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Efficacy of plant protection substances against virus transmission by aphids infesting potato

Wirkung von Insektiziden auf die Übertragung von Viren durch Kartoffel besiedelnde Blattläuse

Abstract

The behaviour of aphids on potato leaf-discs was analysed using time-lapse video-recording. In these experiments the behaviour of both apterous and alate *Aphis frangulae* and *Aulacorthum solani* were studied. The number of probes, the time to the onset of probing, the mean duration and the mean total duration of all probes made within a period of 15 minutes were used to characterize the effect of two insecticides (Ripcord 10 and Confidor).

The behaviour of the two aphid species and the two morphs differed. At a low temperature (16°C) most probes were made by apterae of *A. solani* and the fewest by alate *A. frangulae*.

After exposure to insecticides the aphids showed species specific responses. While testing the leaf surface the successive probes became shorter in both aphid species. This is typical behaviour for aphids on non host plants.

Ripcord 10 induces a strong reaction in both species, which finally adopt a “pain position”, in apparent attempt to reduce body contact with the treated leaf surface. The application of Ripcord 10 caused a reduction in number of probes and duration of probes made by all the morphs except apterous *A. frangulae*. The lowest number of probes was made by the aphids exposed to Ripcord 10 at 25°C.

Confidor did not induce the aphids to adopt a “pain position”. However, at 25°C apterous *A. solani* treated with this insecticide made the fewest probes, whereas the alatae made the fewest probes at 16°C.

The opposite was found for alate *A. frangulae*, which made the fewest probes at 25°C. Exposure of apterous aphids of this species to Confidor at 25°C resulted in more and more prolonged probes than in the control, indicating such treatment might increase the risk of transmission of non-persistent viruses like PVY.

Key words: Potato, insecticides, aphid behaviour, *Aphis frangulae*, *Aulacorthum solani*, PVY transmission

Zusammenfassung

Mit Hilfe von zeitgekoppelten Video-Aufzeichnungen wurde das Verhalten von Blattläusen auf Kartoffel-Blattscheiben untersucht. In den Experimenten wurden sowohl Geflügelte als auch Ungeflügelte von *Aphis frangulae* und *Aulacorthum solani* berücksichtigt.

Als Parameter für die Beeinflussung des Verhaltens von Aphiden durch Insektizide (Ripcord 10 und Confidor) wurden die Anzahl der Probestiche, die Zeit bis zum Beginn des ersten Probestichs, die mittlere Dauer der Probestiche und die mittlere Gesamtdauer aller Probestiche in der Versuchszeit von 15 Minuten registriert.

Zwischen dem Verhalten der geprüften Blattlausarten und dem der beiden Morphen ließen sich Unterschiede nachweisen. Die meisten Probestiche setzten im unteren Temperaturbereich (16°C) ungeflügelte Weibchen von *A. solani*. Die geringste Anzahl von Probestichen wurde für geflügelte *A. frangulae* ermittelt.

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Nach Applikation von Insektiziden reagieren die Blattläuse artspezifisch unterschiedlich. Bei beiden Arten werden die aufeinander folgenden Probestiche während der Prüfung auf Blattscheiben immer kürzer. Dieses Verhalten wird sonst bei Blattläusen auf Nichtwirtspflanzen beobachtet.

Ripcord 10 verursachte eine starke Beunruhigung beider Arten, die letztlich durch eine „Schmerzhaltung“ beantwortet wird. Mit Ausnahme von ungeflügelten *A. frangulae* führte die Anwendung von Ripcord 10 bei den geprüften Morphen zu einer Verringerung der Anzahl von Probestichen und zumindest in der Tendenz zu einer Reduktion der Dauer der Probestiche. Die wenigsten Probestiche setzten die Blattläuse unter Einwirkung von Ripcord 10 im höheren Temperaturbereich.

Der Wirkstoff Imidacloprid verursachte keine Beunruhigung der Versuchstiere, wie sie für Ripcord 10 beschrieben wurde. Während bei höheren Temperaturen die Ungeflügelten von *A. solani* die wenigsten Probestiche setzten, zeigten die Geflügelten dieser Art bei geringerer Temperatur die stärkste Reduktion der Anzahl der Probestiche.

Die Geflügelten von *A. frangulae* zeigten eine entgegengesetzte Reaktion, sie führten die wenigsten Probestiche bei höherer Temperatur aus. Die Ungeflügelten dieser Art realisierten im höheren Temperaturbereich unter Einfluss von Confidor mehr Probestiche als in der Kontrolle und erhöhten auch die mittlere Gesamtdauer der Probestiche.

Die Experimente mit reduzierten Aufwandmengen von Ripcord 10 ließen keine Beeinflussung der Aphiden erkennen, die zu einer verstärkten Infektionsrate von nicht persistenten Viren beiträgt.

Stichwörter: Kartoffel, Insektizide, Blattlausverhalten, *Aphis frangulae*, *Aulacorthum solani*, PVY Übertragung

Introduction

Although farmers spray potatoes with insecticides several times during the growing season this often does not prevent the transmission of Potato virus Y (PVY). There are indications that although potato treated with insecticides are infested with fewer aphids they suffer a higher incidence of virus infection than untreated crops.

As PVY is a non persistent virus, it can be transmitted mechanically and it’s vectors need only probe an infected plant once to acquire the virus and then probe a healthy plant to transmit the virus (RIECKMANN and WÜSTEFELD, 2006).

