VIRUSES CAUSING LOSSES ON PROCESSING BEANS IN THE MIDWEST

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PROBLEM DEFINED

Virus problems in legumes appear to have changed from sporadic occurrences to frequencies that suggest research efforts need to be increased. The virus situation in snap bean has escalated from minor to one of significant concern in Wisconsin and other Midwestern states. Although not fully documented, aphids, including the recently discovered soybean aphid, and other virus transmitting insects appear to be more active in recent years. Many viruses found in snap bean are also present in forage legumes and soybean. Besides seed transmission, forage legumes are viewed as a significant source of virus inoculum from which insects are moving viruses to snap bean and soybean.

Two cases of virus problems in snap beans were brought to our attention in 2000 and multiple samples were evaluated for the presence of plant viruses in 2001. The 2001 samples originated mostly in WI although samples were also processed from as far away as western New York. Symptoms in both years included stunted plants, leaf distortion, yellow and light green mottle and mosaic patterns on leaves, pod distortion and internal pod necrosis. Multiple viruses are known to be present in both the 2000 and 2001 samples as determined by extensive ELISA tests. When healthy bean plants are inoculated with sap expressed from field plants, similar symptoms develop on the inoculated plants within an expected time period. It is not uncommon to find numerous viruses present in a single plant sample. The term "virus cocktail" continues to describe the wide range of plant viruses present in field samples. A common factor is that all viruses detected are transmitted by aphids in a nonpersistent and stylet borne manner. The viruses detected are efficiently transmitted by transitory aphids and do not require an aphid species that colonizes beans to acquire and transmit a specific virus. Soybean aphids were commonly observed in snap bean in 2001. Winged forms of the soybean aphid are attracted to snap bean and give birth to live young. However, immature soybean aphids do not mature on snap bean but rather die. Recent greenhouse experiments indicate that the soybean aphid is a highly efficient transmitting agent of the cucumber mosaic virus (CMV). Previous studies indicated that alfalfa mosaic virus (AMV) is transmitted by the soybean aphid but at a moderate efficiency. Massive numbers of soybean aphids in snap bean may overcome lower efficiency of AMV transmission.

Other factors related to the appearance and severity of virus symptoms have been mentioned by field personnel who view a broad spectrum of cultivars and cultural practices

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on a daily basis over the course of a complete growing season. Specifically, the application of nitrogen and post application of Basigran herbicide have been mentioned in the context of symptom appearance and severity. We speculate that stress imposed by a foliar applied herbicide may interact to modify expressed symptoms such as pod necrosis. Stunted plants could also be more attractive to winged aphids, thus increasing the potential for transmission.

It is not known how the problems observed in 2000 and 2001 relate to the Disease X symptoms observed in previous years. Disease X is a syndrome of snap bean involving leaf fusion, increased numbers of male sterile plants, and other undesirable traits. The Disease X syndrome is associated from generation to generation with the seed. Reduced germination is associated with increased incidence of Disease X plants. A virus has been speculated to be a cause of the Disease X symptoms, but hard evidence that links a specific virus to these symptoms has been elusive.

METHODOLOGY

Snap and lima bean samples exhibiting virus-like symptoms, obtained from growers in the states of Wisconsin and New York, were evaluated by ELISA for AMV, CMV, tobacco streak virus (TSV), clover yellow mosaic virus (CYMV), bean common mosaic virus BCMV), bean yellow mosaic virus (BYMV), bean pod mottle virus (BPMV), clover yellow vein virus (CYVV), soybean mosaic virus (SMV) and potyvirus group. The antibodies used in this study were locally or commercially produced.

To further determine the etiology of the virus-like symptoms, pathogenicity studies were conducted under greenhouse conditions. Healthy bean plants, varieties Top Crop and Hystyle, growing in the greenhouse were inoculated with sap expressed from field plants.

In addition to the previous tests, the reverse transcription polymerase chain reaction (RT-PCR) was also used to reconfirm the presence of the most significant virus affecting snap beans. Qiagen kits were used in the extraction of viral RNA, synthesis of cDNA and PCR reaction. The pair of primers used in the RT-PCR, designed and generously given by Dr. Medhat K. Nakhla, was derived from the capsid protein gene of CMV isolate of bananas. The primer sequences were as follows: 5'GATCGACCATGGACAAATCTGAATCAAC 3' and 5'CTCTGGATGGCGTTTAGTGACTTCAGCAG3'. CMV-positive plants tested by ELISA were used as positive control in the RT-PCR reactions and CMV–negative as negative control.

