Effects of Cotton Leaf Crumple Virus on Cotton Inoculated at Different Growth Stages

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ABSTRACT

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The effects of cotton leaf crumple (CLC) disease on yield components and on symptom expression were examined for cotton plants inoculated at various growth stages. In a field test with three inoculation ages, infection at early and midgrowth stages significantly reduced plant height and yield. Yield losses were expressed as a decrease in the number of bolls set and as a delay in boll maturation. Among nine quality parameters evaluated, only boll weight was reduced as a result of virus infection. In a greenhouse test with five inoculation ages, total leaf area was significantly reduced as a result of virus infection. Regardless of plant age at inoculation, significant yield loss was observed in the form of a decrease in boll set and/or boll weight. Characteristic CLC symptoms were not expressed by plants inoculated at greater than the 14- to 16-leaf stage until midseason; thus, estimates of disease incidence that rely solely on the ability to detect foliar symptoms early in the season are not reliable, because latent symptom expression has not been taken into account.

Cotton leaf crumple (CLC) is a disease of cotton (*Gossypium hirsutum* L.) in the southwestern United States (1,10) and in India (16). The disease is characterized by foliar distortion, mosaic, floral enations, general stunting, and severe yield losses in cotton ranging from 21 to 76% (1,5,7,12,17,18).

All available evidence suggests that the pathogen that incites leaf crumple is a geminivirus (4). It is transmitted exclusively by the sweet-potato whitefly (*Bemisia tabaci* Genn.), not by seed or plant sap (10,15).

CLC was reported only sporadically in the Southwest between 1962 and 1979, but epidemics have become more common (2,3,17) and are believed to be related to the early-season buildup of whitefly populations. Low-level populations of whiteflies are known to overwinter locally on host plants (4,6,8,13) and to increase to maximum levels in the spring or early summer (6,8,19). The ultimate sources of whitefly populations, which appear abruptly and en masse in the spring, are currently unknown.

Previous field studies using graft-inoculated or naturally infected cotton indicated that virus infections resulted in losses of varying magnitudes (1,5,7,17,18). Preliminary greenhouse experiments suggested that, as a general rule, characteristically severe CLC symptoms are not expressed in cotton inoculated after the 10- to 14-leaf growth stage (2). Because visual inspection of foliage for CLC symptoms is currently used to estimate disease incidence, infections that occur after plants have reached critical developmental stages may be overlooked, resulting in inaccurate estimates.

Greenhouse and field studies in which cotton plants were experimentally inoculated (using the whitefly vector) at various stages of growth were initiated to simulate virus infection at different times during a typical growing season. The objectives of these studies were: 1) to investigate the effect(s) of CLC virus (CLCV) infection on various components of yield (plant height, number of flowers, number of green bolls, number of mature bolls, and quality of mature bolls), 2) to determine if characteristic symptom development is dependent on the growth stage of the plant when inoculated, and 3) to determine if the presence or absence of characteristic foliar symptoms can be used to determine disease incidence accurately.

MATERIALS AND METHODS

Cotton (*G. hirsutum* cv. Delta Pine 70) plants were inoculated with CLCV, using viruliferous adult whiteflies as previously described (4). Plants were inoculated at three (2- to 3-, 8- to 10-, and 14- to 16-leaf stage) or five (2- to 3-, 5- to 8-, 8- to 10-, 14- to 16-, and 18- to 20-leaf stage) growth (leaf) stages for field plot (30

