Bee-Mediated Transmission of Blueberry Leaf Mottle Virus Via Infected Pollen in Highbush Blueberry

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ABSTRACT

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Individual highbush blueberry bushes were tested by enzyme-linked immunosorbent assay (ELISA) for blueberry leaf mottle virus (BBLMV) infection in a commercial planting. The pattern of infected bushes was random, suggestive of a mode of spread different than by nematodes. Blueberry aphids allowed either a 72-hr or a 120-hr acquisition access period on BBLMV-infected blueberry failed to acquire detectable amounts of BBLMV when individuals were tested by radioimmunosorbent assay (RISA). Hand pollination of 15 2-yr-old blueberry plants resulted in infection of shoots of one of the plants. Trapping foraging honeybees from bushes in a commercial field containing BBLMV-infected bushes resulted in BBLMV being detected in the pollen from up to 51.4% of the pollen baskets from the bees legs when tested by ELISA. In field experiments, transmission occurred during bloom during a 2- to 3-wk period from BBLMV-diseased source bushes to young, virus-free potted bushes placed around a source bush. A hive of bees was placed within a cage around the bushes. Lower levels of transmission occurred if the hive and bushes were not caged, or if the bushes were caged, but without a hive. Virus-free potted bushes placed around a healthy source bush (in a field known to be free of BBLMV) and caged with a hive of bees inside remained virus-free. Blueberry leaf mottle virus appears to be spread mainly by foraging honeybees, which carry BBLMV-contaminated pollen.

Additional key words: epidemiology, pollen spread.

The association of blueberry leaf mottle virus (BBLMV) with the pollen of highbush blueberry, *Vaccinium corymbosum* L. 'Jersey' has been previously reported (2,3,21). Virus particles were easily removed from the surface of blueberry pollen grains by repeated washings with phosphate-buffered saline (PBS) (4). The virus was readily transmitted to healthy *Chenopodium quinoa* Willd. indicator plants by rub-inoculation of pollen obtained from BBLMV-infected blueberry bushes. However, pollen from infected bushes exhibited reduced viability and germinated poorly in nutrient solutions compared with pollen from virus-free bushes (4). If germ tube formation is necessary for infection, poor germination of pollen may limit the spread of the virus by this means. Virus was detected by enzyme-linked immunosorbent

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assay (ELISA) in 27% of blueberry seeds collected from infected plants, but only 1.5% of the seedlings from infected lots of seed contained virus.

Although BBLMV is placed within the nepovirus group, nematode transmission attempts with *Xiphinema americanum* Cobb and *Longidorus* sp. were not successful (21). Based on a survey of five fields in southwestern Michigan in 1983 and 1984, no association was found between BBLMV-infected bushes and the nematode vectors, *X. americanum* and *Longidorus* sp. In addition, weed species in 17 genera sampled beneath BBLMV-infected bushes revealed that they were not infected by BBLMV (3); infected weeds are usually found beneath woody plants infected by nepoviruses with proven nematode vectors.

The random distribution and rapid spread of BBLMV within highbush blueberry fields is atypical of a nematode-transmitted virus and would suggest a more active vector or rapid mode of spread. Highbush blueberry is well adapted for entomophilous pollination. Insects are attracted to the flower's shape, color, and nectaries. Although blueberry plants are self-fertile, pollination by honeybees (*Apis mellifera* L.) is required for maximum yield. One to three hives per acre are recommended in Michigan blueberry fields (15). Infection of healthy stone fruits as a result of the movement of virus-contaminated pollen during honeybee foraging has been demonstrated for prunus necrotic ring spot virus (PNRSV) (7,8,18) and prune dwarf virus (10,11) in Montmorency cherry orchards.

The blueberry aphid, *Illinoia pepperi* MacG., which has been shown to be the vector of blueberry shoestring virus (BBSSV) (9), was also considered as a possible vector of BBLMV, even though the pattern of spread of BBLMV does not fit that of BBSSV in the field, which is nonrandom and down the row.

The objective of this research was to determine whether honeybees or aphids vector BBLMV under both greenhouse and field conditions.

