Effectiveness of Cross Protection by a Mild Strain of Zucchini Yellow Mosaic Virus in Cucumber, Melon, and Squash

H. L. WANG, Associate Researcher, Fengshan Tropical Horticultural Experiment Station, Kaohsiung, Taiwan 83017, R.O.C.; D. GONSALVES and R. PROVVIDENTI, Professors, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva 14456; and H. L. LECOQ, Directeur de Recherche, INRA, Station de Pathologie Végétale, B.P. 94, 84140 Montfavet, France

ABSTRACT

Wang, H. L., Gonsalves, D., Provvidenti, R., and Lecoq, H. L. 1991. Effectiveness of cross protection by a mild strain of zucchini yellow mosaic virus in cucumber, melon, and squash. Plant Dis. 75:203-207.

A mild variant of zucchini yellow mosaic virus (ZYMV-WK) that had been selected from a severe strain of ZYMV in France was used for cross-protection tests in cucurbit crops in Taiwan. Under greenhouse conditions, ZYMV-WK provided protection in cucumber, melon, and zucchini squash against four severe strains originally from Connecticut, Florida, France, and Taiwan. Cross protection was more effective against the French strain, from which the mild strain was derived. Two field trials with zucchini squash under moderate and high disease pressures showed that ZYMV-WK provided excellent cross protection against the Taiwan strain of ZYMV. Yields of marketable fruit were 2.2 and 40 times greater than those of control plants under moderate and high disease pressure conditions, respectively.

Additional keyword: ELISA

Zucchini yellow mosaic virus (ZYMV) is one of the most destructive pathogens infecting cucurbits in many areas of the world (4,7,8,13,14). The cultivated species squash (*Cucurbita pepo* L., *C. maxima* Duchesne, *C. moschata* (Duchesne) Duchense ex Poir.), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) are particularly affected by this potyvirus, which is efficiently spread by several aphid species in a nonpersistent manner (7,8). Genetic sources of resistance to ZYMV have been found in cultivated species and their wild relatives (11–13), but only a few ZYMV-resistant cultivars are available. Hence, cross protection with mild strains of ZYMV could be a valuable alternative until more numerous and diverse resistant cultivars become available.

Portion of a thesis submitted by the first author in partial fulfillment of the requirement for the Ph.D. degree, Cornell University.

Accepted for publication 18 July 1990 (submitted for electronic processing).

Large-scale applications of cross protection have been attempted for the control of tobacco mosaic virus in tomato in Europe and Japan (10), citrus tristeza virus in citrus in Brazil (9), and papaya ringspot virus in papaya in Hawaii and Taiwan (3,15,16). The discovery in France of a poorly aphid-transmissible strain of ZYMV, inciting mild symptoms on cucurbits, offered a unique opportunity for cross-protection studies (5). Because of the significance of ZYMV in cucurbits in Taiwan, this research was conducted to examine the possibility of cross protection to control ZYMV under greenhouse and field conditions (4).

MATERIALS AND METHODS

Virus isolates and antisera. The mild strain, ZYMV-WK, used for cross-protection tests was derived from a severe strain affecting melon by single-lesion isolations and is poorly aphid-transmissible (5,6). Four known severe strains were used for challenge inoculations: ZYMV-CT (Connecticut), ZYMV-FL (Florida), ZYMV-FR (France), and ZYMV-TW (Taiwan) (4,7,13,14). These viruses, available from previous studies, were maintained and propagated in *C. pepo* 'Zucchini Elite'. Inoculum of each strain was prepared by triturating infected squash leaves in 0.05 M potassium phosphate buffer, pH 7.4. Plants were mechanically inoculated by rubbing leaves of test plants previously dusted with 400-mesh Carborundum. Viral infection was confirmed by direct enzyme-linked immunosorbent assays (ELISA) (2). Antisera, supplied by C. H. Huang, were used to identify cucumber mosaic virus (CMV), cucumber green mottle mosaic virus (CGMMV), melon vein-banding virus (MVBV), papaya ringspot virus W (PRV-W), and watermelon mosaic virus 2 (WMV-2) from material collected in the field (1). Antisera designated as ZYMV-WK, ZYMV-TW, and ZYMV-TW/CT were used to detect ZYMV. Antiserum ZYMV-TW/CT was derived by cross-absorbing antiserum to ZYMV-TW with ZYMV-CT (*unpublished data*). One milliliter of ZYMV-TW antiserum was mixed with 20 ml of crude extract prepared from 10 g of fresh ZYMV-CT-infected leaf tissue ground in 20 ml of 0.1 M potassium phosphate buffer, pH 7.0. Because ZYMV-TW/CT antiserum reacted only with ZYMV-TW and did not react with ZYMV-WK, -CT, and -FL in ELISA tests (2), it allowed the detection of the natural infection of the severe strain in ZYMV-WK-protected plants. ZYMV antisera were prepared in our laboratory at Cornell University.

