

## Some Properties of an Isolate of Broad Bean Wilt Virus from Dogwood (*Cornus florida*)

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### ABSTRACT

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Broad bean wilt virus (BBWV) was isolated from a native *Cornus florida* tree in South Carolina. Serological and host range comparisons with serotypes I and II of BBWV showed the virus to be similar to BBWV-II.

Broad bean wilt virus (BBWV) has been found in Europe, Australia, the United States, Japan, the Middle East, and South America (4,5,13) and occurs as four strains: type strain of Stubbs,

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nasturtium ringspot virus (NRSV), petunia ringspot virus (PRSV), and P.O. pea streak virus (13). These strains infect species from numerous herbaceous dicotyledonous families and have been reported in *Narcissus* (13) and in the woody species *Catalpa bignonioides* Walt. (11). Serotypes I and II of BBWV have been differentiated by Uyemoto and Provvidenti (14).

In the early 1970s, near Clemson, SC, a dogwood tree (*Cornus florida* L.) with a faint yellow mosaic of the leaves was observed. Three viruses were isolated from this tree; cucumber mosaic virus (CMV), arabis mosaic virus (ArMV), and BBWV (9). The BBWV isolate (BBWV-dw) was partially characterized and compared biologically and serologically

with serotypes I and II of BBWV and serologically with NRSV.

### MATERIALS AND METHODS

Isolates of BBWV-I and BBWV-II and antiserum to each virus were gifts from J. K. Uyemoto, Kansas State University, and antiserum to NRSV was a gift from A. J. Cockbain, Rothamsted Experimental Station, Great Britain.

BBWV-dw was isolated by inoculating plants of *Chenopodium quinoa* Willd. or *C. amaranticolor* Coste & Reyn. with sap prepared from young dogwood leaves ground in 2% nicotine solution. Contaminating CMV and ArMV were removed by serial passage of the isolate through *C. quinoa*, with transfer to the next plant being made when systemic symptoms were first detected. Antiserum production and most of the host range studies were done with BBWV-dw isolated by this method. Isolates of each virus strain used as an antigen in the serological comparisons were passaged through single lesions on *Beta patellaris* Moq. three times, with propagation in *Nicotiana clevelandii* Gray between each single-lesion inoculation.

Stock cultures of the three isolates were maintained in either *N. clevelandii* or *C. quinoa*.

The host ranges of the three viruses were compared, using plant species from 15 families. Plants of all species were mechanically inoculated by rubbing sap prepared from *N. clevelandii* leaves ground in 0.03 M sodium phosphate buffer (pH 8.0) containing 0.02 M 2-mercaptoethanol (1:5 w/v tissue) onto leaves dusted with 600-mesh corundum. Symptoms on both inoculated and noninoculated leaves were recorded. Latent infections were tested by inoculating *C. quinoa* with sap from symptomless inoculated plants.

**In vitro properties.** Infected tissue from either *N. clevelandii* or *C. quinoa* was ground in 0.03 M sodium phosphate buffer (pH 8.0) and used to determine the thermal inactivation point (TIP), aging in vitro (AIV), and dilution end point (DEP) of BBWV-dw in crude sap. The tissue was ground 1:5 w/v in buffer for TIP and AIV tests and 1:10 w/v for DEP tests. Crude sap for the TIP test was heated for 10 min at each specified

temperature and cooled rapidly. Aging tests were conducted at 20–22 C.

**Purification.** All three viruses were purified from *N. clevelandii* by the method of Doel (2) modified by removing sucrose from the extraction buffer and substituting glycerol gradients (10–50%) buffered with 0.03 M sodium phosphate (pH 7.0) for sucrose gradients in the rotational centrifugation stage. Gradients were scanned and fractionated using an ISCO model UA 2 ultraviolet analyzer (0.1 o.d. scale, 1 cm path length) linked to an ISCO model D density gradient fractionator. Samples from fractions containing the UV absorbing bands were examined in the electron microscope using 2% sodium phosphotungstate stain (pH 7.0) and in a dual beam GCA/McPherson spectrophotometer with a 1-cm path length. The fractions containing virus particles were combined, sodium azide was added to 0.02%, and the preparation was stored at 4 C.

