

Rice Tungro Disease Management



Edited by T.C.B. Chancellor,
O. Azzam, and K.L. Heong

IRRI

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1999

IRRI

INTERNATIONAL RICE RESEARCH INSTITUTE

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Foreword

The intensification of rice production that began in many countries in Asia during the 1960s resulted in clear benefits for both producers and consumers. Significantly higher grain yields led to increased incomes for farmers and the production gains met the needs of rapidly expanding populations. The new technologies that were associated with this process, however created certain unintended consequences. Some pests and diseases that were previously considered to be unimportant suddenly became major threats to the stability of rice production. Large-scale outbreaks of an unknown virus disease caused massive production losses and seriously affected the livelihoods of rice farmers. This disease, which came to be known as “tungro,” remains a serious problem in intensively cultivated irrigated areas in South and Southeast Asia.

The traditional approach to managing rice tungro disease has been through the use of insecticides to control the leafhopper vector and through the deployment of resistant varieties. The adverse effects of insecticide applications on human health and on the environment, however, are well documented and this approach has severe limitations. The development of resistant varieties, in which IRRI played a leading role, helped to reduce tungro incidence but in some areas the resistance was short-lived due to shifts in the virulence of the vector. Consequently, it was recognized a decade ago that new approaches to managing tungro were needed.

IRRI has long sought to embrace and promote an ecological approach to pest management. Integrated pest management (IPM) uses a combination of control methods to achieve a reduction in insect numbers or disease incidence to levels at which crop damage is minimized. In line with its efforts to help farmers reduce their dependency on insecticides, IRRI has been actively researching appropriate IPM strategies for tungro disease management. Host-plant resistance remains the cornerstone of such strategies and an exciting new development is the availability of advanced lines with resistance to rice tungro viruses. The application of biotechnology to breeding for tungro resistance offers additional opportunities for using diverse mechanisms of resistance. Nevertheless, recent findings on the genetic variability of tungro viruses call for the strategic deployment and monitoring of genes for tungro resistance.

This proceedings summarizes recent research results presented by plant breeders, entomologists, virologists, and sociologists at a workshop on tungro disease management held in Los Baños on 9-11 November 1998. Much of this work was conducted by scientists from IRRI and the Natural Resources Institute of the University of Greenwich in collaboration with partners from India, Indonesia, and the Philippines. Significant progress has been made in key areas of research and studies have been undertaken to ensure that research outputs meet the real needs of rice farmers. Understanding how farmers view tungro, and which factors influence their disease management decisions, is crucial to the development of effective control strategies and much useful information has been gained in this area. It was also encouraging to witness in this workshop a move from tactical control methods such as insecticide sprays to strategic approaches to pest management. Coordinated management strate-

gies implemented by groups of farmers that take into account the whole spectrum of pest and disease problems in a given locality are needed if substantial impact is to be achieved. These changes reflect the clear commitment of IRRI and its partners to increase yields and protect farmers' livelihoods through sustainable production technologies.

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RONALD P. CANTRELL
Director General
International Rice Research Institute

Preface

Providing farmers with options to make rational decisions to manage tungro has been the primary goal of recent research conducted by IRRI in collaboration with partners at the Natural Resources Institute in the United Kingdom and in various research institutes in South and Southeast Asia. A workshop on rice tungro disease management was held on 9-11 November 1998 at IRRI headquarters. The objective was to review the progress made in these research activities conducted in the region in the light of the array of crop management constraints faced by rice farmers. Farmers' varietal preferences and the difficulty they face in modifying cultural practices such as planting dates are highlighted in two papers as being of particular importance in developing appropriate tungro management strategies. Similarly, the need to integrate tungro control measures into an overall pest management strategy practiced at the community level is emphasized.

The development and field evaluation of rice varieties with resistance to rice tungro viruses has been a major focus of recent research as reported by papers describing resistance breeding programs in the Philippines and Indonesia. The potential impact of new virus-resistant lines in reducing tungro incidence in endemic areas is convincingly demonstrated in papers summarizing field trials in these two countries and in India. A cautionary note is sounded in a paper that shows the degree of variation that exists in tungro viruses at the molecular level and suggests that serious consideration needs to be given to how best to deploy the genes with virus resistance. Further breeding work is needed to improve the agronomic characteristics of some of the most promising elite lines, but one line is currently being evaluated by the Philippine Seedboard for release as a stopgap variety for endemic areas.

Genetic engineering approaches to complex diseases, such as tungro, offer an ability to transfer single genes without any linkage to other traits and provide an opportunity for introducing novel genes that might increase the durability of resistance and improve agronomic characteristics. In collaboration with IRRI, several institutions have explored these approaches and rice varieties such as IR64, TN1, TP309, and Kinuhikari were successfully transformed with various antiviral strategies to control tungro. Evaluation of 71 transgenic lines is reported here and results show that effective protection mechanisms must be directed against highly conserved functions or sequences to be successful. Such findings stress the need for a better understanding of the molecular biology of both viruses—rice tungro bacilliform virus and rice tungro spherical virus.

The role of vector control in tungro management was a lively subject of debate at the workshop. Opinions remained divided, as can be seen from the different perspectives presented in the papers on this subject. It was agreed, however, that seedbed protection was only justified in specific cases and that category 1 insecticides should not be used for leafhopper control under any circumstances. It is encouraging to note that leafhopper control through insecticides is no longer a central plank in tungro management strategies recommended for Indonesia and the Philippines, countries where the disease remains a persistent problem.

The development of a simple, robust prototype screening kit for rice tungro bacilliform virus was reported in one paper. This tool will greatly facilitate tungro resistance breeding programs and has further applications for epidemiological studies and for field monitoring. Optimization of the screening kit and application of the technique for rice tungro spherical virus diagnosis were seen by workshop participants as future research priorities. Another fertile area that was identified for future work was the mapping of virus resistance genes and the use of marker-aided selection to advance resistance breeding programs.

We hope that this book will provide a resource for researchers and practitioners in the field of rice pest management and that it will stimulate further debate on the most appropriate areas for future research. We acknowledge the contribution of all the participants at the workshop and thank Ms. Ellen Genil for her assistance in organizing the meeting and in typing the manuscripts.

T.C.B. CHANCELLOR, O. AZZAM, and K.L. HEONG

Rice tungro disease in the Philippines

X.H. Truong, E.R. Tiongco, E.H. Batay-an, S.C. Mancao, M.J.C. Du, and N.A. Juguan

The rice tungro disease scenario in the Philippines, research trends, and current extension activities are briefly explained and discussed in this paper. Farmers' perceptions and knowledge on the subject were also included because farmers make decisions for sustaining farm income and protecting the agroecosystem. Some farmer groups have used green leafhopper (GLH)-resistant varieties for the past several years through the SeedNet and Farmers' Field School, yet rice tungro disease (RTD) remains a major constraint to rice production. For the first time in rice technology development, rice tungro virus (RTV)-resistant advanced breeding lines were identified. A few farmers in RTD-endemic areas evaluated these lines on their farms. Seeds of these lines with yields of 3.5–4.5 t ha⁻¹ are being increased and will be deployed as stopgap materials for farmers during the wet season. Some progress had been made in understanding the genotypes of tungro viruses present during recent RTD outbreaks, and the role of cropping practices and infective GLH or virus inoculum during the past RTD outbreak in Mindanao. More collaborative efforts in research and extension have to be undertaken to strengthen farmers' decision making for appropriate RTD management in the context of a sustainable farming system.

RTD outbreaks

The early outbreaks of rice tungro disease in the 1940s in major rice-growing regions in the Philippines had reduced yield by 1.4 million t annually (Serrano 1957), long before the causal organism of the disease was known (Hibino et al 1978, 1991). Major outbreaks in 1962, 1969, 1971, 1975, 1977, 1984, 1986, and 1989 in areas associated with intensive cultivation of early recommended varieties were reported by municipal agricultural officers (MAO) of the Department of Agriculture (DA), and reviewed by Baria (1997). Recent outbreaks occurred sporadically during 1993-98, affecting areas from 900 to 2,700 ha yearly in Mindanao (Table 1). In addition, about 700 ha were affected in Negros Occidental in 1998. Crop loss estimated by the DA provincial agricultural office from the outbreak in Davao del Norte in 1993 alone reached ₱10.6 million (US\$406,494). Most of the affected locations commonly reported were irrigated lowlands under climatic types III and IV. Dry and wet seasons in type III are not pronounced; it is relatively dry from November to April and wet during the rest of the year, whereas rainfall in type IV (>150 mm monthly) is more or less evenly distributed throughout the year. In most cases, rice tungro disease (RTD) outbreaks were attributed to insufficient irrigation water, which prompted farmers to practice staggered planting. The consolidated report of the DA-MAO showed that early infection at the vegetative stage of IR64 and IR60, BPI Ri10, PSB Rc4, 6, 8, 12, 20, Masipag, and Bugos induced severe crop losses varying from 39% to 65%.

No comprehensive information on the agroecosystem during the reported RTD outbreaks was available. The only known fact was that recent outbreaks occurred during August-October. The average monthly rainfall during the same period recorded

Table 1. Profiles of major rice production areas in Mindanao and outbreaks of rice tungro disease (RTD) in the 1993-98 wet seasons^a.

Location	Province	Area harvested (ha)	Production (t)	Average yield (t ha ⁻¹)	RTD outbreak	Wet season Aug-Oct
Cotabato basin	North Cotabato	42,030	132,993	3.2	Endemic	
	Sultan Kudarat	38,050	121,807	3.2		
	South Cotabato	36,140	98,544	2.7		
Agusan basin	Maguindanao	52,700	91,931	1.7	—	1996
	Davao del Norte	28,550	93,598	3.3	897	1993
	Agusan del Sur	10,880	33,822	3.1		
Valencia/Pulanghi basin	Agusan del Norte	9,460	26,920	2.9		
	Bukidnon	53,510	188,315	3.5	2,000	1996
Kapatagan area	Lanao del Norte	17,890	63,272	3.5	2,702	1998
					1,200	1997
East Lakeshore	Lanao del Sur	14,390	33,243	2.3		
Banay-Banay area	Davao Oriental	4,320	16,356	3.8		
Hagonoy area	Davao del Sur	12,660	47,695	3.8		
Tagao valley	Surigao del Sur	6,330	16,205	2.6		
Sibuguey River basin	Zamboanga del Sur	53,120	198,079	3.7	2,000	1997

^aFrom "Strategic Rice Areas in Research and Development for Mindanao" and consolidated reports from the Department of Agriculture-Provincial and Municipal Agricultural Offices.

at the Philippine Rice Research Institute (PhilRice) experiment station at Midsayap, North Cotabato, for the past 18 years ranged from 170 to 250 mm, which favors green leafhopper movement. Initial data from light traps at this station showed that the GLH population peak coincided with this rainfall level. Monthly rainfall higher than 250 mm or lower than 100 mm gradually decreased the population. Vector population and infective GLH in relation to weather factors in the RTD outbreak areas must be continuously monitored over the year to obtain a better disease forecast. In an analysis of historical survey data from RTD-endemic areas in Mindanao, Savary et al (1993) reported that a high GLH population coupled with a high proportion of viruliferous vectors associated with specific cropping practices increased RTD incidence. In addition to these variables, the epidemic role of the presence of genotypic variations among rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) (Cabauatan and Koganezawa 1994, Arboleda et al 1997, Yambao et al 1997, Villegas et al 1997) in the endemic areas must be investigated. De los Reyes et al (1998) also found mixed infections and variations in RTBV genotypes during the 1997 tungro outbreaks in Zamboanga del Sur and Lanao del Norte and during the 1998 outbreaks in Negros Occidental and Bukidnon. Identification of the selection factors that influence virus variation in the agroecosystem may be the key to RTD management.

Farmers' perceptions, knowledge, and control practices of RTD

RTD is considered as the most important factor limiting rice production by most farmers in endemic areas. Survey information from 658 farmers in five RTD-endemic and RTD-nonendemic provinces (Albay, Davao del Norte, Davao del Sur, North

Cotabato, and Laguna) during 1995-97 showed that most farmers had experienced the RTD problem and could recognize symptoms of RTD (Warburton et al 1996, 1997, Truong et al, this volume). Only a few farmers (8%), however, were aware that GLH is the vector of RTD. Most of them did not know the relationship between the disease infection and the vector spreading the disease from an infected crop to a healthy one. Consequently, they were not aware of the risks posed by a nearby infected crop, which serves as a source of disease inoculum. They usually associated the spread of the disease to factors such as kind of insect on the crop, water, soil, rain, and others. Some confused RTD with nutrient deficiency symptoms because the causal organisms are not observable. Their assessment of crop losses was based on past crop failures. Control practices focused more on preventive measures for all kinds of insect pests because farmers know more about insects than RTD. Most farmers, whether trained or untrained on tungro management technologies in “hot spots,” intensively used insecticides as a tool against RTD and other pests. Cypermethrin, a pyrethroid compound, was effective against GLH in experimental fields (Batay-an and Mancao’ this volume). Its effectiveness, however, was not always observed by farmers because RTD infection had spread. This control strategy aimed more at producing a clean crop than making farming sustainable although some realized that insecticide use was not always effective in controlling RTD.

Meanwhile, most farmers were reluctant to remove the infected crop because they had already incurred costs in purchasing production inputs. In Negros Occidental, farmers preferred to broadcast salt in RTD-infected fields although this practice has no scientific basis, while others practiced roguing infected plants. Tiongo et al (1998), however, pointed out that roguing as a tactical means of control was not effective because it is usually done late, when infected plants are observed. Infected plants without symptoms remain in the field and serve as virus sources. It is thus a challenge for research and extension workers to look for an alternative control technique and to help strengthen farmers’ skills and efficiency in making decisions on RTD management.

On the other hand, farmers in RTD-endemic areas in North Cotabato are very keen on selecting varieties and establishing the crop for RTD management (Truong et al, Community-based rice pest management, this volume). They have more experience with direct seeding (DS), and they claim that a crop established by this method has a reduced risk of pest infestation compared with transplanting. Trained farmers from the Farmers’ Field School (FFS) had adopted GLH-resistant varieties such as IR56, IR62, PSB-Rc 10, PSB-Rc 18, and PSB-Rc 34 to control RTD. Untrained farmers preferred their selections.

Current trends in RTD research and extension

The use of resistant varieties is a cost-effective component of RTD management. The PhilRice Varietal Improvement Program and the Department of Agriculture have focused on developing varieties with high yield and good quality and, at the same time, resistance to insect pests and diseases. Baria (1997) summarized the protocols on varietal selection of the National Cooperative Test network and approval of recom-

mended varieties by the National Seed Industry Council of the Philippines. From 1968 to 1993, 65 varieties recommended for all rice ecosystems were developed by IRRI, Bureau of Plant Industry, the University of the Philippines Los Baños, and PhilRice (Padolina 1995). GLH-resistant varieties were usually selected (Khush 1977, 1989). Their reactions to RTD in hot spots had changed, however, because of changes in GLH virulence (Dahal et al 1990). Among the 44 varieties recommended for the irrigated lowland, seven GLH-resistant varieties (IR56, IR62, IR72, IR74, PSB Rc 10, PSB Rc 18, and PSB Rc 34) were commonly planted by farmers in RTD-endemic areas. IR56 had the least RTD incidence (2-12%). Only a few farmers adopted IR56, however, because it was susceptible to bacterial leaf blight. As expected, IR64 is widely planted because of its good eating quality and high price in the market although it is now susceptible to RTD.

After an immunological test for RTD indexing was established in 1985 (Bajet et al 1985), considerable progress was made in identifying rice accessions with resistance to RTSV (Hibino et al 1987, 1991). Since then, rice tungro virus resistance in Utri Merah (two recessive genes) and Utri Rajapan (one recessive gene), and its eight breeding lines resistant to RTSV has been identified (Sebastian et al, this volume). Promising breeding lines were also derived from wild rice species *Oryza rufipogon* and *O. brachyantha* (Alfonso et al 1996). Likewise, three advanced breeding lines—IR69705-1-1-3-2-1, IR69726-116-1-3, and IR71031-4-5-5-1—among several others with the donor parents of Utri Merah, ARC 11554, and Utri Merah backcrossed to IR64, respectively, were developed by Angeles et al (1998). They showed resistance to RTSV and tolerance to RTBV in endemic areas in the Philippines, India, and Indonesia (Cabunagan et al 1995, 1998). The first two lines were resistant to RTSV but susceptible to GLH, while the third was resistant to both RTSV and GLH. Preliminary trials at the PhilRice Central Experiment Station (CES), Nueva Ecija, and Branch Station at Midsayap showed that IR71031-4-5-5-1 and IR69726-116-1-3 yielded 3.5-4.5 t ha⁻¹. IR71031-4-5-5-1 produced 3.5-4.8 t ha⁻¹ in farmers' field trials at Bual Norte, Midsayap, during the 1998 wet season. These two lines are ideal stopgap materials for farmers in RTD-endemic locations. Seed samples of these lines were given to farmers during the graduation of the Farmers' Field School and Workshop on RTD Management (Natural Resources Institute [NRI]-IRRI-PhilRice-DA) in December 1998. More collaborative efforts on plant breeding between IRRI and the national agricultural research system will be undertaken to broaden the genetic background of donor parents in the development of RTD-resistant lines, and to cope with the urgent need of farmers for seeds.

Seed production

The Seed Production Program of PhilRice plays a vital role in increasing breeder seeds of approved varieties. It maintains seeds of 63 recommended varieties for research purposes of the members of the National Rice R & D Network such as the Technology Demonstration Farm Project (Gintong Ani, 12 Steps in Rice Production, Grain Production Enhancement Program IV, [DA 1996]). It also provides foundation seeds for the National Rice Seed Production Network (SeedNet) and sells these to

seed growers and farmers to increase the registered seed requirements of the country. The area covered by SeedNet increased from 95 ha in 1994 to 354 ha in 1996. SeedNet continues to be an important partner of the PhilRice program to sustain grain production and distribute registered seed requirements of seed growers and farmers all over the country. Among the 10 preferred irrigated lowland varieties, IR62, IR74, PSB Rc 10, PSB Rc 18, and PSB Rc 34 were in great demand in Mindanao because of their tolerance for the RTD vector.

PhilRice's Communication and Training Division and Seed Production Division have collaborated in coordinating training courses on seed production and producing technical data sheets and information on agrocharacteristics of varieties recommended for different rice ecosystems.

Technology promotion

RTD management is a part of the integrated pest management (IPM) curriculum in the Rice Technology Demonstration (Techno Demo) Project of the Gintong Ani Rice Program of the Department of Agriculture. This project was established to demonstrate the latest rice farming technologies in different locations with high-yielding varieties, nutrient and pest management, and farm machinery components. It is spearheaded by PhilRice in collaboration with other agencies of the DA, local government units (LGU), state colleges and universities, and farmers' groups. Macasieb et al (1996) and Javier (1999) describe the project's technical, coordinating, and implementing protocols, as well as the salient features of the technology package. The initial phase started with 1,000 technology demo farms (1 ha each) in 1995 and expanded to 1,469 in 1996 and 2,940 in 1997, with the size reduced to 0.5 ha each. The project was implemented by farmer-cooperators chosen and supervised by municipal agricultural officers and technicians. The supervisors were graduates of the Season-Long Rice Specialist Course and Training of Trainers on IPM Course. They conducted/facilitated the Farmers' Field School for farmer-cooperators and other farmers. The demonstration farms served as venues for farmer-participants to discuss and try out new technologies. The FFS aims to promote the manipulation of the agroecosystem to minimize the disturbance of natural elements in the ecosystem through integrated pest management (Rola et al 1998). From 1993 to 1996, 2,410 rice FFS were established. Survey data from 308 FFS showed that rice farmers who practiced synchronous planting increased from 57% before the project was implemented to 75%. Their preferred varieties were IR60, IR64, IR74, PSB Rc 10, and PSB Rc 18.

One strategic plan of the second phase of the project started in 1998 to focus on synchronous planting as a fundamental step in pest management. This is now carried out on 207 20-ha "compact" and eight village-wide inbred rice demonstration farms, and on eleven 20-ha hybrid rice farms nationwide. As a backup, PhilRice's Crop Protection Division, in collaboration with its Communication and Training Division, has prepared a series of simple technical materials on different components of IPM to enrich the FFS curriculum. These technical bulletins will be translated into local dialects and made into posters by PhilRice.

Cultural practices in RTD management

Cultural practices are the most realistic way to manage RTD because these activities are close to farmers' experiences. An awareness of synchronous planting among farmers in the community is the key to the success of pest management. Synchrony can only be achieved with sufficient irrigation water. Most farmers in irrigated lowlands in northeastern and northwestern Luzon, where dry and wet seasons are distinct (climatic type I), have been effectively practicing synchronous planting. Planting of the first crop begins in December/January, followed by the nonrice crop/fallow period; the second begins in May/June. Irrigation water is provided only during the 4-mo planting period. Farmers could produce high yields even from susceptible varieties. Even in nonendemic locations in Luzon, staggered planting increased the GLH population at later planting dates (Fig. 1A-F). Accordingly, the cumulative incidence of RTD also increased in late planting months (Fig. 1G, H). On the other hand, staggered planting tended to increase GLH densities earlier in the following crops, resulting in high RTD incidence (Fig. 2).

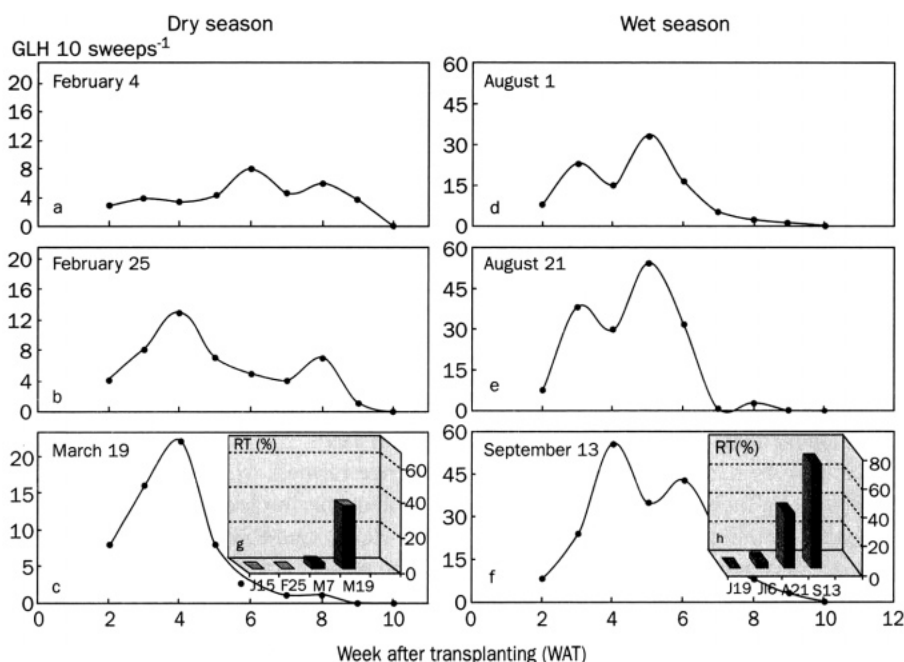


Fig. 1. Population fluctuation of green leafhoppers at different planting dates in the 1994-95 dry season (A-C) and in the 1995 wet season (PF) and the cumulative incidence of rice tungro disease (G and H for dry and wet seasons, respectively) at the PhilRice Experimental Station, Maligaya, Nueva Ecija.

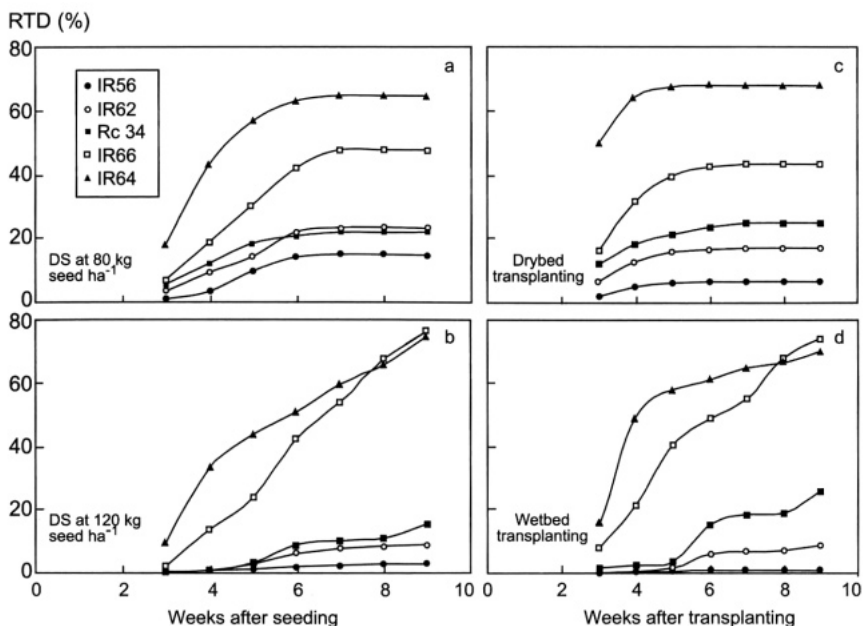


Fig. 2. Incidence of rice tungro disease (RTD) in varieties with different resistance characteristics under four methods of crop establishment. DS = direct seeding.

Synchrony can also be achieved by rotating two rice crops with a nonrice crop such as mungbean, as farmers do in Hagonoy, Davao del Sur. Another important component of cultural practices is the broadcasting method or direct seeding. Crop establishment by direct seeding has become popular in both dry and wet seasons in Midsayap to shorten planting time right after irrigation during land preparation. Farmers consider this method to be easier, faster, and cheaper than transplanting (Truong et al, Community-based rice pest management, this volume). GLH-resistant varieties such as IR56 established by transplanting or direct seeding had the lowest incidence of RTD (2–12%) (Fig. 2A-D). A high seeding rate (120 kg ha⁻¹), however, reduced the infection rate of RTD on GLH-resistant varieties IR62 and PSB Rc 34 and susceptible varieties IR64 and IR66 during the first 4–5 wk after broadcasting (Fig. 2B). Disease incidence on IR64 reached as high as 60–80% as the crop grew older, while disease incidence on IR62 and PSB Rc 34 remained low (8–18%). Likewise, rotation of two to three GLH-resistant varieties after two to three cropping seasons and synchronous transplanting based on communal seedbed preparation were promoted by the Bohol Agricultural Promotion Center (APC), and are being practiced by farmers within the 40-ha contiguous irrigated farms. APC regularly monitored leafhoppers and planthoppers by kerosene light trap, and conducted training for extension workers of LGUs on identifying RTD symptoms and on other diagnostic tests.

Conclusions

RTD has declined markedly in Central Luzon rice production areas since the major outbreak in 1971. However, it remains the most important rice disease in the irrigated lowland ecosystem, especially in locations with insufficient irrigation water and staggered planting practices such as some areas in Mindanao. These are commonly known as “hot spots” or endemic rice tungro areas. Major outbreaks have sporadically occurred in the past 8 yr and affected from 900 to 2,700 ha annually. More research and extension efforts are needed to understand and regularly monitor the agroecosystem to forecast RTD development and to avoid disease outbreaks. RTD management in these areas largely depended on the campaign to practice regular planting with a fallow period or synchronous planting, availability of GLH-resistant varieties, and insecticide application. The introduction of a few advanced breeding lines resistant to rice tungro virus has just begun and is limited to some farmers' groups in rice tungro-endemic villages in Midsayap, North Cotabato.

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Preliminary analysis of genetic variation of rice tungro bacilliform virus in two provinces of the Philippines

M. Arboleda, F. Sta. Cruz, and O. Azzam

A basic understanding of tungro virus populations is a prerequisite for any deployment strategy of conventional or transgenic virus resistance. In the 1996 and 1997 wet seasons, the genetic variability of rice tungro bacilliform virus (RTBV) field populations was monitored in Isabela and North Cotabato provinces of the Philippines. Based on restricted genome DNA profiles and Pearson's correlation coefficient analyses, heterogeneous and distinct RTBV populations were identified in the two provinces. Although members of the populations reoccurred in some sites, the combination of genotypes differed significantly over time, suggesting a rapid evolution of the virus population. This study shows that changes in virus populations need to be continuously monitored to better understand and predict tungro outbreaks and to prolong the life of deployed resistance genes.

In highly intensive irrigated rice ecosystems in Southeast Asia, tungro disease causes considerable yield losses. The rice tungro disease complex is associated with rice tungro bacilliform virus and rice tungro spherical virus. On its own, RTBV causes yellowing and stunting symptoms but it cannot be transmitted by leafhoppers unless RTSV is present. RTBV is a dsDNA belonging to the pararetrovirus group, a group of plant DNA viruses that replicate through an RNA template. A DNA hybridization technique was developed to differentiate the viral genomic DNA of four biological variants of RTBV using total DNA extracts of infected plants (Cabauatan et al 1998). The technique was also applied to examine the variability of natural field populations of RTBV in tungro-endemic areas of the Philippines. Genomic DNA profiles representing single infections based on molecular weight estimates were selected and compared among the surveyed sites.

Materials and methods

Random samples were collected from the tungro hot spot provinces of Isabela and North Cotabato (30–50 samples per field and 4-6 fields per province; Fig. 1). These samples were then assayed by enzyme-linked immunosorbent assay (ELISA) against RTBV and RTSV antisera (Cabauatan et al 1995). Following the procedure developed by Cabauatan et al (1998), total DNA was extracted using a modified cetyl trimethylammonium bromide (CTAB) method.

The pelleted DNA was resuspended in 50 **mL** of sterile distilled water. Three to five micrograms of total plant DNA was then digested with 30–50 units of EcoRV and incubated overnight at 37 °C. The samples were then electrophoresed in 0.8% agarose gel for 5 h at 100 V in 1 x TBE buffer and blotted onto Hybond-N nylon membrane using 2 x SSC. Blots were baked at 80 °C for 2 h prior to hybridization with the full-length RTBV-Ic clone. Chemiluminescence ECL (Amersham) was used for detection following the manufacturer's instructions.

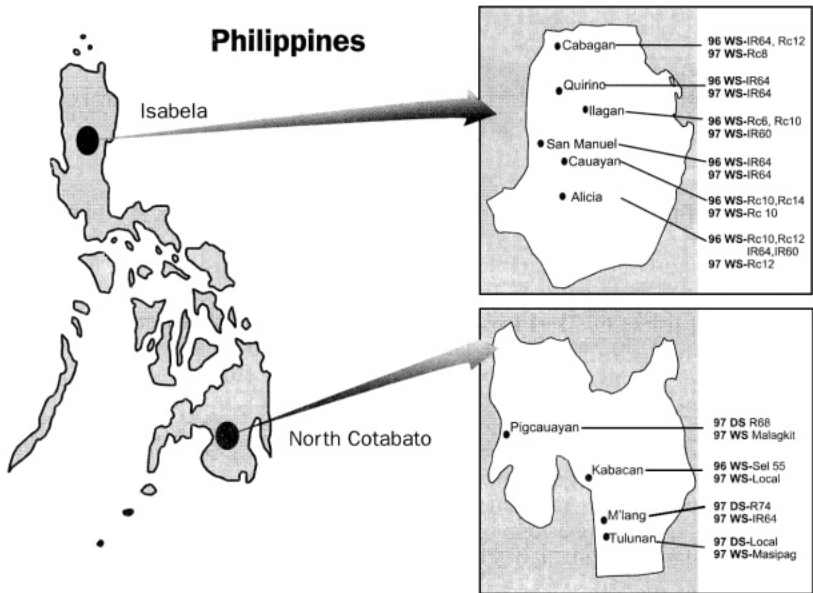


Fig. 1. Sampling sites for the study in Isabela and North Cotabato provinces in the Philippines.

After hybridization, restriction fragment length polymorphisms (RFLP) of different genotypes were used to determine genotype frequencies across locations and times. A dendrogram was also generated using Pearson's correlation coefficient to determine the geographic distribution of the different genotypes.

Results

Following digestion with EcoRV of total DNA extracts from 731 isolates and DNA hybridization, 24 distinct DNA genotypes were identified from Isabela and North Cotabato (Figs. 1 and 2).

High genetic variation was observed among fields and between provinces. Genetic variation was also significantly different over time based on the bulk Chi square analysis of major, minor, and mixed genotypes (Fig. 3, Table 1). Results showed that 2 to 9 distinct genotypes occurred per field and a few dominated at one time. Genotypes 3 and 5 were detected in all sites of Isabela and genotypes 11 and 12 were observed in all sites of North Cotabato. Although some genotypes were common among sites, each site had its unique set of genotypes and genotype frequencies. Data gathered from North Cotabato in the 1997 dry season (DS) exhibited a spatial distribution pattern similar to that of North Cotabato in the 1997 wet season (WS). Between sites, fields sampled in Isabela in the 1996 WS were similar to each other in their set of genotype frequencies, whereas Ilagan and Cauayan in the 1997 WS differed significantly in their set of genotype frequencies.

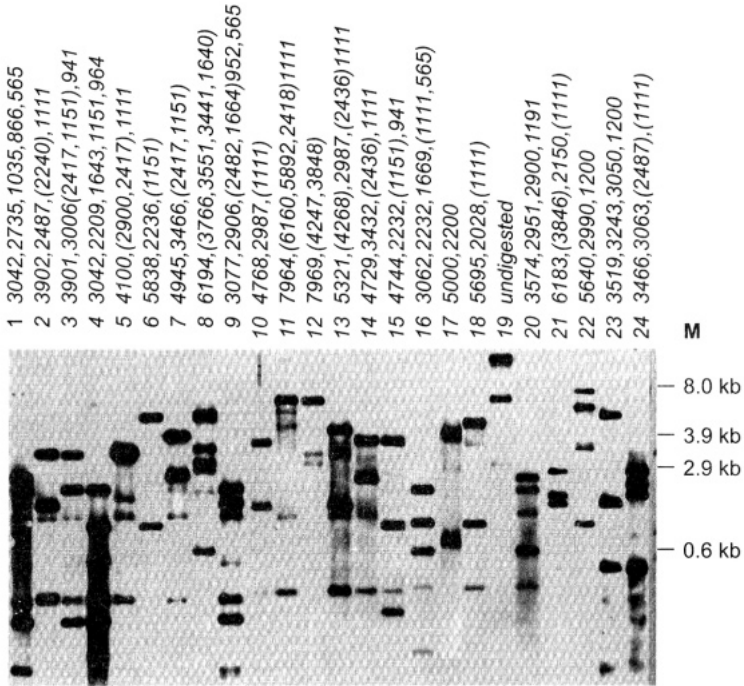


Fig. 2. Distinct rice tungro bacilliform virus (RTBV) genotypes detected in the Philippines. Genotypes 1-9 were initially found in Isabela while genotypes 10-24 were initially found in North Cotabato. Lane marked M is a DNA size marker from the RTBV-Ic3 clone.

RTBV mixed infections (based on the total number of DNA fragments detected >16 kb) were common and accounted for 10–54% of the total sample population. During the 1997 DS, their frequency was even higher than for a single infection in North Cotabato (Fig. 3, Table 1).

Based on the frequency of the genotypes using Pearson's correlation coefficient, RTBV genotypes were distributed geographically (Fig. 4). This showed that the genotype frequencies between Isabela and North Cotabato were significantly different. In Isabela, 10 distinct genotypes (1, 2, 3, 5, 9, 11, 17, 16, 19, and 35) were identified during the 1996 WS and in the 1997 WS. Only five genotypes (3, 5, 9, 19, and 25) were identical to those detected earlier from the same province. Genotypes 3 and 5 generally dominated in the 1996 WS, but these genotypes were augmented with genotypes 9 and 25 in the 1997 WS. A similar pattern was observed in North Cotabato during the 1997 DS and WS. In the 1997 DS, 13 distinct genotypes were observed (2, 3, 10–16, 18–20, and 24) and, in the following season (1997 WS), 11 genotypes were identified (10–16, 18–20, and 24). Genotypes 10–13 were the most frequent in the 1997 DS and remained dominant in the 1997 WS.

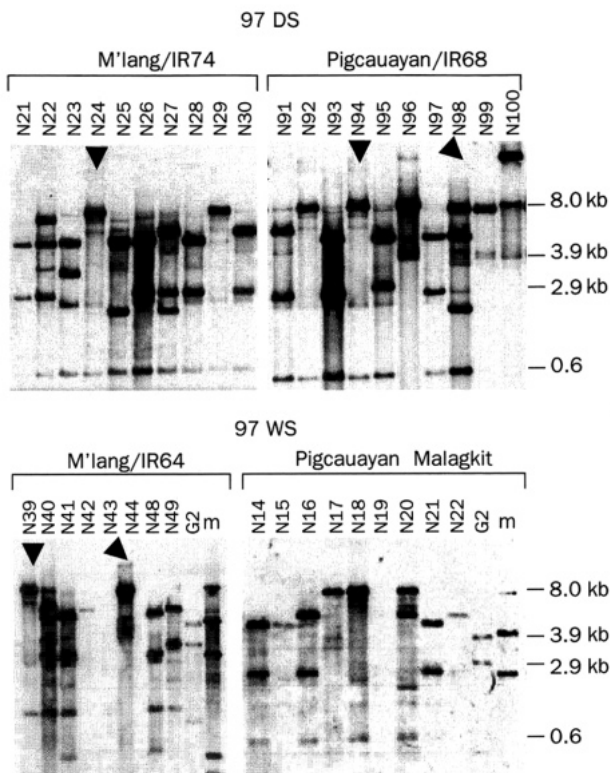


Fig. 3. Representative fields in M'lang and Pigcauayan, North Cotabato, showing the genetic variation of RTBV genotypes between planting seasons (1997 dry season and wet season; 97 DS and WS, respectively), among fields (M'lang vs Pigcauayan) and within fields. Vertical arrows indicate identical genotypes found in the two seasons. Slanting arrows show mixed infections. Lane marked M is a DNA size marker from the RTBV-1c3 clone.

Table 1. Frequency, proportion, and χ^2 for comparing the distribution of major, minor, and mixed genotypes of rice tungro bacilliform virus in North Cotabato, 1997 dry and wet seasons.

Season	Genotypes					Minor	Mixed
	Major						
	10	11	12	13	5		
1997 DS	14 (12.0) ^a	18 (15.5)	14 (12.0)	12 (10.3)	NF ^b (25.0)	29 (25.0)	29
1997 WS	10 (12.0)	22 (15.5)	15 (12.0)	22 (10.3)	10 (7.7)	40 (37.0)	11 (10.2)

$\chi^2=23.17^c$

^aNumbers in parentheses are proportions. ^bNot found in 1997 dry season. ^cFrequency of types differs significantly ($P < 0.01$) across seasons.

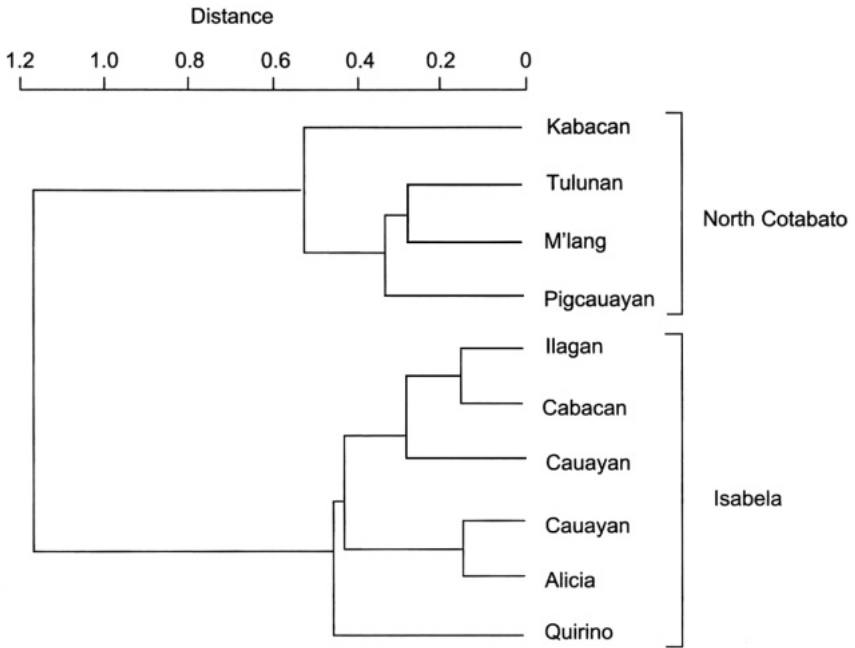


Fig. 4. Dendrogram depicting the relationships among rice tungro bacilliform virus (RTBV) genotypes at different sites in the Philippines. RTBV genotype frequencies were used to calculate Pearson's correlation coefficient between sites, and the correlation matrix was used to construct the dendrogram.

Conclusions

Based on the EcoRV genome profile assay, RTBV populations are genetically variable in the field. The data showed that more than one RTBV isolate could be found in one location. Even with just two planting seasons studied, results showed that RTBV populations exist as a diverse group of variants and are continuously evolving. This study showed that single isolates from a given location are not necessarily representative of or specific to that location. Virus populations need to be monitored continuously to ensure that varietal screening for deployed rice varieties uses appropriate virus isolates in the area.

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Preliminary analysis of genetic variation of rice tungro spherical virus in the Philippines

K.M.L. Umadhay, M.L.M. Yambao, and O. Azzam

This study investigates the genetic variability of rice tungro spherical virus (RTSV) in nine tungro-endemic sites of the Philippines and over the 1996 wet season and 1997 wet and dry seasons. Based on a reverse transcriptase-polymerase chain reaction technique (RT-PCR) followed by restriction enzyme analysis, six distinct genotypes of RTSV were identified and their frequency across all sites determined. Results showed that more than one genotype could exist in a plant and at least two RTSV genotypes are present at one site. Although RTSV population did not change during the sampling, the presence of mixed infections and minor genotypes suggest that the structure and composition of the virus population is not stable. It is essential to continue monitoring these populations over an extended period to identify factors that lead to virus outbreaks or extinction of the current prevailing populations. This approach is critical in achieving durable virus resistance.

In the tungro disease complex, rice tungro spherical virus assists in the semipersistent transmission of rice tungro bacilliform virus (RTBV), the other component, which causes tungro symptoms. Earlier studies showed that rice varieties react differently to RTSV variants. Recently, polymorphic molecular markers were developed to differentiate two variants of RTSV, RTSV-A and RTSV-Vt6 (Yambao et al 1998). These variants are serologically indistinguishable. The molecular markers are based on the amplification of coat protein regions 1 and 2 (CP1 and CP2) of the viral genome using reverse transcriptase-polymerase chain reaction (RT-PCR) followed by a restriction analysis of the PCR product. Using this method, the genetic variation of RTSV natural populations in tungro-endemic regions of the Philippines was investigated in 1996 and 1997.

Methods

Nine tungro-endemic sites were sampled during the 1996 wet season (96 WS) and 1997 dry and wet seasons (97 DS and WS). Figure 1 shows the sampling sites. All samples were tested by enzyme-linked immunosorbent assay (ELISA) against both RTBV and RTSV antisera and ELISA-positive samples for RTSV were processed by RT-PCR and restriction analysis. The RT-PCR of CP regions 1 and 2 included (1) extraction of total RNA using trizol reagent, (2) cDNA synthesis using Superscript II, and (3) PCR using the cDNA as a template mixed with a cocktail of the following reagents: Taq DNA polymerase, primers, MgCl₂, dNTP mixture, and buffer. The reaction conditions were initial denaturation at 95 °C for 3 min, denaturation at 95 °C for 1 min, annealing temperature of 50 °C for 1 min, extension of 68 °C for 5 min, and final extension of 72 °C for 7 min. The generated PCR products were then digested

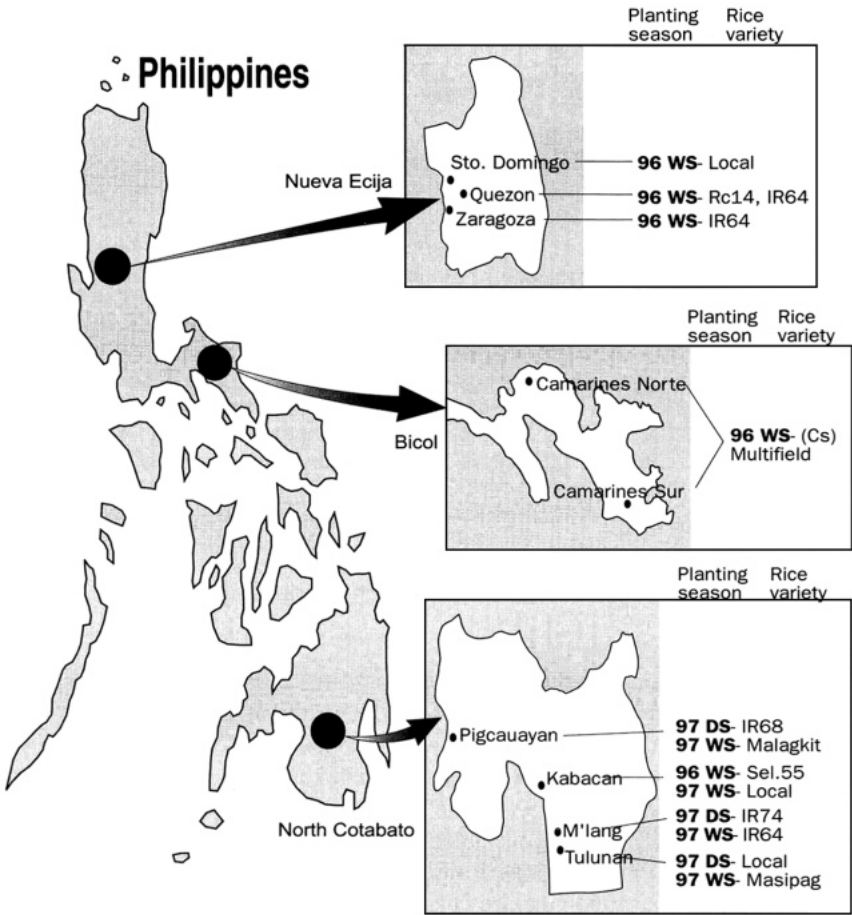


Fig. 1. Sampling sites in tungro-endemic provinces of the Philippines. Planting seasons and rice varieties are indicated on the right side.

with *Hind* III and *Bst* Y I restriction enzymes and run on 1% agarose gel stained with ethidium bromide (Table 1).

