Relationships of Weed Reservoirs of Cucumber Mosaic Virus (CMV) and Broad Bean Wilt Virus (BBWV) to CMV and BBWV in Commercial Lettuce Fields in New York

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ABSTRACT

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The incidence of cucumber mosaic virus (CMV) and broad bean wilt virus (BBWV) in weeds within 10 m of commercial lettuce fields early in the growing season in New York correlated strongly with the incidence of the viruses in lettuce plants in the same fields later in the season ($r = 0.93$ for CMV and 0.88 for BBWV). Both viruses were more common ($P = 0.025$) in weeds within 10 m of lettuce fields than in weeds at distances between 20 and 40 m from the same fields. The 10-m distance always encompassed the drainage ditches that bordered the fields. These studies, conducted during the lettuce-growing seasons of 1984–1987, indicate that removal of weed hosts of CMV and BBWV growing on drainage ditch banks in and bordering commercial lettuce fields in New York would be a prudent practice in any attempt to manage the viruses.

Additional keywords: ELISA, *Lactuca sativa*.

Lettuce (*Lactuca sativa* L.) grown in central New York generally is damaged each year by infection with cucumber mosaic virus (CMV) and broad bean wilt virus (BBWV). The mosaic diseases caused by these viruses generally first appear during early August and then increase rapidly in severity. To minimize damage sustained from late-season outbreaks of the viruses, growers usually avoid planting lettuce after July 15. This allows for harvest of the last crop by mid-September, at which time the threat from the viruses is already high and which is usually about 4 wk before the end of favorable climatic conditions for lettuce production. Some growers even arrange seeding schedules so that all lettuce is harvested by mid-August. Thus, the viruses cause economic losses to growers by direct damage to lettuce crops and also by shortening the profitable lettuce season.

A recent survey of the weed flora associated with lettuce fields in central New York revealed that winter and summer reservoirs of both CMV and BBWV were common in subterranean and aerial structures of weeds that grew near lettuce fields, particularly on the banks of drainage ditches that network through and border the fields (4). Whether removal of the virus reservoirs through weed control in the drainage ditch areas would serve as an effective practice for management of CMV and BBWV in lettuce is not known. Because lettuce fields in central New York are commonly surrounded by either other lettuce fields or wooded and forested areas in which the known weed hosts of CMV and BBWV rarely occur (4), open, noncultivated areas around lettuce fields are limited. It has been suggested that management of the viruses through eradication of their overwintering hosts would be impractical because of the numerous host species and because of the topography of lettuce fields in central New York (3). However, since the major reservoirs of the viruses appear to be those weeds that grow primarily in the drainage ditch areas and seldom in the wooded and forested areas, control of these weeds seems economically feasible and may represent an effective procedure to manage the viruses.

The primary purpose of this study was to evaluate the potential of localized weed host control as a practical method for managing CMV and BBWV in commercial lettuce fields of central New York by characterizing relationships among weed reservoirs of the viruses and occurrences of the viruses in the lettuce fields and by comparing the incidence of the viruses in weeds that grew at different limited distances from the edges of lettuce fields. A secondary purpose of this study was to observe the effect of crop rotation on the incidence of CMV in weed hosts that grew adjacent to cultivated lettuce fields.

MATERIALS AND METHODS

Virus identification. The enzyme-linked immunosorbent assay (ELISA) was used for routine identification of CMV and BBWV in lettuce and weed samples. Detailed descriptions of the ELISA protocols used during this study have been published (4).

Monitoring occurrence of CMV and BBWV in weeds and lettuce fields. Twenty lettuce fields in central New York were monitored routinely for occurrences of CMV during each growing season from 1984 to 1987. Recorded data included the locations of fields where CMV occurred, the locations of the occurrences within each field, and the locations of weed reservoirs of CMV in relation to the lettuce fields. In 1984, weed species known to host CMV and that grew adjacent to lettuce plants with mosaic symptoms were tested for CMV at the same time as the symptomatic lettuce plants were tested. During 1985–1987, weed hosts found adjacent to areas of fields where CMV had occurred the previous season were screened for CMV before or soon after the fields had been seeded.

