Effectiveness of Cross-Protection by Mild Mutants of Papaya Ringspot Virus for Control of Ringspot Disease of Papaya in Taiwan

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ABSTRACT

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Two mild nitrous acid-induced mutants (PRV HA 5-1 and 6-1) of papaya ringspot virus (PRV) were tested under greenhouse and field conditions to study the potential of cross-protection for control of papaya ringspot disease of papaya in Taiwan. Under greenhouse conditions, both mutants caused only mild or symptomless infection on test plants in Chenopodiaceae and Cucurbitaceae families and on the major papaya cultivar of Taiwan, Tainung No. 2. Also, under greenhouse conditions, HA 5-1 and HA 6-1 provided a high degree of protection in papaya against the severe effects of two prevalent PRV strains of Taiwan. Large numbers of papaya seedlings were infected by applying inocula with a spray gun (equipped with a 1.2-mm-diameter nozzle), using pressures of 4–8 kg/cm^2 at distances of 10–20 cm. Field cross-protection trials were started in severely diseased areas in fall 1983. When protected papaya plants were planted randomly with unprotected controls or row by row under high disease pressure, unprotected plants showed severe symptoms 2–3 mo after planting and protected plants showed severe symptoms 1–3 mo after the controls. Under these conditions, cross-protection did not provide economic benefit. However, in a test where protected and unprotected plants were established in solid blocks and where the disease pressure inside the test orchard was minimized by roguing once every 10 days up to the flowering stage, protected trees showed 82% higher fruit yield than unprotected plants. This resulted in a 111% increase in income because of a much higher yield of good-quality fruit from protected trees than from the controls. Because of these initial successes, the government of Taiwan initiated large-scale cross-protection field trials in 1984 and 1985 of 244,000 and 400,000 seedlings planted over 122 and 200 ha, respectively.

Papaya ringspot virus (PRV), a potyvirus (9), is the major limiting factor for economic papaya production throughout the tropics and subtropics (5). This virus was first recorded in Taiwan in 1975 and within 4 yr destroyed much of the papaya production in commercial orchards (18).

Attempts to develop effective control measures in Taiwan and other papaya-producing areas have generally been unsuccessful. Although tolerant selections of papaya have been described (3,4,6), these are not commercially desirable. Resistance to PRV has not been found within *Carica papaya* L. (4,11,18). A diligent roguing program has been practiced successfully to reduce the spread of PRV in certain areas of Hawaii (13). However, roguing of diseased plants is almost impossible in Taiwan, where the disease has become epidemic. Agricultural practices such as planting papaya in the fall, intercropping with corn as a barrier crop, spraying with mineral oils and systemic insecticides, and protecting transplanted seedlings with plastic bags have been ineffective or only marginally beneficial. The unavailability of effective control measures and the restrictive host range of PRV (16,20) make cross-protection an attractive method of controlling this papaya disease.

Cross-protection of plant viruses is a 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 (10). Large-scale application of cross-protection has been reported for the control of tobacco mosaic virus (TMV) in tomato in Europe (7,17) and Japan (14) and for the control of citrus tristeza virus (CTV) in citrus in Brazil (12). The key to these practical applications of cross-protection is the availability of a virus strain that does not cause severe damage and also provides a high degree of protection. The protective mild virus strains used for control of TMV were artifically induced (17) or heat-attenuated mutants (14). In the case of CTV, naturally collected mild virus strains were used (12).

Recently, two mild PRV mutants, designated PRV HA 5-1 and 6-1, were selected after nitrous acid treatment of a severe Hawaiian strain (designated PRV HA) (19). Under greenhouse conditions, a high degree of protection was observed when HA 5-1 was used to protect papaya against PRV HA (19). In this study, we report on the cross-protection effectiveness of PRV HA mild mutants under greenhouse and field conditions in Taiwan.

MATERIALS AND METHODS

Mild virus strains. The nitrous acid-induced mild mutants PRV HA 5-1 and 6-1 (19) were used in this study. The mild mutants were maintained in a susceptible line of jelly melon (*Cucumis metuliferus* (Naud.) Mey.), an excellent propagative host for PRV (15).

Host reactions to mild mutants. Tissues from *C. metuliferus* infected with PRV HA 5-1 or 6-1 were ground in 0.01 M potassium phosphate buffer (pH 7.0, 1 g/10 ml), and the extracts were rubbed on test plants predusted with 600-mesh Carborundum. At least six plants of each species or cultivar were inoculated with HA 5-1 or 6-1. The plants were kept in a greenhouse at 24–35 C without supplemental light. Infection by the mutants was checked visually and by a modified double-antibody enzyme-linked immunosorbent assay (ELISA) (2,8). ELISA reactions were recorded by an EL307 ELISA reader (Biotek Instruments, Inc., Burlington, VT) at $A_{405\ nm}$.

