Rice pests
and diseases

by Mark Stevens
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Foreword

The Australian rice industry is centred in the Murrumbidgee and Murray Valleys of southern NSW, and has developed an enviable reputation as the most water-efficient producer of high-quality rice in the world. Many towns in the production area benefit economically from the industry, particularly due to the milling, packaging and other value-adding activities that occur there.

Water availability is an increasingly significant issue for rice production. Drought in recent years, combined with higher demand for water for non-agricultural uses, has severely affected the industry, which has responded by supporting research on increasing water-use efficiency, and then implementing the findings of that research on a broad scale. While agronomic practices, such as repeat cropping and drill-sowing combined with delayed permanent water, have provided ‘more crop per drop’, these procedures have not been without consequences, particularly in pest and disease management. This report identifies pest and disease issues that are changing in significance in response to a suite of factors dominated by changes in agronomic practices. It also contributes to the development of new approaches for managing those pests that are becoming more problematic.

Some recommendations from this report, such as those involving varietal selection for minimising stem rot resurgence, can be implemented immediately by growers. Much of this report, however, involves studies on alternative methods of chemical pest management. Because pesticide use patterns are regulated federally by the APVMA, this work cannot be legally implemented by growers until pesticide registrations are obtained by the manufacturers concerned. In some instances, further work is required; however, for niclosamide, all developmental work has been completed and a registration application can now be made. To maintain commercial confidentiality, the scope of the work on niclosamide conducted as part of this project is only briefly summarised here, and all resulting data is presented in detail in a separate report.

This report adds to AgriFutures Australia’s diverse range of research publications. It forms part of our Rice R&D program, which aims to improve the productivity and sustainability of the industry by developing better varieties, improving crop protection and farming systems, enhancing extension services and human capital, and supporting cross-sectoral research.

Most of AgriFutures Australia’s publications are available for viewing, free downloading or purchasing online at www.agrifutures.com.au.

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Many farmers and farm managers provided direct support to this project, either by allowing us to sample their crops for armyworms or plant diseases, or by hosting one or more of the nine niclosamide trials conducted during the 2016-17 and 2017-18 seasons. We would like to thank Brian Bourke, Frank Caudero, Pete Chalmers, Ian Dahlenburg, Peter Draper, Barry Kirkup, Garry Knagge, David Lashbrook and Doug Martin and their families for allowing us access to their farms, and also Russell Ford and Antony Vagg for providing us with sites at Rice Research Australia Pty Ltd (RRAPL). Special thanks are also due to the aerial operators who sprayed the niclosamide trials for us with great precision, and also without charging us to do so – Leeton Air, Mas Agworks, Thomson Aviation, Field Air (Finley) and Field Air (Deniliquin).
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AI</td>
<td>active ingredient</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance (statistics)</td>
</tr>
<tr>
<td>APVMA</td>
<td>Australian Pesticides and Veterinary Medicines Authority</td>
</tr>
<tr>
<td>bdl</td>
<td>below detection limit</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>CIA</td>
<td>Coleambally Irrigation Area</td>
</tr>
<tr>
<td>CO1</td>
<td>cytochrome oxidase subunit 1</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>cv.</td>
<td>cultivar</td>
</tr>
<tr>
<td>d</td>
<td>day</td>
</tr>
<tr>
<td>DAA</td>
<td>days after application</td>
</tr>
<tr>
<td>DAS</td>
<td>days after sowing</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>df</td>
<td>degrees of freedom (statistics)</td>
</tr>
<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
</tr>
<tr>
<td>DPI</td>
<td>Department of Primary Industries</td>
</tr>
<tr>
<td>DPW</td>
<td>delayed permanent water</td>
</tr>
<tr>
<td>EC</td>
<td>emulsifiable concentrate</td>
</tr>
<tr>
<td>$F_{A,B}$</td>
<td>$F$ ratio in ANOVA where $A$ and $B$ are degrees of freedom (statistics)</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>ha</td>
<td>hectare</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LC$_X$</td>
<td>lethal concentration for $X$ percent of the test population</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>liquid chromatography – mass spectroscopy/mass spectroscopy</td>
</tr>
<tr>
<td>LSD</td>
<td>least significant difference (statistics)</td>
</tr>
<tr>
<td>m</td>
<td>metre</td>
</tr>
<tr>
<td>mA U</td>
<td>a measure of the activity of a proteinase solution</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>MIA</td>
<td>Murrumbidgee Irrigation Area</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>MRS</td>
<td>Martin’s Rearing Solution</td>
</tr>
<tr>
<td>$n$</td>
<td>number of units within a sample (statistics)</td>
</tr>
<tr>
<td>N/A</td>
<td>not applicable</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>NS</td>
<td>not significant (statistics)</td>
</tr>
</tbody>
</table>
nsd  no significant difference (statistics)
NSW  New South Wales
NTU  nephelometric turbidity units
P    probability value (statistics)
PCR  polymerase chain reaction
R&D  research and development
RT   retention time
SC   suspension concentrate
SE   standard error (statistics)
SPAD soil plant analysis development
  tonne
µg   microgram
µL   microlitre
µS   microsiemen (a unit of conductivity)
VIC  Victoria
WG   wettable granule
w/w  weight for weight
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Executive summary

What the report is about

Rice production in southern Australia faces considerable pressure from reduced water availability. Agronomic practices directed towards maximising water-use efficiency have increased the impact of some pests and diseases. This has occurred at a time when increased regulatory scrutiny of older generic insecticides, particularly organophosphates, threatens their future availability to rice producers.

Repeat cropping, which is used to improve water-use efficiency in the second and subsequent consecutive rice crops on individual fields, leads to an increased incidence of problems with snails and the disease stem rot. There is evidence that water-conserving techniques, such as delayed permanent water and mid-season drying, can make crops more attractive to armyworm.