The same 20 fields were monitored for BBWV during the growing seasons of 1986 and 1987. In both years, the weeds were tested for BBWV before or soon after the fields had been seeded.

Incidence of CMV and BBWV in weed hosts during July compared with incidence in adjacent lettuce plants during September. In 1984 and 1985, it was apparent from monitoring the disease and weed reservoirs that when virus occurred in lettuce plantings, it also was present in weeds that grew adjacent to the infected lettuce. Therefore, an experiment was performed in 1986 to statistically test this apparent direct correlation. Seven lettuce fields chosen for late-planting (July 1 or after) were studied. Before or soon after seeding, line transects were constructed along a ditch bank on one side of each field to determine which weed plants were to be sampled and assayed for the presence of CMV and BBWV. The weed plants closest to the transect at 1.524 m (5 ft) intervals were sampled whether or not they were known hosts of either virus. One hundred samples were collected along each transect. The percentages of the samples that were from

weed species known to host CMV or BBWV, and the percentages of those infected with either virus were determined. During early September, 100 lettuce plants in the third row from the ditch bank, along which the transect line had been constructed in July, were sampled at each field site. The first lettuce plant sampled from the third row was that which was most directly opposite the first weed that had been sampled in the transect. Every eighth plant along the third row of lettuce was then sampled, and the percentages of those plants infected with CMV or BBWV were determined. Correlation coefficients between the percentages of infected weeds and of infected lettuce plants were calculated. The same sampling procedures were repeated in 1987, but only at two sites. These two were the only sites where the viruses had damaged lettuce in 1986 and that were again planted with late-season lettuce in 1987. Also, in 1987, lettuce growers began to eliminate weeds along ditch banks, particularly in areas where virus outbreaks had been common in previous years. Thus, by July 1987, most of the ditch banks included in the 1986 incidence experiment had been treated with herbicides and were relatively weed-free. At the two sites planted with late-season lettuce in 1987, some of the weeds had been killed by herbicides and the remainder were mowed approximately biweekly.

Incidence of CMV and BBWV in weed hosts in two locations at the borders of lettuce fields. A weed was considered to be "near" a lettuce field if it was within 10 m of the outermost row of lettuce. This distance always included the drainage ditches that bordered the lettuce fields. Weeds sampled and considered to be near lettuce fields often grew on the banks of these ditches. A weed was considered to be at a "farther distance" from the lettuce field if it was between 20 and 40 m from the outermost row of lettuce. Distances between 20 and 40 m often included the interface of herbaceous weed communities, which included the known weed hosts of CMV and BBWV (4), and wooded or forest communities, which seldom included known weed hosts of CMV or BBWV.

Two experiments with CMV were conducted, the first in July 1986 and the second in May 1987. The first experiment used four field sites, three of which had relatively high levels of CMV infection in lettuce in at least the preceeding year. The fourth site had not been planted to late-season lettuce for several years. Three weed species, *Asclepias syriaca* L., *Barbarea vulgaris* R. Br., and *Linaria vulgaris* Mill. were chosen as representative weed hosts for these experiments. These species are common hosts of CMV (4) and were relatively abundant in at least two of the four field sites. At field sites 1 and 2, *A. syriaca* and *B. vulgaris* were abundant, and 25 plants of each species located near the lettuce fields and as many located at a farther distance were tested for the presence of CMV. Similar tests were conducted at field sites 3 and 4 with *L. vulgaris* plants, which were abundant at those sites. The percentage of infected plants in each set of 25 plants was determined. The mean percentage of infection of plants near each lettuce field was then statistically compared with the mean percentage of infection of plants at a farther distance from the field; a paired Student's t test was used. The second experiment used the same four field sites and also the same weed species at each of the sites. A paired Student's t test again was used to compare the mean percentages of infection.

A third experiment with BBWV was conducted in May 1987. The three chosen field sites all previously had contained both lettuce plants and weeds infected with BBWV. One host species, *L. vulgaris*, was abundant at all three sites and, therefore, was used for this experiment. At each site, 40 *L. vulgaris* plants located near the lettuce fields and as many located at a farther distance were tested for the presence of BBWV. A paired Student's t test was used to evaluate the difference between the mean incidence of infection by BBWV of plants near lettuce fields and those at a farther distance from the fields.

