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No-Choice Preference of *Cerotoma trifurcata* (Coleoptera: Chrysomelidae) to Potential Host Plants of Bean Pod Mottle Virus (*Comoviridae*) in Iowa

JEFFREY D. BRADSHAW,^{1,2} MARLIN E. RICE,¹ AND JOHN H. HILL³

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ABSTRACT To better understand the naturally occurring host range of Bean pod mottle virus (family *Comoviridae*, genus *Comovirus*, BPMV) and its principal vector *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae), 18 field-collected perennial plant species were tested for the presence of BPMV. By using no-choice assays, we determined the preference of these plants by bean leaf beetle, by measuring their level of herbivory relative to soybean, *Glycine max* (L.). New food hosts for adult bean leaf beetles include *Lespedeza capitata* (Michaux), *Lotus corniculatus* L., *Trifolium alexandrinum* L., *Trifolium ambiguum* Bieberstein, and *Trifolium incarnatum* L. *Desmodium illinoense* Gray is discovered as a new naturally occurring host for BPMV.

KEY WORDS enzyme-linked immunosorbent assay, Fabaceae, insect herbivory, leaf area measurement, reverse transcription-polymerase chain reaction

The bean leaf beetle, *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae), is endemic to North America and a long-known pest of peas (*Vigna* spp.) (McConnell 1915); beans (*Phaseolus* spp.) (Chittenden 1891, Eddy and Nettles 1930, Aguyoh et al. 2004); and soybean, *Glycine max* (L.) (Eddy and Nettles 1930, Higley and Boethel 1994). The recent need to understand the population dynamics (Lam et al. 2001, Carrillo et al. 2005) and management (Lam et al. 2002; Krell et al. 2004, 2005; Koch et al. 2005) of the bean leaf beetle is probably a response to dramatic increases in its abundance (Bradshaw and Rice 2003, Krell et al. 2003). This abundance is positively correlated to Bean pod mottle virus (family *Comoviridae*, genus *Comovirus*, BPMV) incidence in soybean (Hopkins and Mueller 1984); therefore, large vector populations have probably contributed to an apparent increase in this virus in the north central United States (Bradshaw and Rice 2003).

BPMV, discovered in 1947 (Zaumeyer and Thomas 1948), is a common pathogen of soybean in the Americas (Milbrath et al. 1975, Pitre et al. 1979, Hopkins and Mueller 1983, Lin and Hill 1983, Ghabrial et al. 1990, Fribourg and Perez 1994, Michelutti et al. 2002, Anjos et al. 1999, Sikora and Murphy 2005). It is of serious concern for soybean seed production in the United States (Giesler et al. 2002). This viral disease results in yield and quality losses in soybean (Quiniones et al. 1971, Horn et al. 1973, Myhre et al. 1973, Hopkins and

Mueller 1984, Ragsdale 1984, Giesler et al. 2002, Krell et al. 2003); however, "field tolerance" has been reported recently (Hill et al. 2007).

Although the principal vector of BPMV is the bean leaf beetle (Mueller and Haddox 1980), there are other coleopterous vectors within the Chrysomelidae (Horn et al. 1970, Mabry et al. 2003, Werner et al. 2003), Meloidae (Patel and Pitre 1971), and Coccinellidae (Fulton and Scott 1974); however, the susceptible host range of BPMV, by mechanical inoculation, includes plants in the families Apocynaceae, Chenopodiaceae, and Fabaceae (=Leguminosae) (Caesalpinioideae and Papilionoideae). Nonsusceptible plant hosts include species of Compositae, Cruciferae, Cucurbitaceae, Solanaceae, and Fabaceae (Papilionoideae) (Brunt et al. 1996). Although vector transmission has been demonstrated from *Desmodium paniculatum* (L.) to soybean via bean leaf beetles (Waldbauer and Kogan 1976), the range of possible naturally occurring hosts susceptible to both vector and virus is still unknown. This information could be central to the determination of the primary inoculum source of BPMV.

