Mechanical Transmission, Host Range, and Physical Properties of the Beet Yellow Vein Virus

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ABSTRACT

Mechanical transmission of beet yellow vein virus (BYVV), collected from several locations in California and the Great Plains, was facilitated by addition of 0.02 *M* sodium sulfite to the inoculum. *Spinacea oleracea* served as the virus source in all studies. Of 61 species of plants, only *Beta macrocarpa, B. maritima, B. vulgaris* (sugar beet), *B. vulgaris* var. *cicla, Chenopodium capitatum, Senecio vulgaris* (symptomless), and *Spinacea oleracea* became systemically infected with BYVV. Local lesions were formed in all *Beta* spp. except *B. maritima*, and in *C. album, C. amaranticolor, C. capitatum, C. murale, C. quinoa,* and *C. urbicum* in 6-10 days. *Chenopodium quinoa* was used for local lesion assays. Systemic symptoms took 12-14 days to appear in *C. capitatum* and spinach, whereas 25-30 days were necessary in *Beta* hosts.

The dilution end point of BYVV was between 10^{-4} and 10^{-5}, but most infectivity was lost when dilution exceeded 10^{-2}. Thermal inactivation occurred between 65 and 70 C, with most infectivity lost in extracts heated over 50 C. Extracts remained infectious after being stored 48 hr at 25 C or at 3-4C; greater infectivity occurred with extracts stored at the lower temperature. Frozen sap extracts lost infectivity within 24 hr; however, the virus remained viable in frozen tissue for 14 days. Phytopathology 61: 1418-1422.

Additional key words: reducing agent, local lesion assay.

Beet yellow vein was observed as early as 1913 (8), but apparently the disease was first described by Robbins in 1921 (9). It was more completely described by Bennett in 1956 (1). The disease has been difficult to characterize because, until recently (10), grafting was the only known means of transmitting the causal agent. In 1970, Staples et al. (10) reported transmission of the beet yellow vein causal agent by a leafhopper, *Aceratagallia calcaris* Oman, in an apparent circulative manner. However, vector efficiency was low, and transmission was erratic and unpredictable. Thus, characterization of the causal entity was not greatly aided by the leafhopper transmission.

Although yellow vein has not definitely been shown to be caused by a virus, unsuccessful preliminary attempts to control or arrest the disease with tetracycline antibiotics, and failure to isolate a mycoplasma using methods described by Lin et al. (7), indicated that the causal entity is viral in nature (Ruppel, *unpublished data*).

Our paper reports successful mechanical transmission, local lesion and systemic hosts, and some physical properties of beet yellow vein virus (BYVV).

MATERIALS AND METHODS.–BYVV collected from sugar beet in California, Colorado, and Nebraska, and from spinach (*Spinacea oleracea* L.) in California, was used in preliminary mechanical inoculation and host range studies. No differences in symptomatology or transmission between these isolates were apparent. A sugar beet showing typical symptoms of BYVV (1) was transplanted from a field near Fort Collins, Colo., to the greenhouse. Transmission tests with green peach aphids (*Myzus persicae* Sulz.) indicated that the plant was not infected with beet mosaic, beet yellows, beet western yellows, or beet yellow net viruses. This plant served as the source of virus for preliminary transmissions; however, later tests indicated that the virus was more easily transmitted to and from spinach. Thereafter, infected spinach plants were used as the virus source. All extractions were made 21-28 days after inoculation of the source plants.

Mechanical transmission.–Inoculum for host range studies was prepared by triturating infected spinach tissue in 0.01 *M* phosphate buffer (pH 7.2) containing 0.02 *M* sodium sulfite (10 ml buffer/g tissue) with a mortar and pestle. For other experiments, the tissue was ground with a mortar and pestle, and the juice squeezed from the pulp through two layers of cheesecloth. Dilutions, when specified, were made with phosphate buffer containing sodium sulfite, as described above. Inoculations were made by rubbing silicon carbide-dusted (600-mesh) leaves of test plants with inoculum-saturated cheesecloth pads.

