PARASITOIDS AND APHID RESISTANT PLANTS: PROSPECTS FOR *DIURAPHIS NOXIA* (KURDJUMOV) CONTROL

by

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INTRODUCTION AND LITERATURE REVIEW

1.1   The Russian wheat aphid.

1.1.1   Origin of the Russian wheat aphid

The Russian wheat aphid (RWA), *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae) is endemic to the southern parts of Russia and the Iranian-Turkestanian mountain range where it is found on wild and cultivated grasses including wheat and barley (Kovalev *et al.,* 1991). It is also widely distributed from the Mediterranean, to the Middle East and Central Asia (Starý, 1996). It was detected in South Africa for the first time during 1978 (Dürr, 1983) and also spread to Mexico, the USA and Canada between 1980 and 1986 (Kovalev *et al*., 1991).

The outbreak of RWA in South Africa and other countries probably resulted from the spread of an aggressive biotype of the aphid, which is controlled by stabilising selection causing it only to become sporadically a conspicuous pest in its native areas (Kovalev *et al*., 1991). The absence of successful natural enemies in South Africa is probably another prime reason for population explosions occurring regularly in South Africa (Aalbersberg *et al*., 1989). The RWA spread rapidly through the country and became the most serious insect pest of dryland wheat in the summer rainfall region of the country, especially the Free State Province (Du Toit & Walters, 1984; Du Toit, 1986).

The Free State Province is the largest wheat production region in South Africa, contributing between 40 and 50% of the total wheat production in normal years (Marasas *et al.,* 1997).   Included in this province are two areas where small-scale farmers are situated namely Qwaqwa and Thaba Nchu. Maize and wheat are the predominant crops grown by these farmers and account for 90% of the cultivated area on their farms (Marasas *et al.,* 1997).

1.1.2 *Pest status and chemical control*

RWA feeding damage caused yield losses of 80 to 90% on susceptible cultivars not treated with chemical insecticides (Aalbersberg, 1987). Damage assessment studies were conducted and economic injury levels and economic thresholds were determined for use in chemical control of RWA, but farmers tend to spray insecticides routinely (Du Toit, 1986; 1990). Between 1980 and 1990 commercial farmers in the Free State sprayed up to four times annually, costing them about R23/ha for one application, which represented between 7 and 8% of the wheat price per ton at that stage (Du Toit, 1986). Currently one application cost at least R105/ha which represent between 9 and 10 % of the wheat price per ton (Tolmay *et al.*, 2000). The insecticides registered for the control of RWA are all broad-spectrum systemic organophosphates (LD$_{50}$ 2-70mg/kg) (Nel *et al.*, 2002) also killing natural enemies that attack the RWA.

The dependence on chemical insecticides has led to a high frequency of insecticide resistance in some crop systems (Thomas & Waage, 1996). Aphids are also able to develop resistance to insecticides and in the USA the green bug *Schizaphis graminum* (Rondani), attacking both wheat and sorghum, is known to be resistant to organophosphates (Teetes *et al.,* 1975; Siegfried & Ono, 1993). The possible development of insecticide resistance could therefore not be ignored when farmers are spraying RWA on a routine basis. The financial resources and management skills required to ensure economically viable RWA management are high, which is important particular for small-scale farmers in the Free State. In these low input agriculture systems in Qwaqwa and Thaba Nchu financing, necessary equipment, and know-how are not readily available. The use of insecticides is very limited and these farmers suffered severe losses (Marasas *et al.,* 1997).

1.2 Alternative control options

The Agricultural Research Council – Small Grain Institute (ARC-SGI) started with an investigation into the development of a more sustainable alternative control programme for RWA during the early 1980's. Several alternative control methods

like host plant resistance, biological control, attractants and repellents, trap crop barriers and intercropping are available for the control of insect pests in different crops (Kumar, 1984; Thomas & Waage, 1996). In the context of sustainable pest management for RWA, host plant resistance and biological control seem to be the most suitable alternative control methods.

1.2.1 Host plant resistance (HPR)

Several wheat lines, which are resistant to RWA, were identified (Du Toit, 1987; Harvey & Martin, 1990; Smith *et al.*, 1991). A breeding programme to incorporate resistance into good quality bread wheat cultivars was also started. Most successes in breeding of resistant cultivars came from three different sources of resistance PI 137739, PI 262660 and PI 294994 (Tolmay & Van Deventer, 2005). The resistant genes contained in these sources were named as Dn1, Dn2 and Dn5 respectively (Tolmay *et al*., 2006). The ARC-SGI and other seed companies have released many cultivars containing different levels of HPR to *D. noxia.* Approximately 13 cultivars are currently available to farmers in the Free State (Anonymous, 2005) and therefore more than one cultivar is containing the same resistant gene. More than 70% of the wheat farmers in the eastern parts of the Free State are currently planting these effective resistant cultivars and the number of insecticide treatment decreased by approximately 35% between 1990 and 1996 (Marasas *et al*., 1997).

A problem associated with plant resistance breeding has been the tendency for the development of resistance-breaking biotypes (Gould *et al.,* 1990; Stoner, 1996; Thomas & Waage, 1996; Porter *et al.,* 1997). Classical examples of resistance breakdown following large-scale release of resistant cultivars include the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), and green leafhopper, *Nephotettix virescens* (Uhler) (Homoptera: Deltocephalidae) on rice as well as the hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae) and green bug, *S. graminum* on wheat (Thomas & Waage, 1996). As recently as 2003 a resistance breaking biotype of *D. noxia* was reported from Colorado (Haley *et al*., 2004). Except for *S. graminum* and *D. noxia,* six aphid species not feeding on cereals, are also known to have overcome plant resistance (Stoner, 1996).

Because *D. noxia* from various parts of the world differ in their reaction to resistant wheat lines (Puterka *et al*., 1993), the possibility of *D. noxia* to develop a resistant breaking biotype in South Africa cannot be ruled out. Monitoring for biotypes should be considered.

1.2.2 Biological control using natural enemies

Biological control of aphids using natural enemies seems to be limited to a few cases (Van Lenteren, 1991). The RWA, however, seems to be a pest that could be controlled through classical biological control as defined by De Bach (1974). From the distribution of *D. noxia* (Kovalev *et al*., 1991), it is clear that this aphid typically invaded a new area without its effective natural enemies and became a pest. Therefore, it could be controlled by the introduction of natural enemies from the countries of origin of the pest. Although several natural enemies, including ladybirds and parasitoids, attack RWA in SA they are not effective in protecting the susceptible cultivars from damage (Aalbersberg *et al*., 1988). Therefore introduction of natural enemies was started in 1980. Between 1980 and 1994 six natural enemy species were introduced and released (Table 1.1). Two species namely *Adalia bipunctata* (L.) and *Aphidius matricariae* Haliday become established, although not seen regularly on aphid populations on wheat (G. J. Prinsloo, unpublished data).

The parasitoid *Aphelinus hordei* (Kurdjumov) tend to have a narrow host range during laboratory studies (Prinsloo, 2000) and was therefore mass reared and released in the eastern parts of the Free State (Prinsloo, 1998). Mass release of this parasitoid occurred in the wheat growing seasons (September – October) between 1993 and 1994 (Prinsloo, 1998) and each subsequent year until 1999 (G.J. Prinsloo, unpublished data). They have established each year at the release sites, but establishment between seasons could not be confirmed in the wheat fields of the Free State (G. J. Prinsloo, unpublished data).

Table 1.1   Natural enemies introduced and released between 1980 and 1994
for the control of *Diuraphis noxia* in South Africa.

Natural enemy species	Origin	Year imported	Established	Reference
Predators				
Coleoptera: Coccinellidae				
Adalia bipunctata	United Kingdom	1980	Yes	Aalbersberg *et al.,* 1984
Hippodamia convergens	United Kingdom	1980	No	Aalbersberg *et al.,* 1984
Coleomigilla maculata	United Kingdom	1980	No	Aalbersberg *et al.,* 1984
Diptera: Chamaemyiidae				
Leucopis ninae	Pakistan Iran, China	1994	No	Hatting, 1995
Parasitoids				
Hymenoptera: Aphidiidae; Aphelinidae				
Aphidius matricariae	Turkey	1988	Yes	Marassas *et al.*, 1997
Aphelinus hordei	Ukraine	1991	Yes?	Prinsloo & Neser, 1994; Prinsloo, 1998

1.3   Interaction between trophic levels and control methods

According to Price *et al.*, (1980) the study on insect-plant interactions could not
progress realistic without considering the third trophic level as part of a plant's
defence mechanisms against herbivores.   Interaction is present between each
consecutive trophic level (Price, 1986).   Members of the lower trophic level evolve
to reduce feeding by their enemies, while members of the higher trophic level
evolve to increase consumption.   As a result effective attack at each level of
interaction occurs.   An important feature of this trophic system is that members of
alternate levels may act in a mutualistic manner.   Natural enemies of herbivores
may benefit the plants by reducing the herbivore abundance, while plants may
benefit the herbivores by making the herbivores more vulnerable in some way to
the natural enemies (Price, 1986).

For aphids the obvious and traditional order of interaction between the three trophic levels would be the plant A affecting the aphid A in some way, which then has an effect on the natural enemies (Van Emden & Wratten, 1991). There are, however, also examples of at least three other systems: (1) Plant species A can directly influence the natural enemies (2) Plant species B together with an associated aphid species B may affect natural enemies of aphid species A on plant species A (3) Alternatively, plant species B can affect the natural enemy directly or through the aphid population A (Van Emden & Wratten, 1991). It is therefore essential in the integration of plant resistance breeding and biological control to be aware of the influence plants may have on the trophic system. Price (1986) identified three main categories of factors that mediate tritrophic interactions: 1) semiochemically mediated interactions, 2) chemically mediated interactions and 3) physically mediated interactions.

1) Semiochemicals (chemicals that mediate interactions between organisms) (Nordlund *et al*., 1981) are known to play a major role as cues to aid natural enemies in locating and recognising their hosts or prey (Vinson, 1976; Nordlund *et al*., 1981; Vet & Dicke, 1992; Vinson *et al.,* 1998). These chemical cues are divided into two groups: (1) those that are volatile and act at a long distance to attract searching parasitoids and predators and (2) those which are generally non-volatile and act as contact cues, often inducing an arrestment response. It has been demonstrated that parasitoids (including those attacking aphids) use specific stimuli emitted by plants after herbivore damage (herbivore induced synomones) to identify their host habitat (Vet & Dicke, 1992).

2) Plant chemical factors can influence the higher trophic levels in several ways (Price, 1986). Plant resistance and nutrients can influence growth rate and size of herbivores and in turn influence the attack by natural enemies. The survival of herbivores and the numbers available for attack by natural enemies is also influenced. Some herbivores are able to sequester plant allelochemicals in their

haemolymph and thereby alter their suitability for natural enemies (Thomas & Waage, 1996).

3) Plant morphological features can alter the availability of herbivores to natural enemies (Price, 1986). Defence structures such as trichomes and cuticle thickness directly affect natural enemies, while plant architecture can influence the dispersion of herbivores and subsequently searching by natural enemies (Thomas & Waage, 1996).

Parasitoids have evolved behaviours to enable them to find hosts, including the ability to detect chemical signals (Vet & Dicke, 1992). It has been demonstrated that parasitoids can detect specific volatile chemicals released by plants in response to insect feeding (reviewed by Cortesero *et al*., 2000; Dicke, 2000). It is possible that certain plants, or varieties of plants, naturally produce chemicals that attract parasitoids, even when they are not damaged by insect feeding. As part of an integrated system to control stem borers (Lepidoptera: Noctuidae) in Kenya, Khan *et al*. (1997) showed that molasses grass releases a volatile that attracts the stem borer parasitoid *Cotesia sesamiae* Cameron, even when the plant is not infested by the stem borer itself. Aphid parasitoids are known to use feeding-induced signals in host finding (Du *et al*., 1996; 1998). They can also discriminate between different blends of chemicals released by the same plant in response to feeding by different aphid species (Du *et al*., 1996).

1.4 Integration of plant resistance and natural enemies for Russian wheat aphid control.

Tritrophic studies strongly indicated that the application of both HPR and biological control to a particular pest could give significantly better or worse results than expected from the effects of each individual factor (Thomas & Waage, 1996). Host plant resistance and biological control increasingly attracted attention as alternatives for chemical pest control (Thomas & Waage, 1996). There are several reasons why these two methods could to be used together in the context of sustainable pest management in developing countries, namely:

a)   Self–renewing nature: In theory both methods represent self-renewing processes. In the case of HPR the control itself is built into the seed, while in the case of biological control, it is present in the crop environment provided that establishment of the introduced natural enemies occurred. In both cases control could extend between pest generations over cropping seasons. Potentially it can last as long as plant resistance persists in the crop line and the natural enemies stay in the agro-ecosystem. This could fit very well into a commercial farming system, by decreasing input costs. It could also be applied in the small-scale farmer situation, where farmers don't have skills or input costs to control the pest problem (Thomas & Waage, 1996).

b)   Suitability to low input farming: Many farmers in developing countries do not have the resources to buy, and/or skills to apply such pest-control measures as pesticides (Thomas & Waage, 1996). Such farmers are present in the Qwaqwa and Thaba Nchu areas (Marasas *et al.,* 1997). When natural enemies have established in the environment, biological control is present and free to the farmer, while the cost of HPR is included in the seed itself. Therefore, HPR and biological control are suited for low-input insect pest control systems and both commercial farmers and small-scale farmers will benefit from it (Thomas & Waage, 1996).

c)   Ecological evidence for tritrophic effects: The integration of these two methods deserves consideration on the basis of increasing evidence that natural enemies are influenced by properties of the plants that pests attack (Vet & Dicke, 1992; Thomas & Waage, 1996; Chamberlain *et al.*, 2000; Cortesero *et al.*, 2000). Much of this evidence comes from basic behavioural and ecological studies on tritrophic relationships among insects in non-agriculture systems (Van Emden & Wratten, 1991; Vet & Dicke, 1992). This strongly indicates that the application of HPR and biological control to particular pests could give results significantly better than expected from the effects of each factor on its own.

The use of both host plant resistance and biological control seemed to be the most suitable alternative control methods for sustainable control of RWA. If natural enemies are successfully attracted to the resistant plants, the aphids still feeding on these plants will be controlled effectively and therefore diminish the chances for the development of a resistance breaking biotype. If plants, however, repel natural enemies, the feeding aphids are free from natural enemy attack and chances therefore increase for a resistance breaking biotype to develop.

Current resistance breeding and evaluation procedures for different crops (including RWA resistance breeding in South Africa) do not examine the direct or indirect effects of HPR on the third trophic level (Thomas & Waage, 1996). This means that positive or negative interactions between HPR and biological control are not identified. As a result the effects of semiochemicals, sequestration of plant chemicals, or direct physical interactions between host plants and natural enemies are ignored. It is therefore possible that wide scale deployment of resistant varieties could occur that actively interfere with natural enemies, reducing the benefits gained from resistance breeding. These phenomena may have long-term consequences for the persistence of certain key natural enemy species (Thomas & Waage, 1996).

As mentioned above, resistant host plants not only have an effect on the pest aphids feeding on them, but this effect is also passed through the aphid to their natural enemies (Van Emden, 1991; 1995; Feuntes-Contreras *et al.*, 1996; Verkerk *et al.*, 1998). Chemicals such as alkaloids, which are involved in plant resistance, can be toxic to parasitoids developing within hosts or prove to be toxic to aphid predators such as ladybirds and hover fly larvae (Herzog & Funderburk, 1985). These disadvantages, however, are not apparent at low levels of plant resistance. There is potential for more beneficial interactions between biological control agents and partial plant resistance, suggesting that there is a disadvantage in seeking a level of plant resistance greater than necessary when other restraints (natural enemies) are present in a pest management system (Van Emden, 1991).

The compatibility of RWA resistance with natural enemies had been studied in a few cases in the USA. Reed *et al.*, (1991; 1992) found that resistant triticale with

high levels of antibiosis, negatively affected growth and reproduction of both RWA and the parasitoid *Diaeretiella rapae* (McIntosh)*.* During the same study a resistant wheat entry, however, showed a reduction in aphid populations and enhanced parasitoid activity due to the fact that the leaves did not roll close thereby exposing the aphids to parasitism. Brewer *et al.*, (1998) found that RWA was parasitised at approximately equal rates on resistant and susceptible barley lines. Farid *et al.* (1998a; b) found that two different resistant wheat lines had no negative effect on the parasitoid *D. rapae*, even after three parasitoid generations. Compatibility and possible complementary associations were reported between RWA and the ladybird predator *Scymnus frontalis* (Fabricius) (Farid *et al.*, 1997).

1.5   Modification of aphid and parasitoid behaviour

Semiochemicals do not only play a role in the behaviour of natural enemies, but also modify the behaviour of the herbivores itself. There are several examples for the use of behaviour-modifying substances in insect pest control (Pickett *et al.*, 1997). One of the most successful examples has been developed at the Swedish University of Agricultural Sciences and is targeted against cereal aphids (Pettersson *et al.*, 1994). It exploits a range of chemical signals identified from aphid ecological interactions. These volatile substances, which interfere with the behavioural traits aphids use to find host plants, are non-toxic and environmentally benign and should be relatively simple to register. Methyl salicylate is a chemical found in the winter host plant of *Rhopalosiphum padi* (L.), which is host alternating in Sweden (Pettersson *et al.*, 1994; Glinwood & Pettersson, 2000a; b). It is repellent to the aphid because it is used as a signal, which causes the aphids to leave the plant. However, it is also repellent to other cereal aphids because plants commonly produce it as a defence and stress signal (Pettersson *et al.*, 1994; Shulaev *et al.*, 1997). It may cause plants to activate internal chemical defences making them less acceptable to aphids (Glinwood & Pettersson, 2000b).

Semiochemicals such as methyl salicylate may be used to enhance the control of aphids. A cheap, simple formulation has been devised to apply these substances in the field, in which chemicals are put into wax pellets that can be easily distributed in crops by machine or by hand (Ninkovic *et al.*, 2003). Using this

strategy in Swedish cereals, aphid population reductions of 25-50% have been achieved (Ninkovic *et al.,* 2003).   The role that this chemical can play in the control of RWA is unknown and need to be tested. Chemical signals that cause RWA to leave alternative host plants are not known and this could open another field of research.   By reducing initial aphid colonisation of crops, this approach will increase the success of resistant varieties and decrease the potential of aphids to develop resistance-breaking biotypes.

In addition to methyl salicylate as a repellent, a signal consisting of several other repellent volatile substances is produced when *R. padi* colonies reach a high density (Pettersson *et al.*, 1995).   This signal acts as a spacing mechanism for the aphid (Pettersson *et al.*, 1995; Quiroz *et al.*, 1997). These substances may also be effective against other cereal aphid species, since aphids sharing common host plants are known to be able to detect and avoid each other (Pettersson & Stephansson, 1991; Johansson *et al*, 1997).

Aphid parasitoids are known to be attracted to aphid sex pheromones. The sexual females of aphids that have complete life cycles release these pheromones naturally. Parasitoids are also attracted to lures releasing either pheromones synthesised in the laboratory or extracted from the catmint plant, *Nepeta cataria* (Hardie *et al.*, 1991; Glinwood *et al*., 1999). This provides new opportunities to increase the controlling effect of parasitoids by attracting them into crops, especially during the early stages of aphid colonisation. Initial trials have already proved that aphid sex pheromones can be used to increase parasitism in aphid colonies in the field (Glinwood, 1998; Glinwood *et al*., 1998). The pheromone blends of a number of economically important aphid species including *R. padi, Sitobion avenae* (Fabricius)*, Sitobion fragariae* (Walker) and *S. graminum* have already been identified. For the RWA no such record was found in literature. Identification of the correct blend will allow monitoring of aphid populations using traps and attraction of parasitoids into crops.

1.6   Objectives of the study

Interaction between aphid natural enemies and plant resistance could therefore influence the outcome when applied to the same pest and it is therefore of utmost importance to study these interactions.   The objective of this project was to study the interactions between RWA resistant cultivars and two parasitoid species, the specialist *A. hordei* and the generalist *D. rapae* and the response of aphid and its parasitoids to behaviour modifying chemicals. The following aspects were studied:

- Confirmation of establishment of the parasitoid *A. hordei* in South Africa using the polymerase chain reaction.

- Investigation on the impact of an augmentative release of *A. hordei* on resistant cultivars in the field in comparison with susceptible cultivars.

- The influence of volatiles from resistant and susceptible cultivars on the host habitat location of both parasitoid species.

- The response of RWA and both parasitoids to aphid behaviour modifying chemicals in the laboratory.

- The response of aphid behaviour modifying chemicals on RWA population growth on both resistant and susceptible cultivars in the field.

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CHAPTER 2

RELEASE, RECOVERY AND VERIFICATION BY THE POLYMERASE CHAIN REACTION OF THE EXOTIC APHID PARASITOID *APHELINUS HORDEI* (KURDJUMOV) (HYMENOPTERA: APHELINIDAE) IN SOUTH AFRICA [1]

2.1   Introduction

The success of classical biological control programs critically depends on the accurate identification of the natural enemies in both the initial phase where the natural enemies are chosen, and also during release and the subsequent evaluation phases (Delucchi *et al.*, 1976). Identification problems can become particularly difficult when a complex of congeners is released into an area where resident populations of native or previously released species occur. Problems in identifying exotic parasitoids, which were released for the control of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), in the United States, lead to the development of specific polymerase chain reaction (PCR) techniques and their successful use (Zhu & Greenstone, 1999; Zhu *et al*., 2000).

 The parasitoid *Aphelinus hordei* (Kurdjumov) (Hymenoptera: Aphelinidae) has been introduced from the Ukraine into South Africa and the United States for control of *D. noxia,* and other cereal aphids (Prinsloo, 1998; Zhu *et al*., 2000). Other species of *Aphelinus* that attack *D. noxia* in South Africa included *A. nigritus* Howard [= *Aphelinus varipes* (Foerster)], released for the control of *Schizaphis graminum* Rondani*,* and *A. asychis* Walker, which probably occurs naturally in the region (Prinsloo & Neser, 1994).

Aphelinus hordei, showing an oviposition preference for *D. noxia*, co-exists with *A. asychis and A. varipes* in South Africa (Prinsloo, 2000) and in addition to the latter two species, also with *A. albipodus* Hayat & Fatima in the United States. *Aphelinus hordei, A. varipes,* and *A. albipodus* are morphologically very similar, making it difficult to distinguish between them in specimens recovered in the field

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(Hopper *et al.*, 1998; Prokrym *et al.*, 1998; Zhu & Greenstone, 1999). After *A. hordei* was released in South Africa, difficulty was experienced in distinguishing between them and *A. varipes,* and therefore establishment could not be confirmed. This prompted us to develop specific PCR assays to distinguish between them.

The release and recovery of *A. hordei* in South Africa, is reported in this chapter. Furthermore the PCR identification technique developed to distinguish between *A. hordei, A. varipes, and A. albipodus* is described, and how it was used to confirm the successful establishment of *A. hordei* in South Africa and Lesotho.

2.2   Materials and Methods

2.2.1 Insect rearing

Aphelinus hordei parasitoids were mass reared on *D. noxia* at the Agricultural Research Council Small Grain institute (ARC-SGI) in Bethlehem, South Africa. Aphid and parasitoid stock colonies were maintained under temperature controlled greenhouse conditions on winter wheat seedlings (cv. Betta) at 18:6 (L:D) photoperiod and fluctuating temperatures of 15 to 23°C.

Protocols for rearing aphids and parasitoids used in DNA extraction at Stillwater, Oklahoma, USA, was described by Reed *et al.* (1991). Colonies were maintained in cages in a Conviron Model I23 incubator (Controlled Environments, Inc., Pembina, North Dakota, U.S.A.), at 20°C and 16:8 (L:D) photoperiod. Founding stocks for colonies of *A. varipes* from Montpellier, France (voucher number T91/004, Texas A&M University Insect Collection) were provided by K.R. Hopper of the USDA-Agricultural Research Service, Beneficial Insects Introduction Research Unit in Newark, Delaware, USA. *Aphelinus albipodus* (voucher number T92/023, Texas A&M University Insect Collection) were provided by D. Gonzáles of the University of California, Riverside and *A. hordei* from Bethlehem, South Africa, were from the colony of the ARC-SGI, Bethlehem. The *A. hordei* colony descended from material collected at Odessa, Ukraine (Prinsloo & Neser, 1994). In order to reduce the risk of contamination, only one *Aphelinus* colony was maintained at a time.

2.2.2   Voucher Specimens

Vouchers of the *A. hordei* colony and Lesotho populations have been deposited at the National Collection of Insects, ARC–Plant Protection Research Institute, Pretoria, South Africa.

2.2.3   Field studies

Mass reared *A. hordei* were released in South Africa during the 1998 and 1999 wheat growing seasons, at six and four different sites respectively. Wheat is planted between May and July and is harvested between December and January in the areas where the parasitoids were released. Parasitoid numbers and stages released at different localities are given in Table 2.1. These releases were made for establishment purposes. Parasitoid establishment was monitored two to four times at each of the release sites during the wheat-growing season except at Kirklington during 1998 when parasitoids were released at the end of the wheat-growing season. Each time monitoring was conducted, fifty *D. noxia* infested tillers, depending on availability, were collected at random from each site. Mummies present on these tillers were placed in vials for emergence and identification. Live aphids were placed on caged potted plants for 10 days under the greenhouse conditions described in section 2.2.1 and then screened for mummified aphids.

Volunteer wheat and *Bromus catharticus* grass growing in fields at or within 100m from the release sites were monitored for *D. noxia* infestation during the subsequent summer. *Diuraphis noxia* numbers reached very low levels during summer and therefore surveys were conducted on a per plant basis. If available, aphids were collected and checked for parasitism as described above. This was done to determine if the aphids and parasitoids could survive on alternate host plants during summer. In Lesotho the presence of *D. noxia* and parasitoids was monitored on *Bromus* grass, volunteer and planted wheat in the Mokhotlong district (29° 22'S and 29° 38'E) during March 1999 and January 2000.

Table 2.1   Numbers of *Aphelinus hordei* parasitoids released at different localities in the eastern Free State Province during the 1998 and 1999 wheat growing seasons

Locality	Date	Number	Parasitoid stage
1998			
Small Grain Institute	31/08 – 3/12	434 000	Mummies + adults
Meriba	01/09 - 28/10	210 000	Mummies
Paradys	28/08- 16/11	405 000	Mummies
Boomplaas	28/08-27/10	125 000	Mummies
Qwaqwa farmer	28/08 – 3/12	518 000	Mummies + adults
Kirklington	27/10 – 17/11	200 000	Mummies
1999			
Swartfontein	22/9-1/11	651 000	Mummies
Paradys	22/9 – 1/11	183 000	Mummies
Boomplaas	22/9 – 12/10	59 000	Mummies
Kirklington	22/9 – 1/11	187 000	Mummies
Total		2 972 000	

2.2.4 Molecular analysis

After the emergence of adult parasitoids some were preserved in 95% EtOH and shipped to the USDA-ARS Plant Sciences and Water Conservation Laboratory in Stillwater, Oklahoma, U.S.A. for PCR analysis. A similar sample of known *A. hordei* adults from the Bethlehem colony were preserved and shipped at the same time as each year's field samples.

Genomic DNA was isolated from individual wasps, without regard to sex, as previously described (Zhu & Greenstone, 1999). Following RNAase A digestion at a final concentration of 20 μgml^{-1} for 30 min at 37°C, the DNA solution was extracted once with one volume of chloroform/isoamyl alcohol (24:1). DNA was precipitated with two volumes of ethanol overnight at –20°C, pelleted by centrifugation and resuspended in 200 μl of distilled water.

Individual parastioids were subjected to DNA extraction and PCR amplification, using specific primers designed to separate the various *Aphelinus* species by the single base detection technique (Kwok *et al.,* 1990).

Two primer pairs for ribosomal ITS2 DNA, and an additional pair for mitochondrial 16s DNA, were used (Table 2.2). Sequences for the ITS2 primers have been published by (Zhu & Greenstone, 1999; Zhu *et al*., 2000). The 16s primer sequences were:

AphelF	CCTGT TTATC AAAAA CATGG
AphelR	GTCGC AAACT TTTTT ATCAA TA

Table 2.2 Primers used in PCR amplification studies to separate various *Aphelinus* species

Name	Annealing temperature ($^{\circ}$C)	Cycles	Fragment size	Target species
Aho-FAC	50	45	411 bp	*A. hordei*
ITS2-R				
Ava-FA	50	35	300 bp	*A. hordei*
Aalv-R				*A. varipes*
AphelF	51	35	456 bp	*A. hordei*
AphelR				*A. varipes*
				A. albipodus

PCR reactions were performed in a PTC-100 thermocycler (MJ Research, Inc., Watertown, Massachusetts, U.S.A.). DNA was initially denatured for three minutes at 94°C and the PCR amplification was conducted for 35-45 cycles depending on primers, with 30 seconds denaturing at 94°C, 30 seconds annealing at 50-51°C depending on primers, and one minute extension at 72°C. DNA was finally extended for two minutes at 72°C after amplification.

In order to separate *A. varipes* from *A. hordei*, the 456 bp DNA sequences resulting from PCR with the AphelF-AphelR primer pair were searched for restriction endonuclease sites using GCG Wisconsin Package UNIX version 10

(Genetics Computer Group, Madison, Wisconsin, USA). HinfI was selected for its ability to digest the 456 bp PCR product of *A. varipes* into two segments, 336 bp and 120 bp, while leaving the PCR product from *A. hordei* intact. This was accomplished by incubating the PCR product at 37°C for two hours in a digestion solution (40 μl) containing 50 mM Tris-HCl, pH 8.0, 10 mM MgCl$_2$, 50 mM NaCl, 0.05 U μl^{-1} of HinfI (Life Technologies, Rockville, Maryland, U.S.A.).

PCR products (10 μl) were separated on a 1.5% agarose gel, stained with 0.5 μgml^{-1} ethidium bromide, and photographed under UV light. Fragment size was determined by referring to a 100 bp marker ladder (Pharmacia Biotech Products, Piscataway, New Jersey, U.S.A.).

2.3   Results and discussion

Aphelinid mummies were found at each of the release sites during the wheat-growing season in both years (Table 2.3). During both years the majority of the parasitoids that emerged from the mummies collected at the release sites were identified morphologically as *A. hordei* (Table 2.3). *Aphelinus asychis* adults were found at two sites during 1998, while hyperparasitoids (Hymenoptera: Encyrtidae) were found at one site in both years (Table 2.3).

During the surveys carried out in the eastern Free State in February and March 1999, *D. noxia* was found only on one volunteer wheat plant at the Qwaqwa farm. These aphids were not parasitised. In Lesotho *D. noxia* parasitised by an aphelinid parasitoid was found at each of the sites surveyed during 1999 and 2000 (Table 2.4). The parasitoids that emerged from these mummies were identified morphologically as *A. hordei*. Because they were collected between 100 and 200 km from where the parasitoids were released (Table 2.5), they were compared at the DNA level to colony material reared at the Small Grain Institute to determine if they were the same. The specimens from Lesotho were also compared with *A. varipes* and *A. albipodus* to confirm their identity.

Table 2.3 Number of *Aphelinus hordei*, *Aphelinus asychis* and hyperparasitoids that emerged from mummies collected at the different parasitoid release sites in the Free State Province during 1998

Release site	Total mummies	Number not emerged	*A. hordei* numbers	*A. asychis* numbers	Hyperparasitoid numbers
1998					
SGI	162	13	145	4	-
Meriba	304	14	280	9	1
Paradys	398	18	380	-	-
Boomplaas	16	-	16	-	-
Qwaqwa	90	5	85	-	-
1999					
Swartfontein	577	48	527	-	2
Paradys	223	34	189	-	-
Boomplaas	55	5	50	-	-
Kirklington	198	51	147	-	-

Table 2.4 *Diuraphis noxia* infestation and parasitism levels as recorded during a survey in Lesotho during March 1999 and January 2000

Locality	Number of plants examined		*D. noxia* infested plants	Aphelinid parasitism
	Planted wheat/barley	*B. catharticus*		
1999				
Mokhotlong	58		23	No
Mokhotlong		5	1	Yes
Sehlabathebe	10		10	Yes
2000				
Mokhotlong Field 1	50	0	50	Yes
Mokhotlong Field 2	20	0	20	Yes
Mokhotlong Field 3	20	0	20	Yes

Table 2.5   Direct distances (kilometers) between parasitoid release sites and places where parasitoids were recaptured in Lesotho

Release site	Recapturing sites	
	Mokhotlong	Sehlabathebe
Small Grain Institute, Meriba, Paradys	± 143 km	± 215 km
Qwaqwa, Boomplaas	± 117 km	± 193 km
Kirklington	± 145 km	± 194 km

In each of the two years in which recoveries were made, between 14 and 18 individuals from Lesotho and the Free State release sites and from the Bethlehem colony were subjected to PCR. Six individuals each of known *A. varipes, A. albipodus* and *A. hordei* collected from the colonies were also subjected to PCR. Using the ITS-2 primers Aho-FAC and ITS2-R, the percentage of individuals in the various samples identified as *A. hordei* ranged from 22% to 79% (Table 2.6). Due to the highly variable frequency, it was decided to develop the 16s primers and the three-step protocol requiring two PCR reactions and restriction endonuclease digestion. Using the three-step protocol, the frequencies ranged from 93.8 to 100% (Table 2.6).

Table 2.6   Results of PCR assays on colony and field collected material of *Aphelinus hordei, Aphelinus varipes* and *Aphelinus albipodus.*

Collection	Year	N	% identified as *A. hordei* with different primer pairs		
			Aho-FAC/ ITS2-R	Ava-FA/ Aalv-R	AphelF/AphelR plus HinfI
Colony	1999	14	79%	100%	100%
Lesotho	1999	14	79%	100%	100%
Colony	2000	18	61%	100%	94.4%
Lesotho	2000	16	31%	100%	93.8%
Release site	2000	18	22%	100%	100%

The use of the three-step protocol is illustrated on Figure 2.1. Primer pair Ava-FA-AalvR amplified a 300 bp band in *A. varipes* and *A. hordei* but not *A. albipodus* (Fig. 2.1A). When the same individuals are subjected to PCR using primer pair AphelF-AphelR, followed by HinfI restriction endonuclease digestion, a 456 bp band is seen, except in *A. varipes,* in which the band is digested to 336 and 120 bp fragments (Fig. 2.1B).
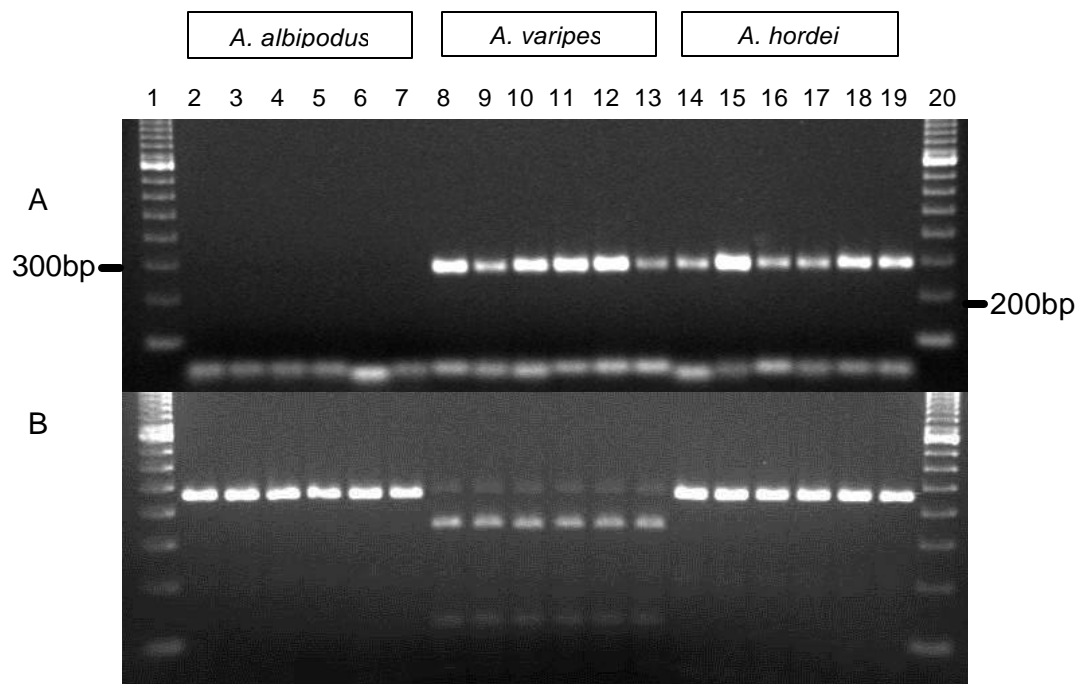
Figure 2.1 PCR amplification of adult aphelinid wasps. Both gels contain known individuals of *A. albipodus*, (lanes 2-7), *A. varipes*, (lanes 8-13) and *A. hordei* (lanes 14-19). Lanes 1 and 20 contain a 100 bp DNA marker from Pharmacia. (A) Primers Ava-FA and Aalv-R were used. (B) Primers AphelF and AphelR were used, followed by HinfI digestion.

