Occurrence and Overwintering of Cucumber Mosaic Virus and Broad Bean Wilt Virus in Weeds Growing Near Commercial Lettuce Fields in New York

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ABSTRACT

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Natural hosts of cucumber mosaic virus (CMV) and broad bean wilt virus (BBWV) near commercial lettuce fields in New York included 18 and four weed species, respectively. Inoculation of diagnostic hosts was useful in discerning whether weeds were infected in cases where results with enzyme-linked immunosorbent assay were borderline. There were no marked differences between the natural hosts of two strains of CMV commonly isolated from lettuce and designated as CMV-L1 and CMV-L2. CMV-L2 was slightly more common than CMV-L1 (55% vs. 45% of the total CMV-positive samples). *Asclepias syriaca*, *Barbarea vulgaris*, *Rorippa islandica*, and *Linaria vulgaris* all harbored CMV in subterranean structures throughout the winter. The rhizomes of *L. vulgaris* also served as overwintering sites for BBWV.

Additional keywords: epidemiology, *Lactuca sativa*.

Diseases of lettuce (*Lactuca sativa* L.) caused by cucumber mosaic virus (CMV) and broad bean wilt virus (BBWV) annually threaten the late-summer lettuce crop on organic soils in central New York (3,11). Outbreaks of these diseases rarely occur before early August but can increase in severity rapidly after that period. Lettuce is seeded daily in New York beginning April 1 and requires approximately 63 days to mature. At present, the only disease-management procedure practiced by growers to limit the damage sustained by these diseases is to avoid seeding lettuce later than mid-July and by harvesting the last lettuce by mid-September, at which time losses of 30% or more can be common on farms where the viruses occur. Climatic conditions favorable for lettuce production generally exist until mid- to late October. Losses due to CMV and BBWV, therefore, are manifested both as reduced yields and as a reduced time period during which lettuce production is profitable.

Management of the CMV and BBWV diseases of lettuce by genetic resistance is not possible at present because no lettuce cultivar is both well suited to production on the organic soils of New York and resistant to CMV and BBWV. Although certain cultivars of commercial lettuce and a few accessions of *Lactuca virosa* Rydb. exhibit tolerance to BBWV (11), this tolerance is not in any of the lettuce cultivars commonly grown in New York. Resistance to CMV was found in *Lactuca saligna* L., a wild species from Portugal, and a program to breed this resistance into commercial lettuce is under way (10). However, this resistance is not effective against all isolates of CMV. Two strains of CMV have been identified from lettuce grown in Oswego County based on the virus's ability to infect *L. saligna* (10). One strain, designated CMV-L1, is incapable of infecting *L. saligna*, whereas the other strain, designated CMV-L2, does so readily. Both strains occur in New York. A source of resistance to CMV-L2 has not yet been identified. Until a suitable resistant cultivar is developed, a disease-management practice other than avoidance of late-season lettuce production is needed to optimize use of farm land and facilitate an entire growing season.

CMV and BBWV both have wide host ranges (7,9,12), and their presence in numerous weed hosts growing adjacent to commercial lettuce fields in New York has been demonstrated (1,4). If infected weeds are important sources of primary inoculum, management of the CMV and BBWV diseases may be feasible through weed control. Bruckart and Lorbeer (4) considered three species, *Barbarea vulgaris* R. Br., *Cerastium arvense* L., and *Rorippa islandica* (Oeder) Borbás, to be likely overwintering hosts of CMV and, therefore, important sources of primary inoculum. However, the presence of CMV in overwintering structures and newly emerging spring shoots of these and other biennial or perennial plants only has been assumed and not verified. Verification that BBWV overwinters in weed hosts near lettuce fields is lacking. Surveys of weed hosts have concentrated on CMV and have been limited to host indexing for virus identification. Incorporation of the enzyme-linked immunosorbent assay (ELISA) into studies of the ecology of these viruses would greatly increase the efficiency of the studies.

