

Cowpea Stunt: Heterogeneous and Differential Reactions of Cowpea Cultivars

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ABSTRACT

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Four major types of disease reactions were observed when 20 cowpea cultivars or test lines were inoculated with an inoculum mixture of cucumber mosaic virus (CMV) and blackeye cowpea mosaic virus (BICMV): severe stunt, severe stunt with necrosis, delayed stunt with necrosis, and mild mottle (CMV only). Heterogeneous disease reactions occurred among plants in 10 of the cultivars; however, progeny from individual plants reacted homogeneously. All seed lots of all cultivars reacted homogeneously (mild mottle) to CMV alone. Although the 10 cultivars reacted heterogeneously to BICMV alone, the reactions of the cultivars were more easily visualized when plants were inoculated with both

viruses because of a synergistic interaction. The mild mottle reaction to mixed inoculum occurred on plants with a high degree of resistance to BICMV. Seed lines selected for homogeneous reaction (susceptible or resistant) to BICMV were used as a differential set of hosts to characterize three other isolates of BICMV and two of cowpea aphid-borne mosaic virus (CAMV). Typical cowpea stunt reactions occurred with all BICMV and CAMV isolates in inoculum mixtures with CMV; the type of reaction was dependent on the cultivar. It is concluded that either BICMV or CAMV can be a virus component of cowpea stunt.

Cowpea stunt was reported to be a potentially important disease of cowpeas, *Vigna unguiculata* (L.) Walp. subsp. *unguiculata*, in Georgia because of the severe synergistic disease reaction caused by its two virus components, blackeye cowpea mosaic virus (BICMV) and cucumber mosaic virus (CMV), both of which are seedborne and aphid transmitted (11). In 1978, the known geographic distribution of the disease was extended to Alabama and South Carolina; the identification was made in cooperation with O. L. Chambliss (Alabama) and O. W. Barnett and M. R. McLaughlin (South Carolina).

A previous study by Brantley et al (2) showed no resistance to CMV in cowpeas. However, Lima (7) reported a few cultivars immune to BICMV, and Kuhn et al (5) found seven cowpea lines immune to a virus previously thought to be bean yellow mosaic virus (BYMV), but now more accurately thought to be BICMV (11). We initiated screening studies which sought resistance to BICMV, CMV, and cowpea stunt. A variety of disease reactions were observed; they were related to cowpea cultivars, cowpea seed lots, and isolates of BICMV found in the southeastern United States.

This article is concerned with characterization of cowpea cultivar reactions to the two cowpea stunt virus components; differential reactions with isolates of BICMV and a serologically related virus, cowpea aphidborne mosaic virus (CAMV); selection of cowpea lines resistant and susceptible to BICMV from cultivars with heterogeneous individual plant reactions to the virus; and application of the selected lines for biological characterization of BICMV isolates and cowpea stunt.

MATERIALS AND METHODS

Virus isolates. The primary isolates of BICMV, designated BICMV-GA, and CMV were obtained in 1974 from field-grown cowpea plants doubly infected with the viruses (11). Additional isolates of BICMV used in the study were BICMV-WA from White Acre cowpea seed produced in Georgia in 1959 (11) (previously named bean yellow mosaic virus [BYMV]), BICMV-S from a

Georgia field isolate which causes a very bright mosaic in cultivar California Blackeye, BICMV-SC from South Carolina, and BICMV-FL from Florida (7). Two isolates of CAMV also were tested: a Moroccan strain (CAMV-MOR) (3) obtained from the University of Florida and a Brazilian isolate (CAMV-BR) from the Universidade Federal Rural de Pernambuco, Brazil. Other viruses used in this study were as follows: cowpea strain of southern bean mosaic virus (SBMV), the type strain of cowpea chlorotic mottle virus (CCMV-T), a strain of CCMV from resistant cowpeas (CCMV-R) (15), and a Brazilian isolate of cowpea mosaic virus (CpMV) which is a member of the Arkansas serogroup. All virus isolates were maintained in California Blackeye cowpeas as previously noted for BICMV and CMV (11).

Seed sources. Seeds from about 25 cowpea lines were obtained from three sources: a plant breeder (B. B. Brantley, Georgia Agricultural Experiment Station); the Southern Regional Plant Introduction Station, Experiment, Georgia; and commercial seed companies. The cowpea lines were tested for virus reaction through two or three generations and there was no evidence that the lines were still segregating.

Evaluation of disease reactions. Evaluations of the disease reactions were made under greenhouse conditions (24–30 C) over a period of at least 30 days after inoculation. Test plants, 8–9 days after seeding, were rubbed with high-titer inoculum obtained from singly infected California Blackeye cowpea plants (9 ml of 0.01 M neutral potassium phosphate containing 1% Celite to 1 g of tissue) inoculated 7–10 days previously.