Using a less sensitive electrophoretic test, Strong (1993) could not find genetic differences between the 'Ukrainian strain' of *A. varipes* that was introduced into South Africa and here referred to as *A. hordei* and a 'German strain'. Based on small taxonomic differences, the Ukrainian strain of *A. varipes* was identified as *A. hordei* after introduction into South Africa (Prinsloo & Neser, 1994), although doubt

has since been expressed as to the reliability of these perceived differences in separating these two species (G. L. Prinsloo, personal communication[1]). The current investigation has revealed that *A. hordei* is extremely close to *A. varipes* and *A. albipodus*, both morphologically (Prokrym *et al*. 1998) and molecularly (Y. Chen, K.L. Giles & M.H. Greenstone, personal communication[2]). The three-stage PCR and digestion protocol developed in the current research appears to be the best methodology presently available to distinguish between them. Using it, made it possible to demonstrate conclusively that *A. hordei* has been recovered. It spread a considerable distance in the field following its release in South Africa for *D. noxia* control.

In its 16s sequence, *A. hordei* differs from *A. asychis* by almost 9%, but its differences from various *A. varipes* and *A. albipodus* populations are less than 0.4% (Y. Chen, K.L. Giles & M.H. Greenstone, personal communication[2]). Considering the close morphological and molecular similarities of these species, it may reasonably asked whether *A. hordei, A. varipes* and *A. albipodus* are three distinct species. Nevertheless, because the name *A. hordei* has repeatedly been used in the literature on the Odessa, Ukraine population, it is important to continue referring to specimens from this stock as *A. hordei,* until the systematics of the various species of *Aphelinus* that are associated with the Russian wheat aphid has been resolved.

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CHAPTER 3

COMPATIBILITY OF *APHELINUS HORDEI* (KURDJUMOV) (HYMENOPTERA: APHELINIDAE) WITH RUSSIAN WHEAT APHID RESISTANT CULTIVARS IN THE FIELD.

3.1   Introduction

Host plant resistance were used effectively in the past as crop protection against several pests on different crops including wheat. The Hessian fly, *Mayetiola destructor* (Say) and the greenbug, *Schizaphis graminum* (Rondani) are two examples of pests on wheat that are controlled by host plant resistance (Porter *et al.,* 1991; Wiseman, 1999). Biological control of aphids using natural enemies seems to be limited to a few cases (Van Lenteren, 1991). The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), however, seems to be a pest that could be controlled through classical biological control as defined by De Bach (1974). Thus, *D. noxia* is typically an insect that invaded a new area without its effective natural enemies and became a pest and natural enemies should be introduced for control. Since both host plant resistance and biological control are generally inexpensive, self-perpetuating and non-pollutant, they are rendered as desirable and sustainable components of integrated pest management (Thomas & Waage, 1996).

Tritrophic studies involving the interactions between plants, herbivores and natural enemies indicate that the application of both host plant resistance and biological control to a particular pest could give significantly better or worse results than expected from each individual factor (Thomas & Waage, 1996). Van Emden & Wearing (1965) proposed that the reduced rate of multiplication of multivoltine insects such as aphids on partially resistant varieties should result in magnification of the plant resistance in the presence of natural enemies. This complementary interaction was validated by Starks *et al.* (1972) in the laboratory, while it was later

also demonstrated on many other occasions (Salto *et al.*, 1983; Kuo, 1986; Ofuya, 1995).

In South Africa both host plant resistance and biological control are used in the control of *D. noxia*, which is the most serious insect pest of wheat in the summer rainfall areas of the country (Du Toit & Walters, 1984). To date 13 cultivars, containing different levels of plant resistance, are available to farmers in the Free State Province (Anonymous, 2004). More than 70% of the wheat farmers in the eastern parts of the Free State Province are planting the resistant cultivars, which resulted in a reduction of 35.8% in insecticide application between 1990 and 1996 (Marasas *et al.*, 1997).

Since 1980 four predators and two parasitoids (Aalbersberg *et al.*, 1984; Hatting, 1995; Marasas *et al.*, 1997; Prinsloo, 1998) were introduced for the control of *D. noxia.* One predatory beetle *Adalia bipunctata* (Linnaeus), origanaly introduced from the United Kingdom (Aalbersberg *et al*., 1984), became established. The parasitoids *Aphidius matricariae* Haliday and *Aphelinus hordei* (Kurdjumov), respectively introduced from Turkey and the Ukraine also became established (Marasas *et al.,* 1997; Prinsloo, 1998). The parasitoid *A. hordei* prefers to oviposit in *D. noxia* rather than in the other wheat aphid species and could be a valuable agent for the control of *D. noxia* in South Africa (Prinsloo, 2000).

The interaction between *D. noxia* resistance and natural enemies has been studied in a few cases in the USA, but not in South Africa. Reed *et al* (1991; 1992) found that resistant triticale with high levels of antibiosis, negatively affected growth and reproduction of both *D. noxia* and the parasitoid *Diaeretiella rapae* (Mcintosh) (Hymenoptera: Aphidiidae). A resistant wheat entry, however, showed a reduction in aphid populations and enhanced parasitoid activity due to the fact that the leaves did not roll close, thereby exposing the aphids to parasitism. Brewer *et al.* (1998) found that *D. noxia* was parasitised at approximately equal rates on resistant and susceptible barley lines. Farid *et al.* (1998a; b) found that two different resistant wheat lines had no negative effect on the parasitoid *D. rapae*, even after three parasitoid generations. Compatibility and possible complementary associations were reported between *D. noxia* and the predatory ladybird, *Scymnus frontalis* (Farid *et al*., 1997).

Current breeding and evaluation procedures for cultivars resistant to *D. noxia* in South Africa do not examine the direct or indirect effects of plant resistance on the third trophic level. This means that positive or negative interactions between host plant resistance and biological control are not identified. It is therefore possible that deployment of resistant varieties could occur that actively interfere with natural enemies, which could reduce the benefits gained from resistance breeding. These phenomena will have long term consequences for the persistence of certain key natural enemy species (Thomas & Waage, 1996). Farmers are using these resistant cultivars on a large scale in South Africa and the objective of this study was to determine the compatibility of resistant Eland and SST 333 and susceptible Betta with the introduced parasitoid *A. hordei* in the field.

3.2 Materials and methods

3.2.1 Aphids and parasitoids.

Aphelinus hordei parasitoids were reared on *D. noxia* on wheat (cultivar Betta) in a greenhouse. Rearing were maintained at a temperature of $23 \pm 2°C$ and ambient light, extended with electrical light to a 14 L:10 D cycle, as described by Prinsloo & Du Plessis (2000).

3.2.2 Field trial

The trial was conducted in two successive seasons during 1998 and 1999. Both trials were conducted on the experimental farm of the ARC- Small Grain Institute outside Bethlehem (28° 10'S and 28° 18'E), South Africa. The trial consisted of a parasitoid and a parasitoid-free main treatment. The experimental fields were approximately 700 m apart, to lower the risk of parasitoids spreading to the parasitoid-free treatment. In each main treatment block, three cultivar sub-treatments viz. the susceptible cultivar Betta and two different resistant cultivars, Gariep and SST 333 were planted in a randomised block design containing four replicates. Each sub-treatment plot size measured 2.7 m X 5 m. The planting

dates for the trials were the 3rd and 5th July 1998 and 1999 respectively. Seeding rate was 20 kg ha^{-1} and fertiliser application at 250 kg ha^{-1}. The resistant cultivar Gariep and SST 333 contained resistance from the PI 137739 and PI 260660 resources respectively (Tolmay & Van Deventer, 2005). The resistant genes in these sources were designated as Dn1 and Dn2 respectively (Du Toit 1987, 1989).

The Russian wheat aphid population development was followed weekly by means of non-destructive aphid counts in the field between Zadock's (Tottman, 1987) growth stage 20 and flowering, which was from 31st August - 4th November 1998 and from 3rd September - 4th November 1999. The number of infested tillers on each of ten randomly chosen plants per plot were counted, as well as the number of aphids per tiller on the first ten infested tillers found in a plot. Temperature and rainfall were recorded on the farm and are presented in Figure 3.1.

On 31/08/1998, a total of 2000 *A. hordei* were released in each plot of the parasitoid main treatment. During 1999 the initial *D. noxia* infestation was low and on the 16th September 1999 a supplementary infestation of approximately 600 *D. noxia* per plot, were released from the greenhouse colony. On the 23rd September 1999, 2000 *A. hordei* per plot were released in the parasitoid main treatment. In both years parasitoids were released as mummies in 500 ml wax-covered paper containers, perforated around the edges of the top, where parasitoids could escape.

Following parasitoid release, the percentage parasitism was monitored at weekly intervals by cutting five randomly chosen infested tillers from each plot after the aphids were counted. In the laboratory, the aphids and mummies on these tillers were brushed off and counted. To determine the number of aphids containing parasitoid eggs and larvae, all living aphids from each plot were placed on clean potted plants and caged individually for ten days, until aphids were mummified. Mummified aphids were counted and added to the original number of mummies to determine the percentage parasitism.
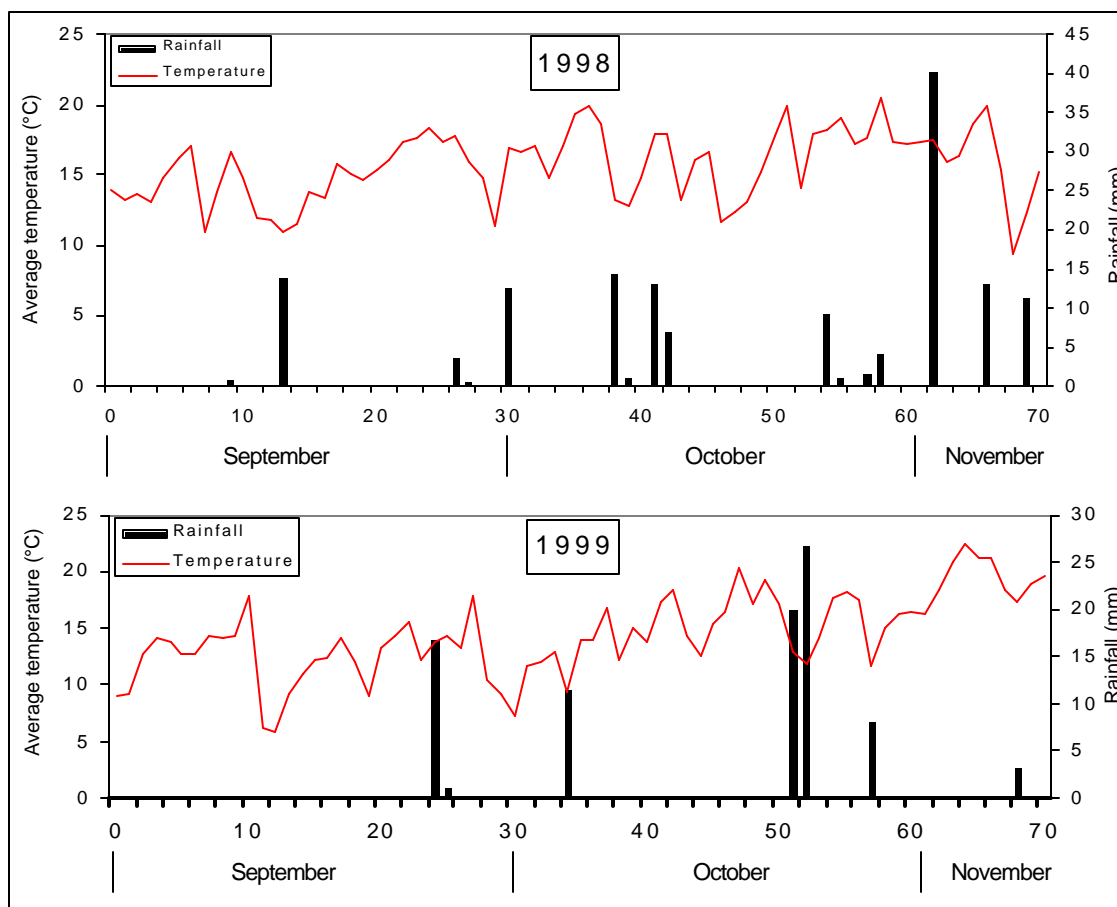
Figure 3.1   Average daily temperature and rainfall for the trial periods during 1998 and 1999

3.2.3 Statistical analyses

Data were analysed in several ways. At first the data were log transformed and the linear relationship between the percentage infested tillers over time was calculated (Van Ark, 1992). These regressions were compared. Differences between cultivars were analysed using an ANOVA for randomised block design (Van Ark, 1992). These analyses, based on Snedecor & Cochran (1967), were performed for each counting date in each of the main treatments. Afterwards the difference in infestation level between the two main treatments was tested for each cultivar on each date, using a t-test for independent samples (Van Ark, 1992).

Following the model of Van Emden (1986) a susceptibility to resistance (S/R) ratio was calculated for the percentage infestation. This direct ratio of performance on the susceptible variety to performance on the resistant variety is an index of the relative resistance of two varieties as a single variable at any point in time. The ratio was calculated for each date and the mean ratio calculated which was used to determine if the resistance was magnified by the presence of the parasitoids, as demonstrated by Van Emden (1986).

3.3 Results and discussion

3.3.1 Susceptible Betta

The mean percentage infestation, mean number of *D. noxia* per tiller and the mean percentage parasitism for both the parasitoid and parasitoid-free treatments during the 1998 trial are shown in Figure 3.2. On 31/08/1998 the percentage infestation between the parasitoid and the parasitoid-free treatments did not differ significantly (Table 3.1) and the parasitoids were therefore released when the populations on both treatments was nearly the same.

The infestation on Betta increased in the typical sigmoidal pattern (Aalbersberg *et al.,* 1989) and *A. hordei* was released early in the lapse phase of population development (31/08/98 – 18/09/98). From 18/09/1998 infestation was visually different between the parasitoid and the parasitoid-free treatments (Fig. 3.2A). The linear regressions for the log percentage infestation versus time of infestation for both Betta treatments were significant (Parasitoid-free: r = 0.8427, F = 73.5, P = 0.000; parasitoids: r = 0.4927, F = 9.619, P = 0.004) indicating population growth was highly correlated with time. When compared, the slopes of the regressions differ significantly (F= 4.7 P = 0.034) showing that the regressions were different. Therefore the population in parasitoid-free treatment grew with a steeper slope than in the parasitoid treatment, which is an indication of the influence of parasitism on aphid population growth. No natural parasitism was found on the parasitoid-free treatment.
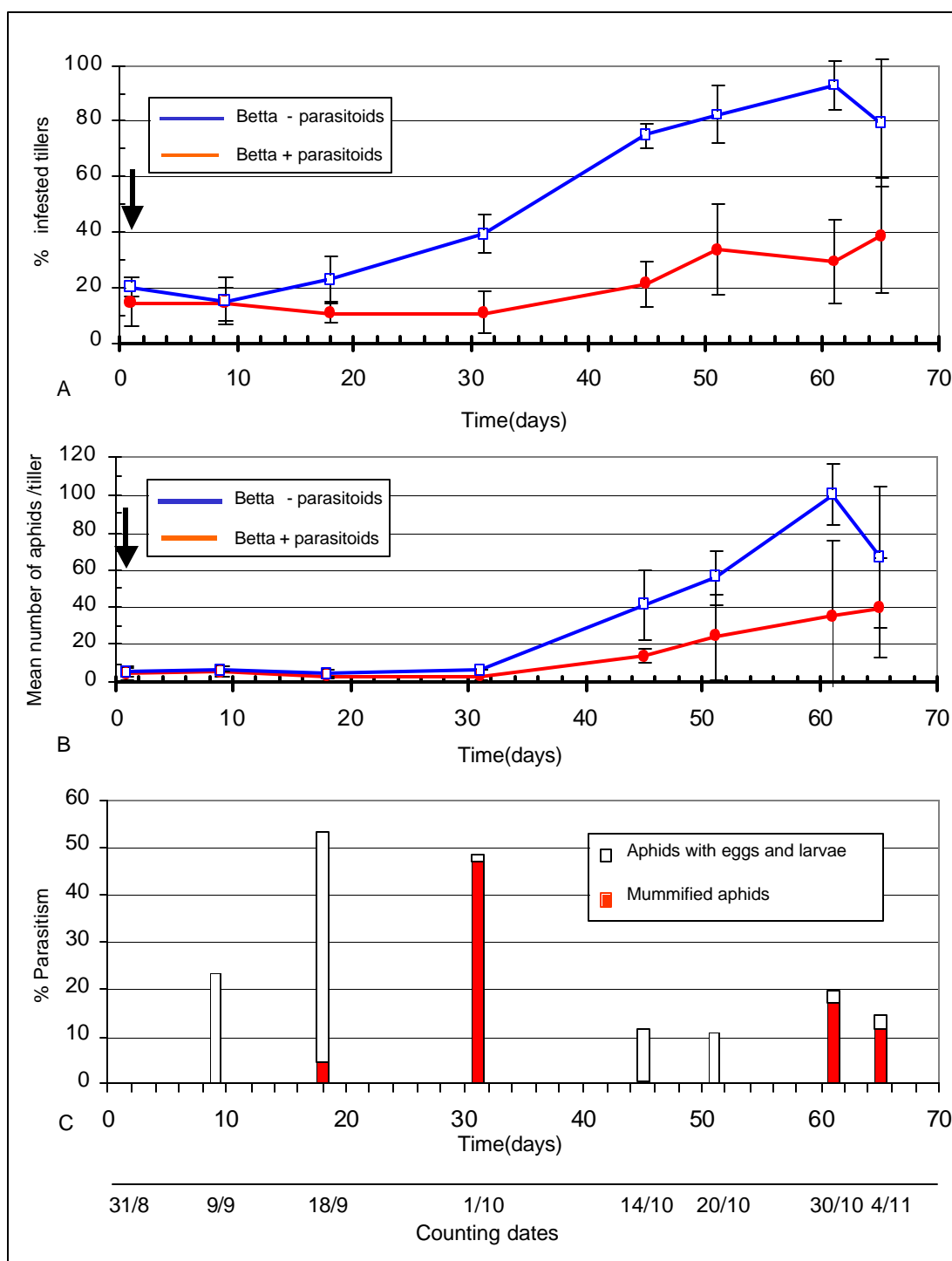
Figure 3.2.   Field trial 1998 – Betta. (A) Mean percentage tillers infested, (B) Mean number of *Diuraphis noxia* per tiller in the presence and absence of parasitoids and (C) Mean percentage parasitism per plot by *Aphelinus hordei* in the parasitoid treatment of the trial. Arrow indicates the date of parasitoid release (I = SD)

The t-test analyses showed further differences between the parasitoid and parasitoid-free treatments. Nine days (9/09/98) after parasitoid release, 23.7% of the *D. noxia* on Betta were parasitised (Fig. 3.2C), although aphids were still alive and percentage infestation was not significantly influenced (Table 3.1).

From 14/10/98 the mean number of aphids per tiller differed visually between the two main treatments (Fig. 3.2B; Table 3.1). This also coincided with the onset of the logarithmic growth phase of the aphid population. Although aphid numbers per tiller were affected due to parasitism, high variance was found in the number of aphids, and the only significant difference was found on 14/10/98 (Table 3.1). The differences on the last three dates were obvious, but not significant (Fig. 3.2).

Table 3.1   Differences in the mean percentage infestation (± SD) for both Betta treatments on all counting dates for 1998 (P = 0.05,t-test, ns = non significant).

	Mean percentage infestation			**Mean number of aphids per tiller**		
Date	**Parasitoid-free**	**Parasitoids**	**P**	**Parasitoid-free**	**Parasitoids**	**P**
31/08/98	21.1 ± 1.7	14.6 ± 8.8	ns	5.6 ± 2.5	4.1 ± 3.3	ns
9/09/98	13.9 ± 10.2	16.0 ± 4.0	ns	6.1 ± 1.4	4.8 ± 1.2	ns
18/09/98	23.1 ± 8.2	10.5 ± 3.6	0.012	6.5 ± 1.7	3.2 ± 1	ns
1/10/98	39.3 ± 7.1	10.9 ± 7.4	0.002	6.5 ± 0.2	3.7 ± 2.4	ns
14/10/98	74.8 ± 4.6	21.2 ± 8.2	0.000	41.2 ± 18.8	14.0 ± 3.8	0.030
20/10/98	82.2 ± 10.4	33.8 ± 16.6	ns	56.1 ± 14.2	24 ± 22.8	ns
30/10/98	92.4 ± 7.9	32.7 ± 20.0	0.002	100.2 ± 16.3	34.7 ± 41.5	ns
4/11/98	79.4 ± 23.0	38.4 ± 20.7	ns	66.5 ± 37.3	43.1 ± 30.5	ns

From 18/09/98 mummification of parasitised aphids commenced and on 1/10/98 almost all parasitised aphids were mummified (Fig. 3.2C). This seemed to coincide with the onset of the logarithmic growth phase of the aphid population. Natural parasitism of *D. noxia* was not recorded in the parasitoid-free treatment but infestation rapidly increased to 92.4% on 30/10/98 (Fig. 3.2A).

Significant differences in the percentage infestation between the parasitoid and parasitoid-free treatments were determined on 18/09/98, 1/10/98, 14/10/98 and 30/10/98, showing the effect of parasitism on the percentage aphid infestation (Fig. 3.2A; Table 3.1). Although a difference in percentage infestation was found on 20/10/98 the variance was high and the statistical analyses showed it was not significant.

Aphelinus hordei completed two generations during the season (Fig. 3.2C). This figure is illustrating the dynamics of biological control where the first generation showed no direct effect of parasitism on the aphid numbers and percentage infested tillers (compare Fig 3.2C, B & A). The control effect became visible in the percentage infested tillers from 1/10/98 when the first generation parasitised aphids became mummified and reduced the number of reproducing aphids.

Due to the aphids added on 16/09/99, data for the 1999 trial were analysed from the date of parasitoid release. Parasitoids were released on 23/09/99, about three weeks later than in the 1998 trial. Similar to the 1998 trial the percentage infestation between the parasitoid and the parasitoid-free treatments of Betta did not differ significantly on the date of parasitoid release (Fig. 3.3, Table 3.2).

Different from 1998 the percentage infestation in the parasitoid-free treatment increased steadily until flowering of the wheat when aphid counting was terminated on 4/11/999 (Fig 3.3 A). The linear regression for the log percentage infestation versus time of infestation was significant for both Betta treatments (Parasitoid-free: $r = 0.8548$, $F = 92.21$, $P = 0.000$; Parasitoids: $r = -0.5588$, $F = 1$, $P = 0.000$).
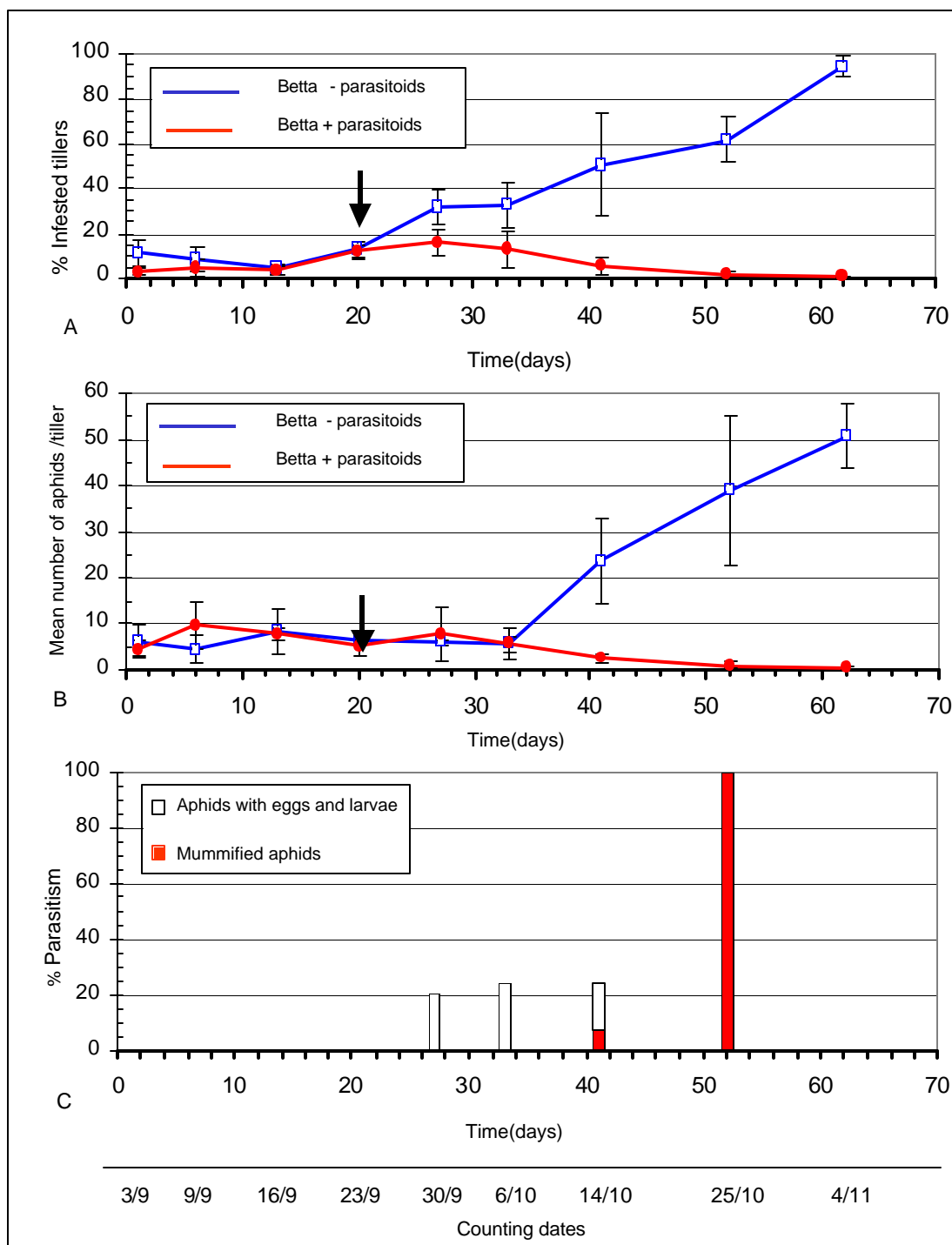
Figure 3.3.   Field trial 1999 – Betta. (A) Mean percentage tillers infested, (B) Mean number of *Diuraphis noxia* per tiller in the presence and absence of parasitoids and (C) Mean percentage parasitism per plot by *Aphelinus hordei* in the parasitoid treatment of the trial. Arrow indicates the date of parasitoid release (I = SD)

The variation attributed to the relationship between percentage infestation and time was however not homogenous for both regressions and they could therefore not be compared. Thus, although growth of both parasitoid and parasitoid-free populations was significantly correlated with time, the line on the parasitoid treatment have a negative slope, showing the effect of parasitoids on the aphid population development (Fig 3.3).

The t-test analyses for each date showed that the parasitoid-free and parasitoid treatments for Betta differed significantly from 30/9/99, seven days after the parasitoids were released (Table 3.2). By this date 20% of the aphids in the parasitoid treatment have been parasitised, although still alive (Fig. 3.3C). No natural parasitism by any other parasitoids was found in the parasitoid-free treatment and the mean number of aphids and the percentage infested plants, increased rapidly after 6/10/99. This coincided with the mummification of the first parasitised aphids on the parasitoid treatment, causing a significant difference in aphid numbers between the two treatments from 14/10/99 onwards (Table 3.2; Fig. 3.3C). Similar to 1998 the control effect started only when the first generation parasitised aphids became mummified.

Table 3.2   Differences in the mean percentage infestation (± SD) for both Betta treatments on all counting dates for 1999. (P = 0.05, t-test, ns = non significant)

	Mean percentage infestation			Mean number of aphids per tiller		
Date	**Parasitoid-free**	**Parasitoids**	**P**	**Parasitoid-free**	**Parasitoids**	**P**
23/09/99	12.8 ± 3.3	11.9 ± 3.5	ns	6.7 ± 0.3	5.4 ± 2.4	0.37
30/09/99	31.5 ± 7.7	15.8 ± 5.5	0.049	6 ± 0.7	7.7 ± 5.8	ns
6/10/99	32.5 ± 10.1	12.7 ± 7.6	0.020	5.9 ± 3.5	5.6 ± 1.6	ns
14/10/99	50.9 ± 23	5.6 ± 4	0.008	23.7 ± 9.3	2.5 ± 1.2	0.020
25/10/99	61.8 ± 9.8	1.5 ± 1.3	0.001	39.0 ± 16.3	0.9 ± 0.7	0.018
4/11/99	94.5 ± 4.7	0.5 ± 0.9	0.04	50.8 ± 6.9	0.3 ± 0.5	0.04

Thus although aphid numbers during 1999 were lower than in 1998, the aphid population development in the parasitoid-free treatment followed the same trend. In both years parasitism by *A. hordei* had a significant suppressing influence on the aphid population development in the parasitoid treatment

3.3.2   Resistant Gariep

The mean percentage infestation and mean aphid numbers per tiller on Gariep during 1998 were much lower than on Betta (Fig. 3.4), showing the effect of resistance. The percentage infestation between the parasitoid and the parasitoid-free treatments did not differ significantly on 31/08/98 (Table 3.3) and the parasitoids were therefore released when the populations on both treatments were nearly the same.

The *D. noxia* population development on this cultivar did not follow the sigmoidal pattern as on Betta (compare Figs. 3.4A & 3.2A). The log percentage infestation was significantly correlated with time only on the parasitoid-free treatment (Parasitoid-free: r = 0.7435, F = 37.08, P=0.000; Parasitoid: r = 0.3299, F = 3.664, P = 0.065). The two populations therefore developed differently which may indicate the influence of parasitism.

Although approximately 41% of *D. noxia* on Gariep was parasitised on 9/09/98, aphids were still alive and infestation was not significantly influenced (Fig. 3.4C), (Table 3.3).   Natural parasitism was absent in the parasitoid-free treatment and aphid infestation increased to 43.5% on 20/10/98, while infestation on the parasitoid treatment increased only to a maximum 19.3% (Fig. 3.4A)

Between 18/9/98 and 1/10/98 when reproducing aphids became mummified, infestation in the parasitoid treatment started to decrease significantly and the aphid population in this treatment developed at a lower level onwards (Table 3.3).

A significant decrease (45.7%) in the percentage parasitism was recorded on 1/10/98 (Fig. 3.4C), which coincides with the mummification of most of the
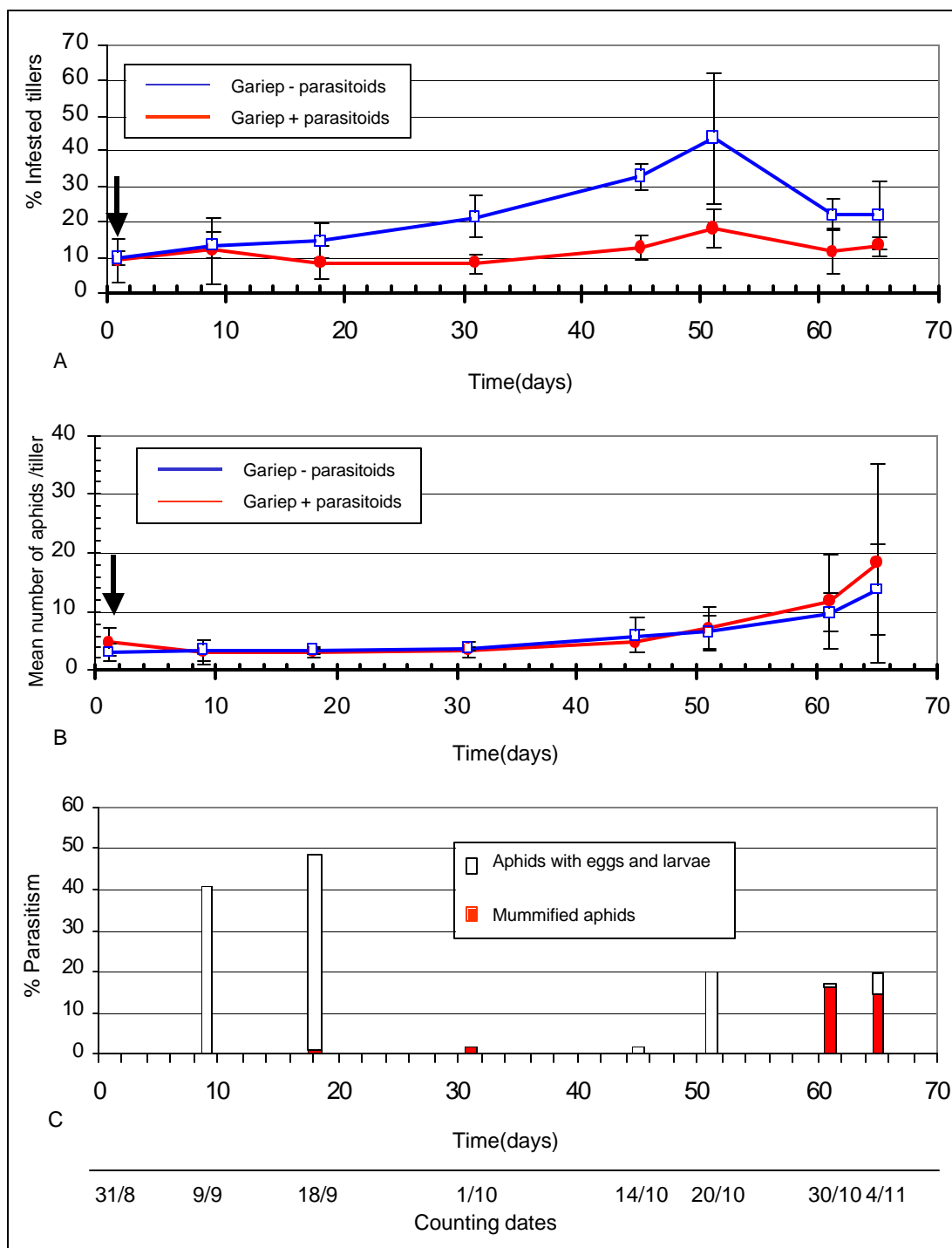
Figure 3.4. Field trial 1998 – Gariep. (A) Mean percentage tillers infested, (B) Mean number of *Diuraphis noxia* per tiller in the presence and absence of parasitoids and (C) Mean percentage parasitism per plot by *Aphelinus hordei* in the parasitoid treatment of the trial. Arrow indicates the date of parasitoid release (I = SD)

parasitised aphids if compared to Betta. Because Betta and Gariep plots were next to each other it could be assumed that, as in Betta, almost all parasitised aphids on Gariep should have became mummified during the same time. An explanation for this decrease might probably be attributed to the physical features of the resistant wheat plant and the mummified aphids. When the aphelinid parasitoid larvae kill an aphid they exude a substance that hardens the aphid integument and turns it to black forming the typical black mummy, which is characteristic of the aphelinid parasitoids (Star?, 1988). These mummies are attached to the plants by the mouthparts and could easily drop from the plants (personal observation).

Russian wheat aphid infestation causes leaf rolling of susceptible cultivars, resulting in the aphids feeding inside these rolled leaves. Leaf rolling is absent in resistant cultivars and the aphid colonies are exposed. Mummified aphids can drop much easier from the open leaves of resistant plants than from rolled leaves of the susceptible plants.

Table 3.3 Differences in the mean percentage infestation (± SD) for both Gariep treatments on all counting dates for 1998. (P = 0.05, t-test, ns = non significant)

	Mean percentage infestation			Mean number of aphids per tiller		
Date	Parasitoid-free	Parasitoids	P	Parasitoid-free	Parasitoids	P
31/08/98	9 ± 1.6	9.1 ± 6.0	Ns	3.2 ± 1.4	4.9 ± 2.3	ns
9/09/98	13.3 ± 3.6	12.0 ± 9.3	Ns	3.4 ± 1.7	3.2 ± 2.1	ns
18/09/98	14.2 ± 4.4	8.5 ± 4.7	Ns	3.3 ± 0.5	3.2 ± 1.0	ns
1/10/98	21.5 ± 5.8	5.1 ± 2.4	0.005	3.8 ± 0.4	3.4 ± 1.2	ns
14/10/98	32.6 ± 5.2	12.6 ± 3.7	0.001	5.9 ± 2.9	4.9 ± 2.0	ns
20/10/98	43.6 ± 18.6	19.3 ± 5.7	0.04	6.7 ± 2.3	7.3 ± 3.5	ns
30/10/98	24.6 ± 6.8	11.8 ± 6.5	0.035	9.7 ± 3.3	11.7 ± 7.9	ns
4/11/98	21.9 ± 9.8	13.0 ± 2.7	0.04	13.9 ± 7.6	18.3 ± 16.8	ns

Between 18/09/98 and 1/10/98 the plants were between Zadock's growth stage 25 and 31 and on these small plants with unrolled leaves mummified aphids were much more exposed and could drop to the ground due to rainy conditions which occurred in this period (Fig. 3.1). This is the most possible reason for the drop in percentage parasitism found on 1/10/98 (Fig. 3.4C). *A. hordei* also completed two generations on the Gariep cultivar during the 1998 season (Fig. 3.4C) and the control effect also started when parasitised aphids became mummified. During the second generation and in spite of more rainy conditions (Fig. 3.1) a drop in the number of mummified aphids did not occur. By this time the plants were in the flag leaf stage and weather conditions might not have such an influence on the mummies on bigger plants.

