Effect of kaolin clay on migrant alate aphids (Hemiptera: Aphididae) in blueberry fields in the context of *Blueberry scorch virus*

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ABSTRACT

The efficacy of kaolin clay (Surround® WP) in reducing the number of migrant aphids on blueberry, *Vaccinium corymbosum* L. (Ericaceae) and the incidence of *Blueberry scorch virus* (BlScV) was determined. Two applications of kaolin clay reduced the number of alatae collected on treated 'Berkeley' plants by as much as a factor of eight between 4 June and 16 August. However, five of 100 test plants located near infected fields and exposed only to migrant alatae between 10 May and 16 August became infected with BlScV: three controls and two treated with kaolin clay. The work demonstrates the importance of migrant alatae in the spread of BlScV; 5% transmission is consistent with previous estimates of annual virus spread by winged and non-winged aphids. Three of the plants became infected between 10 and 27 May (one control and two treated with kaolin clay), indicating the importance of aphid flights in May for virus transmission. Rainfall removed much of the kaolin clay and this may have affected its efficacy. The aphid data demonstrated that migrant alatae are able to discriminate between untreated and kaolin-treated blueberry plants, and that *Ericaphis fimbriata* (Richards), which utilizes blueberry as a host, discriminates better than other migrant species. Water trap data do not necessarily reflect the total migrant aphid composition found on plants in the field. Plant growth was not affected by the kaolin clay, but the fruit had clay residues amongst the bracts of the calyx limiting the use of this product on producing fields to the period before fruit set. Kaolin clay may be best suited to protection of nursery stock, but further work is needed to improve efficacy during wet weather and determine optimal application frequency.

Key Words: *Ericaphis fimbriata*, aphid behaviour, virus transmission

INTRODUCTION

Blueberry scorch virus (BlScV) was detected in 20 fields of blueberry, *Vaccinium corymbosum* L. (Ericaceae), in southwestern British Columbia (BC), Canada in 2000, and this increased to 140 fields by 2004 (Wegener *et al.* 2006). Symptoms

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depend on virus strain (Wegener *et al.* 2006) and blueberry cultivar, and can include severe blighting of flowers and young leaves, twig dieback, and yield reductions of more than 85% in the third year of symptom expression (Bristow *et al*. 2000). The virus is a member of the genus *Carlavirus* and is thought to be transmitted by *Ericaphis fimbriata* (Richards) (= *Fimbriaphis fimbriata*) (Hemiptera: Aphididae) (Remaudière and Remaudière 1997) in a non-persistent fashion (Bristow *et al*. 2000).

Raworth *et al*. (2006) showed that alatae of 87 aphid species out of a known 412 species in BC (Chan and Frazer 1993) fly over blueberry fields. Given the mode of transmission, these alatae may land on an infected plant, probe, then move to an uninfected plant and transmit the virus. Lowery *et al.* (1990) showed that whitewash could be used on rutabaga, *Brassica napobrassicae* (L.) (Brassicaceae), to reduce field populations of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and incidence of *Turnip mosaic virus*, another non-persistently transmitted virus. Liang and Liu (2002) showed in laboratory assays that kaolin particle film applied to the upper surface of melon leaves, *Cucumis melo* L. (Cucurbitaceae), reduced the number of adult silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae), an important vector of viruses. Here, we present the results of an experiment to determine the efficacy of kaolin clay (Surround® WP, Engelhard Corp., Iselin, New Jersey) in reducing the number of alatae found on blueberry plants in the field and the incidence of BlScV.

