Effect of Cucumber Mosaic Virus Inoculation at Successive Weekly Intervals on Growth and Yield of Pepper (*Capsicum annuum*) Plants

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ABSTRACT

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Groups of pepper plants in the field were mechanically inoculated with cucumber mosaic virus (CMV) at successive weekly intervals from 22 June to 10 August. In early inoculations, inoculated leaves developed necrotic rings and oak-leaf-like patterns, whereas systemically infected leaves remained small and narrow and had a fine yellowish green mottle. In later inoculations, few inoculated leaves developed necrotic patterns and only leaves on some branches developed systemic symptoms of any kind. Most of the fruit of early-inoculated plants were small, slightly wrinkled, or bumpy and pale green; a few fruits had dark, depressed spots. The severity of foliar symptoms and the ratio of small, malformed fruit to normal fruit decreased as the date of inoculation was delayed. Plants inoculated in early growth stages were significantly shorter; produced markedly less top weight; and had significantly fewer and smaller leaves, fewer total fruit, and fewer marketable fruit than plants inoculated later in the season or plants remaining uninfected throughout the season. Plant growth and fruit yield improved in almost direct proportion to the lateness of inoculation of the plants with CMV.

The importance of viruses in green peppers has been recognized for many years wherever peppers are grown (2,9,11,15). In most locations, cucumber mosaic (CMV), potato Y (PVY), and/or tobacco etch (TEV) viruses are the most prevalent and cause the most severe damage on peppers. Because all three viruses are brought into and spread in pepper fields by aphids (6,8,14), pepper plants in different geographical areas and under different management treatments may become inoculated with the viruses at different stages of growth (1,16). It has been shown with several plant-virus combinations, eg, tobacco mosaic virus on tomato (5), watermelon mosaic virus on watermelon (7), and beet western yellows virus on sugar beet (13), that generally, the younger the plants are when they become infected the more severely they are affected. No information on such a relationship between timing of virus infection and pepper growth and yield is available even though some of the proposed measures for virus control in peppers (1,3,10) act primarily by delaying the time of infection of peppers by viruses.

Preliminary surveys of pepper fields in 1981 in which a portion of the plants were tested for the three viruses by enzyme-linked immunosorbent assay (ELISA) (1,4,12) showed that 46% of the plants were infected with CMV, 40% with PVY, and 20% with TEV. In some fields, virus symptoms first appeared in mid-July, and after a slow spread to about 10–20% of the plants within the next 2 wk, nearly 100% of the plants had developed virus symptoms 3 wk later (by 20 August). This work was undertaken to determine the effect of CMV on the growth and yield of pepper plants when inoculated at different stages of development.

MATERIALS AND METHODS

Pepper (*Capsicum annuum* L.) 'Lady Bell' seedlings were transplanted in the field on 4 June 1982. Seedlings were planted in double rows with 45 cm between seedlings. Spacing was 1.2 m between rows and 2.4 m between double rows. Forty seedlings were planted in each row. There were eight double rows, each with 80 seedlings. Each group of 10 seedlings per double row (five in each row) constituted a treatment and was inoculated with CMV once in one of eight successive inoculations carried out at weekly intervals beginning on 22 June and ending on 10 August. Treatments were completely randomized within each double row (randomized complete-block experimental design) and the blocks were replicated eight times. CMV inoculum was obtained by grinding symptomatic leaves of Small Sugar pumpkin plants with a mortar and pestle 10 days after inoculation. The infected sap was then applied with the pestle to four Carborundum-dusted pepper leaves of the 10 plants in each treatment and in all eight replicates of each treatment. However, because at the time of later inoculations some plants were already showing viral symptoms from natural infection, such plants were removed and the remaining asymptomatic plants inoculated. Thus, only 67, 64, and 37 plants were inoculated on 27 July, 3 August, and 10 August, respectively. Fruit of all plants were harvested three times, separated into marketable (U.S. fancy, and U.S. No. 1 and U.S. No. 2 combined) and unmarketable fruit, then counted and weighed. At the last harvest (1 October), all fruit were harvested, regardless of size. On 7 October, the height of each plant was measured and the second and third plant of the right row of each double row were cut at the soil line and weighed. Then their leaves were removed, counted, and also weighed. Similar measurements were made on 40 asymptomatic plants. Data were analyzed by Duncan's multiple range test. Significance is expressed at $P = 0.05$.