Krell et al. (2003), upon examination of potential primary inoculum sources, estimated a low frequency of BPMV transmission from seed and overwintered bean leaf beetles of 0.037 and 1.6%, respectively. Additionally, of 23 field-collected plant species tested, only *Desmodium canadense* (L.) was positive for BPMV. Perennial host plants are thought to be an important source for BPMV (Moore et al. 1969, Horn et al. 1970, Stace-Smith 1981, Krell et al. 2003), possibly because of the opportunity for the virus to overwinter within the plant. However, in Iowa, distribution of this

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host plant does not fully explain the temporal appearance and ultimate impact of the disease (Krell et al. 2004).

There is a lack of intensive, replicated sampling for most potential host species for BPMV. Furthermore, bean leaf beetle feeding has been observed on some BPMV hosts (Krell et al. 2003), and host preference has been shown for some Fabaceae (Henn 1989); however, the acceptability of most perennial legumes to bean leaf beetles has not been determined. Therefore, the objective of this study was to investigate the potential host range overlap between the bean leaf beetle and presence of BPMV in nature.

Materials and Methods

Bean Leaf Beetle No-Choice Preference Assays. Female bean leaf beetles, determined by their large size and darkened frons (Kogan et al. 1980, Sims et al. 1984), were field-collected from *Medicago sativa* L. by using a 20-cm sweep net in May 2004 and 2005. They were held in groups of three in 9-cm petri dishes for 48 h (maintained at 24°C under a photoperiod of 16:8 [L:D] h) without access to food or water to cull out weak beetles, to ensure that beetles had similar levels of hunger, and to allow beetles to acclimatize to test conditions.

After acclimatization, beetles (three beetles per petri dish) were given access to one leaflet (with the dish lid and bottom sealed together with electrical tape to prevent moisture loss) from one of the following fabaceous species in 2004 (10 plants of each species were collected the day of the experiment, and six of the 10 plants were assayed): *Amorpha canescens* Pursh; *Astragalus cicer* L.; *G. max* 'Mark RR'; *Lotus corniculatus* L.; *M. sativa*; *Melilotus officinalis* (L.), white sweet clover; *Melilotus officinalis*, yellow sweet clover; *Petalostemum purpureum* (F.); *Robinia pseudoacacia* L.; *Securigera varia* (L.); *Trifolium ambiguum* Bieberstein; *Trifolium hybridum* L.; *Trifolium pratense* L.; and *Trifolium repens* L. In 2005, the following hosts were used (10 plants of each species were collected and three of the 10 plants were assayed): *A. canescens*, *G. max* Mark RR, *L. corniculatus*, *M. officinalis*, *R. pseudoacacia*, *Trifolium alexandrinum* L., *T. ambiguum*, *Trifolium incarnatum* (F.), and *T. pratense*. Dishes were maintained at 24°C under a photoperiod of 16:8 (L:D) h for 24 h (in 2004) or 48 h (in 2005), after which leaflets were removed and pressed until dry. All plants were collected from the Field Extension Education Laboratory, Iowa State University (Boone Co., IA), except for *R. pseudoacacia* (collected from McHose Park, also in Boone Co.).

These plant species were chosen because they are perennials, their vegetative growth overlapped with the emergence of overwintered *C. trifurcata* populations (i.e., they potentially could be primary inoculum sources for BPMV), and they were available free of pesticides. Beetle abundance was limiting to this study during 2004 and 2005; therefore, more plants were collected than assayed. However, all 10 plants were retained for virus assays.

In 2006, bean leaf beetles were collected at a prairie near Ames, IA (42° 07' 48" N, 093° 33' 32" W). Two legumes dominated this location, *Desmodium illinoense* Gray and *Lespedeza capitata* Michaux; they had heretofore not been reported as hosts for *C. trifurcata* or BPMV; however, both had visual evidence of bean leaf beetle injury. Ten plant samples (a nearly complete census from this locality) of both species (one leaflet each) were collected and tested using the conditions described above, except one bean leaf beetle was used per dish and the feeding period was 60 h. For comparison, 10 *G. max* 'Williams' leaflets (grown under glasshouse conditions) were included in this experiment as a control.

