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

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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. As different cultivars of the same crop can vary in their olfactory attractiveness to aphids, selecting an attractive 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* (L.) (Hemiptera: Aphididae), to three cultivars each of maize [*Zea mays* L. (Poaceae)], potato [*Solanum tuberosum* L. (Solanaceae)], and wheat [*Triticum aestivum* L. (Poaceae)] with the aim of selecting an attractive crop border plant to reduce the incidence of the non-persistent *Potato virus Y* [PVY (Potyviridae)] in seed potatoes. Volatiles emitted by the crop cultivars were collected and identified using coupled gas chromatography/mass spectrometry. Quantitative and qualitative differences were found among cultivars. Behavioural responses of alate *R. padi* to odours of the cultivars and synthetic compounds identified from the plants were determined with a four-arm olfactometer. *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 behavioural response from *R. padi* and contributed to differences in plant volatile profiles included (*Z*)-3-hexenyl acetate (attractant) and α-farnesene, (*E*)-2-hexenal, indole, and (3*E*,7*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) (repellents). We conclude that maize cv. 6Q-121 is potentially suitable as a crop border plant based on the behavioural response of *R. padi* 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.

Introduction

The role of olfaction in host plant selection by insects has received considerable attention over the last decades (Hartlieb & Anderson, 1999; Bruce et al., 2005; Webster, 2012; Bergström, 2014). Most 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 them to a trap crop (Cook et al., 2007). In contrast, development of systems incorporating olfactory cues for management of aphid-transmitted non-persistent viruses has received little attention. Crop border plant species with attractive odours could be used to attract aphids to 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 preventingvirus-infected aphids from landing on the main

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crop to reduce the incidence of non-persistent viruses, such as *Potato virus Y* [PVY (Potyviridae)] in seed potato [*Solanum tuberosum* L. (Solanaceae)] fields (Fereres, 2000; Hooks & Fereres, 2006). Aphids are attracted to long-wavelength light reflected by the plant soil interface, causing them to land in higher numbers at the edge of the crop. Through initial probing of the crop border plant, the aphid loses its ability to transmit the virus before entering the main crop (Hooks & Fereres, 2006). Aphids purge mouthparts containing virions through salivation during initial feeding probes before plant acceptance occurs. Offspring are produced and form colonies only after these initial feeding probes have taken place and the plant has been accepted. Therefore, transient aphid species (aphids that do not colonize a crop) play an important role in non-persistent virus spread (Halbert et al., 1981; Powell, 2005). 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 with crop border plant species. For example, Damicone et al. (2007) determined that sorghum was more effective in reducing *Watermelon mosaic virus* (WMV) and *Papaya-ringspot virus* type W (PRSV-W) incidence in pumpkin than in peanut, soybean, or maize [*Zea mays* L. (Poaceae)]. It has been suggested that plant species that aphids prefer over the main crop, should be used to increase the efficiency of crop border plants (Fereres, 2000; Nault et al., 2004; Hooks & Fereres, 2006). An understanding of aphid–host plant interactions is required to optimize 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 volatiles to recognize not only their host plants but also non-host plants (Nottingham et al., 1991; Ahuja et al., 2010; Webster, 2012). For example, both alatae and apterae of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae), were attracted to volatiles of their host plants, wheat (*Triticum aestivum* L., Poaceae) and oats (*Avena sativa* L.) (Quiroz & Niemeyer, 1998). Moreover, *Aphis fabae* Scopoli was found to be attracted to odours from its host plant *Vicia faba* L. and repelled by non-host odours from summer savory (*Satureja hortensis* L.) (Nottingham et al., 1991; Webster et al., 2008). Hence, species should be selected that are host plants of the most important aphid virus vector species and thus attractive to the aphids. The attractive odours from the host plant, in addition to the edge effect, should cause the aphids to land preferentially in the crop border, and thereby reduce the risk of PVY incidence in the main crop.

Volatile compounds released by plants differ not only between species but also between plant cultivars (Storer et al., 1993; Degen et al., 2004). 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 cultivars [derived from *Dendranthema morifolium* (Ramat) Tzvelev and *Dendranthema indicum* (L.) Desmoulins (Asteraceae)] 'Purple Anne' and 'Surfine' over 'Hero' (Storer et al., 1993) and was attracted to odours of *V. faba* var. 'Sutton dwarf', but neither repelled nor attracted by odours of *V. faba* 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 should take cultivar differences into account.

