Feasibility of Cross-Protection for Control of Tomato Mosaic Virus in Fresh Market Field-Grown Tomatoes

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ABSTRACT

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The feasibility of using cross-protection for control of tomato mosaic virus (TMV) in fresh market tomatoes was tested in field plots with random and Latin-square designs. Tomato seedlings (*Lycopersicon esculentum* Mill. 'Ace 55' and '7718') were inoculated in the cotyledon stage with a mild strain of TMV isolated from commercially grown tomatoes in California and with the MII-16 mutant. Sixteen days later they were reinoculated (challenged) with a severe strain of TMV also isolated from California-grown tomatoes. Cross-protected plants were nearly equal in fruit yield and quality to those inoculated only with the mild strain and to buffer-inoculated controls. The yield of cross-protected plants was 50% higher and there was 10% more extra-large fruit than on plants inoculated only with the severe strain. The effectiveness of protection did not differ between the naturally occurring M strain and the artificial mutant MII-16.

A major problem in the production of fresh market field-grown tomatoes in California is tomato mosaic virus (TMV), a member of the tobamovirus group. Yield losses to TMV have been estimated as high as 38% (9) in a California crop valued in excess of $140 million. Because fresh market tomato plants are transplanted and handled frequently during the growing season and since TMV is readily transmitted mechanically, 100% of the plants in a commercial field commonly become infected.

Attempts to reduce spread by sanitation measures have not proved practical. Sprays of antiviral substances (7) or skim milk delay but do not prevent spread (1). Breeding for field resistance has not yielded commercially acceptable cultivars with stable resistance. Thus, alternative control measures are needed. Control of TMV in glasshouses by cross-protection, ie, deliberate inoculation with a mild form of TMV to prevent superinfection with severe forms, has been successful in a number of countries (1). Under commercial conditions young seedlings are inoculated en masse with a symptomless, nitrous acid mutant designated MII-16 (11) by using a paint sprayer. These successes prompted us to test the feasibility of using cross-protection in field-grown tomatoes in California. The state was surveyed for naturally occurring strains of TMV with the intent of isolating a mild strain that might be used for protection and thereby avoid widespread dissemination of the artificial MII-16 that is presenting problems in Europe (6).

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MATERIALS AND METHODS

TMV strains in California. During the summer of 1977, 10 random samples of field-grown tomato leaves were collected from each of eight California counties. Each sample was homogenized with dilute, neutral phosphate buffer (0.05 M, pH 7.0) with a mortar and pestle. Cotton swabs were used to apply the homogenate to *Nicotiana glutinosa* L. to leaves dusted with corundum. When necrotic local lesions developed, they were removed, homogenized, and inoculated onto individual tomato seedlings (*Lycopersicon esculentum* Mill. 'Ace 55'). Inoculated tomato plants that did not show mosaic symptoms were back assayed on *N. glutinosa.*

Plants and virus isolates. Ace 55 and 7718 tomatoes (seeds from Peto Seed Co., Woodland, CA) were used for cross-protection trials. Ace 55 was used for propagation and purification (5) of virus isolates before their use in protection trials. *N. glutinosa* served as an indicator host for detection of the mild strains in tomato.

Three strains of TMV were used: MII-16, originally developed by Rast (11), and M (mild) isolated from a tomato plant in Merced County were mild strains used for protection; a severe strain designated A, which induced yellow mosaic and stunting, was isolated from a tomato plant in a commercial field in San Diego County and was used as the challenge strain.

Inoculation. Tomato seedlings in the cotyledonary stage of growth in seedling pots in the greenhouse were inoculated en masse by spraying them with a suspension containing 2 mg/ml of purified virus in 0.05 M potassium phosphate buffer, pH 7.0, and 2% w/v 400 mesh corundum. Spraying was accomplished with an "H" type airbrush (Paasche) attached to a bottle of compressed nitrogen gas equipped with a regulator valve. More than 95% of the plants became infected when sprayed at a distance of 10 cm and a pressure of 3.5 kg/cm^2.

Cross-protection trials. Five-hundred and four 7718 seedlings were inoculated with strain M, 252 were inoculated with strain A, and the remaining 252 were inoculated with buffer (control). At 16 days after inoculation, samples were taken at random from 50 plants inoculated with strain M and assayed on *N. glutinosa*; all of the plants were infected. At the same time, 252 plants previously inoculated with M were reinoculated (challenged) with the severe A strain. Two days later all seedlings were placed in a shaded area outside the greenhouse for hardening, and 6 days later they were transplanted into the field in a completely random block design.

