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Final report

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Contents

1 Acknowledgments

The assistance of Dorothy Noble with identification of bacteria by fatty acid methyl ester analysis is gratefully acknowledged.

Dr Russell Reinke and Ms Margrit Martin kindly analysed some quality parameters of rice for this project.

2 Executive summary

This project was very successful in accomplishing four of the five intended objectives.

A functioning plant pathology laboratory was established at the Cambodian Agricultural Research and Development Institute (CARDI), equipped with all necessary equipment, consumables and a good library of reference texts.

Training workshops were held in Australia and Cambodia and were followed up with continuing on-the-job training, in Cambodia, in plant pathology survey skills, diagnosis and preliminary identification of pathogens, using rice as a model crop.

A Plant Pathology Herbarium was established at CARDI to provide a permanent record of verified disease samples which not only provide future training material but which also serve to meet Cambodia's WTO obligations under the International Plant Protection Convention whereby countries are required to know the health status of their crops intended for export.

Disease surveys were conducted in the wet and dry seasons in 2006 and 2007, targeting rice but also including other important crops. The incidence of rice blast was found to be low, but bacterial diseases were observed to be causing large yield losses in many crops. Four bacterial pathogens of rice were recorded for the first time in Cambodia. A previously undescribed bacterial pathogen of rice was observed and studied, and a description of a new species of *Pseudomonas* will be published from this work.

The project surveyed for the rice blast fungus in northern Australia over two seasons but it was not found, despite earlier reports of its occurrence. Three bacterial pathogens, similar to those found in Cambodia, were recorded on *Oryza australiensis* in the Northern Territory. The bacterial studies in Cambodia provided a basis for identifying a previously unrecorded bacterial pathogen (*Pseudomonas fuscovaginae*) of rice in the Riverina region of southern New South Wales.

Crop loss assessment studies on rice crops in Takeo Province showed that bacterial diseases caused significant yield and quality losses. Stem disease caused yield losses of up to 50% and high level of disease in florets could reduce grain weight per panicle by >90%. Disease also increased the amount of chalkiness in grain and an increase in grain breakage during milling.

The objective to produce disease management strategies was not accomplished due to the extra time devoted to the unexpected level of bacterial diseases encountered during surveys, and the loss of pathology personnel at CARDI.

3 Background

In Cambodia, rice occupies 90% of the total agricultural area. With an average consumption of about 160 kg per person per year, rice is by far the most important staple food of Cambodia. Rice is also the major agricultural item in terms of farmer income. The per-hectare rice yield in Cambodia is among the lowest in Asia. The average yield for the wet season crop is about 0.95 tonnes of unmilled rice per hectare. The dry-season crop yield is traditionally higher (1.8 t/ha). There is no doubt that, in Cambodia, diseases of rice contribute to the low productivity by reducing yield and grain quality, especially during the wet season.

There are more than 50 important diseases of rice crops in Asia and other tropical and temperate rice growing areas that lower yields and increase production costs. Very little information is known on distribution, prevalence and importance of rice diseases under Cambodian conditions, as there is virtually no plant pathology expertise in Cambodia. Cambodian researchers working at the Cambodian Agricultural and Development Research Institute (CARDI) were, therefore, interested in capacity building in plant pathology.

A visit to CARDI in August 2004 emphasised the complete absence of any plant pathology facilities at CARDI and the almost complete lack of knowledge in the country of plant pathology principles.

The primary goal of the project was to train Cambodian researchers to create and develop local knowledge and expertise in general plant pathology and, in particular, rice pathology. The project will help the development of Cambodia's long term agricultural research capacity.

The Australian rice industry has enjoyed over 80 years of freedom from serious diseases. Few of the debilitating diseases of rice crops found elsewhere in the world have been recorded in the region. However, there are more than 50 important diseases of rice crops in the region that lower yields and increase production costs.

The reciprocal benefits to the Australian rice industry would come from a greater understanding of exotic diseases of rice that are not yet present in Australia and development of strategies that will assist their avoidance. The project, therefore, addressed two of ACIAR's National Research Priorities: an environmentally sustainable Australia and safeguarding Australia from diseases and pests.

4 Objectives

The primary objectives of the project were to:

Objective 1:

Upgrade expertise and CARDI facilities for plant pathology to increase diagnostic capacity and capacity in plant pathology research and development with an emphasis on rice.

- Develop and implement an effective training program in Australia and Cambodia to address Cambodian researcher needs.
- Upgrade the CARDI plant pathology laboratory to support disease identification and plant pathology research and development.
- Support on-the-job training in plant pathology through involvement in various research programs.

Objective 2:

Collect, identify and curate herbarium specimens and isolates of pathogens of rice and other principal crops present in Cambodia.

- Establish a culture collection at CARDI.
- The Australian plant pathologists will travel to Cambodia to assist the local team to survey disease incidence and to collect, identify and lodge specimens into CARDI's new culture collection and herbarium.

Objective 3:

Examine the pathogenicity of rice blast isolates already present in northern and eastern Australia to current rice cultivars.

- Collect isolates of *P. grisea* from northern and eastern Australia.
- Glasshouse and laboratory work will be undertaken to determine the genetic structure and the pathogenicity of the Australian isolates of *P. grisea* collected during the survey. Although there are no quarantine restrictions that would prevent testing the pathogen in Wagga, the pathogenicity testing of the *P. grisea* isolates and DNA extraction will be done at NSW DPI, Orange. DNA characterisation will be done at Charles Sturt University, Wagga Wagga.

Objective 4:

Evaluate the distribution, prevalence, severity and priority for future work of rice diseases occurring in Cambodia.

- Aggregate all data generated by the disease surveys and prepare maps showing the distribution and prevalence of rice diseases in Cambodia (objective 2).
- Identify the most damaging diseases of rice occurring under Cambodian conditions

Objective 5:

Develop, test and, if appropriate, promote some best-bet Integrated Disease Managements strategies for several major diseases.

- Develop and test integrated disease management strategies such as crop rotation, using disease-free clean rice seeds, crop residue removal and variety mixing.
- Produce extension material to help Cambodian extension staff convince Cambodian rice farmers to implement the successful IDM strategies.

5 Methodology

Objective 1. Upgrade expertise and CARDI facilities for plant pathology to increase diagnostic capacity and capacity in plant pathology research and development, with an emphasis on rice

Develop and implement an effective training program in Australia and Cambodia to address Cambodian researcher needs.

Drs Ny Vuthy and Preap Visarto visited Charles Sturt University, Wagga Wagga in November 2005 to participate in 2½ weeks of training in plant pathology principles and methods. The course was structured to meet known and anticipated needs in Cambodia. The course was delivered by Drs Cother, Lanoiselet and Ash, based on material developed by Dr Lanoiselet and teaching aids from Dr Ash.

The workshop covered informal lectures and videos, field visits to onion, tomato and rice crops in the Riverina, and laboratory practices.

A visit was made to the Agricultural Scientific Collection Unit at Orange to look at the plant pathology herbarium and for demonstrations in plant bacteriology techniques. A new plant pathology laboratory at Wagga Agricultural Institute was visited to discuss the principles of lab design and work flow.

Two days were devoted to:

- Planning the workflow in the CARDI plant pathology laboratory, placement of equipment and infrastructure (shelves, cupboards and additional power points) that would need to be acquired for practical operation of the lab. These were overlaid on photographs of the lab taken during our visit in August 2004.
- Planning and logistics for the survey to be undertaken in February 2006. Content and structure of the training workshop to be held at CARDI during the February visit to Cambodia, and an action plan for both parties was prepared. The target audience was deemed to include personnel from CARDI (10-12), DALLI (2), provinces (10), NGOs and AQIP (2) private consultants and farmers (2-4). Scope of instruction and method of delivery was discussed.

A workshop for 29 district and CARDI personnel was held in February 2006, centred on the principles of disease recognition and diagnosis, and how to distinguish disease from other damage.

Upgrade the CARDI plant pathology laboratory to support disease identification and plant pathology research and development

The project provided funds to equip a laboratory at CARDI with all the necessary equipment and consumables to run a functioning microbiology laboratory.

Objective 2. Collect, identify and curate herbarium specimens and isolates of pathogens of rice and other principal crops present in Cambodia.

Although rice was the ‘model’ crop for teaching and surveying, the opportunity was taken to look at diseases in other crops as the principles taught are applicable to all crop species.

Surveys were conducted on six occasions in the wet and dry seasons.

Isolations were made on-the-road at night or in the lab at CARDI. Where identification could not be made during each visit, cultures were carried to Australia under AQIS permits and detailed studies were performed at Orange, NSW on living cultures or by molecular techniques on DNA at Wagga Wagga. Cultures of verified isolates are being stored in the living culture collections of Herb. DAR, OAI, Orange.

Objective 3. Examine the pathogenicity of rice blast isolates already in northern and eastern Australia to current rice cultivars.

Surveys were conducted in 2006 in the flood plains of the Adelaide and Alligator Rivers at the end of the wet season using airboats to access flooded remote regions. In 2007, similar areas were surveyed together with the Mareeba wetlands in northern Qld and various *Oryza* populations in the Kununurra region of NT.

In May 2007, the flood plain below Fogg Dam near Darwin was accessed by airboat and six widely scattered stands of *Oryza* spp. were sampled. Collections included smutted grain, leaf, sheath and panicle lesions. On 21 May, the Mary River was accessed at Shady Camp. Inspection by airboat of the river upstream of the weir did not detect any diseased *Oryza* stands. *Oryza* spp. were sampled at Beatrice Hill Research Station where disease in secondary tillers arising from nodes was widespread and prolific. Drs Cother and Ash also drove from Kununurra north into NT to Legune Station sampling several *Oryza* stands which showed a variety of disease symptoms. The following day we inspected *Oryza* stands between Kununurra and Wyndham and at Parry's Lagoon.

