Ecology and Epidemiology

Aphid Vector Population Dynamics and Movement Relative to Field Transmission of Blueberry Shoestring Virus

K. M. Morimoto and D. C. Ramsdell

Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824.

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ABSTRACT


Illinois pepperi, the aphid vector of blueberry shoestring virus (BBSSV), has been shown to overwinter on highbush blueberry (Vaccinium corymbosum) and to complete its life cycle on that host in caged-bush experiments. Although within-field movement of alatae and apteracae occurred, aphid movement out of the isolated field research site was rare. Field populations of I. pepperi were monitored weekly in yellow-pan water traps, on infected field source plants, and on 2-yr-old blueberry trap plants from early May through September. Populations of alatae and apteracae were greatest in June. Alatae were found throughout the growing season; few apteracae were found after mid-July. Individual I. pepperi were tested for presence of BBSSV by radioimmunosorbent assay. Percentages of virus-positive aphids ranged between 0 and 30% on uncaged source or trap plants throughout the season. There was wide variability in the quantity of virus detected in field-trapped individual aphids. Amounts of BBSSV ranged from 0.5 to 100 ng per aphid. Field transmission of BBSSV occurred from infected source plants to 2-yr-old healthy trap plants surrounding the infected source bushes. The incidence of trap plant infection was highest in May and June when the populations of I. pepperi were greatest.

Additional key words: epidemiology.

Blueberry shoestring disease, which is caused by blueberry shoestring virus (BBSSV), is an economically important virus disease of highbush blueberry (Vaccinium corymbosum L.) in Michigan and New Jersey (11). This is the most widespread and economically important virus-caused disease of blueberries in Michigan. Symptoms of the disease include crescent or strap-shaped leaves and red streaking on current-season and 1-yr-old shoots. BBSSV is a spherical (27-nm diameter), single-component, single-stranded RNA virus (9,10). The only known hosts of BBSSV are highbush blueberry, V. corymbosum (14) and lowbush blueberry, V. angustifolium (Ait.) (6). The virus can be transmitted between blueberry plants by chip budding (6,12) and by rub-inoculation with purified virus (5). However, attempts to transmit purified BBSSV by rub-inoculation to herbaceous plants have been unsuccessful (5). The only known vector of BBSSV is the blueberry aphid, Illinois pepperi (MacGillivray) (9). In the past, control of the disease consisted of roguing infected bushes to remove the source of inoculum. Presently, growers spray insecticides one or two times before harvest to partially control the aphid vectors.

No epidemiological studies of blueberry shoestring disease had been conducted prior to this work.

The objectives of this study were to determine whether the blueberry aphid overwinters on bushes in the blueberry field or immigrates into the field in the spring from alternate hosts; to monitor in-field movement of alate (winged) and apterous (wingsless) aphids; to monitor yellow-pan water traps longer distance egress of alatae from an isolated field; to ascertain seasonal alatae and apteracae population trends on blueberry bushes in a commercial field and on 2-yr-old trap plants placed around them; to determine the percentage of BBSSV-carrying aphids collected weekly from pan traps and blueberry bushes; to determine seasonal infection levels of blueberry by the use of trap plants placed around source plants for 1-mo periods during the growing season; and to test whether infected (source) blueberry plants must be touching for aphid-mediated transmission to occur to adjacent trap plants.

MATERIALS AND METHODS

The test field near Eastmanville, MI, contained about 4 ha of 20-yr-old mature clean-cultivated Jersey blueberry bushes planted on a 3.1 × 1 m spacing. In 1981, the field was mapped for symptomless (latent) BBSSV infection (1). All bushes without blueberry shoestring disease symptoms were tested for BBSSV by ELISA (4,8) prior to establishing the plots in 1982.

Source of overwintering blueberry aphids. A caged-bush experiment was conducted to determine whether blueberry aphids overwinter within the blueberry field. Fourteen BBSSV-infected bushes (hereafter referred to as source plants) were selected and pruned to uniformity. Seven of the source plants were enclosed separately in an aphid-proof screen cage with 1.6 × 1.6-mm openings (16-mesh) before bud break in the spring while the other seven were not caged. The population of I. pepperi was monitored weekly on these source bushes.

