# INVESTIGATION INTO ALTERNATIVE WHEAT APHID CONTROL STRATEGIES FOR EMERGING FARMERS

by

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# DECLARATION

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I declare that the dissertation hereby submitted by me for the *Magister Scientiae* degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university. I further more cede copyright of the dissertation in favour of the University of the Free State.

J.M. Richter

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# ABSTRACT

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In the Qwa-Qwa and Thaba N'chu regions of the Free State Province, South Africa, resource limited farmers that produce wheat are mainly situated in temporary crop environments. They are drastically affected by crop losses that occur during years of serious Russian wheat aphid (*Diuraphis noxia*) (Kurdjumov) and oat aphid (*Rhopalosiphum padi*) (Linnaeus) infestations. Therefore the main objective of this study was to identify simple alternate control methods to be used by small-scale farmers for the control of these aphids.

The focus was on minimizing the numbers of the immigrating individuals. That must happen before they arrive in the crop habitat and decrease the possibility of the pest population reaching damaging levels when the crop is still in its susceptible phase for insect damage. Plant derived semiochemicals, which could modify insect behaviour, were considered as an option to be used since this could be extracted from plants, and were demonstrated to be successful in other countries. These semiochemicals are also known to attract natural enemies of these insects. It was therefore decided to test two types of extracts (an aqueous and a light mineral oil) which could be easily prepared from four plant species, namely Wild wormwood *Artemisia afra* (Jacq. ex Willd.), Big thorn apple *Datura stramonium* (Linnaeus), Khaki bush *Tagetes minuta* (Linnaeus) and Wild garlic *Tulbachia violacea* (Harv.). The plants were chosen due to their availability in the wheat production regions and their possible insect repelling properties known from other species in the same genera. The behavioural response of alate aphids *D. noxia* and *R. padi* and two parasitoids, *Aphelinus hordei* (Kurdjumov) and *Diaeretiella rapae* (McIntosh) to these extracts was tested in olfactometer trials in the laboratory.

The aphid *D. noxia* showed the highest repellence to the aqueous extract of *A. afra* and the oil extract of *T. violacea*. Aphid *R. padi* was also best repelled by the aqueous extract of *A. afra* and the oil extract of *D. stramonium*. The parasitoid *A. hordei* was strongly attracted to the aqueous extracts of *A. afra* and *T. minuta*. *Diaeretiella rapae* on the other hand, was also highly attracted to the aqueous extract of *T. minuta*, but *T. violacea* oil extract had a very strong effect on the parasitoid and would be recommended to farmers. *Artemisia afra* and *T. violacea* are perennials and available as green material for extraction purposes in the winter when wheat is planted. The other two plants are annuals and not available in winter. The *A. afra* aqueous extract will repel both aphid species when sprayed early in the wheat growing season when wheat is still small and aphids are flying into the wheat. This extract will also attract *A. hordei* and this could enhance the biological control of *D. noxia*. The *T. violacea* oil extract could also be used to repel *D. noxia*. It could also be used to attract the parasitoid *D. rapae* later in the season and enhance the biological control of both aphid species. Thus there are potential alternate simple aphid control methods available for small-scale farmers. These methods should be refined and farmers trained to use them effectively.

**Key words**: *Diuraphis noxia; Rhopalosiphum padi; Aphelinus hordei; Diaeretiella rapae;* oil plant extracts, aqueous plant extracts; olfactometer tests; emerging farmers; alternate control.

# OPSOMMING

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In die Qwa-Qwa en Thaba N'chu streke van die Vrystaat Provinsie, Suid Afrika, word hulpbron-beperkte boere aangetref wat koring in tydelik produksie-omgewings aanplant. Hulle lei groot verliese wanneer Russiese koringluise (*Diuraphis noxia*) (Kurdjumov) en hawerluise (*Rhopalosiphum padi*) (Linnaeus) in groot getalle koring in hierdie gebiede besmet. Die hoofdoelwit van hierdie studie was dus om eenvoudige alternatiewe beheermetodes vir boere daar te stel vir die beheer van hierdie luise.

Die fokus was om die aantal plaaginsekte wat in lande inbeweeg, te beperk. Dit moet gebeur voordat hulle die gewas bereik en die moontlikheid verlaag dat die plaagpopulasie skadelikheidsvlakke bereik wanneer die gewas steeds in die vatbare stadium vir insekskade is. Sekondêre chemiese verbindings, wat verkry is van plante wat die potensiaal het om insekgedrag te verander, is oorweeg as alternatief. Dit kan van plante geëkstraheer word en bewyse bestaan dat dit suksesvol in ander lande gebruik word. Hierdie sekondêre chemiese verbindings is ook bekend daarvoor dat hulle die natuurlike vyande van hierdie plaaginsekte aanlok. Op grond hiervan is besluit om twee tipes ekstrakte ('n water en ligte minerale olie) te toets wat maklik vanuit plante voorberei kan word. Vier plante, naamlik Wildeals (*Artemisia afra*) (Jacq. ex Willd.), Olieboom (*Datura stramonium)* (Linnaeus), Kakiebos (*Tagetes minuta*) (Linnaeus) en Wildeknoffel (*Tulbaghia violacea*) (Harv.) is gebruik om ekstraksies van te maak. Die keuse van hierdie plante is op grond van hul beskikbaarheid in die koringproduserende gebiede asook hul moontlike insekafwerende eienskappe wat bekend is by spesies in dieselfde genera. Met olfaktometerproewe in die laboratorium is die gedragsreaksie van die gevleuelde luise *D. noxia*, *R.padi* en twee parasitoïedes *Aphelinus hordei* (Kurdjumov) en *Diaeretiella rapae* (McIntosh) met die ekstrakte getoets.

Die luis *D. noxia* is die beste afgeweer deur die waterekstraksie van *A. afra* en die olie-ekstraksie van *T. violacea*. Die luis *R. padi* is ook die beste afgeweer deur die waterekstraksie van *A. afra* en die olie-ekstraksie van *D. stramonium*. Die parasitoied *A*. *hordei* is sterk aangetrek deur die waterekstraksies van *A*. *afra* en *T*. *minuta*. *Diaeretiella rapae*, aan die ander kant, is ook sterk aangetrek deur die waterekstraksie van *T*. *minuta*, maar *T. violacea* olie-ekstrak het 'n baie sterker aantrekkingsreaksie veroorsaak op die parasitoïed en sal aanbeveel word aan die boere. *Artemisia afra* en *T. violacea* is meerjarige plante. Dit is beskikbaar as groen materiaal vir ekstraksiedoeleindes in die winter wanneer koring verbou word. Die ander twee plante is eenjariges, ryp sensitief, gaan dood en is nie beskikbaar in die winter nie. Die *A. afra* waterekstrak sal beide luisspesies afweer, indien dit vroeg in die koring-groeiseisoen gespuit word wanneer die koring klein is en die luise besig is om in die koringland te vlieg. Hierdie ekstrak sal ook *A. hordei* aantrek en dit kan die biologiese beheer van *D. noxia* aanhelp. Die *T. violacea* olie-ekstrak kan ook gebruik word om *D. noxia* af te weer en kan later in die seisoen aangewend word om die parasitoïed *D. rapae* aan te trek en so die biologiese beheer van beide luisspesies aan te help. Vir die opkomende boer is daar dus alternatiewe luis beheermetodes met potensiaal. Hierdie metodes moet verder verfyn word en daar moet aan boere opleiding verskaf word hoe om dit effektief aan te wend.

**Sleutelwoorde**: *Diuraphis noxia; Rhopalosiphum padi; Aphelinus hordei; Diaeretiella rapae;* olie plantekstrakte; water plantekstrakte; olfaktometer toetse; opkomende boere; alternatiewe beheer.

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# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

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# INTRODUCTION AND LITERATURE REVIEW

## 1.1 Cereal crop production in South Africa

Maize and wheat are the most important grain crops produced in South Africa with an annual consumption of ± 7.99m ton maize and ± 2.85m ton of wheat (Anonymous, 2007). Maize are produced mainly in the following provinces, namely Free State (2.86m ton), Mpumalanga (1.49m ton) and North West (1.39m ton), totalling 7.34m ton. Wheat are produced mainly in the Free State (0.484m ton) and Western Cape (0.813m ton) provinces, as well as in the irrigation areas of Northern Cape, Mpumalanga, Limpopo and KwaZulu Natal, totalling 1.82m ton. The total area in South Africa planted with maize is 2.90m ha and with wheat 0.63m ha ([1] P. Botha, personal communication).

In South Africa with a total population of 47.85m people (38.08m black people) (Anonymous, 2007), grain is imported to feed the nation. Every factor that can contribute to higher grain production, e.g. plant protection, should be investigated and implemented. Resource limited farmers that produce wheat are mainly situated in two areas in the Free State, namely Qwa-Qwa and Thaba N'chu (Marasas, Anandajayasekeram, Tolmay, Martella, Purchase & Prinsloo, 1997).

To be successful, a farmer should manage his farm as a business. Traditionally subsistence farmers are worldwide poorly served by the top-down transfer of technologies. It may be due to its bias in favour of scientific knowledge and its neglect of local participation and traditional knowledge (Altieri, 2002). Another reason may be the fact that new technologies (like new cultivars) are not immediately available to the small-scale farmers. The commercial farmers with more fertile soils and enough resources gained the most from these technologies (Altieri, 2002). Resource limited farmers were also largely excluded from access to financial credit, information and technical support.

[1] Pietman Botha, Senior Research Agriculturist, 2009. Grain SA, P.O. Box 88, Bothaville, 9660.

According to the president of the National African Farmers Union of South Africa (NAFU), some 55 000 commercial farmers own 85.5 million ha of the best farming land, while 12 million developing farmers own some 2.6 million ha of arable land (Matlala, 2008).

Research has been commodity-orientated with the goal of improving yields of crops and livestock, without adequately understanding the needs and options of the poor (Altieri, 2002). Experience, training and addressing the needs of emerging farmers are shortfalls that must be addressed in order to help them (Altieri, 2002).

Pest control on wheat is an essential input to ensure optimum crop production. In South Africa the use of pest control measures on wheat is in many cases lacking amongst the resource limited farmers, especially those in the wheat production areas of the Free State ([2] D. Rose, personal communication). Ignorance, the lack of knowledge, funds and equipment are the main reasons why pest control measures are poorly used. (Marasas *et al.*, 1997).

## 1.2. Wheat aphids as pests in resource limited areas

Aphids are the most common insect pests occurring on wheat in the Free State Province. Six species are involved namely Russian wheat aphid (*Diuraphis noxia*) (Kurdjumov), the English grain ear aphid (*Sitobion avenae*) (Fabricius), common green wheat aphid (*Schizaphis graminum*) (Rondani), bird cherry-oat aphid (*Rhopalosiphum padi*) (Linnaeus), and the rose grain aphid (*Metopolophium dirhodum*) (Walker) (Rabe, Van der Westhuizen & Hewitt, 1989; Prinsloo, Smit, Tolmay & Hattingh, 1997).

The most damaging of these aphids is *D. noxia*, especially on the wheat crop produced in the Free State (Du Toit, 1986; Aalbersberg, 1987; Prinsloo, Ninkovic, Van der Linde, Van der Westhuizen, Pettersson & Glinwood, 2007). *Diuraphis noxia* originated from the southern parts of Russia and the Iranian-Turkestanian mountains where it occurs on wild and cultivated grasses, including wheat and barley. It

[2] David Rose, Extension Officer, 2006. Department of Agriculture, Private Bag X816, Post Office Witsieshoek, 9870.

spread to Mexico, the USA and Canada between 1980 and 1986 (Kovalev, Poprawski, Stekolshchikov, Vereshchagina & Gandrabur, 1991). Since it was discovered in South Africa during 1978 (Dürr, 1983), it spread rapidly through all the wheat-producing areas and became the most important pest of dry land wheat in the country (Aalbersberg, 1987).

Feeding on wheat by *D. noxia* causes white and yellow longitudinal streaks on leaves and leaf roll. Yield loss up to 90% on untreated susceptible cultivars can occur (Aalbersberg, Van Der Westhuizen & Hewitt, 1989). The other aphids are less harmful than *D. noxia* and occur only sporadically as pests of wheat in the summer rainfall area of the Free State (Rabe, *et al*., 1989; Anonymous, 2008).

In the Free State *R. padi*, *M. dirhodum* and *S. avenae* occurs only sporadic on dry land wheat during seasons of high rainfall ([3] G. Prinsloo, personal communication). These aphids are not as harmful as *D. noxia.* Riedell, Kieckhefer, Haley, Lan & Evenson (1999), found that wheat yield could be reduced by 21% due to feeding by *R. padi,* which is much less than the damage caused by *D. noxia*, but could still cause damage of economical importance. Ni, Quisenberry, Markwell, Heng-Moss, Highley, Baxendale, Sarath & Klucas (2001), found that *D. noxia* caused greater fresh leaf weight reduction than *R. padi*. In a comparative study between *M. dirhodum* and *S. avenae* in winter wheat, a significantly higher reduction in dry mass and thousand kernel mass was caused by *S. avenae* on the ear compared to *M. dirhodum* on the flag leaf stage (Niehoff & Staeblein, 1990), which renders *S. avenae* the more damaging of the latter two aphid species.

After the release of Russian wheat aphid resistant cultivars, insecticide treatment in the Free State decreased by approximately 35.5% between 1990 and 1996 (Marasas *et al*., 1997) and even more since then. Following the decreased spraying for *D. noxia*, an upsurge in numbers of *R. padi* happened, especially during seasons when high rainfall occurred before or during winter ([3]G. Prinsloo, personal communication).

[3]Dr. Goddy Prinsloo, Entomologist, 2010. Agricultural Research Council, Small Grain Institute, P/Bag X09, Bethlehem. 9300

Increased spraying for these aphids will be harmful to natural enemies released for the control of *D. noxia*. It is therefore important to investigate alternate control options for both *D. noxia* and *R. padi*.

### 1.3. Control options for aphids

Chemical control of aphids is costly and time of spraying is dependant on aphid population pressure and plant growth stage. Different insecticides (carbamates, organophos-phates and pyrethroids) are registered for the control of aphids, according to the Registrar of Act 36 of 1947. All of these insecticides control both *D. noxia* and *R. padi* successfully, but are also harmful to the natural enemies of these aphids (Nel, Krause, & Khelawanlall, 2002).

For efficient control of insect pests it is necessary to determine an economic threshold value control at which an application will prevent an increasing pest population from reaching the economic injury level. The economic injury level is the lowest population density that will cause economic damage (Vereijken, 1979; Ba-Angood & Stewart, 1980; Van der Westhuizen, 1996; Marasas *et al.,* 1997).

An economic threshold for *D. noxia,* was determined by Du Toit (1986). According to this threshold, spraying should be conducted on susceptible cultivars at GS 31 (Zadoks, Chang & Konzak, 1974) when a minimum infestation level of between 7% and 14% is reached. Spraying at this stage will prevent the aphid from reaching economic damaging populations during the period between GS 38 and GS 60 when the wheat crop is most susceptible for damage by this aphid. For *R. padi* chemical control is necessary when 20 - 30% of the tillers are infested with 5 -10 aphids per tiller at GS 39 (Prinsloo, 2010).