MATERIALS AND METHODS

Virus purification and serological assay techniques. Procedures for virus purification and antiserum preparation were those previously described (19,20,21). *C. quinoa* was used as the propagation host for BBLMV. Virus particles were detected by double antibody sandwich ELISA (5,21). Leaf tissue was homogenized using a Tissuemiser (Tekmar Co., Cincinnati, OH) in a 1:10 (w/v) ratio of tissue to extraction buffer composed of (0.01 M sodium-potassium phosphate buffer, pH 7.4, containing 0.02% sodium azide (w/v), 0.8% sodium chloride (w/v), 0.5% Tween-20 (v/v), and 2.0% polyvinyl pyrrolidone (mol wt 40,000, w/v) (Sigma Chemical Co., St. Louis, MO). A 200-μl quantity of test sample was added to each well of a polystyrene microtiter plate (Dynatech Laboratories, Alexandria, VA), coated with 1 μg of anti-BBLMV-IgG per milliliter of coating buffer and incubated 12–16 hr at 4 C. The alkaline phosphate-IgG conjugate was diluted 1:800 (v/v), in extraction buffer, added to washed plates and incubated 3–6 hr at 37 C. Plates were washed (three to four times) with PBS containing 5% Tween-20 (PBS-Tween). The addition of 1 mg/ml of enzyme substrate buffer (*p*-nitrophenyl phosphate, Sigma Chemical Co.) dissolved in substrate buffer [10% diethanolamine, pH 9.8, in distilled water containing 0.02% sodium azide (w/v)], was incubated for 1 hr or less at room temperature. Color change was read spectrophotometrically at A_{410nm} with a microELISA Minireader (Dynatech Laboratories). Test samples were considered positive for virus, if the A_{410nm} value of a sample well was greater than the mean A_{410nm} value plus three standard deviations of the virus-free control samples.

Radioimmunosorbent assay (RISA) of plant and insect samples. Purified anti-BBLMV-IgG was iodinated according to the chloramine-T method described by Greenwood et al (12).

A modification of the ELISA double antibody sandwich method was used, with the substitution of ^{125}I-labeled IgG for the enzyme-labeled-IgG. Coating-IgG (5 μg/ml) in coating buffer was added to flexible polyvinyl "V" bottom microtitre plates (Dynatech Laboratories) and incubated 3 hr at 37 C. After washing, a 100-μl aliquot of plant tissue, ground in extraction buffer with a Tissuemizer, or insect sample (ground in extraction buffer using a blunt glass rod in a test tube) was added and incubated 14–16 hr at 4 C. To each well was added 100 μl of ^{125}I-IgG [55,000 counts per minute (cpm)] diluted in a bovine serum albumin (BSA)-PBS buffer containing 0.5–1.0% BSA (w/v). Individual wells were cut from the plates with a hot wire knife and placed into plastic scintillation vials (one well per vial). Each vial was counted for radioactivity in a Beckman Biogamma gamma counter for 1 min. Samples that contained radioactive counts greater than the mean of healthy controls plus three standard deviations were considered positive for BBLMV.

Testing of blueberry bushes for BBLMV infection to determine pattern of spread. From a 10-ha highbush blueberry field, a block of 450 25-yr-old bushes was tested in May 1982 and early June 1983 for the presence of BBLMV. Breaks appeared in the field where diseased bushes had been cut to approximately 0.5 m above ground level by the grower. Succulent leaf tissue or blueberry blossoms were sampled at several locations around each bush to assure collection of infected tissue. These tissues were kept on ice, transported to the Michigan State University campus and tested by ELISA.

Transmission of BBLMV in infected pollen to healthy blueberry bushes by hand pollination. In 1981, pollen was obtained from virus-free and BBLMV-infected Jersey blueberry plants as previously described (4). Terminals on 15 virus-free 2-yr-old (pretested negatively by ELISA) bushes were tagged and newly opened blossoms were emasculated by cutting away the anthers. Pollen from infected bushes was applied to the stigmas of marked flowers with a small artist's brush. Four 2-yr-old virus-free, pot-grown bushes were pollinated after emasculation by the application of healthy pollen and were used as controls. The bushes were allowed to set fruit. Leaf tissue, as well as the mature and immature berries, were tested by ELISA for the presence of virus. Berries and leaves on tagged terminals were tested in August 1981, whereas only leaves were tested in June and August 1982 and April 1983. Each year bushes were given a 6-mo cold dormancy treatment (1,000 hr at 4 C).