The primary disease reactions were studied with four groups of plants of each cultivar inoculated with BICMV-GA, CMV, mixture of BICMV-GA and CMV, or rubbed with buffer only. Four selected cultivars, representative of each of the four major types of reaction to a mixture of BICMV-GA and CMV, were used in experiments to determine the effect of single and double inoculations on plant growth and seed yield; the procedure was described previously (11). The reactions of some cultivars and selected lines also were evaluated by single and double inoculations with CMV and the different isolates of BICMV and CAMV. Furthermore, selected cultivars were used to compare the reactions to mixed inoculation of several other unrelated viruses with either BICMV-GA or CMV.

Selection of cowpea lines. Plants from seed lots of 20 cowpea cultivars obtained from one or more sources were inoculated with BICMV-GA, CMV, a mixture of BICMV-GA and CMV, or rubbed with buffer. Resistant and susceptible seed lines of each cultivar were obtained from single plants selected from among large numbers of inoculated plants that exhibited heterogeneous reactions. They were grown under greenhouse conditions for three generations, and the progeny were checked for reactions to the three inocula after each generation.

Analysis of resistance. Resistance of cultivars and selected lines was studied by making three mechanical inoculations, 5 days apart, with BICMV-GA or BICMV-GA plus CMV. The inoculations were made on all expanded leaves. Twenty-four hours before the first inoculation, groups of plants of each line were subjected to three environmental conditions: greenhouse (24–30 C), plants covered with black plastic containers in the greenhouse, or a growth chamber programmed for 33 C and a 16-hr photoperiod. For aphid inoculation of resistant and susceptible plants, apterous individuals of *Aphis craccivora* Koch., cultured on cowpeas and fasted for 3–4 hr in plastic containers, were allowed an acquisition period of 30–60 sec on Knuckle Purple Hull cowpeas infected with BICMV-GA. Then groups of six to eight aphids were transferred to individual plants of the different lines; the aphids were killed 12 hr later with aldicarb.

To test for virus infection in symptomless plants in all experiments, subinoculations were made to California Blackeye and Clay cowpeas. Symptoms for identification of BICMV, CMV, and cowpea stunt were described previously (11).

RESULTS

Major types of disease reactions. When the reactions of 20 cowpea cultivars to an inoculum mixture of CMV and BICMV-GA were evaluated, four major types of disease reactions were observed: severe stunt with necrosis (SSN), severe stunt (SS), delayed stunt with necrosis (DSN), and mild mottle (MM). The first three reactions were the result of mixed infections, and the MM reaction was caused by CMV alone. The four reactions were studied in detail with one representative cultivar from each group (Table 1).

Synergism was observed with the SSN reaction on California Blackeye cowpeas within 5–8 days after inoculation. Necrosis developed on stems, petioles, and leaf veins; and, frequently, plants died (Fig. 1A). Subsequently, the surviving plants recovered from

