Detection of Blueberry Leaf Mottle Virus in Highbush Blueberry Pollen and Seed

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ABSTRACT

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Blueberry leaf mottle virus (BBLMV) antigen was detected by ELISA in the pollen from 13 of 15 mature infected cultivar Jersey bushes. Washing with phosphate-buffered saline followed by centrifugation removed almost all of the antigen from the surface of the pollen. Serologically blocked and washed BBLMV-infected pollen, subsequently ground and tested by ELISA, contained BBLMV. Pollen from infected bushes showed reduced germination in nutrient solutions compared with healthy pollen. Seeds from infected bushes were found by ELISA to contain BBLMV antigen, but seeds ground and inoculated to *Chenopodium quinoa* failed to cause infection. Only 1.5% of the seedlings grown from infected seeds were shown to be BBLMV-infected by radioimmunoassay.

Additional key words: nepovirus, pollenborne virus.

Blueberry leaf mottle disease of highbush blueberry (*Vaccinium corymbosum* L.) caused by blueberry leaf mottle virus (BBLMV) was initially described in Michigan by Ramsdell and Stace-Smith (14) on the cultivar Rubel. The virus was subsequently found in cultivar Jersey (15,16). Systemically infected plants show deformed and/or mottled leaves accompanied in severe cases by a general dieback of the main stems. Physical and chemical properties of the virus have been determined and BBLMV has been placed as a putative member of the nepovirus group (14–16). The Michigan strain of the virus is closely related to the New York State strain of grapevine Bulgarian latent virus (GBLV-NY), described by Uyemoto et al (17) from a cultivar Concord grapevine. Both strains are distantly related serologically to a European strain (GBLV-Eu) (10). BBLMV has a limited natural host range but is sap-transmittable to a number of experimental herbaceous hosts (14,16). The Michigan strain was able to infect a low percentage of grape seedlings (*Vitis labrusca* L.) cultivar Niagara (13), but GBLV-NY did not infect blueberry cultivars (14). Transmission of GBLV-NY through the seed to seedlings of *V. labrusca* (5%) and *Chenopodium quinoa* Willd. (12%) was low (17). Although BBLMV shares similarities in physical and chemical properties with other members in the nepovirus group, nematode transmission has not been demonstrated. In controlled experiments, *Xiphinema americanum* (Cobb) did not transmit BBLMV to *C. quinoa* or *Nicotiana clevelandii* Gray seedlings whether the nematodes were obtained from a Michigan or Arkansas source (16). Blueberry leaf mottle disease is not well associated with vector nematode species in the field. Weeds beneath BBLMV-infected bushes were not infected with BBLMV (2). In bait tests with soil taken from beneath BBLMV-infected bushes containing *X. americanum*, neither *C. quinoa, N. clevelandii,* nor blueberry seedlings became infected after 3 wk of exposure to the nematode in the greenhouse (2).

Transmission of virus through seed and pollen is characteristic of several members of the nepovirus group. Although this mode of dispersal is usually considered to be of secondary importance, it appears to be the primary mechanism of spread for some viruses (4,11). Experiments were conducted to ascertain whether BBLMV is associated with highbush blueberry pollen and to determine the role of pollen in disease spread.

