PATTERNS OF HETEROSIS IN CROSSES BETWEEN AFRICAN STALK BORER RESISTANT AND ELITE MAIZE GENOTYPES

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Patterns of heterosis in crosses between African stalk borer resistant and elite maize genotypes

by

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Dedicated to my beloved mother and to my darling wife who is an incredible woman and my wonderful children for all their love and encouragement

It is not about us
It is about God and His will for us
and His Kingdom.
Do not always expect a positive outcome.
Expect His will
Trust His Heart!

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“Only one life, it will soon be past, only what’s been done for Christ will last”

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CHAPTER 1

INTRODUCTION

Maize (*Zea mays L.*) is the most important summer grain crop in South Africa and annual production may exceed 10 million tonnes during favourable seasons. Mean yield losses caused by pests on agricultural crops are estimated world wide as 35%, with losses in Africa as the highest in the world. The maize stalk borer, *Busseola fusca (*Fuller) (Lepidoptera: Noctuidae) is of special significance, since the borer is of major economic importance. Before synthetic insecticides were available *B. fusca* in South Africa was responsible for total crop losses during epidemic years (Mally, 1920). Information from elsewhere in Africa is often contradictory and not applicable to South African conditions (van Rensburg, *et. al.*, 1987). Infestations regularly occur over large areas at infestation levels too low to warrant expensive spray treatment, but nevertheless causing considerable overall loss in production. Even a limited degree of resistance in commercial maize hybrids could thus be of considerable economic benefit to both commercial maize producers and subsistence farmers (van Rensburg & Malan, 1990). The use of insecticides against the maize stalk borer has become expensive and applications cannot be economically justified for maize production in marginal areas. Development of local maize hybrids with sustained resistance to *B. fusca* seems to be a viable alternative.

Insect resistance specific against the European corn borer *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) originated in Iowa (Robinson *et. al.*, 1978; Guthrie,W.D, 1981). A physiological mechanism that is responsible for the expression of leaf-feeding resistance in these sources was described by Robinson, Klun & Brindley (1978). An antibiotic substance 2,4-dihydroxy-7methoxy-2H-1, 4-benzoxazin-3(4H)-one (DIMBOA) was shown to be present in plant tissue. Barrow (1985) reported that DIMBOA was not effective against *B. fusca*. However, DIMBOA does not present the only mechanism of resistance affecting stem borers, since several exotic maize lines with low DIMBOA-content were found to be highly resistant to *B. fusca* (van Rensburg & van den Berg, 1995; van Rensburg, 1998*).*

Van Rensburg & Malan (1990) found that some Mississippi inbred lines derived from the Caribbean population Antigua group 2 that had been developed for multiple resistance to the south-western corn borer, *Diatraea grandiosella* (Dyar) and the fall armyworm *Spodoptera frugiperda* (Smith) (Davis, 1989) expressed high levels of resistance to *B. fusca*. Some CIMMYT breeding material developed in Mexico for subtropical regions also appeared to offer promise against *B. fusca* from the antibiosis viewpoint (van Rensburg, 1998). Alternative sources developed in Georgia against the maize ear worm *Heliothis zea* (Boddie), showed limited local possibilities (Van Rensburg, 1989).

Since these resistance sources are not adapted to South African conditions (Van Rensburg, 1996; 1997), the resistance had to be introgressed into locally adapted breeding material. The resistance in the Mississippi lines was shown to be additively inherited with low dominance (van Rensburg & Gevers, 1993). Using Mississipi and CIMMYT inbred lines as donor parents in a recurrent selection programme, locally adapted inbred lines with high levels of resistance were eventually released (van Rensburg & Klopper, 2004). From this work it appeared that combining ability for resistance differs between heterotic groups, and that susceptible elite material do not necessarily respond similarly in crosses with sources of resistance from different genetic backgrounds.

The development of conventional resistance was eclipsed worldwide by advances in DNA technology, providing transgenes encoding for *Bacillus thuringiensis* toxins (Bt maize). Deployed commercially for the first time in South Africa during 1998, the use of Bt-hybrids increased to 35% of the national crop in 2006 (James, 2006). In South Africa this culminated in the development of insect resistance to the Cry1Ab toxin (van Rensburg, 2007), which warrants further investigation into conventional resistance sources and the possibility of using conventional resistance in combination with GMO technology to obtain durable plant resistance. The cost of Bt seed is also prohibitive to the small farming community for whom conventional resistance could still present a viable option.

From earlier work conducted under greenhouse conditions (van Rensburg & van den Berg, 1995; van Rensburg, 1998), it was observed that larval developmental rate possibly differs with difference in time of the year. Greenhouse evaluations conducted at different times of the season in which only photoperiod varied, indicated larvae to develop faster during a declining photoperiod than during an increasing photoperiod. This was never further investigated but similar observations were made elsewhere (Personal communications, CIMMYT, Mexico). The question arose to what extent variation in planting date (a common occurrence in South-Africa) could effect the expression of resistance. The possibility exists that resistance may break down under conditions of decreasing photoperiod, which presents practical consequences during years when late spring rains result in late planting. This became of particular importance in recent years due to the availability of so-called super short season hybrids which extends planting dates to as late as Mid-January.

The objective of this study was to determine which combinations of resistant sources with local elite susceptible material could provide the best expression of resistance. A further aspect of the study involved investigation into the effect of variance in planting date in the expression of resistance.

CHAPTER 2

LITERATURE REVIEW

2.1 Historical background

The African maize stalk borer, Busseola fusca (Fuller), technical description and type designation was published by Hampson (1902). Busseola fusca occurs throughout mainland Africa south of the Sahara and has been formally recorded from West Africa (Benin, Burkina Faso, Cameroon, Côte d'Ivoire, Ghana, Guinea, Mali, Nigeria, and Sierra Leone), from eastern Africa (Ethiopia, Kenya, Somalia, Tanzania, and Uganda), and from southern Africa (Angola, Botswana, Lesotho, Malawi, Mozambique, Rwanda, South Africa, Swaziland, Zaire, Zambia, and Zimbabwe). In southern Africa, B. fusca is the dominant stem borer at elevations above 900m in Botswana, Lesotho, Malawi, Mozambique, South Africa, and Swaziland, but it also occurs at lower altitudes in those countries and in Zimbabwe, clearly indicating the ability of this pest to adapt to low-lying and warmer areas (Sithole, 1989).

The biology of this species was reviewed by Harris (1989b) and a detailed study of its ecology on maize in South Africa made by van Rensburg *et al*. (1987b). Earlier key papers include Mally (1920), Wahl (1930), Hargreaves (1939), Lefevre (1935), du Plessis (1936), du Plessis and Lea (1943), Bowden (1956b), Swaine (1957), Ingram (1958), Nye (1960), Smithers (1960), Walker (1960b), and Harris (1962; 1964).

Climatic conditions in South Africa often differ considerably from those in other parts of the continent. Subsequently the life cycle of the maize stalk borer in South Africa differs from life cycle patterns observed in other parts of Africa. This has led to a situation where information gathered outside South Africa may not be applicable to local conditions (van Rensburg *et al.*, 1987). Taxonomic descriptions, diagnoses, and keys for identification were published by Tams and Bowden (1953). There has been no subsequent taxonomic revisionary work on this species. Kaufmann (1983) suggested that sub-speciation may be in progress in Nigeria, but the evidence needs corroboration by further observations and experiments.

The following aspects on the biology of the maize stalk borer are of special significance when control strategies are planned and should be carefully considered:

- The specific periods within which moth flights occur.
- The difference in magnitude of the first and second moth flights.
- The selective behaviour of moths when plants are selected for oviposition.
- The dependency of neonate larvae on soft plant tissue and the tendency of later instar larvae to remain sheltered in whorls.
- The fact that diapause larvae remain in the lower part of maize stalks and that pupation will be induced by specific climatic conditions.

The mentioned aspects on the biology of the maize stalk borer will influence and determine every step taken in the control of this pest.

Busseola *fusca* is a noctuid moth, closely related to the genus *Sesamia*, and its larvae feed inside the stems of grasses and cereal crops, especially maize and sorghum (van Rensburg & van den Berg, 1990). It was first recognized as a pest of maize in South Africa, where much of the early work on its biology and control was done, but it is now known to occur widely in mainland Africa south of the Sahara, but not on the islands of the Indian Ocean. It is not known to occur anywhere outside the African continent, although there must be some danger that it could be accidentally introduced elsewhere (Harris & Nwanze, 1992). The first detailed review of the biology, ecology, and control of this species by Mally (1920) contains 103 references, mostly to work done in South Africa up to 1919, followed by Du Plessis & Lea (1943). Research progress in the 1980s was reviewed by Harris (1989a) who also reviewed the bio-ecology of *B. fusca* (Harris, 1989b).

Two stalk borer species belonging to the family Noctuidae *viz. B. fusca* (Fuller) and *S. calamistis* (Hampson) attack maize in South Africa (Kfir, 1998). Maize crops are also attacked by *Chilo partellus* (Swinhoe), which belongs to the family Crambidae (Kfir, 1998). The maize stalk borer, *B. fusca*, is however the most important noctuid stem borer attacking grain crops in South Africa (Annecke & Moran, 1982), as well as in other mid-altitude production areas of East and Southern Africa (Harris 1989a; Bosque-Pérez & Schulthess, 1998).

The cost of pest control expressed as a percentage of the gross margin is governed by the yield obtained for any specific season (van Hamburg, 1987). This holds true for irrigated as well as dry land maize and it follows that the cost of chemical pest control, as a percentage of gross margin, will increase as yield decreases (van Hamburg, 1987). Maize yields obtained in South Africa can vary considerably from one region to the next. Factors such as recommended plant density (determined by the yield potential of an area), rainfall distribution within any particular season as well as cultivar selection, will have a major influence on harvests. A large percentage of the maize crop in South Africa is planted in marginal areas (van Hamburg, 1987) and it is often not possible for producers to apply chemicals to insect pests.

One of the more significant control tactics for dealing with insect pests of maize is the use of insect and disease resistant hybrids. Since the 1970's significant research progress has provided the basis for implementation of successful maize pest management programmes (Teetes, 1978).

2.2 Resistance breeding strategy

Two distinct phases of maize breeding are necessary for systematic genetic advance. These are the development and improvement of breeding populations and efficient extraction of lines and hybrids from genetically improved backgrounds (Rodriguez & Hallauer, 1991). In maize hybrid breeding programmes, inbred lines are developed from segregating base populations by self-pollinating and testing for grain yield in hybrid combinations (Hallauer, 1990). During inbreeding, visual selection is done for plant and ear traits and disease resistance of the lines *per se*, while grain yield evaluation of the lines is based on their performance when crossed to elite inbred lines or single crosses (Bauman, 1981).

In maize breeding programmes emphasis is placed on a system to ensure high levels of heterosis, whereby parental lines are classified in terms of their ability to perform in hybrid combinations. With this method, the resulting total genetic variation is partitioned into the effects of general (GCA) and specific combining ability (SCA). Significant GCA effects for grain yield and grain yield components have considerable importance in the selection of parents for grain yield improvement in conventional maize breeding programmes. Combinations with significant SCA effects for grain yield may be used in the development of hybrid cultivars (Borghi & Perenzin, 1994). Combining ability analysis of inbred lines is also necessary to exploit the relevant type of gene action.

According to Reeder *et al*. (1987) progressive genetic improvement of maize requires the development and improvement of basic breeding populations and extraction of inbred lines and hybrids from the improved populations. This genetic improvement of maize depends on the availability of favourable alleles for a specific characteristic of interest in the species or population. Favourable alleles are commonly introduced into existing elite maize breeding material through a variety of breeding procedures, by recycling and recombining existing material (Bailey, 1977). In maize hybrid breeding programmes, the identification of inbred lines with superior yield performance in hybrid combinations is costly and time consuming (Bernardo, 1992).

Maize breeders are interested in estimating the magnitude of genetic variance and the type of gene action in their material (Odiemah, 1992). This has implications in choosing the most effective selection and breeding procedures so as to increase the ability to identify the desired genotypes in the studied material. The presence of significant estimates of additive genetic variance indicates that selection of new superior lines from the segregating generations of a given cross may be possible. Odiemah (1992) pointed out that the presence of significant dominant genetic variance for a specific characteristic suggests that the greatest advantage would be in F1 hybrid performance.

Several statistical models have been proposed to determine the components of genetic variance and their partitioning into additive and non-additive genetic components, such as proposed by Griffing (1956) and Mather & Jinks (1982). Several authors (Robinson et al., 1955; Sprague, 1964; Stuber & Moll, 1971) have studied estimates of genetic variance in maize populations. Studies reporting on genetic parameters of additive, dominance and epistatic effects for grain yield and other traits in different maize inbreds have been published by Mariani & Desiderio (1975), Schnell & Singh (1978) and Odiemah & Oraby (1986).

The most important goal of a breeding programme is to increase the grain yield potential of the crop. Kronstad & Foote (1964) indicated that this can be done either by breeding for resistance to one or more of the many adverse factors influencing yield, or by breeding for increased yield itself. Breeding for yield entails genetic manipulation of polygenically inherited yield components such as number of kernel rows per ear, number of ears per plant, etc. or by directly manipulating the target population towards a higher grain yield potential through various possible selection procedures.

Maize is an extremely diverse plant species. It is this genetic variability that makes it such an extremely attractive candidate for breeding programmes aimed at developing insect-resistant varieties (McMillian & Wiseman, 1972). The point must be stressed, however, that insect pest management is a multi-tactic approach and plant resistance constitutes but one of the many available control tactics. Good progress has been made in this area, and it is quite conceivable that the integration of plant resistance and biological control, in conjunction with sound cultural production schemes, will form the most sound and lasting insect control strategy for maize. The fact that plant resistance is compatible with other control tactics is the one feature that makes resistant varieties such a viable component of pest management (Teetes, 1978).

Plant biotechnology is a powerful tool of agriculture research that allows plant breeders to develop plants with special characteristics. Instead of mixing thousands of genes, which is essentially what happens with cross breeding, modern plant breeders select a gene for a specific trait and move it into the cells of another plant. It is more precise, faster and makes it possible to improve plants in ways that conventional breeding cannot (MacIntosh *et al.*, 1991).

Biotechnology, together with other technologies, could provide new solutions for some of the old problems hindering sustainable rural development and achievement of food security (Crickmore *et al.*, 1996).

2.3 Pest status and crop loss assessment

Busseola fusca is of greatest importance as a pest of maize in Africa but it also attacks other cultivated crops, particularly sorghum, pearl millet, sugarcane, and some wild grasses. Damage is caused by the larvae which at first feed on the young leaves but soon tunnel into the stems. During the early stages of crop growth, larvae may kill the growing points, resulting in the production of 'deadhearts' with a consequent loss of crop stand. At later stages of growth, extensive tunneling inside the stems weakens them so that they break and lodge. Maize ears may be directly damaged by tunneling larvae.

2.3.1 Adult emergence, mating and dispersal

Adult moths mostly emerge between sunset and midnight, and soon after emergence the females release a pheromone, consisting of a 10:2:2 mixture of (Z)-11-tetradecyl acetate, (E)-11-tetradecyl acetate and (Z)-9-tetradecyl acetate to attract males (Nesbitt *et al.*, 1980; Hall *et al.*, 1981). Mating behaviour has not been reported in detail. Soon after mating is completed, female moths disperse in search of suitable host plants for oviposition. The period of oviposition continues over three to four successive nights. The extent of adult dispersal during this period has not been established, although the indications are that it is mainly local. Mally (1920) indicated that female moths located and moved to crops from an emergence site at least a mile away. Migration over longer distances has not been reported, although it would seem feasible in some circumstances. Further study of this point is merited, especially since there are occasions when the incidence of *B. fusca* attack on early-sown crops is higher than can be explained by local circumstances (Harris & Nwanze, 1992).