Detection of BBLMV in honeybee pollen sacs. To determine if honeybees carry BBLMV-contaminated pollen in their pollen baskets (sacs), foraging bees were trapped at bloom during the 1981 and 1982 seasons. The hind legs, containing the pollen sacs, were tested using ELISA in 1981, whereas all three pairs of legs were removed and tested in 1982. Honeybee legs were placed in glass vials containing 400 μl (mean weight of legs = 31 mg) of virus extraction buffer and were ground with glass rods for 2 min. Virus-free control samples were obtained from honeybees trapped in BBLMV-free blueberry fields.

Transmission of BBLMV from infected pollen to virus-free blueberry trap-bushes via foraging honeybees. To test the hypothesis that BBLMV may be spread in pollen by honeybees while foraging, containment cages were constructed and placed around known BBLMV-infected and virus-free blueberry bushes in the field before bloom in 1982 and 1983. Ten virus-free (ELISA tested) 2-yr-old, potted Jersey trap-plants (Tower View Nursery, So. Haven, MI) were placed around each caged and selected noncaged bushes. Source and trap-bushes were at the same stage of development, i.e., early bloom. Terminals on the trap bushes, containing developing flower buds were tagged. A small hive or "nuke", containing 2,500–3,000 workers and a queen (Buckfast queen honeybee, Weaver Apiary, Navasota, TX), was placed within some of the cages and near to other noncaged plants. At three times during bloom pollen was collected from pollen-traps attached to the hive entrance, which scraped pollen from the hind legs as bees entered. The pollen was stored at 4 C and was tested by RISA. Before testing by RISA, any maternal tissue was separated from the pollen grains under a steroscopic dissecting microscope.

Trap bushes from the 1982 field experiment were given a cold dormancy treatment (4 C) for 5 mo in 1982 and again in 1983. Bushes were forced in the glasshouse after each cold treatment and succulent leaf tissues was tested by ELISA for BBLMV. Test bushes were held in a cold frame for several years, observed for

symptoms, retested by ELISA and mechanically inoculated to *C. quinoa* in May 1986.

Acquisition access study of BBLMV by the blueberry aphid. Apterous mature and immature aphids, cultured on healthy blueberry bushes in temperature-controlled incubators (18-hr day photoperiod with day and night temperatures of 23 and 18 C, respectively) were obtained for this experiment. Aphids given a starvation preacquisition period of 1 hr were placed on succulent terminals of BBLMV-infected and virus-free 3-yr-old Jersey blueberry bushes, pretested by ELISA. Aphids were allowed to feed for either a 72-hr or 120-hr acquisition access period (AAP). After the acquisition access feeding period, individual aphids were placed in glass vials, containing 200 μl of virus extraction buffer and ground with a glass rod for 2 min (9). Samples were tested by RISA using the same concentrations as described previously, (55,000 cpm per well of anti-BBLMV-^{125}I-IgG) and counted in a gamma counter for 1 min.

RESULTS

Testing of blueberry bushes for BBLMV infection to determine pattern of spread. The experimental block of Jersey highbush blueberry bushes was located in the center of the field and spanned 15 rows of plants (Fig. 1). Blueberry bushes were ELISA-tested for BBLMV in May 1982 and 1983. Of the 450 plants observed, 117 (26%) were missing or cut back by the grower to 0.5 m above the ground. Many suckers grew from these stumps and often blossoms were produced in the spring. Symptoms typical for the disease were obvious on all plants. Twenty-three plants (5.1%) in this block that were not cut back were ELISA-positive and expressed symptoms. No symptomless, but infected bushes were detected. Although the rate of disease spread could not be determined because of such a large number of missing bushes, the pattern of spread appeared to be random. This random pattern was also observed in parts of the field that were not tested, but contained bushes expressing symptoms characteristic of the disease.

