Solanaceous Weeds as Possible Sources of *Cucumber mosaic virus* in Southern Illinois for Aphid Transmission to Pepper

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ABSTRACT

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Over 5,000 individual plants representing approximately 55 species from an area in southern Illinois where *Cucumber mosaic virus* (CMV) has been a major problem in pepper (*Capsicum annuum*) were tested for the presence of CMV by enzyme-linked immunosorbent assay (ELISA). Representative ELISA-positive samples were checked by western blot tests to confirm virus-specific reactions. Nearly all of the infected plants detected were either *Solanum ptycanthum* (eastern black nightshade) or *Physalis* spp. (principally *P. heterophylla*, groundcherry). Over 1,000 pepper transplants and approximately 500 tomato transplants, collected prior to planting, were negative for CMV by ELISA. In aphid transmission (arena) experiments, all five aphid species tested were capable of transmitting CMV from nightshade to pepper: *Aphis fabae* subsp. *solanella*, *Aphis gossypii*, *Myzus persicae*, *Rhopalosiphum padi*, and *Sitobion avenae*. *Aphis fabae* subsp. *solanella*, *A. gossypii*, and *A. nerii* were able to transmit CMV from *P. heterophylla* to pepper. *Aphis fabae* subsp. *solanella* was commonly found colonizing nightshade from May through October in southern Illinois.

Cucumber mosaic virus (CMV) has been causing significant losses in southern Illinois bell pepper (*Capsicum annuum*) production since 1992. Fruit spotting and malformation and reduction in size and number of fruit caused by CMV infection have resulted in some farmers in the area ceasing to grow peppers.

CMV has an extremely wide experimental host range, infecting over 1,000 plant species in 100 families, including both dicots and monocots (7). Natural CMV weed hosts, which are potential virus sources for aphid transmission into crop fields, also belong to many plant families (2,5,6,8,12,13,16,19,20). Some of these weed species are perennial and are likely to be important in the survival of CMV through periods when crop species are absent. CMV can also survive in the seed of at least 17 plant species (7).

At least 86 species of aphids have been reported as vectors of CMV (7). The virus is transmitted in a non-persistent manner with acquisition and transmission times of less than 1 min. This means that plant species involved in CMV epidemiology may not necessarily be preferred aphid hosts. The most common aphid vectors of CMV are *Myzus persicae* and *Aphis gossypii* (14). Raccah et al. (15) implicated *Aphis spiraecola* (syn. *A. citricola*), *A. gossypii*, and *A. craccivora* in CMV infection of peppers in Israel. Sikora et al. (18) believed that *A. gossypii* was involved in CMV epidemics in tomato in Alabama in the early 1990s. Sixteen aphid species reported elsewhere as vectors of CMV have been found in southern Illinois (10,11). *Lipaphis erysimi*, *Rhopalosiphum padi*, *R. maidis*, *Schizaphis graminum*, and *Aphis craccivora* were the most commonly collected (10,11).

The goals of our research were to test plant species for the presence of CMV to identify southern Illinois sources of the virus and to evaluate the ability of these plants to serve as sources for CMV transmission by aphids into pepper plants in aphid transmission (arena) experiments.

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MATERIALS AND METHODS

Virus isolate. The virus isolate used in aphid transmission experiments was obtained by mechanical inoculation from field-infected nightshade (*Solanum ptycanthum*) to tomato (*Lycopersicon esculentum*) plants in the greenhouse. It was identified as CMV by reaction in enzyme-linked immunosorbent assay (ELISA) to CMV-specific antibodies, and by symptomatology in inoculated pepper plants. The CMV isolate was maintained in pepper (*Capsicum annuum*), nightshade, and groundcherry (*Physalis heterophylla*) by mechanical inoculation and aphid transmission.

ELISA. Weeds for assay were collected in and around pepper fields. Weeds with and without virus-like symptoms were collected. Most weed species collected were symptomless. Weeds collected, as well as pepper transplants and cultivated plants grown nearby, were tested for CMV infection in 1996, 1997, and 1998 using ELISA (4) kits (Agdia 1000; Agdia, Inc., Elkhart, IN). Plant samples were kept on ice during transport, then stored at –20 or –80ºC until assayed. The protocol provided by the company for the Agdia CMV "compound direct ELISA" was followed. Absorbance at 405 nm was read using a Dynex MRX ELISA plate reader. The positive threshold value used was twice the absorbance value of the healthy controls. Representative plant samples that were positive for CMV using ELISA were re-tested using western blots.