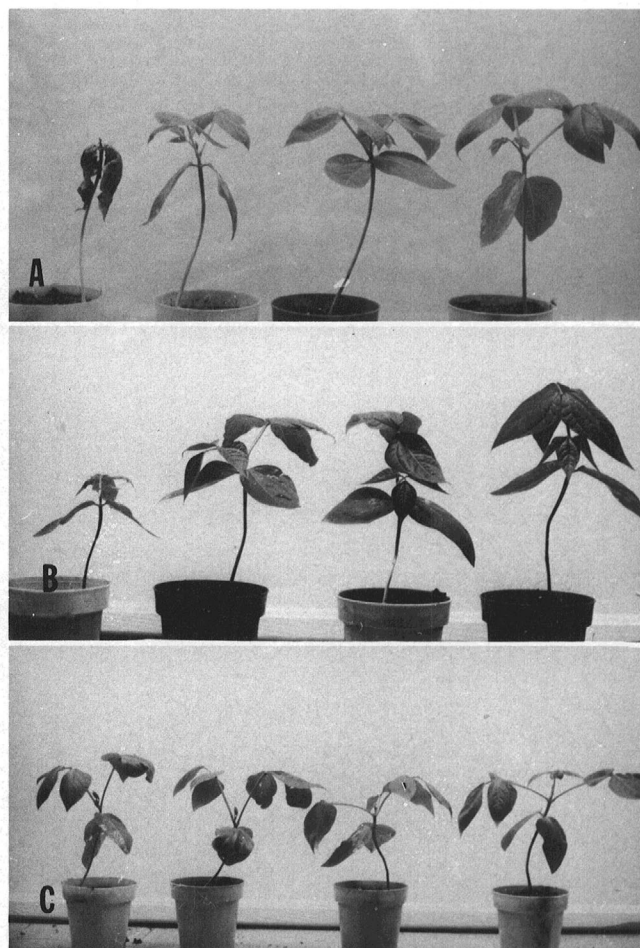


Fig. 1. Disease reactions of three cowpea cultivars to single and double infections with cucumber mosaic virus (CMV) and the Georgia isolate of blackeye cowpea mosaic virus (BICMV-GA) 16 days after inoculation; from left to right CMV + BICMV-GA, CMV only, BICMV-GA only, and buffer-rubbed control. **A**, Synergistic reaction with necrosis on California Blackeye. **B**, Synergistic reaction with no necrosis on Clay. **C**, Additive or less-than-additive reaction on Early Pinkeye.

TABLE 1. Major types of disease reaction of cowpeas to double inoculation (mechanical) with cucumber mosaic virus (CMV) and the Georgia isolate of blackeye cowpea mosaic virus (BICMV)^a

Representative cultivar	Disease reaction ^b			Nature of interaction of viruses	Initiation of synergism (days)	Degree of synergism ^c	
	Local	Systemic	Type			Plant growth at 21 days	Seed yield (%)
California Blackeye	C and N	Strong mottle, leaf distortion, severe stunt, and necrosis	SSN	Double infection, intense synergism	5–8	17%	70–100
Clay	C	Strong mottle, leaf distortion, severe stunt	SS	Double infection, intense synergism	10–15	20%	100 ^d
Early Pinkeye	C and N	Strong mottle, leaf distortion, necrosis, and stunt	DSN	Double infection, delayed synergism	18–25	None	38
Iron	C or C and N (as ringspots)	Mild Mottle	MM	CMV infection only, no interaction	...	None	None

^aPlants inoculated 8–9 days after seeding.

^bLetter designations: SSN = severe stunt with necrosis; SS = severe stunt; DSN = delayed stunt with necrosis; MM = mild mottle; C = chlorosis; N = necrosis.

^cDegree of synergism (%) = (Healthy [100%] – [percent CMV reduction + percent BICMV reduction]) – percent reduction with mixed infection.

^dSometimes, a few seed are produced, but none were produced in a yield test with cultivars Early Pinkeye and Iron.

the necrotic reaction, but they remained severely stunted and produced few or no seeds (Table 1). Although necrosis did not develop on Clay (SSN reaction), synergism was obvious (symptom expression) within 10–15 days after inoculation; plant growth reduction was significant at 16 days (Fig. 1B), and few or no seed were produced (Table 1). The DSN reaction on Early Pinkeye differed from the SSN reaction by having a delayed and less intense necrotic reaction. Necrosis developed 18–25 days after inoculation,

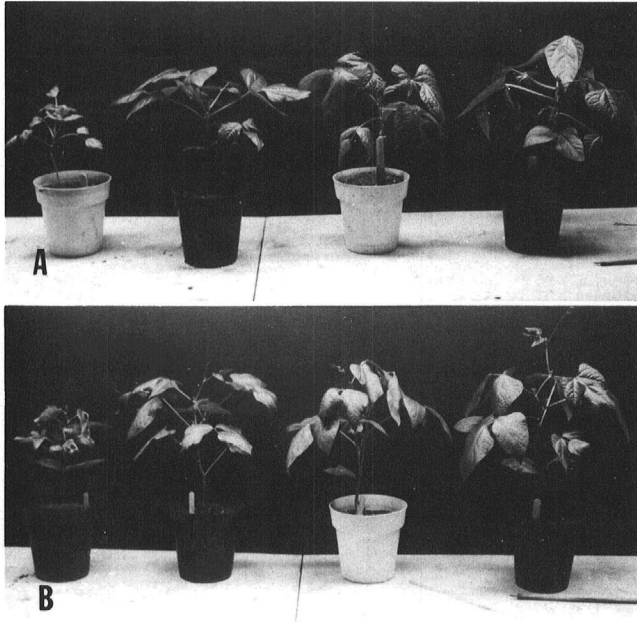


Fig. 2. Disease reactions of two cowpea cultivars to single and double inoculations with cucumber mosaic virus (CMV) and the Georgia isolate of blackeye cowpea mosaic virus (BICMV-GA) 40 days after inoculation; from left to right CMV + BICMV-GA, CMV only, BICMV-GA only, and buffer-rubbed control. **A**, Synergistic reaction with no necrosis on Clay. **B**, Delayed synergistic reaction with necrosis on Early Pinkeye.

but it was not severe enough to cause plant death. However, abscission of young leaves was observed on some plants. After the initial shock reaction, new growth was free of necrosis; however, plants were severely stunted and seed yield was reduced synergistically (Table 1). The plant growth reduction was not significant at 16 days (Fig. 1C), but at 40 days the reduction on Early Pinkeye (Fig. 2B) was similar to that observed on Clay (Fig. 2A).

