J. Appl. Ent. 122, 331–333 (1998)

ISSN 0931-2048

The effects of antifeedant compounds and mineral oil on stylet penetration and transmission of potato virus Y by *Myzus persicae* (Sulz.) (Hom., Aphididae)

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Abstract: Plant-derived antifeedant compounds and mineral oil were applied to tobacco leaves infected by potato virus Y (PVY), in order to assess the effects on stylet penetration behaviour and subsequent transmission of the virus by the aphid vector, *Myzus persicae*. Video recordings of insect movements at the leaf surface showed that the initiation of stylet penetration was significantly delayed when mineral oil (Sunoco 7E) was present, but behaviour was not affected by any of three antifeedant treatments (quassin, hop α-acids or hop β-acids). Each insect was monitored until it had withdrawn its stylets from the leaf, and was then transferred to a healthy tobacco test seedling in order to determine its ability to transmit the virus. The proportion of aphids transmitting PVY from antifeedant-treated plants was similar to that from solvent-treated control plants, but the mineral oil caused a clear reduction in virus transmission efficiency.

1 Introduction

Antifeedants and other semiochemicals provide opportunities for manipulating the behaviour of aphid pests (PICKETT et al., 1994). As phloem-feeders, aphids may need to penetrate the plant for several hours before a suitable ingestion site is reached (TJALLINGII, 1995). Semiochemicals which disrupt the sequence of behaviours which occur before feeding (KLINGAUF, 1987) may therefore reduce plant colonization by these insects. However, aphids initiate brief stylet penetrations ('test probes') of the epidermis when they first encounter plants, and this behaviour may result in the acquisition and inoculation of nonpersistently transmitted plant viruses. Antifeedant compounds useful against such virus transmission must therefore act quickly, before probing, to disrupt these processes. Indeed, a reduction in nonpersistent transmission of potato virus Y (PVY) has been achieved in the laboratory, using the plant-derived drimane sesquiterpenoid, polygodial (GIBSON et al., 1982).

The aim of this study was to use video recording to investigate the immediate effects of potential behaviour-modifying treatments on the responses of *Myzus persicae* (Sulz.) during access to PVY-infected leaves and the consequences for transmission of the virus. The antifeedants tested were quassin and hop resin acids; both of these treatments reduce settling by aphids in long-term bioassays (POLONSKY et al., 1989; POWELL et al., 1997). A mineral oil, which has been shown to delay stylet penetration and reduce virus transmission (SIMONS et al., 1977; POWELL, 1992; POWELL and HARDIE, 1994), was also tested.

2 Materials and methods

2.1 Aphids

A pyrethroid-susceptible clone of *M. persicae* (PICKETT et al., 1987) was reared on Chinese cabbage (*Brassica pekinensis* L. cv. 'Tip Top'), as described previously (POWELL et al., 1993). Adult apterae were collected and starved for 1–2 h prior to use in experiments.

2.2 Virus and plants

The tobacco veinal necrosis strain of PVY was maintained in tobacco (*Nicotiana tabaccum* L. cv. 'White Burley') by aphid transmission. Test seedlings were inoculated with PVY at the 2–3 leaf stage, developed symptoms $\approx$14 days later, and infected plants were used as sources of virus 19–26 days after inoculation. All plants were grown and maintained in a heated glasshouse, kept free of aphids by regular nicotine fumigation.

2.3 Application of treatments

All treatments were applied topically to leaf surfaces in ethanolic solution, using a no. 4 paintbrush to achieve a uniform application of $\approx 15\,\mathrm{nl \cdot mm^{-2}}$ leaf area. The solvent dried rapidly (within 1 min), and experiments were started $\approx$10 min after the chemical treatments were applied. Antifeedants were applied as 0.1% solutions. The hop β-acid mixture contained 48% lupulone and 48% colupulone. The hop α-acid mixture contained 55% humulone and adhumulone, and 43% cohumulone. The mineral oil was Sunoco 7E, tested at 1%.