Cross-protection tests under greenhouse conditions. Papaya seedlings of Tainung No. 2 at the four- to five-leaf stage were mechanically inoculated with HA 5-1 or 6-1 prepared from infected leaves of *C. metuliferus* by manual rubbing as described previously. Infection was confirmed by ELISA 18 days after

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inoculation. Two severe strains, a mosaic type (PRV TM) and a wilting type (PRV TW), that are prevalent in Taiwan were used for challenge inoculations (1,20). Challenge inoculations were done with crude leaf extracts (1 g/10 ml) of *C. metuliferus* infected with the severe PRV strains. Infectivity of these extracts was determined by inoculating *Chenopodium quinoa* Willd., a local lesion host for PRV. Challenge inocula were applied to the three upper fully expanded leaves of protected papaya 15, 20, 25, 30, and 40 days after inoculation with mild strains (19). Ten protected plants were used for each challenge test. The same number of comparable seedlings that were mock-inoculated with buffer only served as controls. Breakdown of cross-protection was judged by the appearance of severe symptoms on test plants, which were kept in the greenhouse for at least 4 mo after challenge inoculation.

Mass inoculation of papaya seedlings. An efficient method for mass inoculation of papaya seedlings is essential for the practical application of cross-protection. Because PRV is mechanically transmissible, the relative efficiency of manual rubbing and pressure spray were compared for mass inoculation. The manual rubbing method was as described previously. Pressure spray inoculations were done with a paint spray gun (standard nozzle of 1.2 mm) attached to an air compressor (85-L tank, 1-hp motor). Inocula were prepared from PRV HA 5-1- or 6-1-infected *C. metuliferus* inoculated 4 wk previously. Leaves were ground with a blender in cold 0.01 M potassium phosphate buffer, pH 7.0 (1 g/10 ml). The extracts were strained through cheesecloth and then mixed with Carborundum (600-mesh, 40 g/1.5 L) in a metal container connected to the spray gun. To determine the optimal spraying conditions, plants were inoculated using pressures of 4–8 kg/cm^2 and at distances between the seedlings and the spray gun of either 10 or 20 cm. Papaya seedlings of Tainung No. 2, Taiwan's most popular commercial cultivar, were raised under greenhouse conditions (24–35 C, without supplemental lights) in plastic bags 10 × 8 cm containing a mixture of soil and manure. Bags with individual seedlings were arranged in a wooden box (60 × 40 × 16 cm, 100 bags per box), which allowed for easy handling and transportation to the field. While inoculating, the spray gun was kept moving steadily back and forth to ensure that each seedling was sprayed twice. Plants were rinsed with tap water immediately after inoculation and then kept in the greenhouse for observation. Papaya seedlings were inoculated at the four- to five-leaf stage. Infection was checked with ELISA 4 wk after inoculation. The threshold for positive ELISA readings was 0.2 and at least twice the optical density of the healthy.

Field trials. Seedlings of Tainung No. 2 were raised in plastic bags 10 × 8 cm, which were arranged in wooden boxes as described before. All wooden boxes were kept in a screenhouse. Papaya seedlings at the four- to five-leaf stage were mechanically inoculated (as described) with PRV HA 5-1 or 6-1 by pressure spray at 6-8 kg/cm^2 air pressure at distances of 10 or 20 cm. The inocula were prepared from infected *C. metuliferus* that had been infected 15 days previously. Inoculated seedlings were kept in a screenhouse and assayed for infection by ELISA 3-4 wk after inoculation. Plants with positive ELISA readings were transplanted into test fields 28 days after inoculation. Seedlings raised under the same conditions and mock-inoculated with 0.01 M phosphate only were used a controls.

The field tests were conducted from November 1983 to December 1984 in southern Taiwan, where PRV is most serious. Three locations were chosen for the tests.

1. Kao-Shu. This plot was chosen to test the effect of high disease presssure. The field was located adjacent to an old orchard in which a tolerant papaya cultivar, a hybrid resulting from crosses of Florida (3,4) and Costa Rican cultivars, had been planted 6 mo earlier. All of the tolerant papaya trees were infected with naturally occurring severe strains of PRV before the test plants were put into the field. A rectangular test field was divided into eight smaller blocks containing 40 or 50 plants. Each block consisted of three rows of protected or unprotected plants. A total of 180 protected and 180 unprotected plants constituted the field test. In this field, PRV HA 6-1 was chosen as a protective virus strain and test plants that became severely diseased were not rogued.

