Detection of Blueberry Shoestring Virus in Xylem and Phloem Tissues of Highbush Blueberry

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This research was supported in part by USDA grant 86-CRSR-2-2758 and a grant-in-aid from the Michigan Blueberry Growers Association, Grand Junction 49056.

This journal article is in partial fulfillment of the requirements of a doctoral thesis by the first author. We thank Janet Besaw for manuscript preparation.

Michigan State University Agricultural Experiment Station Journal Series Article 12570.

Accepted for publication 6 December 1988.

ABSTRACT

Urban, L. A., Ramsdell, D. C., Klomparens, K. L., Lynch, T., and Hancock, J. F. 1989. Detection of blueberry shoestring virus in xylem and phloem tissues of highbush blueberry. Phytopathology 79:488-493.

To study the long-distance movement of blueberry shoestring virus (BBSSV) in highbush blueberry, 3-yr-old healthy bushes were inoculated with BBSSV-diseased buds. The stem was then either girdled below the bud, above the bud, or not girdled. After several months, leaves were tested from above and below the girdled area, using dot blot immunoassay, to determine virus movement. Also, cDNA dot-hybridization and fluorescent antibody labeling were used to determine whether BBSSV moves in the phloem, xylem, or both tissues. No virus was detected in leaves above or below the girdle, when they were tested 1 mo after budding. Two months after budding, one of 15 plants girdled above the diseased bud tested positive for BBSSV antigen. Three and four months after budding, eight and nine, respectively, of 15 plants girdled above the diseased bud tested positive for BBSSV antigen. Six months after budding, leaves above and below the girdled areas were positive for BBSSV antigen. Cross sections (6 μm thick) of young symptomatic stems, made with a cryostat and stained with fluorescent antibody-labeled anti-BBSSV IgG, revealed BBSSV antigen associated with xylem elements. Mechanically separated xylem and phloem tissue were taken from symptomatic stems, extracted, and spotted onto nitrocellulose membranes coated with anti-BBSSV IgG. When probed with ^{32}P-labeled cDNA to BBSSV RNA, BBSSV RNA was identified in the samples, indicating that whole virus was present as well as viral antigen in both phloem and xylem stem tissues.

Additional keywords: immunoassay, *Vaccinium corymbosum*.

Long-distance transport of virus was studied most intensively from the 1930s to the 1960s (1,3,7,13,14). It was thought that long-distance transport occurred mainly in the phloem. In 1934, Sammuels (13), working with tobacco mosaic virus (TMV) in tomato, found that the virus moved downward to the roots first, then upward to the shoot tip. This model of virus movement is still used today. Bennett's work with curly top virus in sugar beets and tobacco led to the suggestion that virus moved in the direction of food transport (3). Schneider summarized virus translocation and distribution in plants in 1965 (14), and more recently a virus-coded protein has been implicated in long-distance transport of TMV (1,7) in plants.

Blueberry shoestring virus (BBSSV) causes an economically important disease of highbush blueberry (*Vaccinium corymbosum* L). Michigan produces more than 40 million pounds of this fruit annually, and shoestring disease causes an annual loss of over $3 million in Michigan alone. Shoestring disease symptoms are typified by small, elongate, reddish streaks (1–2 × 10 mm) on current-year and 1- and 2-yr-old stems. Leaves may be crescent or strap-shaped. Also common are red vein banding and oak leaf patterns on leaves and a reddish purple coloration on immature berries (10,12,19).

BBSSV is found in very low titer and can be latent for up to 4 yr between infection and symptom expression. The virus lacks an herbaceous host (11). Because of our ongoing research interest in BBSSV and our interest in the distribution of the virus in the blueberry plant, we have undertaken the research work reported here, which investigates the long-distance movement in its woody host.

BBSSV is introduced into the phloem tissues of plants by the blueberry aphid, *Illinoia pepperi* MacG. (11), and there is evidence that the virus is limited to the vascular tissue (18). However, whether the virus moves in the xylem, the phloem, or both is not known. The main objective of this research was to determine which of these tissues are involved in virus movement. A second objective was to determine the characteristics of movement from the point of inoculation to other parts of the bush. Virus distribution in woody fruit crops may be irregular (6), and so we also wanted to determine the distribution of BBSSV in its host. Such information would be useful when designing sampling methods for virus detection in virus-tested clean stock programs.

MATERIALS AND METHODS

Grafting and girdling experiments. Stems from diseased blueberry bushes, cultivar Jersey, from a commercial field at Eastmanville, MI, were used as a source of BBSSV-infected budwood. Stems were cut during winter 1985, placed in plastic bags with moist paper towels, and stored in a cold room (4 C) until grafting time.