Incidence of CMV in weed hosts found adjacent to a field where crop rotation was practiced. During the 1984–1986 lettuce-growing seasons, the incidence of CMV was determined in a stand of *Rorippa islandica* (Oeder) Borbás, a common weed host of the virus (4), that grew along the banks of a drainage ditch on one side of a field cropped to late-season lettuce in 1983–1984 and then cropped to onion (*Allium cepa* L.) in 1985–1987. Fifty and 60 plants were tested for CMV during 1984–1985, respectively. In 1986, 50 plants were tested for CMV in June and 45 more plants were tested in July. In 1987, 50 plants were tested for CMV. Similar surveys of *R. islandica* were made each year at several sites where lettuce was grown before and throughout this study.

RESULTS

Monitoring occurrence of CMV and BBWV in weeds and lettuce fields. Infection of lettuce by CMV occurred during each of the growing seasons from 1984 through 1987. In all cases, when CMV was noted at any particular location and when late-season lettuce was again grown at that location the following year, the disease reoccurred at that location, at least to some extent (Table 1).

Symptoms of infection by CMV (verified by ELISA) generally occurred first on lettuce plants grown in the outermost rows of fields. Also, the incidence of infected lettuce plants at harvest often was visibly greater in these outer rows even when the incidence of infection was high throughout the field. This pattern occurred at three locations in both 1984 and 1985, and at four locations in 1986. In 1987, severe outbreaks of CMV and BBWV did not occur and, therefore, no obvious patterns were noted. However, at two locations where low incidence of both CMV and BBWV occurred in 1987, the infected lettuce plants were located in the outermost rows of the fields. At one location in 1984, infection by CMV occurred in clusters of lettuce plants throughout the field; no conspicuous effect on plants closest to the edges of fields was noted.

Patterns of BBWV infection similar to those of CMV occurred during the growing seasons of 1986 and 1987. At the two sites where BBWV occurred in 1986 and late-season lettuce was again planted in 1987, BBWV reoccurred in 1987. Also, at four locations in 1986, and two in 1987, higher incidences of BBWV occurred in lettuce plants near the edges of fields than in lettuce plants farther into the fields.

In all of these situations, the viruses were also present in weed hosts that grew immediately adjacent to the lettuce fields, generally on the banks of the drainage ditches that bordered each field. The presence of both viruses in weeds that grew on banks of drainage ditches was directly correlated with their presence in lettuce plants adjacent to those weeds at numerous field sites during the four lettuce-growing seasons of 1984–1987. This apparent direct correlation between the occurrence of virus in weeds and the occurrence of virus in lettuce grown adjacent to those weeds was tested experimentally during the growing season of 1986. Correlation coefficients were generated from data collected in that year.

Comparisons of incidences of CMV and BBWV in weeds in July with those in adjacent lettuce plants in September. Strong statistical correlations existed in 1986 between the incidences of the viruses in weeds in July and the incidences of the viruses

TABLE 1. Repetition, during 1984–1987, in location of occurrence of cucumber mosaic virus (CMV) in areas of fields where CMV occurred and in which late-season lettuce was planted at least one consecutive year thereafter

Field site	Number of consecutive seasons that late-season lettuce was planted after the first year CMV occurred	Number of years CMV reoccurred at the site
1	3	3
2	3	3
3	2	2
4	2	2
5	2	2
6	1	1
7	1	1
8	1	1

in lettuce grown adjacent to those weeds in September (Table 2, $r = 0.93$ for CMV; Table 3, $r = 0.88$ for BBWV). At sites where the percentage of weeds infected with either virus was zero or very low, the percentage of lettuce plants that became infected was correspondingly zero or very low. Conversely, at sites where the viruses were relatively common in weeds, the percentages of nearby lettuce plants that became infected with the viruses were higher. Also, weed species known to host the viruses generally were more common at the sites where the highest incidences of infected lettuce plants occurred (Table 2, $r = 0.88$ for CMV hosts; Table 3, $r = 0.79$ for BBWV hosts).