Sedimentation coefficients were determined for preparations of BBWV-dw containing 3–4 mg/ml of virus (assuming  $E_{260} = 8.0$  [mg/ml]  $\text{cm}^{-1}$ ) in 0.03 M

sodium phosphate buffer containing 0.005 M magnesium chloride (pH 7.2, viscosity 1.024 cps, density 1.0021  $\text{g}/\text{cm}^3$ ).

**Serology.** A rabbit was immunized against BBWV-dw virions with preparations purified as previously described. A single intravenous injection (7.2 mg of virus in 0.4 ml of buffer) was followed 25, 32, and 46 days later by intramuscular injections of virus preparation (6, 8, and 20 mg, respectively) mixed with 1 ml of Freund's complete adjuvant. Blood samples were taken 7 days after the last injection and at 7-day intervals thereafter for 6 wk. After the third week, an additional intramuscular injection containing 1 ml of virus (5 mg/ml) mixed with complete adjuvant was given. Serum obtained from the fifth bleeding was used in all tests.

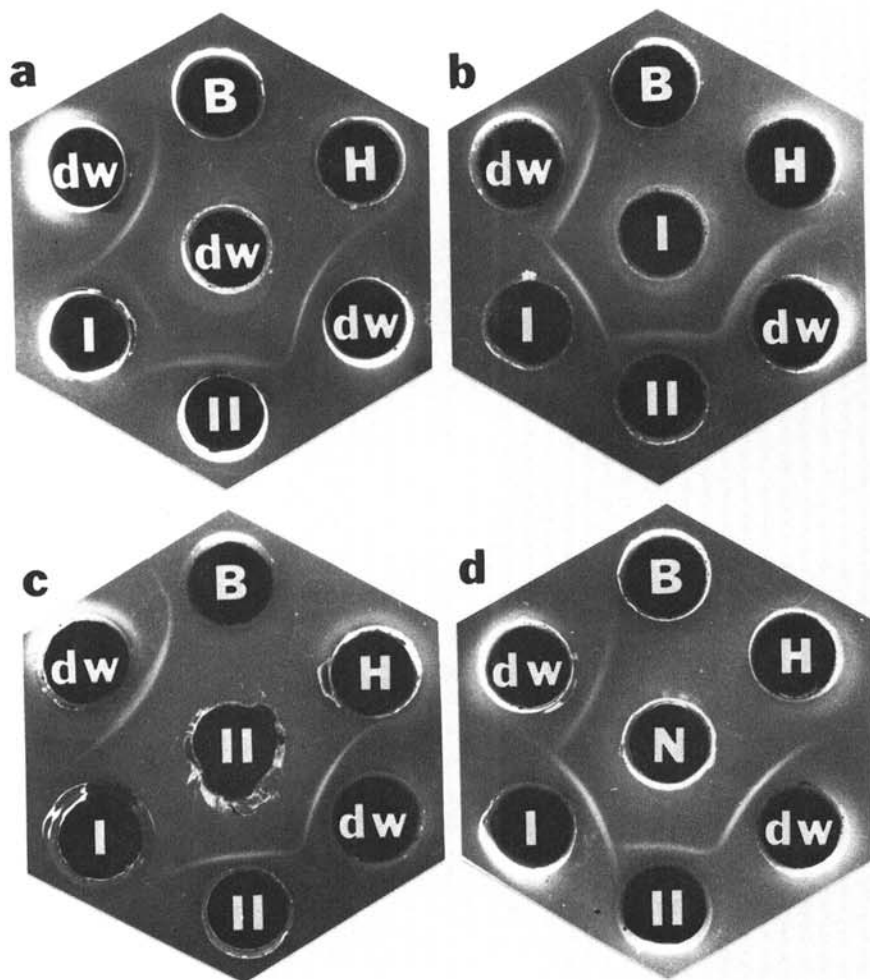
Comparisons of BBWV-dw, BBWV-I, and BBWV-II were completed in gel double-diffusion tests using 0.9% agarose gels made up in 0.07 M phosphate buffer (pH 7.0) containing 0.85% sodium chloride and 0.02% sodium azide. Antigens were prepared by grinding healthy or infected *C. quinoa* (1:1 w/v) in 0.03 M sodium phosphate buffer (pH 7.0) containing 0.02 M 2-mercaptoethanol and were added to their respective wells 4 hr before the antiserum. Healthy *C. quinoa* sap was added to the antiserum well at the same time antigens were applied. Any unabsorbed sap was removed from the well before antiserum was added. Antisera to BBWV-dw, BBWV-I, BBWV-II, and NRSV were used for the comparisons.