Greenhouse tests. Zucchini Elite, Oriental Sweet melon, and Marketer cucumber seedlings at the two-true-leaf stage were mechanically inoculated with crude extracts of ZYMV-WK-infected squash. Fourteen days later, after confirming infection by ELISA, test plants were challenge-inoculated on the three upper fully expanded leaves with crude leaf extracts (1 g/10 ml of 0.05 M potassium phosphate buffer, pH 7.4) of cv. Zucchini Elite infected with ZYMV-CT, -FL, -FR, or -TW. Sets of 10 ZYMV-WK-protected cucumber, melon, and squash plants were used for each of the

Table 1. Cross-protection effectiveness of mild strain of zucchini yellow mosaic virus (ZYMV-WK) against four severe strains in cucumber, melon, and squash plants under greenhouse conditions

Host / Days after challenge inoculation	Control[a]	Number of plants with severe symptoms[b]			
		ZYMV-CT	ZYMV-FL	ZYMV-FR	ZYMV-TW
Cucumber					
0	0	0	0	0	0
10	0	0	0	0	0
20	0	0	0	0	0
30	0	0	2	0	2
40	0	1	2	0	4
50	0	3	3	1	4
Melon					
0	0	0	0	0	0
10	0	0	0	0	0
20	0	0	0	0	0
30	0	0	0	0	0
40	0	0	1	0	1
50	0	0	2	0	2
Squash					
0	0	0	0	0	0
10	0	0	0	0	0
20	0	0	0	0	0
30	0	1	2	0	2
40	0	2	3	0	4
50	0	4	4	1	5

[a] Inoculated with ZYMV-WK only.
[b] Ten plants cross-protected by ZYMV-WK.