Results and discussion

Six distinct coat protein genotypes were identified in the initial 302 analyzed samples from North Cotabato, Nueva Ecija, and Bicol provinces during the 1996 WS and DS, and 1997 WS. The frequency of each coat protein genotype was used as a potential indicator of virus variation per location (Fig. 2). In Nueva Ecija and Bicol, coat protein genotypes II, III, and VI were observed. In North Cotabato, coat protein genotypes II, III, V, and VI were found in the 1997 DS while coat protein genotypes I, II,

Table 1. Size characteristics of the six distinct coat protein genotypes in the natural RTSV population of the Philippines based on restriction analyses of RT-PCR products with *Hind*III and *Bsf*Y1.

Coat protein genotype number	<i>Hind</i> III (kb)	<i>Bsf</i> Y1 (kb)
I	1.15	1.15
II	1.15	1.00
III(RTSV-Vt6)	1.15	0.70, 0.30, 0.20
V	0.58	1.15
VI	0.58	1.00
RTSV-A	0.58	0.80, 0.28
Mixa	Mix	Mix

^aMix = variable and mixed enzyme restriction patterns.

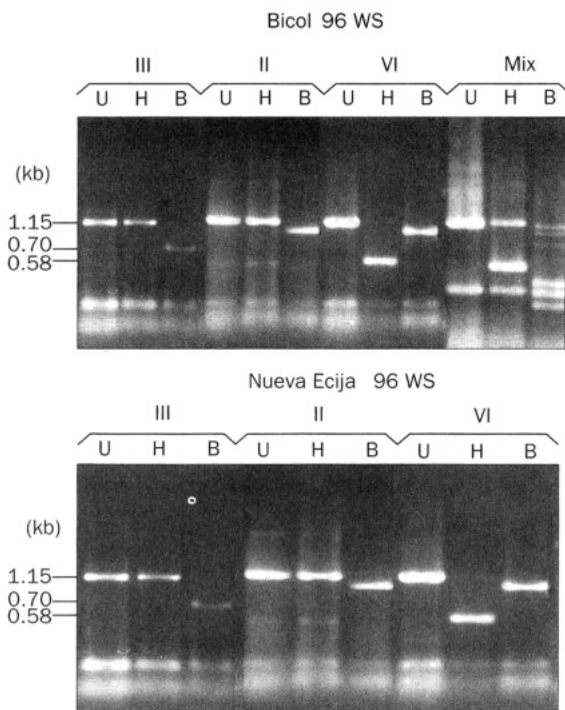


Fig. 2. Diversity of coat protein genotypes in the different tungro-endemic provinces of the Philippines.

III, V, and VI were present in the 1997 WS (Fig. 2). In both the 1996 and 1997 cropping seasons, pattern II dominated the RTSV population in the three locations but minor genotypes and mixed infections were also observed in all locations (Table 2).

Table 2. Distribution of coat protein genotypes of rice tungro spherical virus based on location in the endemic regions of the Philippines.

Location/season ^a	Coat protein genotypes					Mix	PCR (+)	Total analyzed
	I	II	III	v	VI			
Nueva Ecija 96 WS	–	17	2	–	8	–	27	51
Bicol 96 WS	–	4	1	–	1	6	12	12
North Cotabato 97 DS	–	36	5	2	6	17	66	90
North Cotabato 97 WS	4	29	5	1	9	–	48	149
							153	302

^aWS = wet season, DS = dry season.

Table 3. Distribution of coat protein genotypes of rice tungro spherical virus based on variety in the endemic regions of the Philippines.

Variety/location/season	Coat protein genotype					Mix	Total analyzed
	I	II	III	V	VI		
IR74 (M'lang 97 DS)	–	17(56.7) ^a	3(10)	–	–	3(10)	23
IR74 (Bicol 96 WS)	–	–	–	–	–	1(100)	1
IR64 (M'lang 97 WS)	–	18(37.5)	1(2.1)	–	5(10.4)	–	40
IR64 (Nueva Ecija 96 WS)	–	13(41)	2(6)	–	8(25)	–	31
IR68 (Pigcauayan 97 DS)	–	–	–	–	–	5(16.7)	20
PS BRc6 (Bicol 96 WS)	–	–	–	–	1(100)	–	1
PS BRc8 (Bicol 96 WS)	–	–	1(50)	–	–	1(50)	2
PS BRc10 (Bicol 96 WS)	–	3(100)	–	–	–	–	3
PS BRc14 (Bicol 96 WS)	–	1(20)	–	–	–	4(80)	5
RC14 (Nueva Ecija 96 WS)	–	2(29)	–	–	–	–	17
Selection 55 (Kabacan 97 DS)	–	6(20)	2(6.7)	2(6.7)	3(10)	5(16.7)	22
Unknown (Tulunan 97 DS)	–	13(43.3)	–	–	3(10)	4(13.3)	25
Unknown (Nueva Ecija 96 WS)	–	2(67)	–	–	–	–	3
Farmer's 39 (Kabacan 97 WS)	–	1(2.3)	–	–	–	–	31
Masipag (Tulunan 97 WS)	1(1.8)	3(5.4)	4(7.1)	1(1.8)	1(1.8)	–	36
Malagkit (Pigcauayan 97 WS)	3(6.7)	7(15.6)	–	–	3(6.7)	–	42
							302

^a Values in parentheses are frequencies of the corresponding genotypes.

Mixed infections were observed mainly in North Cotabato and Bicol. Coat protein genotype II predominated in both IRRI and local varieties. IR74 and IR64 were susceptible to RTSV coat protein genotypes II and III. On the other hand, local varieties were susceptible to a wide range of RTSV coat protein genotypes (Table 3).

These results suggested that RTSV populations in the field are heterogeneous and more than one RTSV variant could exist in one plant, that at least two variants could coexist at one location, and that the dominant variant may persist over time depending on the selection pressures present in that location. During the sampling, RTSV populations did not change and one genotype seemed to dominate in the Philippines under current conditions. In addition, IRRI varieties were similar to other

varieties and did not seem to exert special selection pressure on current RTSV populations. Because of the presence of mixed infections and minor genotypes, however, our data suggest that the structure and composition of the virus population may change if a different selection pressure such as a host or environmental shift occurs. It is therefore essential to monitor virus populations when virus-resistant varieties are deployed.

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Breeding for rice tungro disease resistance at PhilRice

L.S. Sebastian, E.R. Tiongco, D.A. Tabanao, G.V. Maramara, S. Abdula, and E.B. Tabelin

Breeding for resistance to rice tungro disease is a major objective of the rice breeding program for irrigated lowland and rainfed ecosystems at the Philippine Rice Research Institute. Tungro-resistant donors are identified using visual screening and serological assays and are then crossed with selected high-yielding modern genotypes with good grain quality. Field evaluation of advanced breeding lines is carried out at experimental stations in Nueva Ecija and in North Cotabato. Methods for generation advance and resistance screening are described. As of the 1998 wet season, several F_8 lines from the following crosses showed good resistance to tungro: PSB Rc4 x TI-11-8, BPI Ri10 x TI-11-8, IR64 x TI-11-8. Molecular markers are currently being used to map resistance genes to tungro from Utri Merah and Utri Rajapan and molecular techniques are being developed to characterize strains of rice tungro spherical virus.

The development of tungro-resistant varieties remains a primary concern in the Philippines because tungro is still the most destructive rice virus disease in the country. Almost all resistant varieties so far developed and released are resistant to the insect vector (*Nephotettix virescens*) only. A few varieties have resistance to rice tungro spherical virus (RTSV). Chemical control against the vector is very costly and the disease can still occur even if the vector population is not high. Furthermore, there is no effective chemical control measure against tungro viruses. As such, the development of varieties resistant to this disease is a priority objective of the Philippine rice breeding program.

Rice tungro disease concerns at PhilRice

Breeding for yield and grain quality

The breeding program for irrigated lowland (transplanted and direct-seeded) and rainfed rice ecosystems in the Philippine Rice Research Institute (PhilRice) has always included tungro resistance as an objective. Selection for desirable plant morphology and grain structure starts at F_3 . Segregating generations are advanced with continuous selection for yield, grain quality, and other important traits. Resistance to important insect pests and diseases is incorporated by crossing selections with high yield and good grain quality with breeding lines and traditional accessions carrying the desired resistance traits. For tungro resistance, the most widely used materials are TI-11-8 (a BC₄F_n derived from ARC11554 × TN1) and Utri Merah. To screen for rice tungro disease (RTD) resistance specifically, lines advanced from these crosses are shuttled to Midsayap, North Cotabato, where tungro incidence is consistently high even during the dry season. Each entry is observed for visual symptoms caused by the disease. Lines found to have some amount of resistance are included in multilocation preliminary and advanced yield trials for further and final-stage tests. This method of selection for tungro resistance, however, usually results in the selection of lines that are resistant only to the vector.

Developing breeding lines with RTD resistance

In 1994, a special breeding project began with tungro resistance as the primary trait of interest. The project aims to develop lines with resistance to tungro using various donor sources. By backcrossing and/or selection in the segregating population, resistance genes are transferred to a modern genetic background. The project seeks to produce breeding materials with durable resistance to either RTSV or rice tungro bacilliform virus (RTBV) or to both viruses, with or without resistance to the vector. With the availability of these breeding lines, the efforts of breeders working mainly on high yield and good grain quality will become easier and faster. This prevents the occurrence of undesirable recombinants that arise when landraces are used as sources of resistance. It also disrupts the progress in generation advance by reintroducing many undesirable traits into lines that have been previously considered promising for other traits. Thus, instead of, for instance, using Utri Merah directly, the resistance gene can be transferred into promising selections by way of breeding lines containing the resistance gene but with improved characteristics.

For most of the activities under this project, workers at the PhilRice Central Experimental Station (CES) in Muñoz, Nueva Ecija, work closely with researchers at the PhilRice Midsayap Experiment Station (MES) in North Cotabato. CES acts mainly as a source of materials while conducting field tests on its own and performs the more sophisticated laboratory procedures. MES serves as a satellite station for field tests and as a second source of leaf samples to be analyzed by enzyme-linked immunosorbent assay (ELISA), which is the responsibility of CES. Midsayap is strategically located because of the constantly high insect and disease pressure in the area notably including tungro. With the strengthening of networks, the research group at MES has started performing its own crossing work and is now also equipped with a green leafhopper-rearing nursery for virus strain maintenance and test tube inoculation.

Breeding and screening strategies

Modern varieties, landraces, introductions, and breeding lines are first evaluated for their response to tungro disease using visual screening and ELISA. Selected modern genotypes are then crossed with resistance donors. Table 1 lists materials used in the crossing work conducted from 1994 to 1996.

F_1 and F_2 plants are reared in CES. Individual plant selection begins with F_2 plants, which are space-planted in the field. During the wet season (WS), when disease pressure is high, selection is primarily for resistance (visual screening). During the dry season (DS), when disease pressure is insignificant, selection is primarily for morphology and grain structure.

Starting at F_3 , dual-location testing is carried out at both CES and MES. During wet seasons, generation advance and field screening are conducted at both CES and MES. Only generation advance activities are conducted at CES during dry seasons, however, because of very low occurrence or nonoccurrence of tungro in Central Luzon at this time of the year.

Table 1. Parental materials used in hybridization work for rice tungro disease resistance, 1994-96.

Resistance donors		Modern varieties/lines		
ARC11554	Utri Merah	BPI Ri10	IR65564-22-2-3	CR2
T1-11-8	TK298	C4-63G	IR65597-134-2-3-1	CR3
T15725	TK300	IR24	IR65597-17-4-3-3	CR5
T15850	TK303	IR56	IR65598-112-2	CR93
T15943	TK342	IR64	IR65600-12-3	CR94
T15950	TK352	IR72	IR65600-27-1-2-2	LH422
T15991		PSB Rc4	IR65600-32-4-6-1	LX37
T16000	DS1-1	PSB Rc14	IR65605-6-2-3-2	LX76
T16270	DS1-7	PSB Rc40	IR66158-38-3-2-1	LX77
T16291		IR54883	IR66159-164-5-3-5	LX93
T16393	Kataribhog	IRBB5	IR66160-5-2-3-2	LX99
LS519	Utri Rajapan	IRBB21	IR66165-24-6-3-2	LX136
	LS546	PSB Rc40	IR66738-118-1-2	LX144a
LS551	IR22M series	Habataki	IR66750-6-2-1	LX144b
		Takanarl	IR66764-AC4-8	LX163
		Todorikiwasi	IR66160-121-4-1-1	PR26494

The F_3 materials are planted in small ($2.2-2.8 \times 1.2-1.6$ m), unreplicated plots. Selection is done within plots and seeds are bulked in each plot to constitute the next generation. At F_4 and F_5 , the small plots are replicated thrice. Selection is still within plots and seeds are also bulked, Lines that are consistently damaged by tungro in all three replicates are dropped, whereas lines that show a high degree of resistance in all three replicates are considered as the best selections. At F_6 through F_8 , lines are planted in unreplicated but bigger plots (5.0×5.0 m) to minimize escape and/or preference of insects, which is especially the case when many genotypes are arrayed in succession in the field. At this stage, selections within each plot can be separated into distinct sublines, but each subline can still be composed of several bulked plants. Visual screening is also coupled with ELISA at this stage. Promising selections are then tested in preliminary yield trials.

The ELISA technique is carried out as outlined by Bajet et al (1985). Leaves are sampled at 30 and 60 d after transplanting (DAT). Absorbance is set at 405 nm in a MicroELISA reader. For the field screening, spreader rows consisting of IR64 or PSB Rc14 are planted 1 mo ahead to ensure a sufficient source of virus. Disease incidence (% infection) is observed at 30 and 60 DAT.

Rice tungro disease observation nursery

This part of the project aims to (1) evaluate selected rice collections and advanced lines for resistance to rice tungro disease and (2) identify potential parents for hybridization work. An initial screening of 56 commercial varieties and breeding lines was conducted at CES and MES in the 1995 WS. The entries were assigned to 1.0×2.5 -m plots each in three replications. Visual scoring of tungro incidence and leaf sampling for ELISA were done at 30 and 60 DAT.

In CES, 28 entries had low tungro incidence (0–23%). while in MES all entries had high tungro incidence (47–100%).

In both locations, TI-11-8 showed low virus titers of combined RTBV and RTSV (0%), RTBV (8% in CES, 0% in MES), and RTSV (0% in CES, 13% in MES), indicating a high level of resistance to tungro. In CES, IR74, PSB Rc18, and PSB Rc34 showed low infections with combined RTBV and RTSV (0–4%), with RTBV (0–4%), and with RTSV (0–25%). In MES, LS519, LS551, TK298, and TK300 had low combined RTBV-RTSV (8–25%), RTBV (4–25%), and RTSV (4–13%) infection.

In 1996, the observation nursery in CES handled 399 entries, most of which were F₄ lines. Sixty-seven lines showed a high level of resistance to tungro. In MES, only one entry, IR69705-1-1-3-2-1, exhibited high resistance against tungro out of more than 900 entries evaluated, the bulk of which were F₄ and F₃ lines.

Generating RTD-resistant advanced lines

This task involves the following procedures: (1) advancing early generations of crosses between tungro-resistant genotypes and selected rice cultivars, (2) screening for RTD resistance in early and advanced generations, and (3) purifying and increasing seed of selected lines that are resistant to RTD. Figure 1 shows the scheme for generation advance and resistance screening.

Hybridization work began in 1994, producing 14 crosses. In the succeeding years, many materials were handled in both the dry and wet seasons. In 1995, 70 crosses, 256 F₁ plants from 42 crosses, and 17 F₂ populations were advanced. In 1996, 116 crosses, 270 F₁ plants from 42 crosses, 16 F₂ populations, 1,047 F₃ lines from 24 crosses, and 387 F₄ lines from 4 crosses were advanced. In 1997, 14 crosses, 18 F₂ populations, 334 F₃ lines from 8 crosses, 464 F₄ lines from 16 crosses, 262 F₅ lines from 14 crosses, and 46 F₆ lines from 4 crosses were advanced. In 1998, 6 F₃ crosses, 64 F₄ lines from 8 crosses, 226 F₅ lines from 8 crosses, 276 F₆ lines from 16 crosses, 198 F₇ lines from 14 crosses, and 24 F₈ lines from 4 crosses were advanced.

As of the 1998 WS, several F₈ lines from four crosses have been considered as the best tungro-resistant selections. These include four lines from PSB Rc4 × TI-11-8, three lines from BPI Ri10 × TI-11-8, and one line from IR64 × TI-11-8. Table 2 shows the other crosses with promising lines.

Biotechnology

In support of breeding activities, molecular markers are currently being used to map genes with resistance to tungro from Utri Merah and Utri Rajapan. The molecular markers being used are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and randomly amplified polymorphic DNA (RAPD). Resistance from ARC11554 was mapped earlier (Fig. 2, Sebastian et al 1996a, b). Molecular markers tightly linked to the RTSV resistance gene are now being tested for possible use in marker-aided selection (MAS). Cloning of the RTSV resistance gene is also under way (G.O. Romero, personal communication, 1999).

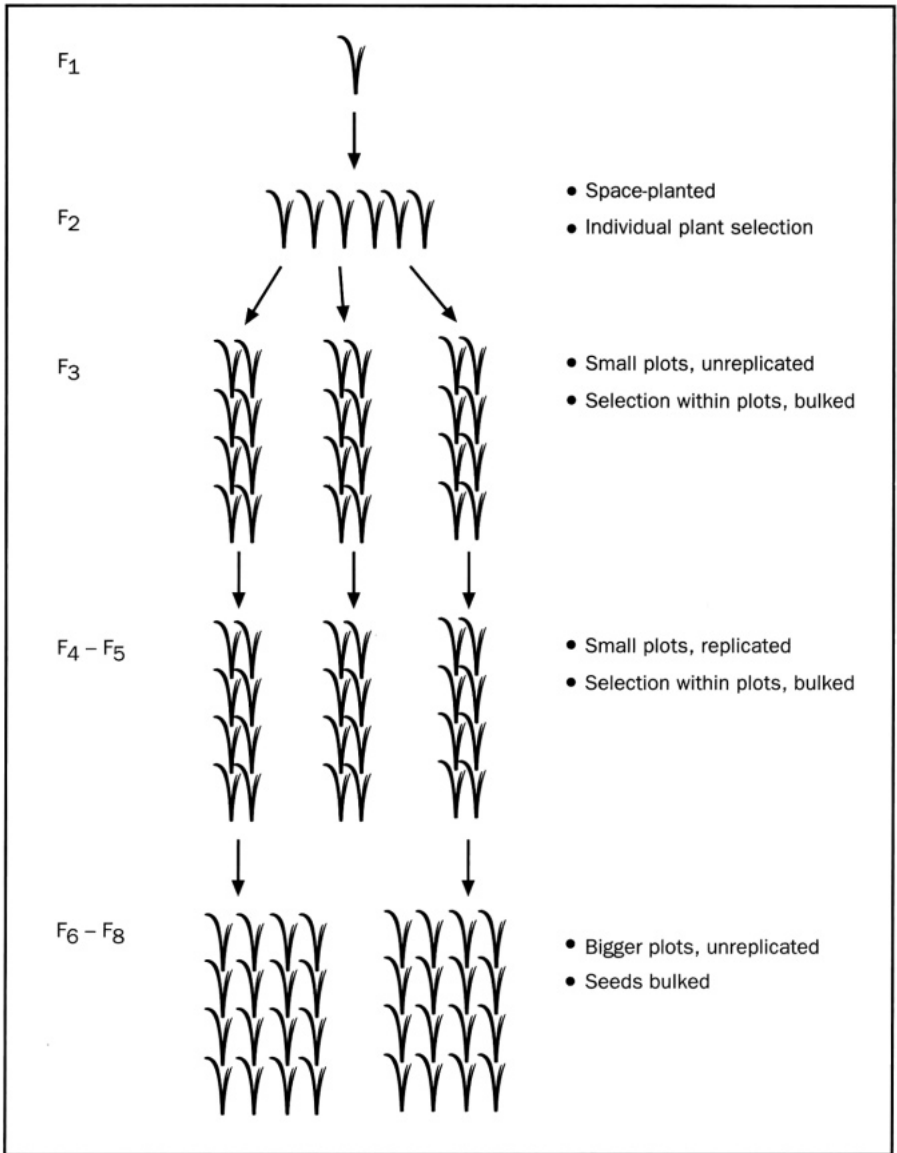


Fig. 1. Scheme for generation advance and screening for tungro resistance.

Furthermore, molecular techniques are also being tested in differentiating RTSV strains in Muñoz, Nueva Ecija, and Midsayap, North Cotabato. This study uses eight differential varieties and detects variation in RTSV strains using the reverse transcriptase-polymerase chain reaction technique.

Table 2. Best rice tungro disease-resistant selections (as of 1998 wet season).

Generation	Pedigree		Lines	
F ₈	PSB Rc4	x	TI-11-8	4
	BPI R110	x	TI-11-8	3
	IR64	x	TI-11-8	1
F ₇	TI-11-8	x	IR56	3
	TI-11-8	x	LX37	1
	TK298	x	IR64	3
	TK298	x	TI-11-8	1
	TK303	x	TI-11-8	1
	TK352	x	TI-11-8	1
	IR22M-2	x	TI-11-8	2
F ₆	LS519	x	IR64	3
	LS519	x	PSB Rc4	6
	Kataribhog	x	PSB Rc4	1
	LS546	x	PSB Rc4	9
	LS551	x	IR64	10
F ₅	IR66158-38-3-2-1	x	TI-11-8	1
	IR66738-118-1-2	x	TI-11-8	3

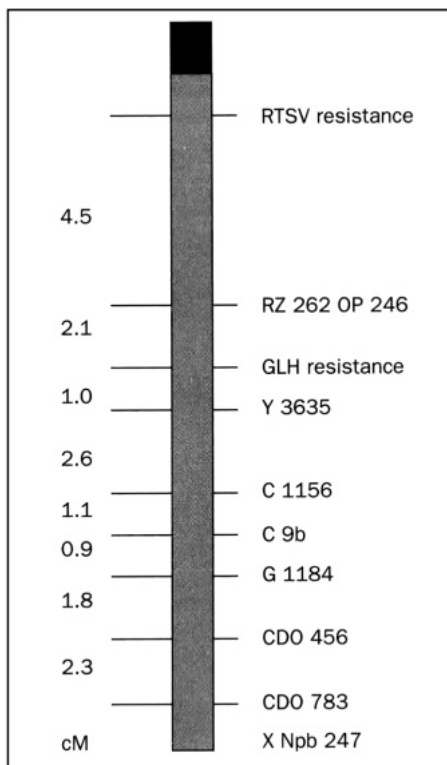


Fig. 2. Molecular map indicating the arrangement of the green leafhopper (GLH) and rice tungro spherical virus (RTSV) resistance genes relative to the molecular markers on chromosome 4 in TN1 × ARC11554 F₂ mapping population, derived using Mapmaker/Exp. 3.0 at LOD 3.0. OP 246 is a RAPD marker; CDO and RZ are Cornell markers; Y, C, and G are Rice Genome Program of Japan markers.

Tungro resistance has also been introgressed into IR64 from *Oryza rufipogon* as part of a wide hybridization project aiming to transfer insect and disease resistance genes from wild species. Several BC₄ lines with resistance to RTSV and RTBV have already been generated.

Future outlook

The development of varieties resistant to tungro will remain a challenge to various breeding programs because of the complexity of the disease. It is hoped, however, that, with new tools and knowledge from concerted research and development efforts, the development of tungro-resistant varieties will become a reality.

In PhilRice, promising F₈ lines will be evaluated in preliminary yield trials for grain yield potential and yield component characteristics. Even materials with below-optimum yields will be maintained, purified, and characterized as long as they have resistance to tungro. Backcrossing to the modern parent may be done to recover as many desirable traits as possible while maintaining the resistance to tungro at the same time.

The development of a marker-aided selection procedure for tungro resistance using the resistance gene from ARC11554 will continue to be undertaken because MAS, as a technique, has become an integral part of other breeding programs for pest and disease resistance. Molecular mapping for resistance genes and characterization of RTSV strains constitute the other thrusts in RTD research, both of which are relevant to the development of strategies for resistance breeding.

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Breeding for rice tungro virus resistance in Indonesia

A.A. Daradjat, N. Widiarta, and A. Hasanuddin

Breeding for rice tungro resistance is one of the major objectives of the rice breeding program in Indonesia. Early studies were directed toward developing rice varieties with good plant type, high yield, and resistance to the green leafhopper (GLH) vector. In recent work, the breeding objectives were redefined to consider two additional traits: grain quality and resistance to tungro viruses. A vigorous hybridization program involving several cultivars with high yield, good plant type, excellent grain quality, and resistance to tungro viruses was implemented. From the initial work, several high-yielding rice varieties with resistance to GLH have been released. Preliminary results from this study indicated that 2,296 accessions have strong resistance to tungro viruses. Based on the range of infection rates with tungro on single-cross populations, it was observed that Utri Merah, Tjempo Kijik, Seratus Hari T36, and M1085c-10-1 were effective donors of tungro resistance. Membramo was the best combiner of the donor cultivars, with high yield and excellent grain quality. The reaction of advanced breeding lines to tungro infection varied with disease pressure and vector population in the area.

Rice tungro disease caused by rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) results in considerable losses in rice production in some irrigated ecosystems in Indonesia. Between 1968 and 1984, the disease damaged an estimated 199,000 ha of rice (Manwan et al 1985).

In 1995, 12,340 ha of rice in Surakarta regency, Central Java, were severely infected, causing yield losses of about US\$1.87 million (Anonymous 1995). Continuous and staggered planting of susceptible cultivars such as Cisadane and IR64 and climatic conditions favorable for both the leafhopper vector and the disease to develop were among the factors that favored the epidemics.

Improved crop production technology that consists of improved cultivars, appropriate cultural practices, and suitable pest management is expected to reduce losses.

In pest outbreaks, the use of resistant cultivars was observed to be the most effective control measure in Indonesian ecosystems. Thus, breeding for resistance to pests and diseases was included as one of the main activities in the breeding program. This paper briefly reviews the efforts that have been made in developing rice cultivars resistant to tungro disease in Indonesia.

Early activities

Early resistance breeding work at the Central Research Institute for Food Crops identified traits associated with virus resistance, such as growth habit, yield, and insect vector resistance.

It was confirmed that tungro disease is transmitted by the green leafhopper (*Nephotettix virescens*). This information led to the adoption of rice cultivars resistant

to green leafhopper as a strategy for reducing tungro disease incidence. Therefore, efforts to reduce losses due to tungro disease focused on developing rice varieties resistant to GLH.

Screening method

The effects of the vector on tungro incidence were studied by field screening in Lanrang Experimental Farm, South Sulawesi. Twenty-one- or 25-day-old seedlings were exposed to natural infection by tungro through GLH infestation in the field. Seedlings were transplanted as two 10-hill rows at a spacing of 30 × 20 cm. To ensure adequate inoculum pressure, single rows of the susceptible check (TN1) were transplanted every 10 rows and in the surrounding field 2 wk before transplanting test seedlings.

Land preparation, fertilizer application, and hand weeding were done as recommended. No pesticides were applied at any stage of plant growth.

Screening results

Up to 1986, 47,503 genotypes had been evaluated. Of these, 6,864 lines were classified as resistant and 8,432 as moderately resistant (Table 1). Table 2 lists some of the promising lines resistant to tungro disease. Selections from crosses involving these promising lines have been released as new improved cultivars (Table 3).

The data in Table 3 indicate that, if a single resistant cultivar of rice is grown continuously in a particular area where there is year-round irrigation, the plant resistance level will be reduced due to the adaptation of the GLH population to the host. Continuous cropping of such GLH-resistant cultivars could increase the selection

Table 1. Rice breeding lines with resistance, moderate resistance, moderate susceptibility, and susceptibility to tungro disease in Indonesia, 1974-86.

Year	Lines tested (no.)	Plant reaction ^a				Lines failed (no.)
		R	MR	MS	S	
1974-75	4,823	975	785	884	1,458	721
1975-76	1,907	335	226	288	415	643
1976-77	1,664	78	280	345	825	136
1977-78	7,622	814	1,367	1,389	2,747	1,305
1978-79	4,314	321	552	1,124	1,718	599
1979-80	4,191	811	954	1,737	689	0
1980-81	6,083	1,438	1,561	1,682	1,402	0
1981-82	4,027	233	931	1,150	714	999
1982-83	5,585	1,303	1,145	1,181	1,886	71
1983-84	1,272	145	115	85	183	744
1984-85	2,753	91	170	354	1,221	917
1985-86	2,812	321	346	439	1,258	448
Total	47,053	6,864	8,432	10,685	14,516	6,583

^aR = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, Source: Anonymous 1988.

Table 2. Promising lines resistant to tungro disease in Indonesia, 1974–84.

Pedigree	Crosses	Source of resistance
B3844-17C-SM-64-2	CR94-13/Pehta I-1// B5436/Pelita I-1	CR94-13, Ptb18, Ptb21
B4076D-PN-20-10	IR3351-38-3-1//IR36	TKM6, Ptb18, Ptb21
B4076D-PN-114-46	IR3351-38-3-1//IR36	TKM6, Ptb18, Ptb21
B4076D-PN-167-63	IR3351-38-3-1//IR36	TKM6, Ptb18, Ptb21
B41080-PN-210-40	B2484B-2-PN-29//IR40	TKM6
B4140C-SM-162-2	B3063/PI-1*2//IR36	TKM6
B4176B-2-IRRI-MR-1	IR36//IR2071//B295J	TKM6
B4180B-22-IRRI-MR-2	IR36//B459B//Paedai Ngulahi	TKM6
B4183B-51-IRRI-MR-4	IR36/PI-1//IR4744/PI-1	TKM6
B4183B-51-IRRI-MR-6	IR36/PI-1//IR4744/PI-1	TKM6
B4196C-IRRI-MR-46	B28508-S1-22//C4-63//Ase Bakk	TKM6, Ptb18, Ptb21, GP15
B4196C-IRRI-MR-47	B2850B-S1-22//C4-63//Ase Bakk	TKM6, Ptb18, Ptb21, GP15

Source: Suprihatno 1985.

Table 3. Rice cultivars resistant to tungro disease released in Indonesia between 1972 and 1984.

Cultivars	Year of release	Reaction against tungro disease within cropping period ^a		
		1974-78	1979-82	1983-84
Serayu	1978	MS	R	R
Semeru	1980	–	MS	MR
Cisadane	1980	–	S	S
Cipunegara	1980	–	S	S
Barito	1981	–	S	S
Krueng Aceh	1981	–	S	S
Sadang	1983	–	MR	S
Bahbolon	1983	–	R	MR
Citanduy	1983	–	–	S
Kelara	1983	–	–	R
IR26	1975	S ^b	MR	MS
IR36	1977	R	MS ^b	S
IR42	1980	–	Sb	S
IR54	1981	–	R	MS ^b

^aR = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible. ^bThe dominant rice cultivar planted within the period. Source: Suprihatno 1985.

pressure on GLH and lead to the development of a new GLH “biotype.” Consequently, the need to rapidly develop and multiply rice cultivars resistant to newly evolving biotypes of GLH is urgent.

Moreover, because a few viruliferous GLH could efficiently transmit tungro disease, it is important that cultivars be resistant to the causal viruses. The strategy of developing virus-resistant cultivars was promoted by identifying several sources of resistance to the disease (Shahjahan et al 1990, IRRI 1996).

Current activities

Currently, several rice cultivars are resistant to GLH, but these are mostly unacceptable to farmers due to their unsatisfactory eating quality. Thus, our breeding objectives emphasized the incorporation of resistance, good plant type, and excellent grain quality to existing GLH-resistant cultivars. Accordingly, breeders at the Research Institute for Rice (RIR) in Sukamandi started a program to breed such cultivars.

Crosses were made between several cultivars with high yield, good plant type, and excellent grain quality and those with resistance to tungro virus (Table 4). Population development was done by single, double, and multiple crossing and backcrossing.

Screening method

Field screening for tungro resistance was conducted in the tungro-endemic area of Tanjungsiang, about 60 km from the Sukamandi Experimental Farm.

To increase the tungro disease pressure on the breeding materials, 15-day-old seedlings seeded in plastic trays were exposed to viruliferous GLH at the rate of two adult vectors per seedling for 24-h inoculation feeding. After 3 wk of exposure, a single inoculated seedling was transplanted in the field in two 15-hill rows for each line at a spacing of 20×15 cm.

Resistant and susceptible parents were planted every 20 rows, and TN1 was established in surrounding plots. Standard agronomic practices were followed, including application of nitrogen, phosphorus, and potassium fertilizer at the rate of 90 kg N ha^{-1} , $60 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, and $30 \text{ kg K}_2\text{O ha}^{-1}$. Hand weeding was done as necessary, and no pesticide was applied at any stage of plant growth.

Six and 8 wk after transplanting, visual scoring was conducted on individual hills by using the scoring method of Hasanuddin et al (1988) where

1 = no symptoms

3 = 1-10% plant height reduction with no distinct leaf discoloration

5 = 11-30% plant height reduction with no distinct leaf discoloration

7 = 31-50% plant height reduction and/or yellow to orange leaf discoloration

9 = more than 50% plant height reduction and yellow to orange leaf discoloration

Percent infection per plant was also assessed visually.

Specifically for F_2 plants at 6 wk after transplanting, leaf samples of healthy plants were tested for the presence of virus particles by enzyme-linked immunosorbent assay. Only plants that showed relatively low concentrations of RTSV and/or RTBV were used in further population development.

Table 4. Rice cultivars used as hybridization parents in breeding for tungro resistance, Indonesia, 1997-98.

Cultivars	Seed source	Donorfor			Amylose (%)	Yield
		Resistance to				
		IRTN96	RTBV	RTSV		
Utri Merah-1 (Acc. 16680)	24	xx	xx			
Utri Merah-2 (Acc. 16682)	25	xx	xx			
Utri Rajapan (Acc. 16684)	26	xx	xx			
ARC 1154 (Acc. 21473)	100			xx		
ARC 10312 (Acc. 124281)	18		xx			
ARC 12596 (Acc. 22176)	19	xx	xx			
ARC 7140	20	xx	xx			
ARC 10343 (Acc. 12337)	60	xx	xx			
Shuli 2 (Acc. 26527)	23	xx	xx			
Seratus Hari T36 (Acc. 5346)	28	xx				
Tjempo Kijik (Acc. 16602)	29	xx				
IR68	6			xx		
IR72	8			xx		
IR74	9			xx		
M1085c-10-1	BS	xx	xx		19	
	IURON95					
CEA 1	1		xx	27		
ITA 323	33			xx		
PR40-1-2-1(87)	36			xx	17	
IR59682-132-1-1-2	43				22 xx	
IR63872-14-2-2-1	68				21 xx	
CT9162-12-8-10-1	98				xx	
IR60819-31-1-3-2	143				23 xx	
IR64	BS96				23 xx	
S969B-265-1-4-1	BS96				23 xx	
S3054-2D-12-2	BS96				xx	
Membramo	BS96				17 xx	
Maros	BS96				22 xx	
Bengawan Solo	BS96				19	
IR66160-121-4-5-3	OBS96				xx	
Taichung Sen 10	PYT96				22 xx	
Taichung Sen Glu 1	PYT96				12 xx	

IRTN96 = International Rice Tungro Nursery 1996, IURON95 = International Upland Rice Observational Nursery 1995, xx = indicator for the presence of resistance or yield potential.

Results of screening

A large population of segregating materials derived from single, double, or multiple crosses was screened. Plants that showed resistant reactions (score: 1–3) and had good plant type were selected.

Based on the range of infection rates with tungro viruses on single-cross populations, Utri Merah, Tjempo Kijik, Seratus Hari T36, and M1085c-10-1 were effective donors for tungro resistance. In contrast, Membramo was the best combiner of the

donor cultivars for high yield and excellent grain quality. In general, all crosses produced offspring with some degree of resistance to tungro, but most of them had poor plant type and a high level of sterility. To overcome the deficiencies of these primary crosses, double and multiple crosses were attempted. Preliminary results of the present study indicated that 2,296 selections have strong resistance to virus based on the screening of more than 143 populations of F_3 single crosses, 200 populations of F_2 double crosses, and 112 populations of F_1 multiple crosses.

Additional breeding populations were also developed during the 1997-98 wet season by backcrossing selected F_2 plants that exhibited resistance with recurrent parents having good plant type and excellent grain quality. The F_2BC_1 progenies were screened for tungro resistance in the 1998-99 wet season before making the next backcrosses.

Comparative reaction of RTD incidence on breeding materials

The first RIR breeding work on tungro screening was done during the 1997 dry season in Celuk, Bali, where IRRI advanced breeding lines were screened. Mass seedling inoculation was done by researchers from the Celuk branch of the Agency for Plant Protection and Pest Assessment. Tungro symptoms in the field trial were assessed visually. Plants that exhibited a resistant reaction and had good plant type were harvested. Resistant selections from this particular nursery and some lines from the IRRI nursery were retested in Tanjungsang, Subang, during the 1997-98 wet season and the 1998 dry season.

Data showed that tungro incidence varied among selections and locations. In Celuk, where IR64 was moderately infected by rice tungro disease (60%), some selections had very low infection rates. In contrast, the same selections showed high infection rates when grown in Tanjungsang. These results indicated that field resistance to tungro in Celuk is not effective in Tanjungsang, and confirmed that tungro incidence varies with inoculum pressure and the vector population prevailing in the area. In the 1998 dry season Tanjungsang nursery, selections were made from the more promising lines. It is hoped that these selections will result in lines homozygous for resistance to rice tungro disease and for other major characters. Observation nurseries of the selected lines are also being planted to determine their yield potential.

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Genetic engineering of rice for tungro resistance

O. Azzam, A. Klöti, F. Sta. Cruz, J. Fütterer, E.L. Coloquio, I. Potrykus, and R. Hull

Genes encoding sense and antisense viral coat proteins, polymerases, and proteases have been successfully used to engineer resistance to several plant viruses. In this study, viral genes of rice tungro bacilliform virus and the coat protein 3 of rice tungro spherical virus were used to engineer resistance in rice against tungro infection. Rice varieties such as IR64, TN1, Taipei 309, and Kinuhikari were successfully transformed and fertile transgenic plants were evaluated at T1 and T2 generations for their ability to confer protection against tungro infection using insect inoculation assays. Unfortunately, none of the 71 transgenic lines tested provided protection against tungro infection. Possible factors for the lack of protection are discussed.

Genetic engineering approaches expand the gene pool from which new and novel virus resistance genes can be selected. For complex diseases of rice, such as tungro, these approaches offer two advantages: (1) the ability to transfer single genes without any linkage to undesirable traits, and (2) the ability to introduce novel genes that have not been explored before in nature and that have potential to increase the durability of resistance. Rice tungro disease incidence is unpredictable, but when it occurs, it can cause catastrophic yield losses in farmers' communities in the irrigated rice ecosystem. For the last 15 yr, several institutions have invested substantial research efforts in studying the molecular biology of the two viruses that cause tungro, rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), and to genetically engineer tungro resistance in rice. In this study, we report on the resistance tests done using insect inoculation assays to evaluate several of these antiviral strategies designed against both RTBV and RTSV. The transgenic rice plants were produced at the Institute of Plant Sciences, ETH, Zurich, Switzerland and John Innes Centre (JIC), Norwich, England, and the evaluation was done at the transgenic CL4 greenhouse facility at IRRI.

Materials and methods

Tables 1 and 2 describe the first 19 transgenic lines from ETH and 20 lines from JIC, respectively. Some additional 32 lines from ETH carrying the antisense RNA constructs of RTBV ORF4 were also evaluated. Seeds from each transgenic line, positive controls (inoculated nontransgenic plants), and negative controls (uninoculated transgenic plants) were sown in sterile soil and seedlings were grown in the CL4 facility at IRRI. At 7–11 d after sowing (DAS), seedlings were inoculated with both tungro viruses by insect feeding using viruliferous green leafhoppers (3–5 insects seedling⁻¹). Inoculated plants were then monitored for symptom expression and assayed for the presence or absence of virus particles at 20 and 40 d postinoculation (DPI). For the evaluation of the first 19 transgenic lines from ETH, two sources of

Table 1. Initial 19 transgenic lines from the Institute of Plant Sciences, ETH, Zurich, Switzerland.

Construct line numbers	Promoter/description of RTBV sequence	Transformed variety	Integrated copies (no.)
5.3	35S/ORF1	TP 309	1
38.2	RTBV/ORF1	TP 309	1
38.7			1
1.3	35S/ORF3 (long coat protein including Cys-His motif)	TP 309	1-2
1.6			>3
1.18			1-2
1.27			1-2
2.31	35S/short coat protein (without Cys-His motif)	TP 309	>3
27.10	RTBV/short coat protein (without Cys-His motif)	TP 309	1
7.6	35S/polymerase with zinc finger	TP 309	3
40.5	RTBV/polymerase with zinc finger	TP 309	1
8.2	35S/polymerase	TP 309	3-5
10.4	35S/protease	TP 309	1
11.8	35S/RNase H	TP 309	2-3
11.11			>6
42.1	RTBV/5'-half of ORF 4	TP 309	>4
K42.1		Kinuhikari	1
K42.1.1			>3
K44.10.9	RTBV/3'-half of ORF 4	Kinuhikari	>3
33.1	RTBV/GUS gene	TP 309	

Table 2. Transgenic lines developed at John Innes Center, Norwich, England.

Line	Promoter/intron/description of the viral gene	Variety transformed	Generation tested
IRI 1	CaMV 35S/RTBV antisense within tRNA binding site (RTBV nt # 7998-550)	IR64	T1/T2
IR2			
IRI 9			
IRI 28			
IRI 37			
IRK 41	CaMV 35S/RTBV antisense within tRNA binding site (RTBV nt # 7998-550) coupled with ribozyme	IR64	T1/T2
IRW 1	CaMV 35S/ubiquitin intron/		
IRW 2	RTBV antisense within tRNA binding site		
IRW 3	(RTBV nt # 7998-550) coupled with ribozyme	IR64	T1
RH 58	Ubiquitin promoter/RTSV coat protein 3	Taipei 309	T1
IRU 1	CaMV 35S/ubiquitin intron/RTSV coat protein 3	IR64	T1
IRU 2			
IRU 4			
TNVP 1	CaMV 35S/ubiquitin intron/RTBV antisense	TN1	T1
TNVP 3	within tRNA binding site (RTBV nt # 7998-550)		
TNVP 4	and ubiquitin/ubiquitin intron/RTSV coat protein 3		
TNVP 6			
TNVP 7			
TNVO 1	Ubiquitin/ubiquitin intron/RTBV antisense		
TNVO 3	within tRNA binding site (RTBV nt # 7998-550) and CaMV 35S/ubiquitin intron/RTSV coat protein 3	TN1	T1

virus inocula were used. For later experiments, only the greenhouse virus inoculum was used.

Results

None of the initial 19 transgenic lines, which used either the greenhouse virus source or a locally collected virus source from Famy, 40 km northeast of Los Baños, showed resistance to either RTBV or RTSV (Tables 3 and 4). In addition, most of the inoculated plants showed severe symptoms such as stunted growth and leaf discoloration at 20 DPI, and their viral coat protein titers, as measured by the enzyme-linked immunosorbent assay (ELISA), were comparable with those titers from the nontransgenic control plants. Titters varied among individual plants from different

Table 3. Percent infection of transgenic lines based on ELISA and visual scores (SS) using mylar test tube inoculation with the greenhouse isolate as inoculum. ELISA results are shown for 20 and 40 days postinoculation (DPI).

Construct line numbers	Plants tested (no.)	Percent Infection				Visual scores ^a (SS)
		20 DPI		40 DPI		
		RTBV	RTSV	RTBV	RTSV	
5.3	20	100	100	100	100	7
38.2	28	100	100	100	100	7
38.7	28	100	100	100	100	7
1.3	28	100	96	100	100	7
1.6	28	100	97	100	100	7
1.18	28	100	100	100	100	7
1.27	28	96	100	96	100	7
2.31	28	100	92	100	93	7
27.1	28	100	100	100	93	7
7.6	28	96	100	96	100	7
40.5	27	100	89	100	100	7
8.2	25	100	100	100	100	7
10.4	28	100	100	100	96	7
11.8	28	100	100	100	96	7
11.11	26	100	89	100	92	6
42.1	27	100	100	100	93	7
K42.1	28	100	96	100	93	7
K42.1.1	27	100	100	100	96	7
K44.10.9	20	100	100	100	96	8
33.1 (construct alone)	28	100	92	100	85	7
TP 309 (nontransgenic)	20	95	100	95	100	7
Kinuhtkari (nontransgenic)	28	100	100	100	96	7
TP 309 (uninoculated)	19	0	0	0	0	1
Kinuhtkari (uninoculated)	28	0	0	0	0	1

^aIRRI Standard Evaluation System (SES), 1=resistant. 9=susceptible.

Table 4. Percent infection of transgenic lines based on ELISA and visual scores (SS) using mylar test tube inoculation with Famy isolate as inoculum. ELISA results are shown for 20 and 40 days postinoculation (DPI).