2.3.2 Timing and size of moth flights

Adult moths are seldom seen in farmers' fields as they are inactive during daylight and are cryptically coloured. However, they are attracted to light traps and are sometimes caught in large numbers. The adult wingspan is about 20-40 mm, with females generally larger than males (Harris & Nwanze, 1992).

Three distinct moth flight periods have been recorded throughout South Africa (Annecke & Moran, 1982; van Rensburg *et al.*, 1985; van Rensburg, 1992). Walters (1979) discussed the probable influence of weather conditions on the flight patterns of *B. fusca* observed over a two-year period. In most cases the first moth flights were considerably smaller than the second flights at various localities (van Rensburg *et al.*, 1985).

According to Du Plessis & Lea (1943) *B. fusca* has a low migratory potential and the damage potential over wide areas in a specific year can not be based on the monitoring of essentially localised populations. The third moth flight is of no economic significance since maize plants that have been planted during the normal planting season are no longer suitable for oviposition after February (van Rensburg *et al.*, 1985). Moth flights occur during specific periods within each season with slight variation between localities as well as between seasons for each locality (Annecke & Moran, 1982; van Rensburg *et al.*, 1985). According to van Rensburg *et al.* (1985), moth flight periods vary most along an east, west distribution line. A shift in locality from east to west will experience a smaller first moth flight, a greater increase in magnitude of second moth flights and a greater tendency for separate flights to overlap.

Extensive light trapping, done by van Rensburg *et al.* (1985) (Figure 1), has shown that first moth flights commence from mid October on the eastern Highveld and from the end of October in western production areas. The second moth flight is however slightly earlier in western parts and commences from the end of December in contrast to mid January for the eastern Highveld areas. Another important factor that should be taken into consideration is the fact that the size of moth flights can vary considerably between seasons for any particular locality. Seasonal variation in moth abundance was shown to be correlated with the rainfall cycle (van Rensburg *et al.*, 1985), with more serious infestations experienced during relatively wet years (Figure 2).

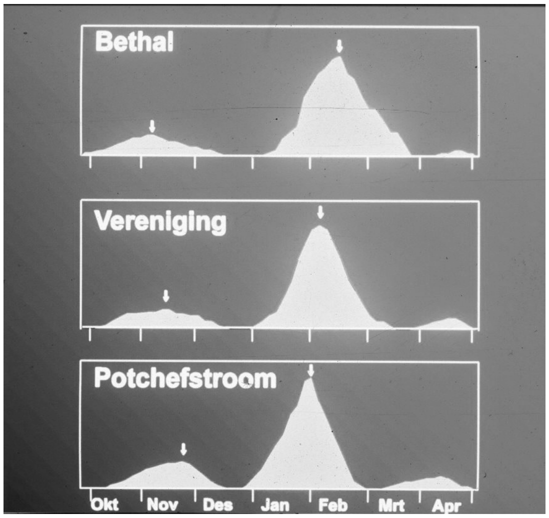

Fig. 1. Geographical variation in moth flight patterns with localities from East to West (van Rensburg *et al.*, 1985).

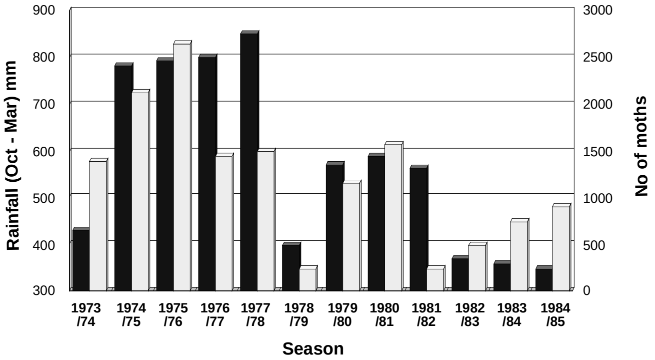

Fig. 2. Association between rainfall (shaded bars) and seasonal abundance of moths (solid bars) (van Rensburg *et al.*, 1987).

2.4 Maize stalk borer life cycle

During the three to four nights following emergence from the pupal stage, females lay eggs in batches of 30-100 under the inner surfaces of leaf sheaths, each female laying about 200 eggs in total (van Rensburg *et al.*, 1987; Harris & Nwanze, 1992).

Larvae hatch about a week later and initially disperse over plants before they enter the leaf whorls and start to feed on the leaves. Once established in their host plants, they bore into stem tissues and feed for three to five weeks, producing extensive tunnels in stems and in maize ears. They then pupate in the tunnels, often after first excavating emergence windows to facilitate the exit of adult moths (van Rensburg *et al.*, 1987; Harris & Nwanze, 1992). Adults emerge nine to 14 days after pupation and the life cycle is completed in seven to eight weeks when conditions are favourable. During dry and/or cold weather, larvae enter diapause for a period of six months or more in stems, stubble, and other plant residues before pupating during the next favourable period.

There is still a lack of adequate studies on the biology of *B. fusca* in many areas; the studies that have been undertaken have not used a uniform approach and are often restricted to a particular crop (Harris & Nwanze, 1992). Van Rensburg (1992) has shown that all final instar larvae are in a state of diapause during the dry season (April to October).

Tams & Bowden (1953) stated that in West Africa, *B. fusca* is probably most serious in the wetter parts of the Tree Savannah. It therefore appears that *B. fusca* is to some extent dependent for survival on the presence of a system of farming which suits the adaptation that the species has acquired in its evolution as a pest (Harris, 1961).

While irrigation may cause definite changes in plant growth and development, which may disrupt pest development, pest problems may also become severe under irrigated conditions. There are no reports available on the effects of water management on *B. fusca* infestations (Harris & Nwanze, 1992).

2.4.1 Selective oviposition pattern by the maize stalk borer

Direct observations of oviposition have seldom been made, mainly because this is a nocturnal activity of the female moths. Van Rensburg *et al.* (1987) reported on selective oviposition on maize where the ovipositional response is related to plant age. Maize plants are most attractive to oviposition three to five weeks after the crop emerges. Van Rensburg *et al.* (1989) indicated that plants younger than two weeks or older than six weeks were not selected for oviposition, although when younger plants were not available during the second-generation flight, oviposition occurred on plants older than six weeks in late plantings. The preferred leaf sheath for oviposition is that of the youngest fully unfolded leaf, so that the oviposition site gradually moves up the plant as the crop matures (van Rensburg *et al.*, 1989).

Evidence of selective oviposition on larger plants was obtained in a later study (van Rensburg *et al*., 1989) by using two maize hybrids with different average stalk circumferences. Significantly more and larger egg masses were laid on the hybrid with thicker stalks. Selection of vigorous plants by ovipositing females in field situations can probably be ascribed to an olfactory response and location of suitable ovipositing sites is probably thigmotactic. Differential oviposition appears to be a mechanism to promote larval survival since larger plants can better tolerate prolonged larval feeding. This phenomenon is also of possible importance in crop loss assessment studies since primary stem borer infestations will tend to be concentrated on potentially higher-yielding plants (Harris & Nwanze, 1992).

Eggs measure about 1mm in diameter. They are hemispherical and have about 70 crenulations (ridges) on the egg shell (chorion). They are generally laid in batches of 30-100 on the inner surfaces of leaf sheaths or on other smooth surfaces (Harris & Nwanze, 1992). According to van Rensburg *et al.* (1987) egg-batches of spring moths were smaller than those of summer moths, the difference being highly significant. A possible explanation is that body reserves of the spring moths may be smaller than those of summer moths since the larvae of the former would have utilised reserves during diapause. Usua (1967) found that spring moths each laid an average of 119.3 eggs compared with a considerably higher average number of 369.9 by summer moths. Walters (unpublished report, Department of Agriculture, Potchefstroom, 1974) found that 78 pairs of spring moths produced an average of 203.4 eggs per pair under laboratory conditions, considerably more than reported by Usua (1973). The results of other workers diverge; Mally (1920) states that the number of eggs per batch can vary from one to 140 with a maximum of 891 eggs/female. Ingram (1958) found an average of 70 per batch and a maximum of 568 per female. No distinction between spring and summer moths was, however, made. Female moths are reputed to produce up to eight egg batches in their life- time and are subsequently capable of infesting more than one maize plant (Ingram, 1958; van Rensburg & Bate, 1987).

2.4.2 The significance of plant age

According to van Rensburg *et al.* (1987), the majority of eggs are laid on plants between four to six weeks old. Oviposition rates decline rapidly on plants older than six weeks and it has been shown that only 9% of the total number of egg batches is deposited on plants older than six weeks (van Rensburg *et al.*, 1987). It must, however, be stressed that high levels of late oviposition can occur if plants have been planted late (van Rensburg *et al.*, 1988a). Severe crop losses can be expected if high levels of oviposition, caused by the second and larger moth flight, take place shortly before tasseling on old plants (up to seven weeks after emergence).

Trials conducted by van Rensburg *et al.* (1987) also confirmed that a high rate of correlation exists between plant age and loci selected for oviposition. No correlation does, however, exist between oviposition loci and the position of larvae on maize plants.

2.5 Larval development and behaviour

The behaviour of first-instar larvae is similar to that described for *Chilo partellus* by Chapman *et al.* (1983) and Bernays *et al.* (1985) but has not been studied in such detail. Soon after hatching, the larvae move up to the leaf funnel and feed on the young leaves before penetrating into the stem. Leaf feeding results in characteristic patterns of small holes that appear on the youngest leaves. During the stage of larval feeding in the stem, the growing point may be killed, resulting in “deadheart”. Van Rensburg & Bate (1987) reported that 81% of larvae up to the fourth instar were found in leaf whorls. The period of larval feeding lasts about 24-36 days and during that time larvae may leave the stem that was initially attacked, especially if it has been severely damaged, and bore into other stems. There is therefore some larval migration within crops. Van Rensburg *et al.* (1987) noted that previous workers underestimated the extent of this migration, and recorded that 4% of the total number of larvae in a planting of maize migrated to adjacent plants immediately after hatching. The fifth-instar larvae were evenly distributed in plants reaching a peak at eight weeks after plant emergence, and that sixth instars were found in considerably larger numbers that previous instars in stems and ears, and were the only instars found in stem bases (van Rensburg *et al.,* 1987).

Before pupation, larvae eat away exit holes to facilitate their emergence as adult moths. These holes are characteristically covered by a thin remaining layer of epidermis and are visible externally, giving an indication that pupation has occurred or is about to occur (Harris & Nwanze, 1992).

Stalk borer larvae normally pass through six instars (Annecke & Moran, 1982) and are dependent on whorls for shelter and soft plant tissue that is necessary to initiate their development (Annecke & Moran, 1982; van Rensburg *et al.*, 1987). Larvae in their fifth and sixth instars are usually forced to leave whorls after tassel appearance (eight weeks after plant emergence) and are normally found inside stems after boring into the sides of stems. Second generation larvae on older plants often seek refuge inside ears (Annecke & Moran, 1982), which are penetrated through husk leaves or ear tips (van Rensburg *et al.,* 1987). The majority of larvae will, however, remain on the plant on which they hatched up to the point of tasseling, after which they will spread to other plants in search of shelter.

2.6 Pupae

Pupae are usually shiny yellow-brown but their colour may vary with location. Female pupae are about 25mm long. Male pupae are generally slightly smaller. They can be sexed by differences in the positioning of the genital scars, found on sternum eight in females and on sternum nine in males. The cremaster bears a single pair of simple spines. *Busseola fusca* pupae can therefore be distinguished from those of *Sesamia*, which have a more complex cremaster with two pairs of thornlike spines (Harris & Nwanze, 1992).

2.7 The importance of diapause and population dynamics

Despite its importance as a pest of African food crops, the population dynamics of *B. fusca* have not been studied in any detail. At most locations, two to three generations are produced (van Rensburg *et al.*, 1985) but in relatively humid areas a small population of larvae may pupate and give rise to a fourth adult generation. Harris & Nwanze (1992) reported that the first generation adults are produced from the diapause larvae of the previous crop season, with moth flights occurring a few weeks after rains have begun, when maize crops are three to five weeks old.

In South Africa, the number of generations on maize increases from two to three from east (Kwazulu Natal province) to west (North West province) (van Rensburg *et al.*, 1985; Barrow 1989). The first generation moths emerge between October and December, the second in January, and the third in March. At all locations, most of the last larval generations of *B. fusca* enter diapause. Although it is thought that the onset of diapause may be favoured by the ageing of maize plants (Usua 1973), there is evidence that the rainfall gradient may contribute indirectly to geographic variation in population dynamics, and in the number of generations produced.

In dry and/or cold conditions larvae enter diapause for six months or more. Usua (1970, 1974) studied the physiology of diapause in detail on maize in southern Nigeria, but at the time there was no clear understanding of the factors inducing and breaking diapause. It was observed that the main factor enabling larvae to survive adverse conditions in diapause seems to be their efficient conservation of water.

Diapause is normally terminated as rainfall increases during the subsequent growing season.

Harris (1962) reported that at the end of the diapause period, the availability of free water, which the larvae drink, facilitates rehydration and stimulates pupation. Subsequent studies by Adesiyun (1983) showed that contact with water in the vapour state (i.e., higher relative humidity), rather than direct intake, promoted diapause termination. Rainfall alone was not considered to be the main factor terminating diapause as pupation continued over an extended period, and some larvae pupated even though they had not been exposed to water. However, more recent findings by Okuda (1988, 1990) confirmed earlier studies by Adesiyun (1983a) and revealed that water contact is more significant than water uptake as a factor in diapause termination. The key factor was eventually identified to be photoperiod and successfully utilised to develop a method for mass rearing of larvae for the purpose of artificial infestation (van Rensburg & van Rensburg, 1993).

Diapause is facultative under unfavourable winter conditions (Usua, 1970), and all stalk borer larvae are in a state of diapause during winter months or during the dry season (April – October). (van Rensburg *et al.,* 1987; Kfir, 1991). Diapause was recorded in June, reaching a peak in July, and completed in September (Ussia, 1970).

Elsewhere in Africa, Ussia (1970) found that some larvae of *B. fusca* go into diapause during each generation, irrespective of the state of the maize plants, suggesting a probable genetic mechanism or heredity. Diapause also occurs in other parts of the continent irrespective of the state of maize stems and in spite of favourable environmental conditions (Usua, 1970).

Sixth instar diapause larvae that are overwintering under South African conditions are found sheltering in subterranean parts of maize stalks since temperatures are slightly higher in these parts (van Rensburg *et. al.*, 1987). Larvae pass through seven stationary moults during this period and lose up to 50% of their body weight (Kfir, 1991). A further weight reduction of 8% occurs during pupation and as a result moths emerging from diapause pupae retain only 30% of the original weight of sixth instar larvae that entered diapause. Comparisons made between diapause and non-diapause moths have revealed a 50% reduction in weight and number of oocytes contained in ovaries of diapause moths (Kfir, 1991). Weight reductions clearly have a dramatic effect on the fecundity of first generation moths (Kfir, 1991), and explain the large population increase experienced with second moth flights, which are caused by non-diapausing females (van Rensburg *et al.*, 1985).

