Eur. J. Entomol. **102**: 303–304, 2005
ISSN 1210-5759

Non-host volatiles do not affect host acceptance by alate virginoparae of *Rhopalosiphum padi* (Hemiptera: Aphididae) settled on the host plant surface

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Key words. Wheat, alfalfa, EPG, host acceptance, feeding behaviour, aphid moods

Abstract. Using electrical penetration graphs to monitor aphid feeding, it was shown that volatiles of a non-host plant (alfalfa, *Medicago sativa* L.) did not disrupt the process of host acceptance by alate virginoparae of the birdcherry-oat aphid, *Rhopalosiphum padi* L., once it was settled on a host plant (wheat, *Triticum aestivum* L.).

INTRODUCTION

Host plant selection by aphids consists of a sequence of four consecutive steps: 1) attraction and landing on the plant, 2) evaluation of the leaf surface and probing of subepidermic tissues, 3) deep probing of plant tissues, and 4) evaluation of the phloem sap (Niemeyer, 1990). During this process the aphids receive different sensory cues including visual, olfactory, gustatory, and tactile stimuli (Dixon, 1998). Several studies have demonstrated that olfactory cues emitted by the host plant are important at the early stage (step 1) of host selection (Pickett et al., 1992). However, the attractiveness of host plant volatiles before aphid landing can be disrupted by the presence of non-host plant volatiles (e.g. Nottingham & Hardie, 1993; Hori & Komatsu, 1997). The aim of this work was to determine if the volatiles of a non-cereal, dicot non-host plant (alfalfa, *Medicago sativa* L.) could disrupt the process of host acceptance (steps 2, 3 and 4) by alate virginoparae of the birdcherry-oat aphid, *Rhopalosiphum padi* L., once it is settled on a host plant (wheat, *Triticum aestivum* L.), using electrical penetration graphs to monitor aphid feeding.

MATERIAL AND METHODS

Insects

A monoclonal colony of *R. padi* was reared parthenogenetically on oat (*Avena sativa* L. cv. Nehuén) under laboratory conditions, at 20 ± 2°C and 16L : 8D photoperiod. Experiments were performed with three-day old alate virginoparae which were in the process of feeding (the rostrum contacted the leaf surface perpendicular to the aphid's body, and the antennae did not wave and pointed backwards forming an angle shorter than 90° with the aphid's body) (Powell & Hardie, 2000).

Plants

Wheat (cv. Nobo) and alfalfa (cv. Palihue) seedlings were grown under the same conditions as the aphids. Wheat seedlings at decimal growth stage 12 (Zadoks et al., 1974) and ca. 9 cm tall were used for aphid stylet monitoring and for emission of volatiles, and two-week old alfalfa seedlings were used for emission of volatiles.

Experimental procedure

Feeding behaviour was monitored using electrical penetration graphs (EPG), where specific voltage changes in the recorded signal can be associated to different stylet activities and stylets' tip position (van Helden & Tjallingii, 2000). The potted experimental wheat seedlings were individually covered by inverted glass test tubes (12.5 cm long × 3 cm diameter), which had a thin slit on their wall (5 cm along the length of the tube × 2 mm width, starting 3 cm from the open end of the tube). Three hours before being connected to the EPG electrode, aphids were removed from the colony and placed in a Petri dish with moist filter paper. Each aphid was tethered with a gold wire electrode (2 cm long × 25 μm diameter) which was attached to its thorax between the wings. Copper electrodes were inserted in the soil where experimental seedlings were potted. Tethered aphids were placed on the seedlings through the slits in the test tubes. Volatiles were injected into the test tubes through small holes (3 mm diameter) in their closed ends. Purified compressed air bubbled through a water glass bottle and then flowed through two glass belljars connected in parallel, one containing a pot with treatment seedlings (alfalfa), and the other containing control (wheat) seedlings. The odour-saturated air leaving the belljars with a flow of 250 ml/min, was supplied to the glass tube as described above. Air flow through the system was started five minutes before placing the tethered aphid on the abaxial side of a wheat leaf and connecting it to the EPG amplifier.