The active substances of insecticides affect the metabolism or nervous system of insects. In most cases they are not killed immediately but die slowly. It is possible that the behaviour of poisoned aphids changes, which results in an increase in probing (THIEME and HEIMBACH, 1998). Therefore, the present study analysed the effect of certain insecticides on aphid behaviour, in particular whether it results in an increase in the transmission rate of PVY.

Material and methods

Plants

Plants of potato cv. ‘Grata’ were used. Sprouted tubers were planted in pots and isolated underneath a gauze tent to prevent contamination by aphids and their natural enemies.

Animals

To determine the effects of plant protection products on the behaviour of adult aphids thriving and long established cultures of *Aphis frangulae frangulae* and *Aulacorthum solani* were used. They were reared at 20 ± 1°C and a photoperiod of 16L:8D on potato (‘Grata’).

Insecticides

Ripcord 10 (active substance: 100 g/l cypermethrin) was applied at a rate of 900 ml/ha and Confidor WG 70 (active substance: 665 g/kg imidacloprid) at a rate of 20 g/ha. In addition Ripcord 10 was also applied at a reduced rate of 180 ml/ha.

Leaf disc test

The underside of mature potato leaves were sprayed with either insecticide or water (control) using an amount of 200 l water/ha. The leaves were allowed to dry for 30 minutes. Then using a cork borer (10 mm diameter) leaf discs were cut and each placed with its underside uppermost on water in a Petri dish. To prevent the leaf discs moving they were impaled on the point of an upturned drawing-pin placed in the centre of each Petri dish.

After starving for 2 ± 1 h a single aphid was carefully placed, with the help of a micro aspirator, in the middle of each leaf disc. Then a 15 minute video recording was made of the behaviour of each aphid.

Unlike the method described by POWELL et al. (1995) the video cameras were not each placed at a right angle above a leaf disc, but at an angle of 45°.

Both adult apterous and alate viviparous aphids of both species were used. In order to standardize alate aphids in terms of their tendency to fly or settle they were taken from the culture and transferred to another cage containing potato plants. After they settled on the plants these aphids were removed and starved prior to use in the experiments. The experiments were done at two different temperatures: 16 ± 1°C and 25 ± 1°C.

Video recording

The behaviour of aphids was studied using simultaneous video recording. With the help of a video splitter (Pieper) the images from two CCD cameras (Pieper FK 2125 with macro zoom 18 - 108 mm) were shown side by side on a monitor. The time lapse pictures were stored on a Mitsubishi Time Lapse Long duration recorder HS - 5168.

Assessments

After transfer of the aphids to the leaf discs the following parameters were recorded for 15 minutes:

– number of probes

– time until the first probe
– mean duration of probes
– mean total duration of all probes during 15 minutes

The results for aphids that did not place their rostrum on the surface of leaf discs during an experiment were not included in the analysis.

Statistical Analysis

All the basic statistical analyses were done using computer programme SYSTAT, Version 10.0. To compensate for the skew in the distribution of the time related assessments they were log transformed. To determine whether the results recorded for the different insecticides, temperatures or morphs differ significantly the data were analysed using U tests (Mann-Whitney) or Kruskal-Wallis tests.

Results and discussion

To determine whether the size of the aphid affected its response, two sizes of adult apterous aphids were evaluated in pre-experiments (*A. frangulae* small: 0.12 – 0.18 mg, large: 0.19 – 0.31 mg; *A. solani* small: 0.51 – 0.89 mg, large: 0.92 – 1.32 mg).

The results indicated that the behaviour of different sized aphids was similar. Both, the number of probes as well as the mean duration of probes did not differ significantly. The adult aphids showed differences in reproduction and in the ability to resist starvation (or water loss). These characters are unlikely to affect the behaviour relevant to the question being addressed.

Large aphids can only be produced by either rearing aphids in isolation, in small colonies or at a low temperature. Because of this and the above results weight of the aphids was not standardized in the following experiments.

Comparison of EPG (electrical penetration graph) and video recordings indicate that the behaviour of aphids with a gold wire attached to their dorsum is affected. *Aphis fabae* that are not attached to a wire start probing sooner and penetrate their host plant (*Vicia faba*) much faster (HARDIE et al., 1992). Although the mean duration of the probes of unwired aphids was shorter their total duration was longer. Experiments with *Rhopalosiphum padi* also indicate that wired aphids ingest less phloem sap and excrete less honeydew (PRADO, 1997). Own experiments with *A. frangulae* and *A. solani* indicate similar effects of wiring on the behaviour of aphids (not presented here). They were restricted in their movement and tried to release themselves from the wire when observed over a long period. Therefore the use of the EPG-technique is inappropriate for studying behaviour, for which video recording is more suitable.

