Program 1 was designed to ensure sustainable use of natural resources in the rice producing areas. The many highlights in this program have included:

- Use of remote sensing to determine crop areas and types. This technology is now in use in the rice producing area.

- Strong indications that remote sensing can be used to measure crop growth at various growth stages.

- Widespread uptake of crops following rice to make use of water left in the profile and so maximise water profitability but reduce additions to the groundwater.

- The methods that were developed for measuring water losses from channels and drains have been used to develop water saving strategies for the Murrumbidgee and Coleambally Irrigation areas.

- Water losses from on-farm channels and drains were measured and strategies developed that can minimise such losses.

- Computer-based strategies were developed to manage net recharge in rice-based systems. These have been transferred to users and are in use.

- Models for water and salt movement in irrigation areas have been developed and made available to irrigation companies.

- Systems for improving the water productivity of rice have been developed. Two systems still under investigation are production in raised beds and mid-season drainage to improve productivity.

- A system has been developed to better predict rice suitable soils by combining sodicity measurement with electromagnetic induction technology. This system is now being used by Murray Irrigation Limited and being evaluated for other irrigation areas.

- A model has been developed for predicting water allocations based on climate forecasts. This has great potential to assist management decisions in all irrigation production systems.
The research programs of the Rice CRC aim at outcomes that will underpin the sustainability of the Australian rice industry. The four research programs address areas associated with resource use and management; production systems; genetic improvement; and processing and product development. Research projects are integrated with the education program to ensure close linkages between these important functions and also between industry and the education program.

The research program also compliment the production orientated research and development funded through RIRDC.

This section summarises research activities and progress during the eighth year, and highlights examples of achievements during the eight years of the Rice CRC.

PROGRAM 1

SUSTAINABILITY OF NATURAL RESOURCES

Program Leader:
Dr Liz Humphreys
CSIRO Land and Water
Griffith

Research in Program 1 is directed towards increasing water use efficiency and protecting the on-farm and regional resource base. Irrigated agriculture in the Murray-Darling Basin is under great pressure to reduce water extraction from rivers and groundwaters, and to change the timing of diversions to help restore river flows towards more natural seasonal patterns. The sustainability of rice-based farming systems is threatened by decreasing water availability, changed patterns of water availability, increasing uncertainty of supply and increasing water price, as a result of National Competition Policy and environmental reforms including The Cap on water diversions, water sharing plans and The Living Murray.

Productivity in the rice growing areas is also at risk from shallow watertables and secondary salinisation and waterlogging. Bulk water licence conditions for irrigation areas include minimum levels of contaminants (salts, pesticides, nutrients) in drainage waters leaving the areas. The regional and national importance of the rice industry, and the requirement for ponding of irrigation water for rice culture to control weeds and reduce cold damage, present unique challenges to management for environmental and economic sustainability of rice-based farming systems. Therefore the objectives of Program 1 are to increase water use efficiency of rice-based farming systems, to prevent soil salinisation and contamination of drains by controlling watertables, and to improve drainage water quality.

The main aims of the research in Program 1 are to:-

* increase water use efficiency of rice-based farming systems;
* prevent salinisation by controlling watertables;
* improve drainage water quality;

while maintaining or increasing the profitability of rice-based farming systems.

This will be achieved through working with stakeholders in the development and application of new knowledge, technologies and tools, including:-

* improved technologies and guidelines for rice soil suitability assessment;
* guidelines for the identification and treatment of leaky on-farm channels and drains;
* rice cultural systems with higher water use efficiency and lower net recharge;
* more flexible cropping systems with greater scope for diversification in response to market opportunities, and greater water use efficiency;
* guidelines and methods for preventing contamination of drainage waters;
* point, farm, district and regional scale models and decision support systems to assess the impacts of changes in management on environmental sustainability and economic viability; and
* remote sensing methods to identify crop type and evaluate water use efficiency across regions.

### 1.1 Measurement and mapping

**Sub-Program Leader:**
Mr Geoff Beecher  
NSW Dept Primary Industries  
Yanco

The aim of the Sub-Program is to develop tools for better land use mapping and land capability assessment.

Tools are required that provide better measurement, in space and time, of factors critical in natural resource management for rice-based farming systems.

Improved land use capability assessment techniques are needed to enable farm planning to minimise groundwater recharge from rice fields, channels and drains.

Better remote sensing tools are required for monitoring crop areas and types, and for identification of groundwater recharge sites.
Remote sensing of irrigated crop types and its regional water balance estimation (1105)

Project Leader:
Dr Tim McVicar
CSIRO Land and Water
Canberra

The project has been focusing on the use of spatial technologies (remote sensing, GIS and spatial interpolation) to better manage rice-based irrigated agricultural industries in southern NSW.

Objectives

There were three main objectives over the last year. These were to:

1. finalise the transfer of technology to CICL (regarding rice administration using satellite imagery);
2. complete broadband (Landsat ETM) research and publications; and
3. inspect the value added to the management of rice-based irrigation farming systems from hyperspectral remote sensing.

Progress

Project progress and achievements are summarised below with respect to the three objectives for this year’s work outlined above.

1. Finalise the transfer of technology for performing rice administration with satellite imagery:

   The rice-based classification system was largely transferred as an operational system to the CIA last reporting period (2003-2004). A detailed Client Report (see “Publications” chapter – “Technical Reports”) was reviewed by both Reuben Robinson and David Klienert of CICL (CICL’s GIS specialists). Their comments were implemented in the client report during this current reporting period and the report was formally published and delivered to CICL in August 2004. This summer, Tracey Singh used this client report to perform the 2004-2005 rice administration using broadband satellite remote sensing. The accuracy of the rice field identification for the 2004-2005 growing season was >98% (estimated from 68 paddocks of known crop types).

2. Complete broadband research and publications:

   The research largely conducted last reporting period using low-cost Landsat imagery to determine the best timing to purchase imagery for crop classification was finished during this current reporting period (see “Publications” chapter – “Refereed Journals”). The last planned broadband research project was also completed this reporting period. This new research concerned defining the relationship between the number of bands and the number
of training sites needed for accurate crop classification. This study has been submitted to Remote Sensing of Environment and responses have been provided to reviewer’s comments. It is expected that the editor will accept this paper soon (see “Publications” chapter – “Refereed Journals”).

3. Inspect hyperspectral remote sensing:

The hyperspectral research associated with the project for this reporting period was mainly concerned with two questions: (1) “What pre-processing is needed before using hyperspectral data in agricultural research?”; and (2) “What extra information is there in hyperspectral data that might be useful in agricultural management?”. We have started to answer these complex questions on the 25 satellite hyperspectral images (Hyperion), and 2 airborne hyperspectral images (HyMap) that were previously acquired and processed (described in previous reports).

Project Successes

Project success is demonstrated by three publications during this past year. Other specific successes are also summarised below with respect to the three objectives for this year’s work.

1. Finalise the transfer of technology for performing rice administration with satellite imagery:

The main project success resulting from this objective was the publication of the client report. The other indicator of success is that these methods developed by Project 1105 have now been operationally used by CICL for three years in a row. During this current year, Tracey Singh (CICL’s new GIS operator) was able to complete the procedure largely unassisted even though it was her first time performing the analysis. This was possible because of the step-by-step user’s manual provided to CICL (and no doubt combined with Tracey’s ability). The fact that the procedure resulted in a very accurate classification (see Figure 1), was proof of concept and demonstrates the success of both the technology transfer and the methods themselves.

![Figure 1. Overall rice identification accuracy vs. ETM+ band 5 digital number threshold. Based on this single band taken from a late November 2004 image, >98% accuracy was achieved for rice paddock identification.](image)

2. Complete broadband research and publications:

The main project success resulting from this objective was an international peer-reviewed journal publication. The results of this research have a potential to impact on the management of the CIA as the results show that both the timing of image acquisition and the methods used to combine multi-date imagery can make a large difference in
classification accuracy (see Figure 2). The new research conducted to define the number
of bands to the number of training sites required for accurate multi-temporal classification
has a potentially high impact in the field of remote sensing, as this issue is quite
fundamental to the utilisation of remote sensing for mapping purposes.

![Figure 2](image)

(a) Per-field producer’s and user’s accuracies for the major crops are shown through the entire 2001-
2002 summer growing season. Number of fields used for validation was 160 for rice (a), 46 for maize (b), 14 for
sorghum (c), and 63 for soybeans (d).

3. Inspecting hyperspectral remote sensing:

The work performed for this objective will lead the way for the future of hyperspectral
remote sensing in the Australian rice industry. For example, initial summaries of the
relationships between soil characteristics and hyperspectral data show promise (see Figure
3). This research revealed what parts of the spectrum have higher correlations to certain
soil chemical properties than others and also demonstrated the amount of processing and
expertise required to perform similar analyses in the future.
Assessing the effect of compaction on seepage from on-farm channels and drains (1107)

Project Leader:
Mr Saud Akbar
NSW Dept Primary Industries
Yanco

Objectives

* An assessment of the effect of compaction and lining materials on the seepage rate in existing on-farm channel and drainage networks.

* Identification of appropriate compaction or lining technologies to allow improved channel distribution efficiency.

* Economic evaluation of the benefits of these technologies to the farmers and the community.

* A Decision Support System (DSS) developed to guide grower actions in response to seepage from channels and drains.

* Development of two and three dimensional channel and drain groundwater interaction models.
A complete set of five years groundwater fluctuation data spreadsheets for Coleambally Irrigation has been prepared for VS2D data analysis.

Complete information and data spreadsheets for four years seepage monitoring at the treated site - including GPS co-ordinates, soil types, compacted areas and costs, has been prepared for economic analysis.

Complete design data spreadsheets have been prepared for statistical analysis.

**Project Success**

Mr Akbar’s work has been presented at national and international conferences and he has provided papers for overseas conferences also. It is anticipated that Mr Akbar will undertake further consultancies and projects resulting from this work. They are likely to be focused on the Murray Valley through the Green Valley and Living Murray program.

**1.2 Net recharge management**

*Sub-Program Leader:*
Dr Liz Humphreys
CSIRO Land and Water
Griffith

Net recharge management seeks to control watertables and salinity by reducing recharge of and/or increasing discharge from watertables.

Projects are in progress that will:-

* assist evaluation of on-farm options to control watertables and root zone salinity, taking into account economic viability and farmer preferences, for individual farms;

* evaluate novel water management and layouts designed to increase irrigation efficiency and/or reduce recharge from rice and rice-based cropping systems.