A field trial aimed to evaluate the reaction of snap bean varieties to the virus complex was conducted at the West Madison Agricultural Research Station. The plot was planted July 19, 2001 and each entry was planted in two row plots, spaced 30 inches apart and 7 feet in length. Each entry was replicated three times. One row was inoculated with a mixture of AMV and CMV at the first trifoliate leaf stage. Foliage was rated for symptoms and leaf

samples were taken twice for virus assays. Five plants were selected and pods assessed for pods per plant, and percentage of plants with internal or external pod necrosis.

Based on the results from the field trial, an experiment designed to evaluate the combined effect between AMV and CMV was established in the greenhouse. This experiment had a split plot design, with four inoculations as main treatment and six varieties as sub-treatments. The inoculations treatments were as follows: control (not inoculated), AMV alone, CMV alone and a combination of AMV and CMV. The snap bean varieties Pix, MV-185, and Minuette with the lowest symptom severity in the field; and the varieties Hystyle, Opus, and B373 with the highest symptom severity were used in this experiment.

RESULTS

Virus detection. Viruses most commonly detected in snap bean included AMV, CMV, TSV and CYMV (Table 1). Single viruses were detected in many samples, but two or more viruses were found in 50% of the samples tested (Table 2). CMV was commonly detected in all regions of Wisconsin (Table 3). Numerous types of symptoms were observed, but specific symptoms could not be linked to specific viruses (Table 3). Bean common mosaic virus, bean yellow mosaic virus, clover yellow mosaic virus, and bean pod mottle virus do not appear to be important in the current snap bean virus problem. CMV is detected in soybean at an extremely low frequency, thus soybean is not considered an inoculum source for CMV. However, AMV and TSV are common in soybean, and this crop, along with forage legumes, is a likely source of both viruses. CYMV is detected in soybean, but at moderate incidence. Red clover, however, is a major source of CYMV.

Pathogenicity test. The snap bean plants inoculated with plant sap extracted from symptomatic field samples developed virus-like symptoms similar to the ones observed on the original field samples. Depending on the virus field isolate, the symptoms included stunting, mosaic leaf deformation and blisters on leaves (Table 4). Interestingly, CMV was detected in all snap bean test plant inoculated with sap from Wisconsin samples (Table 4). Similar results were observed with samples from New York (data not shown). AMV was the second most common virus detected (Table 4), and reflects similar results from field samples (Table 1).

RT-PCR. A strong band with the expected size, 750 base pairs, was present in the lane corresponding to the CMV-positive sample (Figure 1). On the other hand, no DNA was amplified in the negative control. A positive PCR test, plus host range results, is significant proof that CMV is causing symptoms in snap bean in Wisconsin.

Snap bean variety trial. Cucumber mosaic virus was common to all varieties, but varieties expressed differential symptom severity and pod development. Although all varieties expressed symptoms, Pix, Masai, and MV185 expressed low severity of foliar symptoms (Table 5). A goal in 2002 will be to refine methods to evaluate symptoms used to rate snap bean varieties for reaction to viruses.

Table 1: Prevalent viruses in snap bean and lima bean samples from Wisconsin and New York in 2001

Sample source	% Positive samples				
	CMV[1]	**AMV[1]**	**POTY[1]**	**TSV[1]**	**CYMV[1]**
Wisconsin					
Snap bean	78	83	11	40	12
Lima	75	25	0	100	100
New York					
Snap bean	100	40	40	17	20

[1] CMV (cucumber mosaic virus), AMV (alfalfa mosaic virus), POTY (poty-virus group), TSV (tobacco streak virus), CYMV (clover yellow mosaic virus).