2.4 Assessment of behaviour and virus transmission

A tobacco source leaf showing clear symptoms of PVY infection was painted with ethanolic test solution on the upper surface, as above, and allowed to dry. A 16-mm diameter cork

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Table. *Video-recorded stylet penetration parameters and virus transmission efficiencies by* M. persicae: *the effects of hop α- or β-acids, ethanol solvent or no treatment of PVY infected leaf discs* (n = *30*), *and the effects of quassin and mineral oil against ethanol, tested separately* (n = *40*). (*ns = no significant difference, cf. ethanol control group; stylet penetration parameters were* log_{10} *transformed, means ± standard errors are given*)

Treatment of leaf disc	Log mean prepenetration duration (s)	Log mean penetration duration (s)	% virus transmission
Ethanol	1.04 ± 0.05	1.16 ± 0.04	40.0
No treatment	0.98 ± 0.05 ns	1.20 ± 0.03 ns	36.7 ns
α-Acids	1.09 ± 0.06 ns	1.17 ± 0.04 ns	46.7 ns
β-Acids	1.00 ± 0.04 ns	1.09 ± 0.04 ns	33.3 ns
Ethanol	0.85 ± 0.03	1.07 ± 0.04	57.5
Quassin	0.94 ± 0.04 ns	1.09 ± 0.03 ns	45.0 ns
Mineral oil	1.19 ± 0.03***	1.04 ± 0.06 ns	2.5***

borer was then used to cut a disc from the leaf. The disc was floated, treated surface-up, on water in the centre of a transparent dish positioned above a diffuse light source. Aphids were placed near the disc centre using a micro-aspirator. Behaviour was continuously recorded on video, using a camera positioned directly above the disc (Powell et al., 1995), until antennal and body movements indicated that the aphid had initiated and completed a stylet penetration (Hardie et al., 1992; Powell et al., 1993). Following penetration, the insect was transferred to a tobacco test seedling, where it was confined overnight for inoculation access. Aphids were considered to have acquired the virus during the video-recorded stylet penetration if the test plant developed symptoms of infection. Treatments were combined in two separate factorial experiments; hop α- and β-acids were tested against ethanol-treated and untreated controls, and quassin and mineral oil tested against ethanol.

2.5 Statistical analysis

Timed video data were $\log_{10}$ transformed to normalise their distributions (Hardie et al., 1992) and analysed using Student's *t*-tests. The occurrence of virus transmission was compared using χ^2 analysis. Transmission efficiencies represent the proportions of aphids that acquired and inoculated virus, expressed as a percentage.

3 Results and discussion

Behaviour and subsequent PVY transmission efficiency was assessed for *M. persicae*, given access to tobacco leaf discs, subjected to the various treatments, and compared with ethanol control and/or no treatment (table). Video analysis of insect behaviour revealed a delay in the initiation of stylet penetration caused by mineral oil, as found previously (Simons et al., 1977; Powell, 1992; Powell and Hardie, 1994), but no other treatments affected this parameter. The duration of stylet penetration was similar for all treatments, and only the mineral oil significantly reduced virus transmission to healthy test plants. This reduction in virus transmission is apparently caused by direct disruption of the interaction between virus particles and their aphid retention sites by mineral oil (Qui and Pirone, 1989; Wang and Pirone, 1996), and requires higher treatment levels than the concentrations of compounds employed as semiochemicals.

The prospects for using semiochemicals as part of the control of PVY and other nonpersistent viruses are restricted by the tendency of aphids to initiate test probing behaviour even on antifeedant-treated leaf areas. Aphids also exhibit such probes on nonhost plant species, with the consequence that noncolonising aphid species are often important vectors of nonpersistently transmitted viruses (Raccah, 1986). Previous laboratory studies showed that application of polygodial to PVY-infected tobacco leaves reduced transmission of the virus from those leaves by *M. persicae* (Gibson et al., 1982). Video monitoring of insect behaviour indicated that polygodial delays stylet penetration, but when aphids were allowed a single test probe, their subsequent virus transmission efficiency was similar from polygodial- and ethanol-treated leaf areas (Powell et al., 1996). However, in the present study, quassin and hop acids had no effect on the initial behaviour of *M. persicae*, or on PVY transmission.

Acknowledgements

The Biotechnology and Biological Sciences Research Council and the UK Ministry of Agriculture, Fisheries and Food provided financial support.

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