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MATERIALS AND METHODS

Collection and testing of blueberry pollen by enzyme-linked immunosorbent assay (ELISA). Pollen collected during bloom was obtained from healthy and BBLMV-infected Jersey highbush blueberry plants previously tested for virus by ELISA (3). Pollen collected onto glass microscope slides was separated from maternal tissue with the aid of a teasing needle under a stereoscopic dissecting microscope. Each pollen sample (0.2–4.0 mg) was ground with a mortar and pestle in 100 μl of extraction buffer (0.01 M sodium-potassium phosphate buffer, pH 7.4, containing 0.02% sodium azide [w/v], 0.8% sodium chloride [w/v], 0.05% Tween 20 [v/v], and 2% polyvinylpyrrolidone, mol wt 40,000 [w/v; Sigma], and 0.2% ovalbumin [w/v]), then assayed by ELISA. The coating anti-BBLMV-IgG was diluted to 1 μg/ml in 0.5 M sodium carbonate-bicarbonate buffer, pH 9.6, and the anti-BBLMV-IgG-alkaline phosphatase conjugate was diluted to 1:400 (v/v) in extraction buffer. The enzyme substrate, *p*-nitrophenyl phosphate (Sigma) was diluted to 1 mg/ml in substrate buffer (10% diethanolamine, pH 9.8, in distilled water containing 0.02% sodium azide [w/v]). Reaction values in wells were read spectrophotometrically at A_{410nm} with a model MR590 micro-ELISA minireader (Dynatech Laboratories, Alexandria, VA). Test samples were considered positive for virus antigen if the A_{410nm} value of a sample was greater than the mean A_{410nm} value plus three standard deviations of the healthy pollen control samples. A standard curve of purified BBLMV vs. ELISA absorbance values (Fig. 1) was generated for quantification of BBLMV antigen found in and on pollen grains.

Infectivity of pollen. Blueberry pollen collected from diseased Jersey bushes in 1983 was tested for the presence of BBLMV using herbaceous indicator hosts. The samples were obtained from the same infected bushes used for serological blocking experiments discussed later. Samples, diluted 1:5 (w/v) in buffer (0.05 M sodium phosphate buffer, pH 7.2), were ground with a mortar and pestle and rub-inoculated onto Carborundum-dusted (320-mesh) *C. quinoa* plants in the fourth-leaf stage. Plants were kept 14–21

days in a greenhouse (22–30 C) under cool-white fluorescent lights with a 15-hr day length. Leaf tissue showing symptoms was tested by ELISA for BBLMV.

Viability of pollen. If germination of the pollen grain is necessary for BBLMV infection, pollen viability will affect the spread of the disease. To determine the viability of BBLMV-infected pollen, 2–3 mg of healthy and diseased, air-dried pollen was diluted in 1 ml each of 12.5% (w/v) sucrose in distilled water alone or with one of the following additives: 100 μg/ml (ppm) boric acid (H_3BO_3), 100 ppm boric acid plus 300 ppm calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$], 100 ppm boric acid plus 200 ppm magnesium sulfate ($MgSO_4 \cdot 7H_2O$), or 100 ppm boric acid plus 300 ppm calcium nitrate and 200 ppm magnesium sulfate. A portion of each pollen sample was pretested for virus content by ELISA before germination. Cells were counted in a 10× field of view for percent germination, after 3–4 hr of incubation at room temperature, using a steroscopic dissecting microscope. Each treatment was replicated three times.

Serological blocking to determine location of virus in pollen. The location of BBLMV on or in highbush blueberry pollen grains was investigated using a modified serological blocking technique (4). Blueberry pollen was collected in 1983 from four healthy and seven BBLMV-infected bushes as previously described. Twenty-milligram aliquots of each pollen sample collected were suspended in 0.4 ml of a 0.01 M phosphate-buffered saline solution, pH 7.4 (PBS). Pollen grains were examined with a stereoscopic dissecting microscope and appeared to be intact and not contaminated with maternal tissue. After a 30-min incubation at room temperature (21 C), samples were centrifuged at low speed (6,000 rpm, 10 min). Each pellet was given three washes in 0.4 ml of PBS with gentle agitation followed by a low-speed centrifugation after each wash. The supernatant from each wash was tested for BBLMV by ELISA. Pollen integrity of the pellets was checked after each centrifugation by light microscopy. After washing, samples were resuspended in 0.4 ml of PBS and mixed with equal volumes of either 1) PBS containing polyvinylpyrrolidone (PBS-PVP), 2) anti-BBLMV IgG diluted to 0.5 μg/ml in PBS-PVP, or 3) anti-peach rosette mosaic virus (PRMV)-IgG diluted to 0.5 μ/ml in PBS-PVP. Samples were mixed and incubated 3–4 hr at 4 C. After a low-speed centrifugation, samples were rinsed three times in PBS with gentle agitation to remove excess globulin, followed by low-speed centrifugation after each wash. The supernatant was again ELISA-tested and the integrity of the pollen was observed in the pellet. After a final rinse, samples were resuspended in 1 ml of PBS and subdivided. Half of the samples were ground for 30 sec in porcelain spot plates with flat-ended glass rods, whereas the other half were not ground. Microscopic examination revealed that >90% of the ground pollen grains were broken. Both subsamples were tested by radioimmunosorbent assay (RIA). RIAs of pollen resuspended in PBS were conducted with the same IgG concentration as previously described for ELISA, except for the addition of ^{125}I-IgG (55,000 cpm per well) diluted in PBS buffer containing 1% bovine serum albumin. Sample wells were rinsed with BSA-PBS-Tween 20, separated into individual wells by cuts made with a hot wire knife, and the radioactivity counted in a gamma counter for 1 min (Beckman Instruments Inc., Palo Alto, CA).

