Host plant preference of *Rhopalosiphum padi* (Hemiptera: Aphididae) and its role in selecting crop border plants to reduce *Potato virus Y* (PVY) in seed potatoes

by

Michelle Louise Schröder

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Declaration

I, Michelle Louise Schröder, hereby declare that the thesis titled,

Host plant preference of *Rhopalosiphum padi* (Hemiptera: Aphididae) and its role in selecting crop border plants to reduce *Potato virus Y* (PVY) in seed potatoes,

Which I hereby submit for the degree *Philosophiae Doctor* (Entomology) to the University of Pretoria contains my own independent work and has not been submitted for any other degree at any another university.

Signature

Date

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Name of Student: Michelle Louise Schröder

Supervisor: Prof. Kerstin Krüger

Co-supervisor: Dr Robert Glinwood (Swedish University of Agricultural Sciences)
Prof. Rickard Ignell (Swedish University of Agricultural Sciences)

Department: Zoology and Entomology, University of Pretoria

Degree: *Philosophiae Doctor* (Entomology)

Summary

Winged *Rhopalosiphum padi* in search of good quality host plants transmit the non-persistent *Potato virus Y* (PVY) to seed potatoes. Planting a non-virus host plant as a border around the main crop can reduce PVY incidence, because aphids tend to land in high numbers at the edge of a field and the crop border acts as a virus sink. Using a trap crop could increase the number of aphids landing in the border. During host-plant searching behaviour, alate (winged) aphids respond to short (UV) and long (green - yellow) wavelength stimuli and plant volatiles. The present study evaluated *R. padi* preference for maize and wheat cultivars in comparison to potato cultivars to identify a potential crop border plant that is attractive to aphids for landing but does not support a high aphid population density. The study further served to develop selection criteria for potential crop border plants. To this end the landing and settling preference and reproductive rate of *R. padi* on three cultivars each of maize, potato and wheat were compared in choice and no-choice laboratory trials. The attractiveness of different shades of green, relative to differences in spectral reflectance of the crop cultivars, to *R. padi* was determined as well as olfactory responses of this species, to the plant

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cultivars tested. Landing and reproduction of *R. padi* suggested maize cultivars '6Q-121' and '78-15B' are potential crop border plants. In choice and no-choice experiments, *R. padi* produced the highest number of offspring on wheat than on maize. In contrast, wheat cultivars had the greatest potential to be used as crop border plants based on percentage wavelength reflection in the green-yellow wavelength region. *Rhopalosiphum padi* preferred to land on yellow and lime colour targets with a maximum wavelength reflectance of 46% and 26%, respectively. The peak light reflectance of the crop plants ranged between 12% and 20%, with wheat reflecting the highest percentage of light. However, olfactory responses of *R. padi* indicated that maize '6Q-121' may prove to be a suitable crop border plant. The volatiles emitted by maize '6Q-121' did not contain compounds such as α-farnasene, (*E*)-2-hexenal, indole and TMTT which are known to repel *R. padi*. On the other hand, *R. padi* did not distinguish between maize and wheat cultivars prior to landing, which indicates that both visual and olfactory cues are important in host plant selection behaviour and thus for selecting crop border plants. The results of the present study suggest that maize '6Q-121' could be used as a crop border plant in seed potato production regions where *R. padi* is abundant, due to high aphid landing and low reproduction rates. In conclusion, a suitable crop border plant should be a preferred host plant and cultivar of the dominant aphid vector species in a seed potato production region. Potential plant cultivars should reflect a higher percentage of light in the green-yellow wavelength region than the main crop and the volatile profiles should preferably not contain compounds that are known to repel the aphid vector.

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Chapter 1

General introduction

In the potato industry, aphids (Hemiptera: Aphididae) are considered as one of the most serious insect pests because of their ability to transmit more than 25 viruses to potato (*Solanum tuberosum* L., Solanaceae) plants (Swenson 1968; Salazar 1996; Ng & Perry 2004). Of these, *Potato leaf roll virus* (PLRV; Luteoviridae) and *Potato virus Y* (PVY; Potiviridae) are the two economically most important species (Ragsdale *et al.* 2001). *Potato leaf roll virus* is transmitted in a persistent manner, where the insect vector acquires the virus when feeding on the phloem sap for extended periods (Nault 1997). The virus has to pass through the gustatory system of the aphids and has a latent period before it can be transmitted (Nault 1997). *Potato virus Y*, on the other hand, can be acquired and transmitted in less than a minute during brief stylet probes to the epidermis (Bradley 1954). PLRV can be controlled effectively by foliar applied and systemic insecticides due to the post-acquisition latent period and longer feeding probes needed for transmission (Leonard & Holbrook 1978; Hanafi *et al.* 1989; Boiteau & Singh 1999). However, the same cannot be said for PVY. The fast mode of transmission allows the virus to be transmitted before insecticides take effect and therefore alternate control methods are aimed at reducing virus inoculum and reducing the number of potential vectors entering potato fields (Boiteau *et al.* 1985; Lowery & Boiteau 1988; Fereres 2000; Radcliffe & Ragsdale 2002). Therefore, the interaction between the aphid vector and its host plant is central to understanding the spread of non-persistent viruses as well as developing improved management strategies to reduce their incidence.

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1.1 The bird cherry - oat aphid *Rhopalosiphum padi*

The bird cherry - oat aphid, *Rhopalosiphum padi* (L.), is not only an agriculturally important pest of cereal crops, but also a vector of non-persistent plant viruses (Sigvald 1987; Finlay & Luck 2011). *Rhopalosiphum padi* is oval-shaped with a green mottled yellowish green, olive green, dark olive or greenish black appearance (Fig. 1.1) (Blackman & Eastop 2000). This species is heteroecious and able to over-winter holocyclic as well as anholocyclic depending on climatic conditions, genotype and geographical location of the primary host plant, *Prunus padus* L. (Rosaceae), the bird-cherry tree (Leather 1993a). The ability of aphids to alternate between a sexual and parthenogenetic phase of reproduction during their life cycle is considered a primitive feature of Aphididae (Blackman & Eastop 2000).

Figure 1.1: *Rhopalosiphum padi* colony on wheat (**a**) and nymph feeding on a wheat leaf (**b**).

The secondary loss of the sexual phase in certain populations and genotypes within a species is a widespread trait within Aphididae (Blackman & Eastop 2000). Holocyclic populations produce winged migrants, which migrate to the primary host plant the bird cherry tree, *P. padus*, where they produce gynoparae (autumn migrants) and oviposit. In spring the

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eggs hatch, and fundatrices (1st generation, wingless) produce large numbers of parthenogenetic winged (alate) migrants (virginoparae). These migrate to the secondary host plant (Poaceae), which, apart from grasses, includes crops such as barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.). Anholocyclic populations overwinter on the secondary host plant and do not produce sexual morphs (Dixon 1971; Dixon & Glen 1971; Leather 1993b).

1.2 Aphid polymorphism

Each morph has a different reproductive strategy associated with its functional role in the life cycle (Dixon & Wellings 1982; Dixon 1985). Alate aphids invest the majority of their resources in dispersal (Dixon 1985). The spring migrants have well-developed flight muscles (Dixon 1985). They can spread over distances of up to 100 km in search of a host plant (Irwin *et al.* 1988). However, their reproductive fitness is reduced in comparison to the apterous (wingless) morphs (Wratten 1977).

Development to maturity takes longer in alate aphids, which have a lower overall reproductive rate than apterae (Wratten 1977). Spring alate migrants tend to have a higher fecundity but do not reproduce for as long or live as long as alate viviparae from the secondary host plant (Dixon 1976). Gynoparae have the highest initial reproductive rate followed by spring emigrants and alate viviparae (Dixon 1976). In addition, alatae produce smaller offspring than apterae, indicating there may be a trade-off between initial reproductive rate and dispersal (Dixon 1971). The apterae have low dispersal capabilities and movement is restricted to walking within plants and from plant to plant. A large abdomen enables them to invest their resources in reproduction (Dixon 1985). Apterae contain fully developed embryos as soon as they become adults, making it possible to start reproducing

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shortly after the adult moult (Elliott & McDonald 1976). In addition, apterae contain a greater number of oocytes and embryos than alatae (Elliott & McDonald 1976). The development and maintenance of flight muscles is costly, and by not developing them aphids free resources for growth and reproduction (Dixon 1985).

1.3 Transmission of *Potato virus Y* (PVY)

Aphid polymorphism plays an important role in the spread of plant viruses (Swenson 1968). Although *R. padi* is a grass-feeding aphid, it can be a major vector of PVY in seed potatoes (Sigvald 1987; Radcliffe & Ragsdale 2002). In France, PVY epidemics were attributed to the presence of large numbers of cereal aphid migrants such as *R. padi* (Robert *et al.* 2000). In addition, high numbers of *R. padi* as well as other transient aphid species (species that do not colonize potatoes) have been linked to early season PVY incidence in potato in Sweden and the Netherlands (Van Hoof 1977; Sigvald 1987). PVY is mainly transmitted by migrating aphids in search of good quality host plants; it can also be transmitted mechanically and through grafting (Boiteau 1997; Ragsdale *et al.* 2001). The non-persistent nature of PVY enables aphids to obtain and transmit the virus during feeding probes of less than one minute (Bradley 1954). PVY is a *Potyvirus* and belongs to the family Potyviridae (Gray *et al.* 2010). Up to 14 different plant families have been identified as PVY hosts, including a range of solanaceous crops such as tomato (*Lycopersicon esculentum* Miller), potato and wild plants (Robert *et al.* 2000; Kaliciak & Syller 2009; Gray *et al.* 2010).

Globally, PVY is of great concern to seed potato growers, causing yield losses ranging between 10 and 100% due to degeneration of the seed stock (Radcliffe 1982). Infection rates between 0.1 and 1% can reduce the quality of seed and infection rates of 1 to 3% can lead to the rejection of potato seed lots, significantly reducing the value of the crop

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(Ragsdale *et al.* 2001; Gray *et al.* 2010). *Potato virus Y* symptoms include, severe mosaic, leaf drop, stem necrosis, vein necrosis and ring spot necrosis. The different strains of PVY (e.g. PVY^{O}, PVY^{N}, PVY^{NTN} and PVY$^{N\text{-}Wilga}$) differ in the severity of the symptoms they cause in potato, with PVY^{NTN} causing the most severe symptoms that could result in early death of the plant (Blanco-Urgoiti *et al.* 1998; Singh *et al.* 1998).

1.4 Current control of *Potato virus Y* (PVY)

Seed potato growers rely mainly on the application of insecticides to reduce the transmission of PVY (Robert *et al.* 2000). However, due to the non-persistent manner in which aphids transmit PVY, insecticides are not always effective in reducing transmission (Boiteau *et al.* 1985; Lowery & Boiteau 1988). Growers are also advised to make use of cultural control methods that are aimed at reducing virus inoculum in the growing region (Ragsdale *et al.* 2001). Commercial ware potatoes should not be planted in the same region as seed potatoes because cultivation of both may increase the virus inoculum dramatically due to disease tolerances for ware potatoes being much lower than for seed potatoes (Radcliffe & Ragsdale 2002). In addition, only certified virus-free seed potatoes should be used for planting seed potatoes. Furthermore, weed control around fields should be practiced and virus infected potato plants should be removed (roguing) (Radcliffe & Ragsdale 2002). Crop borders (planting a non-virus host plant around the potato crop) may further reduce the incidence of PVY in seed potatoes due to aphids landing in high numbers at the edge of the field and the crop border acting as a virus sink. Aphids probing the crop border lose their ability to further transmit the virus before entering the main crop (Hooks & Fereres 2006).

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1.5 Research objectives and thesis outline

The aim of this study was to evaluate aphid host plant selection behaviour of *R. padi* for maize, wheat and potato with a view to improving the plant selection process for crop borders. To this end, landing preference, settling behaviour as well as reproduction rate was determined for three maize, potato and wheat cultivars each. In addition, behavioural responses of *R. padi* to wavelength reflectance and olfactory cues emitted by these cultivars were determined. The results were used to identify potential maize and wheat cultivars as prospective crop border plants based on aphid behaviour towards visual and olfactory plant cues. Crop border plants can be used to reduce the incidence of non-persistently transmitted viruses such as PVY (Hooks & Fereres 2006). Using a trap crop as a crop border plant may strengthen the edge effect and improve integrated pest management strategies to reduce PVY incidence in South African seed potato production.

The thesis chapters are written in the form of research papers. Therefore, there is some overlap between chapters with regard to parts of the text. Chapter 2 provides a literature review on the role of visual and olfactory plant cues in aphid host plant selection behaviour and how these interactions can be used in developing improved crop protection strategies to reduce the incidence of non-persistent viruses. Chapter 3 examines landing and settling preferences as well as reproduction of *R. padi* on the cultivars studied. Chapter 4 compares the spectral reflectance of the nine crop cultivars as well as landing rates of *R. padi* on colour targets. Chapter 5 identifies the chemical profiles of the cultivars and determines the olfactory preference of *R. padi* for the odour profiles of the cultivars, as well as the individual compounds identified from the crop plants at different concentrations. Chapter 6 provides a general discussion and conclusion of the results and discusses future research possibilities.

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Chapter 2

Literature review: the role of visual and olfactory plant cues in aphid behaviour and the development of non-persistent virus management strategies

2.1 Introduction

Aphids (Hemiptera: Aphididae) are major pests in agro-ecosystems due to their feeding behaviour causing direct damage to plants (Rabbinge *et al.* 1981; Dedryver *et al.* 2010). In addition, they cause indirect damage by transmitting plant viruses (Radcliffe 1982; Dedryver *et al.* 2010), through their piercing-sucking mouthparts, directly to the phloem without causing major damage to the plant (Nault 1997). Aphids account for 50% of all insect-transmitted viruses to plants (Nault 1997) and Ng & Perry (2004) provide an extensive review of the most important aphid-transmitted plant viruses. The family Potyviridae with its large number of species is the most important of these and contains the highest number of aphid-transmitted viruses (Ng & Perry 2004). One of the most economically important viruses of potato (*Solanum tuberosum* L., Solanaceae) is *Potato virus Y* (PVY) in the genus *Potyvirus* (Potyviridae). The virus causes major economic damage to the seed potato industry due to downgrading of seed lots (Radcliffe & Ragsdale 2002).

Potyviruses are transmitted in a non-persistent or non-circulative manner (Nault 1997; Ng & Perry 2004). *Potato virus Y* can be acquired (uptake of virus from infected source) and inoculated (delivery of virions to site of infection) within short feeding times of minutes to seconds, and the aphid is able to transmit the virus for a relatively short time (Bradley 1954; Katis & Gibson 1985; Boquel *et al.* 2011). For example, *Rhopalosiphum padi* (L.), retained PVY^{O} and PVY^{N} (two strains of PVY), for up to six hours, and *Myzus persicae* (Sulzer) for

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up to four hours (Katis & Gibson 1985). Transient aphid species may be more important in transmitting non-persistent viruses than colonizing species (Halbert *et al.* 1981; Tomlinson 1987). Katis *et al.* (2006) found that the majority of aphid vector species able to transmit *Zucchini yellow mosaic virus* (ZYMV) were species that do not colonize zucchini (*Cucurbita pepo* L., Cucurbitaceae). Many of these transient species did not transmit ZYMV efficiently (Katis *et al.* 2006). However, in high numbers, non-colonizing species can play an important role in the epidemiology of non-persistent viruses (Katis & Gibson 1985; Katis *et al.* 2006). For example, *R. padi*, a grass-feeding species, is one of the most important vectors of PVY (Sigvald 1987).

2.2 Current control strategies of non-persistent viruses and their limitations

2.2.1 Insecticides

The main control method of PVY is aimed at reducing the aphid population by means of insecticides and thereby reducing the virus incidence (Robert *et al.* 2000). However, insecticides do not take immediate effect. Due to the fast transmission rate of non-persistent viruses, aphids are able to transmit virus before being killed by the insecticide (Radcliffe & Ragsdale 2002). Systemic organophosphates, such as demeton-S-methyl, did not hinder the transmission of PVY to Swiss chard (*Beta vulgaris* L., Chenopodiaceae) and tobacco (*Nicotiana tabacum* L., Solanaceae) by *M. persicae* (Gibson *et al.* 1982). In addition, imidacloprid (a systemic neonicotinoid) was ineffective in reducing the spread of PVY in the field during a three year study, although it was effective in controlling aphid populations (Boiteau & Singh 1999).

Pyrethroids such as deltamethrin, on the other hand, can assist in the reduction of non-persistent virus spread (Gibson *et al.* 1982). This may be due to toxic effects occurring

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rapidly, paralysing the aphids before transmission or acquisition of the virus can take place (Gibson *et al.* 1982). However, at sub-lethal doses these compounds may enhance virus spread due to a repellent effect, causing an increase in aphid activity (Gibson *et al.* 1982; Robert *et al.* 2000). In contrast, Lowery & Boiteau (1988) did not find a repellent effect caused by pyrethroids but rather a reduced probing behaviour. However, the efficiency of pyrethroids has been found to vary between aphid species and clones (Gibson *et al.* 1982; Lowery & Boiteau 1988). Furthermore, it has been demonstrated that aphids almost always probe pyrethroid-treated leaves once before death occurs. These short probes are sufficient for the acquisition or inoculation of non-persistent viruses such as PVY (Lowery & Boiteau 1988). Despite the varying efficiency of insecticides in controlling non-persistent viruses and the growing concern of aphid resistance to insecticides, they still remain the main management tactic for the control of PVY (Robert *et al.* 2000; Radcliffe & Ragsdale 2002).

2.2.2 Mineral oils

Mineral oils can reduce the incidence of non-persistent viruses such as PVY, and are used commercially in Europe and Israel (Simons & Zitter 1980). They have been shown to delay the onset of virus infestation in pepper (*Capsicum annuum* L., Solanaceae), and once plants are infected can reduce further spread within fields (Simons & Zitter 1980).

In laboratory experiments acquisition and inoculation of PVY between *Nicotiana* plants by *M. persicae* was reduced by mineral oil (Powell 1992). Simons *et al.* (1977) found that contact between the aphid labium and the oil was sufficient to reduce virus transmission by causing an increase in the pre-probing time. However, Powell *et al.* (1992) did not find any change in stylet plant penetration activity and PVY transmission. It has been suggested

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that the oil may have disrupted the interaction between the virus, plant cell and aphid fore-gut (Bradley 1963; Powell 1992).

The ability of mineral oils to reduce PVY incidence varies with inoculation potential (the percentage of infected plants in a field), crop density and application method used (Simons *et al.* 1977; Simons & Zitter 1980). However, the effectiveness of oils in reducing virus incidence diminishes with an increase in inoculation potential (Simons *et al.* 1977). Therefore, oils are considered to be most effective in seed potato production regions at high altitude where aphid activity and abundance is low (Simons & Zitter 1980). In addition, mineral oils are less effective in crops planted at low densities such as watermelon (*Citrillus lanatus* (Thinb.), Cucurbitaceae) compared with crops planted at high densities such as pepper (Simons & Zitter 1980). An even coverage of the oil on the leaves is important to obtain maximum results (Simons & Zitter 1980).

The use of mineral oils in reducing the spread of PVY is further limited due to potential adverse effects on the crop (Simons *et al.* 1977). Mineral oils can be phytotoxic. For example, Simons & Zitter (1980) reported a 20% yield reduction in seed potatoes. On the other hand Boiteau & Singh (1982) did not find phytotoxic effects when spraying potato plots with mineral oils. Phytotoxic effects of mineral oils have been reported when used in conjunction with other chemicals in normal production practices (Simons & Zitter 1980). Although phytotoxic effects can arise when mineral oils are used with insecticides, the majority of chemicals that are incompatible with mineral oils are fungicides (Simons & Zitter 1980; Boiteau & Singh 1982).

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2.2.3 Cultural control

Potato seed certification schemes responsible for grading of seed lots based on the percentage of virus-infected tubers rely mainly on preventative cultural control methods (Radcliffe & Ragsdale 2002). These include practices such as using certified seed, rouging and removing weeds to reduce source inoculum, as well as planting in geographically isolated areas (Weidemann 1988; Robert *et al.* 2000; Radcliffe & Ragsdale 2002). Although these are suitable preventative methods, they need to be used in combination. However, they do not always achieve effective control of non-persistent viruses.

Crop borders, an additional cultural control tactic that relies on the manipulation of the biology of vectors, have been proposed for the control of non-persistent viruses (Hooks & Fereres 2006). Aphids tend to land in high numbers at the edge of a field due to their sensitivity to long wavelength light and the contrast in light reflected from the soil and the plants (Broadbent *et al.* 1951; Minks & Harrewijn 1988). Planting a crop around the main crop displaces the edge of the main field, thereby reducing the numbers of aphids landing in the main crop (Hooks & Fereres 2006). After landing on a plant, aphids evaluate the leaf surface characteristics and briefly probe the epidermis layer of the plant, ingesting small amounts of leaf sap that is evaluated by a small gustatory organ in the gut (Powell *et al.* 2006). During this initial probing behaviour, aphids are able to transmit PVY due to the non-persistent nature of the virus (Broadbent *et al.* 1951; Bradley 1954). Therefore, planting a non-virus host plant (a plant not affected by a virus) as a crop border causes aphid vectors to lose their ability to transmit the virus before entering the field proper (Broadbent *et al.* 1951; Hooks & Fereres 2006).

Several studies have evaluated the efficacy of crop borders with varying results e.g. (DiFonzo *et al.* 1996; Hooks & Fereres 2006). A reduction in PVY incidence has been found

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in seed potatoes planted with crop borders of soybean (*Glycine max* (L.), Fabaceae), sorghum (*Sorghum bicolor* L., Poaceae), wheat (*Triticum aestivum* L., Poaceae) and potato in the Red River Valley of Minnesota and North Dakota (DiFonzo *et al.* 1996). A survey on the use of crop borders in these states in 2005 revealed that on average less than 0.1 out of 152 seed lots entered in seed certification programmes in 2004 were rejected due to PVY infection in summer. In winter, 97% of the seed lots produced with crop borders compared to 54% of seed lost grown without crop border passed the seed certification test (Olson *et al.* 2005). However, factors such as the type of virus spread pattern, height of the barrier crop when maximum risk of infection occurs and the extent of competition between the barrier and the protected crop may affect the outcome of crop borders as a control strategy against PVY (Fereres 2000).

Various plant species have been identified as crop border plants. The effect in reducing non-persistent virus incidence varied between studies. In potatoes planted with wheat, sorghum, rye (*Lolium perenne* L., Poaceae), soybean and potato, the reduction in PVY incidence in potato fields was similar for all crop border plant species used (DiFonzo *et al.* 1996; Olson *et al.* 2005). However, Damicone *et al.* (2007) observed some variation in the reduction of virus incidence between crop border plant species. Crop borders of sorghum were effective in reducing *Watermelon mosaic virus* (WMV) and *Papaya ringspot virus* - W (PRSV-W) in pumpkin (*Cucurbita pepo* L., Cucurbitaceae). However, peanut (*Arachis hypogaea* L., Leguminosae), soybean and maize (*Zea mays* L., Poaceae) did not affect WMV and PRSV-W transmission (Damicone *et al.* 2007). Wheat was effective in reducing WMV-1 and WMV-2 in muskmelon crops (*Cucumis melo* L., Cucurbitaceae) when planted as a crop border (Toba *et al.* 1977). In some cases crop borders had an adverse effect on the main crop. Although Swiss chard reduced WMV-1 and WMV-2 incidence, it caused major yield loss by stunting muskmelon growth and was a host of potato leafhopper, *Empoasca fabae* (Harris),

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(Hemiptera: Cicadellidae), that is a pest on muskmelon (Toba *et al.* 1977). Therefore, the effect of plant species on insect vector behaviour and thus disease incidence, as well as on the crop to be protected should be considered when selecting a crop border plant. In addition, a crop border plant should not be a virus host as it may become a source of inoculum. Furthermore, crop borders should be compatible with current production practices to avoid additional production costs (DiFonzo *et al.* 1996; Radcliffe & Ragsdale 2002).