Local lesion assays.–In preliminary tests, local lesions developed in inoculated leaves of *Chenopodium amaranticolor* Coste & Reyn., *C. capitatum* (L.) Asch., and *C. quinoa* Willd. *Chenopodium quinoa* was the preferred assay host, because: (i) Lesions formed sooner than in other species; (ii) lesions were more distinct and easier to count than in *C. amaranticolor*; and (iii) the virus did not become systemic in this species, but did so in *C. capitatum*. The rosette habit of growth of *C. capitatum*, and the relatively slow growth of *C. amaranticolor*, also made these species less favorable than *C. quinoa*.

Eight- to 10-week-old *C. quinoa* plants were used in all assays. Plants of this age had about 12-14

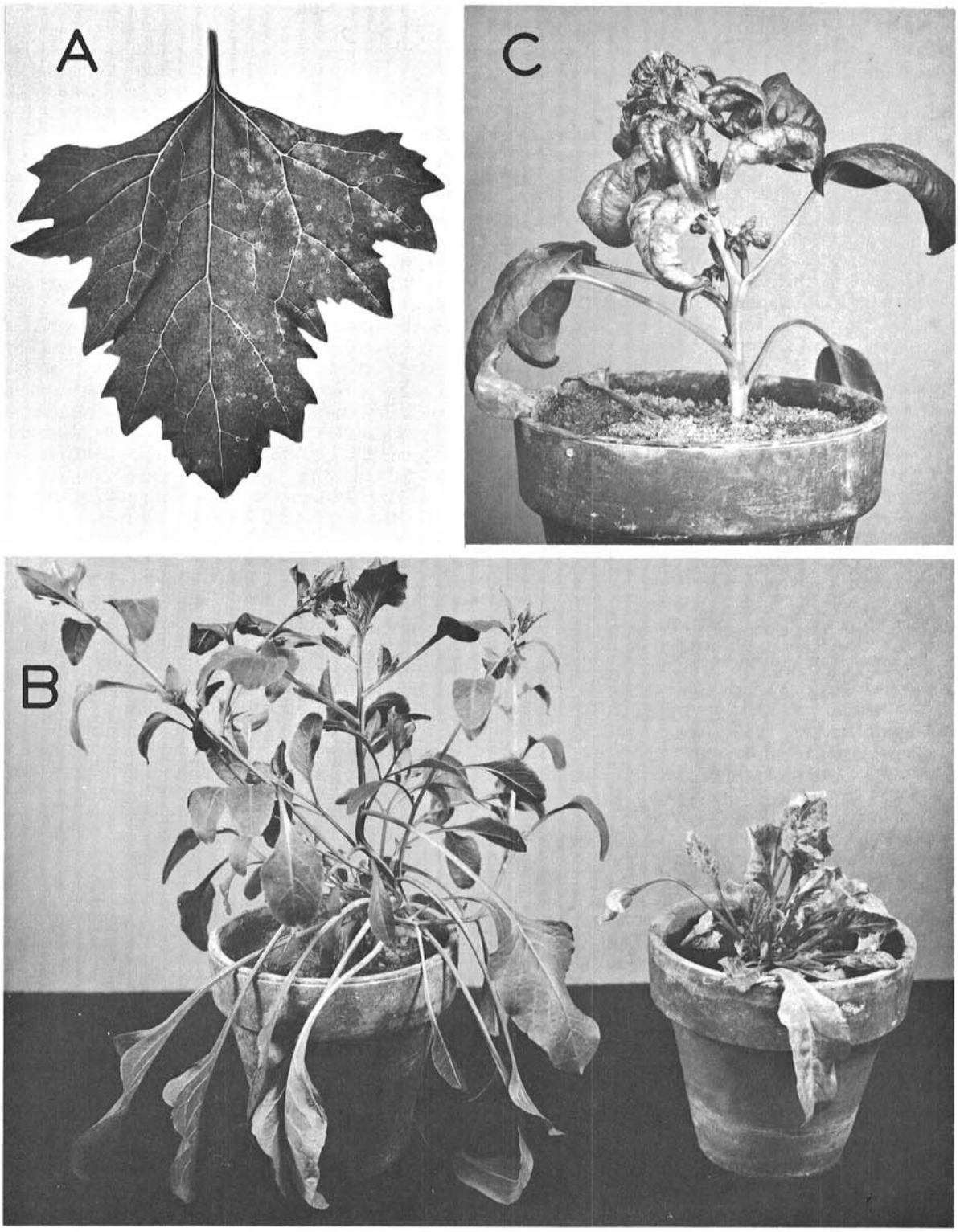

Fig. 1. **A)** Leaf of *Chenopodium quinoa* whose right half was inoculated with a 10^{-1} dilution, whereas the left half-leaf was inoculated with a 10^{-3} dilution of an extract from spinach infected with beet yellow vein virus (BYVV). **B)** Symptoms in *Beta macrocarpa* (right) 30 days after inoculation with BYVV, and noninoculated control (left). **C)** Symptoms in *Spinacea oleracea* (spinach) 25 days after inoculation with BYVV.

expanded leaves, but only the center leaves (i.e., third to ninth) were inoculated. A preinoculation dark period of 24 hr for assay plants increased their sensitivity to BYVV. Lesions appeared in 6-10 days after inoculation (Fig. 1-A). A 6 x 6 Latin square design was used in each of three trials, for the determination of dilution tolerance, thermal inactivation, and longevity in vitro.

Host range.—Sixty-one species of test plants were inoculated with BYVV. At least five plants of each species were inoculated; additional tests were conducted with certain species when results were inconclusive. The species tested included: *Atriplex semibaccata* R. Br.; *Beta macrocarpa* Guss.; *B. maritima* L.; *B. patula* Ait.; *B. trigyna* Waldst. & Kit.; *B. vulgaris* L. (sugar beet and red table beet); *B. vulgaris* var. *cicla* L.; *Brassica