Rhopalosiphum padi, a vector of the non-persistent PVY in seed potatoes, colonizes grasses and cereals (Poaceae) during the summer months in regions where it overwinters holocyclicly (Dixon, 1971; Katis & Gibson, 1985; Blackman & Eastop, 2006). However, in the absence of the primary host, *Prunus padus* L., in South Africa, they overwinter anholocyclicly on a secondary host plant (Dixon, 1971; Blackman & Eastop, 2000; Uusitalo, 2004). In a previous study, aphid landing rates, species composition, and abundance were determined on lucerne, maize, potato, soybean, and wheat in a 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 cultivars each of maize, potato, and wheat, to identify (1) the most attractive host under laboratory conditions, and (2) plant volatile traits that may be used to preselect candidate crop border plants prior to field trials. 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, were 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.

Materials and methods

Plants

Maize cultivars CRN 3505, 6Q-121, and 78-15B, wheat cultivars Duzi, Kariega, and Krokodil, and potato cultivars BP1, Hertha, and Mondial were used for plant volatile entrainments and bioassays. These plants were selected based on a previous study in which maize and wheat were identified as having the greatest potential as crop border plants (Schröder & Krüger, 2014), as well as discussions with farmers. Maize (Monsanto, Fourways, South Africa) and wheat (Obaru, Pretoria, South Africa) 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 ca. 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 (L13:D11 photoperiod). 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 fertilizer (ca. 1.6 g per pot) (Khula Kahle Fruit and Flower food, N:P:K = 3:1:5; Grovida, Durban, South Africa) was added to the soil upon planting. Two weeks after planting, a foliage treatment of micronutrients (Trelmix trace element solution; Hubers cc, Howick, South Africa) 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 L16:D8 photoperiod, and ambient relative humidity. For all experiments, maize and wheat plants were used at BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) growth stages 11 and 12 when 2–3 leaves had unfolded, and the potatoes at growth stages 17 and 18 when 7–8 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 × 55 × 32 cm) in a climate room at 22 °C, natural relative humidity, and a L16:D8 photoperiod. Aphids were separately provided with a mix of maize and wheat cultivars to prevent the aphids preferring a cultivar due to previous experience (Webster et al., 2013). Aphids were reared on the respective host plants for more than 6 months before use in experiments. *Rhopalosiphum padi* takes 6 and 22 days to complete a generation at 13 and 26 °C, respectively (Villanueva & Strong, 1964).

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 the Biosystematics Division of the ARC – Plant Protection Research Institute (ARC-PPRI) to verify species identification.

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%), and α-humulene (>96%), obtained from Sigma-Aldrich (St Louis, MS, USA). 3-Methyl pentadecane and 3-methyl tridecane were obtained from Chiron (Trondheim, Norway). (*E*)-β-Ocimene and TMTT [(3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene] were originally obtained from Rothamsted Research (Harpenden, UK). Dilutions of 1 000, 100, and 10 ng were made from each chemical with dichloromethane (Sigma-Aldrich) as a solvent.

Plant volatile collection and analysis

All equipment was baked overnight before use; glassware was washed with detergent and rinsed with acetone and distilled water prior to baking. Charcoal filters, Teflon tubes 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. Glass tubes containing Porapak Q (50 mg) 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 × 43 cm; Melitta Scandinavia, Helsingborg, Sweden) and the open end tied closed. Charcoal-filtered

air was pushed in from the bottom of the bag at 600 ml per min and pulled out through a Porapak tube at 400 ml per 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 from nine plants of each of the maize and wheat cultivars, six plants each for potato Hertha and Mondial and five plants for potato BP1, were collected for 72 h. Controls (pots with soil covered in aluminium foil) were also included. After 72 h, 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 to achieve a concentration of 1.8 ng μl^{-1} in the final sample.

