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Papaya Ringspot Virus: Disease Eradication and Cross Protection for an Important Subsistence Crop in Northeast Thailand

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PREFACE

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Technology Documentation Series

Documentation of technologies considered appropriate for rainfed agricultural development in Northeast Thailand

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Descriptions and methods of use of proven methodologies and techniques for the planning, analysis and evaluation of research and extension activities for rainfed agriculture.

Problem Definition Series

Situation papers on the problems or constraints currently facing rainfed agriculture and farm families in Northeast Thailand.

All papers in these series are listed in the Appendix of this report and are available on request from the Project Director. The papers are updated at appropriate intervals and NERAD invites comments and discussion from readers on any topic covered in the reports.

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PAPAYA RINGSPOT VIRUS: DISEASE ERADICATION AND CROSS PROTECTION FOR AN IMPORTANT SUBSISTENCE CROP IN NORTHEAST THAILAND

BACKGROUND AND JUSTIFICATION

Papaya (Carica papaya L.) is grown throughout the Northeast as the most important subsistence crop after rice. It is used by the Northeasterners to produce "Somtam", a green papaya salad that is eaten on its own or as a side-dish with rice and other foods. Although papaya is grown throughout the region, total fruit production is insufficient to meet demand and large amounts are imported daily from the Central Plains and sold at a price that reflects transport costs, etc. (Prasartsri et al., 1985).

Papaya ringspot virus (PRV) was discovered in Northeast Thailand in 1974 and is now the most serious limitation to papaya production in the Region. It belongs to the largest and economically most important group of plant viruses. Viruses in this group, often called the potyvirus group, have a flexuous rod shaped morphology of about 700-850 nm in length and generally have a narrow host range, although a wide range of crops are infected by viruses in the group. PRV infects plants in the Cucurbitaceae family, and genus Carica. PRV also causes the most widespread and economically damaging virus disease of papaya on a worldwide basis (Yeh and Gonsalves, 1984).

PRV can infect papaya at any growth stage being transmitted by many aphid species in a nonpersistent manner. The most important vectors are Aphis gossypii (Glov.), A. craccivora (Koch.) and Hysteroneuria setariae (Thom.) (Prasartsri et al., 1985). Infection produces mosaic patterns and distortion of the leaves, ring spots on the fruit and leaves and streaks on the stems and petioles. Diseased plants are stunted and fruit production is drastically reduced. The disease can also be transmitted mechanically but there is no recorded case of transmission by seeds.

Work on PRV has been conducted at the Northeast Regional Office of Agriculture (NEROA) since 1979 and eradication programs have been tested with very variable success rates in a number of villages close to the Center since 1983. In 1984, an RRA with the objective of identifying the most important pest control problems faced by farmers in the Northeast was conducted by members of NERAD's Pest Management Working Group (Katanyukul et al., 1987). As a result of this survey, PRV was identified as high priority research topic and a short-term consultancy was funded by NERAD to obtain the services of Dr. Dennis Gonsalves of Cornell University to assess the potential for using cross-protection as a control measure for PRV in Northeast Thailand. His initial findings were promissing (Gonsalves, D., 1986) and work on cross-protection was initiated under NERAD.

This report, presents a summary of the efforts which have been made to control the disease by eradication and cross protection programs.

RESULTS AND FINDINGS

1. Eradication Programs

Eradication in virology terms refers to the removal of plants infected with a particular virus from a specific geographical area. The area may involve an entire country or a small village. The success of this control measure is dependent on protecting the eradicated area from reinfection, or minimizing the rate of reinfection. This practice appeared possible for controlling PRV in the Northeast since papaya is usually only grown in the backyards of homes in villages which are isolated geographically by stretches of rice paddies or other barriers to the vector.

In 1981, NEROA began eradication experiments in an effort to control PRV infection in villages. The data showed that elimination of infected papaya kept some villages free of PRV infection for as much as two years. Although villages often became reinfected within the first year, the infection incidence was low, which subsequently enabled villagers to harvest a good crop of papaya during the first year. In such villages, subsequent papaya plantings were not as successful because villagers often did not continue to eradicate infected trees before planting new seedlings. In general, the experiments indicated that a diligent eradication program would allow good papaya production for at least a year, but that villages usually became reinfected within the first year. The reluctance of villagers to cut down infected papaya plants which were bearing good fruit has prevented the program from receiving widespread application in the northeast, up until 1985.

However, in 1985 the Governor of Mahasarakarm Province initiated a large scale eradication program. Starting in December of that year, infected papaya trees in villages of the province were cut down and burned in preparation for planting of new seedlings in April, 1986. About 965 of the 1,130 villages of Mahasarakarm province took part in the eradication program. This involved the cutting and burning of over 285,000 infected papaya trees and the planting of 503,000 papaya seedlings (Table 1).

