Beet Western Yellows Virus in Illinois Vegetable Crops and Weeds

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ABSTRACT

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Beet western yellows virus (BWYV) occurs in a wide range of crop and weed hosts throughout Illinois. Volunteer turnip greens and spinach crops and chickweed are overwintering hosts of BWYV in the East St. Louis area and may be sources of inoculum for spring crops. BWYV was detected in spring 1983 crops after aphids were sighted and eventually infected 80% of the turnip greens and 33% of the spinach tested. Green bell peppers and redroot pigweed are summer hosts of BWYV and may be sources of inoculum for fall-planted turnip greens and spinach. High background levels in enzyme-linked immunosorbent assay (ELISA) from some weeds suggest that ELISA results be verified by other methods, such as transmission, when screening a wide range of plants.

Beet western yellows virus (BWYV) is an important virus of vegetable and other economically important crops (8). It causes stunting and chlorosis in a wide range of dicotyledenous plants. In crops such as sugar beet, turnip, spinach, and lettuce, it is known to cause yield reductions (9).

BWYV is transmitted by aphids in a persistent manner, the most efficient known vector being *Myzus persicae* (Sulz.). *M. persicae* has a wide range of host plants, and in areas where its host range overlaps with that of BWYV, the disease can be widespread (7,14). Susceptible overwintering crops can be a major factor in the epidemiology of BWYV, as can many weeds in a wide range of families (6,7). In Illinois, BWYV has been recovered from soybeans (10); however, its incidence and host range have not been studied.

About 15,000 acres in Illinois are used for the production of fresh-market vegetables. Some of this production is for home vegetable stands, "U-pick" operations, or farmers' markets. Many of the known vegetable hosts of BWYV are grown by these farmers, especially crucifers such as turnip and mustard greens, broccoli, cabbage, and cauliflower and noncrucifer crops such as spinach and peppers. This study was conducted to determine the occurrence of BWYV in vegetable crops and weeds of Illinois and the roles played by different hosts in the epidemiology of this disease.

MATERIALS AND METHODS

Sampling. In 1982, samples of vegetable crops and weeds were collected in the Chicago, East St. Louis, and Cobden areas of northern, central, and southern Illinois, respectively. Samples were taken from each area about every third week from June through October. Samples were also collected occasionally from the Champaign-Urbana area. Plant samples included a wide range of vegetable crops and weeds that either showed yellowing symptoms typical of BWY or were known to be hosts of the virus. Each plant sample consisted of leaves from the lower, middle, and upper plant canopy. Samples were placed in plastic bags and kept at 4 C until used.

To determine possible overwintering hosts of BWYV, a large number of volunteer spinach (*Spinacia oleracea* L.) and turnip greens (*Brassica rapa* L.) from the East St. Louis area and spinach from Chicago Heights that had overwintered from the previous fall plantings were collected from March through 18 May 1983, the date of the first aphid sightings. Weeds growing in or near these vegetable fields were also sampled.

On the basis of 1982 results, a more extensive study was made of BWYV in spring- and fall-grown turnip greens and spinach crops from the East St. Louis area in 1983. Ten to 20 plants from spring crops were sampled biweekly from April through June; fall crops were sampled biweekly in September and October. In addition, weeds growing in or near the vegetable fields and green bell peppers (*Capsicum frutescens* L.) were sampled throughout the summer to determine possible oversummering hosts after spring spinach and turnip fields were plowed under. All plant samples collected were tested by enzyme-linked immunosorbent assay (ELISA).

ELISA. In 1982, plant samples were prepared for ELISA by grinding 1 g of tissue in a mortar and pestle with 3 ml of PBS-T, pH 7.4 (phosphate-buffered saline with 1% Tween 80) (2). Coarse plant debris was removed by filtration through cheesecloth. Samples collected in 1983 were prepared by homogenizing 1 g of plant tissue in 3 ml PBS-T with a Janke and Kunkel Ultra-Turrax type TP 18/10SL tissue homogenizer with a 10-N grinding shaft (Tekmar Company, Cincinnati, OH). Coarse plant debris was suctioned off the top of the extract. Plant extract from each sample was used as antigen preparation for ELISA.