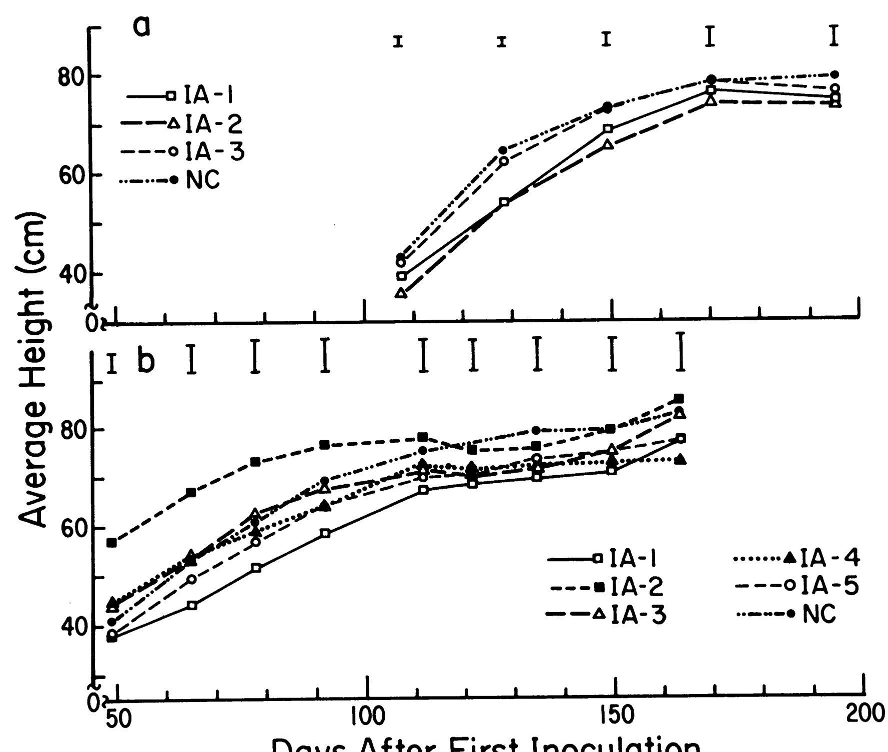

Fig. 1. Effects of cotton leaf crumple on height (cm) throughout the season for **(A)** field-grown and **(B)** greenhouse-grown cotton plants inoculated at different growth stages (IA-1 through IA-5), where IA = inoculation age and NC = noninoculated control. Vertical bars represent LSD values for pairwise comparison of means ($P \leq 0.05$).

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plants per treatment) and greenhouse (20 plants per treatment) experiments, respectively. Groups of plants inoculated at the various growth stages are referenced throughout the text as inoculation ages 1 (IA-1), 2 (IA-2), and 3 (IA-3) for field-plot plants and as inoculation ages 1 (IA-1), 2 (IA-2), 3 (IA-3), 4 (IA-4), and 5 (IA-5) for greenhouse plants. Noninoculated control (NC) plants were maintained under conditions similar to inoculated plants in both field and greenhouse studies. After inoculation, all plants were treated with the systemic insecticide aldicarb (Temik) and maintained in a greenhouse (20–26 C). For the field experiment, cotton was transplanted to a plot at the University of Arizona Campbell Avenue Farm, Tucson, 1 wk after the last inoculation date. A randomized complete block design was used in the plot, and four treatments of 30 plants each were replicated in each of four blocks.

