

# Broad Bean Wilt Virus in Begonia in Minnesota

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## ABSTRACT

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Fibrous-rooted begonia plants (*Begonia semperflorens*) showing leaf mottling, ring spots, and poor growth in a Minnesota greenhouse were found to be infected with broad bean wilt virus. The virus, which had properties similar to those described for other isolates of broad bean wilt virus, was readily transmitted by *Myzus persicae* and belonged to the serotype I group of the virus isolates. This is the first report of broad bean wilt virus infection of begonia and only the third report of its occurrence in North America.

Fibrous-rooted begonia (*Begonia semperflorens* Link & Otto) plants in a greenhouse in St. Paul, MN, were found with viruslike foliar symptoms consisting of leaf mottling and faint ring spots. The diseased plants were also markedly stunted in relation to symptomless plants growing under the same conditions. Color-break occurred in pink- and red-flowered varieties inoculated in the greenhouse. Symptoms were pronounced during winter, but tended to become less discrete during summer. The symptoms were unlike those observed in begonia infected with tobacco ringspot virus (4). Tests were thus made to determine the nature of the disease and the identity of the causal agent.

## MATERIALS AND METHODS

**Transmission and host range.** After initial isolation from infected begonia, the virus was maintained in *Pisum sativum* L. 'Dwarf Gray Sugar.' A range of herbaceous test plants was inoculated mechanically using crude extracts obtained by grinding young, systemically infected pea leaves in cold 0.01 M phosphate buffer, pH 7.2, containing 0.1% 2-mercaptoethanol. *Chenopodium quinoa* Willd. was used as an indicator plant in back-inoculation tests.

Aphid transmission tests were done with nonviruliferous, apterous adults of *Myzus persicae* Sulz. Aphids were starved for 60-90 min, given a 2-5 min acquisition access period on infected begonia, and transferred to healthy begonia or pea. Ten aphids were transferred to each test plant.

**Serology.** Double-diffusion serologic tests were done in 0.9% agarose dissolved

in distilled water. Crude sap from systemically infected pea plants was used in all tests. For comparative serologic tests, the New York spinach isolate (American Type Culture Collection PV 132) and the New York lettuce isolate (ATCC PV 131) of broad bean wilt virus (BBWV) were propagated in Dwarf Gray Sugar pea.

**Purification and electron microscopy.** Virus was purified from inoculated and systemically infected leaves of *C. quinoa* by the method described for parsley and nasturtium isolates (10). After two cycles of differential centrifugation, the virus preparation was subjected to rate-zonal density gradient centrifugation in 5-30% sucrose gradients in distilled water. Purified virus preparations were stained with 2% phosphotungstic acid, pH 6.8, and examined in the electron microscope.

## RESULTS

**Host range.** The virus produced local and systemic symptoms on *C. quinoa*, *Datura stramonium* L., *Spinacia oleracea* L., and *Vinca rosea* L. and systemic symptoms only on *B. semperflorens*, *Nicotiana clevelandii*, *Pisum sativum*, and *Vicia faba* L. Local lesions or ring spots and symptomless systemic infection occurred in *Vigna unguiculata* L. (Walp.), *Phaseolus vulgaris* L. 'Bountiful,' *Ocimum basilicum* L., *Petunia hybrida* Vilm., and *N. tabacum* 'Xanthi.' No symptoms were produced on and no virus could be recovered from *Cucumis sativus* L. 'National Pickling' and 'Lemon,' *Cucurbita pepo* L. 'Fordhook Zucchini,' *N. glutinosa* L., *Tropaeolum majus* L., and *Lactuca sativa* L. 'Buttercrunch' and 'Great Lakes.'

**Aphid transmission.** In a single experiment using a short acquisition access time, the virus was transmitted by *M. persicae* from infected begonia to four of six healthy begonia and to three of five healthy pea test plants.