Construct. line numbers	Plants tested (no.)	Percent Infection				Visual scores ^a (SS)
		20 DPI		40 DPI		
		RTBV	RTSV	RTBV	RTSV	
5.3	14	100	100	100	100	7
38.2	28	100	100	100	100	8
38.7	28	100	100	100	92	7
1.3	27	100	96	100	89	7
1.6	26	100	96	99	96	7
1.18	28	100	100	100	100	7
1.27	28	100	96	100	96	7
2.31	28	100	100	100	99	7
27.1	28	100	100	100	100	7
7.6	28	100	100	100	100	8
40.5	28	100	96	100	97	7
8.2	21	100	100	100	100	8
10.4	28	100	96	100	100	7
11.8	28	100	100	100	100	7
11.11	4	100	100	100	100	7
42.1	26	100	100	100	100	7
K42.1	28	100	100	100	100	7
K42.1.1	28	100	93	100	96	7
K44.10.9	22	95	91	100	85	7
33.1 (construct alone)	27	100	92	100	100	7
TP 309 (nontransgenic)	22	100	100	100	97	7
Kinuhikari (nontransgenic)	28	100	100	100	100	7
TP 309 (uninoculated)	19	0	0	0	0	1
Kinuhikari (uninoculated)	28	0	0	0	0	1

^a IRR1 Standard Evaluation System (SES), 1=resistant, 9=susceptible

lines. Some plants had high RTSV and RTBV titers while others, surprisingly, had low RTSV but high RTBV titers. Based on the ELISA results and visual scores, none of the test lines recovered at 40 DPI. The average symptom severity (SS) was about 7 per line, indicating that most individual plants within a line exhibited stunted growth and leaf discoloration. The 32 remaining lines from ETH were tested using only the greenhouse virus population and results were similar to those obtained earlier. None of the lines showed resistance to RTBV at 20 DPI and plants did not recover after 40 DPI (data not presented).

Furthermore, none of the lines from JIC showed any promising protection against either RTBV or RTSV. Based on ELISA and visual scores, the plants accumulated RTBV at a level similar to that of the nontransgenic control plants. Generation T1 and T2 plants of the IRI and IRK lines did not show any protection against virus infection (Table 5).

Table 5. Percent infection of transgenic lines based on ELISA and visual scores (SS) using mylar test tube inoculation with the greenhouse isolate as inoculum. ELISA results are shown for 21 days postinoculation (DPI).

Line	Clones tested line ⁻¹	Plants tested (no.)	% infection with RTBV	% Infection with RTSV
IRI 1	4	27	64	11
IRI 2	1	38	90	50
IRI 9	1	37	92	78
IRI 28	2	54	89	65
IRI 37	1	40	100	100
IRK 41	6	58	60	19
IRW 1	5	17	95	60
IRW 2	4	92	97	76
IRW 3	2	77	97	74
IR64 inoculated control	Nontransgenic	27	96	74
TN1 inoculated control		40	100	100
IR64 uninoculated		25	0	0
RH 58	3	86	95	87
Taipei 309 inoculated		39	97	74
Taipei 309 uninoculated		20	0	0
IRU 1	3	81	95	69
IRU 2	2	80	85	63
IRU 4	2	63	100	62
TNVP 1	1	39	100	31
TNVP 3	1	37	100	97
TNVP 4	1	40	100	100
TNVP 6	1	39	100	97
TNVP 7	2	32	98	94
TNVO 1	1	22	100	100
TNVO 3	1	34	97	77
TN1 inoculated control		40	100	100
TN1 uninoculated		20	0	0

Discussion

Four rice varieties, IR64, TN1, Taipei 309, and Kinuhikari, were successfully transformed with RTBV and RTSV gene constructs and fertile transgenic plants were generated. The coat protein, polymerase, protease, RNase H, and antisense RNA resistance strategies were used to confer protection against RTBV infection using the cauliflower 35S and RTBV promoters. The coat protein strategy was tried against RTSV infection using the 35S and ubiquitin promoters. Most of these strategies have been successful in other virus systems and they were expected to be effective with DNA and RNA viruses. Unfortunately, the resistance tests showed that none of these strategies was effective against tungro infection.

In the transformation experiments with the coat protein, polymerase, and protease strategies, most constructs were expected to express viral proteins when integrated in rice. Most transgenic plants, however, expressed the transgenes only at a

very low level. In fact, transgene expression was either stopped or only expressed in a subset of cells. Such an irregular expression level could be responsible for the lack of protection. Novel transgene-expression strategies were thus designed and newly generated transgenic plants will be evaluated in the near future.

Another possible factor that could be responsible for the lack of protection is the quasispecies behavior of tungro viruses (Villegas et al 1996, Cabauatan et al 1999). The continuous supply of mutant and recombinant genomes during virus replication may permit great virus adaptability in overcoming selection pressures imposed on its replication or movement within a very short time. Our work on the genetic variation of RTBV and RTSV field populations (see Arboleda et al and Umadhay et al, this volume; Azzam et al 1999) suggests that effective protection mechanisms must be directed against highly conserved functions or sequences, which can be defined only by analyses of large numbers of field isolates, and that other sequence-specific protection mechanisms (like antisense RNA or silencing) are unlikely to be successful.

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Multilocation evaluation of advanced breeding lines for resistance to rice tungro viruses

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Twelve advanced breeding lines with different sources of resistance against rice tungro viruses were tested together with checks IR62 and IR64 in replicated 8 x 8-m plots at six locations in the Philippines, Indonesia, and India from 1995 to 1998. Advanced breeding lines with resistance derived from Utri Merah (IRGC accession no. 16680) had the lowest infection with rice tungro bacilliform virus and rice tungro spherical virus in each of the three countries, suggesting that the resistance is likely to be effective in a wide range of locations. Some of these lines also had promising yield potential. Two lines derived from ARC11554 (IRGC accession no. 21473) showed promising results in the Philippines and Indonesia but not in India.

The rapid spread of high-yielding rice varieties and the intensification of rice cultivation in South and Southeast Asia since the 1960s resulted in outbreaks of several virus diseases. Rice tungro is the most destructive of these diseases and can cause large losses over extensive areas. Breeding for resistance to tungro is an important component of rice varietal improvement programs in South and Southeast Asia and at IRRI (Khush and Virmani 1985). Until recently, the breeding strategy for tungro resistance at IRRI was based on using vector resistance. Since 1969, most IR varieties targeted for the irrigated lowlands have had at least one parent with resistance to the major green leafhopper vector (*Nephotettix virescens*). The main donors have been Ptb18, Gam Pai 30-12-15, and Ptb33. Such varieties escape tungro infection in the field under light to moderate tungro and vector pressure but succumb to infection when there are strong sources of inoculum and vectors are abundant (Cabunagan et al 1987). The reaction of some varieties has changed from resistant to susceptible following a change in the virulence of *N. virescens* in the field (Dahal et al 1990).

Currently, the breeding program for tungro resistance at IRRI uses ARC11554 (accession no. 21473), Utri Merah (accession no. 16680), Utri Rajapan (accession no. 16684), Habiganj DW 8 (accession no. 11751), and some wild rice as virus-resistant donors. Genetic studies have also been conducted to investigate the inheritance of resistance (Imbe et al 1995). New breeding lines have been developed using the most promising sources of virus resistance.

Preliminary studies were conducted where test entries were inoculated by the test tube inoculation method in the greenhouse to select promising lines (Angeles et al 1998). Field evaluation of promising lines was conducted in a tungro hot spot in Midsayap, North Cotabato, Philippines. Some of the most promising lines from test crosses in these trials were selected for further evaluation in replicated field trials in areas with reported high tungro disease incidence in the Philippines, Indonesia, and India. Field trials were conducted in collaboration with PhilRice, the Agency for Agricultural Research and Development (AARD) in Indonesia, and the Indian Council

of Agricultural Research (ICAR). Preliminary results from early trials were reported in Cabunagan et al (1996, 1998). Trial data from India and Indonesia are presented elsewhere in this volume (Astika, Chowdhury, Subramanian et al). In this paper, we summarize results from trials in the Philippines and attempt to provide a synthesis of the findings from the different locations.

Experimental studies

Test locations

Trials in the Philippines were carried out on experimental farms of PhilRice in Maligaya, Nueva Ecija Province, and in Midsayap, North Cotabato Province. In Indonesia, trials were conducted on the experimental farms of the Food Crop Protection Center VI Field Laboratory in Celuk, Gianyar District, Bali, and of the Mares Research Institute for Maize and Other Cereals in Maros and in Sidrap District, South Sulawesi. In India, trials were conducted on the Regional Rice Experimental Farm of Bidhan Chandra Krishi Viswavidyalaya in Chakdah, West Bengal, and on the experimental farm of Tamil Nadu Agricultural University Rice Research Station in Tirur, MGR District, Tamil Nadu. The trials began in 1995 and continued until 1998 (Table 1). At least three trials were conducted at each site, with the exception of Maros (two trials) and Sidrap (one trial). In the Philippines, trials were conducted in both the wet and dry seasons. In India and Indonesia, most of the trials were carried out in the wet season, when tungro incidence was greater. Four sets of trials were conducted, each covering at least three sites, with some lines and varieties evaluated in two or more sets of trials (Table 1, Figs. 1-4).

Test lines and varieties

Advanced breeding lines evaluated in the trials were developed in the IRRI breeding program. Table 2 lists these lines and their parents. Two of the virus-resistant parents used in the crosses, Balimau Putih and Utri Merah, are susceptible to *N. virescens* in greenhouse tests conducted at IRRI using vector populations collected in Los Baños. The other two virus-resistant parents, ARC11554 and *Oryza longistaminata*, are resistant to *N. virescens*. Varieties IR62 and IR64 were used as field-resistant and susceptible checks, respectively. Both varieties were resistant to *N. virescens* when first released, but in many areas IR64 is now susceptible to *N. virescens* and succumbs to tungro disease where inoculum sources are present.

Experimental design and data collection

In each trial, a randomized complete block design was used with four replications. The plot size was 8 × 8 m with a 2-m distance between plots. Two to three seedlings per hill were transplanted at 21 d after sowing at 20 × 20-cm spacing and exposed to natural infection with tungro viruses. In Celuk, Maros, Chakdah, and Tirur, spreader

Table 1. Infection with rice tungro viruses, tungro disease incidence, and green leafhopper (GLH) numbers on susceptible check IR64 at 60 days after transplanting in multilocation field trials in India, Indonesia, and the Philippines, 1995-98.

Set	Location	Season/ year	Infection ^a (%)				GLH (no.)
			BS	B	S	VIS	
1	Maligaya, Philippines	WS 95	69.3	0.3	30.5	62.5	7.0
	Maligaya, Philippines	WS 96	25.0	7.3	9.0	26.5	78.8
	Midsayap, Philippines	WS 95	99.3	0.0	0.8	100.0	56.5
	Midsayap, Philippines	DS 96	63.8	20.0	8.3	99.3	179.5
	Midsayap, Philippines	WS 96	80.0	2.3	14.5	100.0	117.5
	Bali, Indonesia	DS 95	76.0	1.5	18.5	99.8	2.0
	Bali, Indonesia	WS 96	41.0	0.8	29.0	60.3	8.8
	Maros, Indonesia	DS 95	1.0	3.5	5.0	0.5	11.13
	Maros, Indonesia	WS 96	10.3	6.0	19.5	11.0	18.3
	Tamil Nadu, India	DS 96	26.5	2.3	39.5	37.3	nd
	Tamil Nadu, India	WS 96	0.3	0.3	5.8	17.0	9.3
	West Bengal, India	WS 96	4.8	1.5	10.5	32.5	9.8
	Maligaya, Philippines	DS 97	3.0	8.3	6.8	3.5	48.8
	Maligaya, Philippines	WS 97	26.5	1.3	60.3	46.3	28.8
	Midsayap, Philippines	DS 97	53.8	15.0	14.5	91.0	49.8
	Midsayap, Philippines	WS 97	54.5	3.8	33.3	100.0	38.5
	Bali, Indonesia	WS 97	67.8	2.0	20.0	99.8	60.3
	Tamil Nadu, India	WS 97	21.3	5.0	20.3	70.5	12.8
	West Bengal, India	WS 97	27.0	5.5	37.5	64.3	35.8
	Maligaya, Philippines	DS 98	0.5	0.0	3.5	0.5	60.0
	Midsayap, Philippines	DS 98	84.3	3.5	8.8	99.3	85.0
	Bali, Indonesia	WS 98	59.8	4.0	28.0	87.5	30.3
	Maligaya, Philippines	WS 98	0.0	1.5	2.3	0.0	17.8
	Midsayap, Philippines	WS 98	78.0	6.0	12.8	97.3	172.5
	Sidrap, Indonesia	WS 98	44.5	1.5	44.3	100.0	26.0
	Tamil Nadu, India	WS 98	0.0	0.5	2.5	0.0	4.0
	West Bengal, India	WS 98	0.0	0.0	1.5	nd	nd

^aBS = RTBV + RTSV, B = RTBV alone, S = RTSV alone, VIS = visual assessment of disease, nd = no data. WS = wet season, DS = dry season.

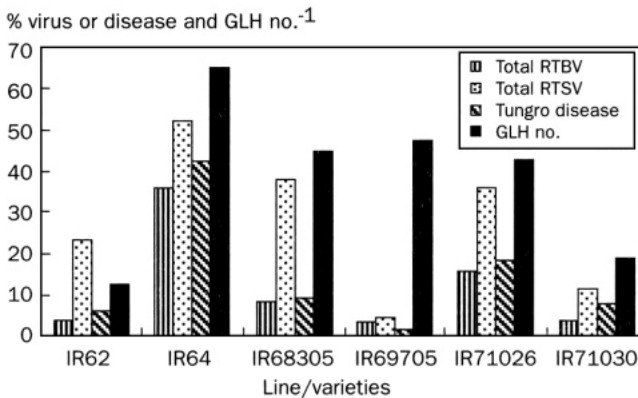


Fig. 1. Mean infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), tungro disease incidence and numbers of green leafhopper (GLH) vectors in the first set of multi-location field trials.

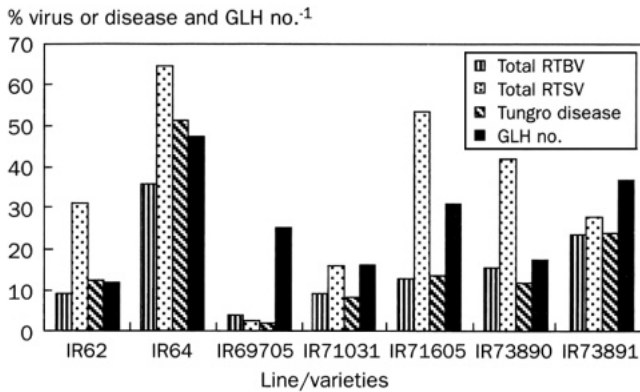


Fig. 2. Mean infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), tungro disease incidence, and numbers of green leafhopper (GLH) vectors in the second set of multilocational field trials.

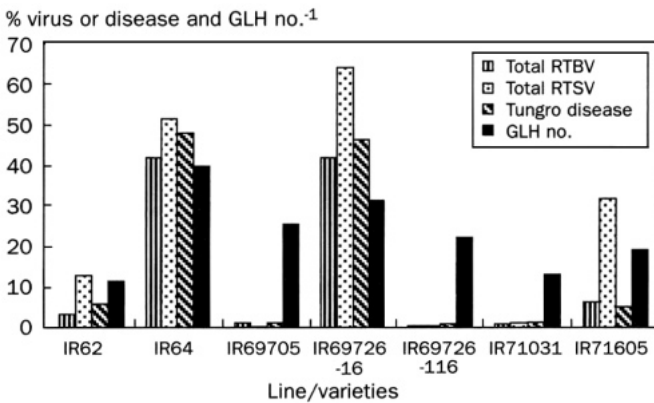


Fig. 3. Mean infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), tungro disease incidence, and numbers of green leafhopper (GLH) vectors in the third set of multilocational field trials.

rows of an *N. virescens*- and tungro-susceptible variety were placed between the four blocks to enhance disease spread. No insecticide was applied to the seedbed or field plots during the trials. Fertilization and other management practices were based on recommendations in the respective test locations.

Plants were assessed for disease symptoms and leaves sampled for detection of tungro viruses by enzyme-linked immunosorbent assay (ELISA) at 30–35 and 55–60 d after transplanting. Disease was recorded and leaves were sampled in six quadrats of 4 × 4-m hills in a "W" pattern in each plot. Disease assessment was based on symptoms of stunting and yellowing. Six- to 8-cm-long leaf samples were taken from rice plants and placed individually in plastic sleeves for temporary storage. Leaf samples were sent by courier to IRRI where the ELISA test was carried out in the virology laboratory. Leafhopper vectors were collected using 10 sweeps of a 30-cm-diameter insect net in each plot on the same dates as for disease assessment.

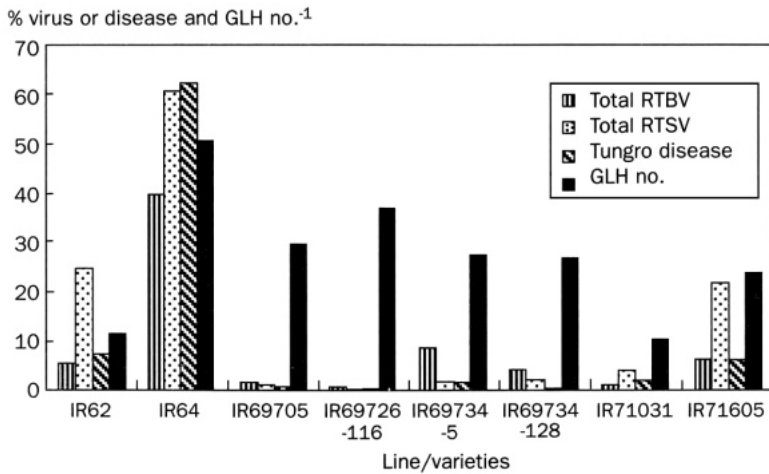


Fig. 4. Mean infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), tungro disease incidence, and numbers of green leafhopper (GLH) vectors in the fourth set of multilocal field trials.

Table 2. Advanced breeding lines evaluated for resistance to rice tungro viruses in multilocation field trials in India, Indonesia, and Philippines, 1995-98.

Breeding line	Cross
IR68305-18-1	IR64 *4/ <u>Balimau Putih</u> ^a
IR69705-1-1-3-2-1	IR1561-228-3-3 *2/ <u>Utri Merah</u>
IR69726-16-3-2	IR61009-37-2-1-2///IR1561-228-3-3/ <u>Utri Merah</u> //IR1561
IR69726-116-1-3	IR61009-37-2-1-2///IR1561-228-3-3/ <u>Utri Merah</u> //IR1561
IR69734-5-1-2	IR44624-127-1-2-2-3///IR1561-228-3-3/ <u>Utri Merah</u> //IR1561
IR69734-128-2-3	IR44624-127-1-2-2-3///IR1561-228-3-3/ <u>Utri Merah</u> //IR1561
IR71026-3-2-4-3-5-2	IR1561-228-3-3 *2/ <u>Oryza longistaminata</u>
IR71030-2-3-2-1	IR1561-228-3-3 *6/ARC11554
IR71031-4-5-5-1	IR1561-228-3-3 *6/ARC11554
IR71605-2-1-5-3	IR1561-228-3-3 *3/ <u>Habiganj DW8</u> //4* IR64
IR73890-1-3-1-4-1	IR1561-228-3-3 *2/ <u>Utri Merah</u> //IR24
IR738912-1-5-1	IR64/ <u>O. rufipogon</u> //3* IR64

^aVarieties underlined are the virus-resistant donors.

In selected trials, yield data were obtained from a 3 × 3-m sample area at harvest. Yields were adjusted to 14% moisture content.

Data analysis

Differences between test lines and varieties in tungro virus infection, tungro disease incidence (percentage of diseased hills), and GLH numbers were analyzed using analysis of variance (ANOVA). For each variable analyzed, values represented the average of two sampling dates. Infection with tungro viruses [rice tungro bacilliform virus

(RTBV) and rice tungro spherical virus (RTSV)] was treated as total RTBV (both RTBV + RTSV and RTBV alone) and total RTSV (both RTBV + RTSV and RTSV alone). Means were separated using Duncan's multiple range test.

Results

Variation in tungro incidence and GLH abundance

Considerable variation occurred in tungro incidence and GLH numbers between test locations, seasons, and years. This is illustrated by data for tungro incidence and GLH abundance on the susceptible check IR64 (Table 1). In some trials (highlighted in Table 1), disease incidence was too low to allow test entries to be evaluated effectively.

In Midsayap and in Bali, tungro incidence was consistently high in both the wet and dry seasons, ranging from 91% to 100%. In Maligaya, tungro incidence was greater in the wet season (WS) than in the dry seasons (DS). At other sites, where most of the trials were conducted only in the wet season, tungro incidence was generally low, except for the 1997 trials in Tamil Nadu and West Bengal and the 1998 trial in Sidrap. Green leafhoppers were often most abundant at each of the two sites in the Philippines, but there was no apparent relationship between leafhopper numbers and tungro incidence and no clear seasonal trend in abundance. For example, tungro incidence in Maligaya was low in both the 1997 and 1998 DS when GLH numbers were relatively high. In contrast, GLH numbers were low in Bali during the 1995 DS and 1996 WS, but disease incidence in both seasons was high.

Reaction of test lines in Maligaya and Midsayap

Table 3 shows the results from the first set of test entries evaluated in three trials in Midsayap and two trials in Maligaya. The resistant check, IR62, had a consistently low infection with RTBV, the highest incidence of tungro in IR62 was 21% in Midsayap in the 1996 WS. Although infection with RTSV was quite high in some trials, the performance of IR62, which has been grown for many years in Midsayap District, showed that leafhopper resistance can play an important role in reducing tungro incidence. In subsequent trials (Tables 4 and 5), tungro incidence in IR62 was also low with the exception of the 1997 WS trial in Midsayap, when it reached 47% under conditions of very heavy disease pressure.

IR69705-1-1-3-2-1 showed consistently low infection with RTSV and RTBV and low disease incidence (Table 3). Because of its potential, this line was included in all subsequent trials with similar results (Tables 4 and 5). Its performance showed that resistance to RTSV and resistance to multiplication of RTBV had been successfully transferred from its parent Utri Merah. IR68305-18-1 had a high RTSV infection and moderate RTBV infection (Table 3). Symptoms on this line, however, were not severe and plants exhibited some tolerance, like resistant parent Balimau Putih.

IR71030-2-3-2-1 had low infection with tungro viruses (Table 3). Tungro incidence in this line was generally low, although it reached 30% in the 1996 DS in

Table 3. Percent infection^a with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), visual disease incidence, and green leafhopper (GLH) vector numbers on rice varieties and advanced breeding lines tested in Maligaya and Midsayap, Philippines, 1995 wet season (WS) and 1996 WS and dry season (DS).

Variable	Variety/line	Maligaya		Midsayap		
		WS 95	WS 96	WS 95	DS 96	WS 96
RTBV ^b infection (%)	IR62	0.3 b ^c	1.3 bc	11.0 d	3.4 c	12.3 c
	IR64	36.0 a	17.9 a	92.3 a	66.1 a	83.9 a
	IR68305-18-1	3.4 b	0.6 c	23.5 c	8.5b	21.9 b
	IR69705-1-1-3-2-1	0.1 b	4.4 b	14.0 cd	2.6 c	2.6 d
	IR71026-3-2-4-3-5-2	0.3 b	1.5 bc	76.5 b	11.9 b	27.9 b
	IR71030-2-3-2-1	0.1 b	1.1 c	12.0 cd	1.4 c	4.9 d
RTSV infection (%)	IR62	58.8 c	9.5 bc	50.3 c	25.6 c	70.6 b
	IR64	99.1 a	35.4 a	98.1 a	75.3 a	94.0 a
	IR68305-18-1	93.0 b	17.4 b	91.4 b	48.9 b	76.5 b
	IR69705-1-1-3-2-1	0.8 e	5.9 cd	11.9 e	3.9 d	2.3 d
	IR71026-3-2-4-3-5-2	55.1 c	1.9 d	97.2 ab	60.1 b	65.0 b
	IR71030-2-3-2-1	19.6 d	2.6 cd	23.6 d	4.5 d	12.0 c
Visual infection (%)	IR62	0.4 b	0.1 b	14.4 c	17.9 c	21.0 b
	IR64	32.1 a	14.1 a	95.6 a	85.1 a	95.5 a
	IR68305-18-1	0.1 b	0.0 b	12.4 c	29.3 b	21.9 b
	IR69705-1-1-3-2-1	0.0 b	0.3 b	3.0 d	1.3 d	1.0 c
	IR71026-3-2-4-3-5-2	0.1 b	0.1 b	73.5 b	24.3 bc	26.9 b
	IR71030-2-3-2-1	0.1 b	0.0 b	15.5 c	29.6 b	17.6 b
GLH (no.)	IR62	41.8 c	7.4 c	6.8 b	4.9 c	18.8 c
	IR64	285.0 ab	53.9 a	51.8 ab	125.3 a	125.0 a
	IR68305-18-1	251.0 b	39.8 a	29.5 ab	46.6 b	49.9 b
	IR69705-1-1-3-2-1	314.5 a	35.0 ab	18.3 ab	40.1 b	47.0 b
	IR71026-3-2-4-3-5-2	257.0 b	12.6 c	63.8 a	31.8 b	38.9 b
	IR71030-2-3-2-1	84.3 c	19.8 bc	7.0 b	12.1 c	19.3 c

^aAverage of 2 observations at 30-35 and 55-60 d after transplanting. ^bRTBV infection = total RTBV + RTSV and RTBV infection alone, RTSV infection = total RTBV + RTSV and RTSV infection alone as assessed by enzyme-linked immunosorbent assay. ^cIn a column for each variable, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range tests.

Midsayap. IR71030-2-3-2-1 is derived from ARC11554, which has both virus and leafhopper resistance. Because of its resistance characteristics, ARC1 1554 is considered one of the most useful donors. Another of its progeny, IR71031-4-5-5-1, was evaluated in subsequent trials (Tables 4 and 5). This line, which has good yield potential, performed consistently well in these trials. IR71026-3-2-4-3-5-2 was heavily infected with both RTSV and RTBV and had high tungro disease incidence in the 1995 WS trial in Maligaya (Table 3).

IR71605-2-1-5-3, derived from Habiganj DW8, was tested in eight field trials in 1997-98 (Tables 4 and 5). Results were variable, with moderately high RTBV infection and high RTSV infection in some seasons. As with IR68305-18-1, this line does show, a degree of tolerance for infection. IR73891-2-1-51, with resistance from *O. rufipogon*, was evaluated in four trials in 1997 (Table 4) and had high levels of tungro incidence in Midsayap. The remaining lines evaluated were all crosses involving

Table 4. Percent infection^a with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), visual disease incidence, and green leafhopper (GLH) vector numbers on rice varieties and advanced breeding lines tested in Maligaya and Midsayap, Philippines, 1997 dry season (DS) and wet season (WS).

Variable	Variety/line	Maligaya		Midsayap	
		DS 97	WS 97	DS 97	WS 97
RTBV ^b infection (%)	IR62	9.0 a ^c	1.1 c	7.5 cd	31.5 b
	IR64	7.4 ab	18.4 a	58.5 a	71.5 a
	IR69705-1-1-3-2-1	2.4 ab	5.7 bc	2.5 d	6.5 c
	IR71031-4-5-5-1	1.9 b	5.8 bc	4.3 d	1.9 c
	IR71605-2-1-5-3	3.1 ab	8.2 ab	20.8 b	31.3 b
	IR73890-1-3-1-4-1	4.6 ab	2.7 bc	14.6 bc	37.2 b
	IR73891-2-1-5-1	5.0 ab	3.0 bc	57.1 a	73.7 a
RTSV infection (%)	IR62	2.3 b	39.5 c	39.4 c	62.2 c
	IR64	5.4 b	91.3 a	79.0 a	90.9 a
	IR69705-1-1-3-2-1	2.4 b	5.3 e	1.4 e	2.3 d
	IR71031-4-5-5-11	1.0 c	17.3 d	8.4 d	5.3 d
	IR71605-2-1-5-3	7.0 a	75.1 b	56.0 b	81.9 ab
	IR73890-1-3-1-4-1	2.9 b	63.1 b	37.8 c	74.3 bc
	IR73891-2-1-5-1	1.4 c	5.5 e	47.8 bc	65.9 c
Visual infection (%)	IR62	0.0 a	0.3 c	12.1 cd	46.8 c
	IR64	0.3 a	23.9 a	53.9 a	100.0 a
	IR69705-1-1-3-2-1	0.0 a	0.0 d	1.1 e	1.4 f
	IR71031-4-5-5-11	0.2 a	0.3 c	8.5 d	1.8 f
	IR71605-2-1-5-3	0.0 a	4.5 b	17.9 c	27.3 e
	IR73890-1-3-1-4-1	0.2 a	0.4 c	12.9 cd	37.3 d
	IR73891-2-1-5-1	0.0 a	0.0 d	40.1 b	74.0 b
GLH (no.)	IR62	4.9 c	20.0 bc	16.0 d	2.7 c
	IR64	27.4 a	54.4 a	68.0 a	57.0 a
	IR69705-1-1-3-2-1	19.0 ab	30.5 b	38.0 bc	26.6 b
	IR71031-4-5-5-11	7.3 bc	13.9 c	24.0 cd	4.5 c
	IR71605-2-1-5-3	26.4 a	32.5 b	38.8 bc	31.6 b
	IR73890-1-3-1-4-1	10.4 bc	26.3 bc	20.0 cd	6.3 c
	IR73891-2-1-5-1	5.4 c	29.3 b	52.8 ab	61.6 a

^aAverage of 2 observations at 30-35 and 55-60 d after transplanting. ^bRTBV infection = total RTBV + RTSV and RTBV alone infection, RTSV Infection = total RTBV + RTSV and RTSV alone infection as assessed by enzyme-linked immunosorbent assay. ^cIn a column for each variable, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range tests.

Utri Merah. IR69726-116-1-3 produced excellent results in four trials in 1998 (Table 5). IR69734-5-1-2 and IR69734-128-2-3 also performed well in the 1998 WS trials (Table 5).

Overall reaction of test lines at all sites

Mean values for tungro viruses infection, tungro incidence, and GLH numbers for each of the four sets of test entries, pooled across all trial sites, are shown in Figures 1–4. In general, the performance of varieties and advanced breeding lines in India and Indonesia was similar to that in the Philippines. Regardless of trial location

Table 5. Percent infection^a with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), visual disease incidence, and green leafhopper (GLH) vector numbers on rice varieties and advanced breeding lines tested in Maligaya and Midsayap, Philippines, and in Sidrap, Indonesia, 1998 dry and wet seasons.

Variable	Variety/line	Season				
		Dry		Wet		
		Philippines		Philippines		Indonesia
		Maligaya	Midsayap	Maligaya	Midsayap	Sidrap
RTBV ^b infection (%)	IR62	0.9 a ^c	6.0 d	3.3 ac	18.6 b	2.6 c
	IR64	0.5 a	80.5 a	3.8 a	60.8 a	57.6 a
	IR69705-1-1-3-2-1	0.8 a	2.1 de	2.7 a	3.4 cd	1.8 cd
	IR69726-16-3-2	— ^d	67.5 b	—	—	—
	IR69726-116-1-3	0.5 a	0.5 e	0.0 a	0.6 d	0.5 d
	IR69734-5-1-2	—	—	0.0 a	19.5 b	9.0 b
	IR69734-128-2-3	—	—	1.4 a	7.8 c	4.4 c
	IR71031-4-5-5-1	0.9 a	2.0 de	1.4 a	2.0 d	1.1 cd
	IR71605-2-1-5-3	0.1 a	15.4 c	1.4 a	18.5 b	—
	RTSV infection (%)	IR62	1.3 b	27.3 c	3.3 a	55.9 b
IR64		2.3 ab	92.3 a	5.9 a	83.1 a	91.8 a
IR69705-1-1-3-2-1		0.0 c	0.9 de	0.0 a	1.8 de	1.1 cd
IR69726-16-3-2		—	90.4 a	—	—	—
IR69726-116-1-3		0.0 c	0.3 e	0.0 a	0.5 e	0.1 d
IR69734-5-1-2		—	—	0.0 a	1.4 de	2.8 c
IR69734-128-2-3		—	—	0.0 a	3.4 d	2.9 c
IR71031-4-5-5-1		0.0 c	3.4d	0.0 a	12.1 c	1.6 c
IR71605-2-1-5-3		1.8 b	61.8b	5.4 a	59.8 b	—
Visual Infection (%)		IR62	0.5 a	13.9c	0.0 a	23.4 b
	IR64	0.5 a	82.6a	0.0 a	82.5 a	97.9 a
	IR69705-1-1-3-2-1	0.0 a	1.0d	0.0 a	1.0 de	1.1 bc
	IR69726-16-3-2	—	72.9b	—	—	—
	IR69726-116-1-3	0.0 a	2.3d	0.0 a	0.3 e	0.3 c
	IR69734-5-1-2	—	—	0.0 a	2.0 cd	2.1 bc
	IR69734-128-2-3	—	—	0.0 a	1.5 cde	0.3 c
	IR71031-4-5-5-1	1.0 a	2.8 d	0.0 a	5.0 c	1.8 bc
	IR71605-2-1-5-3	1.0 a	11.9 c	0.0 a	18.8 b	—
	GLH (no.)	IR62	18.1 c	18.3 d	11.5 bc	20.6 c
IR64		43.1 a	76.0 a	16.5 abc	141.3 a	27.0 a
IR69705-1-1-3-2-1		34.8 ab	33.9 bcd	12.3 bc	65.0 b	24.8 a
IR69726-16-3-2		—	53.8 b	—	—	—
IR69726-116-1-3		27.5 bc	34.1 bcd	10.6 bc	53.8 b	23.0 a
IR69734-5-1-2		—	—	19.8 ab	42.8 bc	28.0 a
IR69734-128-2-3		—	—	17.6 abc	39.4 bc	29.1 a
IR71031-4-5-5-1		21.0 bc	22.0 cd	9.4 c	20.0 c	6.1 b
IR716052-1-53		29.5 abc	40.6 bc	22.0 a	43.1 bc	—

^aAverage of 2 observations at 30–35 and 55–60d after transplanting. ^bRTBV infection = total RTBV + RTSV and RTBV alone infection. RTSV infection = total RTBV + RTSV and RTSV alone infection as assessed by enzyme-linked immunosorbent assay ^cIn a column for each variable, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range tests. ^dNot tested.

and season, infection with tungro viruses and tungro incidence were low on four Utri Merahprogenies: IR69705-1-1-3-2-1, IR69726-116-1-3, IR69734-5-1-2, and IR69734-128-2-3.

GLH numbers were much lower on IR62 and the two ARC11554 progenies, IR71030-2-3-2-1 and IR71031-4-5-5-1, in the Philippines and Indonesia. In India, however, GLH numbers on IR71030-2-3-2-1 and IR71031-1-5-5-1 were comparable with those on susceptible check IR64 (Subramanian et al, Chowdhury, this volume). Similarly, tungro incidence on these two lines was also relatively high in Tamil Nadu and in West Bengal.

Yield of test entries

The highest yielding lines in trials in Bali, Indonesia, were IR68305-18-1, IR71030-2-3-2-1, and IR71031-4-5-5-1, which produced 5 t ha⁻¹; however, these data were from unreplicated plots. Nevertheless, results from Bali do provide an indication of the yield potential of these lines under conditions of high tungro incidence. IR71031-4-5-5-1 generally produced higher yields than other test entries in Midsayap and Maligaya. Data from Midsayap were confounded by the occurrence of feeding damage to rice plants caused by the black bug, *Scotinophara coarctata*. Yields from the promising Utri Merah line, IR69705-1-1-3-2-1, were comparable with those of IR64 in spite of the large difference in tungro incidence. In the 1998 DS trial in Midsayap, however, another Utri Merah line, IR69734-5-1-2, yielded 3.8 (± 0.3) t ha⁻¹ compared with 2.1 (± 0.1) t ha⁻¹ for IR64. IR69726-116-1-3, also derived from Utri Merah, yielded 3.2 (± 0.2) t ha⁻¹ in this trial. In Maligaya, low tungro incidence affected comparisons of yield data between test entries.

Discussion

The potential of tungro to cause severe yield loss and the lack of effective control measures available to rice farmers account for the continuing importance of the disease. Durable resistance to tungro viruses is now regarded as crucial to any long-term solution to the rice tungro disease problem in South and Southeast Asia. Geographical variation in tungro viruses (Dahal et al 1992, Cabauatan et al 1995) and breakdown of resistance because of changes in the virulence of the leafhopper vector (Dahal et al 1990) have been reported. Thus, multilocation testing of germplasm, varieties, and advanced breeding lines with different types of resistance is being undertaken to guide future deployment strategies and ensure durability of resistance.

Results from our studies revealed that there are promising advanced breeding lines that showed low infection with tungro viruses across a range of locations. TR69705-1-1-3-2-1, IR69726-116-1-3, and two other promising Utri Merah-derived lines showed consistently low infection with RTBV and RTSV at all trial locations in the Philippines, Indonesia, and India. These results suggested that resistance derived from Utri Merah is likely to be effective against tungro disease in a wide range of locations. Such varieties with tolerance for RTBV and resistance to RTSV are likely to have a low incidence of tungro and are poor sources of viruses for spread to neighboring fields. Moreover, IR69726-116-1-3 and IR69734-5-1-2 have promising yield potential.

When infected, RTBV-tolerant varieties have mild symptoms, contain a low amount of RTBV in plant tissues (Cabunagan et al 1993), and show low yield reduction (Hasanuddin and Hibino 1989). In our study, IR68305-18-1, a progeny of Balimau Putih, showed some degree of tolerance for RTBV; however, this line had high RTSV infection in Midsayap and Bali when tungro incidence was high in the area. In such a situation, IR68305-18-1 could serve as a virus source for neighboring fields. Farmers in Bali liked this line, however, because of its good eating quality, which is comparable to that of IR64. IR64 is widely grown in the area but is highly susceptible to tungro. Farmers in Karangasem Regency have already begun to cultivate IR68305-18-1 extensively, although it has not yet been released as a variety (Astika, this volume). Similarly, IR68305-18-1 proved to be popular with farmers in Tamil Nadu and West Bengal, India.

The performance of the leafhopper-resistant check IR62 demonstrates that vector resistance can remain effective for long periods. IR62 showed good field resistance at all sites, although RTSV infection was sometimes high in the presence of large amounts of inoculum. Combining vector and virus resistance in a variety may produce a more durable resistance than using only one type of resistance. In IR71030-2-3-2-1 and IR71031-4-5-5-1, progenies of ARC11554, which is resistant to both the GLH vector and RTSV (Sebastian et al 1996), the resistance was effective in the Philippines and Indonesia. These lines, however, did not show strong resistance in India. Consequently, more work needs to be done to determine whether ARC11554, which originates from India, is a suitable donor for tungro resistance in that country.

The other advanced lines—IR71026-3-2-4-3-5-2, a progeny of the wild rice *O. longistaminata*, IR71605-2-1-5-3 (Habiganj DW 8 line), and IR73891-2-1-5-1 (from *O. rufipogon*)—performed well in the preliminary evaluation in the greenhouse at IRRI but showed a very low resistance level in field trials. This may have been due to the presence of different strains of the viruses or differences in GLH populations resulting in increased virulence on these varieties.

In conclusion, substantial progress has been made in the identification and field evaluation of advanced breeding lines that have shown consistently strong resistance to tungro disease across several sites. There is scope for further improvement in these lines to increase their yield potential and to incorporate resistance to other pests and diseases. These lines should prove useful to rice breeders in national agricultural research programs in Asia for crossing with varieties developed to suit local requirements for characteristics such as grain quality.

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Prospects of virus-resistant varieties for controlling rice tungro disease in Bali

I.G.N. Astika

Rice tungro disease has been an important constraint to rice production in Bali, Indonesia since 1980. Tungro incidence is favored by staggered planting dates and the cultivation of susceptible varieties such as IR64 and Krueng Aceh. Since 1995, virus-resistant lines bred at IRRI have been evaluated in replicated field trials in Celuk Gianyar Province. One line with resistance from Balimau Putih, IR68305-18-1, showed useful tolerance to infection. It has proved to be popular with farmers in Karangasem Regency because of its good eating quality and it is now grown widely in this area. Two lines derived from Utri Merah have shown strong resistance in field trials. None of these lines, however, are commercially available as they have not yet been cleared for varietal release.

Introduction

Bali has become not only a tungro-endemic area but also the core of the Indonesian tungro-endemic region where farmers have suffered from tungro attacks since 1980. During a 10-y r investigation (1987-97), tungro incidence fluctuated each year (Fig. 1).

Rice tungro disease (RTD) is caused by two viruses: rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), The main vector of this disease is the green leafhopper (GLH), *Nephotettix virescens*. This is the dominant leafhopper species on rice in tropical areas and it is monophagous on rice (Hibino et al 1978).

The adoption of large-scale synchronous rice planting combined with a fallow period or secondary break crop (*palawija*) is an effective tungro management strategy. This strategy is difficult to implement in Bali, however, because of water supply limitation and sociocultural constraints. Synchronous rice planting is usually conducted in small-scale areas or at the Subak level (50–200 ha), but some Subaks may have staggered plantings. Indeed, Bali Province is still regarded as an asynchronously planted region. Synchronous planting enables GLH numbers to increase continuously in each paddy plot until harvesting time and then decline. In contrast, in asynchronous planting areas, after the first (G1) generation, the population density of GLH decreases sharply because of dispersal activity (emigration). GLH migrate to younger rice plants and transmit RTD from older diseased rice plants. This mechanism facilitates the spread of RTD in asynchronous areas (Fig. 2. Aryawan et al 1993).

Some control strategies have been implemented in Bali, such as a planting regulation enforcing sowing 5 d after first land preparation/plowing, selective eradication, and pesticide use. These measures, however, have not given satisfactory results and RTD remains a big problem in rice production in Bali. Therefore, using RTD-resistant varieties with high yield and good quality is a promising alternative for tungro management in Bali.

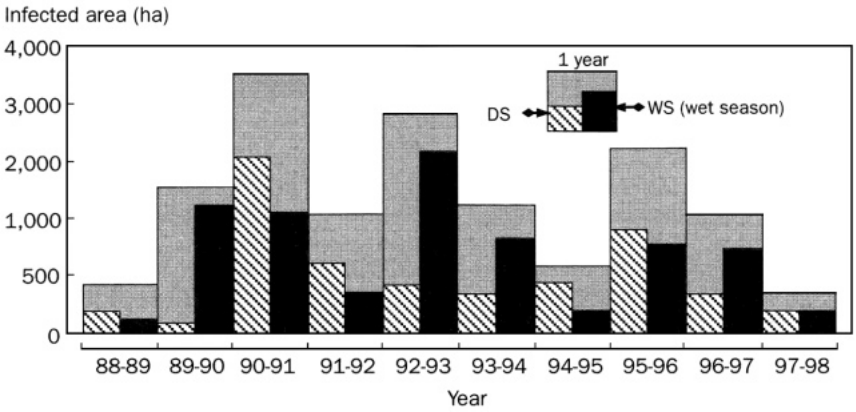


Fig. 1. Seasonal and annual fluctuations in the area affected by rice tungro disease in Bali province, Indonesia, 1988-98.

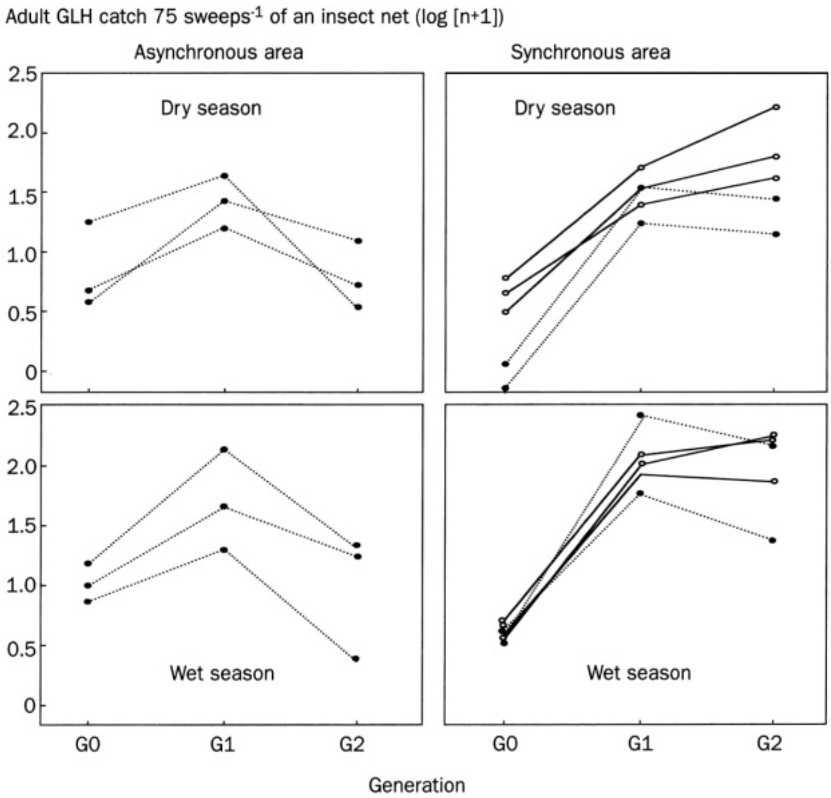


Fig. 2. Population development of green leafhopper (GLH) adults on Krueng Aceh (dotted lines) and IR36 (solid lines) in asynchronous and small-scale synchronous rice cropping areas during the 1987 dry season and the 1987/88 wet season (Aryawan et al 1993).