Fifty 3-yr-old healthy, potted Jersey blueberry plants were obtained from Tower View Nurseries, South Haven, MI. Plants chosen had at least two main stems. All plants tested negative for BBSSV using enzyme-linked immunosorbent assay and were assumed to be uninfected (5,18). In April 1985, three to four chip buds from BBSSV-infected stem material were placed into each of 30 healthy plants. The buds were spaced 1.5 cm apart on a single stem and were wrapped with Parafilm to allow air flow to the wood (18). Three days after inoculation, plants were girdled above the chip buds (designated GA), below the chip buds (designated GB), or were not girdled (Fig. 1). Girdles were approximately 1.5 cm wide. Plants were numbered 1–10 for each category. For controls, three to four chip buds from healthy plants were placed on each of

10 plants, and these buds were wrapped with Parafilm as above. Three days later, three plants were girdled above the chip buds, three plants were girdled below the chip buds, and four plants were not girdled. Plants were designated as previously described. Plants grafted with healthy or diseased buds were placed in similar environments in separate greenhouses.

Ten additional plants were chip-budded with diseased buds and girdled above or girdled below the buds on the same day as budding. Buds were again wrapped with Parafilm. Plants in these two groups were designated GA-1* through GA-5* and GB-1* through GB-5*, respectively. Leaf testing was begun approximately 1 mo after chip-budding, and plants were tested monthly (May–October 1984). Samples consisted of three leaves picked at random from the stem above the girdle in the group of plants that had been girdled above the inserted diseased bud, from above the inserted diseased bud in the group of nongirdled plants, and from below the inserted diseased bud in the group of plants girdled below the bud. In September 1985, leaf samples were taken from inoculated stems and from uninoculated stems. All tissue samples were diluted 1:10 (w/v) with TBS-T80 extraction buffer (2) and ground with a Tissuemizer (Tekmar Co., Inc., Cincinnati, OH).

Plants were placed in an outdoor cold frame in October for a vernalization period of at least 1,000 hr at less than 10 C. In December 1985, chip-budded plants were transferred to a greenhouse that was maintained at 10 C. Root samples from all plants were taken in December 1985 and in January and April 1986 by removal of test plants from the pots and clipping off of feeder roots. These were tested for the presence of BBSSV antigen using the dot-blot immunoassay procedure (2,18). An additional root sampling in May 1986 included only plants that were girdled 3 days after chip-budding. Root samples (1 g) were diluted 1:10 (w/v) in extraction buffer, except in May, when 10 g of roots obtained from the bottom 2 in. of the root mass was tested, diluted 1:10 (w/v), and extracted as described previously.

Plants were moved into a greenhouse maintained at 20–25 C in April 1986. Leaf sampling resumed in May and continued on a monthly basis until September. In 1986, plant material above the girdle was dead; therefore samples were taken from below the girdle and from stems with no chip buds. Roots sampled (1-g samples) in November 1986 from plants girdled on the day of inoculation were tested for the presence of BBSSV antigen using a dot-blot immunoassay procedure.

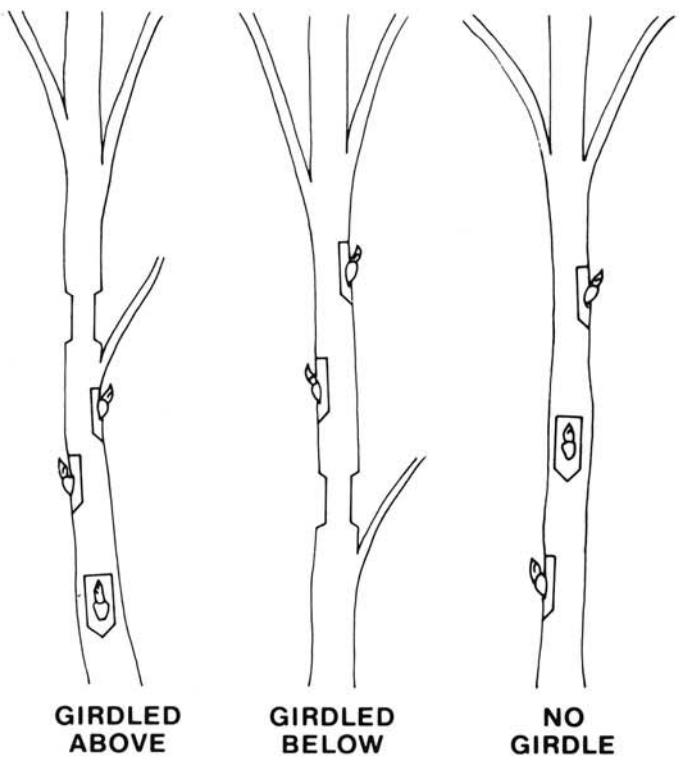

Fig. 1. Location of grafted chip buds and girdling variations used to determine movement of blueberry shoestring virus in the vascular system of the blueberry plant.