Indiscriminate spraying of insecticides is a worldwide phenomenon. In recent decades, insect control has come to depend heavily on chemical insecticides (Thomas & Waage, 1996). The commercial use of synthetic insecticides also led to numerous unforeseen problems (Isman, 2006). Examples are the poisoning of applicators, farm workers, consumers, destruction of fish, bird and wildlife, groundwater contamination, resistance to pesticides in pest populations and a potential threat to the environment. The dependence on insecticides has led in some crop systems to a high frequency of insecticide resistance (Thomas & Waage, 1996). Aphids are also able to develop resistance to insecticides. In the USA the wheat aphid *S. graminum*, attacking both wheat and sorghum, is known to be resistant to organophosphates (Teetes, 1975; Siegfried & Ono, 1993).

## 1.4. Alternative control options

Several alternative control methods are available to control insect pests in different crops. These include methods like host plant resistance, biological control, and the use of plant extracts as biological pesticides. Different cropping strategies, like intercropping, where different crops are planted together to lower insect attack, and the use of trap crop barriers, could also be used. Trap crop barriers include the push-pull strategy, where alternate host plants could be used to repel insects or pull them away from the crop (Kumar, 1984; Thomas & Waage, 1996). In the context of sustainable pest management for *D. noxia*, host plant resistance and biological control seem to be the most suitable alternative control options, though the other methods should also be investigated.

### 1.4.1 Host plant resistance

Host plant resistance (HPR) represents the inherent ability of crop plants to restrict, retard or overcome pest infestations and thereby improve yield and/or quality of the harvestable product. Three different resistance mechanisms are involved, namely antixenosis, antibiosis and tolerance (Thomas & Waage, 1996).

a) Antixenosis (non-preference) describes the inability of a plant to serve as a host to an insect herbivore. The basis of this resistance mechanism can be morphological (hairy leaves, surface waxes, tissue thickness) or chemical (repellents, anti-feedants) (Thomas & Waage, 1996).

b) Antibiosis is the mechanism that describes the negative effects of a resistant plant on the biology of an insect, which has fed on the plant. Both chemical and morphological plant defences can induce antibiotic effects. The consequences of antibiotic resistance may vary from influence on fecundity, development times and body size, reproduction, survival and sometimes death (Thomas & Waage, 1996).

c) Tolerance is the degree to which a plant can tolerate/support an insect that under similar conditions would severely damage a susceptible plant. It means that, when two cultivars are equally infested, the less tolerant one has a smaller yield (Thomas & Waage, 1996).

#### 1.4.1.1 Host plant resistance against *D. noxia*

Several wheat lines, which are resistant to *D. noxia*, were identified (Du Toit, 1987; Harvey & Martin, 1990; Smith, Shotzko, Zemetra, Souza & Schroeder-Teeter, 1991). Some of them, especially those containing an antibiotic type of resistance, are currently used by several breeding institutions to breed resistant cultivars. The Agricultural Research Council - Small Grain Institute (ARC-SGI) and other seed companies have released many cultivars containing different levels of HPR to *D. noxia* (Du Toit, 1990). Approximately 17 cultivars are currently available to farmers in the Free State (Anonymous, 2008) and therefore some cultivars may contain 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 treatments decreased by approximately 35% between 1990 and 1996 (Marasas *et al*., 1997).

A problem, however, associated with plant resistance breeding, has been the tendency for the development of resistance-breaking biotypes (Gould, Wilhoit & Via, 1990; Stoner, 1996; Porter, Burd, Shufran, Webster & Teetes, 1997). A resistance breaking biotype of *D. noxia* was reported from Colorado during 2003 (Haley, Peairs, Walker, Rudolph & Randolph, 2004) and from South Africa during 2005 (Tolmay, Lindeque & Prinsloo, 2007). Except for *S. graminum* and *D. noxia,* six aphid species not feeding on cereals, are also known to have overcome plant resistance (Stoner, 1996).

### 1.4.2 Biological control

Although several natural enemies, including ladybirds and parasitoids, attack *D. noxia* in SA, they are not effective in protecting the susceptible cultivars from damage (Aalbersberg, Van der Westhuizen & Hewitt, 1988). Therefore six natural enemy species were introduced and released between 1980 and 1994. Three of these species, namely *Adalia bipunctata* (L.), *Aphidius matricariae* (Haliday) and *Aphelinus hordei* (Kurdjumov), became established, although not seen regularly on aphid populations on wheat (Prinsloo, 2006). On-farm trials performed in the Free State showed that the application of these natural enemies gives variable control, which is not acceptable for use by resource poor farmers ([4]G. Prinsloo, personal communication). The effective use of natural enemies to control wheat aphids on farms would be complicated and difficult and needs more research and more effective managerial skills from the farmers.

### 1.4.3 Alternate control options based on insect-plant interactions

The majority of food webs based on living plants contain at least three trophic levels namely plants, herbivores and the enemies of herbivores. Members of alternate trophic levels may act in a mutualistic manner. Natural enemies of herbivores benefit the plants by reducing herbivore abundance, and plants may benefit the herbivore's enemies by making herbivores more vulnerable to the natural enemies (Price, 1986).

A strategy using benign, volatile substances that have behaviour-modifying effects on aphids has been developed at the Swedish University of Agricultural Sciences, Uppsala, Sweden (SLU) (Ninkovic, Ahmed, Glinwood & Pettersson, 2003). When applied in cereal fields, these substances dramatically reduce colonisation of the crop by aphids. Preventing aphids from establishing in crops will enhance the effectiveness of resistant varieties. Additionally, a group of natural enemies that attack aphids, parasitic wasps (parasitoids), can have a critical limiting impact on aphid populations, but only if present in sufficient numbers on crops. Host plant resistance can affect parasitoids in ways that are not easily predictable. It is

[4]Dr. Goddy Prinsloo, Manager, 2007. Agricultural Research Council, Small Grain Institute, P/Bag X09, Bethlehem. 9300

important to understand the impact of the release of resistant plant varieties on this group of aphid natural enemies.

According to Price (1986) tritrophic interactions are mediated by three main factors namely semiochemicals, chemicals and physical plant characteristics.

#### 1.4.3.1 Semiochemicals as repellents and attractants

Semiochemicals (a chemical substance that mediates interactions between organisms) are known to play a major role as cues to aid natural enemies in locating and recognising their hosts or prey. These chemical cues are divided into two groups: those that are volatile and act at a long distance to attract searching parasitoids and predators, and those, which are generally non-volatile. It has been demonstrated that parasitoids use specific stimuli emitted by herbivore-damaged plants to identify the habitat where they can find their hosts (Prinsloo, 2006).

#### 1.4.3.2 Plant chemical factors

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 by natural enemies is also influenced. Some herbivores are able to isolate plant secondary chemicals in their haemolymph and thereby alter their suitability for natural enemies (Prinsloo, 2006).

#### 1.4.3.3 Plant morphological features

Many major morphological features of plants are important in altering the availability of herbivores to their natural enemies. For example, *Pieris rapae* (Linnaeus) larvae are heavy parasitized on open-leaf *Brassica* varieties, but much less on heading varieties where the larvae feed within leaf folds (Thomas & Waage, 1996). Plant morphological features can alter the availability of herbivores to natural enemies (Price, 1986). Physical plant defence structures such as trichomes and cuticle thickness can have direct affects on natural enemies. The effect of leaf pubescence in cotton was studied on parasitism of whitefly, *Bermisia tabaci* (Gennadius), by its parasitoids *Eretmocerus mundus* (Mercet) and *Encarsia shafeei* (Hayat). Parasitism was observed to vary significantly between the eight genotypes studied, with glabrous varieties supporting more parasitoid activity (Thomas & Waage, 1996). Plant architecture can influence dispersion of herbivores and searching by enemies, and plant dispersion can have direct effects on natural enemies (Prinsloo, 2006). Hairy leaves, for example, may increase the fall-off rate of a pest species, thus increasing encounters with ground-zone predators (Thomas & Waage, 1996).

### 1.4.4 Plant extracts

The hypothesis for the use of plant extracts is that volatiles exuded by non-host plants are sufficiently strong to repel the host searching insects (Finch & Collier, 2000). Funnel traps, baited with African marigold and sweet pea, were tested for their ability to catch *Helicoverpa armigera* (Hübner). The baited traps with the floral volatiles caught significantly more insects than in unbaited traps. These types of kairomones (chemicals released by plants to initiate communication with insects) show that plant extracts can be useful in attracting insects and their natural enemies (Bruce, Cork, Hall & Dunkelblum, 2002).

Botanical insecticides have long been noted as attractive alternatives to synthetic chemical insecticides for pest management. Botanical insecticides reputedly pose little threat to the environment or to human health. Among five plant extracts at different concentrations tried against the safflower aphid *Dactynotus carthami* (Hille Ris Lambers), *Nicotiana tabacum* (Linnaeus) and *Ipomoea carnea* (Jace.), leaf extracts (2%) were found effective in restricting the development of aphid populations with significant increase in yield (Kulat, Nimbalkar, Nandanwar & Hiwase, 2000).

The real benefit of botanical insecticides can best be realized in developing countries where farmers may not be able to afford synthetic insecticides (Isman, 2006). Pyrethrum- and Neem pesticides are well established commercially. Pesticides based on plant essential oils have recently entered the marketplace, and the use of retonene appears to be waning. A number of plant substances have been considered for use as insect anti-feedants or repellents, but except for some natural mosquito repellents, little commercial success has ensued for plant substances that modify arthropod behaviour (Isman, 2006).

### 1.4.5 Trap cropping

The concept fits into the ecological concept of habitat manipulation of an agro-ecosystem for the purpose of pest management. It is defined as alternate plants or crops that are deployed to attract, divert, intercept, and/or retain targeted insects or the pathogens they transmit in order to reduce damage to the main crop (Shelton & Baderenez-Perez, 2006). Three trap cropping methods exist, namely conventional, dead-end and genetically engineered trap cropping. Conventional trap cropping can be defined as a trap crop planted next to a higher value crop that is more attractive to a pest as a feeding or oviposition site than the main crop. Dead-end trap cropping is used where plants are highly attractive to insects, but on which they or their offspring cannot survive. It serves as a sink for pests, preventing their movement from the trap crop to the main crop later in the season, for example *Barbarea vulgaris* (R.Br.) works as a dead-end crop for the diamondback moth, *Plutella xylostella* (Linnaeus). In genetically engineered trap cropping potatoes that have been genetically engineered to express proteins from *Bacillus thuringiensis* were used on crops to manage Colorado potato beetle *Leptinotarsa decemlineata* (Say) populations (Shelton & Baderenez-Perez, 2006).

### 1.4.6 Intercropping including the push-pull strategy

The push-pull strategy is based on a combination of a trap crop (pull component) with a repellent intercrop (push component). The trap crop attracts insect pests and, combined with the repellent intercrop, diverts the insect away from the main crop. A push-pull strategy was developed for the control of stem borer *Chilo partellus* (Swinhoe). This strategy is based on using Napier grass *Pennisetum purpureum* (Shumach) as the trap crop that is planted around maize as the main crop, and either Silverleaf Desmodium *Desmodium uncinatum* (Jacq.) or molasses grass (*Melinus minutiflora*) (P. Beauv.) planted within the field as a repellent intercrop. It has greatly increased the effectiveness of trap cropping for stem borers in Africa. In addition, the use of molasses grass as a repellent intercrop enhances stem borer parasitoids abundance, thereby improving stem borer control (Shelton & Baderenez-Perez, 2006, Midega, Khan, Van den Berg, Ogol & Pickett, 2006).

## 1.5. What is the most applicable strategy to new emerging farmers?

As stated above, cultivars containing plant resistance against *D. noxia* are available to farmers and this include resource-limited farmers, while natural enemies are present in the areas where these farmers are operating. However, resistant breaking biotypes of *D. noxia* occur in the cropping areas of these farmers and alternates for aphid control should be investigated. Due to their resource-limited status, these farmers need something that is not high in cost, relatively safe and easy to use.

Alternate plants or crops to be used as trap crops or repellents for wheat aphids in a push-pull trap cropping system have not been identified or studied in present times and this option may be studied in future ([5]G. Prinsloo, personal communication). However, volatile substances from plant essential oils were tested in the laboratory and field with *D. noxia*-susceptible and –resistant wheat varieties, and were found repellent to *D. noxia* in olfactometer tests. The impact of the chemicals on aphid numbers and grain quality varied according to plant variety, indicating an interaction between semiochemicals and plant resistance, and semiochemicals and plant variety (Prinsloo *et al*, 2007). Costs of essential oils and availability of these oils could, however, also be problematic to use by these farmers. If they could use plant extracts containing volatiles with the same characteristics that are commonly available, and which is growing in the wheat-growing season, this could, however, be an attractive option for pest control. Therefore the main objective of the study is as follows:

## 1.6. Objective of the study

- To identify plants containing volatiles with potential aphid repellent properties and to prepare extracts.
- Determine the behavioural response of alate *D. noxia* to extracts of these plants in an olfactometer.

[5]Dr. Goddy Prinsloo, Entomologist, 2010. Agricultural Research Council, Small Grain Institute, P/Bag X09, Bethlehem. 9300

- Determine the behavioural response of alate *R. padi* to extracts of these plants in an olfactometer.
- Determine the behavioural response of two parasitoids *Aphelinus hordei* and *Diaeretiella rapae* to extracts of these plants in an olfactometer.

The focus of the study is therefore to determine the potential of plant extracts as aphid repellents in the laboratory. These findings could then be tested and verified during future field studies.

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# CHAPTER 2

# GENERAL MATERIALS AND METHODS

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## 2.1 Introduction

The behavioural response of aphids and natural enemies to different plant extracts was tested in the laboratory using olfactometres. Due to the similarity of the trials it was decided to describe the materials and methods used only once for all the different trials.

## 2.2 Experimental insects

### 2.2.1 Aphids

The aphids *Diuraphis noxia* (Kurdjumov) (Figure 2.1) and *Rhopalosiphum padi* (Linnaeus) (Figure 2.2) were collected from volunteer wheat plants on the experimental farm of the ARC-Small Grain Institute (ARC-SGI), Bethlehem. These aphid species were maintained separately in cages (Fig 2.3) on the aphid-susceptible wheat cultivar Betta in a greenhouse at temperature 23±2˚C, and ambient light conditions with 14L:10D. Alate aphids, which represent new invaders in the field, were used in all the laboratory experiments. These alate aphids were collected from the cages immediately prior to bioassays (Prinsloo, 2006).

### 2.2.2 Parasitoids

*Aphelinus hordei* (Kurdjumov) (Figure 2.4) and *Diaeretiella rapae* (McIntosh) (Figure 2.5) parasitoids were reared on *D. noxia* at the ARC-SGI in Bethlehem, South Africa. Aphid and parasitoid stock colonies were maintained under temperature controlled green house conditions on winter wheat seedlings (cultivar Betta) at 14:10 (L:D) photoperiod and fluctuating temperatures of 15 - 23˚C. Protocols for rearing aphids and parasitoids were followed, according to Prinsloo (2006).


