Comparative Resistance of *Phaseolus vulgaris* Cultivars to Clover Yellow Vein Virus Using Various Inoculation Methods

V. C. DWADASH-SHRENI, Visiting Scientist, and J. R. STAVELY, Research Plant Pathologist, Plant Pathology Laboratory, Beltsville Agricultural Research Center, ARS, USDA, Beltsville, MD 20705

ABSTRACT

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Seventy-eight bean (*Phaseolus vulgaris*) cultivars were mechanically inoculated with clover yellow vein virus (CYVV) and maintained in a greenhouse. Twelve cultivars gave immune, necrotic local lesion, or tolerant reactions to CYVV. Individual plants of these 12 cultivars were inoculated with CYVV by an aphid vector (*Myzus persicae*) or by a hypodermic method. In seven of the 12 cultivars, receptivity varied from 12.5 to 100%, depending on the method of inoculation. The bean cultivar Navajo showed minimum (22.2 and 35.0%) whereas Rufus showed maximum (100 and 90.4%) receptivity to mechanical and aphid inoculations, respectively. The largest difference in percentage of infection occurred in Ouray (0.0, 41.1, 0.0%) and GN 1140 (0.0, 50.0, and 12.5%) for mechanical, aphid, and hypodermic inoculations, respectively. Cultivars Agate, Scout, Gala, Gloria, and GN UI 31 were immune to CYVV regardless of inoculation method. *P. vulgaris* cv. Monroe was identified as an effective local lesion host for CYVV.

Use of resistant cultivars in the United States during the past 50 yr has reduced the impact of many major crop diseases. For initiating a breeding program for disease resistance, sources of resistance are continually being sought among available germ plasm. Crop species can sometimes be screened for resistance to virus diseases in the field by using natural virus spread by vectors or by mechanical inoculation. In greenhouse studies, mechanical inoculation permits rapid screening of germ plasm for disease resistance.

Mechanical inoculation has been used in screening for resistance to some aphidborne bean viruses (1,5,13,17). The site of virus entry into the plants may differ for mechanical and aphid inoculation (15,20,21). Nault et al (16) found that five inbred corn lines differed in susceptibility to maize dwarf mosaic virus (MDMV), depending on whether plants were inoculated mechanically or by the aphid *Schizaphis graminum* Rondani. Screening plants for virus resistance by mechanical inoculation may not be a realistic means for identifying resistance or for screening breeding lines if the virus will be transmitted or vectored by insects in the field.

We have frequently found aphid-transmitted clover yellow vein virus (CYVV) in virus-infected snap beans from the mid-Atlantic states, and we need to identify sources of resistance to this pathogen.

The objective of this work was to identify resistance in beans (*Phaseolus vulgaris* L.) to mechanical inoculation with CYVV and to determine whether the resistant cultivars are also resistant to aphid and hypodermic inoculation of this virus.

MATERIALS AND METHODS

Virus isolate. The isolate of CYVV used was obtained in 1981 from a naturally infected plant of bean cultivar Eagle showing severe virus symptoms in a field plot at the Beltsville Agricultural Research Center, Beltsville, MD. Eagle bean is resistant to the prevalent U.S. strains of bean common mosaic virus. Virus identity was confirmed using CYVV antiserum obtained from O. W. Barnett, Clemson University, Clemson, SC, using enzyme-linked immunosorbent assay (ELISA) (3). This isolate, which was later used for purification, showed no indication of the presence of any of the following viruses in either of two ELISA tests: alfalfa mosaic, bean pod mottle, bean yellow mosaic, peanut stunt, and white clover mosaic. The isolate was mechanically inoculated on *Nicotiana clevelandii*, and within 18 days, systemic chlorotic spots developed on the leaves. Brunt and Kenten's (2) modification of Hollings and Nariani's (7) method was used for purification of the virus from *N. clevelandii*. The resultant preparation was used to mechanically inoculate Eagle beans and peas (*Pisum sativum* L. cv. Dwarf Grey Sugar). Peas inoculated with CYVV and grown at about 24 C developed systemic veinal chlorosis within 8 days, and by 12 days, leaf infection became severe with partial necrosis. On Eagle beans, severe stunting, systemic downward leaf curling, and mosaic-mottling symptoms developed within 12 days. A second purification was made from the peas by using the McLaughlin et al (12) modification of the technique of Jones and Diachun (9). Purified preparations obtained with this procedure produced the above symptoms on Eagle beans and Dwarf Grey Sugar peas at dilutions up to 1:100,000 and were used as the source of infective tissue for inoculation of beans or peas in subsequent studies. The purified CYVV was again tested against Barnett's antiserum with positive results. In addition to the above symptoms, it produced severe veinal chlorosis and a rosette growth habit in *Trifolium incarnatum*, necrotic local lesions on *Chenopodium amaranticolor* and *C. quinoa*, and no symptoms in *P. vulgaris* cv. The Prince, *Vigna unguiculata, Vicia faba,* or *Coriandrum sativum*. Electron microscopy of the purified preparations revealed characteristic CYVV filamentous, flexuous rods of the appropriate dimensions (4).

