# Identification of new aphid vector species of *Blueberry scorch virus*

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## ABSTRACT

Transmission of *Blueberry scorch virus* (BlScV) by the aphid species *Ericaphis fimbriata* (Richards), *Aphis spiraecola* (Patch), *Aphis pomi* DeGeer, *Acyrthosiphon pisum* (Harris), *Myzus ornatus* Laing, *Aphis helianthi* Monell, *Myzus persicae* (Sulzer), and *Rhopalosiphum padi* (L.), was studied in the laboratory using timed aphid acquisition feeding periods and known numbers of aphid vectors. Successful infection of *Nicotiana occidentalis* Wheeler (Solanaceae), a newly identified herbaceous host, and highbush blueberry, *Vaccinium corymbosum* L. (Ericaceae), following brief virus-acquisition feeds lasting less than 5 min, demonstrated that BlScV was transmitted in a non-persistent, non-circulative manner. Based on transfer of 10 aphids per plant, the most efficient vector of BlScV from infected to healthy *N. occidentalis* was *M. ornatus*. Compared with this herbaceous host, infection rates for blueberry were much lower even though higher numbers of aphids (25/plant) were used. The highest rate of infection for blueberry (20%) was achieved when the green colour form of *E. fimbriata* was used to transmit the virus. The relatively low rate of transmission from infected to healthy blueberry suggests that BlScV would spread slowly in the field. Planting of certified virus-free nursery material and aggressive removal of infected plants should help control this economically important disease of highbush blueberries.

**Key Words:** *Blueberry scorch virus*, aphid vectors, virus transmission

## INTRODUCTION

*Blueberry scorch virus* (BlScV) was first reported in New Jersey in the late 1970's as Sheep Pen Hill disease of highbush blueberry, *Vaccinium corymbosum* (L.) (Ericaceae) (Podleckis and Davis 1989). Several distinct strains infect highbush blueberry in the northeastern and northwestern United States and southwestern British Columbia (Cavileer *et al.* 1994, Catlin and Schloemann 2004, Bernardy *et al.* 2005, Wegener *et al.* 2006). BlScV has also been recently reported from Europe (Ciuffo *et al.* 2005). Depending on the virus strain and blueberry cultivar, infection can result in a wide range of symptoms. While some varieties are tolerant to certain strains and display no visible symptoms, infection with other strains can result in severe necrosis of new leaves, twigs and flower clusters and almost complete loss of yield over time (Martin and Bristow 1988, Catlin and Schloemann 2004, Wegener *et al.* 2006). The latent period between infection and development of symptoms for established plants is thought to be one to two years (Caruso and Ramsdell 1995).

There are relatively few previous studies on BlScV; these mostly relate to detection, symptomology and strain differentiation. Although little is currently known about the insect vectors of BlScV, carlaviruses as a group are transmitted primarily by aphids in a non-persistent, non-circulative manner (Ng and Perry 2004). Non-persistent virus transmission is characterized by short acquisition and inoculation feeding times,

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lasting from several seconds to a few minutes in duration (Raccah 1986). In uncontrolled cage studies, Hillman *et al.* (1995) were the first to demonstrate aphid transmission of BlScV. An unidentified aphid collected from blueberry and placed on infected *Chenopodium quinoa* Willd. (Chenopodiaceae), an alternate herbaceous host for the New Jersey strain of BlScV, was shown to transmit the virus to uninfected *C. quinoa*. In a similar manner, Bristow *et al.* (2000) were able to demonstrate infection of containerized highbush blueberry plants in cages supplied with diseased blueberry leaves infested with *Ericaphis fimbriata* (Richards). In the same study, transfer of individual aphids from infected blueberry leaves to containerized potted test plants resulted in a very low rate of infection, less than one percent. These previous studies were not designed to determine if BlScV was transmitted by aphids in a semi-persistent or non-persistent manner. Two carlaviruses vectored by aphids are thought to be transmitted in a semi-persistent manner (Bristow *et al.* 2000).

A better understanding of BlScV epidemiology will aid in the development of effective control measures. To this end, the purpose of our study was to determine the mode of transmission of BlScV and compare aphid transmission efficiencies of *E. fimbriata*, a species that colonizes blueberry, with transmission by several non-colonizing aphid species. Identification of effective aphid vectors will also assist in future laboratory investigations to determine biological differences between the various strains of BlScV.