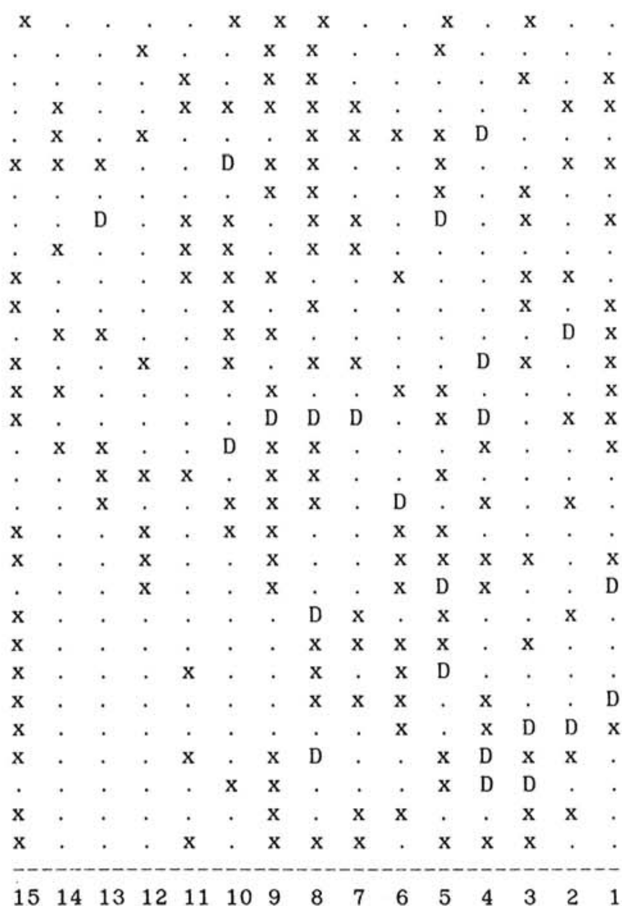

Fig. 1. ELISA-tested highbush blueberry bushes in a commercial field, showing a random pattern of spread of blueberry leaf mottle virus (BBLMV), Agnew, MI, 1983. D = diseased (ELISA-positive), x = missing, cut-off bush, . = healthy bush.

Transmission of BBLMV in infected pollen to virus-free blueberry bushes by hand pollination. Fruit was set on each of the four virus-free control bushes pollinated with virus-free pollen. Fourteen of the 15 test plants pollinated with pollen obtained from BBLMV-infected bushes also set fruit (Table 1). The mean number of blossoms available for pollination was 22.5, with an average of 5.1 corymbs tagged per plant. This resulted in a mean of 9.9 berries set per virus-infected pollen-treated bush. A mean of 35.5 blossoms with 9.8 tagged clusters was obtained from virus-free control bushes. An average of 33.8 berries were set per virus-free control bush. The mean number of blossoms per group was similar for both virus-free and BBLMV-treated bushes. However, twice as many fruits were set per tagged terminal on healthy bushes as on those treated with pollen from diseased bushes. Berries and leaves on tagged terminals were ELISA-tested August 1981, whereas only leaves were tested June and August 1982. Leaves were tested by RISA in April 1983. Blueberry leaf mottle virus was detected by ELISA. June 1982, in leaf tissue sampled from two of the tagged blossom clusters on bush No. 13, pollinated with BBLMV-containing pollen (Table 1). Those two terminals were dead in 1983.

Detection of BBLMV in honeybee pollen sacs. BBLMV was detected in 15.5% (9/58) of the samples tested in 1981 (Table 2). ELISA detected BBLMV in 51.4% (38/74) of the 1982 samples in which all three pairs of legs were tested. The pollen from honeybee legs collected from virus-free fields were negative for BBLMV when tested by ELISA.