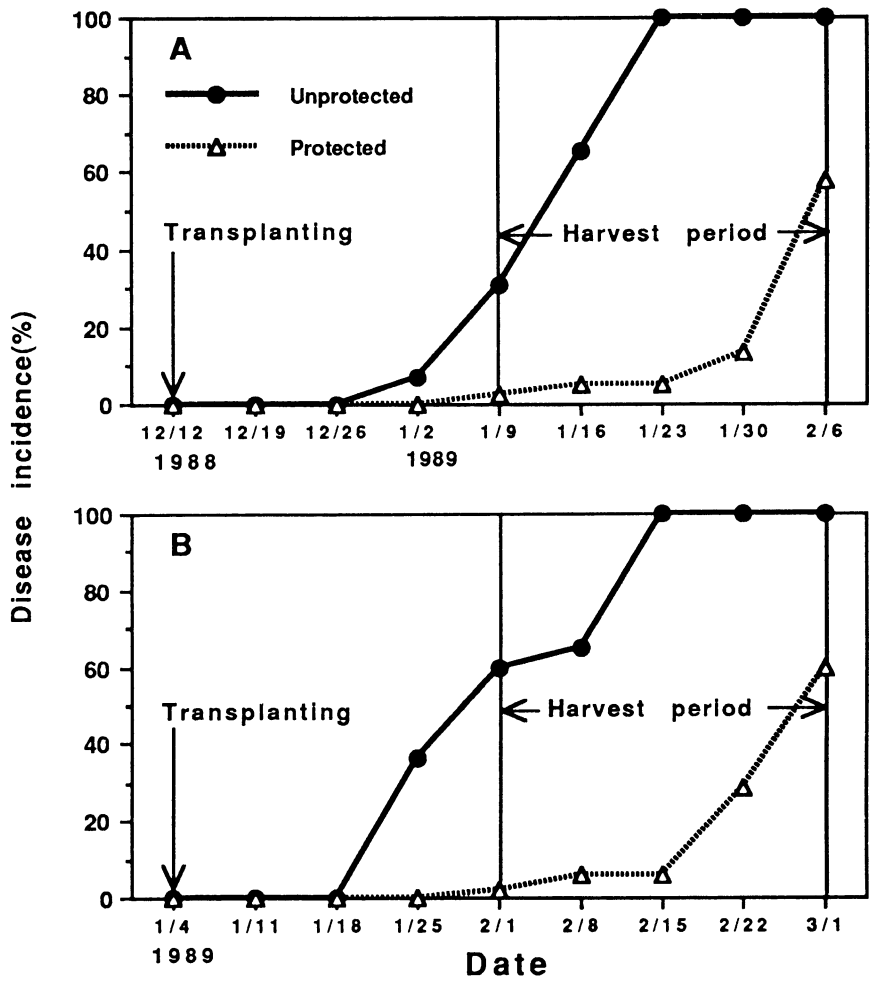

Fig. 1. Cross-protection effectiveness of a mild strain of zucchini yellow mosaic virus (ZYMV-WK) in cv. Zucchini Elite in two fields in Taiwan. Seedlings were inoculated with ZYMV-WK 2 wk before transplantation. Sixty plants were included for each treatment in **(A)** Fengshan A field and **(B)** Fengshan B field.

four severe strains, and one was left as a control. An equal number of healthy plants of each species and of the same age were simultaneously inoculated with each of the four severe strains or mock-inoculated with 0.05 M potassium phosphate buffer, pH 7.4, as healthy controls. Symptom development was recorded every 10 days until 50 days after the challenge inoculation. All of the plants were maintained in a greenhouse in which temperatures ranged from 25 to 35 C.

Field trials. These were conducted at Fengshan with the cultivar Zucchini Elite. Fengshan is situated in southern Taiwan, where ZYMV and other cucurbit viruses are a common occurrence. Zucchini seedlings were initially raised in plastic pots under a screenhouse, where the resulting plants for each of two field tests (Fengshan A and Fengshan B) were randomly divided into groups of 60 plants. The first group was mechanically inoculated with ZYMV-WK on the cotyledons and the first fully expanded leaf; the second group was similarly inoculated with buffer only. Fourteen days later, plants were assayed for viral infection by ELISA and then transplanted to Fengshan A or Fengshan B. Each trial consisted of four blocks of equal size (6 × 6 m), in which plants and rows were spaced 1 m apart. Two blocks (30 plants per block) contained ZYMV-WK-infected plants, and the other two blocks (30 plants per block) contained healthy controls. The protected and unprotected blocks were situated diagonally to each other. The Fengshan A test was conducted from 12 December 1988 to 10 February 1989, and the Fengshan B test was conducted from 4 January 1989 to 1 March 1989. There were many virus-infected cucurbit fields in the vicinity (approximately 500 m away) but not directly adjacent to the Fengshan A test field. To determine the effectiveness of cross protection of ZYMV-WK under high disease pressure, the Fengshan B test field was purposely located directly adjacent to the Fengshan A test field with the assumption that Fengshan A would become severely infected. Cross-protection effectiveness was determined by monitoring the number of plants that showed severe symptoms at weekly intervals. In order to determine which virus(es) caused the severe infection in test plants, leaf specimens were collected from protected and unprotected plants at the time they first showed severe symptoms and were tested for different viruses by direct ELISA (2). At the end of the experiments, plants without severe symptoms were also tested by ELISA. The concentrations of coating and enzyme-conjugated immunoglobulins used were 2 μg/ml and 1/1,000, respectively. ELISA reactions were measured at 405 nm absorbance on an ELISA reader 30 min after the addition of substrate. The reaction was considered postive if it ex-

ceeded the mean plus two standard deviations of the healthy control at the same dilution. Fruits from protected and unprotected plants were harvested every 5 days, weighed, and evaluated for quality (normal or deformed). The beginning and end of the harvest period were 4 wk and 8 wk, respectively, after transplanting.