Crop borders may act as a virus sink, barrier crop or trap crop (Hooks & Fereres 2006). According to the virus sink hypothesis (Hooks & Fereres 2006), aphids land on the border crop first (a non-virus host), probe, and when re-alighting, lose their potential to transmit the virus before landing in the main crop (Toba *et al.* 1977; DiFonzo *et al.* 1996). The non-persistent manner of transmission of PVY causes vectors to lose the ability to transmit the virus when feeding on a non-virus host (Broadbent *et al.* 1951; Katis & Gibson 1985). Tall barrier crops are thought to reduce the number of aphids landing in the main crop by obstructing or closing off a passage to the main crop. However, Boiteau *et al.* (2009) found that the height of the crop border did not make a difference in the number of aphids landing on the edge or border crop and the centre of the potato plot. Trap crops have been defined as crops that are attractive to the pest insect, and therefore actively draw it away from the main crop, where it can be controlled by other means such as pesticides (Hokkanen 1991). The use of trap crops has been suggested to improve the effect of the border by increasing the number of alighting aphids (Nault *et al.* 2004; Hooks & Fereres 2006).

2.3 Aphid flight/migration behaviour

The ability of aphids to disperse over long distances plays an important role in the spread of non-persistent viruses. Aphids that have acquired non-persistent viruses are able to

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disperse into regions where virus inoculum is low, causing unexpected virus outbreaks (Irwin *et al.* 1988). In the past, several virus epidemics have been attributed to long-range dispersal of aphids (Irwin *et al.* 1988). For example, in Eastern Canada *Barley Yellow Dwarf Virus* (BYDV) incidence is generally low. However, in the autumn of 1982 BYDV incidence in winter wheat and barley increased dramatically, reaching levels of up to 100% (Paliwal & Comeau 1984). The dramatic increase was associated with a sudden influx of two cereal aphids, *R. padi* and *Rhopalosiphum maidis* (Fitch), with steady south-westerly winds in Ontario and Quebec (Paliwal & Comeau 1984). However, Loxdale *et al.* (1993) argue that it is possible that many of the virus outbreaks attributed to long distance flight in North America could be due to aphids overwintering anholocyclic. In southern England cereal aphids were able to survive temperatures as low as -7 °C (Williams & Wratten 1987). In sheltered environments, such as grass tussocks and hedgerows, aphids are protected from the wind, rain and snow in winter and they may survive and colonize cereal crops much sooner in spring than migrants from the primary host (Dean 1974; Williams & Wratten 1987). Therefore, anholocyclic populations may be more important in virus spread than holocyclic populations overwintering as eggs, especially early in the season before spring migrations from holocyclic populations take place.

Migratory aphids undergo successive behaviours of pre-flight, flight and alighting to find and settle on a new host plant (Kring 1972). The pre-flight phase involves the teneral or resting period and the take-off response. The teneral period refers to the stage between the final moult of the winged morph and initial flight where the aphid takes off from the plant (Kring 1972). During this phase the wings and the cuticle harden (Kring 1972). Aphids can be flight ready between 8 and 48 hours after the final moult, depending on the species and environmental conditions such as temperature and wind speed (Boiteau 1986). *Myzus* *persicae* and *Aphis nasturtii* Kaltenbach became flight ready within 24 hours after moulting but *Macrosiphum euphorbiae* (Thomas) took up to 36 hours (Boiteau 1986).

In aphids, two types of behaviours in which alate take-off from a plant has been described, taking off from the top or the bottom of the leaf. Alates either move to the highest point of the plant and take-off from the top of the leaf, or drop or kick themselves into flight, usually from the bottom surface of the leaf (Kring 1972). Take-off mainly occurs during day-light hours under favourable environmental conditions. The temperature threshold for take-off is between 14 and 42 °C and may vary slightly between species (Berry 1969; Walters & Dixon 1984; Boiteau 1986). More individuals of *R. maidis* took off at temperatures between 14 and 17 °C and above 31 °C than *Schizaphus graminum* (Rondani) (Berry 1969). *Myzus persicae* has a minimum take-off threshold temperature of 16-17 °C (Boiteau 1986). Aphids are more likely to take off when wind speeds are low (< 1 m s^{-1}). However, when exposed to high wind speeds (up to 4 m s^{-1}) for more than a day, cereal aphids such as *R. padi* become less sensitive to wind speed, indicating that poor environmental conditions may delay but do not inhibit aphid migration (Walters & Dixon 1984).

After take-off, aphids become positive phototactic, orienting themselves upwards into the sky to initiate their dispersal flight (Kennedy & Booth 1963). Aphids are not very strong flyers and are not able to control their flight speed and direction in high wind speeds. In wind speeds above 0.5 m.s^{-1} they are able to drift with the wind currents to disperse over large distances (Johnson 1954; Irwin *et al.* 1988). Similar to the take-off response, flight is affected by temperature and daylight conditions. At temperatures above the minimum threshold required for flight, aphids are able to remain flying at night time; however, when night time temperatures fall below the threshold aphids close their wings and drop from the sky (Berry & Taylor 1968; Halgren 1970).

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Flight duration may vary between species and is dependent on finding a suitable host (Kring 1972). Flight duration in *Aphis fabae* Scopoli varied between 7 and 234 min before settling on a plant (Kennedy & Booth 1963). However, aphids were able to take off and resume a second flight at the same height and time than the first flight, but after the third take-off from a non-host leaf, flight time decreased to 1 min (Kennedy & Booth 1963). After several bouts of short flights the aphids became reluctant to fly and dropped off the non-host leaf to the floor (Kennedy & Booth 1963). In experiments where aphids were tethered, flight duration was much longer, resulting in flight exhaustion. Therefore the flight duration required for an aphid to become responsive to a target and land on it is shorter than the total flight duration till exhaustion occurs. This gives the aphid a chance of still locating a plant if it landed on a poor or unsuitable host plant.

2.4 Aphid host plant selection behaviour

Locating a new host plant is not an easy task for aphids, due to the difficulty in finding a host plant amongst a myriad of other plants. Aphids vary in host plant specificity, only feeding on one or a number of plant families. Many genera of Aphidinae are host alternating and associated with Rosaceae during the sexual phase of the life cycle (Blackman & Eastop 2000). In summer, they may feed on one or numerous plant families, with the pest species usually having a wider host range than species that are of little economic importance (Blackman & Eastop 2000). For example, *M. persicae* and *R. padi,* vectors of non-persistent viruses such as PVY (Bradley 1954; Katis & Gibson 1985; Blackman & Eastop 2000), are two species with a wide host range. *Myzus persicae* feeds on numerous plant families including many crop species of economic importance in the family Solanaceae (Blackman & Eastop 2000). *Rhopalosiphum padi* is oligophagous, feeding on many species of Poaceae

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which include all the major cereals and pasture crops, but has also been recorded on Cyperaceae, Iridaceae, Juncaceae and Typhaceae (Blackman & Eastop 2000). The ability to locate a host plant may differ between aphid species due to their host ranges being different, and oligophagous species may use host plant cues in a different manner than polyphagous species (Hori 1999).

Aphids rely on sensory information such as colour, shape, texture and smell that they perceive from their environment to locate their respective host plants (Bruce *et al.* 2005). This information is gathered in a number of consecutive behavioural steps and integrated within the central nervous system (Bruce *et al.* 2005; Powell *et al.* 2006). The correct combination of plant characteristics will arrest aphids, and reproduction starts shortly after settling (Bruce *et al.* 2005). However, aphids are able to reject a plant at any one of these consecutive behavioural steps and continue searching for a new host plant (Powell *et al.* 2006).

Initial studies on aphid host plant selection behaviour showed that aphids have an indirect host plant selection mechanism (Kennedy *et al.* 1959; Orlob 1961). Aphids have been found to swarm around their host plant while landing and re-alighting before finally settling (Kennedy *et al.* 1959). Settling is the phase during which the aphid accepts a plant, starts feeding on the phloem continuously and reproduces (Johnson 1957; McLean & Kinsey 1968; Tjallingii 1994). For example, *A. fabae* and *Brevicoryne brassicae* (L.) landed on host and non-host plants alike, taking off again in bouts of short flights until the individual became ready to settle on a host plant (Kennedy *et al.* 1959). Similar observations were made by Orlob (1961) during a field study of *R. padi* landing on different species of Poaceae, who argued that aphids are led into their habitable neighbourhood by host plant cues and after landing discriminate between plants by probing. Since these initial studies, much work has

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been done on the role of visual and olfactory plant cues used in aphid host plant searching behaviour, and it is well known that both wavelength reflectance and olfactory plant cues play a role in aphid host plant searching behaviour (Pickett *et al.* 1992; Döring & Chittka 2007; Webster 2012).

After landing and before stylet penetration, aphids evaluate plant surface characteristics (Powell *et al.* 2006). The chemical cues in the plant boundary layer, trichomes, epicuticular waxes, substrate topology and colour may influence their behaviour (Ibbotson & Kennedy 1959; Goffreda *et al.* 1989; Storer *et al.* 1996; Powell *et al.* 1999). However, stylet penetration occurs as a tarsal contact reflex with any solid surface. Stylet penetration starts as brief probes to the epidermal layer during which small amounts of leaf sap are ingested (McLean & Kinsey 1968; Tjallingii 1985; Tjallingii & Esch 1993). The leaf sap is evaluated by a gustatory organ in the food canal providing further information about the plant (Wensler & Filshie 1969). This is followed by longer probes (40 - 60 seconds) to the mesophyll and parenchyma tissue and ends in penetration of the phloem sieve element (Tjallingii & Esch 1993). Watery saliva is injected into the sieve element to supress phloem defensive mechanisms and enable sustained extraction of phloem sap (Tjallingii & Esch 1993). Sustained phloem ingestion of 30 minutes and longer indicates acceptance of the phloem (McLean & Kinsey 1968; Tjallingii & Esch 1993; Tjallingii 1994). However, aphids do not always penetrate the phloem sieve element while probing. McLean & Kinsey (1968) found that *Acyrthosiphon pisum* (Harris) did not ingest phloem sap when penetrating the leaf tissue of a non-host plant. Therefore, aphids may alight in search of another plant shortly after initial probing to the epidermis has taken place.

The restless manner in which aphids land and settle on their host plants may play an important role in virus spread. Aphids perform several bouts of landing and taking off before settling and reproducing on a host plant. The decision to settle is influenced by factors such as landing on a host or non-host plant and their readiness to settle and reproduce (Kennedy *et al.* 1959; Orlob 1961; Kennedy & Booth 1963). In addition aphids probe the plant after landing before making the decision to accept or reject the plant, which is sufficient for aphids to acquire and transmit non-persistent viruses (Powell 1991; Powell *et al.* 1992; Powell *et al.* 2006). This behaviour of settling and probing plants directly after landing increases the risk of virus spread by aphids potentially landing and taking off on virus-infected plants. Therefore it is important to understand how visual and olfactory cues are used by aphids to locate a potential host plant to develop virus control strategies targeted at manipulating aphid behaviour before making contact with the plant.

2.5 Non-persistent virus management strategies: the role of plant visual cues in aphid host plant selection behaviour

Kennedy *et al.* (1961) demonstrated that mainly phototactic cues are involved during the initial phase of aphid host plant selection behaviour. Prior to landing, aphids are attracted to short wavelength light that causes them to fly skywards (Kennedy & Booth 1963; Hardie 1989). However, in the alighting phase following the migratory flight, aphids become responsive to targets, such as colour cards, traps or plants (Kring 1967; Hardie 1989). During the alighting phase aphids also become less sensitive towards short wavelength light, which causes them to move downwards toward the plant canopy. At the same time they become more sensitive to long wavelength light between 500 and 600 nm that are typically reflected by plant foliage and yellow targets (Kennedy *et al.* 1961; Hardie 1989). This behavioural switch does not happen instantly but gradually, causing aphids to alight and take-off several times before being ready to settle on a plant (Kennedy *et al.* 1961). For example, *B. brassicae*

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was more strongly attracted to leaf colours of a non-host plant, sugar beet (*Beta vulgaris* L., Chenopodiaceae), than a host plant, cabbage (*Brassica oleraceae* L., Brassicaceae), which reflected more light in the blue and violet region than the sugar beet leaves (Kennedy *et al.* 1961). Another example is *Hyalopterus pruni* (Geoffroy), which alighted in greater numbers on grey-green leaves of its summer host *Phragmites communis* L., (Poaceae) than on yellow green leaves of sugar beet (Moericke 1969). These studies suggest that plant leaf colour influences aphid landing rates.

Concepts, and many past behavioural and evolutionary studies, on predicting colour vision in insects were based on the assumption that humans and insects have the same sense of colour perception (Bennett *et al.* 1994; Kelber *et al.* 2003). This is possibly due to the misapprehension that humans have the best colour vision among all animals and that most animals' spectral sensitivity lies within the human-visible spectrum (Bennett *et al.* 1994). However, the phototactic response of an insect is governed by the integration of the sensorial inputs by the central nervous system, and visual cells may be sensitive to light from the entire visible spectrum (Vaishampayan *et al.* 1975). Therefore, the type of visual cells (photoreceptors) of the animal under investigation should be known (Endler 1990). To measure colour from the perspective of the observer (insect), the wavelength reflectance should be measured across all wavelengths to which the animal being studied is sensitive to, and not only the human observer (Endler 1990; Bennett *et al.* 1994).

To make behavioural inferences about insect colour vision and understand how other animals may perceive colour differently from humans, it is important to be aware of the different properties of colour. Colour has both chromatic and achromatic properties and can be defined by three parameters, hue, tint and intensity or brightness (Moericke 1969). The chromatic aspect of colour refers to hue and tint, and the achromatic aspect to intensity or

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brightness (Moericke 1969). Hue is defined by the dominant wavelength of the spectrum reflected by the surface (λ_{max}) and is the attribute denoted as yellow, green etc. (Moericke 1969). Tint refers to colour's similarity to a neutral grey/white. Adding white to a hue decreases the saturation, e.g. adding white to yellow increases the wavelength reflectance in the blue-violet region. Furthermore, a grey object with a small yellowish tint may have low saturation, but a yellow object with a grey/white tint has a high saturation (Moericke 1969; Kelber *et al.* 2003). The intensity of a colour refers to the overall reflection and is thus a value on the light-to-dark scale. Intensity can be reduced by adding black (Moericke 1962). A simple measure of intensity of a colour is to compare the spectral sensitivity of the peak area under the curve of reflectance spectrum, with the curve of the white standard, which reflects maximum light across the visible light spectrum (Moericke 1969).

Humans are trichromatic, having three photoreceptors, red, green and blue, absorbing maximum light at wavelengths of 560, 530 and 430 nm, respectively (Maxwell 1860; Bennett *et al.* 1994). Alternatively, they are called long (L), medium (M) and short (S) wavelength sensitive receptors (Kelber *et al.* 2003). Trichromats perceive colour through unique mixtures of these three types of wavelengths. The three photoreceptors produce the perception of many different hues by means of a colour opponency mechanism (Maxwell 1860; Bennett *et al.* 1994). Humans use the summed output of medium and long wavelength photoreceptors to determine brightness. Comparisons of short with long and medium wavelength photoreceptor outputs drives the yellow - blue ($+ M + L - S$ = yellow, $- M - L + S$ = blue) opponency mechanism and the red-green opponency mechanism compares long and medium wavelength ($+L - M$ = Red, $-L + M$ = green) (Bennett *et al.* 1994; Kelber *et al.* 2003).

Aphids, like humans, have three photoreceptor types that include a green and blue photoreceptor. In contrast to humans, they have a UV photoreceptor that is maximally

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sensitive at 350 nm instead of the red photoreceptor present in our own species (Kirchner *et al.* 2005). The opponent processing mechanism thought to be involved in aphid colour vision is therefore slightly different than in humans. Aphids thus possess the basic physiological requirements to have true colour vision, a minimum of two photoreceptor types, and at least one opponent processing mechanism to compare different spectral inputs. However, it has not been determined whether this is used to generate images that share true colour attributes in aphids and it is likely that they only exhibit wavelength-specific behaviour (Döring & Chittka 2007). Therefore, it is obvious that aphids and humans will not perceive colour in a similar manner due to their physiological differences in ability to perceive colour.

To address this problem, Döring *et al.* (2009) developed a colour choice model based on testing 70 different colours in the field and taking both aphid and human photoreceptor characteristics into account. The model suggested that aphids are attracted to yellow, with a tendency to avoid red. This confirms the findings of many studies on aphid colour vision that aphids are generally attracted to yellow (Döring & Chittka 2007). However, species-specific differences in aphid colour preference for different hues and tints have been found. Eastop (1955) and Heathcote (1957) noted that some aphid species were more yellow sensitive than others. For example, *M. persicae* and *A. fabae* were captured more often in yellow traps in comparison to suction traps than aphids from the genera *Rhopalosiphum* and *Sitobion*. In addition *R. padi* was attracted to green leaves of its primary host plant in autumn instead of trees with yellow leaves as predicted by the autumn co-evolution theory (Archetti & Leather 2005). *Hyalopterus pruni* was attracted to unsaturated hues of orange-yellow-green with an optimum at yellow, and *A. fabae* was more attracted to saturated colours that correspond to their respective host plants *Phragmites communis* Tr., (Poaceae) and Swiss chard (Moericke 1969).

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In addition to hue and saturation, the achromatic aspect of colour, light intensity plays an important role in aphid colour preference and may explain the sensitivity towards yellow found in many aphid species. Roach & Agee (1972) caught higher number of alate aphids in yellow compared to green traps and suggested that this could be due to the higher intensity of the yellow traps, which reflected light at a peak percentage of 100% and the green traps at 45%. The preference for yellow observed in *R. maidis* by Eastop (1955) varied with the intensity of sunlight. Likewise, it was found that intensity was an important cue for *H. pruni,* which preferred dull hues when comparing different hues of yellow (Moericke 1969). In addition, Döring & Chittka (2007) argue that the yellow preference found in aphids should not be viewed as a true colour preference but rather a response that is dependent on the actual light intensity. It may further be due to yellow representing a supernormal foliage stimulus to herbivorous insects rather than it being an ecological adaptation (Prokopy & Owens 1983). Yellow may stimulate the opponent processing mechanisms thought to be involved in aphid colour vision more than green. Yellow reflects more light in the long wavelength region (green to red) than in the short wavelength region (UV to blue), resulting in a greater excitation of the opponent mechanism than green (Döring & Chittka 2007).

A better understanding of the role of visual stimuli in aphid host plant selection behaviour could lead to improved management of aphid - transmitted plant viruses, e.g. choice of trap colour for aphid monitoring in crops and use of light reflectance (Döring & Chittka 2007). Yellow traps are frequently used for aphid monitoring, but not all aphid species are equally sensitive to yellow (Broadbent 1948; Eastop 1955; Heathcote 1957; Roach & Agee 1972). This could lead to skewed results where the abundance of yellow-sensitive species is over-estimated and that of species less sensitive to yellow is under-estimated. This can be overcome by using a trap with the same wavelength reflectance spectra than the plant canopy. Irwin *et al.* (1988) found that a lime green tile trap with a

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wavelength reflectance closely resembling that of soybean crops correctly estimated aphid landing patterns compared to direct plant counts. However, when the same trap was used in pepper plants, the abundance of aphid species colonizing pepper was overestimated (Raccah *et al.* 1985). This shows that not only the wavelength reflectance of the plants is important but also the spectral reflectance behaviour of the aphids expected to be captured when developing traps for aphid monitoring.

Management tools such as reflective mulches that rely on aphid vision have been found effective in the reduction of non-persistent viruses (Döring & Chittka 2007). Silver reflective mulches delayed the onset of Cucumber mosaic *cucumuvirus* (CMV), WMV-1 and WMV-2, ZYMV and Squash mosaic *comovirus* (SqMV) in summer squash (*Cucurbito pepo* L., Cucurbitaceae) and muskmelon (Brown *et al.* 1993; Stapleton & Summers 2002). The percentage of coverage by the reflective covers was found to play a role in the reduction of virus incidence. Rows with complete coverage of reflective mulches were more effective than rows with partial coverage and alternate row applications (Stapleton & Summers 2002). Although reflective mulches increased yield and delayed virus infection in pumpkin, the effectiveness of the mulches decreased towards the end of the season (Brust 2000).

2.6 Non-persistent virus management strategies: the role of plant volatile cues in aphid host plant selection behaviour

Aphid host plant selection behaviour, especially the role of olfaction, has received a considerable amount of attention (Pickett *et al.* 1992; Bruce *et al.* 2005; Powell *et al.* 2006; Webster 2012). Although initial studies by Kennedy *et al.* (1959) did not find any evidence for olfactory cues influencing aphid behaviour prior to landing, several studies subsequently demonstrated the use of plant volatiles by aphids during host plant selection (Pickett *et al.*

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1992). Aphids are able to respond to single compounds from their host plants (Pettersson 1970a; Pettersson *et al.* 1994), as well as blends of compounds (Webster 2012).

Electrophysiological studies have shown that aphids are able to detect plant volatile compounds through sensory structures on their antennae (Visser *et al.* 1996). These structures are divided into primary and secondary rhinaria. The primary rhinaria are located in placoid sensilla located on the fifth and sixth segments, and the secondary rhinaria on the third antennal segment of the alate morph (Fig. 2.1) (Bromley *et al.* 1979). The morphology of the aphid antenna is very similar between species although some differences in the structure of the placoid sensilla have been found (Bromley *et al.* 1979). The olfactory receptor neurones in aphids are housed in placoid sensilla on the antennae. The distal and proximal rhinaria are situated on the fifth and sixth segment. Generally, the primary rhinaria on the fifth antennal segment contains at least one large placoid sensilla and four coeloconic pegs (Shambaugh *et al.* 1978; Bromley *et al.* 1979). In winged aphids the secondary rhinaria are mainly located on the third antennal segment but in some species such as *R. padi* and *Pemphigus bursarius* (L.), they may extend up to the fifth and sixth antennal segment (Rogerson 1947; Dunn 1978; Shambaugh *et al.* 1978; Bromley *et al.* 1979).

The primary and secondary rhinaria not only differ morphologically but also in the type of volatile compounds they detect. The primary rhinaria are able to detect plant volatile compounds as well as aphid alarm pheromone (Bromley & Anderson 1982). The function of this receptor does not seem to differ between morphs. Park & Hardie (2002) tested the response of different aphid morphs (*A. fabae* and *R. padi*) to (*E*)-2-hexenal, a green leaf volatile compound. No difference was found in the antennal response and the primary rhinaria on the fifth and sixth antennal segment between the morphs, indicating that the primary rhinaria are responsible for detecting plant volatiles (Park & Hardie 2002). However,

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the role of the secondary rhinaria differs between morphs (Park & Hardie 2002). Pettersson (1970b) suggested that the secondary rhinaria in males were acting as pheromone receivers after a series of behavioural observations. In addition the secondary rhinaria were also found to be involved in detecting the aphid alarm pheromone (*E*)-β-farnesene (Bromley & Anderson 1982). Alate males and gynoparae responded strongly to the sex pheromone components nepetelactone and nepetelactol, but not the alate virginoparae (Park & Hardie 2002). This could be due to their seasonal change in reproduction strategy between parthenogenetic and sexual reproduction (Park & Hardie 2002). The electrophysiologal activity of plant volatile compounds indicates that they are behaviourally active and used by aphids to mediate several behaviours involved in host plant searching and location (Webster 2012). Aphid non-host plant volatiles have been shown to be repellent to aphids. Isothiocyanates, such as 3-butanyl isothiocyanate and 4-pentenyl isothiocyanate, identified from the non-host plants brussels sprouts (*Brassica oleraceae* L., Brassicaceae) and turnip (*B. campestris* Metz., Brassicaceae), repelled alate *A. fabae* (Nottingham *et al.* 1991). When these compounds were presented in an olfactometer together with two varieties of the host plant *Vicia faba* L. (Fabaceae), no significant responses were observed (Nottingham *et al.* 1991). However, when the two varieties were presented alone, alate *A. fabae* were significantly attracted to their odours, indicating that the presence of the repellent non-host compounds (isothiocyantes) masked the attraction of the host plant (*V. faba*) (Nottingham *et al.* 1991). Therefore it is possible that non-host compounds can be used in developing crop protection strategies by masking the odours of host plants.