Recordings lasted 5 h and were performed between 10:00 and 15:00 h at room temperature (25 ± 1°C). The following waveforms were recognised: non-probing (np), pathway phase (C), brief intracellular stylet punctures leading to potencial drops (pd), mechanical difficulties during stylet penetration (F), xylem ingestion (G), phloem salivation (E1) and phloem ingestion (E2). The proportion of time allocated to each of these activities was examined at different times: i) 1, 3, 5, 10, and 15 minutes after the start of the experiment, and ii) until the first sustained phloem ingestion (E2 > 8 min). In addition, the time to first phloem salivation (E1), the time to first phloem ingestion (E2), and the time to first sustained phloem ingestion (E2 > 8 min) were determined. The results were analysed using one-way ANOVA (Zar, 1996) or non-parametric Kruskal-Wallis one-way ANOVA on ranks test (Siegel & Castellan, 1988), depending on the nature of the data.

RESULTS

No significant differences were observed in proportion of time allocated to any individual probing activities independently of the time interval analysed from the start of the experiment and the type of volatiles aphids were exposed to. The statistics for the data until the first sustained phloem ingestion (E2 > 8 min), which included all types of activities, are: C ($F_{(1,28)}$ =

TABLE 1. EPG parameters of feeding behaviour of alate virginoparae of *R. padi* in relation to the type of additional volatiles supplied. Mean and standard errors are shown.

Treatment	n	% np [a]	Time to first E1 (min) [a]	Time to first E2 (min) [a]	Time to first E2 > 8' (min) [b]
Additional wheat volatiles (host plant)	15	15.3 ± 2.4	68.3 ± 14.4	89.6 ± 21.2	149.0 ± 21.2
Additional alfalfa volatiles (non-host plant)	15	9.1 ± 1.7	63.3 ± 10.7	79.9 ± 11.2	116.4 ± 16.5
p*		0.0495	1.000	0.727	0.245

[a] Kruskal-Wallis test.
[b] One-way ANOVA.
* Level of significant differences was $p < 0.05$.

0.057, p = 0.813), pd (H = 0.122, N = 15, p = 0.727), E1 (H = 0.000, N = 15, p = 1.000), E2 (H = 2.143, N = 15, p = 0.143), F (H = 0.080, N = 15, p = 0.778), G (H = 3.098, N = 15, p = 0.078). A barely significant decrease was observed in the proportion of time allotted to non-penetration (np) in aphids that received volatiles of alfalfa (Table 1). Time to first phloem phases (first E1 and E2 waveforms) and to sustained phloem ingestion (first E2 waveform longer that 8 min) were not affected by the nature of additional volatiles supplied (Table 1).

DISCUSSION

Using close-up video recording, Storer et al. (1996) showed that non-host volatiles affected host acceptance by *Aphis fabae* Scop. on *Vicia faba* L. only at the onset (first 5 s) of the period the aphid was on the plant surface. Our results show that the feeding behaviour of *R. padi* was not affected by the supply of extra volatiles from a non-host plant, independent of the time interval tested (all intervals longer than 1 min), indicating that during this phase of host acceptance, where exploration of the surface and subepidermic tissues of the plant occurs, the aphid does not integrate olfactory stimuli with tactile and gustatory stimuli.

It is unlikely that the aphids used in the present study lack the capacity to perceive volatiles. Previous investigations (Ninkovic et al., 2002; Glinwood et al., 2004) have shown that changes in the volatile profile of plants induced by previous exposure to volatiles of another plant are indeed perceived by aphid apterae, which are known to be less endowed with antennal receptors than alatae (Anderson & Bromley, 1987). We hypothesize that the mood of the aphid changes as the process of host acceptance proceeds. Thus, when the teneral period of an alate virginopara is over and the aphid is in an attack mood (Klingauf, 1987), attraction at close range to and landing on the plant occurs mainly in response to plant volatiles (Visser, 1986). Thus, this mood could also be called a "smelling" mood and would correspond to a mood where vision is complemented mainly by the sense of smell. However, once settled on the plant, a fast change to a "tasting" mood occurs, in which the importance of volatiles as repellent or attractant cues seem marginal in comparison to taste stimuli.

ACKNOWLEDGEMENTS. This work was partly supported by FONDECYT (grant 1020561).

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Received June 3, 2004; revised July 7, 2004; accepted August 25, 2004