Figure 2.1 Aphid *Diuraphis noxia* (Kurdjumov) (http://www.ipmimages.org/images/1481029)


Figure 2.2 Aphid *Ropalosiphum padi* (Linnaeus) (http://www.insectimages.org/browse/detail.cfm?imgnum=5422729)

Figure 2.3 Caged insect colonies equipped with potted wheat plants

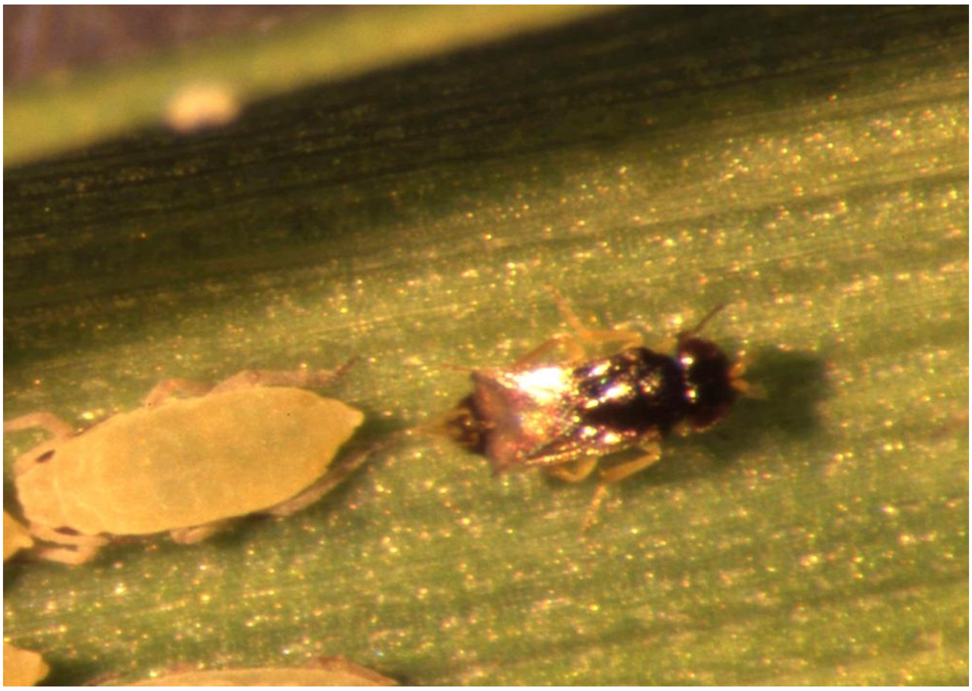

Figure 2.4 Parasitoid *Aphelinus hordei* (Kurdjumov)

Figure 2.5 *Parasitoid Diaeretiella rapae* (McIntosh)

## 2.3 Plant material

Four plants, Wild wormwood *Artemisia afra* (Jacq. ex Willd.), Big thorn apple *Datura stramonium* (Linnaeus), Khakie bush *Tagetes minuta* (Linnaeus) and Wild garlic *Tulbaghia violacea* (Harv.) were used to derive extractions from. They were chosen due to their availability in the wheat production regions, their medicinal properties (Van Wyk, Van Outshoorn & Gericke, 2000) and their possible insect repelling properties known from other species in the same genus (Bruce, Cork, Hall & Dunkelblum, 2002; Isman, 2006; Zehnder, Gurr, Kühne, Wade, Wratten & Wyss, 2007; Halbert, Corsisni, Wiebe & Vaughn, 2008; Işik, & Görűr, 2009). Determination of the mammalian toxicity of the extracts should be done on all plant extracts before being used by farmers.

## 2.4 Plant extracts

It was decided to use uncomplicated aqueous and a light mineral oil extraction methods to make it easy for small-scale farmers. Green leaves and stems of the abovementioned plants were collected before they were flowering. Plants were finely chopped and put in a glass beaker. The water extraction was done by infusing the finely chopped plant parts with boiling water (plant:water solution of 1:3). The beaker was then covered with Parafilm and kept at room temperature for 24h before plant material was sieved out with glass wool. The ratio of 1kg of plant material to 3kg water corresponds with Oparaeke (2006), who used 1kg of plant material with 3.5l water. The oil extraction was prepared by pouring light mineral oil (Citrex 844g/l) (plant:oil solution of 1:3) over finely chopped plant material. The beaker was covered with Parafilm and kept at room temperature for 24 h after which the extract was sieved through glass wool to remove plant material. Both extracts were stored in amber glass at 4°C until it was used.

## 2.5 Olfactometry

A four-arm olfactometer (Pettersson, 1970; Pettersson, 1993) was used to test the response of aphids and parasitoids (Fig. 2.6). It consists of an enclosed Perspex chamber divided into a central arena (A) and four arm zones (12cm diameter). The floor of the chamber was fully covered with white paper. Air was drawn from the centre of the olfactometer using a Hosco vacuum pump at 400-500 ml $min^{-1}$ where discreet air currents were established in the four arms. Odour source vials (B) consisting out of a 40mm x 9mm (inner diameter) glass tube that was connected to each of the four arm zones by means of a 50mm x 1.5mm (inner diameter) piece of Teflon tubing. Each odour source vial contained a piece of Wattman no.1 filter paper (20mm x 5mm) onto which 10µl of the extract, or the control solution, was deposited. An odour field was established by introducing the extract into two neighbouring arm zones, while the opposite two arms contained either oil or water depending on the extract tested. Activated charcoal filters (C) were fitted at the end of each glass vial to ensure clean air entering the odour sources.

The tests were performed in a windowless room with light provided by double fluorescent lamp tubes (20W/640S Cool White, Philips) suspended 50cm directly above the olfactometer.

A single insect (alate aphid or parasitoid) was introduced into the olfactometer and observed for 10 minutes, during which time spend and the number of entries made into either treated or untreated arm zones were recorded using the computer programme OLFA (Olfa: Exeter Software New York, USA). When parasitoids were tested, only mated, honey fed, females were used. Parasitoids were captured in separate gelatine capsules and sexed prior to the tests. Each treatment was repeated with 50 individuals. Olfactometers and odour sources with charcoal filters were changed after every five replications.

Data for both control arm zones and both treated arm zones were pooled to give single figures for the time spent and the number of entries into these zones. The mean duration per entry (seconds) was calculated from this data (number of entries/ total time spend in the arm X 60). The mean number of entries into the arm zones, the time spend in the arm zones and the mean duration of an entry were compared, using matched paired t-tests at 5% test level (Snedecor & Cochran, 1967).

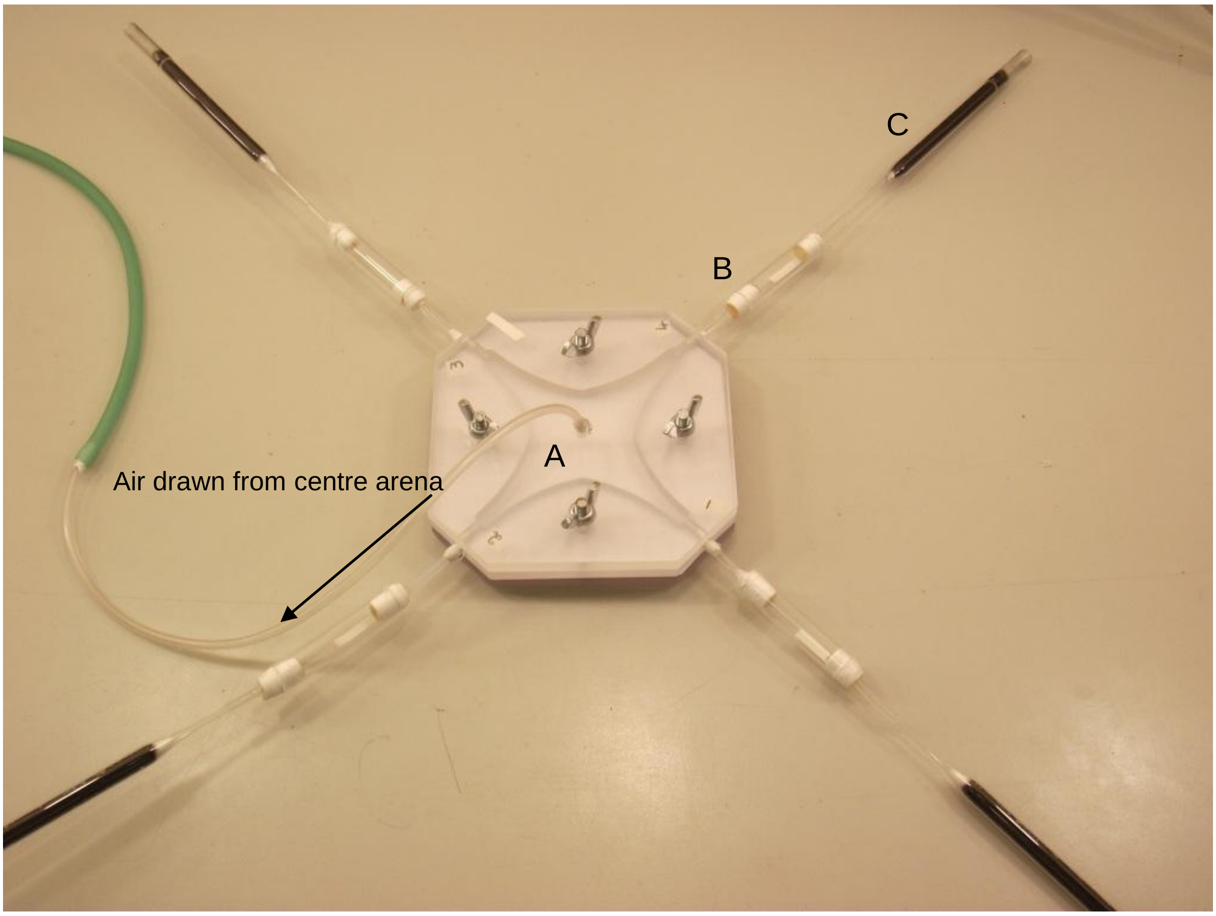


Figure 2.6 A four arm olfactometer, indicating the centre arena A, the odour vial B, and charcoal filter C

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# CHAPTER 3

# THE RESPONSE OF ALATE RUSSIAN WHEAT APHIDS TO PLANT EXTRACTS IN THE LABORATORY

# CHAPTER 3

## THE RESPONSE OF ALATE RUSSIAN WHEAT APHIDS TO PLANT EXTRACTS IN THE LABORATORY

### 3.1 Introduction

Plants have developed direct chemical and morphological defences to limit herbivore attacks. Direct chemical defences include production of toxins, volatile organic compounds and digestibility reducers, while morphological defences include trichomes, spines and tough foliage (Cortesero, Stapel & Lewis, 2000; Dicke & Van Loon, 2000). Since these plant attributes are produced as direct chemical defences, it not only influences the higher trophic levels in several ways, but can also attack new herbivore intruders (Prinsloo, Ninkovic, Van der Linde, Van der Westhuizen, Pettersson & Glinwood, 2007).

Since the early 1980's *Diuraphis noxia* (Kurdjumov) has been the target of an integrated control strategy that has since been actively researched and promoted (Tolmay & Van Deventer, 2005). Resistant cultivars, which are one of the building blocks of this strategy, are being used by more than 70% of the wheat farmers in the Free State Province (Marasas, Anandajayasekeram, Tolmay, Martella, Purchase. & Prinsloo, 1997). Studies over a five-year period with one of these resistant cultivars, Gariep, showed that insecticide treatment was not economically justifiable. Sometimes, however, yields did increase with insecticide application. Infestation of susceptible wheat plants led to a drastic reduction in chlorophyll content which, when combined with the characteristic leaf rolling that occurred, caused considerable loss of effective leaf area (Tolmay & Van Deventer, 2005).

Associated with the use of plant resistance as a control strategy, is the tendency for the development of resistance-breaking biotypes (Gould, Wilhoit & Via, 1990; Stoner, 1996; Thomas & Waage, 1996; Porter, Burd, Shufran, Webster & Teetes, 1997). This happened in 2003, when a resistance breaking biotype of *D. noxia* was reported from Colorado in the USA (Haley, Peairs, Walker, Rudolph & Randolph, 2004) and later from South Africa in 2005 (Tolmay, Lindeque & Prinsloo, 2007). Due to the seriousness of the pest it is necessary to take steps to protect the resistant cultivars and prevent outbreaks of the pest. This merited an investigation to alternate control methods to be used together with plant resistance and natural enemies in an integrated control strategy.

Botanical insecticides that are isolated from plants like French marigolds (*Tagetes patula* L.), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.), chrysanthemum (*Chrysanthemum morifolium* Ramat) and Neem (*Azadirachta indica* A. Juss) have long been noted as attractive alternatives to synthetic chemical insecticides for pest management. This was because botanicals reputedly pose little threat to the environment and to human health (Finch & Collier, 2000; Liu, Li & Lou, 2006; Zaki, 2008). Correspondingly it is known that aphids are highly dependent on chemical cues when searching for and evaluating host plants (Pickett & Glinwood 2007). It was also shown that aphids are susceptible to behavioural manipulation when exposed to repellent cues representing non-host plants. Some European countries are successfully using repellent chemicals of plant origin in pest aphid management (Pettersson, Pickett, Pye, Quiroz, Smart, Wadhams & Woodcock, 1994; Ninkovic, Ahmed, Glinwood & Pettersson, 2003), and resources such as plant essential oils are gaining interest for insect pest management in general (Isman 2006). Authors Liu *et al*., (2006) have shown that botanical preparations, usually from non-host plants, can be used to manipulate the behaviour of insect pests and their natural enemies. For example, extracts from *Nicotiana tabacum* (L.) and *Ipomoea camea* (Jacq.) were found to restrict the development of the safflower aphid, *Dactynotus carthami* (HRL), with significant increase in yield (Kulat, Nimbalkar, Nandanwar & Hiwase, 1998). The rational is therefore to search for plant extracts as a source of repellent volatiles released by non-host plants to be used for aphid control in small-scale farmer systems.

In an experiment where aqueous extracts of nine plant species were tested for efficacy against three insect pests (thrips, legume pod-borers and pod-sucking bugs) of cowpea, all the extracts gave some level of protection (repellence) to cowpea crops relative to the untreated control. Pod density per plant was increased on plots sprayed with extracts of African marigold (*Tagetes* sp.), African Goat weed (*Chromolaena* *odorata* L.) and African curry (*Ocimum gratissimum* L.), in that order. Extracts of African curry, African bush tea (*Hyptis suaveolens* Poit) and African marigold significantly ($p<0.05$) reduced pod damage. Lower pod damage ensured relatively higher grain yield compared with other extracts and the untreated control (Oparaeke, 2006). Odour preferences of *D. noxia,* and its natural enemies, should thus also be taken into consideration when choosing extracts.

Oil distilled from several species of *Artemisia* showed promising results for repellency against aphids (Halbert, Corsisni, Wiebe & Vaughn, 2008). In the Free State Province, Wild wormwood *Artemisia afra* (Jacq. ex Willd.), Big thorn apple *Datura stramonium* (Linnaeus), Khakie bush *Tagetes minuta* (Linnaeus) and Wild garlic *Tulbaghia violacea* (Harv.) are known as traditional medicinal plants (Van Wyk, Van Outshoorn & Gericke, 2000), which could have insect repelling properties. These plants are also growing in the areas where wheat is produced. Therefore the study thus focused on the behavioural response of alate *D. noxia* to volatiles from extracts of these plants tested within a four-arm olfactometer in the laboratory.

## 3.2 Materials and Methods

The materials used and methods followed are discussed in Chapter 2. During data analyses the percentage differences between the control and treated arms for each parameter, namely entry number, total time spend and entry duration, were calculated for each of the treatments. This percentage difference expressed the repellent response of the insect towards the volatile substance. For example, a large percentage difference between entries made into the treatment and control arms meant that it was highly repellent, and the lower the percentage, the less repellent was the substance. If the difference resulted in a negative percentage it meant that there was an attractant effect. A one-way ANOVA and Fisher protected least significant difference (LSD) test (Anonymous 2011) at 5% level were then used to test for differences between the eight extracts for each parameter.