RESULTS

Greenhouse tests. Cucumber, melon, and squash plants inoculated with ZYMV-WK remained symptomless or exhibited a mild green mottle; conversely, those infected with ZYMV-CT, -FL, -FR, or -TW developed severe foliar symptoms and prominent plant stunting within 20 days after inoculation. Cucumber, melon, and squash plants infected with ZYMV-WK and challenge-inoculated with the four severe strains remained free of severe symptoms for 20 days, regardless of the challenge strain used (Table 1). Fifty days later, when the trials were terminated, a significant number of plants were still fully protected. In cucumber, the percentage of plants that remained cross-protected was 90 for ZYMV-FR, 70 for ZYMV-CT and ZYMV-FL, and 60 for ZYMV-TW. In melon, there was full protection against ZYMV-CT and ZYMV-FR, and only 20% of the plants were severely affected by ZYMV-FL and ZYMV-TW. In squash, effectiveness of cross protection was 90% for ZYMV-FR, 60% for ZYMV-CT and ZYMV-FL, and 50% for ZYMV-TW.

Field trials. The incidences of severe infections of unprotected and protected zucchini plants in two field tests, Fengshan A and B, are illustrated in Figures 1 and 2. Severe disease incidences of unprotected plants among Fengshan A and Fengshan B fields were 30 and 60%, respectively, 4 wk after transplanting, and 100% infection 6 wk after transplanting. However, only 5% of the protected plants in both tests showed severe symptoms 6 wk after transplanting. Eight weeks after transplanting, severe infection reached 57 and 60% in the protected blocks of Fengshan A and B, respectively.

On zucchini, several viruses, such as PRV-W, can cause severe symptoms that resemble those caused by ZYMV, making it necessary to determine which viruses were present in plants at the time severe symptoms appeared. Assay results are presented in Figure 3. As expected, ZYMV-WK was detected in all cross-protected plants but not in unprotected plants. Interestingly, PRV-W was detected in all 34 of the cross-protected plants that showed severe symptoms in the Fengshan A plot. Of these 34 plants, ZYMV-TW was detected in only 14 of them, indicating that actual breakdown caused by ZYMV-TW was, at the most, only 23% (14 of 60). In fact, ZYMV-TW was not detected alone in a single cross-protected plant but occurred in 49 of 60 unprotected plants. ZYMV-TW and PRV-W were detected in 10 of 60 unprotected plants. The results from the Fengshan B test were similar to those of the Fengshan A test. CMV, CGMMV, MVBV, and WMV-2 were not detected in any of the test plants.

