Crop and Non-Crop Plants as Potential Reservoir Hosts of *Alfalfa mosaic virus* and *Cucumber mosaic virus* for Spread to Commercial Snap Bean

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Abstract

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Diseases caused by aphid-transmitted viruses such as *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV) have increased in snap bean (*Phaseolus vulgaris*) in the Midwestern United States. Plants immediately surrounding agricultural fields may serve as primary virus inocula for aphids to acquire and transmit to bean crops. The project objectives were to (i) identify potentially important AMV and CMV reservoirs among naturally infected plants and (ii) determine the relationship between the virus inoculum potential (VIP) in adjacent crop field margins and virus incidence in *P. vulgaris*. From 2006 to 2008, surveys were conducted to quantify the virus incidence and percentage cover (2008 only) of plants present within 5 m of the *P. vulgaris* crop. In all, 4,350 individual plants representing 44 species were assayed, with overall AMV and CMV incidences averaging 12 and 1.5%, respectively. A VIP index was developed and used to rank the importance of virus-susceptible plants in adjacent field margins. The overall VIP index for AMV in field margins was weakly associated with AMV incidence in *P. vulgaris* and no relationship was observed between local CMV inoculum and *P. vulgaris* incidence, suggesting that factors additional to local inoculum sources may influence CMV epidemics in *P. vulgaris*.

Since the establishment of the soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae) in 2000, viral diseases have become more prevalent in commercially grown crops in the Midwestern United States. Soybean (*Glycine max* (L.) Merrill), snap bean (*Phaseolus vulgaris* L.), potato (*Solanum tuberosum* L.), and pepper (*Capsicum annuum* L.) are a few examples of cultivated plants where increased incidence of aphid-transmitted viruses have been observed and have resulted in significant crop loss (5,29,34,35,40). In Wisconsin, yield reductions in *P. vulgaris* have been attributed, in part, to the abortion of developing flowers as well as malformed, unmarketable pods caused by infections of nonpersistently transmitted viruses, such as *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV) (17,27).

As members of the virus family *Bromoviridae*, AMV and CMV can infect a wide variety of host species. Over 600 plant species are reported to be susceptible to AMV infection, especially plants of the Fabaceae family. AMV generally incites characteristic yellow, bright-green "mosaic" foliar discoloration throughout the leaf surface and, in *P. vulgaris*, AMV infections are often localized but occasionally produce systemic symptoms such as stunting, yellow spotting on leaves, and pod deformation (22,23,52,53). CMV can infect over 885 plant species, resulting in numerous foliar symptoms, including vein clearing, leaf blistering, and pod distortion in *P. vulgaris* (38,39). Additional symptoms induced by CMV in *P. vulgaris* include green mottling, dark green vein banding, and a zipper-like rugosity along the major veins; however, these symptoms can vary with growth stages of the infected bean plant (18).

Transmission of both AMV and CMV is known to occur through seed and both viruses are readily sap transmitted; however, rapid virus spread is often the result of transmission by aphid vectors (18,23,49). Most aphid species have a narrow host range but will test probe many non-host species, which enables the insect to acquire nonpersistent viruses during brief feeding bouts from a variety of different plant species. Random alightment of winged aphids on a combination of both host and non-host plants followed by brief feeding probes allows for successful transmission of nonpersistent viruses (25). Therefore, adjacent crops, infected alternative weed hosts, and perennial pastures containing legumes may all be considered as candidate sources of inocula for aphids to acquire and transmit AMV and CMV to *P. vulgaris*.

There are previous reports of massive flights of winged aphids leading to viral epidemics in agricultural crops considered to be non-hosts for the insect. In eastern Nebraska, for example, high populations of the pea aphid (*Acyrthosiphon pisum* L.) played an important role acquiring AMV from naturally infected alfalfa (*Medicago sativa* L.) and spreading the virus to nearby *G. max*, a non-host species for the aphid (2). Similarly, large numbers of rusty plum aphids (*Hysteroneura setariae* (Thomas)) also led to a severe outbreak of CMV in non-host sugar beet (*Beta vulgaris* subsp. *vulgaris* L.) plantings (4). More importantly, the relatively high abundance, along with other epidemiological factors such as dispersal time and virus transmission efficiencies, of *Aphis glycines* and yellow clover aphid (*Therioaphis trifolii* (Monell)) link these aphid species with severe virus epidemics in *P. vulgaris* fields (1,8,15,34).

In the Wisconsin agricultural landscape, diverse plant communities are found in roadside ditches and along crop field edges. Plants common to these areas have previously been documented to be naturally susceptible to virus infection and could be potential sources of virus inocula for aphids to acquire and subsequently transmit to *P. vulgaris* plants. Surveys of plants in earlier years along areas neighboring commercial crop fields in Dane and surrounding counties of Wisconsin led to the detection of CMV in nine wild plant species. They include carpetweed (*Mollugo verticillata* L.), catnip (*Nepeta cataria* L.), common milkweed (*Asclepias syriaca* L.), flowering spurge (*Euphorbia corollata* L.), ground cherry (*Physalis* spp.), motherwort (*Leonurus cardiaca* L.), pokeweed (*Phytolacca americana* L.), white cockle (*Silene latifolia* L.), and wild cucumber (*Echinocystis lobata* (Michx.) Torr. & A. Gray) (11,12). A more recent study in Dane county detected AMV in *Medicago sativa*, red clover (*Trifolium pratense* L.), white clover (*T. repens* L.), and bird's-foot trefoil (*Lotus corniculatus* L.), located in non-crop areas (1).

Taken together, these surveys have identified several plants that are susceptible to infection by either AMV or CMV but their epidemiological importance as virus reservoirs in the local environment

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has not been fully characterized. The primary goals of this study were to (i) identify plant species in unmanaged areas surrounding crop fields, (ii) quantify percent infection of AMV and CMV in these plants and adjacent crops, (iii) develop a virus inoculum potential (VIP) index to classify virus-susceptible plants according to their level of importance as local inoculum sources of AMV and CMV, and (iv) establish the relationship between incidence of AMV and CMV in *Phaseolus vulgaris* and adjacent field margins.

Materials and Methods

Plant composition in field margins. In the 2008 growing season, a quadrat sampling was performed to characterize plant community composition in non-crop field margins adjacent to commercial *P. vulgaris* fields. Fourteen commercial fields of *P. vulgaris* in Wisconsin were chosen based on planting date, cultivar, and geographical location (Fig. 1). Only plant vegetation residing in unmanaged areas extending 3 to 5 m from the crop edge was assessed using a square quadrat frame measuring 961 cm^2. The quadrat frame was randomly tossed five times at intervals of 5 m or greater for each of the four field edges, totaling 20 quadrat tosses around the perimeter of each *P. vulgaris* field. For every toss, percent cover of the plant species present within the quadrat was recorded. The number of stems was counted for each plant species and divided by the total number of plant stems within the quadrat to generate percent cover values. Only members of the families Asteraceae and Poaceae were taxonomically grouped at the family level whereas others were classified to species. Unknown plants were identified using weed taxonomic guides and the Wisflora: Checklist of the Vascular Plants of Wisconsin website (www.botany.wisc.edu/wisflora/), along with consultation with weed science specialists (51). Percent cover values were averaged by plant species (or family) across fields (n = 14) and mean comparisons were performed using a mixed-model analysis of variance with quadrat assigned as a random factor (41).