2. Feng-Shan. This area had severely infected papaya orchards in the vicinity but not directly adjacent to the test field. Protected and unprotected trees were interplanted in a randomized block design. Each plot consisted of three treatments: a HA 5-1 protected plant, a HA 6-1 protected plant, and a healthy plant. The test field contained 150 plants per treatment. Papaya plants that became severely infected were not rogued.

3. Ta-Liao. The primary purpose of this test was to determine the effect of protection of PRV HA 6-1 under the conditions of large solid-block plantings and lower disease pressure. The test orchard was planted in a square divided into four equal, smaller square blocks:

Table 1. Reactions of papaya ringspot virus severe strain HA and mild mutants HA 5-1 and HA 6-1 on different hosts

	Symptoms[a]			ELISA reactions[b]		
Test plants	**HA**	**HA 5-1**	**HS 6-1**	**HA**	**HA 5-1**	**HA 6-1**
Caricaceae (papaya)						
Carica papaya						
Tainung No. 2	Ms	Sm	Sm	++++	++++	++++
Chenopodiaceae						
Chenopodium quinoa	L	Sm[c]	Sm[c]	++	++	++
C. amaranticolor	L	Sm	Sm	++	++	+
Cucurbitaceae (cucurbits)						
Citrullus vulgaris						
Chinlan	Mt	Sm	Sm	++++	++++	++++
Chungniang	MMT	Sm	Sm	+++	+++	+++
Juhkuang	Mt	Sm	Sm	++++	+++	+++
Fukuei 1	Mt	Sm	Sm	++++	++++	++++
Cucumis melo						
Hsingjuh	Mt	Sm	Sm	+++	+++	+++
C. melo var. *conomon*						
Oriental Pickling	Mt	Sm	Sm	++	+++	++++
C. metuliferus	Mt	Sm	Sm	++++	++++	++++
Line 35		Vc,Vb	Vc,Vb			
Cucurbita moschata						
Chinese pumpkin	MMt	Sm	Sm	+++	++	++
Cucurbita pepo						
Zucchini	Ms	Sm	Sm	++++	++	++
Cucumis sativus						
National Pickling	Mt	Sm	Sm	++++	++++	++++
Marketer	MT	Sm	Sm	++++	++++	++++
Hsiyen	MMt	Sm	Sm	++	++	++

[a] Ms = mosaic, Mt = mottling, MMt = mild mottling, Sm = symptomless, L = local lesions, Vc = veinclearing, and Vb = veinbanding.

[b] Mean ELISA readings at A_{405nm}: + = 0.101–0.500; ++ = 0.501–1.000; +++ = 1.001–1.500; ++++ = above 1.500; all the uninoculated check had readings below 0.100. The readings were recorded 30 min after the substrate was added. Concentration of globulin to PRV was 2 μg/ml and alkaline phosphatase conjugate was at 1/800 dilution. Each ELISA reading was the mean of at least two wells.

[c] *C. quinoa* and *C. amaranticolor* gave symptomless infections on inoculated leaves, no infection on uninoculated leaves.

two healthy and two infected with HA 6-1. The protected and unprotected blocks were diagonal to each other. Each block contained 200 papaya trees for a total of 400 protected and 400 unprotected trees. To minimize early secondary spread of severe virus in the test orchard, papaya trees showing severe symptoms were rogued every 10 days until flowering. Papaya trees showing severe symptoms after flowering were not rogued in order to compare the yield and quantity of fruit from both protected and unprotected blocks.

All papaya plants in the three test fields were transplanted in a standard way with a planting distance of 2.1 m within a row and 2.4 m between rows. Symptoms of test plants were recorded every 10 days. Yields of papaya trees were measured by the weight and sugar content of three grades of fruit (symptomless, ringspotted, and deformed). Sugar content was determined by Brix readings. The total cash income from fruit of protected and unprotected trees was also recorded.

