CARIBBEAN

FOOD

CROPS SOCIETY

31

Thirty First

Annual Meeting 1995

Barbados

Vol.XXXI

THE EFFECT OF PAPAYA RINGSPOT VIRUS ON PRODUCTION AND FUTURE MEANS OF POSSIBLE CONTROL

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ABSTRACT

Papaya production in most parts of the world has been devastated by the papaya ringspot virus (PRV). This disease costs the papaya industry millions of dollars each year. PRV is transmitted by aphids and causes water-soaked lesions and stunted growth of stems and petioles. Infected leaves are mottled and with severe infection become disfigured. The fruit surface develops ringspots and the fruits are reduced in size, ripen prematurely and become non-marketable. Cultural practices, such as windbreaks, intercropping and regular insect pest control, can at best only delay the outbreak of the disease in the area. Cross protection in which mild virus isolates are used to infect susceptible cultivars has shown variable success. By inserting the coat protein (CP) gene of a PRV strain into papayas, plants can be developed that are immunized to the virus. Papayas have recently been genetically engineered with the CP gene from a virulent Hawaiian strain of PRV. The PRV-CP transgenic plants are resistant to the Hawaiian viral strain but susceptible to PRV strains from other geographical regions, including the Caribbean. The University of the Virgin Islands, in cooperation with the University of Puerto Rico and Cornell University, is working to bioengineer resistance to a Caribbean strain of PRV into papaya cultivars grown in the region.

INTRODUCTION

Papaya production throughout the world has been devastated by the papaya ringspot virus (PRV). This viral disease costs the papaya industry millions of dollars each year and can be a limiting factor to growing papaya in areas of Hawaii, Guam, Florida, the Caribbean, Africa, Australia and the Far East. The *Daily Gleaner* newspaper of Jamaica, last year reported that the agriculture ministry had ordered the destruction of 81ha of papaya valued at $600,000 that were suspected to have PRV. Each year, in south-eastern Mexico, 90% of the papaya in plantations dies from PRV (Mora-Agiulera, 1993). In Taiwan, within 4 years of PRV being first reported, papaya production dropped from 42,000 t to 19,000 t and the export markets to Japan and Hong Kong were lost. The Virgin Islands exported papaya until the early 1980s, when PRV destroyed local plantings. Papaya production in the Virgin Islands is presently sporadic with few fruits being sold at local markets or roadside stands. The demand for papaya is high, both at the local markets and at the hotels and restaurants that cater to tourists.

Papaya ringspot virus is a member of the potyvirus group which have filamentous particles (720 nm) with a protein coat surrounding an RNA core. Multiple strains of PRV exist from varying tropical regions around the world. PRV is closely related to PRV-W, formerly known as water-melon mosaic virus 1. The host range for PRV includes papaya, cucurbits such as pumpkin, squash, cucumber and melon and relatives of beet in the Chenopodiaceae family (Yeh et al., 1984). The virus is principally transmitted by aphids but can also be mechanically transmitted. The virus spreads rapidly once it occurs in an orchard. Symptoms of the virus include: water-soaked streaks or lesions on the stems and leaf petioles; mottled and distorted leaves; ringspots on the fruit; premature ripening and reduced flavour; reduced plant vigour, low fruit set and smaller fruits; and finally death of the plant (Purcifull et al., 1984). Once introduced, PRV has never been successfully eradicated from a production region.

CONTROL OF PRV

Presently, 100% control of PRV is not possible. However, certain factors can delay the outbreak of the virus in a papaya orchard. First, locate the papaya field in an isolated area away from other established or diseased papayas. Do not grow other PRV host plants such as squash, melon, cucumber or beet near papaya. Inspect the orchard weekly for signs of the disease and rogue out infected plants until plants begin to flower. Planting a windbreak around the orchard can act as a natural barrier in isolating the orchard from the insect vector.

PEST CONTROL

Routine pest control will inhibit the insect vector and delay the viral disease outbreak. Maintain a regular spray programme for disease-carrying insects and pests. Apply adequate fertilizer for maximum growth and production in a short period of time. By growing strong healthy plants that have not been subjected to stress, disease symptoms can be delayed in a papaya planting.

VARIETAL SELECTION

Natural resistance to PRV has not been found in *Carica papaya* after screening large collections of papaya lines and cultivars (Conover, 1976). Presently, there are no commercial papaya varieties that are resistant to PRV. However, some varieties have been developed with increased tolerance to PRV such as Cariflora (Conover, 1976; Ramcharan, 1993). Papaya varieties that were screened at the Agricultural Experiment Station of the University of the Virgin Islands on St Croix have indicated varying levels of tolerance to PRV (Table 1). The most tolerant varieties, after 9 months in the field, include 356-3, PR6-65 x Cariflora F2 and Cariflora. An advantage we found in planting Cariflora is that flowering may occur within 3 months after planting while Sunrise and Kapoho took 5 and 6 months, respectively. The solo Sunrise related varieties are very susceptible to PRV with limited production potential for PRV infested areas (Ramcharan, 1993). Growers should plant the

most tolerant papaya varieties available and avoid the most susceptible varieties. The use of ground covers in the papaya field can influence the spread of PRV. PRV spread more quickly throughout a papaya field when mowed grass was used as a ground cover as compared to a chipped wood mulch or tilled soil (Table 2). The grass may harbour aphids that spread this viral disease.