The potential for managing the CMV and BBWV diseases through removal of weed hosts of CMV and BBWV growing in proximity to lettuce fields has been suggested previously (4) but also has been considered impractical because of the numerous host species involved and the topography of the lettuce fields (10). However, control of weed hosts of CMV near cultivated fields has been successful in reducing the incidences of CMV in cucumber (6) and celery (13). A more thorough knowledge of the ecology of CMV (including the strains CMV-L1 and CMV-L2) and BBWV in weed hosts in New York would benefit development of disease-management programs. The purposes of the present study were to define, using ELISA, the weed-host ranges of CMV and BBWV in Oswego County, NY, compare the weed-host ranges and relative frequencies of CMV-L1 and CMV-L2, and determine if CMV and BBWV overwinter in subterranean structures of biennial and perennial weed hosts growing adjacent to lettuce fields.

MATERIALS AND METHODS

Virus identification. Routine identification of CMV and BBWV in both lettuce and weeds was achieved with ELISA (5), using polyclonal antibodies specific for CMV (obtained from D. Gonsalves, New York Agricultural Experiment Station, Geneva, NY) or for BBWV (obtained from Vittoria Lisa, Istituto di Fitovirologia applicata del Consiglio Nazionale delle Ricerche, Torino, Italy). In the field, pieces of leaves (henceforth referred to as specimens) of plants to be indexed for the presence of CMV and/or BBWV were placed into plastic centrifuge tubes containing 6 ml of 0.05 M carbonate coating buffer (5). These tubes were stored in a rack placed in a portable cooler until they were returned to the laboratory where all specimens were ground with a

Brinkman Homogenizer (Brinkman Instruments, Westbury, NY). The tubes containing the specimen/carbonate buffer homogenates (henceforth referred to as homogenates) were stored for approximately 24 hr at 5 C (or in some cases were frozen) while an indirect ELISA protocol (8) was completed. This protocol included the following four steps, each of which were followed by three rinses (3 min each) with phosphate-buffered saline plus 1% Tween-20 (PBST). First, 200 μl of each of the homogenates was placed in a microtitre plate (Imulon II, round bottom, 96 well, Fisher Scientific, Rochester, NY), one homogenate per well. When the plate was filled (the outermost wells were not used), it was incubated in a plastic box at 4 C for 12–14 hr. Homogenates from healthy *L. saligna* plants were used for virus-free controls and routinely added to two wells per microtitre plate. Second, 100 μl of PBST containing rabbit polyclonal antibodies (generally a 1/400 dilution of an antibody stock containing 1 μgm protein/ml) or of PBST containing mouse monoclonal antibodies (same dilution as for polyclonal antibodies) were added to each well that previously had contained an homogenate, and the plates were incubated for 3–5 hr at room temperature. Third, if polyclonal antibodies were used in step 2, 100 μl of goat-anti-rabbit IgG conjugated with alkaline phosphatase (generally, a 1/1000 dilution of a commercially prepared stock, Zymed Laboratories, Inc., South San Francisco, CA) was added to each of the wells involved in the assay. If monoclonal antibodies were used in step 2, goat-anti-mouse IgGAM conjugated with alkaline phosphatase (same dilution, same source) was used in this step. In either case, the loaded microtitre plates were incubated for 3–4 hr at 37 C. Fourth, 50 μl of p-nitrophenyl phosphate (1 mg/ml) in 10% diethanolamine in distilled water (pH 9.8) was added to each well. The loaded plates were incubated at room temperature for 20–60 min and then read spectrophotometrically on an ELISA reader (Dynatech Corp., Burlington, MA). Any specimen generally was considered positive for the presence of CMV or BBWV if the spectrophotometric reading for it was three times that of the average control value (calculated from the two wells that had initially contained healthy *L. saligna* sap) for that plate. Homogenates positive for CMV or BBWV were used to inoculate a zucchini plant (*Cucumis pepo* L. 'Elite') or a broad bean plant (*Vicia faba* L.), respectively. Often, particularly in 1984 and 1985, homogenates with spectrophoto-metric values of less than three but greater than two times the control value also were used to inoculate the appropriate indicator host. This step was taken to determine the reliability of using a value of three times the control value as the threshold for positive values. Inoculated plants were placed in an environmentally controlled growth chamber maintained at 21 C and observed for symptom development.