RESULTS

Host reactions. The host reactions of PRV HA 5-1 and 6-1 are listed in Table 1. Only plants in Caricaceae, Chenopodiaceae, and Cucurbitaceae were tested because the host range of PRV is restricted to these families (16). PRV HA 5-1 and 6-1 caused symptomless or mild symptom infection in Tainung No. 2 papaya, with occasional chlorotic spots along the leaf vein. Both mutants also caused symptomless or mild symptom infection in curbitaceous plants that are commonly grown in Taiwan, such as watermelon (*Citrullus vulgaris* Schrad.), melons (*Cucumis melo* L.), pumpkins (*Cucurbita moschata* Duch.), squash (*Cucurbita pepo* L.), and cucumbers (*Cucumis sativus* L.). However, symptoms of veinclearing with occasional vein-banding were observed on a susceptible line of *C. metuliferus* (15). Infection by PRV HA 5-1 and 6-1 were verified by positive readings of ELISA (Table 1). Both mutants caused symptomless infection on inoculated leaves of *Chenopodium quinoa* and *C. amaranticolor* Coste & Reyn., which are local lesion hosts for most strains of PRV. Symptomless infections were further confirmed by mechanical transfer of the virus from test plants to papaya and *C. metuliferus*, which subsequently tested positive by ELISA. In some cases, ELISA readings from plants infected with HA 5-1 were higher than those with 6-1. However, because no local lesion hosts are available for these mild strains (19), it was not possible to correlate ELISA readings to virus infectivity.

Greenhouse cross-protection tests. Results of cross-protection effectiveness of PRV HA 5-1 and 6-1 against two severe strains from Taiwan, PRV TW and PRV TM, are shown in Tables 2 and 3, respectively. Unprotected plants inoculated with PRV TW or TM showed severe symptoms 10–12 days after inoculation. When challenge inoculations were done 15 or 20 days after the protective inoculations, protection was not observed, or there was only a delay in the expression of severe symptoms. Protection was observed in many plants when challenge inoculation was done 25 or more days after protective inoculation; these plants did not show severe symptoms 20 days after challenge inoculation, no matter which challenge strain was used. However, some protected seedlings gradually showed severe symptoms 1 or 2 mo after challenge inoculations. For example, at 120 days after challenge inoculation with PRV TW or TM, 70% of the of seedlings protected with HA 5-1 still remained free of severe symptoms. Protection effectiveness of HA 6-1 was not as good as that with HA 5-1; only 40–60% of plants protected with HA 6-1 and challenged with PRV TW or TM remained free of severe symptoms 120 days after challenge. The results indicated that both the PRV HA 5-1 and 6-1 mutants protected Tainung No. 2 papaya

Table 2. Cross-protection effectiveness of papaya ringspot virus mild strains HA 5-1 and HA 6-1 in Tainung No. 2 papaya after mechanical challenge with a severe strain (TW) at different time intervals under greenhouse conditions[a]

Days after challenge inoculation	Papaya plants (no.) without severe symptoms, challenged at day[b]									
	15		20		25		30		40	
	HA 5-1	HA 6-1	HA 5-1	HA 6-1	HA 5-1	HA 6-1	HA 5-1	HA 6-1	HA 5-1	HA 6-1
0	10	10	10	10	10	10	10	10	10	10
20	0	0	6	5	10	10	10	10	10	10
40	0	0	0	0	9	9	10	9	10	9
60	0	0	0	0	9	8	9	8	9	8
80	0	0	0	0	8	7	7	6	8	7
100	0	0	0	0	7	5	7	5	7	6
120	0	0	0	0	7	5	7	5	7	6

[a] Papaya seedlings were inoculated with PRV HA 5-1 or HA 6-1, then challenge-inoculated with PRV TW at different time intervals. Ten plants were inoculated per interval. Papaya seedlings inoculated with PRV HA 5-1 or HA 6-1 alone did not show severe symptoms during the same period tested. Healthy papaya seedlings inoculated with PRV TW showed severe symptoms 10–12 days after inoculation.
[b] Example: 15 signifies that plants were challenged with the TW isolate 15 days after protective inoculation with HA 5-1 or HA 6-1.

Table 3. Cross-protection effectiveness of papaya ringspot virus mild strains HA 5-1 and HA 6-1 in Tainung No. 2 papaya after mechanical challenge with severe strain (TM) at different time intervals under greenhouse conditions[a]

Days after challenge inoculation	Papaya plants (no.) without severe symptoms, challenged at day[b]									
	15		20		25		30		40	
	HA 5-1	HA 6-1	HA 5-1	HA 6-1	HA 5-1	HA 6-1	HA 5-1	HA 6-1	HA 5-1	HA 6-1
0	10	10	10	10	10	10	10	10	10	10
20	0	0	6	4	10	10	10	10	10	10
40	0	0	0	0	9	8	9	8	9	8
60	0	0	0	0	8	7	9	7	9	7
80	0	0	0	0	7	6	8	6	9	6
100	0	0	0	0	7	4	7	5	8	6
120	0	0	0	0	7	4	7	5	7	5

[a] Papaya seedlings were inoculated with PRV HA 5-1 or HA 6-1, then challenge-inoculated with PRV TM at different time intervals. Ten plants were inoculated per interval. Papaya seedlings inoculated with PRV HA 5-1 or HA 6-1 alone did not show severe symptoms during the same period tested. Healthy papaya seedlings inoculated with PRV TM showed severe symptoms 10–12 days after inoculation.
[b] Example: 15 signifies that plants were challenged with the TM isolate 15 days after protective inoculation with HA 5-1 or HA 6-1.

seedlings against PRV TW and TM either by complete cross-protection in which symptoms of severe strains were not observed or by partial cross-protection in which the expression of severe symptoms was delayed.

