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The combination of electronic monitoring and video-assisted observations of plant penetration by aphids and behavioural effects of polygodial

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Abstract

Simultaneous electronic and close-up video recordings were made of behaviour during the initial 15 min of plant contact by adult, apterous *Aphis fabae* Scopoli on tick bean seedlings (*Vicia faba* Moench). Electronic techniques accurately determined stylet penetration of plant tissue and there was a close correlation between penetration and periods during which the insect antennae and body were immobile ($r = 0.994$, $n = 60$). Video techniques were then used alone to infer stylet penetration and the behaviour of aphids after various treatments was monitored. In particular, the time to first penetration, the number of penetrations, the mean duration of penetrations and the total time of penetration were observed. Behavioural differences were recorded between tethered (as required for electronic recording) and freely-moving insects as well as between fed and starved insects. The behaviour of starved aphids placed on beans treated with the plant-derived antifeedant, polygodial could not be distinguished from aphids on solvent-treated control beans. However, there were significant differences in behaviour of aphids which had previously been exposed to polygodial on plant or green/yellow paper surfaces for 24 h when compared with insects exposed to solvent alone. The possible modes of action of polygodial are discussed.

Introduction

Since the early 1960s electronic recording techniques have been used to monitor stylet penetration into plants as well as salivation, stylet position and ingestion during penetration (McLean & Kinsey, 1964; Tjallingii, 1988). The sequence of events during penetration seems accurately reflected but the limitations of the technique lie in attempts to determine 'normal' behaviour on a plant surface between penetration activities. These limitations are mostly the result of tethering the insect with a fine wire attached to the dorsal thorax/abdomen by conductive paint. This procedure reduces reproductive performance and longevity (Tjallingii, 1986). Some studies of behaviour after plant contact relied on close observation of the rostrum. If the rostrum contacted the plant surface penetration was assumed although Tjallingii (1976) called this behaviour

'proboscis contact' and did not infer penetration. It has also been noted that the swinging back of the antennae to assume a position parallel to the abdomen, was associated with prolonged stylet penetration and probably ingestion (e.g. Adams and van Emden, 1972). The precise relationship between antennal and body movements and penetration of the stylets into plant material has not previously been made. The present study used simultaneous close-up video and electronic monitoring techniques to study this relationship in detail.

Once body and antennal movement had been shown to provide an accurate record of stylet penetration, video techniques were used to look for differences in the behaviour of freely moving *vs* tethered insects and between fed *vs* starved insects. Behavioural effects of the plant-derived antifeedant compound, polygodial, were also investigated. Previous observations of the initial behaviour of aphids placed on polygodial treated surfaces (Griffiths *et al.*, 1982) revealed no differences that could explain the decrease in the transmission of non-persistent, semi-persistent and persistent viruses (Gibson *et al.*, 1982; Dawson *et al.*, 1986) nor the avoidance of treated areas at the end of a 24-h bioassay period (Asakawa *et al.*, 1988). Video-assisted observations were used to record the effects of this compound on initial contact and after periods of more prolonged exposure. All experiments were carried out using apterous virginoparae of the black bean aphid, *Aphis fabae*.

In this paper periods of penetration are recorded, indicating that plant tissue has been pierced by the aphid stylets. They include brief penetrations associated with host selection probes and prolonged penetrations associated with nutrient intake. Ingestion indicates an uptake of plant material whether for host selection or nutritional purposes (see Klingauf, 1987).

Materials and methods

Insects. The clone of *Aphis fabae* (Kennedy & Booth, 1951) was cultured on tick beans (*Vicia faba*) at 15 °C in long-day conditions (L16:D8). Young (< 48 h) adult apterae were selected and acclimatised to experimental conditions for at least 20 min.