Collections of CMV were identified as either strain CMV-L1 or CMV-L2 based on their ability to infect the differential host plant *L. saligna* (10) or from results of ELISA when using monoclonal antibodies raised against, and specific for, these strains (the facilities of D. Antczack, Baker Institute, College of Veterinary Medicine, Cornell University, were used to raise these antibodies). One monoclonal antibody (designated αL1) specific for CMV-L1 and two monoclonal antibodies (designated αL2a and αL2b) specific for CMV-L2 were used in this study. These antibodies reacted definitively in ELISA with some collections of CMV-L1 or CMV-L2 that had been identified previously using the differential host *L. saligna*. However, αL1 did not recognize all collections of CMV-L1, and αL2a and αL2b did not recognize all collections of CMV-L2. Thus, these antibodies recognize subgroups of the strains of CMV they were raised against. When a collection of CMV was not identifiable as either CMV-L1 or CMV-L2 by use of the monoclonal antibodies, the homogenate containing it was used to inoculate three *L. sativa* 'Summer Bib' plants and three *L. saligna* plants. Virus that infected both *L. sativa* and *L. saligna* was, by definition, CMV-L2. Virus that infected only *L. sativa* was used to inoculate three additional *L. saligna* plants and was considered to be CMV-L1 if it again failed to infect these plants.

Determination of the weed-host ranges of CMV and BBWV. Natural-host ranges of CMV and BBWV in Oswego County, NY, were determined by screening with ELISA numerous specimens of weed species that at least occasionally were observed growing near lettuce fields. Specimens generally were collected from plants growing within 100 yards of the fields and often from plants growing on the banks of drainage ditches that border them. Fifty-five species of weeds from 15 farms operated by seven different growers were screened during the months of April through November in 1984 through 1986. Once sites were located where either virus was common, the subsequent collection of specimens was concentrated in these areas. Collection of numerous virus-free specimens of a given species in these areas was considered evidence that the species was not a natural host.

Determination of the host ranges and abundance of CMV-L1 and CMV-L2. The abundance of strains CMV-L1 and CMV-L2 and the natural-host ranges of these two strains were determined during the growing seasons of 1984 and 1986, when collections of CMV were routinely further identified as either CMV-L1 or CMV-L2. Data pertaining to the abundance of these strains were accumulated from diseased lettuce plants as well as from weed plants.

Determination of overwintering hosts of CMV and BBWV. The overwintering structures of two biennial weed species (*B. vulgaris* and *R. islandica*) and two perennial weed species (*Asclepias syriaca* L. and *Linaria vulgaris* Mill.) found to be commonly infected with CMV in 1984 (Table 1) were collected in early April 1985 and indexed with ELISA to determine if CMV had overwintered in these plant structures. Roots and rhizomes of *A. syriaca* were separated from the bases of a total of 25 dead shoots that remained from the previous growing season at locations where high incidences of infection of this species with CMV had been detected. Similar collections were made of the roots of 15 *R. islandica* plants, 25 rosettes of *B. vulgaris,* and roots and rhizomes of 20 *L. vulgaris* plants at locations where high incidences of infection of these species with CMV had been detected during the previous growing season. Tissue from each overwintering structure was indexed with ELISA for the presence of CMV. Roots or rhizomes from which the samples were positive for the virus were placed in plastic pots and covered with soil. The pots then were placed under a 16-hr photoperiod in an environmentally controlled growth chamber maintained at 21 C. Shoots emerging from these roots were observed for symptoms and indexed with ELISA for CMV. A similar procedure was followed in April 1987 using roots and rhizomes of 15 *L. vulgaris* plants collected where the incidence of BBWV in this species had been high in September 1986. In this case, specimens of the roots and rhizomes, and of the shoots that arose from them, were indexed for the presence of BBWV.

RESULTS

Reliability of ELISA. When homogenates that exhibited, in ELISA, spectrophotometric readings of three times or greater than the control value were used to inoculate an indicator host, symptoms of CMV or BBWV infection invariably developed. Thus, inclusion of false positives in the data was not a problem as long as the "three times the control value" rule was observed and the outermost wells of the microtitre plates were avoided. Occasionally, however, indicator plants also became infected when they were inoculated with homogenates exhibiting spectrophotometric values of less than three times the control value. Thus, errors in the form of false negatives could occur if homogenates with values greater than two times but less than three times the control value were not routinely indexed for virus by inoculations onto suitable indicator hosts.