The infectivity of each challenge inoculum prepared from infected *C. metuliferus* was tested on *C. quinoa*. The number of local lesions that developed 8–12 days after inoculation always exceeded 200 per leaf (average of 20 leaves), indicating high concentrations of infectious PRV TW or TM in the challenge inocula.

Mass inoculations of PRV. PRV HA 5-1 was transmitted 100% from *C. metuliferus* to papaya by manual rubbing. Infection rates of papaya seedlings that were spray-inoculated at various pressures and distances are listed in Table 4. Seedlings sprayed from a distance of 10 cm at pressures of 4, 6, or 8 kg/cm^2 resulted in 100% infection. However, severe damage on leaves of inoculated plants was observed at 8 kg/cm^2 pressure. Infection rates of seedlings inoculated using the same pressure at a distance of 20 cm were 12, 48, and 100%, respectively. The results indicated that 100% infection could be achieved and mechanical damage avoided by spraying plants from a distance of 10 cm and 4–6 kg/cm^2 pressure or at 20 cm and 8 kg/cm^2 pressure. Ten milliliters of leaf extracts and 20 min were needed to inoculate 100 papaya seedlings by manual rubbing. Forty milliliters and only 40 sec were required to inoculate the same number of seedlings by pressure spray. Similar results were also observed when inoculum of HA 6-1 was used (data not shown). The results indicated that pressure spray was a fast and highly efficient method for mass inoculation of papaya seedlings.