Inoculation procedures. Eagle bean seedlings that were inoculated mechanically with CYVV from the second purification 12–15 days previously were used as a source of infective tissue for mechanical and hypodermic inoculations. Infected leaves were ground in a mortar with pestle; the juice was expressed through a double fold of cheesecloth and diluted 1:5 with 0.05 M phosphate buffer, pH 6.5–6.7. Mechanical inoculation of the cultivars was made by rubbing appropriate inoculum on newly expanded, primary leaves previously dusted with silicon carbide (600-mesh). Hypodermic inoculation was done by gently inserting

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the hypodermic needle (25-gauge × 1.59 cm) at the base of fully expanded leaf midribs or primary veins and gently injecting the inoculum into the intercellular spaces of the lamina.

Dwarf Grey Sugar peas that were mechanically inoculated with CYVV from Eagle bean 7 days previously were used as the source of virus for aphid transmission studies. Peas were used because of the greater facility of the aphids for feeding on peas than on beans. Aphid inoculations were performed with an adult, apterous, green peach aphid (*Myzus persicae* Sulzer), which is a known vector of CYVV (7,19). An initial colony of the aphids was obtained from John Neal, Florist and Nursery Crops Laboratory, Beltsville Agricultural Research Center, Beltsville, MD. Aphids were reared on snapdragon cultivar Potomac White in insect cages incubated in growth chambers at 25 C with a 16-hr photoperiod.

The same standard technique was used for inoculating primary leaves of the bean cultivars when they were 25–30% expanded, which was usually 5–7 days after seeding, depending on the season. In all the tests, aphids starved 4–5 hr in covered glass vials were given acquisition access to excised, infected pea leaves that were lying on a moist cotton pad in a petri dish. The viruliferous aphids were then transferred to expanding primary bean leaves with a camel's-hair brush. Transmission test feedings were terminated by spraying malathion on the plants. This was the only time these plants were treated with an insecticide because seed germination and the first days of seedling growth occurred in an insect-free greenhouse. After the aphids died, the test plants were placed in an insect-free greenhouse (21–25 C) or growth chamber (20 C with a 16-hr photoperiod) for 2–3 wk for observation.

Optimum aphid transmission was standardized on Eagle bean and this system was used for all subsequent transmission studies. When 10 aphids per plant were allowed acquisition and inoculation access periods of 2–3 min and 24 hr, respectively, 90–95% of the Eagle plants developed symptoms.

Aphid and hypodermic transmission studies were done only with cultivars that were found immune, necrotic, or tolerant to mechanical inoculation with CYVV. At least 15–20 plants were used in all aphid transmission studies and 8–10 plants were used for hypodermic inoculations. Presence of virus in inoculated plants was indexed by back-inoculating tissue samples onto primary leaves of *P. vulgaris* cv. Monroe. This cultivar, which is a local lesion host for bean common mosaic virus (18), was identified as an effective local lesion host for CYVV early in this work. Seeds from a single lot of each cultivar were used for mechanical, aphid, and hypodermic transmission studies.