2.8 Aspects of the injuriousness of *Busseola fusca*

Previous knowledge of the injuriousness of *B. fusca* is based on studies conducted in East Africa, Nigeria and Zimbabwe by workers such as Ingram (1958), Harris (1962), Rose (1962), Usua (1968) and Walker (1960a; 1977). These workers emphasized yield losses following on the destruction of the growing point of the plant (deadheart), and they attached special importance to the number of larvae per plant as the damage-determining factor (van Rensburg *et al.*, 1988a). It appears that attack by *B. fusca* limits the capacity of the plant to produce rather than stimulating it to compensate for losses. Rose (1962) distinguishes between damage to the leaves and stems but does not quantify the resulting crop loss. In a study conducted by van Rensburg *et al.* (1988a), it was shown that secondary damage, i.e. damage to parts of the plant other than the whorl, is economically more important than primary damage.

Although the injuriousness of insect pests is known to be modified by various factors, including climatic influences (van Rensburg *et al.*, 1988a), the comparative efficacy of various approaches to control *B. fusca* remained similar over years (van Rensburg, 1990).

2.9 The effect of *Busseola fusca* on yield of maize

A survey conducted by Walker (1960a) in East-Africa indicated that infestation at an early stage of plant growth will reduce the yield up to 36 kg grain per ha for every 1% plant infestation in high potential plantings. An infestation at a later stage is less injurious.

Final yields are the result of a number of growth processes, which may be inhibited, retarded or encouraged, directly or indirectly, by the pest (Walker, 1960a). Estimates of this loss vary greatly from place to place and from season to season but in South Africa the average annual loss to the maize crop of the whole country is about 10% (Du Plessis & Lea, 1943). It was estimated as high as 25% in some cases (Mally, 1920), but proved to reach 100% in individual plantings during epidemic conditions such as was experienced during the 1978/79 season (van Rensburg *et al.*, 1989).

Most high yielding maize cultivars are relatively slow growers that spend more time in their vulnerable growth stages and may for this reason suffer more damage than short season hybrids at similar levels of infestation (van Rensburg *et al.*, 1989). Through repeated sampling in maize planted on different dates over different seasons in South Africa, it was shown that damage by *B. fusca* to plant parts other than the whorl had an important influence on yield (van Rensburg, *et al.*, 1988a). Damage to plants in the period after tasselling was shown to be less important unless it involved direct damage to the ears (van Rensburg, *et. al.*, 1988a). The number of larvae in the plant is a weak estimator of expected yield losses. An economic threshold for chemical control based on scouting for eggs and a concomitant method for sequential sampling was subsequently developed (van Rensburg *et al.*, 1989).

2.10 Cultural control strategies

Cultural control strategies often have a direct impact on *B. fusca* numbers because they focus on life cycle disruption. Cultural control often involves no financial inputs but do require an intimate knowledge of stalk borer biology and how it is related to crop cycles.

Nwanze and Mueller (1989) emphasized that stem borer control strategies must be politically practical, socially acceptable, economically feasible, and technically effective (Harris & Nwanze, 1992).

The overall approach to control stem borers on crops in Africa must be to devise and implement an integrated pest management program that must meet local needs and be adapted to local conditions and resources. There is wide scope for the development of such programmes that will be mainly based on non-chemical methods of control. The main elements are summarised below.

2.10.1 The significance of planting date to stalk borer control

Maize stalk borer oviposition is selective (van Rensburg *et al.*, 1989) which results in large moth flights not necessarily giving rise to high infestation levels (van Rensburg *et al.*, 1987). Producers can avoid heavy infestations during the first moth flight and severe crop losses during the second moth flight by ensuring that vulnerable growth stages are not synchronised with moth flight periods (Annecke & Moran, 1982). Repetitive planting trials conducted by van Rensburg *et al.* (1987) have shown that infestation levels were lowest with planting during November since plants will be at their most susceptible growth stage during December (between the first and second moth flights) when no moths are active. Infestation levels do, however, increase progressively as plantings are made earlier than and later than middle of November (van Rensburg *et al.*, 1987).

2.10.2 Carry-over populations

Early observations showed that larvae of this species survive in maize stubble in South Africa (Mally, 1920). Some larvae may also survive on wild grass hosts (van Rensburg & van den Berg, 1990). However, stubble is probably the main source of initial stem borer infestation in subsequent seasons.

2.10.3 Alternative host plants

Busseola fusca belongs to a group of Lepidoptera that has evolved in close association with grasses, and in which the specialised habit of boring into stems has developed (Harris & Nwanze, 1992). The original host plant on which *B. fusca* evolved is not known, but the following indigenous African grasses were recorded as hosts:

Sorghum verticilliflorum (Steud.), Piper (including *Sorghum arundinaceum*), *Pennisetum purpureum* Schum., *Panicum maximum* Jacq., *Hyparrhenia rufa* Nees (Stapf), *Rottboelia exaltata* (L.) and *Phragmites* sp. The original host may well have been one of these, possibly a *Sorghum* or *Pennisetum*. The main crop hosts are maize and sorghum and, to a lesser extent, pearl millet, finger millet, and sugarcane. Of these, all except maize and sugarcane are indigenous to Africa.

The interaction of *B. fusca* with maize is particularly interesting as it dates from about 1550 A.D., from the time of the introduction of that crop to Africa from the Americas. The extension of maize cultivation in Africa may have enabled the borer to follow the crop and become established in new areas, such as South Africa, as suggested by Mally (1920).

Maize stalk borers utilise various indigenous and exotic grass species (family Poaceae and Typhaceae) as host plants (Polaszek & Khan, 1998). A total of four crop species *viz. Zea mays L*, *Sorghum bicolor* (L.) Moench, *Pennicetum glaucum* (L.) R. Br. and *Saccharum officinarum* have been recorded as stalk hosts in South Africa (Polaszek & Khan, 1998). Du Plessis (1936) reported that trap cropping was not effective in South Africa although Jack (1922; 1928) earlier recommended the use of sorghum or maize as trap crops in Rhodesia (Zimbabwe). Later Jack (1931) reported that maize sown as a trap crop was not effective.

Recommendations that wild host plants should be removed as part of stalk borer control programmes might not be the most effective option (van den Berg *et al.*, 1998). Several wild and fodder grass species (Table 1) have been found to have useful properties and must be considered for inclusion in pest control strategies. Van Rensburg & van den Berg (1990) showed that some fodder crops of both the *Sorghum* and *Pennisetum* genera are at least as favourable for egg laying as the grain crops. In a study conducted under laboratory conditions some graminaceous fodder crops were compared to maize for ovipositional preference by *B. fusca*. Based on the ability to sustain larval development, pearl millet (Babala) was found to be superior and Napier grass inferior to maize (van Rensburg & van den Berg, 1990).

Table 1 Important wild and cultivated host grass species of the maize stalk borer (*B. fusca*) in South Africa (van den Berg *et al.*, 1998; van den Berg, 2001).

Grass Species	**Significance to agricultural and stalk borer control**	**Origin**
Desmodium uncinatum	Attract parasitoids	Africa
Echinochloa pyramidalis (Lam.) Hitchc. & Chase	Cereal & pastures	Africa
Hyparrhenia cymbaria (L.) Stapf	Wild host	Africa
Hyparrhenia rufa (Nees) Stapf	Pastures (young plants only)	Africa
Melinis minutiflora Beauv.	Attract parasitoids & pastures	Africa
Panicum deustum Thunb.	Pastures	Africa
Pennisetum glaucum (L.) R. Br..	Pastures	Africa
Pennisetum purpureum Schumacher.	Catch crop & pastures	Africa
Rottboelia cochinchinensis (Lour.) Clayton	Wild host	Africa
Saccharum officinarum	Sugar production	Asia
Setaria incrassata (Hochst.) Hack.	Wild host	Africa
Setaria sphacelata (Schumach.) Moss	Cultivated hay & pastures	Africa
Sorghum bicolor (L.) Moench	Cereal & fodder	Africa
Sorghum versicolor Anderss.	Wild host	Africa
Sporobolus marginatus Hochst. Ex A. Rich.	Pastures	Africa
Sporobolus pyramidalis Beauv.	Wild host	Africa
Vetiveria zizanioides (L.) Nash	Possible catch crop & Vetiver oil	Asia
Zea mays L.	Cereal & fodder	America

The most important qualities found in grasses that have been employed in cultural control strategies are as follows:

- The ability to attract female stalk borer moths and to elicit oviposition behaviour.
- The ability to repel stalk borer moths.
- The ability to attract stalk borer parasitoids.
- The ability to act as a reservoir of natural enemies by hosting non-pest species closely related to harmful stalk borers.

Napier grass (*Pennisetum purpureum* Schumacher) has properties that make it very attractive to stalk borer moths but at the same time unpalatable to stalk borer larvae (van den Berg & Polaszek, 1998). The combination of toughness, hairiness and the production of sticky substances (when plants are damaged) are believed to be responsible for the high mortality rate among larvae feeding on Napier grass (van den Berg, 2001).

Sudan grass (*Sorghum bicolor drummandii* (L.) Moench has the ability to attract stalk borer parasitoids along with stalk borer moths. Trials involving Sudan grass have shown a 5.67% increase in stalk borer parasitism (van den Berg *et al.*, 1998). Molasses grass (*Melinis minutiflora* Beauy) has been reported to attract parasitoids and Silverleaf Desmodium (*Desmodium uncinatum*) to repel *B. fusca* moths (van den Berg, 2001). Dramatic reductions in stalk borer infestations on maize crops were recorded in cases where non-host grasses were intercropped with maize (van den Berg *et al.*, 1998; van den Berg, 2001).

A “push - pull” planting system that has been developed in Kenya is currently under investigation in South Africa (van den Berg, 2001). It involves the planting of highly attractive but unpalatable Napier grass around maize fields, intercropped with a wild host species (such as molasses grass) that is attractive to parasitoids but not to *B. fusca* moths (van den Berg, 2001).

2.11 The importance of sanitation

It was suggested that it might be possible to reduce the pest status of *B. fusca* and *C. partellus* in South Africa by destroying their hibernation sites. This should be done on a national basis, and cooperation of farmers is essential (Kfir, 1990). Crop residues are one of the most important sources for new stalk borer infestations in large commercial plantings (van den Berg & Nur, 1998). Cultural control strategies can be a low cost option but often involves high levels of input and may be labour intensive. Practices such as large scale crop rotation are often not an option in large production systems (van den Berg *et al.*, 1998).

2.11.1 Importance of cultivation as part of a sanitation programme - tillage

Deep ploughing to bury maize stubble was one of the earliest control measures used against this pest in South Africa (Mally 1920). Jack (1918) reported that in Rhodesia (Zimbabwe) moths emerging through 5cm of soil were crippled and that deeper burial of maize stalks under 10-15cm of soil ensured that no adult moths emerged. Du Plessis and Lea (1943) reported that tillage only gave partial control but Walters (1975) emphasised the role of conventional tillage in controlling *B. fusca* in South Africa. More recent work reported by Kfir (1990) showed that slashing maize and sorghum stems destroyed 70% of the stem borer population and that ploughing and disking the crop residues after slashing destroyed a further 24% of the pest population in sorghum and 19% in maize.

Cultural methods of control have recently been reviewed by Verma and Singh (1989) and by Reddy (1985a), but necessarily relate to cereal stem borers in general, rather than to *B. fusca* in particular.

Van den Berg *et al.* (1998) stated that the predictability of the maize stalk borer’s life cycle under local conditions makes the use of cultivation practices in its control a viable option. Du Plessis and Lea (1943) carried out experiments in which maize stems containing larvae were buried in the ground at depths similar to those which would be achieved by the ploughing in of crop residues. They found that stem borer moths were able to emerge from depths of up to four inches, although considerable mortality was affected. Thus the control by ploughing can only be partial.

Trials conducted by Kfir (1990) showed that maize stalk borer numbers were decreased by 89% after a combination of winter slashing, ploughing and disking of plant residues on maize fields. Slashing destroyed larvae that were hibernating in stalks above the soil surface. Ploughing and disking destroyed larvae hibernating inside stalks underneath the soil surface by either crushing them or exposing them to predators and unfavourable climatic conditions. Larvae that survived and managed to complete their life cycle were often unable to re-infest maize fields during the next season because stalks were often buried too deep by cultivation activities and moths could not escape (Kfir, 1990).

2.11.2 The effect of stalk borer survival

Most South African maize producers include livestock as a necessary component of their annual production cycle (van den Berg *et al.*, 1998). Maize residues left on fields after harvesting makes an invaluable contribution in the form of winter fodder and cattle may feed on maize fields for up to four months after harvesting (van den Berg *et al.*, 1998). The effect of grazing animals on stalk borer numbers is, however, minimal since cattle prefer to feed on leaves and upper parts of stalks (van den Berg *et al.*, 1998).

2.11.3 Burning of plant residues

Where destruction by burning or deep ploughing is feasible, it may be possible to take concerted action to reduce carry-over populations and so limit the most damaging early borer infestations in the following season (Harris & Nwanze, 1992).

Of the numerous methods of control examined, a system of dry season burning of stubble showed the greatest promise in countries where pupation occurs within the stems rather than in the stem base. According to Duerden (1953) the burning of crop residues proved to be highly effective in the eradication of overwintering stalk borer populations in Tanzania. A 99.6% reduction in larval counts was recorded after above ground parts of stalks were removed and burned. It must, however, be stressed that the same success rate might not be achieved under South African conditions, since diapause larvae occur almost exclusively in subterranean parts of maize stalks (Kfir, 1991).

Burning of stalks is not recommended for areas where soils have low organic matter content or if wind and water erosion is a problem (van den Berg *et al.*, 1998). The large scale burning of plant residues will also lead to shortages of winter fodder in commercial production systems. The fact that stalks have to be stacked to ensure effective burning (Duerden, 1953) will make this exercise too labour intensive on a commercial scale. According to Duerden (1953) the base of the plant which is often difficult to destroy, could largely be neglected as the number of borers in this zone was negligible.

2.11.4 Volunteer plants

Volunteer plants can normally be found growing in and around maize fields before crop plants have been planted. First generation moths are normally attracted to volunteer plants growing among maize seedlings, which defeats the objective of selecting a planting date to try and avoid infestations (van Rensburg *et al.*, 1987; 1988a). Volunteer plants should be removed since larvae will migrate to crop plants if volunteer plants are left in fields after hoeing (van den Berg *et al.*, 1998). The incidence of growth tip damage ("dead heart") is much higher in cases where seedlings are attacked by stalk borer larvae in advanced stages of development (van den Berg *et al.*, 1998) and will have a greater impact on yield since growth tip damage results in direct crop losses (Annecke & Moran, 1982). Volunteer plants can also be destroyed by allowing animals to graze in fields before the crop is planted (van den Berg *et al.*, 1998).

2.11.5 Crop rotation

Any crop rotation that extends the period between cultivation of successive maize and/or sorghum crops in the same fields may reduce borer infestations, but local dispersal of ovipositing moths is possible and may cancel out any local effects of crop rotation. There appears to be no information available on the effects of different rotation systems on *B. fusca* incidence (Harris & Nwanze, 1992).

2.12 Chemical control

Walker (1960a) stated that larvae do not penetrate the more closely packed leaves in the whorl until about 10 days after hatching. During this period the larvae are exposed, and application of insecticides to the whorl of the plant up to ten days after hatching will give effective control.

Maize stalk borer infestations occur within a limited range of crop growth stages, with the result that re-infestations after treatment of any particular planting is very rare (van Rensburg & van den Berg, 1992). This scenario makes it possible to calculate threshold values for chemical control and to ensure the correct timing of chemical applications.