Electronic and video recording. A DC electronic monitoring technique was used (see Tjallingii, 1988). Thin gold wire (20 μm diam.) was attached to the dorsal abdomen with silver conducting paint (RS Components Ltd) at least 30 min prior to recording. This procedure had no obvious toxic effects and there is evidence for the suitability of this adhesive (Kimmins, 1989). The insect could then be lowered onto a young tick bean seedling (*c.* 2-3 cm tall) growing in damp sand. The copper, indifferent electrode was pushed into the sand and, when the insect's stylets penetrated the plant, a circuit was completed. A low current was passed and the voltage across the sand/plant/insect circuit was monitored via a high impedance amplifier (Murphy Developments, Hilversum, The Netherlands) connected to a hot-pen chart record with suitable frequency response (Graphtec Miniwriter).

Two CCD video cameras (Cohu 4710 series) were linked via an Electro Craft vision mixer (VMC-89) such that both images could be seen on a monitor screen, mirrors were placed so that the insects were always clearly visible. The initial experiments were carried out with one camera observing an electronically monitored aphid and the other focused on the simultaneous output of the chart recorder. The experiments, together with a time base, were recorded on a Sony VO-5800PS U-matic recorder. This initial investigation was undertaken to look for correlations between insect behaviour and stylet penetrations detected electronically. It rapidly became obvious that antennal and body movement provided useful indicators. The duration of penetration, observed on the chart recorder, and the periods during which body and antennal movement ceased, were determined from the recorded tapes.

Once a relationship between antenna/body movement and stylet penetration had been defined it was possible to study penetration behaviour of insects moving freely on germinating tick

beans. For these experiments, the two cameras were used to monitor the behaviour of an experimental and a control aphid simultaneously with external conditions identical for both. Video tapes were then analysed for stylet penetration, defined as the periods when body and antennal movements ceased and the antennae were gradually lowered until parallel to the body. Aphids were observed for 15 min after plant contact, a period during which all experimental insects made at least one penetration.

Treatments. Initially tethered and untethered aphids were compared, as were fed (ie. removed during stylet insertion from the bean host) and starved insects. The antifeedant polygodial was prepared as the pure racemic mixture (±)-polygodial (Asakawa *et al.*, 1988) and stored in sealed glass ampoules. Starved insects were observed on tick beans freshly treated with polygodial (0.1% in 50% aqueous ethanol) or solvent alone. Additionally, insects were exposed to polygodial for 24 h before behavioural observations.

Statistical analysis. The skewed distributions of the time (in seconds) data were normalized by natural log transformations and the differences tested using Student's t tests.

Results

1. Electronic vs *video recording of penetration behaviour.* Simultaneous recording of probing behaviour, using electronic and close-up-video monitoring, showed that penetration times (ranging from 1 to 1007 s) were closely correlated with the cessation of antennation and body movement ($r = 0.994$, $n = 60$). Between penetrations the antennae were waved incessantly and the tips often touched onto the plant surface. During longer penetrations there were occasional brief periods of antennation. These were easily distinguished from antennation in non-penetrating aphids as the frequency of movements tended to be lower and the head could be seen to be anchored to the plant via the stylets. Limited body movement sometimes took place during these periods. Stylet penetration periods recorded electronically and inferred from video were slightly staggered in time. Thus there was an initial 5.5 ± 0.6 s delay between the antennae becoming immobile and stylet penetration indicated electronically, while at the end of the penetration period the antennae began to move 6.0 ± 1.3 s prior to withdrawal of the stylets.

It was possible to infer periods of stylet penetration and non-penetration on the basis of antennal and body movement using only video techniques and to observe the behavioural effects

Table 1. The effects of wire-tether *vs* free movement and previous feeding *vs* starvation on stylet penetration behaviour inferred from video recordings