## MATERIALS AND METHODS

**Plant and aphid culture**. Large highbush blueberry plants from two commercial fields near Abbotsford, British Columbia (BC), that had previously tested positive for BlScV by ELISA using polyclonal antibodies (Agdia, Elkhart, Indiana) were potted into large (~ 60 cm x 43 cm deep) plastic pots and moved to a greenhouse at the Pacific Agri-Food Research Centre, Summerland, BC. These plants also formed the basis for the isolation and molecular characterization of two major strains of BlScV (Bernardy *et al.* 2005).

*Nicotiana occidentalis* Wheeler, recently identified as a herbaceous host for BlScV (Lowery *et al.* 2005), was grown in the greenhouse in 20-cm plastic containers in a 1:1:5 mixture of steam-sterilized field soil, perlite, and commercial potting soil (Pro-Mix BX, Premier Horticulture Ltd., Dorval, Quebec). Temperatures were variable and ranged from daytime highs of 25 °C to nighttime lows of 15 °C, with supplemental lighting supplied by sodium vapour lamps to provide a 16-h photophase. Plants were used at the four- or five-true-leaf stage. Small BlScV-free blueberry plants cv 'Berkeley' were acquired from a commercial supplier (Fall Creek Nurseries, Lowell, Oregon) and grown in the greenhouse in 3.8-litre plastic pots under the same conditions.

Aphids were maintained in vented, Plexiglas ® cages (50 cm x 50 cm x 33 cm wide) in a growth room (18 °C, 16-h photophase) on suitable host plants as follows: red and green forms of *E. fimbriata* on strawberry, *Fragaria x ananassa* Duchesne (Rosaceae); spirea aphid, *Aphis spiraecola* (Patch) and apple aphid, *A. pomi* DeGeer, on apple, *Malus domestica* L. (Rosaceae); pea aphid, *Acyrthosiphon pisum* (Harris), on garden pea, *Pisum sativum* L. (Fabaceae); violet aphid, *Myzus ornatus* Laing, and *Aphis helianthi* Monell on sunflower, *Helianthus annuus* L. (Asteraceae); green peach aphid, *Myzus persicae* (Sulzer), on bok-choi, *Brassica rapa* L. (Brassicaceae); and the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), on barley, *Hordeum vulgare* L. (Poaceae). Host plants were reared in the greenhouse under conditions outlined above.

Except for *E. fimbriata* that were originally collected from commercial fields of highbush blueberry in the Fraser Valley and provided by Dr. D.A. Raworth (Agriculture and Agri-Food Canada, Pacific Agri-Food

Research Centre, Agassiz, BC), all of the aphid species used in these studies, other than *A. pisum*, were collected in Summerland, BC, from the hosts on which they were reared. *Acyrthosiphon pisum* was collected from garden peas in Armstrong, BC. Aphids were identified by Dr. R.G. Foottit (Agriculture and Agri-Food Canada, Eastern Cereals and Oilseeds Research Centre, Ottawa, Ontario).

**Aphid transmission studies.** Fourth instar and adult apterous aphids from the laboratory colonies were placed in small self-sealing petri dishes containing moistened filter paper for a 2- to 3-h preacquisition starvation period. Aphids were allowed to feed for 5 min on BlScV-infected leaf pieces in groups of 10 aphids/petri dish, and then transferred, 25 aphids/plant for blueberry and 10 aphids/plant for *N. occidentalis*, to BlScV-free test plants, which were then sealed in plastic bags to prevent the aphids from escaping. Fine, moistened natural fibre brushes were used to transfer aphids. At least 1 h after the final transfer, plants were sprayed with the aphicide pirimicarb (Pirimor 50WP, Chipman Chemicals Ltd., Stoney Creek, Ontario) to kill any remaining aphids. Plants were held in the bags for a further 24 h to ensure that all aphids were dead. *Nicotiana occidentalis* were then moved to a growth chamber at 20 °C under fluorescent and incandescent lights (approx. 185 $\mu$mol $m^{-2}$ $s^{-1}$ PAR) and a 16-h photophase. After 6 to 8 wk, plants were tested for BlScV infection by enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies (Agdia, Elkhart, Indiana). Blueberry plants were held in the greenhouse under the conditions outlined above and tested for infection approximately 3 mo later. Plants were then moved to cold storage at 4 °C for 3 mo and then re-tested 2 to 3 mo after being returned to the greenhouse. Virus transmission studies for all species of aphids and both species of host plant were conducted concurrently.