CONTROL OF PRV THROUGH CROSS PROTECTION

Cross protection, discovered in 1927, is a system in which plants infected with one strain of a virus are protected from the severe effects of a second related strain of the same virus (McKinney, 1929). The key for practical application of cross protection is the availability of a useful protective virus strain. Nitrous acid was used to induce mutants from the Hawaiian strain of PRV. Two mutants were selected that caused infection with diffuse leaf mottling and no reduction in growth (Yeh and Gonsalves, 1984). Since papaya is propagated by seed, a system was developed to inoculate large numbers of seedlings at the 4 or 5-leaf stage with a pressure sprayer. Initially the growth and fruit set of the inoculated papayas were not affected by the mild mutant PRV strain. The fruit of some plants had a few ringspots that became less apparent at maturity and did not affect the quality of the fruit. The cross protection induced in seedlings persists throughout the plant's life so cross-protected plants do not require reinoculation.

Table 1 Progression of PRV infection through a field of twelve papaya varieties over time

Variety	Total plants	5 months PRV	%	7 months PRV	%	9 months PRV	%
Cariflora	65	1	1.5	17	26.2	28	43.1
SS x CFL F2	41	7	17.1	16	39	27	65.9
Washington	39	0	0	7	17.9	19	48.7
PR 6-65	35	1	2.9	6	17.1	15	42.9
PR 6-65 x CFL F2	32	0	0	6	18.8	11	34.4
Solo 64	25	2	8	7	28	18	72
Barbados Dwarf Solo	23	0	0	10	43.5	15	65.2
Kapoho	23	2	8.7	16	69.6	20	87
Tainung-2	20	2	10	6	30	10	50
Yeun Nong-1	20	2	10	7	35	11	55
356-3	20	2	10	2	10	2	10
Exotica	18	0	0	9	50	17	94.4
Sunrise	13	0	0	5	38.5	13	100
Total	374	19	5.08	114	30.5	206	55.1

Table 2 The influence of ground cover in a papaya field and the spread of PRV infection over time

Variety	Total plants	5 months PRV	%	7 months PRV	%	9 months PRV	%
Grass	118	18	15.3	52	44.1	78	68.1
Mulch	120	6	5	28	23.3	61	50.8
Tilled	136	11	8.1	34	25	67	49.3

Disadvantages of cross protection

Under warm conditions, above 25 °C, papayas inoculated with the mutant PRV strains grow well and do not show conspicuous symptoms. However, when temperatures drop below 20 °C or during rainy and shady conditions, chlorotic spots appear on the leaves and small ringspots appear on fruit set through this period. As the temperatures rise above 25 °C, new developing leaves and fruit have less apparent viral symptoms. The mild symptoms may be repressed by applying more fertilizer to the trees.

Cross protection can only delay expression of severe symptoms and superinfection by severe PRV strains can occur. If cross protection breaks down before flowering, no economic benefit is obtained. Cross protection break-down occurs when: (i) the challenge virus is introduced into the non-expanding young leaves around the apex; (ii) severe challenge pressure surrounds the protected plants; (iii) severe virus strains different from the parental virus of the mutant exist; and (iv) inoculated seedlings escape infection from the mutant strain (Yeh, 1990). The mutant Hawaiian strain is still too virulent for certain papaya varieties, e.g. Sunrise. Cross protection, when tested in Puerto Rico, was not effective in reducing or controlling PRV.

GENETICALLY ENGINEERED VIRAL RESISTANCE

Genetic engineering or transformation involves the transfer of genetic information from one type of organism to another in a way that permits stable incorporation and expression of the foreign genes in the recipient organism. Researchers have demonstrated that the transfer of a gene for the viral coat protein (CP) into virus-susceptible plants can cause resistance to the viral disease (Gonsalves et al., 1992). Through a joint effort of Cornell University and the University of Hawaii, the CP gene from the Hawaiian strain of PRV has been isolated, sequenced, cloned into a vector and genetically engineered into papaya cells that were regenerated into plants. The presence of the PRV-CP gene in transgenic papaya plants has provided resistance to the Hawaiian strain of PRV. However, these transgenic papaya plants are susceptible to strains of the PRV found in other regions of the world, including the Caribbean.

Through a 1994 Caribbean Basin Administrative Group grant, the University of the Virgin Islands (UVI), in cooperation with Bryan Brunner from the University of Puerto Rico and Dennis Gonsalves from Cornell University, are working to bio-engineer resistance to a local PRV strain into papaya cultivars grown in Puerto Rico and the Virgin Islands. Zygotic embryos, from 90-114 days post-anthesis fruits, were cultured on Murashige and Skoog (1962) medium with 40 mM 2,4-D, 2.73 mM glutamine and 175 mM sucrose (Fitch and Manshardt, 1990; Fitch et al., 1993). This medium formulation has been successful on 14 papaya cultivars. Depending on the papaya cultivar, somatic embryos were formed within 4 to 8 wk. After 8 wk, somatic embryos are matured and germinated on 2,4-D free medium.

The coat protein gene has been isolated from PRV infected leaves found in the Virgin Islands and cloned into a vector plasmid construct at Cornell University. The vector plasmid contains the genes for PRV-CP and neomycin phosphotransferase II. This new PRV-CP vector plasmid was obtained by UVI and electroporated into *Agrobacterium tumefaciens*. Cocultivation of 1-week-old embryo cultures with A*grobacterium tumefaciens* containing PRV-CP plasmid is now underway at UVI. Selection for putative transgenic embryos will be on kanamycin-containing medium. Transgenic papaya plants will be evaluated for PRV resistance under greenhouse and field conditions. The final goal of this collaborative research is to develop locally grown papaya cultivars that are immunized against the Virgin Islands strain of PRV by bio-engineering transgenic papayas with the coat protein gene from PRV.

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