Survey of Cowpeas in South Carolina for Six Viruses

M. H. COLLINS, Former Graduate Assistant, J. F. MURPHY, Graduate Assistant, W. WITCHER, Professor, and O. W. BARNETT, Professor, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29631

ABSTRACT

Collins, M. H., Murphy, J. F., Witcher, W., and Barnett, O. W. 1984. Survey of cowpeas in South Carolina for six viruses. Plant Disease 68:561-563.

A survey to determine the occurrence and incidence of six viruses in cowpeas in South Carolina was made in 1981 and 1982. During those 2 yr, 16 counties were involved in the commercial production of cowpeas, but only one county was involved both years. Four viruses were found (blackeye cowpea mosaic, cowpea chlorotic mottle, cowpea severe mosaic, and cucumber mosaic). Incidence within a county ranged from 1 to 76% and plants infected by more than one virus were found in 10 counties. Southern bean mosaic and cowpea mosaic viruses were not detected.

Blackeye cowpea mosaic virus (BlCMV), cowpea chlorotic mottle virus (CCMV), cowpea severe mosaic virus (CSMV), cucumber mosaic virus (CMV), and southern bean mosaic virus-cowpea strain (SBMV-C) infect cowpeas (Vigna unguiculata (L.) Walp. subsp. unguiculata) in the southeastern United States (1,4,7,11-13). Another virus, cowpea mosaic (CPMV), seriously affects cowpea production in other parts of the world (2,3) but has been reported only once in the United States (10). Its presence in South Carolina would be of concern to all cowpea producers in the Southeast. This survey was conducted to determine the presence and prevalence of these six viruses of cowpea in South Carolina.

MATERIALS AND METHODS

Sixteen counties, each with an acre or more of commercially grown fall cowpeas, were surveyed in 1981 and/or 1982. An estimate of acreage and the number of commercial cowpea fields of at least an acre were obtained from county extension personnel in each of the 46 South Carolina counties. In 1981, the acreage reported was 360 in five counties, and in 1982, there were 910 A in 22 fields in 12 counties. Because the survey was done by collecting a uniform number of

Research supported by Cooperative Agreement 58-7B30-0-161 between the South Carolina Agricultural Experiment Station and the USDA Agricultural Research Service.

Contribution No. 2248 of the South Carolina Agricultural Experiment Station. A portion of this paper was presented at the Southern Division of American Phytopathological Society meeting in Atlanta, GA in February, 1983.

Accepted for publication 19 March 1984 (submitted for electronic processing).

samples per acre, comparisons of the virus infection rates were made in different parts of the state. Fields were sampled when cowpeas were near maturity. A single, green leaflet was taken from each of 10 plants per acre, at random throughout the field to determine incidence. Incidence per county was obtained by dividing the total number of plants infected by the total number of plants in the sample per county and year. This is a weighted average of percentage of infections recorded in different fields, with percentages adjusted proportionally to their acreage. To detect virus presence, additional samples were taken from plants with viruslike symptoms not found

in the random samples. Samples consisted of leaflets with symptoms (such as mosaic, mottle, or leaf distortion). Samples were placed individually in moistened paper towels and transported in an ice chest to a refrigerator (4 C). Samples were refrigerated no more than 3 days before analysis.

Sap was expressed from the leaf samples with a leaf squeezer. The grinding buffer used was phosphatebuffered saline with Tween 20 (PBS Tween) and 0.02 M sodium diethyldithiocarbamate (NaDIECA) (9). In 1981, assays for five of the six viruses were by direct enzyme-linked immunosorbent assay (ELISA) according to the method of McLaughlin and Barnett (9). Antiserum against the bean strain of SBMV (SBMV-B) was used in Ouchterlony gel double-diffusion to assay for SBMV-C. This antiserum and the gel diffusion test were used because antiserum to the cowpea strain was not available when the the survey began and SBMV-B antiserum did not detect SBMV-C in ELISA. In 1982, ELISA was used to assay for all six

Gel double-diffusion plates for the first

Table 1. Number of cowpea fields surveyed and incidence of virus infection in 16 South Carolina counties in 1981 and 1982

