

## Evaluation of Induced Mutants of Papaya Ringspot Virus for Control by Cross Protection

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We wish to thank D. K. Hummer and E. D. Williams for technical assistance. This study was supported by the U.S. Department of Agriculture under Agreement 58-9AHZ-1-615.

Accepted for publication 6 April 1984.

### ABSTRACT

Yeh, S.-D., and Gonsalves, D. 1984. Evaluation of induced mutants of papaya ringspot virus for control by cross protection. *Phytopathology* 74:1086-1091.

Efforts to select naturally occurring mild strains of papaya ringspot virus (PRV) by field collection or single-lesion isolation from natural virion populations were not successful. In an attempt to induce mild strains, crude sap from PRV-infected squash was treated with nitrous acid (pH 6.0) and used to inoculate *Chenopodium quinoa*, a local-lesion host. Two mutants, designated PRV HA 5-1 and PRV HA 6-1, that produced no symptoms in papaya were obtained from 663 single-lesion isolations. Papaya seedlings

*Additional key words:* biological control, ELISA, nitrous acid mutants, superinfection.

inoculated with these mild mutants remained symptomless or showed diffuse mottling with no reduction in plant size. Under greenhouse conditions, protection was observed when PRV HA 5-1 was used to protect papaya against different mechanical challenge inoculations with a severe strain. The results indicate that the symptomless mutant could be used as a protectant for control of PRV.

Papaya ringspot virus (PRV) causes one of the most destructive diseases of papaya (*Carica papaya* L.) (7), a fruit tree that is grown throughout the tropics and subtropics. PRV limits papaya production in some areas of Hawaii (17,20), Florida (4,8), Caribbean countries (1,17,35), South America (16,17), Africa (17,18), India (2,34), and the Far East (37). PRV is transmitted by aphids in a nonpersistent manner and has been placed in the potyvirus group (10,15). Host range of PRV is limited to genera in the dicotyledonous families Caricaceae, Chenopodiaceae, and Cucurbitaceae (29). The virus is serologically identical to watermelon mosaic virus 1 (WMV-1) (14,30,38), which is of economic importance wherever cucurbits are grown (36).

Several unsuccessful attempts have been made to develop effective control measures for PRV. Although tolerant selections of papaya have been described (5,6), resistance to PRV does not occur within *C. papaya* (5,6,8,37). Some species of *Carica* are resistant to PRV (5,6,8,22). Unfortunately, these species are incompatible with *C. papaya* and conventional interspecific hybridization has been unsuccessful (22). A diligent roguing program has been practiced successfully in Hawaii to suppress the spread of PRV in certain areas of the state (25). However, roguing is not a permanent solution for other areas that do not have geographic isolation and where the disease has become endemic. Thus, the unavailability of PRV-resistant papaya cultivars and the restricted host range of PRV make cross protection an attractive method of controlling this virus.

Cross protection, first found by McKinney in 1929 (21) with tobacco mosaic virus (TMV), describes the phenomenon in which plants systemically infected with one strain of a virus are protected from the effects of infection by a second related strain of the same virus. However, wide-scale adoption of this technique for control of TMV did not occur until Rast (31) produced a symptomless mutant (MII-16) from a common tomato strain of TMV by using a nitrous acid mutagenic treatment (13). The symptomless mutant has been manufactured commercially (32) and has been applied to a high proportion of glasshouse-grown tomato crops in the

Netherlands and the United Kingdom since 1970 (11,12,32). Successful control of tomato mosaic disease with an attenuated mutant of TMV was also reported in Japan (26). Cross protection is also used on a large scale to control citrus tristeza virus (CTV) (9,23,27). In Brazil, the number of protected sweet orange trees exceeded 8 million in 1980, and no breakdown in protection has been reported (9).

The purpose of this study was to search for and to induce a mild strain (or strains) of PRV that might be used for control by cross protection.

### MATERIALS AND METHODS

**Field collections.** The first attempt to obtain mild strains of PRV was made by selecting naturally occurring isolates from papaya trees with the mildest symptoms in a heavily infected papaya orchard on the island of Hawaii. The 116 isolates collected were mechanically inoculated to *C. papaya* 'Kapoho Solo' and *Cucumis metuliferus* (Naud.) Mey. (Acc. 2459) (28) in the greenhouse. Isolates were evaluated by observing symptom development, and a modified double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) (3,14) was used to check for the presence of PRV. One mild strain, Su-mm (kindly provided by H. J. Su, National Taiwan University, Republic of China) which was selected from 230 PRV isolates in Taiwan (19), was also tested in the same way.