Diuraphis noxia numbers per tiller did not differ significantly between the parasitoid and the parasitoid-free treatments throughout the season (Fig. 3.4B, Table 3.3). It seemed as if the parasitoids mainly influenced the percentage aphid infestation (Fig. 3.4A).

The results from the 1999 trial for Gariep are shown in Figure 3.5. Aphids and parasitoids were released at the same time as for Betta and data were analysed from the date of parasitoid release (23/09/99). On this date the percentage infestation between the parasitoid and the parasitoid-free treatment on Gariep did not differ significantly (Table 3.4) and parasitoids were released at approximately same population levels.

The infestation level in the parasitoid -free treatment of this cultivar followed the a sigmoidal pattern which was different from 1998, although the maximum infestation was lower than in 1998 due to the low natural infestation (compare Figs 3.4A & 3.5A). The log. percentage infestation correlated significantly with time for both the parasitoid and the parasitoid-free treatments (Parasitoid-free: r = 0.4842, F = 10.42, P = 0.003; Parasitoids: r = -0.378, F = 5.66, P = 0.023). The slopes of the lines, however, differed significantly (F = 13.83, P = 0.000) indicating that the two populations developed differently.
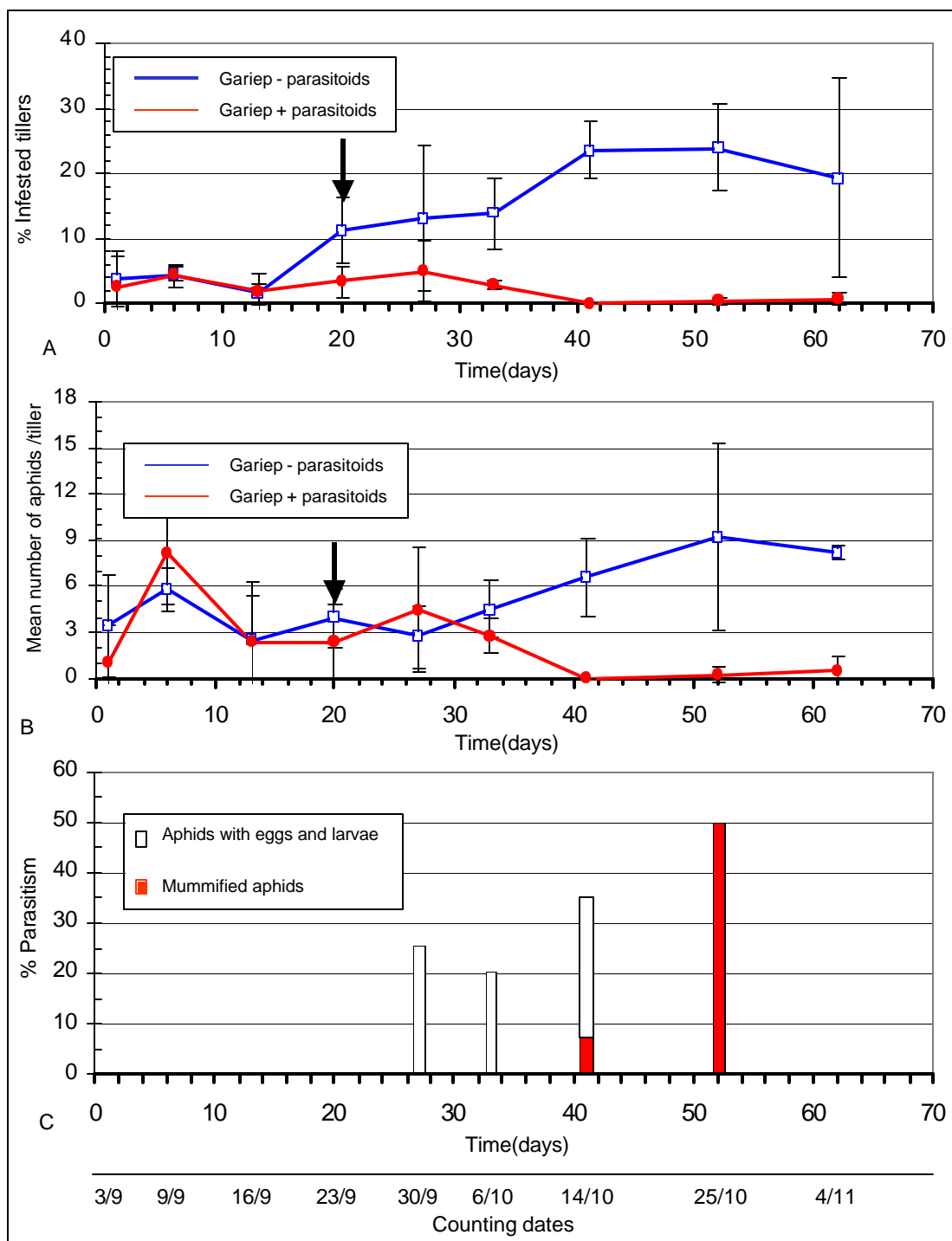
Figure 3.5. Field trial 1999 – Gariep. (A) Mean percentage tillers infested, (B) Mean number of *Diuraphis noxia* per tiller in the presence and absence of parasitoids and (C) Mean percentage parasitism per plot by *Aphelinus hordei* in the parasitoid treatment of the trial. Arrow indicates the date of parasitoid release (I = SD)

Although there was a visual difference in percentage infestation between the parasitoid-free and parasitoid treatments from 23/9/99 (Fig. 3.5A), the differences were not significant due to high variance. The difference became significant form 6/10/99 onwards (Table 3.4) although mummified aphids was recorded only from 14/10/99 onwards.   Similar to Betta in 1999 (Fig. 3.3C), the parasitoids have completed only one life cycle during the trial (Fig. 3.5C).

The aphid numbers per tiller did not differ significantly until 6/10/99. There was, however, a decreasing trend in aphid numbers per tiller in the parasitoid treatment from 6/10/99.   This difference became significant between the parasitoid and parasitoid-free treatments from 14/10/99 onwards (Table 3.4) when the parasitised aphids became mummified, showing the control effect of the parasitoids. Russian wheat aphid population growth on Gariep followed the same pattern in both years with much lower aphid numbers than Betta due to resistance caused by the Dn1 gene (compare Figs. 3.2 and 3.3 with Figs. 3.4 and 3.5).

Table 3.4   Differences in the mean percentage infestation (± SD) for both Gariep treatments on all counting dates for 1999. (P = 0.05, t-test, ns = non significant)

	Mean percentage infestation			Mean number of aphids per tiller		
Date	Parasitoid-free	Parasitoids	P	Parasitoid-free	Parasitoids	P
23/09/99	11.2 ± 5.1	3.3 ± 2.3	ns	3.9 ± 1.92	2.4 ± 2.4	Ns
30/09/99	13.0 ± 11.3	4.9 ± 4.6	ns	2.7 ± 2.0	4.5 ± 4.1	Ns
6/10/99	13.7 ± 5.3	2.8 ± 0.6	0.04	4.5 ± 1.9	2.8 ± 1.1	Ns
14/10/99	23.5 ± 4.3	0 ± 0	0.04	6.6 ± 2.5	0 ± 0	0.04
25/10/99	23.8 ± 6.6	0.3 ± 0.7	0.04	9.2 ± 6.1	0.3 ± 0.5	0.04
4/11/99	19.2 ± 15.3	0.5 ± 0.9	0.04	8.1 ± 0.5	0.5 ± 0.9	0.04

3.3.3 Resistant SST 333

The mean percentage infestation and mean number of *D. noxia* per tiller on SST 333 was lower than on Gariep during 1998 (compare Figs. 3.4 and 3.6) The percentage infestation between the parasitoid and the parasitoid-free treatments did not differ significantly on 31/08/1998 (Table 3.5) and the parasitoids were therefore released when the populations on both treatments were nearly the same.

The Russian wheat aphid infestation did not follow the sigmoidal pattern compared to the other two cultivars (see Figs 3.2A – 3.6A). In spite of this, the log. percentage infestation was significantly correlated with time for both main treatments (Parasitoid-free: r = -0.555, F = 13.371,   P= 0.001; Parasitoid: r = -0.3497, F= 4.180, P = 0.05). This means that the population growth was correlated with time.   The variation attributed to the relationship between percentage infestation and time was, however, not homogenous for both regressions and they could therefore not be compared.

Table 3.5   Differences in the mean percentage infestation (± SD) for both SST 333 treatments on all counting dates for 1998. (P = 0.05, t-test, ns = non significant)

	Mean percentage infestation			Mean number of aphids per tiller		
Date	Parasitoid-free	Parasitoids	P	Parasitoid-free	Parasitoids	P
31/08/98	19.6 ± 3.4	12.0 ± 8.1	ns	2.2 ± 0.3	3.7 ± 1.9	ns
9/09/98	11.4 ± 4.0	13.1 ± 6.6	ns	3.1 ± 0.7	4.2 ± 1.5	ns
18/09/98	12.7 ± 3.7	8.5 ± 4.7	ns	4.4 ± 1.5	3.77 ± 2.3	ns
1/10/98	13.9 ± 3.9	7.0 ± 2.5	ns	3.4 ± 0.5	3.8 ± 2.3	ns
14/10/98	13.8 ± 4.8	10.2 ± 4.0	ns	3.7 ± 1.0	5.0 ± 1.3	ns
20/10/98	10.3 ± 1.4	7.1 ± 6.0	ns	5.3 ± 2.2	2.7 ± 1.5	ns
30/10/98	6.2 ± 5.2	5.1 ± 3.1	ns	8.8 ± 10.9	6.7 ± 5.2	ns
4/11/98	7.3 ± 3.3	4.5 ± 4.4	ns	3.5 ± 1.2	4.8 ± 4.9	ns
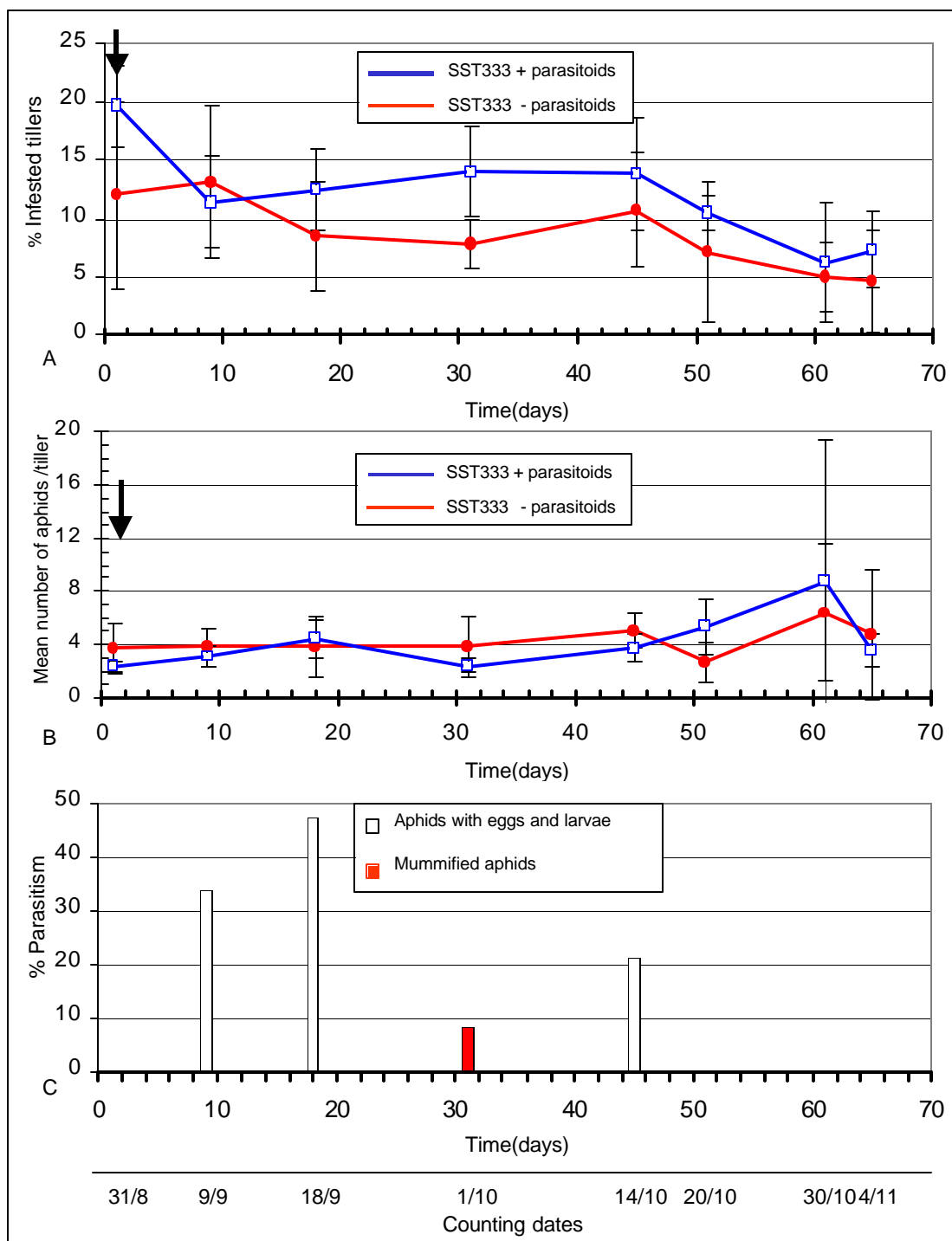
Figure 3.6. Field trial 1998 – SST 333. (A) Mean percentage tillers infested, (B) Mean number of *Diuraphis noxia* per tiller in the presence and absence of parasitoids and (C) Mean percentage parasitism per plot by *Aphelinus hordei* in the parasitoid treatment of the trial. Arrow indicates the date of parasitoid release (I = SD)

Although parasitised *D. noxia* were recorded between 9/09/98 and 14/10/98 (Fig 3.6C), this does not influence the percentage infestation on SST 333 in the parasitoid treatment throughout the season (Table 3.5).   The number of *D. noxia* per tiller on both parasitoid and parasitoid-free treatments followed the same pattern (Fig. 3.5B) and no significant differences between the parasitoid and parasitoid-free treatments could be found (Table 3.5). It therefore seemed as if the parasitism had no influence on the aphid population growth on this cultivar during 1998.   On 1/10/98 all parasitised aphids were mummified and the percentage parasitism decreased from 47.6% to 8.3% (Fig. 3.6C).   The open leaves of this cultivar might also have played a role in the apparent decrease in parasitism due to mummies fallen from the leaves.

The results for SST 333 during the 1999 trial are shown in Figure 3.7. Aphids and parasitoids were released at the same time as Betta and Gariep and data were analysed from the date of parasitoid release (23/09/99). On this date the percentage infestation between the parasitoid and the parasitoid-free treatment of SST 333 did not differ significantly (Table 3.6)

In 1999 *D. noxia* infestation on SST 333 followed a sigmoidal pattern, which is different from 1998 (compare Figs. 3.7A & 3.6A). The log. percentage infestation correlated significantly with time only in the parasitoid treatment (Parasitoid-free: r = -0.247, F= 1.43, P = 0.244; Parasitoids: r = -0.853, F = 58.93, P = 0.000). Therefore, the aphid population growth in the parasitoid and parasitoid-free treatments differed.

Seven days after parasitoid release, 1.6% of the aphids were parasitised (Fig. 3.7C). Although differences in the percentage infestation occurred between the parasitoid-free and parasitoid treatments from 30/9/99, these differences became significant only from 14/10/99 onwards (Table 3.6), when a 33.8% of the parasitised aphids became mummified (Fig. 3.7C) and decreased aphid reproduction.

The number of aphids per tiller also differed significantly from 14/10/99, when most of the parasitised aphids became mummified (Fig. 3.7B; Table 3.6). Although *D.*

Figure 3.7. Field trial 1999 – SST 333. (A) Mean percentage tillers infested, (B) Mean number of *Diuraphis noxia* per tiller in the presence and absence of parasitoids and (C) Mean percentage parasitism per plot by *Aphelinus hordei* in the parasitoid treatment of the trial. Arrow indicates the date of parasitoid release (I = SD)
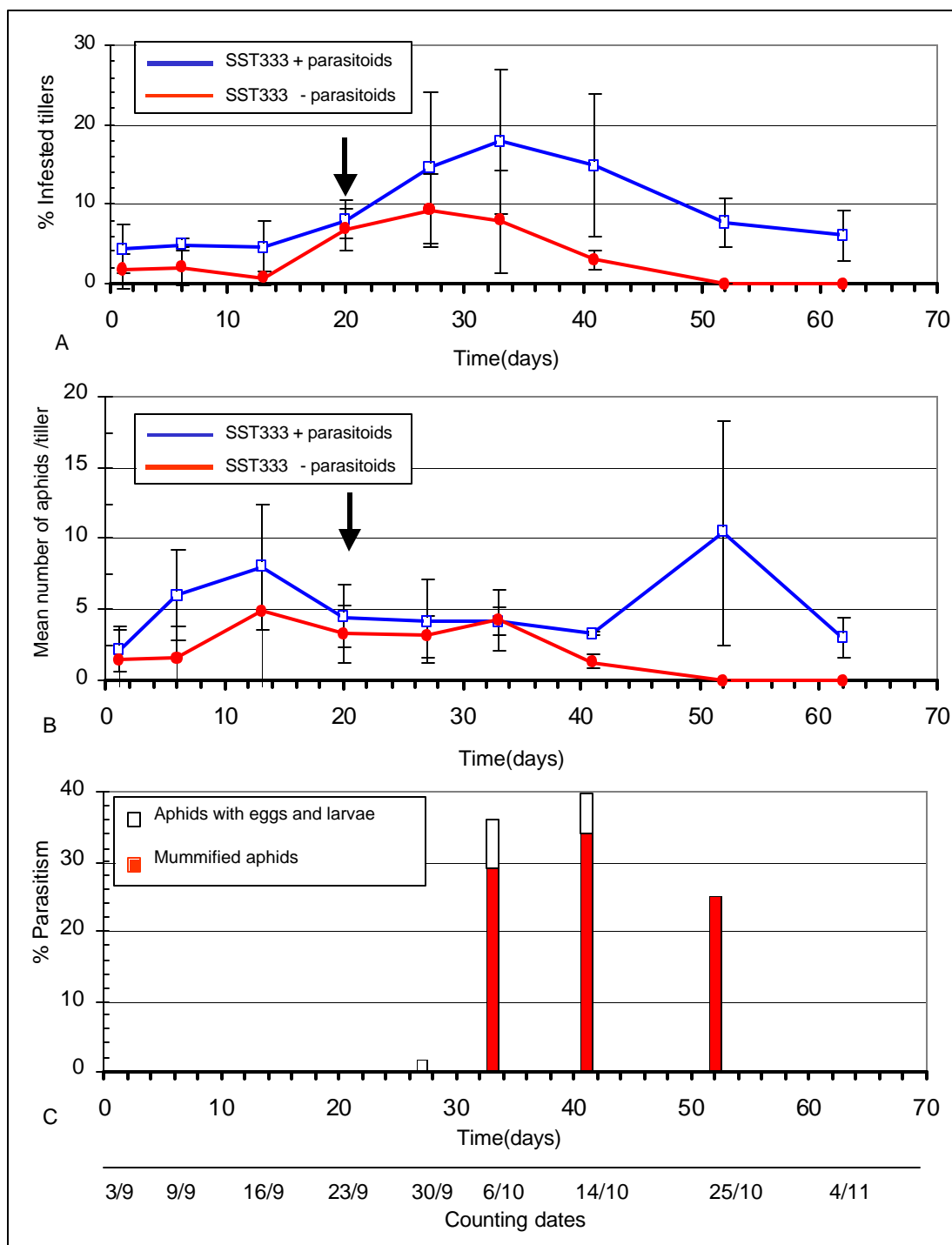
noxia numbers on SST 333 in both years follow approximately the same trend, parasitism seemed not to have any influence on aphid numbers during 1998. A significant influence was, however, observed during 1999.

Table 3.6   Differences in the mean percentage infestation (± SD) for both SST 333 treatments on all counting dates for 1999. (P = 0.05, t-test, ns = non significant)

	Mean percentage infestation			Mean number of aphids per tiller		
Date	Parasitoid-free	Parasitoids	P	Parasitoid-free	Parasitoids	P
23/09/99	8.0 ± 2.44	6.8 ± 2.68	ns	4.5 ± 2.2	3.33 ± 2.0	ns
30/09/99	14.6 ± 9.4	9.2 ± 4.6	ns	4.2 ± 2.9	3.08 ± 1.5	ns
6/10/99	17.9 ± 9.5	7.9 ± 6.5	ns	4.2 ± 1.0	4.33 ± 2.1	ns
14/10/99	14.9 ± 8.9	3.0 ± 1.2	0.004	3.3 ± 0.02	1.33 ± 0.5	0.017
25/10/99	7.7 ± 3.1	0 ± 0	0.04	10.4 ± 7.9	0 ± 0	0.04
4/11/99	6.1 ± 3.2	0 ± 0	0.04	3.0 ± 1.4	0 ± 0	0.04

3.3.4   Comparison between cultivars and susceptible to resistance ratio

During 1998 clear differences in *D. noxia* numbers occurred between the susceptible and resistant cultivars in the parasitoid-free treatment, showing the efficacy of the resistant cultivars to reduce aphid infestation (compare Fig 3.2A, 3.4A & 3.6A). From 1/10/98 onwards, during the logarithmic growth stage of the aphid population, the difference in the percentage infestation between the three cultivars was significant (Table 3.7).

In the parasitoid treatment however, due to parasitism, the aphid population growth was significantly reduced on Betta (Fig. 3.3A) and the infestation was not significantly different from the resistant cultivars. However, some significant differences were found from 20/10/98 onwards (Table 3.7).

Similar to 1998, the percentage infestation on the parasitoid-free treatment of the 1999 trial differed significantly from 30/09/99 onwards during the logarithmic phase

of population development (Table 3.8), showing the efficacy of the resistant cultivars. In the parasitoid treatment, parasitism again controlled the aphids so effectively on Betta that there were no differences in the percentage infestation between the three cultivars except for those found on 23/9/99 when at the date of parasitoid release and on 14/10/99 (Table 3.8).

Both Gariep and SST 333 contain mainly an antibiotic type of resistance (Du Toit, 1990; Tolmay & Maré, 2001) with reduction in fecundity and size of the aphids. From the current study it is thus apparent that the *D. noxia* resistant genes in Gariep and SST 333 strongly influenced the *D. noxia* population development.

Table 3.7 Statistical differences in percentage infestation between cultivars in each of the main treatments during 1998. (Symbols in rows that differ indicate significant differences between cultivars. P = 0.05, LSD-test)

	Parasitoid-free				Parasitoids			
Date	Betta	Gariep	SST 333	P	Betta	Gariep	SST 333	P
31/8/98	a	b	a	0.001	a	a	a	ns
9/09/98	a	a	a	ns	a	a	a	ns
18/90/98	a	a	a	ns	a	a	a	ns
1/10/98	a	b	b	0.002	a	a	a	ns
14/10/98	a	b	c	0.000	a	a	a	ns
20/10/98	a	b	c	0.000	a	ab	b	0.028
30/10/98	a	b	c	0.000	a	a	a	ns
4/11/98	a	ab	b	0.04	a	ab	b	0.04

Similar feeding symptoms e.g. unrolled leaves and small chlorotic spots were found on both resistant cultivars, although aphid numbers differed between the two cultivars. Biological control by *A. hordei* parasitoids reduced *D. noxia* numbers on the susceptible cultivar Betta to such a level that there was for most of the time no

difference between the susceptible and resistant cultivars in the parasitoid treatment. Both plant resistance and parasitoids are therefore effective control measures for *D. noxia*.

Table 3.8 Statistical differences in percentage infestation between cultivars in each of the main treatment during 1999. (Symbols in rows that differ indicate significant differences between cultivars. P = 0.05, LSD-test)

	Parasitoid-free				Parasitoids			
Date	Betta	Gariep	SST 333	P	Betta	Gariep	SST 333	P
23/09/99	a	a	a	ns	a	b	ab	0.028
30/09/99	a	b	a	0.004	a	a	a	ns
6/10/99	a	b	a	0.04	a	a	a	ns
14/10/99	a	ab	b	0.04	a	b	ab	0.04
25/10/99	a	ab	b	0.04	a	a	a	Ns
4/11/99	a	ab	b	0.04	a	a	a	Ns

As far as the interaction between host plant resistance and parasitoids (natural enemies) are concerned, the model of Van Emden & Wearing (1965) was proposed for partial resistant plants. This model was based on the assumption that reduced mortalities on resistant plants with fewer aphids would combine with lower aphid population growth rates on such plants to prevent the population reaching an economic threshold. Although the same absolute mortalities from natural enemies between different varieties are an unlikely assumption, several studies have reported predator or parasitism levels to increase or to be independent of pest density, across cultivars (Thomas & Waage, 1996). Following the model, a susceptibility to resistance (S/R) ratio was calculated for the percentage infestation. The ratio was calculated for each sampling date and subsequently, the mean ratio was used to determine if the resistance was magnified by the presence of the parasitoids, as demonstrated by Van Emden (1986).

During 1998 the mean susceptible to resistance (S/R) ratio in Betta/Gariep was approximately 22% higher in absence of parasitoids than in the presence of parasitoids (Table 3.9). The Betta/SST 333 ratio in the absence of parasitoids was approximately 41% higher than in the presence of parasitoid (Table 3.9).

Table 3.9   The susceptibility to resistant ratios between various cultivars and treatments for the 1998 trial

Date	Betta/Gariep – Parasitoid-free	Betta/Gariep – Parasitoid	Betta/SST 333 Parasitoid-free	Betta/SST 333 Parasitoid
31/08/98	2.4	1.6	1.1	1.2
9/09/98	1.1	1.3	1.2	1.2
18/09/98	1.6	1.2	1.8	1.2
1/10/98	1.8	1.4	2.8	1.6
14/10/98	2.3	1.7	5.4	2.1
20/10/98	1.9	1.7	8.0	4.8
30/10/98	3.6	2.8	15.0	6.4
4/11/98	3.6	3.0	10.9	8.5
Mean	**2.3**	**1.8**	**5.8**	**3.4**

These results were in contrast to the model of Van Emden (1986), where the S/R ratio in the presence of parasitoids was higher than in the absence of parasitoids.

The S/R ratios for Betta/Gariep and Betta/SST 333 for the 1999 trial are shown in Table 3.10. On 14/09/99 for Betta/Gariep parasitoid treatment as well as on 25/10/99 and 4/11/99 for Betta/SST 333 parasitoid treatment it was not possible to calculate an S/R ratio because the aphid infestation measured in the parasitoid treatment was zero. The mean Betta/Gariep ratio in the presence of parasitoids was approximately 23% higher than in the absence of parasitoids (Table 3.10) showing an enhancement of resistance in Gariep by the parasitism of *A. hordei* parasitoids. The mean Betta/SST 333 ratio was still approximately 67% higher in

the absence than in the presence of parasitoids (Table 3.10) showing a negative interaction between the resistance in SST 333 and the parasitoid *A. hordei.*

During the field trials parasitism was monitored constantly as mentioned above. No other parasitism was found through the entire season in both years. Coccinellid predators were present in such low numbers, form approximately mid October onwards and therefore their influence was not taken into account.

Table 3.10 Susceptibility to resistant ratios between various cultivars and treatments for the 1999 trial

Date	Betta/Gariep – Parasitoid-free	Betta/Gariep – Parasitoid	Betta/SST 333 – Parasitoid-free	Betta/SST 333 - Parasitoid
23/9/99	1.1	3.6	1.6	1.8
30/9/99	2.4	3.3	2.2	1.7
6/10/99	2.4	4.5	1.8	1.6
14/10/99	2.2	-	3.4	1.9
25/10/99	2.6	4.5	8.0	-
4/11/99	4.9	1	15.5	-
Mean	**2.6**	**3.4**	**5.5**	**1.8**

Results during both 1998 and 1999 have shown contrasting mean S/R ratios where higher ratios were found in the absence of parasitoids. This contrast was more conspicuous in the Betta/SST 333 ratios while it happened only during 1998 in the Betta/Gariep ratio. This means that instead of an enhancement of resistance in the presence of parasitoids, the resistance decreased.

According to Thomas & Waage (1996) there is a range of conditions under which the interaction between plant resistance and biological control can be sub-additive, or negative. This means that where plant resistance inhibits the contribution of natural enemy control or *vice versa* to such an extent that the overall effect on the pest population is less than the sum of the effect of each individual factor. This sub-additivity may be caused by the interaction of successive effects of plant

resistance and natural enemy control on the pest life-history parameters or through effects of plant resistance on natural enemy effectiveness. It is also possible that natural enemy control and plant resistance can have substantial synergistic effects on reduction of pest numbers in crops.

Reasons for the unexpected inverted S/R reaction was investigated by calculating the percentage difference between (a) Betta with and without parasitoids, (b) Betta and Gariep/SST 333 both without parasitoids and (c) Betta without parasitoids and Gariep/SST 333 with parasitoids. These differences were calculated for both years from the first date after parasitoid release (Tables 3.11 & 3.12).

Table 3.11 Differences in percentage infestation between Betta without parasitoids and other treatments during 1998

Date	Percentage difference between Betta without parasitoids and…				
	Betta with parasitoids	Gariep without parasitoids	Gariep with parasitoids	SST 333 without parasitoids	SST 333 with parasitoids
9/09/98	-15.2	4.3	13.7	18.0	5.8
18/09/98	54.6	38.5	63.2	45.0	63.2
1/10/98	72.3	45.3	87.0	64.6	82.2
14/10/98	71.7	56.4	83.2	81.6	86.4
20/10/98	58.9	47.0	76.5	87.5	91.4
30/10/98	64.6	73.4	87.3	93.3	94.5
4/11/98	51.6	72.4	83.6	90.8	94.3
Mean	**51.2**	**48.1**	**70.6**	**68.7**	**74**

From the differences in 1998 (Table 3.11) it became clear that *D. noxia* on Betta in the parasitoid treatment was parasitised to such an extent that the reduction in infestation on Betta due to parasitism was more than the reduction in infestation on Gariep without parasitoids.

In the parasitoid treatment, infestation on Gariep was reduced by parasitism and the resistance therefore enhanced from 9/09/98 onwards (Table 3.11). When taking the mean percentage difference into account (Table 3.11) adding parasitism to Gariep, reduced the infestation further by 22.5% to become 70.6% less than the infestation on parasitoid free susceptible Betta. This means that parasitism adds on 22.5% to the reduction in infestation on Gariep, which was lower than the reduction in infestation on Betta.

When comparing parasitoid-free SST 333 and Betta it is clear that on 18/09/98 and 1/10/98 the reduction in infestation on SST 333 was lower than the reduction in infestation due to parasitism on Betta. However, from 14/10/98 onwards the effect was inverted, showing that the resistance in SST 333 had a stronger reduction effect on infestation in comparison with the effect of parasitism on Betta (Table 3.11). Adding parasitism to SST 333, the mean reduction in infestation was further reduced by 5.3% to reach 74% less than infestation on parasitoid-free Betta (Table 3.11). This means that parasitism adds on only 5.3% reduction in infestation, which is much lower than in Gariep.

Comparing the means in Table 3.11 it is clear that the reduction in infestation due to resistance in SST 333 was 20.6% higher than in Gariep. When parasitism was added to the resistance the joint reduction in SST 333 was only 3.4% higher than on Gariep. The conclusion could therefore be made that the higher the effect of resistance alone the lower the effect of parasitism when plant resistance and parasitoids were added together. The same conclusion could be made when comparing the big (5.8 vs 3.4) difference between the S/R ratios for Betta/SST 333 compared to the smaller (2.3 vs 1.8) difference in S/R ratios for Betta/Gariep (Table 3.9).

Although enhancement of resistance by parasitoids was shown during 1999 with the Betta/Gariep ratios, the reduction in infestation in Betta due to parasitism was, as in 1998, higher than the reduction in Gariep due to resistance alone (Table 3.12). Adding parasitism to resistant Gariep, reduced the infestation on Gariep by 32,7% to become 95% less than the infestation on parasitoid-free Betta. During 1999 parasitism therefore adds on 32.7% reduction in infestation on Gariep.

The same happened with SST 333 (Table 3.12). Similar to 1998, parasitism reduced the infestation on Betta more than the resistance did in SST 333. The mean reduction in infestation on SST 333 was further reduced by 18% when parasitism was added, to become 88.1% less than parasitoid-free Betta (Table 3.12). This means that parasitism added 18% on to the reduction in infestation on SST 333

Table 3.12 Differences in percentage infestation between Betta without parasitoids and other treatments during 1999

| Date | Percentage difference between Betta without parasitoids and… | | | | |
	Betta with parasitoids	Gariep without parasitoids	Gariep with parasitoids	SST 333 without parasitoids	SST 333 with parasitoids
30/9/99	49.8	58.7	84.5	53.7	70.8
6/10/99	60.9	57.9	91.4	44.9	75.7
14/10/99	89.0	53.8	100	70.7	94.1
25/10/99	97.6	61.5	99.5	87.5	100
4/11/99	99.5	79.4	99.5	93.6	100
Mean	**79.4**	**62.3**	**95**	**70.1**	**88.1**

When comparing the means in Table 3.12 it is clear that reduction in infestation due to resistance was 7,8% higher in SST 333 than in Gariep. Adding parasitism, the joint reduction in infestation on SST 333 was 6,9% lower than on Gariep, showing a sub-additive interaction between plant resistance and parasitoids.

A reason for the inverted S/R ratios could therefore be the huge reduction in infestation due to parasitism on Betta. This reduction was in both years higher than the reduction caused by the resistance alone except for SST 333 in 1998 and

thus resulted in low S/R ratios. On the other hand was it clear that the antibiotic resistance strongly reduced infestation in both SST 333 and Gariep. Although effective reduction in infestation was obtained when plant resistance and parasitoids were added there was a sub-additive effect in the interaction between them resulting in inverted S/R ratios.

Van Emden (1986) reported on contrasting results found where a partial resistant Brussels sprout variety released a lower concentration of volatile substances than a susceptible one, which attracted the parasitoids more towards the susceptible cultivar than to the partial resistant one. The antibiotic nature of resistance mentioned by Du Toit (1989) may in the current study also involve herbivore induced allelochemicals that may interact with the natural enemies. Parasitoids are known to react to volatiles from wheat (Braimah & Van Emden, 1994; Van Emden *et al.*, 2002). However, conditioning of natural enemies to the host plant of the aphid was also recorded (Verkerk, *et al.,* 1998). The reason for the high level of parasitism of *D. noxia* on susceptible Betta may therefore be that Betta released more volatiles than the resistant cultivars. The parasitoids on the other hand may become conditioned to Betta during rearing in the greenhouse and preferred the aphids on this cultivar and this phenomenon should be investigated.

For effective control of *D. noxia* it is therefore recommended to have a positive interaction between resistant cultivars and the natural enemies available. According to Thomas & Waage (1996), theory and practical experience with pest resurgence following pesticide application revealed that natural biological control is an important mechanism in ensuring the durability of plant resistance through its action to reduce pest densities and selection pressure on resistant varieties. The action of natural enemies is therefore a critical component of an effective resistance management system and negative interaction between resistant cultivars and natural enemies may influence the durability of resistance.

In perennial habitats, the remedial action of natural enemies after release could be followed by the long-term prevention of pest resurgence by the natural enemy. In these habitats natural enemies have time to lower epidemic pest populations below a threshold and sustain the lower numbers (Wiedenmann & Smith, 1997).

In ephemeral habitats, however, sequential constrains may prevent long-term establishment and sustainable control. Control in these ephemeral habitats could, however, be possible when the latent phase of population growth is targeted. Early immigrant mortality could keep the pest population size low and thus delay the onset of population growth (Wiedenmann & Smith, 1997). The early release of high numbers of *A. hordei* in both trials demonstrated this effect in spite of the negative interaction that was identified between the resistant cultivars and natural enemies. Thus, if *A. hordei* could not establish somewhere in the agro-ecosystem the use of inundative releases of this parasitoid should be investigated.

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CHAPTER 4

THE EFFECT OF RUSSIAN WHEAT APHID INDUCED VOLATILES FROM DIFFERENT WHEAT CULTIVARS ON THE HOST HABITAT LOCATION BY PARASITOIDS.

