Identification and Incidence of Pepper Viruses in Northeastern Georgia

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ABSTRACT

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Virus diseases are a major constraint to pepper production in northeastern Georgia, where disease incidence was nearly 100% in both 1983 and 1984. Viruses were identified in leaf samples collected from nine fields by serological (enzyme-linked immunosorbent assays and immunodiffusion) and infectivity tests. Tobacco etch virus (TEV) was the predominant virus in each field (more than 96% of plants tested). Cucumber mosaic virus was detected in eight fields, three of which had 20–50% infection. Potato virus Y was detected in eight of 914 samples. Pepper mottle and tobacco mosaic viruses were not positively identified during these surveys. TEV was isolated from perennial *Solanum* and *Physalis* species located in and near pepper fields, suggesting these hosts may be a source of primary inoculum. Lower disease incidence was observed in TEV-resistant pepper lines. Oil sprays applied weekly did not alter disease incidence or severity.

Virus diseases of pepper (*Capsicum annuum* L.) are common in the southeastern United States and cause major yield losses. In the central Piedmont region of Georgia, tobacco etch virus (TEV) and tobacco mosaic virus (TMV) were reported in pimiento pepper (5–7). Virus diseases have been recognized for many years in northeastern Georgia, where both green and red-ripe bell peppers are produced. In the past few years, virus diseases have become increasingly important to commercial producers. Therefore, a study was initiated to identify the viruses involved, determine sources of inoculum, estimate disease incidence, evaluate resistance in pepper lines, and determine the effectiveness of proposed control strategies.

MATERIALS AND METHODS

Virus identification. Samples from northeastern Georgia pepper fields were tested for the five viruses that commonly infect pepper in the southeastern and southwestern United States (2,10–12). Isolates of TEV, potato virus Y (PVY), and pepper mottle virus (PeMV) were obtained from D. E. Purcifull, University of Florida, and maintained in *Nicotiana tabacum* L. 'Xanthi.' TMV (American Type Culture Collection isolate AC-1) was grown in *N. tabacum* 'Hicks.' A strain of cucumber mosaic virus (CMV), which invades *Vigna unguiculata* (L.) Walp. systemically, was cultured in California Blackeye cowpea. All five viruses were purified according to previously described procedures: TEV (9), PVY (9), PeMV (9), TMV (1), and CMV (8). Antisera were produced in rabbits by a series of three to five intramuscular injections of purified virus (1–2 mg/injection) emulsified in Freund's incomplete adjuvant. Furthermore, some serological tests were performed with TEV, PVY, and PeMV antisera obtained from D. E. Purcifull and with TEV antiserum obtained from the American Type Culture Collection.

The enzyme-linked immunosorbent assay (ELISA), described by Clark and Adams (4), was the primary test used to identify the viruses. Sap from leaves of pepper plants was obtained with a leaf press and diluted fivefold to 10-fold in 0.02 M potassium phosphate buffer (pH 7.3) containing 0.15 M NaCl, 0.03 M KCl, 0.05% Tween 20, 2.0% polyvinyl pyrrolidone (mol wt 40,000), and 0.01 M sodium diethyldithiocarbamate. Assay controls were sap from healthy pepper plants and sap from plants with known viruses. Absorbance readings (410 nm) for sap from healthy plants ranged from 0.00 to 0.12, and positive readings were required to be at least three times higher. In some cases, ELISA results were confirmed by immunodiffusion and infectivity tests. Immunodiffusion plates were prepared with 0.8% Noble agar, 0.5% sodium dodecyl sulfate (SDS), and 1% sodium azide; crude sap was diluted twofold with water containing 1% SDS. Pepper, and sometimes cowpea and tobacco, plants were inoculated with sap expressed from field samples of pepper and tested for the presence of virus by ELISA and immunodiffusion.

Source of inoculum. Pepper transplants for farms in northeastern Georgia are normally produced in southern Georgia. Therefore, seven transplant fields were inspected immediately before digging in early May 1984. Leaf samples from 600 plants were collected and tested by ELISA for TEV, PVY, PeMV, TMV, and CMV.

Weeds located in or bordering pepper fields in northeastern Georgia were inspected for viruslike symptoms, and leaves from these weeds were collected, regardless of the presence of symptoms, and tested by ELISA for TEV, PVY, PeMV, TMV, and CMV.

Disease incidence. Incidence of virus diseases was determined visually about 1, 2, 3, and 4 mo after transplanting. In each field, the number of plants with virus symptoms among 25 consecutive plants in a row was determined at 15–20 sites located at random.

Virus resistance. Disease reaction to pepper viruses was determined under natural field conditions in pepper lines obtained from B. B. Brantley, University of Georgia (C 44-14, C 44-14-14, C 44-14-15, C 44-14-16, C 44-14-17, C 44-14-19); B. Villalon, Texas A&M University (Tambel 1, Tambel 2, TAM 800 11-6-B, TAM 8105-2-1); and F. W. Zettler, University of Florida (FL BG-1). All lines were planted at the Georgia Mountain Station near Blairsville, which is centrally located in the northeastern Georgia pepper-producing area. The test plot included 12 pepper lines, each considered resistant to one or more of the five viruses named, and four susceptible cultivars (California Wonder, Keystone Resistant Giant, Yolo Wonder B, and Grande Rio 66). No plants were inoculated artificially; both infection and spread were by natural means. The experimental design was a randomized complete block with four replicates. Ten plants of each of the 16 pepper lines were planted within each replicate with 30 cm between plants and 1 m between rows. The overall plot size was 8 × 36 m.