Nine niclosamide trials conducted under an APVMA permit on commercial farms have generated the data necessary for a commercial partner to register this chemical for snail control in rice. For reasons of commercial confidentiality, only the scope of this work is outlined here, with the results to be provided in a separate report.

Laboratory techniques for rearing the common armyworm *Mythimna convecta* and screening new insecticides have been developed. Two compounds, emamectin benzoate and chlorantraniliprole, have been identified that should move forward to crop residue evaluation and field efficacy trials. A second armyworm species, the sugarcane armyworm *Leucania stenographa*, has been found in NSW rice crops for the first time, and appears to be a significant component of mixed populations on some farms. While the adult moths are readily identified, the caterpillars are largely identical in appearance. DNA barcoding was needed to identify individuals killed by parasitoids during the larval and pupal stages. Parasitism rates as high as 81% were recorded, emphasising the need to conserve beneficial species in the crop through the use of selective chemicals rather than those with broad-spectrum activity. Fermentation traps were effective for monitoring the activity of adult common armyworm moths provided they were placed in or adjacent to rice crops; however, they do not seem to attract adult sugarcane armyworm.

A replicated small-plot trial of fipronil as a direct spray treatment for bloodworm control was highly successful. Existing data associated with the current registration for fipronil as a seed treatment should streamline the registration process, with successful commercial trials under permit the final step needed to make this use pattern available to growers.

Field and laboratory studies on stem rot have demonstrated that inoculation of both pots and isolated stems are effective tools for screening varietal susceptibility to stem rot isolates. No Australian varieties have resistance to stem rot, although there is some variation in vulnerability, with Reiziq showing some tolerance, and Sherpa being among the most susceptible. Effective stubble management remains the key to limiting the impact of this disease.

Who is the report targeted at?

This report covers a wide range of topics, and has some components of immediate relevance to rice producers and commercial agronomists. Other sections of this project involve the evaluation of pesticides that are yet to be registered and legally available for use in rice. While growers will ultimately benefit from this research when they can integrate new products into their cropping systems, in the interim these components of this report will primarily be of interest to those involved in the stewardship of agricultural chemicals within the rice industry, and to other researchers.
Where are the relevant industries located in Australia?

Although small areas of rice are now grown elsewhere in Australia, most rice production in Australia still occurs in the Murrumbidgee and Murray Valleys of southern NSW and northern VIC. The pest and disease management issues investigated in this report are largely specific to this region, with only limited relevance to the cropping systems being developed further north. About 1,500 farm businesses are involved in rice production in the southern industry, with processing occurring locally in towns such as Leeton and Deniliquin. Production is highly variable from year to year, and the current severe drought conditions across much of eastern Australia are likely to limit the 2020 harvest to around 40,000 tonnes.
Introduction

Southern Australia is free of many of the pests and diseases that affect rice production in South-East Asia. Economically significant leafhoppers, planthoppers, and stemborers are all absent from the region, as is rice blast, one of the most significant rice diseases globally. This freedom is the result of a number of factors, including geographic isolation, effective domestic and international quarantine systems and, at least in the case of insect pests, a climate that has been characterised by cold wet winters and hot dry summers. Such a climate does not favour the establishment and persistence of tropical species. Its protective influence on southern Australia is reflected by the presence of many tropical pests in northern Australia that have restricted the viability of rice production there, but that have either not expanded their range to southern Australia or do not reach sufficient population densities in the south to cause economically significant damage.

The pests affecting rice in southern Australia have more in common with those from temperate regions, such as California, Spain and Italy, than they do with those from tropical regions. While some other insects and molluscs are known to feed on rice in southern NSW and northern VIC, only those acknowledged as causing economic losses are discussed here. These are:

- Bloodworms, most notably the rice bloodworm *Chironomus tepperi* Skuse
- The aquatic snail *Isidorella newcombi* (A. Adams and Angas)
- Armyworms, particularly the common armyworm *Mythimna convecta* (Walker)
- The rice leafminer *Hydrellia michelae* Bock
- The aquatic earthworm *Eukerria saltensis* (Beddard).

Bloodworms are the larvae of chironomid midges, small delicate flies that resemble mosquitoes. Many species occur in rice fields in Australia and throughout the world, and colonisation of flooded fields by different species often follows a distinct successional pattern (Stevens et al., 2006). Only a few species are considered to be pests, but they can decimate aerially sown and dry broadcast crops if they are present at high densities. Some species are facultative predators, and many feed on decaying organic matter and algae. None are obligate rice feeders, and all species known from Australian rice crops are also known from other environments. All species found in Australian rice are considered native to Australia, and there is no significant resistance to bloodworm attack in any Australian rice cultivar (Stevens et al., 2000). Control is currently achieved through one or two applications of insecticide shortly after sowing.

The aquatic snail *Isidorella newcombi* attacks the roots of rice plants at their junction with the stem, and can sever plants completely at ground level. *I. newcombi* is well adapted to inland riverine environments, and survives dry periods by entering dormancy in the top layer of the soil, sealing off its shell with a dried mucus plug to prevent water loss. This ability is key to its status as a rice pest, because it allows a significant proportion of adult snails to overwinter between consecutive rice crops. Dormant snails cannot survive over summer except in flooded conditions, so crop rotations are an effective form of management. However, in recent years, the overwhelming imperative of rice growers to minimise water use has led to more repeat cropping, rather than less, because repeat crops can utilise residual water in the soil profile. The only current option, other than rotation-based control, involves the application of copper sulphate; however, this is problematic because efficacy is strongly influenced by water chemistry (particularly, dissolved organic carbon levels). Copper appears to be largely ineffective against the eggs of *I. newcombi*, often necessitating a second or even a third
application during crop establishment (Stevens et al., 2014). Aside from its cost, this process has the potential to lead to unacceptable levels of copper accumulation in the soil.