**Strategies for improving the water use for rice (1204)**

*Project Leader:*
Mr John Thompson
NSW Dept Primary Industries
Deniliquin

**Objectives**

The project aims to investigate opportunities to improve the water use efficiency of the rice crop by comparing irrigation layouts, sowing method and irrigation scheduling.
Progress

A field experiment at NSW Dept Primary Industries’ Field Station at Deniliquin is evaluating mid-season “drainage”, with two intervals of cumulative ETo without surface water (80 and 120 mm) being compared. The experiment also includes a treatment with a similar “drainage” event during grain filling.

Samples of dry matter production (1 m² quadrats) at flowering indicate that those treatments that were drained at mid-season (late tillering) have produced more than the permanently flooded treatment.

Grain yield from the mid-season “drain” treatment was 6% higher than the ponded control (9.9 t/ha v 9.3 t/ha). This difference is not statistically significant. The extreme cold weather in early February 2005 would have influenced the result from this and other treatments.

Sections of ten commercial crops were managed to provide demonstrations of the mid-season “drain” technique. Most of these showed no increase in grain yield. Again the cold weather was experienced at a critical stage of crop development and substantial sterility was evident in all crops.

Adoption pathways for risk-based irrigation demand management under system constraints (formerly “Develop a GIS-based tool for net recharge management in rice-based farming systems”) (1207)

Project Leader:
Dr Shahbaz Khan
CSIRO Land and Water, Griffith/
Charles Sturt University, Wagga Wagga

Objectives

* Refine the climate forecasting tool developed under Project 1207 to make it more widely available through the web.

* Promotion and extension of climate forecasting methods through active stakeholder participation.

Progress

* Developed artificial neural network (ANN) approaches which can learn from historic model simulations and sea surface temperature (SST) predictions can be a way forward to link climate forecasts with risk management. Results of the ANN model show good correlations with the historic water allocation, SST and SOI trends. This tool is now available as a web-based model and can be used to make informed cropping risk decisions.

* As part of this project a stakeholder workshop on climate variability, climate change and adaptation in the Murrumbidgee Basin was organised to examine research ideas on climate research for efficient irrigation management. Participants included a number of interested participants from irrigation companies, NSW DPI, DIPNR, MDBC and the local
community. There is a tremendous interest in climate and water issues due to the recent drought. The farming community needs tools which can link climate forecasts with smarter agricultural water management using a risk-based approach. The key barrier to the adoption of existing climate forecast tools is their lack of proven utility and the risk adverse attitude of water allocation agencies.

* This model has been extended to forecast general security water allocations in the Murray Irrigation Area.

1.3 Surface drainage management

Sub-Program Leader:
Dr Wendy Quayle
CSIRO Land and Water
Griffith

Surface drainage management involves quantity and quality aspects. Salt, suspended sediments (turbidity), nutrients and agrochemicals are major pollutants in surface drainage waters. Release of pollutants from farms can be arrested by methods such as appropriate holding periods on-farm or by amelioration to enhance degradation. This Sub-Program undertakes research to determine practical management options and guidelines for preventing the release of herbicides and pesticides from rice farms.

The persistence of rice pesticides in floodwaters and how this is influenced by water management and layout (1301)

Project Leader:
Dr Wendy Quayle
CSIRO Land and Water
Griffith

Objectives

* To determine the persistence of a range of old and new chemicals in floodwaters on rice fields.

* To determine the effect of water management and layout in rice cropping on the concentration of soluble pesticides and salts in flood waters.

* To develop a model to simulate, and hence predict, the load of pesticides in irrigation waters prior to drainage in rice growing regions of southern NSW.

Progress

* Modelling the dissipation of pesticides in rice bays

RICEWQ (Waterbourne Inc.) is a pesticide run-off model for rice crops developed to evaluate the dissipation of chemicals in rice bays and predict the run-off losses of agrochemicals to
receiving waters. The model has been successfully calibrated against water depths and molinate concentrations measured in a rice field in the MIA (Figure 4).

Figure 4. Molinate concentrations in ponded water.

* Data analysis

Data collected in previous years from field studies has been analysed statistically in preparation for final reporting.

Outcomes

* Modelling of pesticides using RICEWQ

Application efficiency (% of chemical that remains after instantaneous losses between spray tank and field) is the single most sensitive parameter controlling pesticide concentrations in field water. Application should be optimised so that a target concentration is reached which may allow for reduced application rates to be as efficacious as current label rates.

Aerial, ground rig, SCWIIRT and ground rig onto dry bays were compared for effects on pesticide persistence. All concentrations were similar regardless of method after a few days, indicating that application rate is only important if run-off occurs within the first few days after application.
RICEWQ was found to simulate simple water management conditions and adequately predicted pesticide concentrations in a single bay at the supply end of the field. However, this was not the case for bays which were located down the fall of the field receiving water from previous bays.

**The role of sediments in the fate of pesticides in rice bays (1302)**

**Project Leaders:**
Dr Stuart Helliwell/Dr Philip Eberbach
Charles Sturt University
Wagga Wagga

**Objectives**

Flooding of rice bays results in the consumption of oxygen by soil microbes and developing rice plants. Oxygen cannot be replenished rapidly enough by diffusion from the atmosphere and as a result an anaerobic environment develops with strong reducing properties. Pesticide degradation rates can be greatly affected by these reducing conditions as they dictate which soil microbes are able to survive and breakdown the pesticides.

This project is the subject of a PhD studentship (Mr Greg Doran) and aims to better understand the extent to which this anaerobic environment dictates the fate of the rice pesticides thiobencarb (Saturn®) and fipronil (Cosmos®).

Experimental work conducted early in the life of this project indicated that under ponded conditions where vertical water movement was restricted, adsorption of thiobencarb and fipronil to rice bay soils were limited to the top few cm of soil. This raised the question that, under conditions where vertical water flux occurred, could this pesticide leach?

The final experiment in this work focused on resolving if thiobencarb and fipronil could leach under flooded rice conditions.

**Progress**

Work using leaching columns in a glasshouse experiment showed two movement process were responsible for the translocation of both of these chemicals vertically under flooded conditions. Vertical movement was studied in 10 cm deep soil cores collected from rice growing paddocks near Coleambally and Yanco (NSW). Soil cores were glued on top of sand filled drainage assemblies which were fitted with a flow regulation device. Flow through the soil was set at 10 mm per day - a rate normally considered excessive for rice bays but for the present experiment sufficient to exaggerate the leaching phenomenon of these pesticides.

Most of the pesticide applied was found bound in the top cm of soil soon after application. This indicated that diffusion through the soil matrix was a significant driver for movement and that subsequent adsorption to soil solids hampered the downward passage of both chemicals. However, recovery of pesticide in the drainage assembly at the base of the soil cores (10 cm) soon after application, indicated that mass movement of water and chemical via preferential flow paths was also a significant pathway of translocation and that, in soils where deep flow paths existed, leaching of these chemicals may occur.
Greg Doran is in the final stages of completing his thesis. After submission, he anticipates publishing his work as four papers in the scientific literature.

**Enzymatic bioremediation of pesticide residues in irrigation drainage waters (1304)**

*Project Leader:*
Dr John Oakeshott  
CSIRO Entomology  
Canberra

**Objectives**

This study forms part of a larger project to develop an enzymatic bioremediation technology for cleaning up pesticide residues in irrigation tail waters and on horticultural commodities.

Pesticides remaining in irrigation water beyond their necessary life can have non-target impacts with environmental ramifications. Many pesticides have the potential to persist long enough to cause off-site damage when released in drainage water - these include the pesticides “thiobencarb” and “endosulfan”.

The research to be undertaken involves the discovery and characterisation of enzymes that can degrade thiobencarb and endosulfan. The research will focus on isolating and identifying degradative micro-organisms sourced from the environment that can degrade and detoxify these pesticides to metabolites of considerably less ecotoxicity. The research will identify metabolites, attempt to elucidate the degradative pathway and isolate the gene/enzyme system that is responsible for degradation, for the ultimate application of those isolated enzymes to the bioremediation of pesticide residues in drainage water.

**Outcomes**

In the last year PhD student, Kahli Weir, has been finalising her research and writing her thesis which has now been published. An abstract from her thesis “Isolation and characterisation of pesticide degrading enzymes” is provided below.

“This study describes the isolation and characterisation of pesticide degrading micro-organisms and the subsequent cloning and characterisation of genes encoding specific degradative enzymes. The pesticide targets were selected after an extensive review of pesticide residues in drainage water in the rice growing region of Australia.

Two pesticides and a pesticide metabolite of significance to the rice growing industry were selected for this study. The pesticides were thiobencarb, a herbicide used widely in rice agriculture and endosulfan, an insecticide not used on rice but used in the rice growing region. This project also focuses on the toxic metabolite of endosulfan, endosulfan sulfate, which is as toxic as the parent insecticide and more persistent. Endosulfan, endosulfan sulfate and thiobencarb have all been detected in routine monitoring programs in the rice growing region of Australia and this study has been concerned with developing technologies for degrading these compounds for future bioremediation applications.
An *Arthrobacter* strain capable of degrading and detoxifying both isomers of endosulfan and endosulfan sulfate was isolated from a previously described mixed culture of soil micro-organisms (Sutherland *et al.*, 2002a). Catalytic activity was only observed under sulphur-limited conditions, suggesting degradative activity forms part of the sulphur-starvation response of the bacterium. From this strain a gene, *ese*, was isolated that encoded a mono-oxygenase (Ese) that catalyses the metabolism of endosulfan and endosulfan sulfate. This enzyme is a member of the unique two-component flavin diffusible mono-oxygenase family (TC-FDM) that require reduced flavin mononucleotide (FMNH₂) as a co-substrate for activity. Analysis of the gene sequences flanking *ese* suggests that the gene is located in a cluster of open reading frames encoding low sulphur containing proteins, including a flavin reductase, which may provide reduced flavin for Ese mono-oxygenase activity. The cluster of open reading frames was organised into two distinct operon structures located on opposite strands, flanked on one side by an ORF encoding a divergently orientated putative transcriptional regulator.

A secondary aim of this project was to isolate, identify and characterise a pure bacterium capable of metabolising thiobencarb. A different *Arthrobacter* strain was identified with thiobencarb degradative activity. This strain performed the partial mineralisation of thiobencarb under a range of conditions, exhibiting no catabolite repression. The strain was also capable of degrading chlorobenzoates and chloroaromatic metabolites of thiobencarb degradation.

### 1.4 Groundwater management at the regional scale

**Sub-Program Leader:**

Dr Shahbaz Khan  
CSIRO Land and Water, Griffith/  
Charles Sturt University, Wagga Wagga

The aim of this Sub-Program is to develop tools and guidelines, which can be used to manage shallow watertables and soil salinity. The tools developed under these projects will be able to represent the complex interactions between climate, irrigation, crops, soils and groundwater dynamics and salt transport at paddock, farm, irrigation area and regional scales. The specific goals are:-

* estimation of salt transport and salinisation from farm to regional scale;

* development of options and strategies for managing groundwater systems in rice-based areas;

* development of management tools to assist water policy reform in rice-based irrigation areas; and

* geophysics imaging of channels to quantify seepage losses from channels.