MATERIALS AND METHODS

'Berkeley' blueberry test plants were shipped from Fall Creek Farm & Nursery Inc. (Lowell, Oregon) in closed bulk containers in the spring of 2004. The plants were potted in 8-litre pots and held under insect screening at Agassiz, BC, an area isolated from BlScV. On 10 May 2004, 100 plants 42.4 ± 8.5 (SD) cm tall were transported in an enclosed cube truck from Agassiz to a field with BlScV-infected plants in Surrey, BC where they were placed in an open area within the field. One-hundred-forty plants from the same lot were grown in a screenhouse near Abbotsford, BC and served as non-field-exposed controls. Before exposure to the field, half the test plants were sprayed with kaolin clay (50 g per litre of water). The material was applied to the upper leaf surface using a Solo® backpack sprayer with an adjustable plastic nozzle (Solo, Newport News, Virginia) and was re-applied after inspection to ensure adequate coverage. The plants were placed in the field 65 cm above the ground on 7 mm diameter rebar driven 50 cm into the ground. A hose clamp, fastened to the rebar 5 cm from the top, prevented a 20 x 20 x 0.7 cm piece of plywood, a water catch tray, and the 8 litre pot (each with a hole in the centre) from sliding down the rebar. The hole in the water catch tray was sealed with silicone so that excess water applied by hand to the soil in the pot would remain in the catch tray. The rebar was coated every 2 weeks with Stickem Special® (Seabright Enterprises, Emeryville, California) between the ground and the pot to ensure that only flying alatae could reach the plants. Groups of five treated or five untreated plants were established with the supporting rebar posts at the corners of a 60 x 60 cm square and one post in the centre. Groups were placed in a completely randomized design separated by at least 2 m. Because the grower was removing infected plants as they were identified, the experiment was dismantled on 27 May. The test plants were taken back to Agassiz and treated with thiamethoxam (Actara® 25% WG) to kill any aphids. The experiment was then re-established with the same plants on 4 June in an open area 10 m east

of a field in Richmond, BC that had BlScV-infected plants that were not being removed in 2004. The same kaolin-treated and control test plants were placed in groups of five as before. Groups were completely randomized and kaolin clay was applied on 4 and 22 June. In addition, one water trap, a 35 cm diameter x 7 cm deep metal pan coated with Tremclad® yellow paint, was set 1.4 m above the ground, 15 m east of the trial. The trap was maintained weekly by replacing the water and adding 10 ml of extran® 300 detergent (EM Science, Gibbstown, New Jersey) to reduce surface tension, and 50 g of salt as a preservative. The test plants were removed from the field on 16 August, sprayed with thiamethoxam to kill aphids on the plants, and maintained in a screenhouse near Abbotsford, BC.

Alatae observed on the test plants were counted, removed, and stored in 70% ethanol weekly from 8 June until 6 July, then every 2 weeks until 3 August, for a total of seven samples. Alatae from the water trap were collected weekly from 8 June until 15 August and stored in 70% ethanol. The aphids were identified to species; voucher specimens are maintained at the Eastern Cereal and Oilseed Research Centre, Ottawa, Ontario. Colonies of apterae observed on 6 July were removed so that production of alatae on the plants would not confound the estimates of migrant alatae. Plant growth was monitored on one test plant selected at random from each group of five. One stem from each plant was marked and leaves greater than 1 cm long were counted every 2 weeks; on kaolin-treated plants, the numbers of new untreated leaves were estimated by subtracting the leaf counts when the plants were sprayed from the leaf counts at later dates. Rainfall data for the fields at Surrey and Richmond were obtained from Environment Canada meteorological stations at the Surrey Municipal Hall and Vancouver International Airport, respectively.

Because the latency period for BlScV could be as much as 3 years (Bristow *et al.* 2000), the test plants were sampled on 15 March and 16 May 2005, 18 May 2006, and 28 March 2007. Five to seven leaves (buds for the March samples) were collected from each test plant and analyzed for BlScV by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). The March 2005 sample was also analyzed using reverse transcription polymerase chain reaction (RT-PCR), and thereafter each ELISA-positive plant was confirmed by RT-PCR. All BlScV positive material was analyzed for the virus strains BC-1 (GenBank Accession No. AY941198) and BC-2 (GenBank Accession No. AY941199) (Bernardy *et al.* 2005) using RT-PCR. The plants were inspected for virus symptoms — flower blighting and leaf and stem dieback — when sampling.

Because the test plants had been exposed to two fields and there can be different virus strains in different fields (Wegener *et al.* 2006), the field at Richmond was sampled to determine the dominant virus strain; removal of BlScV-infected plants precluded such sampling at the Surrey field. On 9 August 2005, 100 field plants were sampled in a regular pattern from a block of about 800 plants just west of the kaolin clay trial at Richmond, BC, and on 15 May 2007, 13 field plants were sampled from a block of about 60 plants just north of the trial; there were no commercial blueberry plants to the east or south. These samples were analyzed for BlScV in general and specifically for the strains BC-1 and BC-2, using RT-PCR.