Plant virus survey. In summer 2006, 2007, and 2008, virus surveys were conducted to estimate the incidence of AMV and CMV in crop and non-crop plant species abundant in the agricultural landscape. Field selection was based on geographical location and proximity to commercial *P. vulgaris* fields, resulting in 21, 16, and 14 sites for the respective years (Fig. 1). The minimum distance between field sites was 4 km and each field site consisted of two habitat types: (i) commercial field of *P. vulgaris* and (ii) 5 m of field margin adjacent to *P. vulgaris* crop edges. Because *P. vulgaris* is a short-season crop (approximately 60 days), six and seven of the field sites in 2006 and 2007, respectively, were double-cropped (early- and late-season) fields of *P. vulgaris*. In Wisconsin, *P. vulgaris* were characterized as early-season if planted in May or June and harvested prior to 19 July or considered late-season if planted in June or July and harvested on or after 19 July.

Leaf samples were taken from the following areas at a field site: (i) the *P. vulgaris* crop, (ii) other commercial crops bordering the *P. vulgaris* crop, and (iii) the 5-m margin of non-crop area surrounding the *P. vulgaris* crop. Sampling effort in the 5-m field margin included plants in unmanaged areas (i.e., ditches, roadsides, and crop borders) as well as other commercial crops planted immediately adjacent to (within 5 m of) *P. vulgaris* fields. Virus incidence was estimated in *P. vulgaris* fields during the 2007 season only whereas, in 2006, 2007, and 2008, virus incidence estimates were taken for adjacent crop and non-crop plants.

Virus sampling in adjacent field margins. The adjacent non-crop sampling sites used in 2008 for characterizations of the non-crop plant community were the same as those used for virus sampling. At each field site, leaf samples were taken from the predominant plant species present in field margins extending 3 to 5 m from each of the four *P. vulgaris* crop edges. In an effort to estimate virus incidence in plants that matured at different times throughout the growing season, leaf samples were taken at staggered times in each season, beginning in June and ending in September, for a total of two sample points per field site. At least 2 but no more than 15 replicate plants per species were randomly sampled per field edge at a field site. Individual leaf samples were split in two; one half was used for serological assays and the other half was preserved at –20°C for mechanical inoculation experiments (see below). Halved leaf samples were pooled into three sets of five, according to field edge and location, and then serologically tested for the presence of AMV and CMV. For each different crop that bordered the *P. vulgaris* field, leaflets from a minimum of 20 individual crop plants were randomly chosen, grouped into replicate sets of five plants for a total of four (or more) leaf groups, and serologically assayed for AMV and CMV.

Virus sampling in P. vulgaris. Beginning from 10 m inside the *P. vulgaris* crop edge and heading toward the center of the field along a diagonal transect, 100 leaf samples were randomly collected from plants within each *P. vulgaris* field at two time points in the season: (i) seedling emergence and (ii) just prior to harvest. The initial sampling at seedling emergence was conducted to estimate the initial level of AMV and CMV inocula in *P. vulgaris* at the beginning of the season. Excised leaves were pooled into sets of five for a total of 20 leaf groups per field and serologically assayed (see below) for the presence of AMV and CMV.

Serological analysis. Double-antibody sandwich, enzyme-linked immunosorbent assay (ELISA) kits (Agdia Inc., Elkart, IN; AC Diagnostics Inc., Fayetteville, AR) using polyclonal antibodies of AMV or CMV were used to detect virus infection in sampled plant tissue. The testing procedure was followed according to the manufacturer's instructions, with only minor changes. Two sap droplets (approximate volume = 0.1 ml) from the top half portion of leaflet groups (multiples of five plants according to plant species) or individual leaves were extracted using a leaf press (Ravenel Specialties Corp., Seneca, SC) and placed in 2.0-ml polypropylene microcentrifuge tubes (Fisher Scientific, Pittsburgh) with 1 ml of general extract buffer (GEB; Agdia, Inc.). The remaining bottom half portion of leaflet groups (or leaves) was appropriately labeled and kept at 4°C for potential serological retests. Individual test wells consisted of consolidated plant sap of grouped field samples. Healthy (i.e., noninfected) leaf tissue from greenhouse-raised plants (negative control), manufacturer-supplied virus-infected tissue (positive control), and buffer controls of GEB only were included for each microtiter plate. Absorbance readings were measured using an EL800 Universal plate reader (Biotel Instruments, Inc., Winooski, VT) at 405 nm, calculating absorbance values for sample wells based on blank test well readings. A plant sample was considered virus-infected if ELISA absorbance readings were greater than three standard deviations of average absorb-

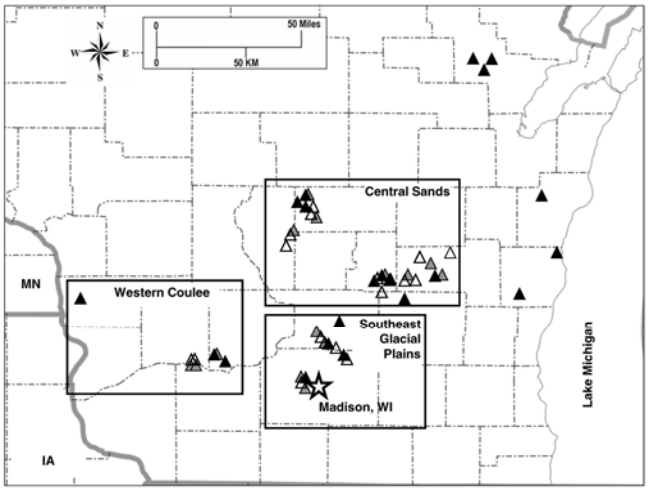

Fig. 1. Geographic distribution of field sites in Wisconsin where virus surveys were conducted in commercial *Phaseolus vulgaris* crops and non-crop field margins. Symbols portray the state capital, Madison (star); plant virus survey field sites in 2006 (black triangles) and 2007 (white triangles); and plant vegetation survey field sites in 2008 (gray triangles). Boxes outline three distinct ecological regions (Western Coulee, Central Sands, and Southeast Glacial Plains) of Wisconsin.

ance values of the negative controls. Positive results of pooled leaf samples were recorded and reserved leaf tissue of individual samples from those sample test wells, with the exception of field samples of *P. vulgaris*, were individually retested for the presence of virus using a similar sap extraction and serological testing procedure.