Volatile blends released by plants in specific ratios have been found to provide aphids with host plant-specific information in addition to single compounds (Campbell *et al.* 1993; Bruce *et al.* 2005; Webster 2012). Three compounds, methyl salicylate, (*E*)-2-hexenal and β-Caryophyllene, were identified from hop (*Humulus lupulus* L., Canabaceae), the host plant of

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Phorodon humuli (Schrank), and found to be behaviourally active in EAG studies (Campbell *et al.* 1993). When these compounds were tested in the olfactometer, *P. humuli* was attracted to (*E*)-2-hexenal and β-Caryophyllene alone. However, the natural blend was more attractive to *P. humuli* than (*E*)-2-hexenal alone, and when the blend was adjusted to a 1:1 ratio it was no longer attractive (Campbell *et al.* 1993). Similar results were found for *A. fabae* and volatile blends from its host plant *V. faba*. Nottingham *et al.* (1991) determined that *A. fabae* was attracted to volatile blends of its host plant *V. faba*. Webster *et al.* (2008a) identified 16 volatile compounds released by *V. faba* and recorded 15 of the 16 compounds to be electrophysiologically active for *A. fabae*. Furthermore, they identified an attractive synthetic blend that resembled the natural ratio of the volatile blend released by *V. faba,* to which the aphids responded (Webster *et al.* 2008a). When these compounds were presented individually to *A. fabae* in an olfactometer, 10 out of the 15 compounds identified previously elicited a negative behavioural response (Webster *et al.* 2008b). In a subsequent study, when the compounds resulting in a negative response were combined in a blend with each compound at the concentration that elicited the most negative response, the blend was attractive to *A. fabae* (Webster *et al.* 2010). In addition, plant volatile blends have been found to play a role in aphid vector-virus interactions (Eigenbrode *et al.* 2002).

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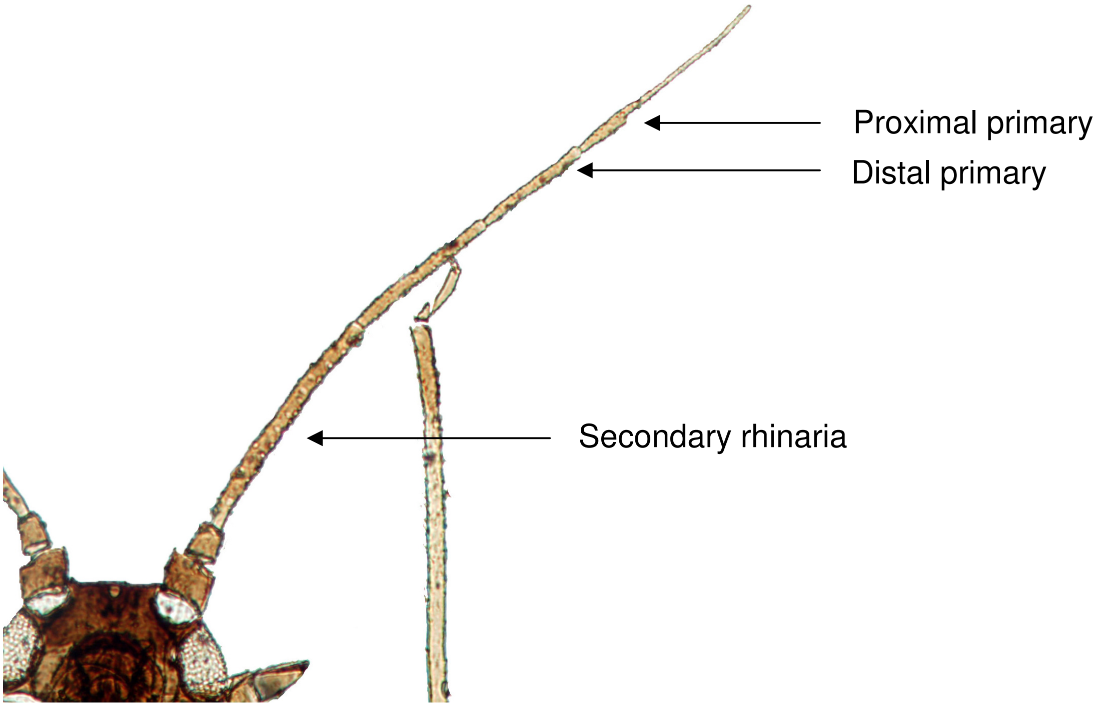

Figure 2.1: Antenna of *Rhopalosiphum padi* indicating position of distal and proximal primary rhinaria and secondary rhinaria.

Plant viruses are known to alter plant volatile emission to attract aphids to virus infected plants (Eigenbrode *et al.* 2002; Ngumbi *et al.* 2007). Eigenbrode *et al.* (2002) found that potatoes infected with the persistently transmitted *Potato leaf roll virus* (PLRV) produced almost double the amount of total volatiles than uninfected plants. Potatoes infected with the non-persistent viruses PVY and *Potato virus X* (PVX) released two compounds in higher concentrations than uninfected plants, whereas PLRV infection resulted in elevated levels of 14 compounds (Eigenbrode *et al.* 2002; Ngumbi *et al.* 2007). In addition, Eigenbrode *et al.* (2002) found that *M. persicae,* the principal vector of PLRV, was attracted by the volatile profile of potato plants infected with PLRV but not to plants infected with PVY or PVX. Similarly Mauck *et al.* (2010) demonstrated that cucumber (*Cucurbita pepo* L., Cucurbitaceae) plants infected with the non-persistent CMV released slightly elevated concentrations of plant volatiles in comparison to uninfected plants. However, in contrast to

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the findings of Eigenbrode *et al.* (2002), Mauck *et al.* (2010) found that two vectors of CMV, *Aphis gossypii* (Glover) and *M. persicae,* were more attracted to cucumber plants infected with CMV than uninfected plants. Although *A. fabae* and *M. persicae* were attracted to volatiles of infected cucumber plants, they did not settle and reproduce in large numbers on these plants, whereas PLRV infected potatoes caused *M. persicae* to settle in high numbers (Ngumbi *et al.* 2007; Mauck *et al.* 2010). This difference has been attributed to the different transmission characteristics of the virus. Persistent viruses are acquired from the phloem and have to pass through the insects' gut and into the salivary glands which requires aphids to feed from the phloem for several hours to days (Sylvester 1980). Non-persistent viruses can be acquired during short probes by aphids and can only be transmitted for a relatively short time (Katis & Gibson 1985; Ng & Perry 2004). Mauck *et al.* (2010) suggested that the odour profile of plants infected with non-persistent viruses mimic the odour profile of strong healthy plants to attract aphids while being a poor host facilitates non-persistent virus spread by causing the aphid to leave the plant in search of a better host plant.

Olfactory cues have also been shown to be involved in other behavioural aspects of aphids such as migration in holocyclic aphids. The switch from primary host in winter to secondary host in spring has been found to be mediated by olfactory signals (Pettersson *et al.* 1994; Glinwood & Pettersson 2000). Methyl salicylate has been identified from the primary host of *R. padi*, the bird cherry tree (*Prunus padus* L., Rosaceae), and plays an important role in *R. padi* host finding behaviour (Pettersson *et al.* 1994). The autumn morphs are attracted to methyl salicylate whereas the spring migrants are repelled, causing them to leave the primary host plant in search of a secondary host (Pettersson 1970a; Pettersson *et al.* 1994; Glinwood & Pettersson 2000; Ninkovic *et al.* 2003). Spring morphs of alatae and apterae *R. padi* were attracted to volatiles from wheat (*Triticum aestivum* L., Poaceae) and oat (*Avena sativa* L., Poaceae) seedlings as well as some of the individual compounds identified from the seedlings, such as (*E*)-2-hexenyl acetate, (*Z*)-3-hexenol, (*E*)-2-hexenol, *n*-heptanol, *n*-octanol, benzaldehyde and linalool (Quiroz & Niemeyer 1998). Similarly, alate virginoparae of *A. fabae* were repelled by odours from the primary host, spindle (*Euonymus europaeus* L., Celastraceae) but gynoparae were attracted (Nottingham *et al.* 1991). Glinwood & Pettersson (2000) found the change in host plant preference to be gradual taking place within 24 hours after *R. padi* spring migrants have left the primary host. When evaluating the behavioural responses of *R. padi* spring migrants that have left the primary host within 24 hours, not all the individuals were repelled by odours from the primary host. This gradual change in *R. padi* behaviour towards methyl salicylate takes place at the level of the individual and not the population, and may require a period of flight (Glinwood & Pettersson 2000). Despite this change being gradual, methyl salicylate significantly reduced immigration, settling and density of *R. padi* in cereal fields, indicating there is definite potential for this compound to be incorporated into novel crop protection strategies. (Pettersson *et al.* 1994; Ninkovic *et al.* 2003).

Other volatile compounds repellent to aphids include those associated with herbivore feeding. These herbivore-induced volatile compounds may act as a signal of competition, or indicate the presence of natural enemies to aphids, resulting in a reduced settling response (Guerrieri *et al.* 1993; Pettersson *et al.* 1995; Bernasconi *et al.* 1998; Dicke & van Loon 2000; Ninkovic *et al.* 2001; Dewhirst & Pickett 2010). Density-related substances, green leaf volatiles and terpenes have been identified as the most common plant volatile substances to affect aphid behaviour (Pettersson *et al.* 1995; Holopainen 2004). Specifically, 6-methyl-5-hepten-2-one (Sulcatone), 6-methyl-5-hepten-2-ol (sulcatol) and 2-tridecanone are associated with high aphid infestations on wheat and barley plants, an increase in the sensitivity of aphids to disturbances and an increase in the mobility of flying aphids (Pettersson *et al.* 1995; Quiroz *et al.* 1997). Ninkovic *et al.* (2003) determined that these compounds significantly

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reduced, immigrating, settling as well as the overall population density of *R. padi* in wheat fields.

Apart from density-related substances, green leaf volatiles and terpenes are associated with the defence mechanisms of plants and are released by the plant upon herbivore feeding. Although Turlings *et al.* (1998) did not find a difference in maize volatile profiles between aphid-infested and undamaged plants, other studies have shown that aphid feeding causes a change in the plants' odour profile and become attractive to aphid natural enemies (Guerrieri *et al.* 1993; Dewhirst & Pickett 2010). Turlings *et al.* (1998) argued that the aphid feeding mechanism causes very little damage to affected plant cells and does not elicit production of herbivore-induced plant volatiles. This contradicts other studies; for example, qualitative and quantitative changes in plant volatile profiles were observed 60-72 hours after *A. pisum* feeding commenced (Guerrieri *et al.* 1999). It is possible that changes in the maize volatile profile observed by Turlings *et al.* (1998) were so subtle that they were not detected. In addition, changes in plant volatile profiles due to aphid feeding have been shown to differ between aphid species (Du *et al.* 1998; Guerrieri *et al.* 1999). *Acyrthosiphon pisum* feeding on *V. faba* induced and increased the concentration of several compounds; although feeding by *A. fabae* induced the same response in the plant, two of the induced compounds, 6-methyl-5-hepton-2-one and geranic acid, emitted in response to *A. pisum* feeding, were absent in the volatile blend induced by *A. fabae.* Behavioural assays indicated that these differences are detected by the aphid parasitoid *Aphidius ervi* Haliday, which was attracted to plant odours released by *V. faba* plants infested with *A. pisum* (host for *A. ervi*), but not to odours released by *V. faba* infested with *A. fabae* (non-host for *A. ervi*).

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2.7 Concluding remarks

Past studies on the use of visual and olfactory cues by aphids during host plant searching behaviour have identified various aspects of aphid behaviour that can be manipulated to develop integrated pest management strategies. However, these signals have proven to be context-specific to aphids, and using only visual cues or olfactory cues alone may not be sufficient to develop management strategies robust enough to control the spread of non-persistent viruses. In addition, chemical control methods that are able to control aphid populations are not always efficient in controlling virus incidence. The manner in which non-persistent viruses are spread and the interaction between virus, plant and vector, enable rapid virus spread. To develop crop protection strategies that are based on manipulating aphid behaviour, it is important to study how aphids respond to visual and olfactory plant cues within the agro-ecosystem. Aphid species composition and vegetation may differ between potato growing regions, and not all crops are equally suited for the different climatic regions and potato growing seasons (pers comm K. Krüger and G. Prinsloo). In the case of crop borders, very few studies have taken aphid species-specific interactions of plant visual and olfactory cues emitted by the main crop to be protected as well as the crop to be used as crop border plant into account. Further work in this regard should therefore be aimed at identifying plant traits that can be selected to develop crop border plants that consider the aphid species composition within the region, for example, in some seed potato growing regions high numbers of cereal aphids are found landing in potato fields.

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Chapter 3

Landing preference and reproduction of *Rhopalosiphum padi* (Hemiptera: Aphididae) on three maize, potato and wheat cultivars

Abstract

Alate *Rhopalosiphum padi* in search of good quality host plants transmit the non-persistent *Potato virus Y* (PVY) to seed potatoes, although they mainly feed on cereals and grasses. Planting a non-virus host plant around the main crop as a crop border can reduce PVY incidence in seed potatoes, because aphids tend to land in high numbers at the edge of a field and the crop border acts as a virus sink. This study evaluated *R. padi* landing and settling preference, and reproductive rate on three maize and three wheat cultivars compared to three potato cultivars in the laboratory. The aim of the study was to identify a potential crop border plant that is attractive to aphids for landing but does not support high population densities. We evaluated aphids reared on maize and wheat to control for bias due to previous experience. Irrespective of origin, alates preferred to land on maize and wheat rather than on potato cultivars in choice experiments; only one aphid settled on potato. Aphid settling on the maize and wheat cultivars depended on aphid origin. In choice and no-choice experiments, *R. padi* produced the highest number of offspring on the wheat cultivars, irrespective of origin. Plant nitrogen content did not influence *R. padi* reproduction, and no relationship was found between aphid population density and trichome density. The study demonstrates that host plant preference of aphids may vary between plant cultivars and can therefore influence the effectiveness of a crop border. The high landing rate but low reproduction suggests that maize '6Q-121' and '78-15B' could be suitable crop border plants in regions where *R. padi* is an important vector of PVY.

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Key words: bird cherry-oat aphid, host plant selection, crop border plants, *Potato virus Y*, trap crops

3.1 Introduction

Potato virus Y (PVY) causes major economic losses in the seed potato industry world-wide (Radcliffe & Ragsdale 2002). PVY is mainly transmitted in a non-persistent manner by aphid (Hemiptera: Aphididae) vectors. Although wingless aphids (apterae) are able to transmit viruses, it is the winged (alate) aphids, when probing a potential host plant, that are mainly responsible for spreading viruses in crops over long distances (Bradley 1954; Powell 1991; Radcliffe & Ragsdale 2002). Hence, control strategies should be aimed at preventing potential virus vectors from landing in potato fields. Current preventative measures include rouging, reduction of virus inoculum and the use of crop borders to reduce the number of aphid vectors landing in potato fields and to decrease virus incidence (Radcliffe & Ragsdale 2002; Hooks & Fereres 2006). These preventative measures, especially crop borders, rely on a good understanding of the host plant searching behaviour of the most abundant aphid vector species.

During their initial pre-alighting behaviour, aphids use visual cues to orientate towards potential host plants, and plant chemical cues to discriminate between host and non-host plants (Kennedy *et al.* 1961; Pickett *et al.* 1992; Powell *et al.* 2006). After landing on a plant, a variety of surface characteristics, such as trichome exudates, epicuticular waxes and topology further influence the aphids' choice (Powell *et al.* 2006). The decision to accept or reject a plant is not only based on plant characteristics, but the plant on which the aphid developed may also play a role (Guldemond 1990; Barron 2001; Gorur *et al.* 2007). Aphid populations only produce alates after several generations of apterae when the colony is larger

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than the plant can sustain (Müller *et al.* 2001). This could cause the aphid to become adapted to the plant species it originated from and it may therefore prefer to reproduce on the same plant species rather than on other species within its host range (Barron 2001; Gorur *et al.* 2007).

PVY is best controlled by combining chemical control with cultural management strategies (Radcliffe & Ragsdale 2002). Crop borders rely on the response of aphids to the contrast in wavelength reflectance between the brown-green interface at the edge of the crop (Minks & Harrewijn 1988). Replacing the edge with a non-virus host plant creates a virus sink (DiFonzo *et al.* 1996; Hooks & Fereres 2006). For example, DiFonzo *et al.* (1996) reported a reduction in PVY incidence in potato fields planted with a crop border of sorghum (*Sorghum bicolor* L., Poaceae), wheat (*Triticum aestivum* L., Poaceae), and soybean (*Glycine max* L., Fabaceae). However, they found no reduction in the number of aphids landing in the fields with crop borders. Trap crops (crop species more attractive to the insect pest than the primary crop), on the other hand, may increase the number of aphids landing in the crop border in comparison to the main crop (Nault *et al.* 2004; Hooks & Fereres 2006). For example, Toba *et al.* (1977) found a reduction in the incidence of *Watermelon mosaic virus* (WMV-1 and -2) when musk melon (*Cucumis melo* L., Cucurbitaceae) was planted with a crop border of wheat. Hence, control methods such as crop borders to reduce the incidence of non-persistent viruses rely on understanding the relationship between vector and host plant.

Rhopalosiphum padi (L.) is an important vector of PVY (Sigvald 1987; Radcliffe & Ragsdale 2002). This aphid species does not colonize potato (*Solanum tuberosum* L., Solanaceae) but transmits PVY to seed potato fields in search of good quality host plants (Boiteau 1997). Although *R. padi* is a less efficient vector than potato colonizing species such as *Myzus persicae* (Sulzer), high numbers of *R. padi* in seed potato fields can result in high

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infection rates (Van Hoof 1977; Sigvald 1987). In addition, early season flights of *R. padi* can advance PVY infection (Van Hoof 1977).

Maize (*Zea mays* L., Poaceae) and wheat have been identified as potential crop border plants, in comparison to lucerne (*Medicago sativa* L., Fabaceae) and soybean in seed potato-producing regions where *R. padi* is abundant (Schröder & Krüger 2014). Although more aphids landed on maize and wheat than potato, the relative attractiveness of *R. padi* to different maize and wheat cultivars was not considered in this study (Schröder & Krüger 2014). It is known that aphid preference may vary between plant cultivars of the same species (Storer *et al.* 1993; Storer & van Emden 1995; Alla *et al.* 2003) and using a more attractive cultivar that supports a low population density may increase the number of aphids landing in the border crop without becoming a source of aphid vectors. Therefore the relative attractiveness of different crop cultivars in comparison to the main crop needs to be considered when evaluating potential trap crops to be used as crop border plants. This study evaluated the landing and settling preferences, as well as reproduction of *R. padi* on three cultivars each of maize, potato and wheat in a laboratory study as a basis for identifying an attractive crop border plant.

3.2 Materials and Methods

Plants

Three cultivars each of maize (cultivars 'CRN 3505', '6Q-121' and '78-15B'), wheat (cultivars 'Duzi', 'Kariega' and 'Krokodil') and potato (cultivars 'BP1', 'Hertha' and 'Mondial') were used in the experiments. Plants were grown in an autoclaved soil mixture consisting of river sand and coco peat in a ratio of 4:1 in 12.5 cm diameter pots. Three maize seeds and two rows of wheat seeds, each row containing 10 seeds, of the same cultivar were

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sown into pots. The seeds were treated with fungicides. In addition, two pre-sprouted potato mini tubers of the same cultivar were planted per pot. The number of seeds and tubers was chosen to ensure that all plants had a comparable leaf area when used in the experiments. No pesticides were applied to the plants during the study. Agricultural lime (5 ml per pot) and slow release fertilizer (*c.* 1.6 g per pot; Grovida, Khula Kahle^{TM} Fruit and Flower, N:P:K (3:1:5)) were added to the soil upon planting. Two weeks after planting, a weekly foliage treatment of micronutrients (Trelmix trace element solution) was applied to the plants according to the manufacturer's instructions. The plants were grown in a climate controlled room at 25 °C, ambient relative humidity (RH) and at a 16 h: 8 h light: dark photoperiod. The maize and wheat plants were used in experiments at growth stages 11 and 12 with two to three leaves unfolded, and the potato plants at growth stages 17 and 18 with seven and eight leaves unfolded (Meier 2001). The leaf area was determined with a leaf area meter (Li-3100C, Li-Cor).

Insects

A culture of *R. padi* was established at the University of Pretoria in 2009 with aphids obtained from a culture maintained on wheat at the Agricultural Research Council - Small Grain Institute (ARC-SGI) in Bethlehem, South Africa. The aphids were originally collected from wheat plants at the Tygerhoek experimental farm, Riviersonderend, Western Cape (34°9'S, 19°54'E), and supplemented with individuals collected from wheat. Aphids were reared in wooden ventilated cages with a glass panel at the top (45 x 55 x 32 cm) in a climate-controlled room at 22 °C, ambient RH and at a 16 h: 8 h light: dark photoperiod. The insects were reared either on mixed cultivars of maize or on mixed cultivars of wheat. Aphids were

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reared on respective host plants for more than six months before used in experiments. *R. padi* takes 6 and 22 days to complete a generation at 13 and 26 °C (Villanueva & Strong 1964).

Alate virginoparae were produced by crowding the aphids on plants. Only actively moving/walking alates of various ages were collected from the top glass panel of the cages. To control for possible effects from previous experience, separate experiments were carried out with aphids reared on maize and wheat, respectively.

Landing and settling preference, and reproduction – choice experiment

For aphids reared on wheat, five rows of plants were placed in a randomized block design in a light grey-walled climate-controlled room with a grey cement floor (Fig. 3.1a). Each row contained nine pots, each with one of the plant cultivars. In total, 115 alate aphids reared on wheat were released evenly within the five rows of plants, in groups of ten aphids for every four plants at plant canopy height, to ensure that each plant had an equal chance of aphids landing and colonizing. The experiment was carried out at 24.1 ± 0.1 °C, 50.6 ± 2.7% RH, and at a 16 h: 8 h light: dark photoperiod (cool white fluorescent lights; Osram, Indonesia). The experiment was repeated five times. Aphids reared on maize produced a considerably lower number of alates than those reared on wheat, and the experimental design was adjusted accordingly. Pots with plants were arranged in a circle consisting of one pot of each plant cultivar placed randomly (Fig. 3.1b). Twenty-three alate aphids reared on maize were released in the centre of the plant circle at plant canopy height. The experiment was carried out in a climate-controlled room under the conditions described above, except that a gauze cage (80 x 80 x 30 cm) was placed over the plants on the floor.

For both aphids reared on wheat and on maize, alates were counted on the plants four hours (landing) and 24 hours (settling) post-release. Aphids were left to reproduce for 14

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days, after which the number of nymphs and apterous adults produced on each plant were counted.

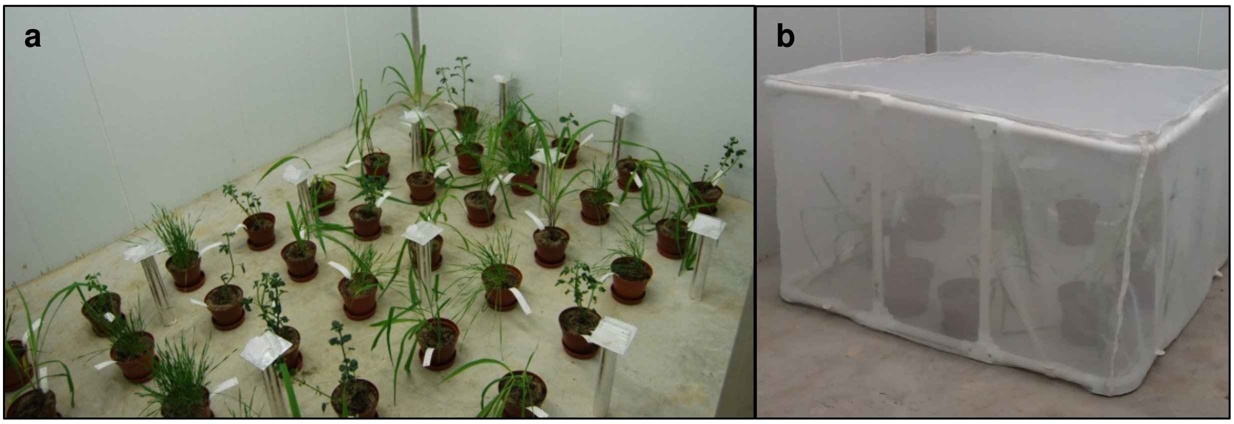

Figure 3.1: Experimental design of landing, and settling preference of *R. padi* reared on wheat (**a**) and maize (**b**) respectively.