During the growing season, at least five plants that had developed systemic symptoms after mechanical inoculation with CMV on 22 June and 6 and 20 July were assayed for CMV by indirect ELISA according to the method of Clark and Adams (4). ELISA was carried out on 14, 22, and 29 July, 8 and 21 August, and 9 September. Two leaves, one approaching the typical leaf size of the plant at that date and one about half that size and still expanding, were collected from each plant on the day they were assayed by ELISA. Healthy leaves used as controls were obtained from virus-free plants kept in the greenhouse. Leaf samples of about equal size were squeezed between stainless steel roller bars. The sap was

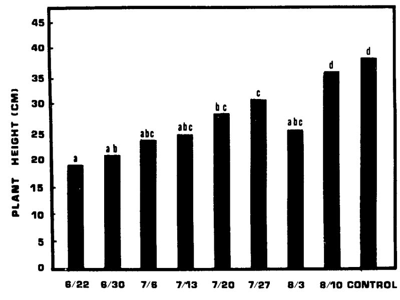

Fig. 1. Average heights of pepper plants inoculated with cucumber mosaic virus at different dates and of uninoculated control plants.

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rinsed off the bars with ELISA "coating buffer" (carbonate buffer, pH 9.6) and collected in tubes in a dilution of about 1:10 (w/v). Sap from each plant was placed in two wells of Gilford microtiter plates, and after the appropriate incubation periods and rinses (4), treatment with purified gamma globulin of CMV antiserum prepared in rabbits in our laboratory, alkaline phosphatase-conjugated antirabbit antiserum produced in goats (Sigma Chemical Co.), and substrate (*p*-nitrophenyl phosphate, Sigma), the plates were read in a Gilford manual ELISA reader at 405 nm.

RESULTS

Symptoms of pepper plants inoculated with CMV. All leaves mechanically inoculated with CMV during the first inoculation (22 June) and most (75 and 60%, respectively) of the leaves inoculated at the next two inoculation dates (30 June and 6 July) developed necrotic rings or oak-leaf-like patterns within 6–12 days of inoculation. Similar symptoms also developed in some of the older uninoculated leaves of the same plants. Many of the directly inoculated, symptomatic leaves later turned yellowish and curled and either fell off or died. Almost all leaves produced after inoculation remained small, narrow, more upright than normal, and had a micropattern of alternating tiny yellowish and green areas that gave the leaf a general macroscopic yellowish green, "dull" appearance. The stems remained small, the internodes short, and the plants markedly stunted. In plants inoculated in subsequent weeks, only some of the inoculated leaves showed necrotic rings or oak-leaf-like patterns, and the number of leaves and plants with such symptoms decreased in the later inoculation dates. In many plants of later inoculations, many of the leaves, especially on the exterior of the plant, remained normal looking, and often, entire branches or whole plants had leaves of normal size, shape, and color. The height and general vigor of the plants were reduced drastically by CMV infection, particularly in early inoculations (Figs. 1 and 2).

Most (70–80%) of the fruit produced by plants inoculated early (22 June through 6 July) with CMV remained small, slightly wrinkled, or bumpy and pale green to yellowish green. Similar fruit were also produced by plants inoculated during the remainder of July and even by those inoculated in early August; however, the proportion of such fruit decreased and their size increased with lateness of inoculation. A small number (3–7%) of both the small, yellowish green and of the normal looking fruit from inoculated plants showed a few to many dark, depressed areas, often on one side of the fruit.

Effect of CMV on pepper growth. At the end of the growing season (7 October), the average height of healthy pepper plants was 38 cm, whereas that of plants inoculated with CMV ranged from 18.8 cm for those inoculated the earliest (22 June) to 35.3 cm for those inoculated the latest (10 August) (Fig. 1). The height increase of plants inoculated with CMV in intermediate weeks was almost proportional to the delay in the date of inoculation (Fig. 1).