Natural-host ranges and related observations. Eighteen weed species were found to host CMV at least occasionally (Table 1), whereas four species were found to host BBWV (Table 2). CMV was found in at least very low incidences in weeds collected from 14 of the 15 farms visited during this study, whereas BBWV was recovered from weeds and lettuce from six of these farms. On farms where both viruses were present, BBWV was generally as common or even more common than CMV.

Generally, CMV was readily recoverable from infected specimens of weed species listed in Table 1. Symptoms of infection

TABLE 1. Weed hosts of cucumber mosaic virus (CMV) in New York and incidence of infection by CMV in these species

Weed hosts	Infected specimens/total specimens[a,b] 1984	1985	1986	1987	Total	Number of farms where CMV-positive specimens were collected/number of farms where this species was sampled[c]
Asclepias syriaca L. Milkweed	15/45	24/147	19/100	10/50	68/342	10/11
Rorippa islandica (Oeder) Borbás Yellow Cress	10/40	52/255	8/40	7/35	77/370	8/9
Barbarea vulgaris R. Br. Yellow Rocket	6/34	24/62	24/100	7/28	61/224	5/7
Linaria vulgaris Mill. Butter and Eggs	···	6/19	14/57	8/25	28/101	3/5
Capsella bursa-pastoris (L.) Medic. Shepard's Purse	4/30	13/43	0/12	2/20	19/105	4/6
Galinsoga parviflora Cav. Frenchweed	0/3	8/15	4/11	···	12/29	3/5
Echinocystis lobata (Mich.) T. & G. Wild Cucumber	1/3	4/8	1/25	3/7	9/43	3/3
Solanum dulcamara L. Nightshade	0/4	1/5	2/5	0/3	3/17	2/4
Stellaria media L. Chickweed	···	···	3/4	···	3/4	2/2
Brassica kaber (Dc) L. C. Wheeler Mustard	2/6	2/8	2/10	0/2	6/24	3/4
Erysimum cheiranthoides L. Worm-seed mustard	2/3	0/3	···	···	2/6	1/4
Unidentified Mustard #1 (small white flowers)	1/2	0/1	···	···	1/3	1/31
Sonchus asper (L.) Hill Sowthistle	···	1/8	0/7	···	1/15	1/3
Amaranthus lividus L. Prostrate pigweed	1/13	2/12	0/15	0/11	3/51	1/4
Urtica procera Muhl. Stinging nettle	0/25	0/20	···	1/1	1/46	1/1
Thlaspi arvense L. Fanweed	1/3	0/7	0/8	···	1/18	1/2
Silene cucubalus Wibel Bladder Campion	0/2	2/3	···	···	2/5	2/3
Lepidium virginicum L. Pepper grass	1/2	0/6	···	···	1/8	1/1

[a] The total number of samples of a given species is an indication of how often that species was observed in this study.
[b] ··· indicates no samples of the species were collected.
[c] The number of farms where a given species was sampled is an indication of the commonness of that species throughout the areas of New York surveyed in this study. At some farms a species may have been sampled only a few times or even once. Thus, an indication that a species was found to be infected in, for example, only one of five farms where it was sampled does not necessarily indicate that this species was not infected in the other four farms, but only that it was not detected in the collections of this species, which may have been few, made at these farms.

TABLE 2. Weed hosts of broad bean wilt virus (BBWV) on lettuce farms in New York and incidence of infection by BBWV in these species

Weed hosts	Infected specimens/total specimens 1986	1987	Total	Number of farms where BBWV-positive specimens were collected/number of farms where this species was sampled
Linaria vulgaris Mill. Butter and Eggs	13/43	12/20	25/63	2/2
Veronica scutellata L. Marsh Speedwell	7/11	2/7	9/18	1/2
Amaranthus lividus L.[a] Prostrate pigweed	8/15	2/11	10/26	2/3
Galium mollugo L. Bedstraw, Cleavers	2/5	0/6	2/11	1/3