## 3.3 Results and discussion

### 3.3.1 Olfactometric response of *Diuraphis noxia* to *Artemisia afra*.

#### 3.3.1.1 Oil extract.

Significantly less time was spent by *Diuraphis noxia* in the olfactometer arms containing volatiles from *Artemisia afra* than in the control arms (Table 3.1). The entries made by *D. noxia* in the treated arms were also significantly less than the entries made into the control arms (Table 3.1). The mean duration per entry was significantly less in the arms containing vapours of *A. afra* than in the control arms containing Citrex oil only (Table 3.1). The extract was therefore repellent to *D. noxia*.

**Table 3.1** Mean response of *Diuraphis noxia* to *Artemisia afra* oil extract in an olfactometer during a ten minute period.

| **Variable** | **N** | **Test statistics** | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 1.49 ± 0.15 | 7.12 ± 0.17 | -18.63 | <0.001 |
| Entries per arm | 50 | 1.78 ± 0.16 | 5.22 ± 0.19 | -12.68 | <0.001 |
| Duration per entry (sec) | 50 | 44.22 ± 4.08 | 86.29 ± 3.40 | -7.59 | <0.001 |

**N** = number of replicates

#### 3.3.1.2 Aqueous extract.

Significant less time was spent in the treated arms by *D. noxia* than in the control arms (Table 3.2). The entries made into the treated arms were also significant less than into the control arms (Table 3.2). The duration time of an entry was significantly shorter in the arms containing vapours of *A. afra* than in the control arms containing water only (Table 3.2). Thus *D. noxia* was repelled by both the aqueous and oil extracts of *A. afra*.

**Table 3.2** Mean behavioural response of *Diuraphis noxia* to *Artemisia afra* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 1.30±0.14 | 7.74±0.16 | -22.36 | <0.001 |
| Entries per arm | 50 | 1.38±0.15 | 4.84±0.16 | -13.96 | <0.001 |
| Duration per entry (sec) | 50 | 48.47±5.56 | 100.44±3.61 | -6.92 | <0.001 |

**N** = number of replicates

### 3.3.2 Olfactometric response of *Diuraphis noxia* to *Datura stramonium*.

#### 3.3.2.1 Oil extract.

No significant differences were found between the number of entries, the time spent and mean duration per entry made by *D. noxia* into the arms containing volatiles from *D. stramonium* and the control arms (Table 3.3). This means that *D. noxia* did not respond to the volatiles coming from this extract.

**Table 3.3** Mean response of *Diuraphis noxia* to *Datura stramonium* oil extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 4.23±0.34 | 4.41±0.33 | -0.29 | 0.772 |
| Entries per arm | 50 | 5.14±0.37 | 4.96±0.34 | 0.44 | 0.661 |
| Duration per entry (sec) | 50 | 54.07±5.48 | 55.83±4.28 | -0.22 | 0.825 |

**N** = number of replicates

#### 3.3.2.2 Aqueous extract.

The aphid responded significantly to volatiles from the aqueous extracts of *D. stramonium.* Significantly less time was spent per arm by the aphids in the treated arms while significantly fewer entries were made into treated arms than the control arms (Table 3.4). The mean duration time of an entry was however not significantly less in the arms containing vapours of *D. stramonium* compared to the control arms containing water only. This means that there was some repellency, though not strong.

**Table 3.4** Mean response of *Diuraphis noxia* to *Datura stramonium* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 3.70±0.13 | 4.64±0.13 | -3.93 | <0.001 |
| Entries per arm | 50 | 5.92±0.21 | 7.32±0.23 | -3.69 | <0.001 |
| Duration per entry (sec) | 50 | 38.36±1.18 | 38.72±0.89 | -0.24 | 0.814 |

**N** = number of replicates

### 3.3.3 Olfactometric response of *Diuraphis noxia* to *Tagetes minuta*.

#### 3.3.3.1 Oil extract.

*Diuraphis noxia* spent significantly less time in the olfactometer arms containing vapours from *T. minuta* than into control arms. Significant fewer entries were made by *D. noxia* into the treated arms, while the duration of an entry in these arms was significantly shorter (Tab 3.5). This indicated that the extract is repellent to *D. noxia*.

**Table 3.5** Mean response of *Diuraphis noxia* to *Tagetes minuta* oil extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 1.82±0.15 | 6.80±0.18 | -16.61 | <0.001 |
| Entries per arm | 50 | 2.14±0.17 | 5.16±0.19 | -12.77 | <0.001 |
| Duration per entry (sec) | 50 | 52.92±4.34 | 83.15±3.16 | -5.51 | <0.001 |

**N** = number of replicates

#### 3.3.3.2 Aqueous extract.

The aphids also responded significantly differently to volatiles emitted by the aqueous extract of *T. minuta.* Significantly less time was spent and fewer entries made into the treated arms (Table 3.6). The entry duration was significantly shorter in the treated arms than the control arms containing water only. Thus *D. noxia* was repelled by both the aqueous and oil extracts of *T. minuta* (Table 3.6).

**Table 3.6** Mean response of *Diuraphis noxia* to *Tagetes minuta* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | | | Test statistics | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 1.51±0.14 | 7.22±0.15 | -21.09 | <0.001 |
| Entries per arm | 50 | 1.96±0.16 | 5.50±0.16 | -14.38 | <0.001 |
| Duration per entry (sec) | 50 | 42.34±3.86 | 81.39±2.43 | -8.42 | <0.001 |

**N** = number of replicates

### 3.3.4 Olfactometric response of *Diuraphis noxia* to *Tulbaghia violacea.*

#### 3.3.4.1 Oil extract.

*Diuraphis noxia* spent significantly less time in the olfactometer arms containing vapours from *T. violacea.* The number of entries made and the entry duration time were significant less in the treated arms (Table 3.7). The aphid was thus significantly repelled by oil extract of *T. violacea*.

**Table 3.7** Mean response of *Diuraphis noxia* to *Tulbaghia violacea* oil extract in an olfactometer during a ten minute period.

| Variable | N | | | Test statistics | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 1.16±0.16 | 7.53± 0.19 | -19.55 | <0.001 |
| Entries per arm | 50 | 1.42±0.17 | 4.44± 0.21 | -10.71 | <0.001 |
| Duration per entry (sec) | 50 | 41.49±6.79 | 119.92±11.42 | -5.64 | <0.001 |

**N** = number of replicates

### 3.3.4.2 Aqueous extract.

The aphid responded strongly to the aqueous extract of *T. violacea.* Significantly less time was spent by the aphids in the treated arms (Table 3.8). The number of entries made into and the duration of an entry in the treated arms were significantly less than into the control arms. Russian wheat aphids are therefore repelled by this extract.

**Table 3.8** Mean response of *Diuraphis noxia* to *Tulbaghia violacea* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 2.73±0.27 | 6.31±0.25 | -7.22 | <0.001 |
| Entries per arm | 50 | 1.90±0.14 | 3.18±0.19 | -4.83 | <0.001 |
| Duration per entry (sec) | 50 | 81.89±8.06 | 137.28±8.86 | -4.65 | <0.001 |

**N** = number of replicates

## 3.4 Comparison between different extracts

When the percentage difference in number of entries was compared between extracts, the aqueous extract of *A. afra* and oil extract of *T. violacea* had a significant higher response than the other treatments (Fig 3.1 A). Although the oil extract of *A. afra* and both extracts of *T. minuta* were significant less repellent than the first mentioned they were still highly repellent to *D. noxia* (Fig 3.1 A). The *T. violacea* oil extract was weekly repellent while *D. stramonium* did not elicit a response (Fig 3.1 A).

The percentage difference in total time spent in the treatment and control arms indicated a strong repellent response from the aqueous extract of *A. afra* and the oil extract of *T. violacea*, and would be regarded as high potential (Fig 3.1 B). The oil extract of *A. afra* (extract 2), and both extracts of *T. minuta* (extracts 5 & 6), were highly repellent to *D. noxia* (Fig 3.1 B). The aphid responded less to the aqueous extract *T. violacea,* although still strong, while both the aqueous- and oil extracts of *D. stramonium* again results in a no response, and would therefore not be considered for use (Fig 3.1 B).

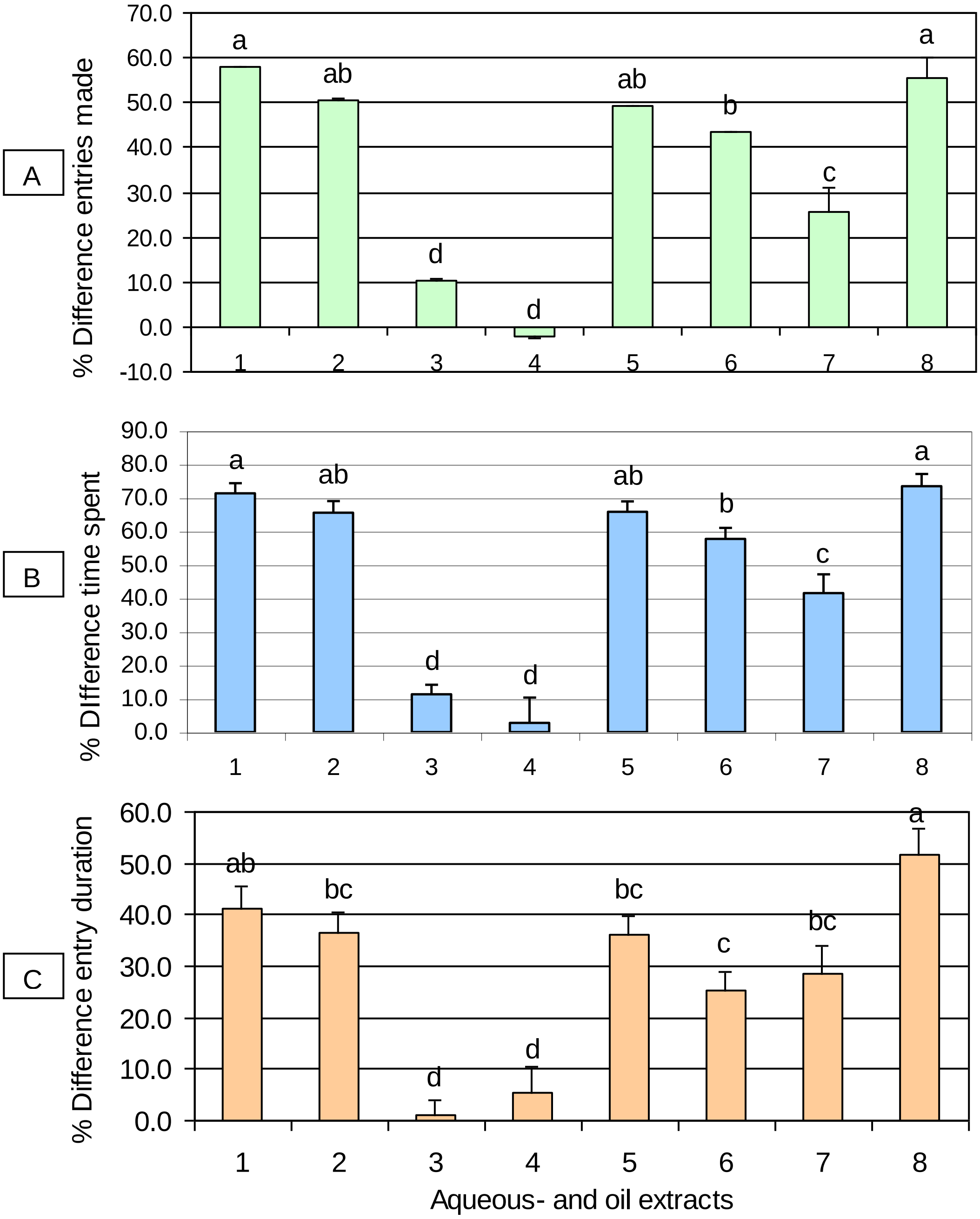


Figure 3.1 Mean percentage differences between the control arms and treated arms for the following parameters: total entries per arm (A) (P = 0.05, LSD = 11.86), time per arm (B) (P = 0.05, LSD = 12.05) and time per entry (entry duration) (C) (P = 0.05, LSD = 13.23) by the Russian wheat aphid *Diuraphis noxia*. Symbols that differ indicate significant differences between extracts. Extract 1 = *Artemisia afra* aqueous extract; 2 = Artemisia *afra* oil extract; 3 = *Datura stramonium* aqueous extract; 4 = *Datura stramonium* oil extract; 5 = *Tagetes minuta* aqueous extract; 6 = *Tagetes minuta* oil extract: 7 = *Tulbachia violacea* aqueous extract; 8 = *Tulbachia violacea* oil extract.

The aqueous extract of *A. afra*, together with both extracts of *T. minuta* and *T. violacea* were highly repellent to *D. noxia*, taking into consideration the percentage difference in entry duration spent in the treatment and control arms (Fig 3.1 C). The strongest response, however, was obtained from the aqueous extract of *A. afra* and the oil extract of *T. violacea* (Fig 3.1 C). These two extracts would be regarded as high potential. Very little response was shown towards both *D. stramonium* extracts and was regarded as a no-response.

It is evident that *D. noxia* was most repelled by the *A. afra* aqueous- and *T. violacea* oil extracts and that these should be recommended for use by farmers. In the second instance the oil extract of *A. afra*, both *T. minuta* extracts and *T. violacea* aqueous extract also elicited strong repellent responses and could also be used. The response to *T. violacea* was lower and would possibly not be as efficient as the others, while the aphid did not respond to both extracts of *D. stramonium,* which could not be used.

*Artemisia afra* and *T. violacea* are perennials and are available throughout the year as green material. Oil extract of *A. afra*, both extracts of *T. minuta* and aqueous extract of *T. violacea* also showed good repellent properties to *D. noxia* and could also be used depending on the response of the natural enemies to them. *Tagetes minuta*, however, is an annual, which die in winter with no green material being available during the wheat-growing season. These extracts should be applied as early as possible in the wheat-growing season to prevent the *D. noxia* from infesting the plants at an early stage (Aalbersberg, 1987). Temperatures are still low during this time of year and *T. minuta* will not be available, especially in the cold eastern parts of the Free State. In the western parts of this province, which is warmer, there may be some smaller plants available, but probably not enough to be used.

The release of odour-masking substances into the air by non-host plant species is considered to provide some protection to the associated host plants. It was found that host plant selection by the cabbage root fly was disrupted when its host plants were surrounded by a range of different plants, including weeds, spurrey, peas, rye grass and clover. Although each of these non-host plants has a different odour profile, it seemed highly improbable that they would all be capable of preventing an adapted specialist insect (like *D. rapae*) from finding its host plant (Finch & Collier, 2000).

The fact remains that one extract type is inadequate to provide an ideal agent for every insect problem. Each insect reacts differently to each plant extract, as was experienced by Nottingham & Hardie (1993). They investigated the flight behaviour of the black bean aphid, *Aphis fabae* (Scopoli) and the cabbage aphid, *Brevicoryne brassicae* (L.) in host and non-host plant odours. Walking alates of these two aphids were presented with odours of steam distilled extracts of the non-host plants Summer savoury (*Satureja hortensis* L.) and Tansy (*Tanacetum vulgare* L.) in an olfactometer. No effects of the extracts were observed on *B. brassicae*, but *A. fabae* was repelled by Summer savoury and Tansy odours. The volatile extracts of Tansy and Summer savoury were also presented to flying *A. fabae* in a wind tunnel with a green plant-mimicking target. No differences in flight behaviour were observed with Summer savoury extract being blown across the surface of the targets. The Tansy extract however, resulted in reduced numbers of alate *A. fabae* landing on the target. The results indicated that plant odours can affect flight and landing behaviour of aphids.