## RESULTS AND DISCUSSION

Inoculations of *C. quinoa* or *C. amaranticolor* with sap from dogwood produced both local and systemic symptoms. Double-diffusion serology tests of crude sap from the two *Chenopodium* spp., using antisera to CMV, ArMV, and BBWV, indicated the presence of all three viruses. CMV caused only local infections in either species. ArMV and BBWV caused systemic infections in both species, but BBWV developed systemically in *C. quinoa* more rapidly than ArMV. Several passages of virus through *C. quinoa*, with transfers to the next plant when systemic symptoms were first detected, yielded BBWV (designated BBWV-dw) free from the other two viruses.

BBWV-dw was isolated from the original dogwood tree at least twice but was not detected in other nearby trees. The virus could be isolated only from young leaves collected in the spring or from leaves forced from dormant buds.

BBWV-dw infected species from the Aizoaceae, Amaranthaceae, Apocynaceae, Chenopodiaceae, Compositae, Cruciferae, Leguminosae, Polemoniaceae, Scrophulariaceae, and Solanaceae but did not infect members of the Convolvulaceae, Cucurbitaceae, Nyctaginaceae, and



**Fig. 1.** Reaction in gel diffusion tests of BBWV-I, BBWV-II, and BBWV-dw to antisera to (A) BBWV-dw, (B) BBWV-I, (C) BBWV-II, and (D) NRSV. Antiserum was placed in the central well and antigens, in the peripheral wells. B = buffer, H = sap from healthy plants, I = BBWV-I, II = BBWV-II, dw = BBWV-dw, and N = NRSV.

Umbelliferae that were tested, namely: *Ipomoea guamoclit* L. 'Cardinal Climber,' *Apium graveolens* L. var. *dulce* Pers., *Mirabilis jalapa* L. 'Petticoat Rose,' *Citrullus vulgaris* Schrad. 'Sugar Baby,' *Cucumis sativus* L. 'Chicago Pickling,' *Cucurbita pepo* L. 'Small Sugar,' and *Momordica balsamina* L. Chlorotic or necrotic local lesions together with systemic symptoms were observed on *Celosia argentea* L. var. *crispata* Kuntze; *B. patellaris*; *B. vulgaris* L. 'Detroit Dark Red'; *Chenopodium album* L.; *C. amaranticolor*; *C. foetideum* Schrad.; *C. murale* L.; *C. quinoa*; *Spinacia oleracea* L.; *Phaseolus vulgaris* L. 'Black Turtle Soup,' 'Bountiful,' and 'Pinto III'; *Pisum sativum* L. 'Bonneville' and 'Ranger'; *Lycopersicon esculentum* Mill.; and *Nicotiana occidentalis* Wheeler. Latent local infections with systemic symptoms were recorded in *Tetragonia expansa* Murr., *Atriplex mulmalaria*, *Lactuca sativa* L. 'Buttercrunch,' *Verbesina encelioides* (Cav.) Benth. & Hook., *Zinnia elegans* Jacq. 'Wild Cherry,' *Lupinus hirsutus* L., *Nicotiana longiflora* Cav., *N. megalosiphon* Heurch. & Muell., and *Petunia hybrida* Vilm. Latent local infections without systemic symptoms were detected in *Brassica nigra* (L.) Koch, *Antirrhinum majus* L., *Nicotiana sylvestris* Speg. & Comes, *N. tabacum* L. 'Burley 21,' and *Physalis alkekengi* L. Latent local and systemic infections were detected in *Lupinus albus* L., *Nicandra physalodes* (L.) Pers., and *N. tabacum* 'Xanthi.' Systemic symptoms without detectable local infection were observed in *Vinca rosea* L., *Phlox drummondii* Hook., *P. sativum* 'Little Marvel' and 'Perfected Wales,' and *Capsicum annuum* L. 'Carolina Hot Pepper.' *Gomphrena globosa* L. developed red local lesions and a latent systemic infection, *Dolichos lablab* L. developed only chlorotic and necrotic local lesions, and *Vigna unguiculata* subsp. *cylindrica* (L.) van Eseltine ex Verdc. developed only necrotic local lesions. *P. sativum* 'Dark Skinned Perfection' and *Vicia faba* L. 'Bell Bean' developed both systemic and local mottles or mosaics. No infections could be detected in *Arabis alpina* L., *Brassica juncea* (L.) Coss, *Desmodium tortuosum* (Sw.) D.C., *Glycine max* (L.) Merr. 'Bragg' and 'Davies,' *Lupinus luteus* L., *Medicago sativa* L., *Trifolium pratense* L., *T. repens* L., *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'California Blackeye,' *Torenia fournieri* Lind., *Datura stramonium* L., *Nicotiana glutinosa* L., and *N. palmeri* Gray.