Double-antibody sandwich ELISA was used according to the procedures of Clark and Adams (2) with the following modifications. Immulon Microtiter plates (Dynatech Laboratories Inc., Alexandria, VA) coated with BWYV-immunoglobulin purified in a DE-22 column (Bio-Rad Chemical Division, Richmond, CA) at a concentration of 4 μg/ml were used in 1982. Nunc-Immuno Plates I-96F (Vangard International, Neptune, NJ) were used, and the concentration of coating antibody was lowered to 3 μg/ml in 1983. Antigen preparation, conjugate, and substrate were added at 200 μl/well. Two wells were used for each plant sample and control in 1982. Samples were not duplicated in 1983 because of the consistency of 1982 results. Plates were rinsed three times for 3 min with PBS-T between each step. Plant extracts were incubated in the microtiter plate overnight at 4 C. Antibody conjugated with alkaline phosphatase diluted 1:200 was incubated at room temperature for 4 hr. Substrate (*p*-nitrophenyl phosphate, Sigma Chemical Co., St. Louis, MO) at 1 mg/ml was incubated for 1 hr at room temperature, after which the reactions were stopped with 50 μl/well of 3 M NaOH. Contents of each well were diluted 1:5 with water filtered with a Milli-Q water purification system (Millipore Corp., Bedford, MA), and the absorbance at 405 nm was determined on a Beckman DU spectrophotometer (Beckman Instruments, Palo Alto, CA) using a Gilford 244 3-A rapid sampler (Gilford Instrument Laboratories, Inc., Oberlin, OH). Positive and negative controls consisted of BWYV-infected and uninfected radish leaves prepared as before. Infection with BWYV was indicated when absorbance readings of plant samples were greater than twice the mean absorbance of two negative controls. Selected plants rated positive by ELISA were used to transmit

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BWYV to indicator hosts to check the validity of ELISA results.

Aphid transmission. For transmission tests, 20–30 *M. persicae* were allowed 48 hr of access to leaf samples in sealed petri dishes or to purified virus preparations adjusted to 20% sucrose. Virus was purified from 25–50 g of plant tissue (5). Aphids allowed access to virus by either method were placed on healthy indicator plants for an inoculation access period of 48 hr. Initial transmission tests in 1982 used Scarlet Globe radish (*Raphanus sativus* L.) as the indicator host; after 19 September 1982, shepherd's purse (*Capsella bursa-pastoris* L.) was used because it is one of the diagnostic species listed for BWYV (8). After the inoculation access period, indicator plants were fumigated with DDVP (Vapona) and kept in a growth chamber at 20C and 12 hr of fluorescent and incandescent light (8,000 lux) for 4–5 wk. Samples were taken from the indicator test plants weekly and assayed by ELISA in 1982. In 1983, plants were examined daily for symptoms and were assayed for BWYV by ELISA when symptoms appeared on individual plants or at the end of the 4- to 5-wk incubation period.

RESULTS

Forty-three samples representing 10 species of crop and weed plants found throughout the state of Illinois in 1982 were rated positive for BWYV by ELISA. The species found positive were bigroot morning glory (*Ipomoea pandurata* L.), redroot pigweed (*Amaranthus retroflexus* L.), smartweed (*Polygonum pensylvanicum* L.), purslane (*Portulaca oleracea* L.), pepper (*C. frutescens* L.), lettuce (*Lactuca sativa* L.), chard (*Beta chilensis* L.), spinach, cabbage (*Brassica oleracea* var. *capitata*), and turnip greens. At least one attempt was made to transmit BWYV from each of these species to either radish or shepherd's purse by allowing aphids access to plant parts in sealed petri dishes. Successful transmission by aphids to shepherd's purse occurred from one turnip green source and one spinach source.

The remaining 346 samples representing 34 species in 13 families that were tested in 1982 for BWYV were rated negative. Those plants are common milkweed, lambsquarter, sugar beet, table beet, broccoli, brussels sprouts, cauliflower, collards, common ragweed, horseradish, kale, kohlrabi, mustard greens, radish, Virginia pepperweed, wild mustard, spaghetti squash, yellow crookneck squash, alfalfa, fava bean, garden pea, snap bean, soybean, velvet leaf, mallow, dock, knotweed, eggplant, goldenrod, horsenettle, nightshade, potato, wild carrot, and wild parsnip.