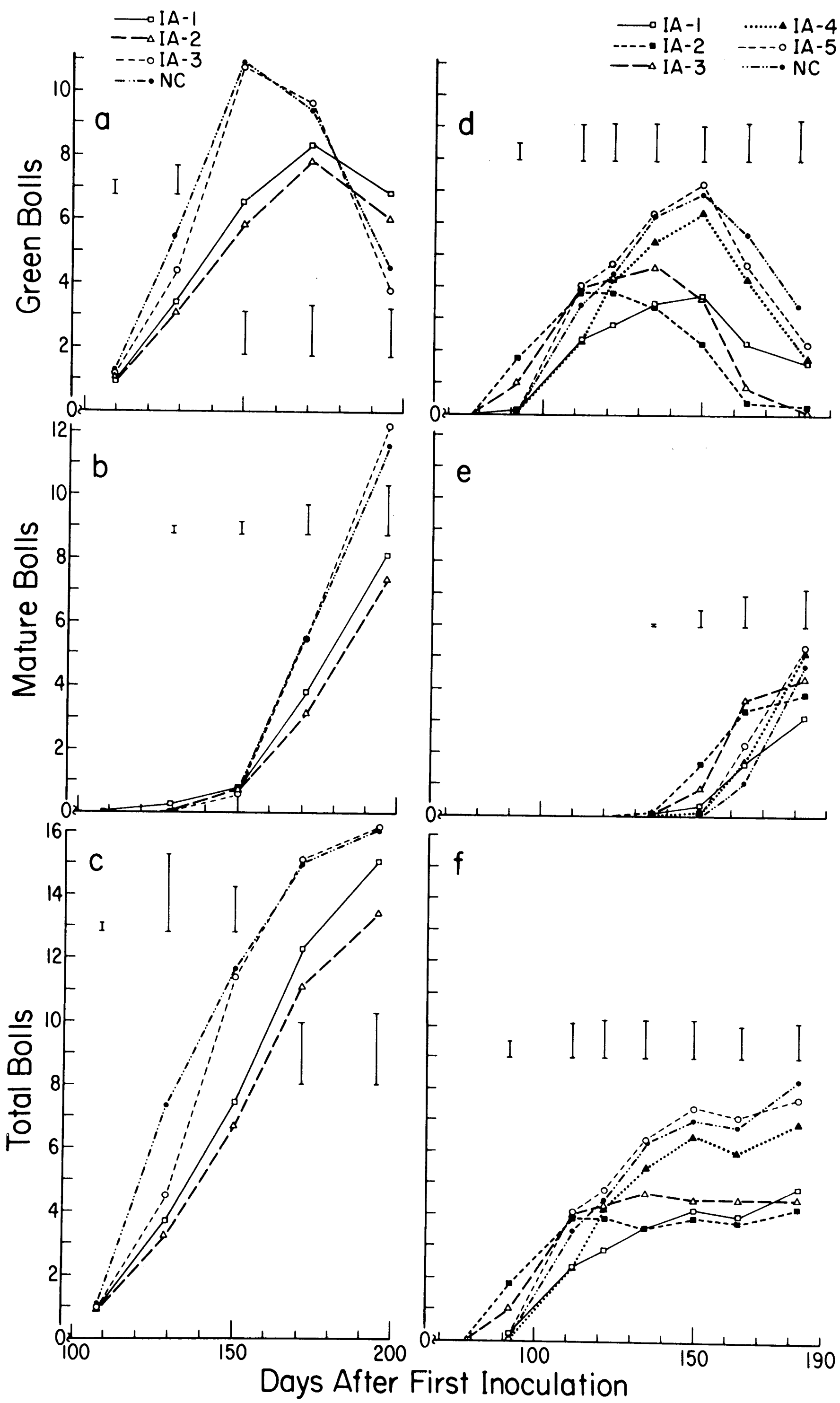

Fig. 2. Effects of cotton leaf crumple on production of **(A)** green, **(B)** mature, and **(C)** total bolls for field-grown cotton plants and on production of **(D)** green, **(E)** mature, and **(F)** total bolls for greenhouse-grown plants inoculated at different growth stages (IA-1 through IA-5), where IA = inoculation age and NC = noninoculated control. Vertical bars represent LSD values for pairwise comparison of means ($P \leq 0.05$).

Yield components. Yield components assessed for field- and greenhouse-grown plants at 2-wk intervals included plant height, number of open flowers, number of green (closed) bolls, and number of mature (open) bolls. The total number of bolls for each plant was calculated by summing the values of the last two variables. Because many flowers aborted and squares (an unopened cotton flower with its enclosing bracts) unexpectedly failed to open and were subsequently shed in the field-plot plants, the effect of the virus on inflorescence (squares plus flowers, collectively) production was assessed in the greenhouse experiment. Data from both experiments were analyzed by one-way analyses of variance and the least significant difference (LSD) mean separation procedures. The term "significantly different" throughout the text implies that compared means were statistically different at $P \leq 0.05$. All other comments refer strictly to qualitative differences, and no implication of statistical difference is intended.

Leaf area analysis. To quantitatively determine the effect of CLCV on leaf area in the greenhouse experiment, the primary leaf at the third internode from the apical meristem was collected every 2 wk from 10 plants per treatment. Leaves were flattened by pressing and were then photocopied. Leaf area was measured by tracing the photocopy of each leaf twice with a polar compensating planimeter.

Standard quality analyses. At the end of the field experiment, mature bolls were collected to assess the effect(s) of the virus on nine standard quality parameters. Each 10-boll sample was collected as a composite of two bolls from each of five plants. One set of samples was taken from plants in each of the four symptom classes (0 = no symptoms, 1 = floral enations and/or one to three symptomatic leaves, 2 = three to five symptomatic leaves, and 3 = more than five symptomatic leaves), regardless of the inoculation age of the plants, and a second set of samples was taken from plants in each of the four treatments, regardless of the symptom class. Quality analyses were conducted by E. L. Turcotte (USDA-ARS Fiber Testing Laboratory, Cotton Research Center, Phoenix, AZ). Under field conditions, boll weight was the only quality parameter affected by the disease and, thus, the only quality parameter evaluated in the greenhouse study.

At the end of the experiment, the green bolls and the mature bolls from each plant were counted. The lint and seeds were harvested from each mature boll and weighed. Individual boll weights were summed, giving the total weight of mature bolls produced by each plant. An average boll weight was determined for each treatment by calculating the average of the mature boll weight for all plants in a treatment. Using the mature boll weight values and the average boll weight to project the weights of immature bolls remaining on plants at the end of the experiment, and assuming that all green bolls matured and contributed to the yield, a maximum harvestable yield (*MHY*) was estimated for each plant with the equation: $MHY = MBW + (ABW \times GB)$, where MBW = mature boll weight, ABW = average boll weight, and GB = green bolls.