Reasons for the differences in response to the different types of extracts of the same plant were not clear. Differences in concentrations of the chemical compounds in the extract, or different volatiles that were released during the different extraction methods might be some of the reasons and should be investigated.

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# CHAPTER 4

# THE RESPONSE OF ALATE BIRD CHERRY-OAT APHIDS IN OLFACTOMETER TO VOLATILES ORIGINATING FROM PLANT EXTRACTS IN THE LABORATORY

## CHAPTER 4

# THE RESPONSE OF ALATE BIRD CHERRY-OAT APHIDS TO VOLATILES ORIGINATING FROM PLANT EXTRACTS IN THE LABORATORY

## 4.1 Introduction

The bird cherry-oat aphid *Rhopalosiphum padi* (Linnaeus) and the English grain aphid, *Sitobion avenae* (Fabricius), are annual pests of wheat in the irrigation areas of South Africa. They are present as very sporadic pests of dryland wheat in the Free State Province. These aphids are not as harmful as the Russian wheat aphid (*Diuraphis noxia*) (Kurdjumov), which is the major pest of wheat in South Africa (Prinsloo, Smit, Tolmay & Hattingh, 1997). This was confirmed by an investigation of the mechanism of feeding by *D. noxia* and *R. padi* on barley and wheat cultivars. The result of the feeding damage on the transport capacity of the phloem showed that *R. padi* infestation does not lead to a significant reduction in the phloem transport capacity during short-term feeding (72 h), while *D. noxia*-infested leaves showed considerable reduction on the transport capacity of the phloem within the same period. However, prolonged feeding (14 days) by *R. padi* induced a considerable reduction of the transport capacity of the phloem on the infested tissues, resulting in crop losses (Saheed, 2008).

Following decreased insecticide spraying for Russian wheat aphid, an upsurge in numbers of *R. padi*, *S. avenae* and *Metopolophium dirhodum* (Walker) occurred between 2000 and 2002 in the Free State Province. When high rainfall was experienced in this province, an upsurge in the occurrence of especially *R. padi* was experienced ([1] G. Prinsloo, personal communication). Increased spraying for these aphids were harmful to natural enemies released for the control of Russian wheat aphid populations. Therefore alternate control options are necessary to keep natural enemy numbers high.

[1]Dr. Goddy Prinsloo, Entomologist, 2010. Agricultural Research Council, Small Grain Institute, P/Bag X09, Bethlehem. 9300

The most obvious method to control wheat aphids is through insecticides. Chemical control of *R. padi* is costly and time of spraying is dependant on aphid population and plant growth stage. Different insecticides (carbamates, organophosphates and pyrethroids) are registered for the control of aphids, according to the Registrar of Act 36 of 1947 (Nel, Krause & Khelawanlall, 2002). Indiscriminate spraying of insecticides is a worldwide phenomenon. In recent decades, insect control became heavily dependant on chemical insecticides, and the dependence on insecticides has led in some crop systems to a high frequency of insecticide resistance (Thomas & Waage, 1996). The commercial use of synthetic insecticides also led to numerous unforeseen problems, for example the poisoning of humans, destruction of fish-, bird- and wildlife, groundwater contamination, resistance to pesticides and a potential threat to the environment (Isman, 2006). Pest control on wheat remains one of the essential inputs to ensure optimum crop production. The use of pest control measures on wheat is in many cases lacking amongst the resource limited farmers, especially those in the wheat production areas of the Free State ([2] D. Rose, personal communication). Several alternative control methods like host plant resistance, biological control, insect-plant interactions, plant extracts, trap crop barriers and intercropping are available (Kumar, 1984; Thomas & Waage, 1996, Isman, 2000).

With the exception of natural enemies attacking *R. padi* it is also known from literature that *R. padi* in Europe could be repelled from grains using a slow release wax pellet formulation of different volatile substances. Preventing aphids from establishing in crops will enhance the effectiveness of resistant varieties. (Pettersson, Pickett, Pye, Quiroz, Smart, Wadhams & Woodcock, 1994; Ninkovic, Ahmed, Glinwood & Pettersson, 2003). Some plant extracts, such as garlic extract, are known to have aphid repellent properties and are generally used by organic farmers in repelling aphids and other insects. Chemicals released by plants to initiate communication with insects, show that plant extracts can be useful in attracting insects and their natural enemies (Bruce, Cork, Hall & Dunkelblum, 2002). According to Price (1986) tritrophic interactions are mediated by inter alia semiochemicals. Semiochemicals are chemical

[2]David Rose, Extension Officer, 2006. Department of Agriculture, Private Bag X816, Post Office Witsieshoek, 9870.

substances that mediates interactions between organisms and are known to act as cues to attract or repel insects and aid natural enemies in locating and recognising their hosts or prey (Price, 1986).

It was therefore decided to determine the effect of the plant extracts from *Artemisia afra*, *Datura stramonium*, *Tagetes minuta* and *Tulbachia violacea* on the behaviour of alate *R. padi*.

## 4.2 MATERIALS AND METHODS

The materials and methods are discussed in Chapter 2. During data analyses the percentage differences between the control and treated arms for each parameter namely, entry number, total time spend and entry duration were calculated for each of the 50 replications. The data were then analysed as discussed in Chapter 3.

## 4.3 RESULTS

### 4.3.1 Olfactometric response of *Rhopalosiphum padi* to *Artemesia afra*.

#### 4.3.1.1 Oil extract.

*Rhopalosiphum padi* spent significantly less time in the olfactometer arms containing volatiles from *A. afra* than in the control arms (Table 4.1). The entries made into the treated arms were also significantly fewer than in the control, while mean entry duration was significantly shorter in the treated arms indicating repellence to *R. padi* (Table 4.1).

**Table 4.1** Mean response of *Rhopalosiphum padi* to *Artemesia afra* oil extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 3.31±0.29 | 5.34±0.31 | -3.48 | 0.001 |
| Entries per arm | 50 | 3.48±0.27 | 4.90±0.32 | -3.28 | 0.002 |
| Duration per entry (sec) | 50 | 57.48±5.09 | 70.42±4.11 | -2.09 | 0.042 |

**N** = number of replicates

#### 4.3.1.2 Aqueous extract.

Significant less time was spent per arm by *R. padi* in the treated arms than in the control arms (Table 4.2). The entries made by the aphid and the mean entry duration in the treated arms were significantly shorter (Table 4.2), indicating repellence to *A. afra*. The aphids were stronger repelled by the aqueous *A. afra* extract than by the oil extract (Table 4.2 & 4.1).

**Table 4.2** Mean response of *Rhopalosiphum padi* to *Artemesia afra* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 2.28±0.24 | 6.55±0.25 | -8.85 | <0.001 |
| Entries per arm | 50 | 3.50±0.29 | 5.50±0.24 | -5.35 | <0.001 |
| Duration per entry (sec) | 50 | 35.88±3.16 | 81.19±7.64 | -5.00 | <0.001 |

**N** = number of replicates

### 4.3.2 Olfactometric response of *Rhopalosiphum padi* to *Datura stramonium*.

#### 4.3.2.1 Oil extract.

Although *R. padi* spent less time in the treated olfactometer arms and made fewer entries into them, it was not significantly different from the control arms (Table 4.3). The mean duration of an entry was also not significantly shorter in the treated arms, indicating that the aphids were not strongly repelled by volatiles of oil extracts of *D. stramonium* (Table 4.3).

**Table 4.3** Mean response of *Rhopalosiphum padi* to *Datura stramonium* oil extract in an olfactometer during a ten minute period.

| Variable | N | | | Test statistics | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 2.33±0.20 | 6.20±0.19 | -10.38 | 0.428 |
| Entries per arm | 50 | 4.08±0.34 | 6.02±0.32 | -5.30 | 0.250 |
| Duration per entry (sec) | 50 | 34.81±3.19 | 75.58±7.34 | -4.82 | 0.723 |

**N** = number of replicates

#### 4.3.2.2 Aqueous extract.

The aphids made significantly less entries into the treated arms containing volatiles of *D. stramonium* (Table 4.4). However, the time spent and the entry duration did not differ significantly between the treated and control arms, indicating that the aphids were not repelled by this extract (Table 4.4).

**Table 4.4** Mean response of *Rhopalosiphum padi* to *Datura stramonium* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | | | Test statistics | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 4.13±0.12 | 4.49±0.13 | -1.51 | 0.138 |
| Entries per arm | 50 | 6.46±0.19 | 7.36±0.24 | -2.83 | 0.007 |
| Duration per entry (sec) | 50 | 39.20±1.14 | 37.30±0.91 | 1.28 | 0.205 |

**N** = number of replicates

### 4.3.3 Olfactometric response of *Rhopalosiphum padi* to *Tagetes minuta*.

#### 4.3.3.1 Oil extract.

*Rhopalosiphum padi* spent significantly less time in the olfactometer arms containing volatiles from *T. minuta* than in the control arms (Table 4.5). Significantly fewer entries were made while the mean entry duration was also significantly shorter in the treated arms, which indicated repellence by *T. minuta* (Table 4.5).

**Table 4.5** Mean response of *Rhopalosiphum padi* to *Tagetes minuta* oil extract in an olfactometer during a ten minute period.

| Variable | N | Treated ±SE | Control ±SE | Test statistics t | P |
|---|---|---|---|---|---|
| Time per arm (min) | 50 | 3.61±0.24 | 5.25±0.23 | -3.57 | <0.001 |
| Entries per arm | 50 | 3.92±0.26 | 5.22±0.25 | -3.26 | 0.002 |
| Duration per entry (sec) | 50 | 55.06±2.76 | 63.22±2.45 | -2.19 | 0.034 |

**N** = number of replicates

#### 4.3.3.2 Aqueous extract.

*Rhopalosiphum padi* did not respond significantly to the treated or the control arms in terms of the number of entries made, the time spend in the arms and the entry duration (Table 4.6). Therefore the aqueous extract of this plant did not elicit any response.

**Table 4.6** Mean response of *Rhopalosiphum padi* to *Tagetes minuta* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | Treated ±SE | Control ±SE | Test statistics t | P |
|---|---|---|---|---|---|
| Time per arm (min) | 50 | 4.49±0.25 | 5.13±0.24 | -1.34 | 0.189 |
| Entries per arm | 50 | 4.63±0.31 | 4.73±0.26 | -0.25 | 0.801 |
| Duration per entry (sec) | 50 | 65.86±4.34 | 70.10±3.70 | -0.95 | 0.347 |

**N** = number of replicates

### 4.3.4 Olfactometric response of *Rhopalosiphum padi* to *Tulbaghia violacea.*

#### 4.3.4.1 Oil extract.

The aphid did not make significantly fewer entries or spent less time in the treated arms of the olfactometer (Table 4.7). No difference in the entry duration occurred (Table 4.7) and therefore *R. padi* did not respond to this extract.

**Table 4.7** Mean response of *Rhopalosiphum padi* to *Tulbaghia violacea* oil extract in an olfactometer during a ten minute period.

| Variable | N | | | Test statistics | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 4.37±0.24 | 4.78±0.24 | -0.87 | 0.387 |
| Entries per arm | 50 | 4.96±0.26 | 5.42±0.29 | -1.22 | 0.230 |
| Duration per entry (sec) | 50 | 58.91±4.35 | 57.51±3.46 | 0.32 | 0.747 |

**N** = number of replicates

#### 4.3.4.2 Aqueous extract

Significantly more entries were made and time spent by the aphid in treated arms than in the control arms (Table 4.8). The entry duration in the treated arm was also significantly longer in the treated arm, which indicated that *R. padi* was attracted to this extract (Table 4.8).

**Table 4.8** Mean response of *Rhopalosiphum padi* to *Tulbaghia violacea* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | | | Test statistics | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 5.73±0.22 | 3.70±0.19 | 4.97 | <0.001 |
| Entries per arm | 50 | 6.02±0.28 | 5.04±0.27 | 2.46 | 0.017 |
| Duration per entry (sec) | 50 | 61.32±3.01 | 46.02±2.40 | 4.07 | <0.001 |

**N** = number of replicates

## 4.4 Comparison between different extracts

The response of *R. padi* varied to a great extent between plants and also between the type of extract from the same plant, some indicated repellence and some affinity. When the percentage differences in the response to the treated and control arms were calculated and used to compare between the extracts for each of the three parameters, namely entries made, time spent and the duration of an entry, certain patterns appeared (Fig. 4.1 A, B, C).

Considering percentage difference in entries made, *R.padi* responded the best to both extracts of *A. afra*, and the oil extracts of *D. stramonium* and *T. minuta* (Fig. 4.1 A). The percentage differences were, however, not very large which indicated that the response of the aphids varied. For the other extracts the responses were low, which indicated that they did not respond to them, while there was a slight attraction to *T. violacea* aqueous extract (Fig. 4.1 A).

The percentage difference in time that was spent in the treatment and control arms showed that the aqueous extract of *A. afra* and the oil extract of *D. stramonium* were highly repellent to *R. padi* (Fig. 4.1 B). This same pattern was observed with the amount of entries made (Fig. 4.1 A). The aphid responded less to the oil extracts of *A. afra* and *T. minuta*, and the aqueous extract of *T. violacea*. Response to aqueous extracts of *D. stramonium* and *T. minuta*, and the oil extract of *T. violacea* differed significantly from extracts 1 and 4, elicited low repellence responses and would therefore not be considered for use (Fig. 4.1 B).