**Serology.** In crude sap from infected pea and *C. quinoa*, the begonia virus did not react with antisera to any of the

following isometric viruses: arabis mosaic, cucumber mosaic, tomato aspermy, cherry leafroll, tobacco ringspot, tomato ringspot, broad bean mottle, bean pod mottle, cowpea mosaic, carnation streak, Tulare apple mosaic, tobacco ringspot, and grapevine fanleaf. The virus reacted positively with antisera to serotype I (Fig. 1A) and serotype II (Fig. 1B) of BBWV. The precipitin line of the begonia virus fused with that of serotype I BBWV when tested against both BBWV antisera (Fig. 1A). The precipitin lines of both the

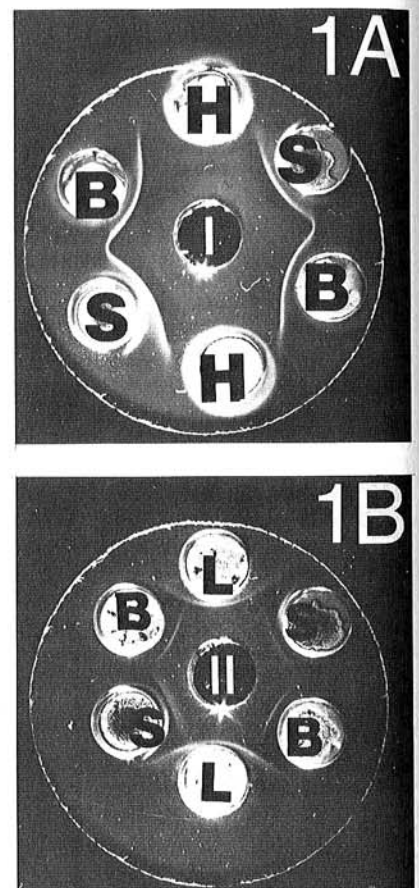


Fig. 1. Reaction of the Minnesota begonia isolate of broad bean wilt virus (BBWV) with antisera to two New York isolates of BBWV. (A) The center well contains antiserum to serotype I BBWV (New York spinach isolate). (B) The center well contains antiserum to serotype II BBWV (New York lettuce isolate). H = undiluted sap from Dwarf Gray Sugar pea; S = New York BBWV spinach isolate (ATCC PV 132) in pea sap; L = New York BBWV lettuce isolate (ATCC PV 131) in pea sap; B = Minnesota BBWV begonia isolate in pea sap.

begonia and spinach isolates spurred with those of the serotype II BBWV when tested against serotype II antiserum (Fig. 1B).

#### Purification and electron microscopy.

The virus was readily purified from infected pea or *C. quinoa*, but better yields of virus, as determined by OD<sub>260</sub> absorption, were obtained from the latter plant. Purified virus contained only isometric particles when viewed in preparations stained with phosphotungstic acid, reacted with antiserum to BBWV, and produced symptoms on pea and *C. quinoa* identical to those produced by the original virus isolate. In rate-zonal density gradient centrifugation in 5–30% sucrose gradients, the virus sedimented as three components: a small, top peak consisting mainly of empty protein shells, and two more rapidly sedimenting nucleoprotein components. The two nucleoprotein components were collected together and found to react with BBWV antiserum and to produce typical symptoms on *C. quinoa*.

#### DISCUSSION

Based on its serologic, physical, and biological properties, the virus isolated from diseased begonia was identified as a serotype I (11) isolate of BBWV. Host range of the virus and symptoms produced on several test plants were slightly different from those reported for several other BBWV isolates (2,5,10). In Dwarf Gray Sugar and Lincoln pea, the Minnesota begonia BBWV produced noticeably milder symptoms than both the New York lettuce and spinach

isolates. The begonia virus also failed to infect the two lettuce varieties tested, but on spinach it produced severe leaf-blight symptoms similar to those caused by the New York spinach isolate (7).

Broad bean wilt virus has been reported from several countries throughout the world, but in North America it has been reported only from New York (3,7,11) and South Carolina (O. W. Barnett, *personal communication*). The occurrence of the virus in Minnesota is therefore of interest, especially in view of the aphid transmissibility of the virus, its wide host range (10), and its potential for causing serious disease on such crops as peas and spinach. In addition, the occurrence of this virus on ornamentals such as begonia, which is maintained indoors during the winter, enhances the possibility of further spread of the virus. It has not been determined whether the virus was introduced into Minnesota on begonia or was already present in weed hosts or other plants. If the latter is the case, it is possible that BBWV may be present but undetected in other areas of North America.

Other viruses reported to infect *Begonia* spp. include tobacco ringspot (4), cucumber mosaic (9), tobacco necrosis (1), arabis mosaic (12), tomato ringspot (12), and tomato spotted wilt viruses (6). There was no evidence to suggest that any of these viruses were present in the plants infected with BBWV. An aphid-transmitted virus was reported to cause ring spot symptoms in *B. tuberhybrida* in Belgium (8), but the causal agent was not identified.

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