Plant volatiles were analysed using coupled gas chromatography (GC)/mass spectrometry. A 1-µl aliquot of the entrained sample was injected into an Agilent 7890A GC (Agilent Technologies, Santa Clara, CA, USA) equipped with a cold-on-column injector and fitted with an HP-5 column (95% dimethyl polysiloxane and 5% diphenyl polysiloxane; 30 m, 0.25 mm i.d., and 0.25 µm film thickness; J&W Scientific, Santa Clara, CA, 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 per min to 250 °C. The carrier gas was helium with a flow rate of 1 ml per min. Volatile compounds were identified by comparing the mass spectra and retention indices against a commercial library (NIST 08; http://www.nist.gov/srd/) and commercial 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 volatile compounds identified from these plants, were tested using a four-arm olfactometer, as described in Pettersson (1970). 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, 20 mm; Merck, Modderfontein, South Africa) was placed on the floor of the chamber for the aphids to walk on and replaced after each experiment. To avoid bias caused by light, an 8-W light bulb ('cool white', Mini Twist® energy-saving lamp; Osram, Midrand, South Africa) 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 (2 mm inner diameter, 4 mm outer diameter). 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. An airstream of 400 ml per 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. 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 was recorded using Olfa software (Udine, Italy). If an aphid did not choose an arm within 3 min it was considered non-responsive and discarded. Each treatment was replicated 20×. Between replicates (each aphid) the chamber was rotated 90° clockwise. Five aphids were tested individually per olfactometer chamber. All equipment was washed with detergent before use (Teepol; Acorn Products, Strubens Valley, 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.

The responses of *R. padi* to volatiles emitted from maize CRN 3505, 78-15B, and 6Q-121, potato BP1, Hertha, and Mondial, and 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 to test whether the odours were either attractive or repellent to *R. padi* (Vet et al., 1983). 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 alate virginoparous *R. padi* to each of the compounds identified from the three maize, potato, and wheat cultivars at three quantities (1 000, 100, and 10 ng) were tested in the following way. Aliquots of 10 µl of each treatment for each test quantity (i.e., 10 µl of 100, 10, and 1 ng μl^{-1} resulting in test doses of 1 000, 100, and 10 ng, respectively) were placed on a filter paper triangle (Whatman no. 2, 90 mm) and allowed to air dry for 30 s and then placed in glass tubes. Two opposing arms contained the test stimulus and the remaining two arms contained dichloromethane solvent (control) to determine whether the compounds tested were either attractive or repellent to *R. padi* (Vet et al., 1983). The olfactometer was divided into four regions corresponding to each olfactometer arm and a central region. Following application of test stimuli/solvent, a single aphid was introduced into the olfactometer and time spent in each region corresponding recorded over a period of 10 min. A significantly greater amount of

time spent in regions corresponding to test stimuli was considered to indicate an attractant response, whereas a significantly greater time spent in regions corresponding to control arms was considered to indicate a repellent response. The relative attractiveness (positive values) and repellency (negative values) of individual volatile compounds was expressed as the percentage of time spent in the treatment arm relative to the control arm [response = (time spent in control − time spent in treatment)/10 min × 100].

Statistical analysis

Intraspecific differences in the quantities of volatiles (total amount and 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 ANOVA. Data were log(x+1) transformed to meet the requirements of normality and homogeneity of the analysis. Means were separated using Tukey's honestly significant difference (HSD) test. Because transformation of data did not result in stabilization of treatment variances the total amount of volatiles released by potato cultivars was analysed with the non-parametric Kruskal–Wallis ANOVA followed by multiple comparisons of mean ranks for all groups to determine intraspecific differences. 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 Tukey's HSD test. 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 (PCA) was used to determine differences and similarities in volatile composition between cultivars for each plant species separately (Warner, 2008). 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 $\alpha = 0.05$. All analyses were performed using STATISTICA v. 11 (1984-2012; StatSoft, Tulsa, OK, USA).