Leaflet Area Measurement. Leaflet images were captured with a digital scanner (Hewlett-Packard Scanjet 4670, Hewlett-Packard Co., Houston, TX) in black and white (i.e., 1 bit/pixel) at 286.12 pixels/cm, uploaded to a computer (Dell OptiPlex GX150 with a Pentium 6 processor, Dell USA, Austin, TX), and saved as an uncompressed TIF by using Adobe Photoshop 7.0.1 (Adobe Systems, San Jose, CA). We previously determined that the measurement of two-dimensional objects acquired at 286.12 pixels/cm is not adversely affected by variations in shape or size (unpublished data). For each image, the "Histogram" function in Adobe Photoshop was used to count pixels. For more intuitive comparisons, digitally scanned areas (in pixels) were converted to square centimeters by dividing the number of black pixels into the number of total square pixels per millimeter in the images as determined by Photoshop as follows:

$$\text{Area (mm}^2\text{)} = \text{black pixels}/(\text{total pixels/mm})^2$$

For 2006 experiments, area measurements were taken as described above, except by capturing leaflet images in 16-bit color. Injured areas were selected with the "magic wand tool" or "color selection tool," because the skeletonization of the *D. illinoense* leaflets was such that a very close-knit leaflet skeleton remained in injured areas, which was not accurately captured as a 1-bit image. The area of the selected area was determined using the image histogram as described above.

BPMV Host Assays. Ten samples each of the aforementioned field-collected plants were tested for presence of BPMV by enzyme-linked immunosorbent assay (ELISA), Western blot assay, and polymerase chain reaction (PCR). For ELISA and Western blot, samples were taken from the same plants used for the no-choice beetle assay, extracted in 0.05 M phosphate-buffered saline with 0.05% Tween 20 (PBST), pH 7.2, and held at -20°C. Samples of plant species (except *D. illinoense* and *L. capitata*) were combined and concentrated using 2-ml filtration devices with a 30,000-mol. wt. filter (Millipore Corporation, Billerica, MA) and centrifuged at 4,500 × *g* for 1 h. The ELISA and Western blot procedures were similar to those used by Krell et al. (2003). *D. illinoense* and *L. capitata* were extracted in PBST, pH 7.2, containing 2% polyvinylpyrrolidone (PVPP) and 1% sodium hydrosulfite. The later extraction buffer was

Table 1. Fabaceae tested by no-choice assay for herbivory and acceptability by adult *C. trifurcata*

