Movement of *Ericaphis fimbriata* (Hemiptera: Aphididae) apterae on blueberry

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ABSTRACT

Blueberry scorch virus is a new and important pathogen of blueberry in British Columbia, Canada of which the blueberry-infesting aphid *Ericaphis fimbriata* is a known vector. In a study of the movement of apterous *E. fimbriata*, significantly more aphids fell when one ladybird beetle was added to *E. fimbriata* infested blueberry branches than when zero, two, or four were added. Similar numbers of aphids fell in the presence or absence of beetles at low aphid density (10-30 aphids per terminal), but more fell in the presence of beetles at high aphid density (50-70 aphids per terminal). The time taken for aphids to move a minimum distance of 60 cm off infested plants onto uninfested plants decreased with increasing aphid density which has important implications for the spread of the virus.

Key Words: *Ericaphis fimbriata*, Blueberry scorch virus, highbush blueberry, apterous aphids, coccinellids

INTRODUCTION

Highbush blueberry, *Vaccinium corymbosum* L. (Ericaceae) is one of the most important agricultural crops in British Columbia (BC). With approximately 4800 ha in production in 2004, BC ranks as the second-highest blueberry producing region in the world (British Columbia Blueberry Council 2006). Blueberry scorch virus (BlScV) was first reported in the Fraser Valley in June 2000 (Martin 2003) and is a serious threat to the BC blueberry industry. As of 2003, over 76 fields were infected with BlScV (Wegener *et al*. 2003). Plants infected with BlScV often do not show symptoms for 1-2 years, but do not recover once symptoms appear (Bristow *et al.* 2000).

Transmission of BlScV by *Ericaphis fimbriata* (Richards) (= *Fimbriaphis fimbriata*) (Hemiptera: Aphididae) (Remaudière and Remaudière 1997), occurs predominantly between mid-April and mid-August, and when aphids are not controlled the number of infected plants in a field can double annually (Bristow *et al.* 2000). While alate *E. fimbriata* can move rapidly from one row to another and potentially spread BlScV through a blueberry field, little is known about the movement of the apterous form of *E. fimbriata*, which predominates in blueberry fields in spring and summer (Raworth 2004). The rate of apterous aphid movement may significantly affect how rapidly a plant virus is transmitted in the field (Bailey *et al.* 1995).

The presence of predators can have a strong influence on the dispersal of aphids and hence on the spread of a virus (Smyrnioudis *et al.* 2001). Predators (e.g. coccinellids) and parasitoids are known to increase aphid movement in the laboratory and the field (Tamaki *et al.* 1970, Niku 1972, Roitberg and Myers 1978, Gowling and van Emden 1994) and have been dem-

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onstrated to increase the spread of plant viruses by aphids (Roitberg and Myers 1978, Sewell *et al.* 1990, Bailey *et al.* 1995). In this study, we investigated whether the number of both red and green apterous *E. fimbriata* that fall off blueberry plants increased in the presence of ladybird beetles and if apterous *E. fimbriata* moved quickly from one plant to another in the field.