Aphid reproduction – no-choice experiment

The three maize and wheat cultivars were randomly arranged in six rows in a greenhouse, with each row containing one of each crop cultivar. A modified ventilated 2 l plastic bottle was placed over the plants in each pot to contain the aphids. Potato was excluded because it is not a host plant for *R. padi* and no offspring was recorded in the choice experiment. Five actively moving adults were collected in small glass vials and thereafter the vials were placed on the soil in each pot where the aphids, to avoid damaging them, were allowed to move freely onto the plants. The study was undertaken separately with alatae from maize and from wheat, as well as apterae from maize and from wheat. The greenhouse temperature was 20.5 ± 0.6 ºC, 43.1 ± 1.5% RH at midday, with average maximum and minimum temperatures of 24.8 ± 0.6 ºC and 10.3 ± 0.5 ºC, a maximum and minimum of 71.9 ± 1.5% and 26.8 ± 1.5% RH, and with natural light conditions from May – August 2012. The

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number of aphids was counted at 24-hour intervals for 14 consecutive days. The experiment was replicated five times.

Nitrogen analysis

To determine the nitrogen content of maize and wheat cultivars, leaves of a subset of plants were weighed and dried in an oven at 55°C for 48 hours. The dried plant material was weighed using a Mettle Toledo PB303-L scale and ground into a fine powder with a Tecator sample mill (Cyclotec 1093). To obtain enough material for the analysis, leaves from five plants were pooled to form a replicate. Five replicate samples of each cultivar were submitted for nitrogen analysis. The nitrogen content of the plants was determined at the UP Nutrilab, Department of Animal Science, University of Pretoria, with the Dumas method (AOAC 2000). Potato was excluded from the nitrogen analysis because *R. padi* did not settle or reproduce on potato during the choice trial.

Trichome density

To determine the leaf trichome density, 1 cm-long sections were cut from the leaf blades across the width of maize and wheat leaves. The trichome density of potato leaves was not determined because *R. padi* did not settle or reproduce on potato during the choice trial. The leaf sections were cleared in a 1:1 (v:v) mixture of phenol and chloral hydrate for 24 hours. Leaf sections were transferred to lactic acid and mounted on microscope slides (Hoxie *et al.* 1975). Photographs of the trichomes were taken using a Nikon Optihot microscope and a Nikon digital camera (DXM 1200F) at 40 x magnification. All trichomes on the abaxial side of the leaf surface along the midvein as well as the leaf mid-section were counted in 1 x

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1 mm squares. Aphids feed on the abaxial side of leaves, therefore the adaxial leaf surfaces was excluded.

Statistical analyses

The numbers of landing and settling aphids, as well as the number of offspring produced on the three cultivars of maize, potato and wheat each in the choice experiment with *R. padi* reared on maize were analysed with a one-way analysis of variance. The data on *R. padi* reared on wheat were analysed with a nested ANOVA with cultivar as factor and landing, settling and reproduction as variates. Fisher's least significant difference (LSD) test was used to separate means. Potato was excluded from all analyses because only one aphid landed on one of the cultivars, and none settled or reproduced on potato. For the no-choice experiment, linear mixed model repeated measurement analysis was used to determine differences in aphid counts on maize and wheat cultivars over 14 days. The counts were transformed by $\log_e(x+0.5)$ to normalize data and stabilize treatment variances. Fixed factors were aphid origin, plant cultivar and aphid origin x cultivar interaction and cage x day was entered as random factor. Fisher's LSD test was used to separate means. Data were analysed with GenStat® (Payne *et al.* 2012).

Leaf areas were analysed with a Kruskal-Wallis ANOVA followed by multiple comparisons of mean ranks for all groups. Separate ANOVAs were used to determine significant differences in plant nitrogen content, as well as in trichome density among plant cultivars. Fisher's LSD test was used to distinguish between means. Statistica (Version 11 $^{©}$Statsoft, Inc. 1984-2012) was used for the data analyses.

The significance level was set at $P < 0.05$ for all analyses.

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3.3 Results

Landing and settling preferences, and reproduction – choice experiment

There was no significant difference in the leaf area among the plant species and cultivars used ($H = 13.1$, d.f. = 8, $P = 0.11$); leaf area ranged between 78 and 127 cm^2.

For *R. padi* reared on maize, the number of alates landing did not differ significantly between the maize and wheat cultivars ($F_{5,41} = 1.73$, $P = 0.16$; Fig. 3.2a). The highest number of alates landed on wheat ‘Krokodil’, followed by wheat ‘Kariega’, maize ‘CRN 3505’, wheat ‘Duzi’, maize ’78-15B’ and maize ‘6Q-121’. However, the number of alates settling on wheat ‘Kariega’ was approximately three and six times higher than that settling on maize ‘6Q-121’, and maize ’78-15B’ and wheat ‘Duzi’ ($F_{5,41} = 2.97$, $P = 0.03$; Fig. 3.2b). No significant differences were observed in the number of adult apterae and nymphs produced after 14 days ($F_{5,41} = 1.34$, $P = 0.274$, Fig. 3.3a).

For *R. padi* reared on wheat, a similar number of alatae landed on the three maize and wheat cultivars ($F_{5,149} = 0.49$, $P = 0.785$; Fig. 3.2c). However, the number of alate aphids that settled on wheat ‘Krokodil’ was more than twice that settling on the three maize cultivars ($F_{5,149} = 3.22$, $P = 0.009$; Fig. 3.2d). After 14 days the number of *R. padi* nymphs and adult apterae was significantly higher on wheat ‘Kariega’ than on any other maize or wheat cultivar, and higher on wheat ‘Duzi’ and ‘Krokodil’ compared to the maize cultivars ($F_{5,149} = 19.80$, $P < 0.001$; Fig. 3.3b). The number of aphids produced on maize did not differ between cultivars, but between wheat cultivars.

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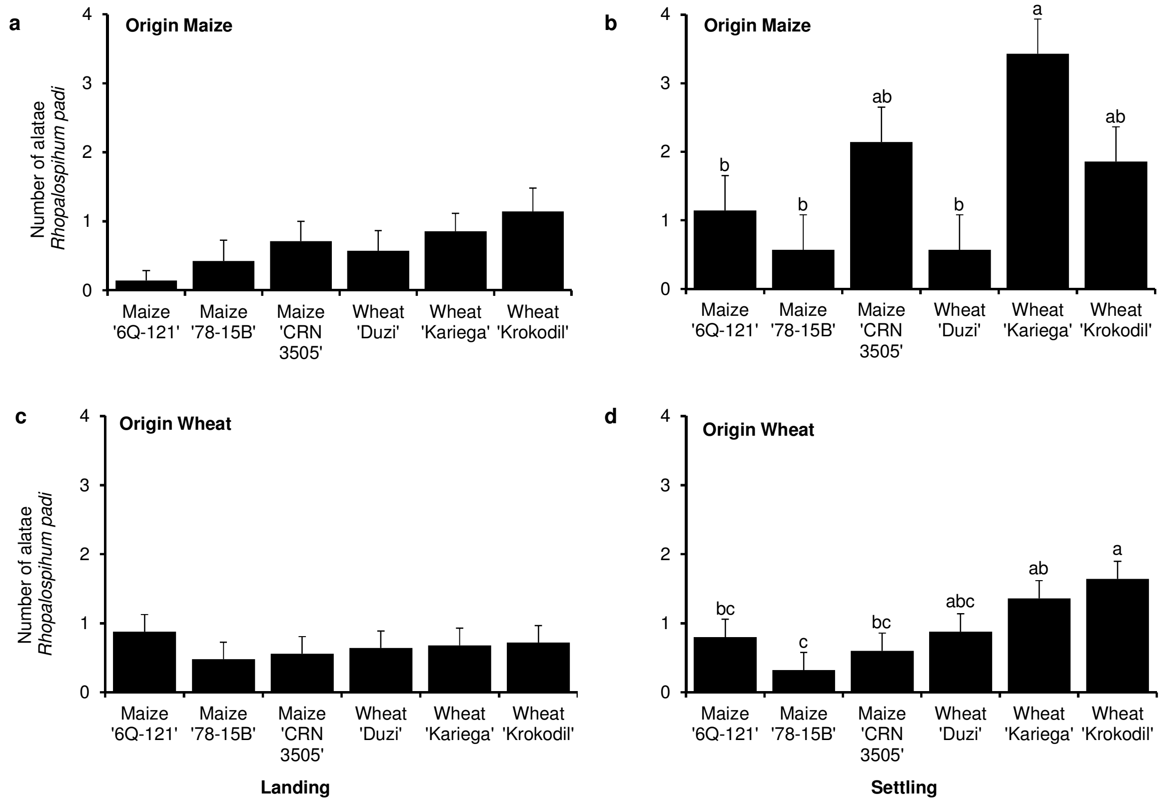

Figure 3.2: *Rhopalosiphum padi* (mean ± SE) landing (**a, c**) and settling (**b, d**) on three maize and three wheat cultivars. Aphids were reared on maize (**a, b**) or wheat (**c, d**). Letters above bars indicate significant differences between means (Fisher's LSD test: P < 0.05).

Aphid reproduction – no-choice experiment

Reproduction of *R. padi* on the three different maize and wheat cultivars was significantly influenced by the plant cultivar, and also the origin of the aphids (morph and plant species). Significant interactions were observed between the number of days and origin as well as the interaction between the origin and the plant cultivars (*P* < 0.05; Table 3.1).

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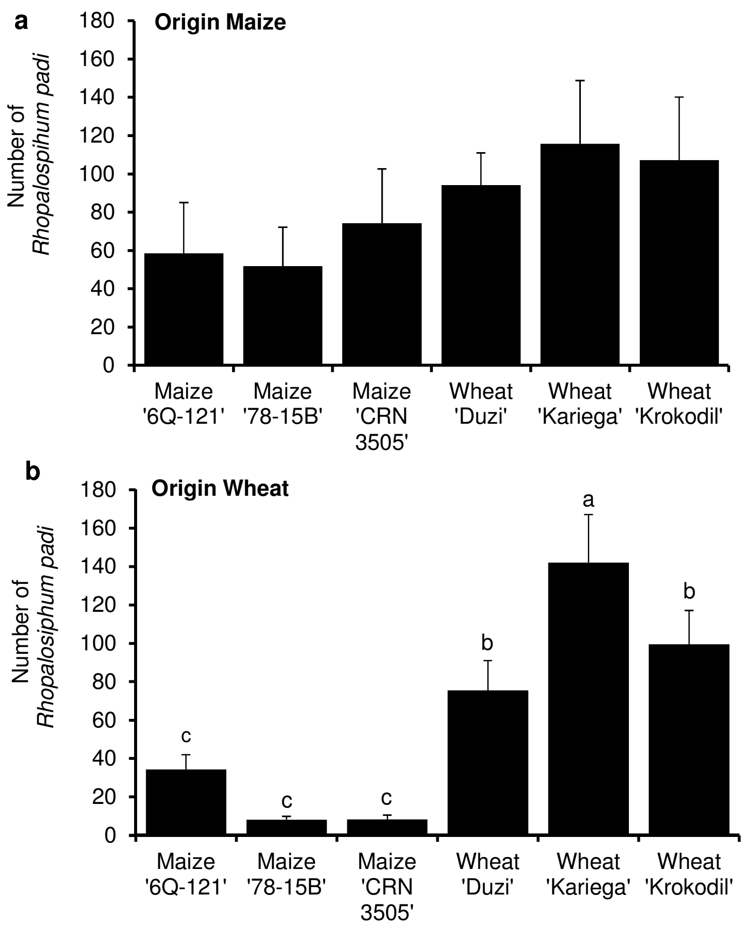

Figure 3.3: Increase in number of *Rhopalosiphum padi* (mean ± SE) after 14 days on three maize, and three wheat cultivars. Aphids were reared on maize (**a**) or wheat (**b**). Letters above bars indicate significant differences between means (Fisher's LSD test: $P < 0.05$).

Over the 14-day trial period significantly higher numbers of *R. padi* were recorded on the three wheat cultivars in comparison to the three maize cultivars (Fig. 3.4). The origin of *R. padi* had a significant effect on the number of adults and nymphs counted over 14 days, with the highest number produced from apterae reared on wheat, followed by alatae reared on maize and wheat and apterae reared on maize (Table 3.1, Fig. 3.4).

R. padi alatae reared on maize and wheat, respectively, produced a significantly higher number of offspring on the three wheat cultivars compared to the three maize cultivars

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(Figs 3.4a,b). Likewise, the number of offspring produced by both *R. padi* apterae origin maize and origin wheat was significantly higher on the three wheat cultivars compared to the three maize cultivars (Figs 3.4c,d). In addition, for *R. padi* apterae origin maize, the number of offspring produced was significantly lower on maize '6Q-121' than maize '78-15B' (Table 3.1, Fig. 3.4c).

Table 3.1: Test statistics generated from the linear mixed model repeated measurement analysis of the number of *Rhopalosiphum padi* offspring recorded over 14 days on maize 'CRN 3505', '78-15B', '6Q-121' and wheat 'Duzi', 'Kariega' and 'Krokodil'. *R. padi* was reared on maize and wheat.

Fixed term	Wald statistic	d.f.	F statistic	P-value
Days	1.93	13	0.15	1.00
Origin	1160.54	3	386.85	<0.001
Cultivar	112.82	5	22.56	<0.001
Days x Origin	57.00	39	1.46	0.034
Days x Cultivar	26.26	65	0.40	1.00
Origin x Cultivar	271.52	15	18.10	<0.001
Days x Origin x Cultivar	102.70	195	0.53	1.00

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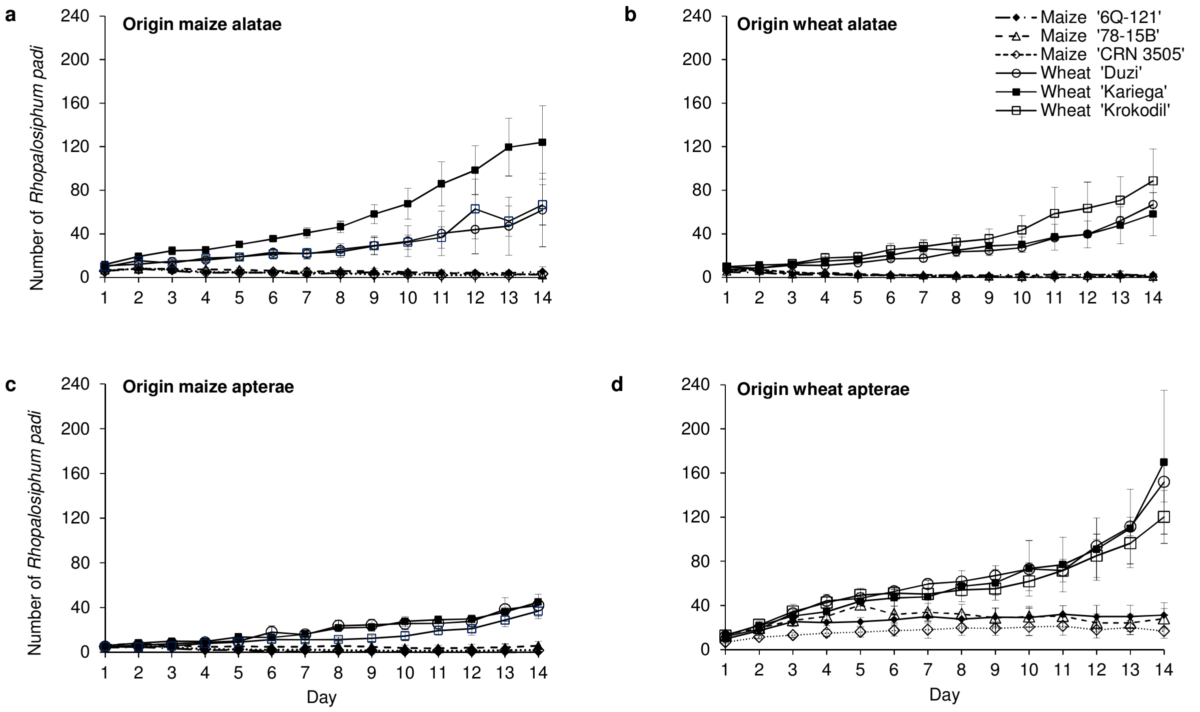

Figure 3.4: Number of *Rhopalosiphum padi* offspring (mean ± SE) from alatae reared on maize (**a**) or wheat (**b**) and apterae reared on maize (**c**) or wheat (**d**) recorded daily over 14 days on three maize and three wheat cultivars.

Nitrogen analysis

No significant differences were found in leaf nitrogen content of the three maize and wheat cultivars ($F_{5,24} = 1.77$, $P = 0.16$, Fig. 3.5).

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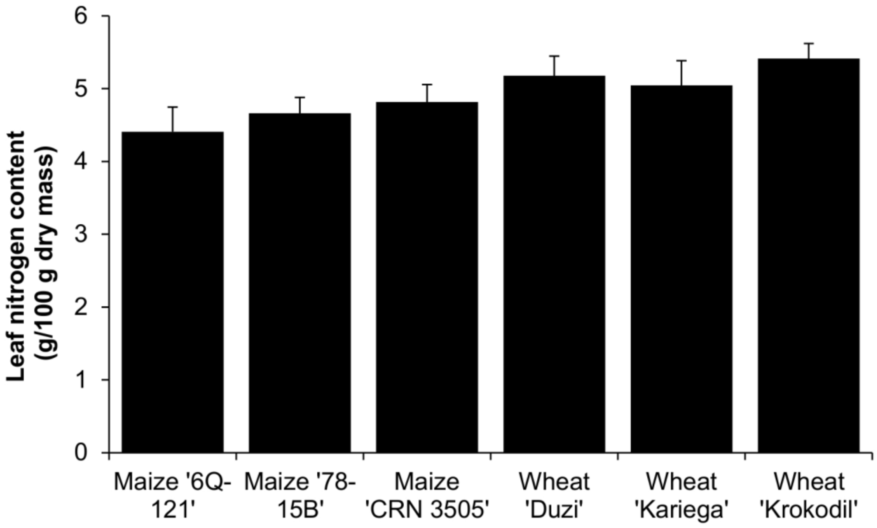

Figure 3.5: Leaf nitrogen (mean ± SE) content of three maize and three wheat cultivars.

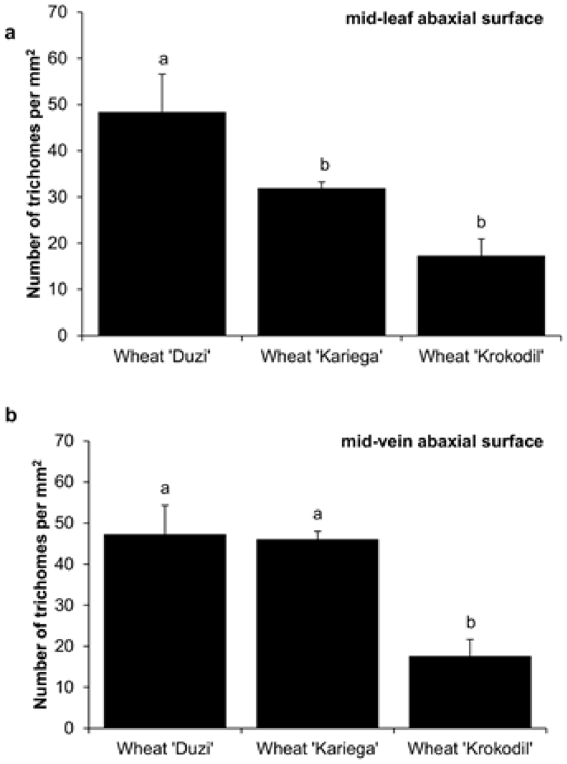

Figure 3.6: Trichome density (mean ± SE) of three wheat cultivars on the abaxial surface along the mid-leaf (**a**) and mid-vein (**b**). Letters above bars indicate significant differences between means (Fisher's LSD test: P < 0.05).

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Trichome density

No trichomes were found on the leaves of the three maize cultivars. However, intraspecific differences in trichome density were found in wheat cultivars for both the mid-vein ($F_{2,27}$ = 8.8, $P < 0.01$; Fig. 3.6a) and the middle section of the leaf ($F_{2,26}$ = 11.83, $P < 0.01$; Fig. 3.6b). Along the mid-vein of the leaves, trichome density of wheat 'Krokodil' was significantly lower than in the wheat cultivars 'Kariega' and 'Duzi' (Figs. 3.7 d,e,f). In the middle section of the leaves between the mid-vein and the edge of the leaf blade, wheat 'Duzi' had the highest trichome density, followed by the wheat cultivars 'Kariega' and 'Krokodil' (Figs. 3.7 a,b,c).

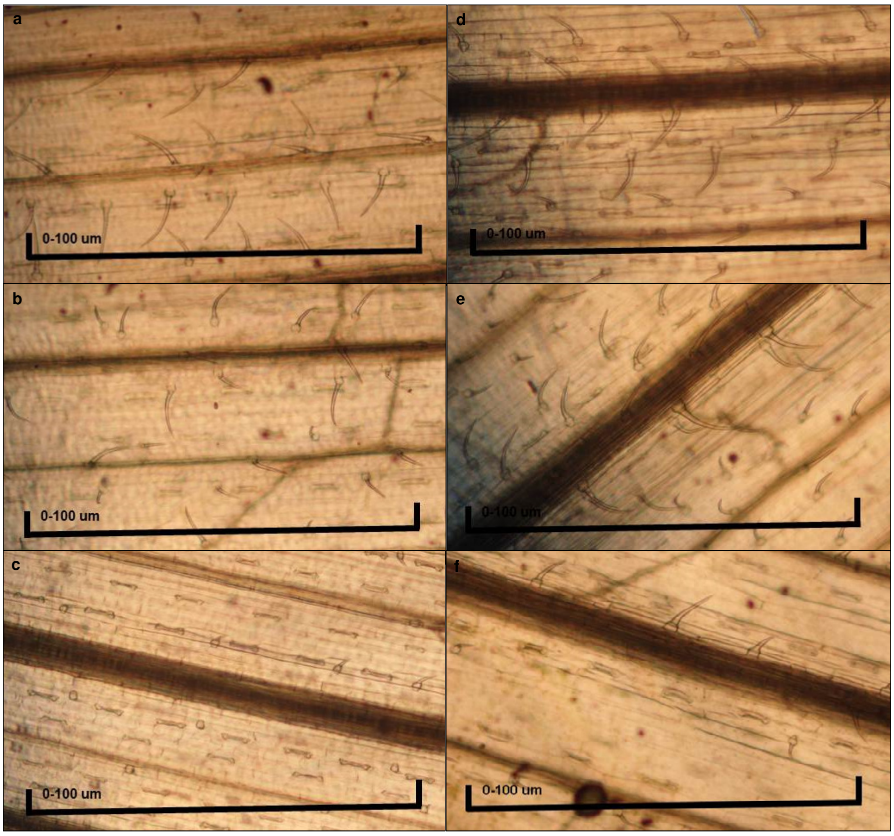

Figure 3.7: Trichomes on the mid-section of the leaf surface of wheat 'Duzi' (**a**), 'Kariega' (**b**), 'Krokodil' (**c**) and along the mid-vein of wheat 'Duzi' (**d**), 'Kariega' (**e**) and 'Krokodil' (**f**).

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3.4 Discussion

Developing non-persistent virus control strategies, such as the use of crop border plants, rely on the understanding of interactions between the insect vector and crop plant. In this study we show that *R. padi* landed in similar numbers on maize and wheat cultivars, regardless of the plant species they originated from, whereas only one individual landed on the potato cultivars. Moreover, *R. padi* showed intraspecific differences in settling and reproduction rates between the maize and wheat cultivars, depending on the plant species they originated from. This suggests that alate *R. padi* use plant cues to orientate towards their host but will only make a final choice among host plants after plant contact and initial probing has taken place. A higher number of *R. padi* landed in maize and wheat and lucerne (non-host plant) than potato plots, but colonized maize and wheat, in a field study on aphid plant preference (Schröder & Krüger 2014). The results of both the laboratory and field study show that maize and wheat appear to be suitable crop border plants for potato.