4.1   Introduction

Plants have developed a number of direct chemical and morphological defences to limit herbivore attacks.   Direct chemical defenses include production of toxins, volatile organic compounds and digestibility reducers, while morphological defences include trichomes, spines and tough foliage (Takabayashi *et al.,* 1998; Cortesero *et al.,* 2000; Dicke & Van Loon, 2000; Farag *et al.*, 2005).   Plants produce mixtures of volatiles that may differ in quantity released per unit of plant biomass and also in the composition of the volatile blend.   The change in composition can be quantitative i.e. changed ratios of the same components, or qualitative, by the release of compounds that do not occur in the blend emitted by the intact plant (Paré & Tumlinson, 1999; Dicke & Van Loon, 2000; Farag *et al.*, 2005).

Herbivore induced plant odours play a major role in the foraging behaviour of predatory and parasitic arthropods (Turlings *et al.,* 1998; Arimura *et al.*, 2000; Fatouros *et al.,* 2005).   Long distance semiochemicals from the host on which natural enemies can rely are difficult to detect because herbivores evolved to be inconspicuous and do not release high levels of volatiles (Powell *et al.*, 1998). Therefore more detectable plant volatiles allow habitat selection although they are not effective in host location (Vet & Dicke, 1992; Powell *et al.* 1998; Cortesero *et al.*, 2000; Kalule & Wright, 2004).   Volatiles from wheat plants are also involved in habitat selection of parasitoids (Powell & Zhang, 1983; Wickremasinghe & Van Emden, 1992; Van Emden, 1995; Quiroz *et al.*, 1997).

Volatiles from different crop cultivars may influence the number of natural enemies responding to these chemical cues (Kalule & Wright, 2004).   Variation in the volatile profile of insect resistant cultivars thus may influence the tritrophic

interactions. Thomas & Waage (1996) indicated that the application of host plant resistance and natural enemies to a particular pest could give significantly better or worse results than expected from the simple combination of the effects of each factor. This is also true for aphids and studies have shown that resistant host plants not only affect the pest aphids feeding on them, but this effect is also passed on by the aphid to their natural enemies (Van Emden, 1991; 1995; Feuntes-Conteras *et al*., 1996; Verkerk *et al*., 1998). Chemicals such as alkaloids, which are involved in plant resistance, can be toxic to parasitoids developing within hosts or prove to be toxic to aphid predators such as ladybirds and hover fly larvae (Herzog & Funderburk, 1985). These disadvantages, however, are not apparent at low levels of plant resistance. There is a potential for more beneficial interactions between biological control agents and partial plant resistance, suggesting that there is a disadvantage in seeking a level of plant resistance greater than necessary when other restraints (natural enemies) are present in a pest management system (Wickremasinghe & Van Emden, 1992).

The compatibility of plant resistance to the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) with natural enemies has been studied in a few cases in the USA. Reed *et al*. (1991, 1992) found that resistant triticale with high levels of antibiosis, negatively affected growth and reproduction of both *D. noxia* and the parasitoid *Diaeretiella rapae* (McIntosh)*.* During the same study a resistant wheat entry, however, showed a reduction in aphid populations and enhanced parasitoid activity due to the fact that the leaves did not roll close thereby exposing the aphids to parasitism. Brewer *et al*. (1998) found that *D. noxia* was parasitised at approximately equal rates on resistant and susceptible barley lines. Farid *et al*., (1998a, b) found that two different resistant wheat lines had no negative effect on the parasitoid *D. rapae*, even after three parasitoid generations. Compatibility and possible complementary associations were reported between *D. noxia* and the ladybird predator *Scymnus frontalis* (Fabricius)(Farid *et al*., 1997).

In South Africa three different sources of plant resistance namely PI 137739, PI 262660 and PI 294994 were most successfully used in breeding resistant cultivars and therefore more than one cultivar are containing the same resistance gene (Tolmay & Van Deventer, 2005). The level of resistance when bred into different

genetic backgrounds differed, causing different resistant levels in cultivars containing the same gene (Tolmay & Van Deventer, 2005). Field studies on the effect of different resistant cultivars in the presence of an introduced parasitoid *Aphelinus hordei* (Kurdjumov), showed some enhancement of plant resistance, but also a difference between the effectiveness of parasitoids on the different cultivars (Chapter 3). Volatiles released by different crop cultivars may influence the host habitat location ability of parasitoids (Kalule & Wright, 2004).

The aim of this study was to determine if volatiles are involved in the host habitat location of parasitoids of *D. noxia*, and what influence volatiles from different plants have on the host habitat location and attack rate of these parasitoids. Two different parasitoids were used namely *A. hordei*, which tends to prefer *D. noxia* as a host (Prinsloo, 2000) and *D. rapae*, which is known to include *D. noxia* in its wide host range (Pike *et al*., 1999).

4.2   Materials and methods

4.2.1 Insect cultures

The *D. noxia* cultures were established from aphids collected from volunteer wheat on the experimental farm at ARC-Small Grain Institute (ARC-SGI), Bethlehem, and new aphids were introduced annually. Aphids were maintained on wheat (variety Betta) in a greenhouse at a temperature of $23 \pm 2°C$ and ambient light conditions with 14 L:10 D. The *A. hordei* and *D. rapae* parasitoid cultures were reared on *D. noxia* under the same greenhouse conditions as the aphids.

4.2.2 Olfactometer

Trials were conducted in the same way for both parasitoid species. A Y-tube olfactometer was used to test the parasitoids response to different plants. It measured 15 mm in diameter and each arm was 150 mm long. Two, one-litre non-transparent, tight sealing PVC bottles were used as odour source containers. The inlet of both containers was connected to the same activated charcoal filter. An airflow rate of 600 ml min^{-1} through the base of the olfactometer was

determined (cold smoke test) to create laminar airflow throughout the tube. The parasitoids, which are not strong fliers, were able to walk easily against this air current.

After emergence, a wheat plant was transplanted into a 100 ml plastic vial, covered with a lid with a hole in it, through which the plant could grow. In this way odour from the soil surface was minimised in the system. Plants were ready for use when they were at the four-leaf stage (25 – 30 days old) and the whole plant was placed into the odour source container. When infested plants were needed, approximately 200 aphids were placed on a plant and left for three days before being used.

For both parasitoid species, mummified aphids were removed from plants and kept together in a mesh covered vial until emergence ensuring no contact with aphids. After emergence they were fed by a drop of honey, and were allowed to mate. Four of these naïve, honey fed and mated parasitoid females were introduced simultaneously at the base of the olfactometer. From introduction the number of parasitoids in each arm of the olfactometer were recorded at 30 sec. intervals for five minutes. A clean Y-tube was used for each group of four females tested. After twenty female wasps were tested the plant in the odour source container was replaced with a fresh one and the collected data were pooled as one replicate. Parasitoids that did not walk into any of the arms and reached a maximum distance of 100 mm from the base of the arm were recorded as not responding. A replicate was discarded and repeated when less than 50 % of the females were responding. To ensure that the same conditions were applied to all treatments, each treatment was repeated once per day in a randomised block design where days were presenting blocks. The data were analysed using an ANOVA for a randomised block design (Van Ark, 1992).

4.2.3 Bias test

To test for bias in the system a trial was run with clean blank odour sources at first. The trial was repeated six times as described above and 120 *A. hordei* females were used.

4.2.4   Clean plant test

Following the bias test, a clean (not infested) Betta plant was tested against a blank odour source, to determine if *A. hordei* and *D. rapae* would respond to volatiles coming from a clean wheat plant. The trial was replicated ten times for each parasitoid species using 200 females.

4.2.5   Cultivar test

Test material consisted of five different cultivars namely Betta (susceptible), Elands (resistant, containing the Dn1 gene), SST 333 (resistant, containing the Dn2 gene), Tugela (susceptible) and TugelaDN (resistant containing the Dn1 gene).   Different choice tests were conducted (Table 4.1) to determine if parasitoids could distinguish between infested and aphid-free plants, as well as between different cultivars. Each test was repeated 15 times using 300 female wasps per test.

Table 4.1   **Different treated plants (A, B) tested against each other (A *versus* B) in the Y-tube olfactometer.**

A	B
Betta aphid free	Betta infested
Elands aphid free	Elands infested
SST 333 aphid free	SST 333 infested
Betta infested	Elands infested
Betta infested	SST 333 infested
Elands infested	SST 333 infested
TugelaDN aphid free	TugelaDN infested
Tugela infested	TugelaDN infested

4.2.6   Plant *versus* aphid test

Parasitoid females were tested to determine if they were responding to odours emitted by infested plants or to chemicals emitted by the aphids. Aphids were gently brushed from an infested Betta plant an hour before the test was conducted. The plant was then thoroughly washed with cold water and left until the leaves were dry. This plant was tested against approximately 0.5 g of living *D. noxia* (approximately 2000) contained in a fine gauze sachet hanging inside the odour source container to ensure airflow through the aphids. The trial was repeated ten times.

4.2.7   Attack rate study

The trial was conducted in three greenhouse cubicles at conditions described in 4.2.1. The cultivars Betta, Elands and SST 333 were used in three combinations namely Betta – Elands, Betta - SST 333 and Elands – SST 333.   Eight pots containing two plants each were used per cultivar in each combination. Four pots per cultivar were infested with 100 adult *D. noxia* aphids, three days before the trial was started and four pots were kept clean. The 16 pots used per combination were randomly arranged in a square block on a bench in the cubicle. Respectively 10 and 20 honey fed, mated, naïve *D. rapae* and *A. hordei* females were released from a 10 ml plastic tube 30 cm above the middle of the trial. They were allowed 24 hours to parasitise the aphids, after which the infested plants were taken out and the parasitoids carefully removed. These pots were caged individually for ten days and the number of parasitised aphids per pot was then determined. Ten replicates were conducted as a randomised block trial over a ten day period. The data were analysed as a randomised block ANOVA, after which the data were pooled and the difference between the total number of mummies on each cultivar was determined using a Chi-square test (Van Ark, 1992).

4.2.8   Collection of plant volatiles

Volatiles were collected from ten potted plants of Betta, Elands and SST 333 respectively, similar to methods followed by Pettersson *et al.* (1994).   Dry air,

which was purified by passing it through 5 Å molecular sieves and activated charcoal, was drawn through a 3 l bell jar containing ten plants of a specific cultivar at a rate of 800 ml min^{-1}. Volatiles were collected over a period of 48 hours from clean plants and plants which were infested with more than ten aphids/plant for three to five days. Volatiles were trapped in Porapak Q and Tenax, which had been purified with approximately 5 ml of diethyl ether and heated for eight hours at 190°C under a constant flow of nitrogen gas.

4.2.9 Analysis of plant volatiles

The Porapak Q and Tenax tubes were analysed differently. For Porapak Q, the tubes were eluted with 500 μl freshly redistilled diethyl ether. Gas chromatography (GC) was then performed on 4 μl of this solution injected onto a HP-1 capillary column (50 m x 0.32 mm i.d. x 0.52 μm film thickness) through a cool-on-column injector with a 1 m-retention gap (0.53 mm i.d. inert capillary). Hydrogen was used as carrier gas and the oven was programmed as follows: 30°C for 0.5 min, 5°C min^{-1} to 150°C, 0.1 min at 150°C then 10°C min^{-1} to 250°C and maintained for 15 minutes. Detection was by flame ionisation detector. For Tenax, the GC conditions were the same, but thermal desorption of the Tenax tubes transferred the entire collection of volatiles onto the entrance of the column. This was achieved by inserting the tube into an OPTIC 2 unit (Anatune Ltd) and heating this at 16°C s^{-1} to 220°C.

4.3   Results

4.3.1 Bias test

The number of *A. hordei* females observed in the right *versus* the left arms did not differ significantly ($F_{1,10}$ = 1.4, P = 0.27)(Fig. 4.1). The total number of females observed in both arms were then compared to the number that remained in the leg of the Y-tube (no response) and again no significant difference was found (Wilcoxon t = 3, P = 0.3)(Fig. 4.1). It was therefore assumed that there was no

bias in the system and that the tests could be conducted under the same conditions.

4.3.2 Clean plant test

Significantly more *A. hordei* females were responding to the clean Betta plants than to the blank odour source (F = 5.62, P = 0.03, LSD = 3,7)(Fig. 4.1). The *D. rapae* females were also responding significantly more to a clean Betta plant than to a empty odour source ($F_{1,19}$ = 10.8, P = 0.004, LSD 16.1) (Fig. 4.2).
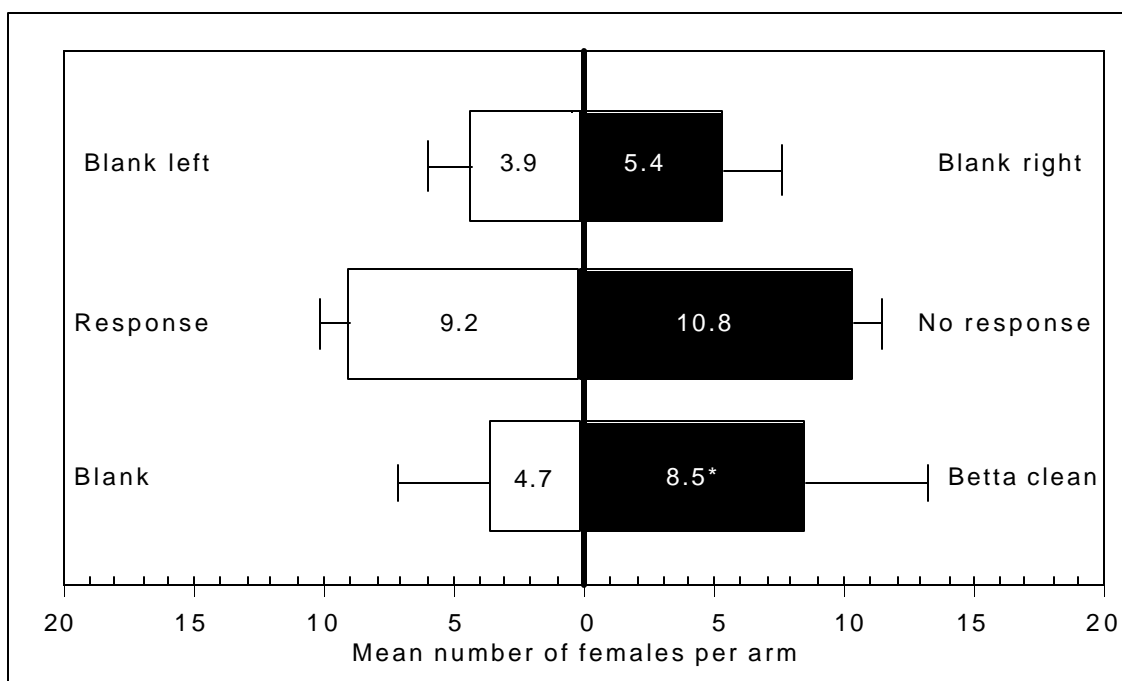
Figure 4.1   Response of *Aphelinus hordei* parasitoid females during the bias and clean Betta plant *versus* blank odour source tests (* = significant difference)

4.3.3 Cultivar test

The results of the response of *D. rapae* are summarised in Figure 4.2 and the results of *A. hordei* are summarised in Figure 4.3. Although the response of the parasitoid females was not very strong significant differences were found in several instances.   The *D. rapae* females were attracted significantly more to
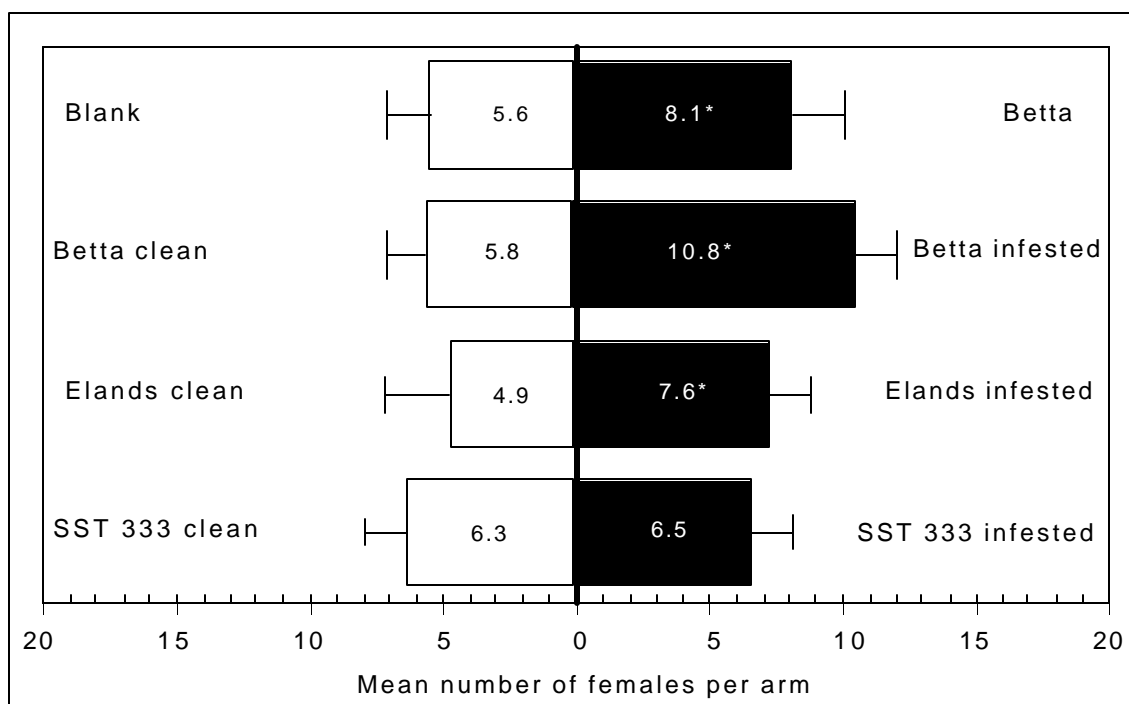
Figure 4.2 Response of *Diaeretiella rapae* females to infested and clean plants of different wheat cultivars in a Y-tube olfactometer (* = significant difference).
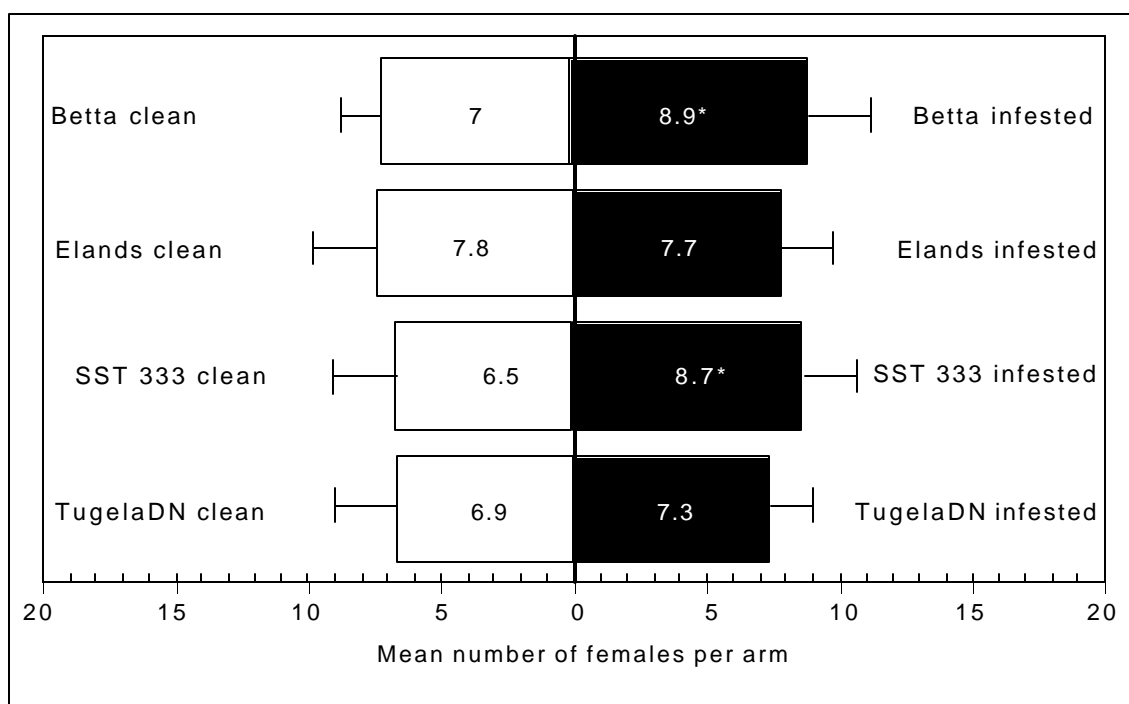
Figure 4.3 Response of *Aphelinus hordei* females to infested and clean plants of different wheat cultivars in a Y-tube olfactometer (* = significant difference).

infested than to clean susceptible Betta plants ($F_{1,19}$ = 46.5, P < 0.001, LSD = 1.45) (Fig. 4.2). This was also true for the *A. hordei* females ($F_{1,29}$ = 5.413, P = 0.036, LSD = 1.81)(Fig. 4.3).

Infested resistant Elands plants, containing resistance from PI 137739, attracted significantly more *D. rapae* females than clean Elands plants ($F_{1,19}$ = 6.82, P = 0.018, LSD = 2.1)(Fig. 4.2), while *A. hordei* was not able to distinguish between these treatments ($F_{1,29}$ = 0.000, P = 0.984)(Fig. 4.3). *Diaeretiella rapae* females, however, did not distinguish between infested and clean plants of resistant SST 333 containing resistance from PI 262660 ($F_{1,19}$ = 0.13, P = 0.72)(Fig. 4.2). *Aphelinus hordei* was significantly attracted to infested resistant SST 333 ($F_{1,29}$ = 5.141, P = 0.04, LSD = 2.1)(Fig. 4.3).

The infested plants of resistant TugelaDN, containing resistance from PI 137739 were also not significantly more attractive to females of *A. hordei* ($F_{1,19}$ = 0.135, P = 0.72)(Fig. 4.3) or *D. rapae* ($F_{1,19}$ = 0.733, P = 0.4)(Fig. 4.4). The near isogenic susceptible Tugela was tested with *D. rapae* only and the females significantly preferred the infested plants to the clean plants ($F_{1,19}$ = 13.9, P = 0.002)(Fig. 4.4).

Aphelinus hordei females did not distinguish significantly between infested Betta and infested Elands plants when tested at first ($F_{1,29}$ = 0.483, P = 0.498). This test was therefore repeated two times more and no significant differences were found in the second ($F_{1,27}$ = 1.149, P = 0.294) and a third test ($F_{1,27}$ = 0.163, P = 0.694)(Fig. 4.5). *Diaeretiella rapae,* however, was significantly more attracted to infested Betta than infested Elands ($F_{1,19}$ = 12.6, P = 0.002, LSD = 1.72)(Fig. 4.6).

Both *D. rapae* and *A. hordei* were significantly more attracted to infested Betta than to infested SST 333 (*D. rapae*: $F_{1,19}$ = 31.82, P = 0.000, LSD = 1.7)(Fig. 4.6) (*A. hordei*: $F_{1,29}$ = 12.5, P = 0.003, LSD = 1.6)(Fig. 4.7). *Diaeretiella rapae* found SST 333 more attractive than Elands ($F_{1,19}$ = 7.14, P = 0.02, LSD = 2.2) (Fig. 4.6), while *A. hordei* found Elands more attractive than SST 333 ($F_{1,14}$ = 6.9, P = 0.02, LSD = 2.6)(Fig. 4.7). Both parasitoid species were significantly more attracted to susceptible Tugela than resistant TugelaDN (*D. rapae*: $F_{1,9}$ = 8.862, P = 0.008, LSD = 2.4) (Fig. 4.6) (*A. hordei*: $F_{1,9}$ =16.8, P = 0.003, LSD = 2.8)(Fig. 4.7).
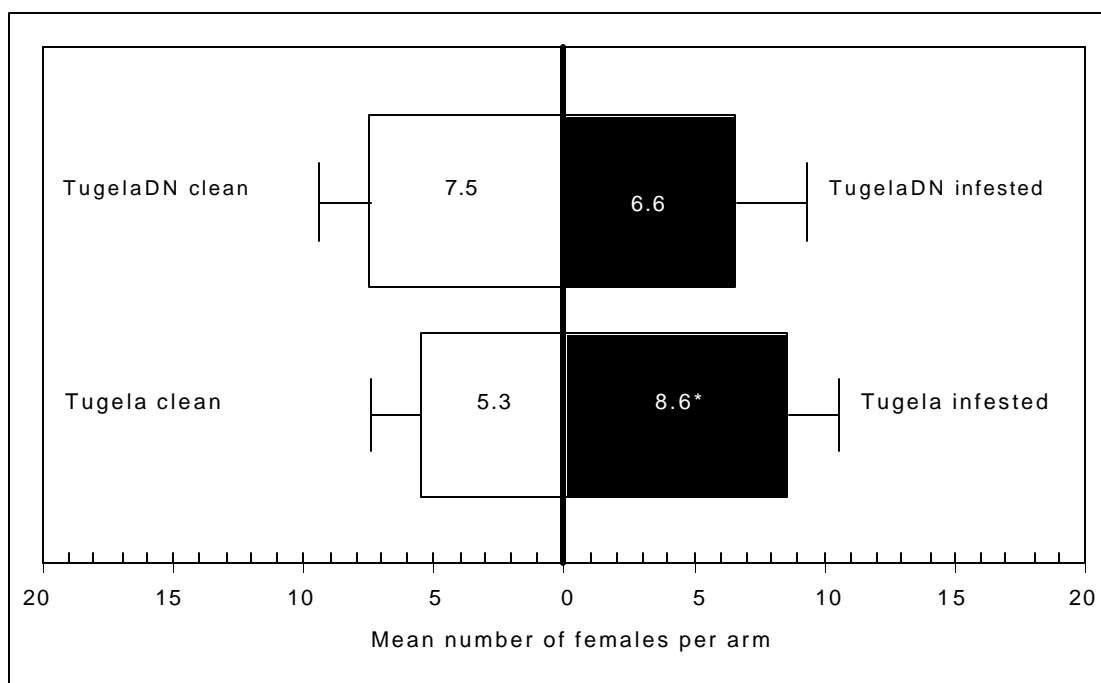
Figure 4.4 The response of *Diaeretiella rapae* to infested and clean plants of susceptible Tugela and resistant TugelaDN (* = significant difference).
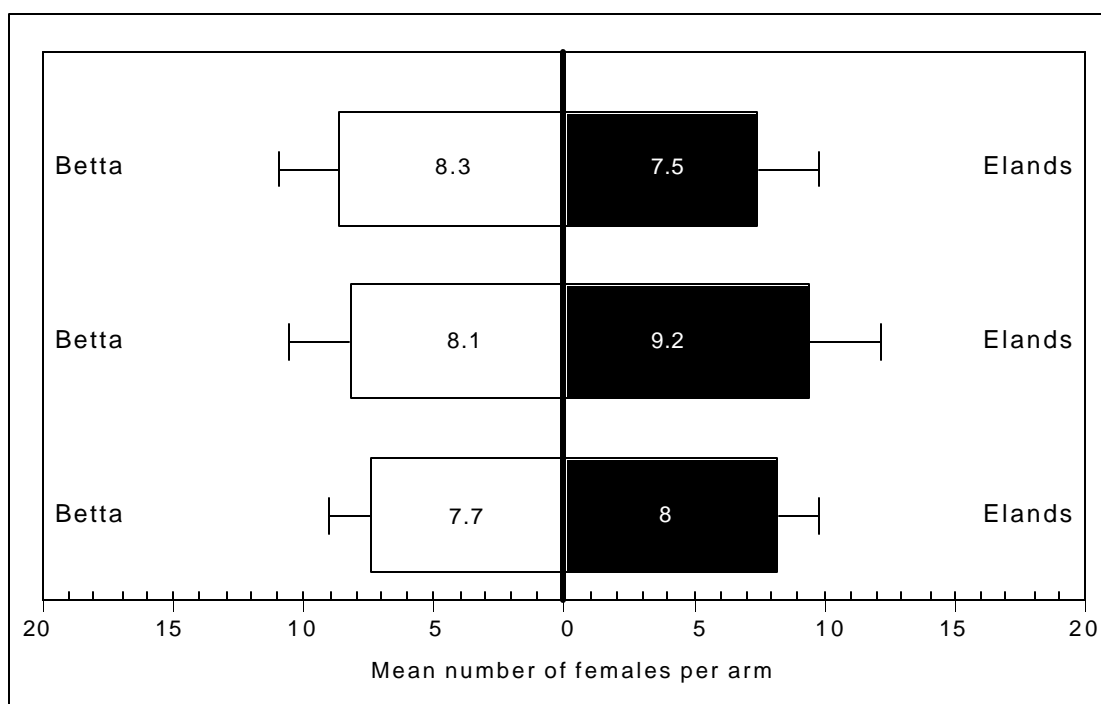
Figure 4.5 Response of *Aphelinus hordei* females to infested Betta and Elands plants in a Y-tube olfactometer (* = significant difference). Test was repeated three times.
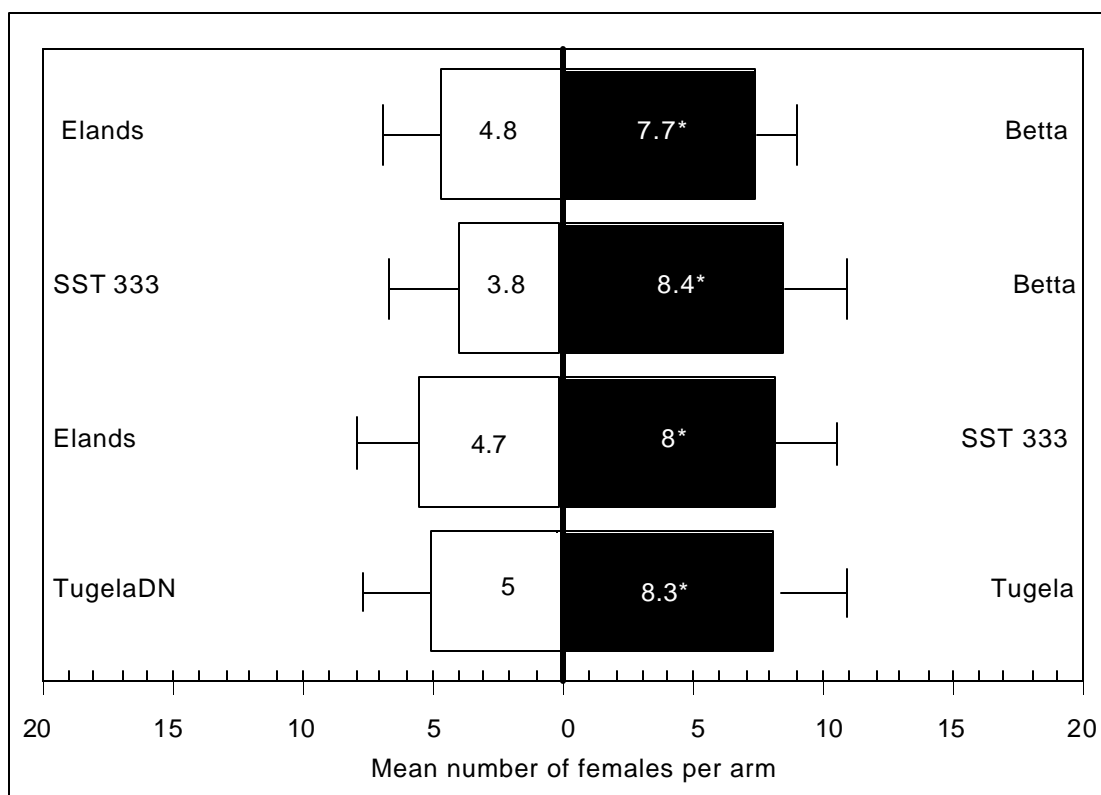
Figure 4.6 Response of *Diaeretiella rapae* to different infested cultivars in a Y-tube olfactometer (* = significant difference).
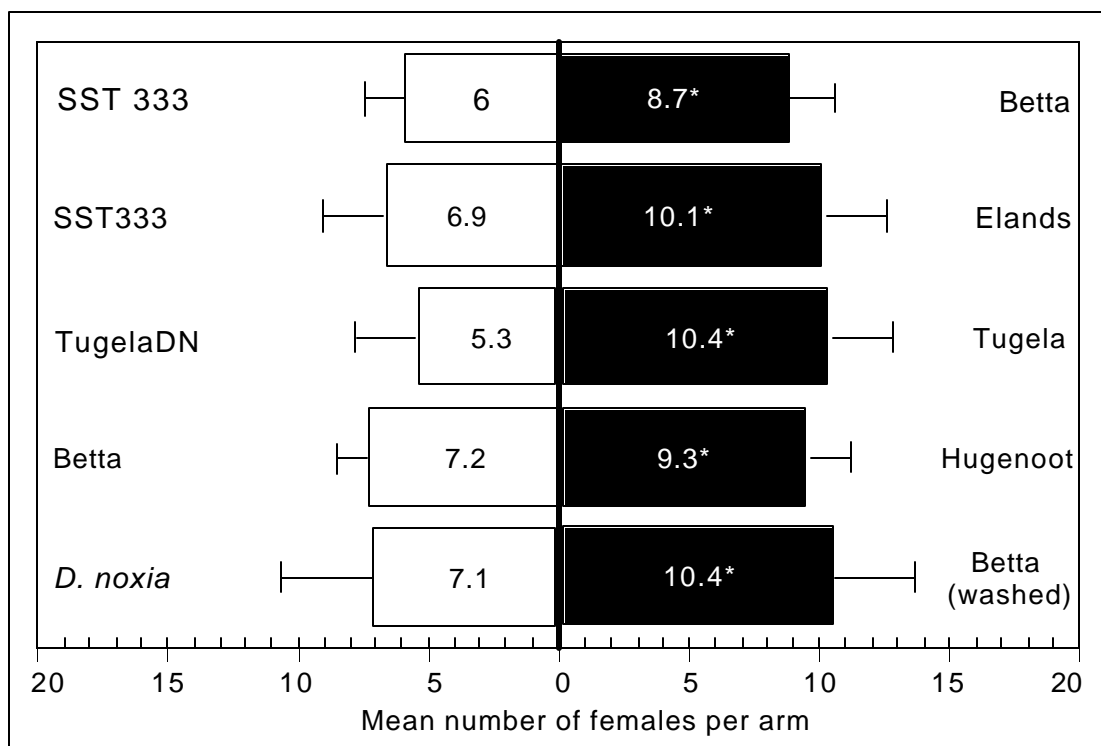
Figure 4.7 Response of *Aphelinus hordei* to different treated cultivars and aphids in a Y-tube olfactometer (* = significant difference).

According to Wickremasinghe & Van Emden (1992) parasitoids became conditioned to the host plant and the aphids they were reared on. The susceptible cultivar Hugenoot was used to test for conditioning of *A. hordei* because this parasitoid was reared on *D. noxia* on Betta. *Aphelinus hordei* parasitoids were more attracted to Hugenoot than Betta ($F_{1, 29}$ = 17.691, P < 0.001, LSD = 10.58)(Fig.4.7) and the possibility of conditioning is thus ruled out.

4.3.4 Plant *versus* aphid test

Infested wheat plants from which all aphids were removed, washed with running water and dried off, were significantly more attractive to *A. hordei* than to the a group of aphids itself ($F_{1,29}$ = 8.28, P = 0.008, LSD = 2.42)(Fig. 4.7).

4.3.5 Attack rate study

4.3.5.1 *Aphelinus hordei*

When the mean number of mummified aphids per replication was analysed no significant differences were found between the numbers of mummies per cultivar. However, when the total number of mummies found on each cultivar for each cultivar combination were analysed differences were found. *Aphelinus hordei* parasitised significantly more aphids on Betta than on SST 333 in the Betta – SST 333 combination (x^2 = 86.8, P < 0.001)(Fig. 4.8). Significant more *A. hordei* mummies were also found on Betta in the Betta - Elands combination (χ^2 = 28.38, P < 0.001)(Fig. 4.8) while no significant difference was found in the Elands – SST 333 combination (Fig. 4.8).   The total number of mummies in the Betta – Elands combination was significantly higher than in the other two combinations (χ^2 = 50.32, P < 0.0001). No difference was found between the Betta - SST 333 and Elands – SST 333 combinations.
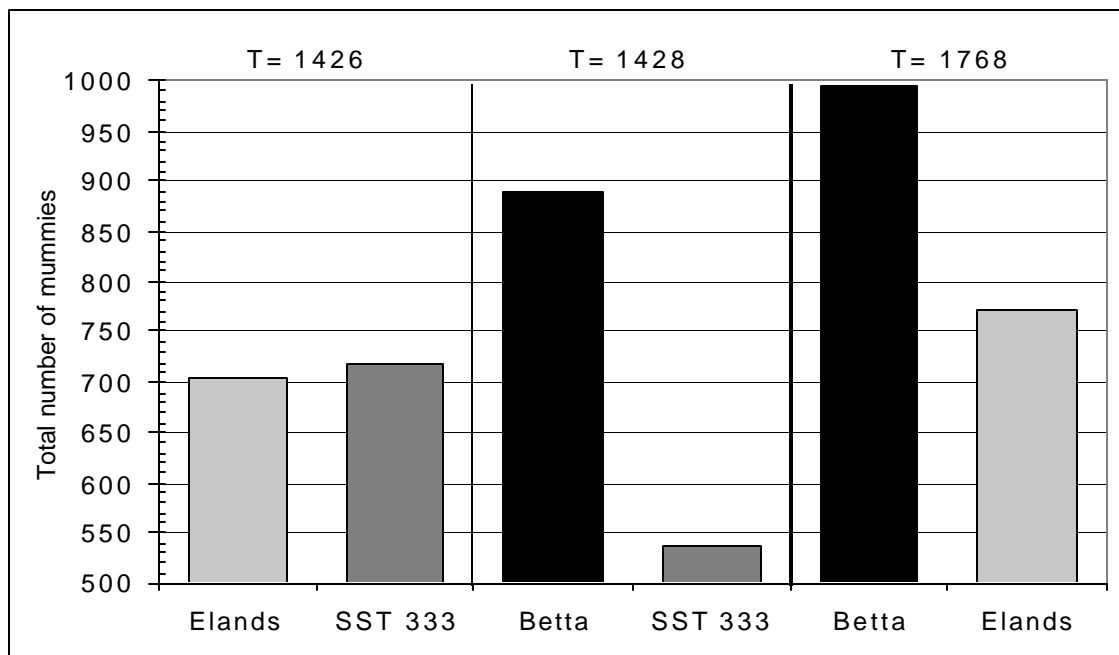
Figure 4.8   Total number of *Aphelinus hordei* mummies found on each of the cultivars for each of the cultivar combinations. T = total number of mummies for the cultivar combination.