Basically, all 20 cultivars reacted with systemic symptoms similar to those caused by CMV. A mild to intense mottle was observed between 3 and 12 days after inoculation. It became less intense as the leaves became older, and after that a very mild mottle was observed on new growth only. On the other hand, the 20 cultivars responded to a single inoculation with BICMV-GA in four ways that were directly related to the four major responses caused by mixed inoculum of BICMV-GA and CMV. For the representative cultivars shown in Table 1, the reactions to BICMV-GA alone were as follows: a mild mottle with limited necrosis of stem and petioles was observed on California Blackeye at 5–8 days after inoculation; a bright mottle occurred on Clay at 7–10 days; a bright mottle occurred on Early Pinkeye at 14–21 days; and no symptoms were observed on Iron.

Local responses. Some cowpea cultivars reacted to single inoculations of BICMV-GA and CMV with both local chlorosis and necrosis and some with local chlorosis only (Table 1). Under greenhouse conditions, the reproducibility of local necrosis was high for CMV and low for BICMV-GA. Cultivar Texas Cream 40 was the one exception; BICMV-GA caused distinct local lesions and severe systemic symptoms (necrotic areas on trifoliolate leaves). When cultivars were doubly infected, both the amount and degree of local necrosis was less than that caused by single infections. However, the local necrosis, particularly that caused by CMV alone, was related to the necrosis observed in SSN and DSN reactions in double infections (Table 1). Cultivar Iron was an exception; sometimes it developed local ringspots (with some necrosis) when inoculated with CMV alone or with both viruses, but the systemic response was not necrotic even when Iron did become infected with isolates other than BICMV-GA; eg. BICMV-WA, CAMV-BR, or CAMV-MOR.

Heterogeneity within cowpea lines and seed lots. During the study of disease reactions, 10 of 20 cowpea cultivars reacted with

TABLE 2. Homogeneous and heterogeneous disease reactions of cowpea cultivars to double inoculation (mechanical) with cucumber mosaic virus and the Georgia isolate of blackeye cowpea mosaic virus^a

Cowpea cultivar and seed lot ^b	Number of plants inoculated	Type of disease reaction (percentage of plants)			
		Severe stunt with necrosis ^c	Severe stunt ^d	Delayed stunt with necrosis	Mild mottle ^e
Calico Crowder	304	6	94
California Blackeye	>3000	95	5
Clay	>2000	...	100
Early Pinkeye	>1000	100	...
Iron	>2000	100
Knuckle Purple Hull	>2000	...	100
Lady Peas	650	2	16	...	82
Monarch	122	80	20
PI 186465	420	25	75
Pinkeye Purple Hull					
Seed Lots 1 to 7	245	100
Seed Lot 8	35	3	97
Seed Lot 9	220	44	56
Seed Lot 10	330	...	1	...	99
Texas Cream 40					
Seed Lot 1	95	5	95
Seed Lots 2 to 5	190	84	16
White Acre					
Seed Lot 1	105	100
Seed Lot 2	92	...	100

^aPlants inoculated 8–9 days after seeding.

^bThe seed lots are different sources, but the exact origin of most of them is unknown.

^cCultivar Coronet reacted similarly to California Blackeye.

^dCultivar Brown Crowder reacted heterogeneously with severe stunt and mild mottle.

^eThe following cultivars reacted with a mild mottle similar to that of cultivar Iron: Big Boy, Blue Goose, G69, Dixiecream, Mississippi Silver, and Worthmore.

more than one type of reaction. Although the seed and plant types were similar for each of the 10 cultivars, the reaction was variable with regard to mixed inoculum. Examples of two types of reaction from seed lots of three different cultivars are shown in Fig. 3, and a summary of the variability is presented in Table 2. Equal numbers of plants of each cultivar also were singly inoculated with CMV and BICMV-GA. All plants reacted uniformly to CMV. The percentage of plants that had different types of reaction to both viruses corresponded very closely to the variability of reaction to BICMV-GA.

Calico Crowder, California Blackeye, and Monarch showed a certain percentage of plants with SSN in mixed infection and a similar percentage of plants susceptible to BICMV-GA (Table 2). A more complex situation occurred with Lady Peas and Pinkeye Purple Hull; there were plants with SSN and SS in the double infection and two kinds of susceptible reactions to BICMV-GA. Clay and Knuckle Purple Hull reacted uniformly with SS to both viruses and were uniformly susceptible to BICMV-GA. Iron was uniformly resistant to BICMV-GA, and only CMV symptoms were observed on plants inoculated with both viruses. All plants of Early Pinkeye had a DSN reaction to both viruses and delayed bright mottle to BICMV-GA. Plant Introduction (PI) 186465 had some plants resistant to BICMV-GA and some with CMV symptoms only in doubly inoculated plants.