A summary of the key outputs of this Sub-Program is given below.

The following tools have been developed:

* Farm scale water and salt dynamics model.
* GIS and web-based SWAGMAN Farm model.
* Net recharge credits model of the Coleambally Irrigation Area (CIA).
* Surface-groundwater interaction model of the Murrumbidgee Irrigation Area (MIA).
* Groundwater models of the Wakool Irrigation District.
* Water and salt balance of the MIA, CIA and the whole Murrumbidgee Catchment in association with Pratt Water Project.
* A new methodology for quantifying seepage losses from channels.

**Risk based spatial modelling to identify regional soil salinity trends in irrigation areas (1403a)**

**Project Leader: Dr Shahbaz Khan**
**PhD Student: Ms Louisa Best**
**CSIRO Land and Water**
**Griffith**

**Objectives**

Soil salinity in irrigation areas undergoes large temporal and spatial variations and is affected by a range of factors. Conventional salinity analysis techniques assume average parameters to forecast spatial and temporal trends of soil salinity in irrigated areas, and therefore fail to provide comprehensive understanding of mechanisms and best management practices. Existing integrated surface and groundwater models need to incorporate stochastic (parametric uncertainty and spatial variability) and risk-based salinity assessment techniques. Specific objectives of this PhD study being undertaken by Louisa Best are to:-

* review and document existing spatial salinity estimation techniques used in the irrigation areas of the Murray-Darling Basin Commission;
* estimate uncertainty of identified physical spatial and temporal parameters responsible for salinity risk in irrigated areas; and
* incorporate uncertainty into existing deterministic salinity risk prediction model using stochastic modelling techniques.

**Progress**

Ms Louisa Best has completed all the field work and has done risk-based salinity modelling work. This PhD has been delayed due to maternity leave by the PhD student. Ms Best is currently writing the PhD thesis which will be submitted early 2006. A summary of the key achievements is provided below.

**Field work:**

* All soil sampling and EM-38 monitoring has been completed. Two intensively monitored sites have had soil samples and EM-38 measurements taken on a monthly basis from September, 2001 to April, 2002, and then bi-monthly from June until December, 2002. Six less-intensively monitored sites have had soil samples and EM-38 measurements twice - pre-irrigation season (September, 2001) and post-irrigation (April, 2002).
* Downloading of loggers in piezometers and test wells has been completed. All sites have been monitored for their watertable and piezometric depths on at least a one-monthly basis.

Analyses:

* EC1:5’s have been completed.

* ECe’s analysis is finished. Relationship between EM-38 and EC1:5 measurements are developed for use in the SWMS_2D modelling process.

* Hydraulic properties for all sites have been measured at three depths to 1m at all sites (moisture characteristics, hydraulic conductivity, bulk density).

* Particle size analysis for each site at 3 depths to 1 m was also done.

Modelling:

* Successful calibration of all sites using SWMS_2D has been done. Ms Best has developed a stochastic spatial framework for analysis salinity trends.

Key results:

Ms Best’s work was presented on June 21, 2005 in a Salinity workshop at Coleambally, NSW. This workshop was attended by representatives of the irrigation companies, Catchment Management Authority and DIPNR. Her results clearly showed that rice rotation is important for maintaining lower groundwater and soil salinity. Poor irrigation management under other crops can result in major salinity increase in the top two meters of soils. More efficient irrigation methods may lead to salinity increase in the unsaturated zone above the shallow watertable. Non-irrigated parts of the CIA show high salinity levels and changes over time. It is important to maintain minimum irrigation intensity to manage these sites. The outputs of this research will have far reaching consequences on where to monitor salinity, choice of irrigation method and minimum irrigation levels to be maintained for keeping salinity below dangerous thresholds.

Risk-based irrigation demand management under system constraints (1404)

Project Leader:
Dr Shahaz Khan
CSIRO Land and Water
Griffith

Project 1404 has become a consolidation of Projects 1201, 1205, 1501 and 1403 to review/revise regional groundwater management of Land and Water Management Plans of Coleambally, Murrumbidgee and Murray Irrigation Limited.

Objectives

* Analyse outputs of Projects 1401 (regional groundwater management), 1403 (quantifying climatic and management impacts on watertables and soil salinity), 1201 (optimising
agronomic options at the farm level) and 1205 (crops after rice) to assess their use for salt management in rice growing areas.

* Run management scenarios using the models developed within these projects to provide an understanding of how these models can value add to decision support systems in the rice-based cropping systems.

**Progress**

* Synthesised key findings of projects in a CRC report and into Land and Water Management Plan review documents.

* Determined applicability of models and overall methodologies for rice-based cropping systems in the Coleambally, Murray and Murrumbidgee Irrigation Areas.

* Defined net recharge limits in the Coleambally Irrigation Area.

* Scenarios analysis to quantify how on-farm and regional water saving options can help improve groundwater and salt management from farm to catchment levels - provided key inputs into the Coleambally Land and Water Management Plan review, Pratt Water Study and Murray Groundwater Management investment decisions in Wakool.

* Integrated project outputs with the Pratt Water Study in the Murrumbidgee catchment to quantify water saving options from farm to irrigation system levels.

Key findings from scenario analysis are summarised below.

A calibrated surface and groundwater interaction model was used to simulate possible water saving scenarios. Using the existing groundwater conditions at September 2000 as "initial conditions", a number of future scenarios were studied to simulate the future groundwater trends under the MIA in response to different water saving options. A stress period length of 18 days was used to enable simulation of off-irrigation and on-irrigation seasons with a computational time step of one day. The regional groundwater model was used to simulate the hydrodynamics of groundwater flow up to the year 2025 under five scenarios:

- scenario 1 - average conditions (similar to 1998-99) continued for the next 25 years;
- scenario 2 - relatively dry conditions (similar to 2001-02) continued for the next 25 years;
- scenario 3 - relatively wet conditions (similar to 1992-93) continued for the next 25 years;
- scenario 4 - reduction in recharge using “high tech” water application techniques continued for the next 25 years; and
- scenario 5 - piping of all channels in the MIA.

The surface-groundwater interaction analysis for the whole of the MIA shows that watertables will drop by 0 to 2 meters but then will fluctuate around a new equilibrium stage within less than five years. This is mainly due to the limited outflow capacity of the aquifers (<20,000 ML/yr groundwater outflow). Drop in groundwater levels due to improved irrigation efficiency will result in decrease in capillary upflows by more than 70,000 ML which will
need to be supplied through additional irrigation. The key benefit of this reduced capillary upflow will be reduced rate of salinisation of soils.

The MIA surface and groundwater management model has been integrated with channel seepage losses studies which has resulted in a new methodology for tracking unaccounted flows and upscaling irrigation water use efficiency. This methodology was adopted by the Pratt Water Project and now CSIRO’s Water for a Healthy Country Flagship program has decided to invest in this methodology to be replicated across the Murray-Darling Basin. This project will provide major inputs into the National Water Initiative aimed at securing 500 GL (MCM) water for environment, largely through irrigation efficiency improvements. The water savings identified by the project will become the basis for infrastructure investments in the Murrumbidgee catchment.

The CIA groundwater model was used to define net recharge limits on a sub-regional basis. This work has provided key inputs into a Market-Based Instruments project for net recharge trading and updating net recharge targets for the CIA Land and Water Management Plan.

**Continuous salinity imaging along canals and drains (1405)**

Project Leader:  Dr Noel Merrick  
PhD Student:  Mr David Allen  
National Centre for Groundwater Management  
University of Technology Sydney  
Broadway

This project is being undertaken by PhD student Mr David Allen.

**Objectives**

* Extend the development of an existing prototype system for continuous conductivity surveying under irrigation canals and drains.

* Write software for rapid processing, display, interpretation and archiving of the image data.

* Perform field trials at sites with Idaho seepage meter, land-based resistivity and electromagnetic control (CRC Projects 1401b and 1107).

**Progress**

* Construction and testing of towed TEM array – transient electromagnetics.

* Software development - Hydro-Geo Imager Software.

* Experimentation with remote controlled airboat.

* Water sealing of electronic components, to reduce current leaks and array crosstalk.

* Completed processing of three years of CRC field data for inclusion in PhD thesis.
Outcomes

Final field survey at Stott’s Farm (Whitton, NSW) through to Murrumbidgee River. Many advances in software development - variable thickness layer inversion, smoothness constrained inversion, sub-noise inversion code, borehole log imaging.

Full details will be included in a thesis, once accepted.

Project Success

Successful testing of towed TEM array for use on land.
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<td>Measurement of soil suitability</td>
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<td>Better prediction of groundwater recharge</td>
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<td>- review indices of recharge</td>
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<td>- experiments</td>
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<td>Water management practices on irrigation farms</td>
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<td>Losses from farm channels</td>
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<td>Remote sensing of crop types</td>
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<td>- appoint student</td>
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<td>- review current methods</td>
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<td>Not achieved. Project Scientist appointed 2000</td>
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<td>Classification of irrigated soils by remote sensing</td>
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<td>- review</td>
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<td>- develop derivative classification maps</td>
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<td>Classification of irrigated soils by remote sensing</td>
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<td>1.2 Net recharge management</td>
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<td>On-farm agronomic options</td>
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<tr>
<td>- model testing and refining</td>
<td></td>
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<td></td>
<td>Achieved prior to Yr.7</td>
</tr>
<tr>
<td>- economic model developed</td>
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<td>Achieved prior to Yr.7</td>
</tr>
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<td>- options assessed</td>
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<td></td>
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<td>Achieved prior to Yr.7</td>
</tr>
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<td>Improved soil salinity assessment and prediction model</td>
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<td>Achieved prior to Yr.7</td>
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<tr>
<td>- model testing and refinement</td>
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<td>- development and application of new models</td>
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<td>Improved water use efficiency</td>
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<td>Achieved prior to Yr.7</td>
</tr>
<tr>
<td>- field trials complete</td>
<td>X ✓</td>
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<td>✓</td>
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<tr>
<td>Improved water use efficiency</td>
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<tr>
<td>- field trials complete</td>
<td>X ✓</td>
<td></td>
<td>✓</td>
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<tr>
<td>Crops following rice</td>
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<td>- survey</td>
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</tr>
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<td>- field monitoring/trials</td>
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<td>Analysis &amp; writeup in progress</td>
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<td>- alternative crops</td>
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<td>X Not commenced - project deferred</td>
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<td>Analysis &amp; writeup in progress</td>
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| **MILESTONES**                                |      |      |      |                 |