Each of the four treatments (M only, M followed by A, A only, and buffer control) was replicated six times. Each replication consisted of 14 plants in each of three rows. Replicates were 220 cm apart, and plants in each row were 45 cm apart.

Plants were staked, tied, weeded, and pruned as is usual in commercial production. To avoid contamination from one plant to another, a new pair of plastic disposable gloves was donned before handling each plant. At termination of the trial, fruit were picked from 12 plants of the middle row of each treatment replicate, sized using U.S. standard size rings (13), and weighed. Data were analyzed by Duncan's multiple range test for mean differences.

The second experiment was done in a field approximately one-half mile from the first. The treatments and numbers of plants were the same as in the first experiment except that MII-16 was the protecting strain, the cultivar was Ace 55, and the experimental design was a Latin square. Fruits were harvested from 20 plants in the middle rows.

RESULTS

Naturally occurring strains. Of 80 samples from commercially grown tomato plants, 39 were infected with TMV as indicated by local-lesion formation on *N. glutinosa*; frequencies of the virus were highest in Stanislaus, Merced, Fresno, Ventura, and San Diego counties. Symptoms in the inoculated tomatoes ranged from mild mosaic to severe stunting and bright yellow mosaic.

The most severe strain, A, was used as the challenge strain in cross-protection trials. One of the inoculated tomato plants showed no symptoms but, when inoculated back to *N. glutinosa*, was found to be infected. Subsequent trials confirmed that the strain multiplied in Ace 55 and 7718 tomatoes but induced no apparent stunting or mosaic symptoms. This mild strain, M, was used as the protecting strain in addition to MII-16 in subsequent protection trials. All of the strains isolated were of the tomato-type since, unlike the tobacco forms, they produced local lesions on *N. tabacum* 'White Burley.'

Cross-protection trials. In the test with 7718 tomatoes in a completely random design, fruit on control plants ripened 12 days earlier than that on plants inoculated with any TMV strain. Average yields for control, M-inoculated, and cross-protected (challenged) plants were 90.1, 85.4, and 85.1 kg per plot, respectively; A-inoculated plants yielded only 33.5 kg per plot (Fig. 1). Differences in yield among the first three treatments were not significant, but the difference in yield between any of those three treatments and A-inoculated plants was highly significant ($P = 0.01$). Yield from A-inoculated tomato plants was reduced more than 50%, whereas M-inoculated and challenged plants yielded only slightly less than the controls. Cross-protected plants were similar in appearance to the unchallenged (M-inoculated) and control plants. In contrast, plants inoculated only with strain A were extremely stunted and showed severe yellow mosaic.

The quality of fruit from cross-protected plants was similar to that of fruit from healthy controls and M-inoculated plants. Fruits were counted and sized as extra small, small, medium, large, and extra large. The percentage of extra-small fruit was significantly higher on A-inoculated plants than on any others; the percentages of extra-large fruit on challenged, M-inoculated, and control plants were significantly higher than on the A-inoculated plants (Table 1). Differences in fruit size among the control, M-inoculated, and challenged treatments were not significant.

Results were similar from the test with Ace 55 tomatoes in the Latin-square design. Plants inoculated with strain A alone were as stunted as those of cultivar 7718. There were no significant differences in yields among control, MII-16-inoculated, and cross-protected plants (147.0, 139.9, 141.8 kg per plot, respectively); however, yields for each of these treatments were significantly higher ($P = 0.01$) than those from A-inoculated plants (77.8 kg per plot, Fig. 2). Fruit size also was very similar among the three treatments, although A-inoculated plants produced 7% fewer extra-large fruit and 10% more extra-small size fruit (Table 1).

Near harvest time, approximately 1% of the protected plants in both experiments exhibited mosaic symptoms.

DISCUSSION

These results extend the findings of Paludan (10), Rast (11), Vanderveken and Coutisse (14), and others on protective control of TMV in tomatoes. Protective inoculation with mild forms of TMV has been effective for control of TMV in glasshouse-grown tomatoes and may also be useful for control in fresh market field-grown tomatoes. Nevertheless, inherent dangers in the use of cross-protection for control have historically suppressed its use (8). The wisdom of artificially disseminating viruses, particularly in the field where containment is not possible, has understandably been questioned. There is the real possibility of the mild strain mutating to a severe form. In recent years severe, necrotic forms of TMV have arisen in commercially grown glasshouse tomatoes that had been inoculated with MII-16 (6).

