

Effects of kaolin particle film on *Myzus persicae* (Hemiptera: Aphididae) behaviour and performance

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Abstract

The emergence of resistance mechanisms to, and revocation of, many insecticides used in the control of the polyphagous aphid pest, *Myzus persicae* (Sulzer), has increased the pressure to develop novel approaches for the control of the pest in many crops. Kaolin-based particle films provide a physical barrier against insect pests and show considerable potential for controlling *M. persicae*. We conducted a series of laboratory experiments to investigate the mode of action of kaolin against aphids. The material appeared to have no direct effect on *M. persicae*; spraying adult aphids with aqueous kaolin suspension had no significant impact on their subsequent survival or reproduction on untreated plants. Similarly, when aphids were placed on kaolin-treated host-plants (*Brassica oleracea*), their performance (survival, growth rate and reproduction) was not significantly different from aphids on untreated plants. However, when *M. persicae* were given a choice between kaolin-treated and untreated (or water solvent-treated) leaf areas, both adults and nymphs exhibited a significant preference for non-kaolin-treated host-plant material. Rejection of kaolin-treated plant material occurred very rapidly (within 20 min) and this behavioural effect may be related to the efficacy of kaolin in controlling aphids under field conditions.

Keywords: *Myzus persicae*, green peach aphid, kaolin, particle film

Introduction

In many countries, there is increasing pressure to develop novel approaches to the management of invertebrate pests of crops. A primary focus target for the development of novel management techniques is the peach-potato aphid or green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). It has a worldwide distribution; even where the primary host plants (*Prunus* sp.) are absent, *M. persicae* can remain anholocyclic on secondary hosts if the climate is

suitable. Over 40 different plant species can act as secondary hosts, many of which are crops of economic importance, including brassicas, sugar beet, sugar cane, tobacco, tomato and potato (van Emden *et al.*, 1969; Blackman & Eastop, 2000). *Myzus persicae* is a very important aphid vector of plant viruses (Kennedy *et al.*, 1962) and can reproduce rapidly in favourable conditions. As a result of its cosmopolitan distribution both geographically and botanically, *M. persicae* has been persistently targeted with different insecticide applications, and this has resulted in the emergence of resistance to organophosphate, organochlorine, carbamate and pyrethroid insecticides (Foster *et al.*, 2000). Insecticide-resistant aphids have been found throughout Europe and the UK (Foster *et al.*, 1998; Fenton *et al.*, 2005) and have highlighted the need for alternative control measures.

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The use of particle film technologies has recently been introduced as a novel approach to suppress arthropod pests of crops (Glenn *et al.*, 1999) and a specific formulation is available commercially as the crop protectant, Surround[®] WP (Engelhard Corporation, NJ, USA). Kaolin, the main component of these films, is a white, non-abrasive aluminosilicate mineral ($\text{Al}_4\text{Si}_4\text{O}_{10}[\text{OH}]_8$), that is purified and finely-sized in these formulations (Glenn *et al.*, 1999; Puterka *et al.*, 2000). The material is sprayed onto the crop as an aqueous suspension that dries to leave a white particle film on the plant surface. The material is chemically inert over a wide pH range, and therefore has no direct toxicity to animals or plants (Glenn *et al.*, 1999; Puterka *et al.*, 2000).

The use of kaolin particle films has been investigated in a number of different crops (mainly fruit) against a range of different pests, such as Diptera, psyllids, Coleoptera and Lepidoptera (Glenn *et al.*, 1999; Lapointe, 2000; Pasqualini *et al.*, 2002; Showler, 2002; Saour & Makee, 2004; Barker *et al.*, 2006). In addition, field studies suggest that kaolin formulations show potential in reducing aphid numbers in a range of crops, including rosy apple aphid, *Dysaphis plantaginea* (Passerini), in apple orchards (Wyss & Daniel, 2004; Bürgel *et al.*, 2005), pea aphid, *Acyrtosiphon pisum* (Harris), in pea fields (Eigenbrode *et al.*, 2006) and *M. persicae* in peach orchards (Karagounis *et al.*, 2006). There have been very few laboratory studies to investigate the mechanisms of action of kaolin against sap-feeding insects. However, there are some indications that aphids and psyllids show decreased preference for kaolin particle film-treated areas of foliage, and decreased survival and reproduction when confined to such foliage (Cottrell *et al.*, 2002; Puterka *et al.*, 2005). The aim of this study was to explore effects of kaolin particle film on *M. persicae* by investigating aphid behaviour and performance in the laboratory.