**Single-lesion isolation from natural populations.** Crude sap of *C. metuliferus* infected with one of four isolates of PRV (HA, HB, F-340, and Su-mm) (14,19) was used to inoculate *Chenopodium quinoa* Willd., a local-lesion host. Single local lesions that developed 20–30 days after inoculation were cut out with a sterile razor blade and placed on a glass spatula with a drop (~15  $\mu$ l) of 0.01 M sodium phosphate buffer, pH 7.0. The lesion was then crushed between two glass spatulas and mechanically inoculated to papaya seedlings at the three-to-four leaf stage. Inoculated plants were rinsed immediately with distilled water and kept in the greenhouse for further investigation. Isolates were evaluated by symptom development and by the ELISA test (3,14).

**Artificial induction of PRV mutants from PRV HA.** Nitrous acid, a powerful chemical mutagen for plant RNA viruses (13,24,33), was used to induce mutants from PRV HA, a severe strain of PRV. Leaf tissue of zucchini squash, *Cucurbita pepo* L. 'President,' infected 3–4 wk previously with PRV, was ground in distilled water (1 g/ml). After it had been strained through

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cheesecloth, the crude sap was centrifuged in a Sorvall SS34 rotor at 8,000 rpm for 10 min. Aliquots of the supernatant were treated with different concentrations of sodium nitrite (0.1–0.5 M) or sodium acetate (0.05–0.125 M), at different pH levels (pH 4.0–6.0, adjusted with acetic acid). The mixtures were incubated at 20 C for 30 min. The reaction was stopped by adding an equal volume of 0.1 M potassium phosphate buffer (pH 7.0) and the mixtures were immediately inoculated to the local-lesion host *C. quinoa*. In the treatment selected for further mutation studies, aliquots of the supernatant were incubated in 0.4 M sodium nitrite and 0.1 M sodium acetate (adjusted with acetic acid to pH 6.0) at 20 C for 30 min. Single local lesions on *C. quinoa* were transferred 20–30 days later to papaya seedlings at the three-to-four-true-leaves stage as described previously. The plants were maintained in the greenhouse and observed for symptom development for 1–2 mo. Papaya plants that did not show visible symptoms or developed only mild symptoms were tested by the ELISA method (3,14) for presence of the virus.

**Evaluation of a symptomless mutant induced by nitrous acid treatment.** Two symptomless mutants, PRV HA 5-1 and PRV HA 6-1, were obtained from artificial induction (see the results section). PRV HA 5-1 was subsequently used to determine whether this mutant would effectively protect papaya plants against challenge inoculations with a severe strain of PRV.

All cross-protection tests were conducted in the greenhouse from October 1982 to April 1983. Papaya seedlings at the five- to six-leaf stage were mechanically inoculated with PRV HA 5-1 prepared from infected tissue of *C. metuliferus* (10 ml of 0.01 M potassium phosphate buffer, pH 7.0, per gram of tissue). A modified double-antibody sandwich ELISA procedure (3,14) was used to confirm the infection by the mutant. Challenge inoculation was performed mechanically with the severe parent strain PRV HA. The challenge inocula were extracted from PRV-infected *C. metuliferus* (10 ml of 0.01 M potassium phosphate buffer, pH 7.0, per gram of tissue) and the infectivities were determined by inoculating *C. quinoa*.

TABLE 1. Infectivity of papaya ringspot virus after treatment with various combinations of acetate buffer and sodium nitrite<sup>a</sup>

Molarity of:			Local lesions (no.) <sup>b</sup>	Survival rate (%)
Sodium acetate	Sodium nitrite	pH		
0.000	0.0	6.8 <sup>c</sup>	295	100
0.075	0.0	6.0	233	79
0.075	0.3	6.0	26	9
0.100	0.0	6.0	208	71
0.100	0.4	6.0	6	2

<sup>a</sup> Incubated at 20 C for 30 min.

<sup>b</sup> Mean of 20 leaves on four plants of *Chenopodium quinoa*.

<sup>c</sup> Untreated control (in distilled water only).

TABLE 2. Properties of single-lesion isolates from populations of papaya ringspot virus treated with nitrous acid

Trials <sup>a</sup>	Lesions transferred (no.)	Papaya plants (no.) showing:			
		Severe symptoms	Mild symptoms	Symptomless but ELISA positive	Symptomless and ELISA negative
1	213	92	11	0	110
2	100	42	5	0	53
3	100	12	0	0	88
4	50	8	0	0	42
5	50	4	0	1	45
6	120	64	3	1	52
7	30	4	0	0	26
Total	663	226	19 <sup>b</sup>	2 <sup>c</sup>	416

<sup>a</sup> PRV HA used in trials 1-6, PRV HB used in trial 7.

