Control of Zucchini Yellow Mosaic Virus in Squash by Cross Protection

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ABSTRACT

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A variant of zucchini yellow mosaic virus ZYMV (ZYMV-WK) that induced mild foliar mottle but no fruit symptoms on cucurbits was selected from a poorly aphid-transmissible isolate that induced severe symptoms. This mild variant could not be differentiated from its originating isolate serologically, by its host range, or by aphid transmissibility. ZYMV-WK reduced the marketable fruit production of zucchini squash only slightly when plants were infected at the seedling stage. In field trials conducted in France in 1988 and 1989, ZYMV-WK proved to be very efficient in protecting two cultivars of zucchini squash from severe ZYMV under intense disease pressure. Increase in weight of marketable fruit in cross-protected plots was up to 14.7 times that in unprotected plots.

Zucchini yellow mosaic virus (ZYMV) is an aphid-borne potyvirus, now regarded a major pathogen of cucurbits in most regions of the world where these crops are cultivated (7,11,13,14). In zucchini squash (*Cucurbita pepo* L.), symptoms are particularly prominent and include mosaic and distortion of leaves and fruit. Infected plants generally cease to produce marketable fruits 1 or 2 wk after infection, resulting in significant economic losses, particularly when infection occurs early after planting. ZYMV is highly variable, and variants differing in symptomatology, host range, virulence toward resistance genes, and aphid transmissibility have been reported (3,4,13).

Control measures by cultural practices, including the use of plastic mulches or oil sprays (8,16), may provide temporary protection, but they are not sufficient to prevent significant economical losses in southern France. Resistance genes to ZYMV have not been identified so far in *C. pepo* germ plasm collections. The only available resistance to ZYMV has been reported in the related species *C. moschata* (Duchesne) Duchesne ex Poir. (12,13). Breeding for resistance to ZYMV in squash, therefore, requires interspecific crosses and will probably be a long process. In addition, the possibility that ZYMV may evolve to overcome this resistance should not be underestimated because it has already been observed in muskmelon (4).

In this context, cross protection appeared to be an attractive alternative approach to control ZYMV. This method has been used successfully in France on a large scale to control tobacco mosaic virus in tomato protected crops (10) and is widely used to control several viruses of perennial crops (2). This paper reports experiments to evaluate the potential of a mild variant of ZYMV to protect zucchini squash from severe isolates of this virus in southern France.

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MATERIALS AND METHODS

Virus isolates. A variant (ZYMV-WK) that induces a mild mottle in melon and squash leaves and no symptoms on fruit was selected in 1986 from a poorly aphid-transmissible isolate (E15-PAT) deficient in the helper component function (3). The E15-PAT isolate incited severe symptoms and was derived in 1983 from the highly aphid-transmissible and severe isolate E15-HAT, isolated in southwestern France in 1979 (6). ZYMV-WK was recovered from a melon plant infected by E15-PAT presenting an axillary branch with attenuated symptoms. Extracts from this branch were mechanically inoculated to a melon plant that subsequently exhibited mild symptoms. This subculture was then passed through two successive single local-lesion transfers on *Chenopodium amaranticolor* Coste & Reyn. and *C. quinoa* Willd. before being propagated in cucurbits and kept in dry tissue over calcium chloride. Other ZYMV isolates used in this study were from our collection.

Transmission experiments. Leaves from infected plants were triturated with 0.03 M Na_2HPO_4 containing 0.2% Na-diethyldithiocarbamate (DIECA) (9) (1:4, w/v) with a mortar and pestle; extracted juice was mixed with 400-mesh Carborundum (75 mg/ml) and activated charcoal (75 mg/ml) before being rub-inoculated on plants at the cotyledonary or first-true-leaf stage. Aphid transmission tests were conducted as previously described (6).

Serological assays. Antisera against papaya ringspot virus type W (PRSV-W), watermelon mosaic virus 2 (WMV-2), ZYMV, IgGs, and alkaline phosphatase-conjugated IgGs were prepared in our laboratory according to standard procedures (1); antisera against cucumber mosaic virus (CMV) were a gift from H. Lot and J. C. Devergne, and antiserum against WMV-Mor was a gift from D. E. Purcifull. Serological assays were done either by the SDS immunodiffusion technique (15) or the double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) (1). Extracts from field samples were prepared with a roll press. A purification procedure described previously (5) was used to obtain purified virus preparations.