The average top weight of healthy pepper plants on 7 October was 379.3 g, whereas the average top weight of CMV-inoculated pepper plants increased from 81.7 g for those inoculated the earliest (22 June) to 345.6 g for those inoculated the latest (10 August) (Fig. 2).

By the end of the growing season (7 October), each uninoculated plant had an average of 232.7 leaves. Plants inoculated with CMV on the earliest date (22 June) had an average of 148.5 leaves each, whereas plants of the last inoculation (10 August) had an average of 279.3 leaves each (Fig. 3A). All or most exterior leaves of plants inoculated in the earlier four inoculations were small and narrow (5–10 cm long by 2–4 cm wide), somewhat thick and leathery, and "dull" yellowish green; in the interior of the plant, there were numerous small (1–3 cm long) leaves in clusters of various sizes. Many plants inoculated in the later four inoculations had exterior leaves of mostly normal shape, size, and color, although some of them appeared mottled. Internally, however, they too had clusters of small leaves 1–3 cm long. Uninoculated plants, of course, had external leaves of normal size, shape, and color, but they too had clusters of small leaves in their interior. An indication of the difference in leaf mass in plants infected for varying lengths of time is given by Figure 3B, which shows that pepper plants inoculated with CMV on 22 June had an average leaf mass of 45.4 g, whereas plants inoculated on 10 August had 192.6 g of leaves and uninoculated plants had 205.1 g of leaves. By taking the ratios of the data shown in Figure 3A,B, it can be seen that the average leaf weight was only 0.3 g in the earliest-inoculated plants, whereas it was 0.7–0.8 g in plants of the last two inoculations and almost 0.9 g in uninoculated plants (Fig. 3C).

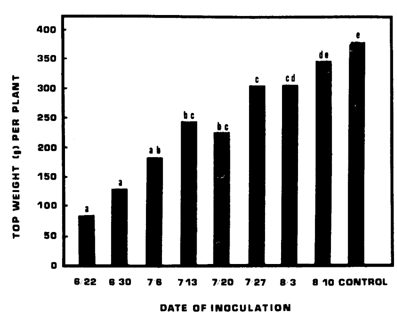

Fig. 2. Average top weights (excluding fruit) of pepper plants inoculated with cucumber mosaic virus at different dates and of uninoculated control plants.

Effect of CMV on fruit yield. The total number of pepper fruits per plant produced throughout the season varied from 6.5–10.5 fruits for plants inoculated with CMV during one of the first 5 wk of inoculations to 12.8–19 fruits for plants inoculated during one of the last 3 wk of inoculations (Fig. 4A). Uninoculated plants produced an average of 19.7 fruits per plant. The total fruit weight per pepper plant was 340 g for plants inoculated earliest (22 June) and ranged from 350 to 606 g for plants inoculated with CMV in the subsequent 4 wk (Fig. 4B). The fruit weight of plants inoculated in the last 3 wk ranged from 915 to 1,165 g, whereas uninoculated plants produced 1,431 g of fruit (Fig. 4B). The average weight per fruit ranged from 39 to 52 g for plants inoculated during the first 3 wk and from 61 to 72 g for plants inoculated in subsequent weeks or left uninoculated (Fig. 4C).

When the pepper fruits were separated into marketable and unmarketable and counted and weighed separately, the effect of the earliress of plant inoculation with CMV on fruit yield and quality became even more striking. Plants inoculated in late June and early July produced an average of 0.5–1.1 marketable fruit, whereas plants inoculated in mid- to