Dot-blot immunoassay. Testing leaves by the dot-blot immunoassay was according to the procedure of Bantarri and Goodwin (2). The procedure was modified by the addition of polyvinylpyrrolidone (1%, w/v) to the extraction buffer (18). A nitrocellulose membrane 0.45 μm thick (Bio-Rad Laboratories, Richmond, CA) was placed in distilled water for 15 sec. The membrane was then transferred to a glass dish containing 60 ml of coating buffer (0.05 M sodium carbonate-bicarbonate, pH 9.6) containing a 1:1,000 (v/v) dilution of anti-BBSSV IgG. The membrane was left covered at room temperature for 4 hr; then the IgG coating solution was replaced with Tris-buffered saline containing 0.05% Tween 80 (TBS-T80), and the dish was placed on a gyratory shaker. This wash solution was changed six times in 30 min. After the last wash, 60 ml of TBS-T80 plus 3% bovine serum albumin (BSA) (w/v) was added to block the remaining available binding sites. The dish was placed in a plastic bag, sealed, and incubated at 37 C for 1.5–2 hr.

The membrane was removed from the BSA solution and placed on a Bio-Rad Bio-dot apparatus (Bio-Rad Laboratories). A vacuum was applied to eliminate leaking of samples from well to well. Extracted tissue samples were applied to the wells of the dot-blot apparatus at a rate of 400 μl/well. The apparatus was placed in a plastic bag, sealed, and refrigerated at 4 C overnight.

The next day the apparatus was disassembled. The membrane was washed with distilled water to remove sample debris, placed in TBS-T80, and shaken for another 30-min wash series. A conjugate of anti-BBSSV IgG and alkaline phosphatase was added to 60 ml of TBS-T80 at a final concentration of 1.25 μg/ml; this replaced the wash solution. The dish was covered and left at room temperature for 3 hr. After 3 hr, the membrane was washed again for 30 min on a shaker with TBS-T80. Enzyme substrate was prepared during the last wash. It consisted of 15 ml of 0.2 M Tris-HCl buffer, pH 8.2, mixed with 6 mg/ml fast red TR salt (Sigma Co., St. Louis, MO). The mixture was filtered, and 9 ml of the same buffer was added to 6 ml of naphthol AS-MX phosphate solution (Sigma Co.) to make a final concentration of 0.1% (v/v). The two solutions were mixed in a glass dish and the membrane added. Color development was stopped after 45–60 min by rinsing with distilled water. The membrane was air dried on filter paper. Samples were judged to be positive if a pink to red dot appeared, or negative if no dot appeared.

A calibration test was run to determine the relative sensitivity of the dot-immunoassay vs. a dot-cDNA probe assay (detailed later). Quantities of purified BBSSV ranging from 300 ng to 0.03 pg were added to either extraction buffer or healthy blueberry leaf sap and spotted onto the membrane. All spotting of samples was done on duplicate halves of the antibody-coated membrane. One half of the membrane was developed in the previously described manner for dot-immunoassay. The other half was probed with alpha ^{32}P-labeled cDNA to BBSSV, exposed to X-ray film, and developed.

Cryostat sectioning of diseased stems and fluorescent antibody staining of vascular tissue. Actively growing succulent, symptomatic stems were frozen and sectioned with a cryostat as follows. An IEC CTF microtome-cryostat (IEC Co., Needham Heights, MA) was cooled to −20 C before sectioning. Tissue-tek cryoprotectant (Miles Scientific, Naperville, IL) was layered onto the specimen stub, to which a water layer had been previously applied, and a small piece of stem was positioned on the cryoprotectant. More Tissue-tek was layered onto the specimen until it was covered; sections were cut with a single-edged, Teflon-coated, injector-type razor blade and placed on a glass slide or into phosphate-buffered saline (PBS, pH 7.0). Sections were refrigerated until they were stained with fluorescent antibody. For staining, a 1:256 dilution of anti-BBSSV IgG in 0.85% NaCl was determined to be appropriate. Preimmune serum was used as a control. Diseased and healthy cryostat sections were placed into spot plate wells using an artists brush. The wells contained anti-BBSSV IgG or preimmune sera. The spot plates were placed in a covered tray and incubated overnight at 4 C. Sections were washed

three to four times with PBS at 10-min intervals.