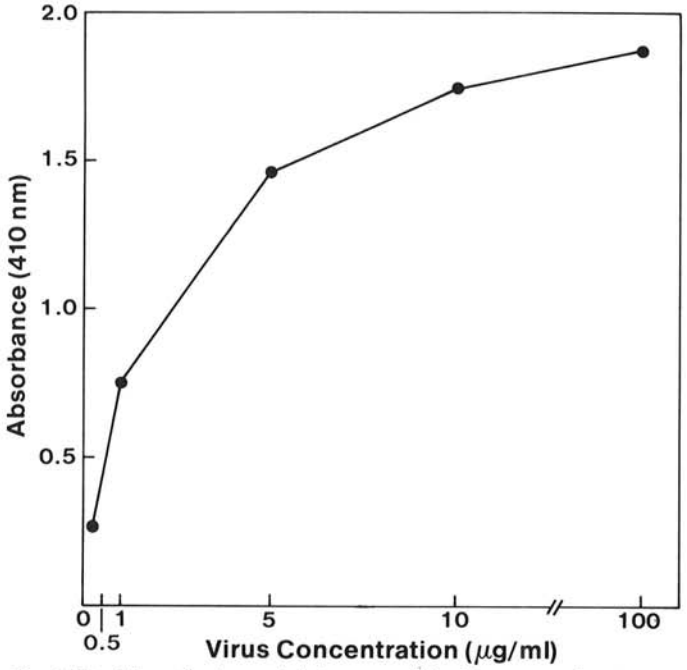

Fig. 1. Double-antibody sandwich enzyme-linked immunosorbent assay standard curve for purified blueberry leaf mottle virus.

Detection of BBLMV in blueberry seed. Mature seeds were extracted from ripe Jersey highbush blueberries harvested from diseased and healthy bushes in the field. Individual seeds were ground in 125 μl of extraction buffer and tested by either ELISA or RIA.

To determine whether the seeds contained infectious virions, seeds obtained from healthy and diseased seed bushes were cut in half transversely with a sterile razor blade. One-half of the seed was tested for virus by ELISA, and the other half was crushed in 200 μl of 0.05 M phosphate buffer and inoculated to *C. quinoa*. Leaves from inoculated plants were tested by ELISA for virus after 2 wk.

Detection of BBLMV antigen in blueberry seedlings. Seedlings from seeds obtained from BBLMV-diseased bushes were tested to determine whether BBLMV survives the germination process. Seeds were extracted from mature blueberries harvested from diseased and healthy bushes and were placed 3.2 mm (1/8 in.) deep in flats of peat and allowed to germinate. The entire seedling was ground in either extraction buffer and tested for virus by ELISA or ground in 0.05 M phosphate buffer plus 2% nicotine (v/v), then mechanically inoculated onto Carborundum-dusted (320-mesh) leaves of *C. quinoa*. One week later, leaves from inoculated plants were ELISA-tested for BBLMV infection.