Incidence estimates of AMV and CMV in *P. vulgaris* were performed based on maximum likelihood calculations from ELISA results of leaf samples collected for each sampling date in 2007 (30). Incidences of AMV and CMV in the surrounding field margins were estimated using the number of ELISA-positive leaf samples divided by the total number of plants sampled in the adjacent commercial crop and non-crop area for a particular field and sampling date. Field estimates of virus incidence in the adjacent field margins were then averaged across years for the summer and fall months of June, July, August, and September.

Mechanical transmission of AMV and CMV. Nonspecific binding of antibodies to plant proteins in weed extracts can be problematic when using serological tests to detect plant viruses (32). As an additional confirmation of virus infections in field-collected plants, a subsample of ELISA-positive leaf samples was mechanically sap-inoculated to healthy *P. vulgaris* as well as a healthy, greenhouse-raised plant of a species similar to the field-collected plants. For each virus (AMV and CMV), at least two ELISA-positive samples were randomly selected from each virus-infected plant species at an individual field collection site to be used in mechanical transmission assays. Virus was mechanically inoculated to two types of host plants: (i) a susceptible cultivar of *P. vulgaris*, 'Hystyle', and (ii) a young, healthy greenhouse-raised plant of the same species as the virus-infected source plant. Mechanical inoculations took place when *P. vulgaris* Hystyle plants were at the expanded unifoliate or first trifoliate leaf stage whereas other plant species were inoculated at early vegetative growth stages, ranging from 1 to 3 weeks post emergence of seedlings. Infectious sap was prepared by individually grinding half portions of reserved field-collected ELISA-positive leaf tissue in phosphate-buffered saline (pH 7.2) at an approximate 1:10 (wt/vol) dilution. Expressed sap was inoculated to primary leaves of recipient plants that had been dusted with carborundum (320-mesh grit powder; Fisher Scientific). Four *P. vulgaris* plants and three plants of wild host species were used for each mechanical inoculation attempt, with an additional two plants of each species serving as noninoculated controls. Inoculated plants were incubated for 10 to 14 days, visually compared with noninoculated control plants for foliar symptoms, and subsequently tested serologically for the presence of either virus. Visual assessment of symptomatic foliage was based on a presence (+) or absence (–) rating scale and virus symptoms used for foliar ratings included any of the following: leaf blistering or curling, mosaic discoloration, or stunted plant growth.

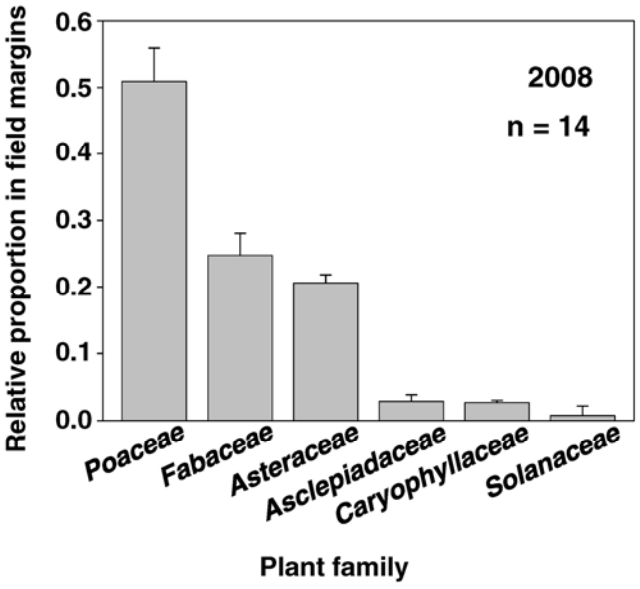

Fig. 2. Relative abundance of plant species at the family level that inhabit unmanaged margins adjacent to 14 commercial *Phaseolus vulgaris* fields in Wisconsin in 2008. Error bars represent the standard error of the mean.

VIP index. A VIP index was created for both AMV and CMV to classify plant species according to their level of risk as epidemiologically important sources of virus inoculum. The VIP index value at a particular field sampling site (i) for plant species (j) is defined as $VIP_{ij} = [(\%\ cover_j) \times (\%\ virus_j)]/1{,}000$, where $i = 1, \ldots, m$; $j = 1, \ldots, n$; m is the number of field sites where virus and plant surveys were conducted; and n is the number of plant species present and surveyed at that field site. This index integrates the relative abundance (percent cover) of a plant species (j) with the natural infectivity (percent virus) with AMV or CMV. The total VIP at a field site (i) is $VIP_i = \Sigma\ VIP_i$, where $i = 1, \ldots, m$; $j = 1, \ldots, s$, and s is the number of plant species surveyed in this study. These overall estimates of virus presence in the local environment of non-crop field margins were then used to examine the relationship between local inoculum pressure of AMV and CMV to virus incidence estimated in the adjacent *P. vulgaris* crop. Maximum likelihood estimates of AMV and CMV incidence in *P. vulgaris* were calculated as previously described using ELISA results from pooled *P. vulgaris* leaf samples at each field location (30). Linear regression analyses and Spearman's rank correlations were performed at a 5% level of statistical significance to compare virus incidence in the *P. vulgaris* crop with relative virus incidence estimates in adjacent field margins (41). Percent virus incidence was arcsine-square root transformed prior to statistical analysis in JMP 8.0.1 (44).

Results

Plant composition in field margins. Vegetation surrounding Wisconsin crop fields in 2008 consisted primarily of members of the families Poaceae, Fabaceae, and Asteraceae (Fig. 2). At a relative abundance of 51%, grasses were most prevalent in field margins, with representative species such as big blue-stem (*Andropogon gerardii* Vitman) and reed canary grass (*Phalaris arundinacea* L.). Legumes (25%) and asters (21%) were also frequent in occurrence. *M. sativa* was the most abundant leguminous plant, at 9% cover, followed by *Trifolium* spp.: *T. repens* (6%), kura clover (*T. ambiguum* M. Bieb., 4%), and *T. pratense* (3%). In the family Asteraceae, common horseweed (*Conyza canadensis* (L.) Cronquist var. *canadensis*), spotted knapweed (*Centaurea biebersteinii* DC.), and thistles (*Cirsium* spp.), are a few examples of frequently encountered species. Plants that were less abundant in the field margins included *Asclepias syriaca* (Asclepiadaceae), *S. latifolia* (Caryophyllaceae), eastern nightshade (*Solanum ptycanthum* Dunal, Solanaceae), and ground cherry (*Physalis virginiana* Mill., Solanaceae). Members of other families were also present but at frequencies of 5% or less.