Sections were stained with sheep anti-rabbit fluorescein isothiocyanate (FITC) at a dilution of 1:32 in PBS, pH 7.0, and incubated overnight at 4 C. Sections were again washed three to four times and mounted in PBS on glass slides. Coverslips were sealed with fingernail polish and held in a moist chamber at 4 C until viewed.

Sections were observed with a Zeiss Universal Research Photomicroscope equipped for epifluorescence (450–490-nm excitation filter, 520-nm barrier filter). Kodachrome 64 film was used at a 4-min exposure at ×100 magnification.

Complimentary DNA synthesis to BBSSV RNA, molecular cloning, and cDNA probe. BBSSV was purified, and RNA was extracted as previously described (10,11). Approximately 85% of the 4-Kb BBSSV genome was cloned using pBR 322 plasmid and the DH5- strain of *Escherichia coli*. Calf thymus DNA hexamers (Pharmacia Fine Chemicals, Piscataway, NJ) were used as a random primer, and avian myeloblastic reverse transcriptase was used to make a cDNA according to the protocol supplied by the manufacturer of the kit (Amersham Biochemicals Co., Inc., Arlington Heights, IL) and per Maniatis et al (9). RNAase H and the Klenow fragment of *E. coli* DNA polymerase I were used, respectively, to degrade the RNA strand and replace it with a second DNA strand. The plasmid was cut with *Bam*HI and phosphorylated; *Bam*HI linkers were added, after which the DNA was ligated to the plasmid. Competent DH5- *E. coli* cells were used as transformation recipients. Clones were selected for ampicillin resistance and then screened for tetracycline sensitivity. Colonies were screened by the Grunstein-Hogness hybridization method (9) and then probed with ^{32}P-end-labeled-BBSSV RNA, made using ^{32}P-adenosine triphosphate and T_4 kinase (9). Those colonies that hybridized with the probe were considered to contain inserts of viral origin and were grown in LB broth. Plasmid preparations made from these individual cultures were cut with *Bam*HI and analyzed by electrophoresis in 1% agarose gels. Lambda phage markers were used to size the *Bam*HI pieces. Southern blotting (9) and hybridization were used to determine three independent classes of virus-specific inserts. Each class was used as a probe against a Northern blot (9) of BBSSV RNA as a final proof that each clone corresponded to a separate part of the viral RNA sequence. A total of three clones were found that did not hybridize to each other. Addition of the sizes of these inserts resulted in about 85% of the viral genome being cloned. A 1-kb virus-specific segment was nick-translated per the protocol of the kit manufacturer (Bethesda Research Laboratories, Gaithersburg, MD) and used as a probe in work reported hereafter.

Comparison of dot-blot immunoassay and cDNA hybridization for detection of BBSSV in separated BBSSV-infected stem phloem and xylem tissues. To demonstrate that BBSSV RNA and antigen move together through the vascular system, both dot-blot immunoassay and cDNA hybridization of immuno-trapped antigen on nitrocellulose membrane were employed.

Stems were cut from BBSSV-diseased bushes in the field in August 1986, placed in plastic bags, and transported on ice. Five diseased and three healthy stems were placed in water and stripped of the outer bark, including the phloem, parenchyma, cortex, phelloderm, phellem, phellogen (these latter three tissues comprise the bark tissues). The inner layers, including the cambium, xylem (xylem vessels and trachieds), parenchyma, and pith, were cut into small pieces (a few millimeters in size). The portions including phloem tissue (0.2 g) and xylem tissue (0.5 g) were ground with a Tissuemizer homogenizer in 5 ml of TBS-T80 buffer as previously described, using 1.25 and 1:10 v/w ratios of buffer to tissue, respectively. Extracts were strained through two layers of cheesecloth and spotted onto a nitrocellulose membrane coated (1:1,000 dilution v/v) with anti-BBSSV IgG in a dot-blot apparatus in 200-μl quantities per well. The left half of the nitrocellulose membrane was spotted with samples for dot-blot immunoassay, and the right half was spotted with duplicate samples to be probed with ^{32}P-cDNA to BBSSV.