Field experiments. An experiment was done to evaluate the effect of ZYMV-WK alone on the fruit production of zucchini squash F_1 hybrid Diamant. Seedlings were raised in plastic pots in an insect-proof greenhouse. Half of these plants were mechanically inoculated with ZYMV-WK at the cotyledonary stage. One week later, two blocks of 16 plants were planted next to two blocks of uninoculated controls. To avoid contamination by other aphid-borne viruses, this experiment was done in early spring, before the major aphid flights start, under a plastic tunnel and with regular aphicide treatments. Fruit was collected every 1 or 2 days for 5 wk, rated for commercial value, and weighed.

Taking advantage of severe natural outbreaks of ZYMV in summer 1988 and 1989 in southeastern France, we designed experiments to evaluate the field efficacy of the protection conferred by ZYMV-WK. Plants were prepared as described previously. Two blocks of 40 zucchini squash F_1 hybrid Diamant plants were planted 1 wk (1989 experiment) or 2 wk (1988 experiment) after mechanical inoculation with ZYMV-WK, next to two blocks of uninoculated plants. Blocks consisted of four lines spaced 2 m apart, with plants spaced 1 m along the line. In 1989, a similar experiment was done with a second F_1 hybrid, Supremo. Fruit was collected and evaluated as previously. Every 2 wk during the production period in both trials, each Diamant plant was individually tested for the presence of CMV, WMV-2, and ZYMV by DAS ELISA. Statistical analysis was done with the analysis of variance.

RESULTS

Characterization of ZYMV-WK. After mechanical inoculation, ZYMV-WK caused systemic infections on the same hosts as E15-HAT and as E15-PAT, from which ZYMV-WK was derived. These included melon (*Cucumis melo* L. 'Védrantais' and 'Doublon'), cucumber (*C. sativus* L. 'Marketer'), zucchini squash F_1 hybrid Diamant, watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), and *Ranunculus sardous* Crantz. On all of these hosts, leaf symptoms were mild and varied from a discrete veinbanding to a mild mottle. Flower production and vegetative growth were not affected, and fruit did not develop symptoms, in contrast to infections by severe ZYMV isolates (Fig. 1). In addition, ZYMV-WK induced chlorotic local lesions on *C. amaranticolor*, while E15-HAT and E15-PAT induced well-defined necrotic local lesions on this host. ZYMV-WK did not systemically infect melon line PI 414723 with the *Zym* resistance gene to ZYMV (4).

When crude plant extracts or purified virus preparations of ZYMV-WK, E15-PAT, or E15-HAT were tested in SDS immunodiffusion against an antiserum to E15-HAT, they all produced precipitation lines that fused without spurs. When the same antiserum was cross-absorbed with ZYMV-WK, no more reaction was observed with E15-PAT or E15-HAT, indicating that ZYMV-WK is serologically similar, if not identical, to its originating isolates.

When dilutions of purified preparations of ZYMV-WK and E15-HAT were tested in DAS ELISA, they resulted in similar titration curves. ZYMV-WK did not react in SDS immunodiffusion with antisera to PRSV-W or WMV-Mor. A weak precipitation line that spurred with the homologous reaction line was observed with some WMV-2 antisera but not with others, as has already been noted for E15-HAT (6).

Aphid transmissibility of ZYMV-WK was estimated with *Aphis gossypii* Glover and *Myzus persicae* (Sulzer): ZYMV-WK was as poorly aphid transmissible as E15-PAT (3), from which it was derived (Table 1).

Squash plants grown from seeds collected from fruit produced on ZYMV-WK-infected plants were tested singly for ZYMV by DAS ELISA 4 wk after planting. None of 308 plants tested were found to be infected.

In a preliminary attempt to evaluate the effectiveness of ZYMV-WK for protecting cucurbits against severe isolates of ZYMV, groups of 10 melon plants were inoculated with ZYMV-WK and then challenge-inoculated mechanically 1 or 2 wk later with severe ZYMV isolates from different pathotypes or geographical origins (E15-HAT and four other isolates from Algeria, Jordan, Spain, and southeastern France). None of the cross-protected plants developed severe symptoms 4 wk after inoculation, while all of the control plants developed severe stunting and leaf deformations. In contrast, no protection was observed when WMV-2 was used as a challenge inoculum.