[a] *A. lividus* plants at several sites also were found to be infected with BBWV during 1984 and 1985.

by CMV (chlorotic spots) were usually visible on cotyledons of zucchini plants that had been maintained at 21 C within 4 to 7 days following their inoculation with homogenates stored at 5 C for 24 hr and that had tested positively for CMV with ELISA. Two exceptions to this were noted. First, expression of symptoms in zucchini cotyledons inoculated with homogenates from infected *Solanum dulcamara* L. plants required up to 3 wk. Spectrophotometric values of wells that had originally been filled with homogenates of infected *S. dulcamara* plants were always lower than values of wells on the same plate that had contained homogenates of other infected weed species. Apparently, CMV was present at lower titre in this species relative to its titre in the other weed species. In the second exception, cotyledons of zucchini plants inoculated with 24-hr-old homogenates from infected *L. vulgaris* plants (as indicated by ELISA) rarely became infected. When *C. pepo* plants were inoculated with freshly prepared homogenates of infected *L. vulgaris*, or with homogenates that had been frozen immediately after their preparation and stored for 24 hr or more, severe infections often developed within 3–5 days. Thus, the longevity of CMV in *L. vulgaris* homogenates is shorter than 24 hr and, therefore, shorter than in homogenates of the other 17 weed species listed in Table 1.

Generally, BBWV was readily recoverable from the weed species listed in Table 2. Symptoms of infection by BBWV (chlorotic mottle, wilting, and/or necrosis) usually were visible on leaves of broad bean plants maintained at 21 C within 7 to 10 days following their inoculation with homogenates that had tested positively for BBWV with ELISA. Homogenates of *L. vulgaris* routinely were frozen following their preparation beginning in 1986 when this weed was first screened for infection by BBWV. Therefore, it is not known if longevity of BBWV in homogenates of this species is reduced relative to that in homogenates of other weed-host species

as was observed for CMV.

Symptoms of infection by CMV or BBWV in weed hosts were sometimes, but not always, useful in detecting infected weeds in the field. Specimens of certain species at times exhibited symptoms that were invariably correlated with infection by CMV or BBWV. A marked chlorotic mottle and ringspotting of leaves of *A. syriaca* was sometimes noted in August and September on plants infected with CMV. Chlorotic mottles of varying degrees also were present on leaves of some specimens of *Echinocystis lobata* (Michx.) T. & G., *R. islandica*, and *B. vulgaris* infected with CMV. Plant stunting and chlorosis as well as thinning and twisting of leaves of young *L. vulgaris* plants were good indications of infection by either CMV or BBWV. Whether differential symptomatology, which would allow the differentiation of infection of *L. vulgaris* by CMV from infection by BBWV, occurs in this mutual host of CMV and BBWV was not critically examined. The presence of BBWV in specimens of *Amaranthus lividus* L. often was correlated with a chlorotic mottle of the middle-aged to young leaves. With the exception of *E. lobata*, which always exhibited systemic chlorotic spots and mottles in samples found to be infected with CMV, and also *Urtica procera* Muhl., which was chlorotic and stunted the single time it was found to be infected with CMV, no weed species screened in this study that was a host species of either CMV or BBWV exhibited discernable symptoms in 100% of the specimens of that species found to be infected by CMV or BBWV.

Comparative host ranges and abundance of CMV-L1 and CMV-L2. There were no marked differences between the natural-host ranges of strains CMV-L1 and CMV-L2. Collections of CMV from *Stellaria media* L., *Sonchus* sp., and *Silene* sp. were not further identified as strain CMV-L1 or CMV-L2. CMV-L2 was found in all of the remaining 15 weed species listed in Table 1. CMV-L1 was recovered from *A. syriaca*, *R. islandica*, *B. vulgaris*, *L. vulgaris*, *Capsella bursa-pastoris* (L.) Medic., *Galinsoga parviflora* Cav., and *E. lobata*. The low frequencies of detection of CMV in the remaining seven host species (Table 1) preclude conclusive statements on whether these species host only strain CMV-L2 and not CMV-L1. In any event, both strains occur in all of the weed species that commonly were found to host CMV. There was, however, a marked correlation between the host plant *B. vulgaris* and the subgroup of strain CMV-L1 which was identifiable with the monoclonal antibody α-L1. In 1984 and 1986, 50 and 48%, respectively, of the collections of CMV-L1 that reacted with the monoclonal antibody αL1 in ELISA were found in *B. vulgaris*.

