A Strain of *Clover yellow vein virus* that Causes Severe Pod Necrosis Disease in Snap Bean

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ABSTRACT

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Soybean aphid (*Aphis glycines*) outbreaks occurring since 2000 have been associated with severe virus epidemics in snap bean (*Phaseolus vulgaris*) production in the Great Lakes region. Our objective was to identify specific viruses associated with the disease complex observed in the region and to survey bean germplasm for sources of resistance to the causal agents. The principle causal agent of the disease complex associated with extensive pod necrosis was identified as *Clover yellow vein virus* (CIYVV), designated CIYVV-WI. The virus alone caused severe mosaic, apical necrosis, and stunting. Putative coat protein amino acid sequence from clones of amplicons generated by reverse-transcription polymerase chain reaction was 98% identical to CIYVV strain no. 30 identified in Japan that has not been reported to cause pod necrosis. CIYVV-WI amplicons were 96% identical to a mild strain of CIYVV from Oregon. A distinguishing feature of this new strain is that it does not react with *Potyvirus* broad-spectrum monoclonal antibody PTY 1. A survey of common bean lines and cultivars revealed that, in addition to UI-31 and US1140 with known resistance to CIYVV, lines with the *bc-3* gene for resistance to *Bean common mosaic necrosis virus* also were resistant to CIYVV-WI. An evaluation of 63 snap bean cultivars and breeding lines revealed just one, Roma 442, with a moderate level of tolerance to CIYVV-WI. Introgression of the *bc-3* gene and resistances from UI-31 and US1140 into snap bean may offer a high level of resistance to extensive pod necrosis disease caused by CIYVV in the Great Lakes region.

Snap bean (*Phaseolus vulgaris* L.) is a major crop in the Great Lakes region of the United States. The most significant production occurs in Wisconsin, Michigan, New York, Illinois, and Pennsylvania. Snap bean crops also are produced in the Pacific Northwest, Georgia, Florida, and Ontario, Canada. In 2005, 124,367 ha of snap bean were harvested with a farm gate value of approximately \$400 million (35). Because most snap bean pods are produced for fresh market, freezing, or canning, pods harvested with imperfections due to disease or mechanical injury are discarded, effectively reducing the growers' yield. Pods harvested with imperfections that exceed allowable thresholds result in rejection by processors of the entire harvest.

Snap bean cultivars in the United States are susceptible to many important plant

viruses that may constrain production in the absence of resistance. The potential negative effect of viruses on snap bean production was fully evident in 2000 when a severe virus outbreak occurred in Wisconsin and other neighboring snap bean production states. The most frequently detected viruses included *Cucumber mosaic virus* (CMV) and many distinct strains of *Alfalfa mosaic virus* (AMV) (8,13). The outbreaks were directly associated with high populations of the soybean aphid (*Aphis glycines*) in adjacent soybean fields (9). A high incidence of mixed infections of CMV and AMV was associated with necrotic lesions or line patterns on pods (8). Other viruses detected included *Bean common mosaic virus* (BCMV), *Bean yellow mosaic virus* (BYMV), *Clover yellow mosaic virus*, *Tobacco streak virus*, and *White clover mosaic virus*. Although BCMV was detected, *Bean common mosaic necrosis virus* (BCMNV) never was detected in any samples evaluated.

Significant virus outbreaks again occurred in 2001 (13) and 2003 to 2005 (R. Larsen, *unpublished*) in Iowa, Illinois, Kentucky, Michigan, Minnesota, New York, Wisconsin, and the province of Ontario, Canada. The same complex of viruses resulted in yield losses up to 100%, especially in fields of late-season processing and fresh-market snap bean crops. During these growing seasons, snap bean

plants in fields were observed showing extensive external and internal pod necrosis, a disease termed "chocolate pod" by local growers. The necrosis frequently affected 75 to 100% of the pod surface. *Clover yellow vein virus* (CIYVV) (family *Potyviridae*, genus *Potyvirus*) was suspected as the causal agent based on preliminary host range response; however, identity of the pathogen was not immediately confirmed. CIYVV causes mild to severe mosaic, leaf malformation and vein necrosis, stunting of the plant, and apical necrosis in highly susceptible cultivars (22). Pods often are mottled, twisted, or malformed. Symptoms in bean plants infected with CIYVV can be highly variable due to the numerous strains of the virus and their interaction with different bean cultivars. Symptoms frequently resemble those caused by the closely related *Potyvirus* BYMV, making visual distinction between the two viruses difficult.