The membrane was cut in half, and the left half was developed for the dot-blot immunoassay as previously described. The right half was washed as described for the dot-blot immunoassay. The membrane was then air-dried, baked under vacuum at 80 C for 2 hr, transferred to a Seal-a-Meal bag (Dazey Products, Industrial Airport, KS) containing 1 ml of 3× SET buffer (20 × SET buffer consists of 3 M NaCl, 0.4 M Tris-HCl, and 20 mM EDTA, pH 7.8) to wet the filter. Ten milliliters of prehybridization solution (9)—5 ml of 50% formamide (v/v), 1 ml of 50 × Denhardt's solution (9), 2.5 ml of 5× SET buffer, 1 ml of distilled H_2O, 50 mM Na_2HPO_4 (pH 6.5), 1% glycine (w/v), and 10 mg of calf thymus DNA—plus 10^6 dpm/ml ^{32}P-labeled cDNA was added to the bag containing the filter. The solutions were incubated with the filter at 42 C overnight. The membrane was washed three times, 5 min for each wash, at room temperature with 2× SET buffer containing 0.1% sodium dodecyl sulfate (SDS). The membrane was then washed twice, 20 min for each wash, at 42 C in 0.12× SET buffer containing 0.1% SDS (9). The membrane was air-dried and exposed to X-ray film (Kodak X-Omat AR) with intensifying screens for 2 days at −70 C.

RESULTS

Comparison of sensitivity of dot-blot immunoassay and cDNA hybridization assay. Dot-blot immunoassay and the cDNA hybridization were of equal sensitivity in detecting purified BBSSV in buffer and healthy leaf sap (Fig. 2). Both assays detected as little as 0.3 pg of BBSSV.

Grafting and girdling experiments. The success of chip-budding, as indicated by healed, green buds, ranged from 47 to 100% 1 wk after the buds were unwrapped. Later in the season some buds dried out and/or fell off. The first plant (girdled above the diseased bud) to test positive for BBSSV antigen was sampled in June 1985, 2 mo after chip-budding. By July and September 1985, 9 and 13, respectively, of 15 plants were positive above the girdle on plants that were girdled above the inserted diseased plants (Table 1). By September 1985, only 1 of 10 plants girdled below the inserted diseased buds was positive for BBSSV antigens. Plants not girdled were positive first in July 1985, and by September, 9 of 10 plants were positive for BBSSV antigen. By August, 2 of 10, and by September, 9 of 10 plants not girdled above or below the diseased band were positive for BBSSV antigen.

Root samples tested negative for BBSSV antigen in December 1985, and in January and April of 1986 when plants were dormant.

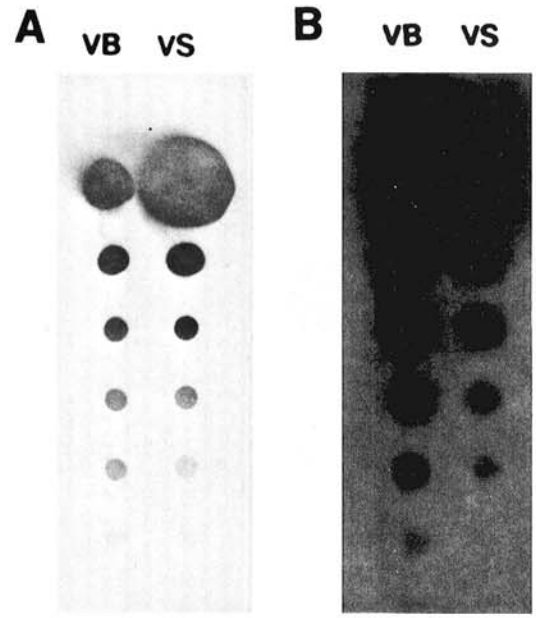

Fig. 2. A, Dot-blot immunoassay and, **B,** autoradiograph of cDNA hybridization assay of purified blueberry shoestring virus. Quantities of purified virus in buffer (VB) and in healthy leaf sap (VS) were (from top to bottom) 300 ng, 30 ng, 300 pg, 30 pg, 0.3 pg, and 0.03 pg, respectively.

In May of 1986, after the breaking of dormancy, the roots of two plants (GB-2 and NG-6) tested positive for BBSSV antigen. Root samples taken in November of 1986 from plants from the group girdled the same day as budded, revealed three of five plants with positive root samples (GA-1*, GA-4*, and GA-5*). Root samples were not taken from the other plants because they were recovering from the severe root pruning they had received in May.