	No. of plants sampled/county	No. of fields	Incidence (%) ^a				Total incidence ^b
County			BICMV	CCMV	CMV	CSMV	(%)
1981							
Fairfield	70°	3	2.9	P	25.7		28.6
Lexington	400	1	0.5	_	0.2	_	0.7
Greenville	195	10	1.5	P	7.7	_	8.7
Oconee	45	2	31.1	P	2.2	_	33.3
Union	19	3	10.5	5.3	10.5	-	21.0
1982							
Abbeville	19	2		P	5.3	_	5.3
Anderson	5	1	20.0	40.0	_	_	60.0
Colleton	55	4	P	P	1.8	P	1.8
Dorchester	30	1	_	_	P	P	P
Georgetown	20	2	P	_	P	75.0	75.0
Greenwood	20	2	P	10.0	10.0	_	15.0
Greenville	85	2	56.5	4.7	5.9	_	61.8
Horry	30	1	_	_	-	76.7	76.7
Marion	. 49	2	36.7	_	2.0	28.6	57.1
Orangeburg	50	3	42.0	_	18.0	22.0	64.0
Marlboro	12(3)	1	5.9	_	11.8	P	17.7
Sumter	-(3)	1	P	_	P	_	

^aPercentage of infections based on 10 random plants per acre sampled in each county. P = virus present in the county but not found in random samples, -= no virus found in random samples or plants collected with symptoms, BlCMV = blackeye cowpea mosaic virus, CCMV = cowpea chlorotic mottle virus, CMV = cucumber mosaic virus, and CSMV = cowpea severe mosaic virus.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

^{© 1984} The American Phytopathological Society

bIncidence per county was obtained by dividing the total number of plants infected by the total number of random plants sampled per county.

^cTo obtain acres per county, divide sample number by 10. Numbers in parentheses signify acres in the county; some samples were lost during processing.

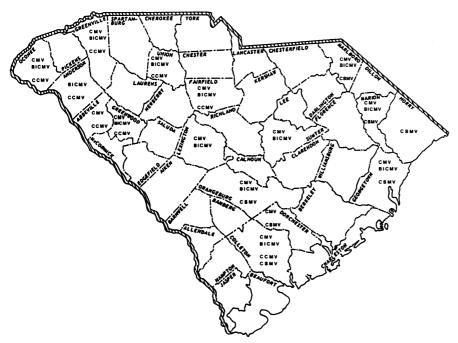


Fig. 1. Locations of South Carolina counties surveyed and the viruses found in cowpea in each county. CMV = cucumber mosaic virus, BlCMV = blackeye cowpea mosaic virus, CCMV = cowpea chlorotic mottle virus, and CSMV = cowpea severe mosaic virus.

329 samples contained 0.8% agarose in 0.03 M sodium phosphate buffer, pH 7.0, with 1% NaN3. Medium for the last 400 samples was changed to 0.8% agarose, 1% NaCl, and 1% NaN3 in distilled water. This change in medium prevented nonspecific reactions. Twenty-four samples were tested per plate. Reactant wells (7 mm in diameter) were spaced 4-5 mm apart from edge to edge. The test sap and antiserum for SBMV-B were incubated in a moist atmosphere for 24 hr at 24 C. Each group of seven ELISA plates and each group of five gel plates included a positive and negative control.

Gel plates were viewed in a light box after 24 hr to detect precipitin bands. All ELISA plates were rated qualitatively after being allowed to develop for 90 min. A strong reaction was rated +++ and a weak reaction, +. All wells that showed a color reaction adequately above the background were recorded as positive and the corresponding leaf sample was recorded as virus-infected. Virus concentration from individual sample leaves was not determined.

RESULTS AND DISCUSSION

A total of 115.4 A of commercial cowpeas in 16 counties was surveyed (Table 1). Neither SBMV-C nor CPMV were detected in any county. Of the four viruses found, CMV was most prevalent (14 counties), followed by B1CMV (13 counties), CCMV (8 counties), and CSMV (7 counties) (Table 1). In individual fields, virus incidence ranged from 100% to zero, even in the samples collected with viruslike symptoms. The median infection per field ranged from 20 to 30%. Highest incidences in individual fields by virus were 100, 92, 40, and 40%

for CSMV, BlCMV, CCMV, and CMV, respectively. Although CMV was more widespread than CSMV (Fig. 1), its incidence was not as high as that of CSMV in any one field.

Mixed infections were detected in 10 counties (Table 2). Although the average incidence of mixed infections per county was not over 9% (Table 2), several individual fields had between 10 and 40% of the plants with mixed infections. Because mixed infections such as BICMV/CMV (stunt disease) and CCMV/SBMV-C have been reported to cause yield reductions in individual plants (6,8), the ultimate effect of 10-40% mixed infections in a field could be considerable. Because mixed infections were common and because viruses that cause cowpea stunt are seedborne and are spread by aerial vectors, growers should plant only virus-free seed in an effort to reduce initial infection.