Alternatives to copper for the chemical control of *I. newcombi* have been difficult to find despite ongoing screening programs. Snails are molluscs rather than insects, and due to differences in their metabolic processes, they do not respond to most insecticides. Only one chemical currently formulated for use against aquatic snails has shown activity against *I. newcombi*; that chemical, niclosamide, was first released commercially in 1961. As a consequence, it has no patent protection. In the 1970s, commercial trials against *I. newcombi* by the major global manufacturer of the product were apparently highly successful, but the product was not registered at that time because the market for it was considered to be too small. After the patent expired, the manufacturer declined to have any further involvement with the product in Australia.

In 2012, Conquest Crop Protection indicated a willingness to pursue the registration of niclosamide for use against rice snails in Australia. This led to a resumption of work on niclosamide, initially involving laboratory toxicology studies and non-target effect trials at Yanco Agricultural Institute, and building on previous studies looking at efficacy, crop residues and environmental fate. These highly successful trials led to Conquest Crop Protection obtaining an APVMA permit for limited commercial-scale trials in time for the 2016-17 rice season.

The registration of out-of-patent compounds for use in Australian agriculture can expose the registrant to significant risk, because placing the data on which the registration relies into the public domain could be seen to negate any commercial confidentiality and allow other manufacturers to quickly enter the market at minimal cost. While this may seem to foster price competition and therefore be desirable, it might actually damage the business case for the initial investment, particularly if the market is relatively small. This project involved substantial work in the 2016-17 and 2017-18 seasons directed at commercial-scale evaluation of niclosamide formulations developed by Conquest Crop Protection in order to support their efforts to make this compound available to Australian rice farmers. While the scope of this work is outlined here, the detailed results of these trials will be provided to AgriFutures in a separate report, ensuring that this report can be circulated as widely as possible among various stakeholders without compromising commercial confidentiality for niclosamide.

Armyworms have long been known as pests of rice in southern and northern Australia. While some species are known to attack rice in the north, only one species, the common armyworm *Mythimna convecta* (Walker), was thought to be present in southern rice crops. During vegetative growth, armyworm caterpillars feed on the plant foliage; however, the most severe damage generally occurs as the crop matures, when the caterpillars strip the panicles by chewing through the spikelets, causing the grain to fall into the water. For many years, the sporadic occurrence of armyworms in southern rice crops effectively precluded both the need and the opportunity to conduct any detailed research on their impact and management. From about 2010, armyworms have gradually increased in significance, with more frequent and more widespread infestations in rice crops across the Murrumbidgee and Murray valleys. The reasons remain unclear; however, agronomic practices, such as delayed permanent water and mid-season drying that lead to plant water stress, could be making the rice crop more attractive to female moths looking for oviposition sites. Climate change may also be playing a role.

Throughout the world, small flies of the genus *Hydrellia* attack rice. Eggs are laid individually on the plant near the waterline during crop establishment, and the developing larvae tunnel within the leaf, eventually killing the section distal to the ‘mine’. In southern Australia, the most abundant species in
rice is *H. michelae*. Leafminers are sporadic pests known mainly from aerially sown and dry broadcast crops. Significant leafminer damage is usually associated with prolonged cold weather during the crop establishment period. In contrast to armyworms, the incidence and severity of leafminer infestations seems to be declining rather than increasing. Armyworms and leafminers are both currently managed through aerial application of insecticides.

The aquatic earthworm *E. saltensis* is the only introduced pest of rice in southern Australia. While beneficial in most environments, within a flooded rice crop, dense populations of aquatic earthworms can cause high levels of turbidity due to their burrowing activity. The mobilisation of nutrients into the water column caused by the burrowing can lead to dense algal growth (Stevens and Warren, 2000). Turbid water remains cold and blocks light penetration, causing seedlings to die before their shoots can emerge into the light. The earthworms do not feed directly on living rice plants, and may attract large numbers of ibis that trample the seedlings while feeding. Damage has always been most severe on dispersive clay soils in the Murray Valley, and has been difficult to avoid in some areas where the formation of hard surface crusts in between flushes has prevented affected growers shifting to drill sowing, which largely eliminates aquatic earthworm damage. No effective chemicals have been found against *E. saltensis* in flooded rice crops (Stevens et al., 2016).

Ecological studies have identified ways to manage *E. saltensis* without chemicals. Uniformly shallow water and small layouts flooded and sown quickly reduce the risk of earthworm-associated seedling loss; however, the best approach to damage reduction is through crop rotations. Irrigated clover-based pastures immediately before rice lead to exceptionally high earthworm populations. Avoiding this transition by following the pasture with an alternative crop before rice strongly suppresses earthworm numbers, at least during rice establishment. While this recommendation has been consistently promoted, the decline in the aquatic earthworm problem to relative insignificance has probably been achieved inadvertently due to irrigated pastures being removed from rotations as a consequence of reduced water availability.

Changes in the way rice is produced have led to changes in the significance of particular pests and diseases. With an overarching goal of minimising water use, the southern rice industry has moved away from aerial and dry broadcast sowing back to the more traditional drill-sowing, which allows the delayed permanent water (DPW) technique to be implemented, increasing water-use efficiency. The other agronomic trend affecting pest and disease incidence is a move away from crop rotations to increased repeat cropping, where rice is grown on the same field for two or more years to take advantage of residual water remaining in the soil profile from the previous rice crop. While there are suggestions that DPW may influence armyworm populations, the major impact of these agronomic changes has been on bloodworms, snails, and the disease stem rot.

To survive, bloodworms need standing water. They have become less significant for many growers because drill sowing involves only intermittent irrigation until the plants are relatively well developed with tougher secondary roots. They often do not require treatment at all after permanent water is applied, or may require only a single chemical application. Crop rotations have no impact on bloodworm activity. The overall impact of changing agronomic practices on snail problems is not as clear, because while drill sowing limits snail activity and might induce significant mortality during drying phases, repeat cropping is known to help snail populations survive from one season to the next.