After plant contact and gustatory evaluation, arrestment or take-off occurs depending on the plant characteristics perceived by the aphids (Bruce *et al.* 2005; Powell *et al.* 2006). In the present study, *R. padi* alatae landed in similar numbers on maize and wheat cultivars but subsequently settled in significantly different numbers on these cultivars, with most individuals settling on wheat ‘Kariega’ and wheat ‘Krokodil’ when reared on maize and wheat, respectively. The difference in the number of aphids settling between the three maize cultivars and wheat ‘Duzi’ was not significant, supporting previous findings that settling is influenced by further evaluation of plant surface characteristics and gustatory cues perceived during initial probing behaviour (Kennedy *et al.* 1959; Orlob 1961; Powell 1991). The change in aphid behaviour between dispersal flight and landing or settling on a plant is gradual, so that an aphid may take several short flights before being behaviourally ready to

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settle (Kennedy *et al.* 1961; Kennedy & Booth 1963). This type of behaviour, repeated alighting and taking-off, favours the spread of non-persistent viruses, such as PVY (Kennedy *et al.* 1959; Kennedy & Booth 1963; Swenson 1968). Once the aphid has landed it probes the plant during the plant contact evaluation phase, which is sufficient for aphids to lose the ability to transmit the virus, and is therefore unlikely to contribute to virus spread when taking off from the crop border plant (Powell 1991; Powell *et al.* 1992; DiFonzo *et al.* 1996). Therefore, planting a crop border plant that is attractive to alate aphids and that is a non-virus host combines two mechanisms of crop borders, i.e. trap crop and virus sink (Hooks & Fereres 2006). Strengthening the edge effect by attracting aphids to the border crop away from the main crop thus has the potential to reduce aphid activity and in turn reduces the risk of PVY incidence in potato fields.

Using trap crops as border crops may cause pest populations to increase in number and become a source of alate aphids (Hokkanen 1991; Müller *et al.* 2001). It is therefore important to select a plant where aphid population increase is low. From among the maize and wheat cultivars combined, *R. padi* reared on wheat plants settled most frequently on wheat ‘Krokodil’, and reproduced most successfully on all wheat cultivars evaluated. Aphids reared on maize, on the other hand, settled most frequently on wheat ‘Kariega’, in comparison to maize ‘6Q-121’, ’78-15B’ and wheat ‘Duzi’. However, no difference was found in the number of offspring produced between the maize and wheat cultivars. The higher number of offspring produced on wheat cultivars by aphids reared on wheat could have been due to the higher number of aphids settling on the wheat cultivars rather than wheat being a more suitable host plant. However, in the no-choice trial, the number of offspring produced was higher on wheat than maize for *R. padi* reared on either maize or wheat. Several studies have reported that *R. padi* prefers barley or rye to wheat, as these cereals are more suitable for reproduction (Leather & Dixon 1982; Farrell & Stufkens 1989).

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However, these studies did not include maize. The results of our study indicate that wheat may be a more suitable host for *R. padi* than maize. However, this could be due to the aphid population being better adapted to wheat than maize.

The feeding and oviposition preferences of an insect can be modified by a host plant that it has been previously exposed to (Guldemond 1990; Barron 2001; Gorur *et al.* 2007). This had a discernible effect on settling and reproductive behaviour of *R. padi*. Aphids reared on wheat preferred to settle in higher numbers on wheat. No preference for maize and wheat was found for aphids reared on maize. Similar observations were made for *Aphis fabae* reared on nasturtium (*Tropaeolum majus* L., Tropaeolaceae) and broad bean (*Vicia faba* L., Fabaceae), where a strong conditioning effect was found for nasturtium but not for broad bean (Gorur *et al.* 2007). The mechanisms involved in the conditioning effect in aphids is not clear but could be the result of an inheritance of feeding preferences, based on either a genetic effect or due to environmental factors such as cues transmitted by the parental host plant (Guldemond 1990; Barron 2001). Our findings suggest that wheat has a stronger conditioning effect than maize.

Both wheat and maize are used as rotational crops (crops planted in the same field after a potato planting to avoid accumulation of pathogens in the soil) in producing seed potatoes, and *R. padi* may therefore originate from both crops as well as grasses. However, wheat is a winter crop and maize a summer crop. Maize would thus be better suited as a crop border plant on which aphids do not rapidly reach high population numbers. However, wheat may be used in regions where potatoes are planted during the winter months.

Plant characteristics such as nitrogen content and trichomes have been found to influence aphid reproduction and population density (Roberts & Foster 1983; Bethke *et al.* 1998; Ponder *et al.* 2001). It is unlikely that plant nitrogen content contributed to the

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observed differences in reproduction in our study because no differences were found in the nitrogen content of the plants tested. Differences in trichome density were found between the wheat cultivars, but no relationship was found between trichome density and reproduction of *R. padi*. In the choice trials, the higher trichome density in wheat ‘Duzi’; may have contributed to the lower number of *R. padi* produced in comparison to wheat ‘Kariega’. However, no difference was observed in the number of *R. padi* produced on the three wheat cultivars in the no-choice trials. Roberts & Foster (1983) observed a negative relationship between trichome density and aphid numbers in the wheat cultivars studied. It is unlikely that plants with a high trichome density will be good crop border plants because aphids may not transmit the virus before leaving the plant. That said, the maize cultivars did not have any trichomes and the reproduction rate of *R. padi* in the present study was generally lower on the maize cultivars than the wheat cultivars, indicating that other factors may also be involved.

In summary, *R. padi* preferred to land on maize and wheat cultivars compared with potato. Wheat ‘Kariega’ and ‘Krokodil’ may be more suitable as crop border plants than the three maize cultivars based on aphid settling rates. However, the three wheat cultivars may be inferior because they supported higher aphid numbers than maize. An accumulation of aphids on the border crop will result in high aphid populations, heightening the risk of virus transmission due to increased aphid activity. Therefore, the maize cultivars may be more suitable as crop border plants in potato-producing regions where *R. padi* is abundant. The study identifies plant characteristics such as trichome density that can aid in selecting potential crop border plants. It would be beneficial to the development of crop border plants to identify further such characteristics that can be used to develop crop border selection criteria, taking aphid behaviour into consideration as well as current farming practices.

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Chapter 4

Visual cues and host-plant preference of the bird cherry - oat aphid, *Rhopalosiphum padi* (Hemiptera: Aphididae)[1]

Abstract

Alate aphids respond to short (UV) and long (green - yellow) wavelength stimuli during host-plant searching behaviour. Although many aphids are attracted to yellow, the bird cherry - oat aphid *Rhopalosiphum padi* is attracted to green. As part of identifying suitable plant species for crop border plants for seed potatoes, the attraction of *R. padi* to different shades of green in relation to differences in spectral reflectance of three cultivars of a non-host, potato, and two host-plant species, maize and wheat, were determined. Choice experiments were carried out under laboratory conditions to evaluate aphid landing preference for stimuli of different colours. *Rhopalosiphum padi* alates preferred to land on the colour targets with the highest reflectance. Significantly more alates landed on yellow and lime colour targets with a maximum wavelength reflectance of 46% from 600 – 610 nm and 26% from 525 – 531 nm, respectively. The peak light reflectance of the crop plants ranged between 12% (potato; 532 – 555 nm) and 20% (wheat; 537 - 553 nm). The results on aphid landing preference for different colour targets suggest that *R. padi* will land in higher numbers on the wheat plants, compared to potato, due to their higher peak percentage reflectance. In addition, the study indicates that the wavelength reflectance curves of plants can be used as a characteristic to select possible crop border plants when making choices between seemingly equally suitable plant species/cultivars.

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[1] Schröder, M.L., Glinwood, R., Ignell, R. & Krüger, K. (2014) Visual cues and host-plant preference of the bird cherry-oat aphid, *Rhopalosiphum padi* (Hemiptera: Aphididae). African Entomology 22(2); in press

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Key words:

Rhopalosiphum padi, crop border plants, wavelength reflectance curves, maize, wheat, potato

4.1 Introduction

Insect herbivores use visual and olfactory cues for host plant selection. Alate aphids (Hemiptera: Aphididae) respond to short (UV) and long (green-yellow) wavelength stimuli during the different phases of dispersion, and consequently landing and host-plant searching behaviour (Kennedy *et al.* 1961; Moericke 1962; Moericke 1969; Roach & Agee 1972). After the teneral period (resting phase), alate aphids take off from the plant and distance flight commences; this is followed by attack flight, which is a gradual change in behaviour where the aphid gradually becomes responsive to yellow and green targets (Kring 1972). The autumn leaf colour co-evolutionary theory postulates that phytophagous insects, such as aphids, migrating to their primary host plant in winter prefer green leaves and dull hues of autumn colours (Hardie 1989; Archetti 2000; Archetti & Brown 2004). In contrast, autumn colours, such as red, serve as warning signals to herbivores (Archetti 2000). Besides evolutionary and ecological perspectives, the co-evolution theory has recently brought interest to the role of colour in aphid host-plant selection behaviour from an applied point of view.

Although several studies have found support for the co-evolution theory of autumn colours (Archetti & Leather 2005; Ramírez *et al.* 2008), many of the studies did not take into account the perception of colour from the aphids' perspective (Schaefer & Rolshausen 2007). To address this problem, Döring *et al.* (2009) developed a colour choice model based on testing 70 different colours in the field, tentatively taking both aphid and human photoreceptor characteristics into account. The model indicated that aphids are able to distinguish between red and green leaves, with a tendency to avoid red. These findings were not species specific and did not make any predictions of aphid colour preference in terms of different light intensities of green or yellow. For example, *Hyalopterus pruni* (Geoffroy) was attracted to unsaturated tints of orange-yellow-green with an optimum at yellow, whereas *Aphis fabae* (Scop.) was more attracted to saturated tints that correspond to their respective host plants *Phragmites communis* (Poaceae) and *Beta vulgaris* (Chenopodiaceae) (Moericke 1969). Furthermore, *Rhopalosiphum padi* (L.) was found to be attracted to green leaves of its primary host plant in autumn instead of trees with yellow leaves as predicted by the autumn co-evolution theory and the aphid colour preference model (Archetti & Leather 2005; Döring *et al.* 2009). These findings could be relevant for aphid control on agricultural crops.

A better understanding of the role of visual stimuli in aphid host plant selection behaviour could lead to improved aphid management, e.g. choice of trap colour and colour intensities for aphid monitoring and use of light reflectance to manage aphid - transmitted plant viruses (Döring & Chittka 2007). Many aphid species preferentially respond to yellow targets, and yellow traps are frequently used for aphid monitoring (Broadbent 1948; Heathcote 1957; Basky 2002; Katis *et al* 2006). However, yellow does not attract all aphid species to the same degree. For example, *Myzus persicae* (Sulzer) is more strongly attracted to yellow than are *Sitobion* species (Eastop 1955; Roach & Agee 1972). This could lead to skewed results where yellow-sensitive species are over-estimated and species that are less sensitive to yellow are under-estimated. Besides colour, the reflectance spectra of plant canopies affect aphid landing patterns. Whereas aphid composition and abundance in lime green tile traps in soybean (*Glycine max* L., Fabaceae), resembling the reflectance spectra of the soybean canopy, correctly estimated aphid landing patterns compared to direct plant counts (Irwin 1980), the green tile trap overestimated aphid species colonizing pepper (*Capsicum annuum* L., Solanaceae) (Raccah *et al.* 1985). Reflective mulches are another

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aphid management tool that relies on aphid vision (Döring & Chittka 2007). Silver reflective mulches have been found to delay the onset of *cucumber mosaic cucumuvirus*, *watermelon mosaic* I and II, *zucchini yellows mosaic* and *squash mosaic* in summer squash (*Cucurbito pepo* L., Cucurbitaceae) and cantaloupe (*Cucumis melo* L., Cucurbitaceae) (Brown *et al.* 1993; Stapleton & Summers 2002).

The use of crop border plants has been proposed as an environmentally friendly method to reduce the spread of non-persistent viruses. Aphids tend to land in higher numbers at the edge of a field due to their sensitivity to the long wavelength light reflected from the soil and the contrasting plants (Broadbent *et al.* 1951; Swenson 1968; Minks & Harrewijn 1988). Planting a non-virus susceptible host plant around the edge of fields replaces the edge and the crop border plant may act as a virus sink, a physical barrier or a trap plant (Hooks & Fereres 2006). Therefore, the choice of plant species used as a crop border plant is important and selecting a crop preferred by aphids may increase the edge effect and strengthen the crop border (Nault *et al.* 2004). For example, DiFonzo *et al.* (1996) showed that virus incidence in potato (*Solanum tuberosum* L., Solanaceae) was reduced when using soybean, maize (*Zea mays* L., Poaceae) potato and wheat (*Triticum aestivum* L., Poaceae) as crop border plants, although the use of potato is unfavourable since this is a virus source. In addition, the efficacy of crop border plants may differ depending on the plant species used. For example grain sorghum (*Sorghum bicolor* L., Poaceae) was more effective in reducing incidence of *watermelon mosaic virus* and *papaya ringspot* virus than soybean and peanut (*Arachis hypogaea* L., Fabaceae) in the main crop pumpkin (*Cucurbita pepo* L., Cucurbitaceae) (Damicone *et al.* 2007).

Rhopalosiphum padi uses herbaceous plant species in the Poaceae as secondary host plants and is commonly found on cereals such as maize, wheat, barley (*Hordeum vulgare* L.,

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Poaceae) and oat (*Avena sativa* L., Poaceae) (Dixon & Glen 1971; Leather 1993). Globally, *R. padi* is an economically important pest feeding on all the major cereal crops (Finlay & Luck 2011). In addition to causing major damage to cereal crops when occurring in high numbers, *R. padi* plays an important role in the transmission of non-persistent plant viruses such as *Potato virus Y* in seed potatoes (Dixon & Glen 1971; Radcliffe 1982). *Potato virus Y* causes considerable economic losses to seed potato growers in many parts of the world due to downgrading of seed lots (Radcliffe & Ragsdale 2002). The virus is mainly transmitted by migrating aphids in search of good quality host plants (Boiteau 1997; Ragsdale *et al.* 2001). Non-persistent viruses can be acquired and transmitted within seconds after aphids alight on a plant. Non-colonizing or transient species alighting on non-host plants are thus able to transmit non-persistent viruses before taking off in search of a host plant (Radcliffe & Ragsdale 2002; Gray *et al.* 2010).

A previous study evaluated maize, wheat, soybean and lucerne (*Medicago sativa* L., Fabaceae) as potential crop border plants for aphids based on abundance and species composition in comparison with potato (Schröder & Krüger 2014). The findings suggested that in areas where cereal aphids such as *R. padi* are abundant, maize and wheat have the potential to be used as crop border plants. The current study aims to determine if *R. padi* is attracted to different shades of green and to relate any preference for the spectral reflectance of three cultivars of the non-host, potato, as well as maize and wheat.

4.2 Materials and Methods

Insects

A laboratory culture of *R. padi* was maintained on wheat plants in ventilated wooden cages with a top glass panel (55 x 45 x 35 cm), in a greenhouse (T_{Max} = 24.8 °C; T_{Min} = 10.3

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°C; RH_{Max} = 71.9%; RH_{Min} = 26.8%) at the University of Pretoria. The culture was established with *R. padi* obtained from the Agricultural Research Council - Small Grain Institute (ARC-SGI), Bethlehem, South Africa. The original culture was established with aphids collected from wheat at Tygerhoek Experimental Farm, Riviersonderend, Western Cape (34°9'0''S, 19°54'0''E) and supplemented with specimens collected from wheat in various wheat-growing regions in South Africa.

Alate aphids were obtained by crowding aphids on plants. Alates of varying ages actively flying or walking in the cage were collected for experiments.

Plants

Maize (cultivars 'CRN 3505', '6Q-121', '78-15B'), potato (cultivars 'BP1', 'Hertha', 'Mondial') and wheat (cultivars 'Duzi', 'Kariega', 'Krokodil') were grown from seed. Three maize seeds per pot (12.5 cm diameter) were planted, two potato mini tubers were placed in a pot and *c*. 20 wheat seeds were sown in two rows per pot, respectively. Pots contained an autoclaved soil mixture of river sand and coco peat in a ratio of 4:1. Slow release fertilizer (*c*. 1.6 g per pot) (Grovida, Khula Kahle^{TM} Fruit and Flower, N:P:K (3:1:5)), was added to the soil upon planting. From two weeks after planting, a foliar treatment of micronutrients (TRELMIX trace element solution) was applied to the plants weekly according to the manufacturer's instructions. The plants were grown at 25 °C with natural relative humidity and a photoperiod of 16 h: 8 h light: dark. Maize and wheat were used at plant growth stage 12 and 13 when two and three leaves were completely unfolded (Meier 2001). Potato plants were used at the plant growth stage 17 and 18 when seven and eight leaves had unfolded from the main stem (Meier 2001).

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Aphid response to stimuli of different colours

Choice experiments were carried out in a glasshouse under natural light conditions and T_{Max} = 24.8 °C; T_{Min} = 10.3 °C; RH_{Max} = 71.9%; RH_{Min} = 26.8%, from May to June 2013. Colour cards consisted of circular sticky traps made from laminated card board, painted with a thin layer of insect glue (^{TM}Plantex) and placed in Petri dishes (13 cm in diameter). Five different shades of green card board were selected: lime green (Sadipal, SIRIO^{TM} 170 g), green (Sadipal, SIRIO^{TM} 170 g), dark green (Elle Erre 220 g), olive green (Grafton paper products, 160 g) and mottle green (Mei-Teintes 160g). In addition, white (Sadipal, SIRIOTM 170 g), yellow (Sadipal, SIRIOTM 170 g), grey (Mei-Teintes 160g) and black (Grafton paper products, 160 g) card board were included as controls. Different types of commercially available cardboard had to be selected due to the limited range of green intensities available within one type of cardboard. The colours were randomized within each trial and the Petri dishes were placed in a circle on a grey cement floor in an equidistant manner (Fig. 4.1). Experiments were carried out in ventilated insect cages (80 x 80 x 50 cm) covered with gauze. The cages, which were open at the bottom, were placed over the Petri dishes. Thirty-six aphids were released in the centre from an aluminium foil covered podium (height 30 cm) in a cage. The number of aphids trapped on each colour card was counted after 24 hours. The experiment was replicated 10 times.

Spectral measurements

(Avalight-DHc). A D65 light spectrum was used for the measurements and an AVANTES WS2 white tile as a white standard.

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Statistical analysis

Aphid counts were analysed with a generalized linear model one-way analysis of variance (ANOVA) with a Poisson distribution and a log link function. Means were separated using Fisher's least significant difference (LSD) test. The mean of the percentage of light reflected by the colour targets in the green-yellow spectrum (500-600 nm) was analysed with a one way ANOVA to determine statistical differences. Means were separated using Fisher's LSD. Statistica (Version 11 ©Statsoft, Inc. 1984-2012) was used for data analysis.

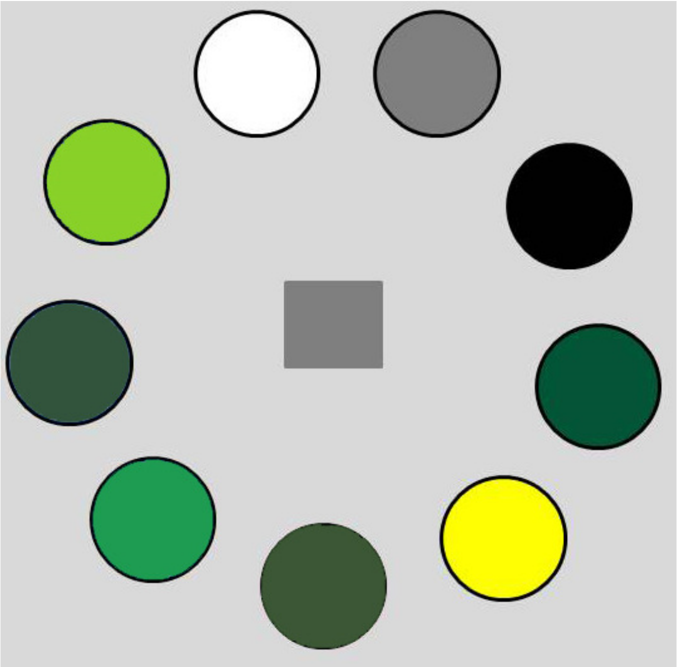

Figure 4.1: Diagram of sticky colour card targets with five different shades of green, white, yellow, grey and black placed equidistantly in a circle on a grey background and an aluminum foil - covered release podium in the centre.

4.3 Results

The landing response of *R. padi* was significantly affected by the colour target (Wald = 95.98; d.f. = 8; $P < 0.01$). Significantly more *R. padi* alates landed on yellow (mean: 4.45 ± 1.35) and lime green (mean: 2.36 ± 0.49) colour targets than on any of the other colour

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targets (Fig. 4.2). The number of alates landing on the yellow and lime green targets did not differ significantly. In addition, there was no significant difference between the number of aphids landing on green, dark green, olive green and mottle green, black, grey or white ($P > 0.05$).

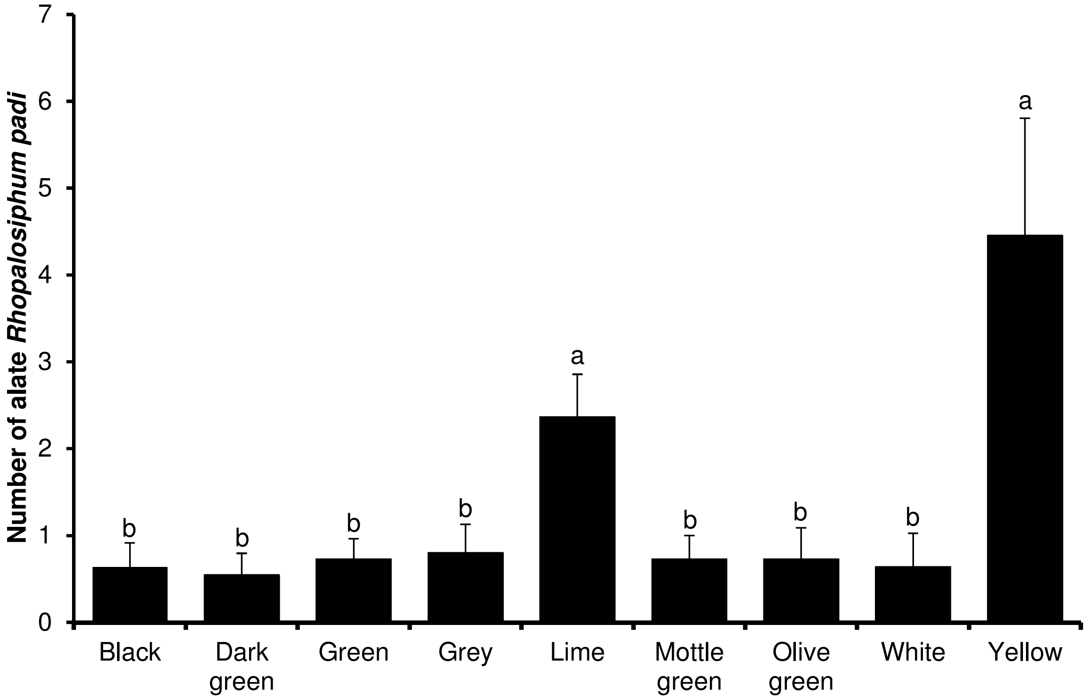

Figure 4.2: Number (mean ± SE) of *Rhopalosiphum padi* alates landing on sticky colour card models used as visual targets. Letters indicate colour targets that are significantly different (Fisher's LSD test: $P < 0.05$).

The yellow colour card's peak percentage reflectance of 46% ranged from 600 to 610 nm, whereas the peak percentage reflection of lime green was 26% at 525 and 531 nm, respectively. Green reflected 19% and 15% at 520 and 550 nm, respectively. The light reflectance curve of the dark green and olive green was similar to the grey model's light reflectance curve, varying between 0 and 10% across the UV-VIS light spectrum. Mottle green reflected less than 10% light between 300 and 659 nm and increased to 12% at 692 nm. White was saturated at 424 – 447 nm. The black and grey colour targets absorbed almost all

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light, reflecting 0 - 3% and 0 - 7% of light, respectively, across the UV-VIS spectrum (Fig. 4.3). Within the long wavelength region (green-yellow, 500-590 nm), grey, dark green and olive green was not significantly different from one another. In contrast, yellow, lime green, green, mottle green, white and black differed significantly from each other and from grey, dark green and olive green in this wavelength region ($F_{8,18}$= 856.8, $P < 0.001$).