MATERIALS AND METHODS

Aphids falling in the presence of ladybird beetles. *Vaccinium corymbosum* 'Duke' plants (approx. 1 year old) purchased from a commercial nursery were used for aphid rearing and movement experiments during May-August 2002. Plants were cultivated in 8 L pots in a vented greenhouse (18-28 ^{o}C), pruned to remove flowers and fertilized with 20-20-20 N-P-K to ensure fresh leaf growth. *Ericaphis fimbriata*, obtained from field samples in 2001 and 2002, were raised on blueberry plants in a 60 x 60 x 60 cm screen-covered cage in the greenhouse. Multi-coloured Asian ladybird beetles, *Harmonia axyridis* L. (Coleoptera: Coccinellidae) were obtained from a commercial insectary (Applied Bionomics Ltd, Sidney, British Columbia) and maintained on pea aphid, *Acyrthosiphon pisum* (Harris), reared on bean *Vicia faba* L. (Fabaceae). One branch from each of 48 'Duke' plants was bent to a 30-45° angle and secured in this position with wire so that it extended beyond the edge of the pot. The branch was cleared of most leaves so that only eight to ten fresh young leaves remained, and the base was coated with StickemSpecial® (Seabright Enterprises, Emeryville, California) to prevent walking insects from moving onto or off the branch. Five randomly selected apterous adult *E. fimbriata*, either of the red or green morph, were placed on one of the leaves on each plant following methods described by Bristow *et al.* (2000). Plants were left in the greenhouse for one, two or four weeks to allow aphids to establish and reproduce for different durations. They were then moved to a table covered with white paper in an observation room (75% RH, 23 ± 1 °C, light intensity 10.1 mE $m^{-2}s^{-1}$) and left undisturbed for 1 h before experimental observations. Zero (control), one, two, or four *H. axyridis* were released on each of 32 plants (eight replicates of each beetle treatment), on or near the leaf that was initially inoculated with aphids one to two weeks previously (without disturbing aphids). Within each beetle treatment, four plants had been previously inoculated with the red morph *E. fimbriata*, and four with the green morph. Among each group of four plants with the respective color morph, two plants had been infested for one week and two for two weeks. In addition, zero or two *H. axyridis* were released on each of 16 plants infested with the green morph for four weeks. Different beetles were used on each plant, and treatments were completely randomized in time. Beetle behaviour was monitored for 20 min and the number of aphids that fell recorded. All observations were conducted within a six week period, and up to four observations were conducted per day. Observations were conducted between 0800 and 1100 h to reduce potential variation in aphid or beetle behaviour resulting from circadian rhythms. After each observation, all aphids were removed from the inoculated branch and placed in 70% ethanol for subsequent counting.

The numbers of aphids fallen were transformed using square-root (x + 0.5) before analysis to stabilize the variance (Southwood 1966). For plants infested for one and two weeks ANOVA (SAS Institute Inc. 1990) was used to determine the effect of the number of weeks aphids were on the plants, the number of ladybird beetles, the color morph of the aphid and the first-order interactions between these terms, on aphid density and the number of aphids fallen. Plants infested for four weeks were not included in this analysis as only green color morphs were used. The analysis was then repeated, pooling color morph data, and including data from plants infested for four weeks. When effects were significant at $P < 0.05$, Fisher's least-significant-difference test was used to separate means. Finally, SAS REG (SAS Institute Inc. 1990) was used to regress aphids fallen on aphid density, ladybird beetles, and the interaction, where ladybird beetles were either present or absent. Data from three plants that were accidentally disturbed during the observations were not included in the analyses.

Aphid movement in the field. Fifteen potted blueberry plants were pruned (45 cm tall and 60 cm wide) and fertilized (as above) to ensure fresh growth. Each plant was inoculated with five aphids and set in screen-covered cages in the greenhouse (as above) for 14 days to prevent predation and parasitism. They were then planted in a row in a freshly tilled field with 1.5 m between plants. On either side of the row of inoculated plants, a row of uninfested plants was planted so that the main stems of the plants were 45 cm apart and there was a 7.5 to 10 cm overlap between one branch of the inoculated plant with one branch of each uninfested plant. Care was taken to ensure that the aphids on the central plant were not disturbed and that the branches of the central plant that touched those of the two uninfested plants had no aphids on them. The minimum combined walking distance for two aphids between an infested and two uninfested plants was 60 cm. The uninfested plants were monitored at 0800 h and 1600 h every day to determine if aphids had moved onto them, and whether they were alate or apterous morphs. There were no other blueberry plants within a 100 m radius. The aphids on the inoculated plants were removed and counted after aphids were observed on both adjacent uninfested plants. The average time for the first aphids to move off the central plant onto the two adjacent plants was regressed against aphid density on the infested terminal using SAS REG (SAS Institute Inc. 1990).