35
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<td>Compaction options</td>
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<td>- alternative techniques assessed</td>
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<td>1.3 Surface drainage management</td>
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<td>Appointment of staff</td>
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<td>Achieved prior to Yr.7</td>
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<td>Downstream impacts on the environment</td>
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<td>X ✓</td>
<td>X retention times confirmed</td>
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<td>- development of techniques to minimise pollutants</td>
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<td>X done in a non-CRC project by CSIRO</td>
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<td>On-farm management options</td>
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<td>Achieved prior to Yr.7</td>
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<td>- desktop study completed</td>
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<tr>
<td>- field trials to assess technology</td>
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<td>- FILTER technology assessed</td>
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<tr>
<td>- DSS system developed</td>
<td>X Not achieved, project commenced late 2000</td>
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<td>Regional management options</td>
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<td>- redundancy of measures defined</td>
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<tr>
<td>- region-wide options defined</td>
<td>X Not achieved and no plans to do this</td>
<td>X Not achieved and no plans to do this</td>
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<td>Smarter farming systems</td>
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<td>- residual toxicity definition</td>
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<td>X field tests evaluated in non-crc projects by CSIRO</td>
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<td>- rapid tests developed</td>
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<td>X Not achieved and no plan to do this</td>
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<tr>
<td>- farming system proposals developed and extended</td>
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<td>1.4 Groundwater management at the regional scale</td>
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<tr>
<td>Estimation of salt transport and salinisation</td>
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<td></td>
<td>Achieved prior to Yr.7</td>
</tr>
<tr>
<td>- recharge estimation and mapping</td>
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<td>- transport models</td>
<td>X ✓</td>
<td>X Not achieved and no plan to do this</td>
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<tr>
<td>- nutrient export and management</td>
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<td></td>
<td>Water allocation prediction model</td>
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<td>- deep groundwater pumping options</td>
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<td>Management model to assist reform</td>
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<td>X ✓ continuing</td>
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<tr>
<td>- allocation strategies optimised</td>
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<td>X ✓ salt only</td>
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<td>- integrated surface &amp; groundwater models developed</td>
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<td>X salt included, not nutrients</td>
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<td>- salt and nutrient strategies included in models</td>
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<td>Milestones for additional Year 8</td>
<td>Year 8 – 2004/2005</td>
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<td>---------------------------------------------------------------------</td>
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<td><strong>Remote sensing:</strong></td>
<td>✓</td>
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<tr>
<td>• Transfer of technology to irrigation companies of remote imaging</td>
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<tr>
<td>techniques developed in the Rice CRC for administration and for</td>
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<td>summer crop mapping.</td>
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<td></td>
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<tr>
<td>• Incorporation of hyperspectral image mapping into crop</td>
<td>✓</td>
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<tr>
<td>management practices for precision management.</td>
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<tr>
<td>• Consolidation of the various streams of remote sensing for use</td>
<td>✓</td>
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<tr>
<td>by farmers and agribusiness.</td>
<td>✓</td>
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<td><strong>Water quality on farm:</strong></td>
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<tr>
<td>• Incorporation of research outcomes into the RGA Environmental</td>
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<tr>
<td>Champions Program.</td>
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<tr>
<td>• Preparation of improved guidelines for on-farm rice pesticide</td>
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<td>management in a form that is practical and readily available to</td>
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<td>growers.</td>
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<td>• Deliver a risk assessment index specifically tailored for</td>
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<td>pesticide management for the rice industry in a format that can</td>
<td>In progress</td>
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<tr>
<td>be recognised in QA programs such as the RGA Environmental</td>
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<td>Champions Program.</td>
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<td><strong>Irrigation research:</strong></td>
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<tr>
<td>• Consolidation of past research outputs to develop scenario</td>
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<td>plans for groundwater and salt management from farm to catchment</td>
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<td>level.</td>
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<td>• Development of a web-based water allocation model and alternative</td>
<td>✓</td>
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<td>options for demand management within irrigation areas.</td>
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<tr>
<td>• Development of recommendations for temporary draining of rice</td>
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<tr>
<td>fields to improve yield and water productivity.</td>
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</tbody>
</table>

X = To be completed (in some cases this exercise is spread over several years).
✓ = Achieved (if not achieved, status provided.)

NB: After obtaining approval from the CRC Secretariat, comments on milestones for Years 1 to 3 inclusive have been removed from this table. Please refer to previous Rice CRC Annual Reports if you wish to view this information or contact the Rice CRC for additional information.
Outcomes from Program 2 will lead to longer term improvement in sustainability of the Australian rice industry, including improved productivity, better use of resources, improved water productivity and improved profitability.

Some of the main highlights include:-

- Definition of the impact of rice growing on soil physical and chemical properties. This was a benchmark study that will provide important information for analysis of better rice management.

- Development of a soil nitrogen test. The test could not be used to provide recommendations across all fields but proved a valuable tool to measure variability within fields. This will be valuable in precision management.

- Better description of the effect of cold on rice, including the interaction between nitrogen and cold; mechanisms of cold damage; and development of cold tolerance selection techniques.

- Demonstration of the potential for raising grain iron and zinc levels through topdressing.

- Improved understanding of straighthead disorder in rice.

- Description of the distribution of wild relatives in rice in Australia and development of a database on the literature of wild relatives.

- Identification of two new diseases of rice in NSW (sheath spot and aggregate sheath spot); demonstration of their importance; and proposed management measures for minimising damage.

- Risk models for “rice blast” disease and a demonstration of its potential as a threat in Australia.
Rice CRC Program 2 has addressed strategic issues relating to the long-term sustainability of rice production over the past eight years. The objective has been to develop a comprehensive understanding of the mechanisms operating in the soil, plant and biological environment that could be manipulated to achieve sustainable high grain yield with high quality, while minimising the impact of intensive rice production on the environment. Ultimately, the sustainability of the rice industry will be judged by its ability to produce more yield per megalitre (ML) and use less water per hectare while not compromising the quality of the produce, most of which is exported, nor jeopardising the no-rice portion of the landscape.

Program 2 has made significant progress in understanding the issues which impact on the sustainability of rice production; has provided a basis for more appropriate management of rice crops; and highlighted questions and opportunities which will prompt future research. The gains in productivity will enable rice producers to meet some of the challenges set by COAG as part of the National Water Agenda.

Program 2 in this CRC has laid the foundation for significant reductions in water requirements per tonne of rice through improved tolerance to cold, a better understanding of nutrient balances in rice-based systems, and options to chemicals to control weeds and pests - safeguarding biodiversity and economic returns.

Specific goals addressed included:-

* an improved understanding of the changes in soils used to grow rice to achieve higher yields per unit of water and fertiliser input;

* an enhanced understanding of the ability of the rice plant to respond to changes in its environment, specifically rice which can withstand minimum temperatures 4ºC lower than current varieties at the reproductive stage;

* the development of reliable rapid tests for nitrogen, nutrients and salinity in rice soils;

* the evaluation of germplasm which will enhance the quality of rice produced in the Australian environment;

* development of a field technique to grow grains with predetermined mineral or quality traits; and

* decreased dependence on agricultural chemicals for weed and insect control and to maintain comparative freedom from major pests and diseases.
2.2 Crop management in relation to environmental change

Sub-Program Leader:
Dr Ranjith Subasinghe
NSW Dept Primary Industries
Yanco

Temperature at the reproductive stage is the most important contributor to the yearly variation in grain yield, with cool night temperatures prior to flowering drastically reducing yields. The average commercial rice yield in 1996, for example, was only 6.5 t/ha, compared with the record 10.3 t/ha in 2003. Some crops in 1996 yielded less than 1 t/ha – a devastating result for those growers. Irrigation water is used to protect rice from cold. During the sensitive reproductive developmental phase, rice growers are advised to increase the depth of water in the crop to at least 20 cm. The aim was to maintain the temperature of the developing panicle above 18°C.

Cold at the reproductive stage particularly affects pollen development. The most sensitive stage is understood to be the early microspore stage, just following pollen mother cell meiosis when single pollen grains are just beginning to fill with starch.

Rice CRC projects in this Sub-Program were particularly aimed at understanding the response of rice to cold at the reproductive stage so that yield stability from year to year can be achieved.

Cold physiology at the plant level (2201)

Project Leader:
Dr Ranjith Subasinghe / Ms Kathryn Fox
NSW Dept Primary Industries
Yanco

Objectives

The aim of the project was to develop a screening technique at flowering to identify low temperature tolerant rice cultivars using a cold water facility.

Progress

The 2004/2005 Cold Water Trial has been successful in differentiating cultivars that are sensitive from those that are tolerant to low temperatures. Two experiments were conducted simultaneously. The first experiment was set up to standardise the time of exposure of rice plants in cold water. The second experiment aimed to standardise the nitrogen (N) rate applied to rice plants so that cold damage is induced. Ten cultivars from various origins (including Australian and international cultivars) were tested using the cold water (bore) facility for experiment one. Both known sensitive and tolerant cultivars were included to determine the correct time of exposure. Three medium grain cultivars (Amaroo, Reiziq and M103) were tested for the N rate experiment.

The plants were subjected to cold treatment by exposing them to a constant water temperature of 19°C and a water depth of 30 cm. There were four exposure times for experiment one
including: (1) panicle (20 mm) to head emergence; (2) panicle (20 mm) to booting; (3) panicle (50 mm) to head emergence; and (4) panicle (50 mm) to booting. All four exposure times ensured the plants were subjected to cold treatment during the critical early pollen microspore stage. The normal exposure time (panicle initiation to head emergence) was used for experiment two.

No conclusion can be made yet as harvesting, data collection and analysis are being continued. However, visual observations have revealed that genotypic variation for spikelet sterility has been achieved. This observation confirms that the application of the cold treatment was successful.

Project success

This project has made significant contributions to the understanding of the impact of cold conditions on rice production and, more importantly, the challenge to increase water use efficiency.

Screening reproductive-stage cold tolerance for the NSW Rice Improvement Program (2206)

Project Leader:
Dr Russell Reinke
NSW Dept Primary Industries
Yanco

Objectives

The principal objective of this project was to establish a repeatable and reliable screening methodology for early generation rice breeding populations. The specific objectives for the series of experiments conducted at both Yanco and Deniliquin during 2004/2005 were as follows:

Deniliquin facility

* Investigate the influence of pot structure on N concentration in the plant at PI.

* Investigate the effect of delaying permanent water application after fertiliser application on plant N concentration at PI.

* Determine if these variations in methodology from the original protocol developed for the Deniliquin facility can influence floret sterility.

Yanco facility

* Determine if there is a more appropriate rate of N fertiliser when applied in a diluted form to the small pots in which only one plant is cultivated.