The light reflectance curve of wheat 'Duzi', 'Kariega' and 'Krokodil' peaked between 537 and 553 nm, reflecting 18%, 19% and 20% light, respectively. The peak percentage reflectance of the three maize cultivars was between 531 and 556 nm, with maize '6Q-121' reflecting the highest percentage of light (14%) and maize '78-15B' and 'CRN 3505' each reflecting 12% of light at the peak. Potato 'BP1' and 'Mondial' reflected 13% and potato 'Hertha' 15% of light at the peak of the reflectance curve between 532 – 555 nm (Fig. 4.4).

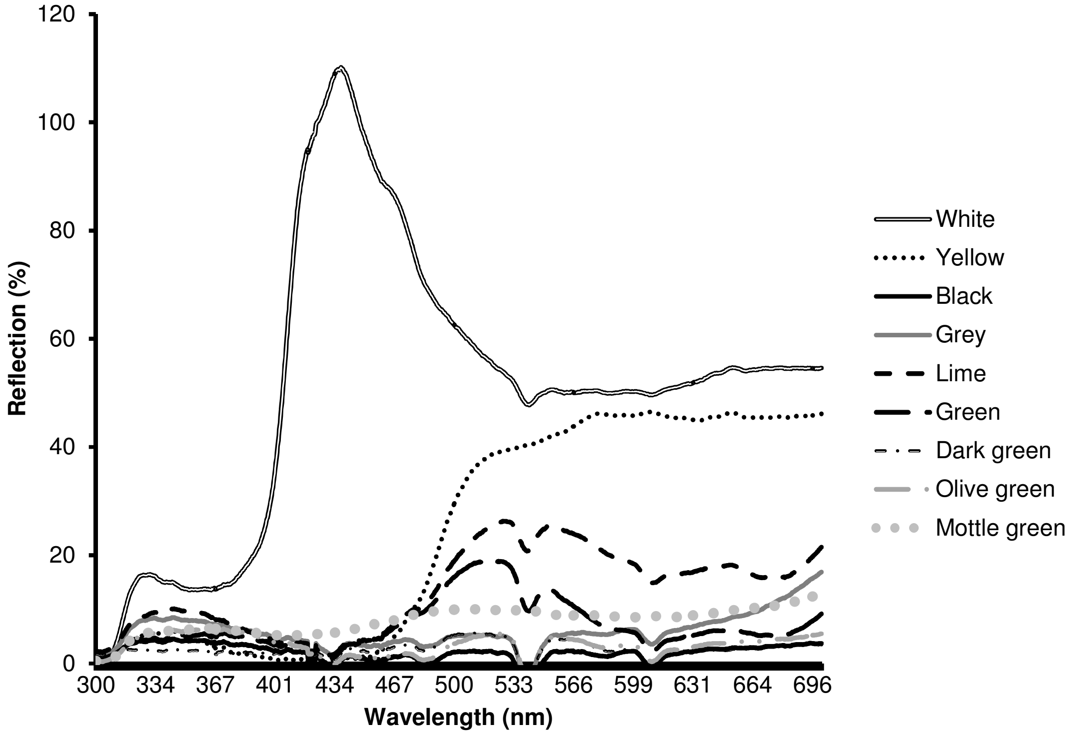

Figure 4.3: Percentage light reflectance of the sticky colour cards used as visual targets for *Rhopalosiphum padi*.

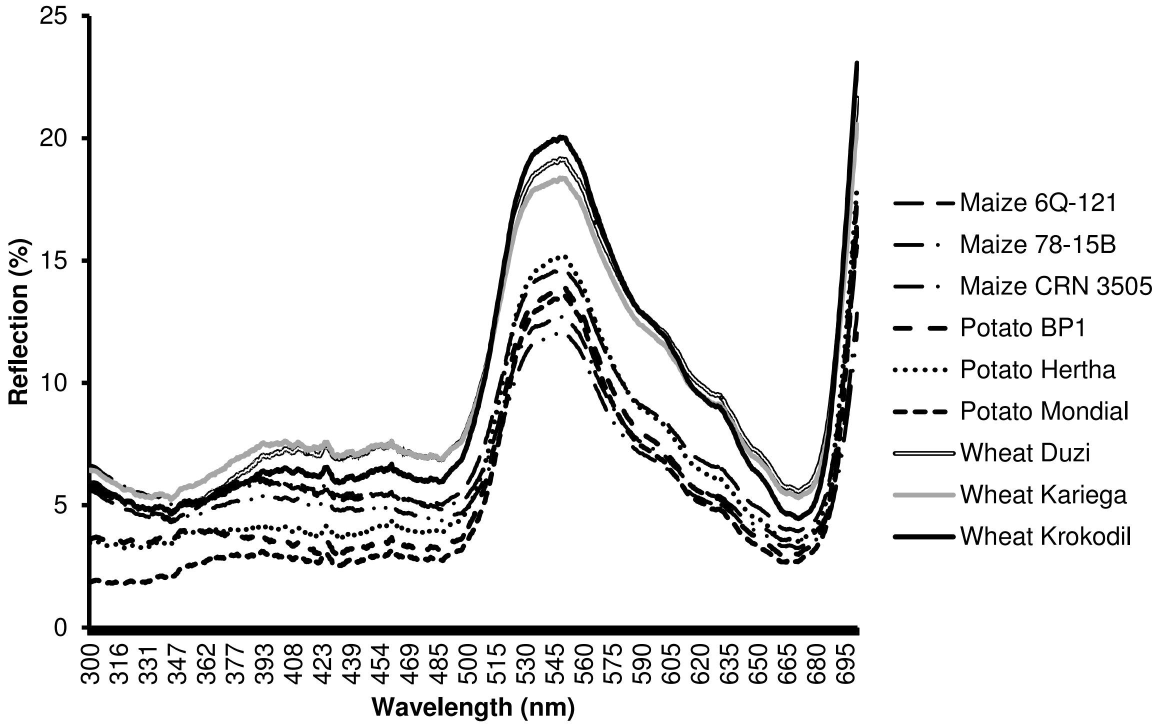

Figure 4.4: Percentage light reflectance of leaves from three maize, potato and wheat cultivars each.

4.4 Discussion

Rhopalosiphum padi preferred light intensities of the yellow and lime green coloured cards to the darker shades of green tested, with similar number of alates landing on the yellow and the lime green cards. Our results are in line with those of Roach & Agee (1972), who observed that *R. padi* summer morphs preferred yellow (Sherwin-Williams Solar Yellow) to green (Sherwin-Williams Vibrant Green). The attraction of *R. padi* to lime green indicates that this species may be able to distinguish between different intensities of green as well as grey, white and black. The lack of attractiveness of white confirms that, like for other aphid species (Kennedy *et al.* 1961; Moericke 1962), *R. padi* prefers long (green – yellow) to short (UV) wavelength light during the alighting phase, as demonstrated in field experiments

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by (A'Brook 1973). The fact that few alates landed on black, grey and the darker shades of green also suggests that *R. padi* requires reflective targets for attraction.

The percentage of light reflected from plants and colour traps play an important role in wavelength discrimination in aphids (Moericke 1955; Kennedy *et al.* 1961). Roach & Agee (1972) suggested that the higher number of *R. padi* alates caught in yellow compared to green traps was due to the increased brightness of the yellow traps reflecting light at a peak of 100% whereas the green traps reflected light at a peak of 45%. Eastop (1955) showed that the preference of yellow observed for *R. maidis* (Fitch) varied with the intensity of sunlight, emphasizing that light intensity (percentage light reflected) may be an important cue in host plant choice. Likewise, Moericke (1969) found that intensity was an important cue for *H. pruni,* which preferred dull hues of yellow over other hues. Similarly, light intensity is an important cue for *R. padi*, which preferred to land on targets with the highest percentage reflectance in the long wavelength region, in our study.

Kennedy *et al.* (1961) proposed that the main function of colour vision in aphids is to distinguish between light reflected from the sky and the plants. During the first phase of dispersal/distance flight, aphids are attracted to short wavelength light, i.e. UV light reflected from the sky, and do not respond to long wavelengths reflected by green or yellow foliage. This behaviour changes during low level/short distance flight when aphids become responsive to long wavelength light (Moericke 1955). Subsequently, it has been suggested that aphids use the ratio between long and short wavelength light to navigate between plants rather than responding to only the one or the other (Kennedy *et al.* 1961; Kennedy & Booth 1963). Döring & Chittka (2007) also argued that the yellow preference showed by aphids should not be viewed as a true colour preference but rather a response that is dependent on the actual light intensity. The yellow preference observed for some aphid species, including

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summer morphs of *R. padi*, may be due to yellow representing a superfoliage stimulus to herbivorous insects rather than it being an ecological adaptation (Prokopy & Owens 1983). Yellow may stimulate the opponent processing mechanisms thought to be involved in aphid colour vision stronger than green does, because yellow reflects more light in the long wavelength region (green to red) than in the short wavelength region (UV to blue), resulting in a greater excitation of the opponent processing mechanism than with green (Döring & Chittka 2007).

Our results contradict previous findings where *R. padi* was shown to prefer green over yellow (Kieckhefer *et al.* 1976; Archetti & Leather 2005). Kieckhefer *et al.* (1976) found that *R. padi* was more frequently attracted to green compared to yellow in an experiment where coloured photographic filters were used as colour stimuli. However, in their study fluorescent and incandescent bulbs supplied light, and the yellow light had a higher intensity than the green light. Archetti & Leather (2005) counted aphids directly on the leaves of *Prunus padus* (Rosaceae), the primary host plant, and did not measure the wavelength reflectance of the leaves. Light intensity (dull green/bright yellow) was therefore not tested unambiguously and olfactory cues emitted by the host plants likely played an important role in host plant selection. The difference found in the light intensity preference between these and our study could also be due to the aphid morph used in experiments.

Heteroecy causes different aphid morphs to behave differently towards the same host plant cues, including wavelength reflectance, depending on the phase of the life cycle (Dixon 1971; A'Brook 1973; Leather & Dixon 1982; Glinwood & Pettersson 2000). Autumn co-evolutionary studies have focussed on autumn aphid morphs that migrate from the secondary host plant to the primary host plant to mate, oviposit and overwinter in the egg stage. Agricultural studies are generally performed with the summer morph that colonizes

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herbaceous plants, such as crops, and reproduces parthenogenetically. Heteroecious species that feed on secondary herbaceous plants during summer tend to have a greater sensitivity for brighter colours such as yellow (Eastop 1955; Heathcote 1957; Kennedy *et al.* 1961). Furthermore, autumn and summer morphs of aphids display morphological and behavioural differences (Leather & Dixon 1982; Leather *et al.* 1983; Simon *et al.* 1991). Spring emigrants and summer virginoparae display distinct host plant preferences. Once the behavioural change has occurred to migrate to the secondary host, the emigrants will not return to the primary host plant (Glinwood & Pettersson 2000). It is possible that the summer morphs of *R. padi* used in our study are more sensitive to brighter colours such as yellow than the autumn morphs which preferred the more dull green in the study by Archetti & Leather (2005).

It has been shown that wavelength reflectance of plant leaves can be of importance for alighting aphids in *Brevicoryne brassicae* L., which was more strongly attracted to wavelength reflectance of sugar beet leaves (*Beta vulgaris* L., Chenopodiaceae), a non-host, compared to cabbage (*Brassica oleraceae* L., Brassicaceae), a preferred host plant (Kennedy *et al*. 1961). The spectral reflectance of cabbage leaves showed that they reflected more light in the short wavelength (UV) region than the sugar beet leaves (Kennedy *et al.* 1961). Another example is *H. pruni*, which alighted in greater numbers on leaves of its summer host *Phragmites communis* L. (Poaceae) than on leaves of sugar beet (Moericke 1969). These studies suggest that plant leaf colour may affect aphid landing rates. There was little difference in the peak reflectance of maize, potato and wheat in our study. However, the results of the colour choice experiment suggest that *R. padi* will land in higher numbers on the wheat plants, due to their higher peak percentage reflectance (20%) compared to the maize and potato cultivars. Furthermore, a landing preference study showed no difference in the number of *R. padi* landing on maize and wheat (Chapter 3), suggesting that other cues also play a role.

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Understanding aphid vision is a key aspect in the selection of crop border plants. Aphids are known to respond to the contrast in long and short wavelength light reflected by the brown – green interface at the edge of a crop field (Kennedy *et al.* 1961; Minks & Harrewijn 1988). Aphids therefore land in higher numbers at the edge of a crop because they become more sensitive to long wavelength than short wavelength light in the alighting phase (Kennedy *et al.* 1961). Our study demonstrates that *R. padi* is able to distinguish between different intensities of green, based on the percentage of light reflected in the green/long wavelength region. In addition, plants that reflect a higher percentage of light in the green region, such as the wheat plants measured in the present study, may be more attractive compared to plants reflecting a low percentage of light in the green region due to an increase in short/long wavelength light. Therefore, wheat is promising as a good crop border plant for seed potatoes to reduce PVY transmission as it reflected the highest percentage of light in the long wavelength region compared to maize and potato. The study suggests that plant wavelength reflectance can be used as a characteristic to select crop border plants and that the plant to be used for the crop border should reflect a higher percentage of light in the long wavelength region than the main crop.

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Chapter 5

Olfactory responses of *Rhopalosiphum padi* (Hemiptera: Aphididae) to three maize, potato and wheat cultivars and the selection of prospective crop border plants

Abstract

Understanding host plant volatile - aphid interactions can facilitate the selection of crop border plants as a strategy to reduce plant virus incidence in crops. Crop border plant species with attractive odours could be used to attract aphids into the border crop and away from the main crop. Since different cultivars of the same crop can vary in their olfactory attractiveness to aphids, selecting an attractive crop cultivar as a border crop is important to increase aphid landing rates. This study evaluated olfactory responses of the bird cherry-oat aphid *Rhopalosiphum padi* to three maize, potato and wheat cultivars each with the aim of selecting an attractive crop border plant to reduce the incidence of the non-persistent *Potato virus Y* (PVY) in seed potatoes. Volatiles emitted by the crop cultivars were collected and identified using coupled gas chromatography and mass spectrometry. Behavioural responses of alate *R. padi* towards odours of the plant cultivars and synthetic compounds identified from the plants were determined with a four-arm olfactometer. Quantitative and qualitative differences were found between cultivars. *Rhopalosiphum padi* was attracted to odours emitted from maize cultivar '6Q-121' but did not respond to odours from the remaining eight crop cultivars. Volatile compounds from maize and wheat cultivars that elicited a response from *R. padi* and contributed to differences in plant volatile profiles include (*Z*)-3-hexenyl acetate (attractant) and α-farnesene, (*E*)-2-hexenal, indole and TMTT (repellents). We conclude that maize '6Q-121' is suitable as a potential crop border plant based on the behavioural response of *R. padi*

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to the olfactory cues emitted by this cultivar. The findings provide insight into selecting crop cultivars capable of attracting *R. padi* to crop border plants.

Key words

aphids, plant volatiles, host plant, olfaction, attractive, repellent, *Potato virus Y*

5.1 Introduction

The role of olfaction in host plant selection by insects has received considerable attention over the last decades (Pickett *et al.* 1992; Bruce *et al.* 2005; Powell *et al.* 2006; Webster 2012). A majority of these studies have aimed to develop novel pest management strategies, such as the ‘push-pull’ strategy, which is based on pushing pests away from the main crop to be protected and attracting pests to an alternate crop (Picket *et al.* 1992; Picket *et al*. 1997). In contrast, selection criteria based on olfactory cues have not been developed for identifying crop border plants in aphid transmitted non-persistent virus management strategies. Crop border plant species with attractive odours could be used to attract aphids into the border crop and away from the main crop.

Non-persistent viruses are transmitted shortly after aphids land on a plant. Crop borders are aimed at preventing virus-infected aphids from landing on the main crop to reduce the incidence of non-persistent viruses, such as *Potato virus Y* (PVY) in seed potato fields (Fereres 2000, Hooks & Fereres 2006). Aphids land in higher numbers at the edge of the crop and initial probing of the crop border plant causes the aphid to lose its ability to transmit the virus before entering the main crop (Hooks & Fereres 2006). The risk of virus spread from the crop border to the main crop is reduced by the selection of a non-virus host plant (plant not affected by a virus) that acts as a virus sink. However, the efficiency of crop borders may vary. It has been suggested that crop border plants preferred by aphids and which act as a trap crop should be used to increase the efficiency of crop border plants (Fereres 2000, Nault *et al*. 2004; Damicone 2007, Hooks & Fereres 2006). An understanding of aphid host plant interactions is required to optimise such a strategy. Thus, the identification of plant volatiles responsible for attracting aphids can aid in selecting a crop border plant.

Plant volatile compounds from potential host and non-host plants may attract or repel aphids, suggesting that aphid species make use of plant volatile compounds to recognize not only their host plants but also non-host plants (Nottingham *et al.* 1991; Webster *et al.* 2008; Ahuja *et al.* 2010). For example, *R. padi* (L.), alatae and apterae were found to be attracted to volatiles of their host plants, wheat (*Triticum aestivum* L., Poaceae) and oats (*Avena sativa* L., Poaceae) (Quiroz & Niemeyer 1998). In contrast, *Aphis fabae* Scopoli was found to be attracted to odours from its host plant *Vicia faba* L., (Fabaceae) and repelled by non-host odours from summer savory (*Satureja hortensis* L., Lamiaceae) (Nottingham *et al.* 1991; Webster *et al.* 2008). Hence, selecting a plant species that is a host plant of the most important aphid virus vector species will likely be attractive to the aphids. The attractive odours from the host plant will cause the aphids to land in the crop border rather than the main crop, and thereby reduce the number of potential virus vector landing in the main crop.

Volatile compounds released by plants not only differ between species but also between plant cultivars (Degen *et al*. 2004, Storer *et al*. 1993). Although these differences may be subtle, aphids vary in their behavioural response to different cultivars of the same species and may be attracted to one cultivar but neither repelled nor attracted by another. For example, *A. fabae* displayed a preference for odours of the chrysanthemum (derived from *Dendranthema morifolium* (Ramat) Tzvelev, and *D. indicum* (L.) Desmoulins, Asteraceae) cultivars ‘Purple Anne’ and ‘Surfine’ over ‘Hero’ (Storer *et al.* 1993) and was attracted to odours of *V. faba* var. ‘Sutton dwarf’, but neither repelled or attracted by odours of *V. faba*

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var. 'tick bean' (Nottingham *et al*., 1991). This demonstrates that a host plant species is not necessarily attractive and that selection of crop border plants could also depend on cultivar differences.

Understanding the relationship between aphids and the volatiles of their host plants offers the opportunity to incorporate these compounds in integrated pest management strategies. For example, methyl salicylate is a volatile compound identified from the headspace of *Prunus padus* L., (Rosaceae), the primary host of *R. padi*, on which it overwinters (Dixon 1971; Pettersson *et al.* 1994). The autumn morphs are attracted to methyl salicylate but the spring morphs that settle on cereal crops are repelled by methyl salicylate (Pettersson 1970a; Glinwood & Pettersson 2000). Releasing methyl salicylate, and the density related substances sulcatone, sulcatol and 2-tridecanone, significantly reduced the settling rate and density of *R. padi* populations in wheat fields (Ninkovic *et al.* 2003). Therefore, it is unlikely that odours of plant species that emit compounds associated with the primary host or herbivore feeding will be attractive to aphids. Although choosing cultivars of the main crop that emits repellent compounds may reduce the number of potential virus infected aphids landing.

Rhopalosiphum padi, the bird cherry - oat aphid, a vector of PVY in seed potatoes, colonizes grasses and cereals (Poaceae) during the summer months in regions where they overwinter holocyclic (Katis & Gibson 1985; Dixon 1971; Blackman & Eastop, 2000). However, in the absence of the primary host, *Prunus padus*, in South Africa, they overwinter anholocyclic on a secondary host plant (Dixon 1971; Blackman & Eastop, 2000; Uusitalo 2004). PVY is a non-persistent virus that causes major economic losses to the seed potato industry world-wide (Radcliffe 1982). In a previous study, aphid landing rates, species composition and abundance were determined on lucerne, maize, potato, soybean and wheat in

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a small-scale field trial to identify potential crop border plants to reduce PVY incidence in seed potato fields. Aphid landing patterns indicated that in regions where cereal aphids such as *R. padi* are abundant, maize and wheat have the greatest potential to be used as crop border plants (Schröder & Krüger 2014).

The aim of this study was to examine the olfactory response of *R. padi* to three maize, potato and wheat cultivars each to identify the most attractive host. To determine key volatile compounds that attract or repel *R. padi*, the volatile profile of each cultivar, as well as qualitative and quantitative differences among the cultivars, was established. The behavioural responses of *R. padi* to different concentrations of volatile compounds identified from the maize, wheat and potato cultivars were evaluated in olfactometer tests.

5.2 Materials and Methods

Plants

Maize cultivars 'CRN 3505', '6Q-121' and '78-15B', wheat cultivars 'Duzi' 'Kariega', 'Krokodil' and potato cultivars 'BP1', 'Hertha' and 'Mondial' were used for plant volatile entrainments and bioassays. Maize and wheat seeds were treated with fungicides but no insecticides were applied to the seeds or plants during the study. For the volatile entrainments one maize seed and *c.* 20 wheat seeds of the same cultivar were planted per pot (10 cm diameter) containing potting soil. The plants were grown in a glasshouse under natural summer daylight conditions. Potato mini tubers, for plant volatile entrainments and bioassays, were pre-sprouted and planted, one tuber per pot (12.5 cm diameter). The potato plants, as well as the maize and wheat plants used in bioassays, were grown in a soil mixture consisting of river sand and coco peat in a ratio of 4:1. The soil was autoclaved before planting to remove all possible pathogens. Agricultural lime (5 ml per pot) and slow release

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fertilizer (*c.* 1.6 g per pot) (Grovida, Khula Kahle^{TM}, Fruit and Flower food N:P:K (3:1:5)) was added to the soil upon planting. Two weeks after planting, a foliage treatment of micronutrients (Trelmix trace element solution) was applied to the plants weekly according to the manufacturer's instructions. The potato plants were grown in a climate controlled room at 25°C, a photoperiod of 16h:8h light: dark and at ambient relative humidity. For all experiments, maize and wheat plants were used at BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) growth stage 11 and 12 when two to three leaves had unfolded and the potatoes at growth stage 17 and 18 when seven and eight leaves had unfolded from the main stem (Meier 2001).

Insects

A culture of *R. padi* was established at the University of Pretoria in 2009 with aphids obtained from a culture maintained on wheat at the Agricultural Research Council - Small-Grain Institute (ARC-SGI) in Bethlehem, South Africa. The original culture was established with aphids collected from wheat at Tygerhoek Experimental Farm, Riviersonderend, Western Cape (34°09'S, 19°54'E) and supplemented with specimens collected from wheat in various wheat-growing regions in South Africa. Aphids were reared in wooden ventilated cages with a glass panel at the top (45 x 55 x 32 cm) in a climate-room at 22 °C, natural relative humidity and 16h:8h light: dark photoperiod. Aphids were provided with a mix of maize and wheat cultivars, separately, to prevent the aphids preferring a cultivar due to previous experience. Aphids were reared on respective host plants for more than six months before used in experiments. *Rhopalosiphum padi* takes 6 and 22 days to complete a generation at 13 and 26 °C (Villanueva and Strong 1964).

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Alate production was induced by crowding the aphids. Only actively moving/walking alates of varying age were collected from the top glass panel of the cages. To avoid bias based on the plant species on which aphids were originally reared, experiments were completed with aphids reared on maize and wheat in equal numbers. Individual aphids from the culture were sent to Ian Millar at the Biosystematics division of the Agricultural Research Council – Plant Protection Research Institute (ARC – PPRI) to verify the identification of the species.

Chemicals

Chemicals used were nonane, β-pinene (purity 99%), acetophenone, methyl salicylate (≥99%), (*E*)-2-hexenal, (*E*)-2-hexenyl acetate, (*Z*)-3-hexen-1-ol, cumene (98%), linalool, limonene (97%), β-caryophyllene (≥98.5%), linalool oxide (≥97%) (*Z*)-3-hexenyl acetate (98%), indole (>99%), (+)-cyclosativene (99%), β-myrcene (90%), (*E,E*)-α-farnesene, (*Z*)-3-hexenal (50%), α-humulene (>96%) obtained from Sigma-Aldrich, St Louis, Missouri, USA. 3-methyl pentadecane and 3-methyl tridecane were obtained from Chrion (Trondheim, Norway). (*E*)-ocimene and TMTT ((3E,7E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene) were obtained from Robert Glinwood (Swedish University for Agricultural Sciences, originally obtained from Rothamsted Research, UK). Dilutions of 100, 10 and 1 ng/µl were made from all the chemicals with dichloromethane (Sigma-Aldrich, St Louis, Missouri, USA) as a solvent.