All plants from seven seed lots of Pinkeye Purple Hull were resistant to BICMV-GA; two seed lots had plants that reacted with SSN to mixed inoculum and one had a few plants that reacted with SS (Table 2). Seed lots of White Acre were either homogeneously resistant to BICMV-GA or homogeneously susceptible with an SS reaction in double infections. No seed source of Texas Cream 40 was homogeneous in its reaction to BICMV-GA, and all doubly infected plants reacted with SSN.

Heterogeneous reactions in the field. A cultivar (Pinkeye Purple Hull) heterogeneous in reaction to BICMV was observed in a commercial field in Georgia in 1978. The plants were produced from seeds that had been grown for several years on the same farm. The incidence of viruses, based on symptoms, was estimated as follows: 30% of plants with BICMV, 20% with CMV, 10% with typical cowpea stunt (both viruses), and 40% with no virus. From each group of plants, seeds were collected and checked for reactions to BICMV-GA and BICMV-GA plus CMV. Plants resistant to BICMV-GA were obtained from some plants with no symptoms and with CMV symptoms only. Susceptible plants reacted to a mixed inoculation with SSN as was observed in some plants in the field.

Nature of resistance. The nature of resistance to BICMV-GA was determined by evaluating the following cowpeas which showed no symptoms after mechanical inoculation: cultivar Iron and progeny from individual resistant plants of California Blackeye, Lady Peas, Pinkeye Purple Hull, Texas Cream 40, and White Acre. Susceptible lines of each cultivar and heterogeneous seed lots of California Blackeye and Pinkeye Purple Hull were used as controls. The following techniques, which were used to enhance plant susceptibility, did not promote infection in the resistant plants: dark treatment for 24 hr before inoculation, inoculation of both sides of leaves, three inoculations at 5-day intervals, and 33 C treatment for 24 hr before inoculation. Subinoculation tests from over 200 symptomless plants to susceptible cultivars California Blackeye and Clay did not detect BICMV-GA. The resistant lines could not be infected by aphid inoculation, even though 30% of control plants (susceptible lines) became infected.

The same lines also were mechanically inoculated with BICMV-GA plus CMV under the same conditions. The addition of CMV did not promote infection by BICMV-GA, and only CMV could be recovered by subinoculation. The susceptible controls displayed the typical strong symptoms characteristic of synergistic interaction of the two viruses.

Progeny from the selected lines mentioned above, both resistant and susceptible ones, reacted homogeneously through three generations to BICMV-GA or BICMV-GA plus CMV. Other cowpea cultivars that had both resistant and susceptible lines were Brown Crowder, Calico Crowder, Coronet, Monarch, and PI

186465.

Differential reactions of cultivars to virus isolates. Several cultivars and selected lines were evaluated for reactions to mixed inocula of CMV with different isolates of either BICMV or CAMV. The types of reaction obtained are summarized in Table 3.

Equal numbers of plants of each cultivar and selected lines were singly inoculated with the different isolates of BICMV and CAMV. Isolates BICMV-GA, BICMV-FL, and BICMV-S produced a similar spectrum of resistant and susceptible reactions, but the BICMV-S symptoms were consistently stronger on selection 1 of California Blackeye, Clay, and Knuckle Purple Hull; selection 2 of Lady Peas; selection 1 of White Acre; and some plants of seed lot 9 of Pinkeye Purple Hull.

These three isolates did not produce symptoms on selection 2 of California Blackeye, Dixiecream, Iron, selection 3 of Lady Peas, selection 2 of Texas Cream 40, selection 2 of White Acre, and some