* Screening of F5 plants having with a least one cold tolerant parent.
Progress

Three experiments were conducted, one in the Deniliquin facility and two in the Yanco facility. The experiments were spread across the two facilities due to continuing problems achieving significant levels of floret sterility in the Deniliquin facility and due to infrastructure requirements to run the experiments concurrently.

Previously a small observational study with two pre-flood nitrogen (N) application rates indicated low levels of N uptake at Panicle Initiation (PI) regardless of N rate. To investigate this outcome the experiment in Deniliquin used different types of pots and also varied the time from fertiliser application to permanent water application. Data was recorded for plant growth duration, PI N concentration and floret sterility as well as temperature throughout the treatment period of development.

Another N experiment was conducted in the Yanco facility in which four different N rates were applied to determine if, in smaller volumes of soil than had been used previously, there is a more appropriate N application rate. Data was recorded for plant growth duration, PI N concentration, leaf N concentration at anthesis, pollen number, floret sterility as well temperature during the treatment period of plant development.

The final experiment in the Yanco facility was the screening of the F5 populations which have been bred with at least one cold tolerant parent. These populations have progressed through F4 screening in the Deniliquin facility and originated from F3 head selections made in the field.

Outcomes

Sample processing for N concentration analysis and measurement of floret sterility is currently underway so results from this series of experiments cannot be presented at this time.

Project success

Analysis of the work from this project will provide an understanding of criteria that have the ability to influence floret sterility in rice plants screened for low temperature tolerance during the reproductive stage of plant development.

2.3 Mineral nutrition and grain quality

Sub-Program Leader:
Prof Graeme Batten
Charles Sturt University
Wagga Wagga

Rice yield, grain quality and human nutrition are all influenced by the minerals available to plant roots and taken up by the plant. There are indications from intensive rice farms in the Murrumbidgee Irrigation Area (MIA) that mineral deficiencies may be impacting on some quality attributes as well as on yield. A better understanding of factors which influence the uptake and translocation of nutrients within the rice plant, especially to the grain, will place the industry in a better position to improve rice yield potential, improve grain quality, and
minimise the impact of rice production on the environment, while meeting the standards demanded by consumers.

The projects in this Sub-Program have examined genotype, environment and management options that impact on production and quality.

**Rice mineral requirements (2302)**

**Project Leaders:**
Prof Graeme Batten and Dr Lindsay Campbell  
Charles Sturt University / University of Sydney  
Wagga Wagga / Sydney

**Objectives**

* Generate nutrient balance models for plant-essential nutrients as aids to crop management and sustainable cropping.

* Develop a protocol to understand the disorder known as “straighthead”.

* Raise the concentrations of iron and zinc in grain to improve its value as a food for humans.

* Evaluate rice production in systems where rice has been grown for extended periods.

**Progress**

In this project we have developed a nutrient balance model which summarises the impact of rice cropping on soil nutrients. The main concerns highlighted from this model are that, on average, all soil nutrients (except sulphur and calcium) are being depleted. This work should alert rice growers to the potential for yield losses due to nutrient depletion. A plant nutrient diagnostic protocol is still required for Australian rice varieties.

A protocol has been developed, and tested over two seasons, to induce the yield-reducing disorder known as straighthead. This will facilitate the design of studies aimed at understanding the cause of this problem. The current theory being tested is that micronutrient deficiencies, eg - copper or zinc, cause the problem. Further testing is required to confirm the findings made up to now.

This project has also demonstrated for the first time that grain Fe and Zn can be increased in rice grains by as much as 44% and 26% respectively following applications of these elements in foliar fertilizers. During this study the value of non-contaminating grain processing equipment for use in the study of micro-nutrients in rice was also demonstrated.

Variation in germplasm is seen as an asset to the breeding program. A literature review of world data from non-cultivated species of the genus *Oryza* has been assembled and will be a valuable source of information for plant breeders and other scientists seeking specific traits. During this study a new taxonomic key has been developed to aid the correct identification of the 5 *Oryza* species which are found in Australia. The threat of diseases carried by some of these species can now be assessed.
Rice accumulates phosphorus (P) to about 0.35% by weight in brown grains. As 85% of Australia’s rice is exported, germplasm was sought to reduce this loss. Samples obtained from a long-term study in Japan clearly demonstrated the impact of P-deficiency on grain yield and grain quality (low P, K and Mg concentrations). Preliminary studies were made at Yanco (NSW) of the mutant rice known as lpa\(^{-1}\). The key feature of lpa\(^{-1}\) is that it deposits more phosphorus into inorganic P but less into organic or phytate P in the grain. We suggest that the line lpa\(^{-1}\) should be incorporated into high yielding Australian rices to produce a rice which could provide a better nutrient intake for humans and monogastric animals.

Linkages have been established with the Yezin Agricultural University in Myanmar and the rice program of the Central Agricultural Research Institute. These linkages have the potential to boost our understanding of the nutrient requirements of rice under long-term cultivation and also provide access to cold-tolerant germplasm from regions with higher altitudes.

**Outcomes**

* Nutrient balance tables have been distributed to the industry and will be published in the nutrition chapter of the manual “Production of Quality Rice in South-Eastern Australia”.

* A protocol which will facilitate the study of the disorder known as straighthead.

* Evidence that rice Fe and Zn density can be manipulated by foliar application of trace elements and that P supply regulate the deposition of K and Mg, as well as P, into the grain.

* A key to the *Oryza* species found in Australia.

* Linkages with scientists in Japan and Myanmar have enhanced our understanding of the impact of rice on the environment.

**Project success**

This project has achieved its objectives by showing:

* the impact of rice production on the soil resource;
* the value of rice to human nutrition can be influenced by management; and
* biosecurity issues have been highlighted by this project.

### 2.4 Sustainable crop protection

**Sub-Program Leader:**

Dr Ric Cother  
NSW Dept Primary Industries  
Orange

Existing crop protection can be maintained in the long term by modifying practices so that the development of resistant pest populations is delayed or avoided altogether. Awareness of disease risks, existing “sleeper” diseases and potential improvements in weed control improve our ability to sustain the rice production system.
Sustainable pest and disease control strategies (2403)

Project Leader:
Dr Ric Cother
NSW Dept Primary Industries
Orange

In August 2004, a competitive grant from the Australia-Korea Foundation funded a visit to Gyeongsang National University in Jinju, Korea by Dr Ric Cother and Assoc Prof Gavin Ash. A survey of Alismataceae weeds in southern Korea was undertaken to obtain isolates of the fungus *Plectosporium tabacinum*, a potential biocontrol agent for several weeds in NSW rice crops. Prof Young Ryun Chung had organised visits to seven sites in southern Korea where *Sagittaria trifolia* was thought to occur. Unfortunately, typhoon activity restricted our visits to only three sites. During these field inspections, the opportunity was taken to inspect rice crops for blast and bakanae, two exotic diseases of rice not present in cultivated rice in Australia.

Outcomes

Forty-five cultures of *Plectosporium*–like fungi were returned to Australia (under AQIS quarantine permits). Five isolates were tested for pathogenicity to *Alisma lanceolatum*, *Damasonium minus* and *Sagittaria graminea* but none were as virulent as local isolates from *A. lanceolatum*.

Pest Risk Assessments and Diagnostic Manuals were prepared (as a draft) for Plant Health Australia for blast, kernel bunt and panicle blight diseases.

Project success

This project has enhanced our understanding of rice diseases and set in place the ability to recognise and respond to threats to sustainable production.

Conservation biology of the Southern Bell Frog in irrigation areas (2408)

Project Leader: Prof Alistar Robertson
PhD Student: Ms Skye Wassens
Charles Sturt University
Wagga Wagga

Objectives

Declining biodiversity in rice growing areas is of great concern to land managers and rural communities. This project aims to create a framework for the integration of threatened species management and biodiversity conservation into successful rice growing practices. The project focuses on the Southern Bell Frog (*Litoria raniformis*), one of a number of threatened species that inhabits rice-growing areas in NSW.
Progress

All field work and laboratory components of this project were completed in July 2004. The final report is due for completion in August 2005.

Outcomes

The project has made a significant contribution to our broader understanding of the ecology and biology of Southern Bell Frogs in Australia with respect to:-

* the historical distribution and patterns of decline in NSW;
* dispersal and movement patterns of the Southern Bell Frog; and
* habitat requirements in irrigation areas.

Findings from this project have been incorporated into:-

* the NSW Southern Bell Frog Recovery Plan;
* the National Southern Bell Frog Recovery Plan; and
* on-farm habitat enhancement projects as part of the Wetlands Incentive Scheme managed by DIPNR.

Project success

This project has enhanced the image of the Australian Rice Industry with respect to its concern for environmental within which it practices.

Stem diseases in rice in south-eastern Australia (2409)

Project Leader:
Dr Gavin Ash
Charles Sturt University
Wagga Wagga

Objectives

The aim of the project is to investigate current diseases of rice with a focus on Aggregate Sheath Spot and Sheath Spot.

Progress

This study was conducted by Dr Vincent Lanoiselet as part of his PhD project at Charles Sturt University. His thesis has now been published. His conclusion were:-

“Aggregate sheath spot and sheath spot of rice were found in south-eastern Australia in 2001. A disease survey revealed that both diseases were well distributed within the Australian rice growing areas and that disease severity could be relatively high in some crops. Aggregate sheath spot and sheath spot of rice were the only leaf sheath diseases of rice found during this
study. None of the other *Rhizoctonia* spp., known to cause rice leaf sheath diseases, were found during the entire research project.

In this study, two fatty acid analysis protocols were investigated for their utility to characterise and differentiate *R. oryzae* and *R. oryzae-sativae* isolates from four different countries. Only the modified MIDI method permitted a clear differentiation between the two species, regardless of the isolates’ country of origin. This study demonstrated that fatty acids profiles, obtained by the modified MIDI protocol, have the potential to be used as an identification tool for both pathogens.

The threat both diseases represent to the Australian rice industry was assessed with two field trials. Aggregate sheath spot caused yield losses as high as 20.3% and sheath spot reduced yields by up to 10%. The field trial results also suggested that the number of grains rather than the size of the grain were affected by the diseases.

Attempts to control both pathogens with fungicides was investigated both *in vitro* and during the field trials. Pyraclostrobin and propiconazole were strong inhibitors of these pathogens *in vitro* and both fungicides significantly reduced disease development in the field, but unfortunately failed to increase rice yield.