Aphids monitor a host plant before colonising it. According to KLINGAUF (1987) this selection procedure consists of several different phases:

– attraction (reaction to optical and olfactory stimuli)
– examination of leaf surface (reaction to optical, olfactory and mechanical stimuli)
– stylet penetration (reaction to mechanical and gustatory stimuli)
– testing phloem sap (reaction to gustatory stimuli)

Each aphid goes through this procedure before accepting a plant and reproducing. For the acquisition or transmission of non-persistent viruses aphids have to penetrate plant tissue with their stylets (probe). The different phases in the selection procedure are correlated with the position of the antennae (Fig. 1). When examining the leaf surface aphids point their antennae in the direction in which they are walking and keep the rostrum ventrally docked (Fig. 1A). When probing aphids keep their antennae upright or place them slowly along their back (Fig. 1B, 1C) (KLINGAUF, 1987; HARDIE et al., 1992; POWELL et al., 1993). Our analyses indicate that aphids not only probe with their antenna in this position, but also often keep their rostrum in a stationary position (ventrally) when their antennae are held along their backs (Fig. 1D). The experimental set-up described by POWELL et al.

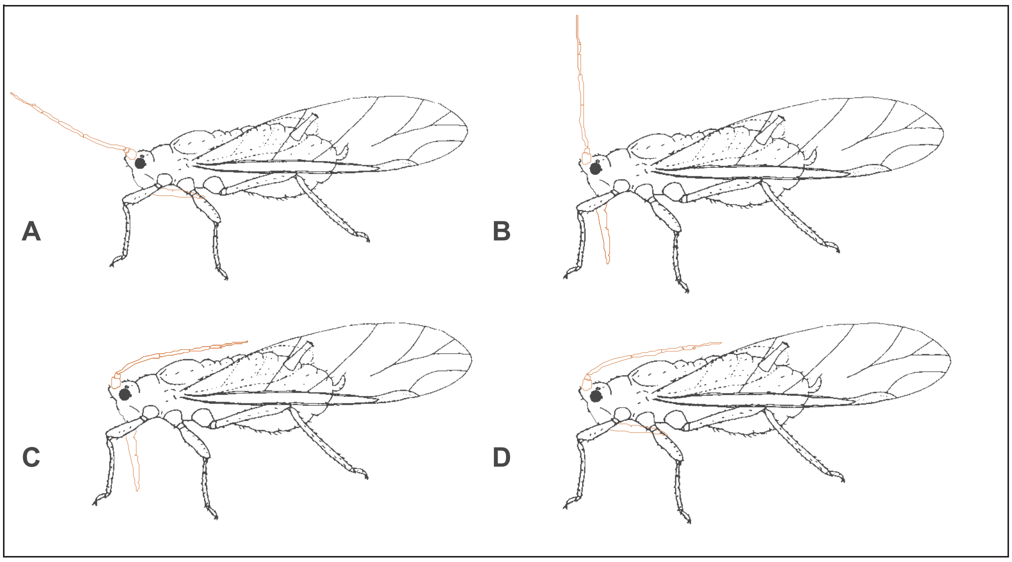

Fig. 1. Classical features used to characterize feeding behaviour (short distance).

(1995) results in an artificially 10% increase in the number of probes recorded (see Fig. 1D). This was avoided in this study by recording the behaviour using cameras placed at an angle of 45°.

The death of an aphid was not recorded in any of the experiments. Not presented here, the first dead aphids (show no response if touched) were noticed after 60 minutes. Exposed to Ripcord 10 the aphids reacted to both dosages (900 and 180 ml/ha) after 10 minutes, as their movements became uncoordinated (staggering, rushing, alternating contraction of legs). Often they adopt a posture that reduces the contact of their body with the leaf surface (Fig. 2). This is described as a "pain position", and is adopted by animals living in hot environments biotopes (e.g. lizards and darkling beetles in the Namib Desert of South-West Africa). Also the sycamore aphid, *Drepanosiphum platanoides*, behaves similar when exposed to strong sunlight and the ambient temperature is high (Dixon, pers. comm.).

After transfer to leaf discs all the aphids paused for a short period in a resting position (Fig. 1D) and then tested the different sources of stimuli. For this they palpated the leaf surface with the distal end of their rostrum

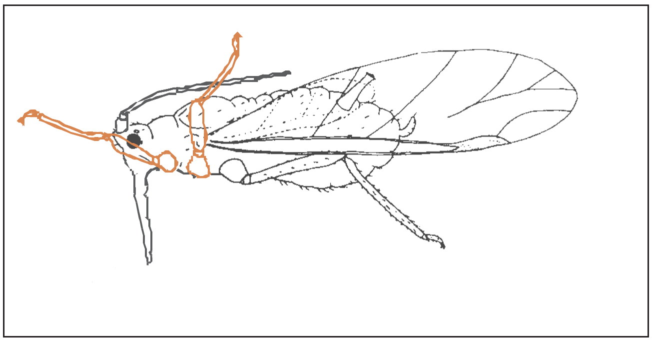

Fig. 2. „Pain position" of aphids on leaf discs treated with Ripcord.

(Fig. 3) and their fast moving antennae were pointed forward. Analyses of EPG-curves show that the stylets at this time are not penetrating the plant tissue. It is possible that, although taken carefully from the culture, their mouth parts were damaged. Such aphids never touched the leaf surface with their rostrum and were not included in the analysis.

Behaviour of the species

Both species used are polyphagous with extensive host ranges (BLACKMANN and EASTOP, 1984). They differ not only in size but also in behaviour.

In relation to *A. solani, A. frangulae* is smaller and less mobile. At 16°C apterous females of *A. frangulae* took longer to start probing (p=0.014) and made fewer probes (p=0.006). The mean duration (p=0.001) and mean total duration of their probes (p=0.001) were longer. During the experiments apterous *A. frangulae* spent 74.8% of the time probing and apterous *A. solani* 44.2% (Fig. 4A and 5A).