nigra* Koch; *B. oleracea* var. *capitata* L.; *B. pekinensis* Rupr.; *B. rapa* L.; *Capsella bursa-pastoris* (L.) Medik.; *Capsicum frutescens* L.; *Celosia cristata* L.; *Chenopodium album* L.; *C. amaranticolor; C. capitatum; C. murale* L.; *C. quinoa; C. urbicum* L.; *Cichorium endiva* L.; *Claytonia perfoliata* Donn.; *Cucumis sativus* L.; *Datura stramonium* L.; *Gomphrena globosa* L.; *Gossypium hirsutum* L.; *Helianthus annuus* L.; *Lactuca serriola* L.; *Lycopersicon esculentum* Mill.; *Malva parviflora* L.; *Medicago sativa* L.; *Melilotus indica* (L.) All.; *Nicandra physalodes* (L.) Gaertn.; *Nicotiana attenuata* (Torr.) S. Wats.; *N. clevelandii* Gray; *N. glutinosa* L.; *N. paniculata* L.; *N. quadrivalis* Pursh; *N. rustica* L.; *N. stocktonii* Brandeg.; *N. sylvestris* Speg. & Comes; *N. tabacum* L. 'Samsun'; *Petunia hybrida* Vilm.; *Phaseolus vulgaris* L.; *Physallis floridana* Rybd.; *Phytolacca americana* L.; *Pisum sativum* L.; *Plantago lanceolata* L.; *Raphanus sativus* L.; *Rumex crispus* L.; *Senecio vulgaris* L.; *Sonchus oleraceus* L.; *Spergula arvensis* L.; *Spergularia rubra* (L.) J. & C. Presl.; *Spinacea oleracea; Trifolium pratense* L.; *T. repens* L.; *Urtica californica* Greene; *Vicia faba* L.; *Vigna sinensis* (Torner) Savi; and *Zinnia elegans* Jacq. An equal number of test plants rubbed with buffer served as controls. Reproduction of BYVV symptoms after back-inoculations to spinach, and local lesions in *C. quinoa*, were the criteria used to determine if a plant was infected. Inoculum for back-inoculations was obtained from individual plants showing definite symptoms. When symptoms were absent or questionable, composite samples of tissue from several plants of the same species were used as inoculum. Back-inoculations were made 30-40 days after the test plants had been inoculated.

Physical properties.—Dilution tolerance.—Sap extracts from infected spinach were diluted 0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} with buffer, and rubbed on the leaves of *C. quinoa* plants.