Stylet oil test. Two field sites at the Georgia Mountain Station were used to evaluate the effectiveness of an oil spray in reducing virus incidence in peppers. One site, a 2-ha rectangular commercial field, was isolated in a valley with no other vegetable production within 400 m; the entire site was sprayed. The second site (30 × 30 m) was next to other pepper

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research plots and was separated into four subplots, two of which were sprayed.

J. M. S. Stylet Oil was mixed with water (0.74 L/100 L) in a Meyers (model 100 TMG) sprayer with constant agitation by recirculation under pressure and was applied with a boom sprayer with 12 cone nozzles (Tee Jet TX4-SS) spanning two rows. The nozzle tips were positioned 30.5 cm above the leaf canopy, and the spray was applied at a pressure of 28 kg/cm^2 with the tractor speed at 4.8 km/hr. All plants were sprayed with oil immediately after transplanting and weekly thereafter. Additional sprays were applied after rains of 0.84 cm or more as soon as the ground would support the machinery.

RESULTS

Virus identification. In 1983, TEV was identified by ELISA in plants from each of 10 pepper fields. Leaves from 140 plants in each field were collected, and sap from 10 leaves (each from a different plant) was combined for one ELISA sample. All 140 ELISA samples were positive for TEV, but TMV was not detected in any sample. Tests for other viruses were not made in 1983.

In 1984, TEV was detected in eight of eight fields, CMV in eight of nine fields, and PVY in three of nine fields (Table 1). ELISA results indicated that PeMV and TMV were present in one plant each; however, these results could not be confirmed by immunodiffusion tests. TEV was detected in 96% of the plants tested, CMV in 21%, and PVY in fewer than 1%. Mixed infection of TEV and CMV occurred in more than 95% of the plants with CMV.

The TEV isolate from northeastern Georgia appeared similar to the TEV isolate obtained from Florida. Both isolates induced leaf mosaic and mild stunting on pepper plants. In immunodiffusion serological tests with TEV antiserum from Florida, precipitin lines of the two isolates fused and no spurs were noted. In greenhouse tests, the CMV isolate from pepper caused chlorosis and severe stunting on peppers and mosaic on California Blackeye cowpea and Xanthi tobacco. These reactions were similar to those caused by a Georgia CMV isolate from cowpea (3). Furthermore, in immunodiffusion tests with antiserum raised to the cowpea isolate, precipitin lines of the two CMV isolates fused and no spurs developed.

Disease incidence. By September 1983, virtually all pepper plants in 10 northern Georgia fields had viruslike symptoms. Monthly surveys in 1984 demonstrated a progression in virus disease incidence from 0% about 3 wk after transplanting (June) to an average of 88% during midharvest in September (Table 2). Incidence varied among fields, with the greatest difference observed during the July and August surveys. On the last survey date (September), two fields had a 60% estimated virus disease incidence and the other six had a 90–100% incidence. Variation in incidence also was noted within fields, but concentrations of diseased plants could not be related to edges of fields or to a specific direction in relation to cultivated or uncultivated areas.

In May 1984, several thousand plants of several pepper cultivars in southern Georgia transplant beds were observed visually, and none was found with viruslike symptoms. When leaves from 600 plants (200 each of three cultivars) were collected at random and tested by ELISA, none of the five test viruses was detected. Furthermore, several thousand plants in two fields were observed carefully 3 wk after transplanting in northeastern Georgia, and no plants with viruslike symptoms were found at that time.

On 12 September 1984, leaves were collected from plants with and without virus symptoms and tested for TEV by ELISA. The virus was detected in 14 of 20 plants without symptoms and in 174 of 176 plants with symptoms.

Source of inoculum. TEV was detected in four Solanaceae species and CMV in one species (Table 3). PeMV, PVY, and TMV were not found in any of the Solanaceae plants. Distinct viruslike symptoms were observed on all of the apple of Peru and jimsonweed plants and most of the black nightshade plants that tested positive for virus. However, very few of the horsenettle plants had symptoms. None of the five suspected pepper viruses was found in more than 600 plants of about 30 other plant species tested.

Virus resistance. In the field resistance study, incidence of TEV, as determined by ELISA, was nearly 100% for each of four cultivars (California Wonder, Grand Rio 66, Keystone Resistant Giant, and Yolo Wonder B). Typical symptoms were mosaic and mild to moderate stunting. On four of the breeding lines (C 44-14, C 44-14-14, C 44-14-15, and C 44-14-17), TEV caused a mild mosaic with little or no stunting on about 50% of the plants. In the C 44-14-16, C 44-14-19, FL BG-1, NV 22, Tambel 1, Tambel 2, TAM 800 11-6-B, and TAM 8105-2-1 lines, TEV incidence ranged from 5 to 32%. Infected plants showed mild mosaic but none was stunted.