At 25°C the differences in behaviour between the species was smaller. Significant differences were found in the mean duration of probes (*A. frangulae* 421 sec. and *A. solani* 589 sec.; p=0.016). During these experiments *A. frangulae* spent 46.6% of the time probing and *A. solani* 63.6%.

At 16°C alate females of *A. frangulae* started probing later (p<0.001) and made fewer probes than those of *A. solani* (p<0.001). Whereas the mean duration of probes of alate *A. frangulae* lasted longer than those of *A. solani* (p=0.011), the differences in the mean total duration are not significant (p=0.277). Alate *A. frangulae* and *A. solani* spent 42.7% and 39.4% of the time probing, respectively, at this temperature (Fig. 4B, 5B).

At 25°C alate females of *A. solani* started probing sooner (p=0.049) and probed more frequently (p=0.001) than *A. frangulae*. Statistically the differences in mean duration and mean total duration of probes are insignificant. The alate females of *A. frangulae* and *A. solani* spent

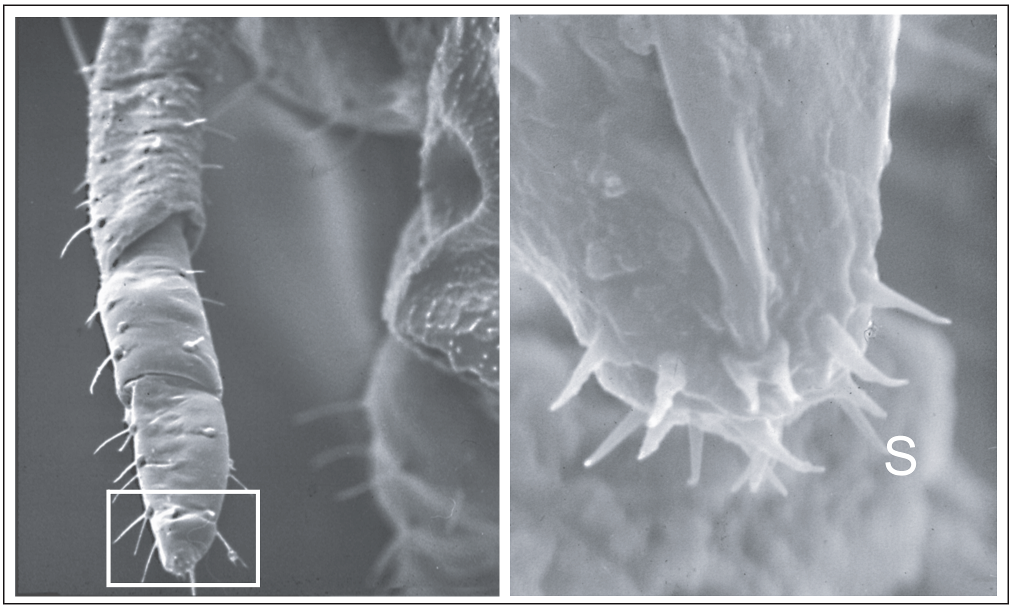

Fig. 3. **Apical** segments of rostrum (left) and enlarged view of last rostral segment (right) with sensillae (S) at the distal end.

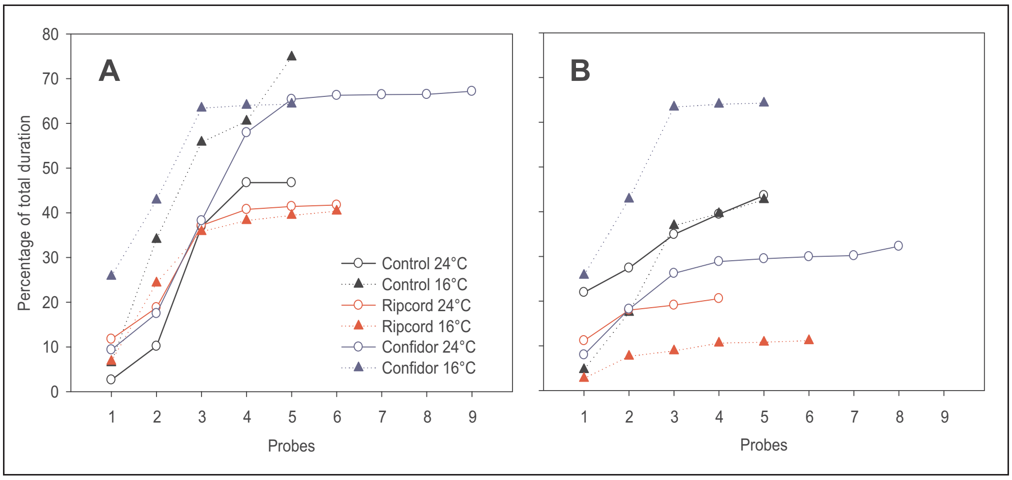

Fig. 4. Effect of insecticides on the number and duration of the probes made by apterous (**A**) and alate (**B**) viviparous *Aphis frangulae* (total duration: 15 min.).