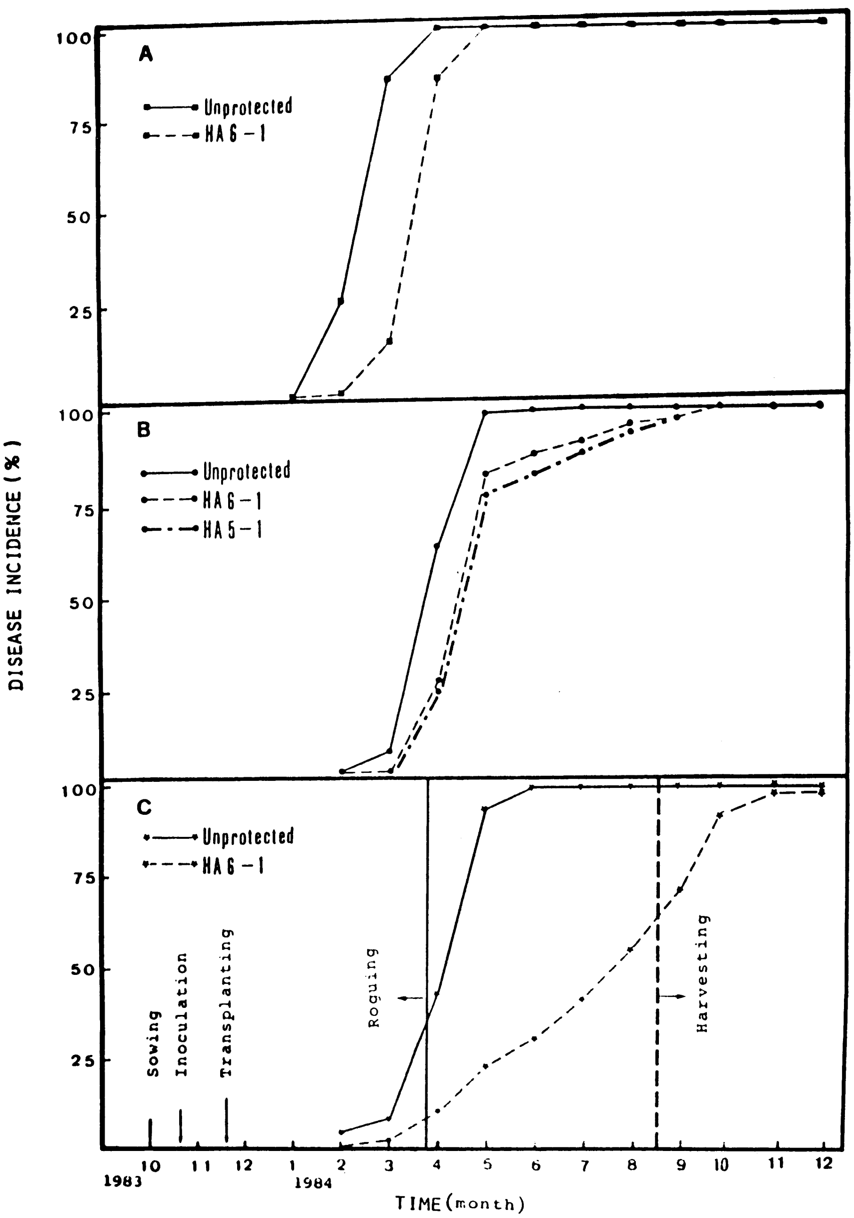

Fig. 1. Cross-protection effectiveness of mild virus mutants, papaya ringspot virus (PRV) HA 5-1 and 6-1, under field conditions in Taiwan from November 1983 to December 1984. **(A)** Kao-Shu area, Taiwan test where the site was adjacent to a papaya orchard 100% infected with PRV. Plants were not rogued. Three rows each of unprotected and HA 6-1 protected plants made up a plot. **(B)** Feng-Shan area, Taiwan test where infected orchards were nearby. Plants were not rogued. HA 5-1, HA 6-1, and healthy plants were interplanted in a randomized block design. **(C)** Ta-Liao area, Taiwan test where the site was located away from PRV-infected papaya orchards. Planting was in a square block consisting of two smaller square blocks of HA 6-1 protected and two of unprotected trees that were diagonal to each other. Plants showing severe symptoms were rogued every 10 days beginning in late March (roguing line in C) until the flowering stage. Note that the disease incidence given at each month represents percent disease at the end of the month; e.g., disease incidence of unprotected plants in Ta-Liao was 41% for April (data point on month 4, representing incidence at the end of April).

Field trials of cross-protection. Plants used in the field trials were inoculated by the pressure spray method. About 70–80% of the papaya seedlings inoculated with HA 5-1 or 6-1 showed positive ELISA readings 3 wk after inoculation. The others gave positive ELISA readings 4 wk after inoculation. The results confirmed that mass inoculation by pressure spray was efficient and practical. Severe damage to inoculated seedlings was not observed.

Unprotected plants showing severe symptoms of PRV were considered to be naturally infected by severe strains of the virus. Initial trials in Hawaii with papaya that were inoculated with HA 5-1 and grown under conditions of low disease pressure indicated that the mild strains were stable under field conditions (data not shown). Thus, severe symptoms of PRV that developed in protected plants (breakdown of cross-protection) were considered to be caused by superinfection with naturally occurring severe strains.

The incidences of severe infection of unprotected and protected plants in the different test fields are shown in Figure 1. At the Kao-Shu field (Fig. 1A), the test

Table 4. Infection rate of papaya seedlings inoculated with mild mutant papaya ringspot virus HA 5-1 by different pressure-spray treatments[a]

Pressure (kg/cm^2)	Distance from nozzle to seedlings (cm)	Infection (%)
4	10	100
6	10	100
8	10	100[b]
4	20	12
6	20	48
8	20	100

[a] One hundred plants per treatment. Infection was checked by ELISA.

[b] Severe damage on leaves of inoculated papaya seedlings was observed.

plot was adjacent to an orchard of tolerant plants that had 100% PRV infection. This provided very high disease pressure for primary infection. Furthermore, secondary spread was possible because infected trees were not rogued. Unprotected plants began to show severe symptoms 2 mo after transplanting, and all of them were infected by the fifth month after transplanting. Protected plants began showing severe symptoms 3 mo after transplanting and reached 100% severe infection 6 mo after transplanting. In this case, the protected plants showed a delay of only 1–2 mo in expressing severe symptoms compared with the controls. For example, the disease incidences among unprotected and protected trees were 28 and 5%, respectively, 3 mo after transplanting and 85 and 15%, respectively, 4 mo after transplanting. Because many plants showed severe infection before flowering, which started 4–5 mo after transplanting, fruit yields from protected and unprotected trees were minimal. This is consistent with our observations that plants that show severe infection before the start of flowering have poor fruit quality and yield.

In the Feng-Shan test, diseased orchards in the vicinity provided high disease pressure for primary infection, as was the case with the Kao-Shu test. Because plants were not rogued, unprotected controls that became infected provided high disease pressure for secondary spread to protected plants that were interplanted among unprotected controls. Under these conditions, cross-protection delayed the expression of severe symptoms 1–6 mo (Fig. 1B) compared with the controls. Because all of the unprotected plants and most of the protected plants were severely infected before flowering, fruit production from protected and unprotected trees was generally low.