Transmission of BBLMV from contaminated pollen to virus-free blueberry trap-bushes via foraging honeybees. Trap bushes were removed from around caged and noncaged BBLMV-infected source plants at the petal fall stage in both 1982 and 1983 and allowed to incubate in isolation for several months before being tested for infection. Shoot terminals were tested by RISA, whereas mature and immature berries were tested by ELISA.

TABLE 1. Hand-pollination of virus-free 2-yr-old cultivar Jersey blueberry bushes with blueberry leaf mottle virus (BBLMV)-contaminated pollen[a]

Virus infected pollen (Bush no.)	Blossoms (no.)/ Tagged corymbs (no.)	Berries set (no.)	BBLMV[b] Infected
1	18/5	8	...
2	17/5	3	...
3	42/7	9	...
4	33/7	17	...
5	8/2	0	...
6	13/1	6	...
7	15/5	3	...
8	38/10	3	...
9	18/4	3	...
10	33/9	26	...
11	13/5	2	...
12	14/3	12	...
13	17/3	10	+[c]
14	26/4	13	...
15	33/6	34	...
Virus-free pollen controls (Bush no.)			
1	24/5	3	...
2	58/19	46	...
3	45/12	68	...
4	15/13	18	...

[a] Pollen transferred with an artist's brush to emasculated blossoms. Leaves and berries tested by enzyme-linked immunosorbent assay (ELISA) in 1981, 1982. Leaves tested by radioimmunosorbent assay 1983.

[b] Mean A_{410nm} value of healthy leaves = 0.05; diseased *Chenopodium quinoa* (positive control) = 1.88. Diseased sample values were greater than the mean plus three standard deviations of healthy controls, A_{410nm} = 0.07.

[c] Leaves from two tagged shoots were ELISA-positive for BBLMV in 1982. A_{410nm} = 0.17, 0.18, respectively.

Twelve of the 138 (8.7%) tagged terminals on the 1982 trap bushes placed around noncaged, diseased bushes with a beehive near were positive for BBLMV in July 1982 (Table 3). From these 20 bushes, only one berry of the 537 (0.2%) tested was ELISA positive. A higher percentage of the terminals (13.4%) and berries (0.5%) on bushes placed in cages with a diseased bush plus a hive were positive for BBLMV. Trap-bushes within cages containing a beehive, set almost twice as much fruit when compared with noncaged trap-bushes with a hive nearby. Only two of the 93 terminals were BBLMV positive (2.2%) on trap bushes placed around caged, diseased source bushes without a beehive inside the cage. It is probable that some pollination and subsequent infection occurred without the aid of honeybees or by other insects, i.e., bumblebees or syrphid flies. None of the mature or immature fruit that developed (0/95) contained BBLMV. A blueberry bush in another field 14.4 km away, without the disease present and previously assayed negative for BBLMV, was used as the control. Leaf tissue from the 55 tagged terminals on trap bushes surrounding this plant assayed virus-free. Virus was not detected in any of the 238 berries that set on these terminals.

Pollen collected from pollen traps placed in front of beehives was tested by ELISA in March 1983 (Table 3). One of the 17 (5.9%) pollen samples collected from hives placed near noncaged, diseased bushes contained BBLMV-infected pollen. Blueberry leaf mottle virus was not detected in any of the six pollen samples collected from hives placed in cages with a diseased source bush, surrounded by trap bushes. Although blueberry pollen may be stored for a year, the virus may have degraded during the storage period between collection and assay. Virus was not detected in the 13 pollen samples collected from the beehive placed within the cage with a healthy source bush.