Epidemiological studies showed that both *R. oryzae* and *R. oryzae-sativae* could overwinter as mycelium on straw debris, regardless of whether the straw was left on the ground or buried. Mycelium of *R. oryzae-sativae* present on rice straw was also found to be able to produce sclerotia, from saprophytic growth, during the overwintering period. These results suggest that overwintered hyphal fragments present in the straw debris supplement the sclerotia as a primary source of inoculum, and also highlight the importance of straw management to reduce the inoculum in rice paddocks. The effect of burning stubble on the survival of laboratory-produced sclerotia of *R. oryzae-sativae* was investigated. Experiments revealed that even if a large proportion of sclerotia present on the soil were killed, many of them survived the stubble burning regardless of whether it was a cold burn or a hot burn, and burning certainly did not eradicate the entire inoculum. It is very likely that these conclusions also apply to sheath spot and to other sclerotial diseases of rice.

Despite my findings and anecdotal evidence from farmers and district agronomists suggesting that significant yield losses caused by aggregate sheath spot and sheath spot do occur in a limited number of commercial rice fields, neither of these diseases have yet become serious problems for the Australian rice industry. The common practice of burning rice straw is thought to have prevented both diseases from becoming more of a problem in south-eastern Australia.

With the possibility of future legislation banning stubble burning, consideration must be given to finding alternative methods of disease management and means to safeguard against economic losses from the diseases. Recommendations are; further fungicide and varietal susceptibility testing, developing and integrating a screening program within the rice breeding program; training agronomists and rice growers in identification of rice diseases for early detection.”

**Project success**

This project has placed the Australian rice industry in an improved position to address current and potential threats from rice diseases and resulted in Dr Lanoiselet being appointed to the Rice Biosecurity Committee.
<table>
<thead>
<tr>
<th>Milestone</th>
<th>Year 4</th>
<th>Year 5</th>
<th>Year 6</th>
<th>Year 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Soil chemical &amp; physical properties</td>
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<tr>
<td>Appointment of post doctoral fellow</td>
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<td>X ✓ Extended</td>
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<td>Achieved prior to Yr.7</td>
</tr>
<tr>
<td>Development of N soil test</td>
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<td></td>
<td>Terminated</td>
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<td>Appointment of technical officer</td>
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<tr>
<td>Soil acidity problem definition</td>
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<tr>
<td>Definition of soil property damages</td>
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<td>X ✓ Continuing</td>
<td>X ✓</td>
<td>X ✓ Continuing to Yr.8</td>
</tr>
<tr>
<td>Evaluation of aerial video as a tool</td>
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<td>X ✓ Continuing</td>
<td>X ✓</td>
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<tr>
<td>Definition of management factors affecting nutrient recovery</td>
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<td>PhD project - spatial analysis</td>
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<td>X Continuing</td>
<td>X Continuing to Yr.8</td>
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<tr>
<td>2.2 Environmental change</td>
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<tr>
<td>Appointment of research scientist</td>
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<tr>
<td>Define flowering test for cold resistance</td>
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<td>X ✓ Continuing</td>
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<td>Achieved prior to Yr.7</td>
</tr>
<tr>
<td>Student projects</td>
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<td>X ✓</td>
<td></td>
<td></td>
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<tr>
<td>Understanding cold and Nitrogen interaction</td>
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<tr>
<td>Lipid metabolism</td>
<td>X Redefined</td>
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<tr>
<td>Understanding cellular response to cold</td>
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<tr>
<td>Application of cold studies</td>
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<tr>
<td>Studies on climate change</td>
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<td>X ✓</td>
<td></td>
<td>X ✓ Program 1</td>
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<tr>
<td>2.3 Mineral nutrition</td>
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<tr>
<td>Appointment of staff</td>
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<tr>
<td>Appointment of student</td>
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<tr>
<td>Review of factors affecting yield and quality</td>
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<td>Achieved prior to Yr.7</td>
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<tr>
<td>Development of techniques</td>
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<td></td>
<td>Achieved prior to Yr.7</td>
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<tr>
<td>Assess mineral changes</td>
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<td>X ✓ Extended</td>
<td>X ✓</td>
<td>X ✓</td>
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<tr>
<td>Assess impact of yield improvement and management changes on mineral/quality relationships in rice and its relatives</td>
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<td>X ✓ Continuing</td>
<td>X ✓</td>
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<tr>
<td>Determine mechanisms influencing translocation of mineral to grain</td>
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<td>X ✓ Continuing</td>
<td>X ✓</td>
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</tr>
<tr>
<td>Modify factors influencing quality in intensive rice growing</td>
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<td>X ✓ Continuing</td>
<td>X ✓</td>
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<tr>
<td>2.4 Sustainable crop protection</td>
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<td>Appointment of PhD student</td>
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<tr>
<td>Appointment of honour students</td>
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<tr>
<td>Biology of Arrowhead and Water Plantain</td>
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<td>RIRDC projects prior to Yr.7</td>
</tr>
<tr>
<td>Better understand biocontrol</td>
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<td>Achieved prior to Yr.7</td>
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<tr>
<td>Identification of dominant bloodworm species</td>
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<td>Achieved prior to Yr.7</td>
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<tr>
<td>Develop lab techniques for at least 1 additional Chironomid</td>
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<tr>
<td>Evaluate Bti* transgenic lines</td>
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<td>X ✓ Continuing</td>
<td>X ✓</td>
<td>X Continuing to Yr.8</td>
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<td>Allelopathy</td>
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<td>Progress towards identification of allelochemicals</td>
<td>Program 3</td>
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<tr>
<td>Improved pathogenicity of R.alsinatis to Alismataceous weeds</td>
<td>X ✓</td>
<td>X ✓ Continuing</td>
<td>X ✓</td>
<td>X ✓</td>
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<tr>
<td>Phenology and host specificity of bloodworm species defined</td>
<td>X reallocated</td>
<td>X ✓ Continuing</td>
<td>X ✓</td>
<td>X Continuing to Yr.8</td>
</tr>
<tr>
<td>Milestone</td>
<td>Year 4</td>
<td>Year 5</td>
<td>Year 6</td>
<td>Year 7</td>
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<td>---------------------------------------------------------------------------</td>
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<tr>
<td>Integration of chemical and biological management of weeds</td>
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<td>✗ Continuing</td>
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<td>✗ Terminated</td>
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<tr>
<td>Determination of susceptibility of major cultivars to exotic pests and</td>
<td>✗</td>
<td>✗ Continuing</td>
<td>✗</td>
<td>✗</td>
</tr>
<tr>
<td>diseases</td>
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</table>

### Milestones for additional Year 8

**Nutrition:**
- Importance of foliar applications of iron and zinc in raising grain levels quantified.
- Nutrient guidelines completed for sustainable rice-based farming.
- The relationship between salinity, nutrient levels and grain quality determined and disseminated.

*\(\text{Bti}\) = Bacillus thuringiensis, a bacterium with insecticidal properties.  
\(\text{X} = \text{To be completed (in some cases this exercise is spread over several years).}\)  
\(\checkmark = \text{Achieved (if not achieved, status provided).}\)  
**NB:** After obtaining approval from the CRC Secretariat, comments on milestones for Years 1 to 3 inclusive have been removed from this table. Please refer to previous Rice CRC Annual Reports if you wish to view this information or contact the Rice CRC for additional information.
A brief snapshot of achievements within Program 3 during the life of the Rice CRC

The CRC coincided with a period of rapid growth in knowledge of rice genomics and genetic control. Rice was being seen as one of the “model” plant species for genetic research. The CRC was able to capitalise on this knowledge to facilitate genetic studies into those areas likely to promote sustainability of rice production in Australia. Main highlights included:

- Description and visualisation of the effects of cold damage to pollen at the cellular level.
- Demonstration of the interaction of carbohydrate metabolism and transporter genes with plant hormones in the development of cold damage.
- Identification of putative genetic markers for selecting cold tolerance.
- Demonstration of links between genomics and proteomics for describing cold response during rice reproduction.
- Success of transformed rice seedlings in controlling bloodworm damage in rice seedlings.
- Breakthrough in microspore culture of rice and use of the technique in describing cold response in rice.
- Demonstration of new ways of measuring, categorising and describing starch components in rice. This will lead to better descriptors of quality characters and rice varieties and products of better quality.
PROGRAM 3

GENETIC IMPROVEMENT FOR SUSTAINABLE RICE PRODUCTION

Program Leader:
Dr Liz Dennis
CSIRO Plant Industry
Canberra

The aim of Program 3 is to use genetic technologies to improve the quality, productivity and sustainability of Australian rice. One focus is overcoming the reduction in yield caused by exposure to low temperature at pollen (microspore) formation which leads to decreased male fertility and lower seed set. As water is used to maintain temperatures during microspore development, production of more cold-tolerant cultivars should lead to lower water use. One of the main elements in Program 3 is the understanding of the cellular and molecular basis for cold-induced sterility.

Tolerance to biotic stress is another objective of the Program. The bacterium *Bacillus thuringiensis var israelensis* can protect rice plants against bloodworm. Transgenic rice plants expressing the native *Cry11A* toxin gene isolated from *B. thuringiensis* were produced but failed to show the production of toxin protein, presumably because this is a bacterial gene being expressed in a plant. The gene has been resynthesised with plant features and introduced into rice callus; the callus now confers resistance to bloodworm.

A number of rice varieties have been shown to suppress the growth of arrowhead, a weed of rice crops. Chemicals in rice responsible for the suppression of arrowhead growth have been analysed and a number of compounds identified which may prevent arrowhead growth.

Breeding for quality attributes is another focus of Program 3. Amylose structure, resistant starch and cooking quality are being investigated. Progress has been made in understanding the structure of amylose in rice. Factors that affect the solubility of the amylose and, hence, its ability to interact with other components of the cooked grain have been determined. Further collaborations aim to develop an understanding of the biochemical processes controlling rice starch. In particular, the techniques for characterisation of amylose and amyllopectin have been further refined in an attempt to obtain information on the molecular architecture of these molecules. These techniques will assist correlation between the physical properties and structure.

3.2 Tolerance to abiotic and biotic stress

Sub-Program Leader:
Dr Liz Dennis
CSIRO Plant Industry
Canberra

The primary abiotic stress investigated in this Sub-Program is cold induced pollen sterility, which can decrease rice yields by as much as 50% in bad years and by a lesser amount in most years. Cellular and molecular approaches are being used. The availability of cold tolerant lines from China will allow a genetic approach to enable identification of markers
that could be used in a breeding program for better cold tolerance. A gene concerned with sugar metabolism invertase has been shown to be down regulated by cold giving an insight into the molecular events occurring.

Biotic stresses investigated include attack by bloodworm, where genes conferring resistance have been isolated from bacteria and weed competition, where a number of rice lines have been screened for their ability to compete with weeds and active chemicals identified.

