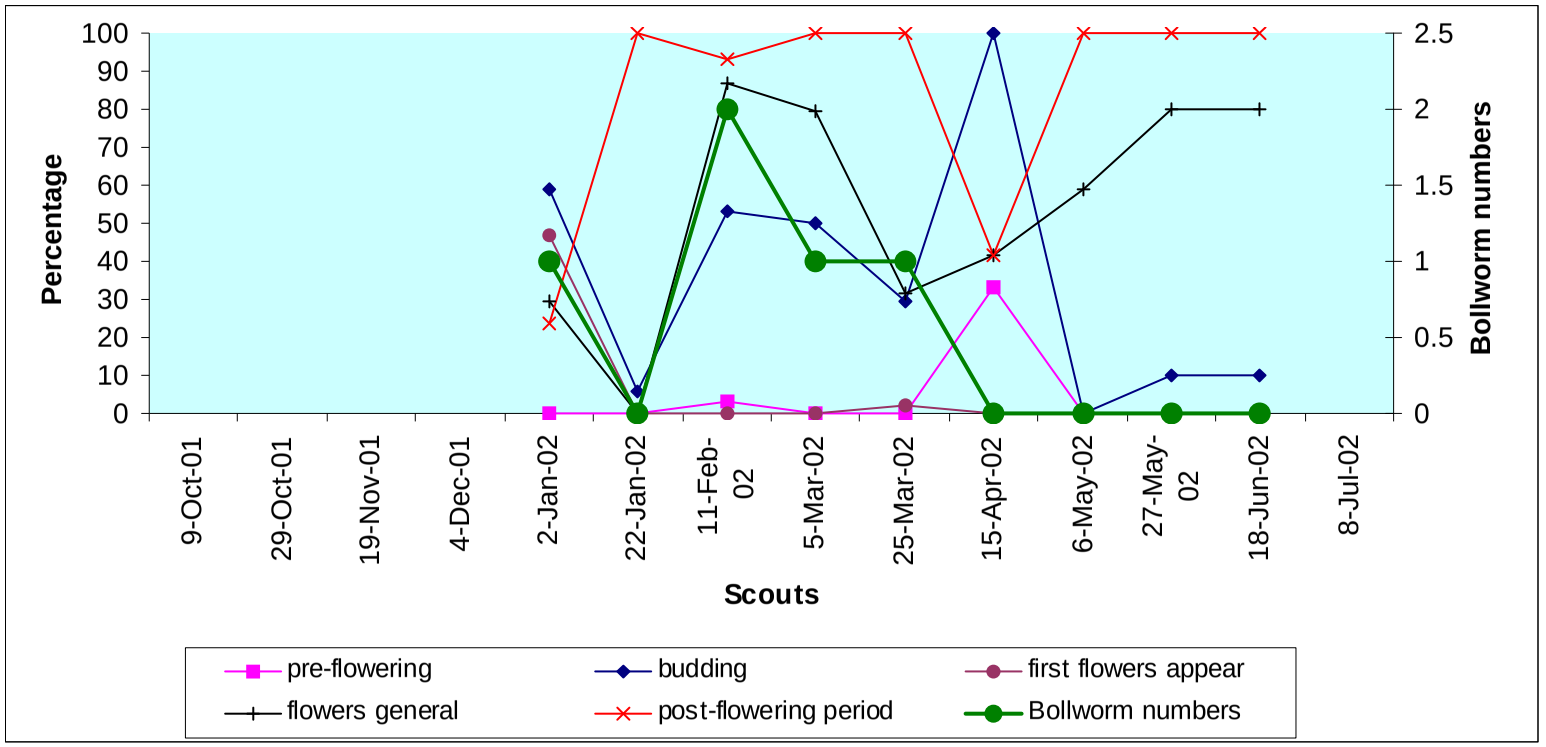


Fig. 6.34. Flowering stages of Acanthospermum hispidum over the season, together with the number of bollworms found during the host plant study 2001/2002.

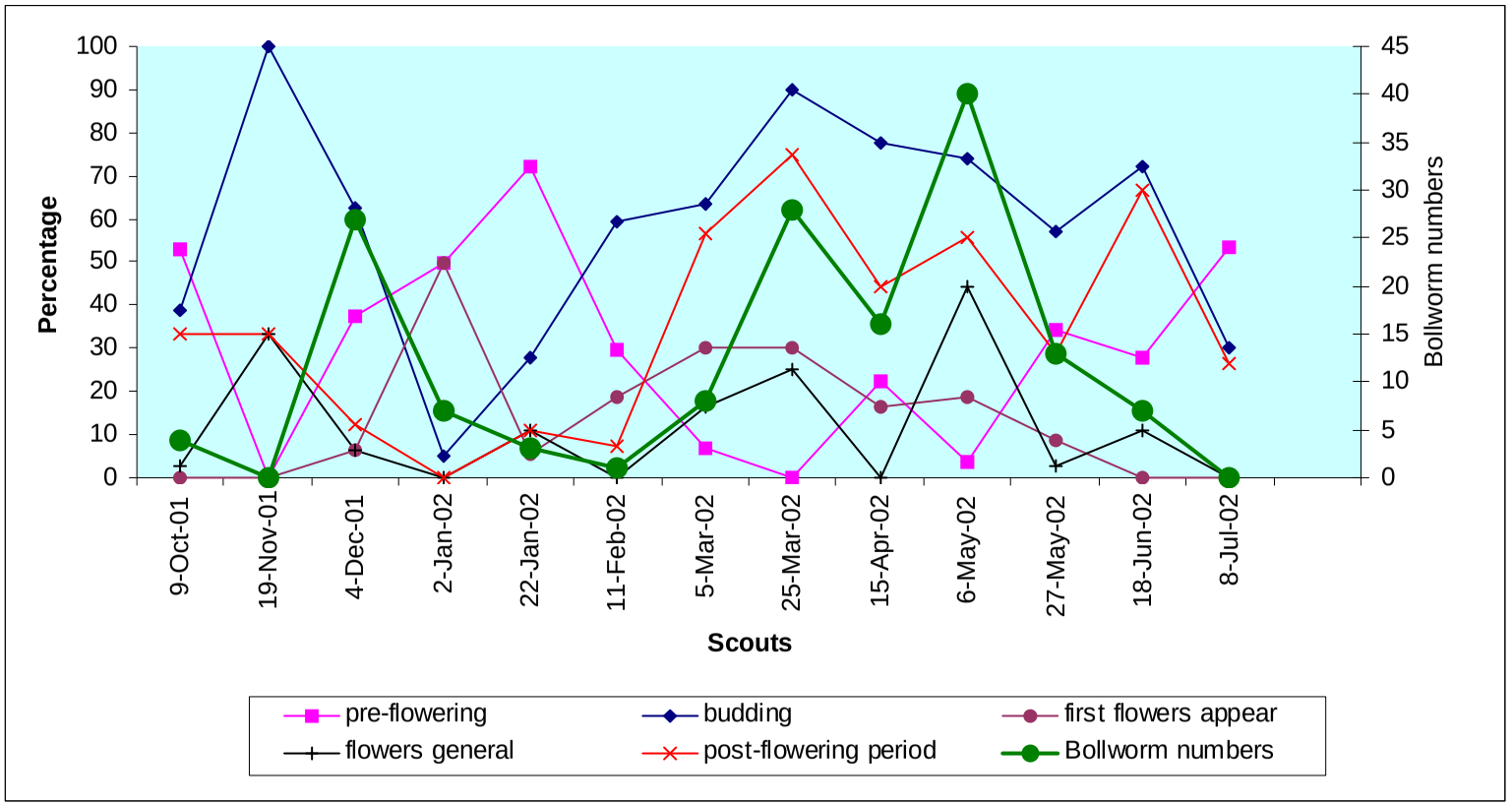


Fig. 6.35. Flowering stages of Cienfeugosia hildebrandtii over the season, together with the number of bollworms found during the host plant study 2001/2002.

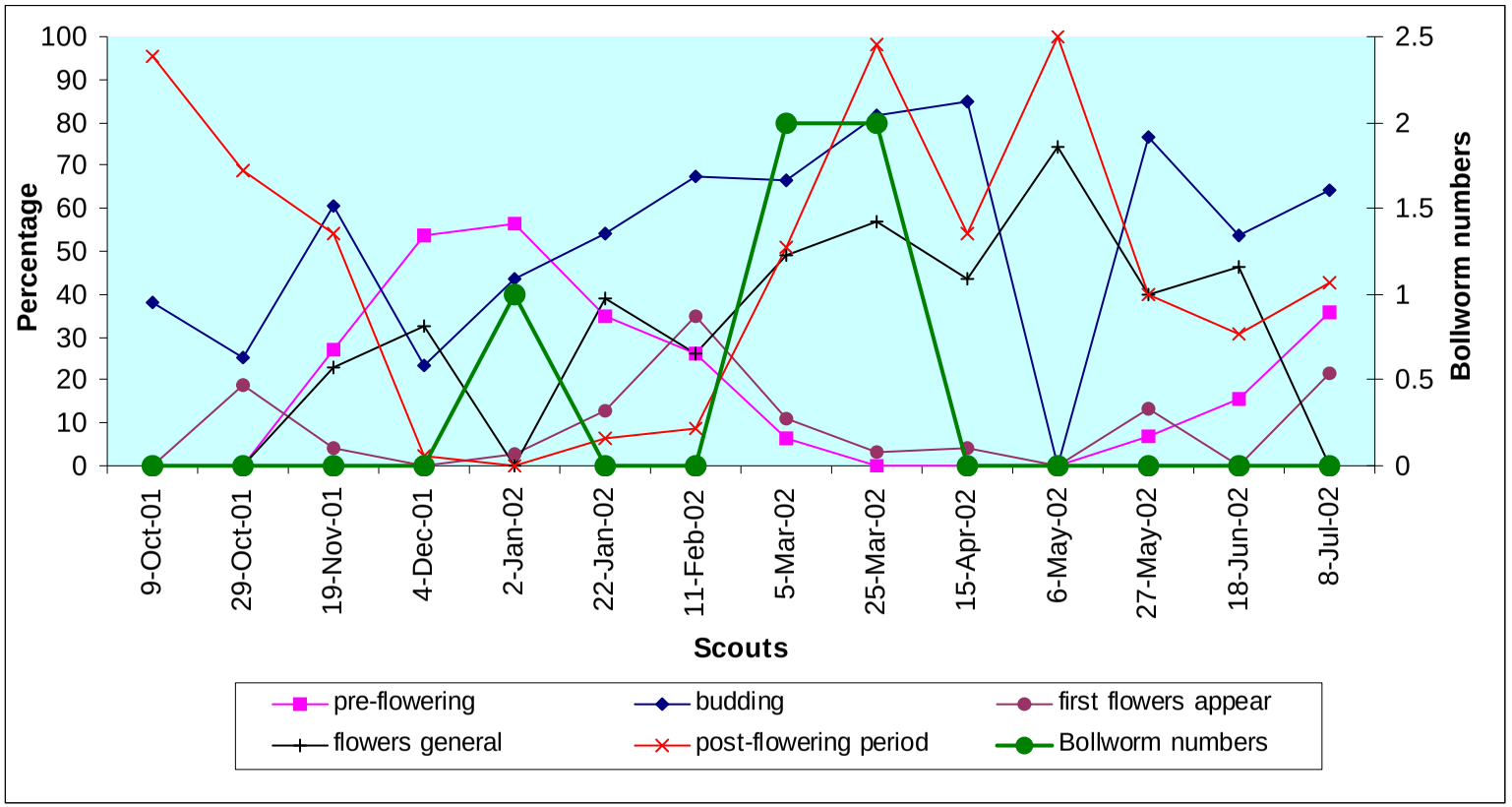


Fig. 6.36. Flowering stages of Corchorus trilocularis over the season, together with the number of bollworms found during the host plant study 2001/2002.

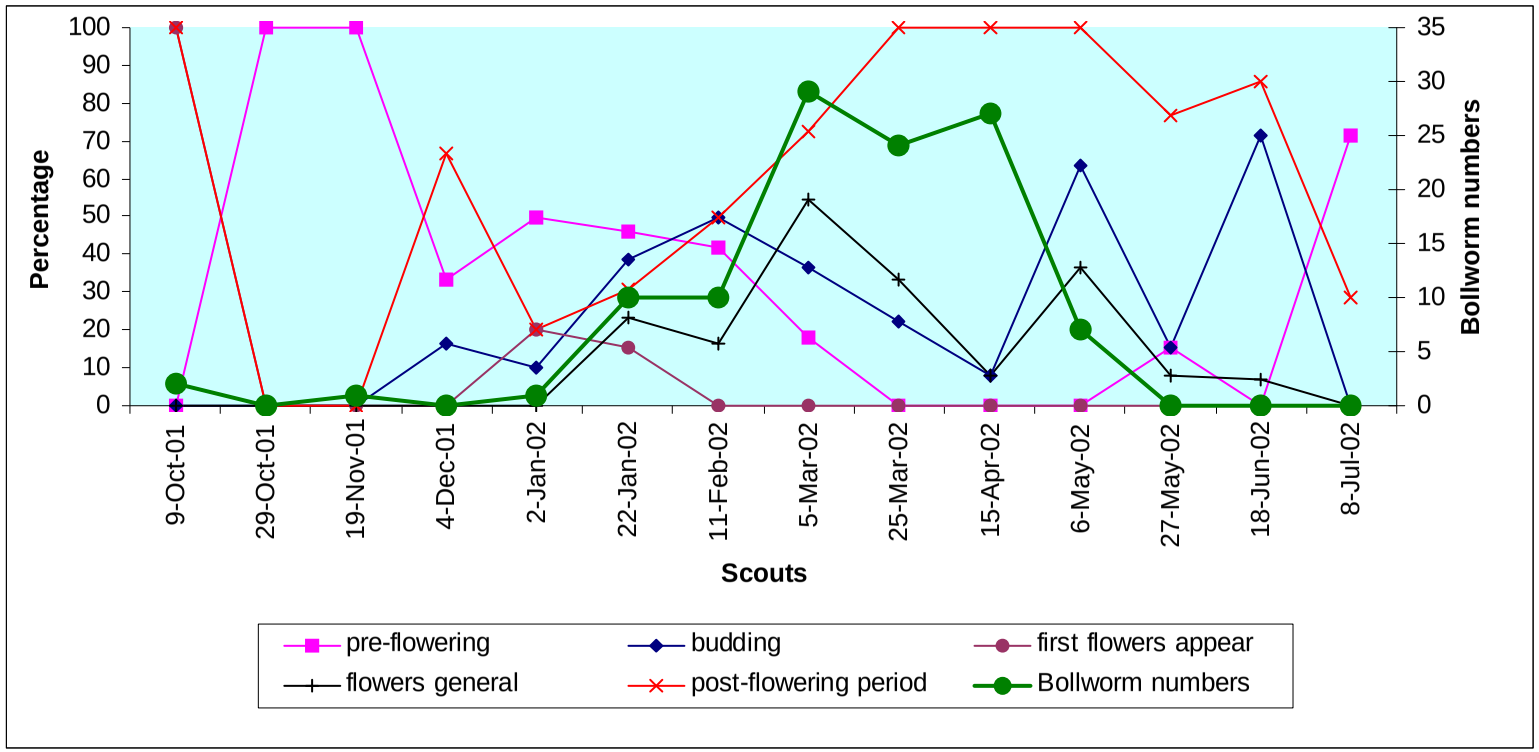


Fig. 6.37. Flowering stages of Gossypium herbaceum over the season, together with the number of bollworms found during the host plant study 2001/2002.

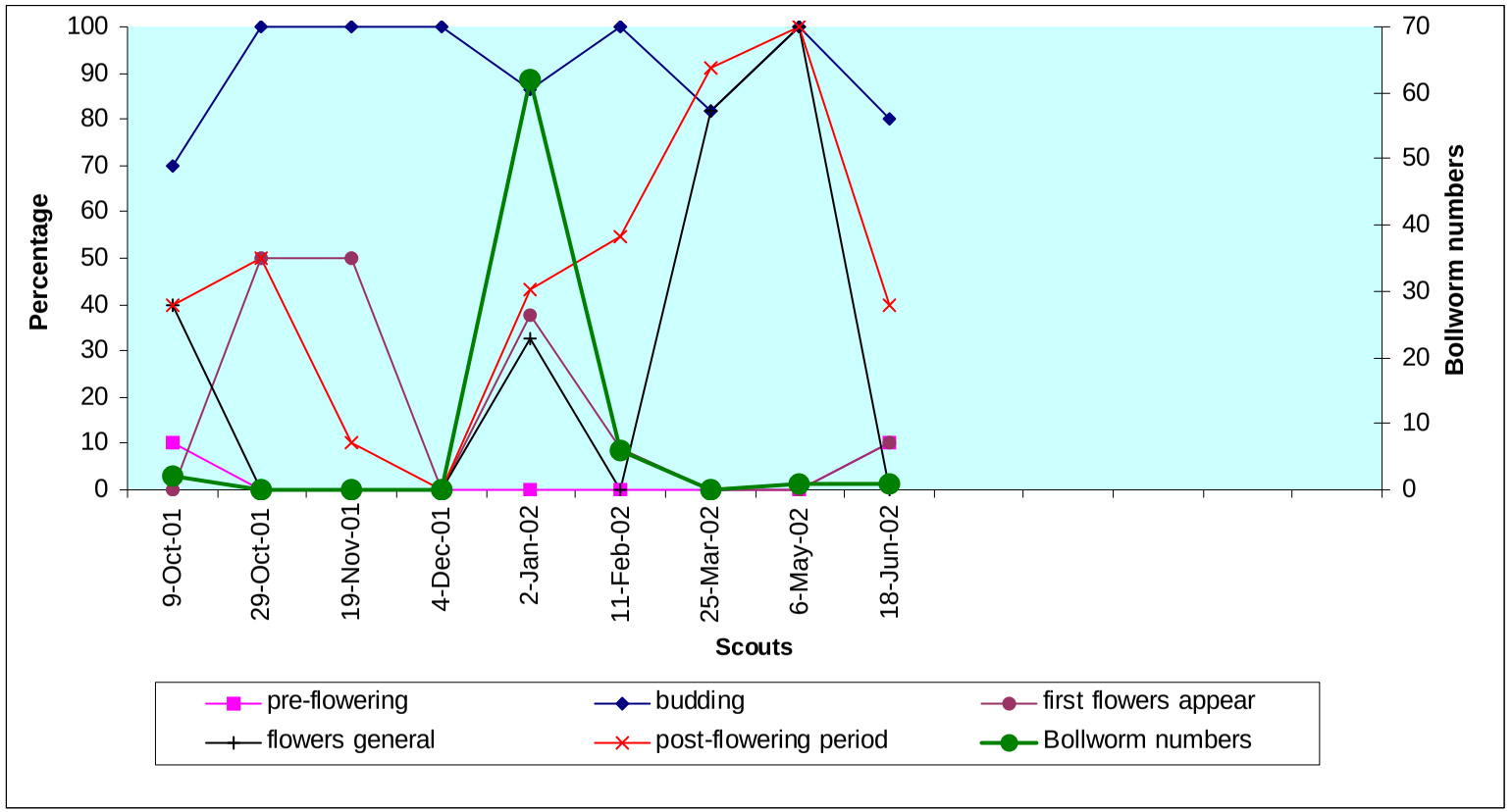


Fig. 6.38. Flowering stages of Hibiscus calyphyllus plotted on dates where it was found over the season, together with the number of bollworms found during the host plant study 2001/2002.

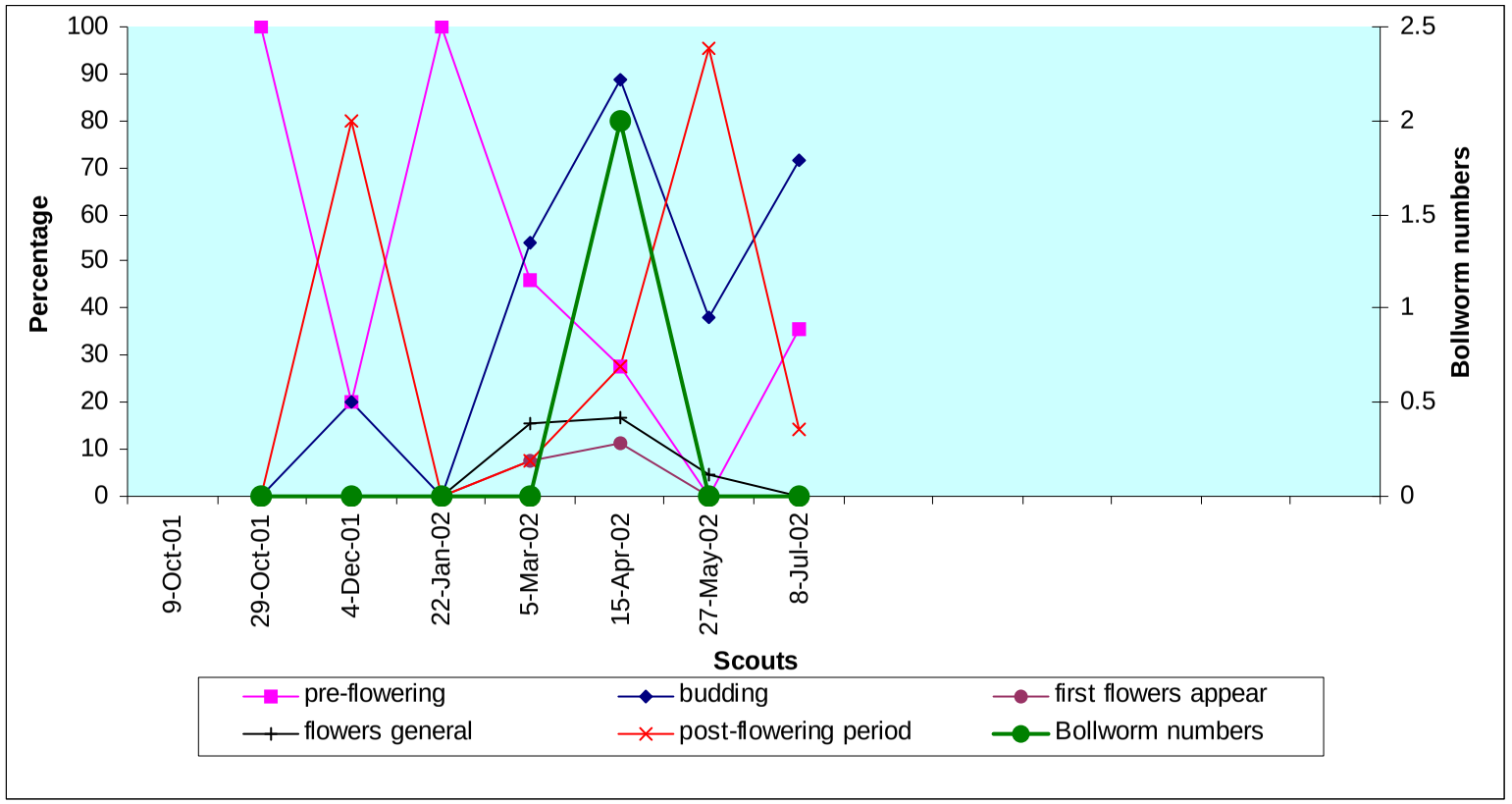


Fig. 6.39. Flowering stages of Hibiscus cannabinus plotted on dates where it was found over the season, together with the number of bollworms found during the host plant study 2001/2002.