**ELISA analysis.** The double-antibody sandwich (DAS) ELISA method used was a modification of the protocol described by Clark and Adams (1977). All reagents were added at 100 $\mu$l per well in microtitre plates. Microtitre plates (EIA Microplate, ICN Biomedicals, Irvine, California) were coated with purified immunoglobulin (IgG) (Agdia, Elkhart, Indiana) diluted (5 $\mu$l $ml^{-1}$) in phosphate-buffered saline (PBS) for 4 h at 37 °C. Plates were washed three times with PBS. Plant samples (0.25 g) were thoroughly ground in Bioreba bags (Bioreba AG, Reinach, Switzerland) with 1.5 ml borate buffer (0.1 M boric acid, 0.01 M sodium borate, 2% polyvinylpyrolidine (PVP 44,000), 0.2% non-fat milk powder, 0.05% Tween-20, 0.5% nicotine), and the bags briefly centrifuged at 2000 rpm to aid pipetting. The liquid extract (25 $\mu$l) and borate buffer (75 $\mu$l) were added to the microtitre plates, which were covered in cellophane and placed overnight on an orbital shaker at 600 rpm. After washing the plates with PBS-Tween and adding a dilute (5 $\mu$l $ml^{-1}$) IgG-enzyme conjugate in PBS-Tween-BSA-polyvinylpyrolidine, plates were incubated at 37 °C for 2 h. After plates were washed with buffer, a dilute (0.5 mg $ml^{-1}$) solution of p-nitrophenyl phosphate buffer was added. Plates were incubated at room temperature on an orbital shaker (600 rpm) for about 1 hr and absorbance was read at 405 nm. A subset of healthy blueberry nursery plants was tested by ELISA to verify that they were free of BlScV.

In order to verify BlScV infections, a subset of blueberry and *N. occidentalis* plants that had tested positive by ELISA were also tested by reverse transcriptase polymerase chain reaction (RT-PCR) as described in Bernardy *et al.* (2005).

**Statistical analysis.** Differences in rates of transmission of BlScV by the various aphid species were determined by contingency table analysis and multiple comparisons for proportions, analogous to a Tukey's test (Zar, 1984). Data were analyzed separately for each combination of infected source and healthy test plants. Infection rates were not included in the analysis if fewer than ten test plants had been inoculated.

## RESULTS

By using *N. occidentalis* for both BlScV-infected and healthy test plants, we were able to compare transmission rates for several species of aphids not previously known to vector this disease (Table 1). Several species, including *A. pomi*, *M. persicae*, and *R. padi*, were inefficient vectors that were able to infect *N. occidentalis* only at low transmission rates ranging from 2% to 4%. The highest rate of transmission from infected to healthy *N. occidentalis* occurred when *M. ornatus* (average transmission rate 69%) or *A. helianthi* (data not shown) were used as vectors. Unfortunately, the latter species was not included in the statistical analysis due to the death of the colony from a fungal infection before the tests could be completed. Both the green and red forms of *E. fimbriata* transmitted BlScV between *N. occidentalis* at intermediate rates of 10% and 8%, respectively. *Acyrthosiphon pisum* and *A. spiraecola* did not transmit BlScV from infected to healthy *N. occidentalis*.

The highest rate of infection of highbush blueberry (20%) was recorded for the green form of *E. fimbriata*, whereas infection rates for *M. ornatus* and *A. spiraecola* were both 7% (Table 1). *Aphis helianthi* was not included in the data analysis, as we were only able to inoculate six blueberry plants with BlScV using this species before the colony collapsed due to a fungal infection. However, the infection rate for this species, which does not colonize blueberry, appeared to nearly equal that for the colonizing species *E. fimbriata*.

Virus transmission tests from infected blueberry to *N. occidentalis* were conducted to evaluate the acceptability of *N. occidentalis* as a trap plant in field studies of BlScV epidemiology. No plants became infected when *M. persicae* was used to vector the virus from infected blueberry to *N. occidentalis* (Table 1), but use of the green form of *E. fimbriata* resulted in an infection rate of 27%.