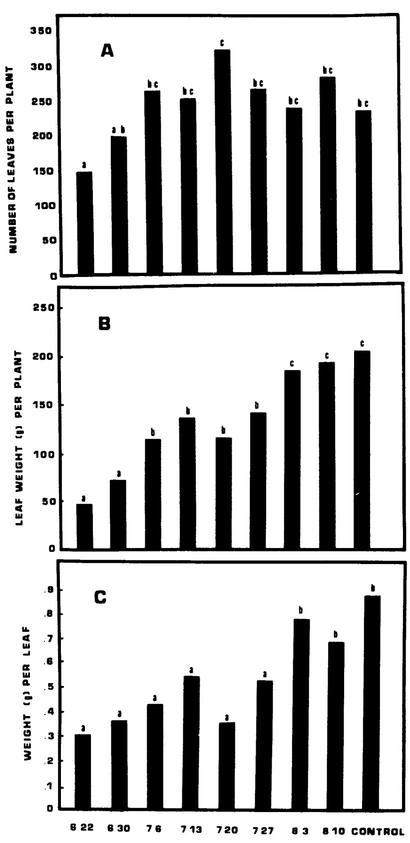

Fig. 3. Average **(A)** number and **(B)** weight of leaves per plant and **(C)** average weight per leaf of pepper plants inoculated with cucumber mosaic virus at different dates and of uninoculated control plants.

late July produced 3.5–4.7 marketable fruit and plants inoculated in early August or left uninoculated produced 6.3–8.3 marketable fruit per plant (Fig. 5A). On the other hand, the average numbers of unmarketable fruit, both those harvested and those present on the plant but still too small to be marketable at the end of the growing season, were quite similar in all treatments (Fig. 5B), although they were smaller in some of the early-inoculated plants than in the late-inoculated and uninoculated plants. The average weight of marketable fruit per plant (Fig. 6A) was significantly lower (55–108 g) for plants inoculated with CMV in late June and early July than in plants inoculated in mid-July (333–380 g). Plants of all treatments, including uninoculated plants, produced considerable numbers of unmarketable fruit (Fig. 6B). Plants inoculated from late June through mid-July produced smaller absolute weights of unmarketable fruit per plant than late-inoculated and uninoculated plants (Fig. 6B); however, plants inoculated in late June and early July had three to five times more unmarketable than marketable fruit, whereas late-inoculated plants had more marketable than unmarketable fruit and uninoculated plants had almost twice as much marketable as unmarketable fruit (Fig. 6A,B).

ELISA of CMV presence and titer in pepper plants. ELISA for CMV, carried out periodically throughout the growing season on the same five or eight plants inoculated at one of three of the inoculation dates (22 June, or 6 or 20 July), showed (Fig. 7) that 1) ELISA detected CMV in all inoculated symptomatic pepper plants if carried out within 2–3 wk of inoculation (14 or 22 July) but not if carried out later; 2) most pepper plants, even when showing severe CMV symptoms from earlier mechanical inoculation, often tested negative for CMV by ELISA beginning with the fourth week after inoculation and continuing for much of the hot summer period (22 July to 21 August); and 3) near the end of the season (21 August, 9 September), most or all plants inoculated early with CMV again tested positive by ELISA, and the detected virus antigen titers were markedly greater than those detected in the same plants during the previous 5 wk (Fig. 7).

DISCUSSION

The severity and frequency of foliar and fruit symptoms on CMV-inoculated pepper plants were drastically greater in plants inoculated while still young and early in the season than in plants inoculated later in the season. Early inoculations generally resulted in severe local and systemic symptoms, whereas successively later inoculations had little or no effect on already existing leaves and caused progressively milder symptoms on new leaves produced subsequently. The data presented here do not distinguish between the effect of plant age and the prevailing environmental conditions (eg, temperature) at and immediately after inoculation on the reaction of the plant to CMV infection. However, June of that year was unusually wet and cool and most of July and all of August were hot and dry. Similarly, the CMV titer as detected by ELISA (Fig. 7) was also greater in plants early in the season (14 July) than in subsequent testing periods, but it increased again near the end of the season (9 September). There are apparent correlations between the young plant age and prevailing low temperatures with higher virus titer and more severe symptoms in the early inoculations on one hand and older plant age and higher prevailing temperatures with lower virus titer and milder symptoms in later inoculations on the other. However, these experiments were not designed to study these relationships.