Genes for resistance to CIYVV have been reported in the literature. The *cyv* gene present in US1140 (23) was transferred to 'Black Knight' (27), UI-31 possesses two recessive genes (32), and the *desc* gene discovered in snap bean cv. Jolanda also was present in snap bean cultivars Imuna and Evolutie (26). The latter three cultivars are not currently grown in the United States. The reactions of these resistant sources against isolates of CIYVV from the Great Lakes region are unknown. To our knowledge, no other commercial snap bean cultivars have been evaluated for resistance or tolerance to CIYVV. The objectives of this work were to validate whether CIYVV was responsible for the chocolate pod disease in snap bean, and to identify sources of resistance to the virus.

MATERIALS AND METHODS

Field isolates, host plants, and serology. Snap bean plants exhibiting leaf mosaic, apical stem necrosis, and pod necrosis symptoms were collected from fields in Wisconsin, Michigan, Minnesota, Ohio, and New York during the 2001–05 growing seasons. Samples were tested routinely by indirect enzyme-linked immunosorbent assays (ELISA) (2) for AMV using a polyclonal antiserum produced at the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) laboratory in Prosser, WA, and for

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CMV using commercially available polyclonal antiserum (Agdia, Inc., Elkhart, IN). The group-specific ELISA for potyviruses used monoclonal antibody (MAb) PTY 1 developed by Jordan (10) and commercially available (Agdia, Inc.). Assays were performed according to the manufacturer's protocols. Reactions were evaluated 30 min and 24 h after the addition of substrate. Leaf tissue exhibiting mosaic symptoms was ground using a mortar and pestle in 50 mM potassium phosphate buffer, pH 7.4, containing 10 mM sodium sulfite. The extract then was used to inoculate a limited selection of diagnostic bean host plants, including 'Hystyle,' 'Sutter Pink,' 'Black Turtle II,' and UI-34. *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reynier were used as local lesion hosts. When other viruses were suspected based on visual diagnosis or detection by ELISA, local lesions were carefully excised from *C. quinoa* as soon as they became visible, ground as above, and inoculated to *C. quinoa*, Sutter Pink, and Hystyle.

Cultures of CIYVV determined to be free of other viruses were inoculated onto UI-34, Hystyle, 92US-1006, and snap bean breeding line PS00-221A from the USDA-ARS, Prosser, WA, bean-breeding program; all were determined to be susceptible to CIYVV. Inoculated plants were maintained in the greenhouse and observed for symptoms of necrosis on developing pods.

Primer design. To differentiate CIYVV from BYMV by reverse-transcription polymerase chain reaction (RT-PCR), DNA primers were designed using available sequences in the National Center for Bio-

technology Information GenBank database for each virus. Target areas for forward and reverse primers were selected from conserved regions in nucleotide sequence alignments of nine different accessions (AB003308, AB011819, AF185959, AF203536, AY169801, D86044, D89539, D89540, and S77521) of CIYVV and eight accessions (AB029436, AB029436, AY178050, AY192568, D28819, D83749, DQ641248, and U47033) of BYMV. The forward (F) primers for CIYVV and BYMV were located in the N1b region of the viral polyprotein. The reverse (R) primer for CIYVV was located at the 3' terminus of the coat protein (CP) gene, and the reverse primer for BYMV was located at the 3' terminus of the nontranslated region. The primer sequences are as follows: CIYVV-F, 5'-TTGATGACAGCCAGATG-3'; CIYVV-R, 5'-GAATCGTGTCCAGCAATG-3'; BYMV-F, 5'-GATGGAGAGGAGCAGGTGCAC-3'; and BYMV-R, 5'-CTCGCTCTACAAAGATCAG-3'.