Table 2: Frequency of viruses in samples

Samples	Total number of samples	No. of samples with		
		One virus	**Two viruses**	**> Two viruses**
Wisconsin				
Snap bean	18	6	8	4
Lima bean	4	3	0	1
New York				
Snap bean	10	5	2	3

Interaction between AMV and CMV. Although the experiment is still in progress, preliminary results suggest that the snap bean varieties being tested have a differential reaction to CMV. The varieties Pix and MV-185 appear to have resistance to CMV, confirming field results. The other four varieties were showing severe CMV symptoms characterized by blisters, dark green vein-banding or the combination of blisters and dark green vein-banding depending on the variety. The variety Opus showed green dark vein-banding only, the varieties Hystyle and B373 had blisters only, and the variety Minuette had both symptoms. It seems that AMV does not have a significant effect on snap beans. Snap bean plants inoculated with AMV alone had mild viral symptoms characterized by slight stunting and yellowing of leaves. The presence of the two viruses in a single plant did not have any effect on the severity of CMV. The reaction of the plants inoculated with both AMV and CMV did not differ from those inoculated with CMV alone.

Table 3: Viruses found in snap bean field samples and relationship to symptoms

Sample code / Location/ Variety	Symptoms	CMV[1]	AMV[1]	CYMV[1]	TSV[1]
A/ WI-Arlington ARS / HMX1945	Leaf curl	+++	-	-	+++
B / Niagra Co. NY/ HMX1945	Mild rugosity/clean pods	+++	++	-	+
C / Orleans Co. NY / HMX1945	Mild rugosity/clean pods	+++	-	-	-
D / Orleans Co. NY / HMX1945	Mild rugosity/clean pods	+++	-	-	-
E / Orleans Co. NY / HMX1945	Mild rugosity/clean pods	+++	-	-	-
F / Orleans Co. NY / HMX1945	Mild rugosity/clean pods	+++	-	-	-
G / Orleans Co. NY / HMX1945	Discolored pods	+++	+++	-	-
H / WI-Central sands / Hystyle	Strong rugosity Clean pods Leaf curl, Herbicide-like injury?	+++	-	-	-
I / WI-Central sands / Hystyle	Strong rugosity/ mosaic Downward leaf roll Pods water soaked, w/ mild discoloration Red nodes	+++	++	-	-
J / WI-Central sands / Hystyle	Same as I, Herbicide-like injury	+++	++	-	-
K / WI-Central sands / Hystyle	Strong rugosity/ mosaic Slight pod and node discoloration	+++	++	-	-
L / WI/ Lima	Chlorotic spots/mosaic Some rusty pod discoloration	+++	+	++	+++
M / WI-Arlington / HMX0944	Bright chlorotic spots	+++	++	+	+++
N / WI-Arlington / Polder	Chlorotic blotches	+++	++	-	+++
O / WI-Arlington / Hystyle	Rugose Mosaic	+++	++	-	-
P / WI-Arligton / Labrador	Chlorosis High incidence of plant death	+++	++	-	-
Q / WI-Arlington / Evergreen	Chlorosis Plant death	-	+	-	++

[1]CMV (cucumber mosaic virus), AMV (alfalfa mosaic virus), CYMV (clover yellow mosaic virus), TSV (tobacco streak virus).

Table 4: Greenhouse Transmission Test of Virus Isolates from Snap Bean Samples from Harris Moran, WI

Plant Test [1]	**Sample** [2] #/tissue	**Foliar and stem symptoms on test plants**	**ELISA RESULTS** [3]					
			CMV	AMV	CYMV	POTY	BYMV	CYVV
25 Hs	Control	No symptoms	-	-	-	-	-	-
26 TC	Control	No symptoms	-	-	-	-	-	-
26 TC	Control	No symptoms	-	-	-	-	-	-
28 Hs	#5 /pod	Stunting	+++	++	-	-	-	-
29 Hs		Stunting	+++	++	-	-	-	-
30 TC		Stunting	+++	++	-	-	-	-
31 TC		Stunting	+++	+	-	-	-	-
32 Hs	#4 /pod	Stunting	+++	-	-	-	-	-
33 TC		Stunting, mosaic, leaf curl	+++	++	-	-	-	-
34 TC		Stunting, rugosity, mosaic	+++	-	-	-	-	-
35 Hs	#2 /leaf	Stunting, severe mosaic	+++	++	-	-	-	-
36 Hs	#2 /pod	Blisters, mosaic	+++	++	-	-	-	-
37 Hs	#2/ pod	Blisters	+++	++	-	-	-	-
38 TC	#2/leaf	Leaf curl, leaf deformation Severe mosaic	+++	++	-	-	-	-
39 TC	#2/ leaf	Stunting, leaf curl	+++	++	-	-	-	-
40 Hs	#1 /pod	Stunting, mosaic	+++	++	-	-	+	-
41 Hs		Stunting, mosaic	+++	++	-	-	-	-
42 Hs		Stunting, mosaic	+++	++	-	-	-	-
43 TC	#3 /pod	Stunting, leaf curl, mosaic	+++	+	-	-	-	-
45 Hs		Stunting, mosaic	+++	-	-	-	-	-