Yr of exp (duration)	Species	Common name	Mean area consumed (mm ²) ^a	Confidence interval (95%)		P value	Fraction of leaflets with herbivory
				Lower	Upper		
2004 (24 h)	<i>Amorpha canescens</i> Pursh	Lead plant	0.12	-0.07	0.09	0.0002	1/6
	<i>Astragalus cicer</i> L.	Cicer milkvetch	0.00	-0.08	0.08		0/6
	<i>Glycine max</i> (L.)	Soybean (Mark RR)	2.29	0.15	0.31		6/6
	<i>Lotus corniculatus</i> L.	Birdsfoot trefoil	<0.01	-0.08	0.08		1/6
	<i>Melilotus officinalis</i> (L.)	Yellow sweet clover	0.00	-0.08	0.08		0/6
	<i>Medicago sativa</i> L.	Alfalfa	<0.01	-0.08	0.08		1/6
	<i>Medicago officinalis</i> (L.)	White sweet clover	0.16	-0.06	0.10		1/6
	<i>Petalostemum purpureum</i> (F.)	Purple prairie clover	0.00	-0.08	0.08		0/6
				-0.08	0.08		1/6
				-0.08	0.08		1/6
				-0.08	0.08		1/6
2005 (48 h)	<i>Trifolium pratense</i> L.	Red clover	0.07	-0.07	0.09	0.0278	3/6
	<i>Trifolium repens</i> L.	White clover	<0.01	-0.06	0.10		4/6
	<i>Trifolium ambiguum</i> Bieberstein	Kura clover	2.07	0.13	0.29		3/6
	<i>Amorpha canescens</i> Pursh	Lead plant	0.01	-0.52	0.52		1/3
	<i>Glycine max</i> (L.)	Soybean (Mark RR)	13.44	0.82	1.86		3/3
	<i>Lotus corniculatus</i> L.	Birdsfoot trefoil	1.57	-0.36	0.68		2/3
	<i>Melilotus officinalis</i> (L.)	White sweet clover	0.02	-0.52	0.52		1/3
	<i>Robinia pseudoacacia</i> L.	Black locust	0.52	-0.47	0.57		2/3
	<i>Trifolium alexandrinum</i> L.	Berseem clover	0.21	-0.50	0.54		3/3
	<i>Trifolium ambiguum</i> Bieberstein	Kura clover	2.20	-0.30	0.74		3/3
	<i>Trifolium incarnatum</i> (F.)	Crimson clover	1.21	-0.40	0.64		3/3
2006 (60 h)	<i>Trifolium pratense</i> L.	Red clover	0.69	-0.45	0.59	0.0001	2/3
	<i>Desmodium illinoense</i> (L.)	Illinois ticktrefoil	1.34	-0.13	0.40		10/10
	<i>Glycine max</i> (L.)	Soybean (Williams)	9.42	0.67	1.21		9/10
	<i>Lespedeza capitata</i> (Michaux)	Roundhead lespedeza	0.15	-0.25	0.28		3/10

See corrected Table 1, following page 814

^a Mean area consumed is estimated from three (years 2004 and 2005) or one (year 2006) beetles per leaflet.

found to eliminate false positives (by ELISA) for many legumes (J.D.B., unpublished data) and simplified the search for BPMV hosts by ELISA.

To further exclude the possibility of a false positive, total RNA was extracted from immunopositive plants and tested by reverse-transcription (RT)-PCR. Plant samples from immunopositive plants were collected into liquid N₂ and stored at -80°C. To extract total RNA, ≈100 μg of frozen plant tissue was added to 1 ml of TRIzol (Invitrogen, Carlsbad, CA), vortexed for 15 min at room temperature, and then 300 μl of chloroform was added, and the sample was vortexed for 10 min. Samples were centrifuged at 3,900 × g at 4°C for 10 min, and the supernatant was extracted twice more with chloroform. The RNA was precipitated from the supernatant by addition of an equal volume of cold (-20°C), ribonuclease-free 70% isopropanol followed by incubation for 30 min at room temperature. The preparation was centrifuged at 18,320 × g at 4°C to recover the pellet, which was washed using 300 μl of cold (-20°C), ribonuclease-free 70% ethanol. The pellet was air-dried for ≈5 min and suspended in ribonuclease-free distilled water.

Reverse transcription and PCR protocols were followed according to Takara, version 3.0 (Takara Bio Inc., Otsu, Japan), by using random 9-mers for reverse transcription primers and BPMV, RNA1-specific forward (3'-TGTGCTACCATTGCAGTTTCTA-5') and reverse (3'-AAGTTTGGTCTACAACATAATGA-5') PCR primers. Avian myeloblastosis virus reverse transcriptase was used for RNA transcription, and Ex Taq-HS (Takara Bio Inc.) was used as a DNA poly-

merase for PCR. The dNTPs for the PCR are supplied in this Takara kit as a separate reagent. Conditions for reverse transcription were 30°C (10 min), 42°C (60 min), 94°C (5 min), and 4°C (hold) and for PCR were 94°C (2 min), 32 cycles [94°C (30 s), 52°C (30 s), 68°C (5 min)], 68°C (15 min), and 8°C (hold) by using a MiniCycler thermocycler (MJ Research, Watertown, MA).