Materials and methods

A clone of *M. persicae* (insecticide susceptible: Pickett *et al.*, 1987) was maintained on *Brassica rapa* var. *pekinensis* cv. Kiansi and *Brassica oleracea* var. *capitata* cv. Pixie in a controlled environment at $18 \pm 1^\circ\text{C}$ in a 16:8 h light:dark cycle. Unless stated otherwise, experiments were also performed under these conditions. Aphids were transferred to new substrates using a fine, moistened paintbrush.

The kaolin-based particle film formulation used in this study was F-01-KV-6, an experimental hydrophilic formulation supplied by Engelhard Corporation (NJ, USA) with similar composition to their Surround[®] WP product (Barker *et al.*, 2006). The material was applied as an aqueous suspension in accordance with the supplier's recommended rate (60 g l^{-1} water). Using a hand-held sprayer, two applications of the kaolin particle suspension were applied to the point of run-off to the plant material or other substrates used experimentally. Unless stated otherwise, the experimental plant material was calabrese, *Brassica oleracea* var. *italica* cv. Fiesta F₁.

Application of kaolin directly onto adults

Adult apterous *Myzus persicae* were placed on filter paper in Petri dishes, ten per dish. Each dish was then sprayed, using a hand-held mister, with either water or the kaolin particle suspension. The paper in each dish was sprayed an equal number of times, which approximated that needed to

spray plant material until run off. Excess liquid in the Petri dishes was allowed to drain off, and the aphids were left for 1 h. After this period of time, the number surviving was assessed. Random samples of the surviving aphids from each dish were then transferred individually using a paintbrush onto single calabrese plants, at the one-to-two true leaf stage. A perforated 'bread' bag was placed over each plant and secured around the base of the pot with an elastic band. Every subsequent 24 h, the bags were removed and the plants examined, until the adult aphid died. At each examination the number, if any, of nymphs produced was noted and these were removed.

Aphids that could still be located 24 h after initially being transferred onto the plants were classed as having settled. The survival time (post-spraying) of settlers, the total number of nymphs produced and the mean number of nymphs produced per aphid per day were compared between treatments using a general linear model procedure (The Lawes Agricultural Trust, 2002). There were five replicates of each treatment at the Petri dish stage and ~30 at the individual survival stage.

Mean relative growth rate

First-instar *Myzus persicae* were weighed using a Sartorius 4303 micro-balance and then individually confined to whole calabrese plants that had previously been sprayed with kaolin on both surfaces, the upper surface only or neither surface. After seven days, the aphids were recovered and weighed again. Their mean relative growth rate (MRGR) was then calculated according to the formula (van Emden, 1969):

$$\text{MRGR}_{(\mu\text{g}\mu\text{g}^{-1}\text{d}^{-1})} = \frac{[\log_e \text{ final weight}_{(\mu\text{g})} - \log_e \text{ initial weight}_{(\mu\text{g})}]}{\times [\text{No. of days over which weight increase is measured}]^{-1}}$$

The experiment was repeated on a total of three occasions (blocks), each one allocating 7–9 aphids to each of the three experimental treatments. The MRGR was compared between treatments using a general linear model procedure (The Lawes Agricultural Trust, 2002) taking into account the effect of block. A chi-square goodness-of-fit analysis (ibid) was used to compare, between treatments, the numbers of aphids that could not be found compared to those which could.

Reproduction and survival

Adult *M. persicae* that had been removed from the culture ~1 h previously were transferred to the apex or petiole of the leaves of calabrese plants (one-to-two true leaf stage) of each treatment type, one per plant: kaolin treated on both leaf surfaces, upper surface only and untreated. Each pot, containing an individual plant, was then covered with a 'bread' bag secured at the base round the pot with an elastic band. After 24 h the numbers of nymphs produced by the adults were counted, and the adult was removed from each plant. After a further 3 days, the plants were checked again; the number of remaining nymphs counted and their position on the stem, the abaxial or adaxial side of a leaf, noted.

The experiment was repeated on a total of three occasions (blocks), and the total number of aphids allocated to each treatment were 43 on untreated plants, 41 on kaolin treated (upper surface only) and 41 on kaolin treated (both surfaces).