Tungro in Bali (1987-97)

Tungro infestation in Bali during the past 10 years has fluctuated from season to season. Tungro incidence is commonly higher in the wet season than in the dry season (Fig. 1). The peak tungro incidence in the dry and wet seasons occurs in May and January, respectively (Fig. 3).

Although all of the regencies in Bali have been attacked by RTD, the endemic regencies are Badung, Tabanan, and Gianyar, where RTD incidence exceeded 200 ha year⁻¹ more than five times during 1987-97 (Fig. 4).

Rice varieties used in Bali

Rice varieties grown and rainfall strongly influence RTD incidence. Rainfall is difficult to forecast, however, and recently it has been difficult to make a clear distinction between dry and wet seasons.

In 1990-91, which was the peak of the RTD problem between 1987 and 1997, the dominant rice variety was IR64. As the performance of IR65 has been disappointing in Bali, the Indonesian Government has recommended growing resistant varieties such as IR66 and IR72. In 1992-93, RTD incidence increased again and this coincided with the dominance (70%) of IR64 in the field (Fig. 5).

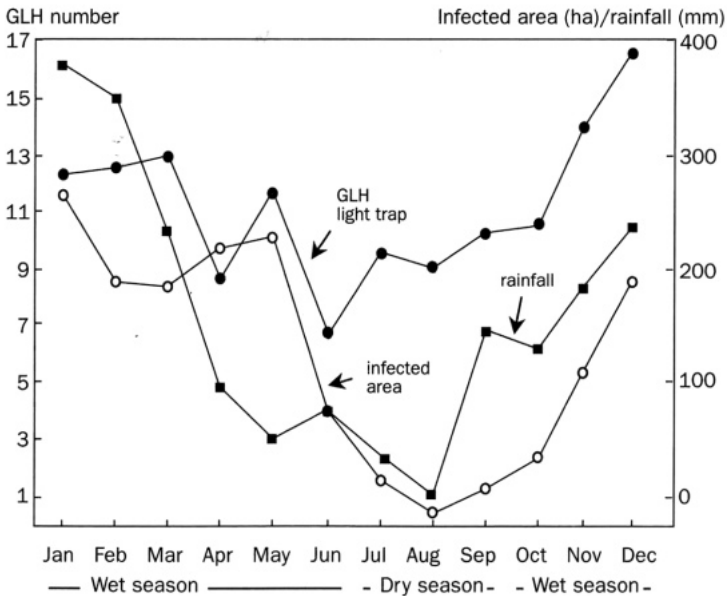


Fig. 3. Monthly populations of green leafhoppers (GLH) caught in light traps and monthly rainfall and tungro incidence in Bali Province, Indonesia, 1987-97.

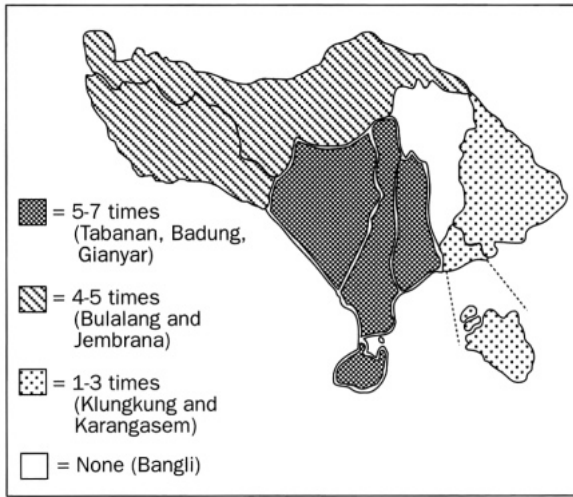


Fig. 4. Frequency of occurrence of tungro in affected areas (exceeding 200 ha yr⁻¹) in Bali Province, Indonesia, 1987-97.

The three regencies of Tabanan, Badung, and Gianyar have been endemic RTD areas since the 1997-98 wet season and the 1998 dry season. An increase in the cultivation of susceptible IR64 was followed by an increase in the incidence of RTD in the field. Although IR64 is very susceptible to tungro in Bali, farmers prefer it because of its high production potential, good taste, and high market price. Farmers still do not have market access to resistant varieties that are high-yielding and have good taste. Moreover, experience has shown that resistant varieties eventually succumb to tungro after being used continuously for several cropping seasons (Table 1). For example, in the 1992-93 cropping seasons, IR66 was moderately resistant to RTD but in 1995-96 it became susceptible. IRRI has produced mainly GLH-resistant varieties, but they are likely to become susceptible after continuous use for several cropping seasons (Inoue and Ruay-Aree 1977). It will be more effective if virus-resistant rice varieties are developed (Dahal et al 1990).

From 1995 until 1998, IRRI, the Natural Resources Institute (UK), and the Food Crop Protection and Horticulture VII in Denpasar collaborated on tungro disease management. In the Celuk field laboratory, rice lines and varieties were evaluated for resistance to tungro viruses (Tables 2 and 3). IR68305-18-1 showed susceptibility to RTBV in the 1995 dry season trial, but tungro disease incidence on the variety was relatively low, indicating that the line has some tolerance for infection. One line derived from Utri Merah, IR69705-1-1-3-2, showed consistently strong resistance to tungro viruses in all trials. Another Utri Merah line, IR69726-116-1-3, performed well in the latest trial in the 1998 wet season and has an excellent plant type.

Yields of these lines ranged from 4.6 to 5.5 t ha⁻¹. In a taste test, 90% of respondents said that the taste of three of the new lines (IR69726-116-1-3, IR69705-1-1-3-2-1, and IR68305-18-1) was excellent and similar to that of IR64. IR68305-18-1 is

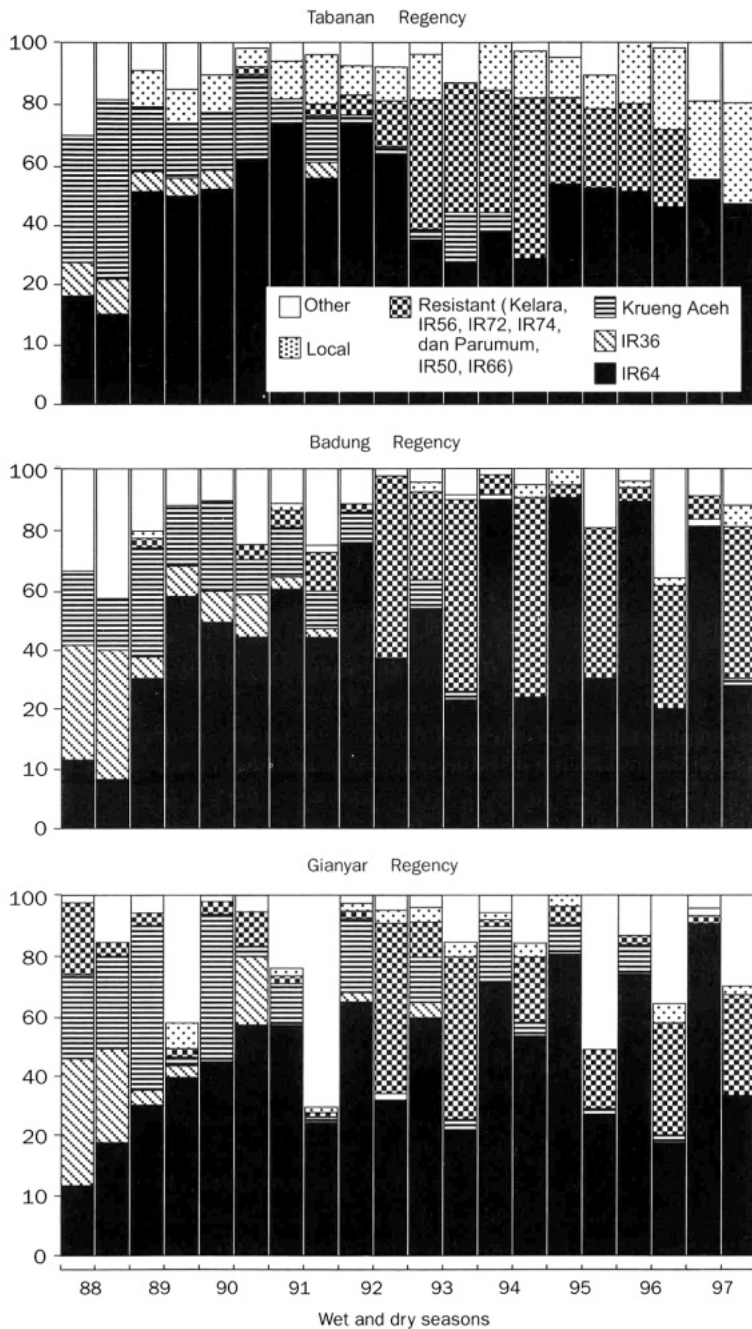


Fig. 5. Proportion of varieties grown in Tabanan, Badung, and Gianyar regencies during 1987-97 in Bali, dry and wet seasons.

Table 1. Reaction of rice varieties to tungro disease based on IRRI standard evaluations^a in five cropping seasons at Badung and Gianyar regency.

Variety	Categories ^b				
	1992-93	1993-94	1994	1995-96	1997-98
Cisadane	S	MR	–	S	S
Cikapunding	–	–	–	S	–
IR26	–	–	–	S	–
Bengawan Solo	–	–	–	S	–
IR36	–	–	–	S	–
IR42	–	–	–	S	–
IR64	S	S	S	S	S
Ciliwung	–	–	–	S	–
IR66	MR	MR	MS	S	S
IR72	R	R	–	S	–
Krueng Aceh	–	MS	–	–	S
Maros	–	–	–	–	S
Sadang	–	–	–	–	S
Membramo	–	–	–	–	S
IR62	–	–	–	–	S
Lariang	S	–	MS	–	–
IR74	R	R	R	–	R
Cendranae	S	–	S	–	–
Barumun	–	R	R	–	–
IR68	–	–	S	–	–

^aChaudhary (1996). ^bR = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible.

Table 2. Percent infection^a with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) on rice varieties and advanced breeding lines at Celuk, Bali, Indonesia, in the 1995 dry season (DS) and 1996-98 wet seasons (WS).

Variable	Variety/Line	DS 95	WS 96	WS 97	WS 98
RTBV ^b	IR62	3.6 d ^c	3.9b	1.5 c	4.1 c
	IR64	72.9 a	23.0 a	69.8 a	65.3 a
	IR68305-18-1	34.8 c	1.5 bc	–	–
	IR69705-1-1-3-2-1	5.4 d	1.9 bc	2.0 c	0.4 d
	IR71026-3-2-4-3-5-2	55.0 b	4.4 b	–	–
	IR71030-2-3-2-1	3.1 d	0.5 c	–	–
	IR71031-4-5-5-1	– ^d	–	1.3 c	0.1 d
	IR71605-2-1-5-3	–	–	10.0 bc	5.5 c
	IR73890-1-3-1-4-1	–	–	24.0 b	–
	IR73891-2-1-5-1	–	–	24.3 b	–
	IR69726-16-3-2	–	–	–	29.9 b
	IR69726-116-1-3	–	–	–	0.6 cd
	RTSV	IR62	12.5 c	5.6 cd	8.8 c
IR64		90.3 a	39.3 a	87.8 a	84.5 a
IR68305-18-1		71.8 b	11.0 c	–	–
IR69705-1-1-3-2-1		2.5 d	1.4 d	3.5 c	0.0 d
IR71026-3-2-4-3-5-2		82.8 a	21.4 b	–	–
IR71030-2-3-2-1		5.6 cd	3.1 d	–	–
IR71031-4-5-5-1		–	–	5.5 c	0.6 d
IR71605-2-1-5-3		–	–	55.0 b	46.0 b
IR73890-1-3-1-4-1		–	–	36.0 b	–
IR73891-2-1-5-1		–	–	34.3 b	–
IR69726-16-3-2		–	–	–	58.1 b
IR69726-116-1-3		–	–	–	0.5 d

^aAverage of 2 observations at 30-35 and 60-65 days after transplanting. ^bValues for RTBV and RTSV are the combined totals of single and double infections. ^cIn a column for each variable, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test. ^dIndicates variety or line not tested.

Table 3. Percent incidence of rice tungro disease (RTD) and numbers of green leafhopper (GLH) vectors^a on rice varieties and advanced breeding lines at Celuk, Bali, Indonesia, in the 1995 dry season (DS) and 1996-98 wet seasons (WS).

Variable	Variety/line	DS 95	WS 96	WS 97	WS 98	
Visual (%)	IR62	3.3 d ^c	2.5 bc	2.3 d	6.6 c	
	IR64	96.0 a	32.3 a	99.8 a	83.9 a	
	IR68305-18-1	29.6 c	1.0 a	—	—	
	IR69705-1-1-3-2-1	1.9 d	0.4 c	0.8 d	0.9 c	
	IR71026-3-2-4-3-5-2	57.6 b	4.9 b	—	—	
	IR71030-2-3-2-1	1.0 d	0.4 c	—	—	
	IR71031-4-5-5-1	— ^d	—	1.3 d	0.9 c	
	IR71605-2-1-5-3	—	—	10.3 cd	6.8 c	
	IR73890-1-3-1-4-1	—	—	18.5 bc	—	
	IR73891-2-1-5-1	—	—	35.3 b	—	
	IR69726-16-3-2	—	—	—	34.5 b	
	IR69726-116-1-3	—	—	—	0.5 c	
	GLH no. ^b	IR62	1.0 a	3.0 a	2.8 c	3.3 c
		IR64	4.3 a	6.9 a	60.3 c	18.8 a
IR68305-18-1		1.8 a	1.0 a	—	—	
IR69705-1-1-3-2-1		2.5 a	2.8 a	11.3 bc	15.6 ab	
IR71026-3-2-4-3-5-2		3.5 a	6.8 a	—	—	
IR71030-2-3-2-1		0.5 a	2.6 a	—	—	
IR71031-4-5-5-1		—	—	1.3 c	2.4 c	
IR71605-2-1-5-3		—	—	18.5 bc	11.4 b	
IR73890-1-3-1-4-1		—	—	21.0 bc	—	
IR73891-2-1-5-1		—	—	39.5 ab	—	
IR69726-16-3-2		—	—	—	18.0 a	
IR69726-116-1-3		—	—	—	15.0 ab	

^aAverage of 2 observations at 30–35 and 60–65 days after transplanting. ^bNumbers of GLH per 10 sweeps of a 30-cm-diameter insect net. ^cIn a column for each variable, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test. ^dIndicates variety or line not tested.

tolerant of RTBV, whereas IR69726-116-1-3 and IR69705-1-1-3-2-1 are resistant to RTSV and tolerant of RTBV. IR68305-18-1 is now grown widely in Karangasem Regency and has shown good field resistance. The three lines have not yet been released, however, and so they cannot be recommended officially to farmers.

Conclusions

Results indicated that these three lines have good potential for controlling tungro disease in Bali: IR68305-18-1 in the dry season and IR69726-116-1-3 and IR69705-1-1-3-2-1 in the wet season.

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Evaluating rice germplasm for resistance to rice tungro disease in West Bengal, India

A.K. Chowdhury

Rice tungro disease is a recurrent problem in areas of West Bengal, India where rice is grown continuously. Rice germplasm developed at IRRI with resistance to tungro viruses were evaluated from 1995 to 1998 at the Regional Rice Research Station of Bidhan Chandra Krishi Viswavidyalaya at Chakdah. Line IR69705-1-1-3-2, with resistance derived from Utri Merah, had consistently low infection with rice tungro spherical virus and rice tungro bacilliform virus in three trials. IR68305-18-1 has a good plant type and excellent eating quality and is also considered to be a promising line. The deployment of tungro-resistant varieties is considered to be the most important strategy for managing tungro disease in the future.

Rice tungro is one of the most common diseases occurring in both aman (winter) and wet-season rice. Rice is the principal crop in the wet season in West Bengal, India, where more than 90% of cultivated land is planted with high-yielding or tall indica varieties. Tungro disease incidence is highest in the aman season, which coincides with the peak abundance of vectors, mostly rice green leafhopper (GLH). The disease occurs sporadically, but it can become widespread. Under field conditions, the most conspicuous tungro symptoms are usually found only in a few high-yielding and local varieties and can easily be confirmed. In other cases, although infection by tungro-associated viruses occurs, plants do not develop any characteristic symptoms, which causes problems in diagnosis by visual observation. In many areas of West Bengal, rice is grown continuously depending on the availability of irrigation water. In these areas, tungro is a recurrent problem, unlike in rainfed single-cropped areas, even though very low populations of GLH occur during the winter and summer months.

In collaboration with IRRI and the Natural Resources Institute (UK), rice germplasm accessions have been tested for resistance to rice tungro viruses since the 1995 wet season. This work has been conducted at the Regional Research Station of Bidhan Chandra Krishi Viswavidyalaya, in a new alluvial zone at Chakdah in West Bengal, India. The methodology of the experiments followed the protocol described by Cabunagan et al (this volume). To coincide with the natural incidence of GLH, transplanting was done in late July. No plant protection chemicals were applied. Indexing of leaf samples by enzyme-linked immunosorbent assay (ELISA) was done in the plant virology laboratory at IRRI.

In the 1995 trial, five rice varieties and one advanced breeding line were evaluated for resistance against tungro disease (Table 1), The susceptible check was IR64 and the field-resistant check was IR62. Two unimproved resistant donors, Balimau Putih and Utri Merah, and IR26, which has resistance to rice tungro spherical virus (RTSV), were included in the trial. In addition, IR68305-18-1, a cross between IR64 and Balimau Putih, was evaluated. Because infection with tungro viruses was low in

Table 1. Percent infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), visual tungro disease incidence, and number of green leafhoppers (GLH) ^a on rice varieties and advanced breeding lines at Chakdah, West Bengal, India, 1995 wet season.

Variable	Varieties/lines ^b					
	IR26	IR62	IR64	IR68305-18-1	Balimau Putih	Utri Merah
BB ^c	9.3 a	4.3 ab	2.1 b	1.8 b	4.4 ab	5.6 ab
SS	13.4 a	3.3 b	17.0 a	10.1 a	16.6 a	2.3 b
Visual	7.0 b	7.8 b	31.1 a	6.3 b	10.6 b	4.6 b
GLH ^d (no. 10 sweeps ⁻¹)	85.3 a	20.6 b	76.4 a	52.1 ab	62.7 ab	68.9 ab

^aAverage of 2 observations at 30–35 and 60–65 d after transplanting. ^bIn a row for each variable, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test. ^cvalues for RTBV and RTSV are the combined totals of single and double infections. BB = total RTBV Infection, SS = total RTSV infections.

^dNumbers of GLH 10 sweeps⁻¹ of a 30-cm diameter insect net.

the trial, it was difficult to assess the performance of the resistant line and varieties. Utri Merah and IR62 had relatively low RTSV infection. In contrast, the level of RTSV infection in resistant IR26 was similar to that in IR64.

In trials between 1996 and 1998, IR62 and IR64 were retained as checks and a range of advanced breeding lines were evaluated for tungro resistance. Infection with tungro viruses was low in both 1996 and 1998, but some differences in the reaction of test lines were detectable in 1996 (Table 2). A line with virus resistance derived from variety ARC11554, IR71030-2-3-2-1, showed relatively high infection of both RTSV and rice tungro bacilliform virus (RTBV) in the 1996 trial. Similarly, IR71031-4-S-S-1, which also has ARC11554 as a parent, was heavily infected with both viruses in the 1997 trial. ARC11554 is of Indian origin and has shown a resistant reaction to tungro viruses in both laboratory and field screening in the Philippines. This variety has been used in many breeding programs as a tungro-resistant donor. Results from field testing its progeny, however, indicated the need for further evaluation under field and laboratory conditions using Indian isolates of tungro viruses.

IR69705-1-1-3-2, with resistance to RTSV and to multiplication of RTBV from Utri Merah, had low infection rates for both viruses in all three trials in 1996–98. Its performance in 1997, when tungro incidence was high, suggested that it has a strong resistance level. Thus, IR69705-1-1-3-2 is considered to have good potential for growing in West Bengal. IR68305-18-1 is also regarded as a promising line, although conditions in the two trials in which it was evaluated were not ideal. This line has a good plant type and excellent eating quality and could be popular with farmers and consumers.

Table 2. Percent infection^a with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) on rice varieties and advanced breeding lines at Chakdah, West Bengal, India, 1996-98 wet seasons.

Variable	Variety/line	WS 96 ^b	ws 97	WS 98
RTBV ^c	IR62	1.5 bc	2.4 e	0 a
	IR64	4.3 ab	21.9 b	0 a
	IR68305-18-1	1.1 bc	—	—
	IR69705-1-1-3-2-1	0.3 c	3.6 de	0 a
	R71026-3-2-4-3-5-2	0.8 c	—	—
	IR71030-2-3-2-1	6.1 a	—	—
	IR71031-4-5-5-1	— ^d	39.3 a	0 a
	IR71605-2-1-5-3	—	19.1 bc	0.3 a
	IR73890-1-3-1-4-1	—	9.4 cd	—
	IR73891-2-1-5-1	—	22.0 b	—
	IR69734-5-1-2	—	—	0.1 a
IR69734-128-2-3	—	—	0 a	
RTSV	IR62	7.8 b	30.1 b	0.3 bc
	IR64	18.0 a	50.4 a	1.5 abc
	IR68305-18-1	5.1 bc	—	—
	IR69705-1-1-3-2-1	1.1 c	1.3 c	0 c
	IR71026-3-2-4-3-5-2	6.0 bc	—	—
	IR71030-2-3-2-1	31.1 a	—	—
	IR71031-4-5-5-1	—	54.0 a	2.6 ab
	IR71605-2-1-5-3	—	64.5 a	2.8 a
	IR73890-1-3-1-4-1	—	50.8 a	—
	IR73891-2-1-5-1	—	22.0 b	—
	IR69734-5-1-2	—	—	0 c
IR69734-128-2-3	—	—	0 c	

^a Average of 2 observations at 30-35 and 60-65 d after transplanting. ^b In a column for each variable. means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test. ^c Values for RTBV and RTSV are the combined totals of single and double infections. ^d indicates variety or line not tested.

A comparison of GLH numbers and incidence of tungro viruses and tungro disease in IR62 and IR64 in the four trials in 1995–98 (Table 3) shows no apparent relationship between leafhopper numbers and tungro incidence. The data reveal that GLH-resistant IR62 still has a strong resistance to tungro disease in the field.

In conclusion, the deployment of resistant varieties is considered to be the most important strategy in tungro management. The causes of sudden disease outbreaks have yet to be determined. This information would enable forecasting of tungro disease and allow the timely application of plant protection measures. Meanwhile, the deployment of virus-resistant varieties offers good prospects for the future.

Table 3. Percent incidence of rice tungro disease (RTD) and numbers of green leafhopper (GLH) vectors^a on rice varieties and advanced breeding lines at Chakdah, West Bengal, India, 1996-98 wet seasons.

Variable	Variety/line	WS 96 ^c	WS97	WS98
RTD (%) incidence	IR62	9.8 b	12.4 b	++ ^e
	IR64	20.0 a	43.3 a	++
	IR68305-18-1	10.8 b	—	—
	IR69705-1-1-3-2-1	9.5 b	4.9 b	++
	IR71026-3-2-4-3-5-2	23.8 a	—	—
	IR71030-2-3-2-1	16.9 ^d ab	—	—
	IR71031-4-5-5-1	— ^d	35.0 a	++
	IR71605-2-1-5-3	—	—	++
	IR73890-1-3-1-4-1	—	10.6 b	—
	IR73891-2-1-5-1	—	11.8 b	—
	IR69734-5-1-2	—	—	++
	IR69734-128-2-3	—	—	++
	GLH no. ^b	IR62	26.6 a	21.1 c
IR64		27.9 a	40.5 ab	++
IR68305-18-1		35.9 a	—	—
IR69705-1-1-3-2-1		24.9 a	24.3 c	++
IR71026-3-2-4-3-5-2		20.5 a	—	—
IR71030-2-3-2-1		26.0 a	—	—
IR71031-4-5-5-1		—	37.3 b	++
IR71605-2-1-5-3		—	38.6 b	++
IR73890-1-3-1-4-1		—	24.5 c	—
IR73891-2-1-5-1		—	46.5 a	—
IR69734-5-1-2		—	—	++
IR69734-128-2-3		—	—	++

^aAverage of 2 observations at 30–35 and 60–65 days after transplanting. ^bNumbers of GLH 10 sweeps⁻¹ of a 30-cm-diameter insect net. ^cIn a column for each variable, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test. ^dIndicates variety or line not tested. ^eNo data.

Notes

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Rice tungro disease resistance and management in Tamil Nadu, India

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Major outbreaks of rice tungro disease occurred in Tamil Nadu in 1984 and 1992 and the disease has continued to appear sporadically in certain districts. Varietal resistance was identified as the most appropriate strategy for managing tungro and collaborative activities were initiated to evaluate the performance of advanced breeding lines with resistance or tolerance to tungro viruses. Field trials were conducted at the Rice Research Station of Tamil Nadu Agricultural University at Tirur in 1996-98 and several promising lines were identified. One tungro-tolerant line, IR68305-18-1, performed well in participatory trials with farmers and is now being used in a resistance breeding program at Tamil Nadu Agricultural University.

Rice tungro disease (RTD) has long been and continues to be a major threat to rice cultivation. It causes great havoc and 30% to 100% losses when it attacks a highly susceptible variety before flowering in conditions conducive to spread. The disease is transmitted by the green leafhopper (GLH), *Nephotettix virescens*. Vector control is the only possible way of managing the disease, but this too is ineffective. Once the disease has become well established in a field, management by vector control is impossible. Hence, there is a strong case for increased dependence on host-plant resistance to tungro disease.

RTD was first reported in India in 1967 (Raychaudhuri and Ghosh) and outbreaks were reported in Bihar in 1969 and in Kerala in 1973. The disease occurred in Tanjore District of Tamil Nadu in 1980 and appeared in Chengalpet District in 1982. Major outbreaks occurred during 1984 and 1992 in Tamil Nadu. In 1984, an epidemic occurred in Tanjore, Chengalpet, and other districts of Tamil Nadu. At the time, varieties IR50, IR36, and CR1009 showed moderate resistance to the disease in Tanjore.

During the 1992 epidemic, RTD was recorded in Thiruvallur, Kanchipuram, Vellore, Villupuram, Thiruvannamalai, and Cuddalore districts. IR50 then showed moderate susceptibility to RTD, although it showed resistance to GLH. Exploiting resistance in rice cultivars against RTD is a continuing process because the potential variability of the causal virus is likely to lead to eventual "breakdown." Moreover, GLH may adapt to vector-resistant varieties by switching from xylem to phloem feeding, which facilitates both leafhopper population development and virus transmission. Consequently, varietal resistance is not stable and a variety that has high resistance to GLH may develop a susceptible reaction to RTD.

To achieve host-plant resistance to disease, there are various options for using and deploying resistance genes. Central to any varietal resistance program is the initial field screening of cultivars under both normal and epidemic conditions. After a resistant line is identified in the field, the material can be used as a donor in a resistance breeding program.

During the 1984 and 1992 RTD epidemics, researchers at the Rice Research Station (RRS) in Tirur identified a few varieties with some resistance to RTD. IR56 and BG 367-3 were moderately resistant in 1984, and IR54 and IET 12888 were resistant in 1992.

RTD has continued to occur sporadically in Thiruvallur and Kanchipuram and there is concern that the presence of inoculum in these areas poses a potential threat to other rice-growing areas, particularly in the Cauvery delta. This led to the development of a collaborative research project among Tamil Nadu Agricultural University, the Department of Agriculture, IRRI, and the Natural Resources Institute (UK).

From 1996 to 1998, experiments were planted at RRS, Tirur, to assess tungro resistance in rice varieties and advanced breeding lines. The test lines and varieties were evaluated in small plots arranged in a randomized complete block design (see Cabunagan et al, this volume). Three trials were laid out during the wet seasons (Samba 1996-98) and one in the dry season (Navarai 1996). RTD incidence was observed and leaf samples were sent to IRRI to be indexed for the presence of rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) by enzyme-linked immunosorbent assay.

On-farm tungro management trials were conducted in 1996-98 in the villages of Vishar and Pudumavilangai to evaluate promising advanced lines under farmers' field conditions. The trials were also used to demonstrate to farmers in village communities the value of tungro resistance as a strategic measure for tungro management. The performance of a leafhopper-resistant variety (T_1) and a virus-resistant or virus-tolerant line (T_2) was compared with that of farmer chosen variety (T_3), which was also planted in the farmer's field. The plot size was 10 x 10 m with a 2-m border row of the leafhopper-resistant variety between plots and surrounding the whole trial area. Each treatment had three replicates laid out in a randomized complete block design. The farmer was requested not to spray T_1 or T_2 plots against leafhoppers or tungro, but he was free to apply pest management practices of his choice to T_3 plots. Tungro incidence was assessed by counting the proportion of diseased hills within five 1 x 1-m quadrats. Leafhopper numbers were estimated by 10 sweeps of a 30-cm-diameter insect net. Yield data were collected from a 5 x 5-m area in the center of each plot.

Results

Field screening for resistance to tungro

The incidence of tungro disease ranged from 0 to 46% on susceptible IR64 in the four trials (Tables 1 and 2). No incidence was recorded in the 1998 wet season and incidence was very low in the 1996 wet season, when there were no significant differences in virus infection rates among test lines and varieties (Table 1). Data from the 1997 wet-season trial could not be analyzed because some treatments had insufficient replications because of problems with crop establishment.

In the 1996 wet season, tungro incidence in ADT37, IR69705-1-1-3-2-1, and IR68305-18-1 was significantly lower than in susceptible check IR64. IR69705-1-1-3-2-1, with resistance to RTSV and RTBV derived from Utri Merah, had low infec-

Table 1. Percent infection^a with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) on rice varieties and advanced breeding lines at Tirur, Tamil Nadu, India, in the 1996 dry season (DS) and 1996-98 wet seasons (WS).

Variable	Variety/line	DS 96	WS 96	WS 97	WS 98
RTBV ^b (%)	IR62/ADT37	4.4 b ^c	0.1 a	4.3	0.3 a
	IR64	19.4 a	0.5 a	14.8	0.4 a
	IR68305-18-1	2.1 bc	0 a	—	—
	IR69705-1-1-3-2-1	0.8 c	0.3 a	2.8	0 a
	IR71026-3-2-4-3-5-2	2.8 bc	0 a	—	—
	IR71030-2-3-2-1	11.6 a	0.1 a	—	—
	IR71031-4-5-5-1	—	—	3.3	0 a
	IR71605-2-1-5-3	—	—	1.0	0.3 a
	IR73890-1-3-1-4-1	—	—	2.8	—
	IR73891-2-1-5-1	—	—	2.5	—
RTSV (%)	IR62/ADT37	—	—	—	0 a
	IR69734-128-2-3	—	—	—	0 a
	IR62/ADT37	25.8 bc	3.1 a	5.8	0 b
	IR64	46.0 a	3.8 a	26.2	1.3 a
	IR68305-18-1	27.0 bc	0.9 a	—	—
	IR69705-1-1-3-2-1	16.8 c	0.3 a	0.7	0 b
	IR71026-3-2-4-3-5-2	30.3 b	2.9 a	—	—
	IR71030-2-3-2-1	30.5 b	1.1 a	—	—
	IR71031-4-5-5-1	—	—	6.1	0.3 ab
	IR71605-2-1-5-3	—	—	11.8	1.1 a
IR73890-1-3-1-4-1	—	—	5.9	—	
IR73891-2-1-5-1	—	—	3.3	—	
IR69734-5-1-2	—	—	—	0 b	
IR69734-128-2-3	—	—	—	0 b	

^aAverage of 2 observations at 30–35 and 60–65d after transplanting. ^bValues for RTBV and RTSV are combined totals of single and double infections. ^cIn a column for each variable, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range tests. ^dIndicates variety or line not tested

tion with both viruses in the 1996 dry- and wet-season trials when disease levels in the trials were relatively high. IR68305-18-1, a cross between IR64 and Balimau Putih, also performed well in the 1996 dry-season trial, although infection with RTSV was relatively high at 27%.

IR71030-2-3-2-1, with resistance to tungro viruses and to GLH from ARC11554, did not perform as well as expected. In the 1996 dry-season trial, infection with both RTSV and RTBV was relatively high. In the 1996 wet season, tungro incidence in this line was similar to that in IR64. Data from another ARC11554 line evaluated in the 1997 and 1998 trials. IR71031-4-5-5-1, were not conclusive. Results, however, suggested the need for further evaluation of the resistance from ARC11553 under field conditions in Tamil Nadu.

On-farm tungro management trials

In general, disease incidence in the trials was low. A tungro outbreak occurred in Vishar in the 1997 wet season and this provided an opportunity for the GLH- and

Table 2. Percent incidence of rice tungro disease and numbers of green leafhopper (GLH) vectors^a on rice varieties and advanced breeding lines at Tirur, Tamil Nadu, India, in the 1996 dry season and 1996-98 wet seasons (WS).

Variable	Variety/line	DS 96	WS 96	WS 97	WS 98	
Visual (%)	IR62/ADT37	1.8 bc ^c	3.1 b	4.8	0 a	
	IR64	23.5 a	10.0 a	36.5	0 a	
	IR68305-18-1	0.5 c	3.8 b	—	—	
	IR69705-1-1-3-2-1	0.9 bc	3.6 b	2.0	0 a	
	IR71026-3-2-4-3-5-2	1.1 bc	6.8 ab	—	—	
	IR71030-2-3-2-1	5.4 ^d b	9.9 a	—	—	
	IR71031-4-5-5-1	—	—	4.0	0 a	
	IR71605-2-1-5-3	—	—	2.0	0 a	
	IR73890-1-3-1-4-1	—	—	0.8	—	
	IR73891-2-1-5-1	—	—	1.0	—	
	IR69734-5-1-2	—	—	—	0 a	
	IR69734-128-2-3	—	—	—	0 a	
	GLH (no.) ^b	IR62/ADT37	—	10.4 a	6.0	4.5 a
		IR64	—	11.3 a	7.3	7.1 a
		IR68305-18-1	—	14.4 a	—	—
IR69705-1-1-3-2-1		—	15.1 a	5.9	2.5 a	
IR71026-3-2-4-3-5-2		—	10.0 a	—	—	
IR71030-2-3-2-1		—	12.4 a	—	—	
IR71031-4-5-5-1		—	—	10.0	2.4 a	
IR71605-2-1-5-3		—	—	6.8	2.8 a	
IR73890-1-3-1-4-1		—	—	7.4	—	
IR73891-2-1-5-1		—	—	6.5	—	
IR69734-5-1-2		—	—	—	1.8 a	
IR69734-128-2-3		—	—	—	2.3 a	

^aAverage of 2 observations at 30-35 and 60-65 d after transplanting. ^bNumbers of GLH per 10 sweeps of a 30-cm-diameter insect net. ^cIn a column for each variable, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range tests. ^dIndicates variety or line not tested.

Table 3. Tungro disease incidence^a, green leafhopper (GLH) numbers^b, and yield for different lines and varieties in an on-farm trial in Vishar, Tamil Nadu, India, in the 1997 wet season.

Line/variety	GLH (no.)	Tungro incidence (%)	Yield (t ha ⁻¹)
ADT37	3.8 ± 0.2	17.4 ± 10.2	4.9 ± 0.1
IR68305-18-1	1.7 ± 0.3	2.6 ± 3.3	6.2 ± 0.4
ADT36	6.1 ± 0.6	31.8 ± 13.3	2.5 ± 0.1

^aNumber of tungro diseased hills at 56 d after transplanting (DAT). Mean of three replications. ^bAverage numbers of adults and nymphs of *Nephotettix virescens* per 10 sweeps of a 30-cm-diameter insect net collected over three sampling dates at 28, 42, and 56 DAT. Mean of three replications.

virus-resistant entries to be effectively evaluated. The majority of varieties affected in the epidemic were the highly susceptible varieties ADT36 and ADT42. ADT36 was the variety chosen in the trial by the farmer-collaborator, Mr. Radha Krishnan. Tungro incidence reached 32% by 56 d after transplanting (Table 3). Incidence in the locally

recommended vector-resistant variety, ADT37, was 17% at the same date and less than 3% in the virus-tolerant IR68305-18-1. This trial stimulated considerable interest among farmers from both Vishar and other villages who were brought to view the trials. As a result, farmers from Vishar and Pudumavilangai grew IR68305-18-1 from seed saved from on-farm trials, which were distributed among them without intervention from researchers.

Conclusions

A line with resistance to tungro viruses derived from Utri Merah, IR69705-1-1-3-2-1, showed good resistance in on-station field trials. This line has good potential for use in breeding programs in Tamil Nadu. IR68305-18-1 performed well in both on-station and on-farm trials. This line has a good plant type and grain yield. In addition, farmers like the white and medium-fine grain. IR68305-18-1 is currently being used in the tungro resistance breeding program at Tamil Nadu Agricultural University.

The trials showed that leafhopper resistance can also play an important role in tungro management. IR62 and ADT37 both showed good resistance to RTD.

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Tungro screen kits for extension agents and plant breeders

O. Azzam, L. Kenyon, and P.D. Nath

For the last 14 years, IRRI has used the enzyme-linked immunosorbent assay (ELISA) method to screen and evaluate rice germplasm for tungro resistance and tolerance. Unfortunately, due to a lack in resources and technical capacities, this technology has not been taken up by national programs. Two years ago, collaborative activities between IRRI and NRI were initiated to develop diagnostic kits that are simpler and more suitable to the needs of national breeding programs and extension services. In this study, we report on the successful development of a Tungro Screen Kit for rice tungro bacilliform virus, one of the two viruses that cause the tungro disease. Five prototype Tungro Screen B kits were assembled and distributed at the Tungro Management Workshop held at IRRI in November 1998 for field testing in India, Indonesia, and the Philippines.

In Southeast Asia, the diagnosis of the two viruses associated with tungro disease, rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), relies mainly on the specific orange-yellow leaf discoloration symptoms exhibited on susceptible cultivars. Several of these cultivars, however, do not produce the specific symptoms in the field or under experimental conditions. Furthermore, if plants are infected with RTSV only, there are no, or only very mild, symptoms.

When enzyme-linked immunosorbent assay was introduced at IRRI in 1985, it revolutionized the approaches to studying the ecology and epidemiology of these two viruses by providing a relatively fast and accurate means of detection. For the last 14 years, IRRI has used the technique to screen and evaluate rice germplasm for tungro resistance and tolerance. More than 35,000 rice accessions have been evaluated and potential sources of resistance against RTSV and tolerance for RTBV have been identified.

In addition to screening germplasm, IRRI assists national research programs in their studies on tungro disease. Unfortunately, because of a lack of resources for establishing and maintaining such facilities and technical difficulties in producing the antisera locally, and optimizing and troubleshooting the technique routinely, the ELISA procedure has not been taken up by the national programs. Commercially produced kits based on 96-well ELISA plates and specific antisera against RTBV and RTSV are available (e.g., Adgen), but these are generally too expensive for routine use in breeding programs or by resource-poor extension services. They also usually require some expertise and the use of expensive equipment for reliable assessment of results.

This report summarizes the collaborative activities undertaken by IRRI and the Natural Resources Institute to try to produce antisera with high titer and specificity suitable for use in simple diagnostic kits by resource-poor national programs and extension services.

Materials and methods

Batches of antisera against the two viruses were produced by using a modification of the usual procedure. Instead of giving the rabbits only three intermuscular and one interveinal injection of the purified virus particles (in adjuvant) at weekly and bi-weekly intervals, they were given nine, with 6-wk intervals between each of the later seven injections. Approximately 20 mL of blood were collected from each rabbit 10 d after each of the later seven antigen injections. This immunization regime is reported to increase the titer and specificity of the resulting antisera (Harlow and Lane 1988).

Antisera titers were first measured using purified virus particles in the ring-interface precipitin test (Van Regenmortel 1982). Immunoglobulins were purified from the sera by ammonium sulfate precipitation and diethylaminoethyl (DEAE)-cellulose chromatography. Alkaline phosphatase was conjugated to the immunoglobulins by the glutaraldehyde procedure (Clark and Adams 1977). The titer and specificity of each of the different batches of purified immunoglobulins were tested in double antibody sandwich (DAS)-ELISA using IgG to trap and IgG-alkaline phosphatase to detect the trapped virions.

The batches of antiserum with the greatest titer and specificity were tested in a nonquantitative membrane-based "tissue-print" assay for their suitability for use in a simple diagnostic kit. Freshly cut stems and leaf midribs were "printed" onto the surface of nitrocellulose or polyvinylidene fluoride (PVDF) membranes. The membranes were then probed either with the virus-specific antisera batch followed by a commercial alkaline phosphatase-labeled antirabbit immunoglobulin or directly with the virus-specific antibody batch conjugated to alkaline phosphatase. Signal development in either case was by using the 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) substrate.

Results and discussion

The need by national programs and extension agents for simple, cheap, and reliable diagnostic tools for rice tungro viruses had been expressed frequently (e.g., Foot 1995). In 1997, Cabauatan and Koganezawa described a simple assay based on the use of colored latex beads bound to specific polyclonal antisera. This procedure, however, was not taken up to any great extent, probably because of the requirement to homogenize and centrifuge samples, and the large quantities of antiserum the test required.

Of the batches of antiserum produced against RTBV and RTSV by the repeated immunization method, RTBV-6 and RTSV-6 showed the greatest titers in the ring interface test. All seven batches for each virus had sufficient specificity and titer for use in DAS-ELISA, although they did present relatively high healthy-background absorbances. This high level of cross-reaction to components from healthy rice plants meant that none of the antisera could be used directly in tissue-print assays because the difference in signal strength between infected and healthy plants was often too small to be reliably observed by the eye.

To try to overcome the problem of cross-reaction to healthy plant components, the batches of antiserum with the greatest titer and least cross-reactivity were cross-adsorbed with healthy sap components. The most effective method for doing this was by first passing the immunoglobulin fractions through a specially prepared healthy-rice-proteins-sepharose column (prepared by treating cyanogen-bromide-activated sepharose with homogenate from healthy rice plants). The resulting solution was then incubated with a piece of nitrocellulose membrane previously saturated with healthy rice plant homogenate. This mopped up any remaining antibodies with affinity for plant proteins.

After this double-cross adsorption, only RTBV-IgG batch 6 retained sufficient titer to be used effectively with little healthy-background reaction in tissue-print assays (Fig. 1). This doubly cross-adsorbed antibody is the basis for the prototype Tungro Screen B diagnostic kits. Because a helper component from RTSV is required for vector transmission of RTBV, a positive reaction with the RTBV detection kit in the field implies the presence of RTSV as well. A simple diagnostic test for the presence of RTSV alone, however, would still be useful.

Five prototype Tungro Screen B kits were distributed at the November 1998 Tungro Management Workshop held at IRRI for field testing in India, Indonesia, and the Philippines. Included with each kit was a question sheet to be filled out and returned to IRRI with the test membranes. The questionnaire asked for details about rice varieties tested, plant age, how plants were stored prior to testing, if there was a delay between printing and developing the membranes, and how easy the evaluator found the test procedure, and comments about the kit or its possible improvement. Based on these responses, it is anticipated that modifications or improvements to the kit will be made in the near future.

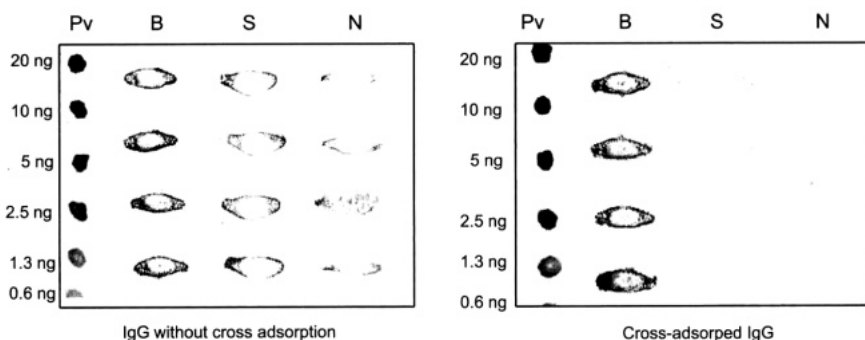


Fig. 1. Tissue printing results of a series of purified rice tungro bacilliform virus (RTBV) dilutions (Pv), RTBV-infected plant prints (B), rice tungro spherical virus (RTSV)-infected plant prints (S), and virus ELISA-negative plant prints (N) when hybridized with RTBV IgG without and with cross-adsorption. IgG and conjugate dilutions used were 10^{-3} .

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Are tungro disease counts repeatable?

K.G.Schoenly

Scientists often assume that the measurements they take are repeatable across observers and sites; however, experience from different and unrelated disciplines indicate that interobserver repeatability varies with the sophistication of the measurement, observer experience, and the measurement scale used. Recent developments in repeatability methodology from quantitative genetics, for example, has produced an easy-to-interpret repeatability index R that varies from 0 (no repeatability) to 1 (perfect repeatability) derived from a one-way ANOVA (or intraclass correlation) that may be useful for plant protection workers. For measurements that are repeatable across a range of observers, the upper confidence interval for R (e.g., 95%) should equal or approach 1. For tungro disease counts, one study from Thailand revealed an R value of 0.1407 and an upper (95%) confidence interval of 0.5501. Although this value is low, without more field counts from more sites, it is premature to ask if tungro disease counts are repeatable. Although no scientific measurement is expected to have perfect repeatability, this Thai study underscores the need for plant protection workers to conduct frequent repeatability trials of their scientific measurements as a routine quality assurance procedure, particularly when multiple persons are required at multiple sites and when data from such studies are pooled for later statistical analysis.