Table 1. Summary of papaya ringspot virus eradication program of Mahasarakarm province, 1986.

Amphur	No. Tambon	No. Villages	No. Farmers	No. Plots Eradicated	No. Plots Planted
Muang	9	73	6817	32718	49819
Borabu	13	159	14447	28909	61650
Kosumphisai	10	82	9856	31309	57311
Payakphamphisai	16	129	10904	26374	60694
Nachiak	9	102	8127	23942	34990
Chiang yuen	8	87	12406	42802	65989
Kuntharawi chai	9	122	10362	38813	58290
Nadoon	7	73	5074	19223	32611
Wapipathum	11	98	9594	30883	67584
Kaedum	5	40	2614	10114	14718
TOTAL	97	965	90201	285087	503656

Source: DOAE, 1986

This was not only the largest PRV eradication project in Thailand but also in the world. Some of the villages of this province were surveyed in November 1986 by the consultand and the results appeared very promising (Table 2).

Table 2. Survey for papaya ringspot disease of papaya in four villages of Mahasarakarm province in which diseased papaya trees had been eradicated prior to April 1986. Survey taken November 1986.

Village	Total No. Plants	No. Infected Plants	Percent Infection
Ban Sansuk	1362	28	2.1
Ban Khing Kang	1920	36	1.9
Ban Nong Hai	898	14	1.6
Ban Loew Ngong	919	110	12.0

The villages have very good fruit production, probably for the first time in several years. Infection ranged from 1.6 to 12 percent; and the degree of infection varied from village to village. Reinfection probably occurred because all plants from a village were not eradicated or because infection came from plants which were outside the village but close enough for aphids to

transmit the virus to plants within the village. In general, the project in Mahasarakarm province has shown that eradication will improve papaya production in the first year, at least.

Next year, the Governor is embarking on a very ambitious program to increase papaya production by encouraging each family to plant a minimum of 5 trees. Also, papaya trees will be planted in schools and other public places. When infected trees are not cut prior to planting, it can be assumed that infection will increase. However, it is likely that Mahasarakarm province will have good papaya production for 1987 because the incidence of PRV will be generally low at the start of planting in April. The low incidence of PRV is due to the eradication efforts made in 1985 and 1986.

2. Cross Protection

Cross protection, as defined in the context of this work, is "the use of a mild strain to protect plants against economic damage caused by a severe strain(s) of the same virus." The mild strain is often referred to as the 'protecting' strain and the severe strain as the 'challenging' strain. A prerequisite for cross protection is the availability of a suitable mild strain, which is evaluated in relation to its ability to limit economic damage relative to the effects of the natural severe strain(s) of the virus. The general procedure in a cross protection trial is to infect the plants (in this case papaya), with the mild strain, and then inoculate them with the severe challenge strain. The plants are then observed, to see if cross protection has occurred fully, partially, or not at all. Additional control experiments help to determine the effects of the mild strain alone, severe strain alone, and challenge by the severe strain under various conditions.

Because of the devasting effects of papaya ringspot disease in Northeast Thailand, it was decided that cross protection should be investigated as a control measure. Experiments were begun in June 1986. Two mild mutants of PRV, which originated from a severe PRV strain from Hawaii, were introduced as potential mild strains. The aims of the experiments were:

1) to test the reaction of the mild strains (HA 5-1 and HA 6-1) on Thailand papaya,

2) to establish optimal conditions for infecting papaya with the mild strains at the NEROA station in Tha Phra,

3) to determine the cross protection effectiveness of the mild strains against PRV isolates from Thailand by mechanical and natural infection, and

4) to start limited field trials using the mild strains.

Numerous experiments showed that infection of papaya under Thailand conditions was most efficient if plants were kept at temperatures of 75-80 degree Farenheit (Table 3), during the summer months. Less than 5 percent of inoculated plants which were kept at 90-100 degree F. in a screenhouse became infected by the mild strains. Inoculated plants kept in the laboratory at 75-80 degree F. resulted in infection rates of 80-90 percent. Following these successes for infecting papaya, experiments were done to evaluate the mildness of HA 5-1 and HA 6-1 as compared to the severe PRV of Thailand. As expected, the mild strains were much milder than the severe PRV strains.

Table 3. Infectivity of PRV HA 6-1, HA 5-1, and TH on papaya under cool and hot conditions.

