Genetic Control of Symptoms, Movement, and Virus Accumulation in Cowpea Plants Infected with Cowpea Chlorotic Mottle Virus

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ABSTRACT

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An inheritance study which evaluated segregating populations of cowpea plants infected with cowpea chlorotic mottle virus (CCMV) indicated that the host genotype conditioned expression of symptoms, virus accumulation, and virus movement. With certain crosses, necrotic etching on inoculated primary leaves was controlled by a single dominant gene, and virus accumulation was related to the presence or absence of necrosis. Systemic symptoms (three types) could not be differentiated objectively enough for quantitative analysis. However, symptomless plants, representing lack of viral movement, were distinguished with ease. Viral movement was controlled by a single dominant gene. Five levels of virus accumulation were found in 10 cowpea lines and in F_1, F_2, and backcross populations. Both the total accumulation of virus in plants and the rate of virus replication appeared to be controlled by the action of several genes. The viral movement gene may be closely linked with one of the genes that controls virus replication. Symptomatology, based on intensity of chlorosis, was unrelated to virus accumulation. Furthermore, yield of pods and seeds was more closely related to virus accumulation than to symptom severity. When different strains of CCMV were tested, the virus genome also was found to control the level of virus accumulation.

The determination of inheritance of resistance usually involves evaluation of disease symptoms. Symptoms probably are a secondary effect of the virus replication process, and very little is known about the physiological processes that elicit them (17). For disease control programs, symptom evaluation will suffice if disease loss is directly related to severity of symptoms, which is usually the case. However, plants may tolerate high levels of virus accumulation and have no loss of yield (5,9), and in rare cases yield enhancement has been noted (10,13).

We believe inheritance studies can lead to a better understanding of the nature of resistance to plant viruses if criteria involving virus-host interactions are evaluated. Therefore, the movement and replication of cowpea chlorotic mottle virus (CCMV) were studied in individual cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) plants in F_1, F_2, and backcross populations. The genetics information was compared to symptomatology and disease loss.

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MATERIALS AND METHODS

Virus and host manipulation. The CCMV isolate used in most of these studies was strain T (6,14), which was transferred each week from single local lesions on soybeans, *Glycine max* (L.) Merr. 'Bragg,' to cowpea cultivar California Blackeye. The transfers were necessary to prevent a major change in the virion population from strain T to strain M (6). Four additional strains of CCMV were used in a study to compare virus concentration in three hosts: M (6), R (15), A (3) and bean yellow stipple virus (BYS) (3). For all tests, CCMV inoculum was prepared by diluting sap from cowpea plants infected 6–10 days with 0.01 M neutral potassium phosphate buffer (1 g of tissue + 9 ml of buffer); the inoculum contained 30–60 μg of virus/ml.

For inheritance and virus replication studies, cowpea plants (two per pot) were grown in a soil-sand-vermiculite mixture (2:1:1, v/v),

and primary leaves were inoculated 8–10 days after seeding in 10-cm-diameter plastic pots. Ammonium nitrate (0.08 g per pot) was added weekly. Plants were maintained in the greenhouse (21–35 C) or in a growth chamber at a continuous 25 C with approximately 10,000 lux illumination for 16 hr per day.

Virus purification. The evaluation of individual plants in the inheritance study required a purification procedure that was both reliable and relatively simple. We anticipated that leaf tissue would weigh 1.0 to 2.5 g per plant and that virus yield would range from 0.03–3.0 mg per plant. The usual purification procedure for CCMV (2,14) includes two cycles of ultracentrifugation. This provides a highly purified virus preparation, but over 50% of the virus is lost during the second cycle. When virus was partially purified through one cycle of ultracentrifugation and then centrifuged through a 10–40% sucrose density gradient column, it was established that about 9% (sometimes less for virus concentrations above 0.3 mg/ml) of the ultraviolet absorbancy was host material that remained near the top of the sucrose column. Similar results were obtained regardless of time of year that experiments were performed.