Strain CMV-L2 was more commonly isolated than CMV-L1 and represented 58 and 52% of the total collections of CMV made during 1984 and 1986, respectively.

Overwintering hosts of CMV and BBWV. Both CMV and BBWV survive in overwintering structures of perennial or biennial weed hosts. Ten of the 25 specimens of roots and rhizomes from *A. syriaca* plants collected in April 1985 tested positively for CMV with ELISA. Similarly, six of the 15 specimens of roots from *R. islandica* plants, 10 of the 25 specimens of overwintering rosettes of *B. vulgaris*, and six of the 20 specimens of roots and rhizomes of *L. vulgaris* tested positively for CMV with ELISA. Six of the 15 specimens of roots and rhizomes of *L. vulgaris* plants collected in April 1987 tested positively for BBWV. In all cases, at least one of the overwintering structures that contained either CMV or BBWV and that was planted under greenhouse conditions produced shoots containing virus.

DISCUSSION

Efficient and reliable techniques for identifying viruses such as CMV and BBWV are essential to field studies of their ecology and epidemiology. Host-range indexing, although reliable, requires several weeks and a substantial area of climate-controlled growth space for suitable development of diagnostic symptoms. Time and space become limiting factors when weekly processing of hundreds of samples is required. Symptoms induced by CMV or BBWV in certain naturally infected weeds sometimes can be used to identify infection by these viruses. However, symptoms are not a reliable means of routinely identifying infection because of inconsistent symptom expression in many hosts such as *A. syriaca* and *R. islandica* and the existence of symptomless host species such as *S. dulcamara*. During the present study, ELISA proved to be a very useful and generally reliable means of screening, for CMV and BBWV, large numbers of samples representing many different plant species. Designation of a threshold spectrophotometric value at which samples are considered, upon completion of ELISA, to be positive for the presence of virus is invariably somewhat arbitrary. Use of a value three times that of the control as the threshold eliminated any problem of false positives, but routine inoculation of indicator hosts with homogenates of specimens with ELISA values approaching this threshold was necessary to avoid false negatives. Also, accommodations for variations, in certain host species, in virus titre and stability in sap are sometimes necessary for successful inoculations. Improvement of ELISA results by reducing the background color development, sometimes observed in virus-free samples, might be achieved by clarifying sap homogenates by centrifugation before their incubation in the microtitre plates. Also, virus-free plants, grown under controlled conditions, of each species of weed to be assayed could be used as controls to compare against field collections of the same species. This could help reduce variability in ELISA results because uninfected samples of some weeds (*Epilobium* sp., *Gallium mollugo* L.) often gave spectrophotometric values of greater than twice the value observed when using *L. saligna* as the control. In large-scale screenings including many species, however, these steps may be impractical.

Both CMV and BBWV infect numerous plant species (9,12), including many that grow in or near the lettuce fields studied during the present investigation. Most of the species listed as hosts of CMV (Table 1) or BBWV (Table 2) have been reported as hosts previously (1,4). However, the present study is the first report of natural infection (occurring in the field) of *L. vulgaris* by CMV and also the first report of natural infection of *L. vulgaris*, *Veronica scutellata* L., and *G. mollugo* by BBWV. Although weeds other than these and the remainder listed in Tables 1 and 2 occasionally may host CMV or BBWV in the areas of lettuce production studied during this investigation, the listed species certainly include the majority that become infected.