CMV was not detected in weeds or lettuce at either of the two sites sampled in 1987. BBWV was found in two and one of the 100 weed samples collected at each site, respectively, and in none and two of the 100 lettuce samples collected at each site, respectively. At the site where none of the lettuce plants collected along the transect line were infected, a few lettuce plants not on the line were symptomatic and were found to be infected with BBWV. Since only two sites were available in 1987, and, therefore, only four data points, correlation coefficients were not determined. However, these data conform to the direct correlation between the presence of virus in weeds and the occurrence of virus in lettuce found in 1984–1986 and characterized by high correlation coefficients in 1986. In these cases, absence of CMV in weeds was coincident with the absence of CMV in lettuce, and low incidence of BBWV in weeds was coincident with low incidence of BBWV in lettuce.

Incidence of infection by CMV and BBWV in weed hosts in two locations at the borders of lettuce fields. Although CMV was sometimes present in weeds that grew between 20 and 40 m from the outer rows of lettuce, at all field sites except one (field 4), more CMV was found in weeds within 10 m from the outer row (Table 4). In the one exception, virus was not detected in any of the weeds at either of the distances from the outer row of lettuce. The paired Student's t test used to analyze these data indicated the mean incidence of infection of weed hosts within 10 m from lettuce fields was significantly greater ($P = 0.025$) than that of weeds located between 20 and 40 m from lettuce fields during both 1986 and 1987.

The pattern for BBWV was similar to that for CMV (Table 5). Although some *L. vulgaris* plants that grew between 20 and 40 m from the outer row of lettuce were infected with BBWV, the incidence of infection was significantly lower ($P = 0.025$) than in plants found within 10 m from the outer row.

Incidence of CMV in weed hosts found adjacent to a field where crop rotation was practiced. The incidence of CMV infection decreased in *R. islandica* plants that grew next to the

TABLE 2. Incidences of weed plants known to host cucumber mosaic virus (CMV) and sampled from transect lines parallel to edges of commercial lettuce fields and incidences of those plants infected with CMV as of July 20, 1986, compared to the incidences of lettuce plants that grew adjacent to the transect lines and were infected with CMV as of September 10, 1986[a]

Field site	Incidence of weed plants known to host CMV[b]	Incidence of weed plants infected with CMV	Incidence of lettuce plants infected with CMV
1	72	19	12
2	60	11	8
3	50	12	7
4	41	8	6
5	29	0	0
6	25	1	0
7	17	0	0

[a] One-hundred weed samples were collected at 1.5-m (5 ft) intervals along each transect line. The plant closest to each 1.5-m increment along each transect line was sampled, whether it was a known CMV host species or not. The weed samples were collected in May, June, or July, generally before, or soon after, planting of the seeds for the lettuce crops from which the infection data were taken. CMV infection incidence in lettuce was determined for each field site on or before September 10.

[b] See Rist and Lorbeer 1989 (4) for a list of plant species found to host CMV within the areas where this study was completed.

TABLE 3. Incidences of weed plants known to host broad bean wilt virus (BBWV) and sampled from transect lines parallel to edges of commercial lettuce fields and incidences of those plants infected with BBWV as of July 20, 1986, compared to the incidences of lettuce plants that grew adjacent to the transect lines and were infected with BBWV as of September 10, 1986[a]

Field site	Incidence of weed plants known to host BBWV[b]	Incidence of weed plants infected with BBWV	Incidence of lettuce plants infected with BBWV
1	25	8	18
2	13	13	10
3	20	3	0
4	8	2	4
5	0	0	0
6	5	0	0
7	0	0	0

[a] One-hundred weed samples were collected at 1.5-m (5 ft) intervals along each transect line. The plant closest to each 1.5-m increment along each transect line was sampled, whether it was a known BBWV host species or not. The weed samples were collected in May and June, generally before, or soon after, planting of the seeds for the lettuce crops from which the infection data were taken. BBWV infection incidence in lettuce was determined for each field site on or before September 10.