For virus quantification a modified purification procedure was used. Infected leaves were harvested 10–12 days after inoculation, a time period which allowed a maximum concentration of virus to accumulate (Table 5, [2], and time course assays performed with more than 10 cowpea cultivars over a period of 15 yr). Virus in two inoculated primary leaves of individual plants was extracted in 10 ml of 0.2 M acetate buffer (pH 4.5) containing cysteine-HCl (0.1 M), sodium diethyldithiocarbamate (0.01 M), and $MgCl_2$ (0.01 M); 10 ml of chloroform; and 10 ml of butanol. Following centrifugation at 10,000 *g* for 10 min, the aqueous phase was frozen, then thawed and the centrifugation was repeated. The virus was concentrated by ultracentrifugation (1 hr at 226,000 *g*) and analyzed spectrophotometrically ($E^{0.1\%}_{260\,nm} = 5.8$). Apparent virus quantities were reduced by 9% to account for nonviral material.

Inheritance studies. Two susceptible cowpea cultivars, California Blackeye and Iron, and one resistant plant introduction (PI) 186465 were used as parents in the following crosses: California Blackeye × PI 186465, PI 186465 × California Blackeye, California Blackeye × Iron, and Iron × PI 186465. The F_1, F_2, and backcross generations were produced and evaluated for disease reaction in the greenhouse.

Seed yield. Plants of California Blackeye, Iron, and PI 186465 were grown in 15-cm-diameter plastic pots in the greenhouse (one plant per pot). Plants were inoculated with CCMV 10 days after seeding, fertilized weekly, and staked for upright growth. Mature pods were harvested between 75 and 100 days after seeding.

TABLE 1. Cowpea chlorotic mottle virus symptoms and concentration in ten cowpea lines

Cowpea line	Virus concentration		Symptom type	
	Class[u]	μg/g[v]	Local	Systemic[w]
PI 147562[x]	I	16 a[y]	None	0
PI 186465	I	30 a	None	0
Pinkeye Purple Hull	III	408 b	Nec[z]	2
White Acre	III	410 b	Nec	3
PI 399419	III	417 b	Nec	3
California Blackeye	III	510 b	Nec	3
Iron	IV	783 c	None	1
Mississippi Silver	IV	846 c	None	2
Knuckle Purple Hull	IV	928 c	Nec	2
Clay	V	1,156 d	Nec	1

[u] I = less than 100 μg/g, II = 100 to 400 μg/g, III = 400 to 700 μg/g, IV = 700 to 1,100 μg/g, V = more than 1,100 μg/g (Class II plants found in F_1, F_2, and backcross populations).

[v] μg/g = Amount of virus per gram fresh weight of inoculated primary leaves; four replications per treatment; each replication consisted of 10 leaves, each from a different plant.

[w] 0 = No symptoms, 1 = mild mottle, 2 = mottle with limited cholosis, and 3 = extensive bright chlorotic mottle.

[x] PI = Plant Introduction.

[y] Treatment means followed by different letters in this column are significantly different according to Duncan's multiple range test, $P = 0.05$.

[z] Nec = necrotic etching.

RESULTS

Classification of phenotypic reactions. When 23 cowpea cultivars were inoculated with CCMV, a variety of symptoms was observed. On inoculated primary leaves, 10 cultivars showed no reaction, seven cultivars showed a trace of necrosis, and six cultivars showed necrotic etching. Uninoculated trifoliolate leaves had no symptoms (two cultivars), mild mottle (four cultivars), mottle with limited chlorosis (eight cultivars), and extensive bright chlorotic mottle (nine cultivars).

It was assumed that the amount of virus replication (milligrams of CCMV per gram of plant tissue) is genetically controlled by both the virus and the host. Therefore, different classes of virus accumulation in inoculated primary leaves were identified by using two criteria. First, about 2,000 plants evaluated individually in the inheritance study were used to prepare histograms which plotted virus concentration vs number of plants. Five distinct classes were observed (see footnote, Table 1). Second, virus concentration was determined in primary leaves of 10 cultivars which represented the different types of symptoms. These cultivars were assigned to four concentration classes which were significantly different from each other (Table 1). Class II virus concentration was not observed in the cultivars tested; it was an intermediate reaction readily observed in F_1, F_2, and backcross populations.

TABLE 2. Local reaction of two cowpea lines and F_1, F_2, and backcross (BC) populations to cowpea chlorotic mottle virus

Parent or cross[a]	Local reaction[b]		Expected ratio (Nec/none)	Chi-square probability
	Necrosis	None		
P - CB	40	0	···	···
PI	0	40	···	···
F_1 - CB × PI	36	0	···	···
PI × CB	36	0	···	···
F_2 - CB × PI	120	42	3:1	0.90–0.75
PI × CB	119	42	3:1	0.75
BC - F_1(CB × PI) × PI	119	114	1:1	0.75

[a] CB = California Blackeye, and PI = Plant Introduction 186465.