Western blot technique. Sample preparation, acrylamide gel preparation, and running buffer for electrophoresis were as described by Sambrook et al. (17). Samples of 10 to 15 µl were run for 1.5 h at 20 milliamps through a 5% stacking and 12% resolving gel. After electrophoresis, band transfer was carried out as described by Cheng et al. (3). The procedure for the detection of CMV protein bands involved the following steps: a 6 h blocking step (5% instant nonfat dry milk in PBS with agitation on a shaker at room temperature), overnight incubation with the primary antibody (2 µg/ml anti-CMV polyclonal antibody, Agdia) under the same conditions, washing of the membrane three times in PBS, incubation with goat anti-rabbit alkaline phosphatase conjugate at a dilution of 1:1000 in PBS for 90 min, and washing three times again. The membrane was then exposed to NBT/BCIP substrate until bands were clearly visible and the

reaction stopped by rinsing with deionized water.

Aphid colonies. Aphid species were identified by D. Voegtlin, aphid taxonomist, and voucher specimens were deposited in the collection of the Illinois State Natural History Survey, Champaign, IL. Aphids used to establish colonies were collected from the following hosts: *S. ptycanthum* (nightshade) for *Aphis fabae* subsp. *solanella*, cucumber (*Cucumis sativus*) for *A. gossypii*, pepper (*Capsicum annuum*) for *Myzus persicae*, various graminaceous plants for *Rhopalosiphum padi*, *R. maidis*, and *Sitobion avenae*. The same hosts were used to maintain caged aphid colonies. *Aphis nerii* was collected from milkweed, *Asclepias syriaca*, and maintained in colony cages on shepherd's purse, *Capsella bursa-pastoris*.

Aphid transmission (arena) tests. *Solanum ptycanthum*, a species of nightshade, and *Physalis heterophylla*, a species of groundcherry, both found to be naturally infected with CMV in southern Illinois, were tested for their ability to serve as CMV sources in laboratory aphid transmission "arena" tests, using procedures slightly modified from Irwin and Ruesink (9). *C. annuum* cv. X3R Camelot was used in both groups of tests as the transmission (or test) plant. Either intact, individual CMV-infected nightshade plants or cuttings from a CMV-infected groundcherry plant were placed in a water-filled tube in the center of and touching a ring of pepper seedling test plants in a 20-cm pot. Nightshade plants were grown from seed in the greenhouse and mechanically inoculated with CMV. The groundcherry plant used in the study was collected from the field and mechanically inoculated with CMV in the greenhouse. Approximately 100 to 400 individuals of an aphid species were placed on the virus acquisition host plant. The pot then was covered with a cylindrical cage for 2 days to allow the aphids time to acquire CMV and move to the pepper test plants. If an aphid species remained very sedentary on the virus acquisition source plant or cutting, after 1 day the plant or cutting was removed from the water in the tube and allowed to wilt. If the aphid species crawled around actively during the first day, the virus acquisition source plant or cutting was kept in the water. The goal was to obtain maximum movement between virus acquisition source plants and test plants. After 2 days of access, some aphids were put in alcohol for later verification of identity, and the rest were killed with an insecticide. Pepper test plants were observed for symptoms and tested using ELISA after 3 weeks of growth in a greenhouse at 26ºC with a 13-h photoperiod.

RESULTS

Except for pepper, the vast majority of CMV-infected plants were *Solanum ptycanthum* (eastern black nightshade) and *Physalis* spp. (groundcherry), both belonging to the Solanaceae (Table 1). These infected plants ranged in symptoms expressed, from strong mosaic to barely visible mosaic or mottling. One tomato plant (*Lycopersicon esculentum*) in 1997 and one *Barbarea vulgaris* plant in 1998 were found to be infected with CMV (Table 1). Three of the species that were tested in very low numbers had virus-like symptoms: *Campsis radicans*, *Desmodium* spp., and *Phytolacca americana*. All were ELISA-negative for CMV, and were therefore presumed to be infected by other viruses. Some individual plants of various species belonging to the Compositae or Cruciferae gave apparently positive ELISA reactions, which were later demonstrated to be host-related false positives using the western blot technique. The *B. vulgaris* plant referred to above was verified as infected with CMV using the western blot technique. Approximately 1,000 pepper and 500 tomato transplants were tested by ELISA prior to potential field infection and were negative for the presence of CMV.