Disease severity ratings. In both field and greenhouse experiments, plants were assigned disease severity ratings (scale of 0–3, given earlier) at each assessment date based on the extent of characteristic CLC symptoms in foliage and/or flower petals.

At the end of the experiments, 196 and 183 days after the first inoculation date for field and greenhouse experiments, respectively, plants were pruned (stubbed) to 0.5 m to promote perennial regrowth (in which severe virus symptoms are expressed, regardless of the severity of symptoms observed on annual foliage) and allowed to grow for 4 wk more. Infection was based on the presence of characteristic CLC symptoms, and data were used to calculate the actual percentage of infection at the end of each experiment.

RESULTS

Effects of CLC on yield components in the field plot. The plant height data collected throughout the field experiment are summarized in Figure 1A. NC plants were significantly taller than the plants inoculated at the first two growth stages (IA-1 and IA-2), but there was no significant difference in height between IA-3 and NC plants (Fig. 1A).

Throughout the growing season, there was no significant difference among the four treatments with respect to the number of open flowers (0.5–2.0 open flowers per plant).

The effects of CLC on cotton boll production in the field plot are shown in Figure 2A–C. Plants inoculated earlier (IA-1 and IA-2) had significantly more green bolls at the end of the experiment than those inoculated later (IA-3) or the NC plants, suggesting a delay in boll maturation (Fig. 2A). The IA-1 and IA-2 plants had significantly fewer mature bolls than the IA-3 and NC plants (Fig. 2B); however, there was no difference among treatments in total boll production at the end of the growing season (Fig. 2C).

Effects of CLC on yield components and leaf area in the greenhouse. Plant heights are summarized in Figure 1B. Early-inoculated plants (IA-1) were significantly shorter than NC plants through day 92; IA-2 plants were significantly taller than NC plants during this period. Between days 112 and 150, there was no significant difference in height among the six treatments.

The number of green, mature, and total bolls produced is shown in Figure 2D–F. From 135 days after the first inoculation, IA-1, IA-2, and IA-3 plants produced significantly fewer green bolls than IA-4 and IA-5 or NC plants (Fig. 2D). By the end of the experiment, plants in the NC group had produced significantly more green bolls than those in the IA-1 through IA-4 treatments. Bolls matured significantly earlier for plants in