Stylet oil test. Incidence of virus disease was estimated three times during the 1984 growing season in the 2-ha field experiment. On 14 June, virus disease incidence in about 10,000 plants was 0%. On 13 July, 199 of 900 pepper plants

Table 1. Identification of viruses in pepper by enzyme-linked immunosorbent assay (ELISA)

Field	TEV[a]	CMV	PVY	PeMV	TMV
1	...	2/20[b]	0/120	0/120	0/120
2	46/46	9/21	0/196	0/196	0/164
3	45/46	2/21	0/46	1/46[c]	0/14
4	46/46	3/21	2/171	0/171	0/139
5	39/46	1/21	0/46	0/46	0/14
6	43/46	0/18	0/46	0/46	0/14
7	43/46	39/169	3/196	0/196	1/164[c]
8	48/48	12/21	0/46	0/46	0/14
9	47/47	2/16	3/47	0/47	0/14
Total	357/371	70/328	8/914	1/914[c]	1/657[c]

[a] TEV = tobacco etch virus, CMV = cucumber mosaic virus, PVY = potato virus Y, PeMV = pepper mottle virus, and TMV = tobacco mosaic virus.
[b] No. of positive reactions/no. of samples tested.
[c] The positive ELISA reaction could not be confirmed by an immunodiffusion test.

Table 2. Incidence of virus disease in pepper in northeastern Georgia in 1984

Survey date	Fields observed	Plants observed	Disease incidence (%)	
			Range[a]	Average
14 June	2	20,000[b]	0	0
13 July	6	1,950	16–74	36
6 August	3	1,360	25–85	56
12 September	8	3,800	60–100	88

[a] Among fields.
[b] Estimated number. Three people observed the fields thoroughly, one field with 50,000–60,000 plants and the other with 30,000–40,000.

Table 3. Tobacco etch virus (TEV) and cucumber mosaic virus (CMV) in four Solanaceae species

Plant species	Plants with virus/plants tested[a]	
	TEV	CMV
Horsenettle (*Solanum carolinense*)	17/36	0/20
Black nightshade (*S. nigrum*)	11/24	0/10
Apple of Peru (*Nicandra physalodes*)	9/9	2/9
Jimsonweed (*Datura stramonium*)	7/7	0/7

[a] Virus identified by enzyme-linked immunosorbent assay.

showed symptoms (22%). By 12 September, 452 of 500 plants were diseased (90%). In the small plot experiment, disease incidence was similar in both the sprayed and unsprayed plots. Numbers of plants with symptoms were 0 of 1,200, 110 of 250, and 300 of 300 on 14 June, 16 August, and 12 September, respectively.

DISCUSSION

The predominant pepper virus disease in northeastern Georgia in 1983 and 1984 was caused by TEV. Nearly all of the pepper plants were infected by the end of the season in both years, with about half infected by the middle of the growing season in 1984. CMV, also detected in peppers, occurred in most fields, and the incidence was high enough in one-third of the fields to speculate that the virus disease was a limiting factor in fruit production. The very low incidence of PVY and the lack of confirmatory evidence for any infection by PeMV and TMV suggests that they are not widespread in northeastern Georgia.

The importance of mixed infections of TEV and CMV in pepper was not determined in this study. Only two plants in the field were observed with symptoms (chlorosis and necrotic line patterns) typical of CMV single infections of pepper in the greenhouse. CMV-infected plants were found by random sampling in the field and subsequent ELISA, and a deliberate attempt to identify CMV-infected plants by observation was unsuccessful. One possible explanation is that TEV infection may have occurred first and masked the symptoms of CMV.

Because no virus could be detected in pepper seedlings either before or 3 wk after transplanting, the source of the pepper viruses in northeastern Georgia pepper fields probably originates from weed species in the area of pepper production. From our studies, two perennial Solanaceae species, horsenettle and black nightshade, were the most likely sources of TEV. Both species were observed in and near pepper fields, although horsenettle was more prevalent. Unless TEV is seed-transmitted in jimsonweed and apple of Peru, these annual species would not be important as sources of primary inoculum, although they could be a factor in the epidemiology of the disease. CMV was found in apple of Peru, but we have insufficient data to estimate the importance of this annual as a reservoir for CMV.

In 1984, virus disease incidence was similar in fields sprayed with stylet oil and those not sprayed. Because this control strategy failed and no other is readily apparent, the use of resistant pepper lines would be highly desirable. Several lines obtained from agricultural experiment stations in Florida, Georgia, and Texas appeared to be resistant to the TEV isolates that occurred naturally in northeastern Georgia. Although TEV was detected in one or more plants of each line, most plants were virus-free according to ELISA results. Plants that were infected showed relatively mild symptoms. Future studies of the resistant lines will determine the nature of resistance and evaluate fruit production and horticultural characteristics.

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