Measurements that scientists take are often assumed to be repeatable and highly precise across a range of observers (Krebs 1989); however, interobserver repeatability varies with the sophistication of the measurement, observer experience, and the measurement scale used. Published trials of repeatability for plant injuries in rice are scarce but revealing. In showing possible causes of varietal reaction to rice tungro disease, Ling (1979) reported that, of 561 rice varieties scored for their reaction to tungro by four observers in 1976, only 49% of within-observer readings (based on two readings of the same varieties) scored identically. Interscorer results from 28 rice varieties, taken by six scorers in 1978, showed that 43% of the varieties differed by 2 points on a 5-point scoring scale. This study also showed that scoring ability can be improved by experience. For field surveys, Ling (1979) recommended that data recording for tungro be restricted to one person using a tape recorder and suggested that a data transfer method be used instead of employing multiple persons to record data at the same site. Studies that require the same data to be collected at multiple sites, however, will likely employ multiple persons. If data from such studies are pooled for statistical analysis, interobserver repeatability becomes an unavoidable scientific issue.

Recent developments in repeatability methodology from quantitative genetics (Becker 1984, Lessells and Boag 1987) have produced an easy-to-interpret repeatability index R (based on a one-way ANOVA classification; see Krebs 1989 for working examples) that varies from 0 (no repeatability) to 1 (perfect repeatability). R is also known as the intraclass correlation, accessible in standard biometrics textbooks

(e.g., Sokal and Rohlf 1995, p 213; Zar 1984, p 323-325). and is calculated as $R = S^2_A / (S^2_E + S^2_A)$, where S^2_A is the variance among items and S^2_E is the variance within individuals. Thus, if measurements are perfectly repeatable. S^2_E is zero and $R = 1.0$. When R is computed with its 95% confidence limits, for example, one hopes that the upper confidence limit of R equals or closely approaches 1. Few scientific measurements are expected to have perfect repeatability, but one hopes no measurement has zero repeatability.

Repeatability methodology is perhaps most useful in the context of quality assurance for identifying both the source and magnitude of correctable error for groups of scientific measurements before they are routinely used in future laboratory, greenhouse, and field trials. In May 1999 at IRRI, for example, a small repeatability study of 20 participants who were asked to individually and independently record several agronomic traits and injuries from the same 10 hills in the field ranked tiller number as the most repeatable measure ($R = 0.8528$ [mean of trained and untrained groups]), followed by plant height (0.7483) and number of whiteheads (0.5856; Schoenly and Domingo, unpublished data). In contrast, counts of tungro-diseased plants recorded by four observers at 10 sampling stations in one farmer's field in Thailand (Disthaporn 1987) gave an R value of 0.1407 and an upper 95% confidence limit of 0.5501. Without additional repeatability results at more sites, it is premature to ask whether multiperson field counts of tungro-diseased plants are repeatable. relative to whitehead counts, for example. These studies, however, underscore the need for plant protection workers to conduct frequent repeatability trials of their scientific measurements before they are put to routine use and to revise or even discard, if necessary, those measurements that are not repeatable.

Other contexts where repeatability methodology is potentially useful include pre- and posttesting exercises in training workshops. This venue gives the principal investigator(s) the opportunity to observe the recorders firsthand and to detect and correct departures in protocol (Kahn and Sempos 1989). Publishing repeatability results alerts colleagues in plant protection disciplines to expected error values and confidence limits for scientific measurements that are gathered under specific laboratory, greenhouse, and field conditions.

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Surveillance scheme for tungro forecasting in Malaysia

A.B. Othman, M.J. Azizah, A.T. Jatil

Rice tungro disease in Malaysia was suspected to occur first in 1933. Serious outbreaks occurred in 1982 and 1983, when more than 20,365 and 12,439 ha, respectively, were affected. A nationwide campaign was launched to control the disease and to strengthen the rice pest surveillance and forecasting system. The techniques used were mapping, field surveillance, mobile nurseries, tests for viruliferous insects, and light traps. As a result of this campaign and the intensification of surveillance activities, the area of tungro infestation was reduced greatly. Green leafhopper-resistant or moderately resistant varieties were widely recommended and adopted. Surveillance for tungro was further refined by incorporating new components such as recording the severity of infestation, using a serological test (ELISA) to detect infection, and using ultraviolet light traps for monitoring GLH abundance and species composition. Economic threshold levels were established for decision making. Other information on natural control, natural enemies, farmers' attitudes and experiences, and availability of pest control equipment was also compiled.

In Malaysia, tungro, or penyakit merah virus (PMV) as it is locally known, was suspected to occur first in Kerian District in Perak State in 1933. At that time, the condition was thought to be caused by a physiological disorder, a soil problem, a nematode, or a combination of several factors. Later, however, it was reported to be caused by a virus (Ou 1965). For a long time, the occurrence of this disease was confined to Kerian District, but in 1980 it was reported in Penang, Kedah, and Perlis. The most serious outbreak occurred in 1982 in Kedah and Perlis when more than 20,300 ha of rice fields were afflicted by tungro and yield loss was estimated to be 34,000 t, amounting to US\$10 million. The 1982 outbreak has been attributed to several factors:

- An increase in staggered planting, which resulted in the presence of a continuous source of tungro.
- A large population of active vectors, which created a greater potential for virus transmission.
- The widespread cultivation of variety MR37, which was susceptible to both the vector and virus.
- The availability of plant stages susceptible to the vector and virus.
- Inadequate staff for pest surveillance and forecasting programs, resulting in ineffective coverage.

Because tungro is disastrous, the Department of Agriculture (DOA) launched a nationwide campaign that involved all agencies dealing with the control of the disease and strengthened the tungro surveillance and forecasting programs in Perlis, Kedah, Pulau Pinang, and Perak (DOA 1983). The surveillance techniques used during this time were mapping, field surveillance, mobile nurseries, testing for viruliferous insects, and light traps.

As a result of this campaign and intensification of the surveillance activities, the area affected by tungro in 1983 decreased to 12,439 ha. Subsequently, in 1984 and 1985, only 3,843 and 978 ha were affected, respectively. From 1986 to 1989, the area infected with tungro was between 330 and 740 ha. In 1990, however, 1,857 ha were infected with the disease (Chen and Othman 1991). This increase in tungro incidence warranted quick action because rice planting was highly staggered due to problems with water management and a high population of the major vector, *Nephotettix virescens*. An intensive survey was carried out to determine the extent of the disease and infected plants and stubbles were destroyed by roguing or with herbicides. Insecticides were used in areas with high vector numbers. GLH-resistant or moderately resistant varieties such as MR77, MR84, MR103, and MR 106 were widely recommended and adopted. Subsequently, the affected area diminished greatly to only 401 ha in 1991, 68 ha in 1992, 24 ha in 1993, 56 ha in 1994, and 22 ha in 1995. The tungro surveillance program was then further refined by incorporating new components to intensify and strengthen surveillance activities and to continuously maintain tungro at very low or zero incidence. In 1996, the diseased area increased slightly to 3 17 ha, but the problem was contained by planting virus-resistant MR 159 in the infected area for one season. As a result, no tungro was recorded in 1997 and 1998 (Fig. 1).

Pest surveillance and forecasting system

The pest surveillance and forecasting system in Malaysia was set up in 1979 by the DOA and implemented in all rice areas in the country. The merits of the surveillance system were fully realized in the light of the devastating outbreak of brown planthopper in Tanjung Karang in 1977 and mixed brown and whitebacked planthopper infestations in Muda in 1978 and 1979. A project proposal with a budget of US\$8.1 million over a 5-yr period was approved by the Malaysian government and the DOA was

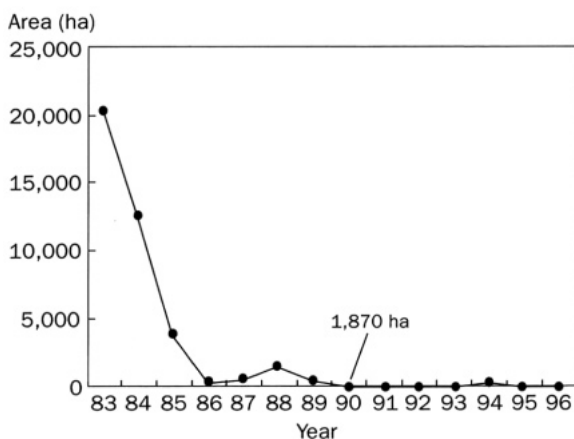


Fig. 1. Annual incidence of rice tungro disease in Malaysia, 1983-96.

entrusted with implementing the project. It was agreed that the Malaysian Agricultural Research and Development Institute (MARDI) would provide the necessary research support. During the same period, the concept of integrated pest management (IPM) was adopted for rice in Malaysia and the pest surveillance and forecasting system became a crucial component. The system gathers information from the 450,000 ha of rice fields in Peninsular Malaysia on the population of insect pests and natural enemies, incidence and severity of rice diseases, infestation of major weeds, and damage by rats and golden apple snail (DOA 1989, Chang 1991). Other factors such as crop age, water level, varieties, weather conditions, and farm practices are monitored. Light traps, net traps, mobile nurseries, and egg parasitism studies were set up to provide additional information.

Location of surveillance system

The establishment of a surveillance and forecasting system involves setting up a practical regional system in all the major rice-growing areas. Each regional system operates from a pest surveillance center. The system is under an agricultural officer who is assisted by assistant agricultural officers, several agricultural technicians, and surveillance scouts. The number of agricultural technicians and scouts varies from one surveillance center to another, depending on the size of the area. Ideally, each agricultural technician supervises an area of 4,000–5,000 ha and is assisted by five trained field scouts. All the regional systems are coordinated by the pest surveillance and forecasting section based at the Crop Protection and Quarantine Services Division headquarters in Kuala Lumpur. There are now 12 pest surveillance centers located in the following areas: Tambung Tulang, Perlis; Telok Chengai and Sungai Petani, Kedah; Bumbong Lima, Pulau Pinang; Parit Buntar, Chenderong Balai, Seberang Perak, and Titi Gantung, Perak; Sungai Burong, Selangor; Gerai, and K. Terengganu, Terengganu; and Lundang, Kelantan.

Surveillance techniques for tungro

The techniques adopted for tungro surveillance and forecasting are field surveys/field scouting, mobile nurseries, tests for viruliferous vectors, and light traps.

Field surveillance/field scouting

In field surveillance, the block surveillance system is used whereby several fields within a 120–240-ha block are examined. All rice surveillance areas are divided into blocks and coded accordingly by state, district, and locality. Ten fields per block are randomly sampled and surveyed every 7–10 d. From each field, 10 hills are chosen randomly and examined. Field scouts detect vector numbers and tungro disease incidence. Visual estimates of GLH are recorded. Visual counting permits rapid examination without subsequent laboratory work, although accuracy probably decreases as pest density increases. At times, sweep nets are also used to estimate GLH numbers.

Initially, tungro incidence was assessed by symptoms and by the starch iodine test. This test detects the increased starch content in rice leaves infected with tungro. The cut edges of infected leaves turn dark blue when tested. This test was abandoned, however, because other virus diseases such as dwarf disease and transitory yellowing as well as other stresses may also give similar results. Subsequently, rice leaves were indexed for infection with tungro viruses by the latex flocculation test, which was carried out at the surveillance centers.

Presently, tungro field surveillance includes an assessment of the disease based on severity of infestation. The index formulated for severity of infestation for tungro is 5%, 10%, 25%, and 50% diseased hills. A field manual has been developed for tungro surveillance and data management. Leaf samples are also collected for indexing for tungro viruses by enzyme-linked immunosorbent assay (ELISA) at the surveillance centers. Whenever required, the examination of rice stubbles for tungro symptoms is also carried out and samples are taken for testing by ELISA. In high-risk areas for tungro, relatively more samples are collected during the field surveillance. For example, in Perak in 1998, more than 5,000 samples were collected for the ELISA test. To complement field scouting, key farmers/farmer-leaders and surveillance brigades that consist mainly of farmers' children have been trained to monitor tungro incidence and report to the surveillance staff. Farmers are encouraged to monitor the disease in their own fields. Infected plants are removed and destroyed. All surveillance data collected are analyzed on the same day.

Mobile nurseries

Mobile nurseries are deployed to detect the presence of any viruliferous GLH 1 mo before planting. The approach involves planting TN1 (Taichung Native 1) rice seedlings in trays, which are exposed to GLH in the field. The seedlings are exposed for 3 days and nights. Ultraviolet lights or inflorescent lamps are used to attract GLH to the rice seedlings in the tray. The tray is then brought back, sprayed with insecticide to kill the GLH, and placed in the glasshouse for 10-15 d. Initially, seedlings were assessed visually for tungro and by using the starch iodine test. Seedlings are now tested serologically for each of the two tungro viruses (rice tungro bacilliform virus and rice tungro spherical virus) by ELISA. The surveillance centers that are equipped with ELISA facilities are Telok Chengai, Parit Buntar, and Lundang, in addition to the Plant Disease Section in Kuala Lumpur. The number of mobile nurseries used depends on the size of the area monitored (Table 1). If the ELISA test gives positive results and there is a high GLH population, the area and field extension officers are immediately informed to allow further action to be taken. Intensive surveys and monitoring are carried out by the surveillance staff during the following season for up to 60 d after planting.

Transmission test for viruliferous vectors

GLH are collected from the field and used in transmission tests to determine their infectivity. After the introduction of ELISA, however, this technique is no longer used.

Table 1. Number of mobile nursery trays (areas y⁻¹).

State	1996		1997		1998	
	Total trays (no.)	Trays w/ tungro ^a	Total trays (no.)	Trays w/ tungro ^a	Total trays (no.)	Trays w/ tungro ^a
Perlis	145	11	88	60	41	1
Kedah	122	13	280	32	245	29
Pulau Pinang	201	23	185	24	71	15
Perak	220	40	310	54	214	20
Kelantan	61	7	18	5	30	0

^aNumber of trays which contained tungro-infected seedlings.

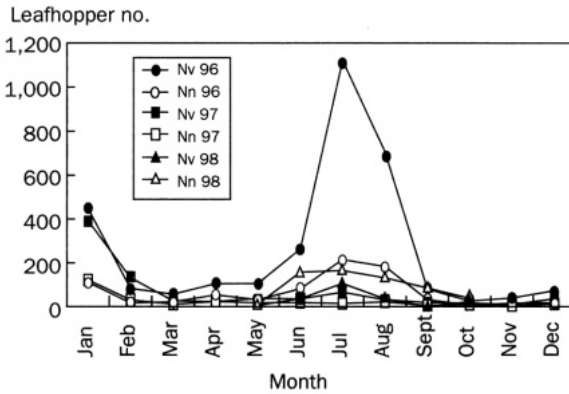


Fig. 2. Monthly light trap catches of *Nephotettix virescens* (Nv) and *N. nigropictus* (Nn) in 1996-98 in Kerian District, Malaysia.

Light traps

Light traps are set up in the surveillance areas to detect the buildup of the GLH population and its species composition. A high population of active vectors increases the potential for virus transmission. In some areas, ultraviolet lights are used because they attract more GLH. Light trap catches are recorded daily. Intensive surveillance activities are carried out in areas previously infected with tungro when large numbers of GLH are caught in the light traps. For example, light trap catches in Kerian were low in 1997 and 1998. This relates to the low or zero incidence of tungro infection found during the same period (Fig. 2). Constant monitoring of light trap catches has enabled the early detection of tungro in rice areas and established a pattern of GLH activities. The number of light traps in the major rice areas are Perlis, 5; Kedah, 20; Pulau Pinang, 4; Perak, 40; Selangor, 7; Terengganu, 6; and Kelantan, 9.

Processing surveillance data

Data collected are examined and compiled on the same day. At the surveillance centers, data are studied by surveillance officers. Relevant information on the field situation is incorporated in the daily surveillance report. Data from light and net traps are also reported as daily and weekly catches.

Tentative economic thresholds

The economic threshold is defined as the density at which control measures should be implemented to prevent an increasing pest population from reaching the economic injury level. The word “tentative” is used to describe the dynamic nature of the threshold values, which are determined by variable factors such as yield, treatment cost, time taken to initiate and carry out treatment, and perception of pest control by farmers. To make sound plant protection decisions, it is necessary to have tentative economic thresholds, however crude these may be initially. An arbitrary economic threshold for tungro is 1 GLH hill⁻¹ (for areas with tungro) and for mobile nurseries 1% infection in ELISA tests combined with a high GLH population.

Decision making

Having processed the surveillance data, the agricultural officers, assistant agricultural officers, and agricultural technicians involved discuss and assess the pest and disease situation. In normal situations where pests and diseases are below threshold levels, farmers and extension officers are informed. This information is also displayed on notice boards at IPM clinics and meeting places for farmers and extension staff. Color-coded indicators are displayed at strategic places where farmers can easily see them. The color codes used are green for safe, yellow for potentially reaching the economic threshold level (ETL), and red for above the ETL with action needed by farmers.

When pest or disease incidence approaches or exceeds the ETL, the information is immediately relayed to the area extension officers and to the Pest Surveillance and Forecasting Section in Kuala Lumpur. The extension officers and surveillance technicians will then advise farmers and their children (surveillance brigades) to check their own fields. An intensive survey is also carried out covering 25–50% of the block area.

Other factors included in the data collected by field scouts, together with the tentative economic threshold, are natural control, farmers' attitudes, past experiences, and availability of pesticides and spraying equipment. At times, only spot spraying or spraying in certain fields is required. Should the pest or disease situation reach outbreak levels in extensive areas, the pest control committee is activated. The committee comprises members from the State Department of Agriculture, extension services, crop protection service, technical branches, and MARDI. In addition to reviewing the pest situation in relation to field conditions and making control decisions, the committee also determines the surveillance for the period and ensures that decisions made

are implemented immediately. The committee is also entrusted with planning pest control when necessary. Any problems that need further research or technical problems that cannot be solved at the ground level are channeled to the IPM Implementation Committee in the state and to the National Rice IPM Technical Committee, if required.

Discussion and conclusions

The success of a surveillance system can be measured by its ability to provide early detection of pests and to prevent a serious outbreak. In this respect, the surveillance system in the major rice-growing regions of Malaysia has been highly successful (DOA 1996, Chen and Jatil 1997). It has successfully contributed to the containment of outbreaks of several pests and diseases through early warning and by aiding extension personnel and relevant agencies to make decisions on control measures. The system has helped to prevent GLH populations and tungro from reaching 1982-83 levels. At present, the surveillance system for tungro very much depends on field scouting and mobile nurseries, which are time-consuming but very effective. Attempts to improve field scouting are currently being carried out using ELISA. Antiserum for ELISA is being produced for detecting rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) with the assistance of staff from IRRI and MARDI. This method would enable better supervision of the field staff because more field samples can be verified. Preliminary observations showed that this method is not only feasible but also more sensitive than visual assessment. The field surveillance data in 1997 and 1998 showed no areas with tungro incidence, but samples analyzed by ELISA gave positive results. Symptoms were not expressed because plants were infected only with RTSV.

Sometimes when symptoms are detected, it is often too late or beyond the action level. What the tungro surveillance system needs is a supply of antiserum for RTSV and RTBV. This will allow the virus to be detected before symptoms are expressed. This approach will identify the areas infected with RTSV and RTBV, either with single or double infections, to map the distribution and spread of tungro. Thus, effective control and eradication measures can be carried out. With this it is hoped that farmers will be able to attain the potential yield of the crop.

Light traps have been used to supplement field data. Light trap catches are known to reflect the field situation of GLH incidence. Besides being used as a monitoring tool, light traps are sometimes used as a control measure. Natural enemies, particularly predators of GLH, are recognized to be important mortality factors. Experience in rice areas has shown that, where predators are common, no insecticides were used. The practice of herbicide application on bunds appears to be highly disruptive to natural enemies of leaf feeders and leafhoppers. It is recommended that bunds be slashed mechanically to maintain refuges for natural enemies.

Rice diseases in Malaysia are controlled whenever possible by incorporating host-plant resistance in MARDI's rice varieties. Eighty percent of the rice planted is variety MR84, which is moderately resistant to tungro and GLH-resistant. The use of clean healthy seeds is a basic requirement in disease control. Field sanitation during

and at the end of the crop cycle is a valuable cultural practice to reduce inoculum sources for the next crop. For tungro control, farmers are urged to destroy ratoon, stubbles, and volunteer seedlings to reduce the survival of hosts for GLH and tungro. The surveillance system allows tungro management programs to be evaluated effectively. Tungro is currently well under control and every effort is being made to maintain this situation.

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Farmers' rice tungro management practices in India and the Philippines

H. Warburton, S. Villareal, and P. Subramanian

Rice tungro disease is a serious disease for farmers because it is difficult to forecast and control, and can cause high yield losses. In this study, we compared the perceptions and practices of rice farmers in India and the Philippines, two areas where tungro is reported as endemic. The aim was to find out what farmers knew about tungro and how they coped with it. We also investigated the factors that influenced their knowledge and management practices.

Farmers and plant diseases

Much has been written about farmers' indigenous knowledge and there are many examples of farmers' detailed knowledge of pests and crop protection methods (for example, Boef et al 1993, Brammer 1980, Brokensha and Riley 1980, Fairhead 1993, Richards 1985). Virus diseases such as tungro, however, might pose problems for farmers because the causal agent (virus) cannot be seen. Bentley (1992) points out that farmers often know more about conspicuous and important pests (for example, weeds, grasshoppers, beetles) but less about inconspicuous pests. With a disease such as tungro, which is difficult to observe but important in the damage that it causes, farmers' understanding may differ considerably from that of scientists (Fig. 1). If researchers are to develop better ways of managing tungro that are acceptable to farmers, they need to find out what farmers already know about the disease and build from there.

Background

The two areas studied were Chengalpattu District, Tamil Nadu, India, and Midsayap, North Cotabato, Mindanao, Philippines. Both are considered tungro-endemic areas by the local agricultural research institutes.

Focused group discussions and semistructured interviews were conducted initially with farmers to discuss their perceptions and practices relating to tungro disease and to gain insights into how farmers viewed the disease. This was followed by a series of surveys of randomly selected farmers using a structured questionnaire. These consisted of a baseline survey with questions on farming practices, pest and disease problems, and knowledge and management of tungro disease, plus follow-up surveys to record actual farming practices for several seasons after the baseline survey (Table 1). In the Philippines, the project had been established for a longer period so it was possible to collect information from a wider range of villages. In addition, actual tungro disease incidence was monitored by researchers on 180 of the total sample farms, so it was possible to compare farmers' and researchers' observations of the disease.

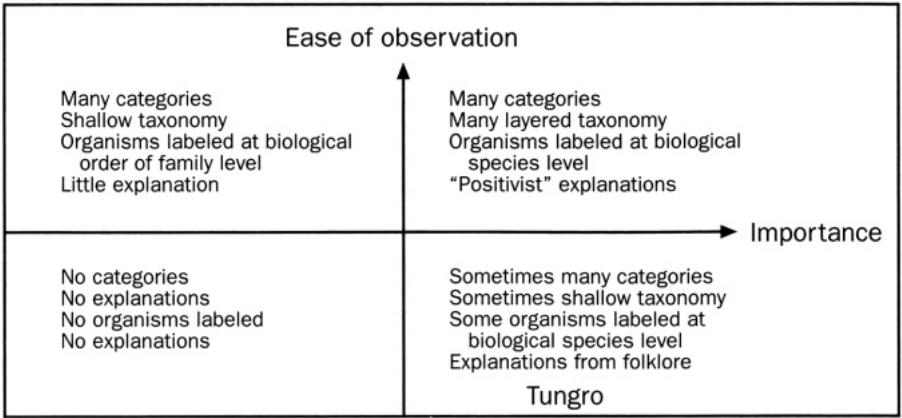


Fig. 1. Characteristics of four classes of farmer knowledge (from Bentley 1991, Fig. 2.).

Table 1. Data collection strategies used.

India	Philippines
Chengalpattu District, Tamil Nadu	Midsayap, North Cotabato, Mindanao
Group interviews in 6 villages	Group interviews in 9 villages
Questionnaire surveys of 90 farmers in 2 villages: baseline plus follow-up surveys for 6 seasons, 1996-98	Questionnaire surveys of 226 farmers in 9 villages: baseline plus follow-up surveys for 4 seasons, 1996-97

Table 2. Farming systems used in India and the Philippines.

Item	India	Philippines
Crops	Rice, sugarcane, groundnut	Rice
Av. no. rice crops year-1	2.3	2.0
Irrigation system	Tank, tube well	Gravity (National Irrigation Administration)
Farm size (ha)	2.78 (std. 3.22)	1.31 (std. 0.98)
Main planting method	Transplanted	Direct-seeded (63.5%)

Results

In both areas, rice was the major staple crop, and multiple crops of rice were grown per year (Table 2); however, India has a more mixed cropping system than the Philippines, with other crops such as sugarcane and groundnut rotated with rice. The irrigation system also differed, with the Filipino farmers relying on a large-scale gravity system, whereas Indian farmers used tanks or wells. India has three distinct seasons (sornavari, samba, and navarai); the Philippines has two main seasons, dry and wet, but farmers can plant rice year-round. The majority of farmers had small farm sizes, with 87% of Indian farmers and 99% of Filipino farmers having 5 ha or less.

Experience with tungro

Although both areas were identified as tungro-endemic, Filipino farmers reported a higher incidence of the disease; 85% of the farmers had experienced tungro at least once, and more than 40% had experienced it two or more times. Seventy-five percent of Indian farmers had experienced tungro at least once, but few could recall more than one disease attack (Table 3). In fact, Indian farmers could only recall one occasion (1990-91) when villages had been badly affected by tungro. In the 1996-98 seasons, tungro incidence in Philippine villages was again much higher, with some incidence found in every season (Table 3). In Indian villages, tungro only occurred in one village in one season (samba 1997) (Table 3). Although the data are based on researchers' measurements in the Philippines and farmers' reports in India, incidence is undoubtedly much higher in the Philippines.

Importance of tungro relative to other pests and diseases

Not surprisingly, Filipino farmers rate tungro as a far more important pest or disease than Indian farmers (Table 4). Indian farmers generally appear to be less "pest-conscious" than Filipino farmers, with many stating that they did not have major pest or disease problems in many seasons (particularly in the dry sornavari season).

Table 3. Farmers' experience of tungro disease.

Percentage of farmers who reported experiencing tungro prior to 1996

No. of tungro attacks experienced	India (n = 90)	Philippines (n = 226)
0	25.6	14.2
1	66.7	43.4
2	7.8	20.4
3	0	13.3
4	0	0.4
5	0	0.4
>5	0	8.0

India: Percentage of farmers reporting tungro incidence, 1996-98 (n = 90)

	96 Samba ^a	97 Navarai	97 Sornavari	97 Samba	98 Navarai	98 Sornavari
Tungro	0	0	0	7.8	0	0
No tungro	100	100	100	92.2	100	100

Philippines: Percentage of farms where tungro incidence was recorded by researchers, 1996-97

Tungro incidence	96 WS	97 DS	97 WS	96-97 WDS
0	18.7	28.5	42.7	31.0
=< 5%	59.1	64.5	50.6	58.1
=< 50%	20.4	5.2	5.4	9.6
> 50%	1.8	1.7	1.3	1.3
Farms (no.)	225	172	239	229

^aSamba = monsoon crop/July-December planting, navarai = low rainfall crop/December-January planting, sornavari = May-early June planting.

Table 4. Most important pests and diseases reported by farmers in baseline survey, 1996.

<i>India</i>			
Sornavari	Samba	Navarai	
Stem borer	Stem borer	Leaffolder	
Leaffolder	Leaffolder	Stem borer	
Neck blast	Tungro	Earhead bug	
BPH ^a hopperburn	Cutworm	Blast	
Ear head bug			
<i>Philippines</i>			
Dry season	Points ^b	Wet season	Points
Stem borer	291	Stem borer	277
Tungro	255	Tungro	275
BPH/hopperburn	204	BPH / hopperburn	189
Black bug	189	Black bug	183
Rat	129	Rat	139
Rice bug	68	GLH	62
GLH	47	Rice bug	55
Cutworm	37	Cutworm	31
Worm	29	Worm	27
Golden snail	19	Golden snail	19
Armyworm	12	Leaffolder	14
Leaffolder	12	Armyworm	12
Neck rot	7	Neck rot	8
Others	27	Others	31

^aBPH = brown planthopper, GLH = greenleafhopper. ^bFarmers' rankings on a scale of 1st = 3 points, 2nd = 2 points, and 3rd = 1 point.

Knowledge of tungro symptoms

All farmers in the Philippines knew of tungro, but 21% of Indian farmers had no idea what it was (Table 5). Farmers had not heard of tungro or its local name. Other farmers were able to give a reasonable description of the disease, with 37% and 50% of farmers describing both the yellowing leaves and stunted appearance in India and the Philippines, respectively. Farmers described tungro as similar to cancer or AIDS because they knew that the plant, once infected, would not recover. In the Philippines, the word “tungro” was used generally to describe a devastating problem, but farmers did not use it to cover any type of damaging rice disease. They could name other conditions and diseases of rice that produced similar symptoms, but that they knew were not tungro (e.g., zinc or nitrogen deficiency). Some researchers observed that some farmers do have difficulty, however, in distinguishing tungro from zinc deficiency.

Farmers' perceptions of causes and mode of tungro spread

Farmers were uncertain about the causes and mode of spread of tungro (Table 6). Half the Indian farmers and 14% of Filipino farmers said they had no idea about the cause of tungro. Insects were identified most often in India as the cause of tungro spread,

Table 5. Farmers (%) reporting tungro symptoms.

Symptom	India (n = 90)	Philippines (n = 226)
Yellowing leaves	11.1	45.1
Stunted appearance	17.8	0
Yellowing leaves & stunting	36.7	50.4
Incompatible symptoms	0	0.4
Do not know	13.3	4.0
Have not heard of tungro	21.1	0

Table 6. Farmers (%) reporting causes of tungro. Some farmers specified more than one cause or mode of spread.

Factor	India (n = 90)	Philippines (n = 226)
Green leafhopper	5.6	36.3
Brown planthopper	0	15.0
insects (general)	36.7	5.3
Other specific insects	0	4.9
Water	0	17.3
Wind/air	2.2	7.1
Soil/roots	0	4.9
Weather	0	5.3
Seeds	0	3.5
Birds	0	0.4
Susceptible variety	0	20.3
Old rice varieties	0	0.4
Continuous cropping	0	0.4
Virus, germ	3.3	15.9
No idea	50.0	14.2

and more than a third of Philippine farmers knew that tungro was spread b) green leafhoppers. Only 6% of Indian farmers knew this. Other modes of spread such as through water, air, and soil were also identified.

Filipino farmers were more aware of the effects of varietal selection on tungro incidence. Through their own observations, they had noticed that some varieties were more susceptible than others to tungro.

Farmer management strategies

From a researcher's point of view, management of tungro is based on controlling the sources of disease inoculum or controlling the disease vector, the green leafhopper. Methods found by researchers to be effective include using resistant rice varieties and using synchronous planting, rotations, or fallow periods to remove rice plants at certain times of the year, thus removing sources of disease inoculum and preventing disease spread. Other methods involve roguing diseased plants and applying fertilizer. Insecticides used to be widely recommended for controlling the green leafhopper, although some researchers doubt their effectiveness.

In the farmers' baseline surveys, farmers were asked what they would do to manage tungro. In addition, actual farming practices that might affect or be affected by tungro, such as choice of variety and insecticide use, were also recorded over several seasons.

Farmers' views on how to manage tungro disease

A significant number of Indian farmers (43%) did not know how to manage tungro. More than half (52%) of the farmers, however, suggested leaving a fallow period (Table 7). (Some said this was recommended by extension people.) Only 2% suggested using insecticides.

In the Philippines, insecticide use was more popular, particularly at the early stages of crop growth. Farmers realized that spraying was not effective at the late stages once the crop was already infected. Many farmers also said that insecticide spraying was not very effective at any stage, but that they used it in the absence of other effective measures.

Filipino farmers frequently suggested using resistant varieties or changing varieties to prevent recurrence of tungro. Cultural controls such as roguing infected plants, fallows, plowing under, and synchronous planting were not commonly used (Table 7).

Farmers' actual farming practices

The rice variety and the amount of pesticide spray used by farmers (and reasons for this) were monitored over a period of 6 and 4 seasons in India and the Philippines, respectively. Information on synchronous planting and rotations was also collected.

Rice varieties used. In India, farmers chose varieties they considered as suitable for the particular season in terms of characteristics such as growth duration, tolerance for cold, and tolerance for pests. For example, IR50 is very popular in the dry, warm sornavari season when there are relatively few pests, but it is not considered so suitable in the wetter or colder seasons.

IR50 was one of the varieties affected by tungro in 1990-91 in the area, but it continues to remain popular with farmers. Besides IR50, other varieties known to be susceptible to tungro are IR36, ADT36, IR64 and, particularly, ADT42. During an outbreak of tungro in one village (Vishar) in the 1997 samba season, 7 out of 10 farmers affected were growing ADT42; the others were growing IR36 or ADT36. In the other unaffected village (Pudumanvilangai), few were growing ADT42. In the following navarai season, farmers switched from ADT42 and many planted ADT37, which has some tungro resistance. Tungro did not recur.

In the Philippines, it was difficult to determine exactly what variety farmers were growing because they are very active in selecting their own seed. Selection was the main variety grown although varieties differed from farmer to farmer (Table 8). Farmers often gave local names to their varieties and it was difficult for researchers to find out the original rice variety. Farmers were aware of which varieties are susceptible to

Table 7. Farmers' reported tungro management strategies.

India				
Control measure	Farmers (%) using control in = 901			
Spray insecticides	2.2			
Practice field sanitation & spraying	1.1			
Use resistant variety	1.1			
Use fallow period	52.2			
fallow & change variety	1.1			
No idea	43.3			
Philippines				
Control measure	Farmers (%) using control (n = 226)			
	When tungro occurs in nearby field	To control tungro at early stages of crop	To control tungro at late stages of crop	To prevent recurrence in next season
Do nothing	9.3	4.4	39.8	1.8
Apply insecticides	66.8	53.5	44.7	5.3
Use resistant varieties	3.5	0	0	47.3
Change variety	2.2	0.4	0	11.1
Rogue Infected plants	0.9	8.0	0.9	0
Replow	0.4	23.9	1.3	0
Add fertilizer	2.7	12.8	4.0	1.3
Prepare land & sanitize	0.9	0	0	14.2
Check water control/drainage	8.0	5.8	3.1	2.7
Practice synchronous/early planting	0.4	0	0	1.8
Observe fallow period	0	0	0	2.2
Plow under	0.4	0	0	0.4
Practice crop rotation	0.4	0	0	0.4
Follow other control measures	0.4	0.9	0.9	1.3
Ask advice	2.2	1.3	1.8	0.9
Do not know/no answer	9.3	8.8	8.4	9.3

tungro, and a significant number were growing varieties with some resistance (such as IR62 and IR74). A few farmers were growing varieties such as the very susceptible IR64; some farmers liked to grow special varieties such as Masipag, despite its susceptibility to tungro.

Insecticide use. The level of insecticide use was far lower in India than in the Philippines, averaging between one and two applications in India and four to five in the Philippines (Table 9), although extension documents in India generally recommend more insecticide use for pest control than do such documents in the Philippines. The high level of insecticide use in the Philippines, however, was due in large part to

Table 8. Rice varieties grown by farmers.

India: Farmers (%) using each variety averaged over 3 yr

Variety	Sornavari	Samba	Navarai
IR50	75.5	5.2	5.6
IR36 ^b	5.5	10.4	10.4
ADT36 ^b	4.6	11.8	23.3
ADT37 ^a	2.1	8.1	13.3
ADT42 ^b	1.8	7.8	4.4
IET1444	0.9	0	6.7
White ponni	0.3	12.2	1.5
ADT39	0	4.8	1.5
ENT2	0	3.3	4.1
IR64 ^b	0	2.6	1.5
Other varieties	1.2	3.0	3.3
No rice crop	10.9	34.4	29.6

Philippines: Farmers (%) using each variety in 1996

Variety	Dry season	Wet season
Selection	27.1	23.8
IR62 ^a	11.5	8.9
IR36	9.9	6.0
Bordagol	7.3	8.5
7 Toner ^b	4.7	2.4
IR78	4.2	6.9
IR60	4.2	4.8
PSB Rc10	4.2	4.0
Korean	4.2	3.2
IR66	4.2	2.4
Masipag ^b	2.1	3.6
California	1.6	1.6
PSB Rc18 ^a	1.6	1.2
IR88	1.0	2.0
555	1.0	1.6
IR74 ^a	1.0	1.2
Series	0.5	2.8
B6	0.5	2.0
Others	9.4	12.1

^a Carries some resistance to tungro. ^b = susceptible to tungro.

the arrival of black bug in the rice-growing area. This new and very visible pest is of enormous concern to farmers, who are not used to dealing with it. A detailed analysis of why farmers sprayed insecticide (Table 10) indicated that black bug was their main reason for spraying. Tungro accounted for less than 5% of the total sprays. There was no correlation between the number of insecticide sprays and tungro incidence.

Table 9. Mean number (standard deviation) of insecticide applications applied over 6 seasons in India and over 4 seasons in the Philippines.

India					
96 Samba	97 Navarai	97 Sornavari	97 Samba	98 Navarai	98 Sornavari
1.0 (0.74)	1.0 (0.89)	0.9 (0.79)	1.6 (1.03)	1.4 (0.90)	1.6 (0.94)
Philippines					
96 WS	97 DS	97 WS	96-97 WDS		
4.6 (2.52)	5.0 (2.96)	4.2 (2.36)	4.5 (2.91)		

Table 10. Reasons of farmers for spraying insecticides (percentage of total sprays), Philippines.

Pest	96 WS	97 DS	97 WS	96-97 WDS
Black bug	53.3	58.2	26.8	70.9
Any pest/for protection	25.3	35.8	56.7	19.7
Stemborer	12.8	9.2	4.0	10.7
GLH	6.0	1.3	7.0	3.7
BPH/hopperburn	5.0	2.3	2.8	2.4
Tungro	4.2	1.0	3.8	1.1
Rice bug	4.1	0.9	1.7	2.0
Worms	3.7	1.0	5.3	1.6
Leaf folder	1.0	0.2	0	0.3
Other	0.5	0	0.5	0.4
Total sprays	1,393	988	1,100	1,161
Total parcels	289	186	244	249

Note: Multiple answers.

Planting dates and other cultural controls

More synchrony in planting dates occurred among Indian farmers than among Filipino farmers. In the Philippines, it was difficult to determine the cutoff point between one cropping season and the next. For example, planting dates for the 1996 wet season varied from 5 April to 24 August; for the 1996 late wet season, planting dates were from 10 August 1996 to 30 January 1997; and for the 1998 dry season, from 18 December 1997 to 30 May 1998. The irrigation water schedule was a major factor in farmers' choice of planting dates.

Indian farmers also used more rotation systems; for example, rice-rice-groundnut or sugarcane for 2 yr, then rice. Many farmed a mix of irrigated, partially irrigated, and rainfed land. There were more rotation and fallow systems in Pudumavilangai (the village with one reported tungro occurrence) than in Vishar (the village where a tungro outbreak occurred in 1997).

Discussion

Factors affecting farmers' perceptions and management practices of tungro included characteristics of the disease, farmers' access to information, plus their own observations and experiments. In addition, farmers' social and economic status and their whole farming and livelihood system affected the management strategies that they can implement.

Tungro is difficult to manage because it is not easily predictable. In India, farmers actually had little experience with it; therefore, their knowledge was more limited than farmers in the Philippines. They had not had the chance to experiment with different management strategies and observe the outcome. They were also much less concerned about the disease because it did not recur season after season, possibly because their farming systems were more mixed. Farmers' information sources for the outbreak in 1990–91 were mainly extension people, who advised some of them to practice fallowing.

Information about differences in varietal susceptibility is something that farmers seem quick to pick up and act on. In the outbreak in one Indian village in 1997, farmers were able to observe the differences between susceptible variety ADT42 and resistant variety ADT37. In the following season and following year, farmers dropped ADT42 and many planted ADT37 (T. Chancellor, personal communication 1998). In the Philippines, farmers are very active in selecting for seeds that they think will give good yield. The speed with which farmers adopt varieties was illustrated by the outcome of an on-farm experiment conducted by researchers at IRRI and PhilRice. This trial was designed to compare a new tungro-resistant line with other varieties. The farmer-collaborator observed the results, selected seed from the new line, multiplied it, rechristened it with a new local name, and then distributed it among other farmers. Farmers, however, do not always fully understand the implications of plant resistance. For example, some believe that tungro disease is in the seeds, or they wonder why a particular variety does well in one field but becomes infected in another field. Although this is a difficult issue, because host resistance can break down over time or in different locations, there is a need for more and clearer information on tungro-resistant varieties for farmers.

Farmers do not generally understand the risks of having infected rice plants around as sources of inoculum. In less intensively cropped areas where other crops can be grown, this is not a big problem. But with asynchronous planting where there is always rice in the vicinity and limited options (because of water control) for alternative crops, this poses a problem for tungro management. Only if farmers realize the potential risks of having infected rice in the field will they have any rationale to aim for synchronous planting or rotation/fallow periods.

In the Philippines, using synchronous planting, fallows, and rotations is likely to be a greater challenge than in Indian villages. These options require control over water, which, in turn, requires negotiations with irrigation authorities and neighboring farmers. Alternative crops (for a rotation system) may not be possible if fields are poorly drained, and Philippine farmers have fewer alternative sources of income (other

than rice), so they may be reluctant to use fallows. Indian villages have greater local control over water supply, and the farming system is more mixed.

The use of resistant varieties is a more straightforward method for farmers to adopt than cultural controls because it does not require group action or agreement. Farmers given training and information on tungro management including the use of cultural controls become aware of the problems of asynchronous planting and tungro incidence, and can try to minimize tungro incidence.

Insecticide use depends on other pests observed by the farmer as well as tungro. Although insecticides are commonly used, particularly in the Philippines, there does seem to be a realization that insecticides are not the complete answer to tungro and cannot cure the disease. Confusion over the tungro vector, the green leafhopper, implies that insecticide application is unlikely to be closely targeted at the vector.

Conclusions

It is apparent that farmers have uneven knowledge of tungro, especially about the vector and sources of inoculum. They can adopt some control measures, however, such as the use of resistant varieties, without having a detailed understanding of the causes of the disease. For management strategies that are difficult to adopt, such as changing planting dates or using fallows, farmers need to understand why these methods could be advantageous. Otherwise they are unlikely to put time and effort into trying them out.

For researchers, it is useful to be aware of what farmers already know. Researchers need to provide relevant information, for example, on resistant varieties, to enable farmers to make informed decisions. Information on the types of virus involved, however important to researchers, is much less useful to farmers because it does not have a direct impact on their management strategies. It may also be useful to reconsider what is meant by “tungro-endemic” area. In the case of India, labeling villages as tungro-endemic (a term applied to the whole district) does not seem appropriate in guiding control recommendations, given the low frequency of tungro outbreaks.

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Community-based rice pest management

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An exploratory interdisciplinary approach with farmers' participation gave an expected and much-needed boost to community efforts to manage pest problems in three villages in Midsayap, North Cotabato. This simple intervention could slowly halt emerging pests to the area. A strategic action plan prepared after informal group dialogue was designed for farmers to help them understand the rationale of the project, learn from their farming experiences from the crisis due to damage caused by rice black bug (RBB) and rice tungro disease (RTD) in 1996, and alleviate fears of threats to their income, while sustaining their rice production. The plan encouraged farmers to participate in establishing their crop simultaneously with their neighbors, choose suitable varieties and crop establishment methods, and monitor pests prior to taking appropriate management action. The community shared ideas and experiences on crop management through a consultative and planning workshop, demonstration, experiments, and a farmers' field day. Based on the participatory rural appraisal method, their indigenous knowledge of farming, pest and control practices, financial constraints, and the irrigation schedule was used to establish an action plan. A total of 140 farmers in Bual Norte, Bual Sur, and Bobonao villages in Midsayap, North Cotabato, participated in the project for two to four cropping seasons in 1996-98. Most farmers (82-97%) realized that the intensive use of insecticides could not control the major pest problems of RBB, RTD, and white stem borer (WSB) in the staggered planting system. They changed gradually from staggered to synchronous planting with a 45-56-d and 30-45-d fallow period in each village. They shifted from transplanting to the broadcast method of planting (76-82%) and selected early maturing varieties (65-79%) and recommended varieties (20-35%). With this practice, farmers in these communities obtained an average rice yield of 3.2-4.6 t ha⁻¹ and reduced RBB density (1.3-2.0 hill⁻¹), RTD-infected farms (2.2-11.5%), and whitehead incidence caused by WSB (3-22%). They reduced the frequency of insecticide application from 5-6 times to 3-3.4 times per season. In general, farmers' perceptions and experiences influence their cultural management and pest control practices, which are also closely related to their sociodemographic characteristics. Thus, most farmers continued searching for varieties resistant to pests through seed exchanges with their friends. A few farmers still used insecticides intensively, especially those with an average income level. Some were aware of the presence of spiders and parasitized egg masses of WSB through information they received, but most farmers have not translated their ideas into farming practices. The results clearly suggest that the project's strategic action plan in the future should focus on the needs of specific target farmer groups.

Rice tungro disease (RTD) has long been considered as one of the most important factors limiting rice production in the irrigated lowland ecosystem, notably in southern Philippines. Outbreaks sporadically occurred in locations commonly associated with staggered planting practices. Besides RTD, farmers in this ecosystem have suffered huge economic losses caused by complex pest problems such as rice black

bug (RBB), *Scotinophara coarctata* (Fabricius), and white stem borer, *Scirpophaga innotata* (Walker). To address farmers' pest concerns, (1) information on rice tungro virus disease (Tiongco 1996) and integrated management of RBB (Justo 1995, and (2) promising technologies have been promoted through training for extension workers and farmers. Most activities were implemented by single or multidisciplinary teams with farmer participation as a basic step leading to pest management. However, such a research approach focused more on pests, in contrast to farmers' pest control practices, which are based on their perceptions, experiences, and socioeconomic resources. As agricultural research becomes increasingly specialized, there is a great need to integrate information across disciplines or among agencies to develop technologies for the total farming system, which is seen as a major challenge.