Several studies on the chemical control of *B. fusca* have been conducted; either to determine the relative efficacy of different chemicals, or to evaluate *B. fusca* control under experimental conditions (Harris & Nwanze, 1992).

The earliest use of insecticides for the control of *B. fusca* was reported from South Africa where maize crops were treated with hycol solution, sheep dip and several other botanical insecticides such as ‘Derrisol®’, ‘Pulvex®’, ‘Kymac®’, etc., that were all based on rotenone, a product of the leguminous plant *Derris chinensis* (USADA, 1922; Chorley, 1932; Ripley, 1928; Ripley & Hepburn, 1928; 1929; Parsons, 1929). Good control of *B. fusca* was achieved by using these chemicals. In the 1950s, DDT at 22.4 kg ha^{-1} was successfully used in Ghana (Bowden, 1956) and in Uganda (Coaker 1956).

With the withdrawel of DDT from the South African market during 1973 (van Rensburg *et al.*, 1978) a host of new and more expensive insecticides became available. Several later studies indicated that a single dose of carbofuran at 1.0-2.5kg a.i. ha^{-1}, applied to the planting furrows of maize in South Africa and in Nigeria, gave good control up to seven weeks after emergence (van Rensburg & Malan 1990; van Rensburg *et al.*, 1978; Egwuatu & Ita 1982; Drinkwater, 1979). Placement of granular dusts of endosulfan, carbaryl, malathion, or fenvalerate in leaf whorls were also reported to control *B. fusca* effectively (Whitney, 1970; Adenuga, 1977;

Adesiyun, 1986; Kishore, 1989). Spray applications of endrin as a 0.03-0.40% emulsion, or as a 2% dust formulation, were effective in eastern Africa (Walker, 1960a).

However, with the changing patterns of maize production in many African countries (i.e., on large-scale and parastatal farms) insecticide use will form a vital component in an integrated approach to stem borer control.

2.13 Determination of threshold values and scouting of maize fields

The economic threshold represents the level of infestation where the value of the expected yield benefit exceeds the cost of chemical control (van Rensburg, 1990). Control measures based on an economic threshold of 10% of the plants showing visible damage proved to be superior to a preventative spray with regard to both larval control and grain yield (van Rensburg, 1990).

Stalk borer oviposition patterns are not linked to planting dates but do remain constant in relation to plant age (van Rensburg & Pringle, 1989). The greatest oviposition intensity will always be reached between three and five weeks after crop emergence (van Rensburg *et al.*, 1989). Scouting efforts should be concentrated between two to seven weeks after crop emergence to ensure that producers have ample time to react (Walters *et al.*, 1975).

Threshold values can be calculated on account of scouting efforts concentrated either on oviposition levels or on plant damage levels (van den Berg & Nur, 1998). Scouting efforts focusing on oviposition levels will involve the counting of egg batches, which are not difficult to spot through the partially transparent leaf sheaths (van Rensburg *et al.*, 1989). Van Rensburg & Pringle (1989) have established a link between the amount of sampling deemed necessary for egg batch counts to reveal whether the economic threshold level has been reached and infestation levels. Sampling suggestions made by van Rensburg *et al.* (1989) include sampling of plants in units of 20, with plants being selected for sampling adjacent to and within the same row at a randomly selected point. This method is a simplified version of a suggestion involving totally randomised sampling and was made to ensure that unskilled labourers will be able to do successful scouting. Walters *et al.* (1975) suggested that egg batches on 5% of plants should be regarded as the economic threshold value for stalk borer control. According to Walters *et al.* (1975) at least 100 plants (five plant units containing 20 plants) must be searched before deciding on control measures. The same recommendation has been made by van Rensburg *et al.* (1989), but only for low to moderate infestations (an average infestation of 2.9%). The recommendation is altered slightly in cases where four egg batches have been found in a single plant unit (20 plants) since this will signify that the economic threshold level has been reached (van Rensburg *et al.*, 1989).

An economic threshold value can also be based on the percentage whorl damage found in a maize field (van den Berg & Nur, 1998). It is important to scout the inner (youngest) leaves of whorls since this will indicate the most recent damage symptoms. The current economic threshold values have been set at very low infestation rates in South Africa to allow for timely applications in fields of high value (van den Berg & Nur, 1998).

2.14 Use of pheromone traps

Pheromone traps can be used to monitor *B. fusca* numbers and are capable of providing maize producers with valuable information (van den Berg & Nur, 1998). Formulations of *B. fusca* sex pheromones are commercially available in South Africa. The action threshold determined for pheromone traps is reached when an average catch per three traps (set in one locality) exceeds two moths per week for three consecutive weeks (van den Berg & Nur, 1998). A sex pheromone-based monitoring system for *B. fusca* moth flight was described by Revington *et al.* (1984). Scouting for *B. fusca* egg masses counts did not coincide with pheromone trap results, but were better correlated with light trap catches (Revington *et al*., 1984).

Van Rensburg *et al.* (1985; 1987) showed that moth numbers three to five weeks after emergence of a given maize planting provide a reliable estimate of the expected infestation level in terms of larval numbers. Most of the variations in infestation levels between plantings (71%) were explained by the variation in planting date and moth numbers (Van Rensburg *et al.,* 1987). While light traps may provide a useful early-warning system by identifying potentially hazardous infestations, extensive use of light traps is prohibited by practical limitations.

While pheromone traps tended to over-estimate moth numbers during periods of diminished moth populations, the possibility of using pheromone trapping systems to identify seasons of potentially severe infestations seems to be feasible. It is anticipated that the ultimate pheromone trapping system used for early-warning will need to indicate only those seasons of above and below average population levels at a given level of probability (van Rensburg, 1992).

If a margin of uncertainty is allowed in between, such a system could be meaningfully integrated with the principle of the economic threshold for chemical control. The application of the current threshold requires regular inspection of the maize crop, while the integrated system could reduce this to only the seasons of uncertain population levels (Van Rensburg *et al.*, 1985; 1987).

The use of pheromone traps does, however, seem to have lost favour in South Africa, with very few, if any, commercial farmers still using them (Personal communication, Professor J.B.J. van Rensburg, Grain Crops Institute, Potchefstroom, 2003).

2.15 Chemicals registered for control of maize stalk borers

A total of 17 chemicals, sold under 64 trade names, have been registered for use against the maize stalk borer (Nel *et al.*, 2007). Products can be classified under three application categories *viz.* pre-emergence preventive, early corrective post-emergence and late corrective.

2.15.1 The importance of timing and methods used for applications

Threshold values are invaluable in determining the need for chemical applications. It must, however, be stressed that the following factors will govern the timing and method used for chemical applications:

- Single applications are in most cases the only economically justifiable option (van Rensburg, 1990).

- Applications made directly into whorls are more effective (van Rensburg, 1990; van den Berg & van Rensburg, 1995).

- Economically significant crop losses are possible after tasseling (van Rensburg *et al.*, 1988a).

- Chemical applications after tasseling will be less effective but can still be economically justified (van Rensburg, 1990).

Walters *et al.* (1975) suggested that chemical applications (early corrective post-emergence) should be made between 10 and 14 days after fields have been scouted for egg batches. The normal practice in cases where scouting has been done for funnel damage, is to apply chemical control measures as soon as the economic threshold of 10% damage has been reached. This practice might, however, necessitate more than one chemical application, since the economic threshold might, in the case of late plantings, be reached before plants are older than six weeks. Late and highly injurious infestations, due to late oviposition, might also occur in late plantings (van Rensburg *et al.*, 1988a) and will necessitate late chemical applications. It has been suggested by van Rensburg (1990) that control measures should be taken after rather than before. This suggestion is based on the fact that whorl applications shortly before tasseling had the same results as applications made at the point of reaching the threshold value (van Rensburg, 1990).

It is advisable that late corrective applications should always be made before growth stage four has been reached (van Rensburg, 1990). Applications made by means of drop arms and aimed at the sides of plants have been shown to be significantly less effective than applications directed into whorls (van den Berg & van Rensburg, 1995). Late infestations might, however, necessitate chemical applications after tasseling. The timing of applications after tasseling becomes crucial and it has been found that yield losses will increase from 15.1% to 42.5% for applications made at 10% and at 50% tasseling respectively (van Rensburg, 1990).

2.16 Biological control

Biological control has been effectively used against stem borers on sugarcane in the Caribbean and might be expected to have potential for use against *B. fusca* and other stem borers of cereal crops in Africa. Ingram (1983) stressed that little is known about predation on stem borers, other than occasional references to ants attacking eggs and first-instar larvae. Skoroszewski and van Hamburg (1987) reported the introduction of *Cotesia flavipes* (Cameron) [= *Apanteles flavipes* (Cameron)] against *C. partellus* and *B. fusca* on maize in South Africa but, although *C. flavipes* became temporarily established, it was not recovered after the winter. Kfir (1989) has also reviewed the prospects of biological and cultural control of lepidoterous stem borers (including *B. fusca*) in South Africa, where a number of different exotic parasitoids have been released. To date, few recoveries of these releases have been made (Harris & Nwanze, 1992).

According to Skoroszewski & van Hamburg (1987) the profit margin for maize production is often low with the result that farmers cannot afford to spray. The cost of one spray application could amount to 28% of the crop value (Van Rensburg & Van Hamburg, 1975). Several biological agents as well as abiotic factors such as drowning are responsible for mortality among immature stages of *B. fusca* (van Rensburg *et al.*, 1988b). Mortality rates among larvae had a tendency to increase as time after plant emergence increased. Several factors - including plant growth stage, the biology of parasitoids, and the spreading of diseases among larvae - are responsible for the observed pattern (van Rensburg *et al.*, 1988b).

2.16.1 The importance of parasitoids as biological control agents

The most important parasitoids of *B. fusca* in eastern Africa are *Cotesia sesamiae [=Apanteles sesamiae], Sturmiopsis parasitica, Procerochasmias glaucopterus,* and *Pediobius furvus* (Mohyuddin & Greathead, 1970). These authors reviewed information on predators and concluded that ants are important.

Only one parasitoid, *Cotesia sesamiae* Cameron, has been consistently recovered (van Rensburg *et al.*, 1988b; Overholt, 1998) and may account for up to 90% of parasitised larvae in South Africa (Kfir, 1995).

Larval parasitism is the single most important mortality factor among *B. fusca* larvae and accounted for 36% of all larval mortalities during trials conducted at Potchefstroom (van Rensburg *et al.*, 1988b). The order Hymenoptera harbours the greatest number of *B. fusca* parasitoids, with 18 species (two remain unidentified) having been recorded in South Africa (van Rensburg *et al.*, 1988a; Kfir, 1995). Parasitoids belonging to the order Diptera are not as well represented, with only three species having been recorded as larval parasitoids (Kfir, 1995). Almost all parasitoids are rare and the local incidence of parasitism is low (van Rensburg *et al.*, 1988b; Kfir, 1995).

Kfir (1995) reported that parasitoids are able to reduce *B. fusca* numbers in South Africa, but have not yet been able to maintain populations below economic threshold levels. The natural enemy complex within South Africa is regarded to be rather impoverished, with very low activity levels being reported early in the growing season (van Rensburg *et al.*, 1988b). Parasitoids found in South Africa are indigenous to Africa and have been noticed to be more habitat specific than host specific (Kfir, 1995). The host plant species on which *B. fusca* larvae feed may play an important part in this phenomenon since attraction between specific host plants and parasitoids has been reported (van den Berg *et al.*, 1998; van den Berg, 2001).

Trials conducted by du Toit (1995) have revealed a predator complex of 56 insect and arachnid species, belonging to 16 orders and 23 families, in maize fields in the Potchefstroom district. Data presented by du Toit (1995) should be applicable to the majority of maize production areas that are affected by *B. fusca* if minor changes in the predator complex are accepted. *Busseola fusca* is the most important maize pest in cooler production areas (more than 900m above sea level) (van Rensburg & Bate 1987), in which the grassland biome is the predominant eco-type (Low & Rebelo, 1996).

Direct predation on *B. fusca* larvae has only been observed on two occasions during trials conducted by du Toit (1995). It is, however, important to note that active predation on immature life stages of *B. fusca* might be more frequent. Ant predation was also reported by van Rensburg *et al.* (1988b), who regarded it as sporadic. Predation undoubtedly plays an important part in the drastic rise in mortality among larvae witnessed after tasseling (van Rensburg *et al.*, 1988b), when migrating larvae are at a greater risk to encounter predatory insects and arachnids. It is however doubtful whether predators will have a significant impact on *B. fusca* numbers because the size of infestations varies between seasons (Annecke & Moran, 1982).

2.16.2 Important diseases

According to van Rensburg *et al.* (1988b), large numbers of *B. fusca* larvae that were collected from fields died without any apparent reason. A Nuclear Polyhedrosis virus was first isolated from dead larvae by Hoechst (van Rensburg *et al.*, 1988b) and is currently regarded as the most common *B. fusca* pathogen (Kfir, 1991).

Beauveria bassiana (Balsamo) is the most prominent entomopathogenic fungus (van Rensburg *et al.*, 1988b; Kfir, 1991). Several important factors will have to be taken into account if pathogens are considered for use as biological control agents. Most *B. fusca* pathogens are active during specific periods within a maize production cycle and are subsequently linked to specific larval densities and or developmental phases. Other agents might also be involved as is the case with *Serratia marcescens* Bizio, where infections as closely related to parasitism (Kfir, 1991).

2.17 The use of plant resistance for stalk borer control

2.17.1 Breeding efforts

Plant breeding for resistance to South African maize insects is a much neglected field of research. Even a limited degree of resistance in commercial maize hybrids could be of considerable economic benefit to both commercial maize producers and subsistence farmers (van Rensburg & Malan, 1990).

Although there were early attempts to develop maize cultivars resistant to *B. fusca* (du Plessis & Lea, 1943; Ingram, 1958; Walters, 1974) it is only within recent years that concerted efforts have been made. Lack of purposeful resistance breeding programmes is to be attributed to insufficient mass rearing techniques for the insect, rather than to a lack of potential sources of resistance. Differential resistance in maize inbred lines to *B. fusca* has been reported by Barrow (1985; 1987) and to both *B. fusca* and the sorghum borer, ***C.** partellus* (Swinhoe) by Fourie (1984), while 50 commercial maize hybrids were shown to display significant differences in tolerance to attack by *B. fusca*.

A physiological mechanism that is responsible for the expression of leaf-feeding resistance against the European corn borer, *Ostrinia nubilalis* (Hübner) was described by Robinson *et al.* (1978). This mechanism is based on the presence of a glycoside, 2-0-glucosyl-4-hydroxy-1, 4-benzoxazin-3-one in undamaged maize leaves that is hydrolysed through enzyme action to the aglucone, 2,4-dihydroxy-7methoxy-2H-1, 4-benzoxazin-3(4H)-one (DIMBOA). Genotypes with DIMBOA-related resistance seem to be less promising as sources of resistance to *B. fusca,* as was also reported from resistance studies with *C. partellus* (Ampofo & Saxena, 1985). This resistance was attributed to non-preference by larvae (Robinson *et al.,* 1978) and was shown to contribute to failure of newly hatched larvae of *C. partellus* to reach the whorls of plants. The presence of DIMBOA in plant tissue which is biologically active against *O. nubilalis* larvae, elicited foraging behaviour and resulted in larvae moving away from plants with this trait (non-preference behaviour) (Robinson *et al.,* 1978). DIMBOA is not the only mechanism of resistance affecting 0. *nubilalis,* since several exotic maize varieties with low DIMBOA-content were found to be highly resistant to this borer species. Van Rensburg & Malan (1990) reported that DIMBOA is not effective against *B. fusca*.