Total nucleic acid preparation and RT-PCR. From bean plants determined not to be infected with CMV or AMV based on ELISA results but suspected to be infected with CIYVV or BYMV, total nucleic acid was extracted using the method described by Dellaporta (4) as modified by Presting et al. (20), except that 100 mg of tissue was macerated in buffer without the use of liquid nitrogen. After washing with 70% ethanol, the final pellet was resuspended in 300 µl of sterile water. Reverse transcription was performed in reactions containing 50 mM Tris, pH 8.0; 75 mM potassium chloride; 3 mM magnesium

chloride; 5 mM dithiothreitol; 500 mM each dATP, dCTP, dGTP, and dTTP; and 1 µM reverse primer. After the addition of 5 µl of total nucleic acid, the final reaction was brought to 20 µl with sterile water. After 3 min at 70°C followed by cooling to 4°C, 200 units of *Moloney murine leukemia virus* reverse transcriptase (Promega Corp., Madison, WI) was added to the reaction and incubated at 42°C for 1 h. The subsequent PCR amplification consisted of 25-µl reactions containing 2 µl of first-strand cDNA template; 10 mM Tris, pH 8.0; 50 mM potassium chloride; 0.1% Triton X-100; 1.5 mM MgCl₂; 150 mM each dATP, dCTP, dGTP, and dTTP; 500 nM each forward and reverse primer; and 1 unit of *Taq* DNA polymerase (Promega Corp.). Thermocycling parameters were optimized and a final profile was employed that consisted of a single cycle of 2 min at 95°C; followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C; followed by a final extension step for 7 min at 72°C. Reaction products were resolved by electrophoresis through 1.5% agarose gels containing 1× Tris-acetate-EDTA buffer (24). DNA was purified from the gel bands using GeneCapsule (Geno Technology, Inc. St. Louis) and cloned into the vector pCR4 using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Plasmid DNA was purified from *Escherichia coli* DH 10β using alkaline lysis (24) and clones containing the RT-PCR product were sequenced using the dideoxy-chain termination method.

Evaluation of common bean for resistance to CIYVV. Once involvement of

Table 1. Reaction of genotypes within *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) host groups to *Clover yellow vein virus* (CIYVV)^a

Host group	Host differential	Resistance genes	Symptoms ^b		
			Primary 11 dpi	Secondary 21 dpi	50 dpi
0	Sutter Pink	None	VN	TN, D	D
1	Stringless Green Refugee	<i>i, bc-u</i>	VN	D	D
2	Redlands Greenleaf C	<i>i, bc-u, bc-1</i>	CLL	sst, sM, D	D
2	Imuna ^c	<i>i, bc-u, bc-1</i>	NS	sst, sM	sst, sM
3	Redlands Greenleaf B	<i>i, bc-u, bc-1²</i>	CLL	sst, sM, D	D
3	UI-59	<i>i, bc-u, bc-1²</i>	NS	NS	M
3	US1140 ^c	<i>i, bc-u, bc-1²</i>	NS	NS	NS
4	Sanilac	<i>i, bc-u, bc-2</i>	VN	D	D
5	UI-114-8 Pinto	<i>i, bc-u, bc-1, bc-2</i>	NS	NS	M
6	UI-31 ^c	<i>i, bc-u, bc-1², bc-2²</i>	NS	NS	NS
6	Othello	<i>i, bc-u, bc-2²</i>	VN	TN	D
7	IVT 7214	<i>i, bc-u, bc-2, bc-3</i>	NLL	sM, st	sM, st, TN
8	Black Turtle I	<i>I</i>	NLL, VN	D	D
9	Jubila	<i>I, bc-1</i>	NLL	M, D	D
9	Jolanda ^c	<i>I, bc-1</i>	NS	sst, sM	sst, sM
10	Red Kloud	<i>I, bc-1²</i>	CLL	sst, sM, BI	sst, sM, BI
11	92US-1006	<i>I, bc-u, bc-2²</i>	NS	NS	TN, pod lesions
12	TARS-VR-1s	<i>I, bc-3</i>	NS	NS	NS

^a All lines were evaluated using CIYVV-WI and CIYVV-OR (strains from Wisconsin and Oregon, respectively) in separate experiments. Host plants reacted similarly with each strain, except symptoms caused by CIYVV-WI were more severe. Only symptoms caused by CIYVV-WI are listed in the table.