4.3.5.2 *Diaeretiella rapae*

In the *D. rapae* trial significant more aphids were parasitised on Betta than on SST 333 in the Betta – SST 333 combination (χ^2 = 86.1, P < 0.0001) (Fig. 4.9). In the Elands – SST 333 combination significantly more aphids were parasitised on Elands (χ^2 = 11.95, P = 0.001) (Fig. 4.9). No significant difference was found in mummy numbers on the different cultivars in the Betta – Elands combination (Fig. 4.9). The number of aphids mummified by *D. rapae* was lower than those of *A. hordei* due to the different numbers of parasitoid females used.

The total number of mummies in the Betta – SST 333 combination was significantly higher than the Elands – SST 333 combination (χ^2 = 13.459, P < 0.001) and the Betta – Elands combination (χ^2 = 54.596, P < 0.001). The total number of mummies in the Elands – SST 333 combination was significantly higher than in the Betta – Elands (χ^2 = 14188, P < 0.001) combination.
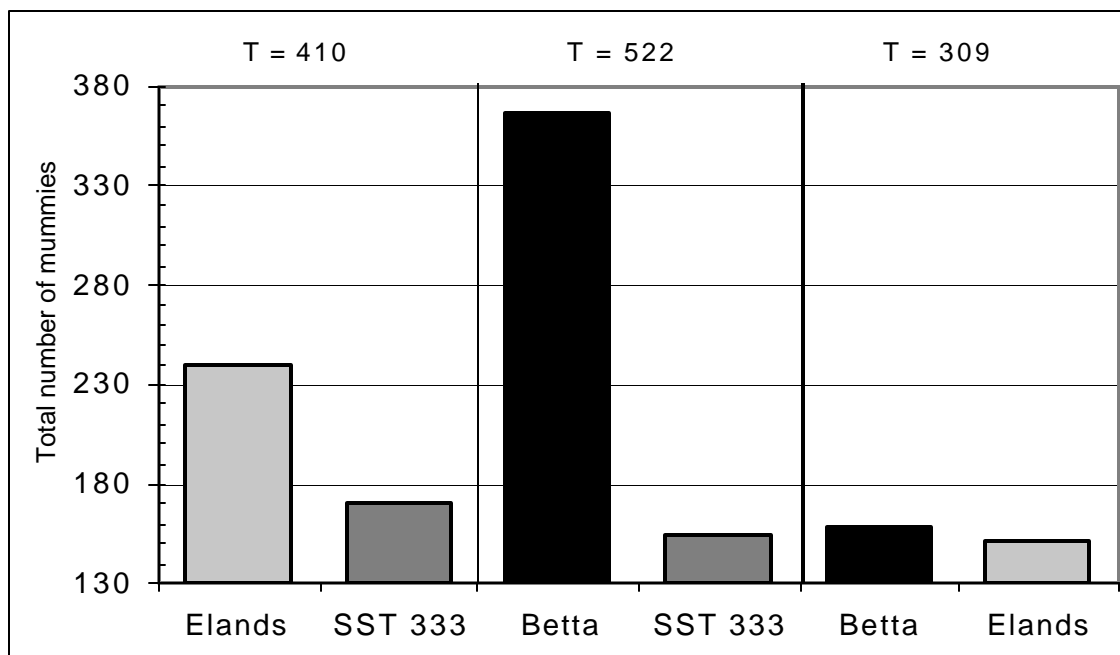
Figure 4.9 Total number of *Diaeretiella rapae* mummies found on each of the cultivar for each of the cultivar combinations. T = total number of mummies for the cultivar combination.

4.3.6 Analysis of plant volatiles.

Although the different substances were not identified yet, clear differences were visible between the number and quantity of volatiles released by the different cultivars (Fig. 4.10). The retention time of some of the most prominent peaks were calculated and marked (A,B, etc.) for comparison between the different infested cultivars. When Betta and Elands were compared, Elands lacked a K peak, while B and P were absent from Betta. In SST 333, A, G, I, N and P were absent while quantitative differences were present in the same peaks between the different cultivars. These volatiles should be identified in the near future to ensure that these volatiles could be tested for attraction of the different parasitoids.
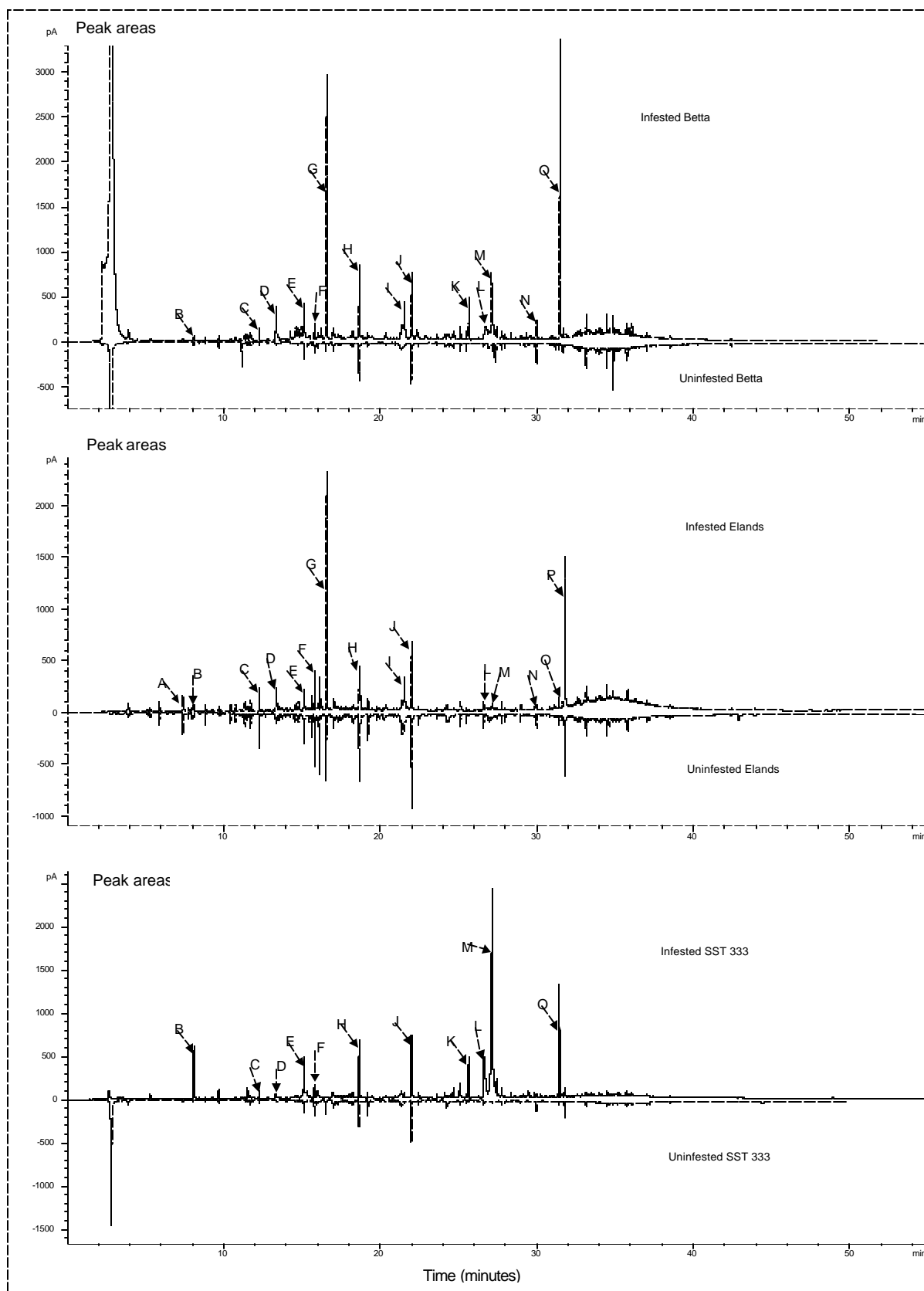
Figure 4.10   Gas chromatograms of volatile profiles of infested and clean plants of the cultivars, Betta, Elands and SST 333 (A-P indicates corresponding peaks i.e. volatiles)

4.4 Discussion

The induced production of volatile cues, which guide natural enemies to their host habitat, is one of various ways in which crop plants influence the efficiency of natural enemies (Takabayashi *et al.,* 1998). Plant breeding may affect these characteristics of plants and thereby positively or negatively contribute to the efficiency of an integrated control programme (Thomas & Waage, 1996).

In all the trials both parasitoid species had emerged from mummies that were collected from the host plant Betta and kept in plastic vials. The response of the females that were used during the trials could therefore not have been affected by the experience on the host plant after emergence. Both *A. hordei* (Fig. 4.3) and *D. rapae* (Fig. 4.2) significantly preferred a clean Betta plant to an empty odour source thus demonstrating that they responded to volatile substances from plants.

It could be expected that *D. noxia* feeding on the different cultivars would induce more volatile chemicals to be released by the infested plants and that parasitoids would be attracted significantly more to them. *Aphelinus hordei* responded significantly more to infested Betta and SST 333 than to the clean plants of these cultivars (Fig. 4.3). They were, however, not able to distinguish between infested and clean Elands and infested and clean TugelaDN (Fig. 4.3). Both Elands and TugelaDN are containing resistance from PI 137737 (Tolmay & Van Deventer 2005). *D. rapae* responded significantly more to infested plants of Betta, Tugela and Elands than to clean plants of these cultivars (Figs. 4.2, 4.4). They did not distinguish between infested and clean SST 333 and clean and infested TugelaDN, containing resistance from PI 262660 and PI 137737 respectively (Tolmay & Van Deventer 2005).

When infested plants of these cultivars were tested against each other, *A. hordei* preferred Betta to SST 333, Elands to SST 333 and Tugela to TugelaDN (Fig. 4.7). This parasitoid was however not able to distinguish between Betta and Elands (Fig. 4.5). It thus seems that when given a choice, *A. hordei* preferred the susceptible to the resistant cultivars, with the exception of Elands. *Diaeretiella rapae* seems also to prefer susceptible to resistance cultivars when a choice is

offered. They preferred Betta to Elands, Betta to SST 333, SST 333 to Elands and Tugela to TugelaDN (Fig. 4.6).

Differences in the volatile profiles from the different cultivars could give some explanation for the different responses from parasitoids. When the volatile profiles of infested and clean Elands plants were compared, it seemed that a large number of volatiles from the infested and clean plants were the same, though higher quantities were released by the infested cultivars (Fig. 4.10). It is possible that *A. hordei* were responding to some volatiles that were present in similar quantities on both infested and clean plants and thus not able to distinguish between infested and clean plants of this cultivar. *Diaeretiella rapae* may also be responding to volatiles that differed in quantities between infested and clean plants to distinguish infested from clean plants. Changes in induced volatile composition can be quantitative i.e. changed ratios of the same components, or qualitative, by the release of compounds that do not occur in the blend emitted by the intact plant (Paré & Tumlinson, 1999; Dicke & Van Loon, 2000; Farag *et al.*, 2005). Therefore, different parasitoids could also respond to different volatiles from the same plant-herbivore blend. This means that both *A. hordei* and *D. rapae*, presenting a narrow and wide host spectrum respectively, use different volatiles or volatile combinations from the same plant-herbivore combination to locate their host habitats. The specificity of herbivore-induced volatiles and the ability of natural enemies to distinguish between different plant-herbivore combinations were demonstrated in several natural enemy species (Takabayashi *et al.*, 1998; Ninkovic *et al.*, 2001). The volatile profiles of infested and clean SST 333 were different from the other cultivars, with approximately four volatiles that were similar in the profile of both (Fig. 4.10). The probability that parasitoids could distinguish between these cultivars should be higher, but the experimental results indicated that *D. rapae* was not able to distinguish.

Diaeretiella rapae females preferred infested to clean plants of another susceptible cultivar, Tugela. Tugela, which was bred in part from Betta (Rachel Oelofse, personal communication[2]), may have some similarities in their volatile profiles,

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[2] Rachel Oelofse, Plant breeder, ARC- Small Grain Institute, Bethelhem, South Africa

which may include some specific compounds to which *D. rapae* responded. Volatiles from this cultivar should be collected in future for confirmation. Both *D. rapae* and *A. hordei* were, however, not able to distinguish between infested and clean plants of the resistant cultivar TugelaDN (Dn1 gene) (Tolmay & Van Deventer, 2005), which was bred from Tugela. *Aphelinus hordei* could not distinguish between infested and clean plants of both Elands and TugelaDN cultivars both containing the same resistance gene. However as expected, *D. rapae,* distinguish between clean and infested plants in Elands, but not in TugelaDN. During breeding of TugelaDN from Tugela, the incorporation of resistance may have changed the volatile profile of the new cultivar in such a way that it has an effect on the host habitat recognition of this parasitoid. This aspect merits further investigation.

The volatile profiles of infested Betta and Elands share several peaks, though they differ in quantities (Fig. 4.10) and this is probably why *A. hordei* did not distinguish Betta from Elands. *Diaeretiella rapae,* however, preferred Betta to Elands significantly more, demonstrating that the parasitoid species responded to different quantities of the same volatiles from the same cultivars. Both parasitoid species preferred Betta to SST 333, but *A. hordei* preferred Elands to SST 333 and *D. rapae* preferred SST 333 to Elands. The volatile profile of SST 333 is clearly different from those of Betta and Elands (Fig. 4.10) and therefore the different parasitoid species could distinguish between these cultivars. Both parasitoids species preferred susceptible Tugela to resistant TugelaDN (Figs. 4.6, 4.7). This may be an indication that differences occurred in the volatile profiles of these two cultivars, after resistance was bred into Tugela. The resistance could also actively interfere with the feeding activity of *D. noxia* and reduced feeding may induce much less volatiles compared to susceptible cultivars.. In this way parasitoids would prefer the susceptible to the resistant cultivar. Van Emden (1986) reported on contrasting results found where a partial resistant Brussels sprout variety released a lower concentration of volatile substances than a susceptible one, which attracted the parasitoids more towards the susceptible cultivar than to the partial resistant one.

A. hordei responded significantly more to volatiles from an infested Betta plant, which was cleaned and washed one hour before the test, than to the group of aphids offered alone. This indicated that parasitoids were responding to herbivore induced volatiles from the host plant. Wickremasinghe & Van Emden (1992) recorded the same results with *Rhopalosiphum padi* (L.) on wheat.

Wickremasinghe & Van Emden (1992) found that parasitoids, which developed on aphids on a certain wheat cultivar, have a preference for volatiles from that cultivar. The same could be true for the current trials, but it was found that *A. hordei* significantly preferred Hugenoot, another susceptible cultivar, to Betta. This is probably an indication that it was not conditioned to Betta. However "emergence conditioning" was demonstrated for *Aphidius rhopalosiphi* De Stephani-Perez (Van Emden *et al.*, 1996) and *Aphidius colemani* (Viereck) (Storeck *et al.,* 2000). This means that emerging parasitoids, while in contact with the mummy case, obtained chemical information, which determined host plant preference.   Such conditioning should have had an influence on the host choice between cultivars in the current trials. However, although they were not reared on Tugela, both *A. hordei* and *D. rapae* distinguished between Tugela and TugelaDN infested, but both of them could not distinguish between infested and clean TugelaDN. Due to the fact that Tugela was partly bred from Betta it is thus possible that a certain volatile or combination of volatiles are present in Betta and Tugela, but not in TugelaDN or Elands. If the volatile profiles of infested Betta and Elands are compared, it is clear that some of the more prominent compounds in Betta marked K is absent in Elands, and that M, N and O are significantly reduced in Elands. This may be a reason why *A. hordei* could not distinguish between Betta and Elands. The difference in induction of volatiles between cultivars merits further investigation.

In the attack rate studies volatile, visual and contact cues were present which may have affected the results.   In both, the Betta-Elands and Betta-SST 333 combinations *A. hordei* parasitised more aphids on Betta than on the resistant cultivars (Fig. 4.8), although the infested leafs of Betta were rolled, showing that they preferred to parasitise aphids on the susceptible cultivar.   On the Elands-SST 333 combination no difference in mummy numbers was found, indicating that

they did not prefer a certain cultivar, or that the chemical information coming from these cultivars differed from Betta on which they were reared. The total number of aphids in the Betta-Elands combination, where the volatile profiles were more similar, was significantly more than the Betta-SST 333 combination, where volatile profiles were much different.

In the Betta-SST 333 and Elands-SST 333 combinations with *D. rapae,* significant more aphids were parasitised on Betta and Elands respectively (Fig. 4.9). This was probably due to a preference for the cultivar on which it developed, Elands, with a similar volatile profile to Betta. In the olfactometer tests SST 333 was preferred to Elands. For Betta-Elands combination no difference was found which is also opposite of the olfactometer results, but could also be due to the little difference in volatile profiles between the two cultivars. In contrast to *D. rapae* the highest total number of aphids per combination for *A. hordei* was found in Betta-SST 333 where the volatile profiles differed the most.

From these results it is clear that both parasitoid species use different induced volatiles or volatile combinations to locate their host habitat. Differences in volatile profiles occur between cultivars and if results from Betta and Tugela are compared it seems that the genetic background of the cultivars is playing a role. In contrast to susceptibility, resistance has a definite affect on the parasitoids, but differences between cultivars are also obvious. On the other hand, if emergence conditioning is taken into account, parasitoids should be able to locate their host habitat without problems. This should, however, be investigated to determine if these natural enemies and the resistant cultivars are compatible in an integrated control programme. Although differences between resistant and susceptible cultivars were evident, Elands (containing the Dn1 gene) seems to be more compatible with the parasitoids than SST 333. It is also important to look for the most compatible plant resistance sources and methodology to enhance the efficiency of natural enemies on these cultivars in order to ensure durable resistance and sustainable control of the aphid.

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CHAPTER 5

THE RESPONSE OF RUSSIAN WHEAT APHID, ITS PARASITOIDS AND THE OAT APHID TO APHID BEHAVIOUR MODIFYING CHEMICALS IN THE LABORATORY.

5.1   Introduction

Phytochemicals are capable of inducing a variety of responses in plants, including induction of defences against pathogens and herbivores (Arimura *et al.*, 2000; Walters *et al.*, 2002), modification of volatile profile (Dicke *et al.*, 1999) and increased attractiveness to herbivore natural enemies (Chamberlain *et al.*, 2000). Examples of volatile phytochemicals are those released by herbivore-infested plants (Dicke & Van Loon, 2000; Farag *et al.*, 2005) and those associated with induced defensive pathways (Farmer & Ryan, 1992; Shulaev *et al.*, 1997).

Semiochemicals that act as insect behaviour-modifying chemicals can be used as tools for management of pest insect populations. It has been successfully applied to aphid populations in barley in the UK and Sweden (Pettersson *et al.*, 1994; Ninkovic *et al*., 2003). Methyl salicylate, slowly released from a wax pellet formulation, reduced populations of bird cherry-oat aphid, *Rhopalosiphum padi* (L.), in barley (Ninkovic *et al.*, 2003). This aphid is host alternating in Sweden and methyl salicylate, released from its winter host plant, *Prunus padus* (Pettersson *et al.*, 1994; Glinwood & Pettersson, 2000a, b) is repellent to the aphid and serves as a host-leaving signal. It is also a repellent to cereal aphids in general, because it is commonly produced by plants that are attacked by pathogens and herbivores as a signal related to defence induction (Pettersson *et al.*, 1994; Shulaev *et al*., 1997). Exposure of cereal plants to this substance makes them less acceptable to *R. padi* (Glinwood & Pettersson, 2000b).

In South Africa where the Russian wheat aphid (RWA), *Diuraphis noxia* (Kurdjumov) is a major insect pest of wheat, more than 70 % of the wheat farmers in the Free State Province are planting cultivars resistant to RWA (Marasas *et al*., 1997). This caused an approximate 35 % decrease in insecticide used by farmers

between 1990 and 1996 (Marasas *et al.*, 1997) and increased the possibility for more efficient integration of host plant resistance and control with introduced natural enemies (Chapter 2).

A problem associated with the use of plant resistance is the tendency for the development of resistance-breaking biotypes (Gould *et al.,* 1990; Stoner, 1996; Thomas & Waage, 1996; Porter *et al.,* 1997).   As recently as 2003, a resistance breaking biotype of *D. noxia* was reported from Colorado (Haley *et al.*, 2004). Since *D. noxia* from various parts of the world differ in their reaction to resistant wheat lines (Puterka *et al.*, 1993) the possibility of *D. noxia* to develop a resistant breaking biotype in South Africa cannot be ruled out. Due to the seriousness of the pest it is necessary to take steps to protect the resistant cultivars and prevent an outbreak of the pest again.   This merits an investigation on the integrated use of plant resistance, natural enemies and behaviour modifying chemicals.

When natural enemies attack herbivores feeding on a plant, the plant benefit from the reduction in herbivore abundance.   The attacked plants that release herbivore induced volatiles may benefit the natural enemies by making the herbivores more vulnerable to the natural enemies (Price, 1986). This mutualism can be very important in the integrated control of RWA. If natural enemies are attracted to resistant plants in an effective way by the release of volatiles, the aphids still feeding on these plants will be controlled effectively. It could, therefore, diminish the chances for the development of a resistance breaking biotype. If natural enemies, however, are not attracted effectively to these plants, the aphids feeding on them are free from natural enemy attack and chances increase for a resistance breaking biotype to develop. The application of methyl salicylate to wheat crop could possibly play a role in modifying the behaviour of RWA and its natural enemies and therefore deserves further investigation.

The aims of this study were:

(i)   Olfactometric testing of the responses of *D. noxia* and South African *R. padi* to aphid behaviour modifying chemicals used against *R. padi* in Sweden. The responses of the South African *R. padi* were also tested,

to compare its reaction to Swedish *R. padi,* since it can obtain pest status in dryland wheat areas in South Africa during wetter years.

(ii)   To test settling of *D. noxia* on susceptible and resistant cultivars treated with these chemicals.

(iii) To test settling of Swedish *R. padi* on South African wheat cultivars treated with these chemicals.

(iv)   Olfactometric testing of the response of the parasitoids *Aphelinus hordei* (Kurdjumov) and *Diaeretiella rapae* (McIntosh) towards these chemicals as well as resistant and susceptible cultivars treated with the formulations.

5.2   Materials and Methods

5.2.1 Insect cultures

The *D. noxia* and *R. padi* cultures were established from aphids collected from volunteer wheat on the experimental farm at ARC-Small Grain Institute (ARC-SGI), Bethlehem, and new aphids were introduced every year. Aphids were maintained on wheat (cultivar Betta) in a greenhouse at a temperature of $23 \pm 2°C$, and ambient light conditions with 14 L:10 D. Aphids used in all the laboratory experiments were mixed-instar apterae and were collected from the cultures immediately prior to bioassays. The *A. hordei* and *D. rapae* parasitoid cultures were reared on *D. noxia* under the same greenhouse conditions as the aphids. Two different parasitoid species were used because *A. hordei* tends to be more host species specific by showing an oviposition preference for *D. noxia* (Prinsloo, 2000). The second parasitoid, *D. rapae,* is a generalist parasitoid with a wide host range (Pike *et al*., 1999). The trial involving Swedish *R. padi* were executed at the Entomology Department of the Swedish University of Agricultural Sciences, Uppsala, Sweden where it was cultured as described by Glinwood *et al.* (2003).

5.2.2 Plant material

Four wheat cultivars, susceptible Betta and Tugela as well as resistant Elands and Tugela-DN, obtained from the seed bank at ARC-SGI Bethlehem, were used for

trial purposes. Plants for use in greenhouse trials at ARC-SGI were sown in one litre plastic pots, with eight to ten plants per pot and were ready to use at the two to three leaf stage. Plants were grown in a greenhouse at a temperature of 23 ± 2°C, and ambient light, extended with electrical light to a 14 L:10 D cycle. The same cultivars were planted in Sweden as described by Glinwood *et al.* (2003).

5.2.3 Chemical formulation and treatments

A slow-release wax pellet formulation (Ninkovic *et al.*, 2003) was used to dispense the active compound. The pellets consisted of a mixture of two paraffins, containing 10% (m/m) active compound. Three different essential oils namely wintergreen, peppermint and eucalyptus oils (Crearome, Sweden) were used to created three respective groups of pellets. The different groups of pellets released volatile methyl salicylate, menthol and 1,8-cineole, respectively. Essential oils in general have been of interest in insect pest control (Isman, 2000; Koschier *et al.*, 2002) and have the advantage of being generally non-toxic and environmentally benign. Initial laboratory experiments indicated behaviour-modifying effects of these substances against *R. padi* ([1]R. Glinwood & V. Ninkovic, personal communication). A product named OX54 was prepared by mixing equal weights the three pellet groups.

5.2.4 Treatment of plants for the aphid settling tests.

Chambers were constructed to expose wheat plants to volatiles, based on the principle described by Pettersson *et al* (1999). Plants were contained in transparent, five litre plastic containers. The containers were connected to an air extraction system, consisting of a fan fixed to a 70 cm X 25 cm plastic tube. Air entered each container through a 10 cm hole close to the base of the container. Chemical containing wax pellets were placed at the base of the plant. In this way air, containing volatiles released by the pellets, passed over the plants and exit

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[1] Drs Robert Glinwood and Velemir Ninkovic, Entomologists – Department of Entomology, Swedish University of Agricultural Sciences,Uppsala, Sweden

through a hole near the top of the container. This air was then vented outside the greenhouse chamber.

In each exposure cage, a pot containing six to ten wheat plants was exposed to either six pellets of methyl salicylate, six pellets of OX54 (two pellets of each substance) or no pellets. Each of the cultivars, Betta, Elands, Tugela and Tugela-DN was treated in this way, for five days before an aphid settling test was conducted.

For *D. noxia* settling tests, three pots of plants per treatment per cultivar were used. Control and treatment cages were placed randomly in a greenhouse at 18 - 22℃, and ambient light with 14 L:10 D. Plants were watered with 100ml water once before the start of the experiment and repeated every second day.

5.2.5 Tests of aphid settling

A no-choice aphid settling test (Ninkovic *et al.*, 2002; Glinwood *et al.*, 2003, 2004) was used to assess aphid settling on plants treated with chemicals. A 50 ml polystyrene tube was placed over the youngest fully developed leaf, which was always the second leaf. The upper end of the tube was covered with a net and the lower end with a foam plastic plug with a slit for the leaf. The tube was supported with a skewer to minimise mechanical damage to the test plant. Ten mixed instar nymphs of *D. noxia* were placed in the tube. After two hours the number of aphids that settled (not walking) on the leaf, was recorded. According to Prado & Tjallingi, (1997) two hours is sufficient time for aphids to settle and penetrate the phloem. Six to nine plants per pot (and therefore per cage since each cage held a single pot) were randomly selected for the test. The percentage of aphids settled on the leaves of plants in different treatments was compared applying the row by column Chi-squared test at a 0.05 significance level (Siegel, 1956).

5.2.6 Olfactometry

The responses of aphids and parasitoids to volatiles were tested using a four-arm olfactometer (Pettersson, 1970) consisting of an enclosed Perspex central chamber and four side arms (12 cm diameter). Air was drawn from the centre of

the olfactometer using a vacuum pump at 400-500 ml min^{-1}, establishing discrete air currents in the side arms. Each side arm was attached to a 50 ml glass vial by means of a 50 mm X 1.5 mm piece of Teflon tubing. Tests were performed in a windowless room, with light provided by a fluorescent lamp suspended 80 cm directly above the olfactometer.

An odour field was established by introducing a chemical substance in pellet formulation into two neighbouring side arms, with the other two arms containing no chemical. Both *D. noxia* and *R. padi* as well as the parasitoids *A. hordei* and *D. rapae* were tested for their response to the different chemicals.   OX54 was presented as a single pellet of each of the three components (total three pellets), and the separate components methyl salicylate, 1,8-cineole and menthol were presented in separate experiments as one pellet.   Release rate studies under constant laboratory conditions indicated that the typical release rate of chemicals from such pellets is 90 µl day^{-1} (R. Glinwood, personal communication). Tests were also done with *D. noxia* and *R. padi* against the three components as 1 mg ml^{-1} solutions in hexane. In this case the stimulus was presented in 4 µl microcapillary tubes (total dose 4 µl arm^{-1}) and a tube containing hexane was used in the control arms.

When parasitoids were tested for their response to different treated plants, each pot was covered with a 3 l container. Each container was connected to two arms of the olfactometer using 250 mm X 1.5 mm of Teflon tubing inserted into small holes near the top of the container. Air entered the container through a 15 mm diameter hole near the base of the plants so that air moved over the plants into the olfactometer.

For both aphid and parasitoid tests, a single insect was introduced into the olfactometer and observed for 10 minutes, during which time the number of entries made into either treated or untreated side arms was recorded. Between 20 and 24 individual aphids or parasitoids were tested for each treatment over a period of 2-3 days. After every two insects, the olfactometer was turned through 90° to compensate for any bias in lighting. After eve ry five insects, a clean olfactometer

was used. The mean number of entries into either treated or untreated arms were compared using Wilcoxon paired t-tests at 5% test level (Snedecor & Cochran, 1967).

5.3   Results

5.3.1   Olfactometric response of aphids.

Diuraphis noxia made significantly fewer entries into the olfactometer arms containing vapours from pellets releasing OX54, methyl salicylate, 1,8-cineole or menthol, than into control arms (Table 5.1).   This indicated that the substances were repellent to *D. noxia*.   When substances were applied as 4 µl solutions, only methyl salicylate and 1,8-cineole were repellent (Table 5.1).

Table 5.1   The response of *Diuraphis noxia* to different volatile chemicals in an olfactometer during a ten minute period.

Treatment	N	Mean number of entries in arm		Wilcoxon test	
		Treated ± SE	Control[1] ± SE	Z	P
OX54 - 3 pellets	22	2.6 ± 0.4	5.6 ± 0.4	3.1	0.001
Methyl salicylate -1 pellet	21	2.5 ± 0.5	5.6 ± 0.4	3.64	0.001
1,8-cineole –1 pellet	24	1.4 ± 0.3	6.1 ± 0.5	3.77	0.001
Menthol - 1 pellet	20	0.8 ± 0.3	6 ± 0.5	3.77	0.001
Methyl salicylate 4 µl	24	4.6 ± 0.6	6.1 ± 0.7	2.01	0.04
1,8-cineole 4 µl	24	2.8 ± 0.6	8 ± 0.8	4	0.001
Menthol 4 µl	24	6 ± 0.5	6.7 ± 0.4	1.2	ns

[1]For experiments with pellets, control was an empty arm. For experiments with solutions, control was 4 µl hexane.   N = number of replicates. ns = non significant.

South African *R. padi* also made significantly fewer entries into olfactometer arms containing vapours from pellets releasing either OX54, methyl salicylate, 1,8-cineole or menthol, than into control arms (Table 5.2).   When the substances were applied as 4 µl solutions, only methyl salicylate was still repellent to this aphid species, but not menthol and 1,8-cineole (Table 5.2).

Table 5.2 The response of South African *Rhopalosiphum padi* to different volatile chemicals in an olfactometer during a ten minute period.

		Mean number of visits in arm		Wilcoxon test	
Treatment	N	Treated ± SE	Control[1] ± SE	Z	P
OX54 - 3 pellets	20	1.7 ± 0.3	6 ± 0.5	3.6	0.001
Methyl salicylate - 1 pellet	24	3.4 ± 0.3	5.2 ± 0.5	2.6	0.005
1,8-cineole -1 pellet	21	3.6 ± 0.3	4.8 ± 0.3	2.03	0.021
Menthol -1 pellet	21	2.3 ± 0.4	3.6 ± 0.3	2.48	0.01
Methyl salicylate 4 μl	21	2.6 ± 0.4	5.1 ± 0.5	2.7	0.003
1,8-cineole 4 μl	21	3.4 ± 0.4	4.4 ± 0.4	1.7	ns
Menthol 4 μl	21	5.7 ± 0.5	6.9 ± 0.4	1.3	ns

[1]For experiments with pellets control was an empty arm. For experiments with solutions, control was 4 μl hexane. N = number of replicates. ns = non significant.

5.3.2 *Diuraphis noxia* settling on resistant and susceptible cultivars exposed to different chemical substances.

During one of the three settling test trials with susceptible Betta, plants exposed to OX54 resulted in a significant reduction in aphid settling (Table 5.3). No significant reduction in aphid settling was found in any of the trials where plants were exposed to methyl salicylate. In the first test significantly more aphids settled on the methyl salicylate treated plants than on the OX54 treated plants, although the number did not differ significantly from the control plants.

Table 5.3. Settling of *Diuraphis noxia* on the susceptible cultivar Betta treated with semiochemicals for five days. Values within rows without the same letters in common differed significantly (test level 0.05).

Percentage aphids settled [1]					
Experiment	Control (N)	OX54 (N)	Methyl salicylate (n)	c^2	P
1	67.2a (18)	63.3ab (18)	78.8a (17)	10.7	0.005
2	61.3a (23)	55.7a (24)	56.7a (23)	1.7	ns
3	57.5a (16)	41.9b (26)	48.5ab (26)	9.7	0.008

[1]Out of ten aphids/settling test. N = number of replicates. ns = non significant.

Susceptible Tugela plants exposed to methyl salicylate resulted in significantly reduced aphid settling in three out of four trials (Table 5.4). Plants exposed to OX54 reduced aphid settling significantly in two out of the four trials (Table 5.4).

Table 5.4 Settling of *Diuraphis noxia* on the susceptible cultivar Tugela treated with semiochemicals for five days. Values within rows without letters in common differed significantly.

Percentage aphids settled[1]					
Experiment	Control (N)	OX54 (N)	Methyl salicylate (n)	χ^2	P
1	64.5a (20)	64.33a (21)	57.5a (20)	2.7	ns
2	59.5a (20)	46.7b (21)	43.4b (21)	11.9	0.003
3	60a (24)	54.2ab (24)	43.3b (24)	13.8	0.001
4	64.3a (23)	48.3b (24)	52.1b (24)	13.2	0.002

[1] Out of ten aphids/settling test. N = number of replicates. ns = non significant.

Possible differences in sensitivity to methyl salicylate also became clear when *D. noxia* settling was tested on RWA resistant cultivars. Elands plants exposed to OX54 showed a significant reduction in aphid settling in two out of the four trials (Table 5.5). Plants exposed to methyl salicylate resulted in significant reduced aphid settling in three of the four trials (Table 5.5).

Table 5.5 Differences in the settling of *Diuraphis noxia* on the resistant cultivar Elands treated with semiochemicals for five days. Values within rows without letters in common differ significantly.

Percentage aphids settled[1]					
Experiment	Control (N)	OX54 (N)	Methyl salicylate [2]	χ^2	P
1	73.4a (21)	55.2b (21)	61.9b (21)	15.2	0.001
2	73.9a (18)	70.6a (18)	57.8b (18)	11.9	0.003
3	61.3a (24)	43.8b (24)	56.7a (24)	15.9	0.000
4	55.6a (27)	44.6ab (24)	44.5b (27)	8.3	0.013

[1] Out of ten aphids/settling test. N = number of replicates.

In Tugela-DN reduced settling of *D. noxia* was found in three out of the four experiments where plants were exposed to methyl salicylate (Table 5.6). No significant reaction, however, was found when plants were exposed to OX54.

Table 5.6   Settling of *Diuraphis noxia* on the resistant cultivar Tugela-DN treated with semiochemicals for five days. Values within rows without letters in common differed significantly.

Percentage aphids settled[1]					
Experiment	**Control (N)**	**OX54 (N)**	**Methyl salicylate (n)**	χ^2	**P**
1	69.5a (21)	58.1ab (21)	50b (21)	16.7	0.000
2	56.2a (21)	46.7a (21)	47.6a (21)	4.6	0.1
3	67.9a (24)	59.6a (24)	44.6b (24)	27.4	0.000
4	62.5a (24)	56.3ab (22)	50b (24)	7.3	0.025

[1] Out of ten aphids/settling test. N = number of replicates.