ELISA was conducted according to the method described by Clark and Adams (1977) with the exception that borate grinding buffer (Martin and Bristow 1988) was substituted. Positive and negative controls were included in each test and samples were considered positive for BlScV if absorbance values were at least three times greater than the mean absorbance value of the negative control samples (Sutula *et al.* 1986; Pataky *et al.* 2004).

The RT-PCR assay utilized a rapid, direct one-tube-RT-PCR procedure (Rowhani *et al.* 2000) for virus detection. For sample preparation, leaf tissue from test plants was homogenized in sample bags (Agdia Inc., Elkhart, Indiana) with grinding buffer, pH

9.6 (carbonate ELISA coating buffer containing 2% PVP-40, 0.2% BSA, 0.1% sodium meta bi-sulfite, and 0.05% Tween 20®, all from Sigma Aldrich Canada, Oakville, Ontario) at a dilution factor of 1:20. A 2 µl aliquot of this plant extract was pipetted into a 0.2 ml PCR tube containing 25 µl of filter-sterile GES buffer (0.1 M glycine-NaOH, pH 9.0, 50 mM NaCl, 1 mM EDTA, 0.5% Triton® X-100, all from Sigma Aldrich Canada, Oakville, Ontario). The sample was heated in a thermal cycler (iCycler, Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ontario) at 95 ºC for 10 minutes, and then chilled in an ice bath for 5 minutes. For the one-tube-one-step RT-PCR procedure, all components for RT and PCR were assembled in 25 µl final volume of reaction mix containing 2.5 µl of 10 X PCR buffer (GeneSys Ltd., Surrey, UK), 0.5 µl of dNTP mixture (10 mM), 0.1 µl of RNaseOUT™ inhibitor (40 U/µl, Invitrogen Corp., Burlington, Ontario), 0.035 µl of MMLV-RTase (200 U/µl, Invitrogen Corp.), 0.25 µl of Taq DNA Polymerase (5 U/µl, GeneSys Ltd.), 1.25 µl dithiothreitol (0.1 M, Invitrogen Corp.), 1.25 µl of each BlScV-specific primer BS32F, CAACCCGACGTTCATATTCA (10 µM) and BS506R, TCTTCAATGCACGATGTTCC (10 µM), and 2 µl of sample extract. RT-PCR was conducted using the following profile: 30 min for RT at 52 ºC, followed by 35 cycles of 94 ºC for 30 s, 53 ºC for 45 s and 72 ºC for 60 s and a final extension step at 72 ºC for 7 min. The amplified fragment (478 bp) was analysed in a 1.5% agarose gel by electrophoresis in 1 X TBE buffer (90 mM Tris-borate, 2 mM EDTA), using 2 µl of the PCR mixture, followed by staining with ethidium bromide (0.5 µg/ml) and visualized with a UV transilluminator (260 nm). Virus strain assays utilized the primers BlScV-BC1-ORF2-F2, AAGGTGAAATCGGGGTTTTG and BlScV-BC1-ORF5-R1, GACTCGGGCAGGGACCTC for strain BC-1, and BlScV-BC2-ORF2-F1, ACCTTCTCTCGACCGAGATC and BlScV-BC2-ORF5-R1, GAGCTTGGACCAGCATCC for strain BC-2, with an annealing temperature of 50-55 ºC.

The mean number of alatae per plant and the number of leaves per stem for each group of five plants (n = 20) on every sample date (n = 7), were analyzed by repeated measures ANOVA (SAS 1990) after transformation by square-root (x + 0.5) and ln (x +1.0), respectively, to stabilize the variance (Southwood 1966). The number of alatae per plant was plotted against day-degrees (dd) above 4.1 ºC, the developmental temperature threshold for *E. fimbriata* (Raworth and Schade 2006), to determine the potential for the earliest progeny of *E. fimbriata* alatae to contribute to the population of alatae given that all aphids were removed by hand on 6 July. Chi-square was used to compare numbers of alate *E. fimbriata* with other migrant aphids as a group on treated versus control plants, or on blueberry plants versus yellow water trap samples.