Plant volatile collection and analysis

All equipment was baked overnight before use; glassware was washed with detergent, and acetone, and rinsed with distilled water prior to baking. Charcoal filters, Teflon tubes,

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and glass rods were baked at 180 °C. A flow of nitrogen was passed through the charcoal filters to prevent oxidization of breakdown products during baking. Glasstubes containing Porapak Q (50 mg) tubes were connected to a flow of nitrogen and placed in a heat block at 150 °C. Polyethylene terephthalate (PET) baking bags and foil were baked at 140 °C. The soil was covered with aluminium foil and two glass rods were placed in the soil in the opposite plane of the plants to keep the bag from constricting the plants. Pots with plants were placed inside PET bags (35 X 43 cm, Melitta Scandinavian AB, Sweden) and the open end tied closed (Fig. 5.1). Charcoal-filtered air was pushed in from the bottom of the bag at 600 ml/min and pulled out through a Porapak tube at 400 ml/min, positioned at the top. The positive pressure in the bag kept air from outside entering the bag and an airtight seal was not required. Volatiles were collected for 72 hours. Controls, pots with soil covered in aluminium foil, were included. After 72 hours, Porapak tubes were eluted with 750 µl redistilled dichloromethane and the eluted solvent concentrated to 50 µl under a gentle nitrogen flow. An internal standard (1-nonene) was added to the eluted sample before concentration of the sample to achieve a concentration of 1.8 ng/µl in the final sample.

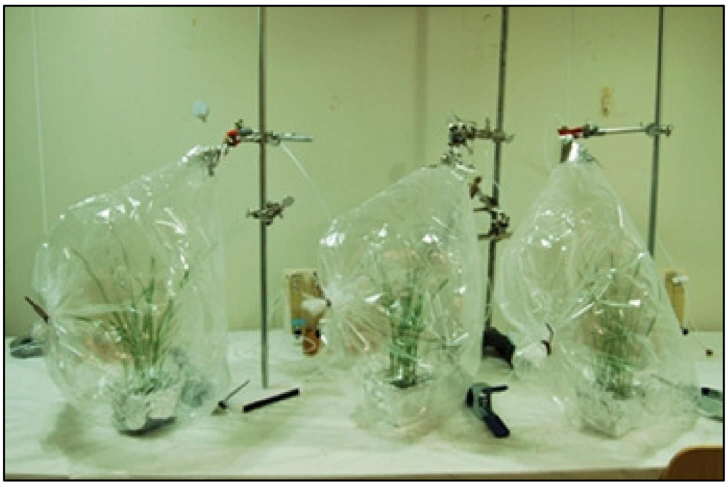

Figure 5.1: Volatile entrainment of wheat plants.

Plant volatiles were analysed using coupled gas chromatography and mass spectrometry (GC-MS). A 1 µl aliquot of the entrained sample was injected into an Agilent

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7890A GC (Agilent Technologies) equipped with a cold-on-column injector and fitted with an HP-5 column (100% dimethyl polysiloxane, 30 m, 0.25 mm i.d. and 0.25 µm film thickness, J&W Scientific, USA) coupled to an Agilent 5975C mass selective detector (electron impact 70 eV, 230 °C). The GC program was set to start at 30 °C for 4 min, and set to rise 8 °C/min to 250 °C. The carrier gas was helium with a flow rate of 1ml/min. Volatile compounds were identified by comparing the mass spectra and retention indices against a commercially available library (NIST 08) and commercially available authentic standards where available. Quantifications were made using ion counts of identified compounds, correcting for injection error by relating to that of the known amount of internal standard.

Behavioural assays

Responses of *R. padi* to plant volatiles of maize, wheat and potato plants as well as to the volatile compounds identified from these plants were tested using a four-arm olfactometer, as described in Pettersson (1970b). The arena consisted of four arms (100 mm in diameter) cut out and placed between two layers of Perspex screwed together. White filter paper (Whatman 2, 90 mm, Merck, (Pty) Ltd, South Africa) was placed on the floor of the chamber for the aphids to walk on, and replaced between each experiment. To avoid bias caused by light, a light bulb (OSRAM, (Pty) Ltd, South Africa, Mini Twist® energy-saving lamp, Cool White, 8 Watt) was placed in the centre, 50 cm above the chamber. Each olfactometer arm had a gauze-covered inlet connected to an odour source chamber (3 l glass jar) with polytetrafluoroethylene (PTFE) tubing (ID = 2 mm; OD = 4 mm). This set-up was used for testing odours from the intact plants. In experiments testing synthetic plant volatile compounds, the odour sources were placed in small glass tubes (45 cm wide, 25 cm at the base and 5 mm at the tip) that connected directly to the olfactometer chamber arms (Fig. 5.2).

An airstream of 400 ml/min was created by removing air from the centre of the chamber with a vacuum pump. Air passed through a charcoal filter to remove any impurities. Filtered air flowed over the odour source into each of the four arms towards the centre of the chamber. The evenness of the airflow in the olfactometer arena was tested before commencing with experiments by lining the arena with litmus paper and using hydrochloric acid (HCl) vapours as an odour source. A single alate aphid was introduced into the centre of the chamber and observed for 10 min, during which the time spent in each of the four arms were recorded using OLFA (Udine, Italy) software. If an aphid did not choose an arm within 3 min it was considered non-responsive and discarded. Each treatment was replicated 20 times. Between replicates (each aphid) the chamber was rotated 90° clockwise. After testing five aphids, the equipment and odour source were replaced with clean glassware and olfactometer chamber and a new odour source. Before each experiment, all equipment was washed with a household detergent (Teepol, Acorn Products (Pty) Ltd, South Africa). The glassware was washed with acetone, whereas the Perspex chambers and PTFE tubing were washed with ethanol. All equipment was then rinsed with distilled water. The glassware and PTFE tubing were baked at 180°C overnight. The Perspex chambers were left to air dry.

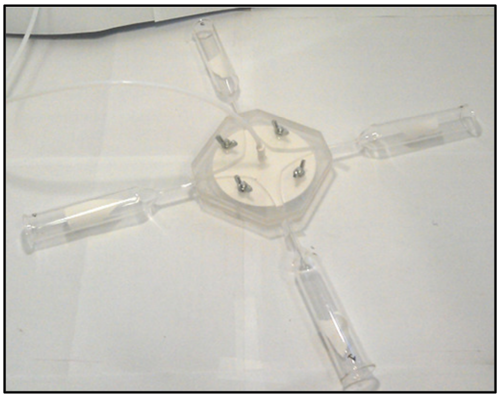

Figure 5.2: Four arm olfactometer testing synthetic plant volatile compounds.

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The responses of *R. padi* to volatiles emitted from maize ‘CRN 3505’, 78-15B’ and ‘6Q-121’, potato ’BP1’, ‘Hertha’ and ‘Mondial’, as well as wheat ‘Duzi’, ‘Kariega’, and ‘Krokodil’ were each tested against a control. The odour source (plant) was placed at the end of two opposing arms and controls (pots with soil) at the end of the remaining two arms. The soil in both the control and plant pots was covered with aluminium foil as much as possible to reduce the effect of soil odours.

Responses of *R. padi* to each of the compounds identified from the three maize, potato and wheat cultivars at three different concentrations, 100, 10 and 1 ng/µl were tested in the following way. Aliquots of 10 µl of each treatment were placed on a filter paper triangle (Whathams No. 2, 90 mm) and allowed to air dry for 30 s and then placed in glass tubes. Filter paper triangles were made by cutting a round piece of filter paper of 90 mm in diameter in 8 equal sections. Two opposing arms contained the test stimulus and the remaining two arms contained dichloromethane solvent (control).

Statistical analysis

Intraspecific differences of the quantities of the total amount of volatiles and the individual compounds identified from the maize, potato and wheat cultivars were determined for each plant species/cultivar separately. Differences in the quantities of total volatiles released by maize and wheat cultivars were analysed with a one-way analysis of variance (ANOVA). Data were $\log_{10}(x+1)$ transformed to meet the requirements of normality and homogeneity of the analysis. Means were separated using Fisher’s least significant difference (LSD) test. The total amount of volatiles released by potato cultivars could not be transformed and a non-parametric Kruskal-Wallis ANOVA followed by multiple comparisons of mean ranks for all groups was used to determine intraspecific differences.

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Separate analyses were performed to determine intraspecific differences in the individual plant volatiles released by each plant species. A one-way ANOVA was used for normally distributed data and means were separated using Fisher's LSD. Non-parametric data were analysed using a Kruskal-Wallis ANOVA followed by multiple comparisons of mean ranks for all groups (cultivars for each species). Principal components analysis was used to determine differences and similarities in volatile composition between cultivars for each plant species, maize, potato and wheat separately. Differences in time spent between the odour and control arms during the four arm olfactometer bioassays were analysed using a student's t-test for dependent samples for normally distributed data. The significance level was set at $P < 0.05$ for all analyses. All analyses were performed using Statistica (Version 11 ©Statsoft, Inc. 1984-2012).

5.3 Results

Plant volatile collection and analysis

Intraspecific differences occurred in the total amount of plant volatiles released by maize ($F_{2,24} = 3.72$, $P = 0.039$) and wheat ($F_{2,24} = 8.9$, $P = 0.001$), but not potato ($H = 1.16$, $P = 0.558$) (Fig. 5.3). Maize '6Q-121' emitted a lower amount of total plant volatiles than maize 'CRN 3505' and '78-15B' (Fig 5.3a). Wheat 'Duzi' released the highest amount of plant volatiles followed by wheat 'Krokodil' and wheat 'Kariega' (Fig. 5.3c).

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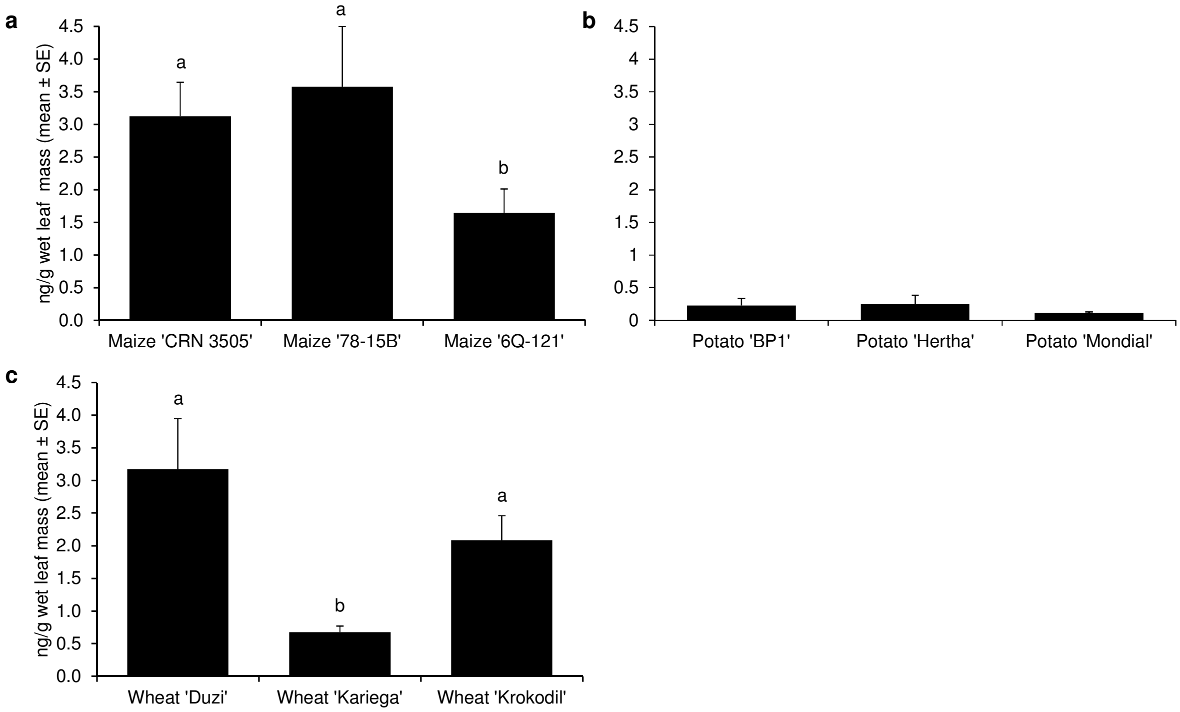

Figure 5.3: Amount of plant volatiles released by three maize (**a**), potato (**b**) and wheat (**b**) cultivars. Letters above bars indicate significant differences (Fisher's LSD test: $P < 0.05$).

Twenty-seven compounds were identified from the three maize cultivars. Linalool oxide, TMTT and two unknown sesquiterpenes, 2 and 12, were identified from maize 'CRN 3505' and '78-15B' but not '6Q-121'. (*Z*)-3-hexenal was identified from maize '78-15B' only. Maize 'CRN 3505' emitted α-farnesene and unknown sesquiterpene 5. α-Humulene and two unknown sesquiterpenes, 6 and 7, were identified from maize '6Q-121' and 'CRN 3505' but not from maize '78-15B'. Linalool and the unknown sesquiterpene 11 were recorded in significantly lower amounts in maize 'CRN 3505' and '78-15B' than '6Q-121' (Table 5.1).

Ten compounds were identified from the potato cultivars with little difference between the compounds identified from the three cultivars, with the exception of limonene, which was recorded from potato 'BP1' and not from 'Hertha' or 'Mondial' (Table 5.1).

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Sixteen compounds were identified from the three wheat cultivars. Wheat 'Duzi' had 15 compounds; wheat 'Kariega' had 13 and wheat 'Krokodil' 14 compounds. (*E*)- 2-hexenal, 3-methyl tetradecane and TMTT was identified from wheat 'Duzi' and 'Kariega' but not wheat 'Krokodil. The plant volatile profile of wheat 'Duzi differed from 'Kariega' and 'Krokodil' by containing higher amounts of (*Z*)-3-hexen-1-ol, β-myrcene, (*Z*)-3-hexenyl acetate and linalool. Indole and α-farnesene were recorded from 'Krokodil' but not from 'Duzi' and 'Kariega'. Significantly lower amounts of 3-methyl pentadecane were recorded from 'Krokodil' (Table 5.1).

Based on the plant volatile profiles it was possible to distinguish between maize, potato and wheat cultivars. The PCA analysis for maize cultivars indicated that factor 1 and 2, accounted for 30% of the variation only (Fig 5.4a). However, maize '6Q-121' was separated from maize 'CRN 3505' and '78-15B', as projected by factor 1 and 2 (Fig. 5.4a). β-myrcene, (*Z*)-3-hexenyl acetate, (*E*)-ocimene and sesquiterpenoid 11 were identified from factor 1, and factor 2 identified sesquiterpenoid 1 and 12 as the compounds mainly responsible for the variation between the maize cultivars. The variation between potato cultivars accounted for by factor 1 and 2 was 59% and 22%, respectively (Fig. 5.4b). The PCA analysis indicated a separation between potato 'BP1', 'Hertha' and 'Mondial'. Nonane, cumene, β-pinene, 2,2,4,6,6,-pentamethylheptane, acetophenone, methyl salicylate, and an unknown sesquiterpenoid 14 contributed to factor 1 and 4-methyl-octane, limonene, and (*E*)-4,8,-dimethyl-1,3,7-nonatrien-6-one (DMNT) contributed to factor 2.

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Table 5.1: Plant volatile compounds (mean ± SE) identified from three maize, potato and wheat cultivars. Letters indicate significant differences in compounds within plant cultivars.

	Quantity ng/g leaf weight								
	Maize			Potato			Wheat		
Compound	**CRN 3505**	**78-15B**	**6Q-121**	**BP 1**	**Hertha**	**Mondial**	**Duzi**	**Kariega**	**Krokodil**
Aldehydes									
(*E*)-2-hexenal							0.003 ±0.002	0.007 ±0.007	
(*Z*)-3-hexenal		0.015 ±0.013					0.025 ±0.011	0.002 ±0.002	0.022 ±0.016
Ketones									
Acetophenone				0.024 ±0.016	0.006 ±0.003	0.013 ±0.010			
(*E*)-4,8-dimethyl-1,3,7-nonatrien-6-one				0.037 ±0.021	0.125 ±0.074	0.070 ±0.053			
Alcohols									
(*Z*)-3-hexen-1-ol	0.049 ±0.016	0.046 ±0.033	0.037 ±0.017				0.119 ±0.037^{a}	0.021 ±0.008^{b}	0.098 ±0.039 ^{ab}
Esters									
(*E*)-2-hexenylacetate							0.008 ±0.003		0.003 ±0.002
(*Z*)-3-hexenylacetate	0.160 ±0.054	0.470 ±0.332	0.165 ±0.055				2.407 ±0.761 ^{a}	0.163 ±0.056^{b}	0.860 ±0.354 ^{ab}
1-Octen-3-ol							0.005 ±0.002	0.005 ±0.003	0.012 ±0.004
Alkanes									
2,2,4,6,6-Pentamethylheptane				0.061 ±0.026	0.014 ±0.008	0.035 ±0.021			
3-Methyl pentadecane							0.097 ±0.016^{a}	0.072 ±0.010^{a}	0.037 ±0.007 ^{b}
3-Methyl tetradecane							0.044 ±0.008	0.038 ±0.006	
3-Methyl tridecane							0.190 ±0.040	0.195 ±0.030	0.107 ±0.032
4-Methyl octane				0.019 ±0.011	0.067 ±0.041	0.014 ±0.010			

				Quantity ng/g leaf weight					
		Maize			**Potato**			**Wheat**	
Compound	**CRN 3505**	**78-15B**	**6Q-121**	**BP 1**	**Hertha**	**Mondial**	**Duzi**	**Kariega**	**Krokodil**
Nonane				0.019 ±0.011	0.011 ±0.005	0.007 ±0.004			
Terpenoids									
(*E*)-ocimene	0.051 ±0.022	0.195 ±0.137	0.003 ±0.003				0.047 ±0.010	0.077 ±0.015	0.031 ±0.019
β-pinene				0.009 ±0.005	0.002 ±0.001	0.002 ±0.001			
Limonene				0.012 ±0.006					
Myrcene	0.091 ±0.027	0.322 ±0.237	0.032 ±0.022				0.044 ±0.009^{a}	0.017 ±0.005^{b}	0.025 ±0.005 ^{ab}
Linalool	0.322 ±0.089^{a}	0.397 ±0.134^{a}	0.042 ±0.019 ^{b}				0.112 ±0.016 ^{a}	0.051 ±0.010 ^{b}	0.087 ±0.014 ^{ab}
Linalool oxide	0.028 ±0.015	0.008 ±0.008					0.056 ±0.018	0.019 ±0.008	0.046 ±0.008
DMNT	0.228 ±0.040	0.179 ±0.113	0.150 ±0.066						
TMTT	0.019 ±0.019	0.020 ±0.017					0.002 ±0.001		0.003 ±0.002
Cyclosativene	0.161 ±0.102	0.084 ±0.026	0.069 ±0.021						
(β)-caryophyllene	0.038 ±0.016	0.015 ±0.008	0.102 ±0.045						
(*E*)-β-farnesene	0.020 ±0.010	0.088 ±0.054	0.033 ±0.027						
α-farnesene	0.263 ±0.086								0.005 ±0.004
α-humulene	0.006 ±0.006		0.028 ±0.011						
Sesquiterpenoid 1	0.120 ±0.036	0.102 ±0.050	0.046 ±0.029						
Sesquiterpenoid 2	0.054 ±0.016	0.016 ±0.007							
Sesquiterpenoid 3	0.025 ±0.014	0.090 ±0.067	0.028 ±0.013						
Sesquiterpenoid 4	0.021 ±0.010	0.069 ±0.028	0.108 ±0.035						
Sesquiterpenoid 5	0.028 ±0.015								
Sesquiterpenoid 6	0.002 ±0.002		0.015 ±0.010						
Sesquiterpenoid 7	0.043 ±0.026		0.047 ±0.024						
Sesquiterpenoid 8	0.099 ±0.016	0.093 ±0.021	0.148 ±0.024						

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	Quantity ng/g leaf weight								
	Maize			Potato			Wheat		
Compound	**CRN 3505**	**78-15B**	**6Q-121**	**BP 1**	**Hertha**	**Mondial**	**Duzi**	**Kariega**	**Krokodil**
Sesquiterpenoid 9	0.010 ±0.010	0.036 ±0.017	0.007 ±0.007						
Sesquiterpenoid 10	0.052 ±0.028	0.002 ±0.002	0.027 ±0.027						
Sesquiterpenoid 11	0.213 ±0.041 ^{a}	0.477 ±0.238 ^{a}	0.051 ±0.033 ^{b}						
Sesquiterpenoid 12	0.083 ±0.031	0.060 ±0.030							
Sesquiterpenoid 13							0.015 ±0.005^{a}	0.011 ±0.002^{a}	0.028 ±0.003 ^{b}
Sesquiterpenoid 14				0.022 ±0.012	0.010 ±0.005	0.017 ±0.013			
Aromatic									
Cumene				0.020 ±0.010	0.003 ±0.002	0.003 ±0.002			
Indole	0.941 ±0.307	0.796 ±0.210	0.503 ±0.232						0.720 ±0.621
Methyl salicylate				0.004 ±0.001	0.007 ±0.004	0.006 ±0.005			

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Similar to the maize cultivars, less than 50% of the variation between the wheat samples was accounted for by factor 1 and 2, 27% and 18% respectively. Wheat 'Duzi' was separated from wheat 'Kariega' and 'Krokodil' on the PCA graph (Fig. 5.4c). Wheat 'Kariega' and 'Krokodil', however showed some separation with only a few samples being similar. Compounds responsible for the separation on factor plane 1 are (*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate and (*E*)-2-hexenyl acetate, and on factor plane 2, are 3-methyl tridecane, 3- methyl pentadecane and 3-methyl tetradecane.

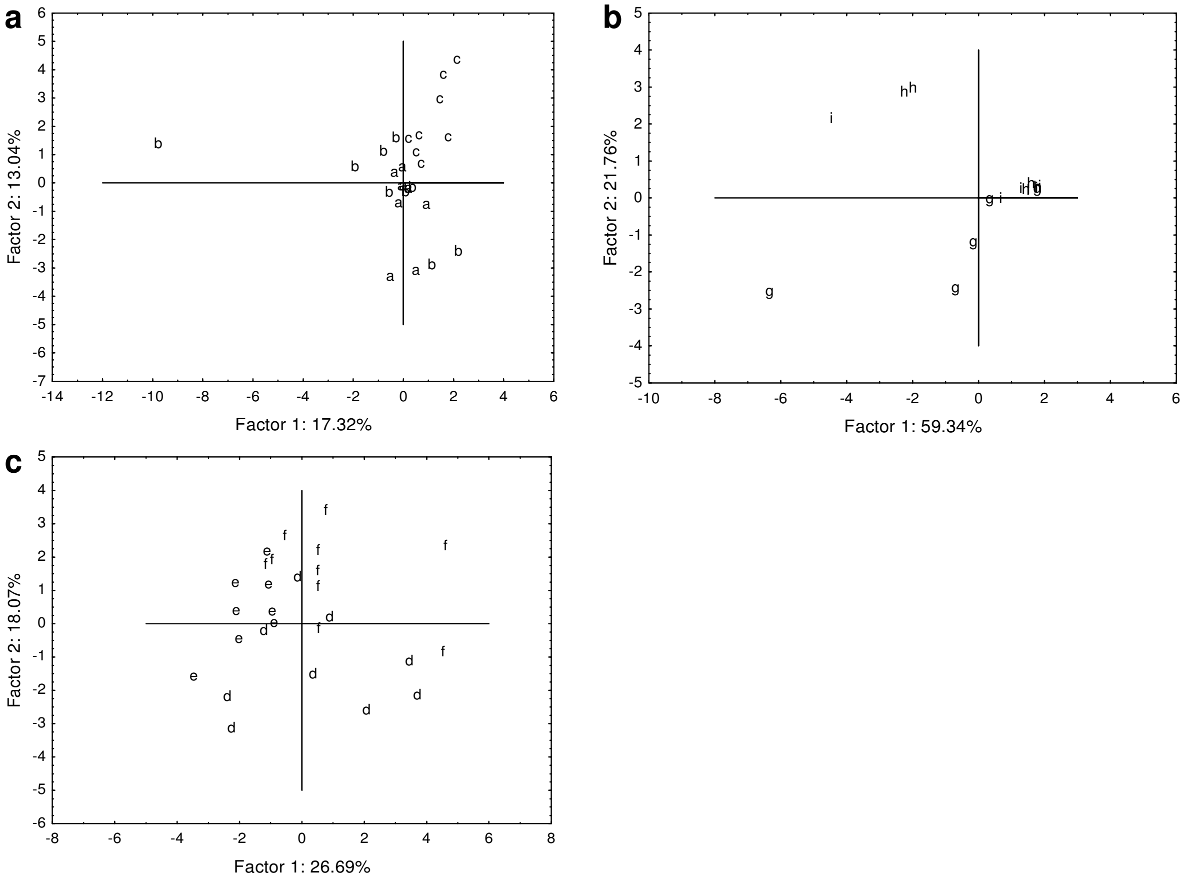

Figure 5.4: Principal components analysis of three maize (**a**), potato (**b**) and wheat (**c**) cultivars. a = maize 'CRN 3505', b = maize '78-15B', c = maize '6Q-121', d = wheat 'Duzi', e = wheat 'Kariega' f = wheat 'Krokodil', g = potato 'BP1', h = potato 'Hertha', i = potato 'Mondial'.