Stem rot is the most serious disease currently affecting the southern rice production area. It proliferates through sclerotia, small propagules that remain on the soil and within rice stubble after a crop is harvested. Good stubble management is critical for preventing the proliferation of stem rot,
which is particularly the case when repeat cropping is being practised. Stubble burning is the best approach for managing stem rot in repeat crops. Wet conditions during winter and spring can result in incomplete burns that lead to more disease pressure in the subsequent crop.

In this project, our laboratory and field trials were directed towards improving the management of three key pests of southern rice – snails, bloodworms, and armyworms – and the one rice disease currently present in the area that is capable of causing economic losses for growers, stem rot. In most cases, this work builds on previous projects; however, the work on armyworms represents the first work conducted on this pest in the context of its detrimental impact on rice production in southern Australia.
Objectives

To confirm that only one armyworm species (*Mythimna convecta*) is present in southeast Australian rice crops.

To quantify armyworm densities within and across rice crops to validate sampling plans and determine whether crops grown using delayed permanent water or mid-season drainage are more vulnerable to armyworm attack, and the basis for that vulnerability.

To determine the level of parasitism in armyworm populations in rice and how that may affect implementation of spray thresholds.

To develop science-based thresholds for armyworm control, incorporating larval density, larval development stage, and crop stage.

To identify newer generation pesticides for further development for armyworm control.

To evaluate duck exclusion netting for use at bloodworm trial sites, and develop fipronil and potentially other compounds for direct spray application against bloodworms

(Alternate objective: subject to APVMA permit availability, conduct commercial trials of niclosamide against rice snails.)

Determine the susceptibility of commercial rice varieties to stem rot.

Test for differences between stem rot isolates from different locations in southern NSW.

Undertake trials to investigate whether saline water increases the infectivity of stem rot.
Methodology, results and discussion

Summary: niclosamide for snail control (results confidential)

Introduction

The development of niclosamide as an alternative to copper sulphate for the control of the snail Isidorella newcombi in rice crops has been a prolonged and sporadic process. The compound was released commercially in 1961 and trialled successfully in NSW in the 1970s, but was never registered. Representations made to the main global manufacturer during the 1980s and 1990s encouraging them to register the material for use in Australia were unsuccessful, so despite knowledge of the product’s potential, it could not be made available to rice producers. The interest and subsequent involvement of Conquest Crop Protection from 2012 onwards stimulated a renewed research effort, and extensive data had to be gathered because there were no existing registrations for this active ingredient in any Australian crop. Most laboratory and small-plot data was gathered during previous projects, leaving only commercial-scale field trials to be conducted as part of this project. These trials were dependent on the APVMA granting a permit to Conquest Crop Protection, which occurred in time for the crop establishment period in 2016. It was initially intended that these trials would take only one season; however, issues with the first niclosamide formulation we evaluated unfortunately necessitated a second group of trials during crop establishment in 2017.

As discussed in the general introduction to this report, the results of our niclosamide trials cannot be included here without prejudicing the business case for product registration. However, it is acknowledged that industry stakeholders need to know the nature and extent of the work conducted to determine whether their investment in this project has been justified. The work on niclosamide as part of this project is therefore outlined below.

Materials and methods

2016-17 field trials

Five commercial-scale trials were conducted under APVMA permit PER81383: three in the MIA, one in the CIA, and one in the Murray Valley. Trial sites were obtained through direct contact with growers after liaison with the Rice Extension Team and, in some cases, commercial agronomists. All five trials sites were being sown as repeat rice crops, and, as such, were expected to harbour significant populations of snails. Small bays generally at the bottom end of each block were chosen for treatment, wherever possible.

Once a trial site was decided, we arranged treatment with the aerial operator who normally serviced that farm. With the date fixed, initial preparations on the day before spraying involved setting up a data logger with an external temperature probe to take temperature measurements at the soil-water interface every 15 minutes. It also involved taking a series of water depth measurements with a ruler while walking along a transect from the low to high side of the trial bay. Six stainless steel mesh cages were then pushed into the sediment at random locations within the trial bay, taking care not to align them with the likely flight path of the spray plane. Each cage was constructed from 3.5 mm aperture stainless steel woven wire mesh, with two edges sown together to form a cylinder 30 cm in diameter, 30 cm in height, and open at both ends. Fifteen mature I. newcombi, collected either from
the trial bay or from adjacent bays and drainage areas, were then placed in each cage. Where possible, snails carrying viable egg masses on their shells were used.

All trials were sprayed using fixed-wing aircraft. Four were sprayed with conventional CP nozzles set to deliver very coarse droplets, while one was treated using a Bickley Boom. Spray volumes varied from a minimum of 20 L/ha up to about 50 L/ha. Initial water samples were collected one hour after spraying ended. Glass tubes (25 mm diameter, 100 mm in length) were treated with Coatasil® glass treatment solution (APS Ajax Finechem, 2% w/w dimethyldichlorosilane in 1,1-dichloro-1-fluoroethane) and allowed to dry for several days. Before sampling, a tube was fitted to an aluminium pole that allowed water samples to be taken from well in front of where the researcher was standing to ensure that turbidity caused by wading across the bay was not incorporated into the sample. A transect diagonally across the aircraft flight path was sampled, with 20 subsamples taken 2-3 steps apart being transferred to a 1 L Coatasil®-treated glass bottle. The composite sample was vigorously shaken before 200 mL was transferred to a smaller Coatasil®-treated glass bottle, the mouth of which was then covered with aluminium foil and capped. The remainder of the composite sample in the larger bottle was then discarded and the process repeated, leading to three composite 200 mL samples, each representing about one-third of the full transect. Water samples were transferred to the laboratory in an insulated cooler containing ice bricks, and were then shaken again, uncapped, and about 80 mL of each sample was discarded. The foil and caps were replaced, and the bottles frozen at an angle at -20 °C until analysis. This approach prevented the samples shattering the bottles as they expanded during freezing. The same sampling procedure was repeated at 3 and 28 days post-spraying at each trial site.