Thermal inactivation.—To counter the effect of inhibitors in spinach sap (6), and to minimize phytotoxicity in *C. quinoa* leaves when concentrated spinach juice was used, sap extracts were diluted 10^{-1} with buffer for thermal inactivation experiments. The diluted extracts were drawn into thin-walled, glass micropipettes (inside diameter = 1.5 mm), and the tapered end of each tube was hermetically sealed. Three tubes/thermal treatment were prepared, each containing about 0.2 ml of extract. Tubes of extract were heated in a constant-temperature water-bath for 10 min at the indicated temperature, then quickly immersed in ice water. Half-leaves were used, with thermally treated sap inoculated to one half-leaf, and unheated, cooled inoculum applied to the opposite half-leaf.

Longevity in vitro.—A sap extract was diluted 10^{-1} with buffer and immediately inoculated on leaves of *C. quinoa*. The extract was divided equally, then stored in stoppered test tubes at room temperature (ca. 25 C) and in a refrigerator at 3-4 C. Aliquots of each were used to inoculate leaves of *C. quinoa* at 3, 6, 12, 24, and 48 hr after extraction.

Freezing tolerance.—A concentrated sap extract, a sap extract diluted 1:2 with buffer, and BYVV-infected tissue were frozen at −15 C. After 24 hr, the sap extracts were quickly thawed by immersion in warm water, and the concentrated extract was diluted 10^{-1} with buffer. Both extracts were inoculated on half-leaves of *C. quinoa*, whereas opposite half-leaves were rubbed with inoculum prepared from fresh tissue. Ten half-leaves were used for each inoculum. Frozen tissue was triturated in buffer with a mortar and pestle 14 days after freezing. The extract was inoculated to 10 half-leaves of *C. quinoa*; opposite half-leaves were rubbed with inoculum prepared from fresh tissue.

RESULTS.—*Host range.*—Only *Beta macrocarpa, B. maritima, B. vulgaris* (sugar beet), *B. vulgaris* var. *cicla, C. capitatum, Senecio vulgaris*, and *Spinacea oleracea* became systemically infected with BYVV. *Senecio vulgaris* was a symptomless host.

Symptoms in the *Beta* spp. were similar to those described by Bennett (1) for sugar beet; however, systemic symptoms of the disease were preceded by local chlorotic lesions in all but *B. maritima*. Local lesions developed in 6-10 days, whereas systemic symptoms usually were not evident for 25-30 days. Percentage transmission was low in all but *B. macrocarpa*. In the latter host, advanced symptoms were quite severe and consisted of stunting, axillary bud proliferation, general chlorosis, and veinbanding (Fig. 1-B). Infected plants died prematurely.

In *C. capitatum*, local chlorotic spots developed in inoculated leaves in 8-10 days. The lesions soon became necrotic. Systemic symptoms developed in 12-14 days, and included vein-clearing, chlorotic veinbanding, and stunting of the youngest leaves. Within 3 weeks, the youngest leaves became yellow with reddish margins; by 6-8 weeks, the plants were dead. In several additional tests with this host, the inoculated plants reacted as above or did not become infected, although the inocula always proved infectious in *C. quinoa* and spinach.

Local chlorotic spots developed in inoculated spinach leaves in 6-10 days. Some spots became sunken and necrotic, whereas others remained chlorotic but enlarged slightly. Coalescence of some necrotic lesions often led to larger areas of necrotic tissue. Systemic invasion by the virus usually was evident in 12-14 days, but disease severity varied

considerably among plants. Young terminal leaves usually exhibited vein-clearing and chlorotic vein-banding of some of the veins. The leaves were reduced in size, curled downwards, and were somewhat lanceolate in shape. Such leaves, and some older leaves, became generally chlorotic and developed brown flecks or spots which often coalesced to form large necrotic areas. These leaves eventually withered and died, whereas inoculated older leaves often persisted for longer periods. In advanced stages, petioles and flower stalks of severely infected plants developed small brown flecks or streaks. Some streaks coalesced to produce rifted, tannish, necrotic troughs along the petioles or stems. Severely infected plants died prematurely. Occasionally, inoculated leaves developed local chlorotic spots, but systemic invasion by the virus was not evident until a few axillary shoots developed that exhibited a striking mosaic. Often, local spots developed without systemic spread of the virus. No virus could be recovered from the noninoculated portions of such plants. Figure 1-C shows a spinach plant with typical systemic symptoms of BYVV.