Data Analysis. Data were analyzed using the mixed model procedure in SAS (PROC MIXED, SAS Institute 2003). For leaflet area consumed, analysis of variance (ANOVA) was used to determine differences between host plant herbivory. Estimates were considered statistically significant if the P value was <0.05, and comparisons were different where their 95% confidence interval for the estimate did not overlap.

Results and Discussion

Bean Leaf Beetle Food Host Assays. Two issues were considered in assessment of bean leaf beetle herbivory. First, the likely host range on perennial legumes, and second, a comparison of herbivory on potential hosts plants with that on soybean. To assess the first issue, the number of leaflets with any feeding was counted and if greater than one, the plant species was assumed to be a likely host (i.e., an acceptable host). To assess the second issue, estimates of leaflet area consumed were compared with soybean to indicate a degree of preference relative to soybean.

The following perennial plants were determined as acceptable hosts for adult bean leaf beetle in Iowa: *D.*

Table 2. Food plants of adult *C. trifurcata*

Family	Scientific name ^{a,b}	Common name(s) ^{a,b}	Reference	
Fabaceae	<i>Amphicarpea bracteata</i> (L.)	American hogpeanut	Chittenden 1897	
	<i>Desmodium canadense</i> (L.)	Sow ticktrefoil	Krell et al. 2003	
	<i>Desmodium canescens</i> (L.)	Hary ticktrefoil	McConnell 1915	
	<i>Desmodium cuspidatum</i> (Muhlenberg ex Willdenow)	Largebract ticktrefoil	Waldbauer and Kogan 1976 ^c	
	<i>Desmodium illinoense</i> Gray	Illinois ticktrefoil	Waldbauer and Kogan 1976 ^{cd}	
	<i>Desmodium laevigatum</i> (Nuttall)	Smooth ticktrefoil	Chittenden 1897	
	<i>Desmodium paniculatum</i> (L.)	Panicledleaf ticktrefoil	Moore et al. 1969	
	<i>Desmodium tortuosum</i> (Swartz)	Dixie ticktrefoil	Chittenden 1898, Eddy and Nettles 1930	
	<i>Glycine max</i> (L.)	Soybean	McConnell 1915	
	<i>Lespedeza capitata</i> (Michaux)	Roundhead lespedeza	— ^d	
	<i>Lespedeza</i> spp.		Chittenden 1891	
	<i>Lotus corniculatus</i> L.	Birdsfoot trefoil	— ^d	
	<i>Phaseolus lunatus</i> L.	Sieva bean	Henn 1989	
	<i>Phaseolus vulgaris</i> L.	Kidney bean	Chittenden 1897	
	<i>Robinia pseudoacacia</i> L.	Black locust	Chittenden 1897 ^d	
	<i>Strophostyles helvola</i> (L.)	Amberique-bean	Waldbauer and Kogan 1976 ^c	
	<i>Trifolium alexandrinum</i> L.	Egyptian clover	— ^d	
	<i>Trifolium ambiguum</i> Bieberstein	Kura clover	— ^d	
	<i>Trifolium pratense</i> L.	Red clover	Davis 1950 ^d	
	<i>Trifolium incarnatum</i> L.	Crimson clover	— ^d	
	<i>Trifolium repens</i> L.	White clover	Henn 1989	
	<i>Vigna aconitifolia</i> (Jacquin)	Moth bean	McConnell 1915	
	<i>Vigna angularis</i> (Willdenow)	Adzuki bean	Henn 1989	
	<i>Vigna radiata</i> (L.)	Mung bean	Henn 1989	
	<i>Vigna unguiculata</i> (L.)	Blackeyed pea	Henn 1989	
	<i>Vigna u. sesquipedalis</i> (L.)	Yardlong bean	Henn 1989	
	<i>Wisteria floribunda</i> (Willdenow)	Japanese wisteria	Staines 1986	
	Celastraceae	<i>Euonymus atropurpureus</i> Jacquin	Burningbush	Helm et al. 1983
	Urticaceae	<i>Urtica dioica</i> L.	Stinging nettle	Helm et al. 1983
		<i>Laportea canadensis</i> (L.)	Canadian woodnettle	Helm et al. 1983
Cucurbitaceae	<i>Cucurbita pepo</i> L. ^e	(i.e., pumpkin 'Magic lantern' and squash 'Turk's turbin')	Koch et al. 2004	
	<i>Cucumis sativus</i> L. ^e	Garden cucumber	Koch et al. 2004	
Poaceae	<i>Zea mays</i> L.	Corn	Metcalf and Metcalf 1993	

^a Scientific and common names taken from the PLANTS database (USDA 2006).