[b] Number of plants with reaction on inoculated primary leaves that developed 2–5 days after inoculation.

TABLE 3. Systemic reaction of three cowpea lines and F_1, F_2, and backcross (BC) populations to cowpea chlorotic mottle virus

Parent or cross[a]	Symptom type[b]				Expected ratio (0:1+2+3)[c]	Chi-square probability
	0	1	2	3		
P - CB	0	0	0	139	···	···
PI	145	0	0	0	···	···
I	0	154	0	0	···	···
F_1 - CB × PI	0	0	0	36	···	···
PI × CB	0	0	0	36	···	···
CB × I	0	0	36	0	···	···
I × PI	0	0	36	0	···	···
F_2 - CB × PI	36	22	23	67	1:3	0.90–0.75
PI × CB	19	7	20	57	1:3	0.25–0.10
CB × I	0[d]	83	50	48	···	···
I × PI	41	27	48	79	1:3	0.25–0.10
BC - F_1 (CB×PI)×PI	80	0	30	36	1:1	0.25–0.10
F_1 (CB×I)×I	0	27	10	34	···	···
F_1 (I×PI)×PI	39	2	6	33	1:1	0.90–0.75
F_1 (I×PI)×I	0	66	38	19	···	···

[a] CB = California Blackeye, PI = Plant Introduction 186465, and I = Iron.

[b] Number of plants with symptoms on uninoculated trifoliolate leaves at 21–28 days after inoculation: 0 = none, 1 = mild mottle, 2 = mottle with limited chlorosis, and 3 = extensive bright chlorotic mottle.

[c] Numbers refer to symptom types.

[d] A few plants were first assigned to this category. Subsequent tests revealed a systemic infection and extremely mild mottle.

Virus accumulation was compared in inoculated primary and systemically infected trifoliolate leaves of 12 cowpea lines which included the parents used in the crosses. Primary leaves were harvested at 10 days after inoculation and trifoliolate ones at 35 days. All 12 lines had significantly more virus in the primary leaves (avg = 0.702 mg/g of tissue) than in trifoliolate ones (avg = 0.334 mg/g). Furthermore, although variation was greater for trifoliolate leaves, there was a positive correlation for the amount of virus in the two leaf tissues ($r = 0.649$; $P = 0.05$).

Since these studies were conducted over a 3-yr period, the influence of seasonal changes, particularly temperature, on the phenotypic reactions was evaluated. The local necrotic reaction was affected the most; frequently, this reaction was not observed if the daily temperature did not decline to 24 C for at least 6 hr. Although the intensity of systemic symptoms decreased during summer months, relative differences among the four types (Table 1) were distinct. Virus accumulation in primary leaves was stable throughout the year, particularly if young plants (8–9 days after seeding) were inoculated with highly infectious virus.

Inheritance of local reaction. Necrotic etching developed on primary leaves of cultivar California Blackeye inoculated with CCMV; no symptoms developed on PI 186465. All F_1 plants of the two lines developed necrotic etching, and F_2 plants segregated 3:1 (necrosis: no symptoms) (Table 2). A backcross between the F_1 and the symptomless parent confirmed that the local necrotic reaction in these two cowpea lines was controlled by a single dominant gene. F_2 plants with local necrosis had approximately three times as much virus (0.348 mg/g of tissue) as plants with no local symptoms (0.118 mg/g).

Reactions with other crosses (California Blackeye × Iron; Iron × PI 186465) indicated that the inheritance pattern may not be simple. Although necrosis did not develop on either Iron or PI 186465, all F_1 plants had the symptom, and there was segregation in F_2 and backcross plants. However, too few F_2 plants were observed in this study for a pattern of inheritance to be determined. With these two crosses, F_2 plants with and without necrosis had similar quantities of virus, 0.497 mg/g of tissue for plants in the Iron × PI 186465 and 0.773 mg/g for Iron × California Blackeye.