A major handicap in breeding for resistance to *B. fusca* has been the lack of efficient screening techniques. Most previous studies had been conducted under natural infestations where several species of stem borers may infest the same crop. Several attempts were made in South Africa to rear *B. fusca* artificially on meridic diets, but these were unsuccessful due to poor survival of first-instar larvae. Van Rensburg & van Rensburg (1993) found that the successful establishment of a colony is dependent on the collection of healthy, vigorous larvae. Larvae parasitized by *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) show characteristic sclerotic patches on the body wall after several weeks in diapause. Parasitised larvae can thus be recognised and immediately discarded if collections are made during August (in the Southern Hemisphere). Allowing for a normal mortality rate due to parasitism and diseases, a collection of 50 000 diapause larvae will, on average, facilitate the artificial infestation of at least 20 000 plants. However, when natural mortality is minimal, and by keeping moths at controlled temperature and humidity, the number of plants can be doubled (van Rensburg & van Rensburg, 1993).

High level resistance to *B. fusca* was first recorded in Mississippi maize lines Mp705, Mp706 and Mp707 (van Rensburg & Malan, 1990), developed for resistance to the fall armyworm *S. frugiperda* and the southwestern corn borer *D. grandiosella* (Williams & Davis, 1989; Davis, 1989). Later evaluation of exotic breeding material identified resistance to *B. fusca* in genotypes from CIMMYT Mexico (van Rensburg & van den Berg, 1995), these being derived from a similar Carribean population as the Mississippi material. The only significant resistance from an unrelated genetic background was recorded in maize lines from Georgia, USA (van Rensburg, 1998), developed as sources of resistance to the corn earworm, *Heliothis zea* (Boddie) (Widstrom *et al.*, 1993). It is possible that further sources of resistance can be obtained from the gene pool with known resistance to *D. grandiosella* and *S. frugiperda*. Interest has subsequently focused on the Mp inbreds due to their possible value in a local resistance breeding programme (van Rensburg & Gevers, 1993).

The genetics of resistance to insects in maize has been investigated for only five insect species. Most studies indicated that resistance is quantitatively inherited (Khush & Brar, 1991). In genotypes closely related to the Mp inbreds, resistance to the south-western corn borer *D. grandiosella* and the fall armyworm *S. frugiperda* was shown to be additively inherited when based on assessments of leaf-feeding damage, whereas both additive genetic effects and dominance were significant when it was based on assessments of larval growth rate (Scott & Davis, 1978; Williams *et al.*, 1978; Williams & Davis, 1989). Selection for resistance to insects in maize is practiced routinely by breeders but is seldom the primary trait in the selection process. When resistance to insects is the primary selection trait, progress is usually made at the expense of other agronomic traits (Widstrom *et al.*, 1993).

Due to poor local adaptation, particularly to drought conditions, the Mississippi and Mexico genotypes could not be used directly in hybrid combinations (van Rensburg, 1997). This necessitated a recurrent selection programme for introgression of resistance (Figure 3) into locally adapted, susceptible breeding material. Van Rensburg and Gevers (1993), and Andre et al. (2003) reported on inheritance of resistance. Until the initiation of this study, resistant genotypes from both Mississippi and Mexico were used indiscriminately as donor parents (van Rensburg & Klopper, 2004).

Figure 3. Difference in the incidence of leaf feeding damage and stunting between a resistant selection (left) and a susceptible standard (right) (van Rensburg & Klopper, 2004).

Due to the resistance being additively inherited, various levels of resistance can be obtained by selective crossing of resistant and susceptible parents in three-way hybrid combinations (van Rensburg, 1997). This may serve to reduce yield losses considerably. A theoretical resistance level of only 25% may potentially increase the economic threshold for chemical control to such an extent that insecticide use can be reduced significantly.

2.17.2 Genetically modified maize

Using modern DNA technology, transgenes derived from the entomopathogenic bacterium *Bacillus thuringiensis* Berliner have been used to modify maize plants (Bt-maize). These genes encode for the production of proteins toxic to Lepidoptera larvae (Venette *et al.*, 2000). The prerequisites for susceptibility in an insect are the presence of the right type of proteolitic enzymes as well as a pH of 9 to 10.5 in the intestine (du Toit, 1995).

This means that toxic proteins produced by *B. thuringiensis* are highly specific in their action and will not be harmful to natural enemies or to humans if ingested (van Rensburg, 2007). Prior to 1994, microbial preparations of *B. thuringiensis,* applied as spray formulations, had been in use for decades without substantial resistance developing in field populations of insects (Tabashnik, 1994). The diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) was the only insect to eventually develop resistance to Bt applied as a biopesticide (Ferre & Van Rie, 2002). However, five of 10 species of moths representing the families Noctuidae, Plutellidae and Pyralidae, selected with Bt under laboratory conditions, developed more than a 10-fold resistance, suggesting that the ability to evolve resistance to Bt is a common phenomenon among the Lepidoptera (Tabashnik, 1994). Recent laboratory studies have shown that this ability to develop resistance to Bt applies to some major agricultural pest species, including the European corn borer, *O. nubilalis* (Chaufaux *et al.,* 2001), the pink bollworm, *Pectinophora gossypiela* (Saunders) (Tabashnik *et al.,* 2002) and the bollworm *Helicoverpa armigera* (Hubner) (Tabashnik *et al.,* 2003).

More than 62 million ha of genetically engineered Bt crops were planted worldwide between 1996 and 2002, and some pest populations are thus considered to be under pronounced selection pressure to evolve resistance (Tabashnik *et al.,* 2003). Resistance monitoring of field populations of the bollworm *H. armigera* in China, *0. nubilalis* and *H. zea* in the United States, in regions with high adoption of Bt-crops has however not as yet detected increases in resistance frequency (Wu *et al.,* 2002; Tabashnik *et al.,* 2003; Stodola *et al.,* 2006). This is attributed to the high-dosage/refuge strategy adopted in the USA to delay pest resistance to Bt-crops, which involves the use of refuges of non-Bt host plants to ensure survival of susceptible individuals (Tabashnik *et al.,* 2003).

During the testing of experimental Bt-events for control of the South African stem borer complex, which commenced in 1994, *B. fusca* was shown to be more tolerant to a specific event than the spotted stem borer *C. partellus* (van Rensburg, 1999). The first Bt-transgenic hybrids all employed the event Mon810 and were grown on approximately 50 000 ha during the 1998/99 growing season. No leaf feeding damage was observed during the vegetative growth stages in any of the plantings. At harvest of the 1999 crop, diapause larvae and damage to the lower stems were noticed at a number of localities, involving various hybrids. Subsequent studies indicated a window of approximately 14 days in the period immediately before and after tasseling in which relatively late infestations may result in increased larval survival on Bt-maize (van Rensburg, 2001). This was attributed to larvae feeding on the silks, of which high water content contributes to a reduced concentration of the Bt-protein, allowing survival of some larvae until completion of the first two instars, and subsequent successful penetration of the stems. During the 2004/ 05 season severe damage to Bt-plantings was caused by *B. fusca* during the vegetative stages of plant development, which was eventually attributed to the development of insect resistance to the Bt-toxin (van Rensburg, 2007).

The possibility that locally developed inbred lines with conventional resistance could be deployed in crosses with Bt-transgenic material as a measure to prevent stem borer populations from developing resistance to the Bt-toxin, deserves further investigation.

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CHAPTER 3

OPTIMISING DONOR SELECTION FOR IMPROVEMENT OF SUSCEPTIBLE BREEDING MATERIAL

Introduction

Van Rensburg & Malan (1990) found that some Mississippi inbred lines derived from the Caribbean population Antigua group 2 that had been developed for multiple resistance to the south-western corn borer, *Diatraea grandiosella* (Dyar) and the fall armyworm *Spodoptera frugiperda* (Smith) (Davis, 1989) expressed high levels of resistance to *B. fusca* (van Rensburg & Malan, 1990). Some CIMMYT breeding material developed in Mexico for subtropical regions also appeared to offer promise against *B. fusca* from the antibiosis viewpoint (van Rensburg, 1998). Since these resistance sources are not adapted to South African conditions (van Rensburg, 1996; 1997), the resistance had to be introgressed into locally adapted breeding material. The resistance in the Mississippi lines was shown to be additively inherited with low dominance (van Rensburg & Gevers, 1993). Using Mississippi and CIMMYT inbred lines as donor parents in a recurrent selection programme, locally adapted inbred lines with high levels of resistance were eventually released (van Rensburg & Klopper, 2004). From this work, it appeared that combining ability for resistance differs between heterotic groups, and that susceptible elite material does not necessarily respond similarly in crosses with sources of resistance from different genetic backgrounds.

The development of conventional resistance was eclipsed worldwide by advances in DNA technology, providing transgenes encoding for *Bacillus thuringiensis* toxins (Bt maize). Deployed commercially for the first time in South Africa during 1998, the use of Bt-hybrids increased to 35% of the national crop in 2006 (James, 2006). In South Africa this culminated in the development of insect resistance to the Cry l Ab toxin (van Rensburg, 2007), which warrants further investigation into conventional resistance sources and the possibility of using conventional resistance in combination with GMO technology to obtain durable plant resistance. The cost of Bt seed is also prohibitive to the small farming community for whom conventional resistance could still present a viable option. The objective of this study was to determine which combinations of resistant sources with local elite susceptible material could provide the best expression of resistance.

Material and Methods

A first cycle of crossing involved various combinations of susceptible and resistant inbred lines. The inbred lines and their derivations are provided in Table 1 and the resultant crosses in Table 2. To facilitate interpretation crosses are shown with resistant parents listed in the first position. Note that this does not necessarily correspond with the direction of crossing. Five susceptible and six resistant lines were crossed in all possible combinations to obtain 30 semi-resistant single crosses with various resistance levels. To serve as resistant standards, various resistant lines were crossed to obtain four resistant crosses. Four susceptible standards were obtained by selective crossing of six susceptible lines. Although closely related and equally resistant to *B. fusca*, three Mississippi inbreds (Mp designation) were included as resistant parents, based on their resistance to leaf feeding damage and assuming that different susceptible genotypes do not benefit equally from the same resistant donor. Other resistant sources were from Mexico (CML 139) and Tifton, Georgia (GT designation), the latter chosen for resistance to ear damage rather than to leaf feeding damage. The inbred lines I137TN and F2834T are representative of the major South African heterotic groups I and F respectively, which are prominent in local yellow maize breeding programmes. R2565Y is a modern derivative of I137TN. The other inbred lines constitute elite material of USA origin representing the two heteroticgroups Reid and Lancaster. The crosses were made at the Burgershall experiment station near Hazyview, a frost-free area in the Mpumalanga Lowveld, during the winter of 2004.

The second cycle of crosses comprised the above 38 single crosses (Table 2) that were crossed selectively in various combinations with the intention to obtain different resistance levels in various genetic backgrounds, providing a total of 431 double crosses. For evaluation purposes two commercial maize hybrids were included as susceptible standards. Evaluation for resistance comprised two field experiments (one each for single and double crosses) conducted over two seasons at Potchefstroom (46

43'S, 27 06'E) in the Northwest Province of South Africa. The trial lay-out was randomsied block designs with three replications per genotype during each season. Each plot consisted of three 5m rows with an inter-row spacing of 1.5m to prevent larval movement between rows. Trials were planted by hand during mid-November with two seeds sown per hill. Plants were thinned by hand to ensure a uniform plant population of 20 plants per 5m. Fertilizer (3:2:1) was broadcast at a rate of 200 kg / ha before planting. Irrigation was applied immediately after planting and during flowering to ensure proper emergence and pollination. Plants were otherwise grown under dry land conditions with the exception that 25mm of irrigation was applied when plant stress was noticed.

Neonate larvae of *B. fusca* used for artificial infestation were obtained from a laboratory colony derived from winter collected diapause larvae in accordance with methods described by Van Rensburg and Van Rensburg (1993). Two rows per plot were artificially infested with 10 neonate larvae per plant four weeks after emergence, using a 'bazooka' dispenser (Wiseman, Davis & Campbell, 1980). The remaining row was treated twice at two-weekly intervals with endosulfan 35 ec at a rate of 227g a.i.ha^{-1} to prevent possible natural infestation. Three weeks after artificial infestation all plants in one infested row were rated on a scale of 1-4 for leaf feeding injury (1 = undamaged; 4 = severely damaged). Frequencies of plants in each category of damage were used to calculate reciprocal indices of attack (R.I.A.) for each genotype (Hanuss, Stelzer, Nietzke, Steudel, Warmbrun & Weigans, 1968) using the model R.I.A. = (f1 + 0.8 f2 + 0.5 f3 + 0.2 f4)/ (f1 + f2 + f3 + f4) where f1 to f4 = frequencies of plants in each category. Despite moderate stand losses experienced with some entries, at least 12 plants per genotype were available for leaf damage ratings. Plants in one infested row were dissected and the numbers of surviving larvae recorded. The total number of surviving larvae was divided by the number of plants sampled per row to obtain the mean number of larvae per plant.

At harvest plants of infested rows were split longitudinally to record the number of damaged internodes. The total number of damaged internodes per row was divided by the number of plants sampled per row to obtain the mean number of damaged internodes per plant. Ear numbers were recorded. The number of damaged ears was divided by the total number of ears per row to obtain the mean percentage damaged ears. Ears were also rated for damage on a scale of 1-4 (as for leaf feeding damage above). Plant height (a sensitive measure of susceptibility to internal damage) of both infested and uninfested rows was recorded, measuring from ground level to the base of the flag leaf. Plant height reduction in terms of the uninfested row was calculated. Grain yield data were recorded separately for infested and uninfested rows. Yield data were converted to t.ha-1 and adjusted to the standard of 12.5% moisture content. Yield loss in terms of the uninfested rows was calculated.

The data for single crosses (trial 1) were subjected to factorial analyses, using genotypes as factor 1 and infestation as factor 2. Data pertaining to double crosses (trial 2) were subjected to principal component analysis (PCA), assessing the importance of the different variables measured to explain multivariate variation (Upadhyaya et al., 2004). PC analyses were performed using Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998).

Results and Discussion

Single crosses

The factorial analyses pertaining to each of the eight variables measured for single crosses are provided in Table 3. Based on main effects, infestation had highly significant effects on those variables related to the vegetative stages of plant development viz. incidence of leaf feeding damage, larval survival and the incidence of stem damage. With the exception of ear damage, infestation did not have significant effects on any variable relating to the reproductive stages of plant development. In contrast entries as main effect differed significantly with regard to those variables relating to the reproductive stages of plant development viz. incidence of ear damage, levels of ear damage, ear numbers, plant height and grain yield. No entry differences were recorded for variables relating to the vegetative stages. Furthermore, none of the interactions was significant, indicating that entries responded similarly to infestation. The result is difficult to explain in view of the historical background of the resistant parents. With the exception of GT-FAW that had been developed as a source of resistance to ear damage the Mississippi and CIMMYT material are all sources of resistance to leaf feeding damage, which should have resulted in crosses being more responsive to damage caused during the vegetative stages.