In the Ta-Liao test field, protected and unprotected papaya seedlings were planted as solid blocks diagonal to each other in the same orchard. The test site was isolated from other diseased orchards. To minimize the disease pressure inside the orchard, unprotected and protected plants showing severe symptoms were rogued every 10 days up to the time of flowering, which began at the end of March 1984. Under these conditions, the effect of cross-protection was much better than in the Kao-Shu and Feng-Shan tests (Figs. 1C and 2). Two months after being transplanted, the unprotected controls began to show severe infection (Fig. 1C). Disease incidence of the unprotected plants reached 100% 7 mo after transplanting, whereas only 28% of the protected plants showed severe symptoms at that time. One year after transplanting, the breakdown reached almost 100% in the protected blocks. Unprotected trees that were nearest the protected trees generally showed severe symptoms much later than those that were farther away. Presumably, natural spread of the mild strain to the nearby unprotected plants gave these unprotected plants a degree of cross-protection.

Total fruit yields of trees in the protected and unprotected blocks up to December 1984 are listed in Table 5. The 374 protected papaya trees produced a total of 6,885 kg of fruit, 82% more than that of the 317 unprotected trees (3,791 kg). Harvested fruit were divided into three categories. The percentages of symptomless, ringspotted, and deformed fruits from protected trees were 25, 58, and 17%, respectively, whereas those from unprotected trees were <1, 63, and 37%, respectively. Symptomless and ringspotted fruit had higher sugar contents than deformed fruit, regardless of whether the fruit came from protected or unprotected trees (Table 6). These data show that protected plants had higher fruit yields and a higher percentage of good-quality fruit (symptomless and ringspotted) than unprotected plants. The total cash income from fruit harvested from the protected blocks was 111% more than those from the unprotected blocks (Table 5).

DISCUSSION

We have shown that the effectiveness of cross-protection by the mild mutants of PRV (HA 5-1 and 6-1) under field conditions is dependent on disease pressure and on the phenological stage of plants when breakdown of cross-protection occurs. For example, cross-protection provided much economic benefit (111% increase in cash income) at the Ta-Liao test (Figs. 1C and 2). In that trial, the test site was isolated from diseased papaya orchards, was planted in solid blocks of protected and unprotected plants, and plants showing severe disease symptoms were rogued up until the flowering stage. Heavy infection did not occur until well after the protected plants had started flowering. On the other hand, cross-protection did not provide economic benefits in the Kao-Shu and Feng-Shan tests (Fig. 1A,B). Both test sites had

Fig. 2. Ta-Liao area, Taiwan test plot. **(Top)** Note that disease incidence in the unprotected block to the right of the arrow is visibly greater than that in the protected block to the left of the arrow (August 1984). **(Bottom left)** Close-up of protected block showing good fruit set (August 1984). **(Bottom right)** View of unprotected block showing poor fruit set (October 1984).

Table 5. Comparison of the fruit yield and the cash income from papaya plants protected by HA 6-1 mild strain of papaya ringspot virus and from unprotected papaya plants at Ta-Liao area, Taiwan, 1984

	Protected with AH 6-1[a]					Unprotected[b]				
Month	Symptomless fruits (kg)	Spotted fruits (kg)	Deformed fruits (kg)	Total harvest (kg)	Cash income (US$)	Symptomless fruits (kg)	Spotted fruits (kg)	Deformed fruits (kg)	Total harvest (kg)	Cash income (US$)
August	18	5	2	28	19	1	4	1	6	4
September	503	732	60	1,295	1,045	3	633	182	818	592
October	646	1,435	412	2,493	1,204	1	890	479	1,370	537
November	349	1,317	362	2,028	600	4	718	445	1,167	270
December	234	493	317	1,044	206	0	149	281	430	51
Total	1,750	3,983	1,153	6,885	3,074	9	2,394	1,388	3,791	1,454

[a] From 374 bearing plants.
[b] From 317 bearing plants.

Table 6. Sugar content (Brix) of papaya fruits from papaya ringspot virus HA 6-1-protected and unprotected plants at Ta-Laio area, Taiwan, 1984[a]

	Protected with HA 6-1			Unprotected		
Month of sampling	Symptomless fruit	Spotted fruit	Deformed fruit	Symptomless fruit	Spotted fruit	Deformed fruit
August	13.0	11.5	9.2	12.0	11.1	8.5
September	11.5	11.0	8.1	10.0	10.5	8.2
October	11.1	11.3	9.2	10.5	10.6	9.1
November	9.7	8.6	8.3	...[b]	8.7	8.5
December	8.0	6.6	6.3	...	7.0	6.5

[a] Fruits were harvested once every 3 days and divided into three categories. Sugar content of four randomly selected fruits from each category was recorded. The data reflect average Brix readings of fruits from each month (means of 40 fruits).
[b] Fruits not available.

infected papaya orchards nearby, the cross-protected and unprotected plants were interplanted, and severely diseased plants were not rogued. Consequently, most protected trees became severely infected before the flowering stage. This resulted in poor fruit production.