Most of the parameters measured in this study were drastically affected by the stage of plant growth at inoculation. Plant height, although significantly reduced in early-infected plants (Fig. 1), does not give as good a measure of the magnitude of the adverse effect of early virus infection as does the total top weight of plants inoculated at different times (Fig. 2) because pepper plants grow proportionately more centripetally than in an upward direction. Late-inoculated or uninoculated plants produced four to five times as much top (leaf and stem) weight as did early-inoculated plants and the difference becomes even more striking when one adds together the corresponding top and fruit weights (Figs. 2 and 4B). Decreases in plant height and in top, leaf, and fruit weight

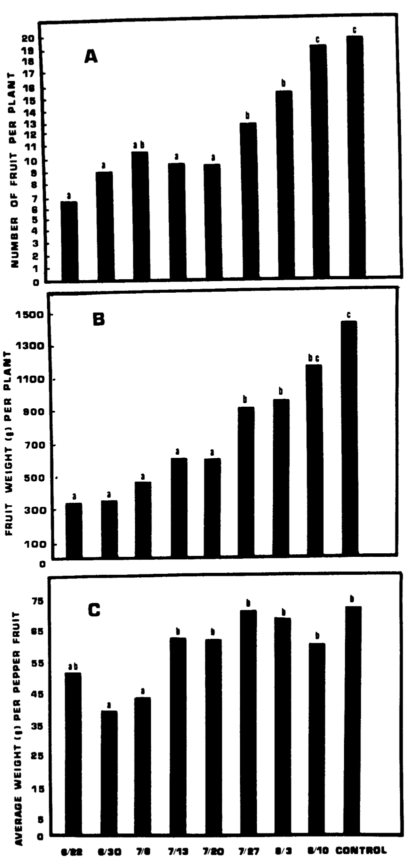

Fig. 4. Average **(A)** number and **(B)** weight of fruit per plant and **(C)** average weight per fruit of pepper plants inoculated with cucumber mosaic virus at different dates and of uninoculated control plants.

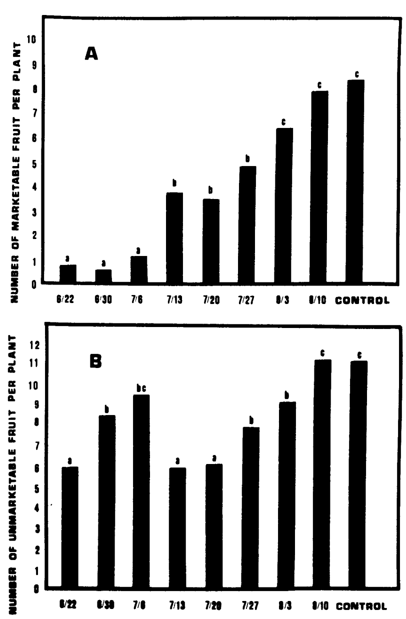

Fig. 5. Average number of **(A)** marketable and **(B)** unmarketable fruit per plant of pepper plants inoculated with cucumber mosaic virus at different dates and of uninoculated control plants.

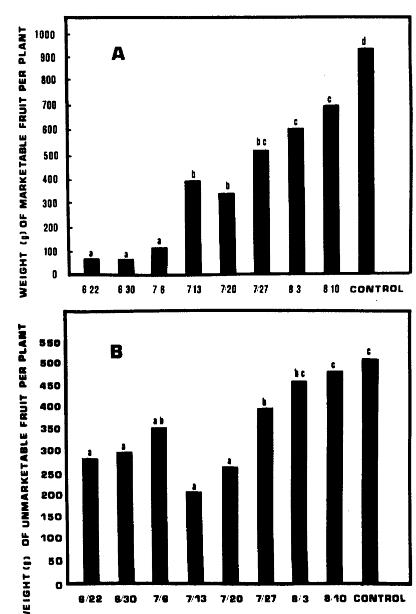

Fig. 6. Average weights of **(A)** marketable and **(B)** unmarketable fruit per plant of pepper plants inoculated with cucumber mosaic virus at different dates and of uninoculated control plants.

per plant are generally proportional to the earliness of plant inoculations, suggesting that it is the stage of plant growth at inoculation that determines the extent of the adverse effect rather than any other factor such as temperature. This may have important implications in efforts to develop virus control measures that delay the introduction of the virus into plants sufficiently to produce a satisfactory yield without requiring exclusion of the virus from the plants for the entire growth season.