Total fruit yields, divided into normal

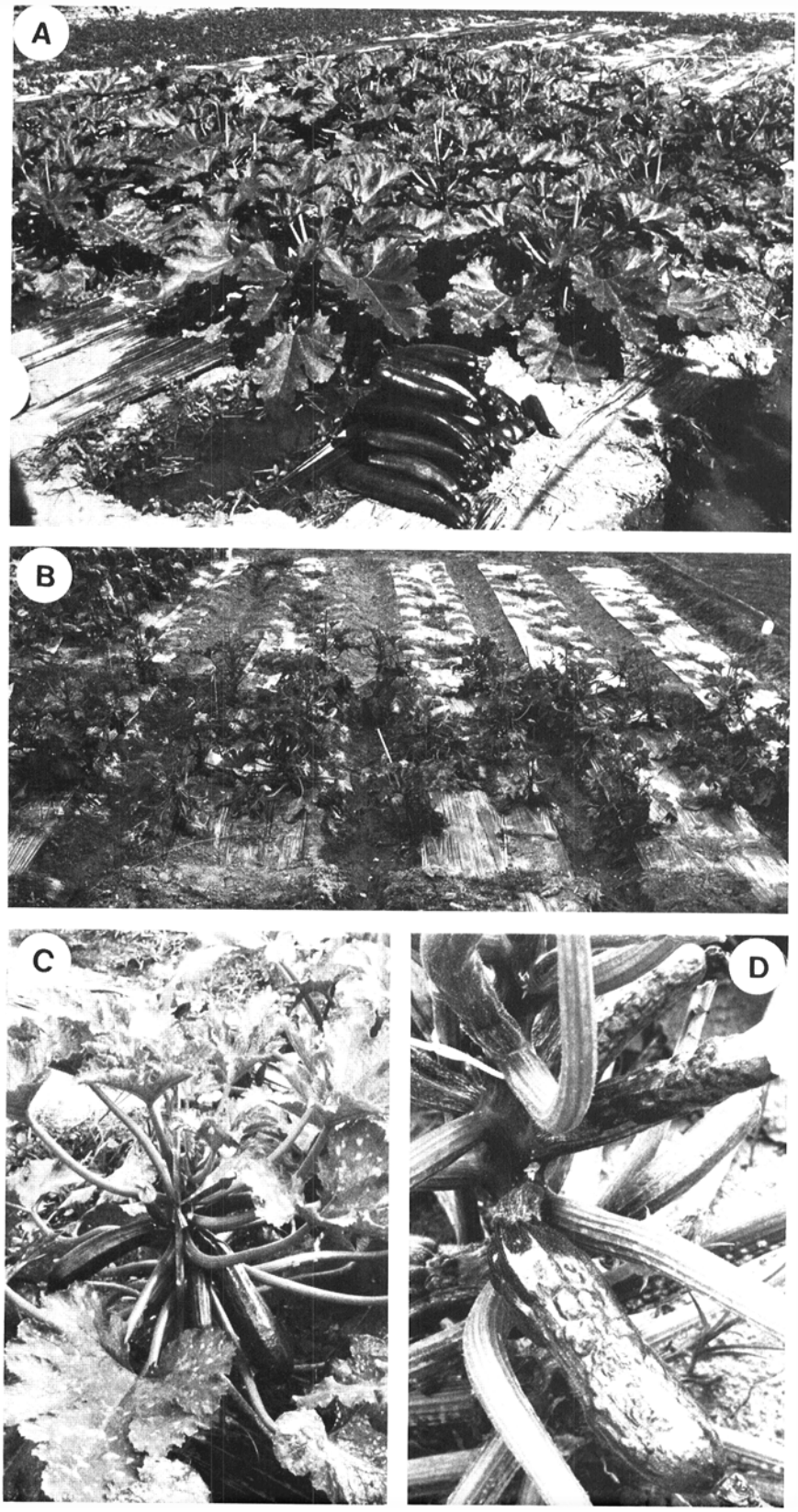

Fig. 2. Field trials of cross-protection effectiveness of a mild strain of zucchini yellow mosaic virus (ZYMV-WK) in Fengshan, Taiwan, during 1988–1989: Zucchini Elite plants in **(A)** protected and **(B)** unprotected plots in Fengshan B field 5 wk after transplantation. **(C)** Protected plant with normal fruit and **(D)** unprotected plant with severely diseased fruit in Fengshan A field.

and deformed, from protected and unprotected blocks in two field tests are shown in Figure 4. In Fengshan A, the protected plants produced a fruit total of 97.1 kg compared with 45.0 kg for the unprotected, whereas in Fengshan B, the yields in protected and unprotected plants were 92.2 and 6.8 kg, respectively.

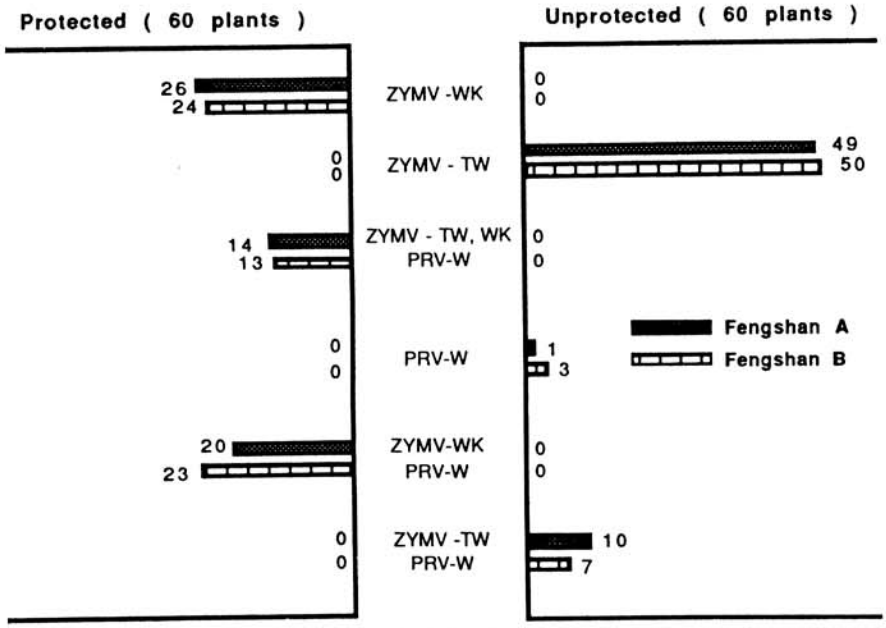

Fig. 3. Naturally occurring viruses in protected and unprotected cv. Zucchini Elite plants in 1989 in Fengshan A and B field tests identified by direct ELISA. ZYMV-TW and ZYMV-WK = zucchini yellow mosaic virus Taiwan strain and mild strain, respectively; PRV-W = papaya ringspot virus watermelon strain. Numbers indicate number of plants infected with a virus or viruses. Only plants infected with ZYMV-TW and/or PRV-W showed severe symptoms.