The use of the naturally occurring M strain may be safer than the use of artificial mutants. Naturally occurring mild viral strains are being used, with no apparent problems, for control of citrus tristeza (12) and apple mosaic (3).

Approximately 1% of the protected plants in each of our experiments developed mosaic symptoms late in the growing season. It is not known whether this was due to those plants not having been infected at the time of protective inoculation, mutation to a severe strain, or the result of a breakdown of protection as reported by others (2,4). Whatever the reason, the small amount of disease had little effect on overall yield and fruit quality. The amount of disease was the same whether the plants were protected with strain M or MII-16.

It has been estimated that TMV can cause 38% loss in yield in commercial fresh market tomatoes (9). In this study, yield losses in unprotected tomatoes exceeded 50%. This uncommonly large

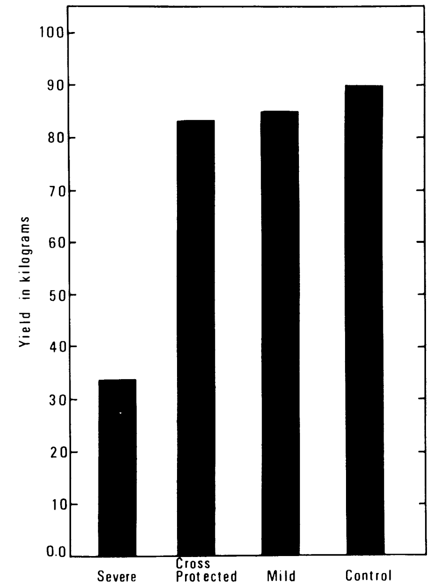

Fig. 1. Effect of protective inoculation with a mild strain of tomato mosaic virus on yield (kg/plot) of 7718 tomato plants that were subsequently inoculated with a severe strain of the virus.

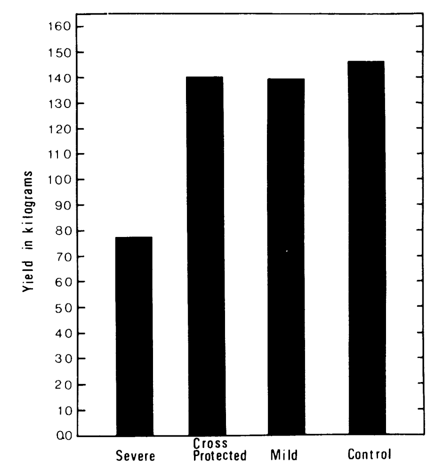

Fig. 2. Effect of protective inoculation with MII-16, a mild strain of tomato mosaic virus, on yield (kg/plot) of Ace 55 tomato plants that were subsequently inoculated with a severe strain of the virus.

Table 1. Number and size of fruit from field-grown tomato plants that were inoculated with a mild strain (M) of tomato mosaic virus before challenge inoculation with a severe strain (A)

Treatment	Total fruit (no.)	Extra large (%)	Extra small (%)
7718 plants			
Control	4,651 y[a]	12.3 y[b]	26.8 y
Inoculated only with M	3,878 y	15.7 y	29.2 y
Inoculated with M and challenged with A	3,725 y	15.0 y	27.9 y
Inoculated only with A	2,012 z	4.4 z	39.2 z
Ace 55 plants			
Control	4,537 y	17.4 y[c]	32.9 y
Inoculated only with MII-16	3,976 y	17.3 y	27.9 y
Inoculated with MII-16 and challenged with A	4,317 y	17.9 y	32.0 y
Inoculated only with A	2,683 z	10.1 z	43.9 z

[a] Values within a column and followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.
[b] Means from 72 plants in six replications.
[c] Means from 80 plants in four replications.

reduction in yield was probably due to the high virulence of strain A and to the fact that all plants were intentionally inoculated at an early stage of growth. Studies are in progress to determine the effectiveness of protective inoculation with the M and MII-16 strains under conditions of natural spread of TMV in commercial operations.

Although the conditions in our experiments were artificial in the sense that all of the challenged plants were intentionally inoculated with a severe isolate, the mild strains conferred a high degree of protection even under extreme infection pressure. That level of disease pressure is unlikely to occur under natural conditions.

ACKNOWLEDGMENT

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