In 1986, 10 plants developed typical symptoms of blueberry shoestring disease, e.g., red-streaked stems, and two plants had strap-shaped leaves. During the vernalization period, all stem material above the girdles died on plants girdled above the grafts. Therefore, in 1986, leaves were sampled from below the girdle, or above the chip buds on stems with no girdles, and from stems other than those bud-inoculated. As the season progressed, stems other than those inoculated with diseased buds began to show positive dot-blot immunoassay results for BBSSV antigen (Table 2). These plants were mainly in the group of plants girdled the same day as chip-budded. The failure of other groups to exhibit this phenomenon may be a consequence of the debilitating effect of root sampling in May of 1986. Groups GB and GB* contained no virus-positive samples in 1986. Plants not girdled above or below the inserted diseased bud were positive for BBSSV antigen at levels similar to those that were girdled above the inserted diseased bud.

Presence of BBSSV in xylem tissue of frozen sections of BBSSV-diseased blueberry stems. Fluorescence was detected in the xylem elements of cross sections of infected stems (Fig. 3A). Sections from healthy plants did not exhibit specific fluorescence (Fig. 3B). The color micrograph from which the black and white figure was made better distinguishes between the virus-specific yellow-green color of FITC and the green autofluorescence of lignin-containing cells of blueberry.

Dot-blot immunoassay and cDNA hybridization of stem tissue. Tissues separated at the vascular cambium from diseased stems were positive by the dot-blot immunoassay and the cDNA hybridization assay (Fig. 4). Both assays were equal in their ability to detect BBSSV in all samples tested. These results show that whole virus or a complex of viral protein and RNA moves through both the xylem and the phloem systemically, instead of viral antigen moving alone.

DISCUSSION

Since BBSSV is aphid-vectored, it was presumed that the virus moved mainly in the phloem. This study has shown that BBSSV moves in both the phloem and the xylem as well as in parenchymatous tissue.

Viruses, in general, are thought to move with normal cell constituents (14). Phloem movement of photosynthate and associated substances is often by a source-to-sink route. Sources in blueberry would be roots in the spring, fruit buds, and expanding leaves. Sinks would include roots in the autumn and fruits, flowers, and meristems in the spring and early summer.

TABLE 1. Dot-blot immunoassay in the year (1985) of inoculation of leaves from blueberry plants budded with blueberry shoestring virus (BBSSV)-infected buds and with girdle variations

	Samples from						
					September[c]		
Group[a]	May[b]	June[b]	July[b]	August[b]	B	A	Plants not girdled[b]
GA-1	–[d]	–	–	–	+	–	
GA-2	–	–	–	–	+	–	
GA-3	–	–	–	–	–	–	
GA-4	–	–	–	–	–	–	
GA-5	–	–	+	+	+	+	
GA-6	–	–	+	–	+	+	
GA-7	–	–	–	+	+	–	
GA-8	–	–	–	+	+	+	
GA-9	–	–	+	+	+	+	
GA-10	–	–	+	+	+	–	
GB-2[e]	–	–	–	–	+	+	
NG-1	–	–	–	–			+
NG-2	–	–	–	–			+
NG-3	–	–	+	+			+
NG-4	–	–	–	–			+
NG-5	–	–	–	–			–
NG-6	–	–	–	–			+
NG-7	–	–	–	–			+
NG-8	–	–	–	+			+
NG-9	–	–	–	–			+
NG-10	–	–	–	–			+
GA-1*	–	–	+	+	+	–	
GA-2*	–	–	+	+	+	–	
GA-3*	–	–	+	+	+	–	
GA-4*	–	+	+	+	+	–	
GA-5*	–	–	–	–	+	–	

[a] Groups included plants girdled above the inserted diseased bud (GA-1–10) or girdled below the inserted diseased bud (BG-1–10) three days after budding, no girdling (NG-1–10), and girdled above (GA-1*–5*) the inserted diseased bud on the same day as budded. Grafting and girdling was done in April 1985.

[b] Samples consisted of three leaves taken at random from above the girdle on each plant except for the plant that was girdled below the diseased bud. In that case, the sample was taken below the girdle.

[c] Samples were taken below the girdle (B) and above the girdle (A) on each girdled plant.

[d] Samples were dot-blot negative (–) or positive (+).

[e] All samples from plants girdled below the chip bud were dot-immunoassay negative for BBSSV, except for plant GB-2.

