

Reactions of 16 Cowpea Cultivars to Six Viruses

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ABSTRACT

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Sixteen cowpea cultivars were inoculated with blackeye cowpea mosaic virus (BICMV), cowpea chlorotic mottle virus, cowpea mosaic virus, cowpea severe mosaic virus, southern bean mosaic virus (cowpea strain), and cucumber mosaic virus and rated for susceptibility. The entire study was conducted three times in the greenhouse with five replicates of two plants for each treatment in the first two studies and with four replicates in the third study. Two weeks after inoculation, symptoms were recorded. All plants were rated for susceptibility. Brown Crowder, Magnolia Blackeye, Mississippi Silver, Mississippi Purple, and Worthmore had promising levels of resistance or tolerance to BICMV. Among the cultivars, there appeared to be some resistance or tolerance to the other five viruses.

In the southeastern United States, cowpeas (edible southern pea form, *Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) are commonly infected with one or more of five viruses: blackeye cowpea mosaic virus (BICMV) (7), cowpea chlorotic mottle virus (CCMV) (5), cowpea severe mosaic virus (CSMV) (3), southern bean mosaic virus (cowpea strain, SBMV-C) (5), and cucumber mosaic virus (CMV) (5). A sixth virus, cowpea mosaic virus (CPMV) (11), which seriously affects cowpea production in other parts of the world, has been reported only once in the United States (8).

The purpose of this study was to determine susceptibility of the 15 most commonly grown cultivars of cowpea. A 16th cultivar, Worthmore, was included because of its reported tolerance to CMV, SBMV-C, and CCMV (4).

MATERIALS AND METHODS

Sixteen cultivars of cowpea (Big Boy, Brown Crowder, California No. 5 Blackeye, Colossus, Colossus 80, Cream 40, Dixielee, Hercules, Knuckle Purple Hull, Magnolia Blackeye, Mississippi Purple, Mississippi Silver, Purple Hull

Pinkeye, Purple Tip Crowder, Worthmore, and Zipper Cream) were grown for inoculation with six viruses in the greenhouse. The virus isolates used (all originally from cowpea) and their sources were BICMV (F. W. Zettler, University of Florida), CCMV-type (C. W. Kuhn, University of Georgia), CPMV-Sb and CSMV-Arkansas (J. P. Fulton, University of Arkansas), SBMV-C (R. W. Fulton, University of Wisconsin), and CMV-CI (cowpea isolate from Clemson).

Carolina Mix and sand in a 1:1 (v/v) ratio were sterilized and 3.7-L metal cans were filled within 1.5 cm of the top with the mixture. Seed were grown at Clemson in plots rogued for off-type plants and plants with viruslike symptoms. Seed transmission was not detected in any seed lot. Six to eight seeds were direct-seeded into each can and seedlings were thinned to two per can when 8-10 cm tall. Two 6-m benches were divided into three sections, each containing plants of all cultivars inoculated with one virus. Controls were placed on a separate bench (except in the third study, when the number of replicates was reduced to four). Total plants inoculated with each virus were 10 in each of the first two studies and eight in the last, making a total of 28. Each section was separated by barriers. Cans of each cultivar were randomized in each section. Eight to 9 days after thinning, plants were dusted with 300-mesh corundum powder and inoculated. Inocula were prepared by grinding infected tissue in a mortar with a pestle with 0.03 M sodium phosphate buffer (pH 8.0) containing 0.02 M 2-mercaptoethanol (2.5 ml/g of tissue). A gauze pad moistened with the inoculum was rubbed gently on both primary leaves. Virus symptoms on the plants were recorded 2 wk after inoculation and several times during the following 4 wk.

All plants not showing symptoms were assayed for virus by inoculation of indicator hosts or ELISA (2).

Aldicarb (Temik) and benomyl (Benlate), 0.2 g each, were added to each can to prevent leaf miner and leaf spot damage. A soluble 20-20-20 fertilizer was applied in the irrigation water once before symptoms were recorded.

Indicator plants (*Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., *Nicotiana tabacum* L. 'Burley 21,' and Clay cowpea) were inoculated to assess infectivity of inocula and to detect possible contamination. Contaminations were not detected in either the inoculum or the test plants. Aphid infestations were not observed during these studies.