The geographic locations of the counties surveyed and the viruses detected in cowpea in each county are shown in Figure 1. The aphid-transmitted viruses, CMV and BlCMV, were found generally over the state; however, the beetle-transmitted viruses, CCMV and CSMV, seemed to be more localized. CSMV was found in the northeastern counties and CCMV was found in the northwestern counties. Both viruses were found in the southern counties (Fig. 1).

Cowpea mosaic virus (probably CSMV) and peanut stunt virus have been reported from cowpea in North Carolina, whereas SBMV, CMV, BlCMV, and CCMV have been reported from cowpea in Georgia (two adjoining states) (5,7). Infection of soybean by CCMV was reported in Georgia but not in North

Table 2. Mixed infections of cowpea viruses detected in nine counties in South Carolina

Virus ^a	County	Infection ^b (%)
BICMV/CMV		
(stunt)	Oconeec	$\mathbf{P}^{\mathbf{d}}$
` '	Fairfield ^c	P
	Greenville ^c	0.51
	Union ^c	5.26
	Colleton	P
	Greenville	2.35
	Marion	2.04
	Orangeburg	P
	Sumter	P
BICMV/CCMV	Union ^c	P
	Greenville	3.53
BICMV/CSMV	Colleton	P
•	Georgetown	P
	Marion	8.16
	Orangeburg	P
BICMV/CMV/		
CSMV	Marion	P
CCMV/CSMV	Colleton	P
CCMV/CMV	Fairfield ^c	P
	Greenwood	5.00
CMV/CSMV	Marion	P
•	Georgetown	P

^aBICMV = blackeye cowpea mosaic virus, CCMV = cowpea chlorotic mottle virus, CMV = cucumber mosaic virus, and CSMV = cowpea severe mosaic virus.

^bPercentage of infections based on 10 random plants per acre sampled in each county.

Surveyed in 1981, others surveyed in 1982.

^dP = mixture present but not found in random samples.

Carolina. It is interesting that CCMV was found on the Georgia side of South Carolina and CSMV on the North Carolina side. This may indicate that South Carolina is in a region between the areas of natural distribution of CCMV and CSMV.

LITERATURE CITED

- Anderson, C. W. 1955. Vigna and crotalaria viruses in Florida. II. Notations concerning cowpea mosaic virus (*Marmor vignae*). Plant Dis. Rep. 39:349-353.
- Chant, S. R. 1959. Viruses of cowpea Vigna unguiculata (L.) Walp. in Nigeria. Ann. Appl. Biol. 47:565-572.
- Chant, S. R. 1960. The effects of infection with tobacco mosaic virus and cowpea yellow mosaic virus on growth rate and yield of cowpea in Nigeria. Emp. J. Exp. Agric. 28(110):114-120.
- 4. Elliott, J. A. 1921. A mosaic of sweet and red clover. Phytopathology 11:146-148.
- Gooding, G. V., Jr. 1977. Host index of plant viruses in North Carolina. Pages 7-12 in: North Carolina Plant Disease Index. L. F. Grand, ed. N.C. Agric. Exp. Stn. Tech. Bull. 240.
- Harrison, A. N., and Gaudauskas, R. T. 1968. Effects of some viruses on growth and seed production of two cowpea cultivars. Plant Dis. Rep. 52:509-512.
- Kuhn, C. W., Brantley, B. B., and Sowell, G., Jr. 1966. Southern pea viruses: Identification, symptomatology, and sources of resistance. Ga. Agric. Exp. Stn. Bull. 157. 22 pp.
- Kuhn, C. W., and Dawson, W. O. 1973. Multiplication and pathogenesis of cowpea chlorotic mottle and southern bean mosaic viruses in single and double infections in cowpea. Phytopathology 63:1380-1385.
- McLaughlin, M. R., and Barnett, O. W. 1978. Enzyme-linked immunosorbent assay (ELISA) for the detection and identification of forage legume viruses. Proc. South. Pasture Forage Crop Improv. Conf. 35th, Sarasota, FL.

- McLaughlin, M. R., Thongmeearkom, P., Milbrath, G. M., and Goodman, R. M. 1977. Isolation and some properties of a yellow subgroup member of cowpea mosaic virus from Illinois. Phytopathology 67:844-847.
- Pio-Ribeiro, G., Wyatt, S. D., and Kuhn, C. W. 1978. Cowpea stunt: A disease caused by the synergistic interaction of two viruses. Phyto-pathology 68:1260-1265.
 Shepherd, R. J., and Fulton, R. W. 1962. Identity
- of a seed-borne virus of cowpea. Phytopathology 52:489-493. 13. Whipple, O. C., and Walker, J. C. 1941. A strain
- of cucumber mosaic virus pathogenic to bean and pea. J. Agric. Res. 62:27-60.