The number of alatae found on the test plants at weekly intervals was a function of numbers attracted to the plant and rate of departure after landing. To distinguish between the two effects, alatae behaviour on kaolin-treated leaves and controls was examined in the laboratory at Agassiz, BC in a windowless room at 20-24 ºC, with overhead fluorescent lighting (6.1 $\mu E \cdot m^{-2} \cdot s^{-1}$; 1 µE = 1 µmol of photons), and 56-67% RH. Alate *E. fimbriata* from an organic blueberry field and from a laboratory colony on 'Berkeley' blueberry were starved for 3-5 h on moist filter paper. An alate aphid was then placed in the centre of a 'Berkeley' blueberry leaf (6.8 ± 0.8 (SD) x 3.8 ± 0.5 cm, abaxial side down) in a glass Petri dish. The aphid was observed for 5 min, recording time settled. Three treatments were run simultaneously: leaves were either dipped in kaolin clay (50 g per litre reverse osmosis [RO] water), sprayed with kaolin clay on the upper side, or dipped in RO water (control). There were 20 replicates of the three treatments, and 15 additional replicates of the kaolin-sprayed versus control leaf. Treatment position was randomized

among replicates. The data were analyzed by ANOVA without transformation. A 24 h variation of the experiment was conducted, examining 14 replicates of kaolin-sprayed versus control leaves (9.1 ± 0.7 x 5.2 ± 0.5 cm, abaxial side down) simultaneously for alatae position (upper or lower leaf surface, or off leaf) 20 times, at various intervals from 15 min to 10 h. When an aphid was found off the leaf, it was placed back on the leaf. The experiment was repeated with 15 replicates of kaolin-sprayed versus control leaves. The proportion of observations of alatae in each position was arcsine square-root transformed and analyzed by ANOVA for differences among treatments, trials, and the interaction treatment by trial.

RESULTS AND DISCUSSION

Kaolin clay significantly ($P < 0.001$) reduced the number of alatae found per plant on groups of five plants at Richmond (Fig. 1), even though rainfall (5, 7, 10-13 June, total 21.8 mm; 2, 6, 10 July, total 16.6 mm; and 3, 4, 6 August, total 19 mm) removed much of the clay, reducing the whiteness of the leaves, and despite the fact that new untreated foliage increased in surface area after application of kaolin clay (6.1 to 49.6 new leaves per stem between 15 June and 3 August). A total of 409 alatae was found on untreated controls and 71 on kaolin-treated plants.

It may be argued that the alatae were not discriminating with respect to attraction, but were discriminating with respect to rate of departure. However, the laboratory experiments provided no evidence that alatae behaved differently on kaolin-treated than on control leaves. In the first experiments, there was no difference in time settled on leaves treated with kaolin clay compared with controls during a 5 min period ($P > 0.05$). In the second experiments conducted during 24 h, alatae tended to move to the underside of the leaves and remain there regardless of treatment. There was no trial by treatment interaction or difference between kaolin-treated leaves and controls with respect to the position of the alatae on the leaf ($P > 0.05$), and the proportion of observations with alatae feeding on the abaxial surface was 0.79 -0.045 +0.042 (back-transformed mean ± SE) versus 0.082 -0.025 +0.029 on the upper surface. There was a trial by treatment interaction with respect to the proportion of observations in which alatae were found off the leaf ($P = 0.037$), but this weak effect merely suggests an inconsistency in the pattern from one trial to the next because there was no overall difference in the proportion off the leaf between treated and control leaves.

It might also be argued that the progeny of the earliest *E. fimbriata* contributed to later populations of alatae found on the test plants, producing biased results. However, alatae generally do not produce alatae directly (Lees 1961), therefore two generations were required for production of alatae on the test plants. Developmental time from birth to adult in June and July requires about 175 dd above 4.1 ºC (Raworth and Schade 2006), so alatae would not arise on the test plants until after the fourth sample (Fig. 1), potentially contributing to the counts in the fifth sample, but this effect would be small because few migrant alatae were found in the first 80 dd, and progeny of alatae produced after 80 dd would have been removed on 6 July.