This report details how an interdisciplinary approach was used to integrate research options and indigenous knowledge (IK) of farmers from three villages around the PhilRice Branch Station at Midsayap, North Cotabato, Mindanao. It has the following objectives:

1. To assess farmers' perceptions and knowledge of pest control practices in rice cultivation as well as constraints in their indigenous rice cultivation,
2. To share with farmers research options that answer their needs to strengthen their decision making in managing the rice crop and pests and
3. To facilitate farmers' participation in sharing ideas and experiences in the community and on their farms.

Methodology

Establishment of baseline survey information

Study sites were selected based on the pest profile of Mindanao reported earlier by Sanchez and Obien (1995). The social science group began a baseline survey to gather information on farmers' perceptions and knowledge of pest control practices. Brainstorming, planning, and orientation among team members across disciplines were done at the study site to determine information needs and how they could be best obtained. These activities were also used to generate a list of potential participants in the community survey from village officials, farmer-leaders, and key informants. One hundred farmers or about 20% of the farmer population in Bantod and Bual Sur were chosen as respondents.

Interagency planning workshop

After gathering information from farmers, a planning workshop was held at the PhilRice Branch Station at Midsayap. Representatives of different agencies under the Department of Agriculture (DA), farmers, cooperatives, community council members and leaders, chemical sales representatives, and municipal agricultural officers at the study sites were invited to discuss and contribute to the action plan and future expansion of the project. They were also asked to examine the performance of newly recommended varieties in the production experiment.

Establishment of farmers' indigenous knowledge and participation

The initial phase of the participatory rural appraisal (PRA) consisted of visits with village officials, farmer-leaders, and key informants. To enhance community participation, the group decided to use the informal dialogue and discussion forum to gather IK on rice cultural practices. Based on the workshop information, questionnaires were redesigned to come up with a conceptual framework. The framework represents a continuing cycle focused on incorporating IK into decision making for sustainable rice production and community pest management (Fig. 1). Seven farmer groups participated in the 5-d survey. Participants were grouped and the discussion covered rice production, the pest scenario, and the project plan.

The last phase of the PRA collected IK through informal focused interviews and created a venue for community awareness and participation in implementing the project (Fig. 2). The sociodemographic profile of respondents in relation to their rice cultural practices was established and analyzed by factor analysis (Statistica).

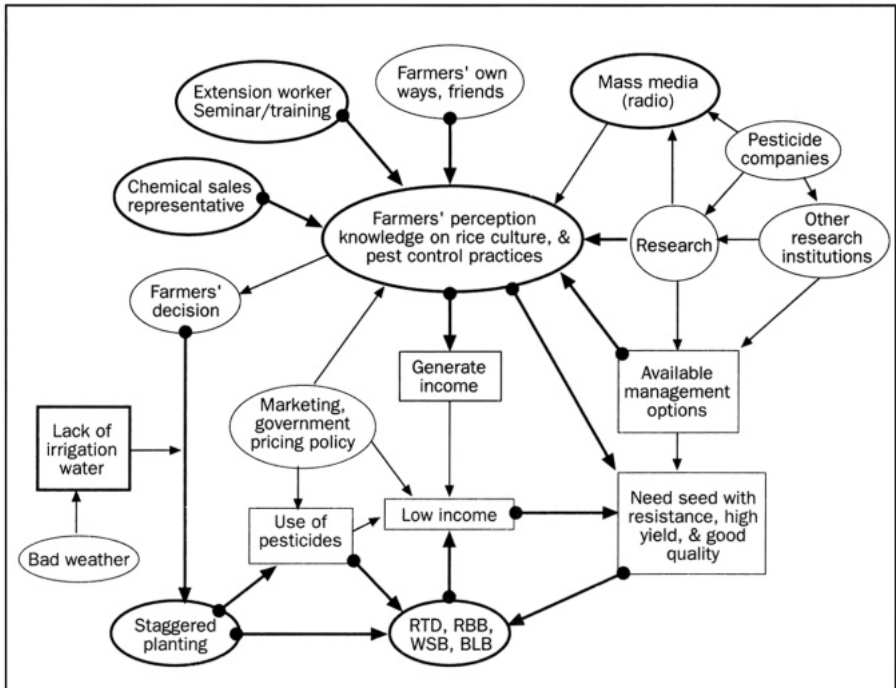


Fig. 1. Conceptual framework showing factors that influence farmers' rice cultivation and pest management practices. RTD = rice tungro disease, RBB = rice black bug, WSB = white stem borer, BLB = bacterial leaf blight.

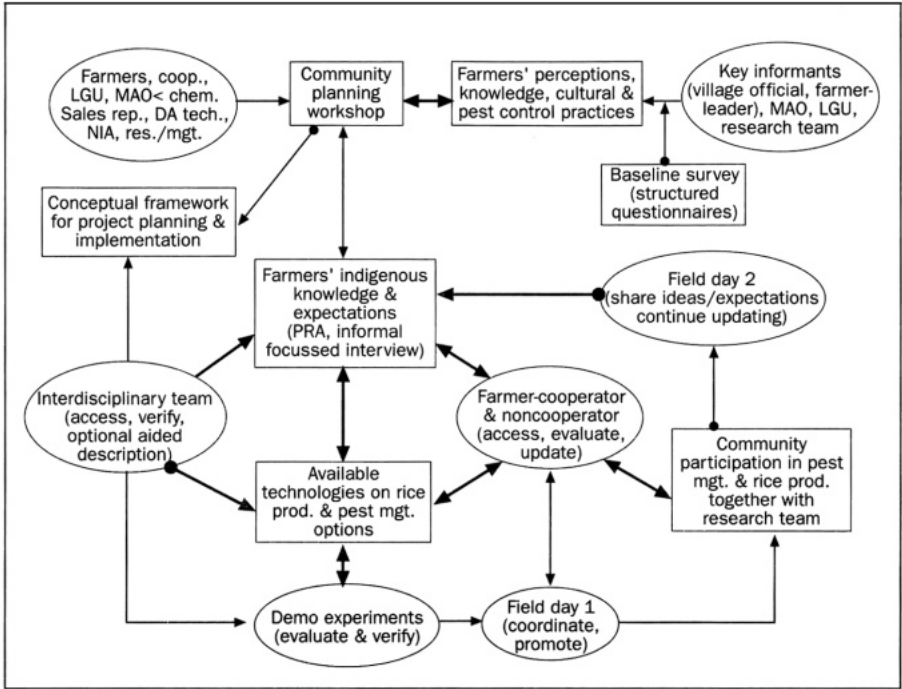


Fig. 2. Diagram of the project implementation strategy in three villages, Bual Norte, Bual Sur, and Bobonao, Midsayap, North Cotabato (1996-98). LGU = local government unit, MAO = municipal agriculture office, DA = Department of Agriculture, NIA = National Irrigation Administration, PRA = participatory rural appraisal.

Verification trials of farmers' IK

Farmers' selected varieties and the direct seeding method, two IK components, were validated in a series of demonstration experiments at the research station. Cooperator and noncooperator farmers were invited to assess varietal performance and evaluate the treatments. Cooperators were offered 10 kg of seeds of their chosen varieties with information on varietal characteristics and performance, and responses to insects and diseases in different rice ecosystems (PhilRice Technoguide Calendar 1997-98).

Monitoring of crop performance, diseases, insects, and natural enemies

Regular monitoring of the green leafhopper, *Nephotettix virescens*; whitebacked planthopper (WBPH), *Sogatella furcifera*; zigzag leafhopper (ZLH), *Recilia dorsalis*; white stem borer (WSB,) RBB; and mind bug (MRB), *Cyrtorhinus lividipennis*, has been carried out by using a light trap (adapted IRRI-EPPD design with a 160-W mercury lamp) set up at the PhilRice Experiment Station at Midsayap since 1997.

Meanwhile, insects, diseases, and natural enemies were monitored by insect net sweeping from three quadrats (2×5 m each) taken from farmers' fields and another three from a 1000-m² farmer's field at 30 and 45 d after planting (DAP). Yields were evaluated based on the cut crop from those quadrats.

Cooperators were also provided with fertilizer (NPK) enough for a 1000-m² experiment. The effect of zinc deficiency was monitored during the rainy season to decide on an amendment. A 1-d field visit (farmers' field day) took place at crop maturity, where farmers were asked to share their experiences. Substantial components of rice cultural practices related to crop yields in each of the three farmer-communities were determined by the principal component analysis (SYSTAT).

Continuing campaign for community synchronous planting

An information campaign was launched to develop awareness about community cooperation in regular planting according to the schedule of water release in the village and fallow period to reduce pest pressure during land preparation; farmers' field days; field visits; and pest monitoring. Increased knowledge was one of the ultimate targets of the project activities.

Results and discussion

Baseline survey information

Community sociodemographic profile. Table 1 summarizes the profile of 100 farmer-respondents (74 men and 26 women) from two villages, Bual Norte and Bual Sur, Midsayap. Most farmers (82%) obtained their income from rice farming. Respondents got loans for production inputs from traders (21%), private agents (18%), cooperatives (15%), and landowners (1%). Only 38% of the farmers were landowners. One young farmer with a college education and three old farmers with a high school education were selected by the community as farmer-irrigators responsible for communicating with the National Irrigation Administration (NIA) to set the water release schedule in their areas. Because of the insufficient irrigation water in Midsayap, particularly during the dry season, NIA recently launched a new irrigation program for the different zones or divisions composed of several villages. The specific week or month of water release, however, depended on the preference of farmer-irrigators in the village. This initiative of NIA encouraged farmers to practice synchronous planting. It benefited both farmers and NIA because NIA was able to collect irrigation fees from farmers who produced more than 2 t ha⁻¹ of rice per season. The limitation of the program was that frequently two nearby villages did not have water at the same time; thus, synchrony cannot be practiced in large contiguous areas.

Farmers' perceptions on insect pests and diseases. Rice tungro was perceived as the most important disease of the rice crop; bacterial leaf blight (BLB) or blast ranked second (Table 2). Crop loss due to RTD ranged from 0% to 91% based on farmers' past experiences. Farmers associated the spread of the disease with farming or weather factors rather than with the disease causal factor. The use of insecticides and GLH-

Table 1. General profile of farmer-respondents (100) in Bual Norte and Bual Sur, Midsayap, North Cotabato, 1996.

General features		Value
Area covered (ha)		129.7
Average farm size (ha)		1.3
Source of irrigation (%)		100
Sociodemographic characteristics		
Average age (yr)		44.5
Yr farming		21.8
Sex(%)	Male	74.0
	Female	26.0
Civil status (%)	Married	96.0
	Single	4.0
Household members (no.)		6.0
Formal education (%)	No schooling	4.0
	Elementary	47.0
	High school	42.0
	College	7.0
Membership in organizations (%)		
Farmers' association		32.0
Farmers' cooperative		4.0
Irrigation		3.0
None		58.0
Socioeconomic characteristics (%)		
Tenure status	owner	37.5
	Certificate of land transfer	33.7
	Tenant	22.1
	Lessee	6.7
Source of financial input	Own	45.1
	Trader	20.6
	Agent	18.3
	Cooperative	14.7
	Landowner	1.0
Farming equipment/animal	Carabao, owned (%)	50.0
	Tractor, owned	6.0
	Thresher, rented	100.0
Source of income	Rice farming	82.5
	Rice-cum-mungbean	0.8
	Animal raising	6.7
	Business	3.3
	Government employee	6.7

resistant varieties were the preventive measures applied. Thirty-eight percent of the farmers claimed that insecticide use was an effective control measure, while 31% said otherwise. A few farmers (5%) confused RTD symptoms with zinc deficiency. A majority of the farmers (71%) used inappropriate control measures for BLB.

Meanwhile, 44% and 20% of the farmers considered RBB and WSB as the first and second major pest concerns, respectively (Table 3). They correctly identified signs of WSB and RBB damage. Crop loss caused by RBB ranged from 0% to 88%, and

Table 2. Most important diseases for farmers.

Disease	Response (%)
<i>Rice tungro</i>	
Perception of causes and spread of rice tungro (n = 45)	
Bad weather/rain/stagnant water	20.0
Insects	18.2
"Tiny creature"	16.4
Pests are contagious	16.6
Overfertilization	1.8
Old variety	1.8
Through seed	1.8
Do not know	24.4
Perception of crop loss (%) due to rice tungro (n = 55)	
0	12.7
7-20	12.7
>20-40	29.1
>40-60	29.1
>60-91	9.1
Respondents' preventive practices for rice tungro	
Effective	
Use insecticides	Yes 38.2
	No 30.9
Dram + add zinc	Yes 5.4
	No 1.8
Leave it	No 3.6
Use water + spray + apply fertilizer	No 9.1
No response	Not applicable 10.9
<i>Bacterial leaf blight (BLB)/blast</i>	
Perception of crop loss (%) due to BLB/blast	
With BLB/blast (n = 35)	
>0-20	42.9
>20-40	37.1
>40-60	14.3
>60	5.7
Without blast/BLB (n = 65)	Not applicable
Perception of causal factors of BLB/blast (n = 35)	
Causes	
Pests	25.7
Fertilizer +/-	25.7
Do not know	22.9
Bad weather	11.4
Disease is air-borne	2.9
No response	11.4
Control practices for BLB/blast (n = 35)	
Drain + use insecticides	71.4
Drain + apply fungicide (hinosan) + add fertilizer	14.3
Add fertilizer	5.7
No response	8.6

Table 3. Pests ranked by farmer-respondents as first and second, and farmers' control practices.

Pest or practice	Crop loss (%)	Response (%)
<i>Rank 1</i>		
Rice black bug (RBB) (n = 44)	0-20	8
	>20-40	16
	>40-60	17
	>60-88	3
White stem borer (WSB) (n = 20)	14-20	3
	>20-40	11
	>40-65	6
Hoppers/hopperburn (n = 4)	42.9	4
Rats (n = 3)	48.1	3
Whorl maggot (n = 1)	29.2	1
Cutworm (n = 1)	28.6	1
Not concerned (n = 27)	Not applicable	
<i>Rank 2</i>		
White stem borer (WSB) (n = 54)	0	5
	8-20	22
	>20-40	22
	>40-50	5
Rice black bug (n = 10)	0-10	4
	>10-20	4
	>20-60	2
Hoppers/hopperburn (n = 16)	10-20	9
	220-40	2
	>40-60	5
Whorl maggot (n = 1)	20	1
No infestation (n = 19)	Not applicable	
Control practices against RBB and WSB		
Practices of respondents who observed pests (94%)		
Used pesticides (99%)	Apply insecticide	45.7
	Spray/adjust timing	45.7
	Irrigate + spray	6.4
	Bait + give poison	1.1
Did not use pesticides (1%)	Irrigate + fertilizer	1.1
Respondents who did not observe pests (6%)	Not applicable	

that caused by WSB from 14% to 65%. Most farmers experienced WSB damage at the vegetative (deadheart) and reproductive stages (whitehead). Some also received information on monitoring of parasitized egg masses, but they said that the activity is time-consuming. The 1996 dry season was the first time in 30 yr, however, that they experienced an RBB outbreak.

Farmers' knowledge of pest control measures. One-half of the respondents had attended seminars on topics related to farming in general, with 40% of the topics focused on pest management (Table 4). The most common information, however, was on how to use pesticides. They received this information from DA technicians (35%) and by radio (11%). The DA (26%) also advised them to monitor insect pests and natural enemies. Water management combined with proper timing of insecticide application was the most common pest control alternative against RBB cited by respondents, whether they attended the seminar or not (23% and 21%, respectively). The second alternative was to check the number of pests and spiders (18% vs 14%) early in the morning or late in the afternoon. Very few farmers applied knowledge gained about beneficial insects. Future training activities for farmers should focus more on practical aspects of pest control.

Table 4. Farmers' information sources on farming practices.

	Topic	Response(%)	
<i>Attendance at seminar</i>			
Respondents who attended (n = 50)	Farming	28	
	Pest management	20	
	IPM ^a	16	
	Rice production	16	
	Cooperative	6	
	Farmers' Field School	4	
	Chemical use	4	
	NIA zonal program	4	
	Home food processing	2	
<i>Technology/information dissemination</i>			
Respondents (n = 65) who received information			
Source Representative (n = 38)	Topic Chemical use	35.4	
		Monitor pests-NE	4.6
		Maintain field	1.5
		No response	16.9
	DA (n = 24)	Monitor pests-NE	21.5
		Chemical use	9.2
		No response	6.2
	NGO/radio in = 3)	Monitor pests-NE	1.5
		Chemical use	1.5
		No response	1.5
	<i>Farmers' pest control practices</i>		(%)
	Respondents who did not attend (n = 43)		
Respondents who did not attend (n = 43)	Observe pests and spiders	14.0	
	Irrigate	9.3	
	Maintain field	2.3	
	Own way	2.3	
	Do not know	2.3	
	No response	48.8	
	Respondents who attended (n = 57)	Irrigate/spray	22.8
Observe pests and spiders		17.5	
Irrigate		15.8	
Own way		1.8	
No response		42.1	

^aIPM = Integrated pest management, NIA = National Irrigation Administration, DA = Department of Agriculture, NGO = nongovernment organization, NE = natural enemies.

Table 5. Rice cultural management practices of 100 farmer-respondents in Bual Norte.

Planting	Planting month/variety	%
Time		
First crop (n = 107 ^a)	January	8.9
	February	13.3
	March	4.4
	April	0.5
	May	5.9
	June	8.9
Second crop (n = 107)	July	12.3
	August	8.9
	September	10.8
	October	6.9
	November	0.5
	December	18.7
Variety planted (n = 105 ^a)	IR62	18.1
	IR74	18.1
	IR56	3.8
	IR66	1.0
	IR8	1.0
	IR1314	1.0
	PSB-Rc 18	12.3
	PSB-Rc 10	1.9
	PSB-RC34	3.8
	PSB-Rc 4	1.0
	PSB-RC6	1.0
	PSB-RC20	0.9
	Farmers' selection number	26.7
	Farmers' selection series	6.7

^aMultiple answers.

Respondents' rice culture management practices. During the first year of implementing the NIA zonal irrigation program in 1996, staggered planting prevailed in Bual Norte and Bual Sur (Table 5). To cope with the irrigation schedule, more farmers shifted from transplanting (TP) to direct seeding (DS). Since then direct seeding has predominated (81%) in most villages of Midsayap. According to farmers, the labor cost was lower in DS than in transplanting. Herbicides were commonly used for both direct seeding and transplanting (1.0 L ha⁻¹). DS was also easier and faster than TP. Under normal weather conditions, most farmers practiced DS in both the dry and wet seasons to shorten crop maturity by about 10 d compared with TP, to get returns on investment more quickly, and to be able to pay back their loans as soon as possible. Some claimed that a crop established by DS had a low infestation of pests such as RBB, WSB, and RTD. The verified trials showed that only green leafhopper-resistant varieties such as PSB Rc 34 established by DS and at a high seeding rate (120 kg seed

ha⁻¹ and above) had a lower RTD incidence than in TP. There was no significant difference, however, in the cumulative disease incidence for GLH-susceptible IR64 at 45 DAT, whether established by DS or TP.

Several farmers used to plant two varieties or change varieties every season to evaluate varietal performance. They looked for resistant varieties to get good harvests. Seed exchange among farmers, friends, and relatives was a common practice. They tried three groups of varieties: those released by IRRI (45%), Philippine Seedboard commercial rice varieties or PSB Rc (22%), and farmer selections (33%). Farmers preferred GLH-resistant varieties such as IR56 (7%), IR62 (18%), IR74(18%), PSB Rc 10 (27%), PSB Rc 18 (12%), and PSB Rc 31 (4%) for rice tungro management. Information came from training or from friends who participated in the Farmers' Field School. Navarro et al (1998) cited a similar observation in a report on the overview of the National Integrated Pest Management Program in the Philippines. Farmers who planted GLH-resistant varieties also applied insecticides at the rate of 1.5 L ha⁻¹ for a broadcast crop and 1.1 L ha⁻¹ for a transplanted crop because they wanted to get a clean crop and avoid crop failure. This practice usually reduced the natural enemies established early in the crop, resulting in pest infestation, which prompted farmers to increase the insecticide application per season.

All farmers in Bual Norte and some from Bual Sur villages practiced duck pasturing and golden snail culture successfully. Not all farmers raised ducks, but a few had large-scale duck farms for egg production, which contributed to the management of golden snail. A farmer with 200 ducks could efficiently grow golden snails on a 1-ha farm.

Influence of sociodemographic factors on respondents' cultural practices. Rice cropping practices of respondents at Bual Norte and Bual Sur villages can be categorized into four groups (Fig. 3). The first group (Fig. 3. top left corner) is composed of young farmers (7%) who owned a farm larger than 1 ha, had less than 10 yr of farming experience, attained the highest education (2-yr college), and benefited the most from technology or information dissemination and training courses. They usually planted the IR varieties mentioned earlier. The second group (Fig. 3. bottom right corner) consists of tenant farmers (30%) who had less than 1-ha farms, 21 to 30 yr of farming experience, planted farmers' selected varieties, and had RTD concerns, but never received any technical information. The third group (top right corner) is the women farmers (16%) who had farms larger than 2 ha, finished high school, practiced synchronous transplanting of PSB Rc varieties, and used to apply insecticides against RBB infestation. Group 4 (bottom left corner) is the resource-poor farmers (41%) who only had elementary education, cultivated their land for more than 30 yr, practiced asynchronous planting and direct seeding, and were concerned about WSB damage. The data showed that technology/information dissemination through training in the past had reached only a small proportion of educated and progressive farmers (group 1). These farmers were tapped as information sources of IK while the campaign paid more attention to the majority and resource-poor farmers (groups 2 and 4). Group 4 farmers were requested to share their experiences on RBB control practices during the farmers' field day.

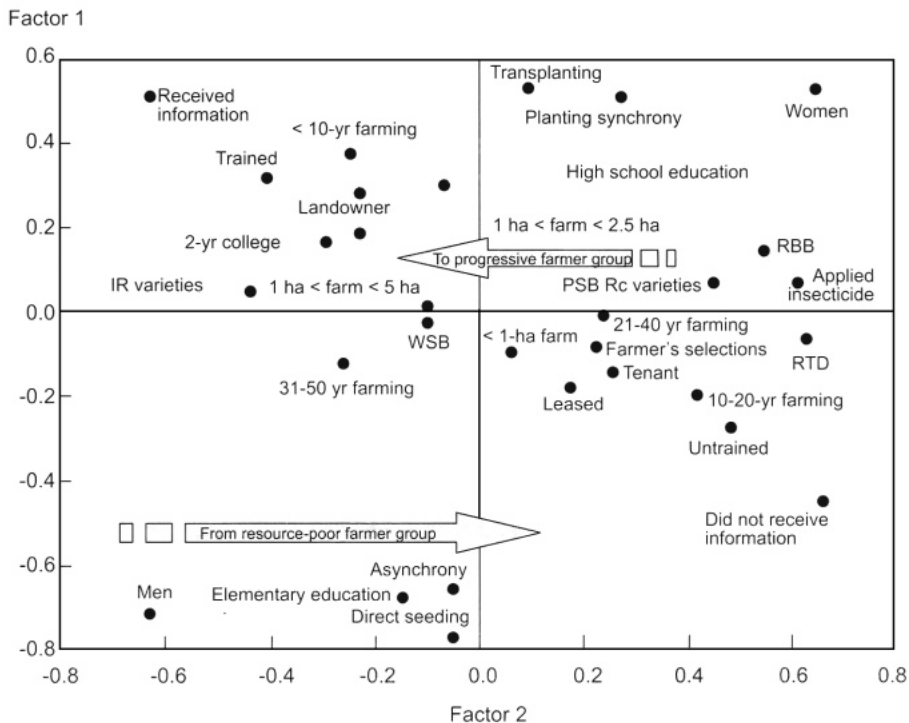


Fig. 3. Correspondence chart showing the coherence of sociodemographic characteristics of farmer-respondents and their rice cultural practices in response to rice black bug (RBB), white stem borer (WSB), and rice tungro disease (RTD) concerns, Bual Norte and Bual Sur, 1995-96: a guide to identifying target farmer-participants.

Monitoring of insects and diseases, and verified trials

The weekly population of RBR, GLH, ZLH, WBPH, and WSB catches was monitored by a light trap and sweeps from farmers' fields. The populations of GLH and ZLH during 1997 were much higher than those in 1998 (Fig. 4, left and right). Hop per catches during 1997 from the light trap had three peaks, in February-March (A), June-July (B), and November-December (C). These population fluctuation patterns mirrored those recorded from farmers' fields, which had been established during peak periods (D) and (F). Frequently, GLH and ZLH were caught together with MRB, and separated from WSB and WBPH (C). The hopper population was lowest in farmers' fields planted during April-May (E). During the second peak of GLH and ZLH, there was a risk of planting susceptible varieties IR60, PSB Rc4, Bugos, Selection #78. Series, and Masipag, which had high RTD incidence (30–80%). This finding strongly suggests that farmers should replace these varieties with GHL-resistant ones during the second crop or in the wet season. Another alternative is the use of RTD virus-resistant lines. Thirty farmer-cooperators in Bual Norte successfully tested IR71031-4-5-5-1 for RTD resistance for the first time in the 1998 wet season. This RTD-resistant advanced line yielded 3.5-4.5 t ha⁻¹ of rice.

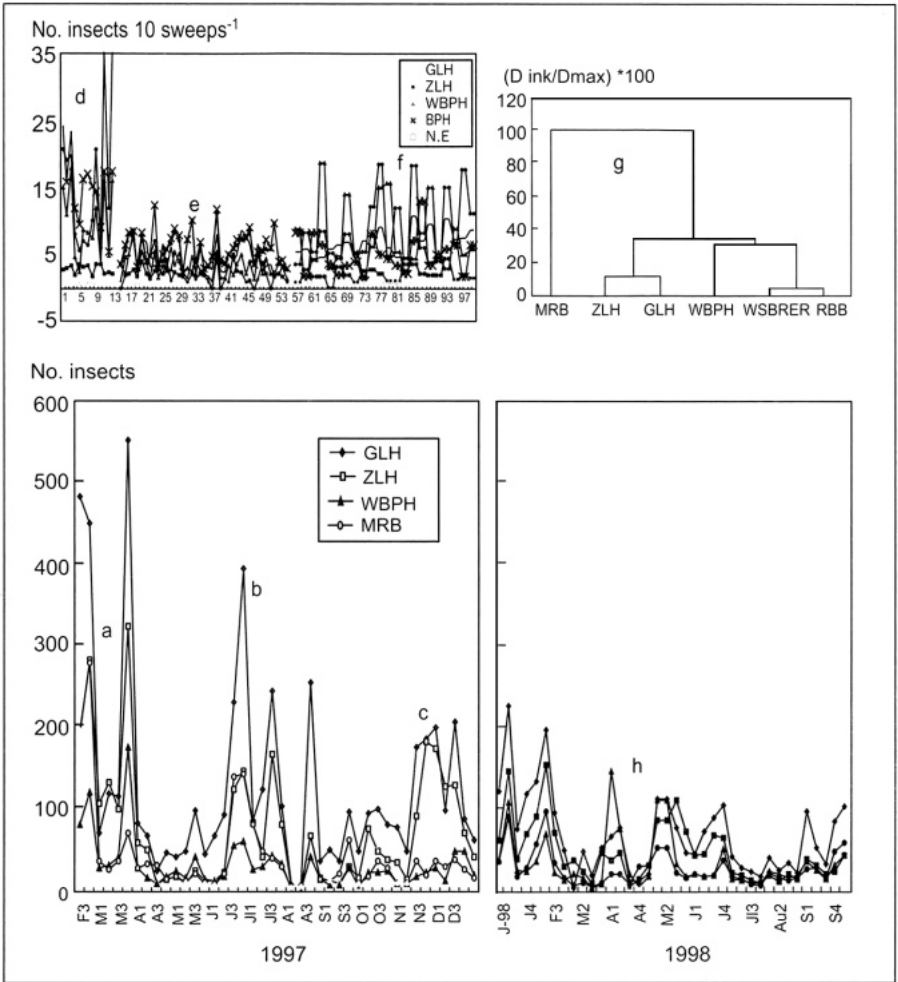


Fig. 4. Three major peaks of green leafhopper (GLH), zigzag leafhopper (ZLH), whitebacked planthopper (WBPH), and mirid bug (MRB) population from light trap (A, B, C) in relation to population on farmers' fields at Bual villages in 1997 (D, E, & F), and tree diagram of their population linkage (G), and weekly population patterns in 1998 (H).

Likewise, RBB catches were also extremely high in 1997 (Fig. 5), with two peaks, in February-March and October-November (A). In a verified trial established during the 1997 dry season, only the stopgap line IR1314, variety C4-137, and a few farmer selections yielded from 1 to 2.5 t ha⁻¹ (B). The field population of RBB reached 10 adults hill and induced 30-45% RBB dead hearts (C). Similar entries planted during the wet season suffered a higher incidence of RBB deadhearts (30-60%), and RBB whiteheads (30-80%), and RBB burns (90-100%) (D). In contrast the crop grown in Bual Norte from the end of March to mid-July in 1998 after a long fallow period (January-March) had a negligible RBB population and RTD incidence (data not shown).

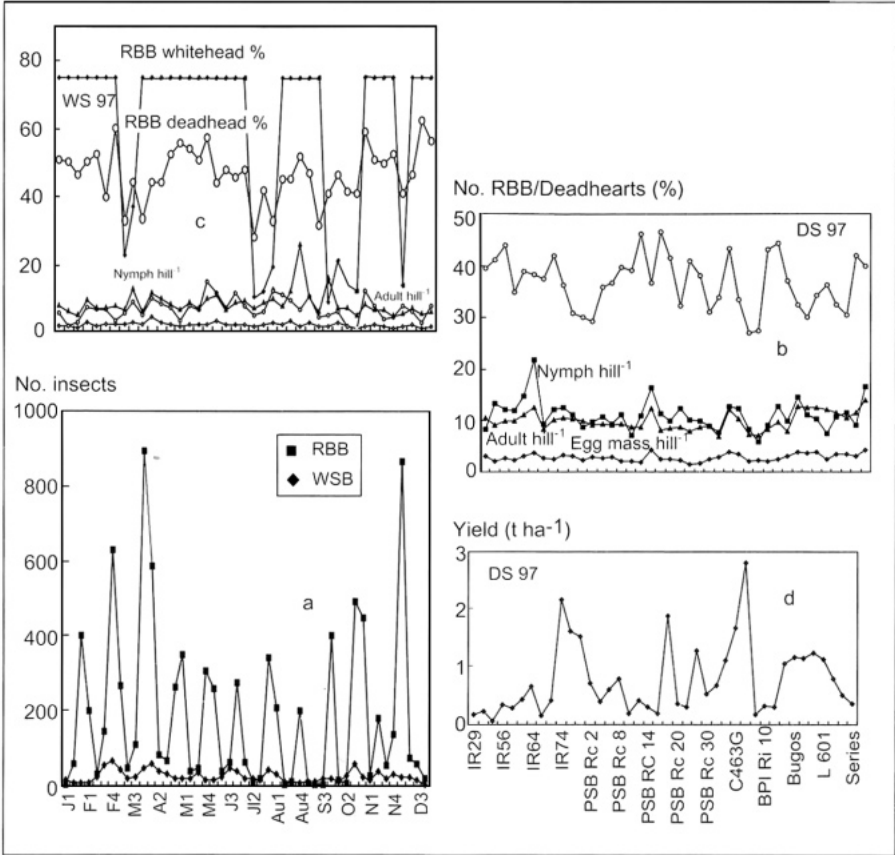


Fig. 5. Monthly population pattern of RBB catches in the light trap (A) and high bug infestation levels in the transplanting trials during dry (B) and wet (C) seasons. Only few varieties were tolerant of RBB (D) (Midsayap Experiments Station, 1997).

Evaluation of community cultural practices and crop performance

Three farmer communities participated in rice pest management activities for four seasons (Bual Norte, 1996-98), three seasons (Bual Sur, 1996-98), and two seasons (Bobonao, 1997-98). A combined data analysis showed that, in all communities the use of insecticides was positively correlated with whiteheads (%) caused by WSB (Figs. 6 and 7). This implies that farmer communities did not benefit from insecticides used against WSB. Their grain yields (3.2–4.6 t ha⁻¹) were not affected by whiteheads below 20%. Grain yields started to decline only when whitehead incidence surpassed 20% (data not shown). Future farmer forums should thus emphasize the use of natural enemies in the management of WSB, particularly during early infestation at the vegetative growth stage, to reduce the insecticide application frequency. In Bual Norte, a combination of varieties and direct seeding with no insecticide use was an effective control component (7% of farmers) in RTD management, with 2

RBB hill^{-1} and 4% WSB (Fig. 6). In Bual Sur, direct seedings effectively reduced the RBB population (1.3 hill^{-1} ; data not shown), but RTD remained a problem in the July 1997 planting (12% of farms). Results indicated that a community strategy in RTD management should focus on varietal deployment and a change in planting schedule.

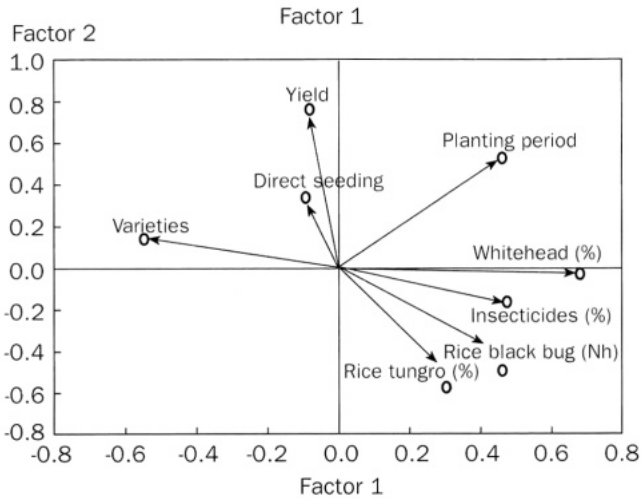


Fig. 6. Principal component analysis of rice cultural practices of farmers in Bual Norte village in relation to their pest control measures from 1996 to 1998 showed that grain yields (not frequency of insecticides used) were positively correlated with a combination of direct seeding and varieties against rice tungro incidence and black bug density under a 56-d planting period.

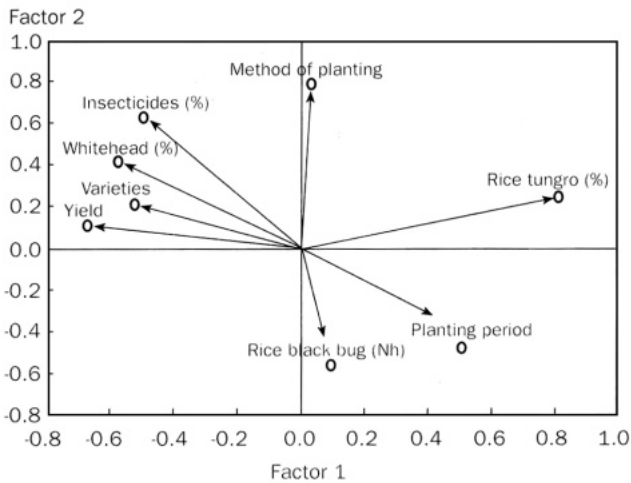


Fig. 7. Varieties planted and frequency of insecticides used by farmers in Bobonao village (not planting period or method) effectively protected grain yield from damage caused by rice black in 1997. Planting method, however, correlated positively with rice tungro incidence, which was low during the same period and did not affect overall the harvest of the community.

In Bobonao, RTD (2% of farms) did not adversely affect community rice production (4.6 t ha^{-1}), as only a few fields were infected with RTD (Fig. 7). Planting dates were positively correlated with the RBB population, which was effectively controlled by a combination of varieties and insecticides used.

Conclusions

1. The project used the PRA method in the baseline survey through the participation of farmer communities at Midsayap, North Cotabato, and collaborative efforts of various agencies concerned about rice production and pest problems to develop a conceptual framework.
2. An informal group-focused dialogue was the key strategy in the PRA method used to discover farmers' IK on rice cultural practices and to design the action plan on community pest management for 1996-98.
3. Little progress has been made in the past 2 yr to enrich farmers' IK on crop-pest management by shortening the period of planting in each village, to shift from transplanting to direct seeding, and to reduce the frequency of insecticide application. The introduction of RTD-resistant lines has just started. The use of natural enemies in crop management in farmer communities has not been widely adopted.
4. Understanding the rice ecosystem through continuous learning would enhance community cooperation, which plays a vital role in managing pests and sustaining rice production.

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The influence of varietal resistance and synchrony on tungro incidence in irrigated rice ecosystems in the Philippines

T.C.B. Chancellor, E.R. Tiongco, J. Holt, S. Villareal, and P.S. Teng

Rice tungro disease is endemic in some intensively cultivated areas in the Philippines where planting dates are highly asynchronous. Results from a survey conducted in Albay Province in 1992-94 showed that tungro disease incidence was significantly lower on rice varieties that are resistant to the main leafhopper vector of tungro, *Nephotettix virescens*, than on susceptible varieties. Late-planted rice crops in both the wet and dry seasons had the highest incidence of tungro. In on-farm trials in North Cotabato Province, a virus-resistant advanced breeding line, IR68705-1-1-3-2-1, showed strong resistance to tungro. These findings are discussed in relation to developing optimal tungro management strategies for endemic areas.

Rice tungro disease was recorded as early as 1859 in Indonesia (Ou 1985) but it only became a serious problem in South and Southeast Asia in the 1960s as a result of major changes in rice production systems during that decade. The introduction of modern, photoperiod-insensitive, semidwarf indica varieties with a good response to fertilizer led to substantial increases in productivity (IRRI 1985). Many of these varieties had significantly shorter maturity periods than traditional varieties. This allowed an increase in cropping intensity, so that two or even three rice crops could be grown in a year. Large yield increases were also due to an expansion in area planted to rice and heavy investment in irrigation schemes that increased water use efficiency and made intensive production possible.

Although changes in rice cultivation practices increased grain yields, the intensification of rice production was associated with more severe insect pest and disease problems. Multiple rice cropping over large areas provided a continuous source of plant hosts and enabled the year-round development of insect pests (Litsinger 1989). For rice virus diseases, such as tungro, the potential survival of pathogens was enhanced by the greater continuity of hosts (Thresh 1989). This was particularly important in areas with highly asynchronous planting dates and overlapping rice crops.

The frequency of major tungro outbreaks appears to have decreased since they peaked in the late 1960s and early 1970s, although large areas of rice were affected by tungro in some eastern coastal areas of India in 1990 and in central Java, Indonesia, in 1995. In the Philippines, the last major tungro epidemic occurred in 1983-84 (Baria 1997), although the disease is present every year in some endemic areas (Savary et al 1993). In a survey of historical data from 1989 to 1993 on insect pest and disease incidence in Mindanao, tungro was considered to be the most destructive disease of rice (Sanchez and Obien 1995). Recent survey data confirm that tungro is a continuing problem in Mindanao (Truong et al, this volume). In this chapter, we discuss the role of two of the most important factors that affect tungro incidence in irrigated rice ecosystems in the Philippines—varietal resistance and synchrony of planting dates.

Varietal resistance

Few commercial varieties with virus resistance are grown in the Philippines, although advanced breeding lines with resistance to tungro viruses are currently being developed at IRRI (Cabunayan et al, this volume). Several different sources of resistance to *Nephotettix virescens* (Distant), the main leafhopper vector, however, have been used in breeding programs and deployed over large areas (Khush 1984). The use of leafhopper-resistant varieties has been one of the major strategies for tungro management and has been highly effective. Rice varieties with resistance to *N. virescens* are not readily infected with tungro viruses except where disease pressure is high. Results from a survey conducted in the municipality of Polangui, Albay Province, Luzon, indicated that farmers clearly benefited from growing leafhopper-resistant varieties because of reduced tungro incidence (Chancellor et al 1996). Nevertheless, adaptation of leafhopper populations to previously resistant varieties has occurred in some areas, particularly in certain provinces in Mindanao (Dahal et al 1990).

In the Polangui survey, adaptation of leafhopper populations is not related to increased leafhopper number. Although the field resistance to tungro of resistant varieties was due to leafhopper resistance, leafhopper number was similar on resistant and susceptible varieties. Figure 1 shows the mean peak numbers of adults and nymphs of *N. virescens* collected by 10 sweeps of a 30-cm-diameter insect net in fields with at least 1% tungro-affected rice hills. The mean peak number per 10 sweeps of *N. virescens* adults was 35 for both resistant and susceptible varieties. Nymphal counts were also similar at 30 and 25 for resistant and susceptible varieties, respectively. Leafhoppers tend to feed in the xylem, rather than the phloem, of resistant varieties (Auclair et al 1982) and this may account for the lower incidence of tungro observed on resistant varieties in Polangui. For all these reasons, it is important that the reaction of resistant varieties to tungro disease be closely monitored after resistance deployment in different rice-growing areas and that information be disseminated to farmers through extension agencies.

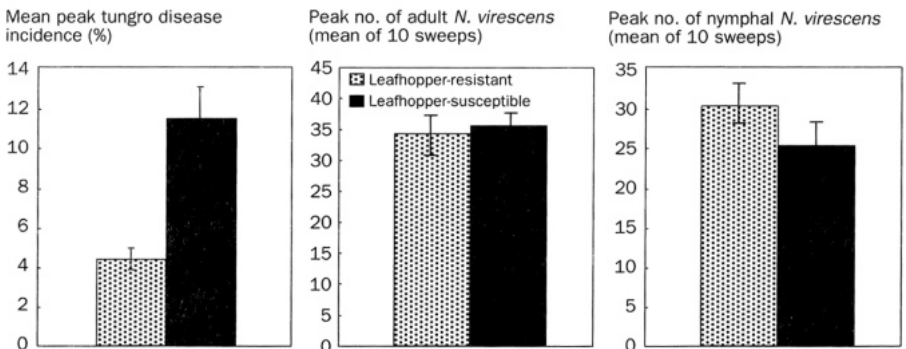


Fig. 1. Tungro disease incidence and abundance of adults and nymphs, respectively, of *Nephotettix virescens* on leafhopper-resistant and -susceptible rice varieties in farmers' fields in Albay Province, Luzon, Philippines, planted between November 1992 and October 1994. Bars indicate 95% confidence limits.

Limited data exist on how resistant varieties perform when grown widely under field conditions. Varieties IR60, IR62, IR66, IR70, IR72, and IR74, which are resistant to *N. virescens* but not to tungro viruses, were classified as “resistant” based on their field reaction to tungro in multilocational varietal trials conducted by PhilRice. All other varieties grown in the study area were classified as susceptible. Figure 1 shows that in fields planted between November 1992 and October 1994 in which at least 1% rice hills were affected by tungro, mean peak disease incidence on resistant varieties was 4.5% compared with 11.5% for susceptible varieties.

On-farm varietal trials were conducted in North Cotabato in 1996 using a methodology similar to that described in Subramanian et al (this volume). Tungro incidence was low in two of the trials but was high in San Pedro, where the highly susceptible variety Masipag, which was chosen by the farmer, was severely affected by tungro at 42 d after transplanting (DAT) and yielded only 0.5 t ha⁻¹ (Table 1). In contrast, green leafhopper-resistant IR62 and virus-resistant IR69705-1-1-3-7-1 had a much lower tungro incidence in spite of the strong sources of inoculum in Masipag plots and surrounding areas. In IR69705-1-1-3-7-1, the result is particularly striking as leafhopper numbers were very high but only 2% of the hills were affected at 56 DAT. The lower than expected yields in IR62 and IR69705-1-1-3-2-1 plots were mainly attributed to damage caused by a late attack of the rice black bug, *Scotinophara coarctata*.

Synchrony of planting dates

Loevinsohn (1984) reported that in the 1981 wet season in an irrigated, double-cropped rice area in Nueva Ecija, Luzon, tungro prevalence in six sites increased with asynchronous planting within 0.6 km of the sites. Sanchez and Obien (1995) considered asynchronous planting dates to be one of the major factors affecting tungro preva-

Table 1. Tungro disease incidence^a, green leafhopper (GLH) numbers^b, and yield in different lines and varieties in on-farm trials in the barangays of Central Bulanan, San Pedro, and Villarica, Midsayap, North Cotabato, 1996 wet season.

Site	Line/variety	GLH no.	Tungro Incidence (%)	Yield (t ha ⁻¹)
Central Bulanan	IR62	64.0 ± 7.5	2.4 ± 0.7	3.2 ± 0.5
	IR68305-18-1	318.7 ± 77.3	4.7 ± 0.9	2.6 ± 0.2
	PSBRc10	287.8 ± 18.7	4.1 ± 0.9	2.9 ± 0.2
San Pedro	IR62	33.7 ± 2.2	16.3 ± 5.7	1.5 ± 0.2
	IR69705-1-1-3-2-1	230.5 ± 28.9	3.7 ± 0.4	1.3 ± 0.1
	Masipag	494.8 ± 41.7	95.0 ± 5.0	0.5 ± 0.1
Villarica	IR62	25.1 ± 2.1	9.6 ± 3.2	3.0 ± 0.1
	IR68305-18-1	183.0 ± 3.5	18.7 ± 2.2	2.1 ± 0.1
	Line 601	68.9 ± 18.4	0.5 ± 0.5	3.5 ± 0.1

^aNumber of tungro-diseased hills at 56 d after transplanting (DAT) or seeding (DAS). Mean of three replications.