Inheritance of systemic symptoms and virus movement. The three parents used to study systemic symptoms (California Blackeye, PI 186465, and Iron) could be categorized into three distinct symptom types (Table 3). In the F_1, all plants of the California Blackeye-PI 186465 cross were like California Blackeye. When Iron was crossed to either California Blackeye or PI 186465, the reaction was intermediate (symptom type 2) between mottle (like Iron) and extensive bright chlorosis (like California Blackeye). The intermediate, type 2 symptom reaction was more severe than either parent in the Iron-PI 186465 cross.

Although segregation of symptom types was observed in the F_2 and backcross populations, no inheritance pattern could be ascertained with the four individual types. The distinction between symptom types 1, 2, and 3 was not definitive and assignment of individual F_2 plants was too subjective for quantitative analysis. However, symptomless type 0 plants were distinguished with ease; it represented lack of viral movement from inoculated primary leaves to uninoculated trifoliolate leaves. The inheritance of viral movement was evaluated by comparing the combined values of symptom types 1, 2, and 3 to type 0 (Table 3). While movement was unrestricted in cultivars California Blackeye and Iron, virus did not move from inoculated leaves of PI 186465. Virus moved systemically in all F_1 plants, in all F_2 and backcross plants of the California Blackeye-Iron cross, and in all plants of the backcross F_1 (Iron × PI 186465) × Iron. With the crosses involving PI 186465, movement occurred in three-fourths of the plants in the F_2 and in one-half of the plants in backcrosses of F_1 progeny to PI 186465 (Table 3). The observed F_2 and backcross distributions did not differ significantly from the ratios expected for control by a single dominant gene.

Virus accumulation in individual plants. The consistency and uniformity of virus accumulation in individual plants were tested in the three parents used in the inheritance study. All plants of PI 186465 were in concentration Class I (Table 4). Seventy-five percent or more of California Blackeye and Iron plants were in Classes III and IV, respectively. Differences in concentration among the parents were statistically significant. $P = 0.01$. Plant to plant variation was evaluated by determining virus accumulation levels in 36 plants of each parent. Calculation of the standard error allowed 95% confidence limits to be set on the population means of three cowpea lines: Iron—0.842 (±0.085) mg of virus per gram of tissue, California Blackeye—0.533 (±0.058), and PI 186465—0.036 (±0.009). A similar degree of variation among individual plants probably occurred in F_1, F_2, and backcross plant populations. However, we considered the variation acceptable, particularly with the certainty of Class I and the use of histograms to aid in establishing the other classes.

Inheritance of virus accumulation. These studies involved crosses between cowpea lines with virus accumulation Classes I and III, Classes I and IV, and Classes III and IV (Table 4). Assignment of individual plants to classes was made objectively; however, variability observed within California Blackeye and Iron (Table 4) probably caused 10–25% of the plants to be placed in an "adjacent" class for Classes II, III, and IV. Plants in Class I were distinctive.

In F_1 plants involving crosses with PI 186465 (Class I) as one parent, the virus accumulation level was intermediate between the parents (Table 4). In the cross between Class III and Class IV, the

TABLE 4. Accumulation of cowpea chlorotic mottle virus in individual plants of three cowpea lines and of F_1, F_2, and backcross populations

Parent or cross[a]	Plants/class[b] I	II	III	IV	V	Percent in classes IV & V	Virus concentration[c] Class I	Classes II-V
P - PI	48	0	0	0	0	0	33	···
CB	0	10	38	0	0	0	···	478
I	0	0	8	27	1	78	···	766
F_1 - CB×PI	0	10	2	0	0	0	···	317
PI×CB	0	10	2	0	0	0	···	300
I×PI	0	9	3	0	0	0	···	306
CB×I	0	0	2	10	0	83	···	815
F_2 - CB×PI	29	42	25	6	1	7	37	396
PI×CB	23	50	26	0	1	1	42	319
I×PI	33	85	48	21	5	14	25	540
CB×I	0	3	30	38	14	61	···	704
BC-F_1(CB×PI)×PI	43	31	5	0	1	1	47	255
F_1(I×PI)×PI	79	59	26	4	1	3	15	441
F_1(I×PI)×I	0	5	14	33	25	75	···	880
F_1(CB×I)×I	0	0	43	34	2	46	···	648

[a] PI = Plant Introduction 186465, CB = California Blackeye, and I = Iron.
[b] I = less than 100 μg/g, II = 100 to 400 μg/g, III = 400 to 700 μg/g, IV = 700 to 1,100 μg/g, and V = more than 1,100 μg of virus per gram of leaf tissue.
[c] Average concentration of virus (micrograms per gram of leaf tissue) from all plants in the horizontal row.