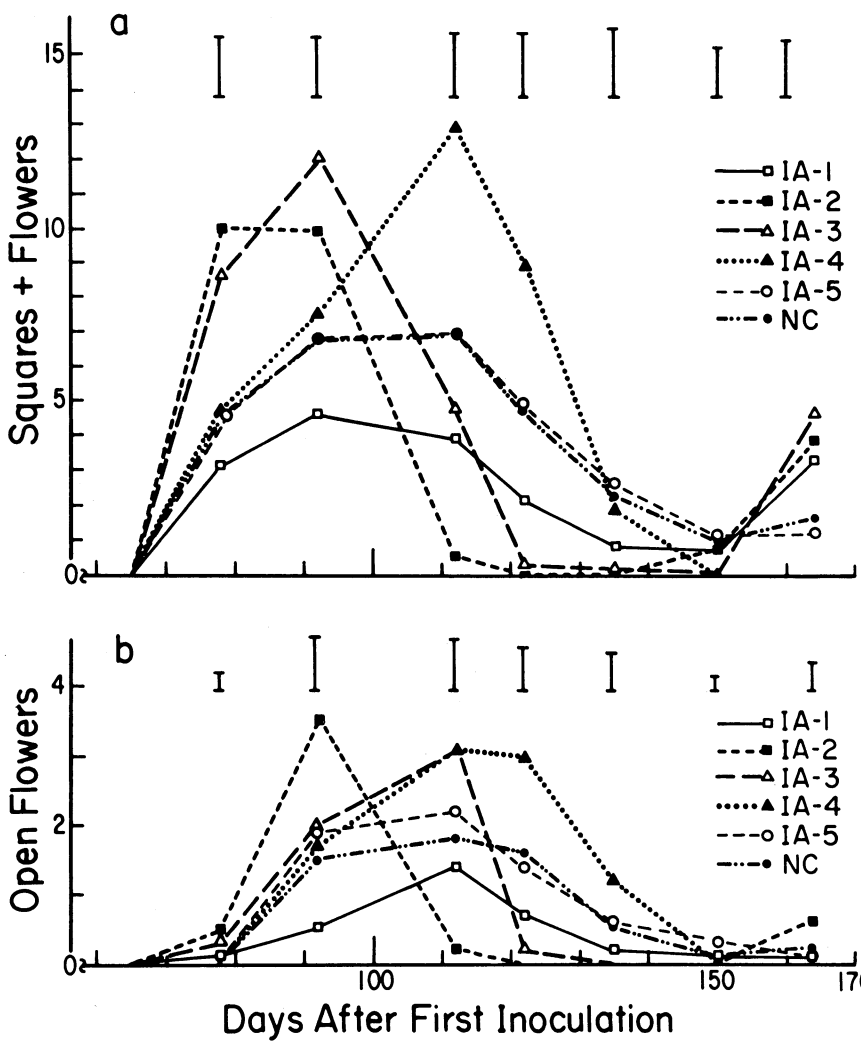

Fig. 3. Effects of cotton leaf crumple on production of **(A)** inflorescences (squares plus flowers) and of **(B)** open flowers for greenhouse-grown plants inoculated at different growth stages (IA-1 through IA-5), where IA = inoculation age and NC = noninoculated control. Vertical bars represent LSD values for pairwise comparison of means ($P \leqslant 0.05$).

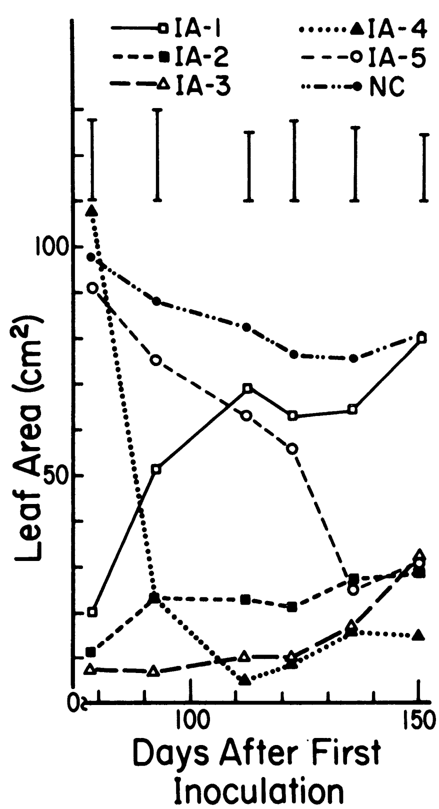

Fig. 4. Effects of cotton leaf crumple on leaf area of greenhouse-grown plants inoculated at different growth stages (IA-1 through IA-5), where IA = inoculation age and NC = noninoculated control. Vertical bars represent LSD values for pairwise comparison of means ($P \leqslant 0.05$).

treatments IA-2 and IA-3 than plants in all other groups (Fig. 2E), as indicated by the significantly greater number of mature bolls for these two groups on day 164. In contrast, IA-1 plants had fewer mature bolls than plants in the IA-4, IA-5, or NC groups at the end of the experiment (Fig. 2E). IA-1 and IA-2 plants yielded significantly fewer total bolls than all other groups beginning 135 days after inoculation (Fig. 2F). By the end of the experiment, plants in the first four inoculation age groups produced significantly fewer total bolls than IA-5 or NC plants (Fig. 2F).

Significantly fewer inflorescences were produced by IA-1 plants throughout much of the experiment when compared with other treatments (Fig. 3A,B). Plants in IA-2, IA-3, and IA-4 treatments produced significantly more squares and flowers than IA-5 or NC plants at various times during the experiment; however, many of these infloresences were subsequently shed. Plants in the IA-1, IA-2, and IA-3 groups seemed to compensate for these losses by initiating additional reproductive structures late in the season (Fig. 3).

The leaves of all inoculated plants were significantly smaller than those of NC plants at some time during the experiment (Fig. 4). Foliar stunting was observed initially with plants inoculated at the earliest growth stage and progressed sequentially to include all groups of inoculated plants, in the order of inoculation (Fig. 4). By the end of the experiment, only the IA-1 plants produced leaves that did not differ significantly in size from those of NC plants (Fig. 4).