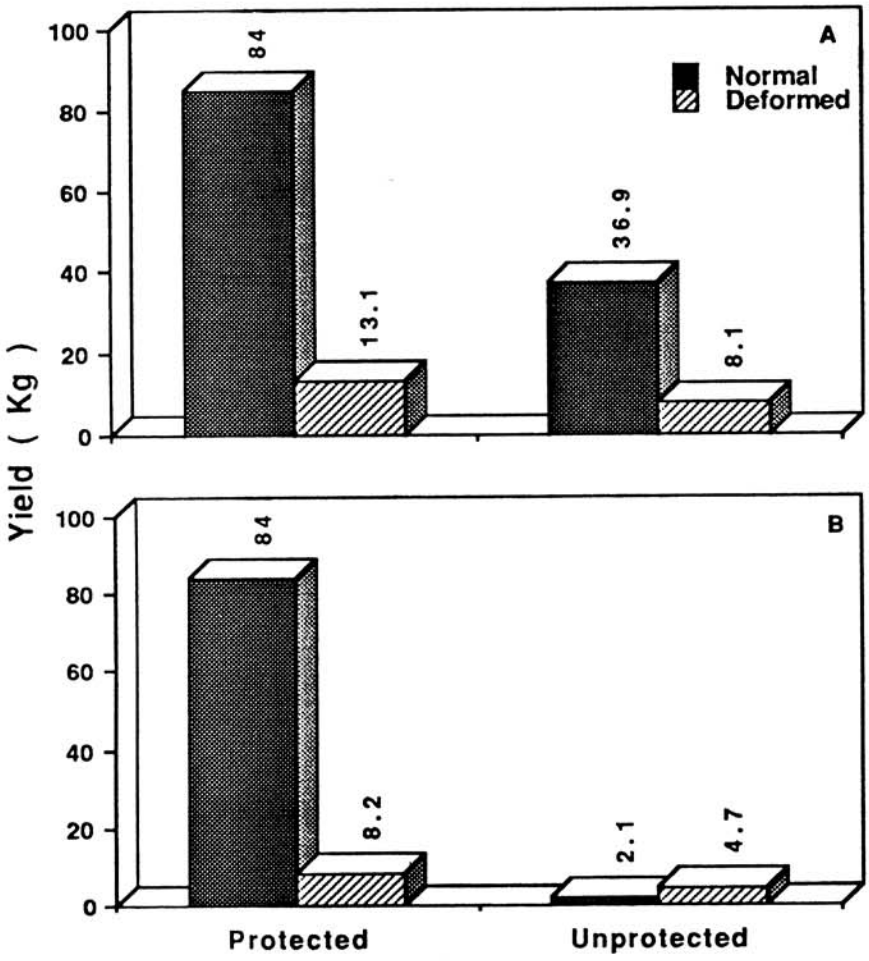

Fig. 4. Comparison of yields and quality of fruit from zucchini plants protected and not protected by a mild strain of ZYMV in **(A)** Fengshan A field and **(B)** Fengshan B field in 1989.

Hence, there was a 116% yield increase attributable to cross protection for Fengshan A and 1,256% for Fengshan B. The percentages of deformed fruits harvested at various times from protected and unprotected plants in Fengshan A and B are shown in Figure 5. In Fengshan A, in the first 10-day harvest period, the percentages of deformed fruits ranged from 0 to 10% for protected and unprotected blocks. However, from 10 days to the end of harvest, the percentages of deformed fruits ranged from 10 to 100% for unprotected blocks, as compared with 10 to 55% for protected blocks. In Fengshan B, no fruit was available from unprotected plants in the first 10 days of harvest. On the other hand, fruits were harvested from protected blocks during this same period, and only 8–10% of them were deformed. From 10 days to the end of harvest, 70–100% of fruits harvested from unprotected plants were deformed compared to 10–45% from the protected plants.