Five field-exposed test plants, three control and two kaolin-treated plants, were infected with BlScV; none of the non-field-exposed plants was infected. This showed that the infections were due to field exposure (5:95 versus 0:140, $\chi^2 = 7.0$, 1 df, $P < 0.01$), and as applied under the prevailing conditions, kaolin clay did not prevent virus transmission. All five plants were BlScV-positive in the first, and subsequent leaf samples during 3 years, and all were symptomatic. The infection rate, 5%, was consistent with the annual rate due to both alate and apterous aphids observed by Wegener

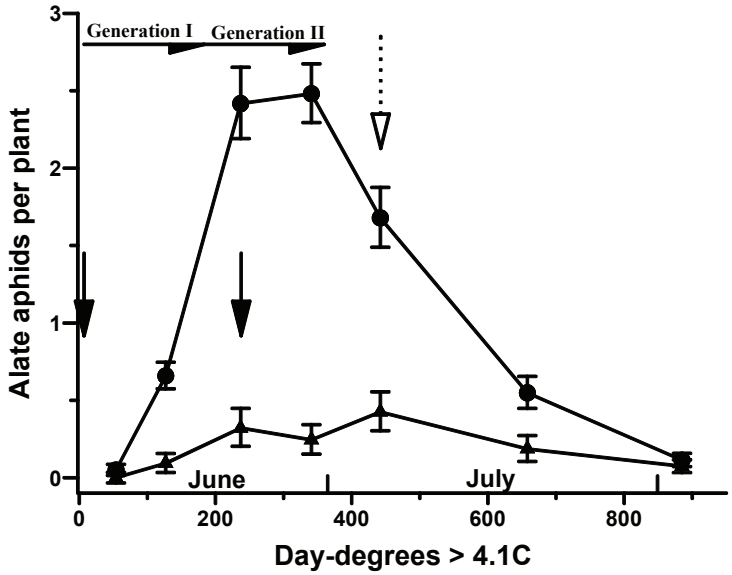

Figure 1. Mean number of alate aphids (± SE, back-transformed data) per control plant (circles) and kaolin-treated plant (triangles) versus time, 2004. Day-degree summations start the day the experiment was set up at Richmond, BC. Dark vertical arrows mark the application of kaolin clay; open vertical arrow marks the removal of all aphids by hand; horizontal arrows mark the first and second aphid generations arising from the earliest alatae to land on the plants.

et al. (2006) in three commercial fields from 2001-2004. Our result therefore demonstrates the importance of alate aphids in the spread of BlScV.

Two of the five BlScV-positive test plants were infected with the BC-2 strain and were negative for BC-1. The remaining three plants were negative for both BC-1 and BC-2 and were therefore infected with unknown BlScV strains. This contrasts with the survey of crop plants just west and north of the trial in Richmond where 46 out of 113 plants were BlScV-positive, all with BC-2 and none with BC-1. There were no BlScV-positive plants with unknown strains. We conclude that the infections in the test plants with unknown BlScV strains probably did not come from the Richmond field (46:0 versus 2:3, Fisher's Exact Probability test $P < 0.001$, 1 df). They probably came from the Surrey field. The Surrey field was known to have plants infected with BC-2 (Wegener, pers. comm.) and BC-5 (Wegener *et al.* 2006). Therefore, at least three of the infections — one control and two kaolin-treated plants — occurred in the Surrey field between 10 and 27 May, a period of 17 days, whereas the two BC-2 infections (both controls) may have occurred during the 17 days in the Surrey field, or between 4 June and 16 August, a period of 73 days in the Richmond field. This demonstrates that May is an important period for virus transmission. During the spring in some years, there are significant flights of *Euceraphis betulae* (Koch) and *Periphyllus testudinaceus* (Fernie) (Raworth *et al.* 2006), but perhaps more importantly, *E. fimbriata*, a known vector of BlScV (Bristow *et al.* 2000) and the dominant aphid on blueberry, produces a high proportion of alatae in May (Raworth 2004).