Alate blueberry aphid activity monitored with yellow-pan water traps. The movement of alate blueberry aphids within and immediately surrounding an isolated blueberry field was monitored with yellow-pan water traps. The traps consisted of goldenrod-colored plastic dish pans (30 × 38 × 16 cm) filled with water to within 3 cm of the rim. The traps were placed on 2-m-high platforms at 100-, 200-, and 300-m intervals from the east, west, and south edges of the blueberry field (Fig. 1). Similar traps also were placed on 30-cm-high boxes and on 2-m-high platforms (the height of the canopy) in the corners and center of a block of the blueberry field. Each week, aphids were collected from the traps and the traps were cleaned and refilled with water. The aphids were placed in test tubes containing 100 µl of RISA (4,8) extraction buffer. Blueberry aphids were identified by using a dissecting microscope prior to
assay. The tubes were corked and kept at 0–4°C for several days until processed for radioimmunosorbent assay (RISA) (4,8). Aphids were assayed in groups of five until 4 June, and then singly thereafter. A standard curve was constructed for BBSSV concentration in aphid extract-amended extraction buffer versus counts per minute (8).

Seasonal trap plant infection relative to aphid populations. Blueberry trap plants were exposed to BBSSV-infected source plants in the field for 4 wk intervals to determine when BBSSV infection occurs during the growing season. The five exposure intervals were 7 May to 4 June, 4 June to 2 July, 2 July to 30 July, 30 July to 27 August, and 27 August to 23 September 1982. Two-year-old Jersey highbush blueberry plants growing in 3.8-L (1-gallon) plastic pots served as trap plants. Prior to placement in the field, the plants were tested for the presence of BBSSV by ELISA and sprayed with DVP (2,2-dichlorovinyl O,O-dimethyl phosphate). A total of 10 plants (five with their leaves touching and five not touching those of the source plants) were placed around each of seven caged (Fig. 2A) and seven uncaged source plants (Fig. 2B). After each 4 wk exposure period, the trap plants were sprayed with DVP and kept in isolation in a cold frame. After a winter dormant period, leaves were sampled and tested for BBSSV infection by ELISA (4,8).

Seasonal blueberry aphid population dynamics and bush-to-bush movement of blueberry aphids. Since aphids move from plant to plant by crawling to touching branches of adjacent bushes, a study was conducted to determine if aphids are likely to move to adjacent trap plants that are touching infected source plants. The same trap plants used to determine the seasonal patterns of BBSSV spread were also used to study aphid movement and population dynamics throughout the growing season. Alate and apterous (late instar nymphs and adult) blueberry aphids on the trap plants and on the source plants were counted at weekly intervals from 7 May to 23 September 1982. Each week, a sample of about 70 aphids were collected at random from source plants and trap plants for testing by RISA (8) to monitor the percentage of BBSSV-carrying aphids (alatae and apterae). The apterous aphids in the samples were tested by RISA in groups of five from 15 May to 4 June; thereafter, they were tested individually. The alate aphids were tested individually throughout the season. Degree-day accumulation (DDA) (base 3.4°C [38°F]) from 1 January to 31 March was calculated from National Oceanic and Atmospheric Administration (NOAA) data collected at Grand Haven, MI (20 km from the field). From 1 April to 23 September, DDA was obtained from an Agricultural Weather observation station (15 km from the field) at Allendale, MI.

RESULTS

Source of overwintering blueberry aphids. Apterous blueberry aphids were first observed on caged source plants on 14 May 1982 (Fig. 3A), and both apterous and alate aphids were found on caged and uncaged source plants on 25 May (Fig. 4A). During most of the season, there were more apterous and alate aphids on the caged than on uncaged source plants (F = 0.001); however, both the caged and uncaged populations had the same seasonal patterns with the population peaks occurring in June.

About 700 degree days with a base of 3.4°C seemed to correlate with the first detectable population of aphids in the spring.

Alete blueberry aphid activity. Alate movement outside the field. Three alatea, one of which contained BBSSV, were caught in yellow-pan water traps outside the isolated blueberry field. This aphid was collected from the trap 100 m east of the field on 29 y 1982 (Fig. 1). The two virus-negative blueberry aphids were collected on 2 July in yellow-pan water traps 200 m south and 300 m east of the blueberry field. Egress of aphids from the field as monitored by yellow-pan water traps seems relatively insignificant.

Alate movement within the field. The distribution of virus-containing blueberry aphids caught in yellow-pan water traps during 1982 is shown in Fig. 1. Most of them were caught early in the season (May through mid-June) but a few were caught in early July. A greater proportion of virus-containing aphids were collected from the low traps (26 of 35) versus the high traps (9 of 35). The northwest and central low traps caught 17 and five virus-

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Fig. 1. Locations and dates that virus-containing blueberry aphids were caught in yellow-pan water traps. Each square represents the location of two traps: one on a 2 m-high platform and one on a 0.5 m-high box. Each letter represents one virus-containing aphid on the designated date. A letter adjacent to the upper square indicates an aphid was caught in a high trap, while a letter adjacent to the lower square indicates that an aphid was caught in the low trap. The squares outside of the field are pan traps at 100-m intervals.