Plant virus survey. In total, 4,250 plants from 44 plant species in 17 plant families were sampled in 11 counties of Wisconsin and assayed for the presence of AMV and CMV from 2006 to 2008 (Table 1). The number of commercial fields sampled varied by year and species. Commercial crop plants that were immediately adjacent to one or more *Phaseolus vulgaris* field locations and included in the survey were *M. sativa* (pasture and volunteer plants), cultivated squash (*Cucurbita* spp.), *G. max*, *S. tuberosum*, and *Zea mays* L. subsp. *mays*. Virus detection varied among plant species. AMV was detected most frequently in *M. sativa* (40%) but was also detected in 13 other plant species belonging mainly to members of the family Fabaceae, including lead plant (*Amorpha canescens* Pursh), *G. max*, *L. corniculatus*, *Melilotus* spp., *T. ambiguum*, alsike clover (*T. hybridum* L.), *T. pratense*, *T. repens*, and hairy vetch (*Vicia sativa* L.) (Tables 1 and 2). Natural AMV infection was also detected in non-leguminous plants such as *A. syriaca*, lamb's-quarter (*Chenopodium album* L.), *Silene latifolia*, and *Solanum ptycanthum*. Detection of CMV was highest in *Cucurbita* spp. and *S. ptycanthum*, at 50 and 14%, respectively (Tables 1 and 2). CMV was also detected in other solanaceous plants such as *P.*

virginiana (11%) and *S. tuberosum* (5%). Natural infections of CMV were also present at low levels (≤5%) in *A. syriaca*, *M. sativa*, *Silene latifolia*, *T. hybridum*, *T. repens*, and *V. sativa*.

Foliar symptoms caused by viral infections were not consistent across naturally susceptible plant species. Of the reported families with AMV-susceptible host species (Table 2, Asclepiadaceae, Caryophyllaceae, Chenopodiaceae, Fabaceae, and Solanaceae), AMV generally incited yellow spotting or a bright yellow mosaic foliar discoloration throughout the leaf surface of infected plants. No visual foliar symptoms were expressed in AMV-infected *A. canescens, L. corniculatus*, *M. sativa*, *V. sativa*, or all members of the family Fabaceae; in addition to *Solanum ptycanthum*, which appeared asymptomatic upon AMV infection. CMV infections often resulted in a combination of foliar symptoms, with leaf mottling, foliar vein banding, smaller leaf size, and an overall stunting of the plant being the most common. Plants in families Caryophyllaceae (*Silene latifolia*), and Fabaceae (*T. repens* and *V. sativa*) did not exhibit obvious foliar symptoms.

Field estimates of AMV-infected plants along adjacent crop margins ranged from 0 to 66% and did not change significantly over the course of the growing season while CMV was not detected in any of the plants sampled in field margins adjacent to *Phaseolus vulgaris* in 2006. AMV infection remained low in both *P. vulgaris* and the surrounding field margins from June to September 2007, with incidence estimates ranging from 1 to 7 and 14 to 20%, respectively (Fig. 3). AMV detection was higher in the field margins than in the bean crop ($F_{1,157} = 3.9$, $P = 0.0495$) but infection levels did not differ significantly over the course of the season ($F_{3,157} = 0.37$, $P > 0.20$). CMV was not detected in any plants during the month of June but CMV infection progressively increased over the remainder of the season ($F_{3,157} = 37$, $P \leq 0.001$) and differed significantly by habitat type (Fig. 3, *P. vulgaris* crop versus non-crop field margin, $F_{3,157} = 30.1$, $P \leq 0.001$). Virus infections were detected in emergent seedlings of *P. vulgaris* at 9 of 23 field sites sampled in 2007. Field estimates of seedborne inoculum of AMV and CMV ranged from 0 to 13 and 0 to 6%, respectively, with overall incidence averaging 1.7% for AMV and 0.6% for CMV.

Mechanical transmission of AMV and CMV. Successful mechanical transmission of AMV and CMV from crude sap inocula-

Table 1. Incidence estimates of *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV) in Wisconsin plant species bordering commercial agricultural *Phaseolus vulgaris* fields from 2006 to 2008[a]

			2006			2007			2008		
Plant family	**Plant species**	**Common name**	**Tested**	**AMV (%)**	**CMV (%)**	**Tested**	**AMV (%)**	**CMV (%)**	**Tested**	**AMV (%)**	**CMV (%)**
Apiaceae	*Daucus carota*	Wild carrot	17	0	0	39	0	0	19	0	0
	Pastinaca sativa	Wild parsnip	–	–	–	17	0	0	–	–	–
Apocynaceae	*Apocynum* spp.	Dogbane	–	–	–	1	0	0	–	–	–
Asclepiadaceae	*Asclepias syriaca*	Milkweed	–	–	–	86	33	0	371	3	7
	Asclepias verticillata	Whorled milkweed	21	0	0	5	0	0	9	0	0
Asteraceae	*Aster* spp.	Aster	–	–	–	3	0	0	21	0	0
	Arctium minus	Burdock	–	–	–	8	0	0	–	–	–
	Solidago canadensis	Goldenrod	–	–	–	1	0	0	–	–	–
	Ambrosia artemisiifolia	Ragweed	–	–	–	9	0	0	–	–	–
	Centaurea biebersteinii	Spotted knapweed	–	–	–	3	0	0	12	0	0
	Ambrosia trifida	Giant ragweed	–	–	–	–	–	–	8	0	0
	Cirsium spp.	Thistle	–	–	–	6	0	0	–	–	–
	Conyza canadensis	Common horseweed	–	–	–	–	–	–	34	0	0
	Galinsoga quadriradiata	Quick-weed	–	–	–	5	0	0	10	0	0
Brassicaceae	*Brassica nigra*	Black mustard	–	–	–	–	–	–	11	0	0
Caryophyllaceae	*Silene latifolia*	White cockle	2	0	0	55	11	0	304	2	0.3
Chenopodiaceae	*Chenopodium album*	Lamb's-quarters	–	–	–	16	19	0	10	0	0
Cucurbitaceae	*Cucurbita* spp.	Squash	–	–	–	14	0	93	12	0	0
Euphorbiaceae	*Euphorbia corollata*	Flowering spurge	–	–	–	3	0	0	14	0	0
Fabaceae	*Medicago sativa*	Alfalfa	200	85	0	209	39	0.5	338	29	0
	Trifolium hybridum	Alsike clover	8	13	0	22	18	0	38	0	3
	Lotus corniculatus	Bird's-foot trefoil	5	0	0	42	12	0	32	3	0
	Robinia pseudoacacia	Black locust	–	–	–	3	0	0	–	–	–
	Medicago lupulina	Black medic	22	0	0	65	0	0	79	0	0
	Coronilla varia	Crown vetch	–	–	–	12	0	0	13	0	0
	Glycine max	Soybean	20	10	0	–	–	–	–	–	–
	Vicia sativa	Hairy vetch	9	0	0	22	32	5	155	0	0
	T. ambiguum	Kura clover	7	14	0	12	58	0	12	0	0
	Amorpha canescens	Lead plant	–	–	–	26	19	0	5	0	0
	T. pratense	Red clover	216	23	0	181	3	0	229	1	0
	Melilotus officinalis	Sweet clover	–	–	–	26	12	0	150	0	0
	T. repens	White clover	40	100	0	167	43	0	245	18	0.4
	Lupinus perennis	Wild lupine	3	0	0	–	–	–	3	0	0
Hypericaceae	*Hypericum perforatum*	St. John's wort	34	0	0	3	0	0	–	–	–
Malvaceae	*Hibiscus trionum*	Venice mallow	20	0	0	1	0	0	–	–	–
Plantaginaceae	*Plantago major*	Plantain	2	0	0	–	–	–	–	–	–
Poaceae	*Zea mays*	Corn	36	0	0	5	0	0	–	–	–
Polygonaceae	*Polygonum pensylvanicum*	Pennsylvania smartweed	33	0	0	8	0	0	–	–	–
Scrophulariaceae	*Linaria vulgaris*	Butter-and-eggs	–	–	–	2	0	0	–	–	–
Solanaceae	*Solanum dulcamara*	Deadly nightshade	–	–	–	15	0	0	21	0	0
	S. ptycanthum	Eastern nightshade	–	–	–	56	5	68	45	0	3
	S. sarrachoides	Hairy nightshade	–	–	–	–	–	–	57	0	0
	S. tuberosum	Potato	–	–	–	133	5	1	0	0	0
	Physalis virginiana	Ground cherry	–	–	–	–	–	–	19	0	11
Total	…	…	567	47	0	1281	18	6	567	7	1