Results

Plant volatile collection and identification

There were intraspecific differences 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

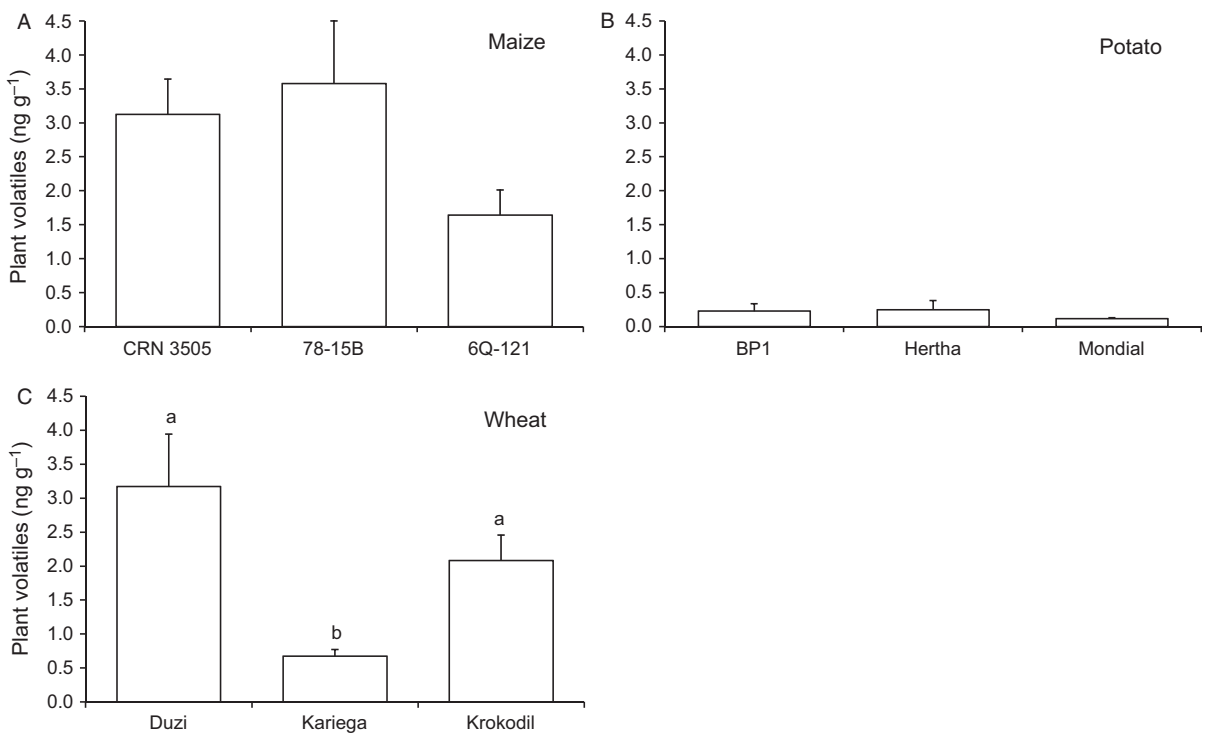

Figure 1 Mean (+ SE) total amount (ng g^{-1} wet leaf mass) of plant volatiles released by three cultivars of (A) maize, (B) potato, and (C) wheat. Bars capped with different letters are significantly different (Tukey's HSD test: $P<0.05$).

(H = 1.16, P = 0.56) (Figure 1). Although the ANOVA showed significant differences at the 5% level, the lower amount of total plant volatiles emitted by maize cv. 6Q-121 compared with CRN 3505 and 78-15B was not significant based on the HSD test (cv. 6Q-121 vs. CRN 3505: P = 0.08; cv. 6Q-121 vs. 78-15B: P = 0.056; Figure 1A). Wheat cultivars Duzi and Krokodil released higher amounts of plant volatiles than Kariega (Figure 1C).

Twenty-seven compounds were identified from the three maize cultivars (Table 1). Linalool oxide, TMTT, and two unknown sesquiterpenes (labelled as 2 and 12; Table 1) were identified from CRN 3505 and 78-15B, but not 6Q-121. (*Z*)-3-Hexenal was identified from 78-15B only, CRN 3505 emitted α-farnesene and an unknown sesquiterpene (5). α-Humulene and two unknown sesquiterpenes (6 and 7) were identified from 6Q-121 and CRN 3505, but not from 78-15B. Linalool and an unknown sesquiterpene (11) were recorded in significantly lower amounts in CRN 3505 and 78-15B than in 6Q-121.

Ten compounds were identified from the potato cultivars, with little difference among the three cultivars, with the exception of limonene. Limonene was recorded from BP1 and not from Hertha or Mondial (Table 1).