Fewer leaf terminals were tagged on all trap bushes used for the transmission study conducted in 1983 (Table 4) than in 1982. Leaf tissue on terminal shoots of trap bushes was assayed for BBLMV in March 1984, following a 4.5-mo dormancy. Two of the 47 marked terminals (4.3%) on noncaged trap bushes surrounding a diseased source bush and a hive nearby were ELISA-positive for BBLMV in March. Leaf tissue on two trap bushes placed within cages with a diseased source bush and beehive were positive for BBLMV in March. However, these bushes were not positive when tested in June of the same year. BBLMV was not detected in leaf terminals from trap bushes surrounding caged, diseased source bushes with no hive, nor surrounding caged, healthy source bushes with a beehive inside. BBLMV was not detected in the berries or

TABLE 2. Detection of blueberry leaf mottle virus (BBLMV)-infected pollen on legs of foraging honeybees trapped from a Jersey highbush blueberry field containing BBLMV-infected bushes, Agnew, MI

Bees trapped from	No. tested	No. with contaminated pollen	% with contaminated pollen
Diseased bushes, May, 1981[a,b]	58	9	15.5
Diseased bushes, May, 1982[b,c]	74	38	51.4

[a] Hind legs (third pair) of honeybees, containing the pollen baskets were ground in 200 μl of extraction buffer for 2 min. Samples were tested by enzyme-linked immunosorbent assay (ELISA). The mean A_{410nm} value plus three standard deviations of the healthy control = 0.1, diseased = 0.1–0.4.

[b] Ten control honeybees obtained from Jersey blueberry fields containing BBLMV-free bushes. BBLMV was not detected in the control pollen.

[c] All three pairs of honeybee legs were assayed by enzyme-linked immunosorbent assay.

TABLE 3. Infection of virus-free Jersey blueberry trap-bushes surrounding blueberry leaf mottle virus (BBLMV)-infected source bushes via virus-contaminated pollen during honeybee foraging, Agnew, MI, 1982

Treatment[a]	Trap bushes (no.)	Infected[b] terminals	%	Infected[c] berries	%	Infected[d] pollen	%
Diseased, noncaged source bush with hive near	20	12/138	8.7	1/537	0.19	1/17	5.9
Diseased, caged source bush with hive inside cage	20	23/172	13.4	6/1,338	0.45	0/6	0.0
Diseased, caged source bush, no hive	19	2/93	2.0	0.95	0.0	0/0	0.0
Virus-free, caged source bush with hive inside cage	10	0/55	0.0	0/238	0.0	0/13	0.0

[a] Virus-free, 2-yr-old trap bushes were placed around BBLMV-infected source bushes, 19 May 1982, at 75% bloom through the end of petal fall.

[b] Number of BBLMV-infected terminals per number tagged on trap plants, tested by radioimmunosorbent assay, 1983. Mean cpm plus three standard deviations of healthy controls = 329; diseased = 368–685 cpm.

[c] Number of berries assayed positive for BBLMV per number of berries tested by enzyme-linked immunosorbent assay, 1982. Mean A_{410nm} plus three standard deviations = 0.07, diseased = 0.09–0.14.

[d] No pollen assayed positive by ELISA, 9 March 1983. Mean A_{410nm} plus three standard deviations of healthy controls = 0.04.

TABLE 4. Infection of virus-free Jersey blueberry trap-bushes surrounding blueberry leaf mottle virus (BBLMV)-infected source bushes via virus-contaminated pollen during honeybee foraging, Agnew, MI, 1983

Source bushes[a]	Trap bushes (no.)	Infected[b] terminals	%	Infected[c] berries	Infected[c] peduncles	Infected[d] pollen	%
Diseased, noncaged source bush with hive near	20	2/47	4.3	0/13	0/10	13/56	23.2
Diseased, caged source bush with hive inside cage	20	2/71	2.8	0/350	0/290	6/20	30.0
Diseased, caged source bush, no hive	20	0/65	0.0	0/3	0/3	0/0	0.0
Virus-free, caged source bush with hive inside cage	10	0/24	0.0	0/125	0/93	0/8	0.0

[a] Healthy 2-yr-old potted trap bushes were placed around BBLMV-infected source bushes, 24 May 1983, at 75% bloom.

[b] Ratio of terminals that assayed enzyme-linked immunosorbent assay (ELISA)-positive for BBLMV/number of terminals assayed in March 1984. Mean A_{410nm} value of healthy control plus three standard deviations = 0.05; diseased = 0.05–0.46.

[c] Number of berries and peduncles that tested positive by radioimmunosorbent assay (RISA) for BBLMV/number individually tested June 1983. Mean value of healthy control plus three standard deviations = 133 cpm. No samples were diseased.