<sup>b</sup> Subinoculations from all these to papaya showed severe symptoms.

<sup>c</sup> Two mutants, designated PRV HA 5-1 and PRV HA 6-1, caused symptomless infection in papaya and *Cucumis metuliferus* (Acc. 2459).

Protection or superinfection was judged from the appearance of symptoms in test plants kept in the greenhouse at least 3–4 mo after challenge inoculation.

To determine the effect of time of challenge inoculation on cross protection, papaya seedlings preinfected with PRV HA 5-1 were mechanically challenge inoculated with PRV HA at 0, 5, 11, 17, 26, 35, and 56 days after the initial inoculation, on the last three fully expanded apical leaves. To determine the effect of different challenge positions, challenge inoculations were made on two leaves each time at four different positions 30 days after the initial inoculation (Fig. 1). The upper positions include the apical nonexpanded leaves, except the apex which was too fragile. In one treatment every leaf of the plant was challenge inoculated. To determine the combined effect of time and challenge position, plants were challenged with PRV HA at 30, 32, 34, and 36 days after the initial inoculation beginning with two basal leaves and continuing to challenge the next two younger leaves at each successive challenge time.

Cross-protection tests were also conducted in *C. metuliferus*. Seedlings at the one-true-leaf stage were infected with PRV HA 5-1 and mechanically challenge inoculated with PRV HA or an isolate of WMV-1 from Florida (WMV-1 F, kindly provided by R. Provvidenti, New York State Agric. Exp. Stn., Geneva 14456) 14 days after the initial inoculation. Challenge inoculations were either on three upper fully-expanded leaves or on three lower leaves. The plants were kept in a greenhouse 1 mo for observation.

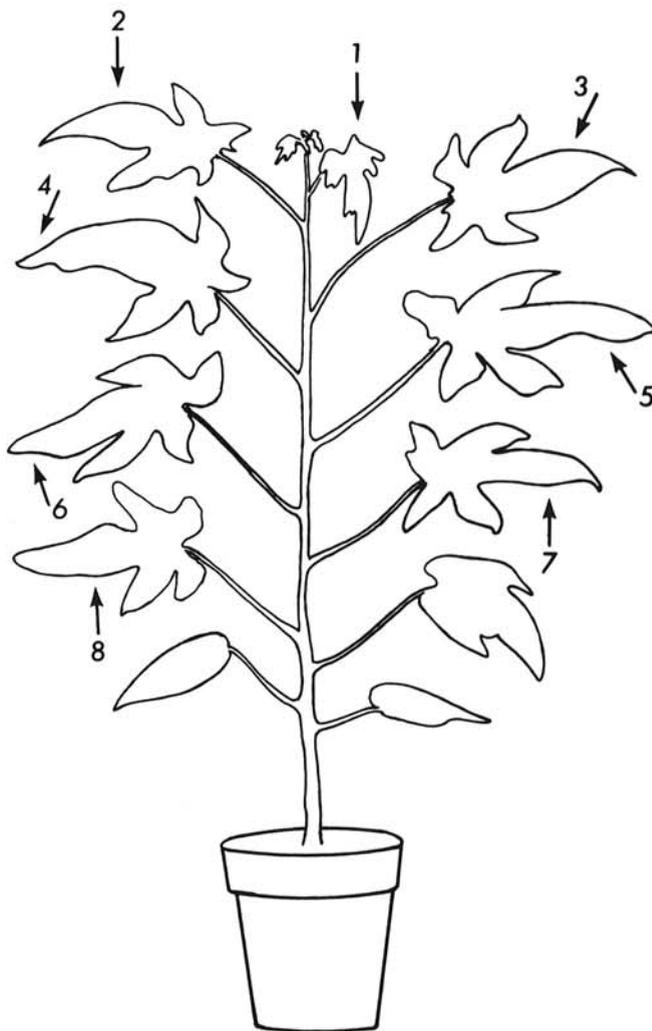


Fig. 1. Sites of inoculation on papaya plants to determine the effect of different challenge positions on cross protection. Challenge inoculations were mechanically made on two leaves each time at four positions (positions 1 and 2, 3 and 4, 5 and 6, and 7 and 8) 30 days after initial inoculation. The nonexpanded young leaf next to the apex was designated as leaf 1.