The aqueous extract of *A. afra* and the oil extract of *D. stramonium* elicited the highest repellent response in *R. padi* in terms of percentage difference in entry duration (Fig. 4.1 C). The oil extract of *A. afra* elicited the second strongest repellent response in terms of duration time though it was not a strong response. The other extracts did not elicit strong repellent responses and even a weak attraction was elicited by the *T. violacea* aqueous extract (Fig. 4.1 C). Depending on the repellence or attractive response of the natural enemies to the aqueous extract of *A. afra* and the oil extract of *D. stramonium* they would be recommended for use by the farmers to

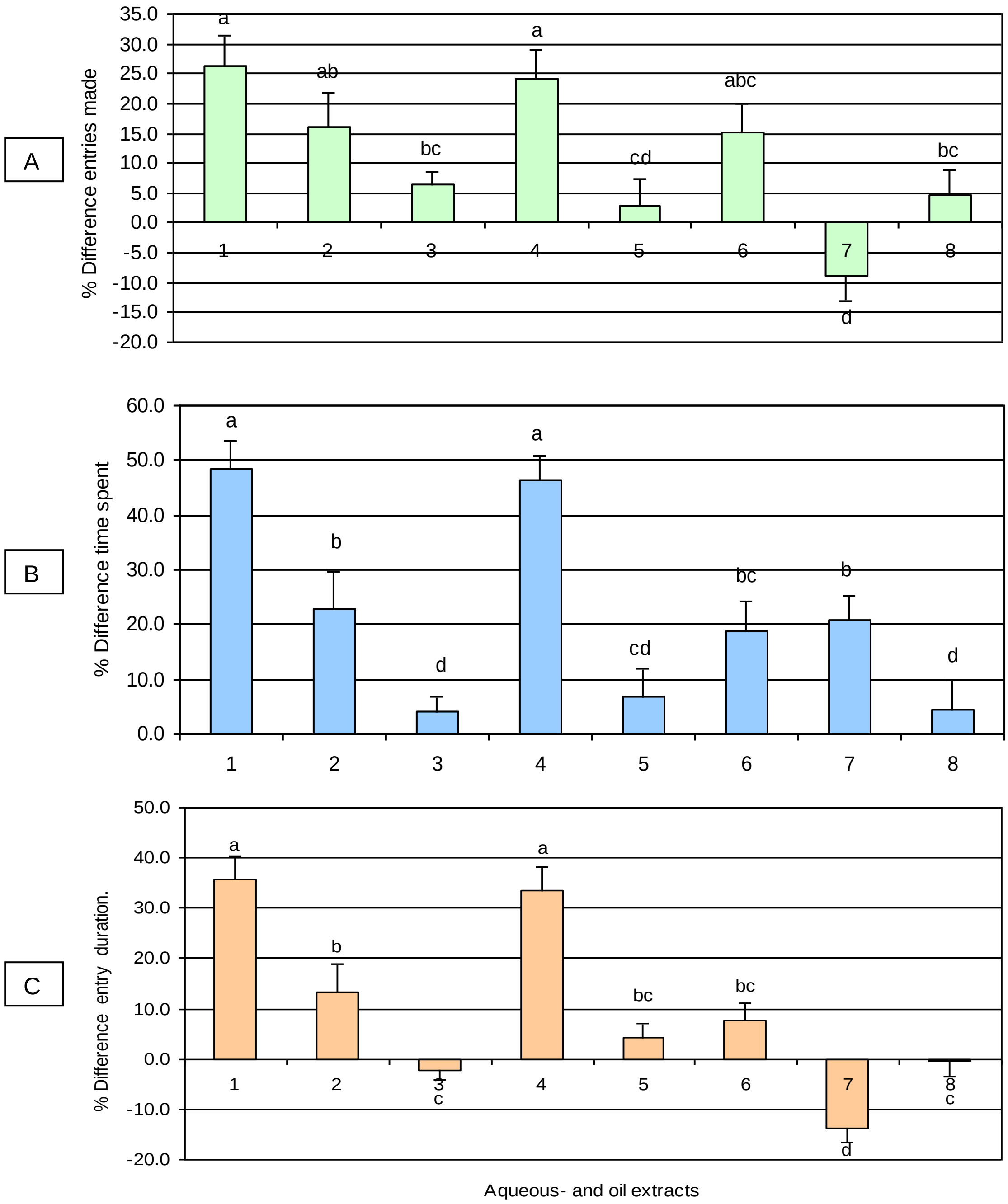


Figure 4.1 Mean percentage differences between the control arms and treated arms for the following parameters: total entries per arm (A) (P = 0.05, LSD = 12.77), time per arm (B) (P = 0.05, LSD = 14.01) and time per entry (entry duration) (C) (P = 0.05, LSD = 10.57) by the bird cherry-oat aphid (*Rhopalosiphum padi*). Symbols that differ indicate significant differences between extracts. Extract 1 = *Artemisia afra* aqueous extract; 2 = *Artemisia afra* oil extract; 3 = *Datura stramonium* aqueous extract; 4 = *Datura stramonium* oil extract; 5 = *Tagetes minuta* aqueous extract; 6 = *Tagetes minuta* oil extract; 7 = *Tulbachia violacea* aqueous extract; 8 = *Tulbachia violacea* oil extract.

repel *R. padi*. The response to the rest of the extracts was low and could be considered as a no- response and would not be used.

*Rhopalosiphum padi* is known to be present on the lower stems of wheat plants near the soil surface before plants are tillering. This is still early in the wheat-growing season and temperatures below zero were experienced regularly. When considering the availability of plants *D. stramonium* is a frost sensitive annual and would not be available. *Artemisia afra* is a perennial and available throughout the year and is therefore the only plant specie which could be used by the farmers.

Reasons for the differences in response to the different types of extracts of the same plant may be due to differences in concentrations of the specific chemical compounds in the extract. It may also be that various volatiles were released during the different extraction methods, which elicit various responses, by *R. padi*.

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# CHAPTER 5

# THE RESPONSE OF APHID NATURAL ENEMIES IN OLFACTOMETER TO VOLATILES ORIGINATING FROM PLANT EXTRACTS IN THE LABORATORY

# CHAPTER 5

# THE RESPONSE OF APHID NATURAL ENEMIES TO VOLATILES ORIGINATING FROM PLANT EXTRACTS IN THE LABORATORY

## 5.1 Introduction

Herbivore induced plant odours play a major role in the foraging behaviour of predatory and parasitic arthropods (Turlings, Bernasconi, Bertossa, Bigler, Carloz & Dorn, 1998; Arimura, Ozawa, Shioda, Nishioka, Boland & Takabayashi, 2000; Fatouros, Van Loon, Hordijk, Smid & Dicke, 2005). Du, Poppy, Powell, Pickett, Wadhams & Woodcock, (1998) found that volatile phytochemicals released by aphid infested broad bean plants *Vicia faba* (Linnaeus), were more attractive to the aphid parasitoid *Aphidius ervi* (Haliday) than those from uninfested plants. Such phytochemicals were capable of inducing a variety of responses in plants, including induction of defences against pathogens and herbivores (Arimura *et al*., 2000; Walters, Cowley & Mitchell, 2002), modification of volatile profile (Dicke, Gols, Ludeking & Posthumus, 1999) and increased attractiveness to herbivore natural enemies (Chamberlain, Pickett & Woodstock, 2000). These phytochemicals, also known as 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, Pickett, Pye, Quiroz, Smart, Wadhams & Woodcock, 1994; Ninkovic, Ahmed, Glinwood & Pettersson, 2003).

The most common insect pests occurring on wheat in the Free State Province are aphids (Prinsloo, Smit, Tolmay & Hattingh, 1997). Six species are involved, of which the Russian wheat aphid (*Diuraphis noxia* Kurdjumov) is the most damaging (Du Toit, 1986; Aalbersberg, 1987; Prinsloo, Ninkovic, Van der Linde, Van der Westhuizen, Pettersson & Glinwood, 2007). Cultivars resistant to *D. noxia* were bred, and more than 70% of farmers in the Free State Province planted these cultivars (Marasas, Anandajayasekeram, Tolmay, Martella, Purchase & Prinsloo, 1997). Natural enemies of the aphid were also introduced, released and became established during the development of an integrated control programme for *D. noxia*. One of the introduced parasitoids *Aphelinus hordei* (Kurdjumov) preferred to oviposit in *D. noxia* (Prinsloo, 2000).

Natural enemies of aphids may benefit the plants by reducing herbivore abundance. Plants may benefit the herbivore's enemies by making herbivores more vulnerable to the natural enemies through the release of volatile substances attractive to these natural enemies (Price, 1986). This mutualism could diminish the chances for the development of a plant resistance breaking biotype in *D. noxia*. If natural enemies, however, were not attracted to these plants, the aphids feeding on them would be free from natural enemy attack, thereby improving chances for a resistance breaking biotype to develop (Prinsloo, 2006).

An example of such mutualism was demonstrated by Ulpah (2006) who studied the effect of volatile substances released by certain weeds on the egg parasitoid *Trichogramma papilionis* (Nagarkatti) of the Asiatic maize borer *Ostrinia furnacalis* (Guen). He found that volatiles from fresh plant material of three different weed species were attractive to the parasitoid. These weeds could therefore be used as part of a natural control programme for the stem borer (Ulpah, 2006).

In the present study the behavioural response of two aphid parasitoid species namely, *Aphelinus hordei* (Kurdjumov) and *Diaeretiella rapae* (McIntosh), to volatiles from four plant species were tested in the laboratory. The four plant species namely, Wild wormwood *Artemisia afra* (Jacq. ex Willd.), Big thorn apple *Datura stramonium* (Linnaeus), Khaki bush *Tagetes minuta* (Linnaeus) and Wild garlic *Tulbaghia violacea* (Harv.) were chosen because they have repellent properties against two wheat aphid species namely *D. noxia* and *Rhopalosiphum padi,* and could therefore be used in the control of these aphids (chapter 3 & 4). If parasitoids could be attracted by these plant extracts, the farmers could use them with more advantage.

## 5.2 MATERIALS AND METHODS

The response of both parasitoids was tested in olfactometers as discussed in Chapter 2. During data analyses the percentage differences between the control and treated arms for each parameter namely, entry number, total time spend and entry duration were calculated for each of the 50 replicates. The data were then analysed as discussed in Chapter 3 and the response of each parasitoid between different extracts compared.

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 *Aphelinus hordei*

#### 5.3.1.1 Olfactometric response of *Aphelinus hordei* to *Artemesia afra*

**A: Oil extract**

*Aphelinus hordei* females spent significantly less time and made significant fewer entries into the treated arms (Table 5.1). The entry duration did not differ significantly between the treated and control arms (Table 5.1). The parasitoid seemed to be weakly repelled by this extract.

**Table 5.1** Mean response of *Aphelinus hordei* to *Artemesia afra* oil extract in an olfactometer during a ten minute period.

| Variable | N | | | Test statistics | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 3.67 ± 0.26 | 5.67 ± 0.26 | -3.68 | <0.001 |
| Entries per arm | 50 | 6.78 ± 0.51 | 8.72 ± 0.48 | -4.12 | <0.001 |
| Duration per entry (sec) | 50 | 40.38 ± 4.00 | 42.95 ± 3.01 | -0.59 | 0.560 |

**N** = number of replicates

**B: Aqueous extract**

Female *A. hordei* spend significantly more time in and made significantly more entries into the treated olfactometer arms (Table 5.2). The entry duration was also significantly longer in the treated arms (Table 5.2), indicating attraction to *A. afra*.

**Table 5.2** Mean behavioural response of *Aphelinus hordei* to *Artemesia afra* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 6.31 ± 0.33 | 3.19 ± 0.31 | 4.93 | <0.001 |
| Entries per arm | 50 | 5.46 ± 0.40 | 4.40 ± 0.42 | 2.31 | 0.025 |
| Duration per entry (sec) | 50 | 86.14 ± 7.06 | 47.66 ± 5.28 | 4.26 | <0.001 |

**N** = number of replicates

### 5.3.1.2 Olfactometric response of *Aphelinus hordei* to *Datura stramonium*

**A: Oil extract**

Significantly more time was spent in the treated arms of the olfactometer (Table 5.3). The number of entries made by *A. hordei* into the different arms did not differ significantly. The time per entry was significantly longer in the treated arms than in the control arms. This indicated that the parasitoids were attracted to the volatile substances present in *D. stramonium* oil extract.

**Table 5.3** Mean response of *Aphelinus hordei* to *Datura stramonium* oil extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 5.29 ± 0.17 | 4.00 ± 0.18 | 3.72 | <0.001 |
| Entries per arm | 50 | 8.70 ± 0.40 | 8.32 ± 0.40 | 0.93 | 0.359 |
| Duration per entry (sec) | 50 | 40.30 ± 3.35 | 31.06 ± 1.80 | 3.46 | 0.001 |

**N** = number of replicates

**B: Aqueous extract**

*Aphelinus hordei* did not respond significantly to either the treated or the control arms in the number of entries made, the time spent or the entry duration (Table 5.4), and was not responding to volatile substances from the extract.

**Table 5.4** Mean response of *Aphelinus hordei* to *Datura stramonium* aqueous extract in an olfactometer during a ten minute period.

| **Variable** | **N** | | | **Test statistics** | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 4.84 ± 0.23 | 4.53 ± 0.24 | 0.67 | 0.505 |
| Entries per arm | 50 | 8.10 ± 0.40 | 8.24 ± 0.52 | -0.34 | 0.737 |
| Duration per entry (sec) | 50 | 40.51 ± 3.41 | 39.73 ± 3.77 | 0.18 | 0.859 |

**N** = number of replicates

### 5.3.1.3 Olfactometric response of *Aphelinus hordei* to *Tagetes minuta*

**A: Oil extract**

*Aphelinus hordei* did not respond differently in the number of entries between the treated and control arms (Table 5.5). They did, however, spend significantly less time in the treated arms. Significantly shorter entry durations were experienced by *A. hordei* (Table 5.5), which indicated a degree of repellence.

**Table 5.5** Mean response of *Aphelinus hordei* to *Tagetes minuta* oil extract in an olfactometer during a ten minute period.

| **Variable** | **N** | | | **Test statistics** | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 3.89 ± 0.19 | 5.21 ± 0.19 | -3.57 | <0.001 |
| Entries per arm | 50 | 9.12 ± 0.45 | 10.10 ± 0.52 | -1.75 | 0.086 |
| Duration per entry (sec) | 50 | 28.52 ± 2.03 | 35.24 ± 2.43 | -3.06 | 0.004 |

**N** = number of replicates

**B: Aqueous extract**

Significantly more entries were made into the treated arms by *A. hordei* females (Table 5.6). They also spent significantly more time with significantly longer entry durations in the treated arms, which indicated that they were attracted to this extract (Table 5.6).

**Table 5.6** Mean response of *Aphelinus hordei* to *Tagetes minuta* aqueous extract in an olfactometer during a ten minute period.

| **Variable** | **N** | | | **Test statistics** | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 6.31 ± 0.31 | 3.06 ± 0.30 | 5.47 | <0.001 |
| Entries per arm | 50 | 6.30 ± 0.46 | 4.80 ± 0.47 | 3.51 | <0.001 |
| Duration per entry (sec) | 50 | 79.24 ± 7.37 | 47.50 ± 6.49 | 3.54 | <0.001 |

**N** = number of replicates

### 5.3.1.4 Olfactometric response of *Aphelinus hordei* to *Tulbaghia violacea*

**A: Oil extract**

*Aphelinus hordei* spent significantly more time in the treated arms (Table 5.7). They entered the treated arms significantly more than in the control arms. The entry duration was also significantly longer, but in both instances less significant (Table 5.7). Since the response was not strong this could be regarded as a no-response.

**Table 5.7** Mean response of *Aphelinus hordei* to *Tulbaghia violacea* oil extract in an olfactometer during a ten minute period.

| **Variable** | **N** | | | **Test statistics** | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 5.13 ± 0.18 | 4.05 ± 0.20 | 2.90 | 0.006 |
| Entries per arm | 50 | 9.96 ± 0.48 | 8.90 ± 0.49 | 2.03 | 0.048 |
| Duration per entry (sec) | 50 | 33.94 ± 2.01 | 28.98 ± 1.49 | 2.04 | 0.047 |

**N** = number of replicates

**B: Aqueous extract**

This parasitoid species spent significantly more time in the treated arms with significantly longer entry durations (Table 5.8). The number of entries, however, did not differ significantly (Table 5.8), and therefore a weak attraction to the volatiles of the extract was indicated.

**Table 5.8** Mean response of *Aphelinus hordei* to *Tulbaghia violacea* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 5.45 ± 0.20 | 3.77 ± 0.19 | 4.50 | <0.001 |
| Entries per arm | 50 | 8.24 ± 0.36 | 7.63 ± 0.42 | 1.32 | 0.194 |
| Duration per entry (sec) | 50 | 44.75 ± 3.15 | 33.26 ± 2.74 | 2.92 | 0.005 |

**N** = number of replicates

### 5.3.1.5 Influence of different extracts on parasitoid *Aphelinus hordei*

From the above-mentioned results it is clear that the response of *A. hordei* varied between the different types of plant extracts, even between aqueous and oil extracts of the same plant species. In order to discriminate more clearly between the different extracts, the difference between time per arm, entries per arm and duration of entries made into treated- and control arms were expressed as percentages (Figure 5.1 A, B & C).

Considering the percentage difference in entries made in the treatment and control arms, the oil extract of *A. afra* was repellent to *A. hordei*, while the aqueous extracts of *A. afra* and *T. minuta* was attractive to the parasitoid (Fig 5.1 A). The percentage difference for the rest of the other extracts was very small and the parasitoids were therefore not responding to them (Fig 5.1 A).