Fig. 2. Arrangement of caged and uncaged blueberry plants for the aphid-mediated virus spread field experiment. A, Blueberry shoestring virus-infected source plants enclosed in a screen cage to contain aphids and surrounded by five touching and five nontouching 2-yr-old cultivar Jersey blueberry trap plants. B, Blueberry shoestring virus-infected source plant (uncaged) surrounded by five touching and five nontouching 2-yr-old cultivar Jersey blueberry trap plants.
containing blueberry aphids, respectively. The northeast and southeast high traps caught two and five virus-containing blueberry aphids, respectively, while the corresponding low traps did not catch any virus-containing aphids. The pattern fits with daily prevailing west winds. Although the yellow pan water traps were set up to trap alate aphids, many nymphs and adult apterous aphids were collected from the traps as well. The total numbers of apterous and alate aphids on 25 June (61 aphids irrespectively of whether or not they contained virus) caught in all of the yellow pan traps for each sampling date in the 1982 season are shown in Fig. 7. Aphids were caught in both high and low traps in roughly equal numbers. Most of the aphids were collected from early June to mid-July. Alate aphid populations occurred in three peaks: late May, mid-June, and early July. None were collected thereafter.

**Populations and percent virus-containing apterous blueberry aphids on source plants.** Seasonal fluctuation of populations of apterous aphids on BBSSV-infected source plants is shown in Fig. 3A. The points represent the mean numbers of apterous aphids counted on seven caged or on seven uncaged source plants on the dates indicated. The apterae populations on the caged source plants were much greater over the season than the corresponding populations on the uncaged source plants. Although the numbers of the apterae were greater on the caged source plants than on the uncaged source plants (P = 0.001), the two populations followed the same general seasonal pattern. The mean numbers of apterae per source plant were maximal the last part of June: 320 apterae on 18 June and 31 apterae on 7 June and source plants did not contain virus on source plants, respectively. The populations then decreased to minimum levels during late July and early August. From mid- to late-June there was a slight increase in the mean numbers of apterae on source plants due to a new flush of vegetative growth after fruit removal; then populations remained very low through September when the experiments were terminated. The incidence of virus-containing apterae aphids on caged and uncaged source plants is presented in Fig. 3B. There was no difference (P = 0.05) in the percentage of virus-containing aphids on caged versus uncaged source plants over the season. Through 9 July, the mean percentages of virus-containing apterous aphids on caged and uncaged source plants were similar and ranged between 0 and 15%. Between mid-July and the end of September these percentages varied widely. The large differences in mean percentages of virus-containing apterae through the season may have been due to the variation in the size, tissues from which aphids were collected, interruption of feeding due to insecticide drift, and the performance of the radioimmunosorbent assay on a given date. After 9 July, there were very few apterae on the source plants, and even fewer apterae that could be collected and used for virus tests on source plants.

**Populations and percent virus-containing alate aphids on source plants.** The caged alate population increased logarithmically to a maximum mean number of 305.6 alate aphids per source bush on 11 June (Fig. 4A). This population then gradually decreased over the next 2 wk before sharply declining prior to 2 July. This sharp drop in the caged alate population may have been due to either natural population decline due to high temperatures or the insecticide applied in the field on 28 June, or both. No alate aphids were observed on any of the source plants after 16 July.

Viruses-containing alatae on source plants were first detected 25 May (Fig. 4B). From 4 June through 2 July (when aphids were individually tested by RISA), the mean percentages of virus-containing alate on caged or uncaged source plants ranged from 3.6 to 25%. No virus-containing alatea were detected on source plants after 2 July.

**Apterae populations and percent virus-containing blueberry aphids on trap plants.** The mean numbers of apterae aphids per trap plant touching and not touching source bushes are shown in Figs. 5A and 6A, respectively. As with the source plants, the population of aphids on the caged trap plants were greater (P = 0.001) than those on uncaged trap plants. In addition, aphids populations on the trap plants followed the same seasonal patterns as the aphids populations on the source plants. The populations on the trap plants were very high the first half of the growing season, through the first week of July. The population numbers were low during late July and then increased again during August before tapering off to the low numbers of apterae found in autumn. The relative decrease in apterae populations found in the trap plants on 11 June, 2 July, 16 July, and 20 August (Figs. 5A and 6A) was due to drift from insecticide applications made by the grower to portions of the field adjacent to the block of bushes being monitored in this study. The mean percentages of virus-containing apterous aphids on trap plants touching and not touching the source plants are presented in Figs. 5B and 6B, respectively. The apterae aphids on the uncaged trap plants ranged from 0 to 18% virus-positive. These percentages fluctuated throughout the season.