[a] Tested = number of plants tested; AMV (%) = number of enzyme-linked immunosorbent assay (ELISA)-positive plant samples/total number of plants tested by ELISA; and – indicates that sample collections were not taken due to absence at field collection site.

tions of field-collected infected plants to healthy greenhouse-grown plants was achieved in particular virus–host plant combinations (Table 3). After repeated inoculation attempts, mechanical transmission of AMV was successful from *A. syriaca*, *Chenopodium album*, *G. max*, *L. corniculatus*, *M. sativa*, *Melilotus* spp., *S. latifolia*, *T. ambiguum*, *T. hybridum*, *T. pratense*, and *T. repens* to *P. vulgaris* (Table 3). Mechanical inoculations of AMV from infected field sample leaf tissue back into original source plant species was also achieved, with the exception of *M. sativa*. Repeated attempts to mechanically transmit AMV from infected *Amorpha canescens*, *S. ptycanthum*, and *V. sativa* to healthy *P. vulgaris* or to the originally infected plant species were unsuccessful; symptom development was not observed in *P. vulgaris* Hystyle and ELISA assays for AMV tested negative.

CMV was mechanically transmitted from *Asclepias syriaca*, *Cucurbita* spp., *M. sativa*, *Physalis virginiana*, *S. ptycanthum*, *Solanum tuberosum*, and *V. sativa* to healthy *Phaseolus vulgaris*. Only mechanical inoculations of field isolates of CMV collected from *Cucurbita* spp., *S. tuberosum*, and one isolate from *A. syriaca* resulted in successful transmission from field-collected leaf tissue back to the original host plant species. CMV infection occurring in *M. sativa*, *Physalis virginiana*, and *S ptycanthum* was not mechanically transmissible to the original host plant species. All mechanical transmission attempts using CMV isolates detected in *S. latifolia*, *T. hybridum*, and *T. repens* were unsuccessful; whereby symptom development was not observed in *P. vulgaris* Hystyle and subsequent ELISA assays for CMV tested negative.

VIP index and snap bean incidence. A VIP index was created for 14 taxonomic plant groups present in adjacent crop and non-crop field margins based on their relative abundance and percent virus infection measured in surveys (Table 4). VIP index values were used to estimate the epidemiological risk of AMV or CMV infections in both adjacent crop and non-crop wild host plants along field margins at experimental field sites. Plants were ranked in increasing order of potential risk as local sources of AMV and CMV inocula, with index values from a high of 3.8 for AMV in *M. sativa* to 0.0 for plants that presumably pose low risk or threat as virus sources. *M. sativa* and *T. repens*, due to their high virus infection levels and regular occurrence in field margins, resulted in the greatest VIP as non-crop sources of AMV, with estimated index values of 3.76 and 1.28, respectively. *T. ambiguum* was also among the top-ranked inoculum sources of AMV (with an estimated value of 1.05); however, it was discretely distributed among fewer field sites in the survey. For CMV inoculum potential, *A. syriaca* ranked

Table 2. Incidence of A*lfalfa mosaic virus* (AMV) and C*ucumber mosaic virus* (CMV) in cultivated and weedy plant species within 5 m of *Phaseolus vulgaris* field edges in Wisconsin, averaging over the years 2006 to 2008

Plant family	Plant species	Common name	Growth habit[a]	Number of plants tested	AMV (%)[b]	CMV (%)
Fabaceae	*Medicago sativa*	Alfalfa	P	747	40	<1
	Trifolium ambiguum	Kura clover	P	31	26	0
	Trifolium repens	White clover	P	452	22	<1
	Amorpha canescens	Lead plant	P	31	16	0
	Glycine max	Soybean	A	20	10	0
	Trifolium pretense	Red clover	P	626	7	0
	Lotus corniculatus	Bird's-foot trefoil	P	79	3	0
	Trifolium hybridum	Alsike clover	P	68	3	<1
	Vicia sativa	Hairy vetch	A/B	186	2	1
	Melilotus officinalis	Sweet clover	A/B	176	1	0
	Medicago lupulina	Black medic	A/B	166	0	0
	Coronilla varia	Crown vetch	P	25	0	0
	Robinia pseudoacacia	Black locust	P	3	0	0
	Lupinus perennis	Wild lupine	P	3	0	0
Cucurbitaceae	*Cucurbita* spp.	Squash	A	26	0	50
Solanaceae	*Solanum ptycanthum*	Eastern nightshade	A	101	3	14
	Physalis virginiana	Ground cherry	P	19	0	11
	S. tuberosum	Potato	A	134	0	5
	S. sarrachoides	Hairy nightshade	A	57	0	0
	S. dulcamara	Deadly nightshade	P	36	0	0
Chenopodiaceae	*Chenopodium album*	Lamb's-quarters	A	26	12	0
Asclepiadaceae	*Asclepias syriaca*	Milkweed	P	457	4	5
	Asclepias verticillata	Whorled milkweed	P	35	0	0
Caryophyllaceae	*Silene latifolia*	White cockle	P	361	2	<1
Apiaceae	*Daucus carota*	Wild carrot	B	75	0	0
	Pastinaca sativa	Wild parsnip	B	17	0	0
Apocynaceae	*Apocynum* spp.	Dogbane	P	1	0	0
Asteraceae	*Aster* spp.	Aster	P	24	0	0
	Arctium minus	Burdock	B	8	0	0
	Solidago canadensis	Goldenrod	P	1	0	0
	Ambrosia artemisiifolia	Common ragweed	A	9	0	0
	Centaurea biebersteinii	Spotted knapweed	B/P	15	0	0
	Ambrosia trifida	Giant ragweed	A	8	0	0
	Cirsium spp.	Thistle	B/P	6	0	0
	Conyza Canadensis	Common horseweed	A	34	0	0
	Galinsoga quadriradiata	Quick-weed	A	15	0	0
Brassicaceae	*Brassica nigra*	Black mustard	A	11	0	0
Euphorbiaceae	*Euphorbia corollata*	Flowering spurge	P	17	0	0
Hypericaceae	*Hypericum perforatum*	St. John's wort	P	37	0	0
Malvaceae	*Hibiscus trionum*	Venice mallow	A	21	0	0
Plantaginaceae	*Plantago major*	Plantain	P	2	0	0
Poaceae	*Zea mays*	Corn	A	41	0	0
Polygonaceae	*Polygonum pensylvanicum*	Pennsylvania smartweed	A	41	0	0
Scrophulariaceae	*Linaria vulgaris*	Butter-and-eggs	P	2	0	0
Total	…	…	…	4,250	12	1.5

[a] Plant life history characterized as A = annual, B = biennial, or P = perennial.
[b] Number of enzyme-linked immunosorbent assay (ELISA)-positive plant samples/total number of plants tested by ELISA.

the highest, with an estimated value of 0.16, followed by *S. ptycanthum* (0.07) and *P. virginiana* (0.02), which were also less common in non-crop field margins.