Effect of ZYMV-WK on zucchini squash production. The effect of ZYMV-WK on yield in the absence of other viruses was ascertained in a trial conducted under a plastic tunnel with regular aphicide treatments in early spring before the major aphid flights. No infection by CMV, WMV-2, or severe ZYMV was detected throughout the experiment. The number of fruit per plant was not reduced significantly by ZYMV-WK, but the weight of fruit per plant was reduced by 11% (Fig. 2A, Table 2). The percentage of unmarketable fruit was low in both treatments (less than 1%) and was generally attributable to physiological improper fruit-set.

Effectiveness of ZYMV-WK to protect zucchini squash in the field. The comparison of either the mean number of fruit or the mean fruit weight per plant indicates significantly higher marketable production regardless of the year or the cultivar in cross-protected plants (Fig. 2B, Table 3). Increase in marketable production was up to 14.7 times that of unprotected plants in Diamant in 1988.

In the Diamant control plots in both the 1988 and 1989 trials, ZYMV spread very rapidly and more than 80% of the plants were naturally infected 2 and 3 wk after planting. All ZYMV-infected plants, except one in 1988 and two in 1989, presented severe symptoms, suggesting a limited spread of the mild isolate, possibly from the neighboring cross-protected plots. WMV-2 spread slightly slower than ZYMV, while CMV did not reach 50% infection by the end of the experiments (Fig. 3).

Patterns of spread for WMV-2 were similar in control and cross-protected plots (Fig. 3). CMV also spread similarly in control and cross-protected plots, but in both years, final infection levels were slightly lower in cross-protected plots than in the control.

In the 1988 experiment, only cross-protected plants that were coinfected with CMV developed severe leaf and fruit symptoms typical of CMV infection. No severe symptoms were noticed on plants infected by ZYMV-WK alone or in mixed infection with WMV-2. WMV-2 alone generally does not induce severe symptoms on leaves or fruit of zucchini squash.

In the 1989 trial, a few cross-protected plants (15%) presented pronounced symptoms by the end of the experiment and were found infected by only ZYMV and WMV-2. A severe isolate of ZYMV could be recovered from such plants, indicating a breakdown of the cross protection in these individual plants.

Acquisition of ZYMV-WK by aphids from field samples. To further investigate the possible spread of ZYMV-WK by aphids, leaf samples were collected from cross-protected or control plants in the field and used as sources for virus acquisition experiments with *M. persicae*. The ZYMV-WK isolate was not transmitted from cross-protected plants infected only with this virus, but it was transmitted at a low frequency from

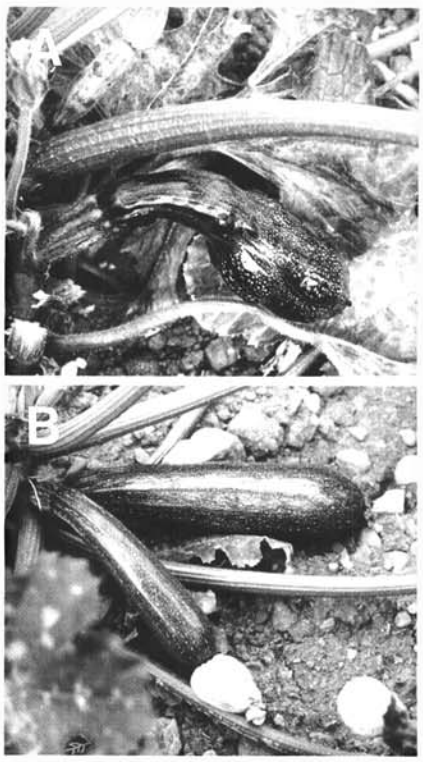

Fig. 1. Zucchini squash fruit produced on F_1 hybrid Diamant plants infected by **(A)** a severe ZYMV isolate or **(B)** the mild variant ZYMV-WK.

Table 1. Comparative aphid transmissibility of three zucchini yellow mosaic virus (ZYMV) isolates

Vector	Isolate		
	E15-HAT	E15-PAT	ZYMV-WK
Myzus persicae	18/30[a]	0/30	0/30
Aphis gossypii	24/30	0/30	1/30

[a] Combined results of three independent experiments in which aphids were allowed a 1-min acquisition period before being transferred by groups of two to test plants for a 2-hr transmission period. Results are expressed as number of infected plants divided by number of inoculated plants.

other cross-protected plants coinfected by WMV-2 (Table 4).