Reservoirs of CMV and BBWV exist throughout the year in the weeds listed in Tables 1 and 2, respectively. Four species, *R. islandica*, *A. syriaca*, *B. vulgaris*, and *L. vulgaris*, are especially important for CMV in this regard (Table 3) because of their abundance, relatively high incidences of infection, and capacity to serve as overwintering hosts of the virus. *L. vulgaris* is especially important for BBWV. These four species frequently colonize the banks of drainage ditches that border and cross lettuce fields. Therefore, reservoirs of CMV and BBWV in these species often exist within a few meters of the cultivated lettuce. We believe that these species are primary sources of the viruses that damage lettuce crops annually. Studies are needed to determine if direct correlations exist between the presence of such reservoirs of CMV and BBWV and the occurrences of the CMV and BBWV diseases in cultivated lettuce growing adjacent to the reservoirs. A study in which the incidence of the viruses in weed hosts growing on drainage ditch banks before emergence of lettuce seedlings is compared to the incidence of infection of lettuce plants at harvest also is needed.

Two of the four species considered as the most important hosts of CMV in the present study, *L. vulgaris* and *A. syriaca*, were not considered important hosts in a previous report that documented the occurrence of CMV in weeds near lettuce fields in New York in 1973 and 1974 (4). In that report, *L. vulgaris* was not listed as a host of CMV but was assayed only four times (2). Infection of this species, therefore, easily could have been overlooked. *A. syriaca* was assayed 116 times and was found to be infected with CMV in only three, or 2.6%, of the samples, whereas in our study from 1984 to 1987, *A. syriaca* was infected with CMV in 68, or 20%, of the 342 samples collected from the same areas (Table 1). *A. syriaca* is a perennial plant that develops extensive systems of rhizomes now known to harbor CMV through the winter. An individual system

TABLE 3. Weed hosts of cucumber mosaic virus (CMV) on lettuce farms in New York ranked according to their potential importance as sources of CMV

Most important[a]	Moderately important[b]	Minimally important[c]
Rorippa islandica (Oeder) Borbás Yellow Cress	*Echinocystis lobata* (A. Mich.) T. & G. Wild Cucumber	*Amaranthus lividus* L. Prostrate pigweed
Asclepias syriaca L. Milkweed	*Galinsoga parviflora* Cav. Frenchweed	*Sonchus* sp. Sowthistle
Barbarea vulgaris R. Br. Yellow Rocket	*Capsella bursa-pastoris* (L.) Medic. Shepard's Purse	*Silene cucubalus* Wibel Bladder Campion
Linaria vulgaris Mill. Butter and Eggs	*Stellaria media* L. Chickweed	*Solanum dulcamara* L. Nightshade
		(others listed in Table 1)

[a] Plant species that commonly grow immediately adjacent to lettuce fields, are commonly infected with CMV, and are known to be overwintering hosts of the virus. These species are sources of primary inoculum.

[b] Plant species that often are found growing near lettuce fields, are sometimes infected with CMV, but are not known to be overwintering hosts of the virus, with the exception of *S. media*. Seed transmission of CMV in this species has been reported (Tomlinson and Carter, 1970), and, therefore, it may be an overwintering host of CMV in New York. However, because it occurred infrequently in the study areas, it is listed as moderately important.

[c] Plant species that seldom grow near lettuce fields and/or are only incidentally infected with CMV, often only late in the growing season.

of rhizomes can produce numerous shoots over areas of 5 m^2 or more, some or all of which may be infected with CMV. These clones can persist for years and produce at least some infected shoots each year. As the clones continue to produce infected shoots and as new clones become infected, the incidence of infected shoots of *A. syriaca* increases annually. This may explain the apparent increase in the incidence of infection of *A. syriaca* over the last 12 years.

Seed transmission of CMV occurs in numerous hosts of this virus (7). Seed transmission of BBWC has not been reported. The present study did not address directly the possibility that seedborne virus may play an important role in the overwintering of CMV or BBWV. It has proven, however, that CMV and BBWV both overwinter in persistent structures, other than seeds, of biennial and perennial host species. Thus, the importance of weed hosts as potential sources of primary inoculum of CMV and BBWV is established without knowing the importance of seedborne virus.

Both strains of CMV, CMV-L1 and CMV-L2, are commonly present in weed hosts and cultivated lettuce. Whether the tendency for CMV-L2 to be slightly more common than CMV-L1 is significant is unknown. In any event, effective management of CMV through use of genetic resistance would require a cultivar with resistance to both strains of the virus. A combination of resistant cultivars and weed control may prove useful for management of the CMV and BBWV diseases of lettuce in the future.

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