	24-h pre-treatment	Penetration parameters				
		Treatment on a tick bean seedling	Number (± SEM)	Log time (s) to first (± SEM)	Log mean duration (s) (± SEM)	Log mean total duration (s) (± SEM)
1	Starved	Free	6.1 ± 0.6	1.15 ± 0.06	1.33 ± 0.03	2.59 ± 0.05
	Starved	Tethered	2.9 ± 0.3	1.68 ± 0.15	1.60 ± 0.06	2.17 ± 0.06
			***	**	***	***
2	Fed	Free	5.1 ± 0.8	1.76 ± 0.12	1.45 ± 0.05	2.55 ± 0.06
	Starved	Free	8.3 ± 0.8	1.19 ± 0.07	1.40 ± 0.03	2.54 ± 0.05
			**	***	ns	ns

Aphids were starved in humid conditions or removed directly from germinating tick beans. n = 15; ns = no significant difference; ** $P < 0.01$; *** $P < 0.001$, Student's tests

associated with the gold-wire tether necessary for electronic monitoring. The tether decreased the number of penetrations, delayed the first penetration, but promoted longer penetration periods, although the total time spent with the stylets inserted was less (Table 1,1). Differences were also found between aphids that had been feeding on tick bean and those that had been starved for 24 h prior to the experiments. Starvation reduced the time to the first penetration, increased the number of penetrations but did not affect the mean duration or total time spent with the stylets inserted (Table 1,2).

2. Behavioural effects of polygodial. No differences in behaviour were recorded when video-assisted observations were made on starved aphids placed on tick beans freshly treated with polygodial or solvent alone (Table 2,1). Attempts were then made to investigate the effects of a 24 h exposure to polygodial but after this time phytotoxic effects (Asakawa *et al.*, 1988) became apparent on young tick beans. Initial experiments were, therefore, carried out with aphids held in clip cages on the more resistant upper surfaces of mature broad bean leaves (*V. faba*, Suttons dwarf) which had been treated with polygodial or solvent. After 24 h these aphids were video-observed on either tick bean seedlings freshly treated with 0.1% polygodial or solvent. The findings indicated that there were significant differences between aphids in the different pre-treatments but not between aphids placed on the differently treated tick beans. These results were difficult to interpret as the antifeedant properties of polygodial could produce a starvation effect but there were indications that polygodial-treated insects behaved more like fed aphids while the solvent controls behaved like starved aphids (see Table 1,2). It is known that *A. fabae* prefers to feed on young and early senescent rather than mature bean leaves (Kennedy & Booth, 1951). Indeed, preliminary observations

Table 2. The immediate effects of polygodial *vs* solvent treatment of tick beans on the behaviour of starved insects (1) and the effects of polygodial 24 h pre-treatments presented on different paper substrates (2, 3) or topically applied (4). Video-assessed stylet penetration behaviour was recorded on clean tick bean seedlings in protocols 2, 3 and 4

	24-h pre-treatment	Penetration parameters				
		Treatment of bean seedling	Number (± SEM)	Log time (s) to first (± SEM)	Log men duration (s) (± SEM)	Log mean total duration (s) (± SEM)
1	Starved Polygodial	Polygodial	8.2 + 0.8	1.17 ± 0.05	1.46 ± 0.02	2.63 ± 0.04
	Starved Ethanol	Ethanol	6.8 ± 0.8	1.24 ± 0.05	1.47 ± 0.03	2.65 ± 0.03
			ns	ns	ns	ns
2	White paper Polygodial	None	6.7 ± 0.5	1.21 ± 0.10	1.37 ± 0.03	2.49 ± 0.05
	White paper Ethanol	None	6.2 ± 0.6	1.21 ± 0.04	1.33 ± 0.03	2.48 ± 0.05
			ns	ns	ns	ns
3	Green paper Polygodial	None	2.8 ± 0.4	1.64 ± 0.07	1.65 ± 0.1	2.78 ± 0.03
	Green paper Ethanol	None	5.8 ± 0.6	1.18 ± 0.04	1.30 ± 0.03	2.34 ± 0.07
			***	***	***	***
4	1.0 µg Polygodial	None	8.1 ± 0.8	1.31 ± 0.05	1.46 ± 0.02	2.62 ± 0.04
	0.1 µl Acetone	None	7.6 ± 0.6	1.23 ± 0.07	1.41 ± 0.03	2.66 ± 0.04
			ns	ns	ns	ns

n = 15; ns = no significant difference; *** $P < 0.001$, Student's t tests

of aphids after 24 h clip-caged to untreated mature bean leaves showed initial penetration behaviour identical to those starved for 24 h.