Seventeen compounds were identified from the three wheat cultivars (Table 1). Duzi had 15 compounds, Kariega 13, and Krokodil 15. (*E*)-2-Hexenal, 3-methyl tetradecane, and TMTT were identified from Duzi and Kariega, but not from Krokodil. The volatile profile of Duzi differed from that of 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 or Kariega. Significantly lower amounts of 3-methyl pentadecane were recorded from Krokodil.

Based on the volatile profiles it was possible to distinguish between maize, potato, and wheat cultivars. The PCA analysis for maize cultivars indicated that factors 1 and 2 together accounted for 30% of the variation and that they separated 6Q-121 from CRN 3505 and 78-15B (Figure 2A). β-Myrcene, (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, and an unkown sesquiterpene (11) were identified from factor 1, and factor 2 identified unknown sesquiterpenes 1 and 12 as the compounds mainly responsible for separation between the maize cultivars. The variation between potato cultivars accounted for by factors 1 and 2 was 59 and 22%, respectively (Figure 2B). The PCA analysis indicated a separation of BP1, Hertha, and Mondial, based on nonane, cumene, β-pinene, 2,2,4,6,6,-pentamethylheptane, acetophenone, methyl salicylate, and unknown sesquiterpene 14 contributing to factor 1, and 4-methyl-octane, limonene, and DMNT [(E)-4,8-dimethyl-1,3,7-nonatriene] contributing to factor 2.

Less than 50% of the variation among the wheat samples was accounted for by factors 1 (27%) and 2 (18%). Duzi was separated from Kariega and Krokodil in the PCA plane, whereas Kariega and Krokodil showed only little overlap (Figure 2C). Prominent compounds in the separation by factor 1 are (*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, and (*E*)-2-hexenyl acetate, and by factor 2: 3-methyl tridecane, 3-methyl pentadecane, and 3-methyl tetradecane.

Behavioural assays

Rhopalosiphum padi was attracted to odours of maize cv. 6Q-121 (Student's t-test: P<0.05; Figure 3). The headspaces of the other eight cultivars did not attract or repel alate *R. padi*. *Rhopalosiphum padi* alates were repelled (aphids spent more time in the control arm than the treated arm) by α-farnesene at 1 000 ng, (*E*)-2-hexenal, and indole at 100 ng, as well as by TMTT at 10 ng (Student's t-test: P<0.05; Figure 4). (*Z*)-3-Hexenyl acetate attracted *R. padi* alates at 1 000 ng. The remaining compounds did not attract or repel *R. padi* at the concentrations tested (Figure 4).

Discussion

Studies on 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 two other maize cultivars, nor to three wheat and potato cultivars. This was unexpected because maize and wheat are host plants for the aphid. The lack of response contrasts with previous findings where *R. padi* was attracted to volatiles from wheat and oats (Quiroz & Niemeyer, 1998), although the cited study did not compare cultivars within plant species. Aphids vary in their behavioural responses 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 cv. 6Q-121, but did not respond to odours from 78-15B and CRN 3505. *Aphis fabae* displayed similar responses towards various cultivars of its host plant *V. faba* and chrysanthemum (Nottingham et al., 1991; Storer & van Emden, 1995). Although the volatile profiles of the various 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 cultivar over another. The present study evaluated *R. padi* behavioural responses to each

Table 1 Mean (± SE) amount (ng g^{-1} leaf weight) of volatile compounds identified from three cultivars of maize ($n = 9$ plants per cultivar), wheat ($n = 9$), and potato ($n = 5–6$)

	Maize			Wheat			Potato		
Compound	CRN 3505	78-15B	6Q-121	Duzi	Kariega	Krokodil	BP1	Hertha	Mondial
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-Pentamethyl-heptane							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
Nonane							0.019 ± 0.011	0.011 ± 0.005	0.007 ± 0.004
Terpenoids									
(*E*)-β-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		
β-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						