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Behavioural assays

Rhopalosiphum padi was significantly attracted to odours of maize '6Q-121' (*P* < 0.05, Fig. 5.5). The remaining maize, potato and wheat cultivars did not attract or repel alate *R. padi*.

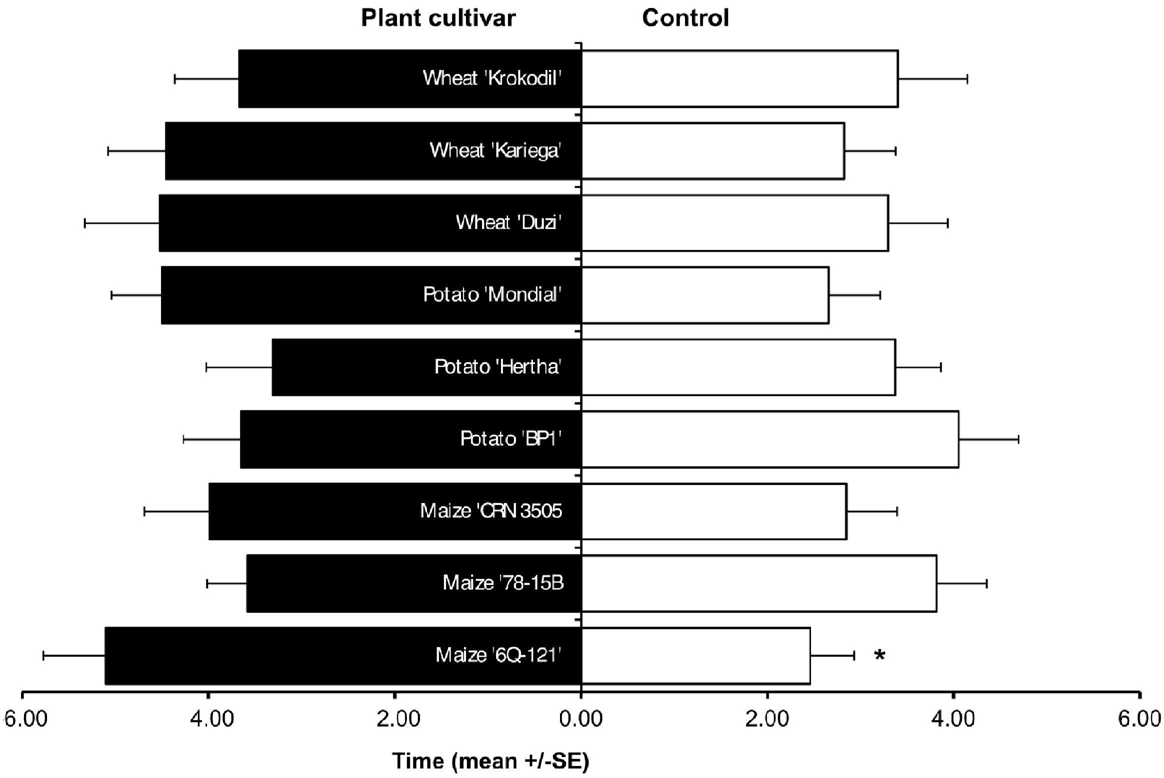

Figure 5.5: Response of alate *Rhopalosiphum padi* to odours emitted from three maize, potato and wheat cultivars each in olfactometer tests. Bars with an asterisk indicate significant differences (Independent t-test: P < 0.05).

Rhopalosiphum padi alates were repelled by α-farnesene at 100 ng/µl and (*E*)-2-hexenal and indole at 10 ng/µl as well as TMTT at 1 ng/µl (P < 0.05, Fig 5.6). (*Z*)-3-hexenyl acetate attracted *R. padi* alates at 100ng/µl. The remaining compounds did not attract or repel *R. padi* at the concentrations tested (Fig 5.6).

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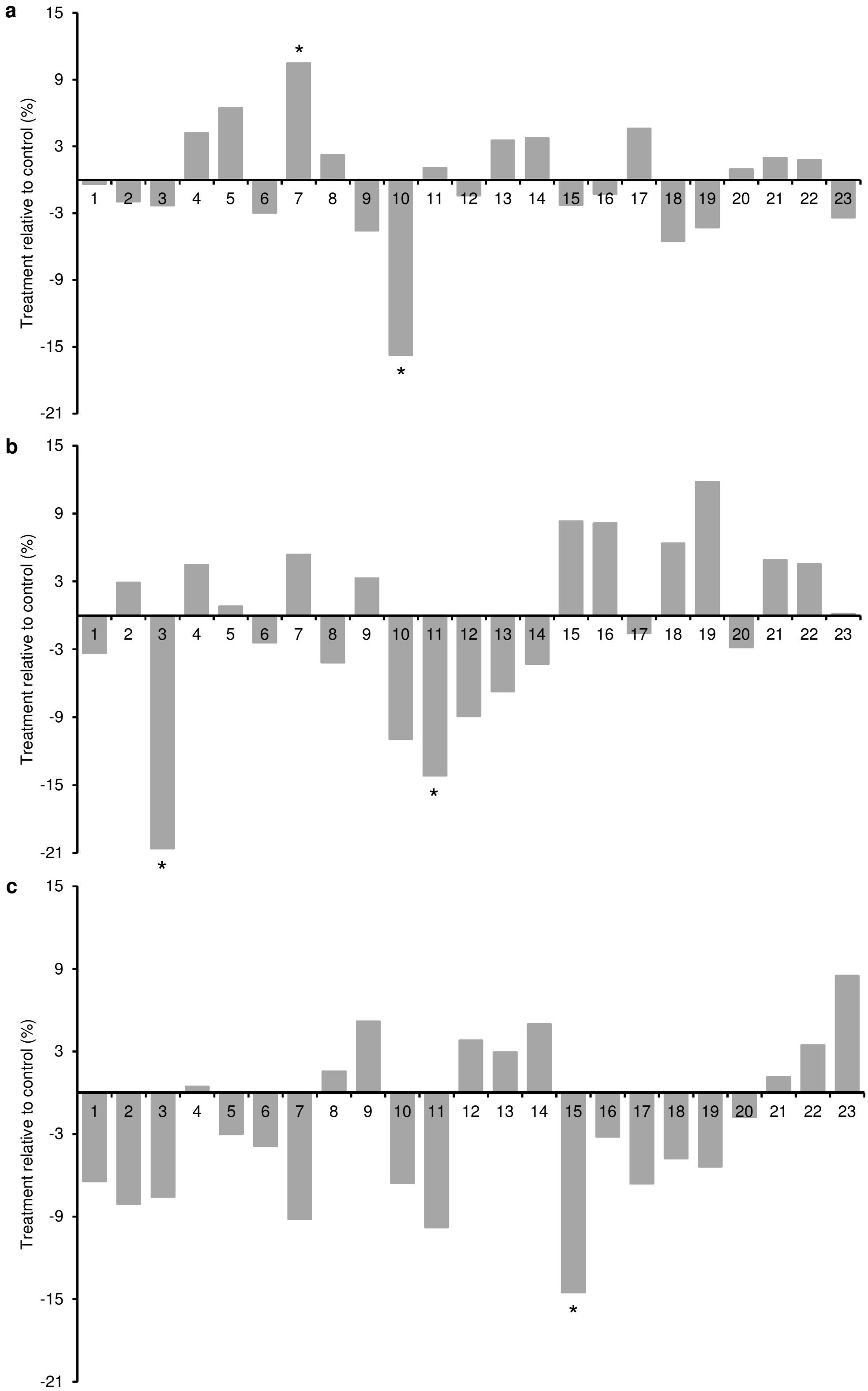

Figure 5.6: Response of alate *Rhopalosiphum padi* to volatile compounds at concentrations of 100 ng/µl (**a**), 10 ng/µl (**b**) and 1 ng/µl (**c**) found in maize, potato and wheat cultivars in olfactometer tests. Twenty three compounds were tested: (1) (*Z*)-3-hexenal, (2) α-humulene, (3) (*E*)-2-hexenal,(4) (*E*)-2-hexenyl acetate, (5) ocimene, (6) (*Z*)-3-hexen-1-ol, (7) (*Z*)-3-

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hexenyl acetate, (8) 3-methyl pentadecane, (9) 3-methyl tridecane, (10) α-farnesene, (11) indole, (12) linalool, (13) linalool oxide, (14) β-myrcene, (15) TMTT, (16) limonene, (17) cyclosativene, (18) caryophyllene, (19) nonane, (20) cumene, (21) β-pinene, (22) acetophenone, (23) methyl salicylate. Bars with an asterisk indicate significant differences (Independent t-test: $P < 0.05$).

5.4 Discussion

Studies aimed at determining aphid behavioural responses to volatiles of different cultivars, especially for *R. padi*, are scarce and direct evidence to account for differences in behaviour is lacking. This is the first study to evaluate the behaviour of *R. padi* in response to odours from maize and the non-host plant potato. *Rhopalosiphum padi* responded to odours of one maize cultivar but not to the other maize and the wheat and potato cultivars. This is unusual because both maize and wheat are host plants of *R. padi.* The lack of response contrasts with previous findings where *R. padi* was attracted to volatiles from wheat and oats (Quiroz & Niemeyer 1998), although the study did not compare cultivars of the same plant species.

Aphids vary in their behavioural response to volatiles emitted by different cultivars of their host plants (Storer *et al*. 1993; Alla *et al*. 2003; PingYan *et al*. 2009). In this study, *R. padi* alates were attracted to volatiles emitted by maize ‘6Q-121’ but did not respond to odours from maize ’78-15B’ and ‘CRN 3505’. *Aphis fabae* showed similar behavioural responses towards different cultivars of its host plant *V. faba* and chrysanthemum (Nottingham *et al*. 1991; Storer & Emden 1995). Although the plant volatile profiles of the different chrysanthemum cultivars were identified, the behavioural response of *A. fabae* to each of these compounds was not determined (Storer *et al*. 1993). Therefore it is not well understood why *A. fabae* preferred one chrysanthemum cultivar over another. The present

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study on the other hand evaluated *R. padi* behavioural responses to each of the compounds identified from the maize cultivars to obtain a better understanding of how aphids respond to plant volatile differences between plant cultivars.

Maize and wheat did not have any volatile compounds in common with potato. In addition, maize and wheat varied more between cultivars than potato. Unlike the other eight cultivars evaluated, no compounds known to repel aphids, such as TMTT and α-farnesene, were recorded from maize '6Q-121'. Maize '6Q-121' differed most from the other two maize cultivars. Many of the compounds identified from the maize and wheat cultivars in the present study were also identified from these plant species previously (Buttery *et al*. 1984; Buttery *et al*. 1985). This high variation between plant cultivars was also observed for maize cultivars in a previous study, which found that a high genetic diversity is conserved within this crop, although no correlation was observed between this diversity and variation in plant volatiles (Degen *et* al. 2004).

Rhopalosiphum padi responded to three compounds identified from maize, TMTT, α-farnesene and (*Z*)-3-hexenyl acetate. Alates were repelled by TMTT at the lowest concentration tested, and maize 'CRN 3505' and '78-15B' released this compound in low amounts (0.019 and 0.020 ng/g leaf weight, each). α-Farnesene repelled *R. padi* at the highest concentration tested and was emitted by 'CRN 3505' in relatively high concentrations (0.263 ng/g leaf weight). (*Z*)-3-hexenyl acetate attracted *R. padi* at high concentrations and was present in all three maize cultivars. It is unusual for TMTT and α-farnesene to be identified from intact plants because both compounds are well-known herbivore-induced plant volatiles (Paré & Tumlinson 1997), but are not commonly identified from undamaged plants (Degenhardt & Gershenzon 2000; Holopainen 2004). However, the presence of TMTT and α-farnesene in maize devoid of herbivore damage has also been recorded in some of the lines

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tested by Degen *et al.* (2004), although these plants had been mechanically damaged. TMTT and α-farnesene indicate the presence of other herbivores on a plant, attract natural enemies of herbivores and repel insect herbivores (Bernasconi *et al.* 1998; Paré & Tumlinson 1999). For example, *Rhopalosiphum maidis* alates and *R. padi* apterae were repelled by herbivore-damaged maize and aphid-infested wheat plants (Quiroz *et al.* 1997; Bernasconi *et al.* 1998). Therefore, the presence of herbivore-induced volatiles together with attractive compounds in the volatile profile of maize 'CRN 3505' and '78-15B' could have caused the aphids not to respond behaviourally to these cultivars.

Although wheat is a host plant of *R. padi*, alates did not respond behaviourally to volatiles from the wheat cultivars. (*Z*)-3-hexenyl acetate, a compound attractive to *R. padi* in high concentrations, was present in the three wheat cultivars and contributed to the quantitative differences between them. However, the repellent compound (*E*)-2-hexenal was present in plant volatiles from wheat 'Duzi' and 'Kariega'. α-Farnesene and indole also repelled *R. padi* and were present in wheat 'Krokodil'. Similar to the maize cultivars, the presence of repellent and attractive compounds in the odour profiles of the wheat cultivars may have masked each other and resulted in the lack of a response by the aphids. In addition, the context (individually or in a blend) in which these compounds are perceived may play a role in the behavioural response of aphids. *Aphis fabae* was attracted to the natural odours emitted by its host *V. faba*; however, many of these compounds repelled this species when perceived individually but became attractive when these repellent compounds were presented in a blend (Webster *et al.* 2008; Webster *et al.* 2010). It is therefore possible that the repellent effect of TMTT, α-farnesene and indole in this study was reduced by the presence of other host plant volatiles. However, further work is needed to explain the difference in the behavioural response of *R. padi* towards plant volatile compounds presented alone and in a blend.

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It is unexpected that *R. padi* was not repelled by odours from the potato cultivars because they released methyl salicylate, a compound previously identified to repel *R. padi* (Pettersson *et al.* 1994). This compound is associated with the primary host plant of *R. padi*, *P. padus* (Pettersson *et al.* 1994). Methyl salicylate has been implicated in mediating migratory behaviour in *R. padi*. The spring migrants of *R. padi* were repelled by this compound (Pettersson *et al.* 1994). However, Glinwood & Pettersson (2000) observed that this repellent effect disappeared in spring migrants after three to four days and was not dependent on contact with the secondary host plant. In our study *R. padi* did not respond to methyl salicylate, possibly because we tested the summer morph. In the anholocyclic populations found in South Africa and in the absence of the primary host plant, the summer morph stays on the secondary host plant all year round (Dixon 1971; Blackman & Eastop 2000; Uusitalo 2004). Therefore, *R. padi* may have developed a reduced sensitivity to methyl salicylate because it does not migrate between summer and winter host plants.

The absence of a behavioural response by *R. padi* observed towards odours of potato may contribute to its role in the spread of PVY. Without olfactory cues mediating landing, the aphids may rely more on visual cues when encountering large potato fields. Therefore, using an attractive plant as a crop border around seed potato fields in seed production regions may increase the amount of sensory information the aphids perceive and cause them to direct their low-level flight towards the attractive crop border plant. For example, high landing rates of *R. padi* on maize and wheat cultivars were observed in the laboratory in comparison to potato (Chapter 3). In addition, our results show that the presence of repellent compounds in the volatile profile of host plant cultivars reduces the attractiveness of the host plant to the aphid. Therefore, the response of aphids to olfactory cues needs to be considered when selecting crop border plants. This study demonstrates that plant cultivars with odours attractive to aphids should be selected.

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In conclusion, this study suggests that maize '6Q-121' has the highest potential as a crop border plant that is effective as a trap crop based on olfactory cues. The results provide an insight into selecting cultivars with the potential to attract aphids to crop border plants. Host plant cultivars that do not contain compounds that may be repellent to aphids should be selected as crop border plants because these may mask other attractive compounds. Behavioural screening of plant cultivars in the laboratory, together with identification of the active volatiles or blends, may be a promising first step towards selecting crop border plants before testing potential candidates in field trials.

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Chapter 6

General Discussion and Conclusions

To facilitate the selection of efficient crop border plants against the spread of aphid transmitted non-persistent viruses, this study examined the role of visual and olfactory cues during the initial phases of host plant selection by the bird-cherry oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) in the laboratory. This species can be an important vector of the non-persistent *Potato virus Y* (Potiviridae) in seed potatoes. In the majority of studies where crop borders were tested with a view to reducing the incidence of non-persistently transmitted viruses by aphids, crop border plants were evaluated as physical barriers or virus sinks (Hooks & Fereres 2006). Furthermore, previous studies concentrated on selecting suitable plant species for crop border plants and did not consider differences in attractiveness to aphid vectors between cultivars. The rationale for this study was that through understanding how aphids use visual and olfactory cues during host plant selection of different plant cultivars of the same species, plant characteristics can be identified that may serve as a basis for selecting efficient crop border plants.

Using host plant cultivars attractive to *R. padi* as crop border plants may cause more aphids to land on the border than on the main crop potato (*Solanum tuberosum* L., Solanaceae). From among each of the three maize and wheat cultivars considered as crop border plants, maize (*Zea mays* L., Poaceae) '6Q-121' and '78-15B' were identified as suitable cultivars based on landing, settling and reproduction of *R. padi*. However, when evaluating visual and olfactory cues separately, results were ambiguous. The experiments on visual cues indicated that *R. padi* preferred targets with the highest percentage of light in the green-yellow wavelength region in comparison to other colour targets. Wheat (*Triticum aestivum* L., Poaceae) reflected the highest percentage of light in the green wavelength region

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compared to maize and potato, suggesting that wheat cultivars would attract the highest number of alate *R. padi* in the landing phase. In contrast, odours from maize ‘6Q-121’ were attractive to *R. padi*, whereas the odours of the remaining eight plant cultivars tested were neither attractive nor repellent. However, in the experiment on *R. padi* landing rates, no distinction was made by alates between maize and wheat cultivars prior to landing. The results confirm that both visual and olfactory cues play a role in *R. padi* host plant selection behaviour. Therefore, visual and olfactory properties of potential crop border plants should be screened for in the laboratory when selecting plant cultivars that are the most attractive to the aphid vector, but do not support high aphid vector densities.

Little variation was found among the spectral properties of the plant cultivars tested. All cultivars had similar hues (chromatic aspect of colour, i.e. wavelength) and only showed differences in the percentage of light reflected (achromatic aspect of colour). It is not known if aphids are able to see colour (Döring & Chittka 2007). Thus, the extent to which these insects use colour during host plant selection is not completely understood. The summer morphs of *R. padi* prefer bright colours, such as lime green and yellow, reflecting light above 25% in the green-yellow region of the light spectrum, which is above the percentage of light reflected by green leaves. It is possible that bright greens and yellow are stronger stimuli for the opponent processing mechanism involved in aphid vision than green and therefore represent a supernormal foliage stimulus to insect herbivores (Prokopy & Owens 1983; Döring & Chittka 2007). The limited knowledge on aphid colour vision, together with aphids having a preference for supernormal foliage stimuli, make it difficult to identify a plant species or cultivar that will be as attractive as the artificial colours used in the aphid vision experiments. Therefore, spectral properties of plants may be of lesser value than olfactory cues as criteria for the selection of crop border plants. However, choosing a plant with a higher reflectance than the main crop may strengthen the crop border plant by providing

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additional host plant cues to aphids for landing. In addition, the increasing knowledge on aphid vision may lead to the improvement of integrated virus management strategies, not only for crop borders but also for other strategies that rely on interfering with aphid behaviour prior to landing, such as the use of reflective mulches.

Although both visual and olfactory cues play a role in aphid host plant selection behaviour, the majority of the plant cultivar odours tested in this study did not elicit a behavioural response from *R. padi*. This suggests that olfactory cues do not play an important role in the landing behaviour of this species because alates did not discriminate between maize '6Q-121' and the remaining maize and wheat cultivars prior to landing. However, it is possible that the attractive compounds were masked by the presence of repellent compounds in the volatile blend of the remaining maize and wheat cultivars. Plant volatile compounds are detected by the proximal (PPR) and distal (DPR) primary rhinaria located on the fifth and sixth antennal segments (Bromley & Anderson 1982; Hardie *et al.* 1994). The rhinaria contain olfactory receptor neurones (ORNs) that are specific to the type of compounds they are able to detect and may not detect all the volatile compounds released from a single plant (Park & Hardie 2002; Bruce *et al.* 2005). For example, methyl salicylate is perceived by DPR, and (-)-(1R,5S)-myrtenal by the PPR of *Aphis fabae* Scop. (Hardie *et al.* 1994). In the present study the compounds identified from potato did not overlap with those identified from maize and wheat. It is possible the olfactory receptor neurones in the PPR and DPR of *R. padi* alates were not sensitive to the compounds identified from potato and thus did not perceive any olfactory information from these plants. The behavioural study showed that *R. padi* was neither attracted nor repelled by any of the compounds identified from potato at the different doses tested. In contrast, maize and wheat cultivars contained several compounds that elicited a behavioural response by *R. padi*. (*Z*)-3-hexenyl acetate was present in all the wheat and maize cultivars and was attractive to *R. padi*. The presence of repellent

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compounds in the remaining cultivars may have contributed to the lack of response in the olfactometer tests, presumably because these compounds did not elicit an avoidance response and thus did not prevent the aphids from landing on the crop. The mechanisms involved in repellent compounds masking host plant volatiles that are attractive to aphids are not known.

The study demonstrates that before commencing with field trials potential crop border plants should be selected according to the following criteria: 1) the crop border plant species should be a preferred host plant of the most abundant aphid vector species in the seed potato production region; 2) different plant cultivars of the most suitable species should be evaluated; 3) cultivars that reflect a higher percentage of light in the green-yellow wavelength region than the main crop should be selected; and 4) plant volatile odour profiles should not contain any volatile compounds that are known to be repellent to the aphid species in question. In addition, the production practices of the seed potato region should be considered. The crop border plant should be cultivated in the same season, not host any diseases that could be transmitted to the main crop that is to be protected, and not lead to an increase in production costs (DiFonzo *et al.* 1996; Radcliffe & Ragsdale 2002).

Apart from evaluating the three maize and wheat cultivars in field trials to support or refute the results of this study, future work should be aimed at determining if aphids can perceive the chromatic properties of plants and artificial colour targets in addition to the achromatic properties. Furthermore, electrophysiological studies are needed to identify physiologically active plant volatile compounds for this species and which types of compounds their ORNs are sensitive to. Extra behavioural studies are required to determine if *R. padi* responds to individual host specific compounds within a blend of odours or different ratios of the same compounds in a blend. In addition, similar studies with other aphid species may be useful to test the conclusions made in the present study.

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Efforts to strengthen non-persistent virus management programmes rely on an understanding of the interactions between the aphid vector, host plants and virus. The approach taken in this study should lead to more efficient crop border plant selection for seed potatoes and other crops and thus advance non-persistent virus management. The results highlighted areas where information is lacking and additional studies are required. In conclusion, this study contributed towards improving crop border selection criteria to manage non-persistently aphid-transmitted viruses.

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