^bAverage numbers of adults and nymphs of *Nephotettix virescens* per 10 sweeps of a 30-cm-diameter insect net collected over three sampling dates at 28, 42, and 56 DAT or DAS (42 and 56 DAT only for Central Bulanan). Mean of three replications.

lence in Mindanao from 1989 to 1993. Continuous planting with no fallow period between rice crops and wide variation in planting dates were regarded as the main causes of tungro problems in Camarines Sur and Albay Provinces (Baria 1997).

Figure 2 shows the distribution of planting dates for rice fields from November 1992 to October 1994 in the survey area in Polangui. Planting was carried out monthly from November 1992 to March 1994. Although planting was continuous, peaks were observed in the number of fields planted within this period, which corresponded to each of the three main cropping seasons. These peaks occurred in December and January (1992-93 dry season), May to July (1993 wet season), and January and February (1993-94 dry season). No clear break between cropping seasons, however, was observed during this period. In contrast, there was a distinct gap between the 1993-94 dry-season and the 1994 wet-season crops, as only one field was planted in April and none in May. Most of the 1994 wet-season plantings were carried out in June and July when few standing rice crops remained in the area.

Figure 2 also shows the mean peak tungro incidence for *all fields* recorded in the survey for each month of planting from November 1992 to October 1994. In both the 1992-93 and 1993-94 dry seasons, tungro disease incidence was greatest in March plantings. In the 1993 wet season, tungro incidence was greatest in August and Sep-

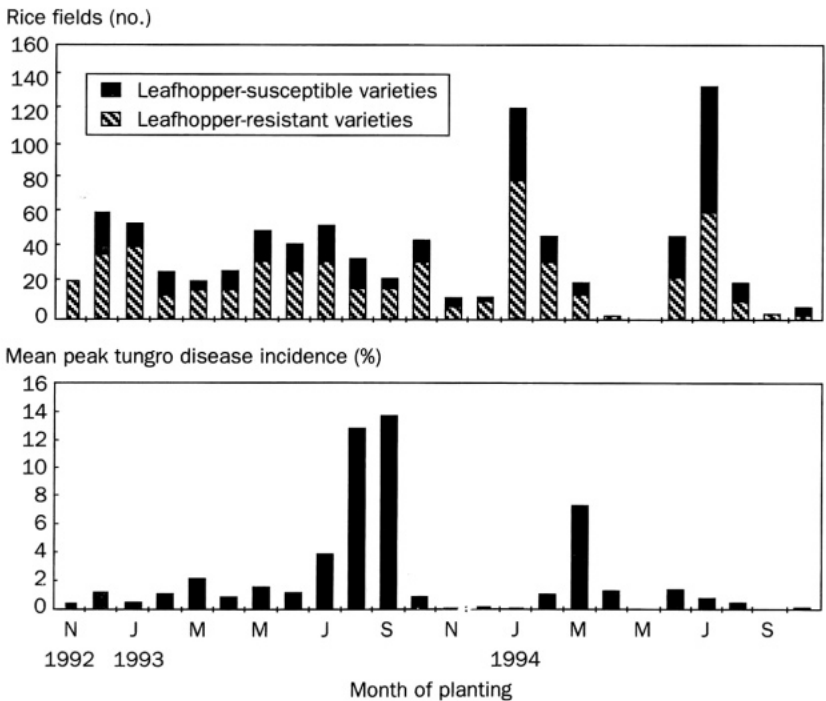


Fig. 2. Frequency of planting of rice crops and mean peak tungro disease incidence in farmers' fields in Albay Province, Luzon, Philippines, planted between November 1992 and October 1994.

tember plantings, reaching 13% and 14%, respectively. Thus, in each of these seasons, late plantings were seriously affected by tungro. Staggered planting allowed the inoculum to be carried over from earlier to later plantings within a season. Disease developed in some of the fields planted during the main planting periods, but incidence rarely reached high levels. This disease increase, however, was sufficient to result in significantly high inoculum levels at a time when later plantings were at a vulnerable growth stage.

Tungro disease incidence was lower in the 1994 wet season than in previous seasons. The fallow period following the 1993-94 dry season reduced the inoculum so that there was less risk of infection for wet-season plantings. Furthermore, most of the 1994 wet-season rice crops were planted in June and July, with few higher risk late-season plantings. Although the effects of environmental factors on tungro incidence were not noted during the survey, the 2-mo break in planting appeared to have helped reduce tungro incidence in the 1994 wet season.

Implications for tungro management strategies

In the 1992 wet season, 340 ha of rice were sprayed with insecticide in Polangui, Albay Province, in a coordinated campaign conducted by the Department of Agriculture to contain what was thought to be a developing outbreak of tungro disease. Spraying insecticide against leafhoppers in response to tungro remains a standard practice in many areas in the Philippines, even though the effectiveness of this approach may be uncertain. In interviews conducted with rice farmers in five provinces in the Philippines in 1994, many respondents thought that insecticide application did not control the spread of tungro very effectively (Warburton et al 1997). The continued practice of spraying is due in part to the perceived advantage of protecting the crop against other insect pests.

Many potentially adverse side effects of spraying insecticides in rice have been well documented. Because of the limited efficacy of spraying insecticides against leafhoppers for tungro control, and the ineffectiveness of other tactical measures such as roguing (Tiongco et al 1998), strategic measures appear to offer the best prospects for tungro management in the Philippines (Holt et al 1996). In South Sulawesi, Indonesia, a tungro management scheme was introduced in the mid-1980s in which planting dates were synchronized and targeted to avoid periods of peak vector populations (Sama et al 1991). The scheme also involved varietal rotation to reduce the potential for leafhopper adaptation to resistant varieties, and selective recommendations for vector control using insecticides. Tungro incidence has remained low since the introduction of the scheme, except in small areas where it has been difficult to synchronize planting dates (S. Sama, personal communication, 1994). The scheme has been quite effective and synchronous planting may have played a key role in its success.

Results from the survey conducted in Polangui are also consistent with the notion that planting rice crops synchronously reduces the risk of tungro incidence considerably. A mathematical model was developed and used to examine the area-wide dynamics of tungro disease (Holt and Chancellor 1997). The potential impact of

changes in cropping synchrony in reducing tungro disease levels was assessed. Model outputs showed that tungro endemicity was determined mainly by planting date variance and disease persisted if this variance exceeded a certain threshold. Where planting dates were only moderately asynchronous, relatively small reductions in planting date variance made a significant contribution to reducing tungro incidence. If the planting was highly asynchronous, however, then a similar marginal increase in synchrony only slightly reduced tungro incidence. Consequently, where rice cropping patterns are highly asynchronous and it is impractical to change them dramatically, a different management approach is needed. In such situations, varietal resistance offers the best prospects for success.

Varietal selection by farmers is often conditioned more by factors such as eating quality and market price of the grain than by pest and disease resistance characteristics. In areas with high tungro incidence, however, resistance characteristics are an important criterion for varietal selection (Warburton et al 1997, Warburton et al, this volume). The mathematical model of tungro dynamics was used to examine issues related to the deployment of resistant varieties (Holt and Chancellor, in press). The most effective strategy, based on model outputs, was to concentrate deployment of resistant varieties in the wet season, the season of greatest disease spread. A relatively large proportion of fields need to be planted with resistant varieties to significantly reduce tungro incidence in fields planted with susceptible varieties. Nevertheless, results from the on-farm trial in San Pedro and the data from many seasons of on-station trials in Midsayap and Maligaya (Cabunagan et al, this volume) show the advantages of growing resistant varieties. Therefore, the deployment of resistant varieties may considerably benefit farming communities, even if the very high levels of adoption possible in South Sulawesi are not obtained.

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Improving IPM technology for rice tungro disease in Indonesia

A. Hasanuddin, I.N. Widiarta, and Yulianto

Rice tungro disease (RTD) is of great economic importance in Indonesia. RTD distribution is still expanding and the disease causes Serious outbreaks in some seasons. In the 1994-95 wet season, RTD severely attacked rice plants in East Java and in the Surakarta region of Central Java. The yield loss was estimated to be approximately 25 billion Indonesian rupiah. RTD is successfully controlled in South Sulawesi by integrated pest management (IPM), combining planting at the appropriate time and the use of green leafhopper-resistant varieties in rotation. This control method needs to be adapted, however, before it can be applied elsewhere, especially in areas planted asynchronously. Some experimental activities have been carried out to improve IPM for tungro such as determining the minimum area necessary to conduct synchronized planting, adjusting planting time, adapting *Nephotettix virescens* to resistant varieties as the basis of variety rotation, and using selective weed sanitation to reduce infection sources. Experimental results indicated that tungro disease spread from a single source of diseased plants reached 200 m. In asynchronously planted fields, RTD incidence at harvest in an observed field correlated positively with RTD incidence at 6-10 wk after transplanting (WAT) in an area within a radius of 101–250 m when rice plants in the observed field were at 3 WAT. RTD incidence was high regardless of planting time in asynchronously planted fields. In synchronously planted fields, however, the later the planting time, the greater the disease incidence. RTD incidence in Bali, West Java, and Central Java was high on Cisadane and IR64, which possess the *Glh5* resistance gene. *N. virescens* colonies collected from West Java, Central Java, Bali, and South Sulawesi were well adapted to IR64. Transmission of rice tungro viruses was achieved in six weed species. This information can be used to improve IPM for RTD in Indonesia.

Rice tungro disease (RTD) is efficiently spread by green leafhopper (GLH) species, especially *Nephotettix virescens* Distant. In the 1994-95 crop season, an outbreak of RTD occurred in East and Central Java. The yield loss approached 25 billion Indonesian rupiah (Anonymous 1995). The area affected by RTD is still expanding, especially in West Java, where RTD was found in the mountainous area but not in the northern coastal lowland until the 1996-97 wet season (Hasanuddin et al 1995, Widiarta et al 1997). In the 1996-97 wet season, RTD was found in Sukamandi (Widiarta et al unpublished). RTD has the potential to cause serious outbreaks.

Sama et al (1991) reported that RTD was controlled successfully in South Sulawesi by integrated pest management practices, combining planting at the appropriate time and the use of GLH-resistant varieties in rotation. Recommended transplanting dates were based on the seasonal fluctuation of rainfall, tungro incidence, and green leafhopper populations. These dates were selected to avoid periods of high disease pressure. Varieties resistant to *N. virescens* are categorized into five groups, based on resistance genes present, for variety rotation. T0 varieties have no resistance gene. The other groups—T1, T2, T3, and T4—have resistance genes *Glh1*, *Glh6*, *Glh5*, and

glh4, respectively. Planting at the appropriate time was considered the most important factor in avoiding periods of high disease pressure. Before the implementation of IPM, the affected area exceeded 1,000 ha, but recently this has dropped to only 100 ha. Implementing IPM for tungro in provinces outside South Sulawesi is more difficult, especially in the asynchronously planted areas of Java and Bali. Consequently, RTD is most prevalent in these islands, which contribute more than 60% of the total rice production in Indonesia. An RTD management strategy suitable for asynchronously planted areas of Java and Bali is urgently needed to minimize yield loss.

Experiments were conducted to estimate the minimum area necessary to undertake synchronized planting, adjust planting dates, adapt *N. virescens* to resistant varieties as the basis of variety rotation, and use selective sanitation to reduce sources of RTD inoculum. The objective of these experiments was to improve IPM implementation in tungro-endemic areas in Java and Bali. The results are reported here.

Materials and methods

Unit of synchronized planting

Synchronized planting within a minimum area is necessary to benefit from implementing recommended transplanting times. The size required was estimated by two field experiments. First, the disease gradient from a single virus source was recorded. Second, RTD incidence in an observation field was correlated with RTD incidence in the surrounding area.

Disease gradient distribution. This experiment was conducted in Sidrap in the 1996 dry season and in Maros in the 1996-97 wet season in a 10-ha rice area that was planted synchronously. Preinfected seedlings of Cisadane, the inoculum source, were planted at a spacing of 20 × 20 cm in the center of the study area in a 10 × 10-m plot. Monitoring plots were established at distances of 100, 300, 300, 400, and 500 m from the inoculum source and were planted with disease-free seedlings of Cisadane at 20 × 20-cm spacing in 10 × 10-m plots. The green leafhopper population was observed at 6 WAT by 10 sweeps of an insect net and RTD incidence was assessed at 8 WAT in the monitoring plots.

Relationship between tungro incidence and diseases in observed fields. This field experiment was conducted in an asynchronously planted area at Subak Padang Galak, Badung regency, Bali, during the 1995 dry season and 1995-96 wet season in a 12-ha rice field. Rice fields in the area were mapped, and rice plants were planted monthly during the wet and dry seasons for observation. The population densities of green leafhopper and tungro incidence were assessed when rice plants in the observed field were at 3, 8, and 12 WAT. At the same time, GLH population density and tungro incidence were observed in surrounding fields 50, 100, 200, and 250 m away. Rice stages in the area were also mapped and categorized into seedling, young rice plant (1–5 WAT), older rice plant (6–10 WAT), and stubble for all surveys.

Time of planting recommendation

Varieties in each of the five group (T0 to T4) were planted at early, normal, and late planting times in the synchronously and asynchronously planted fields. Early planting was at the same time as the earliest plantings by farmers. Normal and late planting were 1 and 2 mo later, respectively. The experiment was conducted in Bali, Central Java, and West Java in the 1996 dry season and 1996-97 wet season. GLH population density and tungro incidence were observed at 4, 6, and 8 WAT. The population density of green leafhoppers was surveyed by 20 strokes of an insect net and RTD incidence was assessed from 100 hills selected randomly.

Variety rotation

Populations of *N. virescens* were collected from rice fields in West and Central Java, Bali, and South Sulawesi from the dominant variety in the particular area. Each colony was reared separately in the greenhouse. Adults from each colony were placed on tungro-infected rice plants in an insect cage for 4 d. Each colony was allowed to transmit virus on a set group of varieties with different genes for resistance to GLH (T0 to T4) for 1 d. The percentage of tungro-infected plants was observed 2 wk after inoculation feeding. The level of resistance to green leafhopper as indicated by the survival rate of colonies was tested by introducing five second or third instar nymphs from each colony into test tubes with 14-d-old seedlings of a set group of varieties with T0-T4 resistance genes. Survival rates and duration until the adult stage were observed.

Selective sanitation

This study involved a two-step experiment. First, weed species that could be successfully inoculated with rice tungro viruses by *N. virescens* were determined. Second, a test was conducted to see whether *N. virescens* could obtain virus from those weeds and successfully transfer the virus to rice plants.

In the first experiment, weeds commonly found in rice fields were collected and transplanted individually in pots. The weed plants were collected from epidemic and endemic areas of RTD in Bali, Klaten, and Yogyakarta. Each weed species was inoculated separately by exposing each plant for 1 d to four infective *N. virescens* that had fed for 4 d on rice plants infected with rice tungro bacilliform (RTBV) and rice tungro spherical (RTSV) viruses. In the second experiment, seedlings of rice variety IR64 were inoculated by *N. virescens* that had fed on weed species infected with RTBV and RTSV. At 35 d after inoculation, extracts of weeds and rice seedlings were indexed by enzyme-linked immunosorbent assay (ELISA) for the presence of RTBV and RTSV.

Results and discussion

Minimum unit for synchronized planting

RTD incidence in the inoculum source field was 89%. RTD incidence was low in all plantings away from the infection source (Fig. 1). The population density of *N. virescens* fluctuated from 12 to 17 per 10 strokes.

RTD incidence at harvest correlated positively with RTD incidence in an area within a radius of 101–250 m at 6–10 WAT, when rice plants in the observation field were at 3 WAT (Fig. 2). Therefore, the area inside this radius can be considered as the unit that should be planted synchronously. Assuming that RTD spread equally in all directions, the size of the synchronously planted area should be about 20–40 ha.

Loevinsohn and Alviola (1991) reported a significant correlation between the degree of asynchrony and tungro spread to plantings within a radius of less than 600 m.

Planting time recommendation

In the asynchronous area, early and late-planted rice had a similar risk of infection by RTD (Fig. 3). In the synchronous planting, early planting showed the lowest risk of RTD infection. Chancellor et al (1996) reported a similar finding in the Philippines. Savary et al (1993), however, found that low RTD incidence was associated with late planting rather than early and mean planting dates in the Philippines. The difference in results may be due to the different methods of assessing planting time. The resistance level of the variety influences RTD incidence in both synchronously and asynchronously planted areas. The resistant variety in group T3 showed tungro incidence similar to that in the susceptible T0 group.

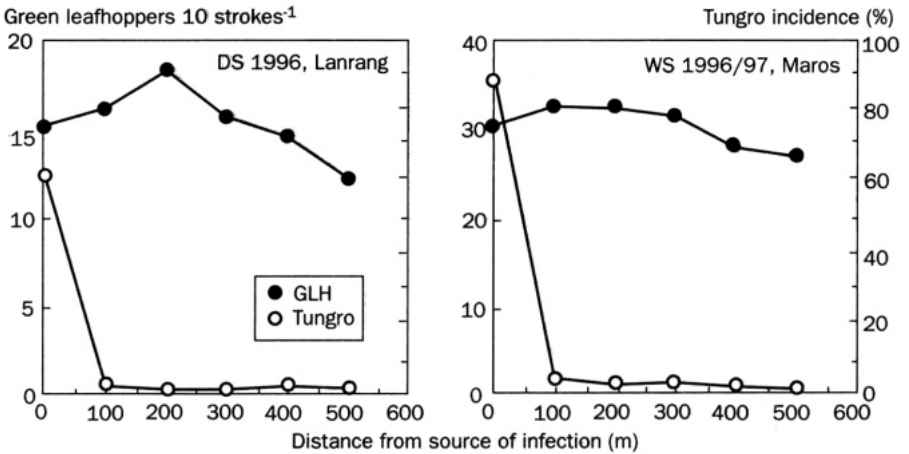


Fig. 1. Incidence of tungro disease and population of green leafhopper (GLH) at different distances from an inoculum source. DS = dry season, WS = wet season.

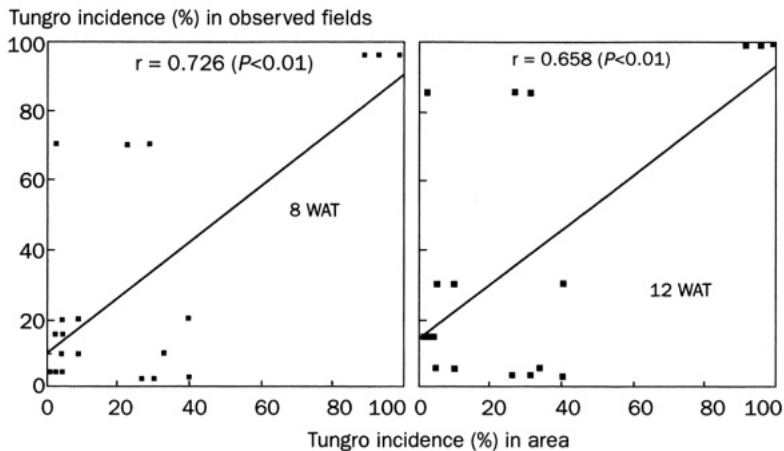


Fig. 2. Correlation between tungro incidence in observation fields at 8 and 12 wk after transplanting (WAT), and incidence within a radius of 101-250 m in the surrounding area when rice plants in the observation fields were at 3 WAT.

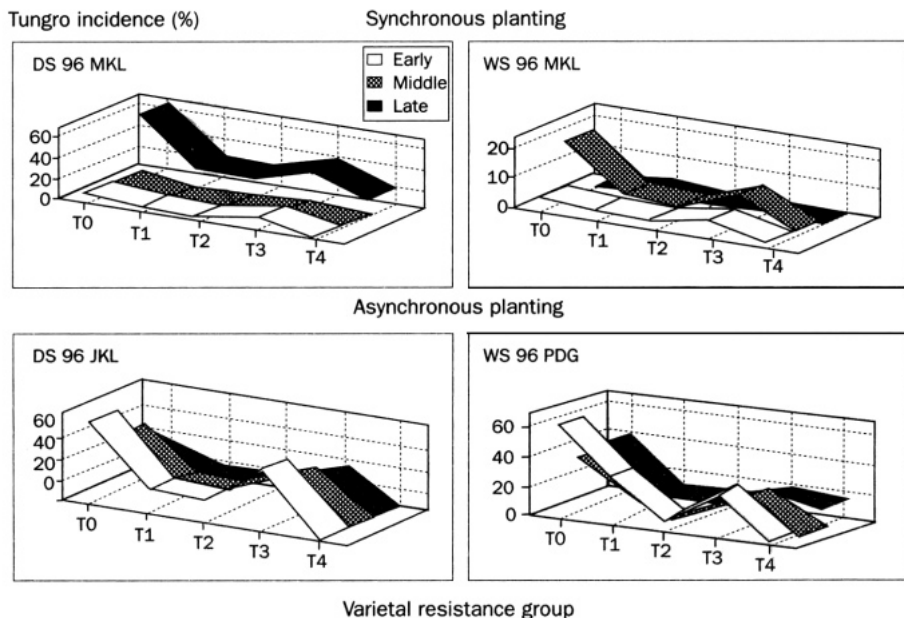


Fig. 3. Tungro incidence in various groups of resistant varieties in synchronously and asynchronously planted fields with rice plants planted in the early, middle, and late crop season. DS = dry season, WS = Wet season, MKL = Mungkul village, PDG = Padanggalak village, JKL = Jaten village.

Tungro incidence (%)

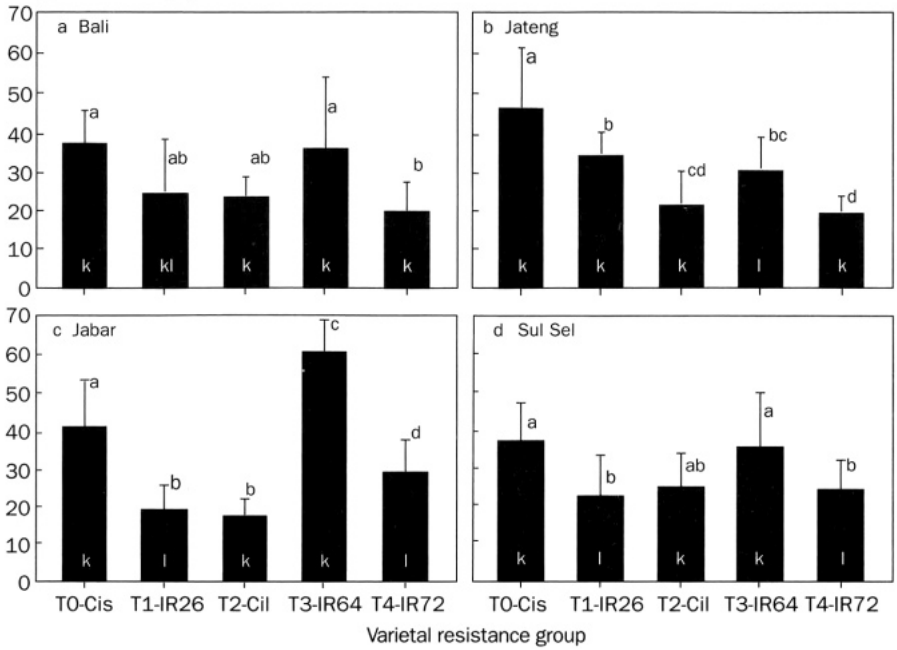


Fig. 4. Transmission efficiency of colonies of *Nephotettix virescens* on rice varieties in five resistance groups. Bars within a variety and colony with the same letter are not significantly different at the 5% level by Duncan's multiple range test (DMRT).

Variety rotation

Figure 4 indicates that some of the resistant varieties tested have now become susceptible. *N. virescens* colonies tested on a set of varieties with different genes for resistance showed variations in their virulence. IR72 was not preferred by *N. virescens* colonies from Bali or Central Java. The survival rate of *N. virescens* nymphs from Bali on IR72 was less than 10%, whereas the survival rate of West Java and South Sulawesi colonies on IR72 was not significantly different from that of the control variety, Cisadane (Fig. 5). IR26 (T1) and Ciliwung (T2) were still resistant to the colony from South Sulawesi. Information on transmission ability and survival rate based on the local situation can be used in choosing suitable varieties for varietal rotation.

Although all *N. virescens* colonies used in this experiment were collected from IR64, the degree of adaptation to the varietal resistance groups was variable. The variety composition in the area probably influenced the variability in the adaptation of *N. virescens*. Bastian et al (1995) showed that the survival rate of *N. virescens* colonies differed according to the variety from which they were collected.

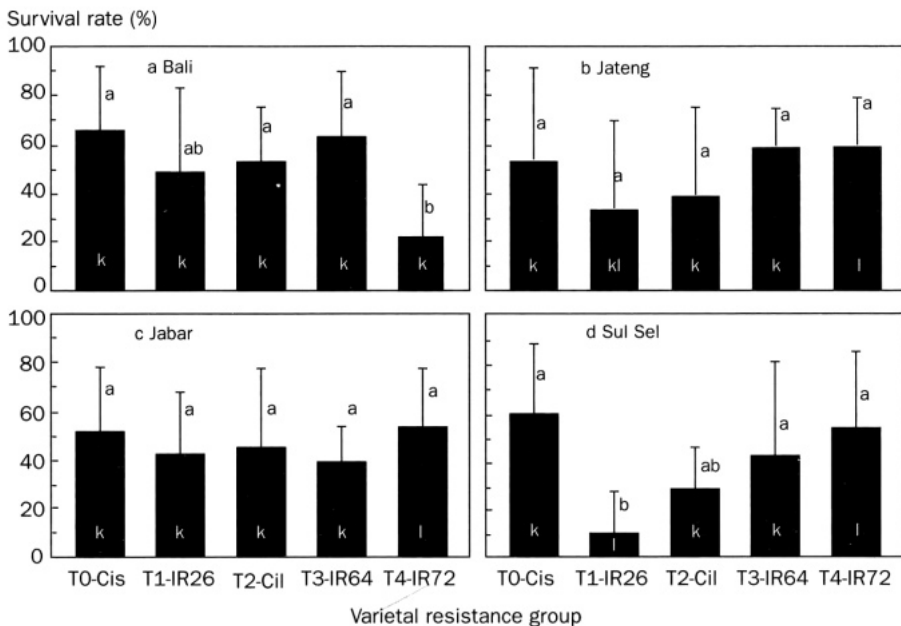


Fig. 5. Survival rate of *Nephrotettix virescens* reared on rice varieties from different resistance groups. Bars within a variety and colony with the same letter are not significantly different at the 5% level by Duncan's multiple range test (DMRT).

Selective sanitation

Among weed species inoculated by infective *N. virescens*, only *Jussiaea repens*, *Trimthema portulacastrum*, *Phyllanthus niruri*, *Cyperus rotundus*, *Monochoria vaginalis*, and *Leersia hexandra* gave positive ELISA readings, suggesting that these weeds can be infected by RTSV and RTBV (Table 1).

Of the five successfully infected weeds (*L. hexandra*, *C. rotundus*, *M. vaginalis*, *J. repens*, and *C. difformis*), only *C. rotundus* and *M. vaginalis* served as an infection source of tungro viruses for rice.

Rice tungro viruses reportedly survive in rice stubble, weeds, and wild rice species, which act as sources for reinfection of succeeding rice crops (Tiongco et al 1992). Anjaneyulu et al (1988) reported that, of some weeds infected with RTSV, only *Brachiaria mutica* and *Axonopus compressus* were infected with RTBV.

Conclusions

The minimum area for synchronized planting is 20–40 ha. Planting time recommendations are difficult to implement in asynchronously planted areas. Rotation of GLH-resistant varieties can be implemented in both synchronously and asynchronously

Table 1. Enzyme-linked immunosorbent assay (ELISA) reactions of sap from plants inoculated with rice tungro spherical (RTSV) and bacilliform (RTBV) viruses by *Nephotettix virescens*.

Weed	ELISA test reaction	
	RTSV	RTBV
<i>Echinochloa colonum</i> (L.) Link.	-	-
<i>E. crus-galli</i> (L.) Deauv	-	-
<i>Leersia hexandra</i> Swartz	+	+
<i>Leptochloa chinensis</i> (L.) Nees.	-	-
<i>Panicum repens</i> L.	-	-
<i>Cyperus difformis</i> L.	-	-
<i>C. rotundus</i>	+	+
<i>C. halpan</i> L.	-	-
<i>C. iria</i> L.	-	-
<i>Jussiaea repens</i>	+	+
<i>Neocharis pellucida</i> Pres.	-	-
<i>Fimbristylis littoralis</i> Gaudich	-	-
<i>Scirpus juncoides</i> Roxb.	-	-
<i>Marsilea crenata</i> Presl.	-	-
<i>Trianthema portulacastrum</i>	+	+
<i>Alternanthera philoxeroides</i> Mart.	-	-
<i>A. sessilis</i> (L.) D.C.	-	-
<i>Limnocharis flava</i> (L.) Buch	-	-
<i>Commelina nudiflora</i> L.	-	-
<i>Eclipta prostrata</i> L.	-	-
<i>Ludwigia adscendens</i> L.	-	-
<i>L. octovalvis</i> (Jacq.) Raven	-	-
<i>Monochoria vaginalis</i> (Burm.) Presl.	+	+
<i>Phyllanthus niruri</i>	+	+
<i>Imperata cylindrica</i>	-	-

planted areas as long as *N. virescens* has not yet adapted to the varieties. Two weeds, *Cyperus rotundus* and *Monochoria vaginalis*, as well as ratoon crops should be eradicated before nursery bed preparation. *N. virescens* is already adapted to IR64, but the other varietal resistance groups can still be used.

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Leafhopper control by insecticides is not the solution to the tungro problem

S. Villareal

Green leafhoppers (GLH) are one of the most abundant canopy arthropods in irrigated rice systems throughout much of South and Southeast Asia. Populations are rarely large enough to cause direct feeding damage to rice, but in some areas they are important pests as vectors of rice tungro disease. In some countries in Asia, chemical control of GLH based on threshold numbers of the insect is still recommended. In the majority of rice-growing areas, however, tungro disease is absent or occurs infrequently. Consequently, routine insecticide applications against GLH cannot be justified. Moreover, spraying insecticides to control GLH does not always result in effective tungro disease management. Insecticides may be harmful to human health and to the environment and indiscriminate use of certain compounds in rice has been shown to cause outbreaks of secondary pests. Alternative methods of managing tungro disease in endemic areas are available and these should be used for GLH control. Currently, virus-resistant varieties are being developed and it is hoped that they will soon be available to rice farmers and that these varieties will provide them with a further option for managing tungro.

The main leafhopper vector of tungro disease, *Nephotettix virescens* (Distant), can be found in almost all irrigated and rainfed rice systems in tropical Asia. In a study conducted at five irrigated sites in the Philippines in 1989, *N. virescens* was the most abundant phytophagous insect in all locations (Heong et al 1989). In the absence of sources of inoculum, however, this insect does not harm the rice crop through direct feeding. There have been reports of “hopperburn” caused by large populations of GLH, especially from India, but these cases are extremely rare. In most rice-growing areas, inoculum sources are few or absent so that attempting to control leafhoppers would be wasting farmers’ time and money.

GLH abundance and tungro disease incidence in endemic areas

In studies conducted in experimental plots on the IRRI farm in 1991-91, there was no correlation between numbers of *N. virescens* and tungro disease (Chancellor et al 1996a). In one season, high tungro incidence was recorded in the presence of low numbers of leafhoppers. The availability of inoculum was considered to be the key determinant of the disease level. Once primary inoculum had been introduced into field plots, rapid secondary plant-to-plant spread occurred. An analysis of factors affecting tungro incidence in endemic areas in Mindanao, Philippines, however, revealed that increases in disease incidence were associated with rising vector numbers as well as with the number of viruliferous vectors (Savary et al 1993). Similar findings were recorded in an endemic area in South Luzon, Philippines, during a survey

conducted in 1993-94 (Chancellor et al 1996b). In view of the impracticability of reducing vector numbers to levels needed to significantly reduce tungro spread, however, the most appropriate tungro management strategy in such areas would be to minimize the amount of initial inoculum by planting resistant varieties or by reducing the variation in planting dates.

Efficacy of insecticide sprays against GLH in managing tungro

Many field trials have been conducted on research stations to evaluate the efficacy of various insecticides against GLH and their effectiveness in reducing tungro disease. Chancellor et al (1997) reviewed the results from some of these trials and concluded that successful control of GLH and tungro disease can be achieved through insecticide sprays but that this may not be possible under certain conditions. A modeling study by Holt (1996) provides an explanation for this. In his study, the author examined the effect on final tungro incidence of applying a single insecticide at 20 d after transplanting, assuming an extremely high mortality rate of 95% to *N. virescens*. The model output indicated that, at very low immigration rates of GLH, a reduction in disease incidence of up to 60% could be achieved. As the immigration rate of infective vectors increased, however, the reduction in tungro incidence became extremely small. Although this case refers to a situation with a single insecticide application, the assumed mortality rate of 95% is unrealistically high and this level of mortality could probably only be achieved with the use of two or more insecticides.

Batay-an and Mancao (this volume) report on an insecticide trial carried out in North Cotabato, Philippines, in which good control of GLH and tungro disease was achieved using synthetic pyrethroids. Where there are widespread sources of inoculum and active movement of GLH between fields, however, such an approach may not be effective. Similar conditions were observed during a tungro epidemic in Negros, Philippines, in the 1998 wet season, where farmers resorted to weekly sprays of cypermethrin in an attempt to control the disease. The strategy was not effective in reducing tungro incidence and the sprays caused the resurgence of populations of the brown planthopper, *Nilaparvata lugens* (Stål), which led to “hopperburn” (Tiongco 1998). Similarly, in an insecticide trial conducted in North Cotabato in 1997, weekly spraying was not successful in preventing tungro from spreading in experimental plots. Tungro disease incidence in surrounding rice fields was very high and presumably there was continuous recolonization of the trial plots by viruliferous leafhoppers similar to the process described by Schoenly et al (1996).

Environmental and health concerns associated with insecticide applications in rice

The cartoon illustrated in Figure 1 was used in training courses for rice farmers on tungro management conducted in India and the Philippines in 1996-98. The cartoon illustrates two of the unwelcome effects of spraying insecticides in rice. First, insect-

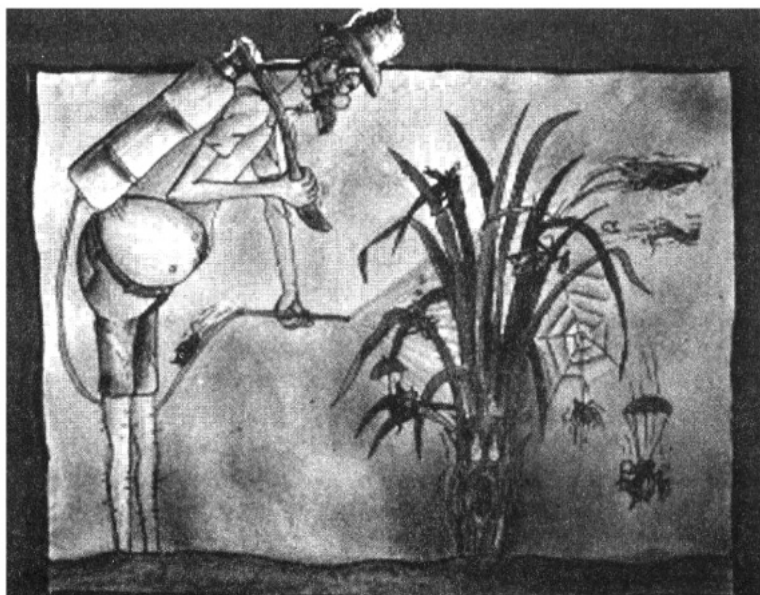


Fig. 1. Cartoon used for tungro management training courses in India and the Philippines showing the negative effects of insecticide application for leafhopper control.

ticide application methods in rice are still very primitive and, with the types of sprayers and nozzles available, the efficacy is doubtful. Most people doing the spraying do not wear protective clothing and they mix the chemical without wearing gloves. They spray in front of them so that they have to walk through the sprayed area. Unfortunately, category 1 and 2 insecticides are still widely available in many Asian countries so the risk to human health through insecticide spraying is serious. Second, most insecticides that are used for insect control in rice are still nonselective and are harmful to the many predators and parasites that are so important for regulating populations of insect pests in rice.

Conclusions

Applying insecticides to control leafhopper vectors of tungro disease cannot be justified in areas where inoculum sources are not present. Even in endemic areas, insecticide applications are often not effective. In a survey conducted in 1994 in the Philippines, some farmers in tungro-endemic areas said that they continued to apply insecticides to control tungro even though they knew that this approach did not work (Warburton et al 1997). They sprayed because they did not know what else they could do and they thought the insecticides might help to control some other insect pests.

Our challenge is to promote the adoption of more effective and environmentally safe tungro management strategies in such areas.

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The role of vector control in rice tungro disease management

N. Widiarta

Tungro disease, which is spread by rice green leafhoppers, especially *Nephotettix virescens*, is one of the most destructive diseases of rice. The disease is successfully suppressed by planting rice at the recommended time to avoid high disease pressure and by rotating varieties with resistance to green leafhopper in synchronously planted areas, for example, in South Sulawesi. Simultaneous planting, however, is difficult to practice by farmers for various reasons. Therefore, until recently, tungro disease expansion and outbreaks have mainly occurred in asynchronously planted areas, primarily in Bali and Java. The population density of green leafhoppers in paddy fields in those areas is maintained at a low level by the dispersal activity of adults. Integrated pest management (IPM) strategies have been developed based on characteristic population dynamics of the vector to reduce the proportion of viruliferous vectors. In this study, the use of antifeedants against *N. virescens* to control tungro spread in synchronously and asynchronously planted fields was also examined. The antifeedant and virus transmission inhibition activities of andrographolide, a major compound of *Andrographis paniculata*, and commercial insecticides such as imidacloprid, pymetrozin, MIPC, and nytenpyram were tested against female adults of *N. virescens*. Imidacloprid and nytenpyram showed better antifeedant activities than andrographolide and pymetrozin. Imidacloprid inhibited acquisition and inoculation of tungro viruses better than the others. The results imply that antifeedants have a potential to reduce virus transmission without directly disturbing the food chain. In asynchronously planted areas, application of diacloden and MIPC successfully decreased vector population density but not tungro spread. In synchronously planted areas, application of diacloden, imidacloprid, and MIPC significantly reduced both vector population density and disease incidence.

Rice tungro disease (RTD) has affected almost all provinces of Indonesia. Recently, the affected area has extended to the northern coastal lowland of West Java in Subang (Hasanuddin et al 1995, Widiarta et al 1997a). Therefore, the important West Java rice bowl is now threatened.

RTD has been successfully controlled in Sulawesi by transplanting synchronously over wide areas at the recommended planting time (Sama et al 1991). This enables rice plants to escape peak periods of disease pressure. Synchronous planting was also combined with rotation of varieties particularly resistant to the tungro vector, *Nephotettix virescens*.

Rice is susceptible to tungro infection during the early vegetative stage. Infection at this stage causes severe yield loss. The older the rice plant is when infected, the lower the yield loss. The recommended planting time is based on the annual cycle of *N. virescens* populations and RTD incidence. In Maros, South Sulawesi, April and October are the months of highest tungro risk because population densities of green leafhopper (GLH) and RTD incidence are high then. Therefore, farmers are recommended not to have young rice plants in the fields at this time to escape damage.

The escape strategy is combined with varietal rotation between seasons. Breeders have identified seven genes with resistance to GLH. Rice varieties are categorized into five groups based on the resistance gene of the parent.

After the combined escape and rotation strategy was implemented, the tungro-infected area in South Sulawesi decreased remarkably. Before synchronous planting was established in 1984, the tungro-infected area exceeded 4,000 ha; now it is less than 100 ha.

The escape strategy is difficult to implement in asynchronously planted areas because it is difficult for farmers to plant simultaneously over wide areas. Varietal rotation reduced disease incidence in asynchronous areas as long as GLH did not adapt to the varieties being used. The problem is, however, that resistant varieties with different resistance genes also differ in eating quality. Farmers need varieties with diverse resistance genes but that are similar in quality. Recommended varieties may not be accepted because of their inferior taste. Almost all Indonesians prefer IR64-type quality.

Following the success of tungro management in South Sulawesi, the main problem has switched from Sulawesi and Bali in 1980-85 to Java and Bali more recently. Unfortunately, Java and Bali contribute more than 60% of the total rice production in Indonesia. Consequently, tungro threatens rice production and self-sufficiency in the country. Other control strategies are therefore required, especially for asynchronously planted areas.

Population dynamics of *N. virescens*

The fluctuating RTD incidence in asynchronously planted areas is closely related to changes in GLH density (Suzuki et al 1992). RTD incidence increases when GLH numbers increase. To establish a control strategy for RTD in asynchronously planted fields, the population dynamics of GLH was studied intensively in Bali using a FARMCOP insect suction trap (Cariño et al 1979, Aryawan et al 1993). All arthropods caught were identified in the laboratory. It was established that the population density of GLH increased only at the early stages of rice growth and decreased thereafter (Widiarta 1992). Some studies showed that population density did not increase at all after immigrants invaded rice fields. The peak density of *N. virescens* was much lower than that of its sibling species, *N. cincticeps*, in temperate paddy fields. The population density of *N. cincticeps* was observed in Okayama, Southwestern Japan, using a procedure similar to that used in Bali (Widiarta 1993). The population density of *N. cincticeps* increases from the time it infests rice fields until just before harvest and its peak densities are much higher than those of *N. virescens*.

A key factor analysis for the life tables of *N. virescens* and *N. cincticeps* was conducted. Key component mortality can be identified with reference to the biggest slope of regression coefficient. Results showed that nymphal mortality including loss of adults after emergence is the key factor for both *N. virescens* and *N. cincticeps*. Egg parasitism is the second key factor for *N. virescens* but not for *N. cincticeps*.

To establish relationships between key component morality and biotic and abiotic factors, regression analysis was conducted using nymphal mortality and predators as well as nymphal mortality and rainfall. Nymphal mortality for virescens was not influenced by spider numbers.

Nymphal mortality for *N. virescens* was not related to physical factors such as rainfall. Therefore, adult loss after emergence is considered to be the key factor.

RTD control strategy

The previously mentioned characteristic population dynamics of *N. virescens* suggest that the population density of *N. virescens* in asynchronously planted fields is kept low by the dispersal activity of adults. Newly emerged adults have short residential periods within fields, probably because they move to other fields of younger rice plants. Therefore, they lay fewer eggs in fields where they emerged. Egg parasites also play an important role in reducing egg survival. Therefore reducing the population density of *N. virescens* is not the most appropriate way to control tungro. The requirement is to reduce the proportion of ineffective vectors.

Virus sources can be reduced by using resistant varieties that restrict virus multiplication and by practicing selective weeding of alternative virus hosts. Antifeedants can be used to reduce the duration of virus inoculation and acquisition. This review presents the role of antifeedants in controlling vector feeding and of insecticides in controlling vector density in relation to RTD.

Control of *N. virescens* feeding activity

The use of an antifeedant reduces virus acquisition and inoculation without killing the insect, which helps maintain the balance with its natural enemies, especially egg parasites. Some substances have been tested in the laboratory (Widiarta et al 1997b). Feeding on artificial diets using the streaked parafilm method was employed. The insects tested were allowed to feed through parafilm. The feeding rate and number of stylet marks on the parafilm were observed. Insect survival after treatment was also recorded to discriminate between antifeedant activity and insecticide activity.

Table 1 shows that andrographolide, an active compound of the tropical plant *Andrographis paniculata*, and pymetrozin insecticide suppressed feeding by females at the lowest concentration of 20 ppm, while imidacloprid and nitenpyram, a neonicotinoid insecticide, did so at concentrations of 0.01 ppm. Insect survival rates at concentrations tested were generally high, but decreased markedly at the highest concentration of 40 ppm for both the andrographolide and pymetrozine. The chemicals tested showed antifeedant activity against *N. virescens*.

Andrographolide at 40 ppm and the insecticides pymetrozine, imidacloprid, and nitenpyram significantly reduced the number of stylet marks (Table 2). The minimum feeding acquisition and inoculation times for transmission of tungro by *N. virescens* were reported to be 5–30 min (Wathanakul and Weerapat 1969). The reduction in number of feeding marks may be used as an indicator of the extent to which virus transmission is impeded.

Table 1. Mean consumption of female *Nephotettix virescens* after feeding 24 h on 5% sugar mixed with various concentrations of test materials through parafilm.

Test material	Concentration (ppm)	Survival rate (%)	Consumption (mg) ^a
Andrographolide	10	93	9.7 ac
	20	100	6.8 ab
	40	70	9.3 a
Pymetrozin	10	90	9.4 ac
	20	80	6.0 ab
	40	53	4.6 b
Imidacloprid	0.01	93	4.6 b
Nytenpyram	0.01	90	5.9 ab
Sugar (5%)		100	19.1 c

^a Means followed by a common letter are not significantly different at the 95% level, Fisher-PLSD test.

Table 2. Mean number of stylet marks by *Nephotettix virescens* on parafilm after 24 h of access to various concentrations of test materials.

Test material	Concentration (ppm)	Stylet marks ^a
Andrographolide	10	61.9 af
	20	75.9 af
	40	37.4 b
Pymetrozin	10	17.9 cd
	20	25.6 bc
	40	15.0 d
Imidacloprid	0.01	9.5 e
Nytenpyram	0.01	21.5 c
Sugar (5%)		93.6 f

^a Means followed by a common letter are not significantly different at the 95% level, Fisher-PLSD test.