Plants were rated S+ if symptoms developed that were typical of those of the virus with which they were inoculated, NS+ if no symptoms developed but the assay indicated infection, and NS- if no symptoms developed and the assay indicated no infection. Each inoculated plant that did not become infected was considered resistant (NS-); all others were susceptible. Susceptible plants that showed no symptoms were considered tolerant (NS+) and those with symptoms were considered sensitive (S+).

RESULTS

The reactions of the 16 cultivars are listed in Table 1. There were variations in symptom expression caused by each virus; symptom variation was often cultivar dependent. Symptoms caused by BICMV included necrotic local lesions, general stunting, mild mosaic, and typical mosaic with veinclearing and green veinbanding (Fig. 1) (7). In the third study, symptoms were particularly cultivar dependent. Six cultivars showed no symptoms; Zipper Cream, Colossus 80, Colossus, and Hercules showed a mild mosaic; and Dixielee, Purple Hull Pinkeye, Knuckle Purple Hull, Big Boy, and California No. 5 Blackeye showed the more typical mosaic pattern with veinclearing and green veinbanding. Symptoms of CCMV-infected plants were not cultivar dependent but ranged from a mild green mottle to almost completely yellow leaflets (Fig. 2). Symptoms in CPMV-infected plants ranged from mild to intense mosaic with blistering of leaflets to death of plants (Fig. 3). Only mild mosaic symptoms were expressed by CSMV-infected plants (Fig. 4). Occasionally, necrotic ring spots

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appeared in the inoculated primary leaves. General symptoms of SBMV-C were mild mosaic on infected leaves but some plants had distorted or rugose leaflets, and inoculated leaves sometimes had chlorotic local lesions (Fig. 5). Symptoms of CMV-CI were a mild mosaic with some ring spotting (Fig. 6), but these tended to disappear 2-3 wk after inoculation.

There were more cultivars resistant to BICMV than to the other viruses; six cultivars had 20 or more resistant plants of 28 inoculated (Table 1). None of the BICMV-inoculated Worthmore plants developed symptoms and only one Magnolia Blackeye and one Mississippi Silver plant developed symptoms (Table 1). Of these, Mississippi Silver seems the most promising for use in breeding programs because only four plants were susceptible. Seven Magnolia Blackeye and eight Worthmore plants were susceptible.

Two cultivars, Mississippi Purple and Mississippi Silver, had promising levels

of resistance to CCMV (11 resistant of 28 inoculated). Four cultivars had promising levels of resistance to CPMV, six to CSMV, and six to SBMV-C (Table 1). Only one cultivar, Brown Crowder, had more than 10 plants resistant to CMV. None of the progeny of these "resistant" plants have been tested to determine if the plants were susceptible but had escaped infection.