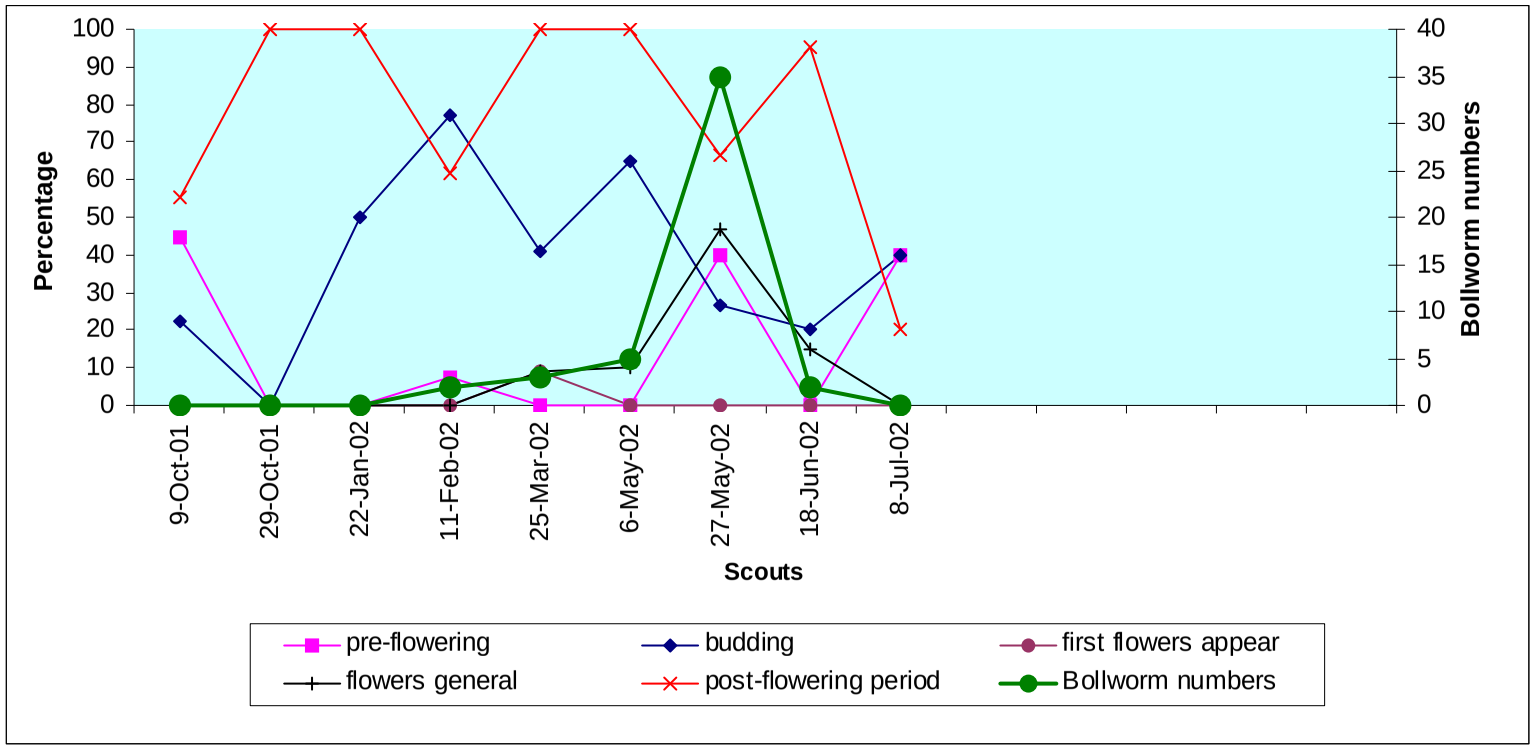


Fig. 6.40. Flowering stages of Hibiscus praeteritus plotted on dates where it was found over the season, together with the number of bollworms found during the host plant study 2001/2002.

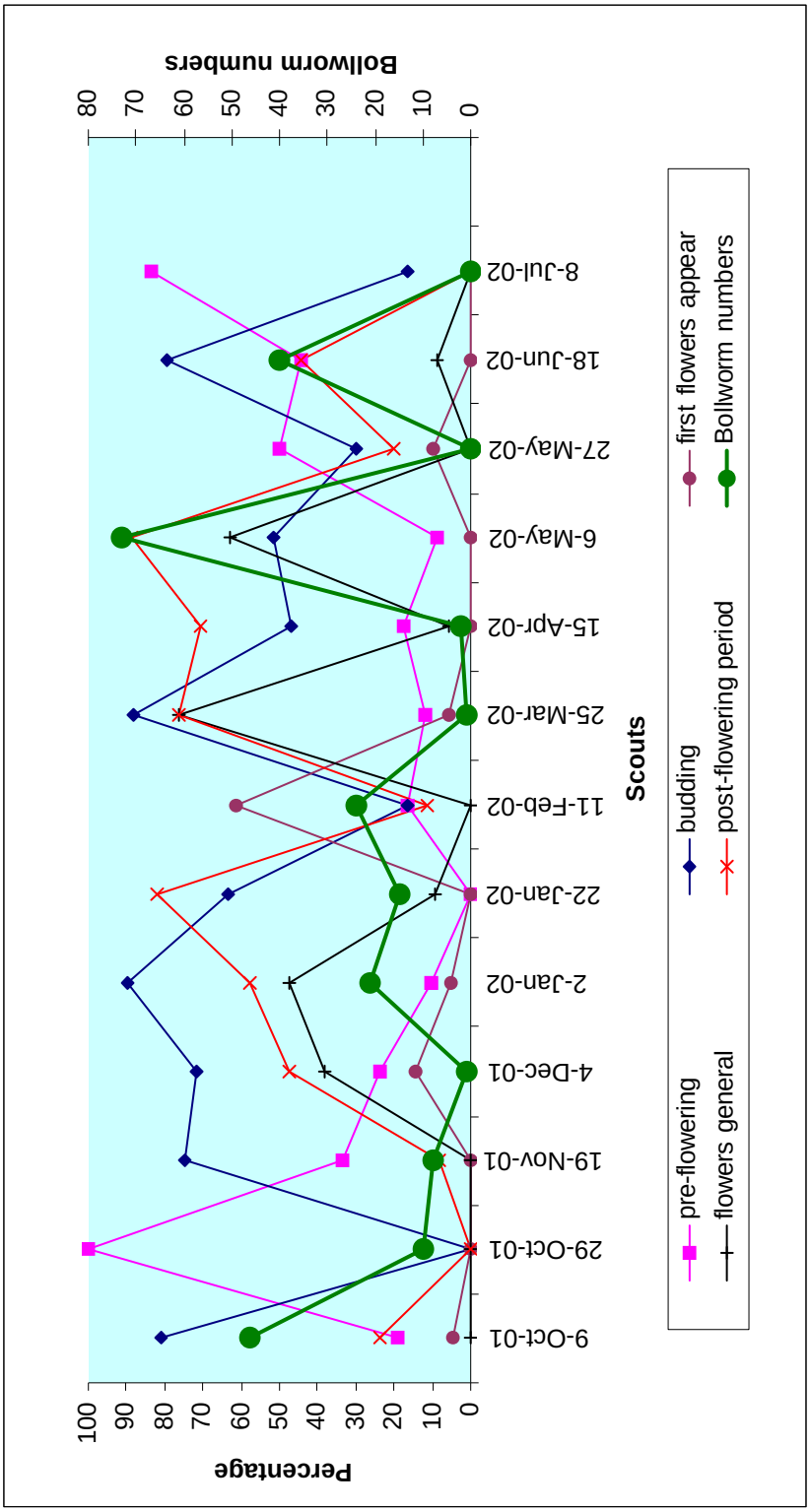


Fig. 6.41. Flowering stages of Hibiscus vitifolius plotted on dates where it was found over the season, together with the number of bollworms found during the host plant study 2001/2002.

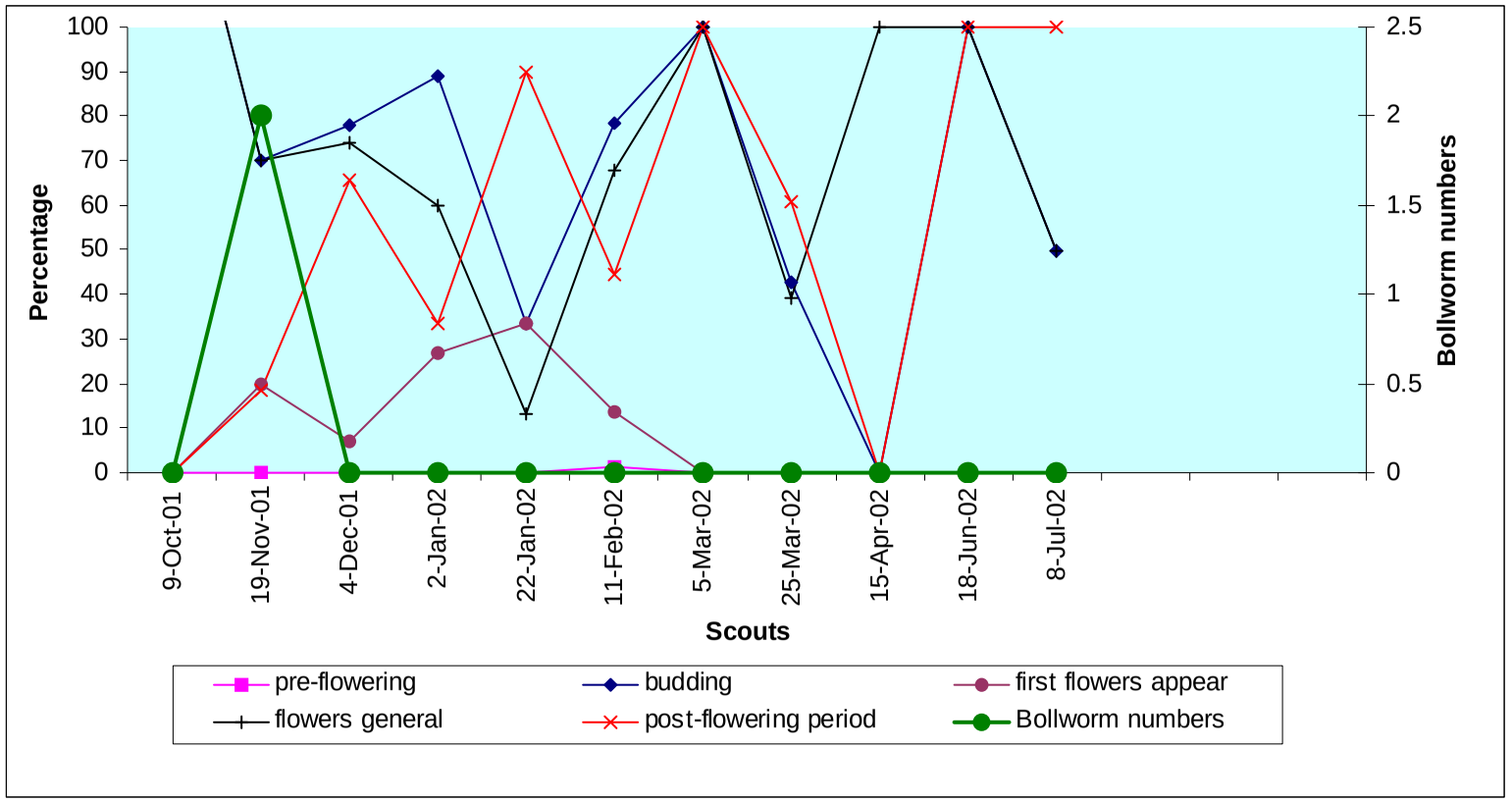


Fig. 6.42. Flowering stages of <u>Justicia</u> sp. over the season, together with the number of bollworms found during the host plant study 2001/2002.

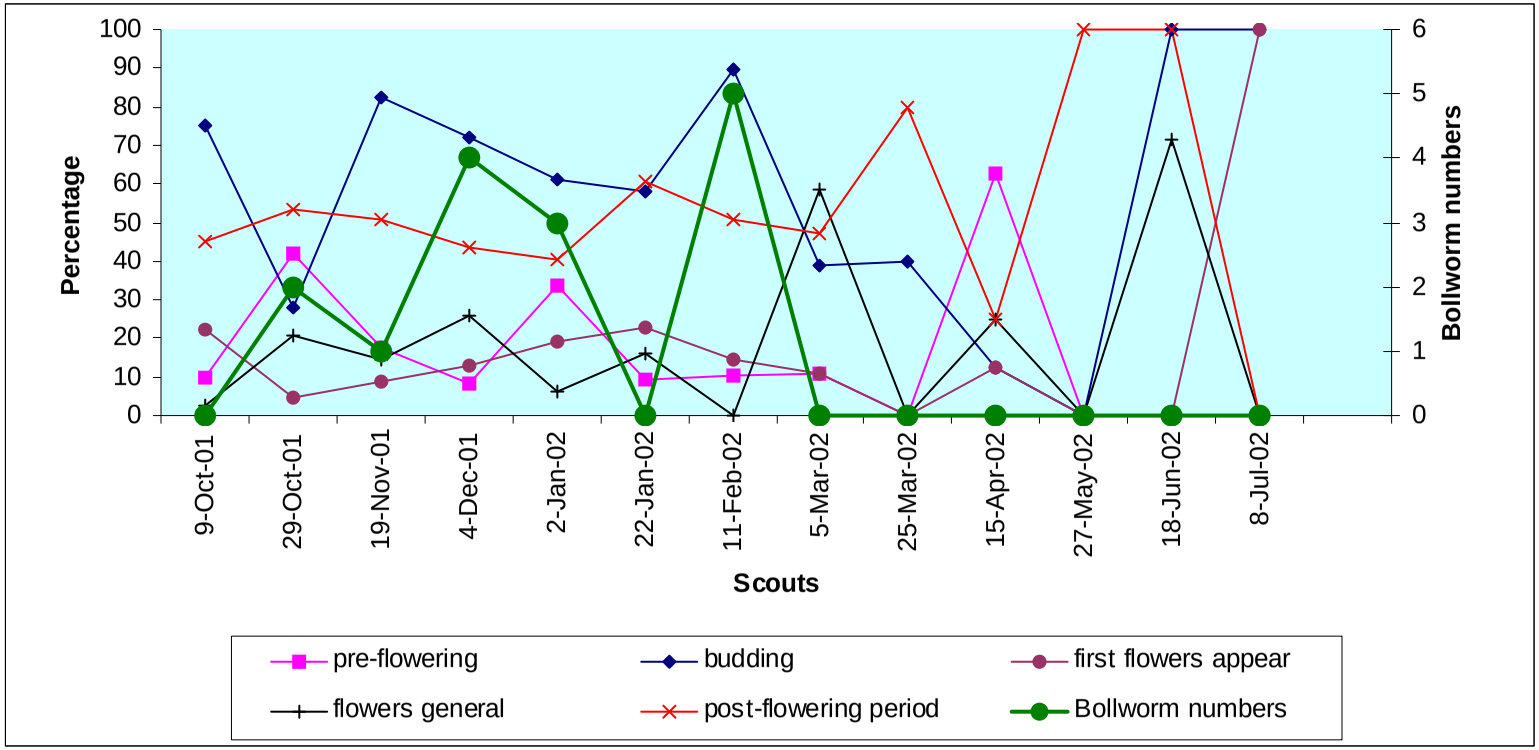


Fig. 6.43. Flowering stages of Malvastrum coromandelianum plotted on dates where it was found over the season, together with the number of bollworms found during the host plant study 2001/2002.

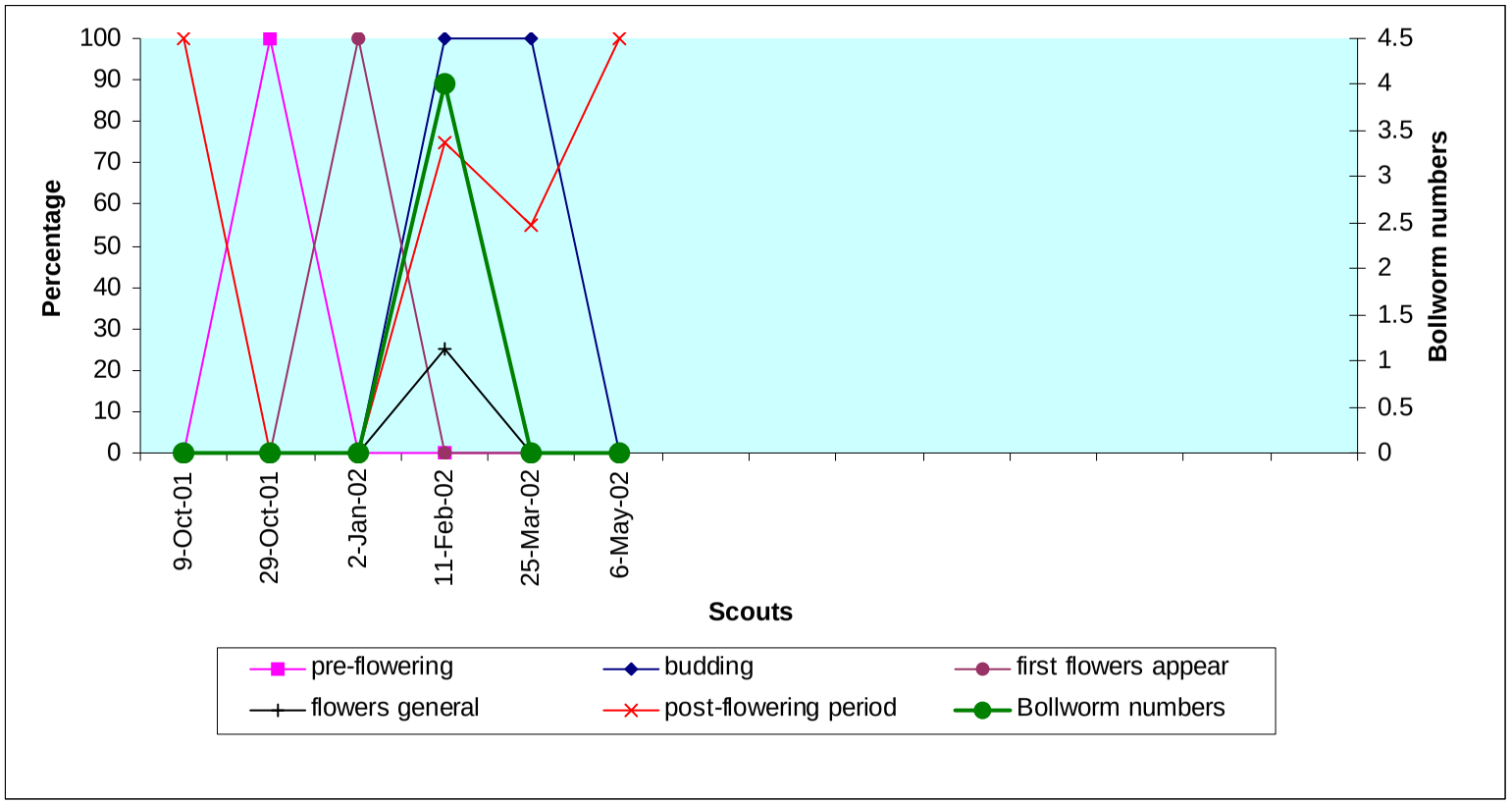


Fig. 6.44. Flowering stages of Melhania forbesii plotted on dates where it was found over the season, together with the number of bollworms found during the host plant study 2001/2002.