	Maize			Wheat			Potato		
Compound	CRN 3505	78-15B	6Q-121	Duzi	Kariega	Krokodil	BP1	Hertha	Mondial
Sesquiterpene 1	0.120 ± 0.036	0.102 ± 0.050	0.046 ± 0.029						
Sesquiterpene 2	0.054 ± 0.016	0.016 ± 0.007							
Sesquiterpene 3	0.025 ± 0.014	0.090 ± 0.067	0.028 ± 0.013						
Sesquiterpene 4	0.021 ± 0.010	0.069 ± 0.028	0.108 ± 0.035						
Sesquiterpene 5	0.028 ± 0.015								
Sesquiterpene 6	0.002 ± 0.002		0.015 ± 0.010						
Sesquiterpene 7	0.043 ± 0.026		0.047 ± 0.024						
Sesquiterpene 8	0.099 ± 0.016	0.093 ± 0.021	0.148 ± 0.024						
Sesquiterpene 9	0.010 ± 0.010	0.036 ± 0.017	0.007 ± 0.007						
Sesquiterpene 10	0.052 ± 0.028	0.002 ± 0.002	0.027 ± 0.027						
Sesquiterpene 11	0.213 ± 0.041a	0.477 ± 0.238a	0.051 ± 0.033b						
Sesquiterpene 12	0.083 ± 0.031	0.060 ± 0.030							
Sesquiterpene 13				0.015 ± 0.005a	0.011 ± 0.002a	0.028 ± 0.003b			
Sesquiterpene 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

DMNT, (*E*)-4,8-dimethyl-1,3,7-nonatriene; TMTT, (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

Means within a plant species and within a row followed by different letters are significantly different (Kruskal–Wallis ANOVA followed by multiple comparisons of mean ranks: $P<0.05$).

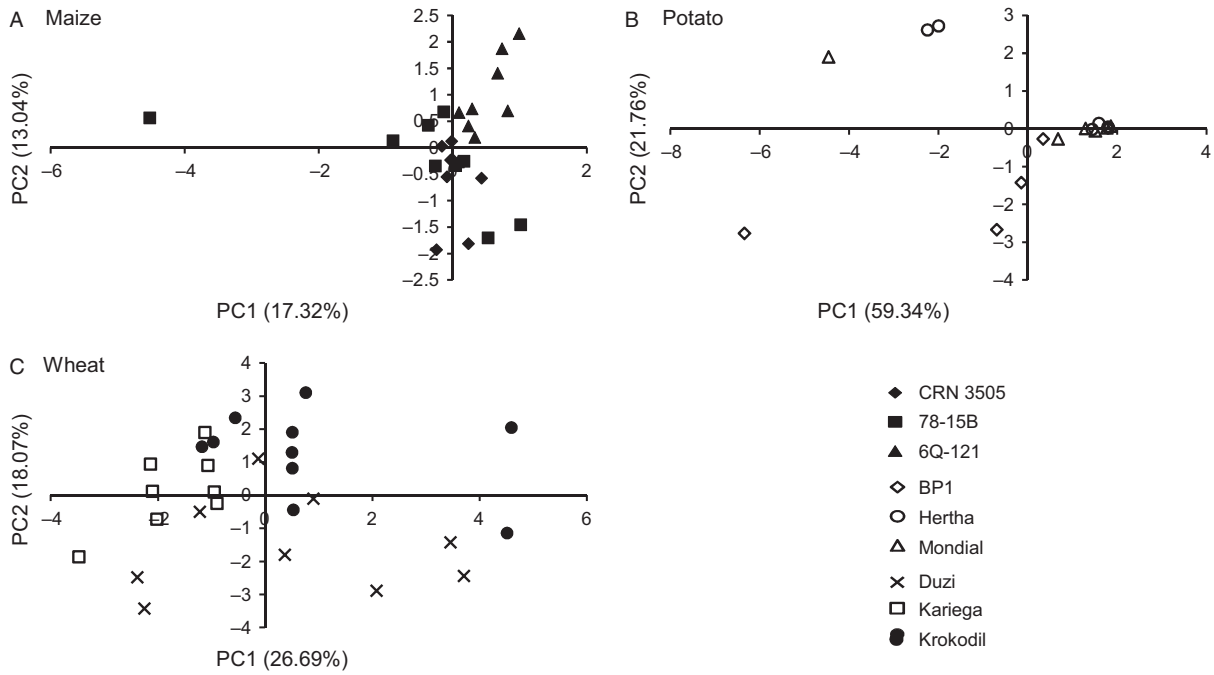

Figure 2 Principal components analysis of the composition of volatile compounds of three cultivars of (A) maize, (B) potato, and (C) wheat.