^b Symptom legend: BI = blistering, CLL = chlorotic local lesions, M = mosaic, mM = mild mosaic, NLL = necrotic local lesions, NS = no symptoms, D = plant death, sM = severe mosaic, st = stunted growth, sst = severe stunting, TN = top (systemic) necrosis, and VN = vein necrosis; dpi = days post inoculation.

^c Lines and cultivars with resistance to CIYVV reported in the literature.

CIYVV in the disease complex was determined and verified, biological assays were initiated to identify potential genes for resistance to CIYVV isolated from infected snap bean from Wisconsin, hereon referred to as CIYVV-WI. Because US1140, UI-31, 'Jolanda,' and 'Imuna' have been reported as resistant to CIYVV (23,25–27), we evaluated their resistance to the newly obtained strain of CIYVV from Wisconsin. Seedlings of each line in several repeated experiments were inoculated at the primary leaf stage and maintained in the greenhouse for at least 50 days.

The limited diagnostic host range described above was expanded to include a set of bean differentials representative of

each of 12 host groups developed by Drijfhout et al. (6) for identification of strains of BCMV and BCMNV. The differentials represent eight resistance genes or combinations thereof and are listed in Table 1. Inoculated plants were maintained in a greenhouse at 18 and 30°C (night and day, respectively) under natural light for symptom development. A collection of 63 commercial snap bean cultivars and breeding lines also was screened for resistance to CIYVV-WI with the intent of identifying a resistance source of immediate use to the snap bean industry. To compare the biological effects of CIYVV-WI with other known strains of CIYVV, the lines were screened additionally with a mild strain of CIYVV from Oregon (CIYVV-OR) iso-

lated from pea (*Pisum sativum* L.) and a strain from New York (CIYVV-NY) described by Provvidenti et al. (23).

Three individual plants of each line or cultivar were mechanically inoculated at the primary leaf stage with each of the strains. The procedure was repeated 2 days following the first inoculation to reduce the possibility of escapes. Plants were maintained under greenhouse conditions and evaluated based on symptom response. A disease severity index (dsi) was assigned as follows: 1 = no disease symptoms; 2 = mild mosaic on secondary leaves and no observable stunting; 3 = mosaic, leaf roll, stunting; 4 = severe mosaic, severe stunting, leaf roll, blistering; and 5 = severe mosaic, systemic vein necrosis, apical necrosis, plant death.

RESULTS

Field observations and host response.

Snap bean samples obtained from the Great Lakes region between 2000 and 2005 with pods showing varying levels of necrosis were evaluated for the presence of viruses, with focus on CIYVV. All pods determined by RT-PCR to be infected with CIYVV from Wisconsin (and other neighboring states) exhibited extensive brown to rust-colored pod necrosis that often affected the entire pod (Fig. 1A). Pods frequently showed internal necrotic areas (Fig. 1B). By comparison, CMV and AMV were detected as a coinfection in pods exhibiting necrosis that was less severe, more localized, and somewhat darker in color (Fig. 2). Reactions on bean plants in grower's fields infected with either CIYVV or BYMV consisted of foliar symptoms typical of these viruses, including severe stunting and mosaic. Apical (top) necrosis occurred with infection by CIYVV in field-grown Hystyle and on greenhouse-grown Sutter Pink, Black Turtle II, UI-34, and Hystyle inoculated with CIYVV-WI. Top necrosis on these hosts never was observed in the field or greenhouse when infected with BYMV only; however, severe mosaic symptoms were typical on leaves and mottling was often observed on the pods.

CIYVV-WI caused local lesions on *C. quinoa* and *C. amaranticolor*. Virus symptoms became systemic on both hosts, causing severe vein and leaf necrosis, malformation, and stunting. Symptoms caused by BYMV in *C. amaranticolor* included local chlorotic lesions followed by systemic vein yellowing and slightly malformed leaves.