Table 1. Number of plants[z] by species in or adjacent to pepper fields in southern Illinois tested for *Cucumber mosaic virus* using enzyme-linked immunosorbent assay (ELISA), 1996 to 1998

Plant family/species	Common name	Number of plants tested
Amaranthaceae		
Amaranthus spp.	Pigweed	168
Apocynaceae		
Apocynum cannabinum	Hemp dogbane	30
Asclepiadaceae		
Asclepias syriaca	Milkweed	169
Bignoniaceae		
Campsis radicans	Trumpet creeper	6
Caprifoliaceae		
Lonicera sp.	Honeysuckle	50
Caryophyllaceae		
Cerastium sp.	Chickweed	331
Chenopodiaceae		
Chenopodium sp.	Lamb's quarters	134
Compositae		
Ambrosia trifida	Giant ragweed	47
Eclipta sp.	Yerba de tajo	21
Erigeron spp.	Fleabane	148
Matricaria matricariodes	Pineapple weed	40
Senecio glabellus	Butterweed	90
Taraxacum officinale	Dandelion	72
Xanthium sp.	Cocklebur	39
Unidentified (without flowers)		166
Convolvulaceae		
Ipomoea hederacea	Ivy-leaved morningglory	104
Ipomoea purpurea	Common morningglory	20
Cruciferae		
Barbarea vulgaris	Yellow rocket	52 (1)
Brassica sp.	Mustard	60
Capsella bursa-pastoris	Shepherd's purse	60
Lepidium sp.	Peppergrass	51
Rorippa sp.	Yellow cress	51
Thlaspi arvense	Field pennycress	6
Euphorbiaceae		
Acalypha ostryaefolia	Three-seeded mercury	53
Geraniaceae		
Geranium sp.	Cranesbill	40
Gramineae		
Secale cereale	Rye	117
Sorghum subglabrescens	Milo	26
Triticum aestivum	Wheat	69

(*continued on next page*)

[z] Number of plants tested using ELISA. In parentheses, number of plants positive by ELISA and verified as virus-infected using western blot.

Physalis spp. plants were not identified to species in 1996 and 1997. Totals for the two years combined were 14 CMV-infected *Physalis* plants of 88 tested. In 1998, emphasis was placed on testing *Physalis* spp. in an area of southern Illinois (near Anna in Union County) in which *S. ptycanthum* was not common. *Physalis* plants tested in 1998 were identified as *P. heterophylla* (168 plants) and *P. longifolia* (29 plants). In that year, CMV was detected only in *P. heterophylla*: 30 of 168 *P. heterophylla* plants were infected with CMV.

Seed was collected from *S. ptycanthum* greenhouse plants infected with CMV and germinated. None of 228 plants from these seeds were ELISA-positive for CMV.

All five of the aphid species tested transmitted CMV from *S. ptycanthum* to pepper (Table 2). *A. fabae* subsp. *solanella* was commonly found colonizing *S. ptycanthum* in southern Illinois from May

Table 1. (*continued*)

Plant family/species	Common name	Number of plants tested
Labiatae		
Lamium amplexicaule	Henbit	15
Lamium purpureum	Deadnettle	232
Leguminosae		
Desmodium spp.	Tick trefoil	12
Glycine max	Soybean	131
Trifolium campestre	Hop clover	51
Trifolium pratense	Red clover	95
Trifolium repens	White clover	95
Trifolium spp.	Clover	427
Vicia sp.	Vetch	73
Liliaceae		
Allium sp.	Wild onion	42
Onagraceae		
Oenothera sp.	Evening primrose	51
Phytolaccaceae		
Phytolacca americana	Pokeweed	4
Plantaginaceae		
Plantago sp.	Plantain	18
Polygonaceae		
Polygonum sp.	Smartweed	143
Rumex sp.	Dock	97
Portulacaceae		
Portulaca sp.	Purslane	64
Rubiaceae		
Galium sp.	Bedstraw	42
Scrophulariaceae		
Verbascum thapsus	Mullein	15
Veronica sp.	Speedwell	53
Solanaceae		
Lycopersicon esculentum	Tomato	202 (1)
Physalis spp.	Groundcherry	285 (44)
Solanum ptycanthum	Nightshade	549 (32)
Solanum carolinense	Horsenettle	84
Solanum melongena	Eggplant	89
Umbelliferae		
Chaerophyllum sp.	Wild chervil	35
Valerianaceae		
Valerianella sp.	Corn salad	40
Vitaceae		
Vitis spp.	Wild grape	10

Table 2. Aphid transmission (arena) experiments with *Cucumber mosaic virus* and with *Solanum ptycanthum* and *Physalis heterophylla* as virus acquisition hosts and pepper as the test host

	Virus acquisition host	
Aphid species	***Solanum ptycanthum***	***Physalis heterophylla***
No aphids (control)	0/44[y]	0/57
Rhopalosiphum padi	6/28	0/30
Rhopalosiphum maidis	—[z]	0/30
Sitobion avenae	3/28	0/28
Myzus persicae	4/43	0/30
Aphis fabae subsp. *solanella*	7/39	9/60
Aphis gossypii	36/42	49/62
Aphis nerii	—	19/32

[y] Number of test plants infected as confirmed by enzyme-linked immunosorbent assay divided by total number of test plants.
[z] Not tested.

through October. Of the five aphid species tested, only *A. gossypii* transmitted successfully in all four individual trials in which *S. ptycanthum* was the virus acquisition host (*data not shown*). Infected pepper plants developed stunting and leaf deformation and were ELISA-positive.