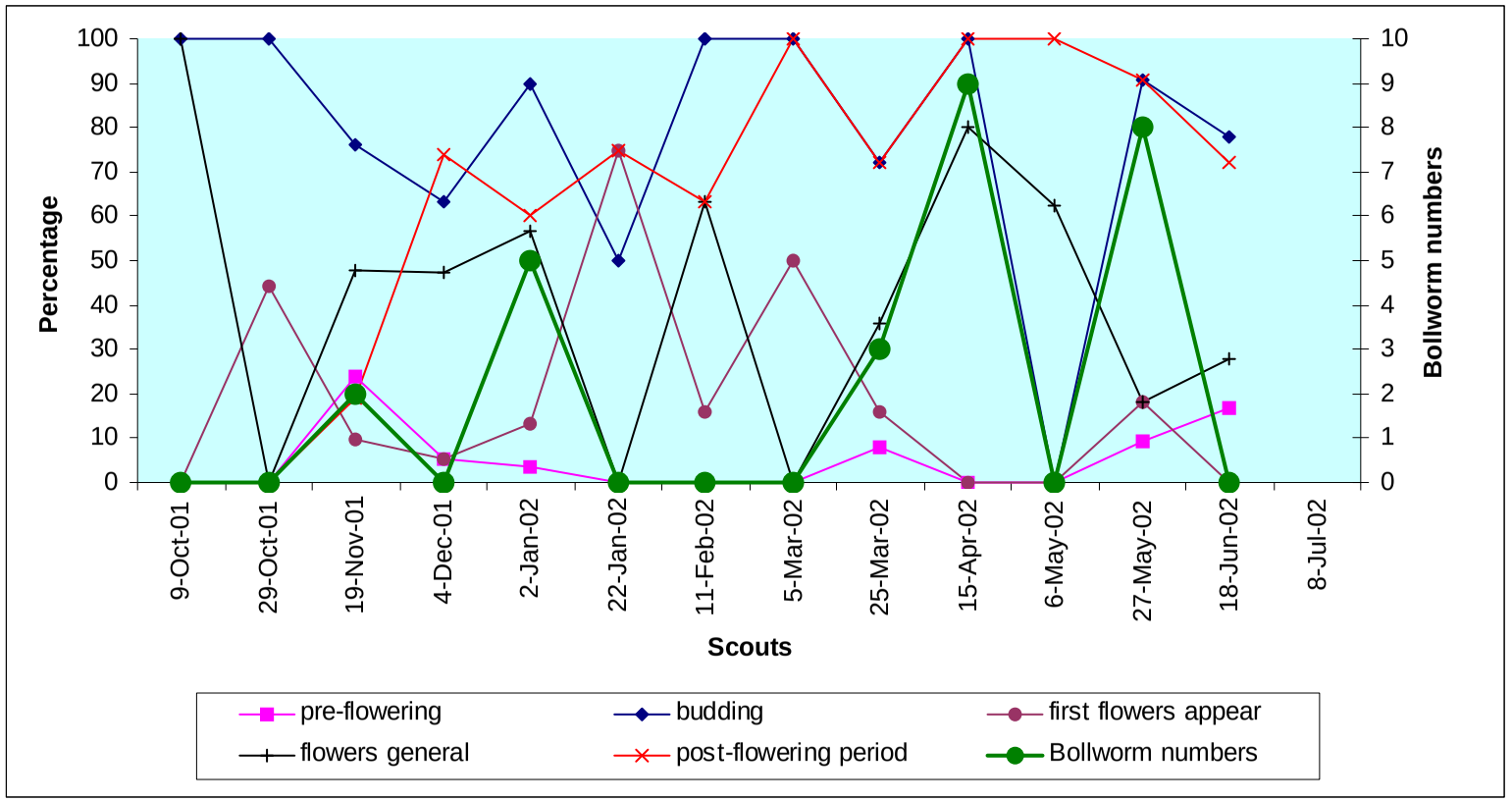


Fig. 6.45. Flowering stages of Pavonia burchellii over the season, together with the number of bollworms found during the host plant study 2001/2002.

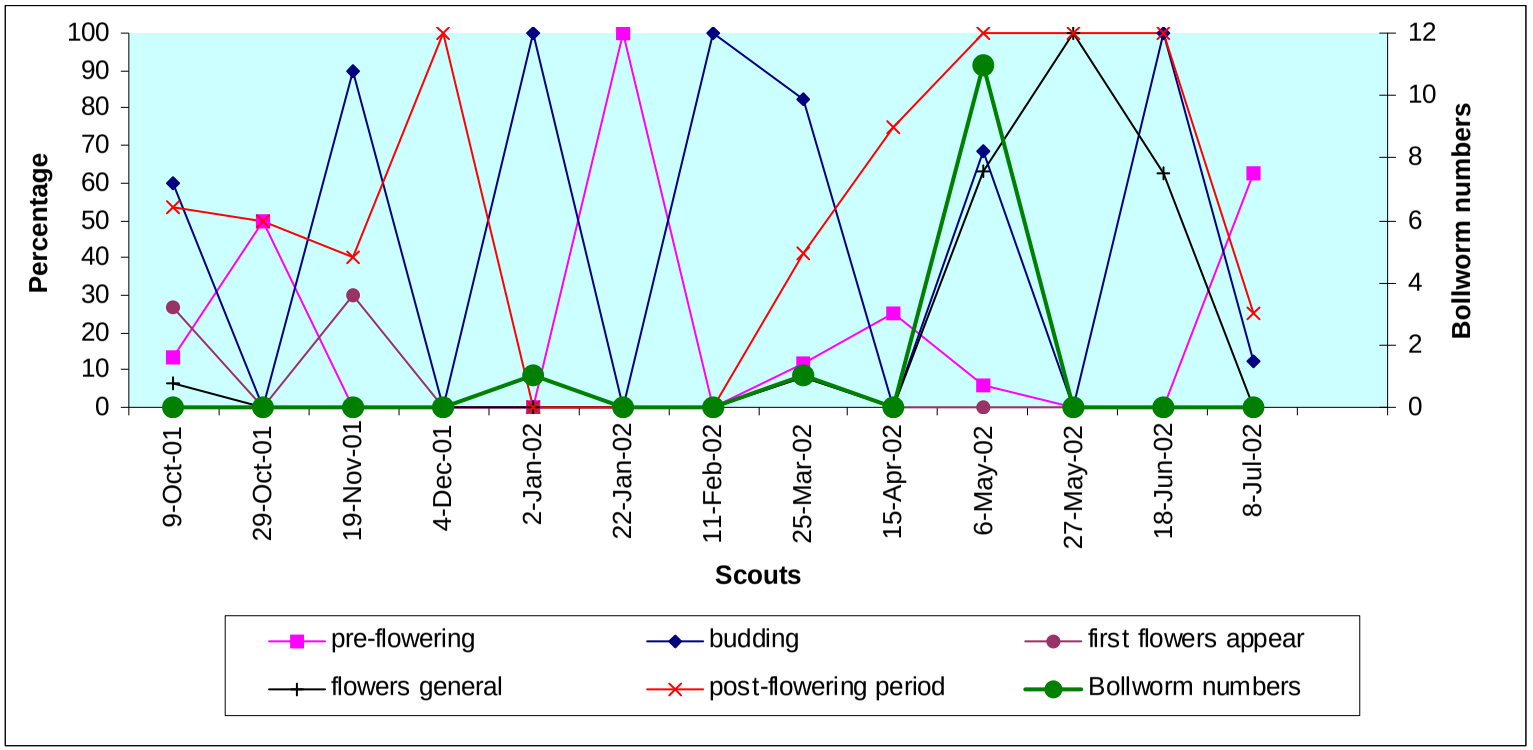


Fig. 6.46. Flowering stages of Sida cordifolia plotted on dates where it was found over the season, together with the number of bollworms found during the host plant study 2001/2002.

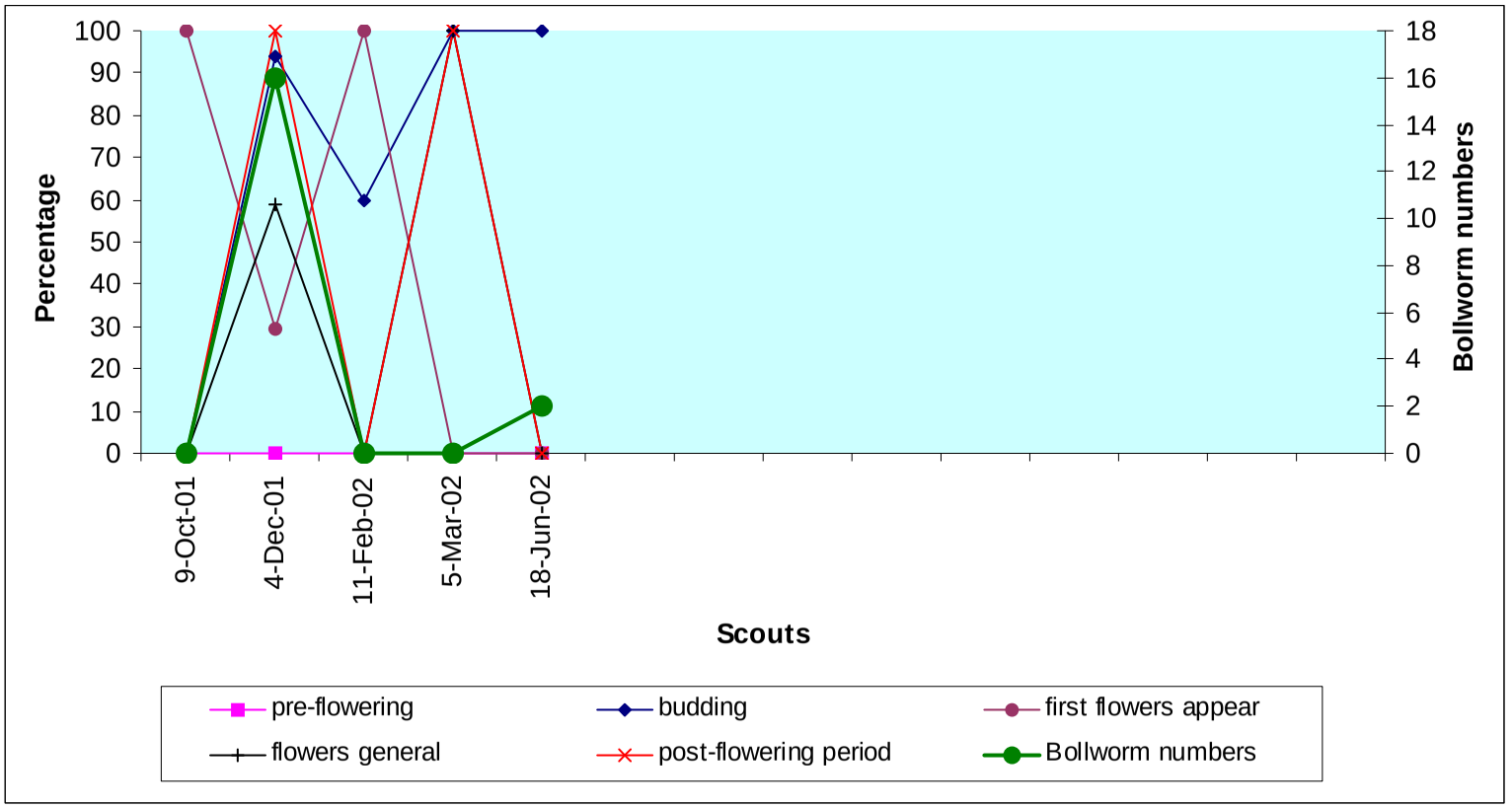


Fig. 6.47. Flowering stages of Sida dregei plotted on dates where it was found over the season, together with the number of bollworms found during the host plant study 2001/2002.

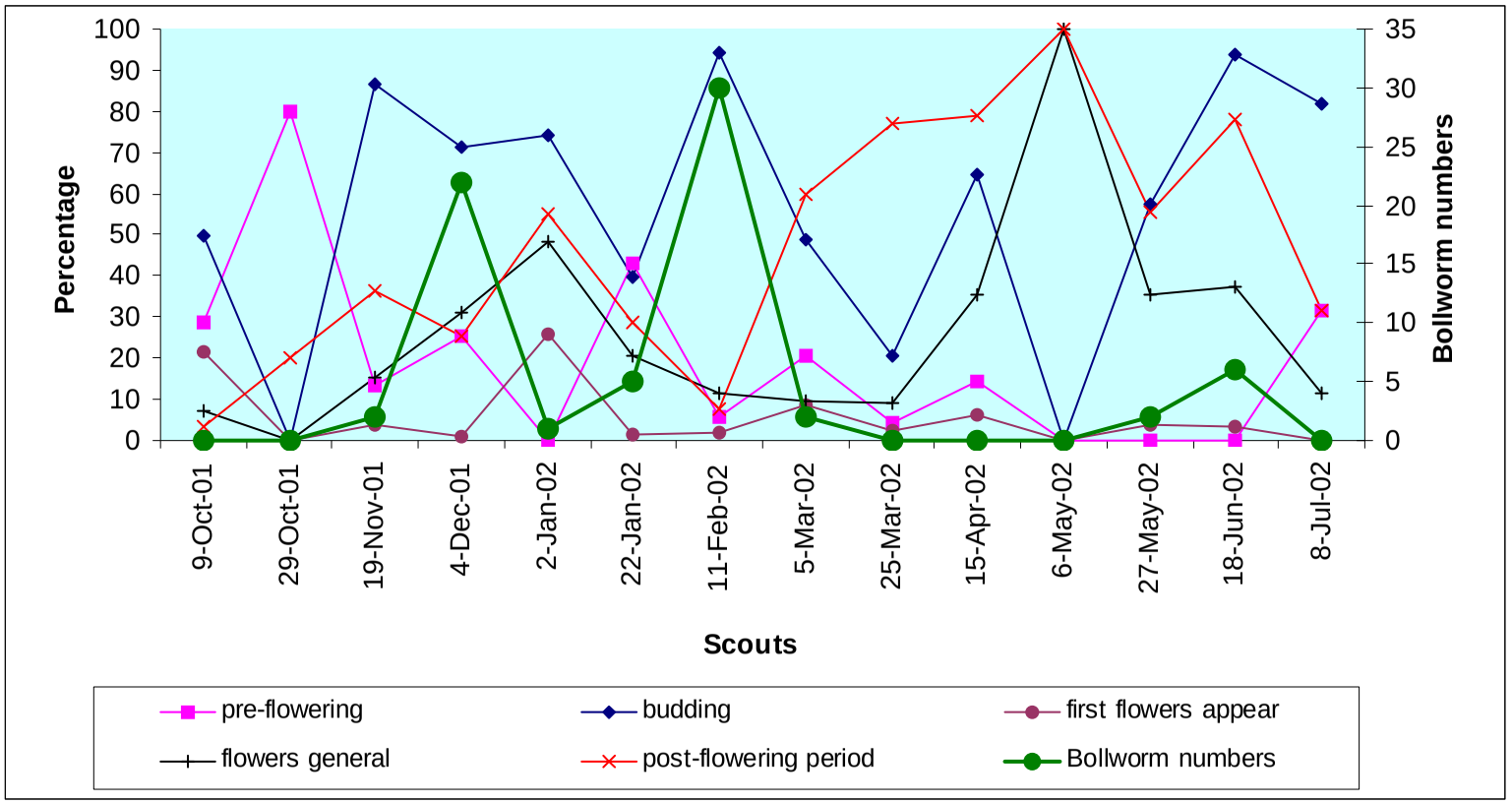
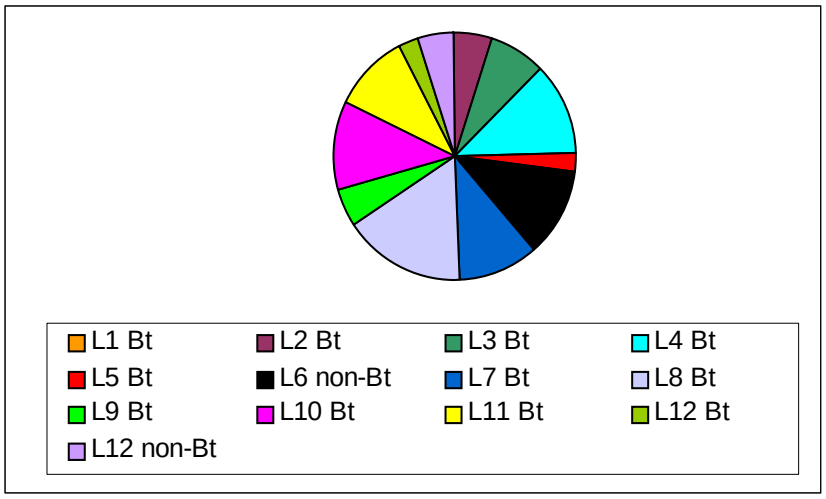
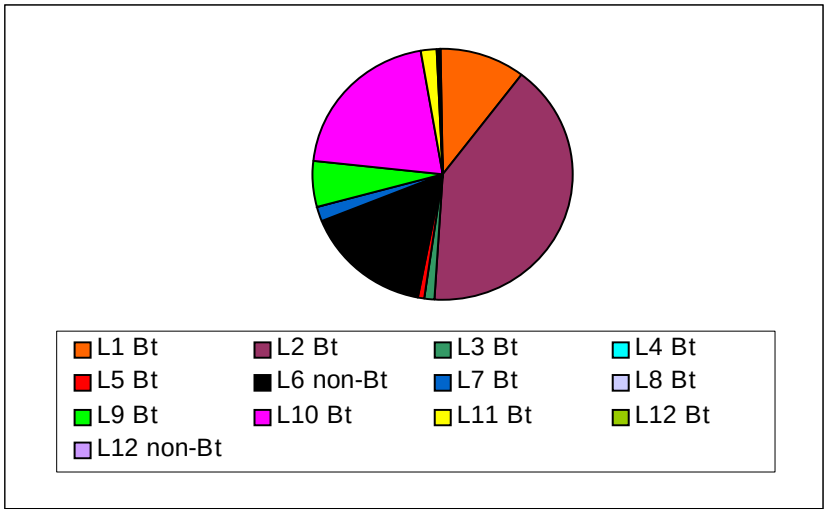


Fig. 6.48. Flowering stages of Sida rhombifolia over the season, together with the number of bollworms found during the host plant study 2001/2002.

a



b



c

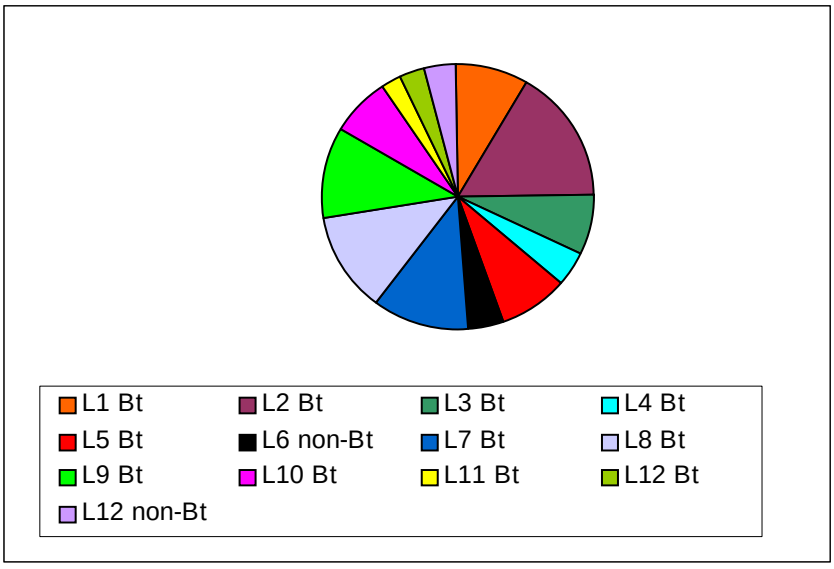
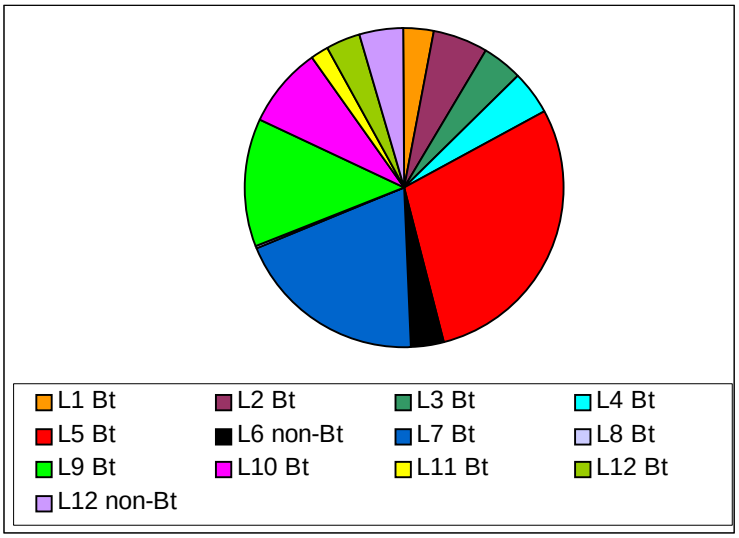
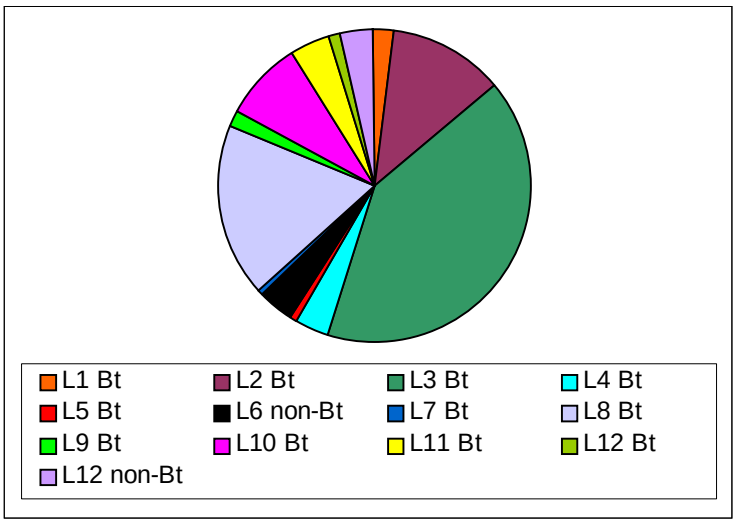