Serology and RT-PCR. Bean samples exhibiting the characteristic pod necrosis disease or foliar symptoms, and infected with CIYVV but not BYMV, did not react positively with the *Potyvirus* group-specific PTY 1 MAb. In contrast, samples exhibiting symptoms typical of BYMV such as leaf mosaic or yellow mosaic reacted positively with the PTY 1 *Potyvirus* MAb. For the purpose of determining the

Fig. 1. A, Extensive external pod necrosis symptoms on snap bean caused by infection with the *Clover yellow vein virus* isolate from Wisconsin. **B,** Snap bean pods cut longitudinally showing internal and external necrotic areas resulting from infection with the *Clover yellow vein virus* isolate from Wisconsin. Uppermost pod is healthy.

effect of CIYVV-WI alone, samples testing positive for BYMV, CMV, or AMV were eliminated from further testing.

In all cases where extracts from symptomatic leaves of plants exhibited pod necrosis but tested negative by ELISA with the *Potyvirus* MAb PTY 1, the plant extract subsequently yielded a positive reaction by RT-PCR using DNA primers designed for the detection of CIYVV. No amplicons were produced with the BYMV primers from any samples with pod necrosis that did not react with MAb PTY 1. In contrast, preparations from samples that exhibited symptoms typical of BYMV tested positive with the *Potyvirus* MAb and produced amplified products (1,113 bp) by RT-PCR using the BYMV-specific primers (data not shown). Neither CIYVV nor BYMV amplicons were produced from leaf samples of bean plants that did not exhibit visible foliar symptoms. Extracts from symptomless plants did not yield any detectable infection of host range plants after mechanical inoculation.

In some cases, CIYVV amplicons were produced from chocolate pod tissue originating from field samples where foliar symptoms were absent. When test plants were inoculated with leaf and pod tissue from these samples, infection was established only from pod tissue. Infection with the virus subsequently was verified by RT-PCR. Interestingly, no symptoms were produced in test plants inoculated with expressed sap from the symptomless leaves, and no amplicons were produced from total nucleic acid extracted from these samples.

Nucleotide sequence of amplicons (835 bp) was determined for two clones each of the CIYVV-WI RT-PCR products obtained from four different snap bean samples after local lesion transfer. Nucleic acid sequence identity was greater than 98% within each pair of clones. The sequence encoding the CP of CIYVV-WI was deposited in GenBank (accession no. EF591473). Nucleic acid sequence identities ranged from 83 to 96% with other CIYVV CP sequences available in GenBank whereas amino acid identities ranged from 91 to 98%. The most common amino acid change between samples was substitution of the proline residue with serine at position 19 in the motif SKDKEP, and the glycine residue with glutamic acid at position 94 in the motif TQEQLG (Fig. 3).

Compared with CIYVV-OR, 5 amino acid substitutions occurred within the first 50 residues of CIYVV-WI at positions 6, 19, 26, 34, and 47 (Fig. 3). CIYVV-WI contained the NAG aphid transmission motif located seven residues downstream of the CP 5' N-terminus, which is consistent with all other known isolates of CIYVV. The amino acid sequence alignment of CIYVV-WI was 96% identical with the CP gene sequence of CIYVV-OR (Fig. 3). When compared with Japanese

strain CIYVV no. 30 (accession no. NP734172), a strain not reported to cause pod necrosis (26,34), only four amino acid substitutions occurred in the CP sequence, corresponding to 98% identity level (Fig. 3).

Evaluation of common bean lines for resistance to CIYVV. Susceptible dry bean lines expressed typical symptoms resulting from infection with CIYVV. All BCMV host differentials screened were susceptible to CIYVV-WI and CIYVV-OR except UI-31 and US1140, and TARS-VR-1s with *I + bc-3* genes (Table 1). Lines 92US-1006 and UI-114-8 Pinto initially appeared to be resistant but subsequently showed characteristic virus symptoms by 50 days post inoculation (dpi). Reactions of the host differentials to inoculations with CIYVV-WI and CIYVV-OR were consistent across resistant and susceptible lines, except that symptoms in susceptible lines were considerably more severe with CIYVV-WI than with CIYVV-OR. CIYVV was not detected by RT-PCR in bean lines that did not develop symptoms; thus, these were considered resistant. Additional dry bean lines with *I + bc-3* (USLK-1, USLK-2, USLK-3, USDK-4, USDK-5, USWK-6, USCR-7, USCR-9, and Raven; 15–19), and with *bc-3* alone (USCR-8; (17) remained symptomless after 50 dpi and tested negative for CIYVV by RT-PCR.