[b] See Rist and Lorbeer 1989 (4) for a list of plant species found to host BBWV within the areas where this study was completed.

TABLE 4. Comparison of the incidences of infection by cucumber mosaic virus (CMV) of weed hosts growing within 10 m of commercial lettuce fields to the incidences of infection by CMV of weed hosts growing between 20 and 40 m of the same fields

Year	Field	Host species	Incidence of infection of 25 plants: Within 10 m from the outer row of lettuce	Incidence of infection of 25 plants: Between 20 and 40 m from the outer row of lettuce
1986	1	*Asclepias syriaca*	5	1
		Barbarea vulgaris	9	2
	2	*A. syriaca*	6	3
		B. vulgaris	12	0
	3	*Linaria vulgaris*	10	3
	4	*L. vulgaris*	0	0
		Mean	7	1.5
1987	1	*A. syriaca*	4	0
		B. vulgaris	6	3
	2	*A. syriaca*	5	2
		B. vulgaris	8	4
	3	*L. vulgaris*	7	2
	4	*L. vulgaris*	0	0
		Mean	5	1.8

TABLE 5. Comparison of the incidence of infection by broad bean wilt virus (BBWV) of *Linaria vulgaris* plants growing within 10 m of commercial lettuce fields to the incidence of infection by BBMV in *L. vulgaris* plants growing between 20 and 40 m of the same fields

Field site	Incidence of infection of 40 plants: Within 10 m from the outer row of lettuce	Incidence of infection of 40 plants: Between 20 and 40 m from the outer row of lettuce
1	6	1
2	9	4
3	8	0
4	13	3
Mean	9	2

field where crop rotation with onion was practiced. In 1984, 17 of 50 samples of *R. islandica* plants collected along the border of the lettuce field were infected with CMV. The next three years, the field was cropped to onion. In 1985, 18 of 60 sample *R. islandica* plants were infected with CMV. In 1986, none of 50 and 45 *R. islandica* plants sampled in June and July, respectively, was infected with CMV. In 1987, none of 40 sample *R. islandica* plants was infected with CMV. Incidence of CMV infection in stands of *R. islandica* adjacent to fields repeatedly cropped to late-season lettuce remained high throughout the time of this study. At one location, infection levels were 22 of 50, 15 of 50, and 19 of 50 in 1984, 1985, and 1986, respectively. At a second location, the infection levels were 7 of 50, 16 of 50, and 13 of 50 during those years, and 12 of 50 during 1987.

DISCUSSION

CMV and BBWV exhibit many similarities in their ecology and epidemiology in the commercial lettuce fields of central New York. Both are nonpersistently transmitted by numerous aphid species (2,5), including *Myzus persicae*, a species common in lettuce fields of the region. Both viruses are common in numerous weed species that grow adjacent to lettuce fields, and both also survive the winter in the overwintering structures of several of these species (4). In this study, both viruses were found to occur at higher incidences in weeds on banks of drainage ditches that network through lettuce fields than in weeds that grew farther from the fields; the presence of such virus reservoirs early in the growing season was strongly correlated with the incidence of infected lettuce plants grown adjacent to the reservoirs later in the season. The actual numbers of infected weeds close to lettuce fields is probably greater than suggested by data in Tables 2–5, because important weed hosts of each virus often colonize drainage ditches much more abundantly than other areas near the fields. For example, *R. islandica*, an important host of CMV (4), often grows in dense stands on ditch banks, but rarely occurs at distances much farther from the field than those banks. Similarly, *Veronica scutellata* L., a host of BBWV (4), is an aquatic species and in our study was seen only in water in the drainage ditches. Also, stands of *L. vulgaris*, an important host of both CMV and BBWV (4), were more common and grew especially dense on ditch banks in comparison with locations farther from the fields.