Fig. 6.49. The total number of african bollworm (a), red bollworm (b) and spiny bollworm (c) host plants per 10m^2 over the season during the host plant study 2001/2002.

a



b



c

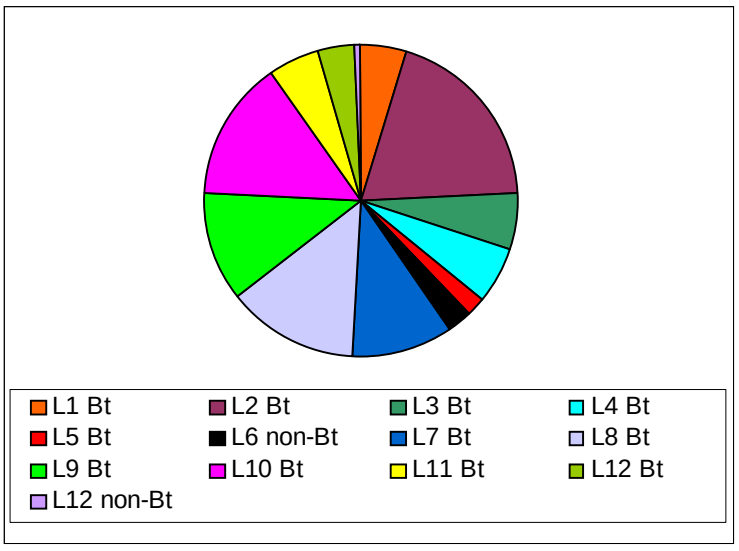
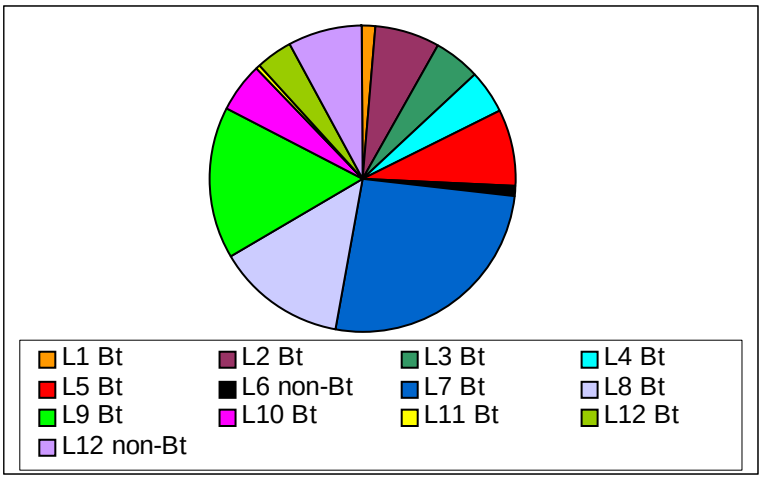
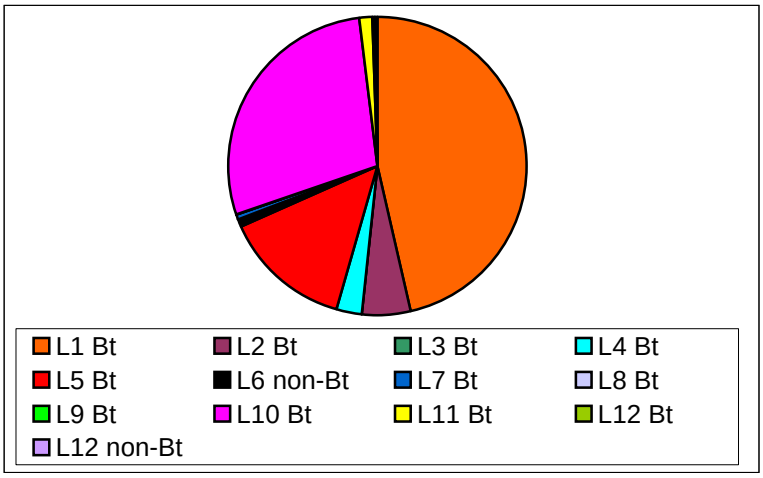


Fig. 6.50. The total number of plants per 10m^2 over the season (a) A. guineense, (b) Hibiscus spp. (c) Sida spp. during the host plant study 2001/2002.

a



b



c

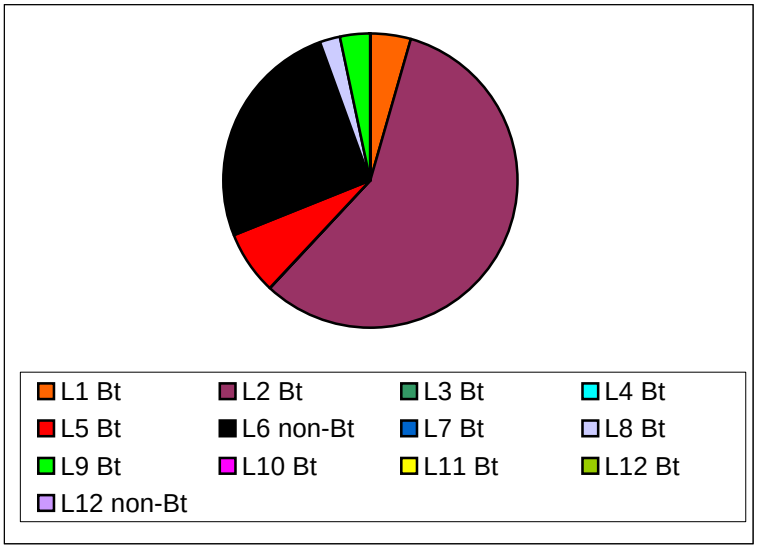


Fig. 6.51. The total no. of plants per 10m^2 over the season (a), C. trilocularis (b) A. austro-africanum (c) C. hildebrandtii during the host plant study 2001/2002.

CHAPTER 7

THE ACCEPTANCE OF GENETICALLY MODIFIED COTTON IN SOUTH AFRICA

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Abstract

This chapter will give a broad overview of the status of genetically modified (GM) crops in South Africa. This paper will deal with the process of registration of cultivars of GM crops in South Africa, and current legislation. In addition, resistant management strategies will be discussed, as well as the mode of action responsible for insect resistant and herbicide tolerance and the penetration of GM crops in South Africa. Cotton will be used as an example to illustrate the benefits of GM crops to the agricultural sector in South Africa. Many opinions and debates exist regarding the benefits as well as the potential risks of GM crops on human health and the environment, but these biosafety issues are beyond the scope of this paper. Information with regard to these issues can be obtained from the different groups mentioned in this paper who deal with lobbying of Biosafety issues.

Introduction

Genetically modified crops refer to crops where a single gene has been inserted into the genetic material of a plant by using a specific technique. In the first method (particle acceleration) or 'gene gun' method, DNA to be introduced into the plant cells is coated onto tiny particles, which are accelerated such that the DNA is "inserted" into the DNA of the recipient plant (*cf.* Padgette et al. 1995). The second method uses a bacterium to introduce the gene(s) of interest into the plant DNA (Madden, 1995). GM crops therefore involve genetic modification which results in the transfer of genetic material from one organism to another with processes other than natural breeding and selection techniques. According to Koch (2003), biotechnology speeds up the ability to transfer genes between species and is therefore a powerful technology that could possibly cause harm if not carefully assessed and monitored. South Africa has developed engineering techniques over the last twenty years, but this technology has not been applied or commercialized to the same extent as it has in other comparable developing countries such as India, China, Taiwan, to name but a few (*cf*. Koch and Webster, 1999). About 55 companies are involved in biotechnology in South Africa and most commercialized products are in the plant and medical sectors. In the case of GM crops, Koch and Webster (1999) are of the opinion, that international technology has been used to breed local varieties. They mention that the number of field trials for genetically modified organisms (GMOs) has rapidly increased from 12 in 1995, to 50 in 1998 and they will increase as time goes on. In South Africa, the first application for the registration of GM crops begun during 1988, when the commercial production of banana plants from tissue culture appeared (Anon 2003a). In 1989, the first application for trials in South Africa, for herbicide-tolerant cotton, was initiated, which was soon followed, in 1990, with field trials (Anon 2003a). This technology has since been accepted by farmers with great eagerness. Currently in South Africa, insect-resistant and herbicide-tolerant cotton (Bollgard® "line 531" and Roundup Ready® Cotton "line 1445" respectively), insect-resistant and herbicide-tolerant maize (YieldGard® "Mon810" and Bt11 (product of Syngenta) - and Roundup Ready® Maize "NK603"

respectively) and herbicide-tolerant soybeans (Roundup Ready® Soya "line 40-3-2") are the only genetically modified crops registered for commercial cultivation. These approvals represent single transgenes. No approvals have been granted as yet for combinations of transgenes in a single plant (the so called "stacked genes"). Bollgard was granted a conditional general release in 1997 and Roundup Ready® Cotton in 2001 (Anon 2003a). There are at present no genetically modified fresh products available in S.A., but imported processed foods can contain genetically modified raw materials such as soya, maize, canola and sugar (Koch and Webster, 1999). GMO pharmaceutical products and vaccines have been available in S.A. for some time (human insulin, human growth hormone and vaccines (Koch and Webster, 1999), but only crops will be dealt with further here. Bt-cotton has been accepted by the textile industry to a large extent. Up to now, no evidence has been found as yet (Jordan and Wakelyn, 2002) to proof that genetically engineered cotton has had a practical effect on fibre quality. It was found that fibre quality is just as likely to have been improved as not. Transgenic cotton varieties are accepted and much sought after by the SA. cotton grower. These would include insect resistant and herbicide resistant varieties. Much interest is created at the moment under SA cotton growers, with the introduction of the stack gene NuOPAL RR, meaning that individual plants will exhibit both insect resistant and herbicide resistant traits.

A set of international and national regulations have been introduced to promote the safe use of these technologies, the Cartagena Protocol on Biosafety and the CODEX Alimentarius guidelines for labeling products derived from GMOs, and on national level, the GMO Act, which was implemented in 1999 (Koch, 2003). In South Africa, regulation of all biotech crops is governed by the GMO Act, (Act 15 of 1997), which was implemented in 1999. This Act provides for a single application to initiate activities with biotech crops, be this research, development testing, import, export or commercialization (Koch 2003). In addition, the Act requires public participation, safety assessment and a risk management assessment, which covers human health and environmental concerns. All applications to the registrar of genetic resources[1] are submitted to an Executive Council. This Executive Council is made up of representatives from the National Department of Agriculture, Department of Environment, Department of Trade and Industry, Department of Labour, Department of Arts and Culture and the Department of Science and Technology. The general public is permitted to make recommendations and comments to the Executive Council. A Scientific Advisory Committee is responsible for safety reviews of an application and serves as a scientific reviewer. The national decision-making process is further explained by Kitch et al. (2002). Close communication exists between the Directorate and the applicant, and the applicant may appeal failing obtaining permission to proceed with the application for a biotech crop.

Specific guidelines have been set out to govern the activities surrounding efficacy testing of biotech crops. These guidelines include the application for permits from the Department of Plant Health and Quality Control (National Department of Agriculture), which makes out part of

[1] The registrar serves as a biosafety administration, produces guidelines for application, sets guidelines and serves as an inspectorate for activities surrounding the application.

the Registrars office, to plant GMO seed under certain conditions. These conditions include: Safety boundaries, by specifying distances from other natural or GM crops; Isolation blocks surrounding the planted crops; Inspections from the National Department of Agriculture, following Good Experimental Practices; Demolishing of the crop within a specific time limit; Providing efficacy data in comparison with non-GM crops to prove its benefits, at least two seasons work on different localities and any additional information on the effects on biodiversity and on the environment.

Insect-resistant crops contain a gene from the soil bacterium Bacillus thuringiensis (Bt). Bollgard™ cotton shows resistance to the bollworm pest species present on cotton in South Africa, the african bollworm Helicoverpa armigera (Hübner), spiny bollworms Earias biplaga Walker and Earias insulana Boisduval and the red bollworm Diparopsis castanea Hampson (Lepidoptera: Noctuidae). Bollgard™ cotton was initially available to growers in two Delta and Pinelands varieties, namely NuCOTN 35^B and NuCOTN 37^B. These have been replaced with the variety NuOpal. All contained the event line 531, which produces endotoxin or protein, Cry1Ac (Akehurst 1993).

Research with a second event (Mon 15985), which produces the Cry2Ab2 protein for bollworm resistance, has been ongoing and the field trials on cotton is discussed in Chapter 2 of this study. Commercial approval of combinations of genes, the so-called stacked gene varieties, where more than one event is present within a single plant has been approved in South Africa in September 2005. These stacked gene varieties, or double gene varieties, express more than one trait, rendering it to be both herbicide-tolerant and insect-resistant at the same time.

A review of the many Bt-crystalline proteins identified is given by Chilcott and Wigley (In: Akehurst 1993). The mode of action of the Bt-protein (endotoxin) is described by many authors, including Knowles (1993), as cited by Akehurst (1993). The Bt-endotoxin is described in Chapter 1 of this study.

HERBICIDE TOLERANT GM CROPS

The herbicide tolerant cotton is tolerant to the application of herbicides containing glyphosate (N-phosphonomethyl glycine). Glyphosate specifically binds to and inhibits the activity of 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) (*cf.* Padgette et al. 1995). EPSPS is an enzyme of the aromatic amino acid biosynthetic pathway. EPSPS is the only physiological target of glyphosate in plants – no other enzymes are affected by glyphosate.

May et al. ((2002) discussed glyphosate as a broad-spectrum herbicide, and mentioned the EPSPS enzyme that controls the production of essential amino acids in the plant, without which the plant would die. They also mentioned that due to its absence in animals, it has very low toxicity. Application of glyphosate thus prevents a plant from making aromatic amino acids which are essential for the synthesis of proteins and some secondary plant metabolites, and the plant dies. Glyphosate-tolerant GM crop plants contain a gene capable of producing a glyphosate insensitive form of EPSPS, namely CP4-EPSPS. This enzyme catalyses the reaction of shikimate-3-phosphate and phosphor-enolpyruvate to form 5-enolpyruvylshikimate-3-phosphate (EPSP) and phosphate in the presence of glyphosate (Fig. 7.1).

AN INSECT RESISTANCE MANAGEMENT STRATEGY

For insect-tolerant crops, the question of possible resistance to the Bt-gene is a critical one, since insects are capable of quickly developing resistance to chemicals. Research has also shown that under specific conditions, bollworms have developed resistance to the insecticidal protein produced by B. thuringiensis. It is thus essential that an Insect Resistance Management (IRM) Strategy be put in place with GM crops to prevent the development of resistance for as long as possible and so preserve the benefits of this technology.

In South Africa, as part of an IRM strategy, the GMO Act, Act 15 of 1997, requires a refuge area to be planted where biotech crops are grown. According to Mallett and Porter (1992), by planting refugia, or toxin-free, non-transgenic cotton plants, on the same cultivated land, the susceptible genes in the bollworm population can be conserved. If bollworms were to reach maturity in a field of Bollgard™ cotton, the specific population could give rise to resistant populations due to the presence of a resistant gene. If the offspring of these potentially resistant larvae were to mate with one another, the survival of the resistant/tolerant gene in the population will be ensured. If, however, a susceptible population has been maintained in the toxin-free refugia, the probability is higher that potentially resistant moths (from the Bt-crop) will mate with moths from the larger susceptible population (from the refugia), resulting in dilution of resistant genes and production of susceptible offspring, ensuring susceptibility to the Bt-toxin for a longer period of time (Anon 1999).

Monsanto SA (the company patenting biotech products in South Africa) offers a choice of two refuge options to be planted with Bollgard® cotton. For each 100 ha of Bollgard planted, the farmer can plant a refuge of either 20 ha sprayed non-transgenic cotton, or 5 ha unsprayed non-transgenic cotton (Anon 1998a and Anon 1998b). Upon purchase of seed, the buyer signs a license agreement with the company stating that one of the two choices will be followed. Refugia must be planted as close as possible to the Bollgard-field and must be planted as a separate block, not mixed with the Bollgard. The refugia should preferably not be further than 500 meters away from Bollgard fields and must be planted at the same time, and managed in the same way, so as to provide a suitable habitat for Bt-susceptible individuals.

Some problems can occur with the planting of refuges in the field, especially in a small-scale farmer set-up, where farmers only plant a few hectares of cotton and so may neglect to plant the refuge areas. A study has recently been completed on the significance of alternative bollworm host plants and the implications thereof on the refuge system in a small-scale farmer setup (Green et al. 2003; Bennett-Nel et al. 2003a and b).

The monitoring of the recommended refuge system is an ongoing process, and to date, there have been no documented cases in South Africa of resistance to bollworm under normal growing practices.

For herbicide tolerant cotton, there are currently no regulatory requirements for a weed resistance management programme. In contrast to many other herbicides, after approximately 30 years of intensive use, very few weed species have developed any tolerance to glyphosate.

Not withstanding this, weed resistance management guidelines for glyphosate have been proposed and are being investigated (Croplife SA).

Several bodies are concerned with biotech crops, and in specific: “The Crop Protection and Animal Health Association (AVCASA) (Croplife SA) (**http://www.croplife.org**)” which is part of a global network (CropLife International) representing the plant science industry. One of the largest challenges facing sustainable agriculture at present is providing sufficient, healthy food for the world’s ever-increasing population. The plant science industry contributes significantly to promoting sustainable agriculture, which is fundamental to food production and poverty alleviation, through balancing environmental, economic and societal concerns.

Also “The South African National Seed Organization (SANSOR) (**http://www.sansor.org**)” is the designated authority that manages and executes all functions pertaining to seed certification on behalf of the government. At present, SANSOR is responsible for the quality of certified seed produced on more than 60 000 hectares. “AfricaBio (http://**www.africabio.com**)” is an independent, non-profitable biotechnology stakeholders association. Their key role is to provide accurate information and create awareness on biotechnology in S.A. and in the regions. Their mandate is to provide accurate information about biotechnology and to encourage an open debate about the concerns and benefits of the new technology and its products. They have an active information, training and education portfolio (Anon 2000), and keep in close contact with the South African Committee for Genetic Engineering (SAGENE), which was established in 1997. “The National Department of Agriculture (**http://www.nda.agric.za**)” has a division, The Department of Genetic Resources, who controls the application of registration of biotech crops. All registrations have to comply with the GMO Act, Act 15 of 1997, which regulates the use of genetically modified crops, the Plant Improvement Act, Act 53 of 1976, and the Plant Breeders Rights Act, Act 15 of 1976. Compliance with the regulations stipulated in these acts places the grower under obligation to comply with license agreements with the patent holders, which, in the case of the registered biotech crops in South Africa (cotton, soya and maize), is Monsanto South Africa (Pty) Ltd.

“The Department of Science and Technology (DST)”, as part of the National Department of Agriculture, is responsible for communication with the general public on biotechnology issues. They formulated a dynamic innovation, the Public Understanding of Biotechnology (PUB) programme (http://**www.pub.ac.za**), which provides funding on a competitive basis for relevant innovative communication training and projects. “The Medical Research Council of South Africa (MRC) (http://**www.mrc.ac.za**)” is interested in studies carried out and literature published on the biosafety of products and safety on human health. They aim to improve the nation's health status and quality of life through relevant and excellent health research aimed at promoting equity and development, and also to develop an appropriate science and technology base and capacity to address current and future health challenges, while at the same time creating sustainability and growth. Research on applied biotechnology is carried out by universities, the Agricultural Research Council (ARC) and private consultants belonging to the South African Association of Pesticide Consultants (SAAPC), on products regulated under Act 36 of 1947 (pesticides,

fungicides, plant growth regulators, performance-enhancing additives, herbicides, et cetera) and Act 15 of 1997 (registration of biotech crops).

THE CARTAGENA PROTOCOL

The Cartagena Protocol on Biosafety (http://www.biodiv.org/biosafety) is a legally binding global protocol that aims to "contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms (LMO's) resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements". The Protocol entered into force on 11 September 2003, ninety days after receipt of the 50th instrument of ratification. The Protocol requires that bulk shipments of LMO commodities intended for use as food, feed or for processing, must be accompanied by documentation that states that such shipments "may contain" LMOs and "are not intended for intentional introduction into the environment" (Anon 2002).