Evaluation of commercial snap bean cultivars. Sixty-three snap bean cultivars and breeding lines inoculated with three

different strains of CIYVV (-OR, -NY, and -WI) exhibited typical symptoms of infection by CIYVV except 'Roma 442,' a Romano type bean that remained symptomless for up to 21 dpi (Table 2). At 50 dpi, this cultivar had developed leaf mosaic but did not exhibit stunting symptoms, apical necrosis, or pod necrosis. Infection in Roma 442 was verified by RT-PCR and by back-inoculation to indicator hosts. In additional experiments, Roma 442 was inoculated with the NL3-D strain of BCMNV. Inoculated plants showed epinasty and vein necrosis on inoculated primary leaves followed by severe stunting and top necrosis, a response indicating *I* gene presence but not *bc-3*. All snap bean lines except Roma 442 were rated susceptible to highly susceptible to CIYVV. All plants with a *dsi* = 5 rating at 7 dpi had responded with vein necrosis on inoculated primary leaves. This was followed by severe stunting, apical necrosis, and death, usually within 14 to 21 dpi. Similar to observation of symptoms in dry bean, CIYVV-OR produced milder symptoms on 46 of 63 snap bean lines relative to those symptoms produced by CIYVV-WI or CIYVV-NY (Table 2).

DISCUSSION

CIYVV isolated from snap bean in Wisconsin and other areas of the Upper Midwest and New York is a previously unreported strain that causes extensive pod

Fig. 2. Pod symptoms on a commercial snap bean cultivar co-infected with *Cucumber mosaic virus* and *Alfalfa mosaic virus*.

necrosis. The necrosis was typically more severe than that caused by the complex of CMV and AMV, and is referred to as chocolate pod by local growers (8,31). These pod symptoms have not been observed or reported on snap bean as a result of infection by previously identified strains of CIYVV. Generally, infection with CIYVV produces distortion and mottling in pods without necrosis (22). Depending on the virus strain, environment, cultivar, and vector populations, CIYVV may or may not have a significant effect on harvest yields in processing bean. For example, a severe outbreak of CIYVV was reported recently in a bean field in California, but pods apparently were not affected (3).

Several samples of snap bean plants were received in different years that showed pod necrosis symptoms associated with CIYVV-WI; however, no symptoms were present in leaves and the virus could not be detected in leaf extracts using RT-PCR. Infection by movement of infected pollen has been considered as a hypothesis for this occurrence. Among the potyviri-

uses, BCMV, *Lettuce mosaic virus*, and *Pea seedborne mosaic virus* have been reported to be pollen transmitted (30) but CIYVV has not. Common bean is self-pollinated and cross-pollination in the field rarely occurs except under high populations of insect pollinators or by wind. Additional studies using CIYVV-WI are required to test this hypothesis. Other pathogens also have been reported to cause necrosis on bean pods. A russet disease on snap bean caused by *Plectosporium tabacinum* described by Dillard et al. (5) was reported recently that produces symptoms on pods similar to those caused by CIYVV or CMV+AMV. However, no pathogenic fungi were isolated from any of the pods we examined that were infected with CIYVV-WI.

Other than its isolation from snap bean grown in the Great Lakes region, the ultimate source of the CIYVV strain causing chocolate pod is unknown. The strain does appear to be specifically associated with high populations of soybean aphid. Alfalfa (*Medicago sativa* L.) was considered a potential source of the virus because it is

widely grown in regions where snap bean crops are produced and is a known host of CIYVV (22). However, during 2002 and 2003 in New York, Shah et al. (29) investigated potential movement of CMV, AMV, and CIYVV or BYMV into snap bean fields from local and remote alfalfa fields. They found no evidence for an increased risk of virus infection in snap bean adjacent to alfalfa, and no correlation was found between virus incidence in alfalfa and adjacent snap bean.