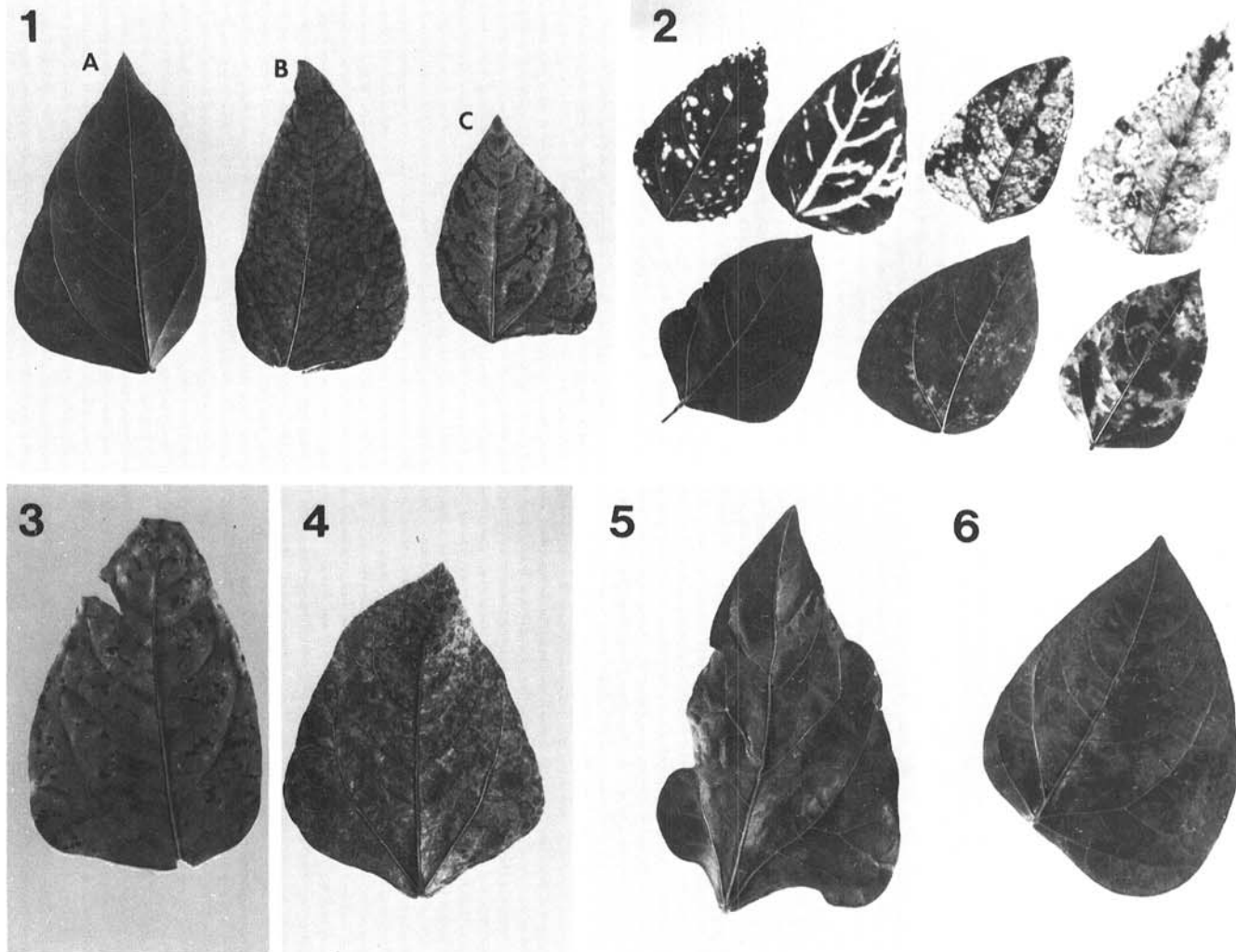
When inoculations with all six viruses are considered, four cultivars, Mississippi Purple, Mississippi Silver, Brown Crowder, and Worthmore, had low levels of infection (40, 49, 33, and 35% resistant and only 50, 42, 43, and 44% susceptible/sensitive plants, respectively, of 168 plants or 28 plants of each cultivar inoculated with six viruses). Magnolia Blackeye and Worthmore had more susceptible/tolerant plants than the other cultivars (more than 20%).

DISCUSSION

Variation of symptom expression among cowpea cultivars or lines is

common for BICMV (7; BYMV in 5), CMV (1), and SBMV-C (9). Symptom variation among cultivars infected with CCMV is not common (5). We have not considered the effects of virus strains on variation of symptom expression or of cultivar susceptibility. For instance, CSMV-Arkansas causes milder symptoms than some other CSMV strains (3).

The cultivars Mississippi Silver, Mississippi Purple, Worthmore, and Brown Crowder, which had the largest proportions of resistant plants, all have common ancestors. W. W. Hare (*personal communication*) found virus field resistance in a plant (4951) of Brown Crowder (Brown Sugar Crowder). This source of resistance, reselected over many years, was used in the parentage of Mississippi Silver and Mississippi Purple. Mississippi Silver is one of the parents of Worthmore (4). This same source of virus resistance (M855) is in the parentage of Magnolia Blackeye, which had a high proportion of susceptible/tolerant plants. Apparently, this field



Figs. 1-6. Symptoms of six viruses on cultivars of *Vigna unguiculata*. (1) Blackeye cowpea mosaic virus-infected leaves of Knuckle Purple Hull showing (A) no symptoms, (B) mild mosaic, and (C) mosaic with vein clearing and green vein banding. (2) Cowpea chlorotic mottle virus-infected leaves of California No. 5 Blackeye showing varying degrees of chlorosis. (3) Cowpea mosaic virus-infected leaves of cultivar California No. 5 Blackeye showing systemic mosaic. (4) Cowpea severe mosaic virus-infected leaves of California No. 5 Blackeye showing systemic mosaic. (5) Southern bean mosaic virus-C-infected leaves of California No. 5 Blackeye showing systemic mosaic and rugosity. (6) Cucumber mosaic virus-infected leaves of California No. 5 Blackeye showing systemic mosaic and ring spotting.