RESULTS

Detection of BBLMV antigen in highbush blueberry pollen. BBLMV antigen was detected by ELISA in 13 of the 15 pollen samples (87%) collected from individual BBLMV-infected bushes (Table 1). ELISA readings $>A_{410nm} = 0.09$, the mean plus three standard deviations of healthy control values, were considered positive. The mean A_{410nm} readings for ELISA-positive pollen was 0.56/mg. According to the standard curve for BBLMV (Fig. 1), 1 mg of infected pollen had about 10 μg of BBLMV on or in the grains.

Infectivity of BBLMV-contaminated blueberry pollen. Infectivity tests were done with 14 of the infected pollen subsamples also tested in the serological blocking experiment. Triturated pollen was rub-inoculated onto *C. quinoa* plants in the fourth-leaf stage of development. Fourteen days after inoculation, 43% (6/14) of the inoculated plants showed leaf mottling and local lesions typical of BBLMV infection; all six of the infective pollen samples were ELISA-positive for the virus. No symptoms were seen and virus was not detected in plants inoculated with buffer or healthy pollen.

Viability of pollen. Percent germination of pollen grains in various nutrient solutions was calculated by dividing the number of cells forming pollen tubes by the number of cells counted in each field. Three subsamples of pollen for each treatment were observed and recorded. Fewer pollen grains from diseased plants germinated in all solutions than pollen from healthy bushes. Only 6% (39/629) of the pollen from diseased bushes germinated in a 12.5% sucrose solution, whereas 44% (266/604) of the healthy pollen germinated (Table 2). Germination in sucrose improved with the addition of boric acid plus nutrient salts for both healthy and diseased pollen samples but decreased with the addition of boric acid alone. Highest germination occurred in sucrose plus boric acid and calcium nitrate and in sucrose plus boric acid, calcium nitrate, and magnesium sulfate. More than one germ tube

per pollen tetrad was formed in these treatments, whereas only one germ tube per tetrad was produced in other treatments. Pollen collected from diseased bushes tested positive for BBLMV antigens by ELISA, with an average A_{410nm} reading of 1.64.

Serological blocking to determine location of BBLMV in blueberry pollen. At least two subsamples of pollen per bush sample was sham-blocked with either anti-PRMV-IgG or PBS, whereas eight subsamples of pollen from diseased bushes were blocked with anti-BBLMV-IgG. Most of the virus antigens were easily washed off the pollen surface as shown by removal of detectable antigens from the pollen tetrads after the three preblocking washes with PBS (Table 3). Virus antigen was not detected in postblocking washings after any of the three blocking treatments. BBLMV antigen was not detected in washings or residual pollen from healthy bushes treated with PBS alone, anti-PRMV-IgG, or anti-BBLMV-IgG.

Pollen samples from infected bushes that were not ground after incubation with any of the blocking treatments were negative for BBLMV when tested by RIA (Table 4). BBLMV antigen was detected in 100% (16/16) of the pollen samples sham-blocked with PBS and ground and in 100% (10/10) of the samples sham-blocked with anti-PRMV-IgG and ground. After pollen samples were ground with a mortar and pestle, eight of the 16 samples (50%) blocked with anti-BBLMV-IgG were positive for BBLMV when tested by RIA. BBLMV was not detected in pollen from virus-free bushes, whether the cells were intact or disrupted by grinding.

Detection of BBLMV in seed and seedlings. BBLMV was detected by ELISA in 31/108 (28.7%) of whole blueberry seeds. The $\underline{A_{410nm}}$ readings for diseased seeds ranged from 0.08 to 0.74, with $\bar{X} + 3$ SD for healthy seedlings = 0.05. Of the seedlings grown from seed obtained from infected bushes, 5/341 (1.5%) were BBLMV-positive when tested by RIA. Values for diseased seedlings ranged from 444 to 515 cpm, with $\bar{X} + 3$ SD for healthy seedlings = 442 cpm.