Unlike other reported common strains of the virus (10–12), CIYVV-WI was undetectable by the *Potyvirus* broad-spectrum-reacting PTY 1 MAb. The inability of PTY 1 to detect CIYVV causing pod necrosis has been problematic with regard to identifying the virus in snap bean each year in the affected regions. PTY 1 has been reported to react to at least 48 different aphid-transmissible potyviruses, including all strains of the BYMV subgroup, including BYMV, *Pea mosaic virus*, *White lupin mosaic virus*, and CIYVV (7,11,12). However, in the absence of BYMV, none of the samples collected that were exhibiting the

CIYVV-WI	1	SDKEKLNAGEQQKSKDKEPRQRDQEGENSNRQIIPDRDINAGTTGTFSVPKLKKISGKLS	60
CIYVV-OR	1	S.....S.....V.....T.....L.....	60
CIYVV No.30	1	V.....S.....	60
CIYVV-WI	61	LPKIKGKGLLNLDHLLVYVPNQDDISNNIATQEQLGAWHEGVKNAYEVDDQQMEIICNGL	120
CIYVV-OR	61	S.....N.....E.....N.....	120
CIYVV No. 30	61	E.....	120
CIYVV-WI	121	MVWCIENGTSGLDQGEWTMMDGEKQVTFPLKPILDFAKPTLRQIMAHFSQAAESYIEFRN	180
CIYVV-OR	121		180
CIYVV No.30	121		180
CIYVV-WI	181	STERYMPRYGLQRNLTDYGLARYAFDFYRLTSKTPARAREAHMQMKA AAIRGKSNHMFGL	240
CIYVV-OR	181	S.....	240
CIYVV No.30	181	...K.....	240
CIYVV-WI	241	DGNVGTDEENTERHTANDVNRNMHHIAGARF	271
CIYVV-OR	241		271
CIYVV No.30	241		271

Fig. 3. Amino acid sequence comparison of the coat protein region of *Clover yellow vein virus* strain from Wisconsin (CIYVV-WI), Oregon (CIYVV-OR), and strain no. 30 (CIYVV No. 30) (26,34) (accession no. NP734172). Amino acids of each strain that differ from those of CIYVV-WI are indicated.

Table 2. Reaction of commercial snap bean cultivars and breeding lines to inoculation with three strains of *Clover yellow vein virus* (CIYVV) under greenhouse conditions

Cultivar	Disease rating ^a		
	WI	OR	NY
Angers	4	3	4
Arena	5	4	5
Banga	5	5	5
Benton	5	4	4
Booster	4	4	3
Bronco	5	4	4
Carlo	4	4	3
Castano	5	4	5
Eden	5	3	5
Envy	5	4	4
Espada	5	4	4
Evergreen	4	3	3
Excalibur	4	4	4
Firstmate	5	5	5
Flo	5	4	5
Foremost	4	3	3
Gold Crop	4	3	4
Goldmine	5	3	3
HB 1745	4	4	3
Hercules	5	4	5
Hialeah	4	3	5
Hystyle	5	4	5
IDMV 19	5	4	4
IDMV 20	5	4	4
IDMV 40	5	3	4
Igloo	5	4	5
Improved Tendergreen	5	5	5
Kentucky Blue	4	4	4
Labrador	5	4	5
Lynx	4	3	3
Masai	5	4	5
Medinah	5	3	5
Minnette	4	3	4
Moncayo	4	3	3
MV 185	5	4	5
Nelson	5	4	4
Newton	4	4	4
Nicelo	5	4	4
NY 6020-4	5	4	5
Opera	5	5	5
Orion	4	4	4
PLS 87	3	3	4
PLS 84	5	3	5
PLS 553	5	4	5
PLS 118	5	3	3
Primo	5	4	5
Rambo	4	3	4
Roma 442	2	2	2
Romano Gold	5	5	5
Sag	5	5	5
Saurus	4	3	3
SB 4243	5	4	4
Sea Biscuit	5	4	4
Serin	5	4	4
Sirio	4	4	3
Spartacus	5	3	5
Summit	4	3	5
Tapia	3	3	3
Titan	4	3	5
Top Crop	5	4	5
Ulysess	4	3	4
Valentino	4	4	4
VR Romano	5	4	4

^a CIYVV strains from Wisconsin (WI), Oregon (OR), and New York (NY). Disease severity index: 1= no foliar or pod symptoms; 2 = mild mosaic, no stunting or apical necrosis; 3 = mosaic, stunting; 4 = severe mosaic, severe stunting, leafroll or apical necrosis; and 5 = vein and apical necrosis followed by death of the plant.