Virus infection levels in bordering crop fields and non-crop areas were compared with the relative incidence of viruses in the adjacent *Phaseolus vulgaris* crop. Specifically, the VIP estimates in the adjacent field margins did not correlate to AMV or CMV levels in the *P. vulgaris* crop: AMV (Spearman's rho) $\rho = 0.1891(23)$, $P \leq 0.3874$; and CMV, $\rho = 0.28(23)$, $P \leq 0.20$ (Fig. 4). However, a weak linear trend was observed between local AMV incidence in field margins and in *P. vulgaris* ($R^2 = 0.152$, $F_{1,22} = 3.77$, $P \leq 0.0658$). There was no clear relationship between CMV-infected *P. vulgaris* and the corresponding levels of CMV in field margins ($R^2 = 0.03$, $F_{1,22} = 0.62$, $P \leq 0.44$).

Discussion

AMV and CMV have an extensive host range encompassing numerous crop plants and weed species, many of which have been implicated as inoculum sources of virus outbreaks in economically important crops such as *P. vulgaris* (7,24,45). Here, we have shown that AMV and CMV are present at varying levels in neighboring commercial crops and wild host plants inhabiting non-crop margins adjacent to *P. vulgaris.* Due to their relative occurrence and incidence of virus, the VIP index ranked *M. sativa* and *A. syriaca* as susceptible plants with the greatest potential risk as local inoculum sources for spread of AMV and CMV, respectively. Local inoculum pressure in the surrounding field margins, however, did not influence virus incidence measured in adjacent *P. vulgaris* crops, suggesting that additional factors may contribute to virus epidemics in *P. vulgaris* in Wisconsin.

Both AMV and CMV are ubiquitous in Wisconsin's agricultural landscape (11,12). This is the first report of AMV naturally occurring in *Silene ptycanthum*, *A. syriaca*, and *Amorpha canescens*, and of both viruses in *V. sativa*. Although present in adjacent non-crop field margins, these susceptible plant hosts also reside in other agricultural regions within the landscape such as forage pastureland and within-crop rows. The sizeable acreage where these plants are present within the state as well as across the Midwest region and their extended growing season as perennials would further create substantial virus reservoirs throughout many of the ecological regions in the state and may provide aphids easy access to viruses to inoculate to young bean seedlings (12,20,42).

Due to differences in virus infection levels in plant species and the abundance of these plants in agroecosystems, a VIP index was developed to determine which plant species may contribute the greatest epidemiological risk as local inoculum sources of AMV and CMV. Previous studies have identified AMV and CMV in weedy hosts along field margins in the Midwest but this is the first attempt at developing a risk index for quantifying virus inoculum levels in unmanaged areas adjacent to bean crop fields (12,20,29). In our research, *M. sativa* had the highest levels of AMV infection in bordering field margins, and AMV incidence in the *P. vulgaris* crop tended to increase as surrounding local inoculum levels increased. Chatzivassiliou et al. (6) identified 12 weed species as potential AMV inoculum sources but observed that AMV was detected primarily in tobacco (*Nicotiana tabacum* L.) crops planted in close proximity to *M. sativa* fields. *M. sativa* has also been implicated in influencing AMV outbreaks in other neighboring cultivated plants such as celery (*Apium graveolens* var. *dulce*) and *Solanum tuberosum*, and is likely a contributing factor to AMV infections in *P. vulgaris* (16,26,37,46,47).

For CMV, multiple surveys have identified more than 50 susceptible plant species as potential virus reservoirs in local non-crop field margins adjacent to commercial crops such as *A. graveolens*, lettuce (*Lactuca sativa* L.), cucumber (*Cucumis sativus* L.), and *Capsicum annuum* (12,20,42,50). These CMV reservoirs can also negatively impact adjacent cropping systems but, as others have shown, may not influence CMV epidemics in *P. vulgaris* on a similar, local scale compared with AMV (43,45). Thus, weed hosts that provide the greatest risk as inoculum sources (e.g., *M. sativa* for AMV and