## RESULTS

**Natural collection.** Ninety-four samples collected from papaya orchards located in Hawaii caused severe symptoms on papaya and *C. metuliferus* 20 days after inoculation. Ten samples caused mild mottling 30 days after inoculation, but all showed severe mosaic or leaf distortion 50 days after inoculation. Twelve samples did not induce any symptom on test plants and the results of ELISA indicated no infection. Thus, no ideal mild strain was isolated from the field collection.

A mild strain (Su-mm) selected from 230 isolates of PRV from Taiwan (19) caused severe symptoms of mosaic, stunting, and leaf distortion on papaya in summer. However, in winter it induced

TABLE 3. Cross-protection effectiveness of PRV HA 5-1 (symptomless) against PRV HA (severe) in papaya after mechanical challenge at different time intervals<sup>a</sup>

Days after challenge inoculation	Papaya plants no. that did not show severe symptoms after challenge at day:					
	5	11	17	26	35	56
15	0	14	14	14	14	14
30	0	9	11	13	13	14
45	0	0	6	13	13	14
60	0	0	4	12	12	13
75	0	0	4	12	11	12
90	0	0	4	12	11	12

<sup>a</sup>In each treatment 14 papaya seedlings were used. All papaya seedlings inoculated with PRV HA (severe) at different time intervals after mock-inoculation with buffer showed severe symptoms 15 days later. Papaya infected with PRV HA 5-1 (symptomless) alone did not show severe symptoms during the period tested.

mild mottling at a time when other isolates were able to cause severe leaf distortion or leaf shoestringing.

**Single-lesion variants from natural populations.** A total of 232 papaya seedlings were inoculated with single lesions from *C. quinoa* infected with PRV HA, PRV HB, PRV F-340, or PRV Su-mm. Various degrees of symptom severity appeared on 130 of the seedlings. None of the isolates, however, caused sufficiently mild symptoms for practical application. The other papaya seedlings showed no indication of infection as judged by lack of symptom development and negative ELISA reactions.

**Condition for mutagenic treatment.** Infectivity of PRV in crude sap of zucchini squash after treatment with 0.1, 0.2, 0.3, 0.4, and 0.5 M sodium nitrite at 20 C for 30 min decreased to 65, 35, 27, 12, and 2%, respectively, compared to the control. The effects of different concentrations of sodium acetate buffer at different pHs were also tested. Lowering the pH from 5.5 to 4.0 reduced virus infectivity drastically (*unpublished*). However, increases in concentration of acetate buffer at a fixed pH only reduced virus infectivity slightly. Treatments with various combinations of acetate buffer and sodium nitrite at pH 6.0 were performed to determine the most suitable conditions for mutation (Table 1). The concentrations of sodium nitrite and sodium acetate that were chosen for further work were 0.4 M and 0.1 M, respectively. The survival rate of PRV was reduced to 2% with this treatment as compared to untreated control. When the sap was treated with 0.1 M sodium acetate alone at pH 6.0, the survival rate remained high (71%). Apparently, the inactivation of the virus was due to the action of nitrous acid, rather than the acetate buffer. All single-lesion isolates from the selected treatment were transferred to papaya seedlings for evaluation.

**Symptomless mutants from mutagenic treatments.** The results from seven mutagenic treatments are summarized in Table 2. Virus was detected in 247 of 663 inoculated seedlings. Of these, 226