High nonspecific absorbance in ELISA was obtained with a total of 43 samples representing 10 species: chickory (*Cichorium intybus* L.), dandelion (*Taraxacum officinale* Weber), field bindweed (*Convolvulus arvensis* L.), giant ragweed (*Ambrosia trifida* L.), horseweed (*Conyza canadensis* (L.) Cronq.), prickly lettuce (*L. serriola* L.), smallflower galinsoga (*Galinsoga parviflora* Cav.), and spurge (*Euphorbia corollata* L., *E. maculata* L., *E. preslii* Guss.) (Table 1).

In March 1983, plants of Seven Tops turnip greens and Ferry Morris hybrids 7 and 621 and Melody spinach were found to have survived the winter in the East St. Louis area. Melody spinach also overwintered in the Chicago area. BWYV was detected by ELISA in 74% of the turnip plants sampled (Table 2). All of the spinach sampled in the East St. Louis area but only 35% of the spinach plants sampled in Chicago had BWYV detectable by ELISA (Table 2). BWYV was transmitted by direct feed on plant parts or from membrane-fed purified virus in three of four attempts from volunteer turnip greens and in three of five attempts from volunteer spinach, one from Chicago and two from East St. Louis.

Nine virus purifications from volunteer turnip greens and 11 virus purifications from spinach tissue resulted in ultraviolet-absorbing bands 3.6 cm from the meniscus of a linear 10–40% sucrose density gradient. The sedimentation of this virus band, relative to that of a known California BWYV isolate from J. E. Duffus, indicated that the two virus isolates had similar sedimentation rates (5).

Three of five chickweed (*Stellaria media* L.) plants collected in East St. Louis and two of seven plants collected in Chicago in the spring of 1983 before the first aphid sightings were rated positive by ELISA. Virus purified from one chickweed sample from East St. Louis and one sample from Chicago banded in linear sucrose gradients in the same location as that from turnip and spinach. The purified virus was transmitted by aphids to *C. bursa-pastoris* and BWYV infection verified serologically. No other early spring weeds collected were determined to be infected with BWYV.

Symptoms initially observed on spring 1983 plantings of turnip greens (Seven Tops) and spinach (Dark Green Bloomsfield) were mild. Reddening and eventually yellowing of leaves in the lower plant canopy became increasingly

Table 1. Examples of high nonspecific absorbance values in enzyme-linked immunosorbent assay (ELISA) of weeds collected in Illinois in 1983

Species	No. of plants	A_{405nm}[a] Mean[b]	A_{405nm}[a] Range	Unsuccessful aphid transmission attempts
Chickory (*Chichorium intybus*)	6	0.160	0.112–0.228	1
Dandelion (*Taraxacum officinale*)	3	0.158	0.068–0.266	1
Field bindweed (*Convolvulus arvensis*)	7	0.109	0.064–0.117	2
Giant ragweed (*Ambrosia trifida*)	2	0.201	0.126–0.276	0
Horseweed (*Conyza canadensis*)	3	0.107	0.083–0.130	1
Prickly lettuce (*Lactuca serriola*)	5	0.146	0.116–0.190	0
Smallflower galinsoga (*Galinsoga parviflora*)	4	0.077	0.066–0.094	2
Spurge (*E. corollata*)	2	0.132	0.126–0.138	2
(*E. maculata*)	2	0.250	0.246–0.255	2
(*E. preslii*)	3	1.210	0.744–1.580	1

[a]Samples diluted 1:5 in distilled water.
[b]Results from several testing dates. Mean negative control over all testing dates = 0.025, range = 0.014–0.033.