In Darwin, wild rice with flag leaf lesions typical of those caused by *M. grisea* was sampled. All lesions of interest were photographed and 157 lesions were surface sterilised and plated out to determine the association of fungi and bacteria with the disease symptoms examined. In addition, leaf lesions were also incubated in a moist environment to stimulate sporulation, or suspended above water agar to detect spore discharge from necrotic tissue.

Dr Vincent Lanoiselet and Dr Ben Stodart travelled to Cairns and surrounding areas to conduct surveys of native rice stands for presence of disease. The Mareeba Wetlands Reserve approx. 15 km north of the Mareeba township consists of approx. 5000 acres of lagoons and savannah grassland and scrub. Staff at the wetlands had mapped eight stands of native rice (*Oryza australiensis)* in 2005, and these were used for the current disease survey. Leaf lesions were present at each site with the following descriptions:

Diamond shaped lesion, 4-5 mm at widest point and 5 mm length, light centre with darkened edge, spread along length of leaf.

Narrow brown lesions, approx 1mm width, >5 mm length scattered along leaf.

In addition, sheath discolouration occurring just below the head was observed on several plants in each stand. Isolations were made from each of the symptoms at QDPI. An additional host was sampled, *Leersia hexandra* (swamp rice grass) which displayed diamond-shaped leaf symptoms similar to those described above. Several other swamp and lagoon sites around Mareeba were examined, but native rice was absent. A second survey was conducted in the surrounds of Mt Surprise, approx. 280 km south west of Cairns. Several stands of *O. australiensis* were located in depressions along roadsides. No presence of disease could be observed, leaves and sheathes were clear of lesions. Seed samples were taken to be used as a source in glasshouse experiments and to provide herbarium specimens and confirm species identification.

Objective 4. Evaluate the distribution, prevalence, severity and priority for future work of rice diseases occurring in Cambodia.

Aggregate all data generated by the disease surveys and prepare maps showing the distribution and prevalence of rice diseases in Cambodia

Data would be based on all surveys conducted. This was contingent on being able to quickly identify all diseases seen.

Identify the most damaging diseases of rice occurring under Cambodian conditions

Data would be based on all surveys conducted.

6 Achievements against activities and outputs/milestones

Objective 1: To upgrade expertise and CARDI facilities for plant pathology to increase diagnostic capacity and capacity in plant pathology research.

no.	activity	outputs / milestones	completion date	comments
1.1	Training in Australia (A)	Drs Vuthy and Visarto appraised of plant pathology principles	Nov 2005	
1.2	Workshop in Cambodia on disease principles(PC)	29 personnel briefed in plant disease recognition	February 2006	
1.3	Upgrade the CARDI plant pathology laboratory (PC)	A functioning laboratory established	Feb 2006-Feb 2008	

PC = partner country, A = Australia

The Wagga workshop was very successful. Drs Vuthy and Visarto were enthusiastic participants who were keen to be involved in hands-on laboratory techniques.

A specimen submission form was developed for future use at CARDI to be translated into Khmer (Appendix).

Drs Vuthy and Visarto were given a lap top computer, copies of presentations in PowerPoint, many plant pathology reference books and numerous forceps, probes, needles and scissors for the CARDI lab.

The workshop covered practical sessions on disease symptoms and laboratory training in media preparation and subculturing.

Drs Visarto and Vuthy showed considerable keenness to master techniques.

Objective 1.2 Conduct a workshop at CARDI on principles of disease recognition

A two day workshop was held at CARDI in February 2006, attended by 30 personnel from CARDI, Provincial Officers, leading farmers and an agricultural company. The workshop was opened by the Director, Dr Men Sarom, who emphasised the greater difficulty in diagnosing plant diseases compared with insect pests. The workshop explained the basis of plant disease, causal organisms and distinguishing signs. Talks were presented in English and translated into Khmer by either Drs Vuthy or Visarto. Questions were encouraged and as much time was allocated to this as to the actual talks.


Objective 1.3: Upgrade the CARDI plant pathology laboratory to support disease identification and plant pathology research and development

CARDI now has the best equipped plant pathology laboratory in the Kingdom.

- A empty room has been furnished and equipped with a laminar airflow cabinet (plus a second cabinet provided on ADB funds), a compound and a dissecting microscope, incubators, balances, an autoclave and all the small equipment and consumables necessary for a microbiology laboratory.
- A considerable number of general and specific plant pathology texts have been purchased for the laboratory.
- The air-conditioning has been upgraded to improve laboratory comfort.
- The laboratory is now fully functional.


The plant pathology laboratory had no facilities at the start of the project.

The plant pathology laboratory is now well equipped and suitable for research and diagnostics and one-on-one training.

Objective 2: Collect, identify and curate herbarium specimens and isolates of pathogens of rice and other principal crops present in Cambodia.

no.	activity	outputs/ milestones	completion date	comments
2.1	Conduct disease surveys (PC)	Six surveys were conducted	November 2007	
2.2	Identify main diseases of rice (A, PC)	New diseases identified	ongoing	
2.3	Establish a plant disease herbarium and curate specimens (PC)	17 specimens lodged in CARDI Plant Pathology Herbarium	ongoing	

PC = partner country, A = Australia

Objective 2.1 Conduct disease surveys

Disease surveys were conducted as follows:

Province	Dates	Targeted crops
Prey Veng	Feb 2006, Aug 2006, Feb 2007, Nov 2007	rice
Svay Rieng	Feb 2006, Aug 2006	rice
Kampong Cham	Feb 2006, Nov 2007	banana, cabbages, peanut, cowpea, mungbean
Kampong Chhnang	Feb 2007	rice
Siem Reap	Feb 2006	mung bean, maize, watermelon
Battambang	Feb 2006	citrus
Takeo	Feb 2006, Aug 2006, Feb 2007, Aug 2007, Nov 2007	rice
Kampot	Feb 2006	Durian, soybean, snake bean, cucumber, tomato
Kandal	Aug 2006, Nov 2007	Rice, cowpea, mungbean
Mondulkiri	Nov 2006	rice
Ratanakiri	Nov 2006	rice
Stung Treng	Nov 2006	rice
Kratie	Nov 2006	rice

Diseased specimens were plated out on to suitable microbiological media at night during survey trips and specimens were pressed for later deposit in the herbarium.

Objective 2.2 Identify main diseases of rice

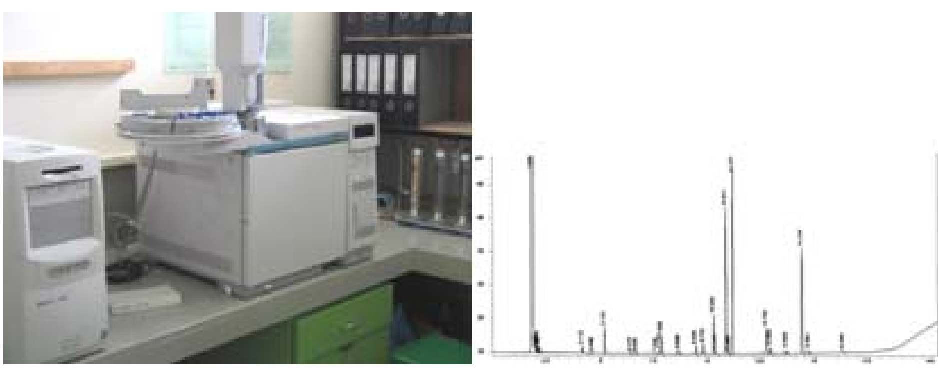
Contrary to expectations, blast was only observed on two occasions. The most common fungal diseases that were confirmed by isolation of the pathogen were:

- narrow brown leaf spot (caused by *Cercospora oryzae*)
- brown leaf spot (caused by *Helminthosporium oryzae*).

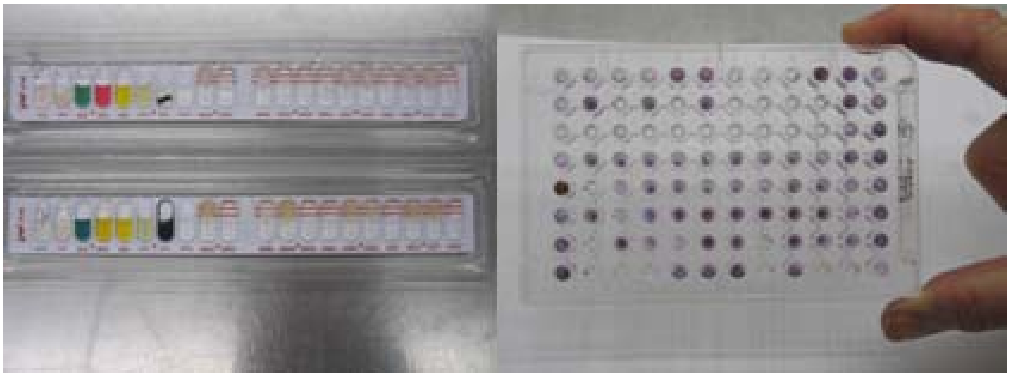
Other diseases observed and confirmed, where appropriate, by isolations were:

- sheath rot (caused by *Sarocladium* sp.)
- Bakanae (caused by *Gibberella fujikuor*i)
- sheath blight (caused by *Rhizoctonia oyzae*)
- false smut (caused by *Ustilaginoidea virens*)
- sheath blight (caused by *Rhizoctonia solani*)
- aggregate sheath spot (caused by *R. oryzae-sativae*).

The pathogens associated with bacterial diseases were identified by cell wall fatty acid methyl ester analysis, and supported where necessary by biochemical methods including Biolog and API kits for metabolic profiles.