Table 1. The longer-term effects of direct application of kaolin particle film onto apterous adult *M. persicae*.

Treatment (no. of aphids transferred to plants)	No. of aphids settled	Survival in days (mean \pm SE)	Total no. of nymphs produced per aphid (mean \pm SE)	Nymphs produced per aphid per day (mean \pm SE)
Water (29)	24	6.21 \pm 0.73	16.75 \pm 3.06	2.26 \pm 0.23
Kaolin treated (34)	28	7.32 \pm 0.69	21.82 \pm 2.80	2.76 \pm 0.22

Statistical analyses of the numbers of adults surviving and the number of surviving adults that produced nymphs were carried out using Pearson chi-square tests (The Lawes Agricultural Trust, 2002). A general linear model procedure was used to analyse the mean number of nymphs produced (ibid) and a binominal logistic regression to analyse the proportion of surviving nymphs after 3 days (ibid). Predicted mean values are presented for both nymph production and survival as the effect of block was taken into account in both of these analyses (ibid).

Settling preference of nymphs

Thirty-five mm diameter leaf discs from calabrese plants (three-to-four true leaf stage) treated either with kaolin or water on both leaf surfaces were placed adaxial side up in 9 cm diameter Petri dishes (one of each treatment type per dish) with a single *M. persicae* second-instar nymph. The position of each aphid (either on the kaolin-treated disc, the water-treated disc or elsewhere) after 2 and 18 h was recorded. Statistical analysis of the effect of treatment was performed by Pearson chi-square goodness-of-fit analysis (The Lawes Agricultural Trust, 2002).

An additional experiment examined the effects of the kaolin particle film on the settling of second-instar nymphs over a shorter timeframe. Single nymphs were placed across the middle of the adaxial side of 35 mm leaf discs, half of which had been treated with two coats of the kaolin, applied with a soft sponge. The other half of the disc was left untreated, the boundary being defined by the midrib. Observations were then made every 100 s over the following 20 min; and each aphid's responses were subsequently classed into one of three categories, dependent on where they had spent the majority of their time: either on the treated leaf portion; the untreated leaf portion; or off the leaf. Statistical analysis of the effect of the location of the aphid was performed by Pearson chi-square goodness-of-fit analysis (ibid). Log-linear modelling was used to investigate these differences further (ibid).

Settling preference of adults

The behaviour of settling apterous adult aphids was investigated using the same methods as those for the nymphs over the 2 and 18 h periods. The settling preference experiments were all performed at $20 \pm 2^\circ\text{C}$.

Results

Application of kaolin directly onto adults

Of the initial 99 and 102 aphids that were sprayed with the kaolin and water, respectively, only one of the kaolin-sprayed aphids and two of the water-sprayed aphids were dead after 1 h. The survival time of the kaolin-sprayed

adults was not significantly different from those sprayed with water ($t=1.10$, 50 df, $P=0.275$) (table 1). No significant difference was found in total number of nymphs produced ($t=1.22$, 50 df, $P=0.227$) or mean number of nymphs produced per aphid per day ($t=1.57$, 50 df, $P=0.124$) (table 1).

Relative growth rate

After one week, 16 of the 21 aphids on untreated plants were still present. This compared to 20 of the 26 on the kaolin (upper surface only) treated plants and 14 of the 23 on the kaolin (both surfaces) treated plants. There were no significant differences in the proportions of aphids that were present between the treatments ($\chi^2=1.87$, 2 df, $P=0.392$).

The MRGR of *M. persicae* was not found to be significantly different between those aphids on the untreated plants and those with kaolin particle film on either the upper leaf surfaces only ($t=0.09$, 45 df, $P=0.928$) or both leaf surfaces ($t=0.27$, 42 df, $P=0.785$). The MRGR values (\pm SE) for aphids on untreated, kaolin particle film-upper surfaces only and kaolin particle film-both surfaces were 0.3052 ± 0.0121 , 0.3077 ± 0.0115 and 0.311 ± 0.0136 , respectively.