Rating procedure. Eight to 10 plants of each of 78 bean cultivars were mechanically inoculated and kept in the greenhouse at 21–25 C for 2–3 wk, during which time symptom development was monitored. Their reactions were classified according to the type and severity of symptoms 10–15 days after inoculation. Four to six control plants were maintained simultaneously. Symptoms that developed after all three inoculation procedures were rated in six severity classes, where I = immune: no symptoms and virus not recoverable by back-inoculation as indicated below. N = necrotic: necrotic local lesions occasionally followed by veinal necrosis on inoculated leaves, no symptoms on trifoliolate leaves. T = tolerant: very mild or no systemic symptoms on trifoliolate leaves, stunting absent or very slight 2–3 wk after inoculation, local lesions obtained with back-inoculation. MS = moderately susceptible: epinasty and moderate stunting, systemic downward curling and moderate mosaic-mottling symptoms on trifoliolate leaves 2–3 wk after inoculation. S = susceptible: epinasty and stunting; small, misshapen, downward-curled trifoliolate leaves with mosaic-mottling symptoms, followed by mild systemic veinal necrosis; plants survived at least 2–3 wk after inoculation. VS = very susceptible: epinasty and severe stunting; development of few, small, downward-curled trifoliolate leaves with mosaic-mottling symptoms, followed by apical or systemic necrosis of the entire plant and death by 15 days after inoculation.

Relative concentration of the virus in each inoculated cultivar was determined by grinding uninoculated, younger leaves 12–13 days after inoculation, diluting 1:5 in 0.05 M phosphate buffer, pH 6.5-6.7, and inoculating the resulting crude sap onto primary leaves of Monroe bean. The numbers of necrotic local lesions on Monroe obtained from the mechanically inoculated cultivars were graded as follows: 0 = none, 1 = 2–5 average local lesions per inoculated primary leaf, 2 = 6–15, 3 = 16–40, 4 = 41–70, and 5 = 71 or more.

Receptivity or the percentage of transmission was determined by dividing the number of plants showing visible symptoms plus the number of plants that showed weak or no symptoms but local lesions on Monroe by the total number of inoculated plants.

RESULTS

Mechanical inoculation. Of the 78 cultivars tested, 66 were susceptible, 2 were tolerant, 3 were necrotic, and 7 were immune. On the basis of the disease rating scale (I–VS) and relative concentration of CYVV indicated by the number of local lesions (grades 0–5) after back-inoculation onto Monroe bean, the reactions of the cultivars were as follows. Pintos: Pinto 114, S(5); Wyoming 166, S(5); Fiesta, N(2); Navajo, T(2); Pindak, MS(5); Ouray, I(0); Agate, I(0); Columbia, T(2); Scout, I(0); and Gala, I(0). Navies: Aurora, VS(5); Sanilac, VS(5); and Swan Valley, VS(3). Dark Red Kidneys: Mecosta, S(4), and Manitou, S(4). Blacks: Domino, VS(1); Black Beauty, VS(1); and Midnight, VS(3). Red Mexicans: Bigbend, N(1), and Rufus, N(2). Great Northerns: Harris, VS(2); Valley, VS(2); GN UI 59, VS(1); GN 1140, I(0); Tara, VS(2); and GN UI 31, I(0). Pinks: Sutter Pink, VS(4); Rosa, MS(2); and Gloria, I(0). Snap beans: Green Isle, VS(3); Triumph, S(3); Del Rey, S(3); Slenderette, MS(3); Wade, MS(4); BBL 47, S(1); Checkmate, S(2); BBL 94, VS(3); Coloma, VS(1); Vitagreen, S(2); Lancer, S(1); Roma II, MS(1); Torrent, VS(1); BLGV 109, S(2); GV 50, S(2); Gallamore, S(1); Aristocrop, VS(3); Blue Crop, VS(2); Mount Hood, VS(2); Bush Romano, MS(4); Tidal Wave, S(4); Avalanche, S(5); Gator Green, MS(4); Cascade, S(5); Lake Superior, VS(5); Rebel, VS(4); Raider, S(5); Conquest, VS(5); Lake Shasta, VS(3); Slenderwhite, MS(4); Greencrop, VS(5); Stretch, S(5); Mountaineer White Half Runner, S(3); McCaslan, VS(1); Kentucky Wonder, S(3); Early Gallatin, S(4); Provider, MS(3); OSU 1604, VS(4); OSU 4091, VS (1); OSU 4883, VS(4); Earlybird, MS(3); Cape, S(5); Code 160, MS(3); and Code 112, S(4). Wax beans: Splendorgold, S(4); Goldroy, S(3); Pencil Pod, MS(5); Eastern Butterwax, S(5); and Earliwax, VS(4). On the basis of the reactions obtained after mechanical inoculation, the 12 most resistant cultivars were selected for comparing relative resistance to aphid and hypodermic inoculations of CYVV. These cultivars included immune Agate, Ouray, Scout, Gala, GN UI 31, GN 1140, and Gloria, necrotic Fiesta, Bigbend, and Rufus and tolerant Navajo and Columbia.