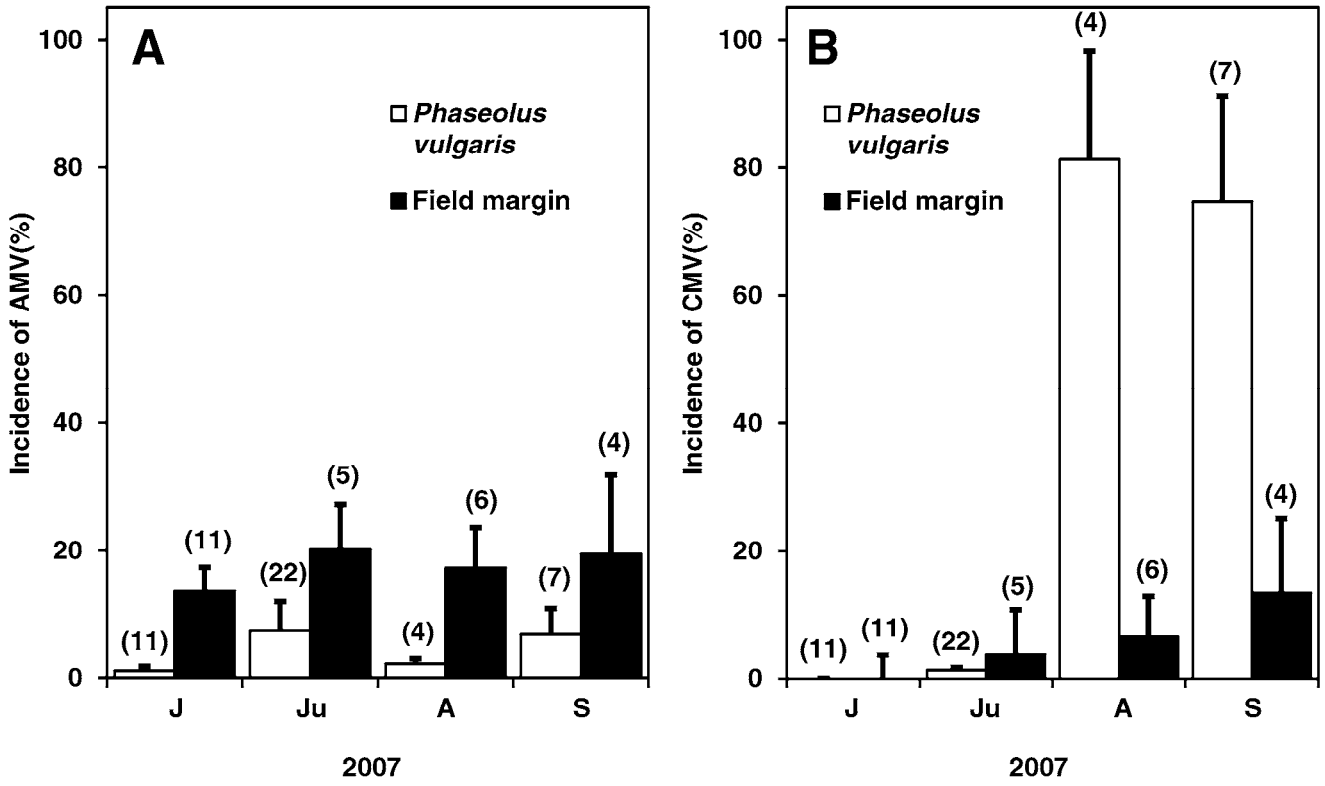

Fig. 3. Mean percent virus detection averaging infection levels of **A,** *Alfalfa mosaic virus* (AMV) and **B,** *Cucumber mosaic virus* (CMV) during the 2007 summer and fall months (June, July, August, and September) in 16 commercial fields with 6 double-cropped fields of *Phaseolus vulgaris* and of wild plants inhabiting non-crop field margins adjacent to *P. vulgaris* crops. Numbers in parentheses correspond to the number of *P. vulgaris* field sites where leaf samples of plants were taken for each specified month. Error bars represent the standard error of the mean.

Table 3. Mechanical transmission experiments of field isolates of *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV) to *Phaseolus vulgaris* and to healthy, greenhouse-raised plants of similar species[a]

Inoculum source	Recipient host	AMV Symptoms[b]	AMV ELISA[c]	CMV Symptoms[b]	CMV ELISA[c]
Amorpha canescens	*P. vulgaris*	– (0/5)	–	NT	NT
	A. canescens	– (0/5)	–	NT	NT
Asclepias syriaca	*P. vulgaris*	+ (9/9)	+	+ (10/10)	+
	A. syriaca	+ (9/9)	+	+ (1/10)	+
Chenopodium album	*P. vulgaris*	+ (3/3)	+	NT	NT
	C. album	+ (3/3)	+	NT	NT
Cucurbita spp.	*P. vulgaris*	NT	NT	+ (7/7)	+
	Cucurbita spp.	NT	NT	+ (7/7)	+
Glycine max	*P. vulgaris*	+ (10/10)	+	NT	NT
	G. max	+ (10/10)	+	NT	NT
Lotus corniculatus	*P. vulgaris*	– (1/1)	+	NT	NT
	L. corniculatus	– (1/1)	+	NT	NT
Medicago sativa	*P. vulgaris*	– (7/30)	+	+ (1/1)	+
	M. sativa	– (0/30)	–	– (0/1)	–
Melilotus spp.	*P. vulgaris*	+ (2/2)	+	+ (1/1)	+
	Melilotus spp.	+ (1/2)	+	+ (1/1)	+
P. vulgaris	*P. vulgaris*	+ (17/18)	+	+ (41/41)	+
Physalis virginiana	*P. vulgaris*	NT	NT	+ (2/2)	+
	Physalis virginiana	NT	NT	– (0/2)	–
Solanum ptycanthum	*Phaseolus vulgaris*	– (0/3)	–	+ (9/10)	+
	S. ptycanthum	– (0/3)	–	– (0/10)	–
S. tuberosum	*P. vulgaris*	+ (1/1)	+	+ (7/7)	+
	S. tuberosum	+ (1/1)	+	+ (6/7)	+
Silene latifolia	*P. vulgaris*	+ (17/17)	+	– (0/1)	–
	S. latifolia	+ (15/17)	+	– (0/1)	–
Trifolium ambiguum	*P. vulgaris*	+ (6/8)	+	NT	NT
	T. ambiguum	+ (6/8)	+	NT	NT
T. hybridum	*P. vulgaris*	+ (2/2)	+	NT	NT
	T. hybridum	+ (2/2)	+	NT	NT
T. pratense	*P. vulgaris*	+ (2/3)	+	NT	NT
	T. pratense	+ (2/3)	+	NT	NT
T. repens	*P. vulgaris*	+ (24/25)	+	– (0/1)	–
	T. repens	+ (23/25)	+	– (0/1)	–
Vicia sativa	*P. vulgaris*	– (0/3)	–	– (1/1)	+
	V. sativa	– (0/3)	–	– (0/1)	–

[a] NT = mechanical inoculations of biological indicator hosts that were not tested in experimental trials.
[b] Foliar symptoms. Visual assessment of symptomatic foliage was based on the presence (+) or absence (–) of common symptoms of virus infection (leaf blistering, curling, mosaic discoloration, or stunted plant growth). Numbers in parentheses indicate Number of enzyme-linked immunosorbent assay (ELISA)-positive viral infections/number of crude sap inoculation attempts.
[c] Positive (+) or negative (–) detection of virus presence in recipient host leaf tissue based on ELISA.

Table 4. Virus inoculum potential (VIP) index to estimate potential risk of common plant species serving as primary inoculum sources for spread of *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV) in *Phaseolus vulgaris*

		Virus incidence[a]			VIP index[b]	
Plant family	Scientific name	AMV	CMV	Plant cover[c]	AMV	CMV
Fabaceae	*Medicago sativa*	40	0.1	9.4 ± 2.6	3.76 (1)	0.011 (4)
	Trifolium repens	21.9	0.2	5.8 ± 2.2	1.28 (2)	0.01 (5)
	T. ambiguum	25.8	0.0	4.1 ± 4.1	1.05 (3)	0.0
	T. pratense	7.3	0.0	2.6 ± 1.0	0.30 (4)	0.0
	Lotus corniculatus	2.5	0.0	0.67 ± 0.53	0.02 (7)	0.0
	Vicia sativa	1.6	0.5	1.0 ± 0.54	0.016 (8)	0.005 (7)
	Melilotus spp.	1.1	0.0	0.87 ± 0.67	0.01 (9)	0.0
	Medicago lupulina	0.0	0.0	0.33 ± 0.16	0.0	0.0
Asclepiadaceae	*Asclepias syriaca*	4.2	5.5	2.9 ± 1.2	0.119 (5)	0.16 (1)
Caryophyllaceae	*Silene latifolia*	1.9	0.3	2.7 ± 1.0	0.116 (6)	0.007 (6)
Solanaceae	*Solanum ptycanthum*	3.0	13.9	0.52 ± 0.40	0.016 (8)	0.07 (2)
	Physalis virginiana	0.0	10.5	0.22 ± 0.22	0.0	0.02 (3)
Poaceae	Grasses	0.0	0.0	51.0 ± 5.0	0.0	0.0
Asteraceae	Asters	0.0	0.0	21.0 ± 3.0	0.0	0.0

[a] Number of enzyme-linked immunosorbent assay (ELISA)-positive plant samples/total number of plants tested by ELISA.
[b] VIP index calculated as [(virus incidence estimate) × (mean percentage plant cover)]/1,000. Numbers in parentheses denote the parenthetical ranking of plant species with the highest overall VIP for each virus.
[c] Relative abundance of plant species in non-crop field margins represented by mean ± standard error.