Rice tungro virus transmission inhibition by antifeedants

The effect of antifeedants on virus acquisition and inoculation was investigated (Widiarta et al unpublished). RTD-affected plants were treated with antifeedant before *N. virescens* was given access to the plants. Healthy rice seedlings were also treated with antifeedant before infective *N. virescens* was given access to them to assess feeding inhibition. The test tube inoculation method was used for both tests.

Results showed that pymetrozin and andrographolide applied to tungro-affected plants significantly decreased the number of vectors that became infective (Fig. 1). Application of imidacloprid at 0.01 to 0.02 ppm completely prevented acquisition.

Pymetrozin and andrographolide applications on test plants at concentrations of 20 ppm significantly reduced virus transmission by *N. virescens* (Fig. 2). Further-

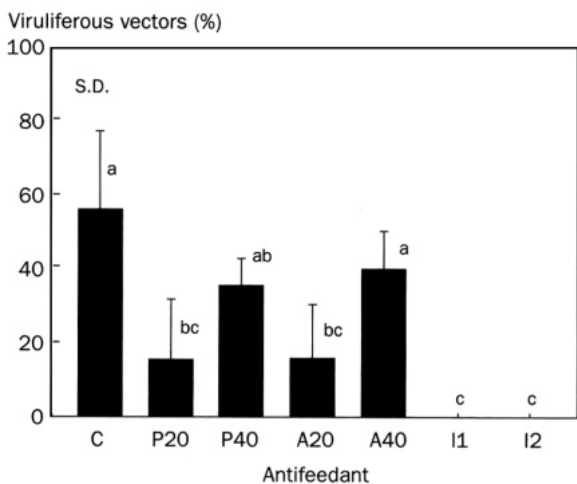


Fig. 1. Influence of antifeedants on acquisition of rice tungro viruses by green leafhoppers C = control; P20 = pymetrozin 20 ppm; P40 = pymetrozin 40 ppm; A20 = andrographolide 20 ppm; A40 = andrographolide 40 ppm; I1 = imidacloprid 0.01 ppm; I2 = imidacloprid 0.02 ppm. Bars followed by a common letter are not significantly different at the 95% level Fisher-PLSD test (Widiarta et al in press).

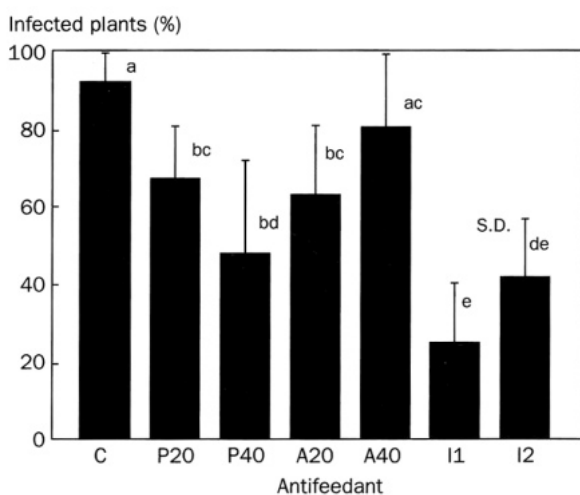


Fig. 2. Influence of antifeedants on the inoculation of rice tungro disease by infective green leafhopper. C = control; P20 = pymetrozin 20 ppm; P40 = pymetrozin 40 ppm; A20 = andrographolide 20 ppm; A40 = andrographolide 40 ppm; I1 = imidacloprid 0.01 ppm; I2 = imidacloprid 0.02 ppm. Bars followed by a common letter are not significantly different at the 95% level by Fisher-PLSD test (Widiarta et al in press).

more, increasing the concentration of pymetrozin to 40 ppm reduced virus transmission, but increasing that of andrographolide did not. Application of imidacloprid at concentrations of 0.01 and 0.02 ppm significantly reduced virus transmission. It was clear that imidacloprid greatly reduced virus transmission by *N. virescens*, while andrographolide and pymetrozin did so only at lower concentrations.

Control of *N. virescens* population

A field experiment was conducted in the 1997 dry-season crop in asynchronously planted fields in Subak Padanggalak, Bali, and in the wet-season crop for synchronously planted fields in Subak Samsam, Bali (Widiarta et al unpublished). Insecticides were applied fortnightly, starting 1 wk after GLH infestations occurred. The insecticides tested were diacloden, imidacloprid, and MIPC. The experiment was conducted using a randomized block design with four replicates of each treatment. The plot size of each replicate was 5 × 8 m. The susceptible rice variety Pelita was transplanted at 25 × 25-cm spacing, 21 d after sowing. The population density of green leafhoppers was observed weekly by counting 39 hills plot⁻¹. RTD incidence was observed at 8 wk after transplanting and before harvest.

In the asynchronous plantings, applications of diacloden and MIPC significantly reduced the population density of GLH (Fig. 3). RTD incidence before harvest, however, was not reduced significantly (Fig. 4). In the synchronous planting, applications

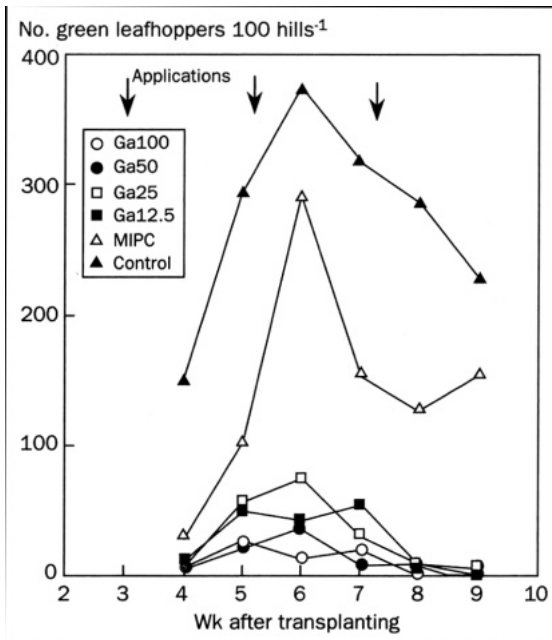


Fig. 3. Population development of green leafhopper in variously treated plots in asynchronously planted fields. MIPC = active ingredient of Mipcin 50 WP, Ga = diacloden at 12.5, 25, 50, and 100 ppm.

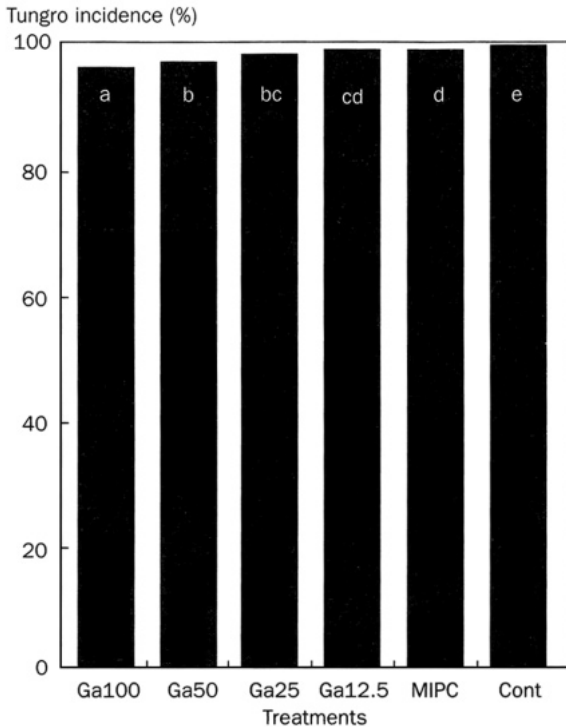


Fig. 4. Tungro incidence before harvest in variously treated plots in asynchronously planted fields. Varietal bars with the same letters are not significantly different at the 5% level by Duncan's multiple range test. Ga = diaclofen at 12.5, 25, 50, and 100 ppm, MIPC = active ingredient of insecticide Mipcin 50 WP, Cont = control.

of diaclofen, imidacloprid, and MIPC significantly reduced not only GLH density (Fig. 5) but also RTD incidence (Fig. 6). Thus, reduced vector density decreased RTD incidence, especially in synchronously planted crops.

Conclusions

Low doses of insecticides such as imidacloprid, nitenpyram, and pymetrozin as well as andrographolide showed antifeedant activity against *N. virescens* and also inhibited transmission of rice tungro viruses. Thus, control of feeding has the potential to check tungro without disturbing the food chain. Population control of GLH by insecticides showed the limitation of this approach in reducing RTD spread in asynchronous plantings.

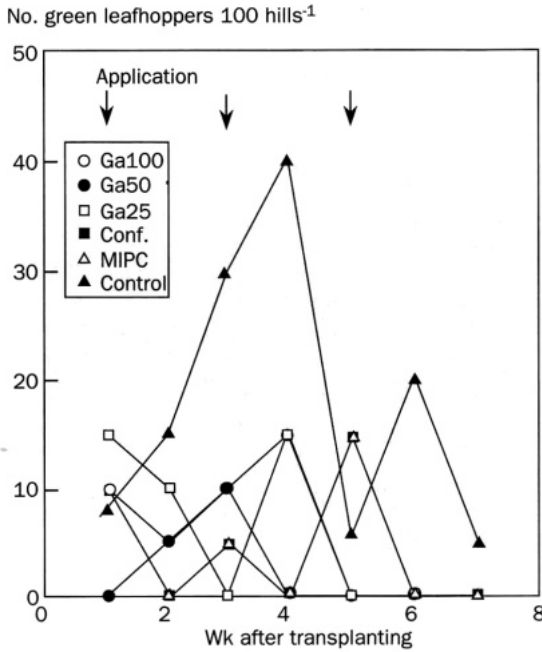


Fig. 5. Population development of green leafhopper in variously treated plots in synchronously planted fields. MIPC = active ingredient of Mipcin 50 WP, Ga = diaclofen at 25, 50, and 100 ppm, Conf = imidacloprid (Confidor 25 WP).

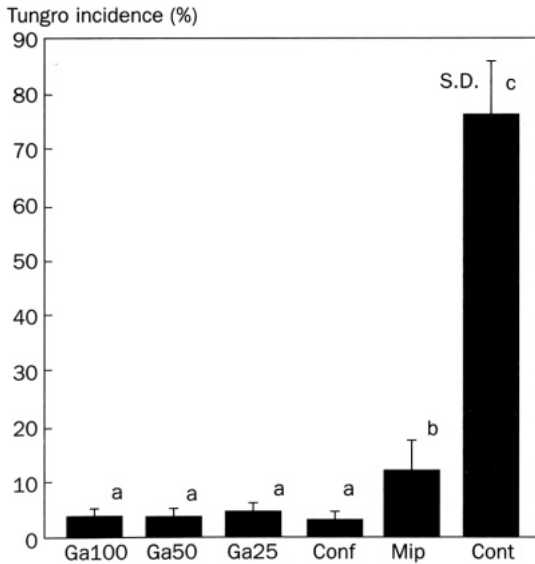


Fig. 6. Tungro incidence before harvest in variously treated plots in synchronously planted fields. Bars with the same letters are not significantly different at the 5% level by Duncan's multiple range test. Ga = diaclofen at 25, 50, and 100 ppm, Conf = imidacloprid, Mip = Mipcin, Cont = control.

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GLH control for the management of rice tungro disease

T. Ganapathy, N. Subramanian, and M. Surendran

Green leafhopper (GLH) is one of the major sucking pests of rice and is capable of transmitting rice tungro disease (RTD), a devastating virus disease of rice. GLH is considered to be more important as a virus vector than as a direct pest of rice. In a seedbed protection trial, application of neem cake in the seedbed followed by spraying of 5% neem seed kernel extract (NSKE) at 30 d after transplanting reduced the incidence of tungro disease by more than 50% and increased rice grain yield. GLH abundance and the presence of a higher percentage of transmitters resulted in high RTD incidence. A significant positive linear relationship was observed between RTD incidence and both log transmitters and percent transmitter population during an RTD epidemic in the northern districts of Tamil Nadu, India, during 1991-93.

Rice tungro disease (RTD) is caused by two morphologically, chemically, and serologically unrelated viruses—rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). The rice green leafhopper (GLH), *Nephotettix virescens* (Distant), is the most efficient vector and can transmit both viruses either together or separately (Hihino et al 1979). RTBV, however, is acquired by GLH only after prior acquisition of RTSV.

GLH does not usually cause any significant feeding damage to the rice crop unless its numbers are extremely high. GLHs that carry tungro viruses, however, may cause extensive damage and crop loss (Anjaneyulu et al 1994).

Where an overlapping rice cropping pattern occurs, GLH prefer young seedlings and migrate from older crops to seedbeds which can then become infected (Mukhopadhyay et al 1984). Tungro spreads very fast during the early growth stage of the crop (Shukla and Anjaneyulu 1981). Hence, it is essential that rice seedlings be adequately protected from tungro infection. This can be achieved by applying insecticides in seedbeds to kill immigrant vectors to prevent virus transmission. A seedbed protection trial was therefore conducted to study the effect of nursery protection on the incidence of GLH and RTD.

The onset of the disease depends on the presence of a susceptible host, a virus source, and the vector. Anjaneyulu (1975) observed that adult GLH plays an active role in introducing primary inoculum to the field, whereas both nymphs and adults help in further secondary spread. The availability of virus inoculum, a high population of GLH, and early growth stage of the crop are responsible for a disease outbreak (Chen and Othman 1991). High vector populations (Vidhyasekaran and Lewin 1986) and a large proportion of viruliferous vectors (Savary et al 1993) also play an important role in disease outbreaks. A direct correlation between vector population and disease incidence has been observed at different locations (Lim 1972, Hibino 1986, Shukla and Anjaneyulu 1981). The presence of viruliferous GLH is one of the most important factors that cause RTD incidence.

Materials and methods

Seedbed protection

Two field trials were conducted to study the effect of seedbed protection with pesticides on the incidence of RTD and yield. A susceptible variety, IR64, was used in the trials, which relied on naturally occurring sources of virus inoculum. Seedlings were raised in a nursery and transplanted into 8×8 -m plots at 24 d after sowing (DAS). There were five treatments. Each treatment was replicated three times in a randomized complete block design: T_1 = carbofuran at 6.25 g m^{-2} at 20 DAS, T_2 = carbofuran in seedbed at 20 DAS and monocrotophos (0.2%) at 25 d after transplanting (DAT), T_3 = neem cake at $1.5.5 \text{ g m}^{-2}$ at 20 DAS, T_4 = neem cake in seedbed and 5% neem seed kernel extract (NSKE) at 25 DAT, T_5 = control.

Numbers of GLH adults were estimated at 14, 28, and 56 DAT using 10 sweeps of a 30-cm-diameter insect net in each plot. Nymphal numbers were counted on 10 hills in each plot. Rice plants were scored visually for tungro incidence on the same dates. After harvest, grain yield was recorded.

Vector indexing

A rice tungro epidemic occurred during 1991-93 in the northern districts of Tamil Nadu. Using data collected at the Rice Research Station (RRS) in Tirur, the relationship between tungro incidence, monthly numbers of GLH collected in a light trap, and the proportion of virus transmitters was examined. The percentage of transmitters in the GLH population was calculated by vector indexing. This was done as follows:

Individual GLHs were placed in a test tube containing 7–10-day-old TN1 seedlings for a 24-h inoculation feeding period. Seedlings were subsequently planted in pots and grown in insect-proof cages. One week after inoculation, the number of seedlings showing typical symptoms of RTD was counted. The percentage of seedlings infected was considered to be equal to the percentage of viruliferous GLH. The following formula was derived:

$$\text{Log transmitters (Lt)} = \log_{10} (\text{Tg} \times \text{Pvv})$$

where Tg = total monthly light trap GLH collection and Pvv = proportion of viruliferous vectors (% vv/10) where % vv = (no. of transmitters/no. of GLH tested) \times 100

The relationship of RTD incidence with log transmitters and percent transmitters was examined using regression analysis.

Results and discussion

Seedbed protection

Protection of the seedbed with insecticides and biopesticides reduced tungro incidence compared with the control (Table 1). Adult GLH numbers at 14 and 28 DAT

Table 1. Green leafhopper (GLH) population, percent rice tungro disease (RTD), and grain yield in seedbed protection trial at Vishar during July to October 1997.

Treatments ^a	GLH count and RTD Incidence									Yield (tha ⁻¹)
	14 DAT			28 DAT			56 DAT			
	GLH ^b		RTD (%)	GLH		RTD (%)	GLH		RTD (%)	
	Adult	Nymph		Adult	Nymph		Adult	Nymph		
T ₁	0.7	0.2	0.0	1.0	1.0	18.1	1.3	0.8	25.5	2.6
T ₂	1.7	0.3	0.0	0.7	1.3	20.4	0.0	0.6	24.4	2.7
T ₃	0.7	0.3	0.0	3.7	1.4	20.0	1.7	1.0	24.5	2.1
T ₄	1.3	0.3	0.0	2.7	0.3	23.5	2.0	0.3	23.3	3.0
T ₅	4.7	0.6	0.0	7.0	2.3	22.3	3.3	1.0	40.8	1.6

^aT₁ = carbofuran at 6.25 g m⁻² at 20 DAS, T₂ = carbofuran at 6.25 g m⁻² at 20 DAS + monocrotophos (0.2%) at 25 DAT, T₃ = neem cake at 15.5 g m⁻² at 20 DAS, T₄ = neem cake at 15.5 g m⁻² at 20 DAS + 5% neem seed kernel extract at 25 DAT, T₅ = control. ^badult = mean no. 10 sweeps⁻¹, nymphs = mean no. 10 hills⁻¹, DAT = days after transplanting.

were lowest in plots where carbofuran and neem cake were applied in the seedbed. but nymphal numbers were similar in all treatments. The highest grain yield was recorded in T₄, where neem cake and NSKE were applied.

These results are consistent with previous findings. Protection of rice seedlings grown in soil incorporated with 150 and 250 kg neem cake ha⁻¹ was effective against rice tungro (Saxena 1987). Also, NSKE and neem cake powder mixed with carbofuran at 1.0 kg ai ha⁻¹ reduced tungro incidence similarly as applying a higher rate of carbofuran alone (Abdul Kareem et al 1988).

Epidemiology

At RRS, Tirur, viruliferous GLH were first observed in August 1991 and initial disease symptoms appeared in September when 16% of rice plants were affected. The disease was prevalent up to December 1991, with a peak incidence of 35% during October, which corresponded to the highest percentage of transmitters. Even though the GLH population increased from January to March 1992, no RTD incidence occurred because of the absence of transmitters. Again, the GLH population increased in May 1992 and, because of the presence of transmitters, symptoms reappeared during June 1992. Numbers of viruliferous GLH and numbers of GLH per hill peaked in August and November 1992. Because of the presence of a high percentage of transmitters. RTD incidence reached a maximum of 96% during August 1992. RTD incidence and the percentage of transmitters started declining at the end of 1992. No further disease was recorded in February 1993.

An analysis of the relationship between log transmitter population and RTD incidence using linear and exponential models revealed that the linear regression model provided the best fit to the data ($r = 0.831$, error mean square = 400.4) (Table 2). The relationship between the percentage of transmitters (percent viruliferous GLH) and RTD incidence was also studied. A highly significant positive relationship between

Table 2. Relationship of RTD incidence with log and percent transmitters at Tirur.

Transmitters	Parameters			EMS ^a
	r	a	b	
<i>Log</i>				
Linear model ^b	0.831	-73.13	44.48	400.4
Exponential model	0.774	-52.71	102.21	483.2
<i>Percent</i>				
Linear model	0.978	2.87	2.99	264.5
Exponential model	0.865	-39.78	33.21	381.8

^aEMS = error mean square. ^bLinear model: $y = a + bx$, exponential model: $y = ae^{bx}$.

the percentage of transmitters and percent RTD incidence was observed in the linear model ($r = 0.978$) (Table 2). Suzuki et al (1992) demonstrated a relationship between the percentage of infective GLH and the percentage of RTD-infected hills in rice fields at 5–7 wk after transplanting. They developed an infective vector index and found it useful in predicting cumulative infection at early stages of crop growth in Indonesia.

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Management of rice tungro disease by chemical control of the green leafhopper vector

E.H. Batay-An and S.C. Mancao

The field efficacy of five synthetic pyrethroid insecticides was evaluated for the control of green leafhoppers (GLH) and rice tungro disease (RTD) and for their effect on natural enemy populations and yield of IR64. In each of two seasons, pyrethroid-treated plots significantly reduced GLH populations and had significantly lower RTD incidence. Among the five treatments, plots treated with cypermethrin and ethofenprox had the lowest GLH population and RTD incidence at all sampling dates. Yields were higher in the treated plots than in the control. Populations of spiders, *Cyrtorhinus lividipennis*, *Conocephalus longipennis*, *Agriocnemis pygmaea*, and coccinellids were significantly affected by insecticide applications 1 d after treatment at 5, 20, and 35 d after transplanting (DAT). Cypermethrin, however, did not affect *C. lividipennis* populations at 20 DAT. Likewise, none of the insecticides reduced *A. pygmaea* populations at 35 DAT in the 1989 wet season or populations of any of the natural enemies at 35 DAT in the 1990 dry season. Sprays of cypermethrin and ethofenprox did not significantly affect the population of spiders, *C. lividipennis*, and *A. pygmaea* at 20 DAT in the 1990 dry season.

Mindanao is one of the largest islands located at the southernmost part of the Philippines. Large areas of rice are grown on the island, which has a relatively even distribution of rainfall throughout the year and is free from typhoons. One of the major rice production constraints in Mindanao is the occurrence of insect pests and diseases. These are brought about by favorable climatic conditions coupled with asynchronous planting, continuous cropping of rice, and planting of susceptible cultivars. Thus, the year-round availability of rice and the warm humid climate are conducive to insect proliferation and survival.

Among the pests in Mindanao, rice tungro disease, which is transmitted by the green leafhopper, *Nephotettix virescens*, is the most destructive disease of rice. Outbreaks of RTD in the Philippines occurred in 1957, 1963, 1969, 1971, 1975, and 1977 (Bergonia 1978). In Central Luzon, IR36 and IR42, both moderately resistant to green leafhopper (GLH), were highly infected with RTD in 1984. Likewise, in Mindanao, high RTD incidences were observed in South and North Cotabato in 1985 and 1986 and again in 1993-98 (Truong et al, "Rice tungro disease in the Philippines," this volume).

At present, the best way to prevent RTD is by using vector-resistant varieties because there is still no variety resistant to the tungro virus. Continuous planting of these vector-resistant cultivars, however, may cause them to succumb to RTD because of the development of virulent GLH populations. Therefore, one possibility for reducing RTD incidence in the field is to control the insect vector with insecticides. Rice yield increases have been attributed to insecticide use under various conditions in nearly all rice-growing countries (Lim and Heong 1984). Greater use of insecti-

cides by farmers is expected because other control strategies such as using resistant varieties and intensifying the role of biocontrol agents take time and are limited in scope. Under laboratory conditions, some commonly used insecticides such as those in the organophosphate and carbamate groups applied as foliar sprays caused 90–100% GLH mortality 24 h after treatment. They cannot prevent infection with tungro viruses (IRRI 1984, 1985), however, because of their slow effect—GLH can transmit virus particles before they die. Thus, rapid-acting insecticides are needed. Synthetic pyrethroids offer some advantages because they are fast-acting and effective at low dosages; thus, residue levels are likely to be low on crops (Ozaki et al 1984). The Midsayap branch of PhilRice conducted this study to evaluate the field efficacy of five synthetic pyrethroid insecticides against GLH and RTD incidence. The objectives were to determine the best insecticide for GLH control, to assess the impact of different insecticides on natural enemies, and to determine the impact on the yield of IR64.

Materials and methods

The experiment was conducted at PhilRice-Midsayap, Bual Norte, Midsayap, Cotabato, during the 1989 wet season (WS) and 1990 dry season (DS). It was laid out in a randomized complete block design consisting of six treatments with a plot size of 5 × 5 m replicated four times.

The treatments were T_1 = plots sprayed with cypermethrin, T_2 = plots sprayed with monocrotophos + cypermethrin, T_3 = plots sprayed with deltamethrin, T_4 = plots sprayed with lambda-cyhalothrin, T_5 = plots sprayed with ethofenprox, and T_6 = untreated control.

Pregerminated seeds of susceptible IR64 were uniformly sown on the seedbed raised under the *dapog* method and immediately covered with nylon mesh after sowing for protection against birds and early insect pest infestation. Ten-day-old *dapog* seedlings were transplanted at 3 to 5 seedlings hill⁻¹ spaced at 20 × 20 cm. Starting 5 d after transplanting (DAT), three insecticide applications by knapsack sprayer were made at 15-d intervals following the manufacturer's recommended dosage with a spray volume of 300 L ha⁻¹.

Fertilizer was applied at a rate of 60-0-0 kg NPK ha⁻¹. One-half of the nitrogen was applied at planting whereas the remaining half was applied 5 to 7 d before panicle initiation. Preemergence herbicide (butachlor) was applied at 3 DAT for weed control.

GLH and natural enemy populations were estimated 1 d before and 1 d after every spray application by 10 sweeps of an insect net per plot. RTD incidence was recorded at 60 DAT by counting the number of infected hills per plot. Rice yield was taken from a 10-m² harvest area per plot, threshed, and dried at 14% moisture content. Data were analyzed statistically using analysis of variance, and Duncan's multiple range test was used for comparison among treatments.

Results and discussion

GLH populations were significantly lower in all plots treated with cypermethrin, monocrotophos + cypermethrin, deltamethrin, lambdacyhalothrin, and ethofenprox than in the untreated control plots at all sampling dates in the 1989 WS (Table 1).

Similarly, RTD incidence was significantly lower in the treated plots than in the untreated control plot (Table 1). There was no significant difference between plots sprayed with cypermethrin, ethofenprox, and lambdacyhalothrin. This result is consistent with findings reported from India (Satapathy and Anjaneyulu 1984, Krishnaiah and Ghosh 1990, Anjaneyulu and Bhaktavatsalam 1986) and from the Philippines (Macatula et al 1987).

The populations of spiders, *Cyrtorhinus lividipennis*, *Conocephalus longipennis*, *Agriocnemis pygmaea*, and coccinellids were considerably reduced by pyrethroid insecticide applications at 5 and 20 DAT in the 1989 WS (Tables 2-6). Cypermethrin and lambdacyhalothrin applications, however, had no effect on *C. longipennis* numbers at 20 DAT (Table 4). Likewise, none of the synthetic pyrethroids tested significantly affected the population of *A. pygmaea* at 20 and 35 DAT (Table 5).

Synthetic pyrethroid insecticides applied at 20 and 35 DAT significantly reduced GLH numbers 1 d after spray application compared with the untreated control in the 1990 DS (Table 1). At 5 DAT, only cypermethrin and ethofenprox significantly reduced GLH numbers.

Table 1. Green leafhopper population and % rice tungro disease (RTD) incidence as affected by foliar sprays of synthetic pyrethroid insecticides on IR64, PhilRice-Midsayap, 1989 wet season and 1990 dry season.

Insecticide ^a	GLH (no. 10 sweeps ⁻¹) ^b						% RTD incidence at 60 DAT
	Application at 5 DAT		Application at 20 DAT		Application at 35 DAT		
	1 DBT	1 DPT	1 DBT	1 DPT	1 DBT	1 DPT	
<i>1989 wet season</i>							
Cypermethrin	12.0 a	1.3 a	11.0 a	3.7 a	10.3 a	1.7 a	18.0 a
Monocrotophos + cypermethrin	16.3 a	4.7 a	11.7 a	6.7 a	14.0 ab	6.7 bc	39.3 b
Deltamethrin	15.0 a	4.7 a	15.3 a	10.3 a	17.0 bc	9.3 c	42.0 b
Lambdacyhalothrin	9.7 a	4.0 a	17.3 a	6.3 a	20.7 c	5.6 abc	29.3 ab
Ethofenprox	8.0 a	3.0 a	13.7 a	3.7 a	8.7 a	2.3 ab	22.0 a
Control	21.7 a	37.7 b	36.0 b	34.0 b	40.7 d	32.0 d	72.0 c
<i>1990 dry season</i>							
Cypermethrin	4.3 a	1.7 b	3.7 c	1.0 c	3.7 ab	1.0 c	
Monocrotophos + cypermethrin	5.0 a	6.0 ab	2.0 c	1.0 c	2.7 b	1.3 c	
Deltamethrin	4.7 a	9.3 a	4.3 bc	1.7 c	4.7 ab	2.3 bc	
Lambdacyhalothrin	6.7 a	4.7 ab	5.0 bc	2.0 c	3.7 ab	2.7 bc	
Ethofenprox	4.3 a	1.7 b	5.3 bc	1.0 c	1.7 b	1.3 c	
Control	5.3 a	11.0 a	11.0 a	11.0 a	9.3 a	7.3 a	

^aInsecticides were applied by knapsack sprayer 3 times at 15-d intervals. Spray volume was 300 L ha⁻¹. ^bAverage of 3 replications. In a column, means followed by the same letter are not significantly different at 5% level Duncan's multiple range test (DMRT). DAT = days after transplanting. DBT = days before treatment, DPT = days posttreatment.

Table 2. Field evaluation of foliar sprays of synthetic pyrethroids against spider populations on IR64, PhilRice-Midsayap, 1989 wet season and 1990 dry season.

Insecticide ^a	Spiders (no. 10 sweeps ⁻¹) ^b					
	Application at 5 DAT		Application at 20 DAT		Application at 35 DAT	
	1 DBT	1 DPT	1 DBT	1 DPT	1 DBT	1 DPT
<i>1989 wet season</i>						
Cypermethrin	12.0 a	3.7 b	17.7 b	7.3 a	12.3 c	4.0 ab
Monocrotophos + cypermethrin	11.3 a	1.7 ab	18.7 b	8.3 a	9.3 bc	7.0 b
Deltamethrin	5.0 a	0.0 a	15.7 bc	6.0 a	3.7 a	1.7 a
Lambdacyhalothrin	5.7 a	0.0 a	11.0 c	1.7 a	6.3 ab	2.3 a
Ethofenprox	11.7 a	9.3 c	26.0 a	11.0 a	14.7 c	7.0 b
Control	21.7 a	14.0 d	25.7 a	30.3 b	20.3 d	16.3 c
<i>1990 dry season</i>						
Cypermethrin	4.7 ab	2.7 b	8.3 ab	4.7 bc	8.0 a	2.0 a
Monocrotophos + cypermethrin	5.7 ab	2.0 b	4.0 b	2.7 c	4.3 a	2.0 a
Deltamethrin	2.0 b	2.0 b	5.0 b	1.7 c	5.7 a	2.0 a
Lambdacyhalothrin	3.3 ab	3.0 b	5.3 b	2.0 c	4.0 a	1.7 a
Ethofenprox	7.3 a	3.0 b	7.3 ab	6.7 ab	5.3 a	3.3 a
Control	7.7 a	9.0 a	13.0 a	11.3 a	10.3 a	3.3 a

^aInsecticides were applied by knapsack sprayer 3 times at 15-d intervals. Spray volume was 300 L ha⁻¹. ^bAverage of 3 replications. In a column, means followed by the same letter are not significantly different at 5% level Duncan's multiple range test (DMRT), DAT = days after transplanting, DBT = days before treatment, DPT = days posttreatment.

Table 3. Field evaluation of foliar sprays of synthetic pyrethroids against *Cyrtorhinus lividipennis* populations on IR64, PhilRice-Midsayap, 1989 wet season and 1990 dry season.

Insecticide ^a	<i>Cyrtorhinus lividipennis</i> (no. 10 sweeps ⁻¹) ^b					
	Application at 5 DAT		Application at 20 DAT		Application at 35 DAT	
	1 DBT	1 DPT	1 DBT	1 DPT	1 DBT	1 DPT
<i>1989 wet season</i>						
Cypermethrin	16.3 ab	9.7 b	12.0 ab	11.3 b	16.3 cd	9.3 b
Monocrotophos + cypermethrin	19.0 abc	7.3 ab	8.7 a	8.3 b	8.3 ab	4.3 ab
Deltamethrin	10.7 a	2.0 a	11.0 ab	3.7 a	4.3 a	1.7 a
Lambdacyhalothrin	12.0 a	2.3 a	7.0 a	3.0 a	7.0 a	6.0 ab
Ethofenprox	10.0 a	5.3 b	4.7 ab	3.0 bc	2.3 a	0.3 a
Control	9.0 a	11.7 a	5.7 a	5.7 ab	2.0 a	1.7 a
<i>1990 dry season</i>						
Cypermethrin	5.3 a	2.3 bc	4.0 ab	2.0 bc	2.3 a	0.0 a
Monocrotophos + cypermethrin	7.3 a	0.7 bc	2.3 ab	0.0 c	1.7 a	0.0 a
Deltamethrin	6.7 a	1.7 bc	1.0 b	0.0 c	1.7 a	0.0 a
Lambdacyhalothrin	6.0 a	0.3 c	3.0 ab	1.0 c	0.3 a	0.3 a
Ethofenprox	10.0 a	5.3 b	4.7 ab	3.0 bc	2.3 a	0.3 a
Control	9.0 a	11.7 a	5.7 a	5.7 ab	2.0 a	1.7 a

^aInsecticides were applied by knapsack sprayer 3 times at 15-d intervals. Spray volume was 300 L ha⁻¹. ^bAverage of 3 replications. In a column, means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test (DMRT). DAT = days after transplanting, DBT = days before treatment, DPT = days posttreatment.

Table 4. Field evaluation of foliar sprays of synthetic pyrethroids against *Conocephalus longipennis* populations on IR64, PhilRice-Midsayap, 1989 wet season and 1990 dry season.

Insecticide ^a	<i>Conocephalus longipennis</i> (no. 10 sweeps ⁻¹) ^b			
	Application at 20 DAT		Application at 35 DAT	
	1 DBT	1 DPT	1 DBT	1 DPT
<i>1989 wet season</i>				
Cypermethrin	5.0 ab	5.0 ab	4.7 ab	1.0 a
Monocrotophos + cypermethrin	3.3 a	1.3 a	3.0 a	0.7 a
Deltamethrin	4.7 a	3.7 a	7.0 abc	2.0 a
Lambdacyhalothrin	2.7 a	2.7 a	6.3 abc	1.3 a
Ethofenprox	3.3 a	3.0 a	7.3 bc	3.7 a
Control	9.3 b	11.0 b	10.0 c	10.3 b
<i>1990 dry season</i>				
Cypermethrin	0.3 b	0.0 b	2.7 a	1.3 a
Monocrotophos + cypermethrin	0.0 b	0.0 a	0.7 a	0.7 a
Deltamethrin	1.3 a	0.3 a	1.0 a	0.0 a
Lambdacyhalothrin	0.7 b	0.0 b	0.7 a	0.0 a
Ethofenprox	0.0 a	0.0 a	3.3 a	2.3 a
Control	0.3 a	1.0 a	1.7 a	1.3 a

^aInsecticides were applied by knapsack sprayer 3 times at 15-d intervals. Spray volume was 300 L ha⁻¹. ^bAverage of 3 replications. In a column, means followed by the same letter are not significantly different at 5% level Duncan's multiple range test (DMRT). DAT = days after transplanting, DBT = days before treatment, DPT = days post treatment.

Table 5. Field evaluation of foliar sprays of synthetic pyrethroids against *Agricoenemis pygmaea* populations on IR64, PhilRice-Midsayap, 1989 wet season and 1990 dry season.

Insecticide ^a	<i>Agricoenemis pygmaea</i> (no. 10 sweeps ⁻¹) ^b			
	Application at 20 DAT		Application at 35 DAT	
	1 DBT	1 DPT	1 DBT	1 DPT
<i>1989 wet season</i>				
Cypermethrin	4.0 a	2.3 a	4.0 a	2.3 a
Monocrotophos + cypermethrin	4.3 a	2.7 a	3.0 a	2.3 a
Deltamethrin	4.7 a	2.3 a	3.3 a	0.7 a
Lambdacyhalothrin	3.0 a	1.0 a	2.0 a	1.7 a
Ethofenprox	4.0 a	3.3 a	2.3 a	2.0 a
Control	7.3 a	7.7 b	5.0 a	5.3 a
<i>1990 dry season</i>				
Cypermethrin	8.0 a	5.7 bc	4.7 a	1.7 a
Monocrotophos + cypermethrin	9.0 a	4.3 c	7.0 a	0.7 a
Deltamethrin	9.7 a	4.3 c	4.0 a	0.7 a
Lambdacyhalothrin	9.0 a	5.1 c	5.3 a	0.3 a
Ethofenprox	9.0 a	6.3 bc	5.0 a	0.3 a
Control	8.7 a	9.0 ab	4.0 a	1.7 a

^aInsecticides were applied by knapsack sprayer 3 times at 15-d intervals. Spray volume was 300 L ha⁻¹. ^bAverage of 3 replications. In a column, means followed by the same letter are not significantly different at 5% level Duncan's multiple range test (DMRT). DAT = days after transplanting, DBT = days before treatment, DPT = days posttreatment.

Table 6. Field evaluation of foliar sprays of synthetic pyrethroids against coccinellid beetle populations on IR64, PhilRice-Midsayap, 1989 wet season and 1990 dry season.

Insecticide ^a	Coccinellid beetle (no. 10 sweeps ⁻¹) ^b			
	Application at 20 DAT		Application at 35 DAT	
	1 DBT	1 DPT	1 DBT	1 DPT
<i>1989 wet season</i>				
Cypermethrin	3.0 a	0.7 a	4.7 a	2.3 a
Monocrotophos + cypermethrin	4.0 a	0.0 a	6.7 a	0.0 a
Deltamethrin	2.0 a	0.0 a	10.0 a	1.0 a
Lambdacyhalothrin	5.7 a	0.0 a	9.7 a	1.0 a
Ethofenprox	3.3 a	2.0 a	5.0 a	2.7 a
Control	5.7 a	6.3 b	12.3 a	8.7 b
<i>1990 dry season</i>				
Cypermethrin	0.3 b	0.0 b	2.3 a	0.0 a
Monocrotophos + cypermethrin	0.0 b	1.7 ab	1.7 a	0.3 a
Deltamethrin	0.0 b	0.0 b	2.3 a	0.0 a
Lambdacyhalothrin	0.7 b	0.0 b	0.7 a	0.0 a
Ethofenprox	2.7 a	2.7 a	1.3 a	0.3 a
Control	0.7 b	2.7 a	4.0 a	0.7 a

^aInsecticides were applied by knapsack sprayer 3 times at 15d Intervals. Spray volume was 300 L ha⁻¹. ^bAverage of 3 replications. In a column, means followed by the same letter are not significantly different at 5% level Duncan's multiple range test (DMRT). DAT = days after transplanting, DBT = days before treatment, DPT = days posttreatment.

Table 7. RTD incidence (%) and yield (t ha⁻¹) of IR64 as affected by foliar sprays of insecticide, PhilRice-Midsayap, 1990 dry season.

Insecticide ^a	RTD incidence (%) ^b		Yield ^c (t ha ⁻¹)
	60 DAT	1 DPT	
Cypermethrin	12.1 b	3.6 a	
Monocrotophos + cypermethrin	19.0 b	2.9 a	
Deltamethrin	23.0 b	2.7 a	
Lambdacyhalothrin	19.1 b	3.2 a	
Ethofenprox	16.8 b	3.3 a	
Control	74.4 a	1.9 b	

^aInsecticides were applied by knapsack sprayer 3 times at 15-d Intervals. Spray volume was 300 L ha⁻¹. ^bAverage of 3 replications. In a column, means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test (DMRT). ^c2 × 5-m yield sample. DAT = days after transplanting, DPT = days posttreatment.

Percent RTD infection obtained visually at 60 DAT was significantly lower in treated plots than in the untreated control in the 1990 DS. There were no significant differences among the five synthetic pyrethroids tested (Table 7).

Yields obtained from plots sprayed with cypermethrin, ethofenprox, lambdacyhalothrin, monocrotophos + cypermethrin, and deltamethrin ranged from

3.6 to 2.7 t ha⁻¹. These yields were significantly higher than the 1.9 t ha⁻¹ yield of the untreated control. This result supports the findings of Macatula et al (1987).

The application of five synthetic pyrethroid insecticides on IR64 by foliar spray at 5 and 20 DAT considerably affected the populations of spiders. *C. lividipennis*, *A. pygmaea*, and coccinellids in the 1990 DS (Tables 2-6). Ethofenprox, however, did not reduce the numbers of spiders and coccinellids, and *A. pygmaea* and *C. lividipennis* adults were not affected by most pyrethroid applications at 20 DAT.

Moreover, none of the five pyrethroid insecticides tested significantly affected natural enemy populations at 35 DAT (Tables 2-6).

Conclusions

Five synthetic pyrethroid insecticides were evaluated for their effect on GLH numbers, RTD incidence, and natural enemy populations at PhilRice-Midsayap. The effect of insecticide application on the yield of IR64 was also assessed.

The evaluation results are summarized as follows:

1. Foliar sprays of cypermethrin, monocrotophos + cypermethrin, deltamethrin, lambdacyhalothrin, and ethofenprox significantly reduced GLH populations and RTD incidence in both seasons.
2. Among the five pyrethroids, cypermethrin and ethofenprox were the most effective in lowering GLH numbers and percent RTD incidence.
3. All plots treated with synthetic pyrethroids yielded significantly higher than the untreated control in the 1990 dry season.
4. In the 1989 wet season, foliar sprays of five pyrethroids significantly affected the populations of spiders, *C. lividipennis*, *C. longipennis*, *A. pygmaea*, and coccinellids at 5 and 20 DAT, except for cypermethrin on *C. longipennis* at 20 DAT. None of the pyrethroids affected the *A. pygmaea* population at 35 DAT.
5. In the 1990 dry season, foliar sprays of cypermethrin and ethofenprox did not affect the populations of spiders, *C. lividipennis*, and *A. pygmaea* at 20 DAT. Moreover, none of the pyrethroids significantly reduced natural enemy numbers at 35 DAT.

The five synthetic pyrethroid insecticides tested were all effective against GLH. Synthetic pyrethroid insecticide application should be started within 5 DAT and repeated at least two times at 15-d intervals to reduce GLH populations and RTD incidence.

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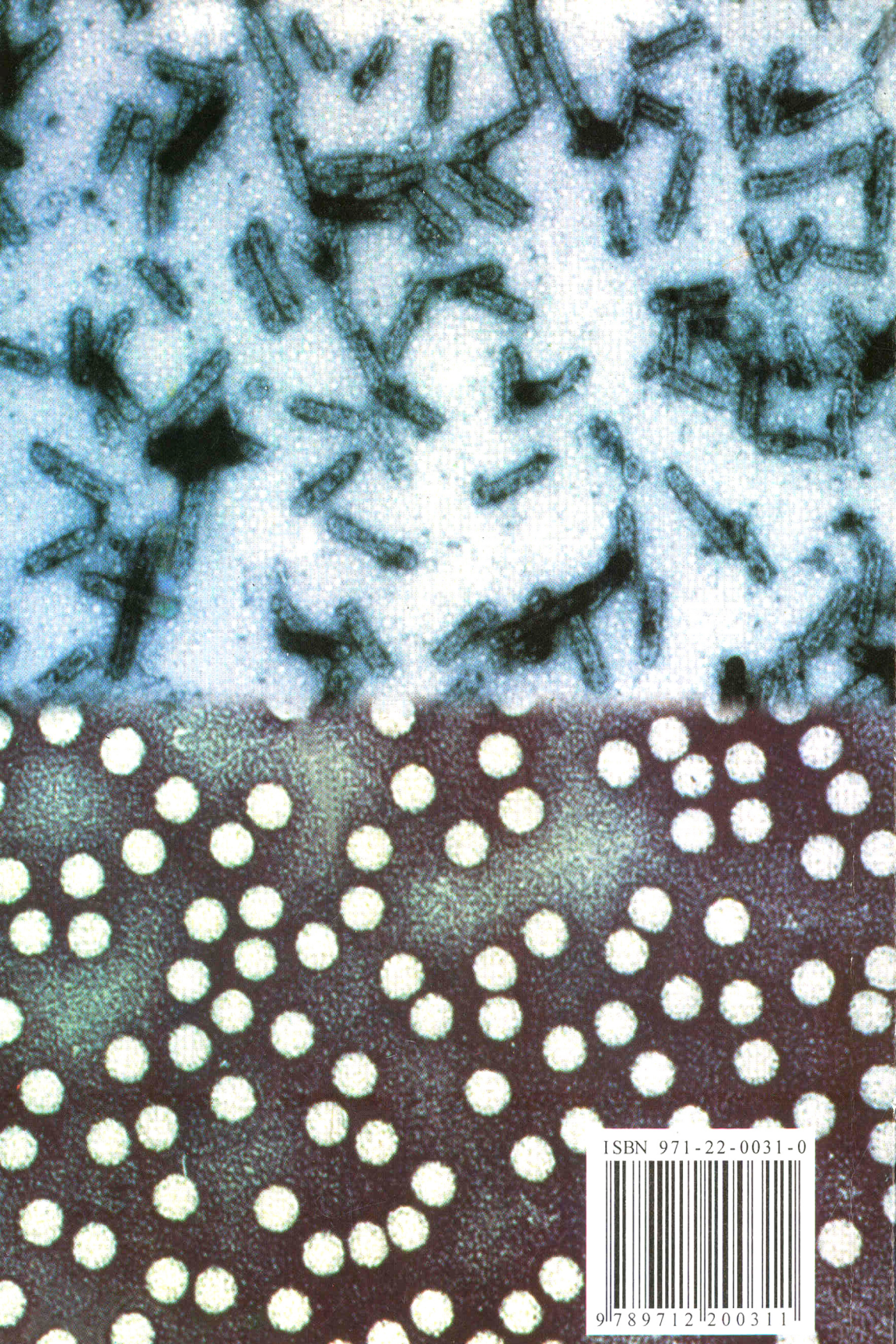
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