Experiment	Virus	Ratio of positive ELISA/ No. inoculated	Percent
	75-80 Degrees Farenheit		
I	HA 5-1A	30/76	39
	5-1B	44/50	88
	6-1A	49/83	59
	6-1B	64/76	84
II & III	HA 5-1	80/100	80
	6-1	103/140	74
	TH	30/30	100
	90-100 Degrees Farenheit		
I	HA 5-1A	0/110	0
	5-1B	1/32	3
	6-1A	0/95	0
	6-1B	1/100	1
	TH	44/45	97

Next, experiments were conducted to determine the effectiveness of the mild HA 5-1 and HA 6-1 strains in protecting plants against severe effects of PRV from Thailand (TH) following mechanical inoculation of PRV TH. Table 4 shows that the degree of effectiveness is dependent on "inoculum pressure." For example, good protection was obtained when one leaf of the protected plant was challenge inoculated; whereas, very poor protection was obtained when all leaves were inoculated. Since inoculation of papaya under natural conditions is caused by aphids, a better evaluation of the protective ability of the mild strain is obtained under field conditions. In one trial where protected and healthy plants were planted at NEROA and subjected

to natural infection, the protected plants showed a much lower incidence of infection by severe strains than did comparable healthy plants (Table 5). Thirteen out of 20 healthy plants became severely infected whereas only 1 out of 13 protected plants showed severe infection.

Table 4. Papaya Plants without severe symptoms after challenge with severe PRV TH at different leaf positions.

Treatment	Days after challenge	Leaf (position) challenged 3	3,4	3,4,5	all
HA 5-1	0	5	5	5	5
	10	5	5	5	3
	21	4	3	0	2
	30	4	2	0	0
HA 6-1	0	5	5	5	5
	10	5	3	1	4
	21	5	2	0	0
	30	4	1	0	0
Healthy	0	5	5	5	5
	10	0	0	0	0
	21	0	0	0	0
	30	0	0	0	0

Table 5. Plants without severe symptoms after mechanical and natural challenge while grown under field conditions.

Treatment	No. plants	Days after transplanting 0	12	46	86
Mechanical challenge:					
Protected + TH	13	13	0	0	0
Healthy + TH	9	0	0	0	0
Natural challenge:					
Protected + TH	13	13	13	12	12
Healthy + TH	20	20	20	9	7

A further trial was conducted to determine the effect of the mild strains on the growth of papaya in the absence of severe PRV infection. A plot of protected plants was established close to

NEROA at Tha Phra. This was an isolated plot which did not have other papaya plants growing in the near vicinity. Other plots containing only healthy plants and plants inoculated with severe PRV were established on the NEROA station at Tha Phra. The protected plants grew as well as the comparable healthy plants in the early stages. Some protected plants showed mild mottling, which is typical of mild strain symptoms on papaya. The plants are flowering and should have mature fruit by March 1987. Plants which were inoculated with the severe PRV strain were very stunted and will not produce any fruit. This trial should give important data on the effect that the mild strains have on yield of papaya.

Several field trials were established in villages in order to provide preliminary information on the feasibility of cross protection for increasing papaya production. Trials were established in villages which had been eradicated of diseased papayas, in villages which had about 50 percent infection, and in villages where infection was 100 percent. Protected plants were established in these villages about 2 months ago, so it is too soon to observe any trends. More data will become available in the next few months when the plants set fruit. Information from these trials will also assist in the planning of larger scale village experiments for the 1987 planting season.

3. Distribution of PRV in cucurbits

As noted earlier, PRV also infects cucurbits and because many cucurbits are also grown extensively in the region, it is important to determine the prevalence of PRV in these plants because they could serve as resevoir hosts for subsequent infection of papaya. If cucurbits play an important role, then the feasability of eradication practices would be questionable.

It should be noted that PRV is classified into two forms, namely PRV-p and PRV-w. PRV-p is the type that infects cucurbits and papaya while PRV-w infects cucurbits but not papaya. The two types are serologically identical; so the serological test (ELISA) which is used to detect PRV in cucurbits cannot be used to distinguish between PRV-p and PRV-w. Hence, inoculations must be made to papaya.

From June to October 1986, numerous cucurbit samples were collected from villages and tested by ELISA to determine which cucurbits were infected with PRV.

Table 6. Serological Detection of PRV in Cucurbits

Cucurbit	Number of Samples	Number ELISA Positive
Pumpkin	98	53
Cucumber	103	2
Watermelon	48	1
Wax gourd	31	1
Snake gourd	28	17
Luffa sp.	67	33
Coccinia indica (gourd)	33	29
Thai melon	43	8
Bitter gourd	26	11
Bottle gourd	4	0
Cantaloupe	13	0
TOTAL	494	155

Data collected from 16 provinces, 92 villages.

Samples which reacted positively for PRV were subsequently inoculated to cucurbits and papaya, to distinguish between PRV-p and PRV-w. A total of 494 samples were collected from 92 villages in 16 provinces of the Northeast. Eleven types of cucurbits were sampled. PRV was detected serologically in nearly all of the cucurbit types and in all of the provinces tested. However, tissue extracts from those samples which gave positive serological reactions to PRV were not able to infect papaya. Several important observations and conclusions can be drawn from this study:

1) many cucurbits that show symptoms in Northeast Thailand give positive serological reactions to PRV,

2) distribution of cucurbits which give positive serological reactions to PRV are not correlated with the ringspot disease of papaya (See Table 7),

3) infectivity data indicate that most of these cucurbits are infected with PRV-w (the type that does not infect papaya), and

4) there is no evidence that cucurbits serve as a primary virus source for infection of papaya.