The utility of *N. occidentalis*, a recently identified herbaceous host of BlScV (Lowery *et al.* 2005), for laboratory studies of aphid transmission efficiencies was demonstrated in this study. Even though fewer aphids (10/plant) were used to inoculate *N. occidentalis* than blueberry (25/plant), overall infection rates were similar. Blueberry plants had to be held for many months to demonstrate virus transmission, and approximately half the plants tested positive only after an intervening 3-month cold period. This was expected since virus titres are generally low in blueberry compared with herbaceous hosts, the virus is often distributed unevenly within blueberry plants, and detection may vary seasonally (Martin and Bristow 1988, Wegener *et al*. 2006). In comparison, unequivocal ELISA results could be obtained for infected *N. occidentalis* within 6 to 8 wk after infection and plants then retained a high virus titre over a period of several months.

## DISCUSSION

Carlaviruses were, until recently, one of the largest and least studied of the plant virus groups (Foster 1992). Diseases caused by these viruses often result in latent infections or they cause indistinct, mild symptoms, which resulted in carlaviruses being largely ignored by pathologists. BlScV is an exception to this general condition, with infections resulting in significant loss of yield and eventual death of certain cultivars of highbush blueberry. For this reason, a number of recent studies have investigated the molecular characteristics, epidemiology, and aphid transmission of BlScV.

Carlaviruses are transmitted largely by aphids in a non-circulative, non-persistent manner (Foster 1992). Certain of them are thought to be transmitted in a semi-persistent manner, however, and at least one member of the group, *Cowpea mild mottle virus*, is transmitted by whiteflies (Harris 1983, Ng and Perry 2004). In the

**Table 1.**
Aphid transmission of *Blueberry scorch virus* from infected *Nicotiana occidentalis* or highbush blueberry, *Vaccinium corymbosum*, to healthy test plants.

| Aphid Species | Infected Source | Test Species | Infected/ Total | % Infection |
|---|---|---|---|---|
| *Acyrthosiphon pisum* | *Nicotiana occidentalis* | *Nicotiana occidentalis* | 0/41 | 0d[1] |
| *Aphis pomi* | *N. occidentalis* | *N. occidentalis* | 1/48 | 2c |
| *Aphis spiraecola* | *N. occidentalis* | *N. occidentalis* | 0/33 | 0d |
| *Ericaphis fimbriata*, green form | *N. occidentalis* | *N. occidentalis* | 4/40 | 10b |
| *Ericaphis fimbriata*, red form | *N. occidentalis* | *N. occidentalis* | 3/40 | 8bc |
| *Myzus ornatus* | *N. occidentalis* | *N. occidentalis* | 11/16 | 69a |
| *Myzus persicae* | *N. occidentalis* | *N. occidentalis* | 2/48 | 4bc |
| *Rhopalosiphum padi* | *N. occidentalis* | *N. occidentalis* | 1/40 | 3bc |
| *Acyrthosiphon pisum* | blueberry | blueberry | 0/18 | 0b |
| *Aphis pomi* | blueberry | blueberry | 0/15 | 0b |
| *Aphis spiraecola* | blueberry | blueberry | 1/14 | 7a |
| *Ericaphis fimbriata*, green form | blueberry | blueberry | 5/25 | 20a |
| *Ericaphis fimbriata*, red form | blueberry | blueberry | 0/7 | - |
| *Myzus ornatus* | blueberry | blueberry | 1/14 | 7a |
| *Myzus persicae* | blueberry | blueberry | 0/24 | 0b |
| *Rhopalosiphum padi* | blueberry | blueberry | 0/23 | 0b |
| *Ericaphis fimbriata*, green form | blueberry | *N. occidentalis* | 4/15 | 27a |
| *Myzus persicae* | blueberry | *N. occidentalis* | 0/30 | 0b |

[1] For each combination of infected source and healthy plant species, infection rates followed by the same letter are not significantly different based on contingency table analysis and multiple comparisons for proportions (Zar 1984).