^b Plant species are listed as food plants where direct herbivory is reportedly observed or where eggs are found near the plant.

^c Indirect evidence—host plant evidence based on the presence of eggs.

^d This record is reported first or confirmed in this manuscript.

^e Only the cucurbit varieties and common names were provided by Koch et al. (2004). *C. pepo* and *C. sativus* are inferred for pumpkin and squash, respectively.

illinoense, *L. corniculatus* (new host), *R. pseudoacacia*, *T. alexandrinum* (new host), *T. ambiguum* (new host), *T. incarnatum* (new host), *T. pratense*, *T. repense*, and *L. capitata* (new host) (Table 1). Additionally, for *L. capitata*, *T. ambiguum*, and *D. illinoense*, one of us (J.D.B.) has observed and found evidence of bean leaf beetle feeding on leaflets of these plants in nature during May and June.

Overall, soybean was the most acceptable host plant in this study, i.e., had the greatest proportion of leaflets with herbivory (Table 1). Additionally, compared with soybean, most perennial hosts support significantly less herbivory (Table 1). However, because of leaflet thickness, the area of herbivory on *D. illinoense* may represent an underestimate. Natural host plants of the bean leaf beetle (e.g., *L. capitata* and *D. illinoense*) received significantly less herbivory than soybean (Table 1, 2006 experiment). Herbivory on *T. ambiguum* was significantly less in 48 h

of exposure than that on soybean, but it was not significantly less within 24 h (Table 1, 2004 and 2005 experiment). This finding indicates that the rate of herbivory may differ between hosts; however, there were half the number of replicates in 2005 as in 2004. Every test plant received some feeding. However, some plants received <0.01 mm² of herbivory in 24 h, which is well within the random error for the scanner used in this study, ±0.06 mm² (unpublished data). Therefore, pairwise comparisons for herbivory ≤0.06 mm² are not meaningful. In the final assay, both *D. illinoense* and *L. capitata* received significantly less feeding than soybean (Table 2, 2006 experiment). However, *D. illinoense* received more feeding than *L. capitata*.

Some species (e.g., *T. repense*) received an average herbivory of <0.01 mm² with more than half of the leaflets receiving some herbivory (Table 1, 2004 experiment). Although leaflets in this experiment ap-

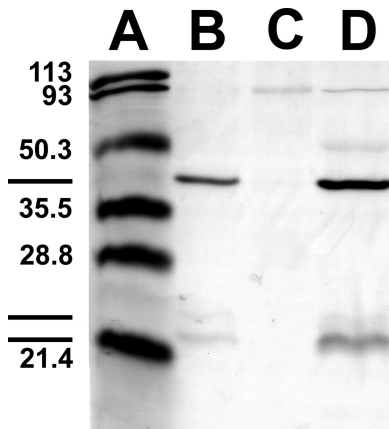


Fig. 1. Western blot for the detection of BPMV in *D. illinoense*. (A) Protein marker, units in molecular mass (kilodaltons). (B) BPMV-infected soybean (Williams) sap. (C) Noninfected soybean (Williams) sap. (D) BPMV-infected *D. illinoense* from total protein extraction. Solid bars to the left of image indicate expected position for the three bands associated with BPMV coat protein: large protein, 41 kDa and small protein, ≈ 22 kDa (consisting of two migration forms).

parently did not differ greatly in observed thickness (unpublished data), physical factors can mediate *C. trifurcata* preference, e.g., trichome density (Lam and Pedigo 2001). The involvement of chemical host factors in *C. trifurcata* host preference has not been studied.