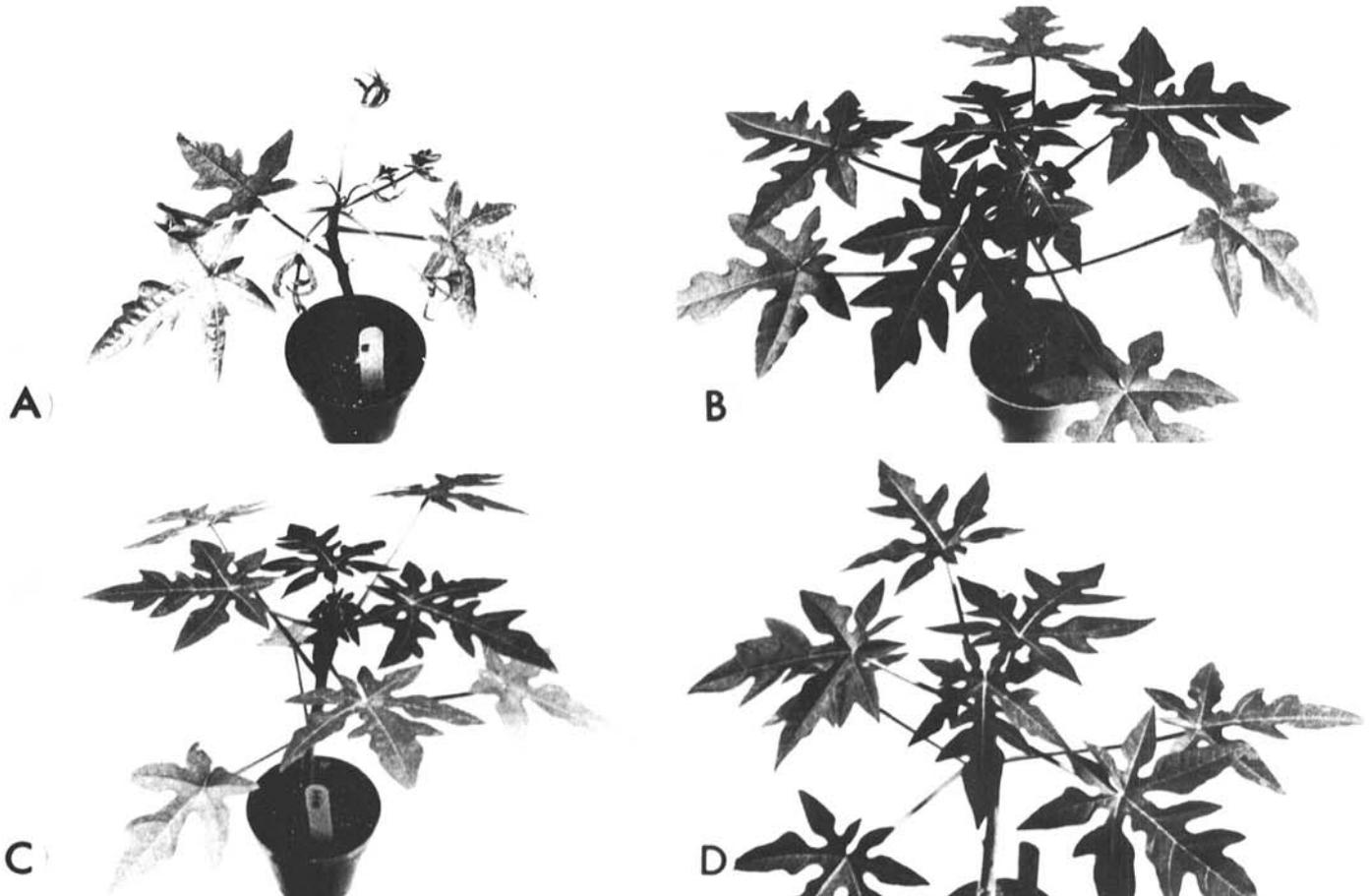


Fig. 2. Symptoms on papaya produced by a severe strain of papaya ringspot virus (PRV HA) and by nitrous-acid-induced mutants (HA 5-1 and HA 6-1). A, Mosaic, distortion, and stunting caused by PRV HA; B, symptomless infection by PRV HA 5-1; C, symptomless infection by PRV HA 6-1; and D, healthy papaya.

papaya seedlings showed severe symptoms of mosaic and leaf distortion. Nineteen papaya seedlings showed mild mottling initially, but subsequently these isolates caused severe symptoms.

Attention was focused on two papaya seedlings that did not show any prominent symptoms but were ELISA positive. Papaya seedlings inoculated with these two isolates, designated PRV HA 5-1 and PRV HA 6-1, remained symptomless or showed diffuse mottling with no reduction in plant size (Fig. 2). Seedlings of *C. metuliferus* and zucchini squash infected with these isolates exhibited light vein-clearing with no reduction in vigor or growth. All the plants infected with PRV HA 5-1 or PRV HA 6-1 had strong positive reactions when tested with ELISA 2-3 wk after inoculation. This indicated that the symptomless infection was not due to low titer or slow multiplication of the virus. Surprisingly, inocula from papaya or *C. metuliferus* that were infected with PRV HA 5-1 or HA 6-1 did not produce local lesions on *C. quinoa*. Because these isolates behaved differently from the parent severe strain HA and caused almost no damage to papaya plants, we considered them to be mutants of PRV HA.

**Cross-protection effectiveness of the PRV HA 5-1 mutant in papaya.** Before each challenge inoculation, symptomless infection by PRV HA 5-1 was confirmed by positive reactions in ELISA tests except that at 5- and 11-day intervals the reactions were either negative or a very weak positive. The virus titer in each challenge inoculum was extremely high (usually >200 local lesions per leaf of *C. quinoa*).