Snails were recovered by hand from the cages three days after chemical application. Where possible, rice seedlings carrying *I. newcombi* egg masses were also collected from within the cages, or from adjacent areas of the trial bay. Snails and plants were placed in 400 mL capacity plastic jars containing water from the trial site. A second group of snails (22–48, depending on availability) were collected from adjacent untreated fields at each site for use as controls, and placed in water from the bays where they were collected. Labelled jars were placed in an insulated cooler and returned to the laboratory. The data loggers were also removed at this time, and when downloaded, the water temperature at time of treatment was recorded. The average water temperatures over the first 6, 24 and 72 hours were calculated. The 72-hour value represents the full period over which the snails were exposed to niclosamide in the field.

Once in the laboratory, snails were assessed for mortality. Snails were considered dead if they made no response when stimulated on the foot with a sharp probe; however, most snails at 3 days post-spraying were either alive and active, or clearly dead and decaying. Live snails were placed in 600 mL capacity beakers (one beaker per field cage plus one beaker per eight control snails) containing ~400 mL of 1x Martin’s Rearing Solution (MRS), an artificial laboratory medium known to be effective for rearing aquatic invertebrates (Martin et al., 1980). They were given a small quantity of fresh garden lettuce. The beakers were maintained for 48 hours at 25 ± 1 °C with a 15L:9D lighting cycle. Aeration was provided to each beaker with glass Pasteur pipettes connected to aquarium aerators using plastic tubing. A final assessment of mortality was made after 48 hours under laboratory conditions, and the number of egg masses produced by the snails was recorded.

Where eggs were attached to the shells of dead snails or on rice seedlings, the individual shells or cut sections of plant tissue were individually placed in 1x MRS in the wells of 12-well tissue culture plates (Cellstar®, Greiner Bio-One GmbH, Austria, each well 23 mm wide x 16 mm deep). The
number of eggs in each egg mass was recorded, and the plates were maintained under the same conditions as the beakers containing the adult snails. The eggs were monitored until all hatching ceased and the embryos within the eggs started to show obvious signs of decay (about 3 weeks in total). Egg hatch data was recorded, both as total hatchability and the proportion of egg masses in which all eggs failed to hatch. The shell heights of all snails from the trial sites were measured and, where feasible, unpaired t-tests were used to determine whether snail size affected response to niclosamide within individual trials.

Liaison with collaborating farmers continued in the period leading up to harvest to ensure samples were taken from drained crops as close as possible to the time of commercial harvesting. Plants were sampled by following a diagonal transect across the line of aircraft flight across each trial bay, with several tillers being cut with a sharp knife about 10 cm above ground level every 2–3 steps along the full transect. Soil samples were taken with an aluminium ring (9 cm diameter, 14 mm height) that was pushed into the soil. A spatula was then used to remove the soil to a depth of about 5 mm within the ring. Samples were again taken along a diagonal transect covering the full width of each trial site. From each site, 16 samples were taken, with the first and second eight being combined to form two composite samples. Plant and soil samples were bagged and transferred to the laboratory in insulated coolers. Plant samples were dried at room temperature for 48 hours and then threshed using a single-head thresher. The grain was then dehulled; the hulls and brown rice were separately ground using a Breville electric coffee grinder. Random tillers were selected for the forage samples and cut into small sections before being finely shredded with a Vita-Prep® commercial blender. Soil samples were air-dried at room temperature and coarsely crushed. All plant materials and soil samples were then stored in glass jars at -20 °C until chemical analysis.

2017-18 field trials
A further four field trials during the 2017-18 season used a different niclosamide formulation. All trials were conducted in the Murray Valley, where conditions are generally regarded as more challenging for the management of aquatic rice pests. Two trials followed the same format as those in 2016-17, including identical procedures for all residue sampling, and with additional plant dry weight comparisons against plants from adjacent bays. Two short-term ring trials used stainless steel cylinder enclosures in individual rice bays. These allowed multiple formulations and application rate to be tested under uniform conditions for soil conditions and water chemistry.

Each ring trial involved 18 stainless steel cylinders covering three replicates of each of six treatments, one of which was an untreated control. The cylinders (47 cm diameter and 38 cm height) were pushed into the sediment in a small area of a single rice bay; depths were measured individually to allow precise calculations of chemical doses. Adult *I. newcombi* were collected from an adjacent untreated area, and 12 individuals were placed in each cylinder. Data loggers were then set to record water temperature within two of the rings (Figure 1). Niclosamide formulations were diluted in deionised water, and cylinders were treated individually with an air displacement pipette. A stainless steel spatula was then used to gently mix the applied chemical into the water column. Single composite water samples were taken from each cylinder one hour after chemical application using a similar protocol to that used in the fixed-wing aircraft trials, but with fewer subsamples due to the small diameter of the cylinders.
Assessment of these trials occurred three days after chemical application. A second set of water samples was taken, and the snails were recovered by hand from each cylinder, with sediment sieving being used to recover snails not initially located. Twelve plants were also removed at random from each cylinder. The snails and plants were placed in 400 mL capacity plastic jars containing clean water from the trial site and returned to the laboratory. Plants were assessed for chlorophyll content using a Minolta SPAD 502 chlorophyll meter, before shoot lengths and dry weights were determined after oven drying at 80 °C. Assessment of snail mortality followed the same protocol used in the aircraft trials.

Charles Sturt University chemically analysed water, soil and plant residues. The LC-MS/MS methodology for the water sample analysis published in the journal *Analytical Methods* (Doran and Stevens, 2014) was used in all analytical determinations of niclosamide and its breakdown products.

Replicated laboratory bioassays assessed the influence of turbidity and dissolved organic carbon on the toxicity of niclosamide to *I. newcombi*, as well as the toxicological equivalence of two further experimental formulations relative to those used in the field trials.
Bloodworm control using direct spray applications of fipronil

Introduction

Fipronil is a phenyl pyrazole insecticide that has been in commercial use since 1993 (Tingle et al., 2003). It was recognised by Stevens et al. (1998) as having high toxicity to Chironomus tepperi, the principal bloodworm species that feeds on rice in southern Australia. It was shown to be highly effective as a seed treatment when applied to pregerminated rice immediately before aerial sowing. The fipronil seed treatment Cosmos® was subsequently registered for use on rice at the rate of 12.5 g AI/ha.