TABLE 2. Dot-blot immunoassay in the year (1986) after inoculation of leaves from blueberry plants budded with (BBSSV)-infected buds and girdled in 1985

	Samples[b] from									
	April		May		June		July		August	
Group[a]	S	O	S	O	S	O	S	O	S	O
GA-1	–[c]	–	–	–	+	–	+	+	+	–
GA-2	+	–	+	–	+	–	–	–	+	–
GA-3	–	–	–	–	–	–	–	–	–	–
GA-4	–	–	–	–	–	–	–	–	–	–
GA-5	+	–	+	–	+	–		–	+	+
GA-6	+	–	+	–	+	–	+	–	–	–
GA-7	+	–	–	–	+	–	+	–	+	+
GA-8	+	–	+	–	–	–	N	N	–	–
GA-9	+	–	+	–	+	+	+	–	+	–
GA-10	+	–	?	–	–	–	N	N	N	N
GB-1–10	–	–	–	–	–	–	–	–	–	–
NG-1	–	–	+	–	+	–	+	–	+	+
NG-2	–	–	–	–	+	+	+	?	+	+
NG-3	+	–	+	–	–	–	–	–	+	–
NG-4	–	–	+	–	–	–	?	–	–	–
NG-5	–	–	–	–	–	–	+	–	–	–
NG-6	+	–	+	–	–	–	+	+	+	+
NG-7	+	–	–	–	–	–	+	–	+	–
NG-8	+	–	+	–	+	–	+	–	–	–
NG-9	–	–	–	–	–	–	N	N	N	N
NG-10	–	–	–	–	–	–	+	–	–	–
GA-1*	+	–	+	–	+	–	+	+	+	+
GA-2*	+	–	+	–	+	+	+	+	+	+
GA-3*	+	–	+	–	+	+	+	+	+	+
GA-4*	+	?	+	+	+	+	+	+	+	+
GA-5*	+	–	+	–	+	–	+	–	+	–
GB-1–5*	–	–	–	–	–	–	–	–	–	–

[a] Groups included plants girdled above (GA-1–10) or girdled below (GB-1–10) the disease bud three days after budding, no girdling (NG-1–10), girdled above (GA-1*–5*), or girdled below (GB-1*–5*) the disease bud the same day as budded.

[b] Samples consisted of three leaves taken at random from below the girdle on each plant. Leaves were taken from the same stem as budded (S) and from a stem other than the one budded (O).

[c] Samples were as indicated: dot-blot negative (–) or positive (+), questionable dot immunoassay results (?), or no sample taken (N). Missing samples were due to dead plants or brown leaves.

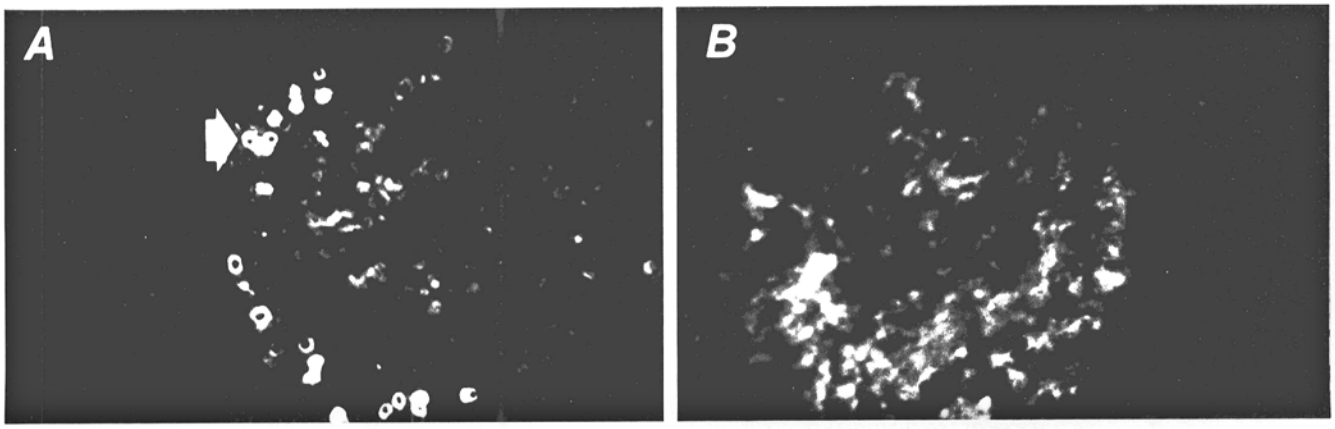

Fig. 3. Cross sections of blueberry stems stained with rabbit anti-BBSSV IgG and sheep anti-rabbit fluorescein isothiocyanate and observed with a UV light microscope (×100). **A,** BBSSV-infected stem, showing BBSSV associated with xylem tissue (arrows). **B,** Healthy stem.