Aphid inoculation. Among the 12 cultivars tested using aphid inoculation, seven reacted with systemic symptoms, and back-inoculations to Monroe indicated five were immune to CYVV (Table 1). Cultivars Navajo, Ouray, and Columbia were moderately susceptible, developing yellow-green, mosaic-mottling symptoms on the trifoliolate leaves, whereas Bigbend, Rufus, and GN 1140 developed necrotic local lesions on the primary inoculated leaves followed by systemic necrotic spots and veinal necrosis on trifoliolate leaves. Cultivar Rufus developed chlorotic local lesions surrounded by a necrotic ring on primary inoculated leaves under greenhouse conditions (21–25 C) and solid necrotic local lesions in the growth chamber (20 C). Agate, Scout, Gala, GN UI 31, and Gloria were immune, producing no symptoms and no lesions upon back-inoculation to Monroe.

Hypodermic inoculation. Only two of the 12 cultivars tested by hypodermic inoculations developed systemic symptoms (Table 1.) Cultivar Columbia showed mild, light and dark green mosaic-mottling symptoms, and GN 1140 developed systemic veinal necrosis on the trifoliolate leaves. Cultivars Navajo, Ouray, Agate, Scout, Gala, Fiesta, Gloria, Bigbend, Rufus, and GN UI 31 were immune to hypodermic inoculation with CYVV.

Receptivity. The receptivity or percentage of transmission (number of infected per number of inoculated plants) obtained among the seven differentially reacting cultivars varied with the inoculation method and cultivar. With mechanical inoculation, it varied 22.2–100%; with aphid inoculation, 35.0–90.4%; and with hypodermic inoculation, 12.5–37.5% (Table 1). Navajo showed minimum (22.2 and 35.0%) whereas Rufus showed the maximum (100 and 90.47%) receptivity to mechanical and aphid inoculation, respectively, with CYVV.

DISCUSSION

We obtained 90–95% transmission of CYVV with *M. persicae* using 10 aphids per plant fed on CYVV-infected *Pisum sativum* cv. Dwarf Grey Sugar as source host and *Phaseolus vulgaris* cv. Eagle as the test host of the virus. This transmission rate was quite similar to that reported by Hollings and Nariani (7) for *M. persicae* and *Acyrthosiphon pisum* when they used other source and test plants. They (7) found that *M. persicae* required 5- to 10-min acquisition and 3- to 24-hr inoculation feeding periods to transmit CYVV from infected *Trifolium incarnatum* to healthy *T. incarnatum*. This duration is longer than that obtained for acquisition feeding (2–3 min), but the inoculation feeding periods (3 and 24 hr) were similar (V. C. Dwadash-Shreni and J. R. Stavely, *unpublished*). The longer acquisition feeding period for *T. incarnatum* could be attributed to the hairy leaf surface that might inhibit the aphids from initiating the feeding process. Similarly, *A. pisum* required longer acquisition feeding (5 min) and inoculation feeding periods (4 hr). Conversely, Singh and Lopez-Abella (19) recorded a very short (30–45 sec) acquisition period by using single aphids of *Acyrthosiphon solani* Kaltenbach (foxglove aphid), *Macrosiphum euphorbiae* Thomas (potato aphid), and *M. persicae* from infected coriander and an inoculation access period of 2–3 hr to transmit the CYVV to test plants of coriander.