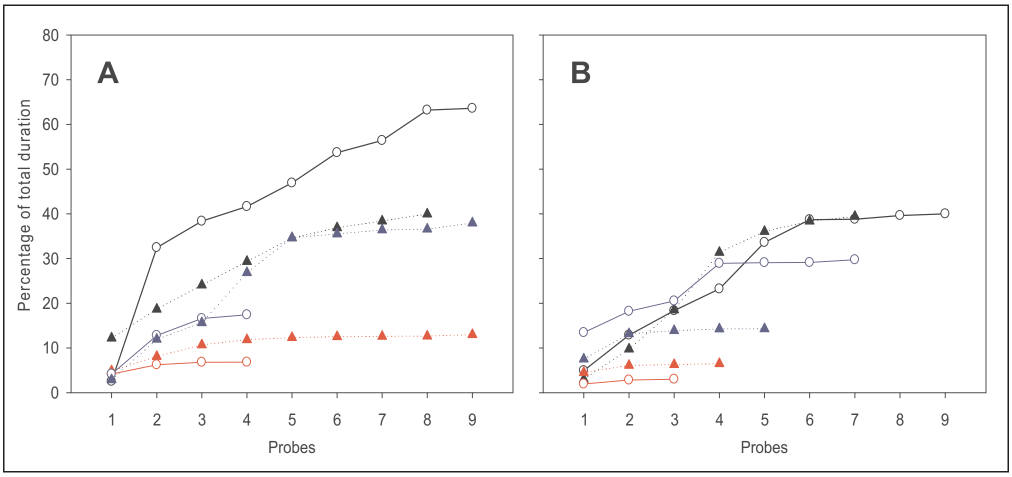

Fig. 5. Effect of insecticides on the number and duration of the probes made by apterous (**A**) and alate (**B**) viviparous *Aulacorthum solani* (total duration: 15 min.).

43.6% and 40.0% of the time probing, respectively, at this temperature.

Effect of temperature on behaviour of the different morphs

The analyses show that there are no significant differences in number of probes the aphids made at 25°C and 16°C (Tab. 1, 2). At 25°C both morphs of both species started probing earlier. But the differences are only significant for alate *A. solani*. At 25°C apterous *A. frangulae* showed a significant reduction in mean duration and mean total duration of probes. The opposite response was recorded for apterous *A. solani*. But the differences are only significant for the mean total duration of probes (Tab. 1).

After the start of the first probe alate aphids did not differ in the duration of probes at both temperatures. This is most likely associated with the dispersal function of this morph. Whereas apterous aphids invest the majority of the energy they consume in reproduction, alatae invest more of this energy in their fat reserves, which fuel their search for a new host plant. By metabolising fat, alate aphids can also obtain water and are thus less affected than apterae by water loss at higher temperatures.

Comparisons of behaviour reveal that alate aphids start probing later than apterous aphids (Tab. 2). There was a tendency for alate to probe more frequently than apterae. In addition, the duration of successive probes increases on suitable host plants.

The differences in total duration of probes presented in Tab. 2 indicate that alatae spent more time testing the leaf surface than apterous aphids. In nature the alatae find new food resources. Therefore after their dispersal flight suitable host plants must be identified and colonized. Apterous aphids are born on a suitable host plant and will only occasionally need to search for a new host. These differences in the function of the two morphs are also reflected in the different numbers of rhinaria on their antennae, which are used to process olfactory stimuli.

Effect of Ripcord 10

The contact insecticide Ripcord 10 had a strong influence on the behaviour of aphids. It caused a fast disturbance of the more mobile morphs after contact to the tarsi. The aphids moved faster and tried to reduce the contact to the treated leaf surface (Fig. 2). At 25°C the onset of probing was delayed and the number of probes was increased with exception of apterous *A. frangulae* (Tab. 3). On leaves treated with Ripcord 10 there was a significant reduction of the mean duration and mean total duration of

Tab. 1. Effect of temperature on the video-registered probing behaviour of *Aphis frangulae* (1) and *Aulacorthum solani* (2) in the control. Values are means ± s.e.

Morph	Temperature (°C)	Probes: Number	Probes: Log of time until first (sec)	Probes: Log of mean duration (sec)	Probes: Log of mean total duration (sec)
1 apterous	25	3.39 ± 0.18	1.17 ± 0.17	2.07 ± 0.06	2.60 ± 0.05
	16	3.50 ± 0.37	1.32 ± 0.07	2.31 ± 0.06	2.83 ± 0.01
		ns	ns	*	***
1 alate	25	2.60 ± 0.36	1.56 ± 0.21	2.07 ± 0.15	2.42 ± 0.13
	16	3.00 ± 0.20	2.06 ± 0.08	2.08 ± 0.06	2.54 ± 0.06
		ns	ns	ns	ns
2 apterous	25	5.08 ± 0.74	0.95 ± 0.07	2.10 ± 0.11	2.74 ± 0.05
	16	5.44 ± 0.48	1.03 ± 0.10	1.87 ± 0.09	2.57 ± 0.04
		ns	ns	ns	*
2 alate	25	4.86 ± 0.44	1.04 ± 0.05	1.81 ± 0.07	2.47 ± 0.09
	16	4.50 ± 0.27	1.38 ± 0.05	1.86 ± 0.05	2.50 ± 0.06
		ns	***	ns	ns

n = 16; ns = not significant; * p<0.05; ** p<0.01; *** p<0.001 Mann-Whitney U-test

Tab. 2. Video-registered probing behaviour of different morphs of *Aphis frangulae* (1) and *Aulacorthum solani* (2) in the control. Values are means ± s.e.