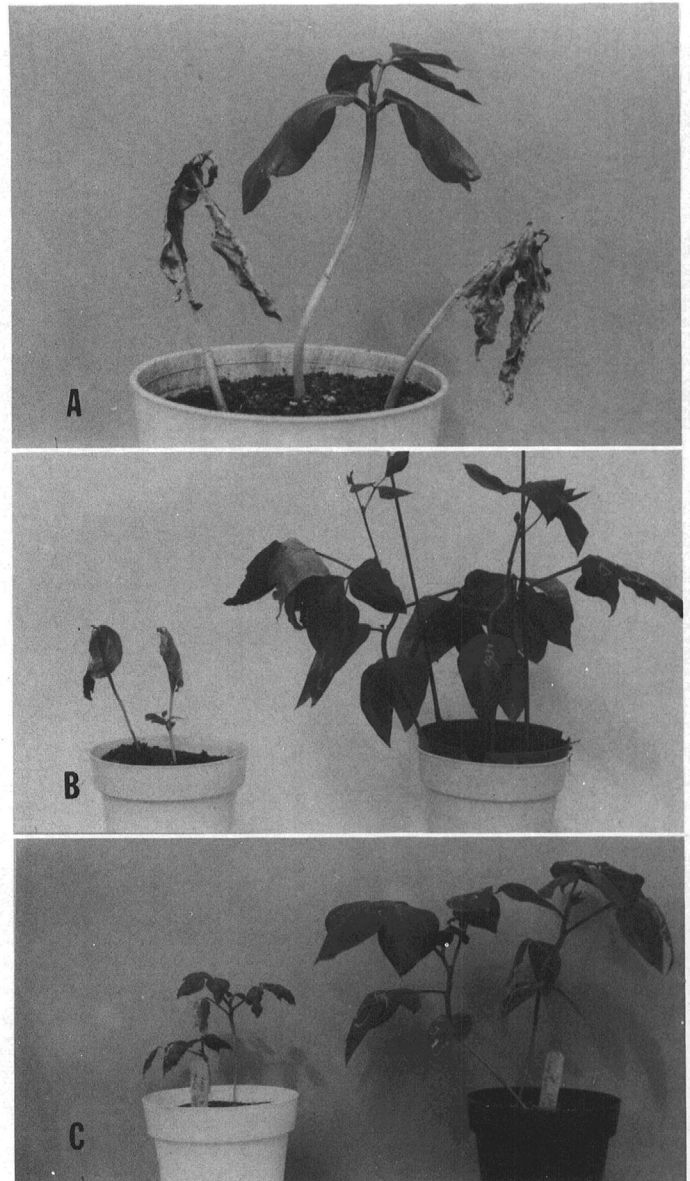


Fig. 3. Heterogeneous reactions of three cowpea cultivars to double inoculation with cucumber mosaic virus (CMV) and the Georgia isolate of blackeye cowpea mosaic virus (BICMV-GA). **A**, Heterogeneous seed lot of California Blackeye; the center plant is resistant to BICMV-GA. **B**, Selected lines of Pinkeye Purple Hull; line susceptible to BICMV-GA on left with strong synergistic necrotic reaction, and resistant line on right with CMV symptoms only. **C**, Selected lines of Lady Peas; susceptible line to BICMV-GA on left with synergistic reaction with no necrosis, and resistant line on right with CMV symptoms only.

TABLE 3. Differential disease reactions of cowpea lines to double mechanical inoculation with cucumber mosaic virus (CMV) and different isolates of blackeye cowpea mosaic virus (BICMV) and cowpea aphid-borne mosaic virus (CAMV)^a

Test lines	BICMV + CMV					CAMV + CMV	
	GA ^b	FL	S	SC	WA	MOR	BR
California Blackeye							
Selection 1	SSN	SSN	SSN	SSN	SSN	SSN	SSN
Selection 2	MM	MM	MM	MM	MM	MM	SSN
Clay	SS	SS	SS	SS	SS	SS	SS
Dixiecream	MM	MM	MM	MM	MM	MM	SSN
Early Pinkeye	DSN	DSN	DSN	DSN	DSN	DSN	SSN
Knuckle Purple Hull	SS	SS	SS	SS	SS	SS	SS
Iron	MM	MM	MM	SS	SS	SS	SS
Lady Peas							
Selection 1	SS	SS	SS	SS	SS	SS	SS
Selection 2	SSN	SSN	SSN	SSN	SSN	SSN	SSN
Selection 3	MM	MM	MM	MM	MM	MM	SS
Pinkeye Purple Hull ^c							
Seed Lot 9	MM and SSN	MM and SSN	MM and SSN	MM and SSN	MM and SSN	MM and SSN	SSN
Texas Cream 40							
Selection 1	SSN	SSN	SSN	SSN	SSN	SSN	SSN
Selection 2	MM	MM	MM	SSN	SSN	SSN	SSN
White Acre							
Selection 1	SS	SS	SS	SS	SS	SS	SS
Selection 2	MM	MM	MM	SS	SS	SS	SS

^aLetter designations: SSN = severe stunt with necrosis, SS = severe stunt, DSN = delayed stunt with necrosis, and MM = mild mottle.

^bGA = Georgia, FL = Florida, S = Severe, SC = South Carolina, W = White Acre, MOR = Morocco, and BR = Brazil.

^cHeterogeneous seed lot.