TABLE 5. Rate of accumulation of cowpea chlorotic mottle virus and time virus accumulation ceased in four cowpea lines and in F_1 plants

Cowpea line or cross	Virus concentration class[a]	Rate of virus accumulation[b] (μg/24 hr)	Time accumulation ceased[c] (days after inoculation)
PI 186465	I	4	No cessation
F_1 - Iron × PI 186465	II	55	6–8
California Blackeye	III	90	6–8
Iron	IV	144	6–8
Clay	V	198	6–8

[a] I = less than 100 μg/g, II = 100 to 400 μg/g, III = 400 to 700 μg/g, IV = 700 to 1,100 μg/g, and V = more than 1,100 μg of virus per gram of leaf tissue.
[b] Rate (average daily accumulation) determined between 2 and 6 days after inoculation.
[c] Time course assay with virus accumulation in inoculated primary leaves: evaluations were made daily through 10 days, then every 3 days through 24 days after inoculation.

F_1 reaction was similar to the Class IV parent.

With PI 186465 as one parent, most of the F_2 plants segregated into concentration Classes I, II, and III (Table 4). Approximately one-fourth (22%) of the plants were in the lowest concentration class (I). With California Blackeye × PI 186465 and its reciprocal cross, approximately one-half (45%) were in Class II, intermediate to the parents; most of the remainder of the plants were in Class III. With Iron as one parent, F_2 plants occurred more frequently in the higher virus concentration classes (III, IV, V), particularly with California Blackeye × Iron (Table 4).

When Class II plants [F_1 (CB × PI) and F_1 (I × PI)] were crossed with PI 186465 (Class I), 49% of the plants had a low concentration of virus (Class I), and there was some segregation over Classes II to V with other plants (36% in Class II) (Table 4). Backcrosses with Iron as the male parent had no plants in Class I and almost all plants were in Classes III to V (Table 4).

The influence of Iron on high levels of virus accumulation can be noted in the six F_1, F_2, and backcross populations involving that cultivar (column 7, Table 4). Forty-three percent of those plants occurred in accumulation Classes IV and V while only 4% of the plants in the other six crosses occurred in those categories. Furthermore, virus concentration (milligrams of virus per gram of tissue) in the six Iron crosses was 1.9 times as much as in the other six crosses.

Rate of virus accumulation. Time-course assays were conducted with cowpea lines representing each virus concentration class. Virus accumulation ceased at 6–8 days after inoculation for all classes except Class I which accumulates very slowly (Table 5). Since the final virus concentration differs with the cowpea line, this means that the rate of accumulation also differs (Table 5).

TABLE 6. Relationship between systemic symptoms and virus concentration in F_2 plants infected with cowpea chlorotic mottle virus

	Symptom type[a]							
	0		1		2		3	
Parents	%[b]	μg/g[c]	%	μg/g	%	μg/g	%	μg/g
California Blackeye and PI 186465[d]	28	40	8	429	13	310	51	369
Iron and PI 186465[e]	21	25	9	829	33	589	37	446
California Blackeye and Iron[f]	9[g]	658	18	757	30	668	43	699

[a] Number of plants with various type symptoms: 0 = no symptoms, 1 = mild mottle, 2 = mottle with limited chlorosis, and 3 = extensive bright chlorotic mottle.
[b] Percent of plants.
[c] μg of virus purified per gram of inoculated primary leaf tissue.
[d] 185 plants.
[e] 87 plants.
[f] 84 plants.
[g] No symptoms were observed at the time of harvest for purification. When the purification results were known, a barely perceptible mottle could be detected.

Systemic symptoms and virus concentration. When cowpea cultivars were evaluated, there appeared to be a possible relationship between mild systemic symptoms and high levels of virus concentration (Table 1). This relationship was analyzed in F_2 plants (Table 6). Truly symptomless plants had low levels of virus in inoculated primary leaves and none in uninoculated trifoliolate leaves. In general, virus concentration was more dependent on the parents in the cross than on the nature of the systemic symptoms. One exception may be plants in the Iron-PI 186465 cross; virus concentration decreased as the symptom intensity increased.