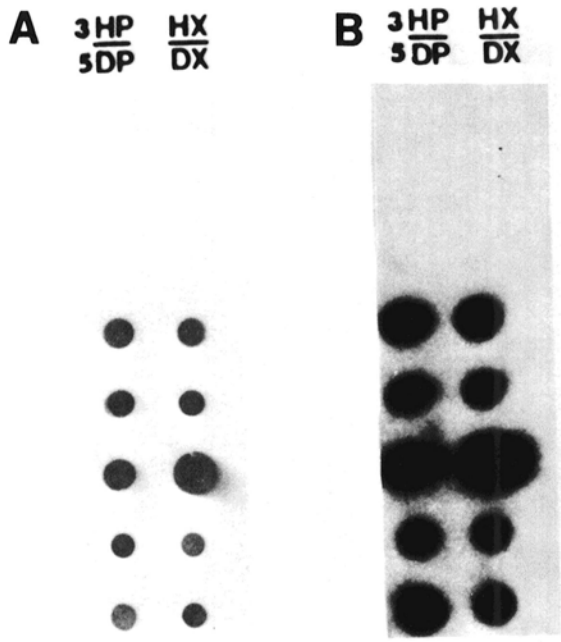

Fig. 4. A, Dot-blot immunoassay and, **B,** autoradiograph of cDNA hybridization assay of blueberry stem xylem and phloem tissue. Extracts of healthy phloem (HP) and healthy xylem (HX) were loaded into the top three wells of the dot-blot apparatus. Extracts of BBSSV-diseased phloem (DP) and diseased xylem (DX) were loaded into the five wells below the healthy samples. Each spot represents a sample from a different plant.

From the data presented in Tables 1 and 2, BBSSV apparently moves down to the root or crown area and subsequently up into uninoculated stems. This statement is supported by the results from the plants girdled below the inserted diseased bud. BBSSV was unable to pass the girdled area, suggesting downward transport in the phloem, as found in the other two plant groups (i.e., those girdled above the inserted diseased bud and those not girdled above or below the inserted diseased bud). Also, movement past the girdle in plants girdled above the grafts is evidence for movement of virus in xylem or pith tissues.

The reported similar levels of virus detection in girdled and nongirdled plants could be due to virus moving in a cell-to-cell manner through ray parenchyma tissue of the pith and xylem. Lateral spread to this area of tissue would take longer than introduction into the phloem and subsequent movement. In the second year of the experiment, the early detection of viral antigen in leaves was possibly due to 1) the virus moving from the roots or crown area via the phloem or 2) the virus having accumulated in the bud tissues, which acted as a sink. Variability of detection from month to month probably indicates that the virus is unequally distributed (17) and that a larger sample is needed for detection of BBSSV in symptomless plants.

Dot-blot immunoassay of known diseased stems confirmed the presence of viral antigen in tissues both inside and outside of the cambial layer. The cDNA hybridization assay, because of the antibody trapping method used, confirmed that viral RNA (possibly less than full length) was associated with viral antigen. Because BBSSV has no known herbaceous hosts (10) we were unable to determine whether this complex was infectious virus. Viral antigen, detected by indirect fluorescent antibody staining in the xylem elements of known diseased stems and leaves (18), provided further evidence for xylem translocation.

Many viruses have been reported to move in the phloem. However, few viruses have been documented as moving in the xylem. Chambers and Francki (4) observed lettuce necrotic yellows virus in young xylem cells of leaf veins. Schneider and Worley (15,16) found that southern bean mosaic virus (SBMV) could travel in the xylem when a local-lesion host was approach-grafted to a systemic host and the stem subsequently steamed to kill phloem cells. However, this local-lesion host would not be a natural host for xylem movement. It is interesting that BBSSV has most of its physical and chemical properties in common with SBMV. Since BBSSV has an aphid vector and SBMV has a beetle vector, BBSSV was not included in the sobemovirus group (11). Fribourg et al (8) have located a tobamovirus, maracuja mosaic virus, in xylem vessels of leaves in an ultrastructural study.

The lack of evidence for xylem translocation of most viruses may have been due to a number of factors, one of which was the few techniques available to facilitate such a study. With the use of cDNA hybridization assays, sensitive immunoassays, fluorescent antibody techniques, and the transmission electron microscope, this type of study has become more feasible.

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