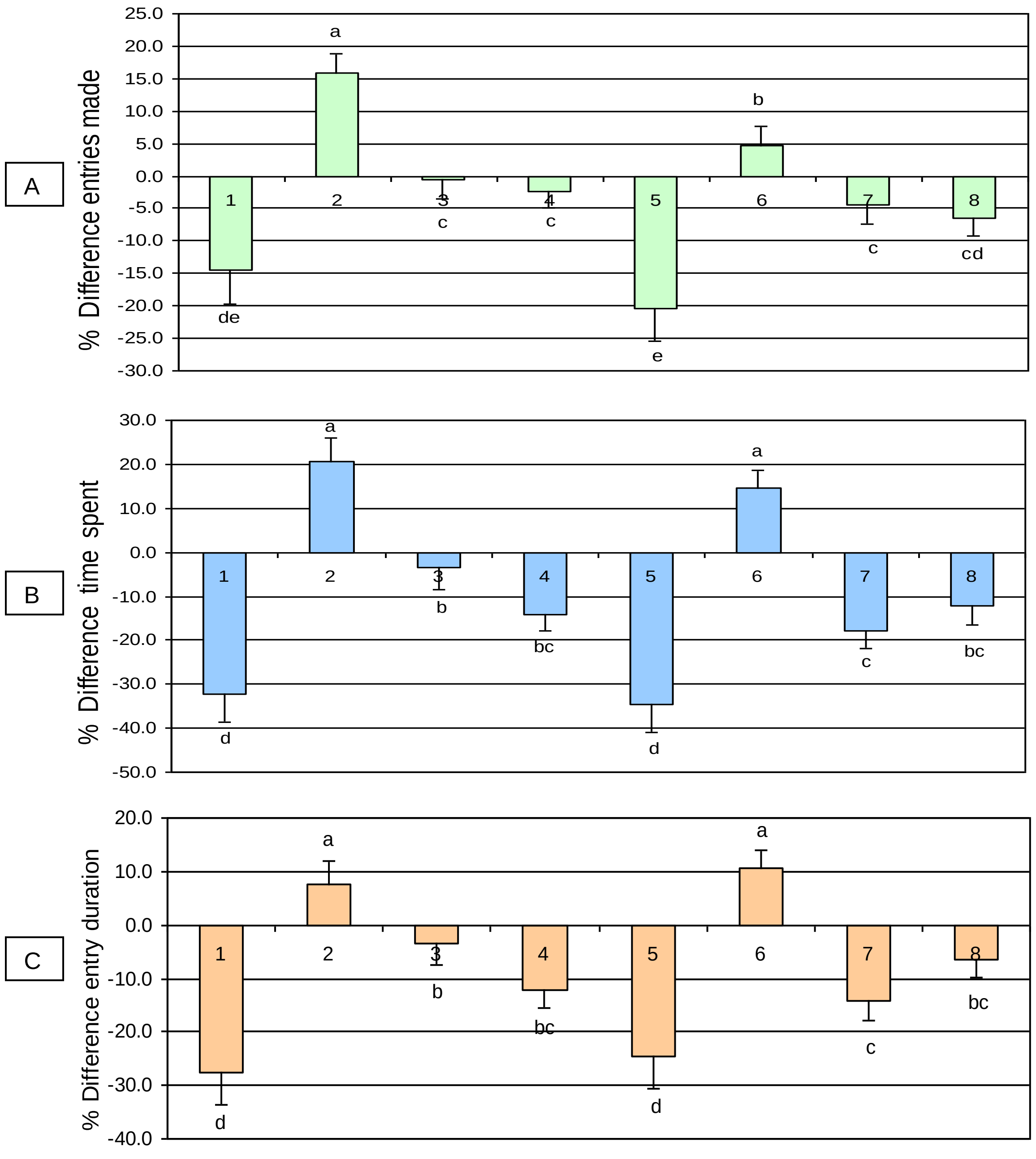


Figure 5.1 Mean percentage differences in response of *Aphelinus hordei* between the control arms and treated arms for the following parameters: total entries per arm (A) (P = 0.05, LSD = 7.87), time per arm (B) (P = 0.05, LSD = 12.33) and time per entry (entry duration) (C) (P = 0.05, LSD = 9.8) by the parasitoid *Aphelinus hordei*. Letters above bars that differ indicate significant differences between extracts. Extract 1 = *Artemisia afra* aqueous extract; 2 = *Artemisia afra* oil extract; 3 = *Datura stramonium* aqueous extract; 4 = *Datura stramonium* oil extract; 5 = *Tagetes minuta* aqueous extract; 6 = *Tagetes minuta* oil extract; 7 = *Tulbaghia violacea* aqueous extract; 8 = *Tulbaghia violacea* oil extract.

The percentage difference in time spent, again indicated repellence by *A. afra* oil extract and attraction by the aqueous extracts of *A. afra* and *T. minuta* through significant higher differences (Fig. 5.1B). They were slightly repelled by the oil extract of *T. minuta* and in the same quantity attracted by both *D. stramonium* and *T. violacea* extracts and therefore not responding to these extracts (Fig. 5.1B).

The percentage difference in entry duration again indicated that *A. hordei* were attracted by the aqueous extracts of *A. afra* and *T. minuta* (Fig. 5.1 C). They were slightly repelled by the oil extracts of *A. afra* and *T. minuta* and slightly attracted by both extracts of both *D. stramonium* and *T. violacea* (Fig. 5.1 C). These results indicated that if the farmers were using the aqueous extracts of *A. afra* and *T. minuta*, it would attract *A. hordei* to the fields. *Tagetes minuta* was available only later in the wheat season, and *A. hordei* was a more specific parasitoid for *D. noxia*. This extract could therefore be prepared and sprayed to enhance biological control of *D. noxia*.

### 5.3.2 *Diaeretiella rapae*

#### 5.3.2.1 Olfactometric response of *Diaeretiella rapae* to *Artemesia afra*

**A: Oil extract**

The parasitoid *D rapae* spent significantly less time in and made significantly fewer entries into the treated arms of the olfactometer in comparison with the control arms (Table 5.9). The entry duration was shorter in the treated arms, but did not differ significantly (Table 5.9). These results indicated that the parasitoid did not prefer to enter the treated arms of the olfactometer, but when some did enter they did not leave quickly.

**Table 5.9** Mean response of *Diaeretiella rapae* to *Artemesia afra* oil extract in an olfactometer during a ten minute period.

| **Variable** | **N** | **Test statistics** | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 3.02 ± 0.14 | 5.73 ± 0.14 | -10.00 | <0.001 |
| Entries per arm | 50 | 4.78 ± 0.15 | 9.62 ± 1.59 | -3.02 | <0.004 |
| Duration per entry (sec) | 50 | 38.44 ± 1.67 | 42.54 ± 1.18 | -1.81 | 0.077 |

**N** = number of replicates

**B: Aqueous extract**

This parasitoid entered significantly fewer times and spent significantly less time in the treated olfactometer arms (Table 5.10). The entry duration was significantly shorter in the treated arms, which indicated that the parasitoid was repelled by the extract (Table 5.10).

**Table 5.10** Mean response of *Diaeretiella rapae* to *Artemesia afra* aqueous extract in an olfactometer during a ten minute period.

| **Variable** | **N** | **Test statistics** | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 2.42 ± 0.26 | 6.29 ± 0.28 | -7.21 | <0.001 |
| Entries per arm | 50 | 4.22 ± 0.37 | 7.44 ± 0.49 | -5.73 | <0.001 |
| Duration per entry (sec) | 50 | 36.04 ± 4.26 | 62.51 ± 5.51 | -3.98 | <0.001 |

**N** = number of replicates

### 5.3.2.2 Olfactometric response of *Diaeretiella rapae* to *Datura stramonium*

**A: Oil extract**

Significantly less time was spent and significantly fewer entries were made by *D. rapae* into the treated arms (Table 5.11). The duration time of an entry was also significantly shorter in the treated arms, indicating that the extract repelled the parasitoid (Table 5.11).

**Table 5.11** Mean response of *Diaeretiella rapae* to *Datura stramonium* oil extract in an olfactometer during a ten minute period.

| **Variable** | **N** | | | **Test statistics** | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 3.32 ± 0.31 | 5.36 ± 0.31 | -3.35 | 0.002 |
| Entries per arm | 50 | 5.50 ± 0.43 | 6.88 ± 0.46 | -2.10 | 0.041 |
| Duration per entry (sec) | 50 | 37.67 ± 3.78 | 56.15 ± 5.63 | -2.80 | 0.007 |

**N** = number of replicates

**B: Aqueous extract**

*Diaeretiella rapae* entered significantly fewer times into and spent significantly less time in the treated olfactometer arms (Table 5.12). The entry duration however did not differ significantly (Table 5.12), indicating that the parasitoid did not prefer to enter the treated arms, but when some did they were slow to leave.

**Table 5.12** Mean response of *Diaeretiella rapae* to *Datura stramonium* aqueous extract in an olfactometer during a ten minute period.

| **Variable** | **N** | | | **Test statistics** | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 2.55 ± 0.37 | 6.23 ± 0.37 | -5.08 | <0.001 |
| Entries per arm | 50 | 3.42 ± 0.46 | 6.26 ± 0.60 | -4.99 | <0.001 |
| Duration per entry (sec) | 50 | 72.24 ± 19.08 | 92.66 ± 14.29 | -0.80 | 0.430 |

**N** = number of replicates

### 5.3.2.3 Olfactometric response of *Diaeretiella rapae* to *Tagetes minuta*

**A: Oil extract**

The parasitoid entered significantly more times into and spent significantly more time in the treated olfactometer arms (Table 5.13). However, the entry duration did not differ significantly (Table 5.13). This indicated that the parasitoid was attracted, and although they entered more times in the treated arms, they did not stay significantly longer than in the control arms.

**Table 5.13** Mean response of *Diaeretiella rapae* to *Tagetes minuta* oil extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 5.77 ± 0.33 | 3.04 ± 0.31 | 4.26 | <0.001 |
| Entries per arm | 50 | 11.05 ± 0.65 | 6.80 ± 0.62 | 4.76 | <0.001 |
| Duration per entry (sec) | 50 | 35.86 ± 3.83 | 29.14 ± 4.44 | 1.13 | 0.264 |

**N** = number of replicates

**B: Aqueous extract**

*Diaeretiella rapae* entered significantly more times into and spent significantly more time in the treated olfactometer arms (Table 5.14). The entry duration was significantly longer in the treated arms, which indicated that the parasitoid was attracted to the extract (Table 5.14).

**Table 5.14** Mean response of *Diaeretiella rapae* to *Tagetes minuta* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 6.01 ± 0.26 | 3.34 ± 0.25 | 5.29 | <0.001 |
| Entries per arm | 50 | 6.78 ± 0.57 | 5.40 ± 0.54 | 3.29 | <0.002 |
| Duration per entry (sec) | 50 | 88.41 ± 13.25 | 57.65 ± 9.49 | 2.19 | 0.003 |

**N** = number of replicates

#### 5.3.2.4 Olfactometric response of *Diaeretiella rapae* to *Tulbaghia violacea*

**A: Oil extract**

*Diaeretiella rapae* entered significantly more times into and spent significantly more time inside the treated olfactometer arms (Table 5.15). The entry duration was significantly longer in the treated arms, which indicated that the parasitoids are attracted to the extract (Table 5.15).

**Table 5.15** Mean response of *Diaeretiella rapae* to *Tulbaghia violacea* oil extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 7.06 ± 0.37 | 1.42 ± 0.31 | 8.65 | <0.001 |
| Entries per arm | 50 | 6.10 ± 0.54 | 2.40 ± 0.35 | 6.94 | <0.001 |
| Duration per entry (sec) | 50 | 115.06 ± 17.27 | 23.98 ± 4.76 | 4.77 | <0.001 |

**N** = number of replicates

**B: Aqueous extraction**

The parasitoid did not enter significantly more into or spend significantly more time through longer entry durations in the treated olfactometer arms, indicating no response towards this extract (Table 5.16).

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**Table 5.16** Mean response of *Diaeretiella rapae* to *Tulbaghia violacea* aqueous extract in an olfactometer during a ten minute period.

| Variable | N | Test statistics | | | |
|---|---|---|---|---|---|
| | | **Treated ±SE** | **Control ±SE** | **t** | **P** |
| Time per arm (min) | 50 | 4.25 ± 0.28 | 4.61 ± 0.28 | -0.65 | 0.525 |
| Entries per arm | 50 | 8.32 ± 0.67 | 8.56 ± 0.48 | -0.34 | 0.734 |
| Duration per entry (sec) | 50 | 36.59 ± 3.21 | 36.53 ± 3.09 | 0.01 | 0.988 |

**N** = number of replicates

### 5.3.2.5 Comparison of the response of *Diaeretiella rapae* to different extracts

The response of *D. rapae* varied between extracts. However, when the difference between time per arm, entries per arm and duration of entries made into treated- and control arms were expressed as a percentage, it is possible to discriminate more clearly between the different extracts (Figure 5.2 A, B & C).

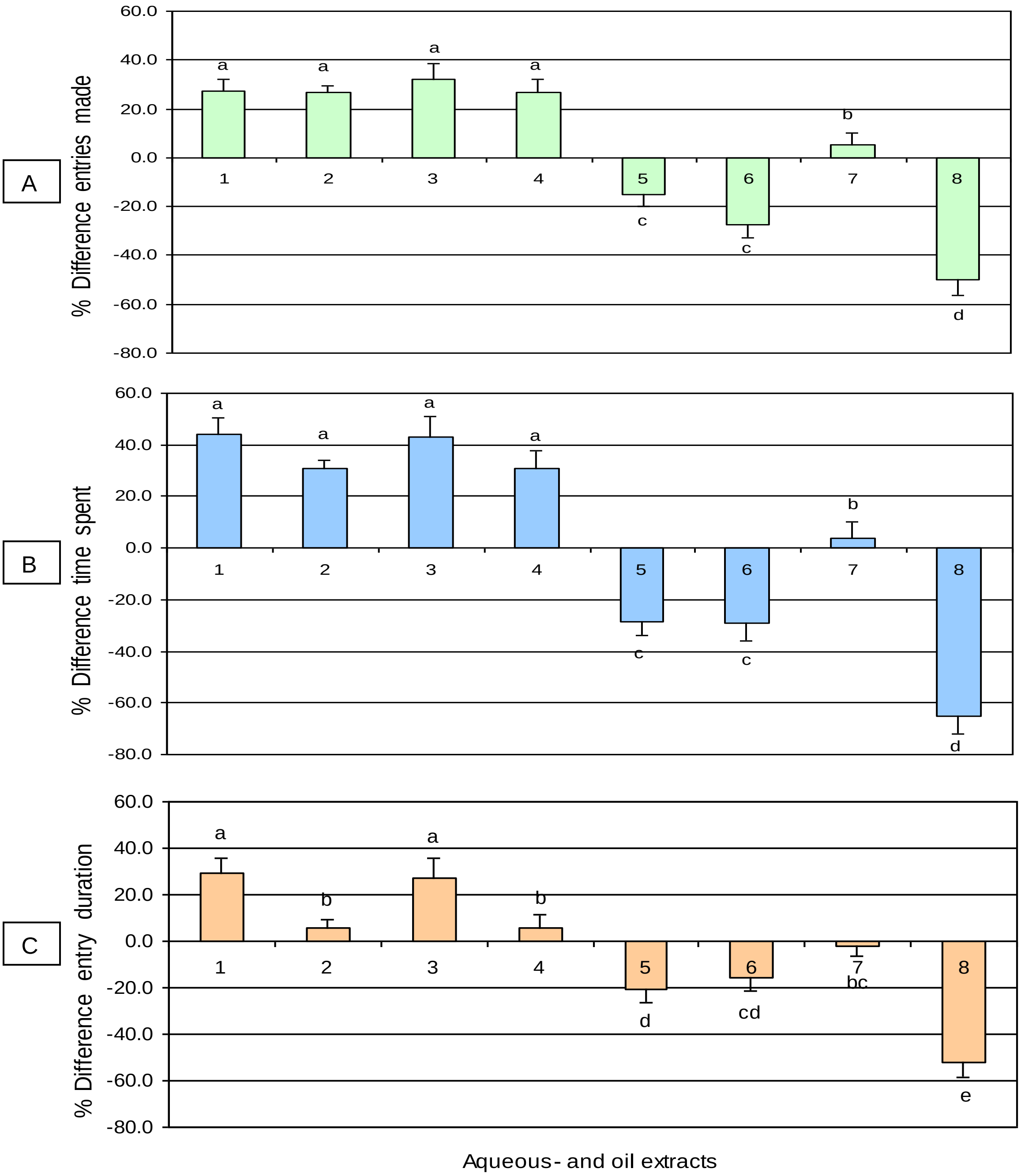


Figure 5.2 Mean percentage differences in response of *Diaeretiella rapae* between the control arms and treated arms for the following parameters: total entries per arm (A) (P = 0.05, LSD = 12.76), time per arm (B) (P = 0.05, LSD = 15.43) and time per entry (entry duration) (C) (P = 0.05, LSD = 14.01) by the parasitoid *Diaeretiella rapae*. Letters above bars that differ indicate significant differences between extracts. Extract 1 = *Artemisia afra* aqueous extract; 2 = Artemisia *afra* oil extract; 3 = *Datura stramonium* aqueous extract; 4 = *Datura stramonium* oil extract; 5 = *Tagetes minuta* aqueous extract; 6 = *Tagetes minuta* oil extract; 7 = *Tulbaghia violacea* aqueous extract; 8 = *Tulbaghia violacea* oil extract.