Table 2. Incidence of beet western yellows virus in volunteer turnip greens and spinach from fall 1982 plantings collected in spring 1983

Species	ELISA-positive/ number sampled	A_{405nm}[a] Negative Mean	Negative Range	Positive Mean	Positive Range	Successful aphid transmissions/ attempts
Spinach (*Spinacia oleracea*)						
East St. Louis	27/27	...	...	0.111[b]	0.089–0.139	2/4
Chicago	9/26	0.032[c]	0.023–0.041	0.138	0.106–0.171	1/1
Turnip greens (*Brassica rapa*)	64/86	0.036[d]	0.019–0.050	0.082	0.053–0.106	3/4

[a]Samples diluted 1:5 in distilled water.
[b]Test date 19 April 1983. Mean negative control = 0.022.
[c]Test date 15 April 1983. Mean negative control = 0.029.
[d]Test date 13 April 1983. Mean negative control = 0.026.

prevalent in turnip greens as the season progressed. BWYV was not detected by ELISA in turnip greens and spinach sampled biweekly in the East St. Louis area until 8 days after the first aphids were seen on the plants (18 May) (Table 3). The percentage of plants with detectable BWYV increased steadily until the crops were plowed under. BWYV was transmitted in six of seven attempts by direct feed from ELISA-positive turnip greens leaves. BWYV was transmitted to *C. bursa-pastoris* from each of two spinach plants tested.

Green bell peppers (Lady Bell) were sampled from May through September. BWYV was detected by ELISA in 20% of the pepper plants sampled on 18 August 1983. Between 0 and 15% of the plants sampled on other dates were positive. No trend of increasing incidence of infection was seen. Virus was transmitted to *C. bursa-pastoris* by *M. persicae* allowed access to ELISA-positive pepper leaves in two of four attempts.

BWYV was regularly detected by ELISA in redroot pigweed, which occurs throughout the summer and fall in the East St. Louis area (Table 4). ELISA results were verified by transmission of BWYV in two of three attempts by aphids given access to pigweed leaves from selected samples. Many samples of smartweed and purslane were rated positive by ELISA (Table 4), but attempts to transmit virus by aphids allowed access to plant parts were unsuccessful.

Fall plantings of turnip greens (White Globe Purple Top) and spinach (Ferry Morris hybrids 7 and 621) were sampled in East St. Louis from August through October 1983. Only 9% (10/107) of the turnip greens sampled had virus detectable by ELISA. Only one spinach plant of 38 sampled during September and October had virus detectable by ELISA. No aphid transmissions were attempted from these collections.

DISCUSSION

ELISA of plant samples in 1982 indicated that BWYV probably occurs throughout Illinois in a wide range of plant families. However, except in turnip greens and spinach, ELISA results were not verified by aphid transmission. Transmission attempts to radish failed, and this species is probably not a host for some Illinois BWYV isolates. When shepherd's purse was used as the diagnostic host, aphid transmissions often were successful.

Fall-planted Seven Tops turnip greens and Ferry Morris 7 and 621 spinach survived the winter of 1982, and a high percentage of these plants sampled in the spring of 1983 were infected with BWYV. Overwintering crop plants may play a significant role in the epidemiology of BWYV in this area by providing overwintering hosts for the virus and thus inoculum for spring grown crops. Overwintered turnip plants may also have a significant role in providing hosts for the buildup of *M. persicae* in the spring, as crucifers are preferred colonization hosts for this aphid (1). One cultivar of turnip greens (White Globe Purple Top) was not able to survive the winter in East St. Louis. Planting cultivars in the fall that do not overwinter can eliminate one source of BWYV inoculum the following spring.

Of the weeds sampled in the spring of 1983, only chickweed was confirmed as a host of BWYV by aphid transmission. In all fields where turnip greens and spinach were sampled, chickweed was abundant in the early spring before aphids were seen on the crops. This weed species may also be a source of BWYV inoculum for spring-grown crops.

In the spring of 1983, the growing season for both turnip greens and spinach extended several weeks before and after the first aphids were found in the fields. No virus was detected in either crop by ELISA until 8 days after the first aphids were sighted in the crops on 18 May. The number of infected plants increased steadily after that date until the crops were plowed under. Although no attempts were made to determine the economic impact of BWYV on these crops, the yellowing and reddening apparent on turnip greens, probably caused by BWYV infection, would make part of the crop unmarketable.