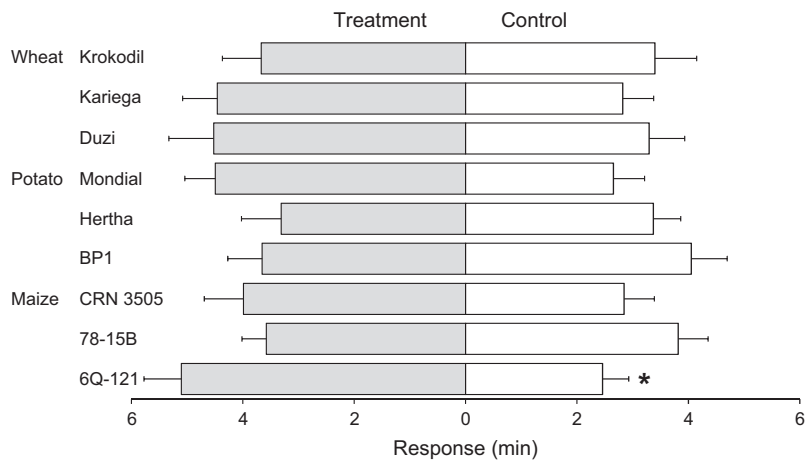

Figure 3 Mean (+ SE) time (min) spent by alate *Rhopalosiphum padi* in the treated and control arms of a four-arm olfactometer in response to odours emitted from three maize, potato, and wheat cultivars each in separate tests. The asterisk indicates a significant difference between the two odour sources (treatment vs. control) (paired t-test: $P<0.05$).

of the compounds identified from the maize, wheat, and potato cultivars to obtain a better understanding of how aphids respond to volatile differences among plant cultivars.

Maize and wheat did not have any detected volatile compounds in common with potato. In addition, maize and wheat varied more among cultivars than did potato. Many of the compounds identified from the maize and wheat cultivars in the present study were also identified from these plant species previously (Buttery & Ling, 1984; Buttery et al., 1985). Variation between plant cultivars was also observed for maize cultivars in a previous study (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 cvs CRN 3505 and 78-15B released this compound in low amounts (ca. 0.02 ng g^{-1} leaf weight). α-Farnesene repelled *R. padi* at the highest concentration tested and was emitted by CRN