[d] Number of pollen samples infected with BBLMV/number of samples collected from beehives, tested by RISA June 1983. Mean value of healthy control plus three standard deviations = 320 cpm, diseased = 322–440 cpm.

peduncles collected from trap bushes surrounding noncaged diseased source bushes or caged diseased bushes with no honeybee hive inside (Table 4). A greater number of berries was set on trap bushes placed within cages with a beehive, compared with bushes surrounding noncaged source bushes. None of these berries or peduncles attached to these berries were ELISA-positive for BBLMV. However, 23% (13/56) of the pollen samples collected from pollen traps placed in front of hives near noncaged diseased source bushes were positive for BBLMV when assayed by RISA (Table 4). Thirty percent (6/20) of the samples obtained from pollen collected from hives caged with BBLMV-infected bushes contained virus. Pollen was not collected from within cages that did not contain a honeybee hive. Virus was not detected in the eight pollen samples collected from the healthy controls. Honeybees did not survive well in the screen cages and the population of 2,500 bees per hive had decreased considerably after 2 wk in the field.

All test plants that had grown in pots in a cold frame were examined for symptoms of blueberry leaf mottle in May 1986. Three of 43 remaining plants from the 1982 tests exhibited stem dieback and leaf symptoms typical of the disease. One plant tested both ELISA-positive and positive on *C. quinoa*, and two plants tested positive on *C. quinoa*, but negative by ELISA. Six of 48 remaining plants from the 1983 tests exhibited leaf symptoms typical of the disease. Three plants tested positive by ELISA, but none tested positive on *C. quinoa*.

Results of BBLMV-acquisition study by the blueberry aphid. BBLMV was not detected in any of the 63 aphids assayed during July 1983 nor in the 80 assayed during September 1983, after an AAP of 72 hr. Blueberry aphids did not appear to acquire the virus when given an AAP longer than 72 hr. None of the 68 and 72 aphids given an AAP of 120 hr and tested on 22 September and 29 September, respectively, were ELISA positive.

DISCUSSION

An estimate of the rate of spread of BBLMV could not be made because of the number of bushes previously removed by the grower. The virus appears to be spread by an active, randomly moving vector either initiating new infection foci or increasing areas of established infection. Mink (18) found patches of cherry trees infected with the cherry rugose mosaic strain of PNRSV. Virus-diseased cherry trees were located around sites of initially infected trees. A similar pattern was observed in highbush blueberry fields not as extensively infected with BBLMV as the field that we indexed. This pattern of disease spread might indicate mediation of BBLMV infection by the foraging activities of honeybees during bloom. It has been documented that honeybees maintain a particular foraging area, i.e., a certain part of the field, group of bushes, or even a single bush where they visit (14). This behavior may be influenced by the size of the foraging area, number of flowers or other foragers, but bees usually return to this same area following each visit to the hive. Honeybees have been observed to maintain a 4- to 5-sq-yd area (14). Therefore, a single honeybee foraging on an infected bush would have a potential to spread virus-contaminated pollen to several bushes in a single season.

Although BBLMV appears to have spread extensively within the field indexed, only two newly infected bushes were detected by ELISA between 1982 and 1983. This may be due in part to a reduction of available sites for infection in this portion of the field. Cameron et al (1) reported a geometric increase in the number of Montmorency cherry trees infected with PNRSV over an initial 4-yr period but a decline after this period. This decline was attributed to the fact that more than 50% of the trees were already infected and had reached a stationary phase of infection with only a few healthy trees remaining to become infected.

Several avenues of virus entry into blueberry via pollen may be proposed: By mechanical inoculation by the germ tube during disruption of the exine as the tube elongates; infection of the stigma by contact with virus-contaminated pollen; infection of the integuments of the ovule, carrying virus internally from the sperm cell to the egg cell, resulting in transmission to the developing zygote, and finally, by transmission through the cytoplasm of the vegetative cell, then to the embryo.