TABLE 4. Reaction of five cowpea lines to five cowpea viruses

Cowpea line	Nature of reaction ^a				
	BICMV ^b	CMV	CCMV-T	CCMV-R	CpMV
Iron	R-U	S-U	S-U	S-U	S-U
Lady Peas	H	S-U	S-U	S-U	S-U
PI 186465	H	S-U	R-U	S-U	H
Pinkeye Purple Hull	H	S-U	S-U	S-U	S-U
Texas Cream 40	H	S-U	S-U	S-U	S-U

^aR = resistant, S = susceptible, U = uniform reaction, and H = heterogeneous reaction.

^bBICMV = Georgia isolate of blackeye cowpea mosaic virus, CMV = cucumber mosaic virus, CCMV-T = cowpea chlorotic mottle virus (type strain), CCMV-R = strain R, and CpMV = cowpea mosaic virus.

plants of seed lot 9 of Pinkeye Purple Hull. They also were the only isolates that produced local necrotic lesions on selection 1 of Texas Cream 40; the other isolates produced local chlorosis. The BICMV-SC and BICMV-WA isolates caused similar symptoms in all cowpeas tested. These two isolates and CAMV-MOR had the same spectrum of susceptible and resistant cultivars and selected lines, but the CAMV-MOR symptoms were more severe in some and milder in others. The CAMV-BR isolate produced severe symptoms in all cowpea lines tested.

Heterogeneous and differential cultivar reactions to other viruses. The variability of virus disease reactions in cowpeas was studied further by singly inoculating five cowpea cultivars with BICMV-GA, CMV, CCMV-T, CCMV-R, and CpMV. As observed previously (Table 2), Iron reacted uniformly to BICMV-GA, and the other four cultivars had plants both susceptible and resistant to BICMV-GA (Table 4). All plants of all cultivars reacted uniformly to CMV, CCMV-T, CCMV-R. The only variability was observed with PI 186465 inoculated with CpMV; 75% of the plants were resistant and 25% were susceptible. This cultivar also showed differential reactions to CCMV: susceptibility to strain R and resistance to strain T.

Cultivar reactions to mixed inoculation of either CMV or BICMV-GA with other viruses. Mixed inoculations with either BICMV-GA or CMV and CCMV-T, CCMV-R, or SBMV showed no synergistic disease reaction on cowpea cultivars California Blackeye, Clay, and Early Pinkeye. The resulting disease reactions were additive or less than additive. Neither CMV nor BICMV altered the bright mottle caused by either strain of CCMV or the

leaf distortion, mottle, and stunt caused by SBMV. Also, the presence of other viruses did not increase the limited systemic necrosis produced by BICMV-GA on California Blackeye. Typical cowpea stunt reactions were observed on controls inoculated with BICMV-GA and CMV. For CpMV, the kind of interaction in mixed infections in most cultivars could not be determined because of the strong reaction caused by the virus alone. In cultivar Iron, the viruses did not react synergistically with CMV, and single virus symptoms of CCMV-T, CCMV-R, and CpMV were obtained when they were in inoculum with BICMV-GA. No symptoms were obtained with SBMV plus BICMV-GA in Iron.

DISCUSSION

Biological specificity has been used as a tool in many plant virus studies, including identification and grouping of different viruses and virus strains, nature of host resistance to the virus, selection of resistant material for control programs, and virus-host relationships. With diseases caused by simultaneous infections of more than one virus, the characterization of the host response frequently is complex. For example, when CMV and BICMV are present in a cowpea field, a variety of disease reactions can be expected. Some plants may have CMV or BICMV alone; others may have both viruses interacting synergistically which causes the cowpea stunt disease (11), with or without necrosis depending on the cultivar involved. Some plants may be free of CMV and/or BICMV either because they were not inoculated or were resistant to the field strains. These assumptions are suggested by the results obtained in the present work. Four distinct types of reaction (SSN, SS, DSN, and MM) were detected when 20 cowpea cultivars were inoculated with a mixture of CMV and BICMV. In three of them (SSN, SS, DSN), the disease reactions were synergistic; only CMV infection was associated with the MM reaction.

Heterogeneous reactions to BICMV and cowpea stunt are very common in cowpea cultivars since both resistant and susceptible seed lines were obtained from 10 of 20 cultivars studied. The heterogeneous aspect was more easily observed and more significant following inoculation with both viruses rather than after single-virus inoculations. A strong contrast of reaction developed between plants of the same cultivar with CMV symptoms only (mild mottle) as compared with those doubly infected plants which normally displayed a strong synergistic reaction (severe stunt; leaf distortion; and in many lines, severe necrosis and plant death) (Fig.

3). Although the plants of several cultivars reacted heterogeneously, the selected lines (seed from individual plants) showed homogeneity for resistance or susceptibility through three generations.