Comparison of the number of leaves per plant shows that the two earliest inoculations resulted in somewhat fewer leaves per plant (Fig. 3A), but generally, in spite of the huge differences in top weight, plants inoculated at different times, and even the uninoculated ones, had similar numbers of leaves. Part of the explanation lies in the fact that the leaves of early-inoculated plants are uniformly much smaller than those of late-inoculated and uninoculated ones (Fig. 3C). Also, plants of intermediate and late inoculations, and uninoculated controls, had two types of leaves: typical large external leaves and several clusters of numerous small internal leaves. The combination of these two types of leaves apparently resulted in overall similar numbers of leaves for the various treatments.

Similarly, there were significant differences in the number of fruits per plant (Fig. 4A), but there appeared to be only a small difference in the size (weight) of fruit in the various inoculation treatments (Fig. 4C). The differences, however, become dramatic when one compares fruit weight per plant among inoculation treatments (Fig. 4B): early-inoculated plants produced only one-fourth as much fruit weight as late-inoculated or uninoculated plants. The differences become even more striking when one compares the number or weight of marketable fruit among inoculation treatments (Figs. 5A and 6A): uninoculated or late-inoculated plants produced eight to 10 times more marketable fruit than early-inoculated plants and at least twice as many fruits as plants inoculated in midseason. On the other hand, plants of all treatments produced considerable and similar numbers and weights of unmarketable fruits (Figs. 5B and 6B). It was apparent at harvesttime, however, that although most of the unmarketable fruit of all plants inoculated early were misshapen and had apparently reached their final size, many (possibly most) fruit classified as unmarketable from late-inoculated plants, and all fruit from uninoculated plants, were simply young but normal-looking and were still growing. These probably would have developed eventually into marketable fruit but (for completing the data of these experiments) were harvested too early. The data presented here suggest that control measures that delay infection of plants with CMV by even a few weeks may have a significant effect on the yield and marketability of the fruit produced.

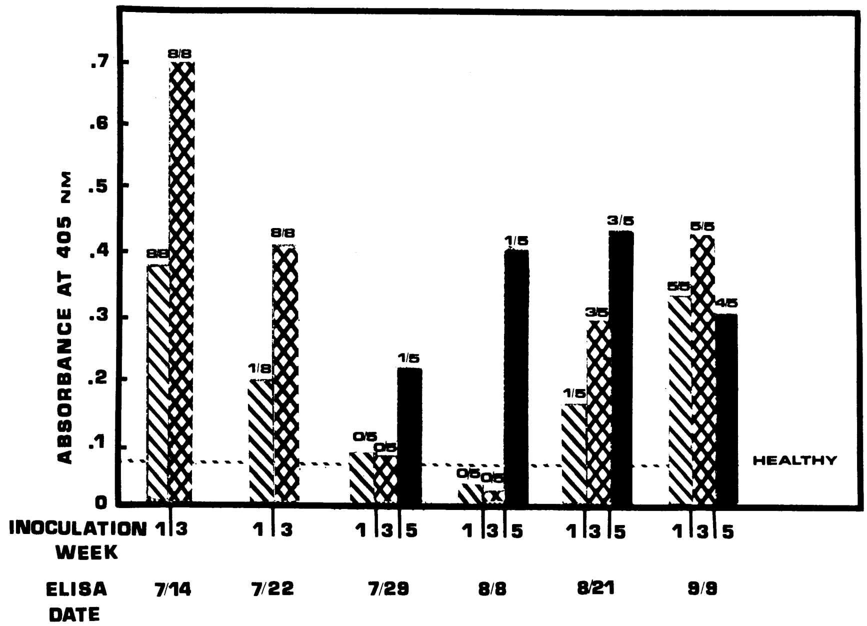

Fig. 7. Presence and approximate relative titers of cucumber mosaic virus (CMV) throughout the summer in pepper plants inoculated with CMV at different dates and tested periodically by enzyme-linked immunosorbent assay (ELISA). Inoculation weeks: 1 = 22 June, 3 = 6 July, and 5 = 20 July. Numbers above bars indicate numbers of plants positive for CMV of those tested by ELISA.

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