present study, the results of earlier uncontrolled cage studies that demonstrated transmission of BlScV by *E. fimbriata* (Bristow *et al.* 2000) were confirmed. Utilizing timed acquisition feeding periods, we found that BlScV is indeed transmitted by aphids in a non-persistent manner, as might be expected for a member of the carlavirus group. Aphids were able to acquire the virus during brief acquisition-feeding periods lasting less than 5 minutes. Additionally, aphids that do not colonize blueberry, such as *M. ornatus*, were equally efficient virus vectors compared to the colonizing species *E. fimbriata*. A pre-acquisition fasting period and short virus-acquisition probes increase transmission of non-persistent viruses, while prolonged feeding leads to greatly reduced transmission rates (Maramorosch 1963). For this reason, under field conditions, non-colonizing aphids are often more important vectors of viruses such as BlScV. Due to low virus titres, however, a slightly longer acquisition feeding period might improve transmission efficiencies when BlScV is acquired from highbush blueberry. Although *E. fimbriata* was not the most efficient vector of BlScV from *N. occidentalis* to *N. occidentalis*, it was the best vector when the virus was ac-

quired from highbush blueberry, possibly because this species was observed to settle and feed more readily on this host plant. Thus, *E. fimbriata* might contribute significantly to the spread of this virus within infected fields, particularly in years with large numbers of these colonizing aphids.

Infection of *N. occidentalis* by viruliferous aphids in this study occurred at a level comparable with that for infections of herbaceous plants with non-persistently transmitted potyviruses. In similar controlled studies, transmission of *Potato virus Y* from infected to healthy sweet pepper by *M. persicae* resulted in an 89% infection rate (Lowery *et al.* 1997), whereas in another study involving several species of aphids the maximum rate of infection of rutabaga with *Turnip mosaic virus* was 55% (Lowery 1997). The highest rate of infection of *N. occidentalis* with BlScV falls within these range of values (Table 1). Successful transmission of BlScV from blueberry to *N. occidentalis* by *E. fimbriata* has also been used successfully to help purify and amplify virus in strain determination studies (Bernardy *et al.* 2005). Compared with highbush blueberry, this herbaceous host should prove useful as a trap or sentinel plant in studies of BlScV epidemiology. It will be necessary, however, to first show that *N. occidentalis* is uniformly susceptible to all strains of BlScV.

During a two year study, Raworth *et al.* (2006) captured alate aphids of 87 species in water pan traps placed in commercial blueberry fields in the Fraser Valley, BC. Our results suggest that many of these species are likely vectors of BlScV. Future virus transmission studies involving aphid species that were captured in large numbers from the middle of June to the middle of July when most trap plants became infected (Raworth *et al.* 2008), which would include several species such as *Euceraphis betulae* (Koch) that develop on trees (Raworth *et al*. 2006), might help identify some of the other major vectors contributing to the spread of BlScV and suggest possible management strategies.

Based on our laboratory results, BlScV is transmitted between highbush blueberry at a rate similar to that for other non-persistent, aphid-borne viruses of woody perennial plants. In comparable transmission tests using 50 *M. persicae* per plant a 'D' strain of *Plum pox virus*, a member of the *Potyviridae*, was transmitted from infected peach, *Prunus persicae* L., to healthy peach seedlings at an average infection rate of 22% (D.T.L. unpublished data). In the present study with blueberry, but using only 25 aphids per plant, a maximum infection rate of 20% was recorded for *E. fimbriata* (Table 1). The relatively low rate of transmission from blueberry to blueberry as compared with infection of herbaceous hosts suggests that a number of years would be required for BlScV to spread throughout a blueberry field from an initial infection locus. Accordingly, mapping of disease incidence in three commercial blueberry fields in the Fraser Valley, BC, showed that BlScV spread only slowly (Wegener *et al.* 2006). Similarly, spread of BlScV throughout two commercial fields of blueberry in the northwestern United States required between 5 to 8 years (Bristow *et al.,* 2000), and Raworth *et al.* (2008) recorded a low rate of BlScV infection for highbush blueberry and *N. occidentalis* bait plants placed weekly throughout the summer in highly infected commercial blueberry fields, indicating a low rate of natural spread. Given the relatively slow spread of the virus under field conditions, these findings suggest that planting only certified virus-free nursery material and aggressive removal of diseased plants might provide an effective means of control of BlScV under field conditions.

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