**Molecular basis of cold-induced pollen sterility in rice (3201)**

*Project Leader: Dr Rudy Dolferus*

*Technical Assistant: Jane Edlington*

CSIRO Plant Industry

Canberra

**Objectives**

* To study the effect of cold on sugar metabolism in rice anthers.

* To study the involvement of plant hormones (ABA and GA) in triggering cold-induced pollen sterility.

* To use microarray technology to compare the cold response between cold-sensitive (Doongara) and cold-tolerant (R31 and R32) rice varieties.

* To identify molecular markers for breeding cold-tolerant rice.

**Progress**

Abscisic acid (ABA) levels were found to increase in response to cold treatment in rice anthers. In the tolerant cultivar R31, it was found that ABA levels were lower, and the increase caused by cold treatment was also lower than in Doongara.

Injection of ABA in rice panicles at the young microspore stage result in the induction of pollen sterility; this was visualised using starch staining. It was also found that ABA injection causes repression of *OSINV4* and *OSMST8* expression, and induction of *OSMST7*. These genes changed their expression pattern in the same way following cold treatment, indicating that ABA mimics the effect of cold treatment. Results suggest that ABA is functioning as a signal used by cold to induce pollen sterility.

Investigation of the role of gibberellic acid (GA) in determining cold tolerance in rice has been initiated, in order to determine the correlation between semi-dwarfism and cold-sensitivity. The cold-sensitive semi-dwarf variety Doongara contains a deletion of the GA20-oxidase gene *SD1*, which results in lower GA levels throughout the plant and smaller plant stature. ABA and GA often act antagonistically in plant development; therefore plants with lower GA content may be more susceptible to increased ABA levels caused by cold treatment. R31 is not a semi-dwarf variety and contains the normal *SD1* gene. The *SD1* gene
from R31 has been cloned and a construct made to introduce this gene in Doongara. This will allow project staff to study in the same genetic background whether cold-tolerance can be improved by complementing the SD1 mutation, and, whether this prevents ABA accumulation to high levels. In addition, they will be able to study whether there is any effect of increased GA levels on grain quality.

Two ABA biosynthetic genes in rice anthers have been identified. One of these genes (OSNCED3) is induced by cold and shows much higher expression in Doongara than in the cold-tolerant cultivar R31. This result proves that in response to cold treatment, the anther induces its own ABA biosynthesis, and this response is stronger in Doongara than in R31. How this difference in OSNCED gene regulation occurs is currently being investigated.

Extensive microarray expression profiling has been used to compare the cold response between Doongara and the two cold-tolerant varieties R31 and R32 at different stages of pollen development, centred around the cold-sensitive young microspore stage. This work revealed extensive differences in gene expression between Doongara and the tolerant cultivars, but also between the two tolerant cultivars. A non-redundant set of 329 genes was identified after DNA sequencing, and the chromosome position of these genes was determined. The potential use of these genes as molecular markers for breeding will be investigated in the future by screening a set of doubled haploid lines (Dr Xiaochun Zhao, University of Sydney) from a Doongara R31 cross. This will allow project staff to identify linkage between the genes identified and the cold-tolerance phenotype, which may ultimately lead to the identification of a set of suitable DNA markers for breeding.

Outcomes

* Identification of the molecular basis of cold-induced pollen sterility in rice: cold causes ABA accumulation and a blockage in sugar metabolism.

* A better understanding of the molecular and physiological basis of cold-induced pollen sterility in rice will enable the design of strategies to produce cold-tolerant rice.

* The development of molecular markers for cold tolerance will greatly facilitate the breeding process of cold-tolerant Australian rice.
Microarray Expression profiling of the cold response of Doongara, R31, and R31 let to the identification of 329 non-redundant genes that are differentially expressed between the three cultivars. The graph shows the distribution of these genes on the 12 rice chromosomes (N/C = non-classified). The numbers above the bars are the number of gene present on each chromosome. Chromosome three is known to contain the strongest QTL markers for cold tolerance in rice.

**Figure 5**

*Cellular biology of chilling-induced pollen damage in rice (3202a, 3202b)/Control of callose degradation and re-use in pollen development in rice (3211)*

**Project Leader:**
Assoc Prof Bruce Sutton  
University of Sydney  
Sydney

**Objectives**

The project aims to:

* investigate and compare control of callose degradation and microspore wall formation between the anthers in different exotic tolerant and local cold susceptible rice varieties to night chilling;

* investigate effect of night chilling on temporal regulation of gene expression for sugar metabolism in anthers;

* investigate the mechanism of sugar upload to the anthers under normal and chilling night temperatures; and
* investigate the detection of cellular viability and potentiality of programmed cell death after chilling in anthers.

**Progress**

* Examination of the physiological consequences of chilling in rice continued along a number of paths.

**Outcomes**

* **Sugar metabolism:**

  Carbohydrate metabolism appears to be affected by chilling during microspore formation. Previous work of in-situ hybridisation of OSINV4 showed down regulation in stressed induced anthers. In complement to previous work, an antibody to cell wall invertase has been used to localise the invertase protein during microspore development and this showed down regulation after chilling, as well as localisation in a different site from the mRNA. mRNA in-situ hybridisation of another two key genes, monosaccharide transporters 7 and 8 (OSMST7 and 8) also shows changes in the expression after chilling.

* **Sugar transport pathway:**

  The fluorescent sugar analogue 2NBDG and indicators of membrane transport have been used to understand the changes of sugar transport pathways in chilling-induced rice anthers. It appears that chilling interrupts sugar transport pathway in the anther.

* **Programmed cell death (PCD):**

  TUNEL (terminal deoxyribonucleotidy transferase (TDT)-mediated dUTP-digoxigenin nick end labelling) assay has been employed to identify nuclear DNA fragmentation, a distinct feature of programmed cell death (PCD) in anthers. Chilling-induced anthers show PCD earlier than expected at the pollen mother cell and tetrad stages. This also suggests that a molecular signal to trigger male sterility in rice might be turned on from the pollen mother cell stage to microspore stage after chilling. RT PCR of three genes associated with PCD in anthers has also shown down-regulation in chilling induced anthers.
Features of programmed cell death

Figure 6. PCD detection in Doongara anthers (transverse sections). A,C showing control anthers at the pollen mother cell and tetrad microspore stages. B and D showing chilled anthers with PCD symptoms (arrows) at corresponding stages.

Engineering rice for bloodworm resistance (3203)

Project Leader:
Dr Peter Hughes
CSIRO Plant Industry
Canberra

Objectives

To produce genetically-modified rice capable of resisting bloodworm in the field.

Progress

The aim this project was to determine if it was feasible to control bloodworm (*Chironomus tepperi*) infestations in rice using transgenic plants expressing an insecticidal toxin. Without
control bloodworm can cause massive damage to the plant stand, up to 85% plant loss in years of high infestation. Currently bloodworm are controlled through the use of chemicals applied at the time the paddy is planted. The advantage of a transgenic plant approach is that it will eliminate, or at least severely reduce, the need for pesticide application and the consequent impact on beneficial insects and animals. Avoidance of pesticide application will improve water quality.

Project staff previously identified Cry11A as a good candidate for expression in rice to produce bloodworm resistant rice, however the native bacterial gene was not expressed well in rice plants. A synthetic Cry11A gene optimised for expression in rice was constructed by Geneart in Germany. The synthetic gene addressed a number of criteria to improve expression, the translatability of the RNA (codon bias), negative expression elements within the gene sequence and secondary structure of the mRNA. This optimised gene is designed to increase the amount of protein produced within rice plants and so obtain plants with enough toxin production to be insecticidal.

The synthetic gene was coupled to a strong constitutive promoter and transformed into rice to ascertain if sufficient expression could be obtained to kill bloodworm. Rice transformation was performed on callus, which is an undifferentiated lump of rice cells not capable of survival outside tissue culture. Ten transgenic callus lines were bioassayed at Yanco. All bar one of the calli had at least some activity against bloodworm and two look to have very good levels of activity against bloodworm. This is significant for several reasons: The expression levels achieved are able to kill bloodworm, meaning that it is possible to express enough of the toxin to be lethal to insects. The resynthesised gene retains the toxicity of the parental gene.

The large number of lines that are demonstrating toxicity indicates that even moderate expression in planta enables control of bloodworm at least in the laboratory. This gives a greater range of options for deciding on the level of expression acceptable in the field. The speed at which the insects die implies that they stop feeding quickly. In some insecticidal plants it takes days to stop feeding and die, in this time they are able to do considerable damage to the plant. With a rapid acting toxin, it is more likely that the damage caused between feeding and death will be minimal.

The demonstration of bloodworm activity is a major step forward. Plants expressing Cry11A have been generated and demonstrated to have high level bloodworm activity. All insects exposed to the Cry11Asyn transgenics were killed. Figure 7 shows the level of protection afforded by Cry11A expression in GM rice. A root specific promoter has been cloned and used to drive the expression of Cry11Asyn. Root specific expression is seen as a desirable characteristic because it limits the expression of the transgene in seed and therefore people’s exposure to Cry11A protein. Genes with potentially interesting expression profiles were identified through EST expression data in public databases, the expression pattern and level were experimentally determined using real time PCR and subsequently cloned. Expression analysis will be performed once plants are generated.

**Outcomes**

The project has delivered GM bloodworm resistant rice, Figure 7 demonstrates the degree of protection afforded by expression of Cry11A in rice against bloodworm.
Objectives

The overall aim of this thesis was to find out more about the molecular signalling processes that lead to cold-induced sterility in rice. This study focused on two aspects which have not been thoroughly explored in the literature: firstly, the effect of cold on sugar metabolism in rice anthers and its possible association with causing sterility, and secondly, the role of ABA in cold-induced sterility.

The major aim of this work was to identify and characterise genes that are involved in regulating sugar metabolism in rice anthers. Initially, biochemical measurements of anther sugar levels and invertase activity levels were performed in order to establish the effect of cold on sugar metabolism in anthers. This study then focused on the identification and characterisation of genes that encode enzymes involved in apoplastic sugar transport in rice anthers, namely invertases and monosaccharide transporters. The effect of cold on the expression of these sugar metabolic genes was examined in order to determine their possible role in causing cold-induced pollen sterility.

This thesis also aimed to investigate whether ABA-induced signalling mechanisms are involved in causing cold-induced pollen sterility. This study focused on establishing whether
ABA biosynthesis could be involved in causing pollen sterility, and explored the link between ABA signalling and sugar metabolism in anthers. ABA-mediated regulation of the expression of the invertase and monosaccharide transporter genes was studied in order to understand the cold-induced signal transduction events involved in regulating these sugar metabolic genes.

Conclusions

This study has shown that cold-induced sterility is associated with a disruption in the sugar metabolism and accumulation of ABA anthers at the young microspore stage.