BPMV Host Assay. Of all the tested plants, only *D. illinoense* was positive for BPMV by ELISA, Western blot (Fig. 1), and RT-PCR (Fig. 2). On a Western blot, sap extracted from *D. illinoense* gave bands that corresponded to the large and small coat protein subunits of BPMV and corresponded to similar bands in a BPMV-infected soybean plant. Additionally, of the 10 *D. illinoense* tested, all 10 plants were positive for BPMV. Furthermore, RT-PCR of the total RNA from *D. illinoense* leaflets yielded the

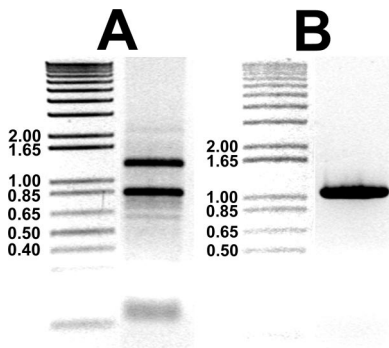


Fig. 2. Total extracted RNA (A, marker, left lane in kilobases [kb]; sample, right lane) and cDNA from RT-PCR of BPMV RNA1 (using a primer pair with an expected product size of 1.037 kb) from *D. illinoense* (B, marker, left lane [kb]; sample, right lane) in ethidium bromide-stained agarose gel.

expected product size for an RNA1 cDNA-specific primer pair (Fig. 2B). This is the first report of BPMV from *D. illinoense*.

Many plants (e.g., *A. canescens*, *T. pratense*, and *R. pseudoacacia*) yielded false positives by ELISA, as was similarly noted by Krell et al. (2003), via PBST sap extraction. However, sap extracted from *R. pseudoacacia* (used as a false-positive control in this test), *D. illinoense*, and *L. capitata*, using PBST containing PVPP and sodium hydrosulfite, resulted in no false positives compared with sap extracted in PBST alone. Similar results have been noted when extracting sap from various legumes (unpublished data).

The bean leaf beetle may have a broader host range (Table 2) than the natural host range for BPMV. Searches for the natural reservoir for this virus have often found *Desmodium* spp. as an important source for this virus (Moore et al. 1969, Walters and Lee 1969, Lee and Walters 1970, Krell et al. 2003). Of the plant species listed on the Virus Identification Data Exchange database (Brunt et al. 1996) as hosts of BPMV, ≈ 16 species are susceptible and 21 species are nonsusceptible; however, these conclusions are based primarily on mechanical inoculations. Species such as *T. incarnatum* are listed as susceptible hosts, whereas *T. pratense* and *T. rapens* are nonsusceptible. In this study, BPMV was not found to occur naturally in any of these hosts. If BPMV requires the activity of certain ribonucleases for efficient transmission (Gergerich et al. 1986; Gergerich and Scott 1988a, 1988b), and if some legumes contain factors that inhibit ribonuclease activity, there may be a discontinuity between the natural host range of BPMV by mechanical inoculation and that by beetle transmission.

Plants such as *T. ambiguum* and *L. capitata* were negative for BPMV, even though bean leaf beetle herbivory and many beetles were found on these plants. If such plants truly represent nonsusceptible hosts for BPMV, it may be possible that bean leaf beetles "clean" themselves of virus in nature. Furthermore, such BPMV nonhosts could be used as a trap crop for both virus and bean leaf beetles.

Sixteen species of *Desmodium* are found in Iowa (USDA 2006), and, of these species, three species are now known to be susceptible to BPMV in nature (Moore et al. 1969, Krell et al. 2003). Other *Desmodium* spp. should be assayed to determine the wild host range of this virus. The distribution and abundance of these hosts are not well known in Iowa; however, this knowledge may be helpful in understanding BPMV epidemics. Furthermore, simply identifying a susceptible host plant is insufficient to determine its potential impact on the pathosystem. BPMV exists as more than one subgroup population in nature (Gu et al. 2002) that is associated with varying degrees of symptom severity. The *Desmodium* BPMV isolate found in this study is currently being characterized.