Gas chromatography of bacterial cell wall fatty acids produces characteristic profiles that aid the identification of bacteria



API (left) and Biolog (right) kits were used to provide nutritional profiles of suspected pathogens

The diseases observed that were causing the most damage to rice were caused by bacteria. Bacterial blight of rice (caused by *Xanthomonas oryzae* pv. *oryzae*) is the most common bacterial disease in other tropical countries but it was observed on only one occasion in a single variety during our surveys. The bacterial diseases observed were, in most instances, causing considerable damage to stems and panicles and resulted in serious yield loss in many crops. The pathogens were confirmed by isolation and completion of Koch's Postulates. There are no generally accepted common names for the diseases caused by these pathogens:

- *Pantoea ananatis*
- *Burkholderia cepacia*
- *Burkholderia gladioli/cocovenenans* – this species complex contains lethal toxin-producing strains that cause high fatality in food poisoning events. Characterisation of isolates from rice has not yet been undertaken
- *Acidovorax avenae* subsp. *avenae*
- *Pseudomonas (fuscovaginae ?)* – this pathogen has been commonly found causing, or associated with, panicle disease and up to 100% sterility of florets. The identification is questioned because of the heterogeneity of isolates studied and 24 isolates are presently the subject of extensive biochemical, physiological and molecular analyses. **It is likely we are dealing with a previously undescribed pathogen. Work is in progress to publish a description of this pathogen**
- Experiments have been conducted on pathogenicity and the transmission of the pathogens in seed.

Suspected fungal and bacterial pathogens were tested for pathogenicity to rice at CARDI to test Koch's Postulates. Only pathogenic isolates were chosen for identification to the species level.

Objective 2.3 Establish a plant disease Herbarium

An important outcome of this project was the establishment at CARDI of the first Plant Pathology Herbarium in Cambodia. Specimens deposited in the herbarium represent a verified record of disease occurrence, which is essential for quarantine purposes, WTO negotiations and national obligations to various agreements under the International Plant Protection Convention.

Dr Vuthy with the first deposited voucher specimen of a plant disease lodged in the new CARDI Plant Pathology Herbarium.

Objective 3. Examine the pathogenicity of rice blast isolates already present in northern and eastern Australia to current rice cultivars.

no.	activity	outputs/ milestones	completion date	comments
3.1	Examine the pathogenicity of rice blast isolates already in northern and eastern Australia to current rice cultivars. (A)	No blast disease detected but several bacterial diseases were identified together with a new smut species.	May 2007	

PC = partner country, A = Australia

Objective 3.1. Survey for blast disease in northern Australia

Diseased plants were observed in all locations. Although many symptoms observed were similar to those caused by the blast fungus, no conidia of *Magnaporthe grisea* were observed, nor was the fungus isolated, from any of the samples over 2 years. Species of *Helminthosporium* and *Curvularia* were detected frequently.

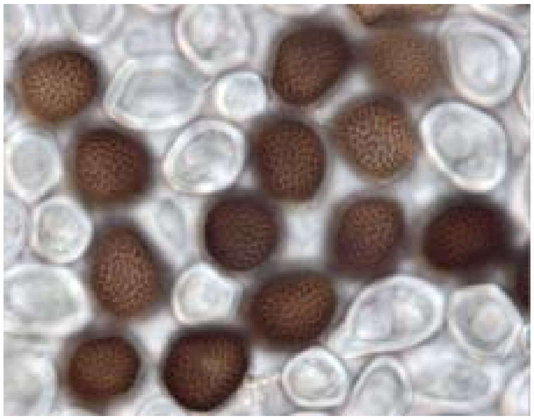
From the 16 sites sampled, 23 fungal and 19 bacterial cultures of interest were examined to confirm presumptive diagnoses.

We have discovered a new disease of *Oryza* grain caused by a previously undescribed species of the smut fungus *Tilletia*.

Tilletia australiensis R.G.Shivas, Cother, Ash, Ray & Vánky in K Vánky & RG Shivas, Fungi of Australia: The Smut Fungi. ABRS, Canberra. CSIRO Publishing, Melbourne (2008).

T: Fogg Dam, N.T., on *Oryza australiensis*, 20 May 2007, J.D.Ray, E.J.Cother & G.J.Ash; holo: BRIP 49645; iso: HUV 21452.

Sori destroying the ovaries, concealed by the coriaceous lemma and palea. Spore globose, subglobose, ovoid, ellipsoidal, or angular and polyhedral, 18–29 × 17–24 ?m, dark yellowish brown to dark chocolate brown; wall with 1.0-1.5 ?m high blunt warts which, in surface view appear as darker irregular areas, 10–16 per spore diam. Sterile cells globose to irregular, sometimes with a flattened side, 12–30 ?m long, hyaline, guttulate; wall smooth, uneven, 1–4 ?m thick.



Pathogenicity tests on a limited number of rice plants, cv. Amaroo, resulted in some seedling death, and panicle emergence 2 weeks earlier than the non-inoculated control plants. Although no smut formed on these plants, it is clear that plant physiology was disrupted.

Disease lesions on leaves of wild rice were collected and treated by standard plant pathology methods to isolate causal organisms on agar for later identification. Pathogenicity tests on cultivars of domestic rice (*O. sativa*) were conducted at Charles Sturt University, Wagga Wagga, NSW.

- Although many samples displayed leaf lesions very similar to those reported to be caused by the blast fungus, *Magnaporthe grisea*, no conidia resembling this pathogen were observed on field material and the fungus has not been isolated from any lesion type.
- Three fungal genera, *Bipolaris, Curvularia* and *Nigrospora* have been isolated from leaf lesions and identification is in progress together with pathogenicity testing.

Four bacterial pathogens of rice, *Pantoea ananatis*, *Acidovorax avenae*, *Burkholderia cenocepacia* (possibly *B. gladioli*) and *B. cepacia*, were found consistently associated with diseased flag, sheath and node lesions. These pathogens have been shown from our work in Cambodia to cause disease in domestic rice cultivars.

Wild *Oryza* species were accessed and surveyed by airboat.

Although a range of disease symptoms were observed on leaves, conidia of *Magnaporthe grisea* were never observed, nor was the fungus isolated.

Fungi isolated from *Oryza* spp. in the Northern Territory were pathogenic to domestic cvs of *Oryza sativa*.

Objective 4. Evaluate the distribution, prevalence, severity and priority for future work of rice diseases occurring in Cambodia

Aggregate all data generated by the disease surveys and prepare maps showing the distribution and prevalence of rice diseases in Cambodia

no.	activity	outputs/ milestones	completion date	comments
4.1	Prepare disease distribution maps (PC)	Disease incidence maps available	On-going	Distribution maps were considered to be unnecessary as diseases (except for blast) were widespread in all Provinces surveyed.
4.2	Identify the most important rice diseases (A, PC)	Important diseases identified	June 2008	Contrary to expectations, blast disease was neither common nor severe. Several severe bacterial diseases were identified.

PC = partner country, A = Australia

There appears to be no particular spatial pattern to rice diseases in Cambodia. Although intensity varied between Provinces and season, all diseases were regularly observed. The only exceptions were rice blast which was only observed twice and bacterial blight (caused by *Xanthomonas oryzae* subsp. *oryzae*) which was only observed on one occasion.

Map of localities surveyed during the project

Intensive collections were made at the sites indicated. Many smaller collections of diseased plants were made en route between these locations.

Objective 4.2. Identify the most damaging diseases of rice occurring under Cambodian conditions

Bacterial diseases were by far the most common diseases observed and they caused considerable damage in many of the crops surveyed.

Table .Summary of bacterial isolations from diseased rice

Locality	Symptoms	Pathogen isolated
August 2006		
Kori Vung, Takeo	Glume discolouration	*Pantoea ananatis*
		Acidovorax avenae subsp. *avenae*
	Collar lesion	*P. ananatis*
		Burkholderia cepacia
Chaka Chang village, Prac sdach district, Prey Veng		*B. gladioli*
November 2006		
Seima Biodiversity Conservation Area, Mondulkiri Province	Discoloured florets	*Pseudomonas* (*fuscovaginae* (?))*
		P. ananatis
		B. gladioli
Andoung Kraleng village, Sen Monorom Commune, O-Rang district	Collar lesions	*Ps*. (*fuscovaginae* (?))
	Leaf sheath lesions	*B. gladioli*
		A. avenae subsp. *avenae*
Bu Klare, Mondulkiri	Discoloured florets	*Ps*. (*fuscovaginae* (?))
		B. gladioli
Bu Tang, near Sen Monorom	Lesioned distal portion of florets	*Ps*. (*fuscovaginae* (?))
		B. gladioli
Sam Khou village, Sam Khou commune, Stung Treng Province	Glume lesions	*Ps*. (*fuscovaginae* (?))
Memai village, Prak Bat commune, Stung Treng Province	Leaf sheath/collar lesions	*Ps*. (*fuscovaginae* (?))
		B. cenocepacia
Day Low village, Lum Phat district, Ratanakiri Province	Discoloured florets	*A. avenae* subsp. *avenae*
		B. gladioli
Patang Commune, Lum Phat district	Collar lesions and discoloured florets	*P. ananatis*
CARDI	Discoloured florets	*Ps*. (*fuscovaginae* (?))
	Leaf sheath lesions	*Ps*. (*fuscovaginae* (?))
		A. avenae subsp. *avenae*
February 2007		