Thus, the data indicate that papaya is the most important virus source for the disease on papaya. This information will help immensely in planning eradication and cross protection strategies for the Northeast region of Thailand.

Table 7. Summary of PRV Survey Results.

PRV in Papaya:	Number of Villages	Reacting Positive to ELISA Papaya	Cucurbits
Not Observed	27	0/27	27/27
Eradicated	3	0/3	3/3
Observed	62	0/62	62/62

FURTHER RESEARCH REQUIRED

Large scale inoculations of the mild strains to papaya were conducted in April 1987 and experiments were initiated in 18 villages to assess the potential for integrating cross protection with eradication for ringspot virus control. Experimental treatments comprised: cross protection combined with eradication (6 villages); cross protection only (6 villages) and readication only (6 villages). Because of the severe drought experienced this year, many of the papaya seedlings died and this will undoubtedly affect results. However, it is anticipated that there will be sufficient data to determine the potential for integrated control as means of controlling ringspot when the results of this experiment are available later this year once fruit harvesting begins.

Data from other countries (Taiwan especially) show that cross protection is even more effective when it is combined with the use of a tolerant papaya variety which was selected in Florida. This Florida line is now being tested at NEROA for its productivity and potential for making cross protection more effective in Thailand. This line is also being used as a genetic base for breeding papaya which are suitable for Thailand conditions. Early work has shown that the taste and eating quality of the Florida papaya meets local requirements, that it is capable of good growth under Northeast conditions and is tolerant to the Thailand severe strain of the virus. However, the fruit shape is round and long fruits are preferred by the Northeasterners and therefore breeding work has been initiated to cross the Florida tolerant type with the local Thai type of papaya in order to produce a fruit shape acceptable to local consumers while retaining the former's tolerance to ringspot.

Data indicate the HA 5-1 and HA 6-1 mild strains protect against Hawaiian PRV strains better than it does against PRV strains from Taiwan and from Thailand. In Taiwan, the use of HA 5-1 as a protecting strain has allowed farmers to produce papaya economically. It is hoped that the same will be true for Thailand but efforts are also being made to select mild strains

which are derived from PRV strains from Thailand. Ten attempts at chemically inducing mutant mild strains have been conducted to date, without success. One mutant posessing mild strain characteristics was isolated but it later reverted to the severe strain and chemically inducing mutations will therefore have to continue

If promising research results continue to be obtained, then an extension program will be initiated in 1988. However, thorough research must be completed before extension can begin and if problems are encountered during the trials that are currently being conducted, then the extension phase will be postponed until the problems can be solved.

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APPENDIX

The following NERADICS Working Papers are available on request from the Project Director:

NERADICS Problem Definition Series

P1 Effects of Paddy-bund-planted Eucalyptus Trees on the Performance of Paddy Field Crops. Craig, I.A. and Wasunan, S., 1987. (English)

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P3 The Upper Paddies in Northeast Thailand: The Current Situation and Implications for Development. Craig, I.A., and Baker, G.P., 1986. (English)

P4 Current Pest Management Problems Facing Farmers in Northeast Thailand: Key Research and Development Priorities. Katanyakul, W., Amaritsut, W., Keerati-Kasikorn, M. and Craig, I.A., 1987. (English)

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T0 Executive Summary: NERAD Promising Technologies. Thamabood, S. (Editor), 1986. (Thai)

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T3 Modified Shallow Wells: a Farmer Developed Technology for Northeast Thailand. Craig, I.A., Phensupha, N. and Ragland, J.L., 1986. (English)

T4 Pre-rice Green Manuring: a Technology for Soil Improvement Under Rainfed Conditions in Northeast Thailand. Craig, I.A., 1987. (English)

T5 Papaya Rinspot Virus: Cross Protection for an Important Subsistence Crop in Northeast Thailand. Gonsalves, D. and Prasartsri, V. (English)

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M3 NERAD Project Agricultural Development Information and Coordination System (NERADICS): A Project Description. Hopkins, J., 1987. (English)

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M5 Key Characteristics of the NERAD Full-cycle, Integrated Development Models. Songlin, R., 1987. (Thai)

M6 The NERAD Logical Framework: a Project Design Summary for Planning, Monitoring and Evaluation. NERAD, 1987. (English)

M7 Crop Protection and IPM for Rainfed Cropping Systems in Northeast Thailand. Amaritsut, W., Prasartsri, V. and Craig, I.A., 1987. (English)