**Population levels and percent virus-containing alate aphids on trap plants.** Populations of alatae were greatest from 29 May through 25 June (unpublished). Except for a mean number of 0.1 alate per trap plant (four aphids for 35 trap plants not touching source plants) found on 6 August, no alate were found after 23 July. The maximum mean number of alatae on caged touching trap plants was 14.9 on 18 June. For uncaged trap plants there was no definite maximum peak population; the mean numbers were never greater than one alate aphid per trap plant. Alate aphids were relatively much less numerous on trap plants than were apterae. Virus-containing aphids on caged and uncaged trap plants were first found 21 May. They were last found on 4 June and on 25 June on uncaged and caged plants, respectively. The percentage of virus-positive alatae ranged from 5 to 30% for uncaged trap plants and from 4 to 84% for caged trap plants. Whether or not the trap plants touched source plants did not affect the percentage of aphids found to be virus-positive.

**Variation in quantity of BBSSV in individual aphids.** Most of the virus-carrying aphids contained relatively small quantities of BBSSV (0.5 to 1.5 ng per aphid) (Table 1). Quantities of BBSSV (100 ng) were detected in a low percentage of apterous and alate individuals on trap plants as well as on source plants. The quantity of BBSSV detected in individual aphids for each sampling date varied greatly.

**Seasonal trap plant infection.** The percentages of infection of uncaged trap plants touching and not touching infected source plants are presented in Fig. 8. There were no significant differences (P = 0.001) in infection rate between trap plants touching and not touching source plants whether caged or not. The aphids were able to move to and transmit virus to trap plants adjacent to source plants regardless of physical contact between the bushes. The greatest amount of transmission occurred during May and June. As the season progressed through July and August there was less BBSSV infection. A slight increase in trap plant infection during September corresponded to the resurgence in apterous aphid populations during this time. The percentage of trap plants that became infected correlated with the size of apterous aphid populations during the season. The greatest amount of infection occurred in May and June when populations were maximal and conversely, little infection occurred when populations were low, which was the case in mid-August.

**DISCUSSION**

In these field studies, large populations of blueberry aphids were found on caged source bushes, suggesting that blueberry aphids do indeed overwinter within the blueberry field and have monoeocious aphid life cycle characteristics. These findings are in agreement with those of Elesner (3) who found oviparae and eggs in late autumn on basil blueberry shoots.

Throughout the season there were significantly greater populations of apterae and alatae on caged versus uncaged plants. This probably resulted from the protection that the screen cages provided the aphids against wind, rain, and predators. Aphid populations within the cages were an indicator of the potential number of aphids possible since they were partially protected from mortality factors.

Drift from the grower's insecticide sprays applied to the bushes adjacent to the portion of the field that was monitored had some deleterious effects on the aphid populations. However, the populations regained previous levels soon after the sprays were
Figs. 3-6. Seasonal blueberry aphid populations on blueberry shoestring virus (BBSSV)-infected source plants or on 2-yr-old blueberry trap plants surrounding the source plants. The degree day base is 3.4 C (38 F). Narrow arrows indicate spray application of Guthion insecticide at 0.28 kg active ingredient per hectare. Wide arrows indicate grower application of Aqua Malathion at 2.24 kg per hectare with an air-carrier-type sprayer within 3 m of the test area. Aphids were tested for the presence of BBSSV by radioimmunosorbent assay in groups of five for the samples taken 14 May through 4 June. Aphids were assayed singly thereafter. 3. Apterous blueberry aphids on source plants: A, seasonal aperous blueberry aphid populations on caged and uncaged BBSSV-infected source plants; B, seasonal distribution of virus-containing aperous blueberry aphids on caged and uncaged BBSSV-infected source plants.

made. In fact, the last two sprays of malathion had little effect on population levels. The aphid populations on the caged source and trap plants received some protection against the insecticides.

The yellow-pan water traps may not have been optimal for monitoring the alate populations. The numbers of alatae caught in yellow pan traps were low even when there were large populations of alatae on source plants. Elsner (3) also reported low numbers of trap catches with the same type of traps. He suggested that blueberry leaves and traps compete as attractive stimuli to the aphids. In addition, over the season, aphids may have been dislodged from the bushes and deposited in the traps by wind or rain. The apterous aphids trapped on 13 August (Fig. 7) may have been an example of this. These aphids were trapped during a very windy and rainy period when aphid populations were relatively low.