Standard quality analyses. Bolls collected from the field plot were analyzed using nine standard quality parameters. Parameters analyzed were boll weight (grams per boll), percent lint, lint index, seed index, seeds per boll, fiber length, uniformity ratio, strength, and fineness. Of these parameters, only boll weight (grams per boll) was significantly different among treatments. When collection of samples was based entirely on symptom severity ratings, the boll weights were 4.7, 4.9, 4.3, and 4.0 g/boll for symptom classes 0, 1, 2, and 3, respectively. When the same analysis was performed on the basis of inoculation age of plants, boll weights were 3.8, 4.2, 4.7, and 4.8 g/boll for IA-1, IA-2, IA-3, and NC plants, respectively.

In the greenhouse experiment, the average boll weights for plants in the IA-1 and IA-2 treatments did not differ significantly from the NC plants, whereas average boll weights for plants in IA-3, IA-4, and IA-5 groups were significantly lower than those of the NC plants (Table 1). Significantly fewer total bolls were produced by the IA-1, IA-2, and IA-3 groups than by the IA-4 and IA-5 groups or the NC plants (Fig. 2F). Thus, in the greenhouse, boll weight was reduced for IA-3, IA-4, and IA-5 plants, while fewer bolls were produced by IA-1, IA-2, and IA-3 plants. A computation of the estimated yields (based on the formula given earlier for maximum harvestable yield) indicated that all inoculated treatments had lower overall harvestable yields than the NC plants (Table 1).

Disease severity ratings in field and greenhouse. Severity ratings are summarized in Figure 5. In the field plot, ratings were highest and thus symptoms were most severe for IA-1 and IA-2 plants (Fig. 5A). Extremely mild virus symptoms were associated with the IA-3 plants (Fig. 5A) early in the season, but severity ratings increased as the season progressed. The observation that NC plants were symptomless until day 109 while 72% were infected by the end of the season (Fig. 5A) supports the argument for natural virus spread to NC plants late in the season. Severity ratings in the greenhouse study were consistently high (3.0) for the IA-1, IA-2, and IA-3 groups (Fig. 5B) throughout the experiment. Mild symptoms (1.0–2.2) were observed about 2 and 3 wk after inoculation of IA-4 and IA-5 plants, respectively, whereas typically severe CLC symptoms were not observed for IA-4 and IA-5 plants until midway through the season (Fig. 5B).

DISCUSSION

The results of this study indicate that yield reduction in cotton results from infection of plants by CLCV at virtually any developmental stage. Losses are expressed in the forms of a reduction in boll weight, a reduced number of bolls set, and/or a delay in maturation of bolls. The magnitude of yield reduction appears to depend on the developmental stage of cotton plants when virus infection occurs. Boll weight and the number of set bolls are of primary consideration when determining the total

Table 1. Effects of cotton leaf crumple on boll weight and plant yield for greenhouse-grown cotton plants inoculated at different growth stages

Test plant group[x]	Maximum harvestable yield[y] (g/plant)	Average boll weight[z] (g)
IA-1	20.7 ab	4.20 a (59)
IA-2	16.3 ac	3.88 a (77)
IA-3	16.0 c	3.53 bc (88)
IA-4	22.2 b	3.22 b (99)
IA-5	27.0 d	3.54 c (101)
NC	33.4 e	4.18 a (93)

[x] Groups of test plants were inoculated at five growth stages (IA-1 through IA-5), where IA = inoculation age and NC = noninoculated control.

[y] Maximum harvestable yield (*MHY*) was calculated on a per plant basis as $MHY = MBW + (ABW \times GB)$, where MBW = the sum of weights of mature bolls per plant, ABW = the average weight per boll for the entire treatment, and GB = the number of green bolls per plant. *MHY* values followed by the same letter are not statistically different at $P = 0.05$.

[z] Average boll weight was calculated using the weights of all mature bolls in each treatment. Total numbers of mature bolls are indicated parenthetically. Values followed by the same letter are not statistically different at $P = 0.05$.