Temperature (°C)	Morph	Probes: Number	Probes: Log of time until first (sec)	Probes: Log of mean duration (sec)	Probes: Log of mean total duration (sec)
25	1 apterous	3.39 ± 0.18	1.17 ± 0.17	2.07 ± 0.06	2.60 ± 0.05
	alate	2.60 ± 0.36	1.56 ± 0.21	2.07 ± 0.15	2.42 ± 0.13
		ns	ns	ns	ns
16	1 apterous	3.50 ± 0.37	1.32 ± 0.07	2.31 ± 0.06	2.83 ± 0.01
	alate	3.00 ± 0.20	2.06 ± 0.08	2.08 ± 0.06	2.54 ± 0.06
		ns	***	*	***
25	2 apterous	5.08 ± 0.74	0.95 ± 0.07	2.10 ± 0.11	2.74 ± 0.05
	alate	4.86 ± 0.44	1.04 ± 0.05	1.81 ± 0.07	2.47 ± 0.09
		ns	ns	ns	*
16	2 apterous	5.44 ± 0.48	1.03 ± 0.10	1.87 ± 0.09	2.57 ± 0.04
	alate	4.50 ± 0.27	1.38 ± 0.05	1.86 ± 0.05	2.50 ± 0.06
		ns	**	ns	ns

n = 16; ns = not significant; * p<0.05; ** p<0.01; *** p<0.001 Mann-Whitney U-test

probes for apterous *A. solani*. The mean duration of probes made by alate *A. solani*, which were also very short on the control leaves, was unaffected by this insecticide.

At 16°C there were no effects of Ripcord 10 to the onset of probing by any morph (Tab. 3). The number of probes as well as the mean duration and the mean total duration of probes were significantly reduced for all morphs tested with exception of apterous *A. frangulae*.

Effect of Confidor

The systemic insecticide Confidor did not affect aphid behaviour in the same way as Ripcord 10. In no case did the

Tab. 3. Effect of Ripcord 10 on the video-registered probing behaviour of *Aphis frangulae* (1) and *Aulacorthum solani* (2) at 25°C and 16°C. Values are means ± s.e.

25°C

Morph	Treatment	Probes: Number	Probes: Log of time until first (sec)	Probes: Log of mean duration (sec)	Probes: Log of mean total duration (sec)
1 apterous	Control	3.39 ± 0.18	1.17 ± 0.17	2.07 ± 0.06	2.60 ± 0.05
	Ripcord 10	3.11 ± 0.31	1.32 ± 0.10	2.02 ± 0.09	2.47 ± 0.08
		ns	ns	ns	ns
1 alate	Control	2.60 ± 0.36	1.56 ± 0.21	2.07 ± 0.15	2.42 ± 0.13
	Ripcord 10	1.35 ± 0.28	2.20 ± 0.14	1.97 ± 0.12	2.16 ± 0.13
		*	*	ns	ns
2 apterous	Control	5.08 ± 0.74	0.95 ± 0.07	2.10 ± 0.11	2.74 ± 0.05
	Ripcord 10	1.50 ± 0.25	1.59 ± 0.11	1.55 ± 0.06	1.76 ± 0.08
		***	***	***	***
2 alate	Control	4.86 ± 0.44	1.04 ± 0.05	1.81 ± 0.07	2.47 ± 0.09
	Ripcord 10	0.44 ± 0.20	2.15 ± 0.09	1.77 ± 0.04	1.93 ± 0.06
		***	***	ns	**

n = 16; ns = not significant; * p<0.05; ** p<0.01; *** p<0.001 Mann-Whitney U-test

16°C

Morph	Treatment	Probes: Number	Probes: Log of time until first (sec)	Probes: Log of mean duration (sec)	Probes: Log of mean total duration (sec)
1 apterous	Control	3.50 ± 0.37	1.32 ± 0.07	2.31 ± 0.06	2.83 ± 0.01
	Ripcord 10	3.27 ± 0.35	1.34 ± 0.06	2.02 ± 0.08	2.49 ± 0.07
		ns	ns	*	***
1 alate	Control	3.00 ± 0.20	2.06 ± 0.08	2.08 ± 0.06	2.54 ± 0.06
	Ripcord 10	1.67 ± 0.39	2.20 ± 0.11	1.67 ± 0.41	1.84 ± 0.15
		**	ns	**	***
2 apterous	Control	5.44 ± 0.48	1.03 ± 0.10	1.87 ± 0.09	2.57 ± 0.04
	Ripcord 10	4.00 ± 0.50	1.02 ± 0.09	1.42 ± 0.06	1.97 ± 0.09
		*	ns	***	***
2 alate	Control	4.50 ± 0.27	1.38 ± 0.05	1.86 ± 0.05	2.50 ± 0.06
	Ripcord 10	1.81 ± 0.26	1.53 ± 0.18	1.44 ± 0.10	1.68 ± 0.10
		***	ns	***	***

n = 16; ns = not significant; * p<0.05; ** p<0.01; *** p<0.001 Mann-Whitney U-test

aphids attempt to reduce their contact with the treated leaf surface ("pain behaviour"). At 25°C the onset of probing was delayed but not significant in alate *A. frangulae* (Tab. 4). Whereas Confidor caused a significant reduction in the number of probes by both, apterous and alate morphs of *A. solani*, this insecticide caused a significant increase in the number of probes (p=0.028) and an increase in the mean duration and mean total duration of probes made by apterous *A. frangulae*. Confidor did not have a significant affect on the duration of probes of alate *A. frangulae*.