The results of cross protection in papaya seedlings between the symptomless strain HA 5-1 and the severe HA strain of PRV after challenge inoculation at different time intervals are shown in Table 3. Fourteen plants were used in each treatment. All papaya seedlings inoculated with the severe strain at different time intervals after mock-inoculation with buffer showed severe symptoms 15 days later (Fig. 3A). When challenge inoculations were made 5 days after initial inoculation, protection was not observed. However,

when the time intervals were increased to 11 or 17 days, severe symptoms of HA were either delayed or not expressed. A high proportion (79-93%) of the plants remained symptomless (Fig. 3B) even 60 or 90 days after challenge inoculation when the time interval was increased to 26, 35, and 56 days.

Results of cross protection between PRV HA 5-1 and PRV HA after challenge inoculation to different leaf positions are shown in Table 4. Papaya plants inoculated with buffer first and

TABLE 4. Cross-protection effectiveness of papaya ringspot virus (PRV) mutant PRV HA 5-1 (symptomless) against PRV HA (severe) in papaya after mechanical challenge at different positions on preinfected papaya seedlings<sup>a</sup>

Days after challenge inoculation	Plants (no.) without severe symptoms					Multiple challenge inoculations <sup>b</sup>
	Leaves inoculated with the challenge strain					
	1 + 2	3 + 4	5 + 6	7 + 8	Whole plant	
15	10	10	10	10	10	10
30	8	10	10	9	8	10
45	6	10	10	9	8	10
60	5	10	10	8	7	10
75	2	9	9	6	6	9
90	2	9	9	6	4	8

<sup>a</sup>Ten papaya seedlings were used in each treatment. The plants mock-inoculated with buffer first and re-inoculated with PRV HA at different leaf positions 30 days later showed severe symptoms 15-25 days after HA inoculation. The plants inoculated with PRV HA 5-1 alone on whole plant at the stage of five to six leaves did not show severe symptoms during the period tested. Leaf age increased with leaf number (see Fig. 1).

<sup>b</sup>The plants were challenged with PRV HA at 30, 32, 34, and 36 days after initial inoculation, beginning with two basal leaves and continuing to challenge two leaves moving toward apex.

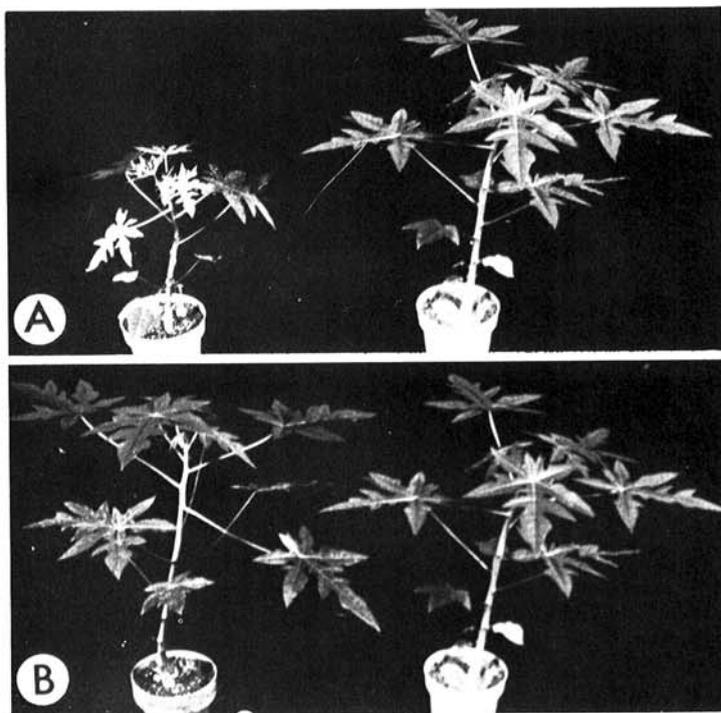


Fig. 3. Cross-protection effectiveness of PRV HA 5-1 (symptomless) against PRV HA (severe) in papaya (A and B) and *Cucumis metuliferus* (C). A, Left, papaya infected with PRV HA showing severe symptoms; right, healthy papaya. B, Left, papaya first infected with PRV HA 5-1 and then challenged with PRV HA showing no reduction in growth 45 days after challenge inoculation; right, healthy papaya. C, Left to right: *C. metuliferus* infected with PRV HA showing severe symptoms, a plant first infected with PRV HA 5-1 and then challenged with PRV HA showing no reduction in growth 30 days after challenge inoculation, a plant infected with PRV HA 5-1 alone, and a healthy plant.