The development of fipronil as a seed treatment occurred in response to a refusal by aerial operators to continue using malathion (maldison) seed treatments because of their strong odour and the movement of fumes from the seed hopper into the aircraft cabin. Although Cosmos® largely solved these issues, seed treatments for aerially sown rice never regained their previous popularity because of delays associated with treating the seed using specialised equipment, and also because of the cost of the product. Fipronil remains a highly effective but little-used active for bloodworm control in rice.

Fipronil is no longer under patent protection, and commercial products containing this active are now substantially cheaper. In addition, the registration of Cosmos® required the establishment of maximum residue limits and allowable daily intakes for fipronil in rice. Because these parameters have been established, the development of an alternative fipronil formulation for direct spray application will be simpler and cheaper than registering an entirely new active compound. Although some other actives are currently registered for bloodworm control, all except alphacypermethrin (Dominex®) are organophosphate compounds under increasing regulatory scrutiny. Only one of them, chlorpyrifos (Lorsban®), is considered by growers to be highly effective.

Trials of fipronil as a direct spray treatment for bloodworm have been undertaken. Stevens (2000) reported the results of a trial of Regent® 300EC in the 1997-98 season, and another trial used Regent® 200SC at Yanco in 2001-02 (Stevens, 2003). Although these trials produced useful data on chemical residue issues, poor colonisation of the trial sites by the target bloodworm species mean that the efficacy data was insufficient to support an application for an APVMA commercial trial permit. In this study, we evaluated a newer fipronil formulation, Maestro® 200SC, in the laboratory for its acute toxicity to final instar C. tepperi larvae, and then conducted a replicated field trial to assess its efficacy and residue profile at rates from 12.5 g AI/ha (applied once) to 22.5 g AI/ha (applied twice).

Materials and methods

Rearing of C. tepperi larvae

C. tepperi larvae were reared using the Stevens (1992) technique. Glass aquariums containing 10 L of 1x Martin’s rearing solution (MRS, Martin et al., 1980) supplemented with thiamine hydrochloride at 1.2 mg/L were lined with ethanol-washed paper tissues to provide dietary bulk and tunnel building material. Fresh C. tepperi egg masses were collected from rainwater pools at Yanco Agricultural Institute NSW (34°37’S, 146°26′E) and added, along with a small quantity of ground TetraMin Tropical Flakes® fish food (Tetra Holding (US), Inc., Blacksburg, VA). The aquariums were covered with plastic film to prevent contamination and reduce evaporation. They were maintained in a controlled environment room at 25±1 °C with a 15L:9D lighting cycle. Aeration was provided to each aquarium through a hypodermic needle attached to an aquarium aerator via plastic tubing.
Bioassay procedure
Flat-bottomed glass specimen tubes (100 mm height and 25 mm internal diameter) were washed thoroughly and used in the bioassays. Paper tissues used as a substrate were cut into thin, short strips, soaked in ethanol for 24 hours, and then drained and air dried for at least 3 days before use. Four to six strips were then placed in each glass bioassay tube. Sets of 21 tubes were used in each replicate, with three tubes assigned to each of six fipronil test concentrations, and another three used as untreated controls. Maestro® 200SC (200 g/L fipronil Batch No. 07, 2015) was obtained from Nufarm Australia Pty Ltd, Laverton North, VIC. Initial dilutions were made in distilled water, with the final dilutions being made with 1x MRS before 20 mL of test suspension was added to each of the tubes. Preliminary bioassays were used to locate a range of concentrations that provided partial (10–97%) mortality. Control tubes contained 20 mL of 1x MRS only.

Ten final instar *C. tepperi* larvae were then added to each tube. The tubes were placed in a controlled environment room set to 25±1 °C with a 15L:9D lighting cycle. No food or aeration was provided during pesticide exposure. Two separate bioassays were conducted, each replicated four times and each exposing a total of 120 larvae to each concentration of pesticide. Larvae were assessed for mortality after 24 hours in one bioassay, and after 48 hours in the second.

Bioassay data were analysed without replicate pooling using a standalone probit program following the same approach as that developed by Barchia (2001) for use in the Genstat statistical environment. The program applies the method outlined in Finney (1971), including data adjustment for natural mortality using Abbott’s formula (Abbott, 1925). This approach was taken to ensure variability between replicates was considered during the analysis. Significant heterogeneity was identified using a π² test of residual deviance. When heterogeneity was significant (5% level), the variance of the estimated parameters was scaled by the corresponding heterogeneity factor equal to the residual mean deviance (Finney, 1971). Lethal concentration ratios plus the associated 95% confidence intervals calculated, as described by Robertson et al. (2007), were used to determine the significance of differences between LC values.

The results from the Maestro® bioassays were compared to those with other fipronil formulations during earlier projects. These were Regent® 300EC (Rhône-Poulenc Rural Australia Pty Ltd, Baulkham Hills, NSW) and Regent® 200SC (Aventis Crop Science Pty Ltd, East Hawthorn, VIC). These earlier bioassays were conducted using identical methods and conditions.

Field trial design
During the 2018-19 rice season at Yanco Agricultural Institute in south-west NSW, a replicated small plot trial was conducted on a Birganbigil clay loam soil (van Dijk, 1961). Fipronil as Maestro® 200SC (200 g AI/L, Batch No. 07, 2015) was obtained from Nufarm Australia Pty Ltd, Laverton North, VIC).