In order to avoid these problems, experiments were designed to examine the pre-exposure effects of polygodial in the absence of plant material. Insects were caged on white paper treated with polygodial or solvent alone for 24 h before video observations were made on untreated tick beans. There were no differences in behaviour between these pre-treatments (Table 2,2). However, when the experiments were repeated using a green/yellow coloured paper (Mecanorma normacolour system 022 255) there were significant differences in behaviour (Table 2,3). However, aphids topically treated with 1.0 μg of polygodial in 0.1 μl acetone or 0.1 μl acetone and then starved for 24 h showed no differences in penetration behaviour (Table 2,4).

Discussion

The development of electronic recording techniques and the elaborate studies used to correlate electrical patterns with a variety of associated activities has greatly enhanced our knowledge of aphid host selection and feeding behaviour (Tjallingii, 1988). Although some problems with the technique can be overcome (e.g. potential toxicity of conductive adhesives) or minimalised (e.g. effect of electrical current) the aphid's natural behaviour remains severely restricted by the insect-amplifier tether (Tjallingii, 1986). For this reason observations of free aphids offer some advantages. So far there has been no attempt to correlate directly observed behaviour with stylet penetration, a prerequisite for plant damage and probably for virus transmission. In addition, the close proximity of the experimenter and the need to manipulate the insect/plant or the microscope/hand lens to obtain the required viewing angle (e.g. to observe proboscis contact: Lowery & Boiteau, 1988; Tjallingii, 1976; Walker, 1987) may also affect behaviour. The use of close-up video recording, therefore, has distinct advantages and a similar system has been used to observe the behaviour of the brown planthopper, *Nilapavarta lugens* (Cook *et al.*, 1987). The technique is, however, made more powerful by coupling it with simultaneous electronic monitoring. This has allowed us to infer accurately stylet penetration from the more readily observed movements of antennae and body.

The current study has used video-assisted observation to demonstrate differences in behaviour between tethered and freely moving, apterous *A. fabae*. The results are similar to those obtained by Tjallingii (1986) from *Brevicoryne brassicae* and *Acyrthosiphon pisum*; free aphids penetrated more frequently, had a shorter time before first penetration and a reduced mean duration of penetration, than tethered aphids. Tjallingii's observations were made over 30 min periods but he also noted a longer-term reduction in fecundity and longevity. The video technique also revealed a more rapid initiation and an increase in the frequency of penetration by starved aphids, a finding that had been noted previously in *A. pisum* by Nault & Gyrisco (1966) using proboscis contact as a measure of probing. However, the mean and total penetration times were unaffected by starvation over the present experimental period.

A number of studies have indicated that polygodial has an antifeedant effect on aphids. Gibson *et al.* (1982) showed that a 0.1% ethanolic solution of natural (−)-polygodial reduced the numbers of adult *Myzus persicae* settling on and the numbers of progeny deposited on treated halves of host plant leaves over a 4 h period. Asakawa *et al.* (1988), using a similar settling assay over 24 h, found the natural (−)- and the synthetic (+)-polygodial equally effective at 0.05% in ethanol. They also showed both enantiomers to be effective at these concentrations when painted on the synthetic membranes covering artificial diet. The latter observation suggests that although polygodial has a phytotoxic action on some plants (Asakawa *et al.*, 1988) this is not directly related to its deterrent properties.