CMV and BBWV also are similar in that they induce similar and indistinguishable symptoms in lettuce plants. Thus, lettuce infected by either virus commonly is referred to by growers as having resulted from a single disease. In terms of disease management, this view is not necessarily disadvantageous. Results from the present study indicated that both viruses spread from overwintering weeds that grew adjacent to lettuce fields. Incidence of plant infections by nonpersistently transmitted viruses such as CMV and BBWV sometimes decreases sharply as the distances from primary inoculum foci increase (6). Therefore, a way to achieve appreciable reductions of CMV and BBWV in lettuce in central New York would be through the elimination of the major weed reservoirs of the viruses, which are found in those weeds that grow near lettuce fields, particularly on the banks of drainage ditches. Management of CMV through weed control measures in other instances has been effective (1,7). Given that lettuce fields in central New York are closely surrounded by forests and wooded areas in which the weed hosts of CMV and BBWV seldom occur, this practice might even eliminate the viruses as an economic threat. The potential for success in this instance is supported by three facts established during our study. First, CMV and BBWV were detected mostly in weeds that grew near (within 10 m) lettuce fields (Tables 4 and 5). Second, late-season incidences of infection of lettuce plants were directly correlated with early-season incidences of infection of weeds on ditch banks (Tables 2 and 3). Third, in some fields the viruses occurred in the same locations in two, three, or even four consecutive years while other areas of the same fields remained virus-free (Table 1). Therefore, virus that originated from weeds immediately adjacent to the lettuce plants was much more likely to have initiated the outbreaks than was virus that originated from a greater distance and that was spread by chance to the same areas of the fields year after year. Weeds infected with CMV or BBWV and located at extended distances from lettuce fields may represent a potential threat to lettuce. However, elimination of weed hosts on drainage ditch banks and in the immediately adjacent areas would be a sound first step toward management of CMV and BBWV in commercial lettuce fields in central New York.

Practices aimed at reducing the incidence of weeds infected with virus near fields also would be beneficial in management of CMV and BBWV, either with or in the absence of practices aimed at reducing weed densities. Lettuce plantings with high percentages of infected plants represent relatively vast reservoirs of virus. Movement of aphids between the infected lettuce plants and adjacent weeds may be common and could result in spread of virus from lettuce plants to the weeds. This would result in the infection of more overwintering weed hosts and, therefore, the occurrence of more virus in the weeds the next growing season. The fact that both CMV and BBWV occurred at higher incidences in weeds immediately adjacent to fields that had contained diseased lettuce crops (Tables 4 and 5) corroborates this hypothesis. The cycle could then continue as virus spreads from weeds to lettuce (Tables 2 and 3). Avoiding the production of late-season lettuce at the same location in consecutive years either by rotating crops or manipulating planting schedules could prevent this cycle.

A few growers in New York rotate lettuce crops with onion, but this practice is not common. At the only field site in our study where several years of late-season lettuce production were followed by 3 yr of onion production, data collected were consistent with the concept that crop rotation can result in lower incidences of weeds with virus at the borders of cultivated fields. At this site, the biennial CMV host species *R. islandica* had extensively colonized the banks of the drainage ditch that bordered the north end of the field. In August 1984, incidence of CMV was very high both in *R. islandica* that bordered the northern half of the field as well as in lettuce plants in that section of the field. In July 1985, when onions were planted, the incidence of CMV remained high in *R. islandica*. This would be expected because plants (arising from seeds) that became infected in 1984 would still be infected in the summer of 1985 when they were second year shoots sprouted from roots in which CMV had overwintered (4). These plants would have died naturally at the end of the 1985 growing season, and, therefore, would not have been part of the population of *R. islandica* in 1986. Accordingly, in both June and July of 1986, the incidence of CMV in the *R. islandica* plants sampled at this site was zero. Thus, in this case, a marked decline in the incidence of infection of a weed host by CMV occurred when crop rotation was practiced. In contrast, at sites where late-season lettuce was grown in 1984–1986, the incidence of infection by CMV remained relatively high in *R. islandica* through September 1987. Crop rotation or avoidance of late-season lettuce at the same location in consecutive years obviously prevents the late-season buildup of virus in lettuce, and thereby would interrupt the cycle of virus spread from weeds to lettuce and subsequently back to weed hosts that grow on ditch banks that border the fields.

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