The observed host ranges of the three viruses (BBWV-I, BBWV-II, and BBWV-dw) were generally similar to those reported for BBWV and its strains (4,5,12-14). BBWV-dw, however, differed

from the other two isolates in that it did not infect either *D. stramonium* or *V. unguiculata* 'California Blackeye,' species cited as being useful local-lesion and assay hosts for BBWV (13). As in other reports concerning BBWV (4,7), serotype II isolates (BBWV-II and BBWV-dw) infected *L. sativa* but the serotype I isolate (BBWV-I) did not.

BBWV-dw had a DEP of  $10^{-4}$ - $10^{-5}$  and a TIP between 50 and 60 C and survived in sap for 7-8 days at temperatures of about 20 C.

Preparations of all three viruses sedimented as three bands in glycerol gradients. The upper (T) component consisted of capsids apparently devoid of RNA (empty capsids). The middle (M) and bottom (B) components contained whole particles but also a few empty capsids. The  $A_{260nm}/A_{280nm}$  ratios (corrected for light scattering) of the T, M, and B components of BBWV-dw were about 1.0, 1.7, and 1.78, respectively. Using the relationship described by Gibbs and Harrison (3), we calculated that the M and B components contained 25.5 and 35.5% RNA, respectively. The ratios varied slightly among preparations and were similar to those observed for BBWV-I and BBWV-II (about 1.0, 1.68, and 1.79 for BBWV-I and about 1.0, 1.7, and 1.79 for BBWV-II). Mean values for the  $A_{260nm}/A_{280nm}$  ratio of unfractinated preparations of BBWV-I, BBWV-II, and BBWV-dw were  $1.63 \pm 0.2$ ,  $1.58 \pm 0.3$ , and  $1.57 \pm 0.3$ , respectively.

The sedimentation coefficients of the T, M, and B components of BBWV-dw at infinite dilution were calculated to be 59S, 97S, and 117S, respectively.

In all double-diffusion tests, the BBWV-dw precipitation line was continuous with that of BBWV-II but not with that of BBWV-I (Fig. 1). With BBWV-dw antiserum (Fig. 1A), spurs could be inferred but the reaction line of BBWV-I did not actually contact that of BBWV-II or BBWV-dw. With BBWV-I antiserum (Fig. 1B) and NRSV antiserum (Fig. 1D), BBWV-I produced definite spurs with both BBWV-dw and BBWV-II. BBWV-II antiserum (Fig. 1C) did not react with BBWV-I.

BBWV has been identified frequently in other areas of the world (4,5,10,13), but only three previous records of the virus in the United States exist. In New York (1,14), the virus was isolated from pea (*P. sativum*), spinach (*S. oleracea*), lettuce (*L. sativa*), pigweed (*Amaranthus retroflexus* L.), sowthistle (*Sonchus asper* (L.) Hill), and ribgrass (*Plantago lanceolata* L.); in Minnesota (7), it was isolated from *Begonia semperflorens* Link & Otto. The BBWV-isolate in this report from South Carolina originated from dogwood. The occurrence of the

virus in three widely separated locations and in diverse species may indicate that the virus is more prevalent in the United States than is generally believed.

BBWV-dw has properties similar to those reported for BBWV and the other strains of the virus (4,13) and is a serotype II isolate similar to that isolated from *P. lanceolata* (14). Thus, the heterologous reaction of BBWV-dw to both NRSV and BBWV-I is to be expected. Uyemoto and Provvidenti (14) and Gracia and Feldman (4) have reported that NRSV and BBWV-I are closely related serologically, as are NRSV and the type strain of BBWV (6). Serotype II isolates have previously been reported from the United States (14), Argentina (4), Australia (12), and Great Britain (8) but appear to occur less frequently than serotype I isolates.

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