A single row of 12 rectangular bays with earthen banks (each about 94 m²) was used in the trial. Each bay was supplied with water from an adjacent channel via a plastic siphon. A water depth of about 10.7 cm (mean of 12 measurements/bay) was maintained in all bays throughout the trial. Treatments were allocated randomly within each block of four adjacent bays (three bays total per treatment). Bays were measured individually before calculating chemical dosages. Six days after flooding (on 14 November 2018) Maestro® 200SC was applied at 12.5, 17.5 and 22.5 g AI/ha. The other three bays were maintained as untreated controls. A second application of Maestro® at 22.5 g AI/ha was applied to the top-rate bays only 16 days after the first application. All chemical applications were made using
a single nozzle hand sprayer in a water volume of 3 L/bay.

All treatment and control bays were sown with pregerminated rice (cv. ‘Langi’, 160 kg (dry)/ha) by hand broadcasting within two hours of chemical treatment. Large mesh cages were placed in each bay to protect sections of the crop from duck damage.

**Monitoring of environmental conditions**

Temperatures at the soil-water interface in two of the fipronil-treated bays (one 12.5 and one 17.5 g AI/ha) were recorded at 30-minute intervals using data loggers, with readings taken from immediately after pesticide application until 21 days after application (DAA). Conductivity, pH and turbidity were assessed twice on every second day throughout the trial until 21 DAA. A single 250 mL water sample was taken from each of the temperature logger bays twice on each sampling day (0750–0920 and 1605–1740 daylight saving time). Measurements made in the laboratory used regularly recalibrated electronic meters. Rainfall was monitored at a nearby automated weather station.

**Chironomid population assessments**

Soil core sampling combined with magnesium sulphate flotation was used to quantify larval chironomid populations. Samples were obtained by pushing a 96 mm diameter plastic cylinder 50 mm into the sediment, sealing the top, and then digging around the outside of the cylinder and sliding a thin perspex sheet underneath. The complete assembly was then lifted out, and the water and sediment within the cylinder was transferred to a 1 L capacity plastic container and frozen at -20°C until sample analysis. Magnesium sulphate flotation of larvae from thawed samples followed the procedure of Stevens and Warren (1992). Three samples were taken from each bay at 4, 9, 14, and 21 DAA. Extracted larvae were divided into two groups: Chironominae/Orthocladiinae (the target group) and Tanypodinae (non-target species).

Data on larval numbers (bay totals on each sampling occasion) were transformed as $y' = \log_e(y + 1)$. Target and non-target data sets were analysed separately for each trial. ANOVA was used to assess the significance of treatment, day, and block effects, as well as treatment x day interactions. A significant treatment x day interaction was identified for both target and non-target larvae, so ANOVA was also used to detect significant treatment differences on individual sampling days after removal of block effects. Fisher’s LSD test was used to separate means where treatment effects were significant at $P < 0.05$.

**Plant establishment**

Plant counts were made using a 35 cm internal diameter sampling ring. To quantify rice plant establishment, eight random counts were taken from under the duck-proof cages in each bay 34 days after sowing. Data were square-root transformed to stabilise variances, and analysed using two-way ANOVA to separate treatment and block effects. Fisher’s LSD test was used to separate means.

**Chemical residue analysis – sample collection**

To assess chemical behaviour, concentrations of fipronil were measured in the surface water of each bay at regular intervals after treatment application. Composite water samples were taken from all control and treatment bays. All glassware used in sample collection and storage of water samples was treated with Coatasil® glass treatment (APS Ajax Finechem, 2% w/w dimethyldichlorosilane in 1,1-dichloro-1-fluoroethane) to minimise pesticide adsorption. Small (40 mL) clear glass tubes were fitted to an aluminium handle and used as dippers, with 8 x 20 mL subsamples taken from random points in each bay being used to make up each composite sample. Single composite samples were collected
from each bay on each sampling occasion. Samples were collected 1, 2, 5, 10, 15, and 28 days after the initial chemical application; additional samples (controls and 22.5 x 2 g AI/ha treatments only) were taken 44 days after the initial chemical applications (28 days after the second spray application to the 22.5 x 2 g AI/ha treatment). Samples were mixed thoroughly in the laboratory, and about 50 mL was discarded from each bottle, allowing the samples to be frozen at an angle and stored at –20 °C until analysis.

Plants were randomly harvested from each bay on 29 March 2019 (135 DAA) by cutting them just above ground level. Thirty to forty randomly selected stems were manually threshed; green and empty hulls were removed from the grain samples by hand. The cleaned grain samples were dehulled mechanically, and the hulls and brown grain were ground with a commercial coffee grinder before being frozen in Coatasil®-treated amber glass jars at –20 °C until analysis. Foliage samples were coarsely chopped before being shredded in a commercial kitchen blender, and also stored at –20 °C.

After the trial had been drained, sediment was collected on 5 April 2019 (142 DAA). A 9 cm diameter aluminium ring was pushed into the soil at a random point and the sediment within collected to a depth of about 4 mm. Three samples were collected from random points in each bay, combined, and air dried at room temperature in darkness before being coarsely ground and frozen in Coatasil®-treated amber glass jars at –20 °C until analysis.

Chemical residue analysis – quantification of fipronil concentrations in water
At Charles Sturt University, Wagga Wagga, LC-MS/MS analysis was used to determine concentrations of fipronil and fipronil sulphide (the primary fipronil metabolite under anaerobic conditions). The LC column was 2.1 x 100 mm x 2.7 µm SB-C18 at 30 °C. The injection volume was 10 µL. The mobile phase was water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), and the flow rate was 0.3 mL/min. Initial conditions were 90% phase A held for 0.5 min, then gradient to 90% phase B over 5 min, held for 5 min. RTs were 9.15 (fipronil) and 9.209 (sulphide). Calibration was performed over the 0.3–13 ng/mL concentration range. The range was linear and remained so up to the 50 ng/mL maximum tested. No interference between fipronil and fipronil sulphide was observed as a result of their co-elution. MS detection parameters are given in Table 1. The internal standard used was 50 µL of 1.5 µg/mL heavy fipronil (\(^{15}\)N\(_2^{13}\)C\(_2\) fipronil) in 1500 µL of water.