DISCUSSION

The variant ZYMV-WK caused mild symptoms both in cucurbits (melon, cucumber, squash, or watermelon) and in noncucurbits (*C. amaranticolor*) and, as with the isolate E15-PAT from which it derives, was poorly aphid transmissible. These two properties made ZYMV-WK an attractive candidate for cross-protection experiments to protect zucchini squash against ZYMV, a virus for which no satisfactory control measure is currently available.

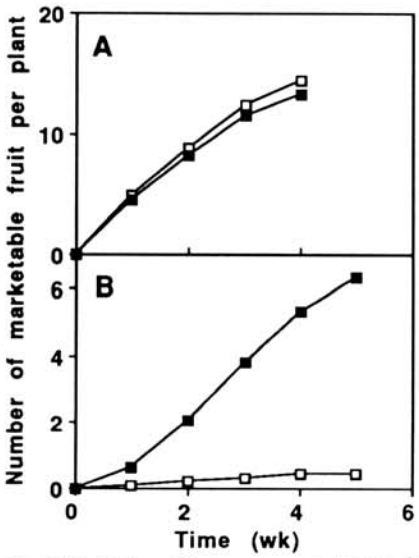

Fig. 2. Evolution of the mean marketable fruit production per plant of zucchini squash F_1 hybrid Diamant inoculated (■) or not inoculated (□) by ZYMV-WK either **(A)** under protected conditions in the absence of other virus (spring 1989) or **(B)** in the field under severe ZYMV epidemic conditions (summer 1989).

ZYMV-WK alone only slightly affected the production of zucchini squash, and in the field, under severe ZYMV epidemic conditions, it provided effective protection and a significant yield increase. Late in the season in 1989 (but not in 1988), some Diamant plants (15%) developed more severe symptoms on leaves and moderate symptoms on fruit that could not be associated with a virus other than ZYMV. It is not yet known whether the apparent partial breakdown of the cross protection observed in 1989 and not in 1988 is attributable to differences in the prevalent severe isolates encountered in the field or to the time interval between inoculation by the mild isolate and planting dates (2 wk in 1988 and 1 wk in 1989). This point will be further investigated. Similar symptoms were also observed in the F_1 hybrid Supremo plot but in fewer number (less than 5%). This suggests a possible role of the host genotype in the field efficacy of cross protection.

Although ZYMV-WK is poorly aphid transmissible, it was transmitted to a significant extent from plants grown in the field and coinfected by WMV-2. This indicates that under certain circumstances, ZYMV-WK may be disseminated by the aphids, and this was probably the case for the three control plants found infected by this isolate in 1988 and 1989 Diamant experiments. ZYMV-WK is derived from E15-PAT, which has been shown to be deficient in the helper component function, because this isolate could be transmitted by aphids if a functional helper component was provided (3 and H. Lecoq, *unpublished*). Therefore, it is assumed that the WMV-2 helper component may assist ZYMV-WK transmission from coinfected plants, as it was demonstrated that the WMV-2 helper component was efficient in mediating ZYMV transmission (5). Nevertheless, our data suggest that the dissemination of ZYMV-WK remains very limited and will depend upon the presence of another potyvirus to provide an efficient helper component.

CMV and WMV-2 disease progression curves were very similar in control and cross-protected plots, suggesting that an