RESULTS AND DISCUSSION

Aphids falling in the presence of ladybird beetles. Aphid density did not differ significantly ($P > 0.05$) between color morphs, the number of weeks plants were infested, or for the interaction between color morph and week, for plants infested for one and two weeks. Similarly, the number of fallen aphids did not differ significantly ($P > 0.05$) between color morphs, the number of weeks plants were infested, or for the interaction between color morph and week, for plants infested for one and two weeks. This suggests that the variation in aphid density was large enough to mask an extra week's reproduction by the aphids on plants infested for two weeks. It also suggests that reproduction by red and green color morphs was similar and confirms that allocation of beetle treatments to plants infested for one and two weeks was random. An effect of ladybird beetles on the number of aphids falling was almost detectable (F = 2.97; df = 3,19; $P = 0.058$) at the aphid densities utilized (mean density = 30.6 ± 2.9 (SE) aphids per terminal, n = 32).

When the data for red and green color morphs were pooled and data from the plants infested for 4 weeks included in the analysis, aphid density was significantly different between plants infested for different numbers of weeks (F = 5.28; df = 2,45; $P < 0.01$; mean number of aphids per terminal for one, two, and four weeks of infestation were: 30.4 ± 4.1; 30.7 ± 4.1; and 50.7 ± 4.6, respectively). Aphid density was not significantly affected by the number of ladybird beetles or by the interaction between beetles and weeks of infestation ($P > 0.05$). This shows that maintaining aphids on the plants for different lengths of time was eventually effective in creating different aphid densities and that beetle treatments were allocated at random to plants infested for different numbers of weeks.

For the combined data (plants infested for one, two, and four weeks, pooling red and green forms), zero to four aphids fell per plant in each 20 minute replication. There was a significant effect of ladybird beetles on the number of aphids falling (F = 3.95; df = 3,35; $P < 0.05$). Significantly ($P < 0.05$) more aphids fell when one ladybird beetle was added to the branches (2.13 ± 0.44, n = 8) than when zero, two, or four were added (0.47 ± 0.17, n = 15; 1.14 ± 0.36, n = 14; 0.75 ± 0.37, n = 8, respectively). The analysis of variance indicated no significant effect of the number of weeks plants were infested or of the interaction between the number of beetles and the weeks of infestation ($P > 0.05$). Regression of the number of aphids falling against aphid density in the absence of ladybird beetles indicated that significantly more aphids fell as density increased (t = 2.86, $P < 0.01$). When beetle presence or absence was added to the regression, the interaction between aphid density and ladybird beetles was significant (t = 4.12, $P < 0.001$). Similar numbers of aphids fell in the presence or absence of beetles at low aphid density (10-30 aphids per terminal), but more fell in the presence of beetles at high aphid density (50-70 aphids per terminal); $y = 1.01 - 0.0104\ x - 0.118\ z + 0.0116\ xz$; where y = the number of apterous *E. fimbriata* falling; x = aphid density; and z = absence (0) or presence (1) of *H. axyridis*; $P < 0.01$, $R^2 = 0.30$, df = 41; $SE_{(slopes)}$ = 0.0122, 0.276, and 0.00692 respectively. While neither main effect was significant when combined with the interaction, both were retained for completeness of the regression.

Hodgson (1991) reported that apterous forms of the green peach aphid, *Myzus persicae* (Sulzer), the cabbage aphid, *Brevicoryne brassicae* (L.) and the vetch aphid, *Megoura viciae* Buckton, move off acceptable host plants in the absence of crowding and argued that the dispersal of apterous aphids may be common. Apterous aphids usually move off plants either to escape predators or to search for new host plants (Robert 1987), and this behaviour would likely evolve if new host plants are easy to find (Hodgson 1991) and if there is little probability of mortality when aphids have left a plant (e.g. desiccation). Movement of individual apterous aphids off plants may maximize the overall fitness of the aphid clone by distributing the clone over different resource units (Roitberg *et al.* 1979). Aphids do this by producing alate forms when the quality of the resource plant decreases, but movement by apterous aphids eliminates the one-generation lag time required to produce alates (Roitberg *et al.* 1979). The mortality of apterous *E. fimbriata* that fall in a blueberry field may be low as the aphids are likely to fall onto other blueberry plants or find new host plants quickly. While little is known about the survival of *E. fimbriata* on the soil, Alyokhin and Sewell (2003) demonstrated that *M. persicae*, the potato aphid *Macrosiphum euphorbiae* (Thomas), and the buckthorn aphid *Aphis nasturtii* Kaltenbach, can all survive at least 24 h off host plants and move at least 180 cm on the soil surface.