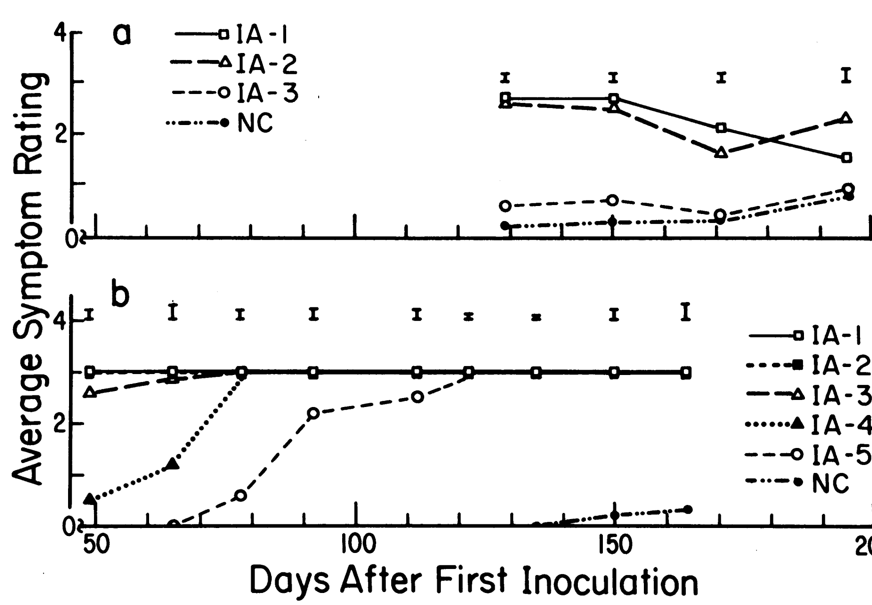

Fig. 5. Effects of cotton leaf crumple on symptom expression for **(A)** field-grown and **(B)** greenhouse-grown cotton plants inoculated at different growth stages (IA-1–IA-5), where IA = inoculation age and NC = noninoculated control. Symptom ratings were: 0 = no symptoms, 1 = floral enations and/or one to three symptomatic leaves, 2 = three to five symptomatic leaves, and 3 = more than five symptomatic leaves. Vertical bars represent LSD values for pairwise comparison of means ($P \leq 0.05$).

yield; however, delayed maturation of bolls may be of serious consequence only when fields are not harvested multiple times.

Plants inoculated at the early developmental stages were initially stunted and, in some cases, appeared to have recovered by the end of the experiment (Fig. 1). An exception was observed in which the greenhouse-grown IA-2 plants were taller than the NC plants (Fig. 1B). In no case, however, did the IA-2 plants appear normal and vigorous when compared with healthy plants in that an increase in internode length, which resembled etiolation, and a dramatic reduction in leaf size were observed with infected plants (Fig. 4). Observations made from the greenhouse data suggest that there may be one or more specific developmental stages at which the normal appearance and growth habits of cotton plants are most critically affected by virus infection.

Among the nine standard quality parameters analyzed in the field study, only boll weight was negatively affected by virus infection. Parameters not affected by the disease included percent lint, lint index, seed index, number of seeds per boll, fiber length, fiber uniformity ratio, fiber strength, and fiber fineness. Our results confirm those of previous studies, which report no difference in the quality of cotton harvested from virus-infected and healthy NC plants (1,17,18).

In the greenhouse experiment, plants in the IA-2 through IA-4 groups flowered sooner and produced more inflorescences than the NC plants (Fig. 3); however, many squares and flowers were shed abruptly before boll set (J. K. Brown and J. D. Mihail, *personal observation*). The end result was that either the same number or fewer flowers remained on the infected plants (depending on inoculation age) (Fig. 3) to ultimately develop into intact, mature bolls (Fig. 2F).

In the greenhouse experiment, the total leaf area of virus-infected plants was generally reduced. An exception was noted with the IA-1 plants, which developed apparently normal-sized leaves at the end of the experiment (Fig. 4). A decrease in the effective photosynthetic leaf area is a primary factor contributing to square shedding and flower drop (11). The number of bolls set and, thus, the ultimate yield are directly related to the total amount of effective leaf area. The abrupt loss of squares and flowers by infected plants was probably due to the reduction in total effective photosynthetic leaf area and the subsequent inability of plants to support all potential bolls.

Although plants that become infected at early developmental stages express characteristic CLC symptoms, midseason and late-season infections result in mild, latent symptom expression. In nonepidemic years, CLC symptoms are not associated with annual foliage, whereas late-season axillary branches and perennial regrowth show characteristic CLC symptoms. Such field observations and others made during this study suggest that, in most years, virus infection of cotton probably occurs at less critical developmental stages. Furthermore, the results of this study indicated that symptom severity is not directly related to, nor can it be used to, predict the magnitude of yield loss resulting from virus infection.

Accurate determination of disease incidence, based on visual detection of symptoms, appears to be feasible only when infection occurs in the early developmental stages. A more useful strategy could possibly involve the monitoring of whiteflies to determine when seasonal influxes occur and, thus, the potential time of virus infection. Precisely timed application of insecticides to prevent the initial establishment of whitefly populations and subsequent infection loci would then be feasible. Influxes of *B. tabaci* appear to follow general but imprecise trends (6,8,9,13,14,19), and local collection data are currently available for a few consecutive years. Thus, long-term evaluation of whitefly migration behavior, as well as an effective means of diagnosing leaf crumple infection, are needed to aid in the development of a method by which disease incidence may be determined and strategically timed control measures may be implemented.