There were 320 *E. fimbriata*, 28 *Aphis fabae* Scopoli, 8 *E. scammelli* (Mason), 11 *Euceraphis betulae* (Koch), 23 *Wahlgreniella nervata arbuti* (Davidson) and one or two individuals of 10 other species collected from the test plants at Richmond. *Ericaphis fimbriata* was affected more by the kaolin clay than the other aphids taken as a group; 284 and 36 *E. fimbriata* alatae were collected on controls and kaolin-treated plants, respectively, but 60 and 21

alatae of other species were collected on the respective plants (χ^2 = 11.5, 1 df, P < 0.001). Assuming that the effect of kaolin clay on alatae departure was equal among species, this result suggests that *E. fimbriata*, which utilizes blueberry as a host, can better differentiate between untreated and kaolin-sprayed blueberry than other species which do not utilize blueberry as a host.

Only nine *E. fimbriata* alatae were found in the water trap compared with 232 alatae of other species. This was different from the pattern on blueberry (320 *E. fimbriata* and 81 other species; χ^2 = 339.6, 1 df, P < 0.001), reflecting lower attraction to blueberry, a higher turnover rate, or both, for migrant species that don't utilize blueberry as a host compared with *E. fimbriata* which does. In the water trap, there were 35 *A. fabae*, 12 *Brachycaudus helichrysi* (Kaltenbach), 77 *Calaphis flava* Mordvilko, 12 *E. betulae*, 16 *Myzocallis coryli* (Goeze) and fewer than 10 individuals for each of 29 other species, a different mix of aphids than on the test plants. Only one *B. helichrysi* and no *C. flava* or *M. coryli* were collected on the test plants, suggesting that either they are not attracted to blueberry, or that their residence time on blueberry is very short. Studies of the attraction and alighting behaviour of several aphid species with respect to blueberry would be useful. Using water trap data, Raworth *et al.* (2006) suggested that *B. helichrysi* requires study as a vector of BlScV, however, this would not be necessary if the aphid is rarely attracted to blueberry. On the other hand, no *E. scammelli* were collected in the water trap in this study, or in a more extensive 2-year study (Raworth *et al.* 2006), but this species was found on the test plants. This result shows that water trap data do not necessarily reflect the total migrant aphid composition.

Plant growth was not affected by the kaolin clay (P > 0.05). The number of leaves per stem increased from 30.3 ± 3.0 (SE) on 11 May to 131.8 ± 16.2 on 10 August (P < 0.0001), and the interaction of time and treatment was not significant. Spiers *et al.* (2004) observed increased plant growth in blueberry treated with Surround WP. However, their study was conducted in Mississippi, and they speculated that the clay protected the plants from heat stress and insect pests.

Given the low virus transmission rate in the blueberry system, further work is needed on a much larger scale — in the order of 5000 plants — to determine the efficacy of kaolin clay with respect to virus transmission. However, it is clear that even if a difference is detected, BlScV will still be transmitted to kaolin-treated plants unless efficacy can be improved by repeated applications or addition of an effective surfactant-sticker. Lowery *et al.* (1990) applied whitewash weekly, significantly reducing aphid numbers and infection rates of *Turnip mosaic virus*, which is transmitted in a non-persistent manner. In our study, kaolin clay was applied only three times in nearly 13 weeks. This consideration is particularly important in May when rainfall tends to be higher than the subsequent 3 months (66.6 versus 49.6, 44.1, and 28.8 mm, respectively, ± 8.6 overall SE; data from the Vancouver International Airport 1997-2006). In our study, rainfall at Surrey on 21, 22, and 25 May removed most of the clay film on the leaves. However, weekly sprays later than bloom in May would not be practical in commercial fields because clay residues which we observed on the fruit amongst the bracts of the calyx from our sprays in June would render the fruit unmarketable. In the final analysis, unless an effective surfactant-sticker is added, kaolin clay may not be a useful IPM tool for this crop, except perhaps on nursery stock where repeated sprays are possible throughout the spring and summer. The difference in numbers of alatae collected on kaolin-treated plants compared with controls suggests that further studies to determine efficacy and optimal application frequency for nursery stock are warranted. These studies should also examine the effect of plant-group size, and distance between treatment and control groups, on the numbers of alatae collected from each group.

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