The increase in apterous aphid movement in the presence of predators has been reported for other aphid species (Niku 1972). Aphid falling in response to predators is an integral part of aphid escape behaviour for some (McAllister and Roitberg 1987; Bailey *et al.* 1995) but not all aphid species. Roitberg *et al.* (1979) found that the black bean aphid *Aphis fabae* does not drop in response to predators. The increase in apterous *E. fimbriata* movement in the presence of adult coccinellids would increase their dispersal and hence likely increase the local spread of BlScV through a field. This suggests that biological control of *E. fimbriata* via the introduction of adult coccinellids may increase the spread of BlScV.

Aphid movement in the field. Apterous *E. fimbriata* moved quickly from infested blueberry plants onto adjacent uninfested plants in the field (Fig.1). The first apterous aphids were detected on the uninfested plants after 24 h and aphids had moved onto all of the uninfested plants after 120 h. The time taken for aphids to move a minimum distance of 60 cm off infested plants onto the uninfested plants decreased with increasing aphid density (t = 6.09, $P < 0.0001$) (Fig. 1).

Raworth (2004) showed that peak aphid density varied between 300 and 9000 per

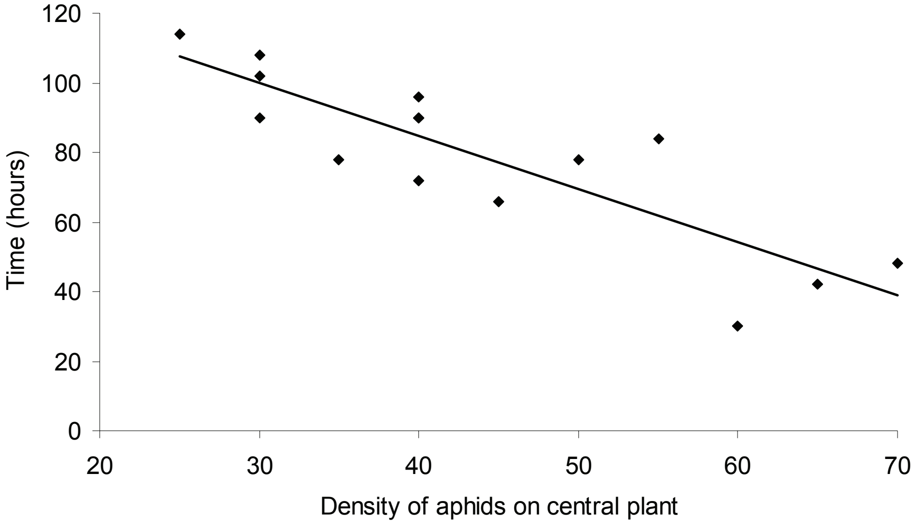

Figure 1. Average time required for apterous *Ericaphis fimbriata* to move a minimum of 60 cm onto branches of adjacent plants in the field versus aphid density on the central plant. $y = -1.52 x + 145.7$; $P < 0.0001$, $R^2 = 0.74$, df = 13, $SE_{(slope)} = 0.25$.

blueberry plant in commercial fields. Given that branches overlap between plants within a row, the results of our current work suggest that there is probably significant movement of apterous *E. fimbriata* from one plant to another in the field. Bristow *et al.* (2000) showed that *E. fimbriata* is not an efficient vector of BlScV, but given that high aphid densities can occur, there is significant potential for plant-to-plant, within-row transmission of the virus by apterae. Although coccinellids are generally considered beneficial, they may exacerbate the problem by increasing aphid movement. Further work is needed to determine the effect of various aphid controls and removal of BlScV-infected plants on the local spread of the virus.

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