On some leaves of California Blackeye, completely chlorotic areas were interspersed with normal green areas. The virus concentration was 490 μg/g in the chlorotic tissue and 184 μg/g in the green tissue. Furthermore, sap from the former tissue was three times as infective as sap from the latter.

Symptoms, virus concentration, and yield. Pod and seed yield was compared with three cowpea lines which differed in symptom type and level of virus concentration (Table 7). Virus infection caused no significant yield reduction in either PI 186465 or California Blackeye, despite the occurrence of extensive bright chlorosis on the latter. In Iron, however, seed weight was reduced 37% for plants infected with CCMV.

Strains and virus concentration. The virion concentration of five strains of CCMV was compared in three cowpea lines which differed in symptomatology (Table 8). For all strains, the most virus was produced in Iron and the least in PI 186465. The virus concentration of strains T, M, and BYS were relatively similar in the three lines. Strain R produced the least amount of virus in California Blackeye and Iron, but, as shown previously (15), it could overcome the resistance to replication and to movement in PI 186465. Approximately twice as much of strain A was synthesized in California Blackeye and Iron as the other four strains.

DISCUSSION

In a previous study based on symptomatology, Rogers et al (11) concluded that resistance to CCMV in cowpeas is controlled by one major recessive gene pair, with possible unidentified minor genes. Our studies, which utilized different cowpea lines and additional phenotypic characteristics, partially support the conclusion, but they also provide a more in-depth understanding of the nature and inheritance of resistance.

In PI 186465, resistance to CCMV involves a low level of virus replication and a restriction of the virus to inoculated leaves (12,14). Virus movement is controlled by a single dominant gene (proposed designation *Mv*). California Blackeye and Iron were homozygous dominant (*Mv Mv*), and PI 186465 was homozygous recessive (*mv mv*). No restriction of virus movement occurred among the F_1. These results support conclusions made by Rogers et al (11). It is highly probably that the resistant plant introduction (255811) used in their studies has the same alleles (*mv mv*) controlling virus movement as PI 186465. On the basis of a CCMV strain derivation study (15), we believe the appearance of systemic symptoms on homozygous recessive plants (*mv mv*) in any

TABLE 7. Seed and pod yield of three cowpea lines infected with cowpea chlorotic mottle virus[a]

	Classification			Average per plant		
Cultivar	Systemic symptoms[b]	Virus concentration[c]	Treatment	Pod no.	Seed no.	Seed wt (g)
Iron	1	IV	Control	5.7	68	9.9
			Virus	3.3[d]	43[d]	6.2[d]
California Blackeye	3	III	Control	5.8	33	8.1
			Virus	5.7	31	6.8
PI 186465[e]	0	I	Control	9.3	100	13.1
			Virus	9.7	103	12.9

[a] Experimental design: five replications per treatment, three to five plants per replication, and one plant per 15-cm-diameter pot.
[b] 0 = None, 1 = mild mottle, 2 = mottle with limited chlorosis, and 3 = extensive bright chlorotic mottle.
[c] I = less than 100 μg/g, III = 400 to 700 μg/g, IV = 700 to 1,100 μg/g.
[d] According to an unpaired *T*-test, reductions were statistically significant as follows: number of pods at $P = 0.042$, seed no. at $P = 0.092$, and seed wt. at $P = 0.033$.
[e] PI = Plant Introduction.

generation probably is the result of viral variants whose genomes (specifically RNA 1) have been altered from the type strain (T).

The concentration of virus in segregating progenies appeared to be controlled by the action of several genes. Virus accumulation levels in F_1 hybrids of crosses between resistant PI 186465 (Class I level) and susceptible cultivars California Blackeye (Class III level) and Iron (Class IV level) were intermediate between the parental levels. The F_2 progenies were dispersed over all five classes with a bias toward the classes with low concentration levels. Virus levels in progenies of two backcrosses involving the resistant parent and F_1 plants were biased toward the lower concentration classes; one backcross between a susceptible parent (Iron) and F_1 plants resulted in plants biased toward the higher concentration classes (Table 4). In crosses between the susceptible parents, almost all progeny of the F_1, F_2, and backcross were in the higher virus concentration classes, and there seemed to be a slight bias toward Class IV when Iron was involved.