5.3.3 Settling of Swedish *Rhopalosiphum padi* on Russian wheat aphid resistant and susceptible cultivars exposed to different chemical substances.

After one day of exposure to methyl salicylate and OX54, both susceptible Tugela and resistant Tugela-DN plants significantly reduced *R. padi* settling (Table 5.7). Elands, however, did not significantly reduce *R. padi* settling. After five days of exposure, only Tugela-DN plants exposed to methyl salicylate as well as to OX54 significantly reduced *R. padi* settling (Table 5.8).

Table 5.7   Settling of Swedish *Rhopalosiphum padi* on three different wheat cultivars treated with semiochemicals for one day. Values within rows without letters in common differed significantly.

Percentage aphids settled[1]					
Cultivar	**Control (N)**	**OX54 (N)**	**Methyl salicylate (n)**	χ^2	**P**
Tugela	77.3a (15)	58.8b (16)	51.9b (16)	22.7	0.0000
Tugela -DN	72a (15)	58.8b (16)	56.7b (15)	14.1	0.001
Elands	71.2a (16)	62.5a (16)	63.8a (16)	3.70	ns

[1] Out of ten aphids/settling test. N = number of replicates. ns = non significant.

Table 5.8 Settling of Swedish *Rhopalosiphum padi* on three different wheat varieties treated with semiochemicals for five days. Values within rows without letters in common differed significantly.

	Percentage aphids settled[1]				
Cultivar	**Control (n)**	**OX54 (n)**	**Methyl salicylate (n)**	χ^2	**P**
Tugela	67.5a (16)	56.9a (16)	61.9a (15)	1.8	ns
Tugela-DN	80a (16)	53.1b (16)	61.9b (16)	26.4	0.000
Elands	73.1a (16)	68.1a (16)	65.6a (16)	2.2	ns

[1] Out of ten aphids/settling test. ns = non significant

5.3.4 Olfactometric response of parasitoids to different chemical substances

The parasitoid *A. hordei* made significant fewer entries into the olfactometer arms containing vapours from pellets releasing OX54 (N=24, Z=2.1, P=0.018), methyl salicylate (N=21, Z=3.9, P=0.000) and menthol (N=21, Z=3.8, P=0.000) (Fig. 5.1). They, however, did not distinguish significantly between the control arms and those containing 1,8-cineole (N=21, Z=1.8, P=0.38).
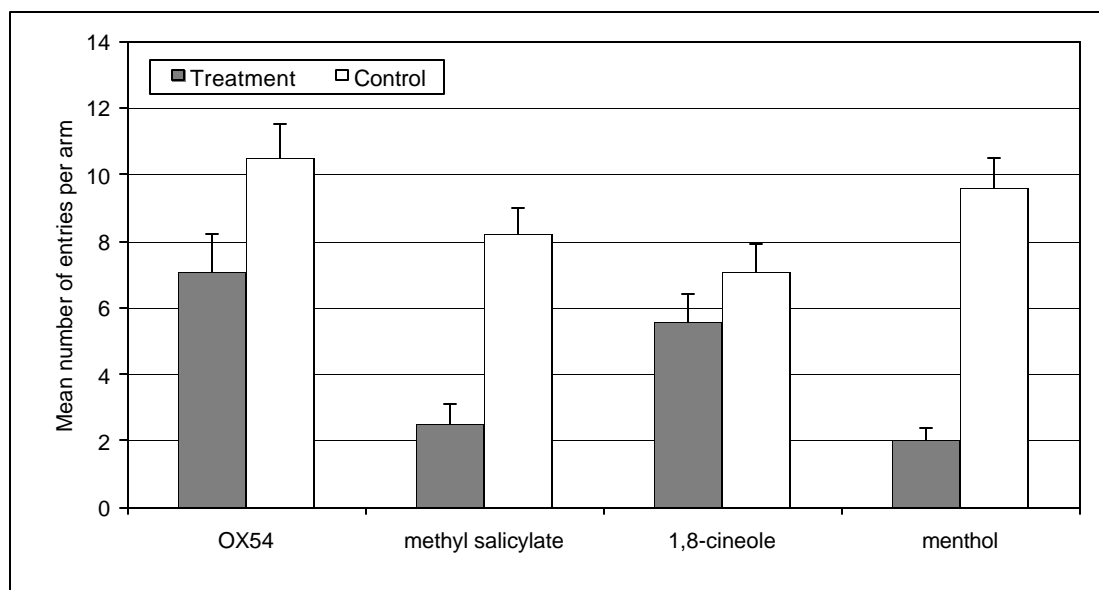
Figure 5.1 Mean number (±SE) of visits by *Aphelinus hordei* into olfactometer arms containing a chemical stimulus or an empty control during a ten minute period

Diaeretiella rapae made significant fewer entries into the olfactometer arms containing pellets releasing OX54 (n=24, Z=2.5, P=0.006), methyl salicylate (n=24, Z=2.8, P=0.002) and 1,8-cineole (n=24, Z=3.5, P=0.0002) (Fig. 5.2). They did not distinguish significantly between the control arms and those containing menthol (n=24 Z=3.5, P=1.123) (Fig. 5.2).
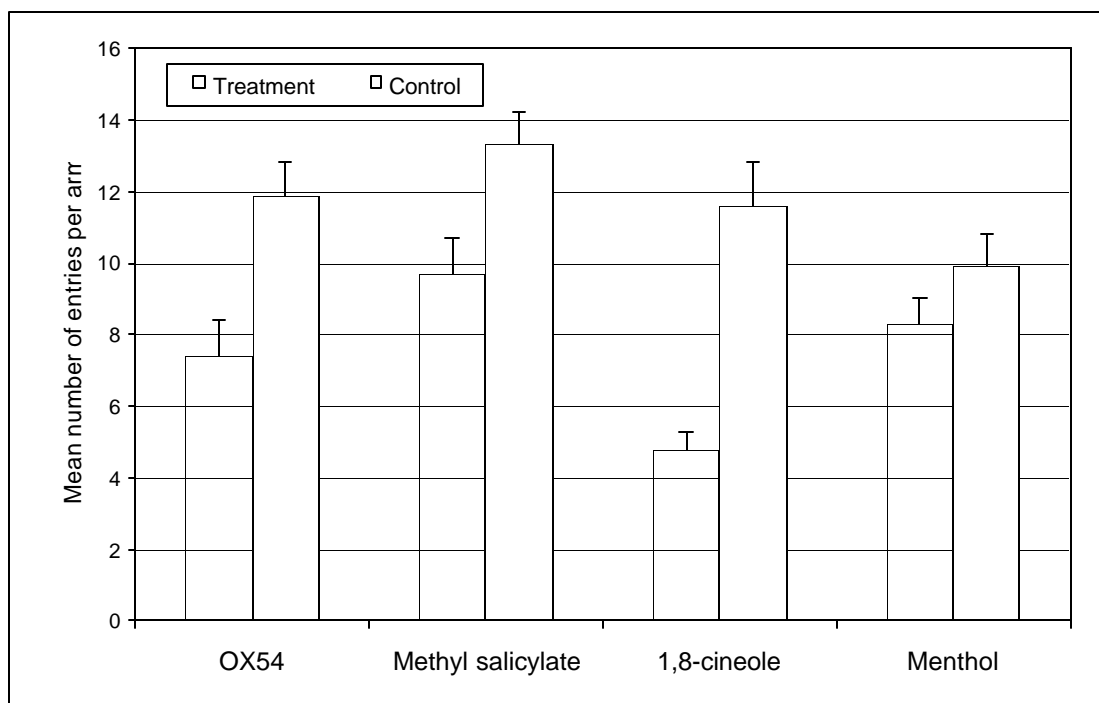
Figure 5.2 Mean number (±SE) of visits by *Diaeretiella rapae* into olfactometer arms containing a chemical stimulus or an empty control during a ten minute period

5.3.5 Olfactometric response of parasitoids to Russian wheat aphid susceptible and resistant cultivars exposed to different chemical substances.

The parasitoid *A. hordei* made significantly more entries into the olfactometer arms containing Tugela plants which were exposed for five days to either methyl salicylate (n = 15, t=9.5, P=0.005), or OX54 (n=21, t=58, P=0.023) than into the control arms (Fig. 5.3). Similar results were obtained with Tugela-DN treated with methyl salicylate (n=15, t=6.5, P=0.001) and OX54 (n=21, t=20, P=0.002) (Fig. 5.3).
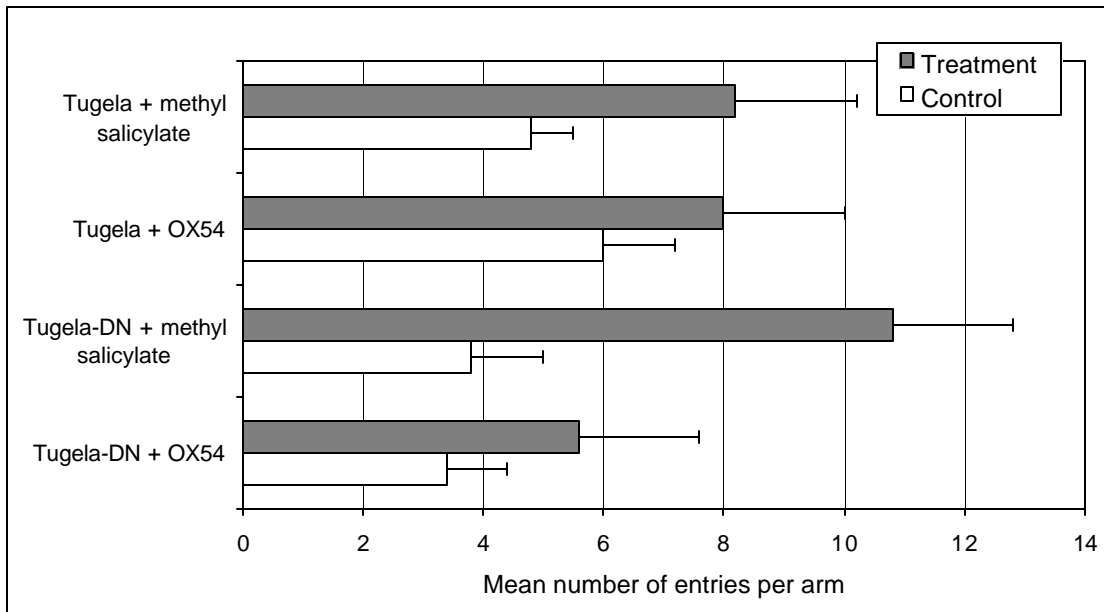
Figure 5.3   Response of *Aphelinus hordei* in olfactometer to resistant (Tugela-DN) and susceptible (Tugela) plants exposed with different semiochemicals for five days .

The parasitoid *D. rapae* also made significantly more entries into the olfactometer arms containing Tugela plants exposed to either methyl salicylate (n=20, t=19, P=0.018), or OX54 (n=15, t=13, P=0.005) (Fig. 5.4). A similar response was elicited by Tugela-DN exposed to either methyl salicylate (n=20, t=26, P=0.004) or OX54 (n=15, t=15.5, P=0.004)(Fig. 5.4).
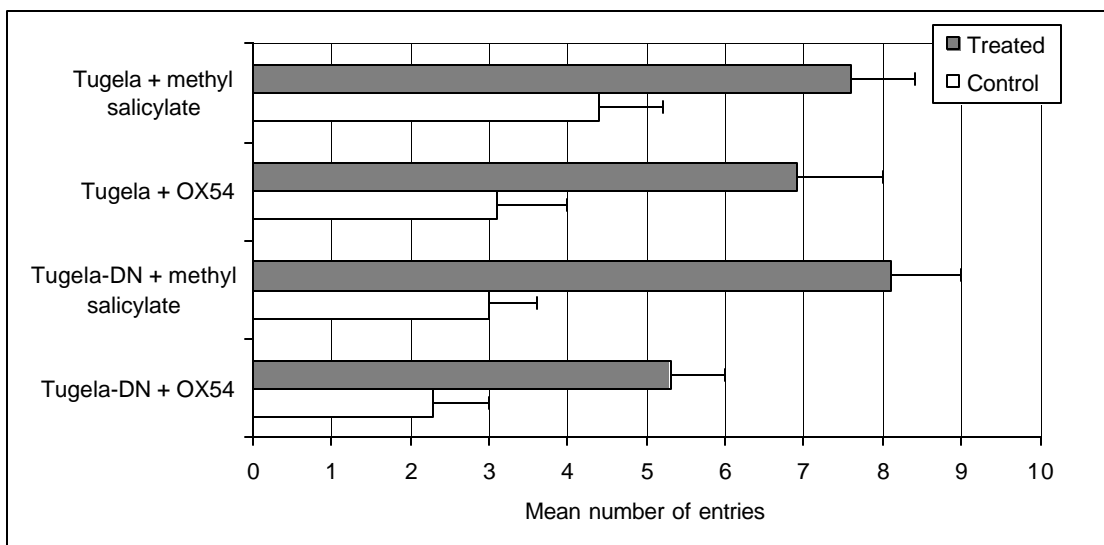
Figure 5.4   Olfactometric response of *Diaeretiella rapae* resistant (Tugela-DN) and susceptible (Tugela) plants treated for five days with different chemical stimulus.

5.4   Discussion

The European alternate host plant of *R. padi*, *Prunus padus,* is releasing methyl salicylate which repels the aphid from this plant (Pettersson *et al*., 1994).   From the current results it is clear that, although the European alternate host plant of this aphid is not present in South Africa (Millar & Dürr, 1985), it is also repelled by methyl salicylate.   The alternate host range of *D. noxia* in its countries of origin as well as in South Africa includes only grass species (Kovalev *et al*.,1991; Millar & Dürr, 1985).   Similar to *R. padi, D. noxia* was also repelled by methyl salicylate although it is not a known volatile component of grasses or cereals (Glinwood & Pettersson, 2000b; James, 2003) and therefore possibly not produced by any of the alternate host plants of *D. noxia*.   Results from the current study thus confirm behaviour modification of induced phytochemicals on aphids and their natural enemies.   These results are in accordance with the general repellency of methyl salicylate towards cereal aphids (Pettersson *et al*., 1994).

Both menthol and 1,8-cineole in the pellet formulation were also found to be effective repellents during olfactometer tests, although the lower concentrations were less effective.   According to Chamberlain *et al*., (2000) some olfactory neurons on aphid antennae did not respond to host plant volatiles but are stimulated by non-host plant volatiles.   The reason why *D. noxia* and South African *R. padi* reacted to menthol and 1,8-cineole could therefore simply be in avoidance of a poor quality host plant or a non-host plant.

According to Shulaev *et al*., (1997), methyl salicylate, however, act as an airborne signal mediating induced plant resistance against pathogens in tobacco. Glinwood & Pettersson (2000b) mentioned that changes in oat plants after exposure to methyl salicylate resulted in reduced attraction of *R. padi* to these plants.   This could also be true of wheat cultivars, because reduced settling of *D. noxia* on different wheat cultivars was found in the current study.   Methyl salicylate could therefore also serve as a signal to induced defence mechanisms in the wheat plants.   Aphid settling was, however, reduced at different levels depending on the formulation and the cultivar.   The susceptible cultivars Betta and Tugela seemed to be not equally sensitive towards these chemical signals.   A significant reduction

in aphid settling was found only once on Betta when treated with OX54. Significant reduction in aphid numbers on Tugela was recorded more often with methyl salicylate, than with OX54. Similar to the reaction of susceptible Tugela, significant less aphid settling was found more often with methyl salicylate than with OX54 treatment on both resistant Elands and Tugela-DN. The reason for the difference in reaction when treated with either methyl salicylate or OX54 is not clear, but the presence of 1,8-cineole and menthol in OX54 may not contribute to the signalling function and may have a masking effect on the methyl salicylate in this formulation.

Plants respond to feeding damage of herbivores with the production of many novel compounds, resulting in the emission of complex volatile blends (Dicke, 2000). Many authors reported on these volatiles with reference to its attraction affect on natural enemies such as parasitoids and predators (Vet & Dicke, 1992; Powell & Pickett, 2003). For example, the lacewing, *Chrysopa nigricornis* Burmeister, is attracted by methyl salicylate, which is induced in hops (*Humulus lupulus* L.) by feeding of the hop aphid *Phorodon humuli* Schrank (James, 2003).

In the current study, both *A. hordei* and *D. rapae* were, however, repelled by some of the semiochemicals tested, but differences occurred between the two species. *A. hordei* was strongly repelled by methyl salicylate and menthol individually, while the reaction towards OX54 (three compounds combined) were less strong but still significant (Fig. 5.1). *Diaeretiella rapae* was strongly repelled by 1,8-cineole while less strongly but still significantly by methyl salicylate and OX54 (Fig. 5.2). The fact that the parasitoids are repelled by these semiochemicals and not attracted as expected may be an indication that these are not induced by *D. noxia* feeding on wheat. The amount of volatile substance released by the pellet in the olfactometer may also be high which could have a repellent effect. *Aphelinus hordei* did not respond to 1,8-cineole while the *D. rapae* was not responding to menthol thus showing that the different parasitoids differ in sensitivity to other semiochemicals. A possible reason for this reaction could be the differences in their host ranges. *Diaeretiella rapae* with a wide host range and therefore a wider range of host plant volatiles, reacted differently from *A. hordei* with a narrow host range and few host

plant volatiles. Parasitoids could, however, also react to different volatiles from the same plant as was demonstrated by James (2003).

Tugela and Tugela-DN plants treated with either methyl salicylate or OX54 were attractive to both parasitoid species (Figs. 5.3, 5.4). According to Chamberlain *et al.* (2000) certain volatile compounds from plants can be used to elicit induced defence in plants prior to attack. Both methyl jasmonate and methyl salicylate were used to demonstrate the effect, though methyl salicylate was used to demonstrate induction of pathogen defence when externally applied (Shulaev *et al.*, 1997). (Z)-jasmone, a well known plant volatile was also shown to be repellent to certain morphs of the lettuce aphid *Nasonovia ribis nigri* (Birkett *et al.*, 2000). It, however, attracted a coccinellid predator and an aphid parasitoid. Uninfested bean plants, exposed to (Z)-jasmone also became attractive to these natural enemies (Birkett *et al.*, 2000). Therefore, it can be assumed that the exposure of both Tugela and Tugela-DN to methyl salicylate and OX54 induced a defence reaction resulting in the production of volatiles that are attractive to both *A. hordei* and *D. rapae*. According to Van der Westhuizen (Botha *et al.*, 1998; Van der Westhuizen *et al.*, 2002) it was found that RWA resistance response is not a wounding response but more a typical hypersensitive response, characteristic of pathogenesis. This hypersensitive response was developed by plants to rapidly detect biotic intruders including viruses, bacteria, nematodes and insects. This also confirms the above-mentioned assumptions that changes induced by treatment of wheat cultivars with methyl salicylate reduced RWA settling.

Both Tugela-DN and Elands contain the same source of resistance (DN1 gene) to *D. noxia* (Tolmay & Van Deventer, 2005). The regulatory effect that RWA resistant cultivars might have on other wheat aphids is not known. Regular infestation of these cultivars by *R. padi*, and *Sitobion avenae* (Fabricius) were observed in greenhouses and wheat fields (personal observation). The reduced settling effect of a RWA resistant cultivar (Tugela-DN) on the Swedish *R. padi* is therefore new. However, if the reduced *R. padi* settling on susceptible Tugela after one day of exposure, is taken into account, characteristics from the Tugela background in combination with the DN1 resistance gene, may be responsible for the reduced settling. Van der Westhuizen *et al*, (1998a; b) showed that genetic background into

which the DN1 gene is bred, have an influence on the level of resistance of the new cultivar and thus confirms the above-mentioned assumption. Therefore, Elands not being related to Tugela ([4]R. Oelofse, personal communication) have no reduced settling effect on *R. padi*.

Barley susceptible to *R. padi* was used by Pettersson *et al.* (1994); Glinwood & Pettersson, (2000b) and Glinwood *et al.* (2003) during their tests. It can be assumed that reduced settling is a feature of susceptible crops. Therefore, tests using *R. padi* susceptible wheat cultivars should have shown the same response. Both Tugela and Elands showed no significant reduced settling of Swedish *R. padi* after five days of methyl salicylate treatment (Table 5.8). Methyl salicylate therefore did not elicit a reaction in these cultivars that would affect the settling of *R. padi.* Experiments with South African *R. padi* should be conducted to determine their response. The outcome of such experiments may have implications for the use of methyl salicylate to control this aphid.

Results of the current study indicated that methyl salicylate has a repellent effect on *D. noxia* while plants exposed to this semiochemical became less attractive to the aphid, though not at the same level in all cultivars. Although the parasitoids of *D. noxia* are repelled by the semiochemical itself, exposed plants became attractive to these parasitoids possibly due to the induced defence response. This may have an influence on parasitism in the field. The use of methyl salicylate in an integrated control programme against *D. noxia* in the field is therefore feasible. The difference in efficiency on different cultivars merits further investigation before implementation of this strategy.

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CHAPTER 6

THE EFFECT OF BEHAVIOUR-MODIFYING CHEMICALS ON THE CONTROL OF *DIURAPHIS NOXIA* (Kurdjumov) ON RESISTANT AND SUSCEPTIBLE WHEAT CROP IN SOUTH AFRICA

6.1   Introduction

Herbivore damage to plants induces certain plant defence responses, which can include the accumulation of among others polyphenol oxidases, proteinase inhibitors as well as the release of volatile organic compounds (Farag *et al.*, 2005). The volatile compounds can serve as a signal for beneficial insects. This is also true of aphids that do not cause extensive mechanical damage to plants (Powell *et al.*, 1998). The phenolic compound, methyl salicylate, is a herbivore induced volatile released by several plants (James, 2003).   It is also released by the winter host plant of the bird cherry oat aphid, *Rhopalosiphum padi* (Linnaeus)*,* which is host alternating in Sweden (Pettersson *et al.*, 1994; Glinwood & Pettersson, 2000a, b).   Methyl salicylate is used as a host-leaving signal and repels *R. padi.* It is however, also a repellent to cereal aphids in general. This is probably because it is commonly produced by pathogen and herbivore-attacked plants, as a signal related to induce defence (Pettersson *et al.*, 1994; Shulaev *et al*., 1997). Laboratory studies have shown that methyl salicylate itself is also repellent to Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Chapter 5), which is a serious pest of wheat in the summer rainfall region of South Africa (Du Toit & Walters, 1984).

Evidence is also growing that herbivore induced volatiles such as methyl jasmonate, methyl salicylate, ethylene and green leaf volatiles can induce defence responses in intact neighbouring plants (Chamberlain *et. al*, 2000; Ruther & Kleier, 2005). Methyl salicylate, as a semiochemical, has been successfully applied to control aphid populations in barley in the UK and Sweden (Pettersson *et al.*, 1994; Ninkovic *et al.*, 2003). Being repellent to cereal aphids in general, this compound may be masking the attractive volatiles released from the aphid's host plants, thereby preventing them from landing on these plants (Hardie *et al.,* 1994).

Exposure of barley plants to this substance makes them less acceptable to *R. padi*, which is an indication of an induced defence effect in the plant (Glinwood & Pettersson, 2000b). Early application of a wax pellet formulation reduced *R. padi* infestations by between 25% and 50% during field trials (Ninkovic *et al.*, 2003). In spite of being repelled by methyl salicylate, *D. noxia* also found wheat plants exposed to these chemicals less attractive than the unexposed controls (Chapter 5).

Cultivars resistant to *D. noxia* were bred and released and more than 70% of farmers in the Free State Province are planting these cultivars (Marasas *et al.*, 1997). Natural enemies of the aphid were also introduced, released and became established during the development of an integrated control programme for *D. noxia* (see Chapter 2). One of the introduced parasitoids *Aphelinus hordei* (Kurdjumov) became established (Chapter 2) and prefers to oviposit in *D. noxia* (Prinsloo, 2000). Recently, a resistance breaking biotype of *D. noxia* developed in the USA (Haley *et al.,* 2004). *Diuraphis noxia* from various parts of the world was found to differ in their reaction to resistant wheat lines (Puterka *et al.*, 1993). Therefore the possibility of *D. noxia* to develop a resistant breaking biotype in South Africa cannot be ruled out. Due to the seriousness of the pest it is necessary to take steps to protect the resistant cultivars and prevent an outbreak of the pest again.

The main objective of an integrated or alternate control programme for this aphid should be the reduction of the pest population below its economic threshold value. This could be achieved either by suppressing a pest population already exceeding a threshold. A delay in initial infestation of the crop could also prevent a population from reaching a threshold during the vulnerable crop stage (Wiedenmann & Smith, 1997). To prevent a pest population increase, sufficient mortality must occur during the latent phase of population growth. In contrast, delaying a pest problem differs because the aphid population can still reach the epidemic phase, but does so out of phase with the vulnerable crop stage (Wiedenmann & Smith, 1997). Due to the fact that aphids are repelled by methyl salicylate, it is thus in theory possible that it could delay the arrival of the pest population in wheat or barley fields. The *D. noxia* population on susceptible

cultivars could still reach the epidemic phase, but at a later crop growth stage that may have less impact on the crop.

The aim of the current study was to determine the response of *D. noxia* to the use of different methyl salicylate formulations on resistant and susceptible cultivars in the field.

6.2   Materials and Methods

6.2.1   Field conditions

Field experiments were carried out in winter-sown wheat on the experimental farm of the ARC - Small Grain Institute (ARC-SGI) at Bethlehem (28° 10'S 28° 18'E), South Africa. Wheat was planted in rows with an inter-row spacing of 40 cm, at a seeding rate of 20-25 kg ha^{-1}, which is standard for dry land wheat production in Free State province.   The crop was sown on 4 July 2003 and 6 July 2004 respectively. Fertiliser was applied during planting at 250 kg ha^{-1}. No herbicides were used and trials were manually weeded when necessary. Maximum daily temperatures during the field trials were measured on the experimental farm and are shown in Figure 6.1. Due to dry conditions each of the two trials were irrigated on 15 October of each respective year. Sprinklers were used, delivering 4 ml h^{-1} rain equivalent for four hours to all plots simultaneously.

In 2003 quellea finches badly damaged the trial when the wheat kernels was still in the soft dough stage and the trial was therefore not harvested. In 2004, the entire trial was covered with bird netting and yield and quality data were obtained.

6.2.2   Field experimental design

A randomised block design was employed in both trials, with six plots for each treatment. Plots measured 2.3m x 5m and each contained five rows of plants. Plots were separated along all edges by 2m wide paths.   During 2003 (first trial) the resistant cultivar Elands and two susceptible cultivars, Betta and PAN 3349 were planted, while in 2004 (second trial) only Elands and Betta were planted.
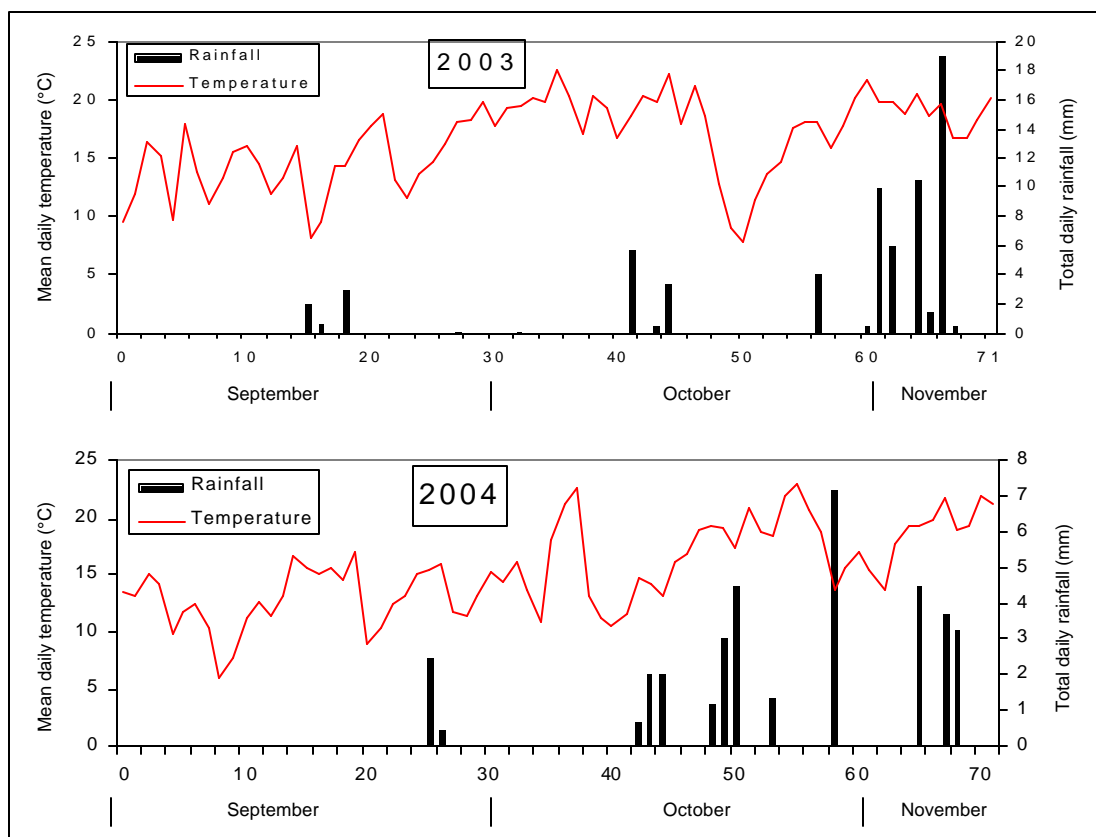
Figure 6.1   Maximum temperature and total daily rainfall for the field trial periods in 2003 and 2004.

Application of the various chemical treatments resulted in six cultivar x treatment combinations in both experiments, as detailed below.   To ensure aphid populations, both experiments were artificially seeded with *D. noxia* on 10 September 2003 and 7 September 2004 respectively.   Ten pots with ten wheat plants (cultivar Betta) infested with *D. noxia* were placed along each edge of the trial (40 plants total), about 2m away from the edge of the sown plots. These plants were left for two weeks.

6.2.3   Chemical formulation and treatments

A slow-release wax pellet formulation was used to dispense the active compound. The pellets consisted of a mixture of two paraffins, containing 10% (m/m) active compound (Ninkovic *et al*., 2003). Three different essential oils were included in the formulation. Essential oils in general have been of interest in insect pest

control (Isman, 2000; Koschier *et al.*, 2002). They have the advantage of being generally non-toxic and environmentally benign. Initial laboratory experiments indicated behaviour-modifying effects of all these substances against *R. padi* ([5]R Glinwood and V Ninkovic, personal communication). The plant derived essential oils used in the formulation were wintergreen, peppermint and eucalyptus (Crearome, Sweden), respectively releasing the volatiles, methyl salicylate, menthol and 1,8-cineole. Three groups of pellets, each containing a single volatile were produced. The OX54 mixture was prepared by mixing equal weights of these three groups of pellets.

In the first trial, the mixture OX54 was compared against an untreated control on each of three cultivars. During the second trial, both OX54 and methyl salicylate alone was compared against an untreated control on two cultivars. Since wax pellets alone (not loaded with any chemical) have previously been shown not to influence aphid populations (Ninkovic *et al.*, 2003), this treatment was not included in the experiments. The following treatments were included in the trials:

First trial (2003): Elands + OX54, Elands untreated; Betta + OX54, Betta untreated; Pan 3344 + OX54, Pan 3344 untreated.
Second trial (2004): Elands + OX54, Elands + methyl salicylate, Elands untreated; Betta + OX54, Betta + methyl salicylate, Betta untreated.

Pellets were applied by hand to experimental plots by spreading them evenly along the rows of wheat plants, leaving the pellets on the soil around the bottom of the plants. Each treated plot received 63 g of pellets (i.e. 6.3 g of active substance per 12.5 m^2 per application), giving the equivalent of 5 kg active substance per hectare per application. The first application of pellets to the first trial was made on 8 September 2003, at the 6-8 leaf stage, about ten weeks after planting, and four subsequent applications were made at 14 day intervals until aphid counting ended. In the second trial, the first application of pellets was made on 19 August 2004, at the 2-3 leaf stage, about six weeks after planting. This was followed by six subsequent applications were made. The first four applications of second

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trial were made at 14 day intervals, while the last three applications were made at approximate ten day intervals.

The release rate of volatiles from the OX54 formulation was determined in each trial. Fifty grams of OX54 was placed in each of two aluminium foil plates. The plates were placed outside the trial on bare soil surface. They were covered with fine gause to prevent soil mixing with the pellets and secured to the soil with steel wires. The pellets in each plate were weighed after nine days and the difference in weight was expressed as percentage volatiles released. In the first trial, OX54 release rates of 4.3% and 5.5% was respectively measured for September and October 2003, indicating the effect of higher temperatures on the release of the volatiles. During the second trial 4,6% OX54 and 6.6% methyl salicylate were released by the end of September 2004.

6.2.4   Assessment of aphid populations

Aphid population assessment began on 16 September 2003 and 10 September 2004 respectively (at the stem elongation growth stage) and continued at weekly intervals until 10 November 2003 and 12 November 2004 (at the end of flowering growth stage). Non-destructive sampling was used. On each sampling occasion, the number of infested tillers on each of ten randomly chosen plants per plot were counted, as well as the number of aphids per tiller on the first ten infested tillers. From these data the percentage infested tillers and plants were calculated. The mean number of aphids per plant was also calculated by multiplying the mean number of infested tillers/plant with the mean number of aphids/tiller.

6.2.5   Parasitoid release

On 1 October 2004 approximately 1500 *A. hordei* parasitoids were released. Parasitoids were released in the mummy stage on two opposite sides of the trial. Two longitudinal waxed carton containers that were perforated close to the top were used to release the parasitoids near the middle and about 1m outside the trial. Parasitism in the trial was determined by counting mummified aphids found

on the tillers during the aphid counts. The total number of mummified aphids per treatment were determined and analysed with a Chi-squared test (Van Ark, 1992).

6.2.6 Yield and quality

In 2004, the innermost three rows of each experimental plot were harvested using a small plot harvester. The yield, hectolitre mass, thousand kernel mass and protein content was determined for each plot.

6.2.7 Statistical analysis

Aphid population assessment data was analysed for each counting date by performing an ANOVA for randomised block design and Fisher protec ted least significant difference (LSD) test (Anonymous, 2000) at the 5% level. During this test data for all the treatments were analysed at the same time, showing differences between treated and untreated plots of the same cultivar and between different cultivars. It was sometimes found that although the ANOVA was significant, no significant differences occurred between the treated and untreated plots of a specific cultivar according to the LSD test. Yield and quality data were also analysed using an ANOVA for randomised block design and Fisher protected least significant difference test. Aphid population assessment data of all the counting dates were then pooled per treatment and analysed in the same way to determine differences between treated and untreated plots. Data were most of the time parametric, but where necessary data were transformed using log (X+0.5).