One of the major concerns for practical application of cross-protection is whether the mild strain would cause severe damage to the protected crop and to other crops in the vicinity. PRV HA 5-1 and HA 6-1 were previously reported to cause mild or symptomless infection on Kapoho, the most important papaya cultivar grown in Hawaii (19). In this study, our greenhouse results showed that neither mutant caused visible damage to the major cucurbitaceous plants and papaya cultivar Tainung No. 2 of Taiwan. These results indicate that the possibility of HA 5-1 and 6-1 causing damage to papaya and commercial cucurbits in the field is minimal.

The effect of the mild mutant on fruit yield was observed only at the Ta-Liao test field. It was not possible to determine the effect that PRV HA 6-1 alone had on fruit yield because comparable healthy plants were not available. However, from the number of symptomless fruit produced and the higher sugar content of the protected fruit, it seems that damage caused by the mutant alone is minimal compared with reduction in fruit yield and quality caused by severe PRV strains, especially if infection occurs before the flowering stage. In this study, only the most popular papaya cultivar, Tainung No. 2, was tested. The mutants may behave differently on other cultivars.

The stability of the mild strain is another factor that must be considered in the practical application of cross-protection. PRV HA 5-1 and HA 6-1 were obtained in 1982 (19) and have been investigated intensively under greenhouse conditions. In all tests of manual and spray inoculations, we have not seen severe symptoms caused by possible revertants. However, we have had a very few cases where *C. metuliferus* and papaya developed severe symptoms after being inoculated with extracts made directly from long-term-stored dry tissue of mild strain-infected *C. metuliferus*. We have no explanation for this. It may be that some of our inocula of the mild strains that were stored were actually contaminated with severe strains and that these strains survive storage much better than the mild mutants. Thus, one would always need to take the necessary precautions, continually evaluating the actual inocula used for the cross-protection studies.

The mild mutants used in this study were derived from PRV HA, a severe strain from Hawaii (8). In fact, a Hawaiian strain was chosen for mutagenesis treatments because our cross-protection research on papaya was initially started with the aim of using cross-protection in Hawaii should the Puna district, the main papaya-growing area of Hawaii, become infected with PRV. Fortunately, this has not yet occurred.

Although the Taiwanese and Hawaiian strains of PRV we have tested so far are serologically indistinguishable, they do differ in severity of symptoms on papaya and cucurbits. Thus, in addition to high disease pressure, the early breakdown at Kao-Shu and Feng-Shan might have been due to the fact that the mild mutants that we used were derived from a Hawaiian PRV strain that is biologically distinct from Taiwanese PRV strains. Our data from this and previous work (19) suggest that HA 5-1 provides better protection against the severe PRV HA than against PRV TW or TM strains from Taiwan. We previously showed that complete protection was observed in 79–93% of the HA 5-1 protected plants that were challenged by PRV HA 26 days or more after mild strain inoculation (19). In this study, complete protection was observed in only 40–70% of the protected plants that were challenged with PRV TW or TM 25 or more days after mild strain inoculation. Mild mutants derived from local PRV strains might therefore provide better protection in Taiwan than PRV HA 6-1 and HA 5-1.

Because papaya is normally propagated by seed, an efficient method for infecting large numbers of seedlings must be available to achieve a practical application of cross-protection. The method of pressure-spray inoculation meets this criterion. The results suggest that one person could inoculate 10,000 seedlings within 2 hr. In Taiwan, the additional cost of spraying is minimal because seedlings are routinely raised in plastic bags arranged in wooden boxes for easy handling during transplanting. It is not practical to assay each inoculated seedling when large numbers of plants are inoculated. To ensure that inoculated seedlings are infected, randomly selected ones could be tested by ELISA. Also seedlings may be inoculated more than once to increase the chances of infection by the mild mutants.

The successful results of the Ta-Liao test suggest that using cross-protection to control papaya ringspot disease could be a key to restoring a higher level of papaya production in Taiwan. Based on these initial trials, the government of Taiwan commenced large-scale solid-block field tests using 244,000 plants (122 ha) in 1984 and 400,000 plants (200 ha) in 1985. Results of these tests look very promising.

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