Honeybees foraging in BBLMV-infected fields were shown to contain virus-contaminated pollen, either associated with the corbicula (pollen baskets) or with all three pairs of legs. The anthers of highbush blueberry flowers are located in front of the nectaries and nectar cannot be gathered without pollen becoming attached to the honeybee's body. Mink (18) observed that honeybees leaving commercial hives in cherry orchards had pollen attached to their bodies. He postulated that PNRSV-containing pollen may be spread for several days in this manner without pollen viability being important. It was found that, although the infectivity of PNRSV decreased with time on pollen stored in the hive, it remained infectious on pollen collected within the field for 14 days.

Pollen collected from pollen-traps placed in front of hives located within cages containing BBLMV-infected source plants or near to uncaged source plants produced symptoms if rub-inoculated to *C. quinoa*. It would be of interest to determine the length of time BBLMV remains infectious in the field. It is also possible that virus-contaminated pollen is spread throughout the bee colony through physical contact alone. However, Mink (18) found that, although more than 50,000 hives were transported from PNRSV-infected cherry orchards in California to orchards in Washington state, only a small number of new infections were observed. When one of these hives was placed within a cage with a healthy cherry tree in full bloom, only five seeds from the 120 fruits that set contained prune dwarf virus and none contained PNRSV. A similar low occurrence of virus transmission was observed on virus-free trap-bushes surrounding caged or noncaged BBLMV-infected blueberry source bushes with honeybee hives nearby. The highest percentage of infected leaf shoots (13.4%) and berries (0.5%) was on trap bushes in cages enclosing a hive of foraging bees, in 1982. Only a small number (2.5%) of the leaf shoots on trap bushes associated with honeybee activity were infected with BBLMV when tested the following year in 1983. None of the berries or peduncles tested in 1983 were infected with BBLMV. Symptoms were not observed on these infected leaf shoots and several years may be necessary for symptom expression to occur.

The random and relatively rapid spread of BBLMV does not resemble that characteristic of virus spread by a nematode vector, but rather, it fits with a bee-mediated pollen-borne pattern of spread. Cherry leaf roll virus (CLRV) has also been placed as a member of the nepovirus group, although transmission by a nematode vector has not been conclusively demonstrated (16). The virus has been associated with pollen. Evidence suggests that CLRV has evolved a more efficient mode of spread, presumably by pollen. Although transmission to healthy *Prunus* species via CLRV-contaminated pollen has not been successful in the United States, Mircetich et al (17) have demonstrated seed and pollen transmission of the CLRV-W strain causing black line disease in English walnut (*Juglans regia*) in California orchards. Several different strains of CLRV have been found naturally infecting many woody plant species in Europe, North America, and New Zealand (6). Pollen may represent an alternate mode of BBLMV transmission to highbush blueberry in the absence of the nematode vector, or may represent an ecological adaptation for a more rapid mode of spread. Honeybees foraging for nectar and pollen do wound the floral tissue, and BBLMV may be directly introduced to the parent plants through these wounds. Virus spread through the fertilization process and developing seed and seedlings seems to be a less efficient mechanism. Hamilton et al (13) described several factors affecting the efficiency of virus spread via pollen: Transmission would be higher in open-pollinated than self-pollinated plants; high virus titer in pollen and an effective method of attachment to the pollen grain would result in more efficient transmission; windblown pollen is a less efficient vehicle or mode of transmission than that mediated by a pollinator; and in most cases, transmission of virus to seed is more efficient when virus is introduced to the egg during the fertilization process. The BBLMV-incited disease of highbush blueberry appears to be a prime candidate for virus being effectively spread via pollen.

Blueberry plants may be either self- or open-pollinated. BBLMV maintains a high titer in the infected leaf tissue and pollen grains. Virus-contaminated pollen is transmitted to susceptible hosts via honeybees. Finally, BBLMV is readily transmitted to the seed but the mode of virus entry may be via wounds induced during honeybee foraging rather than by fertilization of the ovule, because survival in germinated seedlings is low (4). Further examination of the last point would be interesting in terms of seed transmission and the potential role of birds as mediators of long distance spread of BBLMV.

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