Entries as main effect is provided in Table 4. Note that some variables (plant height, ear numbers and grain yield) when presented as main effects are indicative of heterotic patterns rather than resistance levels. For these variables interactions (differences between infested and uninfested) are more meaningful. For the other variables resistant cross Mp706 x Mp704 provided the highest level of resistance when based on larval survival and the incidence of damaged ears whereas the resistant cross CML139 x Mp706 provided the highest resistance when based on internal stem damage. For crosses between resistant and susceptible parents the highest levels of resistance were obtained with Mp706, Mp707 and CML139, all of which seemed to combine favorably with susceptible parents I137TN, F2834T and Mo17. The cross I137TN x Mp706 rated notably high (compared to resistant crosses) for most variables. In general the Georgia lines, GT-FAW in particular, did not provide for particularly high levels of resistance. As expected susceptible crosses, e.g. B73 x F2834T and F2834T x I137TN tended to rank lowest in resistance. However, some exceptions occurred, e.g. Mo17 x B73 showed a low incidence of damaged ears. Infestation as main effect is provided in Table 5. Based on confidence intervals infestation had a significant effect for all eight variables measured, despite non-significant F-values indicated for some variables in the factorial analyses (Table 3). Interaction data on the eight variables are provided in Table 6. Note that the uninfested category is not meaningful for some variables due to zero figures, whereas for others only the difference between infested and uninfested is meaningful. The interaction table is therefore presented showing only the infested category, except for ear numbers, plant height and grain yield where the reductions in terms of the uninfested were calculated as percentages.

With regard to the vegetative stages of plant development, only three entries showed a pronounced reduction in the incidence of leaf feeding damage viz. the resistant cross I137TN x Mo706 as well as crosses Mp706 x I137TN and Mp704 x R2565Y. Most damage was observed for two susceptible standards and two crosses involving the GT-lines. Only limited differences in larval survival were observed between entries, with one susceptible cross and two semi-resistant crosses showing increased larval survival. The data on the incidence of stem damage showed some inconsistencies. Reduced injury occurred in all crosses involving Mp704 and Mp706 as well as in two resistant crosses. However, less damage was recorded for two of the four susceptible standards.

The results pertaining to the reproductive stages of plant development were clouded by discrepancies. Reduced ear damage was recorded for three resistant crosses and all crosses involving Mp 704 and Mp706. However, reduced ear damage also occurred in two of the four susceptible standards. Similarly, levels of ear damage as indicated by R.I. A. were notably reduced in only one of the four resistant crosses whereas ear numbers were not significantly reduced in two of the four susceptible standards. It should be noted however, that ear injury was not particularly severe due to the relatively early time of infestation. With late infestation (closer to tasseling) injury levels in susceptible genotypes may approach values as low as 0.2 (Van Rensburg, 1998). Plant height reduction is a sensitive measure of genotype susceptibility. However, in this study plant height was not significantly reduced in three of the four susceptible standards whereas the highest reductions were recorded with crosses involving Mp706 and CML 139. Similarly yield losses caused by infestation were notably high in two resistant crosses but in only one suscesptible cross.

Double crosses

Principle component analysis for double crosses is provided in Table 7. Principle component 1 (PC1) declared 32.89% of the variation and PC2 24.07% of the remaining variation. Plant height reduction and the incidence of damaged internodes were identified as the variables providing the highest correlations. A plot of PC2 on PC1 is provided in Fig. 1. Quadrant A presents low plant height reduction in combination with low stem damage, therefore denoting the most resistant entries. Quadrant B presents a combination of high plant height reduction and low stem damage, therefore denoting intermediate resistance. The most susceptible entries are indicated by quadrant C, presenting a combination of high plant height reduction and high stem damage. Entries of similar resistance classification should therefore group together. However, in this study no cluster occurred and most entries (including the susceptible standards) tended to centre around the origin.

Conclusions

In single crosses between resistant and susceptible breeding material, resistant lines Mp704 and Mp706, particularly the combination Mp706 x I137TN seemed to provide particularly high levels of resistance. However, the study did not successfully identify which resistance source should be used in combination with which heterotic group, neither did resistance derived from different sources (eg. Mississippi and Georgia) seem to compliment each other to the effect that increased resistance levels were obtained if resistance to leaf feeding damage and ear damage were combined.

With double crosses no pattern could be observed that indicated crosses comprising more than one resistant parent to be more resistant than those in which only one resistant parent featured, whereas variance in resistance did not seem to relate to heterotic patterns. Van Rensburg (1997) successfully obtained different levels of resistance in three-way hybrid combinations. It is possible that a more comprehensive result could have been obtained in this study if evaluations were based on either three-way hybrids or on segregating progenies as was suggested by Andre *et al.* 2003.

Table 3.1 Genotypes used in single crosses and their derivations

Genotype	**Derivation**	**Resistance designation**
I137TN	Teko Yellow x Natal Yellow Horsetooth	S
R2565Y	Capture of I137TN x L16 backcross	S
B73	Iowa Stiff Stalk Synthetic	S
Mo17	187-2 x C103	S
F2834T	Teko Yellow	S
D940Y*	[B060W(A166N x B556Y - B559Y)] B557Y	S
Mp706	Mp SWCB-4(1)	R
Mp707	Mp SWCB-4(1)	R
Mp 704	Mp SWCB-4(1)	R
CML139	Antigua Gp2	R
GT-FAW	Mexican, Caribbean & Brazilian background	R
GT115	DSSynA (C3)	R

*Used in one susceptible cross only

Table 3.2 Single crosses evaluated in this study

No	**Entry**	**No**	**Entry**
	Resistant x Susceptible		**Resistant x Susceptible**
1	Mp706 x I137TN	21	Mp707 x I137TN
2	Mp706 x R2565Y	22	Mp707 x R2565Y
3	Mp706 x B73	23	Mp707 x B73
4	Mp706 x Mo17	24	Mp707 x Mo17
5	Mp706 x F2834T	25	Mp707 x F2834T
6	Mp704 x I137TN	26	GT115 x I137TN
7	Mp704 x R2565Y	27	GT115 x R2565Y
8	Mp704 x B73	28	GT115 x B73
9	Mp704 x Mo17	29	GT115 x Mo17
10	Mp704 x F2834T	30	GT115 x F2834T
11	CML139 x I137TN		**Resistant x Resistant**
12	CML139 x R2565Y	31	Mp704 x MP706
13	CML139 x B73	32	Mp707 x CML139
14	CML139 x Mo17	33	GT115 x Mp704
15	CML139 x F2834T	34	CML139 x Mp706
16	GT-FAW x I137TN		**Susceptible x Susceptible**
17	GT-FAW x R2565Y	35	F2834T x I137TN
18	GT-FAW x B73	36	Mo17 x B73
19	GT-FAW x Mo17	37	D940Y x R2565Y
20	GT-FAW x F2834T	38	B73 x F2834T

Table 3.3 Factorial analyses for each of eight variables, single crosses, trial 1.

Variable	**Source**	**F**	**P**
Leaf feeding damage (R.I.A.)	Entries	1.35	0.1092
	Infestation	374.62	<0.0001
	Interaction	1.35	0.1092
Surviving larvae / plant	Entries	0.93	0.5889
	Infestation	78.72	<0.0001
	Interaction	0.93	0.5889
Ear damage (R.I.A.)	Entries	1.70	0.0140
	Infestation	6.90	0.0095
	Interaction	1.05	0.3996
Numbers of ears / plant	Entries	1.67	0.0171
	Infestation	0.06	0.8082
	Interaction	0.83	0.7404
Numbers of damaged internodes / plant	Entries	1.27	0.1579
	Infestation	318.57	<0.0001
	Interaction	1.27	0.1579
Damaged ears (%)	Entries	1.45	0.0640
	Infestation	2.13	0.1463
	Interaction	0.82	0.7569
Plant height (cm)	Entries	6.24	<0.0001
	Infestation	0.66	0.4176
	Interaction	0.66	0.9266
Grain yield (t ha^{-1})	Entries	1.72	0.0125
	Infestation	0.11	0.7400
	Interaction	1.07	0.3804

Table 3.4 Entries as main effect (averaged over infestation levels)

Entries	**RIA leaves**	**Surv larvae**	**Dam intern**	**Dam ears**	**RIA ears**	**Ear numbers**	**Plant height**	**Grain yield**
Mp706 x I137TN	0.965a	0.018c	0.232b	4.844c	0.988a	1.382b	197.23b	6.640a
Mp706 x R2565Y	0.938a	0.038b	0.338b	8.377b	0.981a	1.464b	179.85b	6.683a
Mp706 x B73	0.897b	0.042b	0.416b	5.288c	0.989a	1.213b	197.11b	5.951a
Mp706 x Mo17	0.929a	0.009c	0.277b	3.936c	0.992a	1.225b	161.08bc	5.375ab
Mp706 x F2834T	0.896b	0.008c	0.269b	5.663c	0.989a	1.230b	187.22b	4.586b
Mp704 x I137TN	0.935a	0.049b	0.324b	4.543c	0.991a	1.583b	197.98a	5.682a
Mp704 x R2565Y	0.971a	0.043b	0.352b	10.56b	0.966a	1.576b	176.72b	5.541a
Mp704 x B73	0.892b	0.024b	0.443b	4.285c	0.990a	1.660b	199.98a	6.395a
Mp704 x Mo17	0.903b	0.027b	0.449b	3.813c	0.992a	1.548b	191.47b	5.060b
Mp704 x F2834T	0.915b	0.033b	0.446b	8.266b	0.983a	1.322b	185.70b	4.585b
CML139 x I137TN	0.915b	0.116a	0.571ab	15.982a	0.961a	1.208b	134.40c	3.611b
CML139 x R2565Y	0.934a	0.038b	0.467b	9.572b	0.981a	1.703a	195.53b	6.313a
CML139 x B73	0.892b	0.014c	0.313b	6.167c	0.988a	1.849a	185.59b	6.861a
CML139 x Mo17	0.926a	0.037b	0.231b	11.220b	0.975a	1.516b	186.55b	5.648a
CML139 x F2834T	0.934a	0.084a	0.619a	5.098c	0.975a	1.416b	197.43b	6.020a
GT-FAW x I137TN	0.902b	0.055b	0.438b	10.888b	0.969b	1.465b	202.18a	6.142a
GT-FAW x R2565Y	0.901b	0.061a	0.756a	7.672b	0.979a	1.543b	213.22a	6.081a
GT-FAW x B73	0.899b	0.035b	0.645a	8.880b	0.980a	1.350b	182.47b	5.214b
GT-FAW x Mo17	0.827b	0.073a	0.698a	15.133a	0.954b	1.459b	171.32b	5.435a
GT-FAW x F2834T	0.922a	0.028b	0.315b	8.747b	0.980a	1.210b	189.21b	4.797b
Mp707 x I137TN	0.915b	0.014c	0.422b	8.514b	0.978a	1.286b	157.28bc	4.764b
Mp707 x R2565Y	0.915b	0.052b	0.806a	13.066b	0.959b	1.303b	187.43b	5.883a
Mp707 x B73	0.903b	0.001c	0.671a	18.445a	0.947b	1.979a	147.11bc	4.340b
Mp707 x Mo17	0.903b	0.040b	0.407b	13.643b	0.969b	1.299b	176.93b	4.253b
Mp707 x F2834T	0.914b	0.036b	0.452b	7.051b	0.984a	1.343b	159.66bc	4.992b
GT115 x I137TN	0.911b	0.044b	0.359b	6.160c	0.982a	1.373b	152.90bc	4.831b
GT115 x R2565Y	0.871b	0.011c	0.446b	12.538a	0.971b	1.567b	189.62b	5.291b
GT115 x B73	0.887b	0.022c	0.414b	4.733c	0.991a	1.480b	180.42b	5.361b
GT115 x Mo17	0.881b	0.020c	0.574ab	5.447c	0.985a	1.355b	163.94b	4.786b
GT115 x F2834T	0.901b	0.010c	0.512b	17.562a	0.955c	1.393b	165.44b	4.675b
Mp704 x MP706	0.930a	0.056b	0.328b	3.000c	0.994a	1.138c	140.75bc	2.843c
Mp707 x CML139	0.920b	0.042b	0.525b	11.657b	0.969b	1.667ab	149.43bc	5.339a/b
GT115 x Mp704	0.926a	0.072a	0.893a	15.327a	0.966b	1.579b	153.88bc	4.667b
CML139 x Mp706	0.983a	0.016c	0.031c	6.845c	0.955b	1.231b	161.67bc	5.148b
F2834T x I137TN	0.862b	0.095a	0.841a	7.425b	0.983a	1.412b	195.58b	5.797a
Mo17 x B73	0.889b	0.046b	0.461b	3.666c	0.978a	1.415b	189.07b	5.775a
D940Y x R2565Y	0.911b	0.072a	0.501b	14.351b	0.958b	1.686ab	204.81a	6.650a
B73 x F2834T	0.799c	0.019c	0.480b	14.405b	0.968b	1.321b	153.12c	4.838b

Means within columns followed by different letters differ at P=0.05 according to confidence intervals

Table 3.5 Infestation as main effect (averaged over entries)

Variable	**Infested**	**Not infested**
Surviving larvae / plant	0.078a	0.001b
RIA ears	0.817a	1.0b
Damaged internodes / plant	0.932a	0.001b
Damaged ears (%)	9.873a	8.168b
RIA ears	0.972a	0.983b
Ear number / plant	1.435a	1.447b
Plant height (cm)	176.871a	178.985b
Grain yield (t ha^{-1})	5.374a	5.303b

Means within columns followed by different letters differ at P=0.05 according to confidence intervals

Table 3.6 Interactions (shown for uninfested only or as % reductions in terms of the uninfested sub-treatment).