The identification and characterisation of genes affected by cold treatment in rice anthers in this study could be useful in molecular marker and future transgenic approaches to breeding cold-tolerant cultivars in Australia.

The similarities between the mechanisms of cold- and water deficit-induced sterility could mean that strategies for developing cold-tolerant rice may also be useful for the development of drought-resistant rice in Australia.

The development of cold-tolerant rice cultivars in Australia will be of great benefit to the Australian rice industry.

The incorporation of cold tolerance traits into Australian cultivars will mean that consistently high yields will be maintained without the threat of yield losses by cold weather conditions. Nitrogen application will be able to be applied without the risk of cold damage. Cold-tolerant rice crops will not require such a high water level at the young microspore stage to buffer against cold air temperatures, and so growers will be able to reduce the amount of water used to grow their crop.

### 3.3 Enhancing the technology base for rice improvement

**Sub-Program Leader:**
Dr Norm Darvey
University of Sydney
Cobbitty

The main objective of this Sub-Program has been the development and enhancement of a microspore culture system for rice.

**Enhancing the technology base for rice improvement (3301)**

**Project Leader:**
Dr Norm Darvey
University of Sydney
Cobbitty
Objective

This project is being carried out by Dr Xiaochun Zhao. The main focus has been improvements to the methodology for isolated microspore culture.

Doubled haploid lines were to be produced from a number of crosses involving cold-tolerant germplasm from China.

At the molecular level, a collaborative project with CSIRO, microarrays were to be used to identify genes associated with cold tolerance.

Progress

The following was achieved during 2004/2005:

* On-going developments with the microspore culture procedure. A low number of calli regenerated green plants (up to 50 per panicle), with a moderate level of success across the majority of F1s and parent genotypes. The anther culture procedure has now been replaced with isolated microspore culture for the production of double haploid plants.

* Crossing to cold tolerant Chinese genotypes. The Australian cultivar “Doongara” has been crossed and back-crossed to cold tolerant Chinese genotypes, and some double haploid lines from these crosses have already been produced. Others are still being processed.

* Cold tolerant upland rice germplasm is being quarantined and some crosses to Australian genotypes have already been made.

* Identification of genes for cold tolerance using molecular approaches was carried out in collaboration with colleagues at CSIRO Plant Industry, Canberra. Several chromosomes have been identified by molecular mapping to carry important genetic factors for cold tolerance. Investigation of cold tolerance by microarrays has also revealed the genes involved in cold tolerance. These findings, in turn, will lead to the development of specific molecular markers for cold tolerance in breeding programs.

3.4 Breeding for quality attributes

Sub-Program Leader:
Mr Tony Blakeney
Cereal Solutions
North Ryde

The quality attributes of rice include physical descriptors of the milled white grains and characteristics of the cooking quality of rice. In this Sub-Program, research is addressing quality attributes that are difficult to measure and to breed for: amylose structure, and heritability and expression of isoforms of the genes of starch synthesis. Cooking quality is difficult to breed for since we don’t fully understand the factors that influence it. By knowing the variation in the sequences of the genes of starch synthesis, programs can be targeted to
reveal the function of those genes, both in starch synthesis, and further along the track, for cooking properties.

The findings of the projects on amylose structure and on the isoforms of the genes of starch synthesis will contribute to our understanding of cooking quality, and our capacity to breed rices that contain the quality attributes that markets desire.

**Customising molecular architecture of starch for rice quality - biochemistry aspects (3404)**

*Project Leader:
Prof Bob Gilbert
University of Sydney
Sydney*

**Objectives**

* Customisation of the molecular architecture of rice polymers to improve rice cooking quality.

* Concentrate on biochemistry and identify what are the biochemical processes controlling rice molecular microstructure.

**Progress**

PhD student, Jeff Castro, is currently finishing writing up his thesis.

Work undertaken in 2004/2005 was:-

(a) devised a general method for deconvoluting distributions from size-exclusion chromatography to obtain quantitatively accurate molecular weight and hydrodynamic volume distributions of starch, both branched and debranched;

(b) *in vitro* growth of linear starch using phosphorylase-a, and relating the molecular weight distributions from this to the enzymatic processes to obtain the first rate coefficients for these processes which do not rely on traditional low-conversion data.

**Customising molecular architecture of rice polymers for rice cooking quality - characterisation aspects (3405)**

*Project Leader:
Prof Bob Gilbert
University of Sydney
Sydney*
Objectives

* Customisation of the molecular architecture of rice polymers to improve rice cooking quality.

* Concentrate on characterisation and identify how this microstructure can be characterised and how does this architecture affect observables relevant to cooking qualities such as viscosity, glass-transition temperature and gelatinisation.

Progress

PhD student, Herbert Chiou, has completed his thesis (“Structure-Property Relationship to rice starch”) and an abstract from his report is provided below.

Rice starch, like other native cereal starches, contains both branched and unbranched polymers. Not only the proportion of these branched polymers in the starch, but their structure, can affect cooking and processing properties. A better understanding of starch structure is required to understand these properties.

In this work, various “snapshots” of starch structure and processing properties were taken and examined individually and as part of the big picture. Starch was isolated from rice grains and examined by Capillary Electrophoresis for chain length distributions, Dynamic Light Scattering for molecular size and Rapid Visco Analysis for processing properties. Various other experimental techniques were used to obtain more structural and physical information on different rice starches.

An improved purification process was developed such that the starch structure was not significantly damaged compared to the previous alkaline purification process. It was found that the chain length distribution is related to the way the starch expanded in increasingly concentrated salt solutions. Shorter chains tend to expand gradually while the presence of long chains gives an initially steep expansion. It was postulated that the expansion and contraction of the starch molecules on addition of salt is due to the formation of hydrophobic and hydrophilic domains within the starch molecule to maximise favourable interactions with the surroundings. This expansion was found to affect the viscosity in a starch-salt-water paste.

Pearson’s correlation was applied to all data collected to see if there are any linkages between structural-structural, structural-physical and physical-physical information. It was found that linkages between some “snapshots” could be physically explained to give a self-consistent picture of how starch structure is related to behaviour.
## MILESTONES

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Year 4</th>
<th>Year 5</th>
<th>Year 6</th>
<th>Year 7</th>
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<td><strong>3.1 Improved yield efficiency</strong></td>
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<td>Evaluation of hybrid lines</td>
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<td>- develop assay methods for pollen sterility</td>
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<tr>
<td>- ABA assay</td>
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<td>- insert and test useful constructs</td>
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<td>- feasibility investigated</td>
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<td>X ✓</td>
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<td>- mechanics studied</td>
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<td>- characters incorporated</td>
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<td>X ✓ In progress</td>
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<td><strong>3.3 Breeding methods</strong></td>
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<td>Isolated microspore culture</td>
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<td>- conditions optimised</td>
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<td>Achieved prior to Yr.7</td>
</tr>
<tr>
<td>- optimise response</td>
<td>X ✓</td>
<td>X ✓</td>
<td>X ✓</td>
<td>X ✓</td>
</tr>
<tr>
<td>- evaluate cultivar response</td>
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<td></td>
<td></td>
<td>X ✓</td>
</tr>
<tr>
<td>- doubled haploid production</td>
<td>X ✓</td>
<td>X ✓</td>
<td>X ✓</td>
<td>X ✓</td>
</tr>
<tr>
<td>- extension of technique</td>
<td>X ✓</td>
<td>X ✓</td>
<td>X ✓</td>
<td>X ✓</td>
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<td>Development of markers for semi-dwarf and fragrance</td>
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<td>X Continuing to Yr.8</td>
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<tr>
<td>Extension of markers to other traits</td>
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<td>X ✓</td>
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<td>Development of male sterility</td>
<td>X RIRDC project</td>
<td>X RIRDC project</td>
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<td>X Did not work</td>
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<tr>
<td><strong>3.4 Breeding for quality attributes</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appointment of research officer, PhD student</td>
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<td></td>
<td>Achieved prior to Yr.7</td>
</tr>
<tr>
<td>Appointment of a technical officer</td>
<td></td>
<td></td>
<td></td>
<td>Achieved prior to Yr.7</td>
</tr>
<tr>
<td>Evaluate factors promoting chalkiness</td>
<td>X RIRDC project</td>
<td></td>
<td></td>
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<tr>
<td>Evaluate factors promoting suncracking</td>
<td>X ✓</td>
<td></td>
<td></td>
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<tr>
<td>Evaluate novel quality characteristics</td>
<td>X ✓</td>
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## Milestones for additional Year 8

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<tr>
<th>Milestone</th>
<th>Year 8 2004-2005</th>
</tr>
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<tbody>
<tr>
<td><strong>Cold tolerance:</strong></td>
<td></td>
</tr>
<tr>
<td>• The cellular, metabolic and genetic mechanism for cold tolerance defined and disseminated.</td>
<td>✓</td>
</tr>
<tr>
<td>• Genetic markers and cold tolerance screening methods finalised.</td>
<td>✓</td>
</tr>
<tr>
<td>• Cold tolerance screening incorporated into the Australian rice breeding program.</td>
<td>Will be implemented from 2005/2006</td>
</tr>
<tr>
<td><strong>Insect tolerance:</strong></td>
<td></td>
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<tr>
<td>• Testing of options to optimise gene expression for bloodworm tolerance for targeted tissues only.</td>
<td>✓ In Progress</td>
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<tr>
<td>• Development of IPM strategy for bloodworm control.</td>
<td>✓</td>
</tr>
<tr>
<td>• Search for genes other than <em>Bti</em> toxin genes.</td>
<td></td>
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<tr>
<td><strong>Breeding technology and population development:</strong></td>
<td></td>
</tr>
<tr>
<td>• Further improvement in microspore culture technique.</td>
<td>✓</td>
</tr>
<tr>
<td>• Use of the microspore culture technique to deliver four doubled haploid populations.</td>
<td>✓ Scheduled in 2005/2006</td>
</tr>
<tr>
<td>• Incorporation of the technology into the Australian rice breeding program.</td>
<td></td>
</tr>
<tr>
<td><strong>Starch chemistry:</strong></td>
<td></td>
</tr>
<tr>
<td>• Application of CRC developed techniques into the rice breeding program.</td>
<td>✓ To be funded in 2006</td>
</tr>
<tr>
<td>• Explanation of the relationship between resistant starch and glycaemic index.</td>
<td></td>
</tr>
</tbody>
</table>

X = To be completed (in some cases this exercise is spread over several years).
✓ = Achieved (if not achieved, status provided.)

**NB:** After obtaining approval from the CRC Secretariat, comments on milestones for Years 1 to 3 inclusive have been removed from this table. Please refer to previous Rice CRC Annual Reports if you wish to view this information or contact the Rice CRC for additional information.

See Parts C & D for remainder of Report