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Corrections. In the article, “No-Choice Preference of *Cerotoma trifurcata* (Coleoptera: Chrysomelidae) to Potential Host Plants of Bean Pod Mottle Virus (*Comoviridae*) in Iowa,” by Jeffrey D. Bradshaw, Marlin E. Rice, and John H. Hill, published in the [Journal of Economic Entomology \(100: 808–814\)](#), the *Confidence interval (95%)* columns for [Table 1](#), p. 810, are all one decimal place off. The corrected [Table 1](#) is below. The authors regret the error.

Yr of exp (duration)	Species	Common name	Mean area consumed (mm ²) ^a	Confidence interval (95%)		P value	Fraction of leaflets with herbivory
				Lower	Upper		
2004 (24 h)	<i>Amorpha canescens</i> Pursh	Lead plant	0.12	-0.7	0.9	0.0002	1/6
	<i>Astragalus cicer</i> L.	Cicer milkvetch	0.00	-0.8	0.8		0/6
	<i>Glycine max</i> (L.)	Soybean (Mark RR)	2.29	1.5	3.1		6/6
	<i>Lotus corniculatus</i> L.	Birdsfoot trefoil	<0.01	-0.8	0.8		1/6
	<i>Melilotus officinalis</i> (L.)	Yellow sweet clover	0.00	-0.8	0.8		0/6
	<i>Medicago sativa</i> L.	Alfalfa	<0.01	-0.8	0.8		1/6
	<i>Medicago officinalis</i> (L.)	White sweet clover	0.16	-0.6	1.0		1/6
	<i>Petalostemum purpureum</i> (F.)	Purple prairie clover	0.00	-0.8	0.8		0/6
	<i>Robinia pseudoacacia</i> L.	Black locust	0.02	-0.8	0.8		1/6
	<i>Securigera varia</i> (L.)	Crown vetch	<0.01	-0.8	0.8		1/6
	<i>Trifolium hybridum</i> L.	Alsike clover	0.01	-0.8	0.8		1/6
	<i>Trifolium pratense</i> L.	Red clover	0.07	-0.7	0.9		3/6
	<i>Trifolium repens</i> L.	White clover	<0.01	-0.6	1.0		4/6
	<i>Trifolium ambiguum</i> Bieberstein	Kura clover	2.07	1.3	2.9		3/6
	2005 (48 h)	<i>Amorpha canescens</i> Pursh	Lead plant	0.01	-5.2		5.2
<i>Glycine max</i> (L.)		Soybean (Mark RR)	13.44	8.2	18.6	3/3	
<i>Lotus corniculatus</i> L.		Birdsfoot trefoil	1.57	-3.6	6.8	2/3	
<i>Melilotus officinalis</i> (L.)		White sweet clover	0.02	-5.2	5.2	1/3	
<i>Robinia pseudoacacia</i> L.		Black locust	0.52	-4.7	5.7	2/3	
<i>Trifolium alexandrinum</i> L.		Berseem clover	0.21	-5.0	5.4	3/3	
<i>Trifolium ambiguum</i> Bieberstein		Kura clover	2.20	-3.0	7.4	3/3	
<i>Trifolium incarnatum</i> (F.)		Crimson clover	1.21	-4.0	6.4	3/3	
<i>Trifolium pratense</i> L.		Red clover	0.69	-4.5	5.9	2/3	
2006 (60 h)		<i>Desmodium illinoense</i> (L.)	Illinois ticktrefoil	1.34	-1.3	4.0	0.0001
	<i>Glycine max</i> (L.)	Soybean (Williams)	9.42	6.7	12.1	9/10	
	<i>Lespedeza capitata</i> (Michaux)	Roundhead lespedeza	0.15	-2.5	2.8	3/10	

^a Mean area consumed is estimated from three (years 2004 and 2005) or one (year 2006) beetles per leaflet.