6.3   Results

6.3.1 Aphid populations

6.3.1.1   Betta

During the first five counting dates in the first trial the percentage infested plants in the OX5 4 treated Betta plots were lower than in the untreated Betta plots although not significantly (Fig. 6.2A). The percentage infested plants reached a maximum
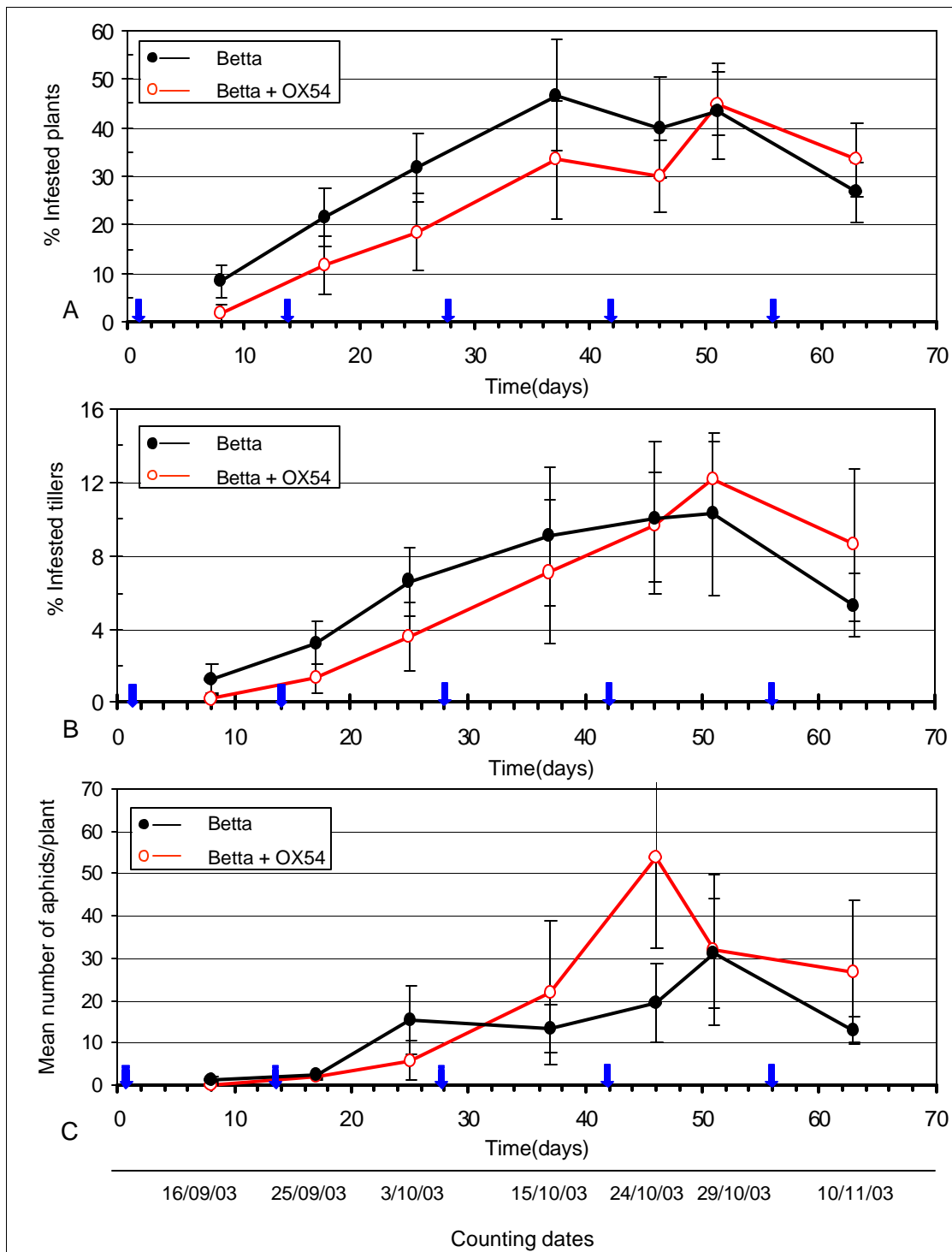
Figure 6.2   Field trial 2003 – Betta (±SE) (A) Mean percentage infested plants (B) mean percentage infested tillers per plant and (C) mean number of *Diuraphis noxia* per plant. Arrows indicate pellet application times.

by 15/10/03 in the control plots, 14 days before the treated plots. On the sixth counting date the percentage infested plants reached a maximum but no differences occurred between the treated and control Betta plots (LSD = 19.5).
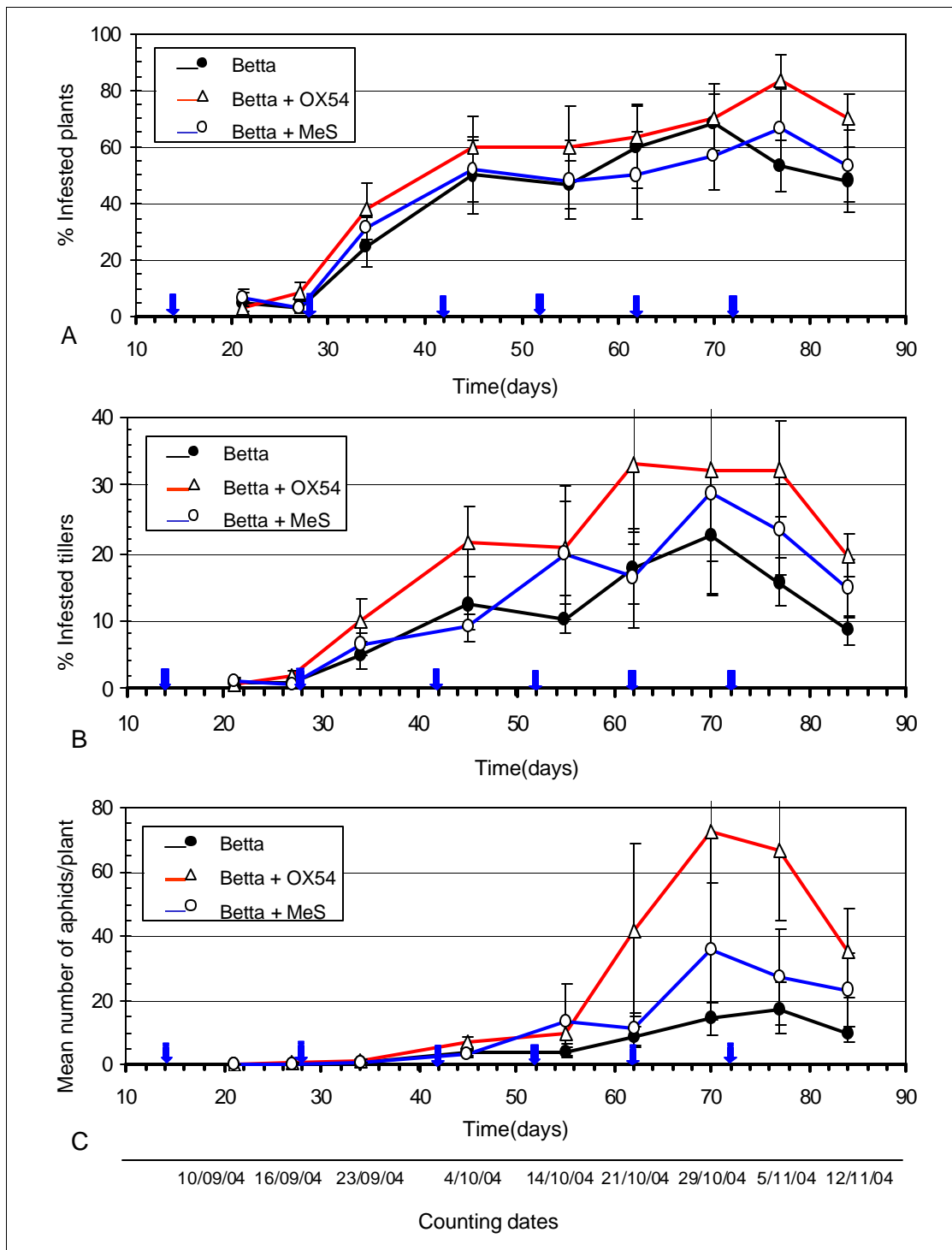
The mean percentage infested tillers on the first four counting dates were lower in the OX54 treated than control Betta plots (Fig. 6.2B). From 24/10/03 the percentage in the treated plots increased faster than in the untreated plots (Fig. 6.2B) although not significantly. The percentage infested tillers in the treated plots reached a higher peak on 29/10/03 than those in the control plots but not significant (LSD = 7.8).

The mean number of aphids/plant, increased rapidly in the OX54 treated Betta plots, reaching a peak approximately five days before the untreated plots (Fig. 6.2C). Although aphid numbers/plant were higher on the treated plots on 15/10/03 and 24/10/03, the ANOVA was not significant different in both cases (P = 0.199 and P = 0.097 respectively) and no LSD test was performed.

During 2004 different Betta plots were treated with methyl salicylate and OX54 respectively and compared to an untreated control (Fig. 6.3). Almost 50% of the flag leaves have emerged by 14/10/04, while most of the plants have completed flowering by the 5/11/04. Similar to 2003 the percentage infested plants in the control plots reached a maximum about seven days before those in the OX54 and methyl salicylate treated plots (Fig. 6.3A). When the percentage infested plants on the treated plots reached maximum numbers on 5/11/98 significantly more plants were infested in the OX54 treated plots than in the control ($F_{5,25}$ = 25.37, P < 0.001, LSD = 18.1). This coincided with the end of the flowering stage of the plants.

The percentage infested tillers in the control plots were most of the time lower than in the treated plots (Fig. 6.3B). From the onset of the flag leave stage (14/10/04) the number of infested tillers in the OX54 treatment seemed to increase more rapidly. Significantly more tillers were infested in the OX54 treatment on 21/10/04 than on the methyl salicylate treatment and the control (F = 4.9, P = 0.003, LSD = 14.9,).

Figure 6.3 Field trial 2004 – Betta (±SE) (A) Mean percentage infested plants (B) mean number infested tillers per plant and (C) mean number of *Diuraphis noxia* per plant. Arrows indicate pellet application times. (MeS = methyl salicylate)

The percentage infested tillers on OX54 treated plots were also significantly more than the control on 5/11/04 ($F_{5,25}$ =12.5, P < 0.001, LSD =10.6) and 12/11/04 ($F_{5,25}$ = 11.2, P < 0.001, LSD = 6.7). No significant differences occurred, however, between the methyl salicylate treatment and the control on the respective dates.

Similar to first trial, aphid numbers per plant increased faster on the treated plots during the second trial, reaching a maximum, seven days before that of the control plots. Data were skew and were transformed as described in section 6.2.7. Between the onset of the flag leaf stage and the end of flowering a rapid increase in the number of aphids per plant were observed especially on the treated plots. During this period the temperature seemed to increase gradually (Fig. 6.1) and probably did not cause such a rapid increase. On both 21/10/04 and 5/11/04 aphid numbers/plant in the OX54 treated plots were significantly higher than on the methyl salicylate treated plots and the control (21/10/04: $F_{5,25}$ = 8.28, P < 0.001, LSD = 1.07; 5/11/04: $F_{5,25}$ = 30.26, P < 0.001, LSD = 0.91). Differences on 29/10/04 between the OX54 and methyl salicylate treatments and the control seemed significant, but high variance within treatments influenced significance. On 12/11/04, significantly more aphids were present on the OX54 treatment than on the control ($F_{5,25}$ = 22.28, P < 0.001, LSD = 0.984), which is also true of the pooled data for all the counting dates ($F_{5,25}$ = 23.73, P < 0.001, LSD = 0.89).

6.3.1.2   Elands

During the first four counting dates in 2003 the percentage plants infested on the treated plots were lower than the control plots, although not significantly (Fig. 6.4A). The number of infested plants on the control plots reached a maximum by 3/10/03, 12 days earlier than on the OX54 treated plots (Fig. 6.4A).

From the first till the third counting dates, the percentage infested tillers in the treated plots were lower than in the untreated plots although not significantly (Fig. 6.4B). The percentage infested tillers on the control plots also reached a maximum, 12 days before the treated plots. No significant differences were found on any of the sampling dates between the treated and untreated plots.
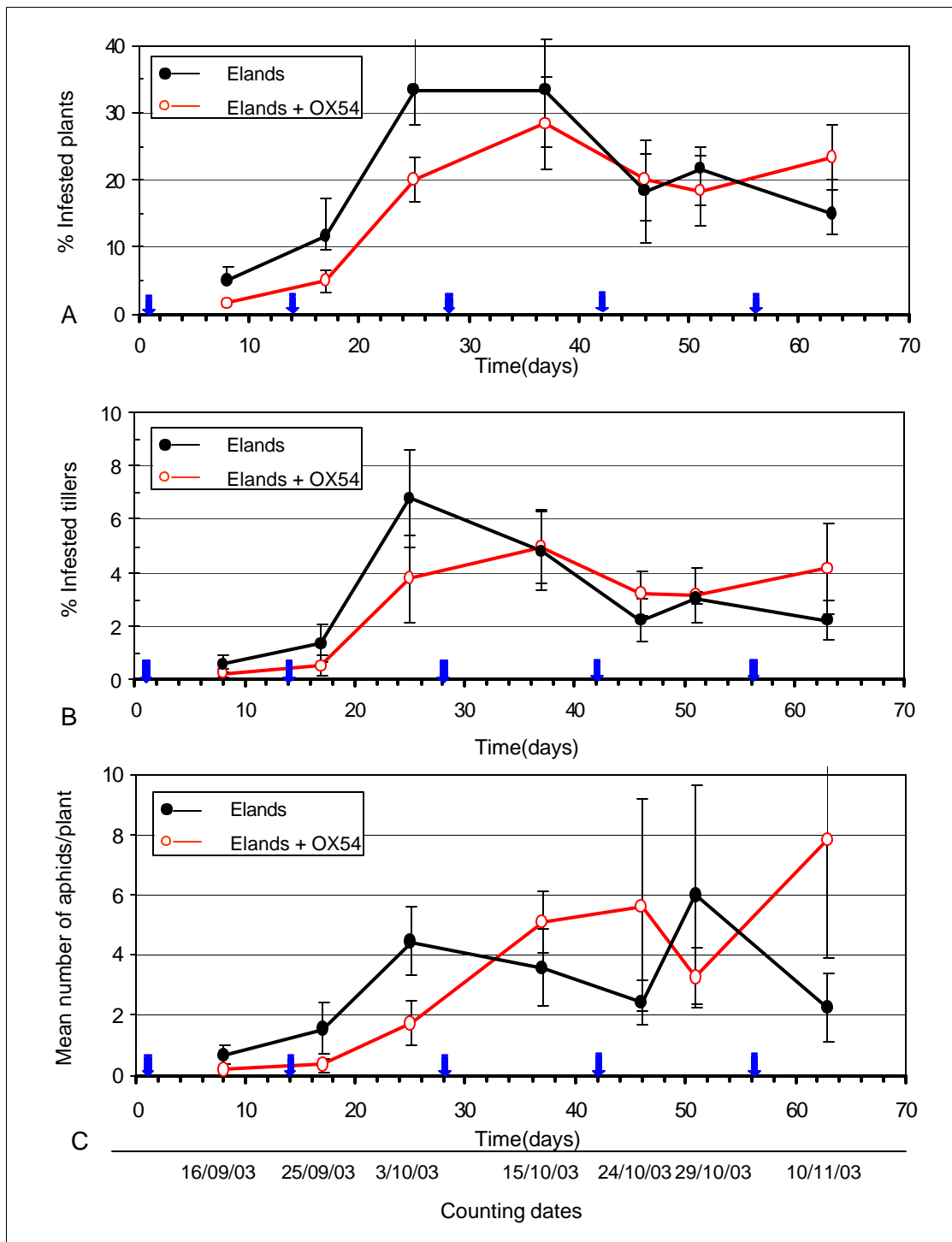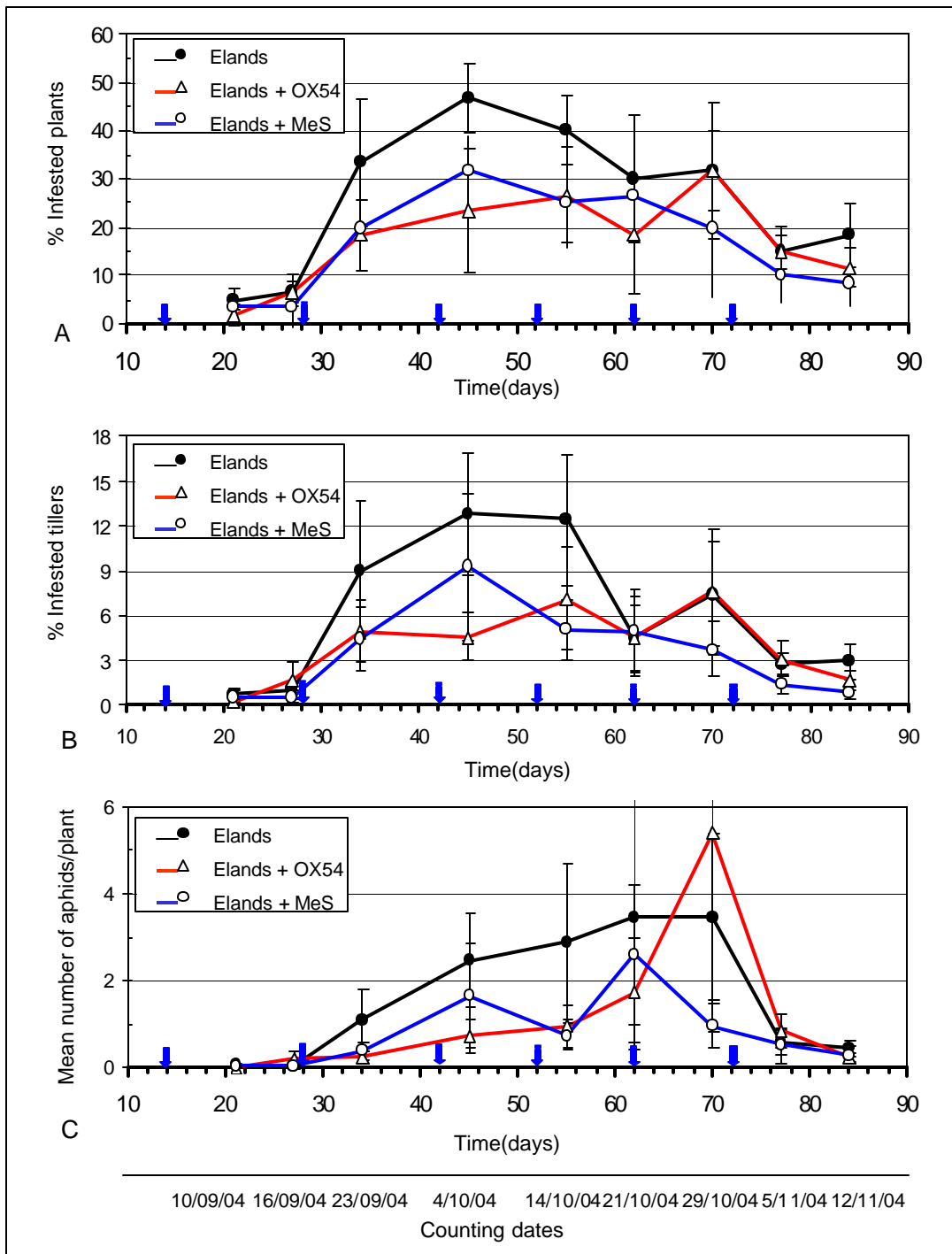
Figure 6.4 Field trial 2003 – Elands (± SE) (A) Mean percentage infested plants (B) mean number infested tillers per plant and (C) mean number of *Diuraphis noxia* per plant. Arrows indicate pellet application times.

The mean number of aphids/plant on Elands was low, compared to susceptible Betta (compare Fig. 6.4 C and Fig. 6.2 C). The increase in aphid numbers/plant in the control plots was unstable reaching a maximum on 29/10/03 approximately 11 days before the treated plots (Fig. 6.4 C).

During the second trial the percentage infested plants in the untreated control reached a maximum on 4/10/04 (Fig. 6.5A), which is 10 days before the onset of flag leaf stage. The percentage plants infested in the methyl salicylate treated plots reached a maximum at the same time, although at a lower level (Fig. 6.5 A). The increase in percentage plants infested in the OX54 treatment increased gradually until it reached a maximum on 29/10/04 (Fig. 6.5A), which is during the flowering stage.   The mean percentage infested plants in both treatments was lower than the untreated control from 23/09/04 until 21/10/04, although not significantly.

The percentage infested tillers in both treatments and the control followed almost the same trend as the percentage plants infested (Fig. 6.5B). Both the control and methyl salicylate treatment reached a maximum percentage infested tillers on 4/10/04, about 10 days before onset of flag leaf stage (Fig. 6.5B). The maximum percentage tillers in the OX54 treatment was infested on 29/10/04, which coincided with flowering of the plants.   No significant differences were found between the treatments and the control at any of the dates.

The mean number of aphids per plant for the control plot increased steadily until 21/10/04, which are 17 days after the tillers reached their maximum percentage infestation (Fig. 6.5 C). On the methyl salicylate treated plots the aphid numbers also reached their maximum on the same date (21/10/04) as the untreated control although at a lower level. On the OX54 treated plots, number of aphids increased gradually at a lower rate and then increased rapidly from 21/10/04 to reach a peak on 29/10/04, which was higher than the untreated control. This occurred between the flag leaf stage and the end of flowering.   The differences between the treatments and the control were only significant on 4/10/04 when OX54 had significantly less aphids per plant than the control ($F_{5,25}$ = 5.81, P =0.001, LSD = 0.815).

Figure 6.5 Field trial 2004 – Elands (± SE) (A) Mean percentage infested plants (B) mean number infested tillers per plant and (C) mean number of *Diuraphis noxia* per plant. Arrows indicate the date of pellet applications. (MeS = methyl salicylate)

6.3.1.3   PAN 3349

This cultivar which is *D. noxia* susceptible was only included in the first trial. The percentage infested plants in the control plots increased rapidly and reached its maximum on 15/10/2003 (Fig.. 6.6 A).   In the treated plots percentage infested plants reached a maximum about 14 days later than the untreated control (Fig. 6.6 A).   The differences between the treated and control plots were however not significant on any of the dates.

The percentage tillers infested in the treated plots reached a maximum on 29/10/03, which is five days after the untreated plots (Fig. 6.6 B).   Significant more tillers were infested in the treated plots on this date ($F_{5,25} = 6.17$, $P < 0.001$, LSD = 7.8).

The mean number of aphids per plant increased slower from 3/10/05 in the treated plots and reached a peak also five days after the untreated plots (Fig.. 6.6 C). The number of aphids per plant did not differ significantly between the treated and control plots on any of the counting dates.

6.3.2   Parasitism

Mummified aphids were found only between 14/10/04 and 12/11/04.   More mummified aphids were counted on the treated Betta than on treated Elands (Fig. 6.7). Significantly more mummified aphids were found on OX54 treated Betta than on Betta and Betta treated with methyl salicylate ($\chi^2 = 23.7$, $P = 0.001$). Except for the *A. hordei* mummified aphids, 11 aphids mummified by *Diaeretiella rapae* (McIntosh) were also found only on Betta treated with OX54 on 5/11/04. The *D. rapae* parasitoids were not released near the trial and probably came from the surrounding environment.
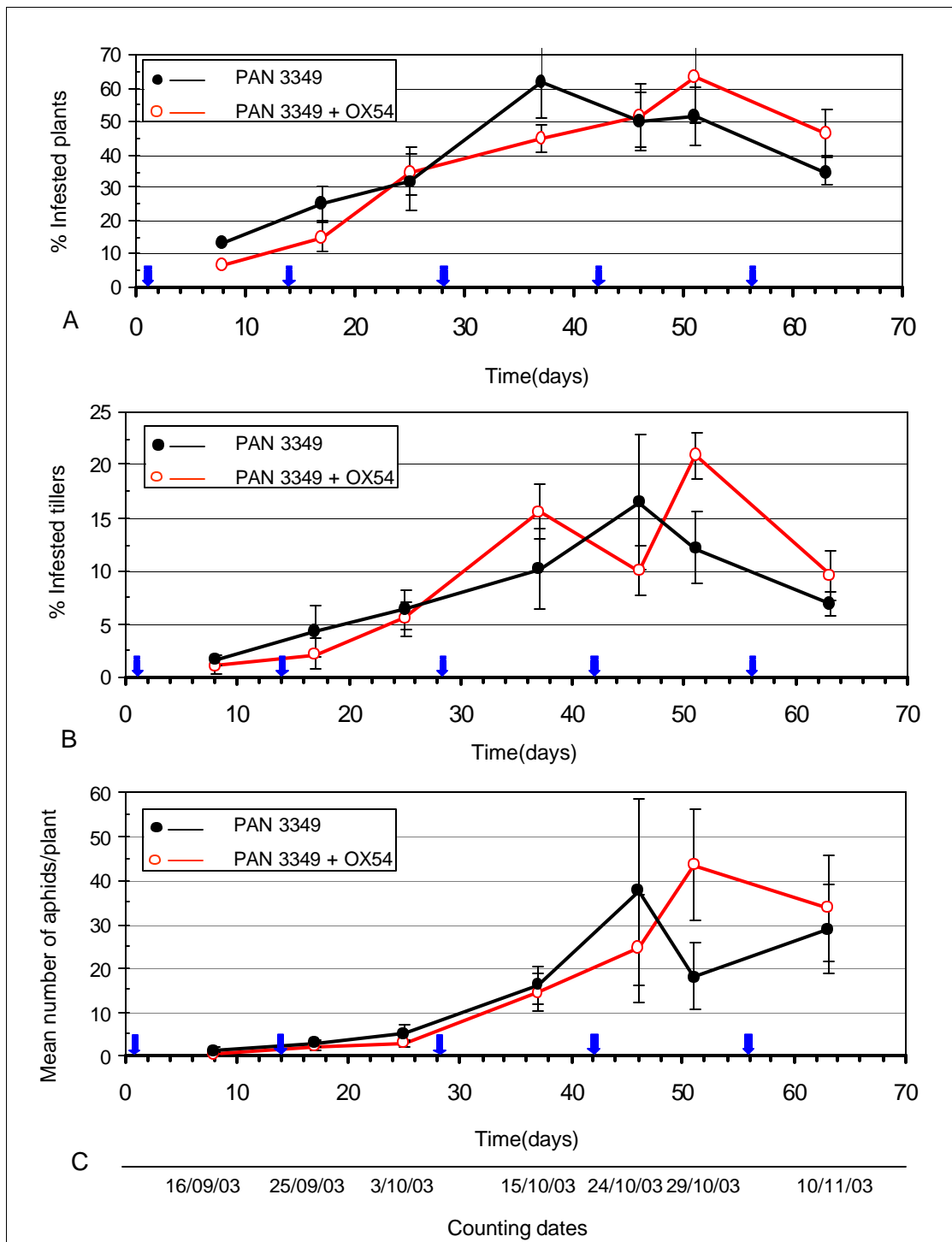
Figure 6.6 Field trial 2003 – PAN 3349 (± SE) (A) Mean percentage infested plants (B) mean number infested tillers per plant and (C) mean number of *Diuraphis noxia* per plant. Arrows indicate the date of pellet applications.
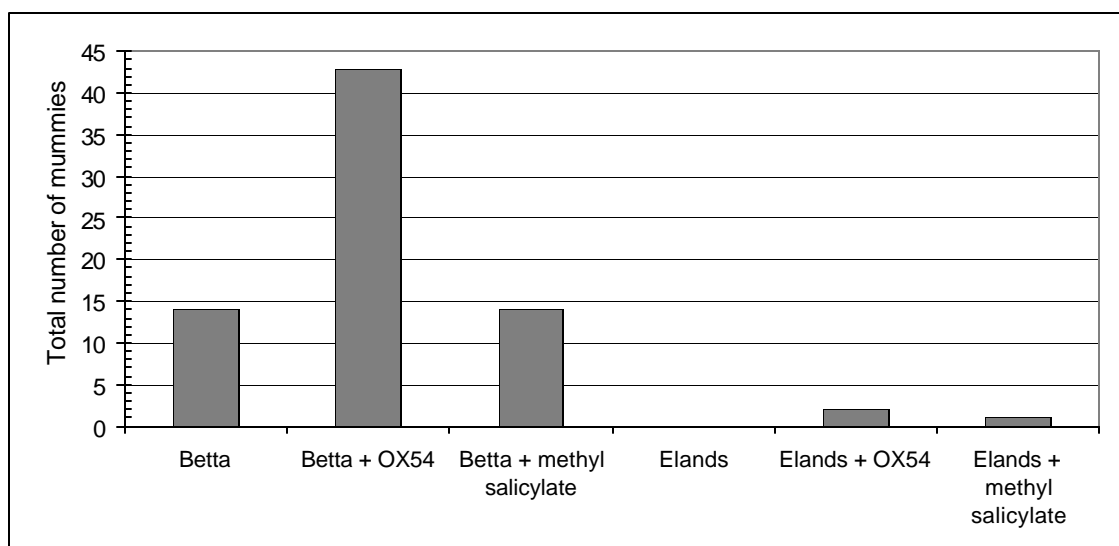
Figure 6.7   Total number of *Aphelinus hordei* mummies found on different treatments during 2004.

6.3.3   Comparison of cultivars

Data of all the counting dates in each trial were then pooled for the categories percentage plants infested, percentage infested tillers and mean number of aphids per plant.   During the first trial treated and untreated PAN 3349 and untreated Betta had a significantly higher percentage infested plants than treated and untreated Elands ($F_{5,25}$ = 3.18, P = 0.023, LSD = 14.8). No significant differences were, however, found between the treated and untreated plots of the same cultivar.   No significant differences were found between the percentage tillers infested in any of the cultivars.   Treated and untreated Elands had significantly less aphids than any of the Betta and PAN 3349 treatments ($F_{5,25}$ = 4.97, P = 0.003, LSD = 0.956).

In the second trial Betta treated with OX54 had a significantly higher percentage infested plants than methyl salicylate treated and untreated Betta as well as any of the Elands treatments ($F_{5,25}$ = 18.22, P < 0.001, LSD = 9.7).   The Elands treatments did not differed significantly from each other.   The Betta - OX54 treatment had a significantly higher percentage infested tillers than methyl salicylate treated and untreated Betta ($F_{5,25}$ = 7.2, P < 0.001, LSD = 6.7) and any

of the Elands treatments. The percentage infested tillers in Elands did not differ significantly between the different treatments. The mean number of aphids per plant on the Betta - OX54 treatment was significant higher than on untreated Betta and all the Elands treatments ($F_{5,25}$ = 16.1 P < 0.001, LSD = 6.7).

The application of methyl salicylate and OX54 had different effects on the different cultivars. The magnitude of these differences was determined by the calculation of the percentage differences between (a) untreated Betta and Betta treated with either OX54 or methyl salicylate, (b) untreated Betta and untreated Elands (c) untreated Betta and Elands treated with either OX54 or methyl salicylate.   These differences were calculated for both trials and for the whole counting period (Tables 6.1 & 6.2).

Table 6.1   Percentage differences in mean number of aphids per plant between untreated Betta and other treatments during the first trial.

	Percentage difference between untreated Betta and…		
Date	Untreated Elands	OX54 treated Betta	OX54 treated Elands
16/9/03	52	88	88.
25/9/03	32.4	14.5	85.5
3/10/03	71.2	62.3	88.8
15/10/03	73.1	-66	61.5
24/10/03	87.6	-177.3	71
29/10/03	80.8	-2.7	89.6
10/11/03	82.6	-106.9	39.5
Mean	**68.5**	**-26.9**	**74.8**

During the first trial the mean percentage difference in aphid numbers per plant between untreated Elands and untreated Betta was 68.5% (Table 6.1). The lower aphid numbers on Elands is due to plant resistance. The mean percentage difference between treated and untreated Betta was negative (Table 6.1), showing the increase in aphid numbers on the treated Betta. The untreated Betta differed from OX54 treated Elands by 74.8% which is 6,3% higher than the difference between untreated Betta and untreated Elands (Table 6.1). The application of OX54 therefore enhanced the resistance on Elands by 6.3% during this trial.

The mean percentage difference between untreated Betta and untreated Elands in the second trial was 28.7% (Table 6.2). This was much lower than during the first trial (Table 6.1). Treatment of Betta with either OX54 or methyl salicylate, increased the number of aphids per plant (Table 6.2), hence the negative values, but the effect was smaller with methyl salicylate (Table 6.2). Aphid numbers on both Betta treatments increased drastically in the second trial compared to the first trial (compare Table 6.2 with Table 6.1). The OX54 and methyl salicylate treated Elands also enhanced the effect of resistance by 25.3% and 31.4% respectively during the second trial (Table 6.2).

Table 6.2 Percentage differences in mean number of aphids per plant between untreated Betta and other treatments during the second trial

Date	Percentage difference between untreated Betta and				
	Untreated Elands	OX54 treated Betta	Methyl salicylate treated Betta	OX54 treated Elands	Methyl salicylate treated Elands
10/9/04	-100	0	-175	50	0
16/9/04	0	-140	40	-40	40
23/9/04	-7	-13.	1.7	47.8	31.4
4/10/04	35.4	-83.9	-13.2	68.3	54.4
14/10/04	26.5	-85.2	-112.9	53.6	79.1
21/10/04	52.5	-322.9	31.1	66.5	66.5
29/10/04	71.4	-338.6	-121.6	60.1	86.6
5/11/04	88.8	-133.8	-10.7	88.6	91.4
12/11/04	90.9	-135.4	-84.1	91.5	91.3
Mean	**28.7**	**-139.2**	**-49.4**	**54**	**60.1**

6.3.4 Yield and quality

Due to cultivar differences the yield of all Betta treatments were significantly lower than all Elands treatments ($F_{5,25} = 2.8$, $P = 0.038$) (Table 6.3). The yield of the Betta control plots was higher than the Betta - methyl salicylate treatment and Betta - OX54 treatments. None of these differences were significant. The yield of the Elands control plots was lower than the Elands - methyl salicylate and Elands - OX54 treatments although not significantly.

Hectolitre mass between all Elands and Betta treatments as groups was significantly different ($F_{5,25}$ = 3.2, P = 0.022). Between different treatments in each cultivar no significant differences were found.

The thousand kernel mass did not differ significantly between treatments ($F_{5,25}$ = 1.38, P = 0.25). Due to cultivar differences the protein content of Betta and Elands as groups, irrespective of treatments, differ significantly ($F_{5,25}$ = 3.1, P = 0.027, LSD 1.318). However, no significant differences were found between the treatments of the different cultivars.

Table 6.3   Yield and quality data for Elands and Betta cultivars treated with different semiochemicals in the 2004 trial

Treatment	Yield (kg/ha) ± SE	Hectolitre mass (kg/hl) ± SE	Thousand kernel mass (g) ± SE	Protein content (%) ± SE
Elands	1563 ± 110a	77.7 ± 1a	31.3 ± 0.5a	13.9 ± 0.4a
Elands + OX54	1625.8 ±186.9a	78.2 ± 0.5a	31.5 ± 1.2a	14.6 ± 0.5ab
Elands + MeS	1636.9 ± 127.6a	77.8 ± 0.7a	31.7 ± 1a	13.9 ± 0.4a
Betta	1242.4 ± 86.1b	75.6 ± 1b	30.2 ± 0.8a	15.7 ± 0.4b
Betta + OX54	1188.2 ± 107.8b	73.8 ± 1.3b	28.8 ± 0.9a	15.3 ± 0.5b
Betta + MeS	1219 ± 109.3b	76 ± 0.9b	30.1 ± 1.2a	15.4 ± 0.4b

MeS = methyl salicylate

6.4   Discussion

Wheat is grown in South Africa in a typical temporary crop environment, over a period of six to seven months per year. Therefore, insect pests of wheat must immigrate from alternate host plants in the vicinity. A possible control option for a pest population in such a temporary crop environment is to decrease the numbers of the immigrating pest before they arrive in the crop habitat (Wiedenmann & Smith, 1997). With fewer immigrants the pest population remains longer in the latent growth phase and decrease the possibility of the pest population reaching the threshold value when the crop is still in its susceptible phase for insect damage (Wiedenmann & Smith, 1997).

Plant derived semiochemicals that act as insect behaviour-modifying chemicals can be used as tools for management of insect pest populations (Powell & Pickett, 2003). These chemicals are capable of inducing a variety of responses in plants, including induction of defences against pathogens and herbivores (Arimura *et al.*, 2000; Walters *et al.*, 2002) and modification of volatile profiles (Dicke *et al.*, 1999). The modification of the volatile profile of plants could repel herbivores including aphids. In this way the immigration of aphids into a wheat field could be limited. This will result in a longer latent population growth phase and the aphid population could reach a damage threshold outside the susceptible phase of the crop.

Response of the aphids in the field was lower than expected and almost no significant differences occurred between treated and untreated plots within each cultivar. Results from the field trial showed that both OX54 and methyl salicylate cause some retarded *D. noxia* population growth on the resistant cultivar Elands although it was not significant (Figs. 6.4 & 6.5). Whether this delay was related to a lower immigration rate or a physiological effect from the plant, or both is not clear. Both the repellence of *D. noxia* and induction of physiological effects in the plants by methyl salicylate and OX54 were demonstrated in Chapter 5. These results could be an indication that *D. noxia* behaviour could be modified by methyl salicylate in wheat similar to that of *R. padi* in barley (Pettersson *et al.*, 1994; Glinwood & Pettersson, 2000b).

During the second trial, the maximum number of aphids per plant on the control and the methyl salicylate treated Elands occurred approximately at the same time after flag leaf stage. The aphid numbers per plant on the OX54 treatment reached their maximum population approximately eight days later and the aphid numbers were higher than the rest (Fig. 6.5 C). The extent of the delay is similar to that recorded for *R. padi* on barley in Sweden (Ninkovic *et al.*, 2003). The reduction in *R. padi* in this instance is important due to the fact that early damage to the barley crop when the plants are still small had a higher economic impact compared to later damage (Ninkovic *et al.*, 2003).

The effect of delay in *D. noxia* numbers on Elands was also reflected in the yield although no significant differences occurred. The untreated control with higher

infestation in the vegetative growth stages and higher aphid numbers/plant extending into the reproductive growth stages had the lowest yield. The OX54 treated plots with low infestation in the vegetative growth stages and high aphid numbers per plant had a slightly higher yield. The methyl salicylate treated plots with low infestation in the vegetative growth stage as well as low aphid numbers per plant during the reproductive growth stages had the highest yield.   The treatments had no effect on the baking quality of the different cultivars.

The treatment of Betta plants with methyl salicylate or OX54 caused an opposite effect than on Elands and stimulated *D. noxia* population development on this cultivar (Figs. 6.2 & 6.3). From the small differences in percentage infested plants and tillers during the first two counting dates on this cultivar (Figs. 6.2A,B; 6.3A,B), as well as the rapid increase in aphid numbers per plant (Figs. 6.2C & 6.3C), it seemed likely to be a physiological effect on the plant.