DISCUSSION

Greenhouse and field tests demonstrated the usefulness of a mild strain for the control of severe strains of ZYMV. Under greenhouse conditions, 50 days after challenge inoculations, full protection was observed against mechanical inoculation in melon for ZYMV-CT and ZYMV-FR and 80% for ZYMV-FL and ZYMV-TW. In cucumber and squash, the effectiveness of cross protection against the four severe strains varied from 50 to 90%. In all three crops, cross protection was most effective against ZYMV-FR, from which ZYMV-WK was derived. Conversely, the lowest effectiveness of cross protection was for the local strain, ZYMV-TW. In conclusion, mild strains selected from local severe strains in the area may provide better cross protection.

In field trials, plants showing severe symptoms were not rogued in order to increase infection by secondary spread. As expected, the rate of infection increased with time, but 8 wk after transplanting, when both trials were terminated, there was a remarkable difference in disease incidence in protected (58–60%) and unprotected blocks (100%). The results also revealed that effectiveness of cross protection was good under different levels of disease pressure.

As noted above, data from Figure 1 refer to severe disease incidence but do not accurately reflect cross-protection breakdown. By definition, cross protection refers to the protection afforded by an attenuated strain against severe strains of the same virus (3). Analysis of the data on this basis (Fig. 3) shows that cross-protection breakdown could have ranged from 0 to 23%; the latter percentage refers to plants that had

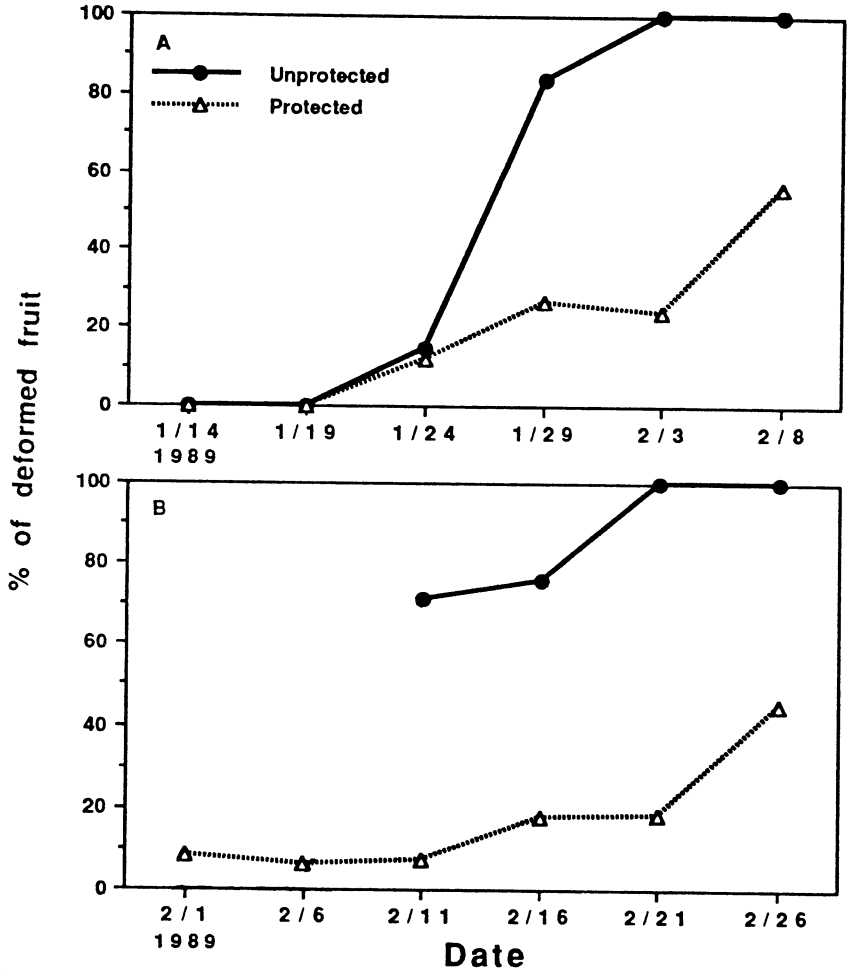

Fig. 5. Percentage of deformed fruit harvested at various time intervals from protected and unprotected zucchini plants in 1989 Fengshan A and B field tests.