Reproduction and survival

The number of adults that survived 24 h post-transfer onto plants did not differ significantly between treatments ($\chi^2=0.73$, 2 df, $P=0.692$; table 2). Similarly, the number of adults which produced nymphs did not differ significantly between treatments ($\chi^2=1.76$, 2 df, $P=0.415$; table 2). The mean number of nymphs resulting from both the total number of surviving adults and the nymph-producing adults only was found to be higher on the water only treatment compared to the kaolin treatments; however, treatment was not found to have a significant effect ($F=1.40$, 111 df, $P=0.252$; $F=1.24$, 88 df, $P=0.294$, respectively; table 2). After 3 days, the proportion of nymphs that had survived was high and not significantly different between treatments (deviance ratio = 1.25, $P=0.288$; table 2).

The position of the *M. persicae* nymphs after 3 days was such that none were found on either the plant stem or leaf petioles, all being on either an abaxial or an adaxial leaf (including cotyledons) surface. There was no significant difference between treatments in the proportion of nymphs on the adaxial compared to the abaxial leaf surfaces (data not shown, $\chi^2=0.99$, 2 df, $P=0.610$).

Settling preference of *M. persicae*

After both 2 and 18 h, there were significantly less nymphs ($\chi^2=157.03$, 2 df, $P<0.001$; $\chi^2=183.99$, 2 df, $P<0.001$, respectively) and adults ($\chi^2=88.91$, 2 df, $P<0.001$;

Table 2. The effects of kaolin particle film-treated plant material on *M. persicae* reproduction and survival. *Mean \pm SE.

Treatment (No. of replicates)	Adults present (a) after 24 h	No. producing nymphs (b) after 24 h	No. of nymphs per (a)*	No. of nymphs per (b)*	Prop. out of 1 of nymphs surviving at 3 days*
Untreated (43)	40	32	2.85 \pm 0.29	3.52 \pm 0.27	0.90 \pm 0.03
Kaolin-upper surface (41)	37	32	2.42 \pm 0.30	2.93 \pm 0.27	0.92 \pm 0.03
Kaolin-both leaf surfaces (41)	39	29	2.17 \pm 0.29	3.19 \pm 0.28	0.89 \pm 0.03

$\chi^2 = 100.49$, 2 df, $P < 0.001$, respectively) on the kaolin-treated discs compared to the water-treated discs (fig. 1a, b).

Of 200 second-instar nymphs observed for a 20 min period, 104 were observed for the majority of times on the untreated half of the leaf disc. This was significantly more than the 60 which were observed more frequently on the kaolin-treated half ($\chi^2 = 99.71$, 2 df, $P < 0.001$). Thirty-five of the aphids spent the majority of the time points off the leaf disc and one was observed an equal number of times off the disc and on the treated half of the leaf disc.

Discussion

A direct coating of kaolin particle film does not appear to directly affect the survival of *M. persicae* as may have been

the case if the residual coating (which was still visible on the kaolin-treated aphids throughout) had blocked feeding or respiration, or limited movement (Glenn *et al.*, 1999; Cottrell *et al.*, 2002). Similar style direct application experiments with the pear psylla, *Cacopsylla pyricola* (Foerster) also indicated that the particle films had no immediate contact toxicity (Puterka *et al.*, 2005). However, in contrast to the *M. persicae* data, increased mortality was reported after 72 h for *C. pyricola* adults and just 3 h for nymphs. The mechanisms for these effects are uncertain (Puterka *et al.*, 2005).

In contrast to studies with the spirea aphid (*Aphis spireacola* Patch) where a nearly 50% reduction in numbers occurred within a 24 h period after insects were placed on treated apple leaves in no-choice experiments (Glenn *et al.*, 1999), survival of *M. persicae* was not affected by kaolin