Baty Commune, Peareang district, Prey Veng Province	Flag leaf sheath lesions	*A. avenae* subsp. *avenae*
		P. ananatis
August 2007		
Smouk Commune, Takeo Province	Lesioned glumes	*A. avenae* subsp. *avenae*
	Stem lesion	*P. ananatis*
		Ps. (*fuscovaginae* (?))
		A. avenae subsp. *avenae*
Prekompok Commune, Takeo Province	Lesioned glumes	*A. avenae* subsp. *avenae*
		P. ananatis
		Ps. (*fuscovaginae* (?))
Kirichong Kaa Commune, Takeo Province	Lesioned glumes	*Ps*. (*fuscovaginae* (?))
Chikhma commune Takeo Province	Stem lesion	*B. cenocepacia*
		A. avenae subsp. *avenae*
CARDI	Stem lesions	*B. cenocepacia*
	Panicle lesions	*P. ananatis*
November 2007		
Prau Put Commune, Kandalsteung District, Kandal Province. cv. Mak Saroi	Discoloured glumes	*B. gladioli*
	Flag leaf sheath lesion	*B. gladioli*
Prau Put Commune, Kandalsteung District, Kandal Province. cv. Pkar Rumduol	Margin on bleached glumes	*A. avenae* subsp. *avenae*
	Speckled glumes	*P. ananatis*
	flag leaf sheath lesion	*B. cenocepacia*
	Necrotic glumes, just emerged from boot	*P. ananatis*
	mottled stem lesions, no pan emerge	*B. gladioli*
	Dark blotch lesions on glumes	*P. ananatis*
		A. avenae subsp. *avenae*
Vihea Loung commune, Tbong Khmum district, Khampong Cham Prov	Discoloured glumes	*P. ananatis*

*Some of the isolates originally thought to be *P. fuscovaginae* are in the process of being described as a new species of *Pseudomonas,* tentatively named *P. nyii.*

Fungal diseases were widespread but were generally of low incidence.

Table . Summary of fungal pathogens isolated from rice

Locality	Symptoms	Pathogen isolated
August 2006		
Kori Vung, Takeo	Narrow leaf spot	*Bipolaris oryzae*
	Sheath lesions	*Rhizoctonia oryzae*
Svey Rieng province	Brown leaf spot	*B. oryzae*
	Narrow leaf spot	*B. oryzae*
	Sheath blight	*R. oryzae*
Orey Chhor district, Kampong Cham		*R. oryzae-sativae*
November 2006		
Seima Biodiversity Conservation Area, Mondulkiri Province	Crown and root lesions	*Gaeumonnomyces graminis* var *graminis*
Andoung Kraleng village, Sen Monorom Commune, O-Rang district, Mondolkiri	Collar necrosis and sporulating sheath lesion	*Sarocladium oryzae*
	Narrow brown spot	*B. oryzae*
	False smut	*Ustilaginoidae virens*
Bu Klare village, Sen Monorom district, Mondulkiri	Brown leaf spot	*B.oryzae*
Sam Khou village, Sam Khou commune, Stung Treng Province	Dark upper sheath lesion	*S. oryzae*
Memai village, Prak Bat commune, Stung Treng Province	Blast like lesions, patchy distribution	*Pyricularia grisea*
Bey village, Trapaang Krahom commune, Konmom district, Stung Treng Province	Dark upper sheath lesions	*S. oryzae*
	Narrow elongated lesions on leaf	*Phaeoseptoria oryzae*
O Cham commune, O Cham district, Ratanak Kiri Province	Leaf lesions, blast like less diamond shaped	*B. oryzae*
CARDI	Leaf lesions, similar to brown spot, on farmer variety	*Nigrospora oryzae*
	Pot trial – upper sheath lesions	*S. oryzae*
February 2007		
Kirichongkok commune, Kirivong district, Takeo province	Brown leaf spot, some blast like, wide spread	*B. oryzae*
		N. oryzae
Baty Commune, Peareang district, Prey Veng Province	Brown leaf spot, light dispersal	*B. oryzae*
		N. oryzae

Kompong leave commune, Kompong leave district, Prey Veng Province	Light coloured brown leaf spot	*N. oryzae*
Cheung Turk commune, Cheung Turk district, Prey Veng Province	Leaf lesions similar to narrow brown leaf spot	*N. oryzae*
		Curvularia lunata
Tonle Bet commune, Thong Khmom district, Prey Veng Province	Isolated lower sheath lesion	*R. oryzae*
August 2007		
Prekompok Commune, Kirivong district, Takeo Province	Leaf and collar lesions, small, circular and darkened	*Curvularia sp.*
Kirichong Kaa Commune, Kirivong district, Takeo Province	Small circular stem lesion, just below collar	*Curvularia sp.*
	Discoloured seed	*S. oryzae*
Un-recorded location	Lower stem lesion	*R. solani*
November 2007		
CARDI	False smut	*U. virens*
Svey Antor village, Svey Antor commune, Prey Veng	Lower stem lesion, bleached with dark margin	*R. solani*
	False smut	*U. virens*
Pochrey village, Svay Antor commune, Prey Veng Province	Cluster of darkened sheath lesions below collar	*N. oryzae*
	False smut	*U. virens*
Khnal Tmey village, Vihea Louing commune, Tbong Khmom district, Kampong Cham Province	Brown leaf spot across field	*B. oryzae*
		N. oryzae
	Discoloured seed	*Alternaria* sp.
	Darkened sheath lesions	*S. oryzae*

A crop loss assessment study was carried out on diseased rice plants. In August 2007, we sampled 12 sites at 4 localities, between Takeo and the Vietnamese border, from crops infected by one or more of the bacterial pathogens listed in 6.2.2. This revealed significant yield and quality losses due to disease.

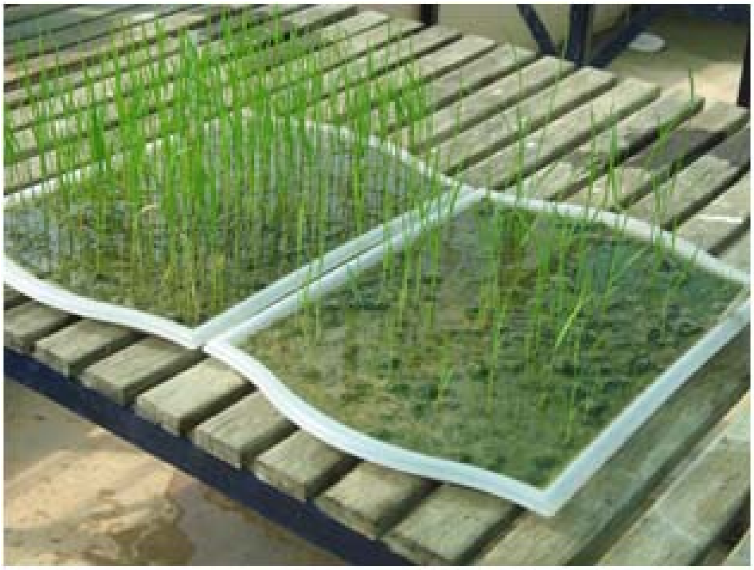
Diseased rice plants at maturity were harvested within a 0.2m^2 quadrat ring. Twelve samples were taken at random at one location and 6 from each of the other 11 sites. Where possible, the cultivar was recorded. Three university students were employed for 10 days to assist with sorting each sample at CARDI. Stems were rated as ‘diseased’ or ‘healthy’ and then each panicle was examined. Sterile florets were segregated as diseased or healthy, then grain was segregated as healthy or with low, medium or high level of disease on the glumes. Each category was counted and the grain in each class was weighed. In all, over 4000 stems and panicles were assessed yielding 77 430 sterile florets, and 115 400 grains which were categorised as shown below.

Milled grain was analysed at Yanco Agricultural Institute using a Cervitec 1625 Grain Inspector. Parameters measured were % broken grain, % chalkiness and grain size.

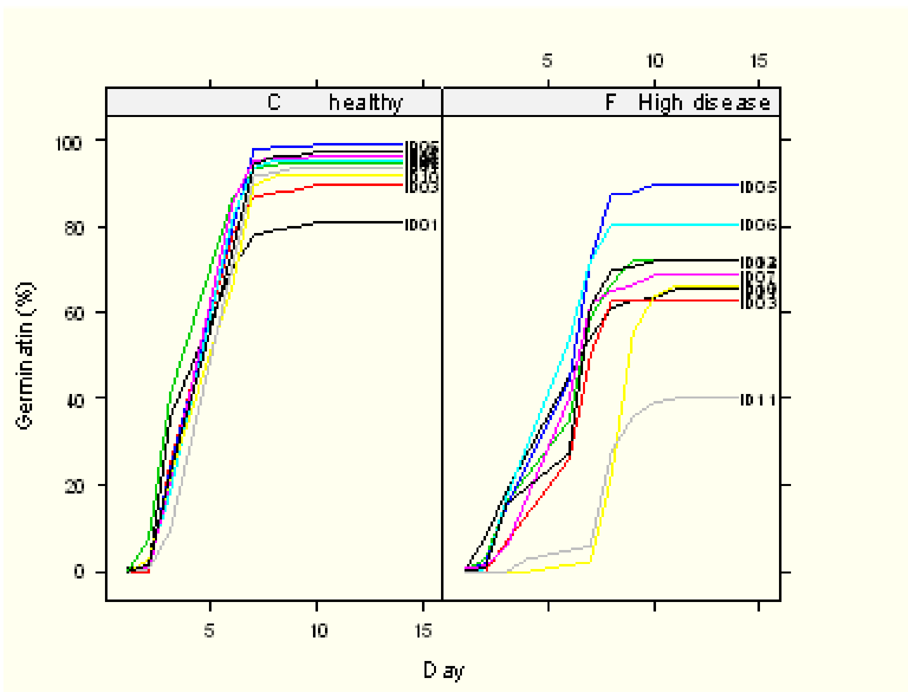
Categories of disease status of florets and grain separated for the crop loss assessment study

Small samples of seed of class C (healthy) and class F (high disease), ranging from 150 to 400 seeds from nine of the August 2007 sites, were sown on 16 October 2007and assessed at regular intervals for emergence. Heights were measured on two rows selected at random from each sample category after 23 days and dry weights were determined on samples cut at soil level and dried overnight at 55ºC.