chocolate pod disease caused by CIYVV-WI and related isolates in 2000–05 from Wisconsin or other states, including Michigan, Minnesota, Ohio, or New York, resulted in positive reactions to this antibody by ELISA (*data not shown*). Likewise, the Oregon and New York strains used in our evaluations also were not detected. It is not immediately clear why CIYVV-WI or other isolates evaluated from this region did not react with the PTY1 MAb, although similar results have been reported for other *Potyvirus* isolates. For example, PTY1 did not detect 3 of 15 isolates of *Papaya ringspot virus* in Florida (1) and *Peanut mottle virus* isolates M and VS were not detected by Li et al. but another isolate of the virus was detected by the antibody (11,14). A possible reason for lack of detection by PTY 1 may involve weakened avidity due to a difference of one or more of the target amino acids in the core region of the CIYVV-WI CP.

Recent reports have focused on placement of CIYVV isolates into subsets or strains based on serology, host response, and sequence comparisons (25,33). Sasaya et al. (25) described variabilities in 10 isolates of CIYVV collected in Japan that allowed them to be divided into two subsets based on serology, host response, and sequence comparisons. The predicted CP amino acid sequence of CIYVV-WI was 98% identical with seven isolates in subset 1 and ranged from 91 to 93% identity with three isolates composing subset 2. CIYVV-WI also produced systemic symptoms of mosaic, severely malformed leaves, and necrosis in *C. amaranticolor* and *C. quinoa*, which was consistent with host responses for the strains reported in subset 1. Although we were not able to compare serological reactions with the MAbs produced by Sasaya et al. (25), the biological data and molecular sequence comparisons suggest that CIYVV-WI are similar to their isolates in subset 1. It is unknown if any of the Japanese isolates produced symptoms on snap bean comparable with those caused by CIYVV-WI because pod necrosis was not cited as a symptom (25).

Bean differential hosts UI-31 and US1140 did not develop symptoms in response to inoculation with CIYVV-WI. Provvidenti and Schroeder (23) proposed a single recessive gene, *by-3*, the symbol of which was later changed to *cyv* (21), that conferred a high level of resistance to CIYVV in US1140. The gene has been introgressed into some dry bean cultivars (27) but it is not known to be present in any snap bean breeding lines or cultivars. In addition, US1140 possesses *bc-1²* and UI-31 possesses *bc-1²* and *bc-2²*, which confer resistance to BCMV. However, the susceptibility of other host differentials with *bc-1²* or *bc-2²* resistance genes (Table 1) indicates that resistance to CIYVV, in the case of UI-31 and US1140, is not de-

termined by these genes alone that confer resistance to BCMV.

Jolanda and Imuna both possess the *desc* gene for resistance to CIYVV as well as gene *bc-1* for resistance to BCMV. The two genes are unrelated because other lines with *bc-1* were susceptible to CIYVV, as determined by Sato et al (26). Jolanda and Imuna both were susceptible to CIYVV-WI, but exhibited symptoms less severe than their BCMV host group counterparts in this study. The susceptibility of lines with reported resistance to CIYVV (28) provides additional evidence that CIYVV-WI may be a unique strain of virus.

Except for IVT 7214, genotypes with the *bc-3* gene for resistance to BCMV and BCMNV were resistant to CIYVV-WI. These results suggest that *bc-3*, or a gene tightly linked with *bc-3*, may be associated with resistance to CIYVV-WI.

Because CIYVV is transmitted by aphids in a nonpersistent manner, the use of pesticides to manage the spread of the virus is generally ineffective. Hence, the most effective management strategy is to develop cultivars with resistance or tolerance to CIYVV. Unfortunately, no snap bean cultivars currently in production exhibit resistance to the virus. It is likely that Roma 442 may be grown successfully in areas with a high incidence of CIYVV, although evaluation under field conditions is a prerequisite for this recommendation. In addition, because it is a flat-pod processing Romano type, production is limited compared with the more popular round-podded snap bean types. Introgression of the *bc-3* gene into current snap bean cultivars may offer resistance against CIYVV, but further investigation is warranted to verify that *bc-3* confers resistance to CIYVV.

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