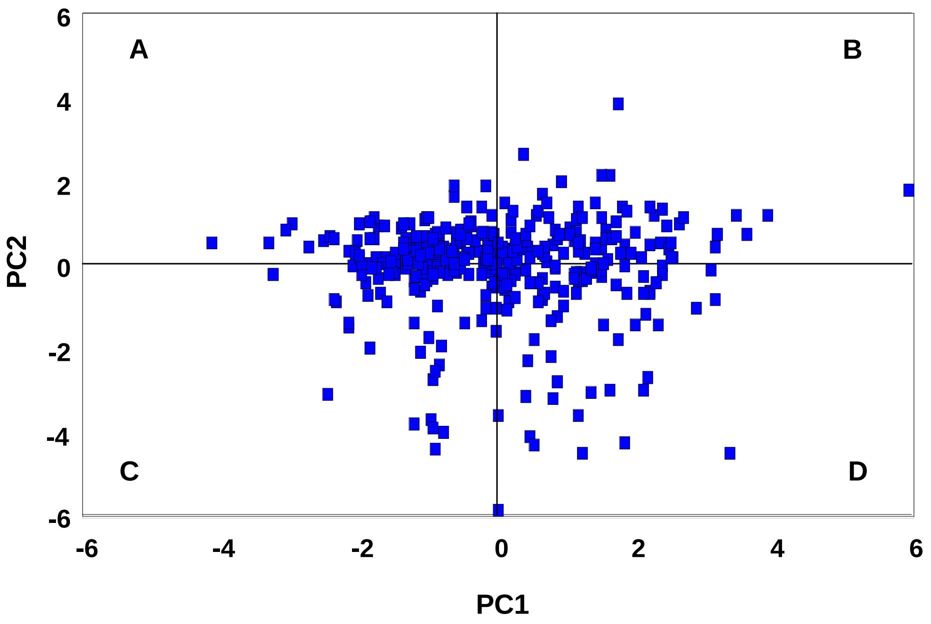
	Entries	**Surv larvae**	**RIA leaves**	**Dam Intern**	**Dam Ears**	**RIA ears**	**Ear no red (%)**	**P height red (%)**	**Y loss (%)**
1	Mp706 x I137TN	0.035a	0.930a	0.463c	6.790c	0.982a	8.9b	0.0c	11.1a
2	Mp706 x R2565Y	0.073a	0.876b	0.675c	8.931c	0.978b	16.8a	7.7a	22.6a
3	Mp706 x B73	0.085a	0.795b	0.832c	7.576c	0.985a	0.0c	0.0c	8.6b
4	Mp 706 x Mo17	0.017a	0.859b	0.553c	5.911c	0.988a	0.0c	2.7b	15.1a
5	Mp706 x F2834T	0.015a	0.793b	0.538c	3.548c	0.993a	0.0c	17.2a	0.0c
6	Mp704 xI137TN	0.097a	0.870b	0.647c	5.159c	0.990a	0.0c	0.0c	0.0c
7	Mp704 x R2565Y	0.085a	0.942a	0.701c	10.809c	0.966c	0.0c	0.0c	0.5c
8	Mp704 x B73	0.047a	0.784b	0.885c	3.448c	0.990a	0.0c	0.0c	6.3b
9	Mp704 x Mo17	0.053a	0.806b	0.897c	1.149c	0.998a	9.4b	2.4b	13.9a
10	Mp704 x F2834T	0.066a	0.830b	0.891c	11.597c	0.977b	0.0c	2.0b	1.9c
11	CML139 x I137TN	0.231b	0.829b	1.142b	19.048a	0.948c	17.7a	13.4a	28.3a
13	CML139 x R2565Y	0.075a	0.868b	0.929c	10.974c	0.978b	18.2a	0.0c	12.6a
14	CML139 x B73	0.026a	0.784b	0.625c	8.333c	0.983b	1.6c	8.5a	0.0c
15	CML139 x Mo17	0.073a	0.852b	0.460c	2.737c	0.990b	0.0c	1.0b	0.0c
12	CML139 x F2834T	0.167b	0.868b	1.236b	3.252c	0.984b	0.0c	0.8b	0.0c
16	GT-FAW x I137TN	0.109a	0.805b	0.874c	9.843c	0.969c	7.1b	4.7b	0.0c
17	GT-FAW x R2565Y	0.123a	0.802b	1.512b	10.313c	0.967b	0.0c	7.7a	0.0c
18	GT-FAW x B73	0.069a	0.798b	1.290b	8.712c	0.978b	0.0c	9.7a	4.0b
19	GT-FAW x Mo17	0.145a	0.655c	1.396b	23.355a	0.922c	0.0c	4.7b	8.6b
20	GT-FAW x F2834T	0.054a	0.844b	0.630c	5.673c	0.984b	0.0c	2.4b	3.8c
25	Mp707 x I137TN	0.026a	0.829b	0.843c	3.846c	0.992b	0.0c	3.3b	0.0c
23	Mp707 x R2565Y	0.102a	0.829b	1.610a	15.825b	0.942c	1.5c	0.0c	0.0c
24	Mp707 x B73	0.001a	0.806b	1.341b	19.583a	0.937c	20.9a	0.0c	0.0c
22	Mp707 x Mo17	0.079a	0.807b	0.813c	12.000b	0.968c	0.0c	2.9b	0.0c
21	Mp707 x F2834T	0.071a	0.828b	0.903c	6.189c	0.985b	0.0c	0.0c	19.0a
30	GT115 x I137TN	0.088a	0.822b	0.717c	7.692c	0.977b	0.0c	2.5b	0.0c
28	GT115 x R2565Y	0.020a	0.742c	0.890c	16.149b	0.963c	5.0b	9.4a	7.7b
29	GT115 x B73	0.042a	0.775b	0.826c	3.262c	0.993a	8.7b	0.0c	17.5a
27	GT115 x Mo17	0.040a	0.761b	1.148c	6.944c	0.977b	0.0c	0.8c	0.0c
26	GT115 x F2834T	0.019a	0.802b	1.022b	20.439a	0.952c	9.8b	1.9b	5.2b
31	Mp704 x MP706	0.110a	0.860b	0.656c	2.667c	0.995a	25.8a	2.7b	32.5a
32	Mp707 x CML139	0.084a	0.840b	1.049b	7.953c	0.952c	18.9a	0.0c	24.2a
33	GT115 x Mp704	0.142a	0.852b	1.785a	13.232a	0.974c	27.5a	0.0c	15.9a
34	CML139 x Mp706	0.031a	0.967a	0.061c	9.524c	0.924c	0.0c	0.0c	0.0c
35	F2834T x I137TN	0.188b	0.723c	1.681a	14.850c	0.966b	21.4a	0.0c	31.0a
36	MO17 x B73	0.091a	0.777b	0.920c	4.624c	0.977b	0.0c	0.7c	0.0c
37	D940Y x R2565Y	0.144a	0.823b	1.001b	12.906b	0.960c	16.0a	0.0c	7.7b
38	B73 x F2834T	0.038a	0.598c	0.959c	20.318a	0.983c	0.0c	5.6a	1.6c

Means within columns followed by different letters differ at P=0.05 according to confidence intervals

Table 3.7 Principal component analysis of five variables pertaining to 431 double crosses.

Latent roots	**1**	**2**	**3**	**4**	**5**
	1.973	1.444	1.031	0.692	0.610
Percentage Variation	**1**	**2**	**3**	**4**	**5**
	32.89	24.07	17.18	11.53	10.17
Latent Vectors	**1**	**2**	**3**	**4**	**5**
R.I.A. leaves	-0.4142	0.3813	0.2395	0.3826	-0.6896
Damaged internodes	0.6206	0.1686	0.1395	-0.0471	-0.3173
R.I.A. ears	0.2091	-0.5492	-0.2785	0.7518	-0.1092
Yield loss	-0.0179	-0.2382	0.8982	0.2073	0.3019
Pl height reduction	-0.0676	-0.6570	0.1259	-0.4932	-0.5446
Correlation matrix	**R.I.A. leaves**	**D internodes**	**R.I.A. ears**	**Yield loss**	**Pl height**
R.I.A. leaves	1.000				
Dam internodes	-0.269	1.000			
R.I.A. ears	-0.297	0.080	1.000		
Yield loss	0.032	-0.008	0.011	1.000	
Pl height reduction	-0.175	-0.118	0.237	0.173	1.000
Principal components	**P height**	**R.I.A. leaves**	**D internodes**	**R.I.A. ears**	**Yield loss**
Pc1	-0.582	0.872	-0.025	-0.095	0.294
Pc2	0.458	0.203	-0.286	-0.790	-0.660
Pc3	0.243	0.142	0.912	0.128	-0.283

Figure 3.1 The plot of PC2 on PC1 for five variables in 431 double crosses



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CHAPTER 4

THE EFFECT OF PLANTING DATE ON GENOTYPE BY ENVIRONMENT INTERACTION FOR THE EXPRESSION OF STEM BORER RESISTANCE

Introduction

From earlier work conducted under greenhouse conditions (van Rensburg *et al.*, 1995; van Rensburg, 1998), it was observed that larval developmental rate differed at various times of the year. Greenhouse evaluations conducted at different times of the season in which only photoperiod varied indicated that larvae developed faster during a declining photoperiod than during an increasing photoperiod. This was never further investigated, but similar observations were made elsewhere (Personal communication, CIMMYT, Mexico). The question arose as to what extent variation in planting date (a common occurrence in South-Africa) could affect the expression of resistance. The possibility exists that resistance may break down under conditions of decreasing photoperiod, which presents practical consequences during years when late spring rains result in late planting. This became of particular importance in recent years due to the availability of so-called super short season hybrids which extends planting dates to as late as mid-January for the western parts of the production area. This study investigated the effect of variation in planting date on the expression of resistance.

Material and methods

A field trial consisting of five planting date treatments *viz.* 15 October, 30 October, 13 November, 27 November and 11 December was conducted at Potchefstroom (46°43'S, 27°06'E, North-West Province, South Africa) during the 2002/2003 planting season. The lines, hybrid combinations and designated resistance levels are provided in Table 4.1. The entries comprised of three resistant and three susceptible lines as well as a susceptible hybrid and two 50% resistant single cross hybrids.

Table 4.1 Experimental lines and maize hybrid combinations derived from crosses between insect-susceptible (S) and insect-resistant (R) inbred lines

Entries	**Resistance designation**
Mp706	R
Mp704	R
CML139	R
P6528	S
I137TN	S
P28	S
I137TN x Mp706	SR
I137TN x CML139	SR
B73 x I137TN	SS

The trial was conducted under dry-land conditions except for irrigation applications made directly after sowing and during tasseling. The five plantings were done in the same field using a randomised complete block design with three replications per genotype for each planting. Plots consisted of three rows 10m long with 1.5m row spacing to avoid lateral movement of larvae between rows. The trial was planted by hand with two kernels per hill and thinned after emergence to obtain a uniform stand of 20 plants per 10m. A 2m space separated planting dates.

Neonate larvae of *B. fusca* used for artificial infestation were obtained from a laboratory colony derived from winter collected diapause larvae in accordance with methods described by van Rensburg and van Rensburg (1993). Plants in two rows per plot were artificially infested with 10 to 12 neonate larvae per plant five weeks after plant emergence, which approximates early season levels of natural infestation. One row per plot served as an un-infested control, kept free of natural infestation with insecticide (beta-cyfluthrin) applied at fortnightly intervals during the vegetative stages of plant development.

Leaf feeding damage was evaluated 14 days after infestation on a scale of 1-4 where 1 = no damage and 4 = severe damage. From the frequencies of plants in each category reciprocal indices of attack (Hanuss *et al.*, 1968) were calculated for each genotype.

Plants in one row per plot were dissected. Numbers of surviving larvae and larval mass were recorded.

Plant height (a sensitive measure of plant susceptibility to internal damage) was recorded at harvest, measured from ground level to the base of the flag leaf. From these measurements the percentage plant height reduction was calculated. Plants were dissected and the incidence of internal damage and ear damage recorded. Grain yield was determined for all plants in both infested and uninfested rows. Yield data were adjusted to the standard of 12.5% moisture content before conversion to t ha^{-1}.

Data on leaf feeding damage, larval survival, larval mass and plant height reduction were subjected to factorial analyses, using planting date as factor 1 and genotypes as factor 2. Factorial analysis pertaining to data on internal plant damage, ear damage and grain yield involved infestation (infested vs. not infested) as third factor.

Results and discussion

Data on leaf feeding damage are provided in Table 4.2. The resistant cross I137TN x Mp706 and the two resistant Mp lines suffered significantly less damage than the other entries. There was a tendency for later plantings to suffer less damage, but entries did not respond equally, as indicated by the significant interaction.

Data on larval survival are provided in Table 4.3. Significant differences were observed with regard to both planting date and entries. The two resistant Mp lines and the resistant crosses showed lower larval survival, with a tendency for less larvae to survive in later plantings. The interaction was not significant, indicating that entries responded similarly. The response was more pronounced with regard to larval mass gain (Table 4.4). The three resistant lines and both resistant crosses provided for much reduced larval mass but all entries responded similarly to planting date as indicated by the non-significant interaction. Larval mass increased dramatically in the last planting. Plant height reduction (Table 4.5) was in accordance with data on larval survival and mass gain with regard to both genotype differences and planting date. Data confirm less damage with later planting, but without a clear pattern. Genotypes responded similarly to planting date.

Data on internal plant damage are provided in Table 4.6. All three main effects were highly significant, with resistant entries consistently suffering less damage than the susceptible entries. Entries responded similarly to planting date (interaction not significant) but differently to infestation (as expected). Infestation resulted in significantly different levels of damage occurring in different plantings (interaction significant), with the two latest plantings suffering significantly less damage.

The incidence of ear damage is provided in Table 4.7. In contrast to stem damage, ear damage was more pronounced with later planting date. Entries responded similarly to planting date, but not to infestation. The resistant cross I137TN x Mp706 suffered an unexpectedly high incidence of ear damage in comparison to the resistant lines. Note that ear damage would have had a more pronounced effect on yield than stem damage (known response).

Data on grain yield are provided in Table 4.8. The observed relationship between yield and planting date is a known response for the area, with mid-November being optimal and later planting as a rule providing inferior yields. The value of plant resistance in combination with good combining ability for yield is indicated by the superior yield performance of I137TN x Mp706 compared to the susceptible cross B73 x I137TN, despite the former hybrid suffering a higher incidence of ear damage.

A further result was obtained by calculating yield losses in terms of the un-infested sub-treatments (Table 4.9). Since the yield performance of inbred lines is not meaningful in relation to that of crosses, these should be ignored since a small quantitative loss reflects as a relatively large percentage. Based on crosses only, losses seemed to decrease with planting date until late November. The increase with later planting was possibly due to a breakdown of resistance where direct ear damage is concerned. This applies to both resistant and susceptible crosses as reflected in the non-significant interaction.

It is concluded that the expression of resistance was significantly affected by planting date. The incidence of leaf feeding damage, internal plant damage, larval survival and plant height reduction decreased with an increase in planting date, whereas larval mass and the incidence of ear damage increased with later planting. The latter is a common occurrence in commercial plantings, until now attributed to increased infestation at late plant growth stages derived from the greater magnitude of the second seasonal moth flight (van Rensburg *et al*., 1985; 1987). From this study it appears that increased ear damage with later planting may result at least in part from an increase in larval developmental rate as affected by a decreasing photoperiod. The result was, however, not of an order to be regarded as a breakdown in plant resistance since the resistant cross still managed to out-yield its susceptible counterpart whereas resistant and susceptible genotypes responded similarly to planting date.