At 16°C the inhibition was stronger for alate than apterous *A. solani* (Tab. 4). The alate aphids started probing later and made fewer and shorter duration. Confidor

Tab. 4. Effect of Confidor on the video-registered probing behaviour of *Aphis frangulae* (1) and *Aulacorthum solani* (2) at 25°C and 16°C. Values are means ± s.e.

25°C

Morph	Treatment	Probes: Number	Probes: Log of time until first (sec)	Probes: Log of mean duration (sec)	Probes: Log of mean total duration (sec)
1 apterous	Control	3.39 ± 0.18	1.17 ± 0.17	2.07 ± 0.06	2.60 ± 0.05
	Confidor	4.64 ± 0.46	1.51 ± 0.09	2.12 ± 0.07	2.76 ± 0.04
		*	ns	ns	**
1 alate	Control	2.60 ± 0.36	1.56 ± 0.21	2.07 ± 0.15	2.42 ± 0.13
	Confidor	2.08 ± 0.20	1.58 ± 0.18	1.97 ± 0.12	2.30 ± 0.14
		ns	ns	ns	ns
2 apterous	Control	5.08 ± 0.74	0.95 ± 0.07	2.10 ± 0.11	2.74 ± 0.05
	Confidor	2.09 ± 0.32	1.24 ± 0.07	1.96 ± 0.08	2.34 ± 0.07
		***	**	ns	*
2 alate	Control	4.86 ± 0.44	1.04 ± 0.05	1.81 ± 0.07	2.47 ± 0.09
	Confidor	2.10 ± 0.31	1.61 ± 0.12	2.00 ± 0.08	2.33 ± 0.08
		***	***	ns	ns

n = 16; ns = not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$ Mann-Whitney U-test

16°C

Morph	Treatment	Probes: Number	Probes: Log of time until first (sec)	Probes: Log of mean duration (sec)	Probes: Log of mean total duration (sec)
1 apterous	Control	3.50 ± 0.37	1.32 ± 0.07	2.31 ± 0.06	2.83 ± 0.01
	Confidor	2.57 ± 0.31	1.83 ± 0.09	2.37 ± 0.07	2.73 ± 0.05
		ns	***	ns	ns
1 alate	Control	3.00 ± 0.20	2.06 ± 0.08	2.08 ± 0.06	2.54 ± 0.06
	Confidor	2.21 ± 0.53	1.85 ± 0.16	2.12 ± 0.07	2.56 ± 0.06
		*	ns	ns	ns
2 apterous	Control	5.44 ± 0.48	1.03 ± 0.10	1.87 ± 0.09	2.57 ± 0.04
	Confidor	4.41 ± 0.45	1.06 ± 0.10	1.94 ± 0.07	2.55 ± 0.07
		ns	ns	ns	ns
2 alate	Control	4.50 ± 0.27	1.38 ± 0.05	1.86 ± 0.05	2.50 ± 0.06
	Confidor	1.61 ± 0.33	2.05 ± 0.12	1.72 ± 0.12	1.97 ± 0.14
		***	***	ns	**

n = 16; ns = not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$ Mann-Whitney U-test

delayed the onset of probing by apterous *A. frangulae*. Alate aphids of this species showed a significant reduction in the number of probes.

Comparison of the effects of both insecticides

A comparison of the behaviour of the two aphids treated with Ripcord 10 and Confidor is presented in Tab. 5. At 25°C apterous *A. frangulae* on Confidor treated leaf discs made significantly more probes of longer mean duration (Tab. 5). On Confidor treated leaves alate *A. frangulae* started probing sooner than on leaves treated with Ripcord 10. The Ripcord 10 treatment prolonged the duration of the probes of apterous *A. solani* more than Confidor. Clearly Ripcord 10 treatment caused alate *A. solani* to maker fewer probes.

Tab. 5. Comparision of the effect of insecticides on the video-registered probing behaviour of *Aphis frangulae* (1) and *Aulacorthum solani* (2) at 25°C and 16°C. Values are means ± s.e.

25°C

Morph	Treatment	Probes Number	Log of time until first (sec)	Log of mean duration (sec)	Log of mean total duration (sec)
1 apterous	Ripcord 10	3.11 ± 0.31	1.32 ± 0.10	2.02 ± 0.09	2.47 ± 0.08
	Confidor	4.64 ± 0.46	1.51 ± 0.09	2.12 ± 0.07	2.76 ± 0.04
		**	ns	ns	**
1 alate	Ripcord 10	1.35 ± 0.28	2.20 ± 0.14	1.97 ± 0.12	2.16 ± 0.13
	Confidor	2.08 ± 0.20	1.58 ± 0.18	1.97 ± 0.12	2.30 ± 0.14
		ns	*	ns	ns
2 apterous	Ripcord 10	1.50 ± 0.25	1.59 ± 0.11	1.55 ± 0.06	1.76 ± 0.08
	Confidor	2.09 ± 0.32	1.24 ± 0.07	1.96 ± 0.08	2.34 ± 0.07
		ns	ns	**	***
2 alate	Ripcord 10	0.44 ± 0.20	2.15 ± 0.09	1.77 ± 0.04	1.93 ± 0.06
	Confidor	2.10 ± 0.31	1.61 ± 0.12	2.00 ± 0.08	2.33 ± 0.08
		*	ns	ns	ns

n = 16; ns = not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$ Mann-Whitney U-test