In tests of seeds from diseased bushes where one-half of a seed was assayed for BBLMV by ELISA and the other half was rub-inoculated to *C. quinoa* plants, the following results were obtained: 35% of the seeds (14/40) assayed positive for BBLMV by ELISA

TABLE 1. Detection of blueberry leaf mottle virus (BBLMV) in cultivar Jersey highbush blueberry pollen collected from ELISA-tested healthy and infected bushes in Agnew, MI[a]

Pollen sample	Weight (mg)[b]	ELISA values (A_{410nm})[c]
From infected bushes		
1	4.0	0.08
2	4.6	1.61
3	6.0	1.92
4	2.0	1.90
5	2.0	1.94
6	0.2	1.26
7	1.1	1.87
8	1.8	0.14
9	1.8	1.85
10	7.2	1.73
11	2.5	1.90
12	1.1	0.82
13	1.0	0.07
14	1.0	1.86
15	2.0	0.58
From healthy bushes		
1	1.0	0.06
2	1.0	0.05
3	1.0	0.07
4	1.0	0.06
From infected leaf tissue		
1	1.0	1.96
2	1.0	1.97
3	1.0	1.93

[a] Samples were collected at full bloom, May 1981.
[b] Samples were diluted in 100 µl of a 0.05 M sodium-potassium phosphate buffer, pH 7.4
[c] Absorbance readings at A_{410nm}, represent a mean of two subsamples. The values of the diseased samples were considered positive if they were greater than the mean plus three standard deviations of healthy controls (X+ 3 SD = 0.09).

TABLE 2. Viability of pollen obtained from healthy and blueberry leaf mottle virus-infected blueberry in several nutrient solutions

	Germination[b] (%)							
	Healthy plant				Diseased plant			
Germination solution[a]	1	2	3	Mean[c]	1	2	3	Mean[c]
Sucrose	39	35	58	44	5	10	4	6
Sucrose, boric acid	20	16	21	19	13	10	18	14
Sucrose, boric acid, calcium nitrate	90	78	81	83	50	66	52	56
Sucrose, boric acid, magnesium sulfate	42	58	72	57	26	31	30	29
Sucrose, boric acid, calcium nitrate, magnesium sulfate	80	77	94	83	60	57	58	58

[a] A 12.5% sucrose solution in sterile distilled water alone or containing one or a combination of the following additives: 100 mg/ml H_3BO_3, 300 mg/ml $Ca(NO_3)_2 \cdot 4H_2O$, or 200 mg/ml $MgSO_4 \cdot 7H_2O$.
[b] Pollen samples (2 mg) were incubated in 1 ml of the treatment solution for 3–4 hr at 21 C. Samples were pretested by enzyme-linked immunosorbent assay (ELISA).
[c] Mean of three subsamples; percentage of pollen grains forming germ tubes per number of cells counted with a light microscope (×250).

TABLE 3. Enzyme-linked immunosorbent assays of phosphate-buffered saline washings of blueberry pollen before and after blocking of the pollen surface with anti-BBLMV-IgG

			Mean ELISA values (A_{410nm})					
			Preblocking washings[c]			Postblocking washings[d]		
Pollen source[a]	No. subsamples tested	Blocking treatment[b]	1	2	3	1	2	3
Healthy	5	PBS	0.06	0.05	0.06	0.04	0.06	0.04
	2	Anti-PRMV-IgG	0.05	0.05	0.05	0.04	0.04	0.04
	6	Anti-BBLMV-IgG	0.07	0.06	0.05	0.05	0.04	0.05
BBLMV-infected[e]	9	PBS	0.54	0.22	0.09	0.05	0.05	0.05
	6	Anti-PRMV-IgG	0.54	0.10	0.06	0.05	0.05	0.04
	12	Anti-BBLMV-IgG	0.46	0.19	0.10	0.04	0.04	0.04