Earlier studies could not distinguish between the initial behaviour of aphids walking on plant or other surfaces treated with polygodial compared

with control surfaces (Griffiths *et al.*, 1982). Similarly, in the present work, starved *A. fabae* showed no behavioural differences between polygodial or solvent treated tick beans. However, previous bioassay results, which showed antifeedant effects, were obtained over longer periods (i.e. 4 and 24 h, see above). Preliminary observations in the present study suggested that extended exposure to polygodial resulted in marked behavioural differences. In addition, although polygodial was not effective when painted onto a white paper surface it was effective on a green/yellow paper surface. Thus, it appears that not only is plant material unnecessary for polygodial's action but neither is stylet penetration. Likewise, if a vapour effect was involved, identical results would have been expected on white and green/yellow paper. Prolonged contact with the compound appears to be necessary for its action with the green/yellow paper surfaces encouraging the aphids to stand and attempt penetration. Certainly, yellow and green surfaces initiate this behaviour in *M. persicae* and *Macrosiphum euphorbiae* (Moericke, 1950; Pelletier, 1990) and our *A. fabae* behaved differently on the white and green/yellow paper. During a 5 min observation period only 12/15 insects on white paper showed proboscis contact while 15/15 did so on green/yellow; the number of contacts differed 2.2 ± 0.5 *vs* 6.2 ± 0.5; log time (s) to first contact differed 1.27 ± 0.09 *vs* 0.98 ± 0.06 but the log mean durations were similar 1.06 ± 0.03 *vs* 1.11 ± 0.02.

No direct toxicity was recorded after topical application of 0.8 μg polygodial to individual *M. persicae* (Griffiths *et al.*, 1989) but 1.0 μg applied to newly moulted adult *A. fabae* (compared with *c.* 25 μg applied to the treated surfaces in the present experiments) did increase mortality such that 50% survival occurred after *c.* 15 days for polygodial treated insects and *c.* day 28 for solvent controls (J. Hardie & R. Isaacs, unpublished). These topical treatments did not, however, produce any behavioural changes in the aphids after 24 h indicating that polygodial does not have a general contact effect. It may act via gustatory receptors and interferes with contact chemoreceptors in larvae of *Pieris brassicae* (Schoonhoven & Yan, 1989). There was no immediate effect on these chemoreceptors but a prolonged 30 min exposure of maxillary sensilla resulted in a decreased sensitivity to phagostimulants, and the deterrent receptor was marginally stimulated. These delayed effects may explain why there are no immediate behavioural effects recorded for aphids on initial contact with treated surfaces. Contact chemoreceptors are found on the antennae (Slifer *et al.*, 1964; Bromley *et al.*, 1980) and the tarsi (Hardie unpublished) of aphids but the proboscis tip has only mechanosensory hairs (Wensler, 1977; Tjallingii, 1978). The principal gustatory organ is the epipharyngeal organ (Wensler & Filshie, 1969) which lies in the head and sensory neurones make direct contract with imbibed fluids. The present results indicate that ingestion for nutritional purposes is not necessary for the effect of polygodial but it is possible that the chemical may reach the epipharyngeal organ if salivary secretions placed on the green/yellow paper surface are reimbibed.

It is not immediately obvious how the behavioural changes induced after prolonged exposure to polygodial can explain its antifeedant effect or its effect on virus acquisition and spread. Nevertheless, previous studies have shown that polygodial decreased acquisition of the non-persistently transmitted potato virus Y and the semi-persistently transmitted beet yellow virus by *M. persicae* (Gibson *et al.*, 1982). It also decreased field infection of the persistent barley yellow dwarf virus (Dawson *et al.*, 1986). The transmission of non-persistent viruses requires only brief penetrations while persistent viruses require longer contact and phloem penetration (e.g. Scheller & Shukle, 1986). There are no obvious alterations in penetration parameters on initial contact with polygodial that could account for a decrease in the acquisition of non-persistent viruses. The effect of prolonged exposure to polygodial is to decrease the number of penetrations and to delay the first penetration. This might assist in an explanation of why polygodial decreased virus transmission but the increased mean and total penetration times would be expected to have the opposite

effects particularly with persistent viruses. The precise mechanism of action of polygodial requires further investigation.

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