Healthy seed (left) and high disease seed (right) graded in the crop loss assessment experiment were sown to assess germination and vigour.



Seedling germination and vigour from seed classed as healthy (left) compared with that from seed with high disease levels (right)



Effect of disease on germination vigour of nine samples of seed from Takeo Province.

Affect of stem disease on seed health:

Stem disease category	% sterile florets	% healthy seed	% seed with disease
Healthy stems	35.4	44.4	20.2
Diseased stems	39.5	39.3	21.2

Affect of disease on panicle yield:

Stem disease category	Seed health	LSD ranking	Predicted seed weight per panicle (g)	Standard error
Diseased	High disease	a	0.40	0.13
Healthy	High disease	ab	0.57	0.14

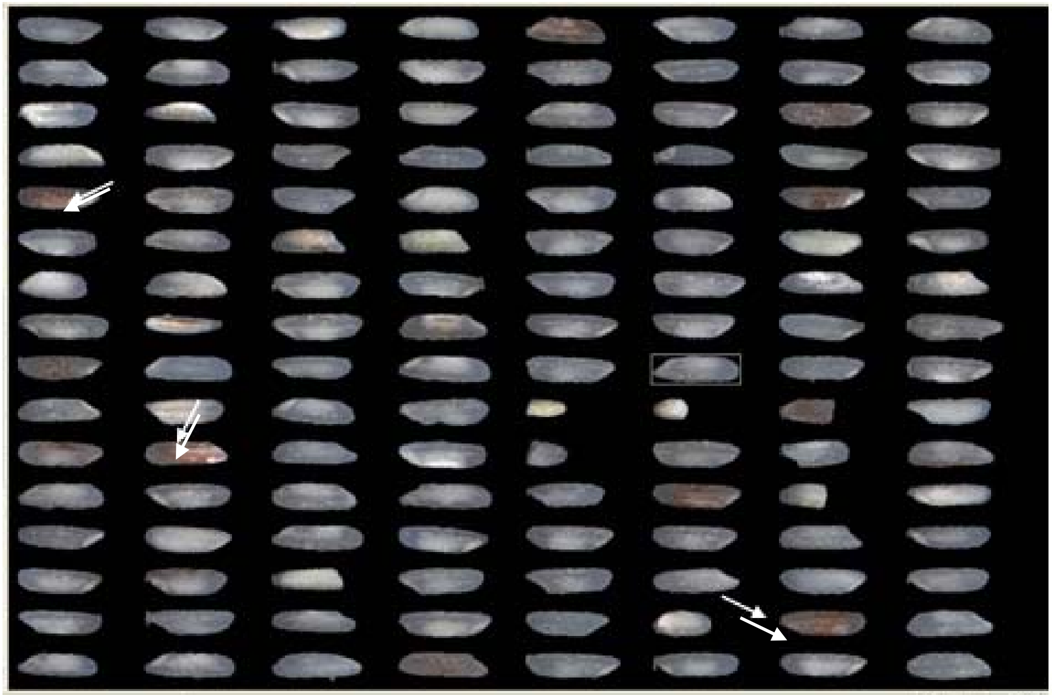
Diseased	Medium disease	b	0.82	0.17
Healthy	Medium disease	c	1.44	0.23
Diseased	Low disease	d	2.9	0.32
Healthy	Low disease	e	4.7	0.56
Diseased	Healthy	f	8.1	0.92
Healthy	Healthy	g	16.8	1.8

Affect of disease on grain yield as measured by 1000 grain weight:

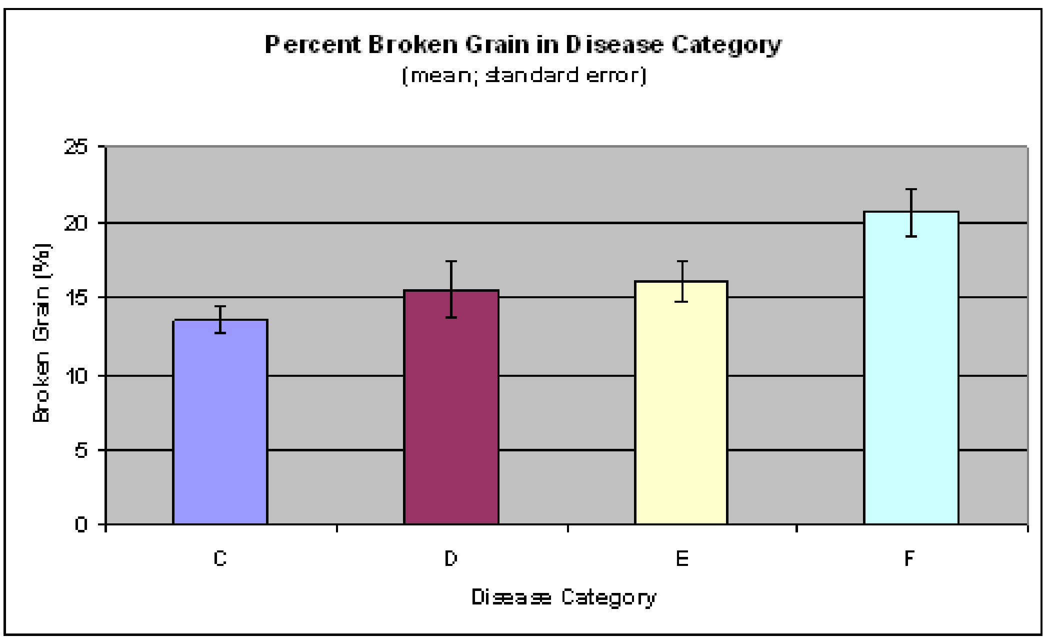
Stem disease category	Seed health	1000 grain weight (g)	LSD ranking
Diseased	High disease	18.1	a
Healthy	High disease	18.9	b
Diseased	Medium disease	19.8	b
Healthy	Medium disease	20.6	c
Diseased	Low disease	21.6	c
Healthy	Low disease	22.4	d
Diseased	Healthy	23.9	e
Healthy	Healthy	24.7	f

Statistical analysis shows an increase in chalkiness and an increase in grain broken during milling as the level of disease in grain increases. ACIAR is funding a project in the Philippines on molecular markers for chalkiness in rice (CIM/2006/176). Our data is showing that this important quality parameter is being further exacerbated by disease.

Many of the crops sampled in August 2007 from farmers' fields were contaminated with red rice. This is only noticeable after dehulling. At CARDI, this was attributed to variable seed moisture levels, indicating a possible lack of awareness of the occurrence and importance of red rice.



After polishing, remnants of the red pericarp (arrowed) can remain, resulting in a product of lower quality.



As disease increases, so does the percentage of grain that breaks during milling.

In Takeo Province, the average yield in 2005-06 from 174 900 ha was 2.69 t/ha (MAFF data). Our data show an average 20% reduction in seed weight. This translates to an average 120 kg/ha weight loss due to disease. At 800 riel/kg, this equals about $24 loss/ha to the farmer. If we assume that only have the crops in Takeo Province experienced an average 20% loss, this would still amount to a loss of 10 500 tonnes or $2.1 million for the Province alone.

Objective 5. Develop integrated disease management strategies

This objective is now not appropriate as it was predicated on detecting widespread occurrence of blast, about which much is known on its aetiology and management.

We have determined that bacterial diseases occur much more widely and more intensely than anticipated and crop loss assessment studies have shown their role in yield/quality to be very important. Extra resources were taken in bacterial identification and time did not permit development of IDM approaches.

7 Key results and discussion

Establishment of a Plant Pathology discipline Laboratory:

The plant pathology laboratory at CARDI is now well equipped with two laminar airflow cabinets, compound and dissecting microscopes, refrigerator, balances, incubators, an autoclave and all the small items necessary for daily operation in a plant pathology laboratory. In addition, many specific and general plant pathology texts have been provided.

CARDI is now the repository for the Cambodian Plant Pathology Herbarium. This will function as an on-going, verifiable record of plant disease specimens that will be essential for Cambodia's obligations under the WTO Agreement on Sanitary and Phytosanitary Measures.

Training of Key Scientists in Disease Recognition and Identification:

At the conclusion of this project, three CARDI scientists have received extensive training in plant pathology discipline. In addition, two others have been introduced to the basics of plant pathology based work. Unfortunately, two of the participants who received substantial training are no longer at CARDI. Mr Kohn Hen passed away mid 2007 and Mr Pak Dolar was placed in a situation in which he felt he needed to leave CARDI late 2007. The remaining trained plant pathologist, Dr Ny Vuthy, was placed as an assistant to deputy director Dr Ouk Makara, and has since resigned. Therefore, CARDI and, to our knowledge Cambodia, are back in the position of having no trained pathologists actively working in the discipline.

While the above have had a negative affect, these results were entirely out of the control of the project. The key results of the project cover the aspects of plant pathology as a discipline.