In 1998 and 1999, colonies of *A. nerii* were observed on milkweed, *Asclepias syriaca*, in Union County in the areas where CMV-infected *P. heterophylla* plants were present. Because no aphid colonies were found on *P. heterophylla*, the possibility that *A. nerii* could be involved in CMV transmission from *P. heterophylla* to pepper was explored. *A. nerii* was included in the trials with *P. heterophylla* as virus acquisition host (Table 2).

With *P. heterophylla* as virus acquisition host, *A. gossypii*, *A. fabae* subsp. *solanella*, and *A. nerii* transmitted CMV (Table 2). *A. gossypii* transmitted in six of the six trials, *A. fabae* subsp. *solanella* transmitted in four of the six trials, and *A. nerii* transmitted in all three of the three trials in which it was included (*data not shown*). The remaining aphid species tested (*R. padi, R. maidis, S. avenae*, and *M. persicae)* did not transmit CMV when *P. heterophylla* was used as the virus acquisition host (Table 2).

DISCUSSION

Many weed hosts of CMV believed to be virus sources for crop infection have been reported. These have represented a number of plant families, including Asclepiadaceae, Caryophyllaceae, Cruciferae, and Scrophulariaceae in New York (2,16); Commelinaceae (a monocot family), Compositae, Cucurbitaceae, Gerianaceae, Phytolaccaceae, and Solanaceae in Florida (1,13,20); and Caryophyllaceae, Cucurbitaceae, Euphorbiaceae, Labiatae, Phytolaccaceae, and Solanaceae in Wisconsin and Illinois (5,8,19). It is interesting to note that in the work of Doolittle and Walker (5) and Walker (19) in Wisconsin and Illinois, *P. heterophylla* was reported as a source for CMV infection of cucumber. In Arizona, *P. wrightii* was believed to be an important CMV source for aphid transmission to sugar beet (6).

In our study, with the exception of a single CMV-infected *B. vulgaris* (Cruciferae) plant, all sources of the virus were solanaceous weeds of the genera *Solanum* (species *ptycanthum*) and *Physalis* (primarily species *heterophylla*). The predominance of *S. ptycanthum* in Johnson County and of *P. heterophylla* in Union County suggests that the principal CMV weed hosts at the two locations could be different. Infected plants of both *S. ptycanthum* and *P. heterophylla* were mostly found on the edges of or within the boundaries of fields planted to peppers in previous years. Since weed populations in any given area are so large, particularly those of species with small plants like *Cerastium* spp. (chickweed), and because of the localized nature of virus infection of weeds in some cases, it is possible that infected plants of other weed species were missed during sampling.

Knowledge of CMV weed hosts in an area could be the basis for CMV control by their removal from in and around crop fields. Doolittle and Walker (5) and Wellman (20) reported good virus control following eradication of suspected CMV weed hosts.

We included seven of the 16 species of CMV-vectoring aphids reported to occur in southern Illinois (10,11). The remaining nine species have not been tested with *S. ptycanthum* or *P. heterophylla*, using CMV isolates from southern Illinois.

The current results show that *A. fabae* subsp. *solanella*, the aphid commonly colonizing *S. ptycanthum* from May to October, is capable of transmitting CMV from that host. Although *S. ptycanthum* is generally considered to be an annual in the southern Illinois region, we found roots of

the species that appeared to be perennial. *S. ptycanthum* has also been observed to be a short-lived perennial in Kentucky (M. Crotser, *personal communication*). *A. fabae* subsp. *solanella* could transmit CMV from *S. ptycanthum* to peppers in the spring.

P. heterophylla is a perennial. We found no aphids on this species. CMV is transmitted in a non-persistent manner, however, and efficient vectors, such as *A. gossypii* and *A. nerii*, could transmit CMV from *P. heterophylla* to peppers during movements through the area. *A. nerii* colonies were observed in mid-July on milkweed plants, which were growing in abundance in the vicinity of CMV-infected *Physalis* plants. *A. nerii* does not overwinter in southern Illinois but instead migrates from more southerly regions (11). It is possible that during these migrations some *A. nerii* individuals may land on infected *Physalis* plants, probe, and then fly to pepper plants and probe, before finally finding their milkweed hosts. However, whether the timing of *A. nerii* arrival in the area coincides with the likely time of initial CMV transmission to pepper, about mid-June, remains to be determined.

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