[a] Pollen was collected from seven ELISA-tested BBLMV-infected and four healthy Jersey blueberry bushes, May 1983.
[b] Antigenic sites were blocked on the surface of BBLMV-infected pollen with anti-BBLMV-IgG or sham-blocked with phosphate-buffered saline (PBS) or anti-PRMV-IgG, as controls.
[c] Pollen grains washed three times (0.4 ml PBS) and centrifuged (6,000 rpm, 10 min, Beckman No. 40 rotor) to remove BBLMV from the pollen surface before antivirus IgG-blocking or sham-blocking.
[d] Pollen grains washed three times (0.4 ml PBS) and centrifuged (6,000 rpm, 10 min, No. 40 rotor) to remove excess antivirus-IgG.
[e] The ELISA values were considered positive if they were greater than the mean plus three standard deviaitons of healthy control values ($\bar{X}$+ 3 SD = 0.10). The range of healthy pollen absorbance values was 0.04–0.07.

TABLE 4. Assay of healthy and blueberry leaf mottle virus-infected blueberry pollen for virus localization using radioimmunoassay after pretreatment with anti-virus-specific and nonspecific IgG

Pollen source	Subsamples tested (no.)	Mean preblocking ELISA value[a]	Infectivity[b]	Blocking solution[c]	Postblocking treatment(cpm)	Mean RIA[f] (%)	Samples RIA-positive
Healthy	2	0.07	−	PBS	Washed, not ground[d]	1,470	0
	4	0.07	−	Anti-BBLMV	Washed, not ground	1,593	0
	8	0.07	−	PBS	Washed, ground[e]	1,209	0
	4	0.09	−	Anti-PRMV	Washed, ground	1,418	0
	8	0.07	−	Anti-BBLMV	Washed, ground	1,422	0
BBLMV-infected	2	1.60	+	PBS	Washed, not ground	1,556	0
	2	1.82	+	Anti-PRMV	Washed, not ground	1,759	0
	8	1.07	+	Anti-BBLMV	Washed, not ground	1,983	0
	16	1.26	+	PBS	Washed, ground[e]	6,487	100
	10	1.01	+	Anti-PRMV	Washed, ground	5,549	100
	16	1.25	+	Anti-BBLMV	Washed, ground	5,002	50

[a] Diseased sample values are greater than the mean plus three standard deviations of healthy samples ($A_{410nm} = 0.10$).
[b] *Chenopodium quinoa* was mechanically inoculated.
[c] Either anti-BBLMV-IgG was incubated with pollen to block surface antigens or anti-PRMV-IgG or phosphate-buffered saline (PBS) was added as a sham-block control.
[d] Pollen samples washed three times in 0.4 ml PBS but not ground with a mortar and pestle.
[e] Pollen samples washed three times in 0.4 ml PBS and ground with a mortar and pestle to disrupt cells.
[f] Samples tested by radioimmunoassay (RIA). Values shown are counts per minute (cpm). Samples with cpm greater than the healthy sample $\bar{X}$+ 3 SD (= 2,650 cpm) were considered BBLMV-positive.

(diseased A_{410nm} = 0.11–0.75; healthy A_{410nm} = 0.03–0.05). However, none of the seeds induced symptoms on inoculated *C. quinoa* plants.

DISCUSSION

ELISA of highbush blueberry pollen demonstrated that a high percentage of the samples collected in the field from BBLMV-infected bushes contained BBLMV antigen. BBLMV antigen was easily removed from the pollen surface by washing several times with PBS.

Cole et al (4) detected Prunus necrotic ringspot virus (PNRSV) on the surface of cherry pollen, which was also easily removed by serial washing with a saline solution. Hamilton et al (8) also detected PNRSV on or in the exine of sweet cherry pollen by washing and ELISA or by the use of PNRSV antiserum-coated latex spheres and scanning electron microscopy. Kelley and Cameron (9) reported that both PNRSV and prune dwarf virus (PDV) antigens were present on and in sweet cherry pollen, as demonstrated by washing and centrifugation followed by ELISA of supernatants. In addition, they observed virus particles 25 nm in diameter inside pollen grains from PDA-infected sweet cherry trees.

Our work reported herein has shown that BBLMV antigen located on the surface of blueberry pollen grains was easily removed by washing and that RIA of disrupted cells detected significant amounts of BBLMV antigen either within the pollen grains or possibly released from the lipid layer of the pollen exine.