LITERATURE CITED

1. Allen, R. M., Tucker, H., and Nelson, R. A. 1960. Leaf crumple disease of cotton in Arizona. Plant Dis. Rep. 44:246-250.
2. Brown, J. K., Butler, G. D., Jr., and Nelson, M. R. 1983. Occurrence of cotton leaf crumple associated with whitefly infestations in Arizona. (Abstr.) Phytopathology 73:787.
3. Brown, J. K., and Nelson, M. R. 1982. Whitefly-transmitted pathogens in Arizona. Ariz. Plant Pathol. Coop. Ext. Serv. Newsl. 2(2):2-3.
4. Brown, J. K., and Nelson, M. R. 1984. Geminate particles associated with cotton leaf crumple disease in Arizona. Phytopathology 74:987-990.
5. Butler, G. D., Jr., Brown, J. K., and Henneberry, T. J. 1986. Effect of cotton seedling infection by cotton leaf crumple virus on subsequent growth and yield. J. Econ. Entomol. 79:208-211.
6. Butler, G. D., Jr., and Henneberry, T. J. 1985. *Bemisia tabaci* (Gennadius), a pest of cotton in the southwestern United States. U.S. Dep. Agric. Tech. Bull. 1707. 19 pp.
7. Butler, G. D., Jr., Henneberry, T. J., and Brown, J. K. 1985. Cotton leaf crumple disease of Pima cotton. Pages 158-159 in: Cotton—A College of Agriculture Report. Ser. P-63. Coop. Ext. Serv./Agric. Exp. Stn. Univ. Ariz./U.S. Dep. Agric. 195 pp.
8. Butler, G. D., Jr., Henneberry, T. J., and Natwick, E. T. 1985. *Bemisia tabaci*: 1982 and 1983 populations in Arizona and California cotton fields. Southwest. Entomol. 10:20-25.
9. Byrne, D. N., Von Bretzel, P. K., and Hoffman, C. J. 1986. Impact of trap design and placement when monitoring for the bandedwinged whitefly and the sweetpotato whitefly (Homoptera: Aleyrodidae). Environ. Entomol. 15:300-304.
10. Dickson, R. C., Johnson, J. M., and Laird, E. F., Jr. 1954. Leaf crumple, a virus disease of cotton. Phytopathology 44:479-480.
11. Elliot, F. C., Hoover, M., and Porter, Jr., W. K. 1968. Advances in Production and Utilization of Quality Cotton: Principles and Practice. Iowa State University Press, Ames. 532 pp.
12. Erwin, D. C., and Meyer, R. 1961. Symptomatology of the leaf crumple disease in several species and varieties of *Gossypium* and variation of the causal virus. Phytopathology 51:472-477.
13. Gerling, D. 1967. Bionomics of the whitefly-parasite complex associated with cotton in southern California (Homoptera:Aleyrodidae; Hymenoptera:Aphelinidae). Ann. Entomol. Soc. Am. 60:1306-1321.
14. Gerling, D., and Horowitz, A. R. 1984. Yellow traps for evaluating the population levels and dispersal patterns of *Bemisia tabaci* (Gennadius) (Homoptera:Aleyrodidae). Ann. Entomol. Soc. Am. 77:753-759.
15. Laird, E. F., and Dickson, R. C. 1959. Insect transmission of the leaf crumple virus of cotton. Phytopathology 49:324-327.
16. Mali, V. R. 1977. Cotton leaf crumple disease—a new record for India. Indian Phytopathol. 30:326-329.
17. Russell, T. E. 1982. Effect of cotton leaf crumple (CLC) disease on stub and planted cotton. Pages 43-47 in: Cotton. Coll. Agric. Rep. Ser. P-56. Coop. Ext. Serv./Agric. Exp. Stn. Univ. Ariz./U.S. Dep. Agric. 170 pp.
18. van Shaik, P. H., Erwin, D. C., and Garber, M. J. 1962. Effects of time on symptom expression of the leaf-crumple virus on yield and quality of fiber of cotton. Crop. Sci. 2:275-277.
19. Zalom, F. G., Natwick, E. T., and Toscano, N. C. 1985. Temperature regulation of *Bemisia tabaci* (Homoptera:Aleyrodidae) populations in Imperial Valley cotton. J. Econ. Entomol. 78:61-64.