[1] Snap bean varieties (Hystyle 'Hs' and Top Crop 'TC') used as test plants.
[2] Field samples (pods or leaves) used as source of inoculum for the transmission test.
[3] Inoculated plants were tested by ELISA for CMV (cucumber mosaic virus), AMV (alfalfa mosaic virus), CYMV (clover yellow mosaic virus), POTY (potyvirus group), BYMV (bean yellow mosaic virus, and CYVV (clover yellow vein virus). The magnitude of the optical density is represented as:
'+' 0.1<OD<=0.2 '++' 0.2<OD<=0.5 '+++' OD>0.5 '-' OD<0.1

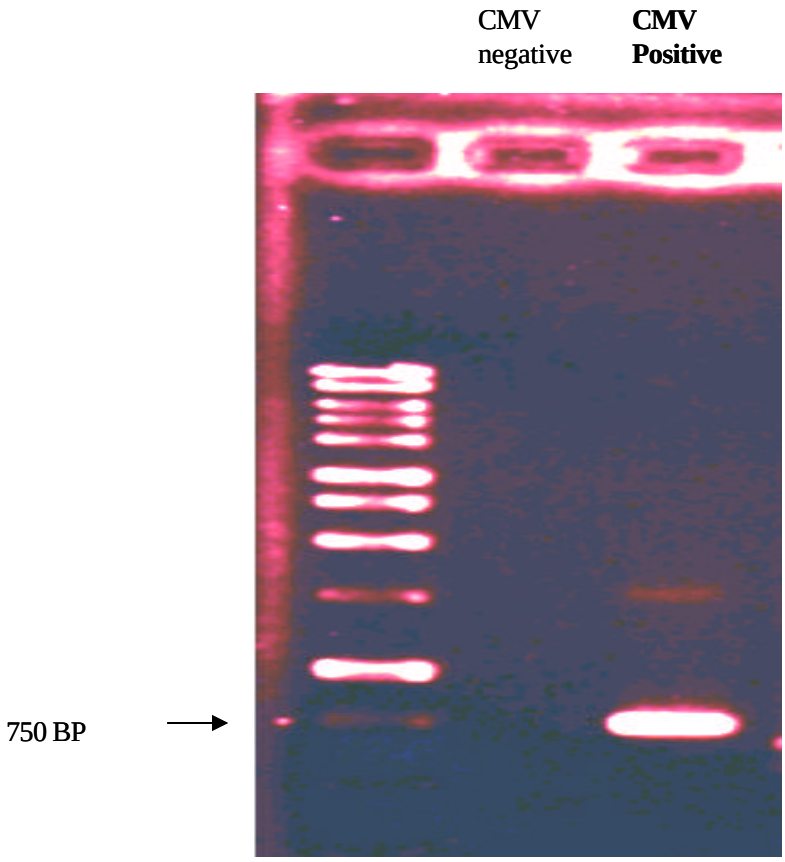

Figure 1. Cucumber mosaic virus (CMV) RT-PCR results

Table 5. Snap Bean Variety Virus Field Evaluation -2001

			ELISA		Pod Traits		
Entry	**Company**	**% Foliar symptoms**	**CMV**	**AMV**	**N. Pods**	**%External necrosis**	**%Internal necrosis**
Bronco	Asgrow	62.5	+++	+	3.6	55	35
B378	Asgrow	39.5	+++	++	3.7	80	25
K. King	Asgrow	60.5	+++	++	3.2	70	10
Cyclone	Asgrow	54.2	++	-	3.1	90	10
B373	Asgrow	33.2	+++	++	3.6	70	45
B402	Asgrow	45.8	++	-	3.2	80	15
Pix	Asgrow	4.5	+++	++	4.4	30	0
Opus	Asgrow	63.7	+++	++	2.7	85	20
Hurricane	Asgrow	80.2	+++	+	3.4	40	15
Grenoble	Asgrow	31.2	+++	+	3.2	60	30
Fandango	Asgrow	17.0	+++	-	3.7	75	30
Flo	Asgrow	52.2	+++	-	3.7	75	30
BBL156	Syngenta	54.2	+++	-	3.6	65	15
SB4243	Syngenta	45.8	+++	-	3.6	55	15
R6004	Syngenta	62.5	+++	-	2.7	35	20
Masai	Syngenta	15.3	+++	-	3.2	55	15
Medinah	Syngenta	98.5	+++	+	2.4	30	0
Indy Gold	Syngenta	37.5	+++	+	3.4	100	5
Charon	Syngenta	68.8	+++	-	3.8	70	10
Castano	Syngenta	75.2	+++	-	3.2	55	15
SB4249	Syngenta	87.8	+++	+	3.3	85	0
SB4247	Syngenta	91.0	+++	+	3.0	55	15
CTD 34	Del Monte	60.5	+++	-	3.4	95	5
CTD 88	Del Monte	93.5	+++	+	3.2	45	20
CTD 94	Del Monte	68.8	+++	+	3.4	30	5
PLS-72	Chiquita	60.5	+++	+	2.6	40	30
BBL-156	Chiquita	78.3	+++	++	3.6	15	10
Hercules	Chiquita	45.8	+++	-	3.6	85	20
Hercules	Chiquita	33.2	++	-	3.6	65	10
Arena	Brotherton	87.8	++	+	3.0	35	10
Evergreen	Brotherton	37.5	+++	-	2.6	25	5
Saurus	Brotherton	78.3	+++	-	2.8	30	10
Dorint	Brotherton	47.8	+++	-	3.4	35	25
Newton	Brotherton	60.5	+++	-	3.5	55	10
Pension	Brotherton	42.7	+++	-	3.6	30	0
Nelson	Brotherton	84.0	+++	++	3.4	40	5
True blue	Harris M.	33.2	+++	-	3.7	35	5
Envy	Harris M.	68.8	+++	+	3.4	25	10
Hystyle	Harris M.	54.2	+++	+	4.4	80	15
FM658	Harris M.	60.5	+++	++	3.4	65	5
Minuette	Harris M.	26.8	+++	-	3.8	30	5
Hialeah	Harris M.	75.2	+++	-	3.4	55	10