Methyl salicylate is known as a plant stress signal (Shuleav *et al*., 1997, Chamberlain, *et al.,* 2000; Ninkovic *et al.*, 2003). Glinwood & Pettersson (2000b) stated that changes in oat plants after exposure to methyl salicylate resulted in reduced attraction of *R. padi* to these plants.   In laboratory studies it was demonstrated that methyl salicylate and OX54 respectively could induce a response in the exposed plant (par. 5.3.5). Methyl salicylate could have induced a response in the treated Betta plants, making them more favourable for aphid development and therefore caused a faster population growth of aphids on the plant. Treated plants could also be more attractive to aphids and aphids repelled in other plots could have moved into the Betta treated plots. These assumptions should be tested in future. The rapid increase in aphid numbers per plant on Betta occurred during the reproductive growth phase of the plant and therefore could have an effect on the grain produced by the plant. Although no significant differences in the yield between the different treatments could be found during 2004, the untreated Betta carrying the lowest number of aphids resulted in a higher yield, while OX54 treated Betta containing the highest number of aphids per plant had the lowest yield.

Different cultivars responded differently to the effect of methyl salicylate in the OX54 formulation. On the second *D. noxia* susceptible cultivar, PAN 3349, aphids responded differently to OX54. No increase in aphid numbers occurred in the treated plots compared to Betta. There was, however, little difference between the treated and untreated plots in the percentage plants and tillers infested, but a delay in the populations on the treated plots was visible (Figs. 6.6 A, B). This delay was especially clear in the number of aphids per plant where the population maximum on the treated plots was delayed by five days compared to the untreated control (Fig. 6.6C).

Both OX54 and methyl salicylate reduced the aphid numbers on Elands to some extend and could therefore enhance the effect of the resistance. This was demonstrated by the percentage differences calculated between untreated Betta and treated and untreated Elands (Tables 6.1 & 6.2). The calculation of these differences also showed the increase in aphid numbers on the treated Betta plants. The differences between untreated Betta and Elands differed between years (compare Tables 6.1 & 6.2) probably due to differences in climatic conditions between the trials (Fig. 6.1). It is however apparent that the enhancement effect on Elands also differed between trials (Tables 6.1 & 6.2). During September and the first half of October wheat crop are young and not covering the soil surface. According to Ninkovic *et al.* (2003), high temperatures experienced during this time could influence the release rate of pellets. In the first trial temperatures was high during this period and an increase in release rate from 4.3% to 5.5% was experienced. Although a similar release rate was experienced during the second trial, the exact reasons for the difference in enhancement is not clear.

Herbivore induced volatiles from plants are known to increase the attractiveness to herbivore natural enemies (Vet & Dicke, 1992; Chamberlain *et al.*, 2000). Laboratory tests have shown that both *A. hordei* and *D. rapae* parasitoids were repelled by pellet formulations of both methyl salicylate and OX54 (par. 5.3.4). It was also shown that susceptible and resistant cultivars became more attractive to parasitoids after the plants were exposed to either methyl salicylate or OX54 (par. 5.4.5). The same response was found during the second field trial. More aphids

were parasitised on susceptible Betta than on resistant Elands . From the results it is clear that *A. hordei* parasitised aphids more readily on Betta than on Elands. Parasitism was increased on the OX54 treated Betta by approximately 67% compared to the untreated control. The methyl salicylate treatment was not different from the control. Although some parasitised aphids were found on the treated Elands, numbers were low and could be due to the low aphid numbers present on this cultivar. Enhancement of parasitism is therefore possible when cultivars are treated with OX54.

From the results it is clear that using semiochemicals together with a resistant wheat cultivar could reduce the already reduced aphid numbers feeding on Elands. The lower infestation level and delayed the population maximum resulted in a slight increase in yield compared to the control. The increase in aphid numbers and the decrease in yield on the treated Betta plots is and indication that all cultivars available should be screened before these semiochemicals could be used on a broad basis . The current pellet formulation was developed for use under Swedish conditions. Due to differences in climatic conditions and wheat production methods, the amount released may not be adequate for the more harsh conditions in the Free State Province production area.

As stated earlier methyl salicylate may have two modes of action. The first is the masking of the aphid attractive volatiles from plants and therefore preventing the aphids to identify there host plants. The second is the induction of a defensive response in plants, which could have an effect on the aphids. The initial infestation of the wheat plants could be influenced by masking of the attractive volatiles, but once succeeded in landing on a treated plant, the induced effect may have a bigger effect on the aphids. This was first identified in the laboratory with the aphid settling tests. These aphids were put together with the leaf in a small cage and they only should climb on the leaf and start feeding. Reduced settling thus indicated a response in the plant. The induced response on Betta plants in the field increased the plants susceptibility causing an increase in infestation. The fact that parasitoids also responded positively to treated plants is also a confirmation of an induced plant response.

These semiochemicals do not only modify the behaviour of *D. noxia*, but also induces reactions in the plants, causing them to release volatiles that are attractive to the parasitoids of these aphids. Further investigations into the successful use to these semiochemicals under field conditions in the Free State Province are recommended.

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Chapter 7

General Discussion

7.1   Importance of tritrophic studies in pest control

Pest control in modern agriculture is increasingly moving away from the reliance on exogenously applied pesticides, towards more environmentally friendly methods. This move is the result of several factors like, awareness of the negative effect of broad spectrum pesticides, an increasing level of resistance to common pesticides in many agricultural pests, economic considerations and consumer pressure (Gatehouse, 2002). Host plant resistance and biological control by means of natural enemies are dealt with for many years as high potential alternatives to chemical control. Additionally tritrophic interaction studies (plant–herbivore-parasitoid) indicated strongly that the application of host plant resistance and biological control to a particular pest could give significantly better or worse results than expected from the simple combination of the effects of each factor (Thomas & Waage, 1996).

Farmers in the wheat production areas of the Free State Province in South Africa are using cultivars, resistant to *Diuraphis noxia* (Kurdjumov)*,* since 1992 (Marasas *et al.*, 1997). The introduction of parasitoids for biological control of this pest automatically led to the development of an integrated control programme where both components are involved. An impact analysis on the control programme for *D. noxia* was conducted during 1996-1997 (Marasas *et al.*, 1997). It was estimated that an effective biological control component even under the most conservative scenario, could improve the internal rate of return for the investment in research by 4.7 % (Marasas *et al.*, 1997). The improvement in the net present value at the time of the study was R22.1 million (Marasas *et al.*, 1997). This was in addition to the anticipated positive environmental consequences and emphasised the importance of a positive interaction between host plant resistance and natural enemies.

The introduced parasitoid *Aphelinus hordei* (Kurdjumov) did establish in the mountains of Lesotho, Southern Africa (Prinsloo *et al.,* 2002) and was still active during 2006 (personal observation). However, in the vicinity of the original release sites in the Free State Province it could not be recovered (unpublished data).   In wheat growing regions of the Free State Province, wheat is planted between mid-May and mid-August (Anon., 2005). *Diuraphis noxia* is invading the wheat fields normally from the end of August, depending on environmental temperature and reaches a maximum population between mid-October and mid-November (Aalbersberg *et al.* 1989)(see Chapter 3). When the *D. noxia* population is reaching a maximum, wheat plants are normally in the heading stage and became unfavourable for *D. noxia.* The aphids then move to *Bromus catharticus* (Vahl) grass, which is growing alongside most of the wheat fields. *B. catharticus* and volunteer wheat are the only known alternate host plants for *D. noxia* in this area (Aalbersberg *et al.*, 1988).   During mid-summer (January) *B. catharticus* is flowering and the aphids leave these plants for unknown host plants. From approximately mid-March onwards they are found again on volunteer wheat and new *B. catharticus* in certain regions of the wheat production areas (personal observation).

The wheat-growing season in the lowlands of Lesotho is similar to that in the eastern Free State. However, in the highlands of Lesotho (approximately 2500m above sea level), due to the cold climate wheat is planted during November and harvested from mid-March onwards. High *D. noxia* numbers are also present during February and March in these wheat fields (personal observation). The establishment of *A. hordei* in Lesotho indicated that it moved probably with *D. noxia* from the release sites in the Free State Province. It is thus possible that *D. noxia* could move from the *B. catharticus* with air currents to the mountains and survive there on the planted wheat. The wheat production in the Lesotho highlands could therefore serve as a green bridge for the survival of the *D. noxia* during the summer period in the Free State Province.

Hirose (1998) described a mobile insect pest as an insect that moves freely over large areas and between crops in diversified agro-ecosystems, in ephemeral habitats. These pests are also polyphagous and multivoltine. According to this

description, mobile pests are major pests because their natural enemies have difficulties in changing their habitats to follow the seasonal movement of the pest. Although *D. noxia* is multivoltine but not very polyphagous, moving from planted wheat to *B. catharicus* and probably other grass species, to volunteer wheat and then back to planted fields seems to fit into the description of a mobile pest.

According to Landis & Menalled (1998) many of the immediate factors that are limiting the efficacy of parasitoids in agricultural systems, e.g. the lack of alternative hosts can be the direct result of ecological disturbance imposed on these systems. Disturbance can be defined as any event in time that disrupts the ecosystem, community or population structure and changes resources, substrate availability, or physical environment (Landis & Menalled, 1998). Several intense disturbances, occurring over large areas in agro-ecosystems each growing season, are due to human activities. To effectively conserve parasitoids for biological control may require that disturbance should be actively managed on several levels, e.g. within crops, within farms and at landscape level. For example, if pesticide treatment is eliminated within a field to permit establishment of a parasitoid population and viable metapopulations (patches in natural vegetation where parasitoids can survive) are not available at landscape level to provide immigrants, the intra field effort might not be effective (Landis & Menalled, 1998). This could probably have happened with the parasitoid *A. hordei* where it was released in the eastern parts of the Free State Province. The lack of a continuous population of *D. noxia* hosts throughout the summer prevented the establishment of metapopulations and thus no immigrants were available to parasitise aphids in the fields. *Aphelinus hordei* has moved with *D. noxia* into Lesotho and found a more diverse environment in which a population could survive and became established. The alternate hosts of *D. noxia* in the mountains as well as possible alternate hosts for *A. hordei* are not known and merits investigation. This could give valuable information on the diversity, which is needed for the survival of natural enemies and enhancement of natural enemies of *D. noxia* in the Free State Province.

7.2   Integration of host plant resistance and natural enemies.

Biological control by means of natural enemies and breeding of resistant crop cultivars were mostly been parallel, but independent pest management practices in the past (Cortesero *et al.*, 2000). Therefore, resistance breeding and evaluation procedures for different crops, including *D. noxia* resistance breeding in South Africa, did not examine the direct or indirect effects of host plant resistance on the third trophic level (Thomas & Waage, 1996). This means that positive or negative interactions between host plant resistance and biological control were not identified. It means also that the effects of semiochemicals, sequestration of plant chemicals, or direct physical interactions between host plants and natural enemy were ignored. It is therefore possible that wide scale deployment of resistant varieties could occur that actively interfere with natural enemies, which in turn could reduce the benefits gained from resistance breeding (Thomas & Waage, 1996). These phenomena may have long-term consequences for the persistence of certain key natural enemy species.

Some tritrophic studies demonstrated that resistant triticale, containing high levels of antibiosis, negatively affected growth and reproduction of *D. noxia* and the parasitoid *Diaeretiella rapae* (Reed *et al.*, 1991, 1992). However, other studies on the compatibility of resistant wheat and barley lines with natural enemies resulted in equal parasitism rates on resistance and susceptible lines (Brewer *et al.*, 1998; Farid *et al.*, 1998a, b) and complementary associations between resistant host plants and ladybird predators (Farid *et al*., 1997).

Field trials during 1998 and 1999 were conducted at ARC-Small Grain Institute (ARC-SGI) to determine the interaction between parasitoids and plant resistance. In both years the susceptible cultivar Betta and two different cultivars Gariep and SST 333 were subjected to *D. noxia* infestation in the absence and presence of the parasitoid *A. hordei*. Due to plant resistance, the percentage infested tillers on resistant cultivars were approximately 50% lower on the resistant cultivars than on the susceptible Betta (see Chapter 3).   Parasitism by *A. hordei* reduced percentage infested tillers on Betta by more than 50 and 70% respectively during both years (par. 3.3.4). However, on the resistant cultivars parasitism reduced the

percentage infested tillers by between 5 and 30%.   Parasitism therefore reduced the infestation on Betta to a level where there was no significant difference between the resistant and susceptible cultivars (par. 3.3.4).   When multivoltine insects such as aphids are feeding on partial resistant cultivars, their increase rate is reduced at a certain level (Van Emden & Wearing, 1965).   In the presence of parasitoids the increase rate of aphids is further reduced and therefore the reduction effect on partial resistant cultivars is enhanced. Enhancement of plant resistance in the presence of parasitoids therefore should result in a higher susceptibility to resistance (S/R) ratio than in the absence of parasitoids. During the 1998 trial a lower S/R ratio in the presence of parasitoids showed a negative effect when plant resistance and parasitoids were combined, while in 1999, enhancement of resistance was shown on Gariep and a negative effect was shown on SST 333.

A reason for the inverted S/R ratios could be the high level of parasitism (51.2% and 79.4% respectively for 1998 and 1999) on Betta causing a huge reduction in infestation. This reduction was in both years higher than the reduction caused by the resistance alone, except for SST 333 in 1998. However, it was clear that the antibiotic resistance strongly reduced infestation in both SST 333 and Gariep. Although effective reduction in infestation was obtained when plant resistance and parasitoids were added there was a sub-additive effect (where plant resistance inhibits the contribution of biological control) in the interaction between them resulting in inverted S/R ratios.   Van Emden (1986) reported on contrasting results found where a partial resistant Brussels sprout cultivar released a lower concentration of volatile substances than a susceptible cultivar, attracting the parasitoids more towards the susceptible cultivar than to the partial resistant one. Parasitoids are known to react to volatiles from wheat (Braimah & Van Emden, 1994; Van Emden *et al.*, 2002). The antibiotic nature of resistance mentioned by Du Toit (1989) may in the current study also involve herbivore induced allelochemicals that may interact with the natural enemies. However, conditioning of natural enemies to the host plant of the aphid was also recorded (Verkerk *et al.,* 1998). The reason for the high level of parasitism of *D. noxia* on susceptible Betta may therefore be that Betta released more volatiles than the resistant cultivars. The parasitoids may experience emergence conditioning to Betta during rearing in

the greenhouse and preferred the aphids on this cultivar, a phenomenon that should be investigated (Van Emden *et al*., 1996; Storeck, *et al.,* 2000).

Although good reduction in aphid infestation resulted from the augmentative release of the parasitoids, some negative interaction between host plant resistance and the parasitoids was experienced. This sub-additive effect probably originated from sequential effects of interaction between host plant resistance and biological control on life-history parameters of *D. noxia.* Host plant resistance e.g. could caused a prolonged life cycle and reduced reproduction in *D. noxia*, while the parasitoid only host feed on a certain aphid size. However, the sub-additive effect may also originate through an effect of host plant resistance on natural enemy efficacy, e.g. less volatiles being produced by resistant cultivars, which reduce the host habitat location of the parasitoid.

The action of natural enemies was also capable of substantially modifying the efficacy and durability of host plant resistance (Thomas & Waage, 1996). Pest resurgence following pesticide use revealed that natural biological control, through its action to reduce pest densities and selection pressure on resistant varieties, is an important mechanism in ensuring the durability of host plant resistance. Thus the action of natural enemies is a critical component of an effective resistance management strategy (Thomas & Waage, 1996).

7.3   Semiochemicals involved in tritrophic interaction

Semiochemicals are known to play a major role as cues to aid natural enemies in locating and recognising their hosts or prey (Nordlund *et al.*, 1981; Vet & Dicke, 1992; Barbosa & Benrey, 1998; Vinson *et al.,* 1998; Chamberlain *et. al*, 2000; Ruther & Kleier, 2005). Intact plants maintain a baseline level of volatile metabolites that are released from the surface of the leaf and from accumulated storage sites in the leaf. They include monoterpenes, sesquiterpenes and aromatics and form part of the constitutive or 'static' defence mechanism of plants, which are often accumulated in specialised glands or trichomes (Paré *et al.*, 1999; Gatehouse, 2002). When plants are damaged by herbivores, an 'active' or

induced mechanism release volatile compounds which are synthesised and released in response to herbivore attack (Paré *et al.*, 1999; Gatehouse, 2002).

Different cultivars could influence the efficacy of parasitoids that are responding to chemical cues from the plants (Kalule & Wright, 2004). The efficacy of parasitoids could be influenced by using plant varieties with desired characteristics e.g. plants that release greater amounts of volatile compounds that attract parasitoids (Barbosa & Benrey, 1998; Powell & Pickett, 2003).

Volatiles were found to be involved in host habitat location of both parasitoids *A. hordei* and *D. rapae*. Volatile profiles emitted by infested plants of susceptible Betta and two different resistant cultivars, Elands and SST 333 differed qualitatively (different volatiles) and quantitatively (concentration of a certain volatiles) (par. 4.3.6).   These differences resulted in different behavioural responses by the parasitoids (par. 4.3.3). For instance, *A. hordei* were not able to distinguish between infested and clean Elands while *D. rapae* did. *Diaeretiella rapae,* however, did not distinguish between the infested and clean SST 333 while *A. hordei* did so significantly. Substantial similarities in volatile profiles of infested Betta and Elands may be the reason why *A. hordei* did not distinguish Betta from Elands (par. 4.3.6). *Diaeretiella rapae,* however, significantly preferred Betta to Elands, demonstrating that the parasitoid species responded to different volatiles from the same cultivars. This means that both *A. hordei* and *D. rapae*, presenting a narrow and wide host spectrum respectively (Pike *et al*., 1999, Prinsloo, 2000), use different volatiles or volatile combinations from the same plant-herbivore combination to locate their host habitats. The specificity of herbivore-induced volatiles was demonstrated in several natural enemy species. Natural enemies can also distinguish between different plant-herbivore combinations (Takabayashi *et al*., 1998; Ninkovic *et al*. 2001).

Conditioning of the parasitoids during emergence, to chemicals present in the mummy shell (Van Emden *et al*., 1996; Storeck *et al.,* 2000), could be involved in the preference of both parasitoid species for the susceptible cultivars Betta, Tugela and Hugenoot. Although these cultivars may have some similarities in their genetic background, they are still different cultivars. It could be possible that

some key volatiles are released by most susceptible cultivars, which may be not detectable in resistant cultivars and therefore the breeding of resistant cultivars have a definite influence on the host habitat location of parasitoids. The specific volatiles involved in the host habitat location of these parasitoids should be identified and their abundance in resistant cultivars determined. This could contribute to the manipulation of plants in order to promote the third trophic level.

7.4 The application of semiochemicals to enhance aphid control through behaviour manipulation

Semiochemicals that act as insect behaviour-modifying chemicals can be used in insect pest population management (Powell & Pickett, 2003). This approach is initially based on the conservation of natural enemy populations within agro-ecosystems by means of habitat manipulation. However, it is also linked with the manipulation of insect behaviour to exploit tritrophic interactions. Most of these interactions between the plant, the aphid and the natural enemy involve insect behavioural responses to semiochemicals (Powell & Pickett, 2003). Apart from serving as signals for beneficial insects, volatile compounds released by plants are capable of inducing a variety of responses in plants, including induction of defences against pathogens and herbivores (Arimura *et al*., 2000; Walters *et al*., 2002). Manipulation of plant signals offers the most promising perspective for enhancing the efficacy of biological control agents in the field (Cortesero *et al*., 2000)

The herbivore induced compound, methyl salicylate, is released by several plants (James, 2003), including the winter host plant (*Prunus padus)* of the bird cherry oat aphid, *Rhopalosiphum padi* (Linnaeus) in Sweden (Pettersson *et al*, 1994, Glinwood & Pettersson, 2000a, b). Methyl salicylate is repellent to *R. padi* and is used as a host-leaving signal.

Despite the fact that some artificial attractants were not successfully applied in the field (Cortesero *et al.,* 2000), methyl salicylate was successfully applied to aphid populations in the form of a slow release wax pellet formulation and spray application on barley in the UK and Sweden (Pettersson *et al*., 1994; Ninkovic *et*

al., 2003). It was demonstrated as a repellent to cereal aphids in general and is commonly produced by plants that are attacked by pathogens and herbivores as a signal related to defence induction (Pettersson *et al.*, 1994; Shulaev *et al.*, 1997). Exposure of cereal plants to this substance makes them less acceptable to *R. padi* (Glinwood & Pettersson, 2000b).

Laboratory studies confirmed the general repellency of methyl salicylate towards aphids by repelling *D. noxia* and South African *R. padi*, although none of their alternate host plants are known to release this compound. Included in the slow release pellet formulation were two other semiochemicals, menthol and 1,8-cineole, which were also found to be effective repellents of *D. noxia* during olfactometer tests, although lower concentrations were less effective. According to Chamberlain *et al.* (2000) some olfactory neurons on aphid antennae did not respond to host plant volatiles, but are stimulated by non-host plant volatiles. The reason why *D. noxia* and South African *R. padi* reacted to menthol and 1,8-cineole could simply be an avoidance reaction of a poor quality host plant, or a non-host plant species (Chamberlain *et al.*, 2000)

Although herbivore induced plant volatiles are known to attract natural enemies (Vet & Dicke, 1992; Cortesero *et al.*, 2000; Powell & Pickett, 2003), the parasitoids *A. hordei* and *D. rapae* were also repelled by some of the semiochemicals tested, but differences occurred between the two species. *Aphelinus hordei* was strongly repelled by methyl salicylate and menthol respectively, while the reaction towards OX54 (three compounds combined) was less strong but still significant. *Diaeretiella rapae* was strongly repelled by 1,8-cineole but less strongly by methyl salicylate and OX54. The fact that the parasitoids were repelled by these semiochemicals and not attracted as expected is probably an indication that these are not induced by *D. noxia* when feeding on wheat. *Aphelinus hordei* did not respond to 1,8-cineole while the *D. rapae* was not responding to menthol indicating that the different parasitoids differ in sensitivity to other semiochemicals. A possible reason for this reaction could be the differences in their host ranges. Parasitoids could, however, also react to different volatiles from the same plant as was demonstrated by James (2003).

Glinwood & Pettersson (2000b) stated that changes in oat plants, after exposure to methyl salicylate, resulted in reduced attraction of *R. padi* to these plants. This could also be true for wheat cultivars, because reduced settling of *D. noxia* on different wheat cultivars was found in the current study. Methyl salicylate could probably serve as a signal that induces a defensive response in wheat plants. Not all cultivars responded in the same way to these semiochemicals with very little effect on susceptible Betta. Significant reduction in aphid numbers on susceptible Tugela was recorded more often with methyl salicylate than with OX54. Similar to the reaction of susceptible Tugela, significant less aphid settling was found in more occasions with methyl salicylate than with OX54 treatment on both resistant Elands and Tugela-DN. The reason for the difference in reaction when treated with different formulations is not clear, but the presence of 1,8-cineole and menthol in OX54 may not contribute to the signalling function but rather have a masking effect on the methyl salicylate in this formulation.

Tugela and Tugela-DN plants treated with either methyl salicylate or OX54 were all becoming attractive to both parasitoid species. According to Chamberlain *et al.* (2000) certain volatile compounds from plants can be used to elicit induced defence in plants prior to attack. Both methyl jasmonate and methyl salicylate were used to demonstrate the effect, though methyl salicylate was used to demonstrate induction of pathogen defence when externally applied (Shulaev *et al.*, 1997). Different feeding guilds of insects activate different responses in plants. Chewing insects i.e. caterpillars, predominantly activate the jasmonic acid mediated signalling pathway, while aphids and other phloem-feeding insects frequently activates the salicylic acid signalling pathway typically associated with responses to many pathogens (Mohase & Van der Westhuizen, 2002; Kaloshian & Walling, 2005). (Z)-jasmone, a well known component of plant volatiles was repellent to certain morphs of the lettuce aphid *Nasonovia ribis nigri* (Birkett *et al.*, 2000). It was attractive towards a coccinellid predator and an aphid parasitoid. Uninfested bean plants, exposed to (Z)-jasmone became attractive to these natural enemies (Birkett *et al*, 2000). Therefore, the assumption could be made that the exposure of both Tugela and Tugela-DN to methyl salicylate and OX54 induced the defence reaction in these plants causing them to produce volatiles that are attractive to both *A. hordei* and *D. rapae*. According to Van der

Westhuizen & Pretorius (1995) it was found that *D. noxia* resistance response is not a wounding response, but more a typical hypersensitive response, characteristic of pathogenesis. This also confirms the assumptions that changes induced by treatment of wheat cultivars with methyl salicylate reduced RWA settling.

Field trial results have shown that semiochemicals could cause a delay in the immigration of *D. noxia* into wheat fields planted with resistant cultivars, delaying the increase in aphid numbers by a few days. Methyl salicylate as a plant stress signal may have two modes of action (Chamberlain *et al.,* 2000; Ninkovic *et al.*, 2003). The first is the masking of the aphid attractive volatiles from plants, consequently preventing the aphids to identify there host plants. The second is the induction of a defence response in plants making the plants less attractive to the aphids or more attractive to aphid natural enemies. The initial infestation of the wheat plants could be influenced by masking of the attractive volatiles, but once succeeded in landing on a treated plant, the induced effect may have even bigger effects on the aphids. This was first identified in the laboratory with the aphid settling tests. *Diuraphis noxia* were put together with an intact leaf in a small cage and they could climb on the leaf and start feeding. Reduced settling was found indicating a response in the plant. The fact that parasitoids also responded positively to treated plants is also a confirmation of an induced plant response. The induced response on Betta plants in the field increased the susceptibility of the plants causing an increase in infestation. The results confirm that aphid behaviour could be modified by these semiochemicals in wheat, similar to barley (Ninkovic *et al.*, 2003).

The application of semiochemicals to the resistant wheat cultivar Elands could reduce the already reduced *D. noxia* numbers feeding on this cultivar causing a synergistic effect in aphid control. The lower infestation and delayed population growth resulted in a slight increase in yield compared to the control. The opposite response that was found on Betta, suggested the screening of all cultivars available before semiochemicals should be used on a broad basis. The current pellet formulation was developed for use under Swedish conditions. Due to differences in climatic conditions and wheat production methods, the amount

released may not be adequate for the more harsh conditions in the Free State Province production area.

The use of semiochemicals thus not only modified the behaviour of *D. noxia*, but also induced reactions in the plants, causing them to release volatiles that were attractive to the parasitoids of these aphids. Further investigations into the successful use to these semiochemicals under field conditions in the Free State Province are recommended.

7.5 Conclusion

Tritrophic studies revealed the occurrence of extensive interactions between *D. noxia*, different wheat cultivars and its natural enemies. The positive integration of host plant resistance and biological control can have two objectives namely synergistic reduction of pest densities and the protection of durability of resistance. In highly resistant cultivars where resistance could break down due to its limited genetic base, the primary value of biological control will be ensuring durability of resistance. Some of the wheat cultivars resistant to *D. noxia* seem to be highly resistant and the efficacy of natural enemies in these wheat fields is of utmost importance. The application of semiochemicals in these cases should also be investigated. Where resistant cultivars are less effective and hence have more durable resistance, the value of biological control are to augment the effect of plant resistance on the reduction of the pest population in such a way that the effects of plant resistance and biological control are sufficient to prevent damage. The understanding and effective manipulation of agro-ecosystems in the wheat production areas of the Free State Province is therefore essential for the successful establishment of natural enemies.

7.6   References

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SUMMARY

Host plant resistance and biological control by means of natural enemies are becoming more favourable as high potential alternatives for chemical control of insect pests. Tritrophic studies (plant–herbivore-natural enemy) indicated that the application of host plant resistance and biological control to a particular pest could give significantly better or worse results than expected from each component respectively.

Russian wheat aphid *Diuraphis noxia* (Kurdjumov) is a serious pest of wheat in South Africa since 1978.   Plant resistant cultivars are being used against *D. noxia* since 1992. The introduction of parasitoids and predators for biological control of this pest automatically led to the development of an integrated pest control programme involving both control strategies. Nothing is known about interactions between resistant cultivars, *D. noxia* and natural enemies in South Africa. These interactions could have substantial influence on the efficacy of the control programme.

The parasitoid *Aphelinus hordei* (Kurdjumov), introduced from the Ukraine, established in the Lesotho highlands after being released in the wheat production areas of the Free State Province. This parasitoid together with a native parasitoid *Diaeretiella rapae* (McIntosh), also parasitising *D. noxia* in South Africa, was included in a study on tritrophic interactions. *A. hordei* and *D. rapae* respectively have narrow and wide host ranges.

Field studies on the interaction between *A. hordei* and resistant and susceptible cultivars indicated reduction in aphid population growth on each of the cultivars. *Diuraphis noxia* was highly parasitised on a susceptible cultivar Betta, while a positive interaction on resistant Gariep occurred, resulting in the enhancement of the resistance. A slightly lower percentage control was found on SST 333 in the presence of *A. hordei*.

Volatile profiles emitted by infested Betta plants and resistant Elands and SST 333 plants, differed qualitatively (different volatiles) and quantitatively (concentration of volatiles). These differences caused behavioural differences between parasitoid species e.g. *A. hordei* could not distinguish infested from clean Elands, while *D. rapae* did. *Diaeretiella rapae* could not distinguish infested from clean SST 333 while *A. hordei* did. *Aphelinus hordei* could not distinguish between infested Betta and Elands, while *D. rapae* significantly preferred Betta to Elands. Parasitoids therefore responded to different volatiles from the same cultivars. This means that both *A. hordei* and *D. rapae*, use different volatiles or volatile combinations from the same plant-herbivore combination in host habitat location.

Semiochemicals e.g. methyl salicylate, that act as insect behaviour-modifying chemicals, was tested in the laboratory and the field as potential control options against *D. noxia*.   Volatile compounds released by plants could serve as signals attracting beneficial insects and induce a variety of responses in plants. A slow release wax pellet formulation named OX54 releasing methyl salicylate, menthol and 1,8-cineole was tested. Olfactometric studies showed that *D. noxia* and *R. padi* was repelled by each of the compounds although not released by their alternate host plants in South Africa.

Both parasitoid species were repelled by some of the semiochemicals tested, but differences occurred between the two species. *Aphelinus hordei* did not respond to 1,8-cineole, while *D. rapae* was not responding to menthol indicating that the different parasitoids differ in sensitivity to other semiochemicals. The repellence of the parasitoids by methyl salicylate indicated that these volatiles are not induced by *D. noxia* when feeding on wheat. Different host range of the parasitoids may be a reason for this reaction. OX54 and methyl salicylate respectively caused a delay in the immigration of *D. noxia* into resistant cultivar Elands during field trials, but on susceptible Betta an increase in infestation was found. Lower infestation on treated Elands resulted in a slight increase in yield compared to the control.

The positive integration of host plant resistance and biological control can have two objectives namely synergistic reduction of pest densities and the protection of durability of resistance. Some resistant wheat cultivars to *D. noxia* seem to be

highly resistant and the efficacy of natural enemies in these wheat fields is of utmost importance. The application of semiochemicals in these cases should also be investigated. Where resistant cultivars are less effective and hence have more durable resistance, the value of biological control is to enhance the effect of plant resistance on the reduction of the pest population in such a way that the effects of plant resistance and biological control are sufficient to prevent damage. The understanding and effective manipulation of agro-ecosystems in the wheat production areas of the Free State Province is therefore essential for the successful establishment of a successful integrated pest control programme.

Keywords: *Diuraphis noxia*, *Aphelinus hordei, Diaeretiella rapae*, chemical ecology

OPSOMMING

Gasheer plant weerstand en biologiese beheer met behulp van natuurlike vyande neem in gewildheid toe as alternatiewe beheermetodes vir chemiese beheer. Trofiese vlak verwantskapstudies (tussen plant, herbivoor en natuurlike vyand) het aangedui dat die gesamentlike gebruik van plantweerstand en biologiese beheer op 'n sekere plaag betekenisvol beter, maar soms ook swakker beheer tot gevolg het as wanneer die beheermetodes afsonderlik gebruik word.

Die Russiese koringluis *Diuraphis noxia* (Kurdjumov) is sedert 1978 die belangrikste insekplaag van koring in Suid Afrika. Weestandbiedende koring kultivars word sedert 1992 as beheermetode vir *D. noxia* gebruik. Die invoer van parasitoïede en predatore vir biologiese beheer van *D. noxia* het vanselfsprekend tot die ontstaan van 'n geïntegreerde plaagbeheerprogram gelei, waarin beide beheermetodes betrokke is. Geen inligting is bekend oor die wisselwerking tussen weerstandbiedende kultivars, *D. noxia* en natuurlike vyande in Suid Afrika nie. Hierdie wisselwerkings kan verreikende gevolge inhou vir die doeltreffendheid van die beheerprogram.

Die parasitoïed *Aphelinus hordei* (Kurdjumov), wat uit die Oekraïne ingevoer is, het na vrystelling in die koringproduserende gebiede in die Vrystaat Provinsie en die hooglande van Lesotho gevestig. Hierdie parasitoïede is saam met 'n plaaslike parasitoïed, *Diaeretiella rapae* (McIntosh), wat ook *D. noxia* in Suid Afrika parasiteer, in die studie gebruik. *Aphelinus hordei* en *D. rapae* het onderskeidelik 'n meer beperkte en 'n wye gasheerreeks.

Veldproewe oor die interaksie tussen *A. hordei,* weerstandbiedende en vatbare kultivars het aangedui dat luispopulasies se groei afneem in die teenwoordigheid van die parasiatoïed. 'n Groot aantal *D. noxia* is op vatbare Betta geparasiteer, terwyl daar 'n positiewe interaksie met weerstandbiedende Gariep was wat die versterking van die weerstand tot gevolg gehad het. Die beheer van *D. noxia* op SST 333 in die teenwoordigheid van *A. hordei* was effens swakker as op die kontrole wat op 'n klein negatiewe interaksie dui.

Vlugtige reukstof profiele wat deur besmette Betta, weerstandbiedende Elands en SST 333 plante afgeskei word, verskil in kwaliteit (verskillende reukstowwe) en kwantiteit (konsentrasie van reukstowwe). Hierdie verskille het gedragsverskille tussen parasitoïed spesies veroorsaak en wat daartoe gelei het dat parasitoïede swakker op sommige weerstandbiedende cultivars gereageer het. Die verskillende parasitoïede het ook verskillend op reukstowwe van dieselfde kultivars gereageer. Dit beteken dus dat *A. hordei* en *D. rapae* verskillende reukstof of reukstofkombinasies vanaf dieselfde plant-herbivore kombinasie gebruik om hulle gasheerhabitat te identifiseer.

Reukstowwe wat as seine dien bv. metiel-salisilaat, kan ook as insekgedrags-veranderende stowwe dien en is ook in die laboratorium en veld getoets vir 'n moontlike beheerstrategie teen *D. noxia*. Reukstowwe wat deur plante afgeskei word, kan as seine om natuurlike vyande aan te lok dien, maar kan ook 'n verskeidenheid van reaksies in plante ontlok. 'n Waskorrelformulasie wat reukstowwe stadig vrystel is getoets. Hierdie formulasie genaamd OX54, bevat metiel-salisilaat, mentol, en 1,8 cineole. Olfaktometriese toetse het bewys dat *D. noxia* deur hierdie stowwe afgeweer word, hoewel geeneen van hierdie stowwe deur alternatiewe gasheerplante in Suid Afrika geproduseer word nie.

Beide parasitoïed spesies is ook deur die reukstowwe afgeweer, maar verskille het tussen spesies voorgekom. *Aphelinus hordei* het nie op 1,8-cineole gereageer nie, terwyl *D. rapae* nie op mentol gereageer het nie. Die afwering van die parasietoïede deur metiel-salisilaat, dui daarop dat die stof nie geïnduseer word wanneer *D. noxia* op koring voed nie. Verskille in gasheerreeks samestelling van parasitoïede mag veroorsaak dat hulle verskillend op hierdie stowwe reageer. Gedurende veldproewe het beide OX54 en metiel-salisilaat 'n vertraging in die immigrasie van *D. noxia* na weerstandbiedende Elands teweeg gebring. Op vatbare Betta is egter 'n verhoging in die besmetting gevind. Die laer besmetting op Elands het 'n geringe verhoging in opbrengs in vergelyking met die kontrole tot gevolg gehad.

Die positiewe integrasie van gasheerplantweerstand en biologiese beheer het twee doelwitte naamlik die sinergistiese vermindering van plaagpopulasies en die beskerming van standhoudende plantweerstand tot gevolg. Sommige kultivars het 'n sterker weerstand en die doeltreffendheid van natuurlike vyande in die lande is belangrik vir beskerming van die weerstand. Die toediening van reukstowwe in hierdie gevalle moet ondersoek word. Waar die weerstand swakker is en meer standhoudend, moet natuurlike vyande bykomend die effek van plantweerstand verhoog en voorkom dat skade aangerig word. Kennis oor doeltreffende manipulasie van landbou eksosisteme in die koringproduserende gebiede van die Vrystaat Provinsie is dus belangrik vir die vestiging en doeltreffende werking van 'n geïntegreerde plaagbeheerprogram.