Pollen grains of many entomophilous flowers have a sticky coating, enabling the pollen to either adhere to the insect for subsequent dissemination, adhere to the style during pollination, to serve as a protective layer against desiccation, or possibly be involved in pollen-style recognition. Dickinson (5) has discussed the role that tapetal plastids play in the composition of the exine layer of the pollen grain. Tapetal plastids disintegrate and the microspore may be covered with a mixture of lipid, proteins, and tapetal fragments, depending on the type of pollen development. In addition, the surface of the pollen exine contains a fibrous layer of protein that facilitates the compaction of this sticky coating. It is interesting that Dickinson (5) observed that the coating does not usually cover the orbicules or bacula portions of the pollen grain. Hamilton et al (7) used the electron microscope to show southern bean mosaic virus (SBMV) associated with the bacula of the exine and coating the surface of the exine in mature bean (*Phaseolus vulgaris* 'Bountiful') pollen. Virus was not located within all of the anthers observed, and 100% infection was not obtained when pollen from SBMV-infected bean was inoculated to healthy Pinto bean.

The results of our serological blocking experiment suggest that BBLMV may be located within the bacula or other parts of the pollen grain not covered by exine and can be easily removed during gentle agitation in PBS. Disruption of the exine, either mechanically or during the agatitation process, may be required to release unblocked virus particles from within the exine. Observations of pollen from BBLMV-infected bushes with the transmission electron microscope (TEM) would be helpful in determining whether virions are located within the cells of the tetrad. Yang and Hamilton (19) used the TEM to locate tobacco ringspot virus in the intine of the pollen wall as well as to show its association with parts of the generative and vegetative cells of soybean pollen. They also observed that infected soybeans produced less pollen and that fewer pollen cells germinated or the germ tube did not elongate as rapidly as in pollen from healthy plants.

The microspore of *Vaccinium* species is composed of four cells, usually a tetrahedron. Each cell is capable of germination, but in highbush blueberry, usually only one germ tube is produced. Pollen collected from BBLMV-infected bushes and germinated in a 12.5% sucrose solution produced considerably fewer germ tubes than pollen collected from healthy plants. These results suggest a decreased viability of virus-contaminated pollen caused by the presence of the virus directly or by the infected condition of the parent plant. Reduced pollen viability, abnormalities, or pollen abortion has been described for several virus-host combinations (6,12,18). Cole et al (4) postulated that morphological and physiological alterations of cherry and almond pollen by PNRSV may depend on the cultivar of the host and the virus isolate. They also concluded, however, that viable pollen may not be necessary for PNRSV infection and that mechanical transmission could take place via wounds of the flower parts created during honeybee foraging.

The delay in germ-tube formation of highbush blueberry pollen from BBLMV-infected bushes would indicate that contaminated pollen could not compete as well as pollen from healthy plants. This would be a disadvantage for transmission of virus through infection of the ovule during the fertilization process. Transmission through wounding of the undeveloped ovary or other flower parts directly during honeybee foraging would be a more efficient mode of transmission.

Several avenues of virus entry into blueberry plants via pollen may be proposed: 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, transmission through the cytoplasm of the vegetative cell, then to the embryo. Carroll et al (1) found that barley stripe mosaic virus is found more often in the cytoplasm than in the nucleus of the vegetative cell. The virus was also found in more than 41% of the sperm nuclei of barley pollen, indicating virus infection may occur in the seed by either maternal transmission through the vegetative cells or during pollination.

Only 1.5% of the blueberry seedlings arising from virus-contaminated seed assayed positive for BBLMV by RIA. It is possible that the seedlings became infected as a result of virus contamination of the germinating seedling via the seed coat during germination. Infected seedlings would only be of epidemiological significance if they were allowed to grow and to flower. This rarely happens in commercial blueberry fields.

Research dealing with pollen transmission of BBLMV from blueberry to blueberry by bees will be reported in a subsequent paper.

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