Shade	Harris M.	54.1	+++	-	3.4	70	40
Hmx0104	Harris M.	78.3	+++	-	2.6	40	20
Savanna	Harris M.	18.5	+++	-	3.6	45	15
MV185	Harris M.	9.0	+++	-	4.0	35	10
Probability %		< 0.1			4.7	1.4	5.6
LSD 10%		29.4			0.6	24	19

CONCLUDING REMARKS

Cucumber mosaic virus and alfalfa mosaic virus were found to be the most prevalent virus in snap bean samples as well as in the field trial. Results from these studies, suggest that CMV is one of the prime candidates responsible for deterioration of snap bean observed in Wisconsin fields. Alfalfa mosaic virus alone does not appear to have a significant effect on snap bean productivity. The presence of CMV was revealed by ELISA and reconfirmed by RT-PCR. An additional evidence of the detrimental effect of CMV on snap bean was shown by the transmission test. Greenhouse grown snap bean plants inoculated with sap from field samples showed symptoms that were similar to the ones observed in field. Considering the fact that other viruses were also detected in the field samples, and that a wide variation in symptomatology was observed, it raises the question of whether CMV acts alone or in combination with other viruses. Although it is assumed that beans are universally susceptible to CMV, we found varieties that appear to have with partial resistance to CMV, such as Pix and MV-185.

To our understanding, cucumber mosaic virus is not a new pathogen for beans in Wisconsin. Cucumber mosaic virus affecting beans has been reported in previous reports. The virus was first reported as a pathogen to common beans in 1941 in Wisconsin by Whipple and Walker. CMV also has been isolated from pea plants and was capable of infecting several other legumes including beans under a controlled environment. In 1974, a strain of CMV in naturally infected bean plants was found near Geneva, New York (Provvidenti, 1976) and in Illinois (Milbrath et al., 1975). A more recent report by Kostova and Poryazoc (1995) cited that the lack of resistance to CMV in beans is of great concern for breeders in Bulgaria.

It seems that CMV epidemics are sporadic in nature. A better understanding on the role of the newly introduced soybean aphid as well as the effect of seed transmission in the epidemiology of CMV is a crucial element towards the control of this virus.

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