Multigenic control of virus accumulation is supported by other data. Although Clay (choice of parents was made before its significance was realized) was not used in crosses in this inheritance study, numerous experiments, including the one in Table 1, showed that it has significantly more virus than other cowpea lines. Furthermore, the rate of virus accumulation (Table 5) clearly is distinctive for the cowpea lines in the five concentration classes. In fact it may be significant that the rate intervals between the classes is similar (35–55 μg/24 hr). When Iron, California Blackeye, and PI 186465 are compared to Clay, it seems apparent that each of the three lines has a different genotype acting to retard virus replication. The reduced virus concentration levels are cumulative from Clay to Iron to California Blackeye to PI 186465 and may be equal. Since virus accumulation is inhibited after 6 to 8 days in Clay, Iron, California Blackeye, and F_1 plants, the virus replication cycle in inoculated primary leaves has been completed, or nearly so. This involves both virion maturation and movement within the inoculated leaf. Furthermore, preliminary studies (16) demonstrated that the rate of virus accumulation in individual protoplasts isolated from infected PI 186465 plants was slower than protoplasts from infected California Blackeye. Therefore, it seems likely that it is the rate of virus replication and spread which is being controlled by host genes, and virus accumulation (concentration) is a result of the two phenomena.

Although other explanations may be plausible, the lack of systemic symptoms in any concentration Class I plants suggests the movement gene (*mv*) may be closely linked with one of the genes that retard virus replication. This is supported by data from the backcross of F_1 (California Blackeye × PI 186465) × PI 186465; 55% of the progeny had no symptoms (Table 3) and 54% were in Class I (Table 4). Assuming random assortment of genes, one-half of the plants with Class I concentration would be expected to have systemic symptoms. Previously (15), we investigated the possibility of a threshold virus level being required for virus movement. However, virus accumulation in PI 186465 (Class I) plants could be increased to levels similar to Class III plants without viral movement occurring.

When a new CCMV variant, strain R, developed from strain T in PI 186465, its properties included systemic movement and a Class II concentration level which was intermediate between strain T concentration in PI 186465 and in California Blackeye (15). This indicates that strain R was able to overcome the two closely linked resistant genes in PI 186465.

A few inheritance studies have been conducted which relate virus concentration to host genes; however, we found no reports of attempts to quantify virus levels in individual plants to various genetic populations. Moreover, virus concentrations were based on local lesion assays which determine biological activity, a virus property not necessarily related to virion replication and accumulation. In three studies (1,4,7), each involving different hosts and different viruses, the infectious virus concentration in F_1 plants was intermediate between the parents, a finding similar to CCMV in cowpeas. Since crosses between only two plant lines were evaluated, there was no suggestion of multigenic control of virus replication. In a fourth study, Pelham (8) established isogenic tomato genotypes which included three genes for resistance to tobacco mosaic virus. Each gene could limit virus replication; however, they also were involved in other facets of disease resistance, such as hypersensitivity, and it is difficult to interpret the results with regard to replication.

The intensity of the chlorosis associated with a CCMV infection is unrelated to the virus quantity in cowpeas. In fact, the brightest, most extensive chlorosis occurred in California Blackeye, which consistently had 35 to 55% less virus than Iron and Clay which had much milder symptoms. Since the nature of symptoms was controlled by the host, it appears the chlorosis symptom reaction functions independently of the virus replication process. This phenomenon with CCMV and cowpeas, however, probably does not exemplify all virus-host relationships. For example, Cohen et al (1) reported a relationship between relative recovery of infectious melon mosaic virus in F_2 plants of cucumbers and severity of disease symptoms.

Yield of pods and seeds is another way to measure the disease severity. In this study, yield was found to be more closely related to level of CCMV accumulation in cowpeas than to symptom severity (Table 7). Therefore, virus concentration and not severity of foliar symptoms should be used to evaluate cowpeas for resistance to CCMV.

Replication of CCMV is controlled by both host and virus genes. Pseudorecombinant studies (15) established that CCMV RNAs 1 and 3 influence replication in resistant cowpeas (PI 186465). In susceptible cowpeas (California Blackeye and Iron), CCMV strain A caused approximately twice as much virus to be produced as four other strains (Table 8). Host control of CCMV replication was demonstrated in the inheritance study (Table 4). Moreover, the host determined the relative virus concentration, regardless of virus strain (Table 8). Additional studies should elucidate the interaction between host and virus genes and established gene-for-gene functions.