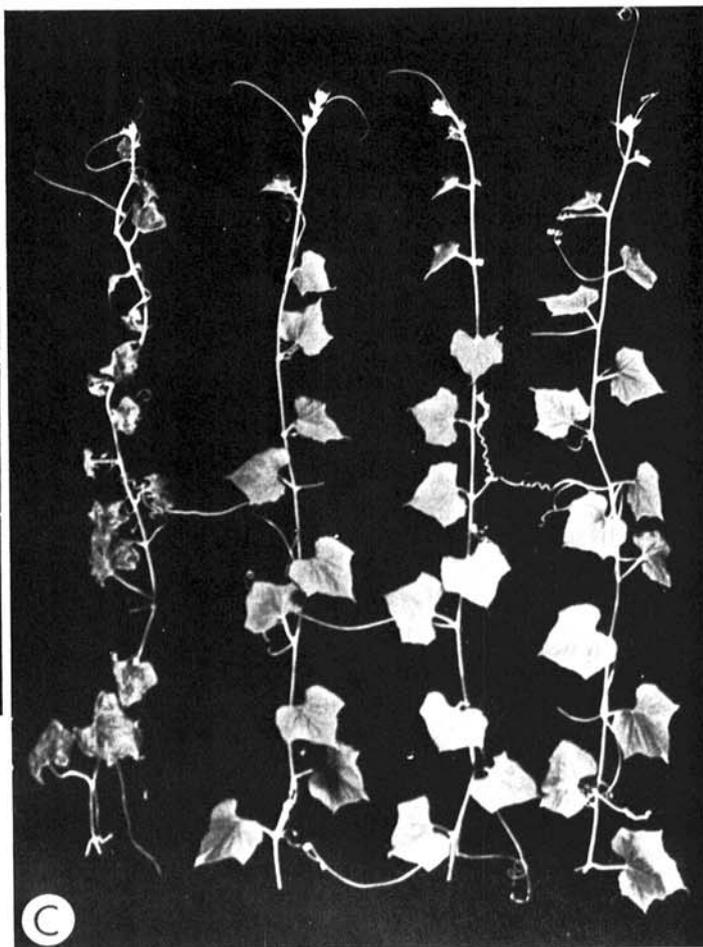


TABLE 5. Cross-protection effectiveness of PRV HA 5-1 (mild) against PRV HA (severe) and WMV-1 F (severe) in *Cucumis metuliferus* (Acc. 2459)

Challenge virus	Days after challenge inoculation	Challenge inoculation at:			
		Upper leaves		Lower leaves	
		Control <sup>a</sup>	Treatment <sup>b</sup>	Control	Treatment
PRV-HA	15	0/20 <sup>c</sup>	24/24	0/20	24/24
	30	0/20	24/24	0/20	24/24
WMV-1 F	15	0/20	30/30	0/20	30/30
	30	0/20	6/30	0/20	13/30

<sup>a</sup>The plants were mock inoculated with buffer and reinoculated with the severe strain 14 days later.

<sup>b</sup>The plants were inoculated with PRV HA 5-1 and then challenge inoculated with PRV HA or WMV-1 F 14 days later.

<sup>c</sup>Number of plants protected per total number of plants used.

reinoculated with HA showed severe symptoms 15–25 days later. In all cross-protection treatments, severe symptoms were not observed for at least 30 days in 80% of the plants. A high proportion of plants that were challenge inoculated on expanded leaves remained protected throughout the test period. However, a majority of the plants that were challenge inoculated on the upper nonexpanded young leaves or all leaves developed symptoms 60–90 days after challenge inoculation.

When the plants preinfected with PRV HA 5-1 were continually challenged at different leaf positions 30, 32, 34, and 36 days after initial inoculation, nearly all plants remained symptomless 90 days after the first challenge inoculation (Table 4).

**Cross-protection effectiveness of the PRV HA 5-1 mutant in *C. metuliferus*.** Complete cross protection was observed with all test plants when PRV HA 5-1 was used against PRV HA in *C. metuliferus* (Table 5). Thirty days after challenge inoculation, no superinfection was noticed when PRV HA was introduced either on upper or lower leaves. The control plants infected with PRV HA alone showed severe symptoms 12 days after inoculation (Fig. 3C). Cross protection was less effective against WMV-1 F. Although plants did not show symptoms 15 days after challenge inoculation, most plants had WMV-1 symptoms 30 days after challenge inoculation (Table 5).

## DISCUSSION

The primary objective of this study was to obtain mild strains of PRV that could protect papaya against damage by severe strains. We have isolated two mild mutants, designated PRV HA 5-1 and PRV HA 6-1, following treatment of sap from PRV HA-infected squash tissue with nitrous acid. Papaya seedlings inoculated with these mutants remained symptomless or showed diffuse mottling with no reduction in plant size. Either complete or a high degree of protection was observed when PRV HA 5-1 was used to protect papaya against the severe effects of infection by the parent strain PRV HA under various mechanical challenge treatments. The results indicate a good potential for the use of the mutant as a protectant for control of PRV.