Other cases of heterogeneous disease reactions to legume viruses have been reported previously. Williams (14), checking large numbers of cowpea lines for resistance to cowpea (yellow) mosaic virus, found several types of reactions: resistance, different degrees of susceptibility, mixed or segregating, and resistance to one virus strain, but susceptibility to another. In *Phaseolus vulgaris* L. 'Black Turtle,' the number of plants susceptible to watermelon mosaic virus 2, bean common mosaic virus, soybean mosaic virus, and a cowpea seed-borne virus (13), now known as BICMV, varied from 30-70%, depending upon the seed lot being tested (12, and R. Providenti, *personal communication*).

The nature of resistance in Iron and in several selected lines (California Blackeye, Lady Peas, Pinkeye Purple Hull, Texas Cream 40, and White Acre) to BICMV-GA appeared to be immunity since no virus could be recovered from inoculated plants, and only CMV could be detected in doubly inoculated ones. Furthermore, techniques to enhance susceptibility were ineffective. Previous studies (5,6) demonstrated immunity to BYMV (referred to as BIMV-WA in this article) in Dixiecream and several plant introductions. Iron, however, was susceptible to BYMV (5), and in this study, four Iron plants (of approximately 3,000 tested) were susceptible to BICMV-GA. When variants of BICMV and CAMV were tested, it was clear that resistance in cowpeas could be overcome by changes in the pathogen (Table 3). Therefore, the term "immunity" probably should not be used to describe the cowpea resistance to BICMV and CAMV.

BICMV and CAMV have been considered similar or synonymous (1,8). Direct comparison between a Florida isolate of BICMV, here referred to as BICMV-FL, and a Moroccan strain of CAMV-MOR by Lima (7) indicated several differences between the two. In our studies, however, BICMV and CAMV were equally able to interact with CMV to cause cowpea stunt. Differential reactions of some cultivars and selected lines were observed for isolates of both viruses, and a similar synergistic phenomenon with CMV was observed when double infection obviously had occurred. This viewpoint is supported by the fact that the type of synergistic reaction (necrotic or non-necrotic) was observed regardless of the isolate that was mixed with CMV. The reaction on California Blackeye (formerly known as Early Ramshorn) confirmed similarity between cowpea stunt and the synergistic disease reaction observed by Fischer and Lockhart (3,4) in a Moroccan cowpea field. Some cowpea cultivars were resistant to cowpea stunt when BICMV-FL, BICMV-S, or BICMV-GA were in the inoculum. The same cultivars were susceptible with either BICMV-WA, BICMV-SC, CAMV-MOR, or CAMV-BR in the inoculum.

A preliminary report (10) indicated that an additive disease effect of BICMV-GA plus CMV was dependent on the cowpea cultivar, Early Pinkeye. The conclusion was based on symptoms and dry weight of plant tissue at 3 wk after inoculation on one cowpea line. Further observation, however, indicated that the BICMV-GA symptoms normally developed slowly, and the synergistic reaction became evident only after 3 wk (delayed synergism) and this was substantiated by seed yield measurements (Table 1). A similar reaction was observed when the other isolates of BICMV and CAMV-MOR were used instead of BICMV-GA. However, an SSN reaction was obtained in Early Pinkeye following double inoculation with CMV and CAMV-BR. Thus, the cowpea stunt evaluations at 3 wk after inoculation are adequate for many, but not all, cultivars and virus strains.

Sometimes the same virus disease symptoms can be caused by one virus in paired mixtures with different unrelated viruses. For example, corn lethal necrosis is caused by maize chlorotic mottle virus in combination with either maize dwarf mosaic virus or wheat streak mosaic virus (9). Apparently, this is not the situation with cowpea stunt, since cowpea viruses unrelated to either CMV and BICMV, such as CCMV, SBMV, and CpMV, did not interact synergistically on cowpea stunt test lines when doubly inoculated with either cowpea stunt component. The synergism of either BICMV or CAMV isolates with CMV, especially when associated with field occurrence in the USA (11) and Morocco (3,4), is a characteristic that demonstrates biological similarity between the two viruses; either can be a component of cowpea stunt.

The cowpea lines selected for resistance to BICMV-GA are valuable biological material tools for identification of BICMV (or CAMV) and cowpea stunt, and they can be applied in control programs. However, the occurrence of cowpea stunt in a cultivar is dependent on the reaction to the specific isolate of BICMV or CAMV; cultivars that are resistant to BICMV and react with MM to a mixed inoculum of strains of BICMV and CMV native in one geographical area may be highly susceptible to the strains of both viruses in another area and a severe synergistic stunt may occur.

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