- Surveys of rice growing regions for disease affected plants

  Practically all regions within Cambodia were visited for the purposes of disease identification and sampling. During these visits, Ny Vuthy, Kohn Hen and Pak Dolar were exposed to the process of identifying symptoms associated with disease. Often abiotic stress, nutritional stress and other factors can produce symptoms similar to those associated with disease. Repeated exposure to the differences is essential to gain the experience to determine if an observable symptom is disease associated or an indictor of another problem. On most occasions, the survey team was accompanied by the local district agronomist who directed the team to sites of interest. On several of these occasions, the team was taken to a rice crop affected by nitrogen deficiency and drought and asked what could be causing the problem. While advice can be given, this is not a position a pathologist likes to be in. Sample collection and preservation techniques were devised and improved upon over the course of the project, enabling excellent collection of diseased materials. By the completion of the survey work, CARDI participants were able to independently identify and assess potential disease symptoms and collect and preserve these appropriately for laboratory examination.

Key result – CARDI personnel were able to competently conduct disease surveys of rice and have the ability to transfer this to any crop CARDI wishes to focus on.

- Isolation and identification of disease-associated organisms

  Fungi – project participants were introduced to the steps necessary to identify the most probable fungi associated with disease symptoms. Media preparation was introduced as a regular process required in any pathology laboratory, and early in the project participants were capable of, and were routinely, preparing media for use in isolations of fungal species. For each collection trip, participants were required to isolate from diseased material, using appropriate aseptic techniques and understand the rational for using a different media or approach. For example, the isolation from leaf material requires less sterilisation time than isolation from root materials. During the course of the project, CARDI participants achieved a level of successful isolation, allowing for uncontaminated cultures of fungal specimens to be produced even while travelling in remote provinces.

In the early stages of the project, the main effort of identifications was performed back in Australia, where access to taxonomic keys and internet resources allowed for a more extensive evaluation of cultures. In addition, the use of molecular techniques was available. This then provided reference materials, such as permanent-mount slides and digital photographs of spores and other structures, which were then used to train CARDI participants in the steps required to identify the common fungal species from rice in Cambodia. By the conclusion of the project, CARDI personnel were successfully able to identify *Bipolaris, Sarocladium, Rhizoctonia, Nigrospora* and *Curvularia* isolates from diseased rice material. Dr Ny Vuthy also used the experience he gained during the project to independently isolate and identify the blast pathogen, *Pyricularia grisea*, and to isolate and culture several organisms responsible for disease on citrus in Cambodia. In addition, Dr Vuthy has been approached on several occasions by staff from other units within CARDI to assist in and identify disease issues observed in rice, legume crops and citrus.

Bacteria - Contrary to expectations, bacterial diseases in rice were more common and serious. Bacteria are more difficult to identify than fungi because they lack the morphological characteristics commonly used in fungal identification. In general, Identification relies on biochemical, phenotypic and nucleic acid characteristics.

Based on symptoms alone, presumptive diagnoses were made that the diseases caused by the recognised pathogens *Pseudomonas fuscovaginae* and *Burkholderia glumae* would occur in Cambodia. Accordingly, four semi-selective media were prepared in Australia and conveyed to Cambodia for use in isolations. These media contain antibiotics and are reported in the literature to be selective for various pathogens associated with rice. Forty four isolates obtained from colonies which grew on these media were studied further in Australia by FAME analysis, carbohydrate utilisation and other biochemical tests using the Biolog GN system and API 20NE test strips. As these systems rely on comparison with their proprietary database libraries and which may contain a limited number of plant pathogens, further supplementary tests are usually necessary to identify plant-associated bacteria. The Type and other cultures of *Burkholderia glumae* and *Pseudomonas fuscovaginae*, and cultures of *Acidovorax avenae* subsp. *avenae* from overseas were used as known standards in all biochemical tests.

No isolates of *B. glumae* were identified despite considerable colony diversity on several of the media. *Ps. fuscovaginae* was identified on one occasion. Four recognised bacterial pathogens, *Acidovorax avenae* subsp. *avenae*, *Burkholderia gladioli, B. cepacia* and *Pantoea ananatis*, isolated from a variety of symptoms, were identified. However symptoms alone are not sufficient for accurate diagnosis of some of the diseases caused by these pathogens.

Key result – CARDI personnel are able to competently isolate fungal and bacterial species from diseased plant material. In terms of fungi, CARDI personnel are able to identify common disease-causing organisms, some to the species level.

- Preliminary trials to determine pathogenicity of potential causal organisms

Fungi – In order to determine if the isolated fungal specimens were responsible for the associated disease symptom, CARDI participants were required to perform pathogenicity trials under glasshouse conditions. Isolates of each of the identified fungal species were examined on common rice varieties, trialling a range of inoculation techniques. While disease expressions was evident on several occasions, the process seemed to be plagued by variability. Several reasons for this exist. The glasshouses used for the experiments have rudimentary temperature control and therefore the temperature inside is often 10-20°C above the ambient. Many of the fungi trialled have a limited temperature range in which they can grow, and thus this will have a dramatic effect on their ability to survive and infect a host. Humidity in the glasshouse is difficult to control, despite the use of plastic bags to promote a humid environment (which also adds to the temperature increase). As host plants were grown in pots, the water level for each was much less that what would be expected in a field situation and therefore, the humidity level would be much less. While misting of the host may assist this, difficulties were encountered in terms of whose responsibility it was to maintain the trails. More often than not, general glasshouse workers were assigned to check the trials. In general, these workers are unskilled and have no real background in conducting trials for research purposes. While not all trials were a success, CARDI participants were able to reproduce similar disease symptoms in the glasshouse as those observed in the field, therefore providing evidence that the isolated specimen was the causal agent of the disease.

Bacteria – similar pathogenicity tests were conducted with representative isolates of the pathogens identified on the basis of biochemical tests. Koch's postulates were completed for the pathogens listed, including several isolates of a bacterium that does not fit the species description of known bacterial pathogens.

Key result – CARDI personnel have the ability to conduct pathogenicity trails on rice hosts.

Other activities conducted through the term of the project included workshops, delivering education materials to a wide range of candidates. These provided information on disease recognition, preliminary identification and provided information on where to go for assistance. While these generally were directed towards district agronomists and members of other research/advisory bodies, the need to further disseminate information exists. However, until there is an established "place to go" for assistance, it seems pointless to have farmers attempting to diagnose an issue.

Association of fungal sp. with disease symptoms of rice in Cambodia

Surveys conducted across Cambodia resulted in the collection of diseased material exhibiting characteristics associated with several common fungi. While blast, caused by the pathogen *Pyricularia grisea*, was initially thought to be wide spread in Cambodia, the pathogen was identified only on a few occasions. There may be several possible explanations for this, with the most likely that outbreaks are not reported and, therefore, collections not made until too late. Lack of disease recognition by farmers and district agronomists would play a major role in this. During the surveys, disease symptoms that could be taken as blast associated did not result in isolation of the pathogen. Therefore, the likelihood that blast has been mis-identified and, therefore, over reported as a disease is moderately high. The most recent confirmed outbreak of blast was 2005, prior to this project beginning. Environmental conditions throughout the duration of this current project may not have been conducive to a serious outbreak. In addition, the dissemination of IRRI rice varieties will have likely resulted in the introduction of some blast resistance to Cambodia. Having said this, there remains a need for appropriate screening of breeding material and continued surveillance for blast, particularly when neighbouring countries report an outbreak.

The most common fungal species isolated from rice material were *Bipolaris, Curvularia* and *Nigrospora* sp. *Bipolaris oryzae* was isolated from all regions surveyed, while both *Curvularia* and *Nigrospora* sp. were detected in the southern provinces of Takeo, Prey Veng and Kandal. In general, the three species were associated with leaf disease on a low to moderate scale and did not appear to be resulting in serious yield decline. On several occasions these species were isolated from darkened and discoloured seed, generally in association with bacterial infection. This represents a more serious situation in terms of affecting seed quality and, therefore, price for the commodity. In addition, many farmers retain seed for following plantings, thereby running the risk of carrying over diseased seed and having a reduced seedling emergence and vigour (Van Nghiep and Gaur, 2004). Other common fungi were *Sarocladium* and *Rhizoctonia* sp. Both were isolated on several occasions, with *Sarocladium oryzae* being identified in provinces in the south and north east. *S. oryzae* presents an interesting case as this fungus was generally isolated in association with bacteria and from plants exhibiting disease symptoms associated with bacterial disease. Literature suggests that *S. oryzae* may persist in rice seed for up to 7 months (Gopalakrishnan and Valluvaparidasan, 2007). Once infected, the fungus has the potential to repress head formation and to affect seed quality. While not seen in extensive areas of rice, this pathogen presents a potential disease issue for Cambodia. *Rhizoctonia* species (*R. solani, R. oryzae* and *R. oryzae-sativae*) were all isolated on one or two occasions. In general, these were taken from stem and sheath lesions observed on individual plants, with little evidence for major outbreaks. However, the potential exists, from experiences in other countries, for this to occur.

Key result - *Sarocladium oryzae, Rhizoctonia oryzae* and *R. oryzae-sativae* are not listed on the Endemic and Quarantine Pests List Of Rice Of Cambodia (DAALI/PPPIO 2003). Given the impact of these diseases elsewhere in the world, their detection during this project indicates an important step towards Cambodia maintaining the requirements for WTO membership.

Association of bacterial sp. with disease symptoms of rice in Cambodia

As stated above, bacterial diseases were widespread in both the wet and dry seasons. Symptoms were not characteristic of the causal pathogen and hence a particular disease can only be confidently identified by isolation and identification of the organism. *Burkholderia gladioli* was commonly found in association with *Acidovorax avenae* or *Pantoea ananatis*. Pathogenicity testing with each pathogen alone often produced identical symptoms.