BWYV can oversummer in green pepper. Although the incidence of virus infection in green bell pepper was not high in this study, pepper could be a source of BWYV inoculum for fall-grown crops.

BWYV was regularly detected in redroot pigweed throughout the summer, as confirmed by aphid transmission. Smartweed and purslane also may be possible sources of BWYV for infection of fall crops. Although attempts to transmit virus from purslane and smartweed were unsuccessful, the prevalence of these weeds in and near turnip greens and spinach fields throughout the summer suggests that further studies should be made of these species as hosts of BWYV.

The advantages of ELISA over other serological methods for the detection of

Table 3. Percentage of 1983 spinach and turnip greens samples infected with beet western yellows virus as detected by enzyme-linked immunosorbent assay (ELISA)

Samples collected (date)	Turnip greens		Spinach	
	Percent positive by ELISA	Aphid transmissions/ attempts	Percent positive by ELISA	Aphid transmissions/ attempts
13 April	0	...	...	...
25 April	0	...	...	...
18 May[a]	0	...	0	...
26 May	15	...	20	1/1
10 June	65	...	27	...
25 June	50	3/4	33	1/1
12 July	80	3/3	...	...

[a] First aphid sightings on crops.

Table 4. Weeds sampled in 1983 for beet western yellows virus by enzyme-linked immunosorbent assay (ELISA) and aphid transmission

Species	ELISA-positive/ number sampled	A_{405nm}[a] Negative Mean	Negative Range	Positive Mean	Positive Range	Successful aphid transmissions/ attempts
Chickweed (*Stellaria media*)	5/12	0.034[b]	0.023–0.047	0.133[c]	0.123–0.143	2/2
Redroot pigweed (*Amaranthus retroflexus*)	13/17	0.030[d]	0.028–0.033	0.061[d]	0.059–0.063	2/3
Purslane (*Portulaca oleracea*)	2/7	0.036[e]	0.025–0.048	0.064[e]	0.060–0.069	0/1
Smartweed (*Polygonum pensylvanicum*)	11/28	0.015[f]	0.012–0.019	0.064[g]	0.049–0.081	0/4

[a] Samples diluted 1:5 in distilled water.
[b] Test date 11 May 1983. Mean negative control = 0.029.
[c] Test date 25 April 1983. Mean negative control = 0.044.
[d] Test date 18 August 1983. Mean negative control = 0.019.
[e] Test date 14 September 1983. Mean negative control = 0.028.
[f] Test date 12 July 1983. Mean negative control = 0.016.
[g] Test date 18 August 1983. Mean negative control = 0.019.

plant viruses in large-scale testing of field samples have been summarized by Clark and Adams (2). Several researchers have used this method successfully for screening crops for plant virus diseases (3,4,12). In a previous study, ELISA was used to screen a wide range of plants as potential overwintering hosts of maize dwarf mosaic virus (13). Purslane speedwell (*Veronica peregrina* L.), field bindweed, and dandelion produced high nonspecific absorbance levels. In the BWYV-ELISA system used in this study, the weeds listed in Table 1 produced consistently high nonspecific absorbance levels.

Johns (11) found that older leaves of dandelion gave high background levels in an ELISA system because of alkaline phosphatase produced by bacteria in older leaves. In our study, no attempt was made to determine the cause of the high background levels. At least one attempt to transmit BWYV by direct feed on plant parts from chickory, spurge, horseweed, dandelion, and smallflower galinsoga was unsuccessful. Aphids survived the 48-hr acquisition access period on all plants except smallflower galinsoga, suggesting this species may not be a host of *M. persicae*. Because high nonspecific absorbances in ELISA were anticipated from some of the weeds sampled because of the previous reports, plants were considered positive for BWYV by ELISA only if both positive and negative representatives from a species were included in the test.

ELISA was considered a better method than aphid transmission to screen for BWYV in this study because of the number of samples that could be processed at one time. A great deal of information was obtained about the incidence of BWYV in two crops and the occurrence of this virus in several crops and weed species during a single growing season. It is important to stress that when ELISA is used to screen a wide variety of plants, results rated positive should be verified by some other method, such as transmission tests.

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