Only local lesions developed in inoculated leaves of *B. patula, B. trigyna, B. vulgaris* (red table beet), *C. album, C. amaranticolor, C. murale, C. quinoa*, and *C. urbicum*. Faint, reddish, lesionlike flecks developed in inoculated leaves of *G. globosa* about 25 days after inoculation, but BYVV could not be recovered from the plants.

No symptoms were observed in the other test plants, and BYVV was not detected in back-inoculations to spinach or *C. quinoa*.

Physical properties.–Dilution tolerance.–The dilution end point of BYVV was between 10^{-4} and 10^{-5}; however, most infectivity was lost in dilutions greater than 10^{-2}. Average numbers of local lesions per leaf (18 leaves) were 261, 481, 49, 10, 2, and 0 for dilutions of 0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}, respectively. The increased infectivity with the 10^{-1} dilution of concentrated sap is typical when source-plant inhibitors of the virus are present in the inoculum (6).

Thermal inactivation.–Although thermal inactivation occurred between 65 and 70 C, most infectivity was lost in sap heated over 50 C. Average numbers of local lesions per leaf were 111, 64, 5, 1, 1, and 0 for inocula heated at 45, 50, 55, 60, 65, and 70 C, respectively. Unheated inoculum induced an average of 127 lesions/leaf.

Longevity in vitro.–Sap extracts stored up to 48 hr at room temperature or at 3-4 C were still infectious. Generally, the trend was for increased longevity of BYVV in extracts stored in the cold compared to those held at room temperature. Average numbers of local lesions per half-leaf (18 half-leaves) were 43 and 49, 26 and 52, 15 and 40, 2 and 13, and 3 and 5 for extracts stored at room temperature and at 3-4 C for 3, 6, 12, 24, and 48 hr, respectively. Extracts inoculated to test plants immediately after preparation induced an average of 84 lesions/half-leaf. Lesion numbers among the various treatments (storage times) are not directly comparable because of variations in host susceptibility which undoubtedly occurred at the different times of inoculation. Paired comparisons, however, can be made between infectivities of the inocula stored at room temperature and in the cold for any given duration of storage.

Freezing tolerance.–Frozen sap extracts, when thawed, were noninfectious. However, the virus remained viable in frozen leaf tissue for at least 14 days without apparent loss in infectivity.

DISCUSSION.–It is not clear why Bennett (1) or Howell & Mink (5) failed to mechanically transmit BYVV, whereas our isolate was relatively easily, although inconsistently, transmitted by juice inoculation. Strain differences between isolates, or our use of a reducing agent in the inoculum, may account for the apparent discrepancy. Also, since symptoms in sugar beet in the field are the only criteria for distinguishing yellow vein, it is conceivable that the disease referred to as "yellow vein" by us and others (4, 5, 10) may be incited by different viruses. Bennett's isolate no longer is available for comparison with our isolate; however, the striking symptoms that we have observed in sugar beet in California and the Great Plains were identical to those described by Bennett. The fact that isolates from California and several locations in the Great Plains, selected as typical BYVV in those areas, were mechanically transmissible indicates that these isolates were indeed BYVV, and that the virus may be mechanically transmitted.

BYVV seems to be relatively unstable. At least, most infectivity was lost with dilution beyond 10^{-2} or heating above 45-50 C. Such properties might make purification attempts difficult. However, if instability is associated with the presence of inhibitors in the sap of its chenopodiaceous hosts, techniques to separate inhibitors from the virions might improve the stability of BYVV.

Although the virus seems to have a very narrow host range, failure to obtain infection by juice inoculation does not preclude susceptibility in the test species. The low percentage transmission in *Beta* spp., and the occasional failure of systemic invasion of spinach after local lesions developed in inoculated leaves, illustrate the inefficiency of transmitting BYVV mechanically. Then, too, chenopodiaceous source plants are known to contain virus inhibitors in sap extracts.

As far as can be determined, BYVV, potato yellow dwarf virus (3), and sowbane mosaic virus (2) are the only leafhopper-vectored viruses that have been transmitted from plant to plant by the rubbing method of juice inoculation. However, further work is needed to clarify the relationships of the reported vector (10) to BYVV.

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