This situation highlights the need for plant disease diagnosis to be supported by laboratory identification of the causal organism. Otherwise incorrect disease identification could lead to the (incorrect) listing of a disease on a national register that may have trade implications later on. For example, a recent IRRI/ACIAR publication “Rice in Laos” (Eds Schiller *et al*.) 2006, lists several rice diseases that, given the proximity of the two countries to each other, should occur in Cambodia. We have not observed several of these diseases and it appears that the identification of rice diseases in Laos was by visual inspection only and was not supported by laboratory isolations (Seng Praseut, pers. comm.). Thus, several listed diseases may not occur in Laos or, if they do, their significance may be over- or under- estimated.

The importance of plant pathology seems to be lost on senior management at CARDI. While we need to consider that there are political ramifications for the declaration of certain diseases, it is surely better for Cambodia to publicly state which diseases are present than for a major trading partner to identify the disease and use it to block future trade. Plant pathology needs to be seen as more than disease diagnosis. The discipline has essential roles in plant breeding, quarantine services, human health and social welfare.

Key results– Only one bacterial disease (bacterial leaf blight) is listed on the current Endemic and Quarantine Pests of Rice list for Cambodia.

CARDI staff now have more confidence in distinguishing bacterial from fungal diseases in rice and are confident in the procedures of isolation of the suspected pathogen that will allow future presumptive identification of the bacterial pathogen.

Identification of fungi and bacteria associated with native wild rice in Australia

The majority of fungi and bacteria isolated in Cambodia were also present in northern Australia. Blast disease occurred infrequently in Cambodia and was not observed at all in Australia, despite some records of its occurrence in the past.

Yield loss data

It was demonstrated that bacterial diseases caused significant yield and quality losses to rice in Takeo Province and it is expected that similar losses would occur where ever these diseases occur in Cambodia.

8 Impacts

8.1 Scientific impacts – now and in 5 years

Relevance to Australia

Disease symptoms observed in Cambodia and the resulting bacterial isolates rekindled interest in a bacterial disease observed in Leeton, NSW in 2005. Bacteria isolated in 2005 were re-examined and compared with Cambodian isolates. We have been able to identify the cause of diseased panicles as *Pseudomonas fuscovaginae*, a pathogen which has not previously been reported in Australia. In glasshouse tests, this pathogen causes extensive spikelet disease and sterility, and stem lesions.

The Australian rice industry is now aware of the presence of a new disease and the susceptibility of some parental breeding lines - this can be taken into account when selecting breeding material for future crosses.

Relevance to Cambodia

Laboratory with capacity for diagnosis and research in plant pathology/crop protection

Herbarium established. A collection was established during a USAID sponsored project running form 1958-1960, based at the Ministry of Agriculture, Phnom Penh (Litzenberger et al. 1962). We have been unable to verify if this collection still exists, but specimens are accessible from the US National Fungus Collections (BPI). Thus, the herbarium collection at CARDI is thought to be the only viable collection operating in Cambodia. While the collection has a rice base, it provides the start for a broad range herbarium. This collection provides verifiable reference material for both scientific and trade purposes.

Training of CARDI staff has provided competent personnel to conduct identifications of fungi and, hopefully in future, the preliminary identification of plant pathogenic bacteria. Again, this provides Cambodia with the only operational laboratory capable of performing routine identifications and research in the discipline.

Compilation of genetic database of ITS sequences for common fungal pathogens, along with 16s rDNA and rpoB gene sequences of bacterial isolates from rice will provide a reference resource for future studies in Cambodia.

8.2 Capacity impacts – now and in 5 years

Given the current situation in Cambodia in relation to plant pathology skills, the impacts of this project have been somewhat diluted. However, we believe significant inroads have been made which will allow future development of the discipline. CARDI has a fully equipped and operational laboratory and staff with the capability to receive and process plant material with suspected disease symptoms. In addition, CARDI staff have experience in field based analysis of suspected disease issues which will be important for any continuing project. While very limited in bacteriology, CARDI have the staff with the capacity to isolate and partially identify disease-causing organisms. While this may currently be a preliminary diagnosis, it is at least to a level where initial management options can be advised.

The current skills set is ready to be built upon. Presumptive identifications of plant disease followed by systematic identification will allow the development of robust management systems in the future. CARDI do not have the overall capacity to achieve the required roles in plant pathology. Field based surveys may need to conducted by DAALI field staff, with CARDI having an initial role in the training required to achieve this. Ideally, samples collected by DAALI staff would come to CARDI for identification. Cooperative participation is the only option if Cambodia is to progress in plant pathology. Any new project proposals must aim to achieve this.

Key impacts:

Now

Ability of scientists to perform presumptive identification of organisms associated with plant disease and therefore provide basic information on management strategies.

CARDI staff capable of conducting research activities to determine the effect of organisms on plant hosts

Five years

With further training:

1. CARDI staff and the established laboratory have the capability to determine potential risks in imported plant products.
2. CARDI staff have the capability to provide services to other disciplines eg plant breeding, seed multiplication, quality assurance.

8.3 Community impacts – now and in 5 years

This current project took a top down approach to plant pathology, aiming to establish a central laboratory to which farmers, agronomists and other research organisations could go for assistance. During field surveys, local villagers continually approached and asked questions, indicated problems and showed great interest that CARDI was offering help with disease issues. While this does not provide measurable community impact directly, the potential exists in future projects to provide this

There are limited impacts for community now. This is due to the fact that no recent work had been conducted on plant pathology specifically. Surveys and accurate identification was required to determine which pathogens were causing significant disease occurrence in Cambodia. Appropriate training (if continued) of personnel to identify pathogens and therefore recommend appropriate management will have significant impacts in 5 years. As farmer knowledge and awareness increases over time, they will be able to implement these management strategies with limited input from advisory groups.

The impacts below would be expected to develop in 5 years if further training is provided:

8.3.1 Economic impacts

Compliance with WTO regulations in regards to accurate pest and disease lists. The current list published by DAALI appears to be based upon literature reviews and on limited examination of seed.

An improvement in the productivity of key crops in Cambodia will see economic benefit on several scales. Cambodia’s participation in the WTO agreements has a reliance on issuing accurate pest and disease lists. This is only possible when effective surveys are conducted, followed by the competent diagnosis of pathogens and pests. A reduction in yield loss due to disease and the associated increase in quality of the commodity will see an increase in farmer income. Linked to this is a reduction in operational costs related to pesticide application

There exists the potential to establish a quarantine inspection facility to examine plant commodity imports, thereby preventing incursions of disease not present in Cambodia. Thus potentially damaging outbreaks can be avoided or managed at reduced expense.

Accurate identification of plant pathogens plays a role in rice intensification and increased production. In addition, appropriate management assists in the maintenance of quality.

This has several flow-on effects affecting farm scale economy:

Reduced reliance on pesticides – appropriate identification reduces the risk of spraying in-effective chemical.

Increased yields results in better farm returns for effort.

Increased quality results in increased farm profits for effort.

As farm productivity increases a flow on to education of children generally occurs, resulting in better prospects for families.

8.3.2 Social impacts

Improving plant pathology skills and applying this to non-rice crops would see a benefit of improved farm production. Support for disease issues in field crops such as soybean, mung bean and peanut, as well as in horticulture, will see an impact on the sustainability of the farm enterprise.

Increasing rural investment, particularly in agriculture, has been identified as a key element for poverty reduction. A reduction in the yield and quality loss caused by disease has the potential to increase farm productivity, thereby increasing rural investment. Projects conducted elsewhere in the world have demonstrated that increased production which improves total farm income invariably leads to improved educational opportunities for rural children and has a positive impact on rural health.

Development of disease management strategies can lead to a reduction in the use of pesticides, which will have direct impacts on social health.

8.3.3 Environmental impacts

Correct disease diagnosis and management practices to reduce disease impacts will reduce the need for pesticide applications and ensure that such chemicals are applied only when needed, at the right time and are appropriate for the target pathogen.

8.4 Communication and dissemination activities

In general, dissemination has occurred in the field. The team met with a journalist group in Phnom Penh and several meetings were held with NGOs and other institutes to discuss our work.

Laboratory based methods and protocols were written for the CARDI laboratory.

9 Conclusions and recommendations

9.1 Conclusions

Success in this project can be measured in two areas. It has highlighted the almost complete absence of plant pathology skills in the Kingdom and it has established a functioning plant pathology laboratory to support this discipline. The project has also identified at least 4 major bacterial pathogens of rice and is leading to the description of a new rice bacterial pathogen, previously not known to science. Further success has been compromised by the unwillingness of CARDI to acknowledge the seriousness of diseases in rice and to actively support the training of the Kingdom's only qualified plant pathologist. Training recommenced with 2 new staff in the last 6 months of the project and it is hoped these people will have a more permanent presence and support at CARDI.

9.2 Recommendations

We have demonstrated that the most important diseases of rice occurring in Cambodia are caused by several bacteria: *Pantoea ananatis, Burkholderia cepacia, B. gladioli, Acidovorax avenae* subsp. *avenae*, *Xanthomonas oryzae* pv. *oryzae*, and *Pseudomonas* (*fuscovaginae* ?). Little information is known regarding the epidemiology of these diseases and any possible management strategies.

The fungal diseases occurring in Cambodia are less important than those caused by bacteria, and management strategies for these are relatively well known by CARDI staff.

It should be noted that our numerous disease surveys over the past three years have also clearly shown that diseases on legume crops (soybean, peanut, cowpea) are important in Cambodia.

Future work

Several ACIAR projects on crop diversification are currently in progress, with a focus on crop suitability and insect pests. Very little is being conducted on plant pathology, whether by design or lack of knowledge. This is a major issue, because Cambodia currently lacks the capacity to deal with new host-pathogen interactions that may emerge when alternative crops are introduced.