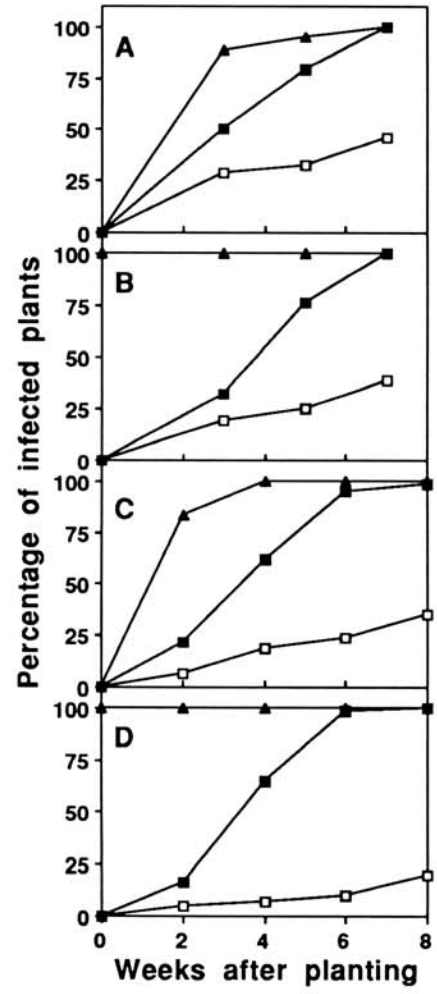

Fig. 3. Progress of virus infections in zucchini squash F_1 hybrid Diamant: **(A)** control plants in 1988, **(B)** cross-protected plants in 1988, **(C)** control plants in 1989, and **(D)** cross-protected plants in 1989. Plants in cross-protected plots were inoculated by the mild variant ZYMV-WK 2 and 1 wk before being transplanted to the field in 1988 and 1989, respectively. All plants were individually tested for CMV (□), ZYMV (▲), and WMV-2 (■) by DAS ELISA.

Table 2. Comparison of marketable and total production of zucchini squash plants inoculated or not inoculated with a mild isolate of ZYMV under protected conditions

	Mean yield per plant[x]			
	Number of fruit		Weight (kg)	
Treatment	Marketable	Total	Marketable	Total
Healthy control[y]	15.0 a[z]	15.1 a	2.94 a	2.95 a
ZYMV-WK infected[y]	14.0 a	14.2 a	2.59 b	2.62 b

[x] Mean values obtained for 32 plants per treatment.
[y] No CMV, WMV-2, or severe ZYMV was detected in these plants during the experiment.
[z] Data in the same column followed by the same letter are not significantly different ($P = 0.05$).

Table 3. Comparison of marketable and total production of zucchini squash plants cross-protected or not, in the field under severe ZYMV epidemic conditions

	Mean yield per plant[y]			
	Number of fruit		Weight (kg)	
Trial	Marketable	Total	Marketable	Total
Diamant, 1988				
Control	0.31 a[z]	1.67 a	0.09 a	0.41 a
Cross-protected	4.78 b	5.98 b	1.33 b	1.61 b
Diamant, 1989				
Control	0.49 a	1.02 a	0.11 a	0.18 a
Cross-protected	6.12 b	7.21 b	1.18 b	1.33 b
Supremo, 1989				
Control	1.32 a	3.89 a	0.26 a	0.61 a
Cross-protected	7.85 b	8.80 b	1.58 b	1.72 b

[y] Mean values obtained for 80 plants per treatment.
[z] Data in the same column and for a same experiment followed by the same letter are not significantly different ($P = 0.05$).

Table 4. Transmission by *Myzus persicae* of zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus 2 (WMV-2) from zucchini squash plants grown in the field

Source plant[a]	Virus in source leaf	Plants tested (no.)	Virus transmitted (%)[b]	
			ZYMV[c]	WMV-2
Control	ZYMV	3	83.3[d]	0.0
	ZYMV + WMV-2	12	44.0[d]	15.0
Cross-protected	ZYMV	3	0.0	0.0
	ZYMV + WMV-2	12	12.0[e]	31.6

[a]Control plants were naturally infected by severe ZYMV isolates, whereas cross-protected plants were mechanically inoculated by the mild variant ZYMV-WK 1 wk before planting. WMV-2 infections were natural.
[b]Aphids were allowed a 1-min acquisition period before being transferred by groups of two to 10 zucchini squash test plants for each source plant tested.
[c]Identity of the viruses transmitted was established by DAS-ELISA.
[d]Test plants with severe ZYMV symptoms.
[e]Test plants with mild ZYMV symptoms.

infection by ZYMV-WK did not specifically interfere with the spread of these viruses.

Preliminary results indicate that ZYMV-WK is not seed transmitted. If this is confirmed with a larger number of seedlings, ZYMV-WK may be very useful to plant breeders who encounter difficulties in breeding programs or in seed production because of the severity of ZYMV epidemics.

ZYMV-WK proved to be effective in protecting zucchini squash against severe ZYMV infections under field conditions in southern France. Promising results recently obtained by Wang et al (17) in Taiwan with the same isolate suggest that it might be useful to protect cucurbits against severe ZYMV isolates under different ecological conditions. Further investigations are now needed to demonstrate the feasibility of using ZYMV-WK to control ZYMV on a large-scale basis.

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