Cross-protection effectiveness was affected by the time of challenge inoculation. If the challenge inoculation was made <18 days after the initial inoculation, no cross protection or incomplete cross protection (which only delayed the expression of severe symptoms) were observed. However, if the time interval was increased to 26 days or more, complete or a high degree of cross protection was obtained. The results, coupled with the negative or weak reactions in ELISA tests at 5- and 11-day intervals, indicate that 17–26 days are required for PRV HA 5-1 to build up to a sufficient titer in the plants to provide protection against the severe strain.

Superinfection also was noticed in a large proportion of the test plants when the challenge inoculations were made on the very top nonexpanded young leaves or the whole plant. ELISA tests indicated that the titer of the mutant in the young apical leaves are lower than in the fully expanded leaves (*unpublished*). Thus, low

virus titer of the protectant in the very young leaves and the high challenge pressure on the whole plant may be the cause of incomplete cross protection in these tests.

In cases in which superinfection occurred, the expression of severe symptoms of PRV HA on most plants was delayed 1–2 mo compared to the unprotected control. This slower disease-development rate might reduce crop loss significantly. To minimize the chance of superinfections developing in the field, papaya seedlings should be inoculated with the protectant strain at the very young (one-true-leaf) stage, and the plants should be kept in the greenhouse for about 1 mo before being transplanted to the field.

The mild mutants were initially isolated from discrete lesions on *C. quinoa* after nitrous acid treatment. However, the mutants did not induce local lesions on *C. quinoa* when the inocula were prepared from infected papaya or *C. metuliferus*. We do not have a good explanation for this observation. One possibility is that the buffer conditions used in the initial inoculation following nitrous acid treatment differed from those used for subsequent inoculations to *C. quinoa*. Nevertheless, this makes further selection of milder or more stable mutants difficult.

Several lines of evidence indicate that PRV HA 5-1 and 6-1 are mutants of PRV HA: HA 5-1 and 6-1 are serologically indistinguishable from HA; numerous attempts to obtain symptomless strains through single-lesion isolation without mutagenic treatment failed; we have not observed any mild strains in the field; and we were able to purify HA 5-1 from infected *C. metuliferus* by using the same procedures as for HA. Nevertheless, the possibility that the mutants were selected, rather than mutated, from a heterogeneous population of PRV cannot be entirely excluded. Direct biochemical analyses of the coat proteins and RNAs of mutants compared to those of the parental strain are needed to prove that they are mutants derived from PRV HA.

Because of the restricted host range of PRV (29,39), the possibility of the mild mutants damaging other crops in the vicinity of a papaya orchard is very low. The only consideration is cucurbitaceous plants, which have been reported to be natural hosts of PRV (37). The symptomless mutants, obtained by artificial induction, produced almost no symptoms on zucchini squash and *C. metuliferus* (Acc. 2459). However, the latter develops severe symptoms following inoculations by wild-type PRV isolates. The possibility of damaging cucurbitaceous plants would be minimal if the mild mutant is not aphid transmitted. However, the aphid transmissibility of HA 5-1 and of HA 6-1 has not been tested.

The cross-protection tests indicate that PRV HA 5-1 can protect *C. metuliferus* from the severe effects of infection by PRV or WMV-1. The latter is serologically identical to PRV (14,30) and is of great economic importance wherever cucurbits are grown (36). Thus, the mild mutants of PRV might be useful as protectants in cucurbitaceous crops for the control of WMV-1.

The cultivar Kapoho Solo papaya used for the tests is currently a major commercial cultivar in Hawaii and is very sensitive to infection by PRV. Although Kapoho Solo plants inoculated with the mutants were nearly symptomless, further studies should be done to test their pathogenicity to this and other commercial papayas under field conditions. Previous studies established that nine PRV isolates from Hawaii, Florida, Taiwan, and Ecuador were the same serotype and shared an identical host range (39). These PRV strains demonstrated little variation and, therefore, it is reasonable to think that the mutants will protect papaya against other PRV strains.

The use of aphids as a challenge vector and the development of an efficient mass-inoculation method for the mild mutants are being studied. The long-term effect of the mild mutants on horticultural properties of papaya and their ability to cross protect papaya against severe strains are also being investigated under field conditions. The practical value of the symptomless mutants should become known in the near future.

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