Both types of extracts of *A. afra* and *D. stramonium* were repellent to *D. rapae*, considering the percentage difference in entries made (Fig. 5.2 A). Both extracts of *T. minuta* and the oil extract of *T. violacea* were attractive to *D. rapae*. The strongest response was obtained from the oil extract of *T. violacea*, and would be regarded as high potential (Fig. 5.2 A). A small repellent response to the *T. violacea* aqueous extract could be regarded as a no-response.

Another important parameter to evaluate attractiveness or repellence from the different extracts is the percentage difference in time spent in the treated and control arms of the olfactometer by *D. rapae*. The parasitoid again was attracted to both extracts of *T. minuta* and the oil extract of *T. violacea* (Fig. 5.2 B). The *T. violacea* oil extract was, however, highly attractive, and would be highly recommended as an attractant (Fig. 5.2 B). Both types of extracts of *A. afra* and *T. minuta* elicited a repellent response while the aqueous extract of *T. violacea* did not elicit a response (Fig. 5.2 B).

When considering the percentage difference in entry duration, *D. rapae* was highly attracted by the oil extract of *T. violacea* (Fig. 5.2 C). This means that the parasitoid stayed much longer after entering the treated arms than in the control arms. This extract can be used as to enhance biological control in wheat fields. They were also attracted by both extracts of *T. minuta*, but not as efficient as *T. violacea* oil extract (Fig. 5.2 C). The parasitoid did not respond to the aqueous extract of *T. violacea* and the oil extracts of both *A. afra* and *D. stramonium.* They were, however, repelled by the aqueous extracts of the latter two plant species (Fig. 5.2 C).

## 5.4 RELATION BETWEEN PARASITOIDS AND DIFFERENT EXTRACTS

The parasitoids differed in their response to the same group of plant extracts, which could be expected, because of various host ranges feeding on different plants. Extraction of volatiles using boiling water would extract volatiles that are chemically different from those extracted through cold mineral oil. This was also responsible for the varying responses observed. This variation complicated the decision to which extract should be used and when. The results, however, clearly indicated some good attractants which should be investigated. The type of parasitoid, which is locally abundant in wheat fields, should be identified. This is to determine the corresponding extract (attractant) before spraying any plant extract on wheat fields.

Elucidating the chemical ecology of natural enemies, herbivores and host plants are important in the development of successful integrated pest management (IPM) strategies. Abundance and distribution of natural enemies could be manipulated by semiochemicals for improved conservation biological control. In response to attack by herbivores, plants produce semiochemicals called Herbivore-Induced Plant Volatiles (HIPVs). These volatiles act to repel pests and attract their natural enemies. This natural delivery of semiochemicals for conservation biological control is currently being exploited by smallholder farmers in eastern Africa in the management of cereal stem borers in grain crops (Zevaur, James, Midega & Pickett, 2008).

The parasitoid *A. hordei* was most attracted to the aqueous extracts of *A. afra* and *T. minuta* with the best response from A. afra aqueous extract (Fig. 5.1). In Figure 5.2 parasitoid *D. rapae* was best attracted by the oil extract of *T. violacea* and both extracts of *T. minuta*. The reasons for the differences in response to the different types of extracts of the same plant are unknown. It may be due to differences in chemical compound concentrations of the extracts. Different volatiles were released during the different extraction processes. The plant extracts consist of many different volatile chemical compounds, some unknown, but some did show promises of attraction and should be investigated further.

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# CHAPTER 6

# GENERAL DISCUSSION

# CHAPTER 6

## GENERAL DISCUSSION

Arthropod pests are responsible for global pre- and post-harvest crop losses of approximately 20-50% of potential production (Thacker, 2002). In South Africa wheat is grown in a typical temporary crop environment, over a period of six to seven months per year. Resource limited farmers who produce wheat under such circumstances in the Free State Province are mainly situated in the Qwa-Qwa and Thaba N'chu regions. They could be sincerely affected by such losses which could occur during years of serious Russian wheat aphid infestations (Marasas, Anandajayasekeram, Tolmay, Martella, Purchase & Prinsloo, 1997).

Since wheat is produced in such a temporary crop environment, insect pests, including wheat aphids, 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 individuals before they arrive in the crop habitat (Wiedenmann & Smith, 1997). With fewer immigrants the pest population remains longer in the latent growth phase and reduce the possibility of the pest population reaching damaging levels 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, Ozawa, Shioda, Nishioka, Boland & Takabayashi, 2000; Walters, Cowley & Mitchell, 2002) and modification of volatile profiles (Dicke, Gols, Ludeking & Posthumus, 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.

Plant tissue or crude products of plants, such as aqueous or organic extracts, are used directly by farmers in China as botanical insecticides when pest are already present in their crops. These practices are labour intensive, but are often economically and ecologically sound and do not require sophisticated technology (Yang & Tang, 1988). The real benefit of botanical insecticides can best be realized in developing countries where farmers may not be able to afford synthetic insecticides (Isman, 2006). In Nigeria water and oil extracts of seeds, leaves, barks and roots of various plant species have been used amongst resource-limited farmers in traditional agriculture to protect crops from insect pest infestation (Oparaeke, 2006).

Several examples, however, also exist where behaviour-modification through plant derived semiochemicals are being used in insect pest control (Pickett, Wadhams & Woodcock, 1997). One such chemical is being used with success in Sweden to repel the oat aphid *Rhopalosiphum padi* (McIntosh) from barley fields (Glinwood & Pettersson, 2000). Although this method is based on the basic principles of plant defence mechanisms, sophisticated technology is involved which would not be easy to apply in a small scale farmer system. However, if such semiochemicals could be extracted from plants through a basic extraction method, farmers could use this to their benefit. The focus is therefore not to use the extracts as insecticides, but to keep aphids out of the fields by early application of these extracts.

Two types of basic plant extracts, namely an aqueous and light mineral oil, were tested for their aphid repellent and natural enemy attractant properties in the present study. Four plant species were extracted, namely Wild wormwood, *Artemisia afra* (Jacq. ex Willd.), Big thorn apple, *Datura stramonium* (Linnaeus), Khaki bush, *Tagetes minuta* (Linnaeus) and Wild garlic *Tulbaghia violacea* (Harv.). They were chosen due to their availability in these wheat production regions, their medicinal properties (Van Wyk, Van Outshoorn & Gericke, 2000) and their possible insect repelling properties known from other species in the same genus (Bruce, Cork, Hall & Dunkelblum, 2002; Isman, 2006; Zehnder, Gurr, Kühne, Wade, Wratten & Wyss, 2007; Halbert, Corsisni, Wiebe & Vaughn, 2008; Işik, & Görűr, 2009).

In the present studies *Diuraphis noxia* (Kurdjumov) was highly repelled by the aqueous extract of *A. afra* and the oil extract of *T. violacea*. Oil extract of *A. afra*, both extracts of *T. minuta* and aqueous extract of *T. violacea* also showed good repellent properties to *D. noxia* and could be used. The aphid *R. padi* was also strongly repelled by the aqueous extract of *A. afra* and the oil extract of *D. stramonium*. The oil extract of *A. afra* and both extracts of *T. minuta* could also be used, as they showed good repellent properties to *R. padi*.

Both aphid species will fly into wheat fields during July and August when wheat are still small, which is the time when plant extracts should be applied. Both *A. afra* and *T. violacea* are perennials and available throughout the year as green material and could therefore be used. *Datura stramonium*, on the other hand, is an annual that is frost sensitive and no green material is available during winter when *R. padi* can infest wheat fields, and would therefore not be used. An aqueous extract of *A. afra* could be easy to prepare and used for both aphid species. *Artemisia* species are known for their insect repellent properties and distilled oil from several species showed promising results as aphid repellents (Halbert *et al*., 2008).

Herbivore induced plant odours play a major role in the foraging behaviour of predatory and parasitic arthropods (Turlings, Bernasconi, Bertossa, Bigler, Carloz & Dorn, 1998; Arimura *et al.*, 2000; Fatouros, Van Loon, Hordijk, Smid & Dicke, 2005). The control of pests by their natural enemies is important in regulating stability of crop ecosystems (Zhang & Swinton, 2009). The presence of aphid natural enemies will reduce aphid populations and damage to wheat crop. If natural enemies are not attracted to the wheat crop, the aphids feeding on them are free from natural enemy attack and increase chances for a resistance breaking biotype to develop (Prinsloo, 2006). It should, however, be taken into account that early in the wheat growing season, when aphids are invading the wheat crop, natural enemies are not present. During these stages the effect of plant extracts on the natural enemies is not important, but as the wheat is growing and aphids may appear on the crop, extracts eliciting attraction in parasitoids and predators would be beneficial.

The parasitoid *A*. *hordei* was strongly attracted to the aqueous extracts of *A*. *afra* and *T*. *minuta*. Both the extracts of *D. stramonium* and *T. violacea* also revealed good attractant properties to *A. hordei* and could be used. *Diaeretiella rapae*, on the other hand, was highly attracted by the aqueous extract of *T*. *minuta*, and the oil extract of *T*. *violacea*. The aqueous and oil extract of *T. minuta* could also be used when available, as they showed good attractant properties to *D. rapae*. The plants, *A*. *afra* and *T. violacea,* are perennials and available during the wheat growing season as green material. That is not the case of *D. stramonium* and *T. minuta* that die in winter and is then only available as dry material. The aqueous extract of *T*. *minuta* attracted both parasitoids, but is not available during winter time. The parasitoid *A*. *hordei* will then only be attracted by *A. afra* aqueous extract and parasitoid *D*. *rapae* by *T*. *violacea* oil extract.

The parasitoids differed clearly in their response to the same group of plant extracts which could be expected, because of various host ranges feeding on different plants. This was substantiate by *A. afra* aqueous extract that repelled *D. rapae*, but showed strong attraction for *A. hordei*, but both parasitoids were repelled by *A. afra* oil extract. Extraction of volatiles using boiling water would extract volatiles different from those extracted through cold mineral oil, which was also responsible for the varying responses observed. This variation complicates the decision to which extract should be used and when. However, the results clearly indicated some good attractants that should be investigated further.

Based on these laboratory results recommendations could be made on which plant extracts to be used against the different aphids, and which would effectively attract natural enemies (Table 6.1).

Table 6.1 Corresponding plant extracts to be used in a wheat crop situation in the presence of aphids and parasitoids.

| **Plant extract** | ***Diuraphis noxia*** | ***Ropalosiphum padi*** | ***Aphelinus hordei*** | ***Diaeretiella rapae*** |
|---|---|---|---|---|
| *Artemesia afra* (Aqueous) | √√ | √√ | √√ | X |
| *Artemesia afra* (Oil) | √ | √ | X | X |
| *Datura stramonium* (Aqueous) | X | X | √ | X |
| *Datura stramonium* (Oil) | X | √√ | √ | X |
| *Tagetes minuta* (Aqueous) | √ | √ | √√ | √ |
| *Tagetes minuta* (Oil) | √ | √ | X | √ |
| *Tulbachia violacea* (Aqueous) | √ | X | √ | X |
| *Tulbachia violacea* (Oil) | √√ | X | √ | √√ |

**√√:** Extracts with high potential
**√:** Extracts with lower potential
**X:** Extracts unsuitable

The table is compiled in such a way that every farmer should be able to know what extract to use to produce a desired response to specific aphid or parasitoid specie:

- Aqueous extract of *A. afra* should be sprayed on a regular basis when wheat is at the tillering stage. This could help to reduce early infestations of both *D. noxia* and *R. padi*
- In the presence of *D. noxia*, spray *A. afra* aqueous extract (it will also attract *A. hordei*) or *T. violacea* oil extract (that will attract *D. rapae*) for repellency.
- Spray *A. afra* aqueous extract in the presence of *R. padi* to repel them. Mixture with aqueous T. violacea could help to lure *D. rapae.*
- An extract with high potential for parasitoids *R. padi* and *D. rapae* in winter is not available.

Potential for the use of plant extracts was determined. These results should be confirmed under greenhouse conditions on plants and then be tested under field conditions. Application methodology, including phytotoxicity tests and application rate, should be sorted out before any recommendations could be made to farmers.

Short comings of this study can be summarised in the following questions which should be addressed to complete a holistic view of this study:

- When spraying and aphids are already on wheat plants, will their responses be the same to the extracts as the aphids in the olfactometer?
- What will the effect be of the extracts on other insects, like parasitoids, from the vicinity?
- What will the behaviour be of these insects towards the extracts?
- How will the wheat plants react to these extracts?
- Will the farmers continue using it on the long term?
- What constraints do farmers have that could possibly affect the adoption of this technology?
- How long will the repellent/attractant effect last in field after a spray?

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# CHAPTER 7

# APPENDICES

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## 7.1 Seminar and Conferences contributions

Parts of this study were presented at the following seminar and congresses:

i) Poster at XXIII International Congress Entomology (Durban) 2008:

Title: The potential use of plant extracts as control option for Russian wheat aphid.

The author also acted as session moderator (Behaviour and Neurobiology) at this congress.

ii) Oral presentation at the post graduate seminar of the University of the Free State (Bloemfontein) 2009:

Title: Plant extracts: alternate control measures for the Russsian wheat aphid, *Diuraphis noxia* (Kurdjumov).

iii) Oral presentation at the 16th Entomology Congress of Southern Africa (Stellenbosch) 2009:

Title: Plant extracts: Potential use as a control strategy against Russian Wheat Aphid.