The protocol aims to promote international cooperation to help developing countries acquire resources and capacity to use biotechnology safely and regulate it efficiently. Member governments are encouraged to assist with scientific and technical training to promote the transfer of technology, knowledge and financial resources. Governments are also expected to facilitate greater involvement of the private sector. Member governments must commit themselves to promoting public awareness, insuring public access to information, and public consultation. The Protocol recognizes that national measures are important to make procedures effective. Nations must also take measures to prevent illegal shipments or accidental releases of LMOs (Anon 2002a and b). The Biosafety Protocol assists developing countries in building their capacity for managing modern biotechnology. It creates an advanced informed agreement (AIA) procedure that requires exporters to first seek consent from importing countries, and so must be followed before the first international transboundary movement of an LMO into the environment of the importing country. Further information can be obtained from the website address, mentioned above. Some of the problems involved with the implementation of the Protocol include: (a) The Protocol depends on full information for its effective implementation. Labeling and traceability are therefore essential, however, countries like the United States refuse to label GMO products and it is therefore impossible to tell whether GMOs are being imported; and (b) Very poor and poverty-stricken countries are too poor to allocate adequate resources for Biosafety and if risks did occur, these countries would also be unable to find the financial and technical capacity needed to combat it (Egziabher 2003)

In South Africa, the Directorate for genetic resources[2] is responsible for biotech crops and is currently in the process of implementing the Cartagena Protocol and abiding by the strict regulations thereof. At present, the Cartagena Protocol is the only law governing the environmental impacts of GMOs. The World Health Organization is responsible only for the safety to human health of pharmaceuticals, GMOs or otherwise, and not for their environmental

[2] Directorate of Genetic Resources, Nat. Dept of Agriculture, Dr. J. B. Jaftha., P.O. Box 138, Pretoria, South Africa.

impact (Egziabher 2003). All new applications for GMO plantings, be it for commercial release, seed production, or research purposes have to comply with these strict regulations set by the Dept. of Genetic Resources, which is part of the National Department of Agriculture of S.A.

THE EFFICACY OF GM COTTON IN SOUTH AFRICA

In South Africa, cotton growers are currently divided into either large-scale commercial growers or small-scale emerging farmers. The commercial farmers need training in financial management and the intelligent use of biotechnology, while the small-scale farmers need basic production information and training in the use of advanced technology. Benefits of planting Bt-cotton include: the increased profits due to reduced insecticide and herbicide applications, also reducing the handling of dangerous chemicals; the savings of time and labour; an alternative solution for the grower to combat resistance to pyrethroids in bollworms; a reduction in the influence of weather conditions on application efficiency; safety to humans and livestock; and environmentally friendly control of pests and conservation of natural enemies of pests by supporting and encouraging an IPM approach (Anon 1999, 2004). Cotton is a crop ideally suited to peasant production since it involves the whole family and has a guaranteed income, especially in the more arid regions where rainfall is uncertain. Bennett et al. (in press) were of the opinion that the main benefits to small-scale growers include savings in time and labour, a reduction in the handling of dangerous chemicals, and improved levels of bollworm control resulting in significantly higher yields. Once the benefits of GMO technology become more apparent over time, it will become even more widely accepted. Many authors have already dealt with the acceptance of Bt-cotton and the socio-economic impact of Bt-cotton in South Africa, specifically in the Makhathini area (KwaZulu-Natal). These references are not necessarily quoted here, but reference thereto is made in Annexure IV.

Insect-resistance expressed by Bt-cotton is consistent for the whole season under normal seasonal conditions (i.e. average rainfall and daylight hours for a specific region within S.A.), and is even expressed for a second season in the case of ratoon or stand-over cotton (Van Jaarsveld[3], pers comm.). Monsanto does not, however, recommend the ratooning of cotton and the Technology Agreement signed by every grower is valid for one season only. Gene expression may vary under plant stress conditions, and fourth and fifth instar bollworm larvae have been observed in isolated cases during experimental trials. The expression of the gene in the flowers can vary between individual plants and between localities, and this phenomenon emphasizes the importance of following an Integrated Pest Management approach by scouting (Bennett-Nel, 2003c), in order to observe and monitor first and second instar larvae at the earliest possible stage. The use of Bollgard® cotton in S.A. has been shown to require fewer insecticide applications while at the same time producing higher yields (see Chapter 5).

[3] Van Jaarsveld, M. Agricultural Research Council - Plant Protection Research Institute (ARC-PPRI). Private Bag X134, Pretoria, 0001.

HERBICIDE-TOLERANT COTTON (ROUNDUP READY® COTTON)

According to May et al. (2002), herbicide-tolerant GM crops present the grower with specific benefits, including an opportunity to manage weeds in such a way so as to provide environmental benefits; to reduce weed control inputs; and it also provides the farmer with an opportunity to use best management practices, such as zero tillage. Weed conditions vary widely from farm to farm and it is therefore very difficult to generalize with respect to monetary or economic benefits delivered by this product. In general, yield is not affected (positively or negatively) by Roundup Ready Cotton. Benefits of this technology tend to be intangible and include ease of management, the ability to control previously uncontrollable weeds, replacement of persistent herbicides with non-persistent herbicides and compatibility with reduce tillage practices. In particular, it has been found that sequential applications of between 2.5 and 3 liters of Roundup per hectare effectively control Cypress sp. (nutsedges). These weeds were previously very difficult and costly to control. Compatibility with reduced tillage practices also reduces the impact of mechanical cultivation on the soil environment.

THE PENETRATION OF GMO CROPS

According to James (2003), during 2003, six principal countries, compared with four in 2002, grew 99% of the global transgenic crop area, this reflects the broadening participation of the lead GM countries with ten countries now growing 50 000 ha or more GM crops. James (2003) mentioned that South Africa and China had the highest year-on-year increase with a 33% growth rate and that South Africa increased its combined area of GM maize, soybean and cotton, to 0.4 million hectares in 2003, characterized by a sharp increase in white maize production from 6 000 ha in 2001 to 84 000 ha in 2003. Current figures representing the plantings of GMO crops in S.A. (Bennett, 2003) are 300 000 ha Bt-maize, 20 000 ha Bt-cotton, 4 000 ha Roundup Ready (RR) cotton and 30 000 ha RR soya (Fig. 7.2).

In South Africa the current situation for cotton is about 10 000 ha Bollgard, 5 000 ha Roundup Ready, and 2 000 ha conventional cotton. In the small-scale cotton production areas, dry land cotton is produced and it accounts for about 3 % of the cotton production in South Africa, whereas small-scale cotton production in neighboring countries accounts for about 90 % of the production (cf. Anon 2003b). The area planted to cotton in 2002/03 in this sector has fallen significantly, but this is due as much to the restrictions on credit introduced by the lending institutions as to falling cotton prices (there are less options more attractive than cotton in marginal rainfall areas, where most of this cotton is grown). Credit restrictions also influence the quantity and quality of chemicals applied for pest control, so reduced spraying often occurs by default rather than intention. Whereas the large-scale producers tended to be “spoon-fed”, the small-scale producers (outside of parastatal irrigation projects, many of which have collapsed) have had to fend for themselves. This situation with regards to production is reflected in the production statistics for the past few years. Bennett (2001) discussed the adoption rate of Bt-Technology with regards to cotton under smallholder users, and mentioned that in 2001, the growth in the number of smallholder users has increased 16-fold. He further mentioned that their

main reason for adoption of the technology is the expected increases in yields, while the savings in chemicals and labour was additional drivers for adoption of the technology (Kirsten et al. 2002). At present about 80% or the total annual production in South Africa is derived from GM cotton. Farmers do have a choice in the selection of cultivars, but the benefits they obtain from planting GM varieties far out-compete the consideration of alternative conventional varieties. The core statistics (Table 7.1) provided by Cotton SA mentions that the hectares planted under irrigation has decreased considerably over the past 5 years, while the yield per hectare under irrigation has increased somewhat to become stable at about 3 000 kg/ha seed cotton. The seed cotton yield for dry land has been under 500 kg/ha, and the number of hectares planted has decreased considerably. This can probably be attributed to the past seasons where rains came very late for planting, and many small-scale farmers and commercial farmers did not plant cotton. The need to increase yields of crops cultivated in Africa, is clearly expressed by Wambugu (2001): "…the greatest challenge is to raise the yields of the most widely grown crops, since these are the mainstay of the region's subsistence farming systems. This must be done because there is no longer any room to expand the area cultivated…".

South Africa produced only about 16 000 T lint during 2003, which was far below the general consumption of 46 000 T lint (Cotton SA, Core statistics, 2004). The cotton growing industry experienced a major decline during the past 4 years, with the area planted to cotton falling from about 124 000 ha in 1998 to about 30 000 ha in 2002/03 (Cotton SA, 2006) (Table 7.1). This has been due to the significant increase in costs of inputs (chemicals about 8 % p.a.) and a fall in the world cotton price to an all time low in 2001/02 (S.W. Broodryk[4], pers comm.).

Of the total of 22 551ha planted under cotton in South Africa, more than 80% have been planted with Bt-cotton, or a cotton type, with the Bt-gene (A.L. Bennett[5], pers comm.) The impact that this technology has on a cotton management programme is discussed below.

THE IMPACT OF GM TECHNOLOGY

The current impact of Bt-technology was described by AL. Bennett (sic) in (Bennett-Nel, et al., 2005) and can be illustrated with the following example, indicating the clear benefits of Bt-technology. Currently, approx. 30 000 ha of Bt-cotton is planted. By using Bt-cotton, a grower saves about 10 sprays made us as follows: 3 endosulfan applications at 1.5 liters/ha; 4 monocrotophos applications at 0.8 liters/ha, and 3 cypermethrin sprays at 0.25 liters/ha, corresponding to 8.45 liters/ha of pesticides. This results in a reduction of 254 000 liters of product used, equivalent to 103 000 kg a.i., and having a value of 13.6 million Rand (Bennett, 2003) (Fig. 7.3.).

It is expected that the acceptance of GM cotton will grow, since a new strategic plan is presently being formulated by the National Department of Agriculture, in collaboration with Cotton SA and other role-players, encouraging the stabilization and expansion of cotton production in S.A. A training programme for cotton growers has been suggested and undertaken by a training

[4] Dr. S.W. Broodryk was a consultant and cotton specialist in S.A., in specific with regards to Bt-technology and cotton, but has passed away in 2005.
[5] A.L. Bennett is the Biotech Marketing Lead of Europe-Africa in Switzerland, and formerly from SA.

unit under the umbrella of Cotton SA. Due to the large percentage of growers purchasing GM technology, the principles with regards to production and pest control have been included in the training programme. In addition, various aspects of the AGOA (The African Growth and Opportunity Act) initiative have been enforced by an inter-agency group, the U.S. Committee for the Implementation of Textile Agreements (CITA), and it has been determined that trade in handmade, hand loomed and folkloric items, specifically clothing, are eligible for duty-free status. African countries that have satisfied the AGOA conditions of eligibility include Namibia, Zambia, DRC (Democratic Republic of Congo), Botswana, Ghana, Mauritius, Tanzania, Uganda and South Africa amongst the 19 approved Sub Saharan African Countries. These countries can trade duty-free in exporting clothing to the U.S, while duty-free trade in regional fabric between the African countries is allowed. This agreement ensures that some of the lesser-developed countries can obtain cotton material or yarn duty-free from third world countries, and this should encourage cotton production locally to supply cotton to Botswana in accordance with demand (Dr. B. Brink[6], pers comm.). Since the largest bulk of cotton produced in S.A. originates from GM cotton and no distinction is currently made between conventional or GM lint, it is expected that this free-trade agreement will also encourage the use of GM cotton in order to reap the benefits thereof. Cotton research and extension will be an important facet of institutional support to the industry. What this agreement holds for the future of GM crops, and in specific cotton in South Africa, is unsure. However, the hope is expressed that this would encourage small-scale farmers to take Bt-cotton as a first choice crop, and that this would not only promote the acceptability of Bt-cotton, but would also benefit the individual farmer and the industry as a whole.

The acceptance of the existing varieties will depend on the level of product information concerning the technology and management of the technology that is transferred to extension officers and to people in the industry involved with training. Training and extension is one of the approved strategies of the New National Cotton Strategy, with the aim of improving and expanding current systems. Farmers are trained by means of the development of a learnership programme and skills training that would enable the industry to transfer knowledge to emerging farmers. This outcome based training programme will be in agreement with unit standards on biotech crops for primary agriculture, which are implemented by the Primary Agricultural Education Authority of South Africa (PAETA), working within the National Qualifications Framework (NQF) as provided for in the South African Qualifications Association (SAQA) Act, 1995. This system will enable people to gain recognition for all competencies and skills as per nationally agreed requirements.

There is an interest developing, under commercial cotton farmers, and in specific the South African Cotton Producers Organization (SACPO) to cultivate GMO cotton. This sentiment is also felt by other organizations that promote grain plantings and soya. SACPO controls orderly marketing and quality levels in the interest of growers and processors. Its duty is promoting

[6] Dr. B. Brink is a director of the Textile Federation of S.A. and a specialist in his field of trading statistics on cotton in South Africa.

expansion of the national crop and facilitating training and research. SACPO supports the introduction of new transgenic varieties and has expressed a commitment to continue and encourage cotton farmers to plant the new stacked gene variety NuOPAL RR. NuOPAL RR has now become commercially available in South Africa (October 2005), and double gene combinations of other Bt-genes and genes providing tolerance to glyphosate are now being tested (pers obs.) with the aim of submitting these cotton types for registration. The acceptance of new developments and technologies, such as the introduction and success of the new stacked gene varieties in the future will depend on scientific evidence of the effect of the gene on the immediate environment where cotton is planted, on the social stability of the farmers concerned and on the natural resources related to farming. Research on the impact of the Bt-gene on the environment and non-target organisms is a continuous process and the costs thereof are high (W.M. Green[7], pers comm.). The patent holders continue an interest in bringing this technology to the farmer, and ensuring the safety thereof, which emphasize the eagerness with which the single gene trait was accepted. Most small-scale growers plant Bt-cotton, and they communicate their hectares planted to Cotton SA during training. Seed supplied on demand to small-scale growers includes a special packaging of 5kg Bt-cotton seed with additional conventional seed to be used as a refuge (pers comm. F. Friss[8]). This seed is treated with an insecticide applied as a seed treatment which is aimed at sucking pests. The fact that nearly all small-scale farmers (about 90%) (pers comm. C. Matlou[9]) from the largest area of small-scale cotton growing (Makhathini area, KwaZulu Natal) purchase these small packages of seed provide evidence for the acceptance of this technology.

More than 75% of the annual cotton crop in S.A. is still produced by commercial cotton farmers and it is presumed that many of the more efficient large-scale producers have persevered with cotton since they have been able to trim input costs and, at the same time, increase yields by using GM cotton varieties (*cf*. S.W. Broodryk[10], Anon 2003b). The genetically altered varieties therefore present farmers with the opportunity of producing higher yields, even under unfavorable economic circumstances.

CONCLUSION

Transgenic crops are here to stay. These crops benefit both the commercial and the small-scale farmer in South Africa, mainly by increasing yields and enabling farmers to farm more cost-effectively. These crops also provide the farmers with a new outlook on promoting cleaner production and so conserving the environment, by making use of an Integrated Pest Management approach and using the minimum amount of hazardous chemicals, which also lessens the exposure of farmers to these insecticides. In the case of herbicide tolerant crops,

[7] Wally Green was the Regulatory Manager at Monsanto SA until Dec, 2007, but now acts as a private consultant for Crop and Plant Biotechnology Services cc..
[8] Fanie Friss is a plant breeder for Delta and Pinelands, SA.
[9] C. Matlou was formerly employed by Monsanto SA as the small-scale farmer representative in the Makhathini Flats (KwaZulu-Natal).
[10] The late Dr. S. W. Broodryk was a cotton specialist, and known for his experience with GM cottons. He was a consultant for Stoneville (later integrated as part of Monsanto SA) doing trials on GM cotton, and an Integrated Pest Management specialist under the DANIDA funded project at Cotton SA (Pretoria, SA.).

there is a considerable saving in the repeated use of a variety of pre-plant and post-plant herbicides, it enables broad-spectrum weed control and there is no reduction in yield due to the weed control programme followed. Apart from cost savings, GM crops enable the grower to follow a simple crop management programme, therefore saving in both time and labour.

Final conclusion on the acceptance of biotechnology amongst South African farmers

The decrease in the use of insecticides and the use of non-persistent herbicides will, in time, benefit residual build-up in the environment. Beneficial insects and other arthropods are also not affected by the use of this technology. S.A has strict legislation with regards to biotech crops, which requires more thorough testing than any other chemical, bio-product, or conventional crop registered, and as long as these regulations are in place to promote biosafety, these crops will benefit not only the farming community but the economy of S.A. as a whole. The fact that very little, of the cotton planted by South African farmers are conventional cotton varieties confirms the acceptance of Bt-technology amongst the cotton growers. Technology transfer is now an important aspect applied in the cotton training programme to cotton growers and the viewpoint of the seed producers to provide seed of the single gene trait (NuOPAL) and double gene trait (NuOPAL RR) emphasizes the demand placed on the seed company by the producers.

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Table

Table 7.1. South Africa Cotton Statistics (Cotton SA Core Statistics, 2006).

***HECTARES PLANTED PERTAINING TO SEED COTTON PURCHASES BY RSA AND SWAZILAND GINNERS**

PRODUCTION YEAR	IRRIGATION			DRYLAND			TOTAL		
	RSA	SWAZIL.	OTHER**	RSA	SWAZIL.	OTHER**	RSA	SWAZIL.	OTHER**
	ha			ha			ha		
1995/96	17609	385		72809	19007		90418	19392	
1996/97	15954	0		67017	40473		82971	40473	
1997/98	20361	400	1733	69578	30998	2781	89939	31398	4514
1998/99	31263	450	1585	67356	32932	6325	98619	33382	7910
1999/00	10486	0	1346	40282	23875	2879	50768	23875	4225
2000/01	18539	0	1565	38153	11301	2050	56692	11301	3615
2001/02	9791	0	802	28897	9606	2400	38688	9606	3202
2002/03	10322	0	790	12252	4500	2016	22574	4500	2806
2003/04	18269	0	364	17450	6500	760	35719	6500	1124
2004/05	12935	0	280	8866	5000	750	21801	5000	1030
2005/06 ***	12910	0	0	9641	2500	0	22551	2500	0

** Botswana, Namibia and Zimbabwe / Figures prior to 1997/98 not available

*** ESTIMATE

Figures

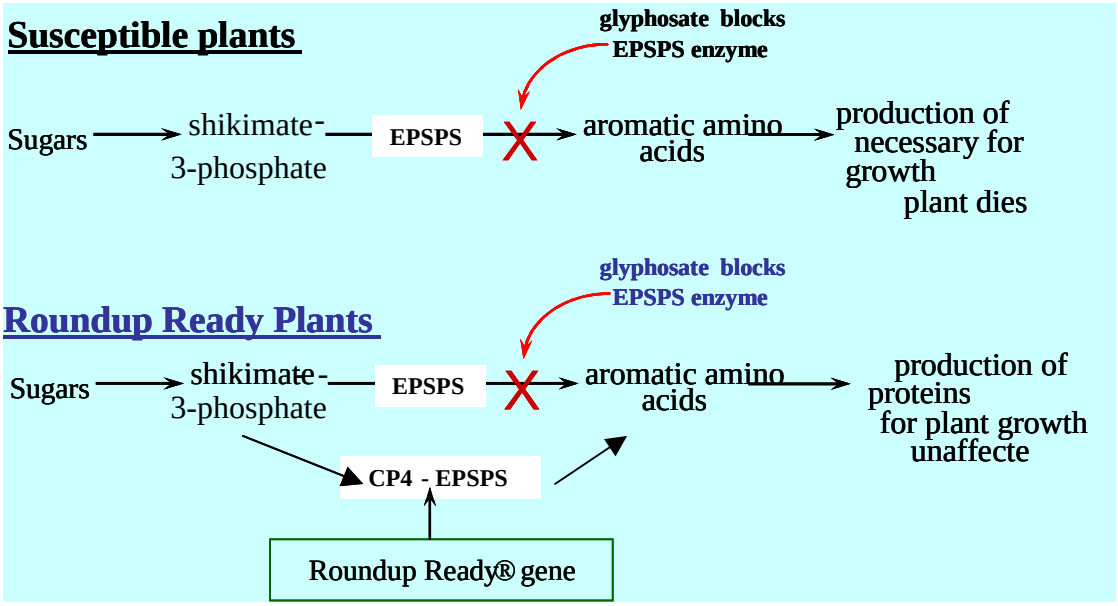


Fig. 7.1. The mechanism of the Roundup Ready gene, CP4-EPSPS in herbicide tolerant plants (after Bennett 2003).