As stated before, Dr Ny Vuthy is the only scientist in Cambodia with any university-accredited training in plant pathology. Further capacity building in plant pathology/crop protection is, therefore, urgently needed in Cambodia. To address this issue, we suggest that any new project in plant pathology should continue working with CARDI and include collaboration with the Royal University of Agriculture to:

- develop an appropriate curriculum in plant pathology for Cambodian undergraduate students
- provide training to lecturers and demonstrators
- provide laboratory equipment for teaching and research in plant pathology
- assist RUA postgraduates students to apply for John Allwright Fellowships to study plant pathology and pesticide residue sciences in Australia.

The current teaching in Plant Pathology at RUA is conducted by a MAFF staff member who is not a plant pathologist by training. The above proposals result from several discussions held at RUA with Mr Chuong Sophal, Dean of Agronomy and Drs Adrian Bolliger and Ms Ines Kannewischer (German Development Service).

Drs Men Sarom, Ouk Makara and Preap Visarto (CARDI) have also indicated their support for a continuation of our Plant Pathology project. They recommended that the project should focus on legume and legume-based rotations (soybean, mungbean, cowpea, peanut, maize, cassava). They also underlined the fact that the project should strongly focus on disease management:

- year 1: survey and diagnostic
- year 2: testing management techniques
- year 3-5: on farm demonstration plots / farmers' clinics / extension

Dr Makara also suggested that another component of the new proposed project should focus on developing and testing management techniques for the new bacterial diseases on rice identified in our current project.

10 References

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Gopalakrishnan, C., Valluvaparidasan, V (2007) Survival of Sarocladium oryzae in rice seeds as affected by length of storage period. *Pest Science and Management* 32: 20-21.

Van Nghiep, H., Gaur, A (2004) Role of Bipolaris oryzae in producing abnormal seedling of rice (Oryza sativa). *Omonrice*, 12: 102-108.

10.2 List of publications produced by project

Vuthy N, Stodart B, Cother E, Lanoiselet V, Ash G, Visarto P. 2007. Building plant pathology capacity in Cambodia. 16th Biennial Australasian Plant Pathology Conference, Adelaide, September 2007.

Stodart B, Cother E, Lanoiselet V, Ash G. 2007. Diseases occurring on *Oryza* species in Northern Australia. 16th Biennial Australasian Plant Pathology Conference, Adelaide, September 2007

Shivas RG, Cother EJ, Ash GJ, Ray JD, Vanky K. 2008. *Tilletia australiensis*. In Fungi of Australia: The smut fungi. K. Vanky and RG Shivas. pp148-149. ABRS, Canberra.

Cother EJ, Reinke R, Noble DH, Stodart B, van de Ven RJ (2009). Identification of *Pseudomonas fuscovaginae* causing sheath and glume lesions on rice in Australia and comparison with *P. fuscovaginae* strains from world culture collections. *Australasian Plant Pathology* (submitted)

Stodart B, Vuthy N, Lanoiselet V, Ash G, Visarto P, Cother E (2009). Incidence of rice pathogens in Cambodia, Australasian Plant Pathology (in preparation)

11 Appendixes

11.1 Plant Disease specimen submission form

CARDI

SPECIMEN DEPOSITION FORM

Insert a brief description of the service that the CARDI Plant Pathology unit provides for farmers

Please provide as much information as you can

1. CONTACT INFORMATION

(Name of person sending sample)

Position Title — (Company)

Address

(Tel) — (Fax) — (Email)

(Name of farmer)

(Address) — (Tel)

2. SAMPLE INFORMATION

(Common name of plant)

(Scientific name of plant, if known)

(Date of Sampling) — (Date of Sowing)

3. CROP INFORMATION

(Variety) — Rootstock — Scion

(Soil type)

(Part of plant affected)

- ☐ (Root / tuber)
- ☐ (Leaf)
- ☐ Fruit
- ☐ (Stem)
- ☐ (Flowers)
- ☐ (Whole plant)

4. DESCRIBE THE PROBLEM

- ☐ Leaf spot
- ☐ Wilt
- ☐ Dieback
- ☐ Mosaic
- ☐ Yellowing
- ☐ Soft rot
- ☐ Canker
- ☐ Other (Please provide details)

Details

5. WHERE IN FIELD ARE PLANTS AFFECTED?

- ☐ High areas
- ☐ Patchy in groups
- ☐ Scattered
- ☐ Low areas
- ☐ Centre of field
- ☐ Widespread

6. HOW MANY PLANTS ARE AFFECTED?

- ☐ Few
- ☐ Some
- ☐ Many

7. WHEN WAS THE PROBLEM FIRST OBSERVED? — Date

Continued over the page

CARDI

SPECIMEN DEPOSITION FORM continued

8. WHAT TREATMENTS HAVE BEEN APPLIED TO THE PLANTS?

- ☐ None
- ☐ Insecticide
- ☐ Fungicide
- ☐ Herbicide
- ☐ Fertiliser

When were they applied?

9. SOURCE OF PLANTING MATERIAL

- ☐ Own seed
- ☐ Seed from neighbour
- ☐ Seed from market
- ☐ Seed from private company
- ☐ Seed from AQIP
- ☐ CARDI
- ☐ DALI

10. WHAT OTHER PESTS ARE PRESENT IN THE CROP?

- ☐ Snails
- ☐ Crabs
- ☐ Rodents
- ☐ Insects (specify)

11. PREVIOUS CROPPING HISTORY

Have weather conditions been normal in the past 2 weeks? ☐ Yes ☐ No

If no please provide details

12. OTHER INFORMATION

Signature ____________________ Date ____________

So that we can provide you with a speedy, accurate identification please:

- DO NOT send dead plants
- Collect and send samples as

PLANT PATHOLOGY
CARDI
National Road N° 3

CARDI

SPECIMEN DEPOSITION FORM continued

soon as possible

- [illegible] ៦-១០ Collect a range of symptoms from different plants (6 to 10 preferred)
- [illegible] Avoid leaving samples in direct sunlight or hot places
- [illegible] Wash roots to remove soil
- [illegible] Remove surface moisture from plants with old newspaper
- [illegible] Wrap the sample in clean, dry newspaper
- [illegible] Send completed form and specimens to:

Prateah Lang Commune Dangkor
Phnom Penh
TEL: (855 23) 219 693

11.2 Poster accepted for 16th Biennial Australasian Plant Pathology Conference, Adelaide, September 2007

EH GRAHAM CENTRE for Agricultural Innovation

an alliance between Charles Sturt University & NSW Department of Primary Industries

Australian Government — Australian Centre for International Agricultural Research

CHARLES STURT UNIVERSITY

NSW DEPARTMENT OF PRIMARY INDUSTRIES

BUILDING PLANT PATHOLOGY CAPACITY IN CAMBODIA

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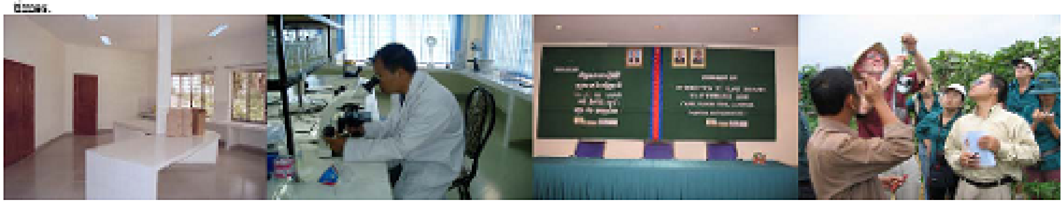
In Cambodia rice is by far the most important staple food source, occupying approximately 90% of agricultural land. The per-hectare yield is amongst the lowest in Asia, with a major contributor to this being pests and diseases. Very little is known about the distribution, prevalence and severity of rice diseases in Cambodia and the knowledge base for plant pathology is limited. The main aims of this project are (1) capacity building in plant pathology; and (2) to survey and characterise the diseases commonly associated with rice crops.

Capacity Building: CARDI had established buildings and laboratories but had no laboratory equipment. In 2005-06 the plant pathology laboratory at CARDI was equipped and is now fully functional. Workshops and training have been provided in basic plant pathology techniques, with over 50 participants attending at various times.



Disease Surveys: Fourteen provinces (blue stars) have been visited during the growing seasons. Random crops were examined for the presence of disease symptoms. Staff from the District Agronomy offices provided information on disease reports, weather conditions and issues leading up to disease occurrence, as well as direct translation from farmers.

Rice Disease: Field samples were collected for isolation and identification of causal organisms. Table 1 lists the fungi and bacteria isolated from disease symptoms on rice. Bacterial diseases appear to be more prevalent than anticipated. Type specimens have been lodged in Cambodia's first disease herbarium at CARDI.

Table 1: Disease symptoms, associated organism and their occurrence on rice in Cambodia

Symptom	Isolated Pathogen	Occurrence
Brown spot - leaf	*Bipolaris oryzae*	Frequent
	Curvularia lunata	Frequent
	Nigrospora oryzae	Frequent
Sheath rot	*Sarocladium oryzae*	Moderate
Sheath lesion	*Rhizoctonia oryzae-sativae*	Moderate
	Rhizoctonia solani	Low
Blast	*Pyricularia oryzae*	Low
False smut	*Ustilaginoidea virens* (Cooke) Takah	Low
Sheath lesions	*Burkholderia cepacia*	Frequent
	Burkholderia glumae	Frequent
	Pantoea ananatis	Frequent
	Pseudomonas fuscovaginae	Low
	Pseudomonas putida	Low
Discoloured glumes	*Acidovorax avenae* subsp. *avenae*	Moderate

Future direction:

- Develop management strategies for the main fungal and bacterial diseases on rice.
- Develop and disseminate extension materials to district agronomy offices.
- Increase farmer participation in disease recognition and management workshops.
- Expand surveys to include upland crops such as soybean and mungbean.

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