Asclepias syriaca for CMV) may be more important indicators of broader, area-wide occurrences of viral epidemics in *P. vulgaris*.

Additional factors, such as overwintering capability of viruses in different host plants, could further contribute to their likelihood of serving as primary virus inocula in a subsequent year's crops. Virus persistence through infected weed seed, such as CMV-infected *S. media* seed, could also augment virus inoculum levels in the field margins over time (50). Our research demonstrated that *M. sativa* and *A. syriaca* may pose greater risk as sources of AMV and CMV, respectively. Interestingly, both plant species have been pre-

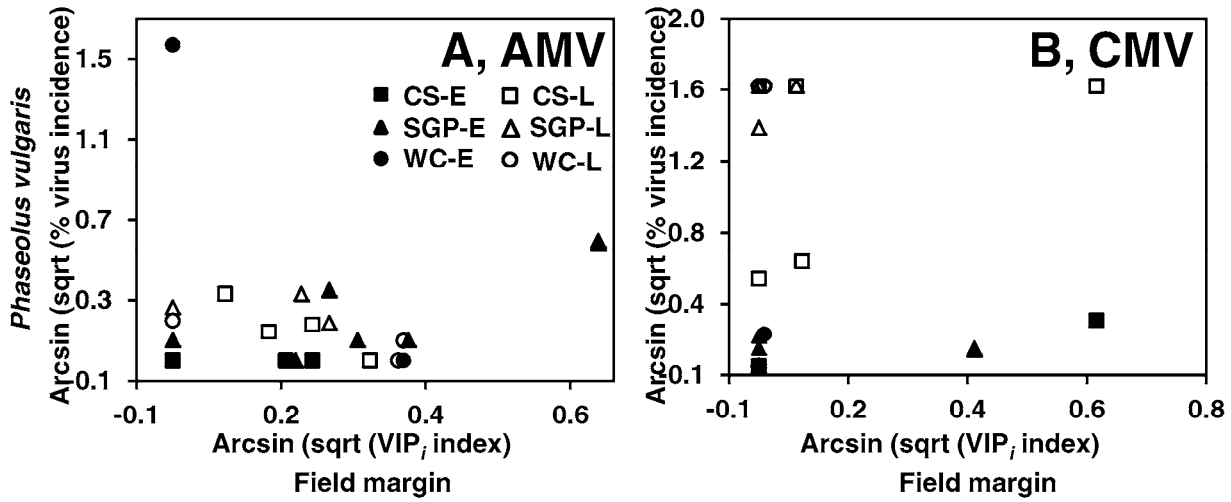

Fig. 4. Relationship between local inoculum pressure in field margins and maximum likelihood incidence estimates of **A,** *Alfalfa mosaic virus* (AMV) and **B,** *Cucumber mosaic virus* (CMV) in commercial *Phaseolus vulgaris* in three agricultural regions—Central Sands (CS, squares), Southeast Glacial Plains (SGP, triangles), and Western Coulee (WC, circles)—of Wisconsin. Hollow symbols represent the early-season *P. vulgaris* crop (planting date prior to 16 June) and late-season double-cropped *P. vulgaris* fields are shown with solid symbols.

viously implicated as reservoirs of nonpersistent viruses, specifically AMV and CMV, of commercial crops in the Midwest and northeastern United States (36,42). The ability of these viruses to reside in belowground tap roots and tuberous rhizomes of these herbaceous perennials could partially explain why AMV and CMV are prevalent in these species.

VIP of naturally infected hosts may also be influenced by mechanisms involved with host specificity and transmission success. In this study, mechanical transmission of AMV and CMV was not consistent across host species, with virus infections occurring more frequently in *P. vulgaris* than in other susceptible host plants. Virus transmission within the same host species is often more successful than transmission across botanically unrelated host plants, which may further enhance or restrict particular plant species as potential sources of AMV and CMV inocula in *P. vulgaris* (3).

Virus presence in weed hosts is important for understanding disease spread but insect vectors can also influence the potential effects of these local reservoirs on virus epidemiology in commercial crops (48). Non-random alightment patterns of aphids in different habitat types indicate that certain virus-susceptible plants are visited more frequently than others (13,30). For example, the rare occurrence of *Aphis glycines* in *S. ptycanthum* suggests that other susceptible plants, such as cultivated *Cucurbita* spp. or perhaps *C. annuum*, are more significant inoculum sources of CMV in *P. vulgaris*. Aerial dispersal of aphids between virus acquisition and subsequent transmission periods indicates that these vectors can transmit virus from both local and distant inoculum sources (21,54,55).

Inoculum potential of these virus-susceptible plants is also affected by the seasonal abundance and efficiency by which alighting aphids can acquire and subsequently transmit virus to the agricultural crop. Virus incidence in *P. vulgaris* drastically increased after massive flights of commonly encountered aphid species *A. glycines* and *Therioaphis trifolii*, both of which are efficient vectors of AMV and CMV (15,28,30,34). In aphid transmission experiments, Hobbs et al. (20) showed that aphid species differed in their ability to transmit CMV from solanaceous weeds to *C. annuum*. In *P. vulgaris*, *A. glycines* is reported as one of the more efficient CMV vectors; however, the rate of CMV transmission from reservoir hosts to *P. vulgaris* is not well known (15).

P. vulgaris is not the only economically important crop of the Midwest negatively impacted by increased infections of AMV or CMV. Other crops negatively impacted by viral diseases include AMV in *M. sativa* and *G. max*, CMV in *Cucurbita* spp. and *C. annuum*, and both AMV and CMV in *S. tuberosum* (9,10,14,19, 31,33,45,56). Based on our findings, local field margins did not play an important role as sources of primary virus inocula for spread into adjacent bean crops. However, the increased frequency of virus detection in several Wisconsin crops suggests that there may be a broader influence of these naturally infected plants on virus epidemiology within agroecosystems. Understanding additional components important for viral disease spread among crop fields and their neighboring habitats, such as aphid flight phenology and aphid transmission efficiencies, may be useful for developing an area-wide management approach to minimizing virus outbreaks in Midwestern susceptible crops.

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