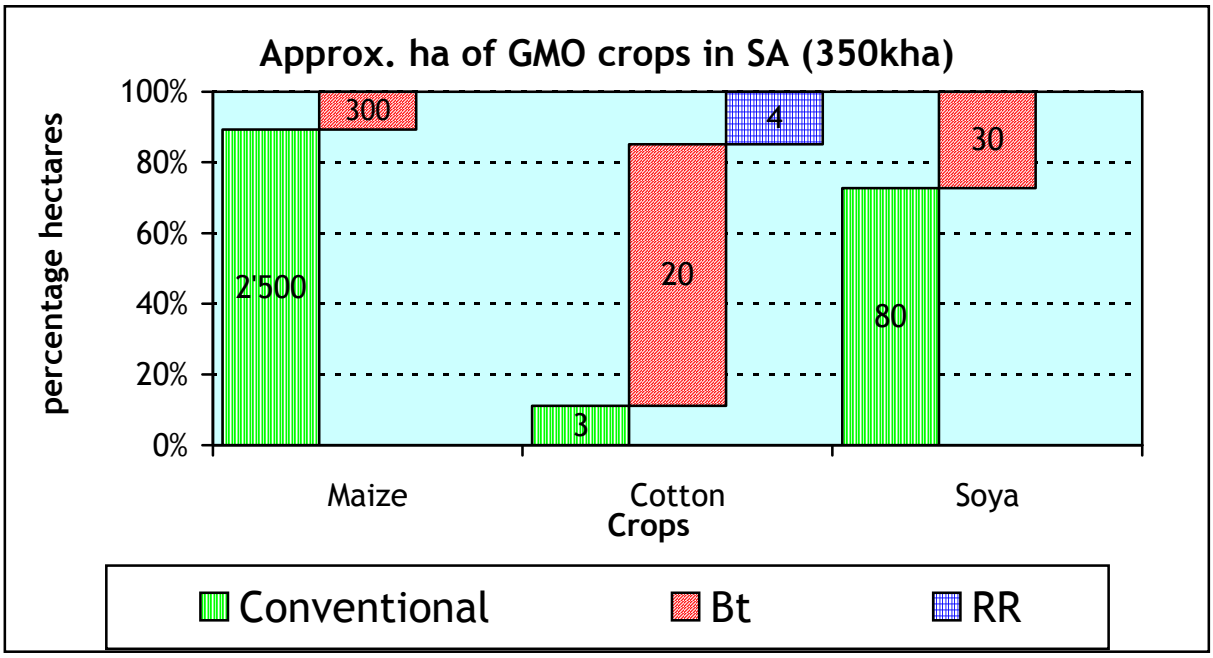
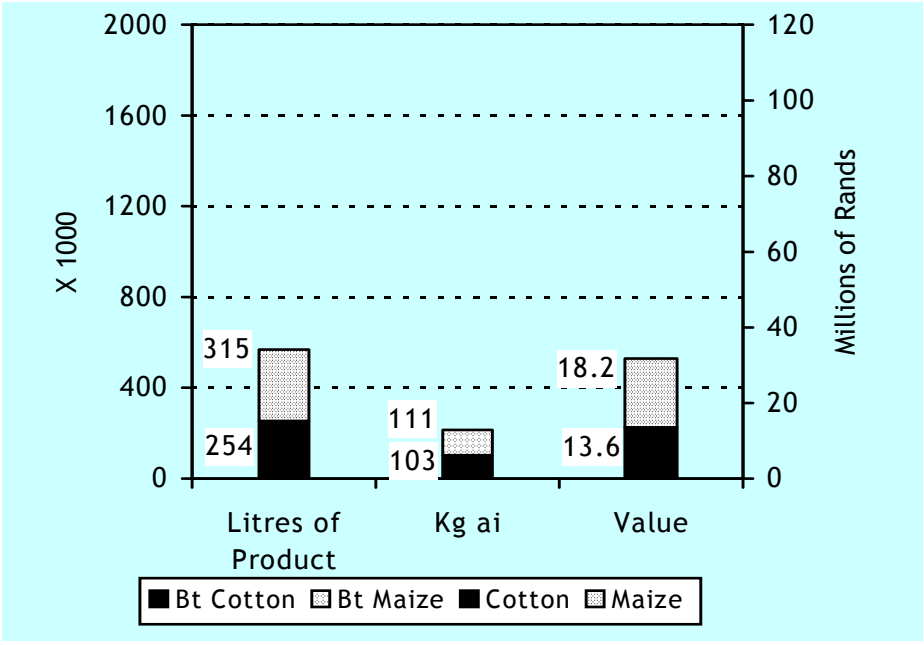


Fig. 7.2. Estimated area (hectares) planted under GMO crops in South Africa (2003) (Bennett, 2003).



(after Bennett, 2003)

Fig. 7.3. The impact of Bt-technology on cotton and maize in South Africa (Bennett, 2003).

OVERALL CONCLUSION

The study contains the initial results of the registration trials for the first introduction of single gene Bt-cotton that exhibits only bollworm resistance, in South Africa as well as the results from the initial registration of the first stackgene Bt-cotton in South Africa, which not only exhibits bollworm resistance but also herbicide tolerance. The study has brought certainty to the efficaciousness of Bt-proteins in combating the bollworm complex on cotton and provided strong evidence on the lack of effect on non-target organisms, such as secondary pests. It also confirmed its narrow selectivity to certain lepidopterans by showing no adverse affects on beneficiary insects Bt-technology in cotton production has proved to be not only effective against the target pests, which are the bollworms on cotton. The effect of the Bt-gene on non-target organisms is minimal if any in most instances and beneficial arthropods flourish in the presence of their other insect hosts, irrelative of the cotton type planted. These interrelations between predators and secondary pests are a function of the shift in the rate of applying insecticides to control these pests and the population dynamics of especially secondary pests. Fewer bollworm sprays result sometimes in a tendency for some secondary pests like leafhoppers and aphids to increase, which might in return result in an increase in aphid predators, as a factor of predator-host availability. Even when additional sprays for secondary pests are applied, the benefit of the farmer is still valuable in the form of higher yields and lower input costs reflected in non or fewer bollworm sprays. This work illustrates the extensive research that was undertaken, in this case only with the first registration of the *cry1Ac* and *cry2Ab2* gene by the patent holders. The benefit of planting Bt-cotton amongst cotton growers reflected in an increase in yield production has been illustrated in this study, and it clearly demonstrates the acceptance of Bt-cotton in South Africa. Another important contribution is the identification and categorization of weed and other plant species and the evaluation of bollworm numbers on these plant species in the largest small-scale cotton production region of South Africa, the Makhathini Flats. Insect monitoring with regards to the prevention of resistance to the Bt-gene by bollworms, and the significance of alternative host plants in an area where cotton is cultivated, provided evidence for a possible alternative refuge to conventional cotton. In the latter study, the results showed that bollworms occur on natural host plants in adequate abundance so that the “wild population” or Bt-unexposed population of bollworms can mate with Bt-exposed individuals originating from the Bt-cotton. The establishment of many organizations in Biotechnology in many areas of science, and in particular the interest paid by growers in Bt-technology, supports the statement that Bt-cotton is now widely accepted by most cotton growers. The results are of particular importance to South African commercial cotton producers as part of an Integrated Pest Management strategy on cotton, and to small-scale cotton growers on the African continent, where small-holder cotton growing forms a critical part of the cotton industry globally.

ANNEXURES

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Annexure I (Chapter 2): Statistical analyses of bollworm larvae from Rustenburg (1997/1998)

Table A.2.1. Accumulated analysis of variance of the log (total number of bollworm larvae) from Rustenburg (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	13.367	13.367	5.44	< 0.027
Treatments	3	31.828	10.609	4.32	0.013
Time Treatments	3	3.936	1.312	0.53	0.663
Residual	28	68.639	2.459		
Total	**35**	**117.971**	**3.371**		

The non-significant interaction effect implies that separate parallel regression lines represent the different treatment responses. The regression equations were calculated ($y = mx + c$), where x =days after planting); from the estimates of the regression coefficients and for the 4 treatments they are as follows:

$\log y_{(1)\ \text{Bollgard unsprayed}} = -2.095 + 0.0277\ x$

$\log y_{(2)\ \text{Bollgard sprayed}} = (-2.095 + 0.472) + 0.0277\ x$
$= -1.623 + 0.0277\ x$

$\log y_{(3)\ \text{DpAC 90 unsprayed}} = (-2.095 + 2.115) + 0.0277\ x$
$= 0.02 + 0.0277\ x$

$\log y_{(4)\ \text{DpAC 90 sprayed}} = (-2.095 + 2.058) + 0.0277\ x$
$= -0.037 + 0.0277\ x$

By studying the t-values and t-probability associated with the estimates of the regression coefficients in the analysis for the best model, it can be deduced that Treatment one (Bollgard unsprayed) differed significantly from Treatments three and four (DpAC 90 unsprayed and sprayed) with regard to the y-intercept. In order to compare the other remaining regression lines with one another, t-statistics were calculated:

$t_{ij} = (\text{slope}_I - \text{slope}_j) / (\text{var}(\text{slope}_I . \text{slope}_j))^{1/2}$
$= (\text{slope}_I - \text{slope}_j) / (S_i^2 + S_j^2 - 2 S_i S_j)^{1/}$

(the above equation is not applicable in this case, since slopes are equal and there is no significance between treatments and time)

or $t_{ij} = (\text{interc}_I - \text{interc}_j) / (\text{var}(\text{interc}_I . \text{interc}_j))^{1/2}$
$= (\text{interc}_I - \text{interc}_j) / (S_i^2 + S_j^2 - 2 S_i S_j)^{1/2}$

The necessary variances/co-variances were obtained from a variance/covariance matrix in the analyses.
The t-statistics for intercept for lines 2 and 3 were calculated as:
Treatments two and three
$t_{2;3} = ((-2.095 + 0.472) - (-2.095 + 2.115))/ ((0.5217 + 0.5217 - 2(0.2608))^{1/2}$
$= -1.643/ 0.72236$
$= -2.274$

The t-statistic was compared against a tabulated t-value obtained from an extended table of critical Bonferroni t-values.

t_{31} 0.05: α /2p; where $P_{(\text{number of pairs})} = k(k-1)/2 = 4(3)/2 = 6$

and α = significance level of 0.05 (5%).

t'_{31} 0.05/2(6)

= 1.697 (tabulated value = absolute value)

Since $t_{2;3} = -2.274 < -1.697$, Treatments two and three differ significantly.

[The calculated value for the t-statistic (-2.274) lies outside the absolute critical values of the t distribution (t=-1.697), independent of whether it is negative or positive. If in this case, t = -1.5, this would have been inside the critical values for the t-distribution and would have indicated no significance between treatments.]

Likewise, Treatments two and four were compared:

$t_{2;4}$ = $(0.472 - (0.2608))/ ((0.5217 + 0.5217 - 2(0.2608))^{1/2}$

= -1.586/ 0.72236

= -2.1955

Since $t_{2;4} = -2.1955 < -1.697$, it was deduced that Treatments two and four differed significantly.

Treatment two gave similar results to Treatment one. This was expected, since Treatments one and two were the same. The threshold for bollworm larvae (12.5/ 60 plants) was never reached in Treatment two and no insecticide was ever applied to this treatment during the performance of this trial.

Likewise, Treatments three and four (intercepts) were compared:

$t_{3;4}$ = 2.115-2.058/ 0.72236

= 0.0789

Table A.2.2. Accumulated analysis of variance of the log (total number of larvae) from Potgietersrus (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	32.860	32.860	15.97	< 0.001
Treatments	3	22.215	7.405	3.60	0.026
Time Treatments	3	5.188	1.729	0.84	0.483
Residual	28	57.624	2.058		
Total	**35**	**117.886**	**3.368**		

The non-significant interaction effect implies that the different treatment responses are represented by parallel regression lines (Fig. 2.3). The regression equations were calculated ($y = mx + c$), where x = days after planting); from the estimates of the regression coefficients and for the 4 treatments (see Annexure I).

$\log y_{(1)\ \text{Bollgard unsprayed}} = -0.274 - 0.0434x$

$\log y_{(2)\ \text{Bollgard sprayed}} = (-0.274 + 0.692) - 0.0434x$
$= 0.418 - 0.0434x$

$\log y_{(3)\ \text{DpAC 90 unsprayed}} = (-0.274 + 1.530) - 0.0434x$
$= 1.256 - 0.0434x$

$\log y_{(4)\ \text{DpAC 90 sprayed}} = (-0.274 + 2.054) - 0.0434x$
$= 1.78 - 0.0434x$

Table A.2.3. Accumulated analysis of variance of the log (number of Bt-cotton larvae) from Rustenburg (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	4.647	4.647	3.68	< 0.065
Treatments	3	62.853	20.951	16.60	< 0.001
Time Treatments	3	2.359	0.786	0.62	0.606
Residual	28	35.334	1.262		
Total	**35**	**105.192**	**3.005**		

The regression equations for the comparison of Bt-cotton larvae were calculated as previously described and for the 4 treatments they are as follows:

$\log y_{(1)\ \text{Bollgard unsprayed}}$ = -3.544 + 0.01633 x
$\log y_{(2)\ \text{Bollgard sprayed}}$ = (-3.544 + 2.000) + 0.01633 x
= -1.544 + 0.01633 x
$\log y_{(3)\ \text{DpAC 90 unsprayed}}$ = (-3.544 + 3.402) + 0.01633 x
= -0.142 + 0.01633 x
$\log y_{(4)\ \text{DpAC 90 sprayed}}$ = (-3.544 + 3.032) + 0.01633 x
= -0.512 + 0.01633 x

Subsequent t-tests for the comparison of the other treatments gave the following results:

Treatments two and three can be compared:
$t_{2;3}$ = (2 - (3.402))/ ((0.2702 + 0.2702 - 2(0.1351))$^{1/2}$
= -1.402 0.51981
= -2.697
compared with t_{31} 0.05 = 1.697.
Treatment two differs significantly from Treatment three (as explained under “total number of bollworm larvae”).

Likewise, Treatments two and four can be compared:
$t_{2;4}$ = (2 - (3.032))/0.51981
= -2.697
Treatment two differs from Treatment four.

Likewise, Treatments three and four can be compared:
$t_{2;4}$ = 0.370/ 0.51981
= 0.712
Treatment three does not differ from Treatment four.

Table A.2.4. Accumulated analysis of variance of the log (total number of Bt-cotton larvae) from Potgietersrus (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	71.116	71.116	65.48	<0.001
Treatments	3	1.503	0.501	0.46	0.711
Time Treatments	3	0.078	0.026	0.02	0.995
Residual	28	30.410	1.086		
Total	**35**	**103.107**	**2.946**		

The significant effect over time implies that the different treatment responses each follow the same trend over time and this can be represented by a general regression lines:

logy = 0.464-0.06387 x

Table A.2.5. Accumulated analysis of variance of the log (number of red bollworm larvae) from Rustenburg (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	26.062	26.062	11.08	< 0.002
Treatments	3	29.892	9.964	4.24	< 0.014
Time Treatments	3	6.701	2.234	0.95	0.430
Residual	28	65.877	2.353		
Total	**35**	**128.533**	**3.672**		

The significance between treatments implies that the different treatment responses each follow a separate parallel regression line. The regression equations are as follows:

$\text{logy}_{(1)\ \text{Bollgard unsprayed}} = -2.461 + 0.0387\ x$

$\text{logy}_{(2)\ \text{Bollgard sprayed}} = (-2.461 - 0.254) + 0.0387\ x$
$= -2.715 + 0.0387\ x$

$\text{logy}_{(3)\ \text{DpAC 90 unsprayed}} = (-2.461 + 1.425) + 0.0387\ x$
$= -1.006 + 0.0387\ x$

$\text{logy}_{(4)\text{DpAC 90 sprayed}} = (-2.461 + 1.888) + 0.0387\ x$
$= -0.573 + 0.0387\ x$

Table A.2.6. Accumulated analysis of variance of the log (total number of red bollworm larvae) from Potgietersrus (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	22.724	22.724	10.47	0.003
Treatments	3	23.878	7.959	3.67	0.024
Time Treatments	3	5.004	1.668	0.77	0.521
Residual	28	60.794	2.171		
Total	**35**	**112.401**	**3.211**		

The significant effect between treatments implies that the different treatment responses are represented by separate parallel regression lines:

$\log y_{(1)\ \text{Bollgard unsprayed}} = -0.624 - 0.0361\ x$

$\log y_{(2)\ \text{Bollgard sprayed}} = (-0.624 + 0.261) - 0.0361\ x$
$= -0.363 - 0.0361\ x$

$\log y_{(3)\ \text{DpAC 90 unsprayed}} = (-0.624 + 1.227) - 0.0361\ x$
$= 0.603 - 0.0361\ x$

$\log y_{(4)\text{DpAC 90 sprayed}} = (-0.624 + 2.053) - 0.0361\ x$
$= 1.429 - 0.0361\ x$

Table A.2.7. Accumulated analysis of variance of the log (number of spiny bollworm larvae) from Rustenburg (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	7.3012	7.3012	9.79	< 0.004
Treatments	3	89.4449	29.8150	39.98	< 0.001
Time Treatments	3	7.6793	2.5599	3.43	0.030
Residual	28	20.8804	0.7457		
Total	**35**	**125.3063**	**3.5802**		

The significant interaction effect implies that the different treatment responses each follow a different trend over time and this can be represented by separate non-parallel regression lines:

$\text{logy}_{(1)\ \text{Bollgard unsprayed}} = -3.00 + 0.0\ x$
$= -3$

$\text{logy}_{(2)\ \text{Bollgard sprayed}} = (-3.00 + 0.0) + 0.0\ x$
$= -3$

$\text{logy}_{(3)\ \text{DpAC 90 unsprayed}} = (-3.00 + 2.297) + 0.0343\ x$
$= -0.703 + 0.0343\ x$

$\text{logy}_{(4)\text{DpAC 90 sprayed}} = (-3.00 + 1.214) + 0.0475\ x$
$= -1.786 + 0.0475\ x$

Table A.2.8. Accumulated analysis of variance of the log (total number of spiny bollworm larvae) from Potgietersrus (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	40.612	40.612	29.94	<0.001
Treatments	3	1.080	0.360	0.27	0.850
Time Treatments	3	2.689	0.896	0.66	0.583
Residual	28	37.983	1.357		
Total	**35**	**82.364**	**2.353**		

The significant effect over time implies that the treatment responses each follow the same trend over time and this can be represented by a regression line:

$$\log y = -0.393 - 0.04827\ x$$

Table A.2.9. Accumulated analysis of variance of the log (number of first instar larvae) from Rustenburg (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	11.487	11.487	4.85	0.036
Treatments	3	24.792	8.264	3.49	0.029
Time Treatments	3	3.186	1.061	0.45	0.721
Residual	28	66.381	2.371		
Total	**35**	**105.845**	**3.024**		

The significant treatment effect implies that the different treatment responses each follow a different trend over time and this can be represented by separate parallel regression lines:

$\text{logy}_{(1)\ \text{Bollgard unsprayed}} = -2.028 + 0.0257\ x$

$\text{logy}_{(2)\ \text{Bollgard sprayed}} = (-2.028 + 0.443) + 0.0257\ x$
$= -1.585 + 0.0257\ x$

$\text{logy}_{(3)\ \text{DpAC 90 unsprayed}} = (-2.028 + 1.887) + 0.0257\ x$
$= -0.141 + 0.0257\ x$

$\text{logy}_{(4)\text{DpAC 90 sprayed}} = (-2.028 + 1.813) + 0.0257\ x$
$= -0.215 + 0.0257\ x$

Table A.2.10. Accumulated analysis of variance of the log (number of second instar larvae) from Rustenburg (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	9.2765	9.2765	10.93	0.003
Treatments	3	96.7791	32.2597	38.00	< 0.001
Time Treatments	3	4.5930	1.5310	1.80	0.169
Residual	28	23.7682	0.8489		
Total	**35**	**134.4168**	**3.8405**		

The significant effect implies that the different treatment responses each follow a different trend over time and this can be represented by separate parallel regression lines:

$\text{logy}_{(1)\ \text{Bollgard unsprayed}} = -3.769 + 0.02307\ x$

$\text{logy}_{(2)\ \text{Bollgard sprayed}} = (-3.769 + 0.333) + 0.02307\ x$
$= -3.436 + 0.02307\ x$

$\text{logy}_{(3)\ \text{DpAC 90 unsprayed}} = (-3.769 + 3.454) + 0.02307\ x$
$= -0.315 + 0.02307\ x$

$\text{logy}_{(4)\text{DpAC 90 sprayed}} = (-3.769 + 3.420) + 0.02307\ x$
$= -0.349 + 0.02307\ x$

Table A.2.11. Accumulated analysis of variance of the log (number of third instar bollworm larvae) from Rustenburg (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	19.6868	19.6868	20.87	< 0.001
Treatments	3	57.2209	19.0736	20.22	< 0.001
Time Treatments	3	14.3818	4.7939	5.08	0.006
Residual	28	26.4117	0.9433		
Total	**35**	**117.7012**	**3.3629**		

The significant interaction effect implies that the different treatment responses each follow a different trend over time and this can be represented by separate non-parallel regression lines:

$\log y_{(1)\ \text{Bollgard unsprayed}}$ = (-3.00 + 0.0) x
= -3 x

$\log y_{(2)\ \text{Bollgard sprayed}}$ = (-3.00 + 0.004) + (0.0 + 0.0111) x
= -3.004 + 0.0111 x

$\log y_{(3)\ \text{DpAC 90 unsprayed}}$ = (-3.00 + 0.987) + (0 + 0.0549) x
= -2.013 + 0.0549 x

$\log y_{(4)\text{DpAC 90 sprayed}}$ = (-3.00 + 0.272) +(0 + 0.0684) x
= -2.728 + 0.0684 x

Table A.2.12. Accumulated analysis of variance of the log (total number of first instar larvae) from Potgietersrus (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	75.565	75.565	71.33	<0.001
Treatments	3	0.935	0.312	0.29	0.829
Time Treatments	3	0.101	0.034	0.03	0.992
Residual	28	29.662	1.059		
Total	**35**	**106.263**	**3.036**		

The general trend over time for all treatments can be described by:

$\log y = 0.479 - 0.06584\ x$

Table A.2.13. Accumulated analysis of variance of the log (total number of second instar bollworm larvae) from Potgietersrus (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	35.168	35.168	16.98	<0.001
Treatments	3	12.310	4.103	1.98	0.140
Time Treatments	3	1.454	0.485	0.23	0.872
Residual	28	57.991	2.071		
Total	**35**	**106.923**	**3.055**		