TABLE 8. Virus concentration of five strains of cowpea chlorotic mottle virus in three cowpea lines[a]

Virus strain	Cowpea line[b]	Symptom type[c]	Virus concentration[d] (μg/g)
T	CB	3	500
	I	1	950
	PI	0	37
M	CB	1	450
	I	1	920
	PI	0	6
R	CB	2	324
	I	1	671
	PI	2	188
A	CB	3	930
	I	1	1,650
	PI	0	46
BYS	CB	3	530
	I	1	780
	PI	0	26

[a] Three replications per treatment; each treatment consisted of eight primary levels, each from a different plant.
[b] CB = California Blackeye, I = Iron, and PI = Plant Introduction 186465.
[c] 0 = None, 1 = mottle, 2 = mottle with limited chlorosis, and 3 = extensive bright chlorotic mottle.
[d] Virus concentration in inoculated primary leaves 10 days after inoculation (average of three replications). For each strain, differences among the three cowpea lines are significant according to Duncan's multiple range test, $P = 0.01$.

LITERATURE CITED

1. Cohen, S., Gertman, E., and Dedar, N. 1971. Inheritance of resistance to melon mosaic virus in cucumbers. Phytopathology 61:253-255.
2. Dawson, W. O., and Kuhn, C. W. 1974. Kinetics of multiplication,

inactivation, and particle-breakdown of cowpea chlorotic mottle virus in cowpea. Phytopathology 64:951-957.
3. Fulton, J. P., Gámez, R., and Scott, H. A. 1975. Cowpea chlorotic mottle and bean yellow stipple viruses. Phytopathology 65:741-742.
4. Karchi, Z., Cohen, S., and Govers, A. 1975. Inheritance of resistance to cucumber mosaic virus in melons. Phytopathology 65:479-481.
5. Kuhn, C. W., Paguio, O. R., and Adams, D. B. 1978. Tolerance in peanuts to peanut mottle virus. Plant Dis. Rep. 62:365-368.
6. Kuhn, C. W., and Wyatt, S. D. 1979. A variant of cowpea chlorotic mottle virus obtained by passage through beans. Phytopathology 69:621-624.
7. Lewellen, R. T. 1973. Inheritance of beet mosaic virus resistance in sugarbeet. Phytopathology 63:877-881.
8. Pelham, J. 1972. Strain-genotype interaction of tobacco mosaic virus in tomato. Ann. Appl. Biol. 71:219-228.
9. Posnette, A. F. 1969. Tolerance of virus infection in crop plants. Rev. Appl. Mycol. 48:113-118.
10. Posnette, A. F., and Swait, A. A. J. 1968. The incidence of virus diseases in English sweet cherry orchards and their effect on yield. Ann. Appl. Biol. 61:351-360.
11. Rogers, D. M., Norton, J. D., and Chambliss, O. L. 1973. Inheritance of resistance to cowpea chlorotic mottle virus in southern pea, *Vigna sinensis*. J. Am. Soc. Hortic. Sci. 98:62-63.
12. Sowell, G., Jr., Kuhn, C. W., and Brantley, B. B. 1965. Resistance of southern pea, *Vigna sinensis*, to cowpea chlorotic mottle virus. Proc. Am. Soc. Hortic. Sci. 86:487-490.
13. Way, D. W. 1965. Further observations on the field performance of heat-treated strawberry clones. J. Hortic. Sci. 40:167-174.
14. Wyatt, S. D., and Kuhn, C. W. 1979. Replication and properties of cowpea chlorotic mottle virus in resistant cowpeas. Phytopathology 69:125-129.
15. Wyatt, S. D., and Kuhn, C. W. 1980. Derivation of a new strain of cowpea chlorotic mottle virus from resistant cowpeas. J. Gen. Virol. 49:289-296.
16. Wyatt, S. D., and Wilkinson, T. C. 1981. Movement of virus within cowpeas resistant to cowpea chlorotic mottle virus. (Abstr.) Phytopathology 71:266.
17. Zaitlin, M. 1977. Replication of plant viruses—an overview. Pages 33–46 in: J. A. Romberger, J. D. Anderson, and R. L. Powell, eds. Virology in agriculture. Beltsville Symposia in Agricultural Research, Volume 1. Allanheld, Osmum & Co. Publishers, Inc., Montclair, NJ. 293 pp.