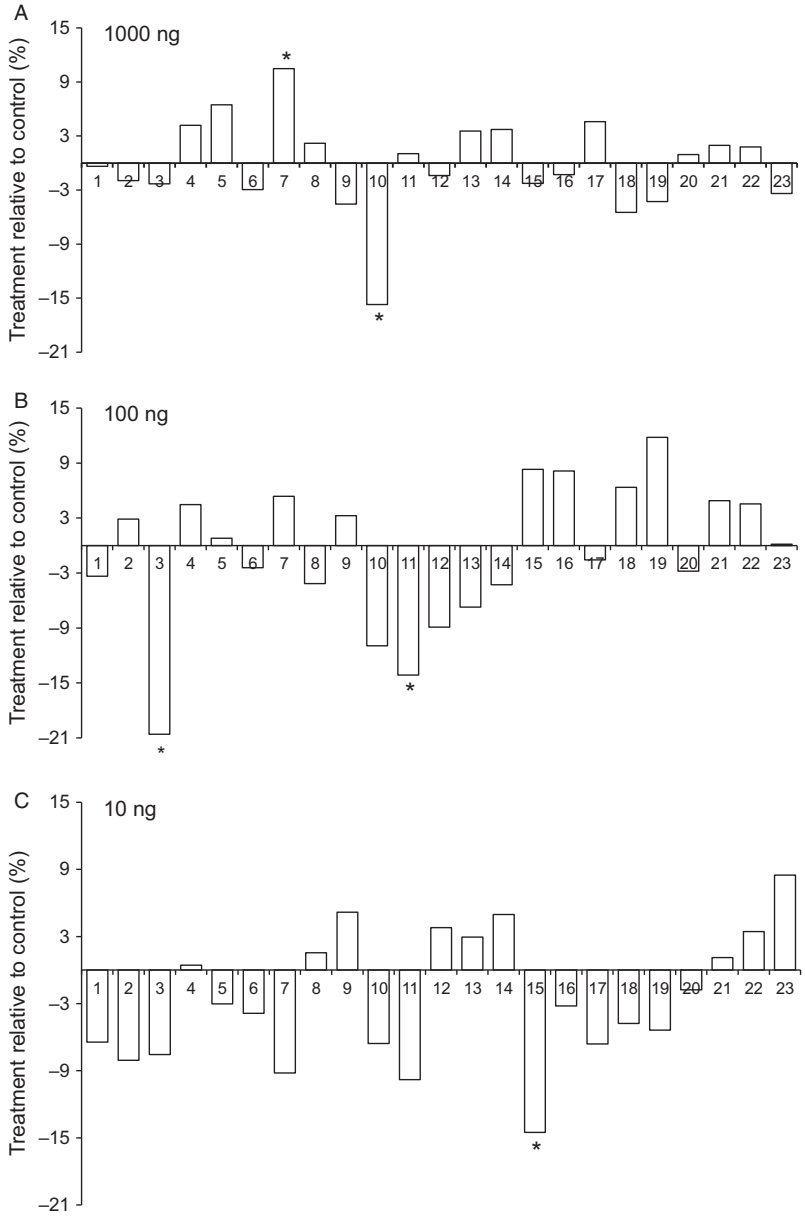

Figure 4 The relative attractiveness (positive values) and repellency (negative values) of individual volatile compounds at quantities of (A) 1000 ng, (B) 100 ng, and (C) 10 ng found in maize, potato, or wheat cultivars to *Rhopalosiphum padi* in separate olfactometer tests. Twenty-three compounds were tested: (1) (*Z*)-3-hexenal, (2) α-humulene, (3) (*E*)-2-hexenal, (4) (*E*)-2-hexenyl acetate, (5) (*E*)-β-ocimene, (6) (*Z*)-3-hexen-1-ol, (7) (*Z*)-3-hexenyl acetate, (8) 3-methyl pentadecane, (9) 3-methyl tridecane, (10) α-farnesene, (11) indole, (12) linalool, (13) linalool oxide, (14) myrcene, (15) (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, (16) limonene, (17) (+)-cyclosativene, (18) β-caryophyllene, (19) nonane, (20) cumene, (21) β-pinene, (22) acetophenone, and (23) methyl salicylate. Asterisks indicate significant differences between treatment and control odour sources (paired t-test: $P<0.05$).

3505 in relatively high concentrations (0.26 ng g^{-1} 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, as both compounds are well-known herbivore-induced plant volatiles (Paré & Tumlinson, 1997), that 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 tested by Degen et al. (2004), although these plants had been mechanically damaged. In the current study, the plants were handled with great care to ensure that no mechanical damage occurred. 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* (Fitch) alates and *R. padi* apterae were repelled by herbivore-damaged maize (Bernasconi et al., 1998) and aphid-infested wheat plants (Quiroz et al., 1997). Therefore, the presence of herbivore-induced volatiles together with attractive compounds in the volatile profile of CRN 3505 and 78-15B could have masked the attractive compounds and

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 cvs Duzi and Kariega. α-Farnesene and indole also repelled *R. padi* and were present in Krokodil. Similar to the maize cultivars, 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 were attractive when presented in blends as emitted naturally by the host (Webster et al., 2008, 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. Further work is needed to explain the difference in the behavioural response of *R. padi* towards plant volatile compounds presented alone vs. in a blend.

It was 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 3-4 days and was not dependent on contact with the secondary host plant. The lack of response of *R. padi* to methyl salicylate may be related to the summer morph not making use of methyl salicylate as a host plant cue. 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* 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 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 (Schröder et al., 2015), although no distinction was made between maize and wheat prior to landing, indicating that other plant cues may also be involved in aphid pre-alighting behaviour. Aphids are attracted to yellow targets and display wavelength-dependant behaviour (Moericke, 1969; Döring & Chittka, 2007). *Rhopalosiphum padi* has been found to be attracted to colour cues reflecting more than 20% light in the long-wavelength region. Therefore, other plant cues such as wavelength reflectance should not be excluded when evaluating potential crop border plants (Schröder et al., 2014). Our results suggest 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 in addition to visual plant cues when selecting crop border plants. This study demonstrates that plant cultivars with odours that are attractive to aphids are candidate crop border plants.

In conclusion, this study suggests that maize cv. 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 repellent to aphids should be selected as crop border plants because these compounds may reduce landing and subsequent probing. 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 the field.

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