The significant effect over time implies that all treatment responses follow the same trend as already stated:

logy = 0.395 - 0.0449 x

Table A.2.14. Accumulated analysis of variance of the log (total number of 3rd instar larvae) from Potgietersrus (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	8.797	8.797	3.61	0.068
Treatments	3	22.851	7.617	3.13	0.041
Time Treatments	3	1.975	0.658	0.27	0.846
Residual	28	68.157	2.434		
Total	**35**	**101.780**	**2.908**		

The significant effect between treatments, can be represented by separate parallel regression lines:

$\text{logy}_{(1)\ \text{Bollgard unsprayed}}$ = -1.551 - 0.0225 x
$\text{logy}_{(2)\ \text{Bollgard sprayed}}$ = (-1.551 + 0.386) - 0.0225 x
= -1.165 - 0.0225 x
$\text{logy}_{(3)\ \text{DpAC 90 unsprayed}}$ = (-1.551 + 1.514) - 0.0225 x
= -0.037 - 0.0225 x
$\text{logy}_{(4)\text{DpAC 90 sprayed}}$ = (-1.551 + 1.951) - 0.0225 x
= 0.4 - 0.0225 x

Table A.2.15. Accumulated analysis of variance of the log (total number of bollworm eggs) counted in Rustenburg (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	5.51992	5.51992	74.94	<0.001
Treatments	3	0.23719	0.07906	1.07	0.376
Time Treatments	3	0.12239	0.04080	0.55	0.650
Residual	28	2.06238	0.07366		
Total	**35**	**7.94187**	**0.22691**		

The significant effect over time can be described by:

logy = 0.7058 + 0.01779 x

Table A.2.16. Accumulated analysis of variance of the log (total number of bollworm eggs) counted in Potgietersrus (1997/1998) in the Cry1Ac trial.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Time (days)	1	111.013	111.013	110.05	0.001
Treatments	3	0.786	0.262	0.26	0.854
Time Treatments	3	0.111	0.037	0.04	0.990
Residual	28	28.244	1.009		
Total	**35**	**140.154**	**4.004**		

The significant time effect can be described by a general regression line:

logy = 2.219 - 0.07980 x

Annexure II (Chapter 2): Cry2Ab2 study: IPC line selection

Table A.2.17. Analysis of variance on the transformed total number of african larvae of four localities (transformation: counts$^{0.25}$) (1998/1999) in the Cry2Ab2 trials.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Locality	3	4.8845	1.6282		
Locality Rep.	12	2.1983	0.1832	0.59	
Treatments	3	16.5808	5.5269	17.77	<0.001
Treatment Locality	9	3.3329	0.3703	1.19	0.331
Residual	36	11.1986	0.3111		
Total	**63**	**38.1952**			

Table A.2.18. Analysis of variance on the transformed total number of red bollworm larvae of four localities (1998/1999) in the Cry2Ab2 trials.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Locality	3	4.5423	1.5141		
Locality Rep.	12	0.6668	0.0556	0.34	
Treatments	3	8.7267	2.9089	17.55	<0.001
Treatment Locality	9	2.5256	0.2806	1.69	0.127
Residual	36	5.9681	0.1658		
Total	**63**	**22.4296**			

Table A.2.19. Analysis of variance of the transformed mean number of spiny bollworm larvae of four localities (1998/1999) in the Cry2Ab2 trials.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Locality	3	0.6840	0.2280		
Locality Rep.	12	0.9807	0.0817	0.54	
Treatments	3	11.1357	3.7119	24.57	<0.001
Treatment Locality	9	1.3850	0.1539	1.02	0.444
Residual	36	5.4376	0.1510		
Total	**63**	**19.6230**			

Table A.2.20. Analysis of variance of the transformed total number of bollworm larvae (african, red and spiny bollworms) of four localities (1998/1999) in the Cry2Ab2 trials.

Change	d.f. (degrees of freedom)	s.s. (sum of squares)	m.s. (mean sum of squares)	v.r. (variance)	F pr. (significance)
Locality	3	3.6343	1.2114		
Locality Rep.	12	1.5344	0.1279	0.38	
Treatments	3	21.3877	7.1292	21.39	<0.001
Treatment Locality	9	2.5844	0.2872	0.86	0.567
Residual	36	11.9997	0.3333		
Total	**63**	**41.1404**			

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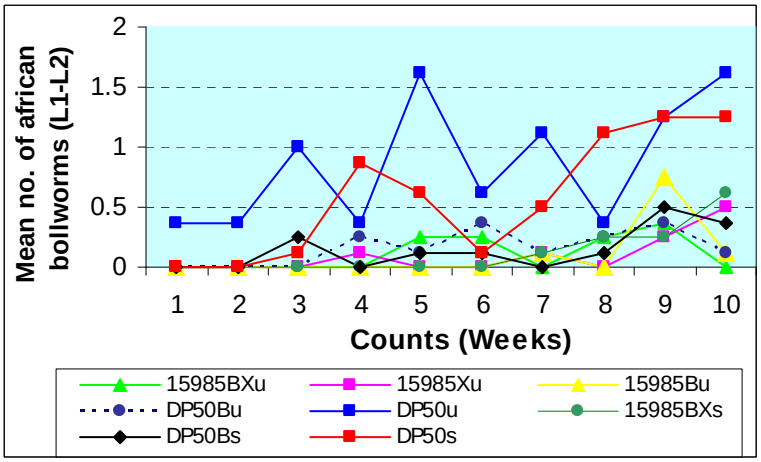
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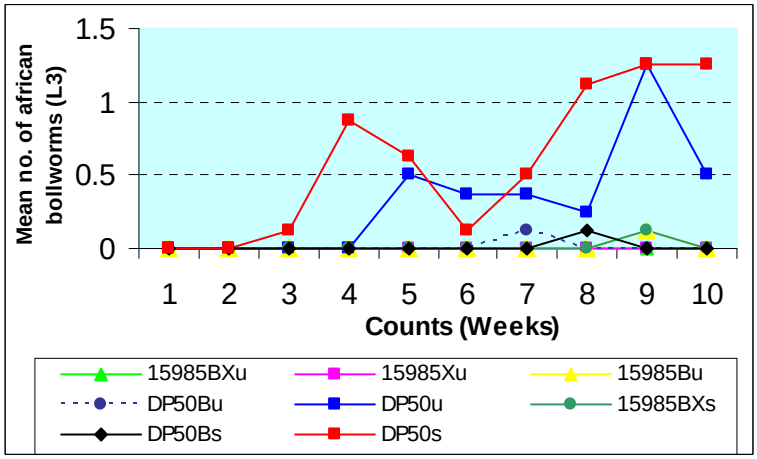
Figures

Annexure V (Chapter 2): Figures of the different bollworm instars

a)



b)



c)

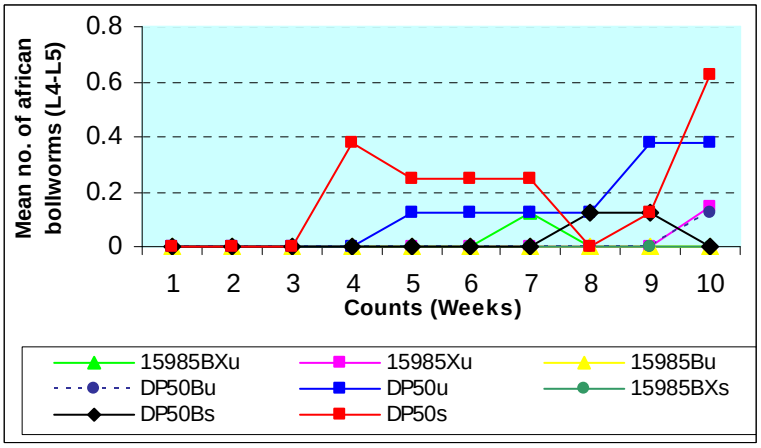
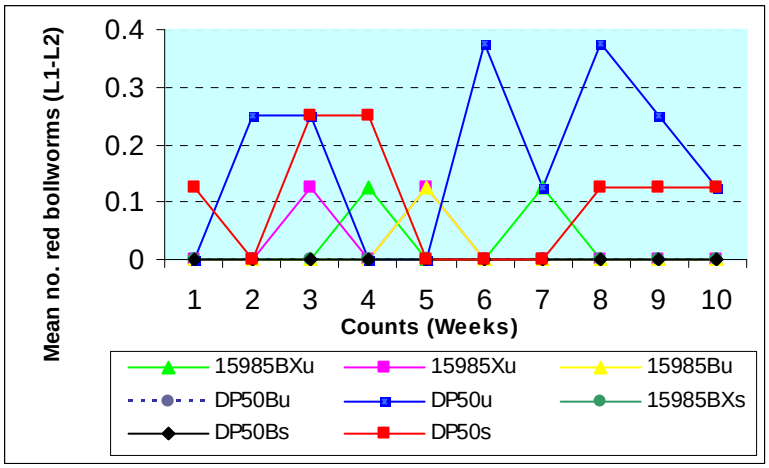
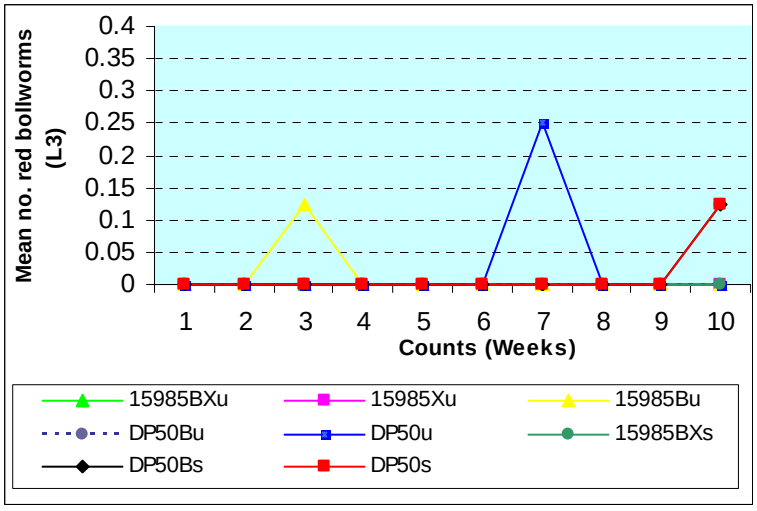


Fig. A.2.1 The mean number of african bollworm instars on 10 plants per plot at Rustenburg (2000) in the stacked Cry1Ac and Cry2Ab2 trial (a) (L1-L2); (b) (L3); (c) (L4-L5).

a)



b)



c)

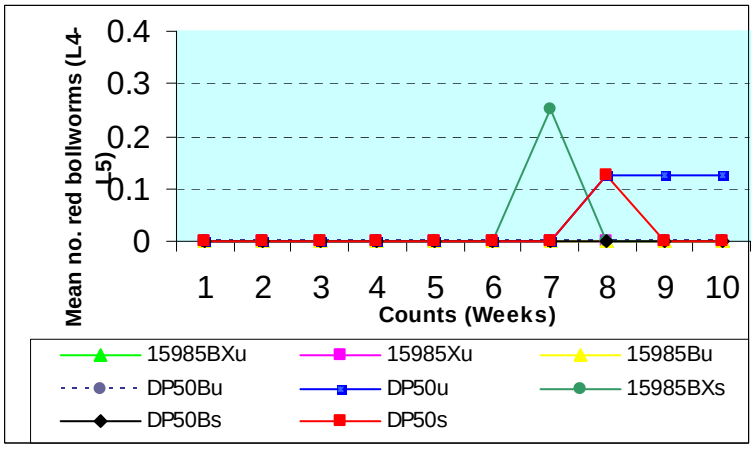
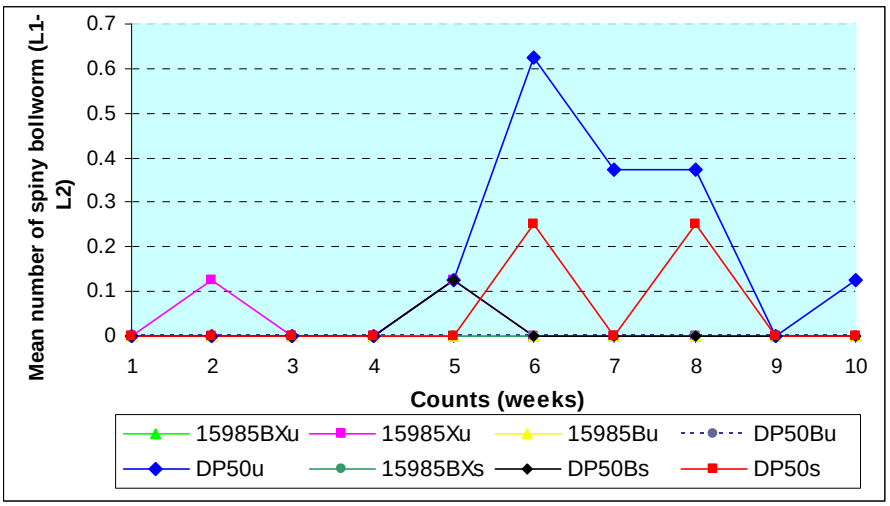
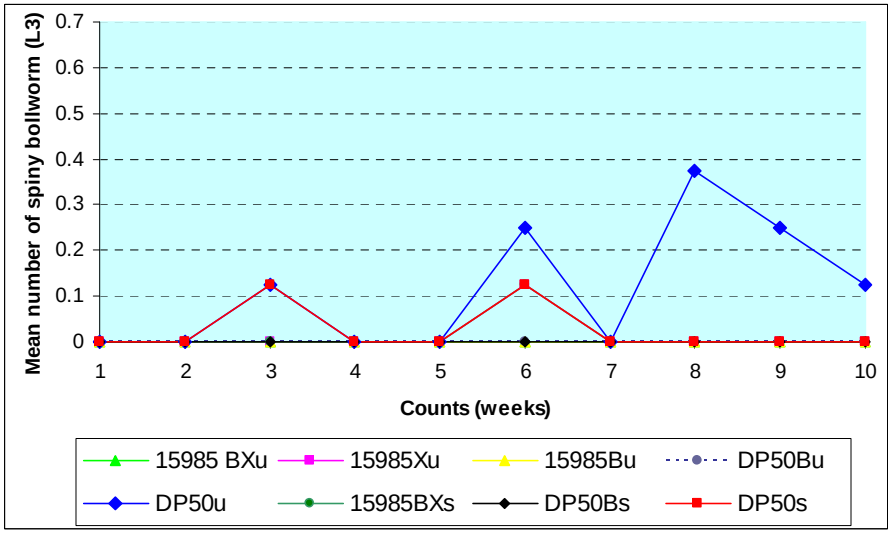


Fig. A.2.2. The mean number of red bollworm instars on 10 plants per plot at Rustenburg (2000) in the stacked Cry1Ac and Cry2Ab2 trial (a) (L1-L2); (b) (L3); (c) (L4-L5).

a)



b)



c)

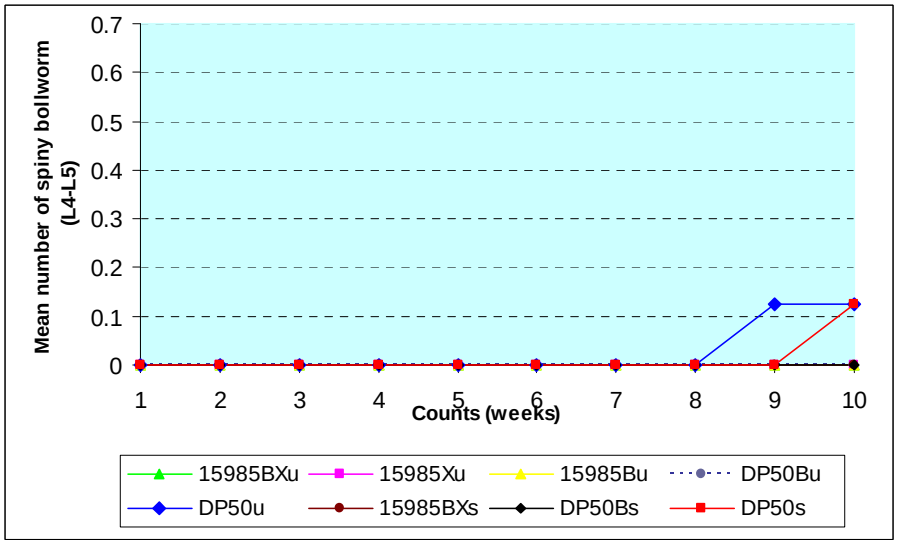
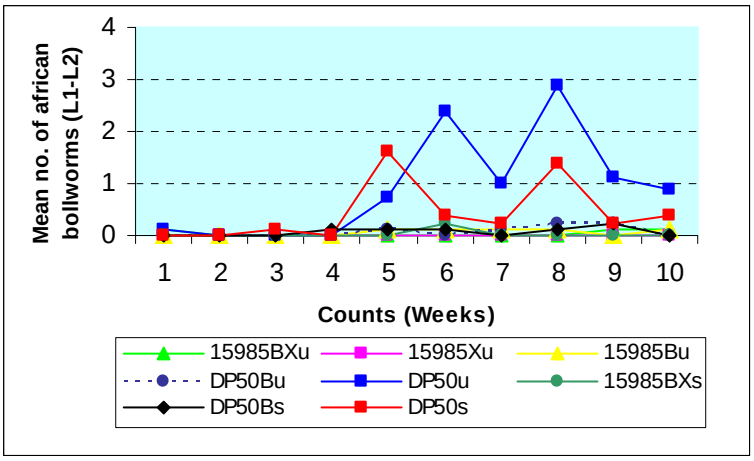
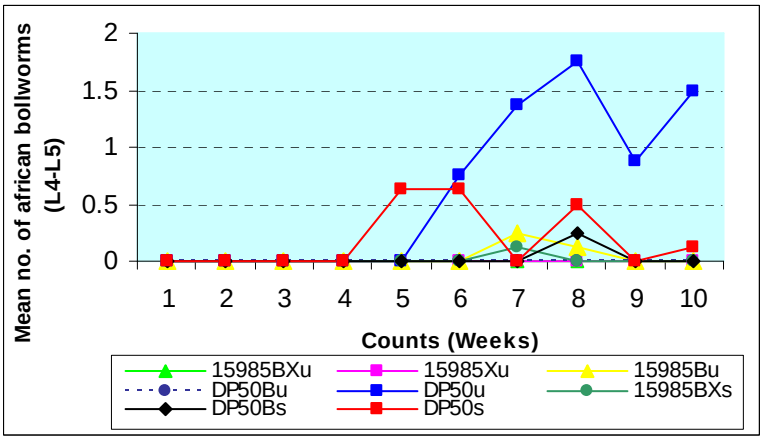


Fig. A.2.3 The mean number of spiny bollworm instars on 10 plants per plot at Rustenburg (2000) in the stacked Cry1Ac and Cry2Ab2 trial (a) (L1-L2); (b) (L3); (c) (L4-L5).

a)



b)



c)

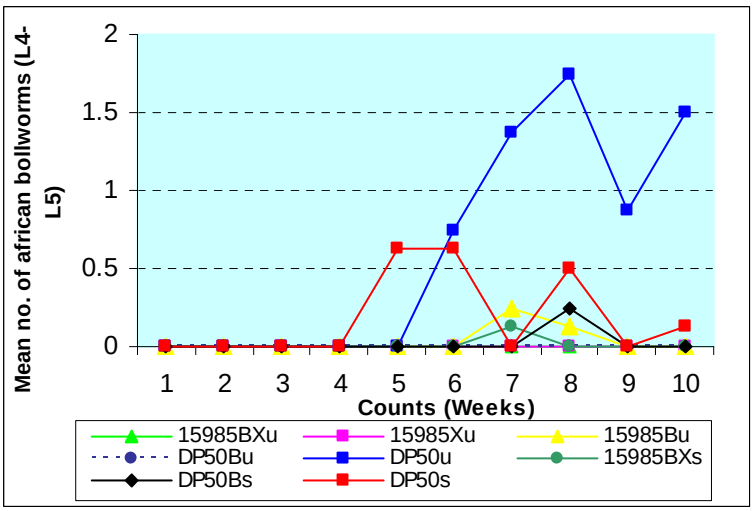
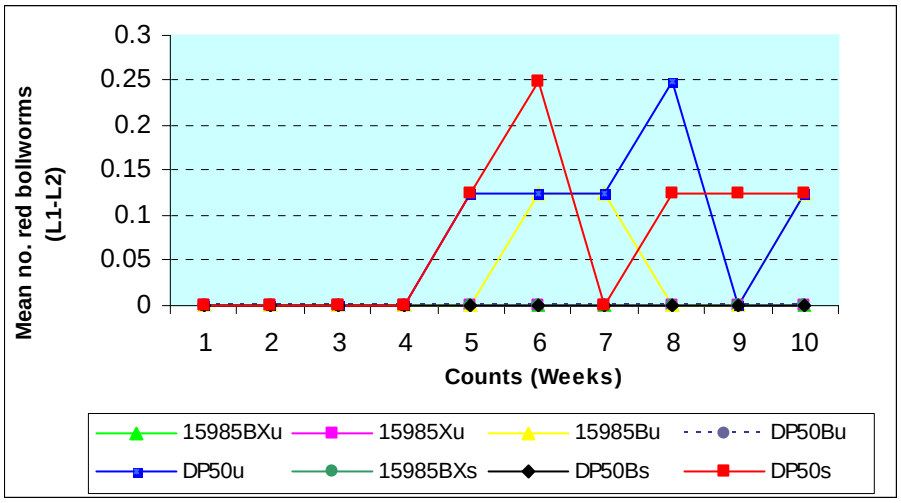
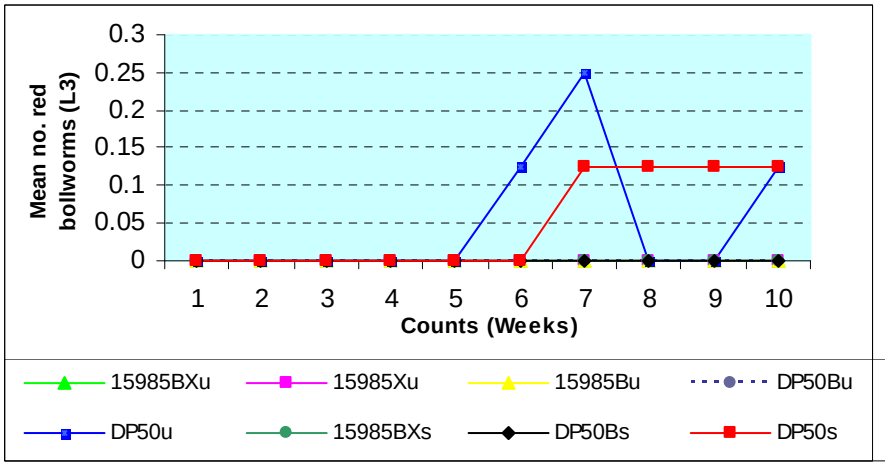