Table 1. Reactions of 16 cowpea cultivars to inoculation with six viruses^a

Cultivar	BICMV ^b			CCMV			CMV			CPMV			CSMV			SBMV-C			Totals		
	NS- ^c	NS+	S+	NS-	NS+	S+	NS-	NS+	S+	NS-	NS+	S+	NS-	NS+	S+	NS-	NS+	S+	NS-	NS+	S+
Cream 40	13 ^d	2	13	2	3	23	2	0	26	0	0	28	5	0	23	2	6	20	24	11	133
Zipper Cream	13	0	15	1	2	25	3	1	24	0	0	28	5	2	21	15	3	10	37	8	123
Dixielee	0	0	28	0	0	28	8	2	18	17	2	9	9	3	16	0	2	26	34	9	125
Purple Hull																					
Pinkeye	8	3	17	1	2	25	8	0	20	0	0	28	8	7	13	2	1	25	27	13	128
Knuckle Purple																					
Hull	0	0	28	3	0	25	8	0	20	2	0	26	11	2	15	1	0	27	25	2	141
Big Boy	20	3	5	0	1	27	6	0	22	0	0	28	10	4	14	8	3	17	44	11	113
Mississippi Purple	21	3	4	11	4	13	9	3	16	0	0	28	9	2	17	17	5	6	67	17	84
California No. 5																					
Blackeye	4	3	21	0	0	28	4	0	24	0	0	28	1	0	27	3	0	25	12	3	153
Mississippi Silver	24	3	1	11	4	13	7	0	21	15	2	11	13	2	13	12	5	11	82	16	70
Colossus 80	0	1	27	2	0	26	1	0	27	3	0	25	9	3	16	3	5	20	18	9	141
Colossus	2	0	26	2	0	26	6	0	22	6	1	21	10	1	17	11	9	8	37	11	120
Brown Crowder	21	4	3	0	4	24	12	0	16	11	0	17	0	3	25	11	2	15	55	13	100
Magnolia Blackeye	21	6	1	9	5	14	8	1	19	5	1	22	2	20	6	2	15	11	47	48	73
Hercules	2	1	25	2	1	25	9	3	16	0	1	27	13	2	13	13	5	10	39	13	116
Purple Tip																					
Crowder	17	9	2	1	0	27	7	1	20	0	0	28	5	1	22	9	5	14	39	16	113
Worthmore	20	8	0	3	11	14	3	0	25	13	2	13	11	5	12	9	9	10	59	35	74

^aSymptomless plants were tested with either local lesion hosts or enzyme-linked immunosorbent assay to determine infectivity. Values are based on a total of 28 plants, 10 each in the first two greenhouse tests and eight in the third.

^bBICMV = blackeye cowpea mosaic virus, CCMV = cowpea chlorotic mottle virus, CMV = cucumber mosaic virus, CPMV = cowpea mosaic virus, CSMV = cowpea severe mosaic virus, SBMV-C = southern bean mosaic virus (cowpea strain).

^cNS = no symptoms, S = symptoms, + = virus infected, - = not virus infected; NS- = resistant, NS+ = susceptible/tolerant, and S+ = susceptible/sensitive.

^dValues indicate the number of plants of each cultivar with given reaction.

resistance is expressed both as resistance to virus infection and as tolerance to symptom expression in different cultivars. The presence of several resistance mechanisms in cowpea should be expected because Kuhn et al (6) found that different cowpea genes were responsible for control of CCMV symptom expression, movement, and virus accumulation.

None of these commonly used cultivars were resistant to CMV, with the possible exception of Brown Crowder, and resistance to BICMV ranged from 0 to 86%. Yield of plants doubly infected with these two viruses can be reduced 86% (10). The disease "cowpea stunt" (10), resulting from double infection with CMV and BICMV, has the potential to

cause serious losses in most of the common cultivars tested.

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