16°C

Morph	Treatment	Probes Number	Log of time until first (sec)	Log of mean duration (sec)	Log of mean total duration (sec)
1 apterous	Ripcord 10	3.27 ± 0.35	1.34 ± 0.06	2.02 ± 0.08	2.49 ± 0.07
	Confidor	2.57 ± 0.31	1.83 ± 0.09	2.37 ± 0.07	2.73 ± 0.05
		ns	***	**	**
1 alate	Ripcord 10	1.67 ± 0.39	2.20 ± 0.11	1.67 ± 0.41	1.84 ± 0.15
	Confidor	2.21 ± 0.53	1.85 ± 0.16	2.12 ± 0.07	2.56 ± 0.06
		ns	ns	**	**
2 apterous	Ripcord 10	4.00 ± 0.50	1.02 ± 0.09	1.42 ± 0.06	1.97 ± 0.09
	Confidor	4.41 ± 0.45	1.06 ± 0.10	1.94 ± 0.07	2.55 ± 0.07
		ns	ns	***	***
2 alate	Ripcord 10	1.81 ± 0.26	1.53 ± 0.18	1.44 ± 0.10	1.68 ± 0.10
	Confidor	1.61 ± 0.33	2.05 ± 0.12	1.72 ± 0.12	1.97 ± 0.14
		ns	*	ns	*

n = 16; ns = not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$ Mann-Whitney U-test

At 16°C apterous *A. frangulae* exposed to Confidor showed a delay in the onset of probing. In both morphs of this species the duration of the probes was shorter after exposure to Ripcord 10 than to Confidor. In contrast to Confidor, Ripcord 10 caused a stronger reduction in the mean duration of probes made by *A. solani*, but only so for apterous aphids.

The effect of Ripcord 10 and Confidor on the number and duration of probes is presented in Fig. 4 (*A. frangulae*) and Fig. 5 (*A. solani*). When exposed to Confidor at 25°C apterous and alate *A. frangulae* made more probes than on the control leaves and those treated with Ripcord 10. *A. solani* did differ in its behaviour. Whereas both insecticides caused a strong reduction of the number of

Tab. 6. Effect of reduced dosage of Ripcord 10 on the video-registered probing behaviour of *Aulacorthum solani* at 25°C. Values are means ± s.e.

Morph	Treatment	Probes			
		Number	Log of time until first (sec)	Log of mean duration (sec)	Log of mean total duration (sec)
1 apterous	Control	5.08 ± 0.74 a	0.95 ± 0.07 a	2.10 ± 0.11 a	2.74 ± 0.05 a
	900 ml/ha	1.50 ± 0.25 b	1.59 ± 0.11 b	1.55 ± 0.06 b	1.76 ± 0.08 b
	180 ml/ha	0.50 ± 0.16 c	2.06 ± 0.16 b	1.81 ± 0.06 a	1.86 ± 0.07 c
1 alate	Control	4.86 ± 0.44 a	1.04 ± 0.05 a	1.81 ± 0.07 a	2.47 ± 0.09 a
	900 ml/ha	0.44 ± 0.20 b	2.15 ± 0.09 b	1.77 ± 0.04 a	1.93 ± 0.06 b
	180 ml/ha	0.69 ± 0.22 b	1.75 ± 0.24 ab	1.31 ± 0.09 c	1.22 ± 0.97 c

n = 16; values within a column for each morph with different letters are significantly different p<0.01, Kruskal-Wallis test

probes made by apterous aphids at both insecticides caused a strong reduction of number of probes at 25°C, but not at 16°C. Alate *A. solani* also made fewer probes when exposed to both insecticides, but Ripcord 10 had a much stronger effect than Confidor.

Confidor is taken up by the plants and translocated there. Aphids also take it up when feeding. This exposure route would need another test design than used here.

Effect of reducing the dosage

In an additional experiment the effect of a reduced dosage of Ripcord 10 on the behaviour of *A. solani* was analysed (Tab. 6). Exposure to a reduced dosage resulted in apterous aphids delaying the onset of probing to a similar extent as exposure to a high rate but had a more marked effect in reducing the number of probes. The mean total duration of the probes made by this morph was reduced by Ripcord 10, most strongly by the higher dosage. The mean duration of probes made by apterous aphids was significantly reduced only after exposure to the reduced dosage.

In comparison to the control the reduced dosage reduced the time to the start of probing by alate aphids. For both dosages tested the number and mean total duration of probes was significantly reduced compared with the control.

These results indicate that application of reduced dosages will not result in higher transmission rates of non-persistent viruses. But this is only true for this experimental set up. There is no evidence that other formulations would differ in their effect on the behaviour of these aphids. Furthermore, it is clear that Ripcord 10 is a very effective contact insecticide. Further experiments should be done using lower dosages or insecticides with other modes of action and other aphid species.

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