Fig. A.2.4. The mean number of african bollworm instars on 10 plants per plot at Brits (2000) in the stacked Cry1Ac and Cry2Ab2 trial (a) (L1-L2; (b) (L3); (c) (L4-L5).

a)



b)



c)

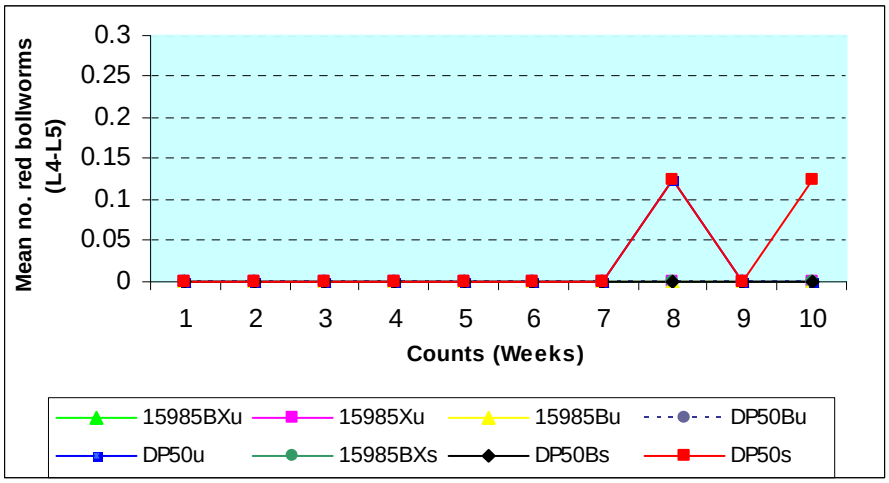
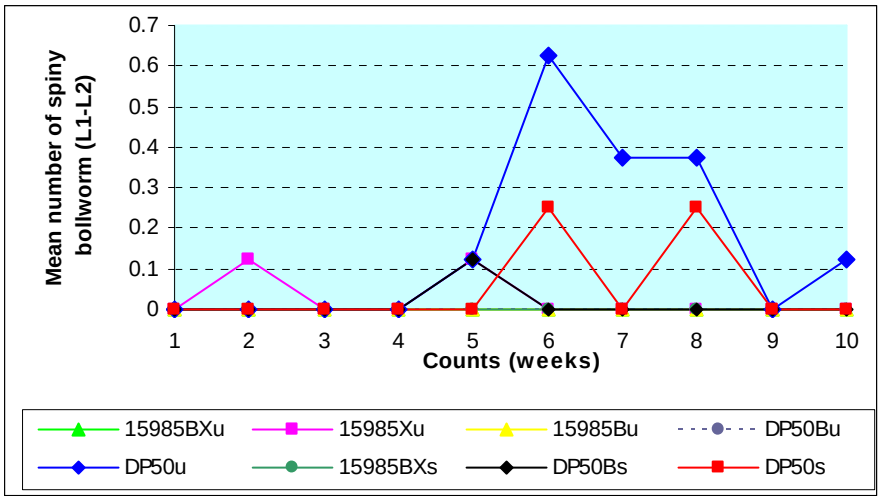
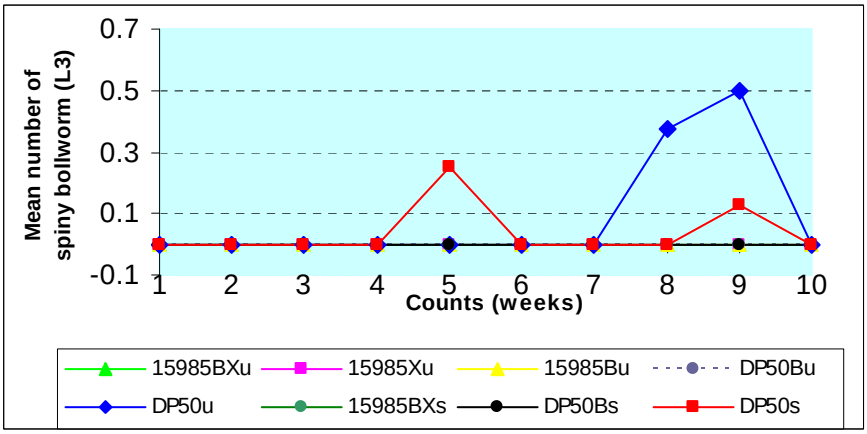


Fig. A.2.5. The mean number of red bollworm instars on 10 plants per plot at Brits (2000) in the stacked Cry1Ac and Cry2Ab2 trial (a) (L1-L2); (b) (L3); (C) L4-L5.

a)



b)



c)

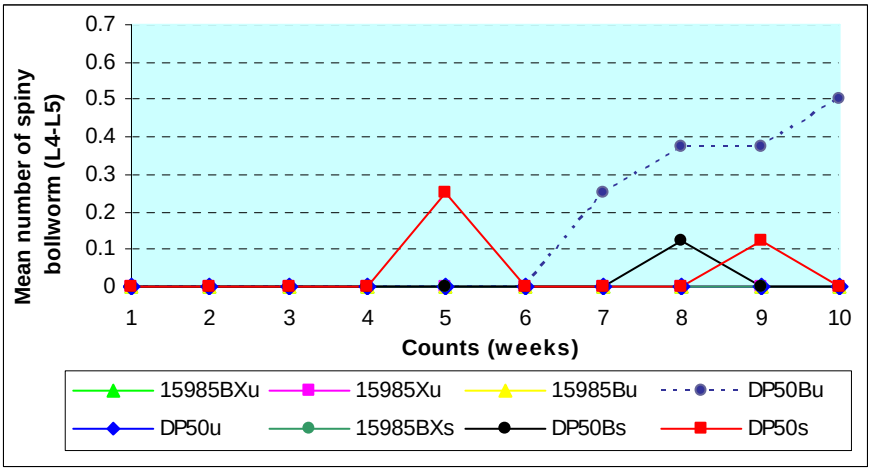
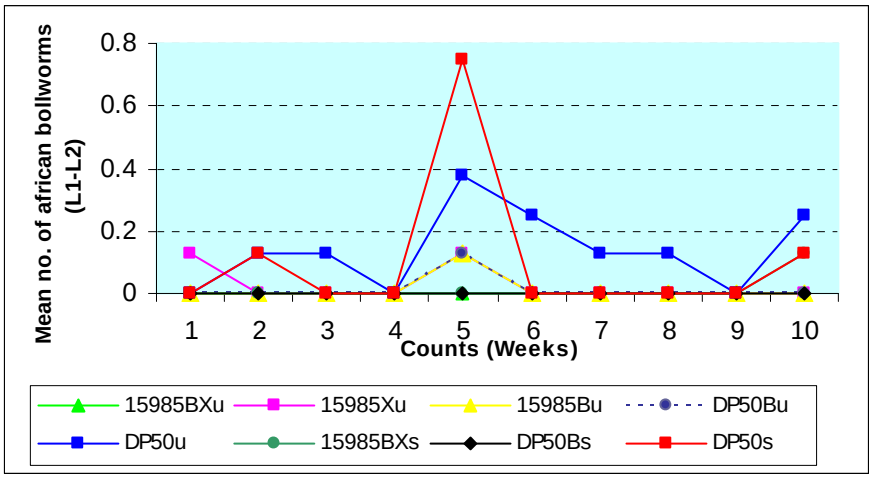
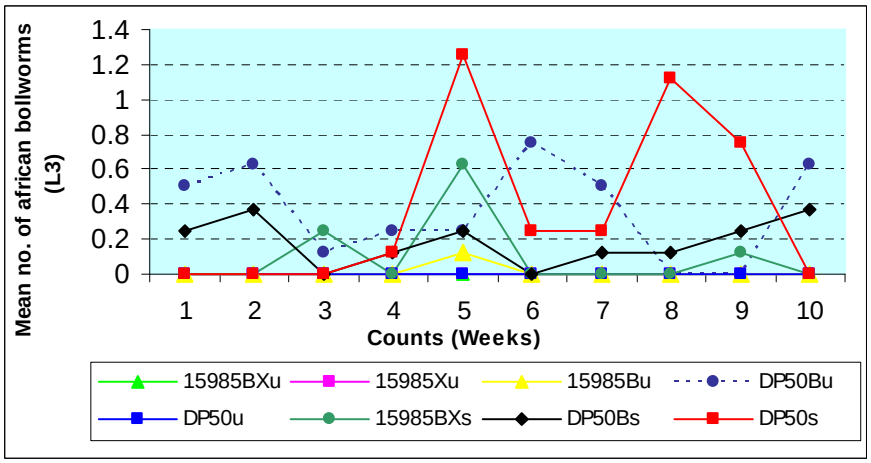


Fig. A.2.6. The mean number of spiny bollworm instars on 10 plants per plot at Brits (2000) in the stacked Cry1Ac and Cry2Ab2 trial (a) (L1-L2); (b) (L3); (c) (L4-L5).

a)



b)



c)

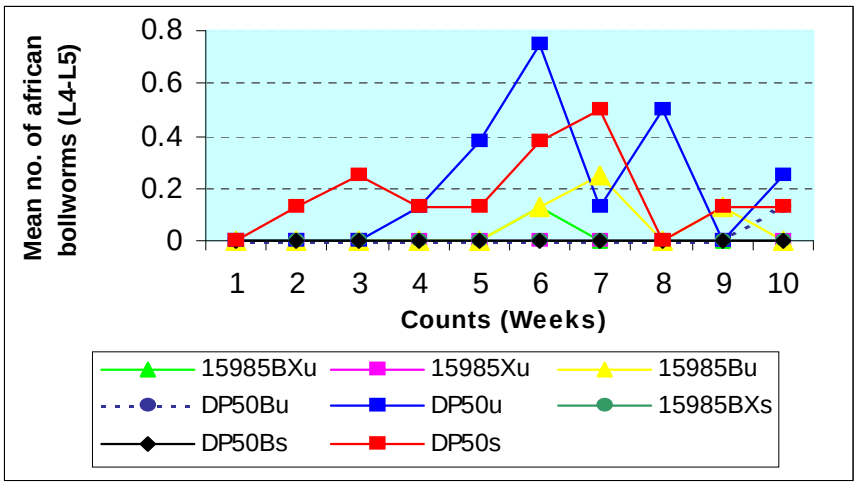
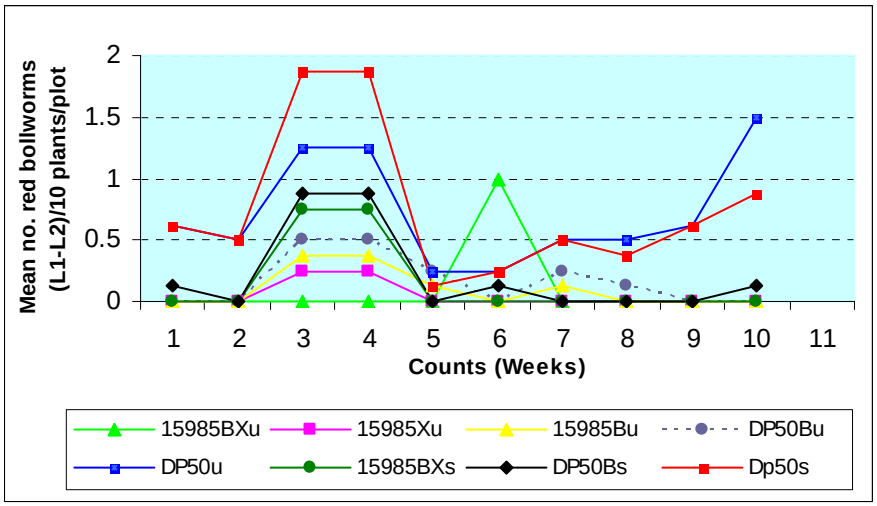
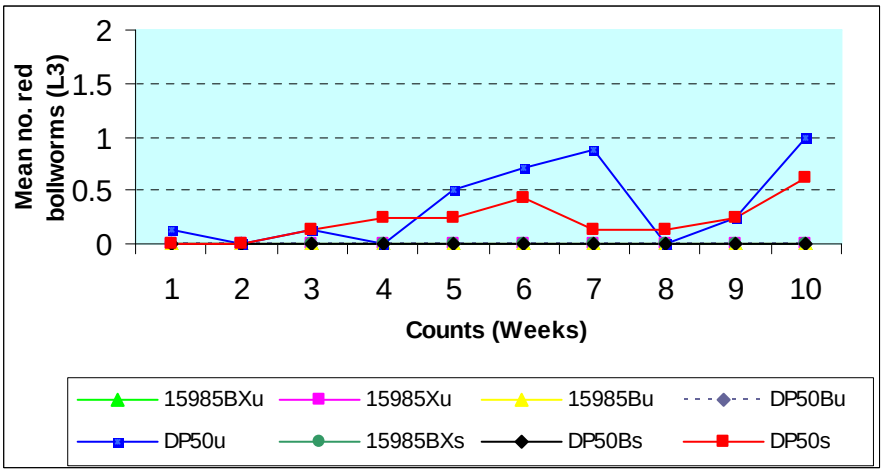


Fig. A.2.7. The mean number of african bollworm instars on 10 plants per plot at Nelspruit (2000) in the stacked Cry1Ac and Cry2Ab2 trial (a) (L1-L2); (b) (L3); (c) (L4-L5).

a)



b)



c)

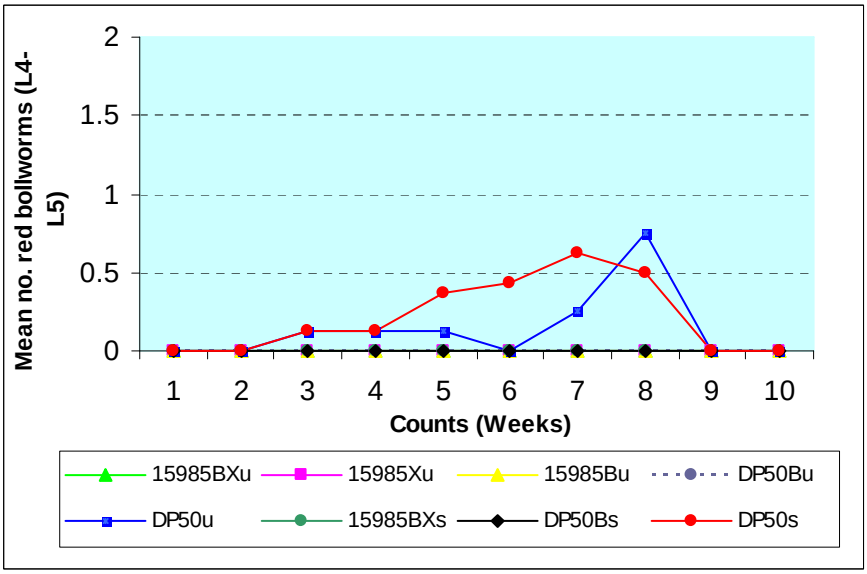
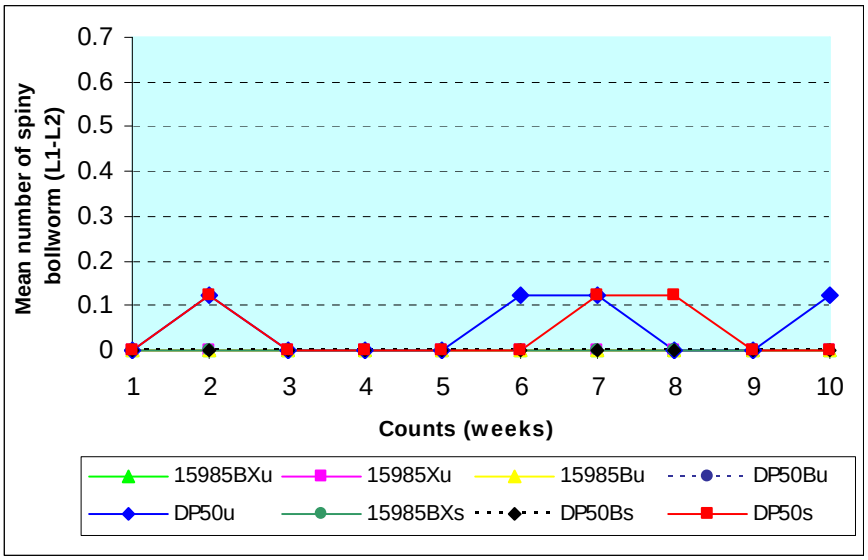
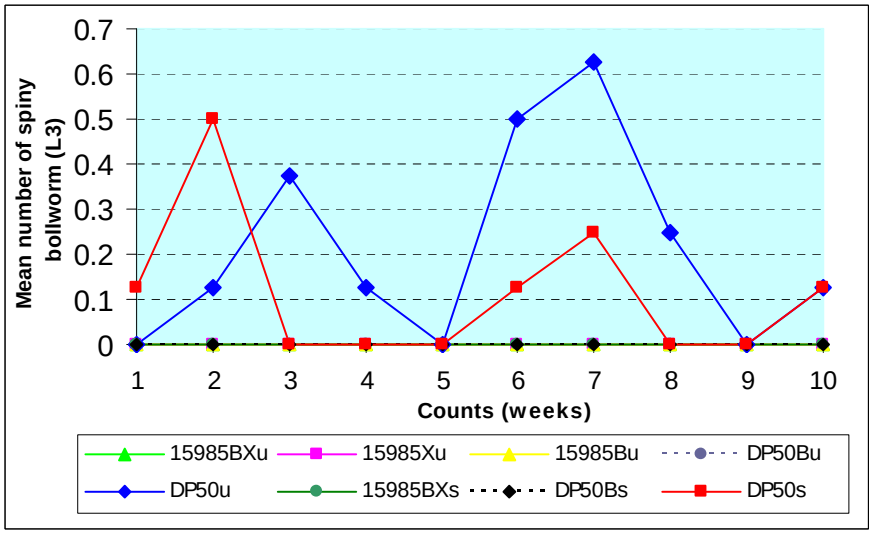


Fig. A.2.8. The mean number of red bollworm instars on 10 plants per plot at Nelspruit (2000) in the stacked Cry1Ac and Cry2Ab2 trial (a) (L1-L2); (b) (L3); (c) (L4-L5).

a)



b)



c)

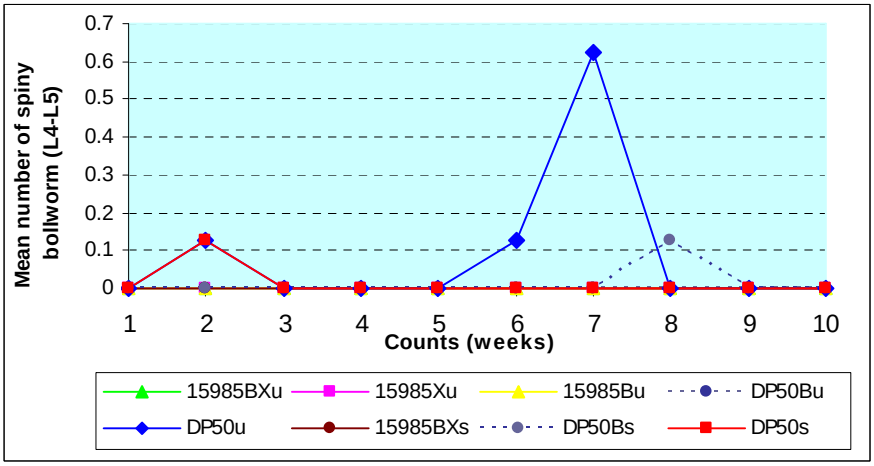


Fig. A.2.9. The mean number of spiny bollworm instars on 10 plants per plot at Nelspruit (2000) in the stacked Cry1Ac and Cry2Ab2 trial (a) (L1-L2); (b) (L3); (c) (L4-L5).