Pea leaves were found to be more suitable for aphid transmission studies than bean leaves. Limited tests indicated that CYVV-infected pea and bean leaves were equally suitable as sources of infective tissue for mechanical inoculation. In the past, several workers (6,8,11) have reported that the curved epidermal hairs typical of bean leaves ensnare aphids, thereby preventing them from feeding and establishing colonies.

Cultivars Navajo, Ouray, Columbia, Bigbend, Rufus, and GN 1140 all differed in their susceptibility to CYVV under the various methods of inoculation. The largest differences occurred in Ouray and GN 1140 among mechanical, aphid, and hypodermic inoculations. As we found for CYVV on bean cultivars, Nault et al (16) reported that the reactions of five inbred corn lines showed differences in degrees of susceptibility to MDMV, depending on whether inoculation was done mechanically or by aphids.

Results from recent studies have shown that the site of virus entry into plants may differ for mechanical or aphid inoculation. Thomas and Fulton (20) reported a positive correlation between number of demonstrable ectodesmata in the outer walls of the epidermal cells of tobacco leaves and susceptibility to mechanically inoculated tobacco mosaic or cucumber mosaic virus. They postulated that ectodesmata in the outer walls might be receptive to mechanical but not to aphid inoculation with the virus. It has been suggested (15,21) that aphids transmit virus into the plasmodesmata when their stylets pass between epidermal cells. The numerous ectodesmata that occur in the area between epidermal cells are also favorable sites for aphid inoculation (20). The distribution, number, and character of ectodesmata and plasmodesmata in epidermal cell walls may vary independently from one plant species to another and may differentially affect the susceptibility of plants to aphid and mechanical inoculation.

The variation among the responses of bean cultivars to mechanical and aphid inoculation with CYVV may thus be due to differences in the sites of virus inoculation into the bean leaves. With mechanical inoculation, the CYVV may be inoculated into the ectodesmata, as has been suggested for tobacco leaves with tobacco mosaic and cucumber mosaic viruses (20), or into ruptures in the outer walls of epidermal cells. During aphid inoculation, the virus may be inoculated into plasmodesmata (15,21) or through ruptures in transverse cell walls (10,14) as the aphid's stylet penetrates between the cell walls. CYVV could not be transmitted by either of the three methods of inoculation to the cultivars Agate, Scout, Gala, Gloria, and GN UI 31. The differential reactions of Navajo, Ouray, Columbia, Bigbend, Rufus, and GN 1140 indicate that they are resistant to one mode of infection but not to the other.

Table 1. Cultivars differing in their reactions and susceptibility to clover yellow vein virus when inoculated mechanically, with aphids, or hypodermically

Class / Cultivar	Inoculation method: Mechanical[a] Symptoms[c]	Mechanical[a] Back-inoculation grade[d]	Mechanical[a] Receptivity[e] (%)	*Myzus persicae*[b] Symptoms	*Myzus persicae*[b] Receptivity (%)	Hypodermic[a] Symptoms	Hypodermic[a] Receptivity (%)
Pinto							
Navajo	T	2	22.2	MS	35.0	I	···
Ouray	I	···	···	MS	41.1	I	···
Columbia	T	2	70.0	MS	50.0	MS	37.5
Fiesta	N	2	60.0	N	44.6	I	···
Red Mexican							
Bigbend	N	1	57.1	SN	72.2	I	···
Rufus	N	2	100.0	SN	90.4	I	···
Great Northern							
GN 1140	I	···	···	SN	50.0	SN	12.5

[a] Based on eight to 10 inoculated plants.
[b] Based on 15–20 inoculated plants.
[c] I = immune, no symptoms and local lesions not obtained on back-inoculation; N = necrotic; T = tolerant; SN (systemic necrosis) = local lesions on inoculated primary leaves, followed by systemic necrotic spots and veinal necrosis on trifoliolate leaves; and MS = moderately susceptible.
[d] Based on a grading scale of 0–5 for local lesion numbers on Monroe bean, where 0 = none, 1 = 2–5 average local lesions per inoculated primary leaf, 2 = 6–15, 3 = 16–40, 4 = 41–70, and 5 = 71 or more. Based on an average of eight primary inoculated leaves of bean cultivar Monroe.
[e] Based on no. of plants infected/no. of plants inoculated.

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