PRV-W and ZYMV-TW infections. Conceivably, the severe symptoms could have been caused by PRV-W alone. In fact, none of the severely infected plants had only ZYMV-TW.

From a practical standpoint, severe disease incidence will reduce yield regardless of the virus(es) involved. Thus, under Taiwan conditions, plants that are cross-protected with the mild strain of ZYMV and PRV should have the best protection. It would be of interest to determine if cucurbit plants infected with ZYMV-WK and the mild strain of PRV that is used for papaya (15,16) would have better protection in Taiwan.

The effect of ZYMV-WK cross protection on fruit yield and quality was also notable. Yield increase was 116% for protected plants growing in Fengshan A and 1,256% for those in Fengshan B. Similarly, the fruit quality was far superior in the protected blocks. A higher percentage of deformed fruit was obtained from unprotected plants in Fengshan A and B. The results from the Fengshan B test (high disease pressure) also revealed that the effectiveness of cross protection with ZYMV-WK was even more dramatic when fruit yield and quality were measured.

The results of greenhouse and field tests are encouraging. Furthermore, concurrent tests done in France with ZYMV-WK have given similar results (6). Thus, it now appears that cross protection may be a practical way to control ZYMV. ZYMV is one of major limiting factors for production of cucurbits in Taiwan, hence, large-scale cross-protection trials are contemplated for the future.

LITERATURE CITED

1. Chang, Y. M., Hsiao, C. H., Yang, W. Z., Hseu, S. H., Chao, Y. J., and Huang, C. H. 1987. The occurrence and distribution of five cucurbit viruses on melon and watermelon in Taiwan. J. Agric. Res. China 36:389-397.
2. Clark, M. F., and Adams, A. M. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
3. Gonsalves, D., and Garnsey, S. M. 1989. Cross-protection techniques for control of plant virus diseases in the tropics. Plant Dis. 73:592-596.
4. Hseu, S. H., Wang, H. L., and Huang, C. H. 1985. Identification of zucchini yellow mosaic virus from *Cucumis sativus*. J. Agric. Res. China 34:87-95.
5. Lecoq, H. 1986. A poorly aphid transmissible variant of zucchini yellow mosaic virus. (Abstr.) Phytopathology 76:1063.
6. Lecoq, H., Lemaire, J. M., and Wipf-Scheibel, C. 1991. Control of zucchini yellow mosaic virus in squash by cross protection. Plant Dis. 75:208-211.
7. Lecoq, H., and Pitrat, M. 1984. Strains of zucchini yellow mosaic virus in muskmelon (*Cucumis melo* L.). Phytopathol. Z. 111:165-173.
8. Lisa, V., and Lecoq, H. 1984. Zucchini yellow mosaic virus. No. 282 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
9. Müller, G. W., and Costa, A. S. 1987. Tristeza control in Brazil by preimmunization with mild strains. Proc. Int. Soc. Citric. 3:868-872.
10. Oshima, N. 1975. The control of tomato mosaic disease with attenuated virus of a tomato strain of TMV. Rev. Plant Prot. Res. 8:126-135.
11. Pitrat, M., and Lecoq, H. 1984. Inheritance of zucchini yellow mosaic virus resistance in *Cucumis melo* L. Euphytica 33:57-61.
12. Provvidenti, R. 1987. Inheritance of resistance to a strain of zucchini yellow mosaic virus in cucumber. HortScience 22:102-103.
13. Provvidenti, R., Gonsalves, D., and Humaydan, H. S. 1984. Occurrence of zucchini yellow mosaic virus in cucurbits from Connecticut, New York, Florida, and California. Plant Dis. 68:443-446.
14. Purcifull, D. E., Adlerz, W. C., Simone, G. W., Hiebert, E., and Christie, S. R. 1984. Serological relationships and partial characterization of zucchini yellow mosaic isolated from squash in Florida. Plant Dis. 68:230-233.
15. Wang, H.-L., Yeh, S.-D., Chiu, R.-J., and Gonsalves, D. 1987. Effectiveness of cross-protection by mild mutants of papaya ringspot virus for control of ringspot disease of papaya in Taiwan. Plant Dis. 71:491-497.
16. Yeh, S.-D., Gonsalves, D., Wang, H.-L., Namba, R., and Chiu, R.-J. 1988. Control of papaya ringspot virus by cross protection. Plant Dis. 72:375-380.