The small number of alatae caught in traps outside of the field indicated that there was little movement of alatae outside of the field and that transmission of BBSSV from field to field by flying aphids is unlikely. This corroborated the study by Lesney et al (5), who used a formula of Vanderplank (13) to obtain evidence that the inoculum source was within, rather than outside of, the field. In addition, Elsner (3) found very few blueberry aphids outside of blueberry fields even when acceptable alternate hosts were present.

Apterae were found only during the first 9 wk of the growing season, while apterae were found throughout the season. Therefore, alatae were only available for potential long distance virus spread during the first part of the season.

The mean percentage of virus-conducting apterae (uncaged) usually ranged between 0% and 15% throughout the season. Since aphid populations were greatest early in the season, the potential numbers of virus-containing aphids were also greatest during that time.

There were no differences in populations of apterae or alatae on touching versus nontouching trap plants over the season. This indicated that although aphids moved to adjacent touching plants they also moved easily to nontouching plants. Pruning bushes to avoid branch contact between adjacent bushes would not be an effective method of control.

Trap plant infection occurred throughout the entire season. The greatest incidence of trap plant infection was during the first two exposure periods from May through July. This was to be expected since it was during this period that the greatest aphid populations were present. The decrease in percentage of infection in August likewise corresponded to a drop in aphid population. However, 30% of the trap plants were infected at the end of the season in September when very few aphids were present. This may be because the 2-yr-old trap plants were in better growing condition at the end of the season than were the source plants. Aphids would have been attracted to the more succulent trap plants than the source plants planted in the field. It is likely that the attractiveness of the growing trap plants over the field source plants resulted in a higher than expected percentage of BBSSV-infected plants in September.

Earlier results (7) suggest a circulative, persistent virus-vector relationship. Therefore, well-timed aphicide applications beginning at about 700 degree days (base, 3.4°C) should effectively control the spread of BBSSV in a field containing BBSSV-infected bushes. If no shoestring-diseased bushes were present in the field, a minimal spray program for aphids which allows natural predators and parasites to control aphids, would be sufficient. However, if shoestring-diseased plants were present, a well-timed spray program beginning at the first appearance of aphids with repeat applications to maintain populations near zero, would be necessary to prevent further spread of the disease. Aphid population and seasonal trap plant infection data provide information for timing the insecticide sprays. It is important that at harvest, the populations should be at or near zero levels since Rubidium-labeled aphids have been shown to be carried up to 64 bushes down the row from a source bush by mechanical over-the-row harvesters (M. Whalon, unpublished) which are used to harvest 95% of Michigan's crop. Growers should wash out the harvesters before moving them to another field.

TABLE 1. The quantities of blueberry shoestring virus (BBSSV) in individual virus-containing *Illemonia pepperi* collected from BBSSV-infected source plants and healthy trap plants. Eastmanville, MI, in 1982

<table>
<thead>
<tr>
<th>Quantity of BBSSV per aphid (ng)</th>
<th>Apterous aphids</th>
<th>Alate aphids</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
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<tr>
<td>0.5</td>
<td>18</td>
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<tr>
<td>0.5 to 1.5</td>
<td>28</td>
<td>45.9</td>
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<td>6</td>
<td>9.9</td>
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<tr>
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<td>1</td>
<td>1.6</td>
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<tr>
<td>15 to 50</td>
<td>2</td>
<td>3.3</td>
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<tr>
<td>50 to 100</td>
<td>3</td>
<td>4.9</td>
</tr>
<tr>
<td>&gt;100</td>
<td>3</td>
<td>4.9</td>
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* Aphis were tested individually for the presence of BBSSV by radioimmunoassay assay. Values shown are from a standard curve using purified BBSSV-amended with aphid extract.

![Fig. 7](image1.jpg)  
**Fig. 7.** Seasonal distribution of apterous and alate blueberry aphids caught in 10 yellow-pan water traps placed within an isolated blueberry field of about 4 hectares. Each bar represents the total number of apterous or alate aphids collected in five low (0.5 m) and five high (2 m) traps placed within the field for each calendar date.

![Fig. 8](image2.jpg)  
**Fig. 8.** Percentage of uncaged 2-yr-old cultivar Jersey blueberry trap plants infected with blueberry shoestring virus (BBSSV) as a result of being placed around a BBSSV-infected source plant for a 1-mo period. Trap plants were either touching the source plant, or not touching it, and 1 m away from it. Trap plants were tested by radioimmunoassay assay after being held in isolation after the 1-mo period in the field.
An ideal long-term control strategy would be to plant blueberry bushes that are resistant either to the virus or to the aphid. The highbush blueberry cultivar Bluecrop has already been identified as having field resistance to blueberry shoestring virus (10).

LITERATURE CITED