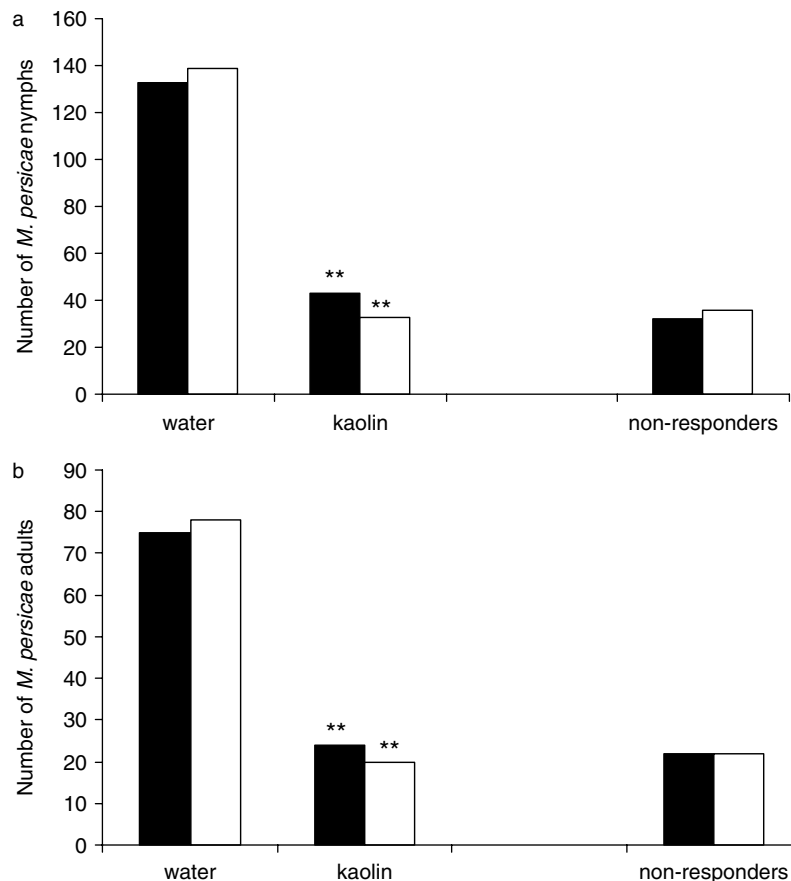


Fig. 1. The leaf disc treatment type on which nymph (a), and adult (b), *M. persicae* were positioned after 2 (■) and 18 (□) hours in a choice situation. ** Significantly different from water-treated disc at $P < 0.01$ level.

particle film treatment of plants. Production of progeny by adults was also not affected by the treatment, and this is congruent with studies of the black pecan aphid, *Melanocallis caryaefoliae* (Davis) (Cottrell *et al.*, 2002). However, in contrast, nymph production by *A. spiraeicola* adults was inhibited on kaolin-treated plant material (Glenn *et al.*, 1999).

These studies show that, under laboratory conditions, the kaolin particle film treatment does not appear to affect *M. persicae* in terms of growth rate, reproduction or survival. The lack of effect of the kaolin particle film on aphid performance contrasts with investigations of leaf-chewing pests such as *Plutella xylostella* (L.), *Spodoptera exigua* (Hübner) and *Macrodactylus subspinosus* (F.) (Knight *et al.*, 2000; Showler, 2003; Isaacs *et al.*, 2004; Sackett *et al.*, 2005; Barker *et al.*, 2006), and this may be a consequence of the feeding strategy of aphids. The needle-like stylets may be able to penetrate between the particles, so that any disruption by the kaolin particle film is minimal or negligible. However, previous laboratory studies, with other species of aphids and psyllids, indicate that kaolin applications may reduce the performance of other sap-feeding insects (Glenn *et al.*, 1999; Cottrell *et al.*, 2002; Puterka *et al.*, 2005). *Myzus persicae* survival may be unaffected by the kaolin particle film for a number of reasons: it could be due to the specific formulation of the film used in these studies, or a particular feature of this aphid.

The results reported here, therefore, provide no evidence that kaolin treatment affects *M. persicae* performance, but kaolin application to plants has been reported to reduce *M. persicae* infestations under field conditions (Karagounis *et al.*, 2006). It seems likely that effects on aphids in the field involve some degree of behavioural rejection of kaolin-treated plant surfaces. Congruent with investigations of other sap feeders, our studies of *M. persicae* show a highly significant decreased preference for the kaolin-treated leaf material. However, previous studies assessed insect preference over relatively long-term choice tests: the earliest assessments of insect numbers were made at 24 h (Puterka *et al.*, 2005) and 72 h (Cottrell *et al.*, 2002). Our small-scale leaf disc bioassay revealed that aphids show a clear avoidance of kaolin-treated leaf areas very rapidly, certainly within the first 20 min of plant contact. The early change in aphid behaviour may be related to altered visual or tactile cues from the plant material (Glenn *et al.*, 1999; Bürgel *et al.*, 2005), and further studies are necessary to elucidate behavioural effects of kaolin in detail.

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