Table 4.2 Incidence of leaf feeding damage 14 days after artificial infestation as indicated by Reciprocal Indices of Attack (less damage is closer to unity)

Entries	**Planting date**					**Mean***
	15 Oct	**30 Oct**	**14 Nov**	**28 Nov**	**12 Dec**	
Mp706	0.54	0.68	0.56	0.88	0.65	0.66 ab
Mp704	0.55	0.76	0.65	0.89	0.70	0.71 a
CML139	0.52	0.60	0.56	0.53	0.54	0.55 c
P6528	0.52	0.50	0.53	0.76	0.64	0.59 b
I137TN	0.53	0.53	0.50	0.56	0.64	0.55 c
P28	0.50	0.49	0.41	0.46	0.59	0.49 d
I137TN x Mp706	0.59	0.82	0.60	0.68	0.79	0.70 a
I137TN x CML139	0.51	0.68	0.54	0.56	0.80	0.62 b
B73 x I137TN	0.60	0.61	0.54	0.54	0.69	0.59 b
Mean*	0.54 c	0.63 b	0.54 c	0.65 ab	0.67 a	
Source	**F**	**P**				
Planting date (a)	9.57	<0.0001				
Entries (b)	7.43	<0.0001				
Interaction (a x b)	1.82	0.0150				

**Means followed by different letters differ significantly at P=0.05 according to confidence intervals*

Table 4.3 Surviving larvae per plant as recorded 14 days after artificial infestation

Entries	**Planting date**					**Mean***
	15 Oct	**30 Oct**	**14 Nov**	**28 Nov**	**12 Dec**	
Mp706	2.00	1.70	2.02	0.55	0.78	1.41 d
Mp704	1.13	1.27	1.80	0.38	0.36	0.99 d
CML139	2.00	1.58	1.77	1.73	2.18	1.85 c
P6528	3.36	2.47	2.39	0.93	1.33	2.09 c
I137TN	5.24	4.73	4.91	2.34	3.62	4.17 a
P28	3.90	6.00	6.23	3.68	4.10	4.78 a
I137TN x Mp706	1.27	1.27	3.56	0.72	1.62	1.68 c
I137TN x CML139	2.73	2.33	2.28	1.26	1.57	2.03 c
B73 x I137TN	3.15	3.42	5.05	1.12	2.22	2.99 b
Mean*	2.75 b	2.75 b	3.33 a	1.41 c	1.97 c	
Source	**F**	**P**				
Planting date (a)	5.71	0.0004				
Entries (b)	9.19	<0.0001				
Interaction (a x b)	0.47	0.9906				

**Means followed by different letters differ significantly at P=0.05 according to confidence intervals*

Table 4.4. Mean larval mass plant^{-1} (mg) as recorded 14 days after artificial infestation

Entries	**Planting date**					**Mean***
	15 Oct	**30 Oct**	**14 Nov**	**28 Nov**	**12 Dec**	
Mp706	1.41	0.80	1.95	0.25	3.05	1.49 c
Mp704	0.70	0.42	1.44	0.13	0.87	0.71 c
CML139	1.41	0.54	1.77	0.76	7.9	2.48 c
P6528	4.33	1.09	2.47	0.44	18.79	5.42 b
I137TN	9.32	3.25	10.85	1.75	24.76	9.99 a
P28	7.13	3.96	15.35	2.19	29.12	11.55 a
I137TN x Mp706	0.85	0.49	4.25	1.46	6.64	2.74 c
I137TN x CML139	2.11	0.86	2.48	0.66	8.59	2.94 c
B73 x I137TN	4.16	1.76	7.41	0.79	15.20	5.87 b
Mean*	3.49bc	1.47 c	5.33 b	0.94 c	12.77 a	
Source	**F**	**P**				
Planting date (a)	6.38	<0.0001				
Entries (b)	2.22	0.0330				
Interaction (a x b)	0.46	0.9922				

**Means followed by different letters differ significantly at P=0.05 according to confidence intervals*

Table 4.5 Plant height reduction (%) as recorded at harvest

Entries	**Planting date**					**Mean***
	15 Oct	**30 Oct**	**14 Nov**	**28 Nov**	**12 Dec**	
Mp706	13.8	0.7	13.8	6.6	8.9	8.8 de
Mp704	11.4	1.0	2.6	3.7	1.1	3.9 e
CML139	12.8	1.8	6.0	5.7	16.7	8.6 de
P6528	17.5	23.7	11.1	3.5	15.0	14.2 cd
I137TN	27.2	25.8	30.6	18.5	23.8	25.2 b
P28	41.9	17.0	44.7	23.4	26.2	30.6 a
I137TN x Mp706	11.8	1.2	5.6	6.4	8.3	6.7 de
I137TN x CML139	15.9	5.4	8.5	7.4	11.9	9.8 d
B73 x I137TN	15.9	15.9	21.2	15.6	15.9	16.9 c
Mean*	18.7 a	10.3 c	16.0 ab	10.1 c	14.2 b	
Source	**F**	**P**				
Planting date (a)	3.84	0.0064				
Entries (b)	12.37	<0.0001				
Interaction (a x b)	0.86	0.6820				

**Means followed by different letters differ significantly at P=0.05 according to confidence intervals*

Table 4.6. Damaged internodes per plant as recorded at harvest

Entries	**Planting date**					**Mean***
	15 Oct	**30 Oct**	**14 Nov**	**28 Nov**	**12 Dec**	
Mp706	1.7	0.9	0.9	0.8	1.1	1.07 b
Mp704	1.2	0.8	0.7	0.5	0.6	0.78 a
CML139	0.9	0.9	0.9	0.9	1.0	0.94 a
P6528	2.1	1.6	2.1	0.7	0.8	1.46 d
I137TN	2.3	2.3	2.7	1.8	1.4	2.11 e
P28	2.5	2.3	2.1	1.9	1.5	2.06 e
I137TN x Mp706	1.4	1.0	1.2	1.2	1.3	1.23 b
I137TN x CML139	1.5	1.0	0.9	1.2	0.9	1.13 b
B73 x I137TN	1.7	1.3	1.7	0.9	1.1	1.36 c
Mean*	1.73 a	1.35 b	1.46 b	1.11 c	1.09 c	
Source	**F**	**P**				
Planting date (a)	6.20	0.0001				
Entries (b)	10.52	<0.0001				
Infestation (c)	580.79	<0.0001				
Interaction (a x b)	0.88	0.6539				
Interaction (a x c)	3.10	0.0170				
Interaction (b x c)	5.39	<0.0001				

**Means followed by different letters differ significantly at P=0.05 according to confidence intervals*

Table 4.7. Incidence of damaged ears (%) in lines and hybrids

Entries	**Planting date**					**Mean***
	15 Oct	**30 Oct**	**14 Nov**	**28 Nov**	**12 Dec**	
Mp706	0.8	2.4	2.9	16.1	4.8	5.42 b
Mp704	0.7	0.0	3.0	0.0	3.6	1.45 a
CML139	3.9	3.3	1.3	2.1	1.7	2.46a
P6528	6.7	4.1	1.7	3.7	6.5	4.54 b
I137TN	7.1	6.4	5.5	15.9	5.5	8.08 b
P28	4.9	1.8	6.1	8.0	8.3	5.82 b
I137TN x Mp706	8.7	13.9	5.3	7.8	15.6	10.28 c
I137TN x CML139	8.1	8.2	4.9	13.7	8.2	8.64 c
B73 x I137TN	4.4	6.4	10.4	8.7	13.4	8.67 c
Mean*	5.01 a	5.18 a	4.56 a	8.45 b	7.52 b	
Source	**F**	**P**				
Planting date (a)	2.64	0.0353				
Entries (b)	3.81	0.0004				
Infestation (c)	88.08	<0.0001				
Interaction (a x b)	0.91	0.6107				
Interaction (a x c)	2.55	0.0407				
Interaction (b x c)	3.40	0.0012				

**Means followed by different letters differ significantly at P=0.05 according to confidence intervals*

Table 4.8. Grain yield of lines and hybrids (t ha^{-1})

Entries	**Planting date**					**Mean***
	15 Oct	**30 Oct**	**14 Nov**	**28 Nov**	**12 Dec**	
Mp706	1.670	1.456	1.540	1.360	0.646	1.334 b
Mp704	0.703	0.669	0.992	0.550	0.254	0.634 a
CML139	1.423	0.499	0.977	0.737	0.407	0.809 a
P6528	0.933	1.031	1.871	1.002	1.193	1.206 b
I137TN	2.166	2.94	1.681	2.295	2.420	2.300 c
P28	1.155	1.058	1.749	1.312	0.440	1.143 ab
I137TN x Mp706	7.108	7.752	8.680	6.280	4.800	6.924 f
I137TN x CML139	5.492	5.065	5.186	4.441	1.812	4.399 e
B73 x I137TN	3.508	3.495	3.265	3.471	1.240	2.996 d
Mean	2.684 a	2.663 a	2.882 a	2.383 b	1.468 c	
Source	**F**	**P**				
Planting date (a)	17.37	<0.0001				
Entries (b)	133.64	<0.0001				
Infestation (c)	5.78	0.0173				
Interaction (a x b)	2.75	<0.0001				
Interaction (a x c)	2.83	0.0261				
Interaction (b x c)	2.88	0.0049				

**Means followed by different letters differ significantly at P=0.05 according to confidence intervals*

Table 4.9 Yield losses (%) in terms of un-infested sub-treatments

Entries	**Planting date**					**Mean***
	15 Oct	**30 Oct**	**14 Nov**	**28 Nov**	**12 Dec**	
Mp706	38.9	42.5	14.3	0	14.5	22.0
Mp704	24.8	0	20.7	0	32.2	15.5
CML139	52.4	0	22.2	29.1	59.4	32.6
P6528	18.7	34.9	54.8	8.1	45.7	32.4
I137TN	44.2	46.9	36.6	19.0	33.6	36.1
P28	61.7	45.6	94.2	20.5	75.9	59.6
I137TN x Mp706	8.44	1.4	0.0	0.0	23.5	6.7
I137TN x CML139	19.5	0.0	0.0	0.0	39.3	13.9
B73 x I137TN	27.3	17.0	25.6	10.5	34.4	20.9
Mean*	32.9	20.9	29.9	9.7	39.8	

Source	**F**	**P**
Planting date (a)	6.12	0.0002
Entries (b)	6.21	<0.0001
Interaction (a x b)	1.08	0.3774

**Means followed by different letters differ significantly at P=0.05 according to confidence intervals*

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CHAPTER 5

GENERAL CONCLUSIONS

Due to the polygenic nature of conventional plant resistance to stem borers, it seems feasible that it can be combined with GMO technology to avoid that insect populations become resistant to the Bt-toxin deployed in GM maize. Combining ability for resistance possibly differs between heterotic groups, and susceptible elite material does not necessarily respond similarly in crosses with sources of resistance from different genetic backgrounds. Although this study confirmed high levels of resistance in some resistance sources previously used in a resistance breeding programme, neither single crosses nor double crosses between resistant and susceptible genotypes could identify which resistance source should be used in combination with which heterotic group, despite the use of eight measures of stem borer injuriousness and a large number of cross combinations. Future efforts should rather be based on evaluations of resistance levels in segregating progenies of crosses following one cycle of self pollination.

From earlier work conducted under greenhouse conditions it was observed that larval development rate possibly differs with difference in time of the year. This was confirmed in the present study. The expression of resistance was significantly affected by planting date. The incidence of damage to the vegetative stages of plant development decreased with an increase in planting date, whereas larval mass and the incidence of ear damage increased with later planting. Until now this has been attributed to increased infestation at late plant growth stages derived from the greater magnitude of the second seasonal moth flight. From this study it appears that increased ear damage with later planting may result at least in part from an increase in larval developmental rate as affected by a decreasing photoperiod. The result has application in resistance assessments. It indicates that evaluations of ear damage should be regarded as high priority for genotypes with short season growing requirements since these will necessarily be subject to late planting and a consequent increase in levels of infestation.

The resistant cross I137TN x Mp706 and the two resistant Mp lines suffered significantly less damage than the other entries. There was a tendency for later plantings to suffer less damage, but entries did not respond equally, as indicated by the significant interaction. The value of plant resistance in combination with good combining ability for yield is indicated by the superior yield performance of I137TN x Mp706 compared to the susceptible cross B73 x I137TN, despite the former hybrid suffering a higher incidence of ear damage. Based on crosses only, yield losses seemed to decrease with planting date until late November. The increase with later planting was possibly due to a breakdown of resistance where direct ear damage is concerned. This applies to both resistant and susceptible crosses as reflected in the non-significant interaction.

CHAPTER 6

SUMMARY

Since conventional sources of resistance to stem borers are not adapted to South African conditions, resistance has to be introgressed into locally adapted breeding material. Combining ability for resistance possibly differs between heterotic groups, and susceptible elite material do not necessarily respond similarly in crosses with sources of resistance from different genetic backgrounds. From earlier work conducted under greenhouse conditions it was observed that larval development rate possibly differs with difference in time of the year. The objective of this study was to determine which combinations of resistant sources with local elite susceptible material could provide the best expression of resistance. Susceptible and resistant lines were crossed in various combinations to obtain semi-resistant single and double crosses with various resistance levels. These were compared to susceptible and resistant standards in field trials. Plants were artificially infested with neonate larvae. Evaluations of leaf feeding damage, stem damage, ear damage, plant height reduction and grain yield were subjected to factorial analysis, using planting date and infestation as factors. Two resistant lines and one semi-resistant single cross were identified that seemed to provide particularly high levels of resistance. However, the study did not successfully identify which resistance source should be used in combination with which heterotic group, neither did resistance derived from different sources seem to compliment each other. Using principal component analysis of data on double crosses, no pattern could be observed that indicated crosses comprising more than one resistant parent to be more resistant than those in which only one resistant parent featured, whereas variance in resistance did not seem to relate to heterotic patterns. A further aspect of the study involved investigation into the effect of variance in planting date on the expression of resistance. Various lines and hybrid combinations with different resistance levels were evaluated in a field trial comprising five planting date treatments and artificial infestation of plants. Data were subjected to factorial analysis as above. The expression of resistance was significantly affected by planting date. The incidence of leaf feeding damage, internal plant damage, larval survival and plant height reduction decreased with an increase in planting date, whereas larval mass and the incidence of ear damage increased with later planting. It appears that increased ear damage with later planting may result at least in part from an increase in larval development rate as affected by a decreasing photoperiod.

Keywords: *Busseola fusca*, maize, plant resistance, stem borers

OPSOMMING

Konvensionele bronne van weerstand teen mieliestamruspers is nie aangepas vir Suid-Afrikaanse toestande nie en moet dus geintegreer word in plaaslik aangepaste teel-materiaal. Kombineervermoë vir weerstand verskil moontlik tussen heterotiese groepe, en vatbare elite materiaal reageer nie noodwendig eenders in kruisings met bronne van weerstand van verskillende genetiese agtergronde nie. Uit vroeëre werk wat onder glashuistoestande gedoen is, is bevind dat die larwale ontwikkelingstempo moontlik verskil met verskille in die tyd van die jaar. Die doel van die studie was om te bepaal watter kombinasies van weerstandsbronne met plaaslike elite vatbare materiaal die beste moontlike bestandheid kan lewer. Vatbare en bestande lyne is gekruis in verskillende kombinasies om semiweerstandbiedende enkel- en dubbelkruisings met verskillende weerstandsvlakke te verkry. Die kruisings is in veld-proewe vergelyk met vatbare en bestande standaarde. Plante is kunsmatig besmet met pasuitgebroeide larwes. Evaluasies van blaarbeskadiging, stamskade, kopskade, planthoogteverskille en graanopbrengs is aan faktoriaalontledings onderwerp waar plantdatum en infestasie as faktore gebruik is. Twee bestande lyne en een semi-bestande enkelkruis is geiidentifiseer wat hoë vlakke van weerstand gelewer het. Die studie het egter nie suksesvol geiidentifiseer watter weerstandsbronne in watter kombinasies met verskillende heterotiese groepe gebruik moet word nie. Dit het ook nie kon bevestig dat weerstandsbronne uit veskillende agtergronde mekaar komplimenteer nie. Hoofkomponent analise van die data uit dubbelkruisings het geen weerstandspatrone kon aantoon nie en kruisings waarin meer as een bestande ouer gebruik is, het nie hoër weerstand gehad as kruisings waarin slegs een ouer gebruik is nie. Variasie in weerstand het ook nie met heterotiese patrone verband gehou nie. ‘n Verdere aspek van die studie het gehandel oor die effek van variasie in plantdatum op die uitdrukking van weerstand. Verskeie lyne en basterkombinasies met verskillende weerstandsvlakke is ge-evalueer in ‘n veld-proef bestaande uit vyf plantdatum-behandelings en kunsmatige besmetting van plante. Data is aan faktoriaalanalise soos hierbo onderwerp. Weerstandsuitdrukking was betekenisvol deur plantdatumverskille beiinvloed. Die insidensie van blaarbeskadiging, inwendige plantskade, larwale oorlewing en planthoogteverskille het afgeneem met ‘n toename in plantdatum, terwyl larwale massa en die insidensie van kopbeskadiging toegeneem het met latere plantdatum. Dit wil voorkom asof toenemende kopskade met latere plantdatum minstens ten dele deur ‘n toename in larwale ontwikkelingstempo in reaksie op ‘n afnemende fotoperiode veroorsaak word.

Sleutelwoorde: *Busseola fusca*, mielies, plantweerstand, stronkboorders