<table>
<thead>
<tr>
<th></th>
<th>Ion transition (m/z)</th>
<th>Quant</th>
<th>Fragmentor</th>
<th>Collision</th>
</tr>
</thead>
<tbody>
<tr>
<td>fipronil 1</td>
<td>435.6 → 330.5</td>
<td>Y</td>
<td>113</td>
<td>15</td>
</tr>
<tr>
<td>fipronil 2</td>
<td>435.6 → 251.2</td>
<td></td>
<td>113</td>
<td>30</td>
</tr>
<tr>
<td>fipronil sulphide 1</td>
<td>419.5 → 384.5</td>
<td>Y</td>
<td>120</td>
<td>12</td>
</tr>
<tr>
<td>fipronil sulphide 2</td>
<td>419.5 → 263.5</td>
<td></td>
<td>120</td>
<td>30</td>
</tr>
<tr>
<td>heavy fipronil IS</td>
<td>439.4 → 333.8</td>
<td>Y</td>
<td>122</td>
<td>15</td>
</tr>
</tbody>
</table>

Results

Laboratory bioassays
Results of the laboratory bioassays comparing Maestro® 200SC to earlier formulations (Regent® 300EC and 200SC) are provided in Table 2. The Regent® data, provided for comparative purposes, was generated during previous projects.
Table 2 – Toxicity of fipronil formulations to final instar Chironomus teppei larvae in laboratory bioassays. Larvae unfed and maintained at 25 ± 1 oC, 15L:9D. LC values for 24 h exposures in individual columns with different superscript letters are significantly different (P < 0.05). Figures in brackets are 95% fiducial limits; Exp. = exposure period; df = degrees of freedom.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Exp. (h)</th>
<th>LC\textsubscript{50} µg Al/L</th>
<th>LC\textsubscript{90} µg Al/L</th>
<th>Slope (SE)</th>
<th>$\chi^2$ (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regent® 300EC</td>
<td>24</td>
<td>0.41 (0.39 – 0.43)\textsuperscript{a}</td>
<td>0.68 (0.61 – 0.78)\textsuperscript{a}</td>
<td>5.8 (0.5)</td>
<td>31.5 (22)</td>
</tr>
<tr>
<td>Maestro® 200SC</td>
<td>24</td>
<td>0.52 (0.48 – 0.56)\textsuperscript{b}</td>
<td>1.01 (0.90 – 1.19)\textsuperscript{b}</td>
<td>4.4 (0.4)</td>
<td>41.6 (22)</td>
</tr>
<tr>
<td>Regent® 200SC</td>
<td>24</td>
<td>0.66 (0.58 – 0.74)\textsuperscript{c}</td>
<td>1.41 (1.17 – 2.02)\textsuperscript{c}</td>
<td>3.9 (0.6)</td>
<td>69.9 (22)</td>
</tr>
<tr>
<td>Maestro® 200SC</td>
<td>48</td>
<td>0.38 (0.33 – 0.43)</td>
<td>0.66 (0.58 – 0.79)</td>
<td>5.4 (0.8)</td>
<td>86.5 (22)</td>
</tr>
</tbody>
</table>

In the 24-hour bioassays, the three formulations all had statistically different levels of toxicity, with the Regent® 300EC formulation showing the highest activity (lowest LC values). However, higher activity of emulsifiable concentrates in comparison to suspension concentrates is not uncommon when the two formulation types are compared in terms of their activity against aquatic organisms. In the comparison between the two suspension concentrates, the Maestro® 200SC formulation showed a higher level of activity than Regent® 200SC. Reasons are unclear, however it should be noted that the data reflects nominal rather than measured concentrations of active ingredient, and formulated products often contain concentrations of active ingredient that vary slightly from label specifications. As anticipated, the 48-hour LC values for Maestro® 200EC were substantially below those for the 24-hour exposures.

**Environmental conditions**

Environmental conditions during the field trial are summarised in Table 3.

Table 3 - Environmental conditions during the Maestro® fipronil trial, first 21 days after spraying.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water temperature (°C)</td>
<td>22.3</td>
<td>10.3 – 35.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.16</td>
<td>5.9 – 8.8</td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>99.5</td>
<td>66.5 – 132.8</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>130</td>
<td>19 – 615</td>
</tr>
<tr>
<td>Rainfall (mm)</td>
<td>1.1</td>
<td>0 – 8.2</td>
</tr>
</tbody>
</table>

Average pH levels were close to neutral, with strong diel variation being apparent. Conductivity remained relatively low initially, but increased progressively throughout the trial as a consequence of ongoing evaporation. There was minimal rainfall (0.2 mm in total) during the first six days after the initial spraying. Turbidity was highly variable, but often reached high levels due to duck activity at the site, which was adjacent to the main canal at Yanco.

**Response of chironomid populations**

The response of chironomid larval populations to the Maestro® treatments is shown in Figure 2, and summarised in Table 4.

Table 4 - Response of target and non-target chironomid larval groups to Maestro® treatments over the full 21-day trial period. The uppermost group of values in each column represents the percentage difference in populations relative to the untreated controls. In each column, values with different letters are significantly different (ANOVA,
LSD Test, P < 0.05). The lower group of values in each column is the multifactor ANOVA values for the analysed parameters.

<table>
<thead>
<tr>
<th>% change</th>
<th>Target group</th>
<th>Non-target group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 a</td>
<td>0 a</td>
</tr>
<tr>
<td>12.5 g Al/ha</td>
<td>-85.4 b</td>
<td>-93.0 b</td>
</tr>
<tr>
<td>17.5 g Al/ha</td>
<td>-86.9 b</td>
<td>-96.8 bc</td>
</tr>
<tr>
<td>22.5 g Al/ha x 2</td>
<td>-88.8 b</td>
<td>-99.5 c</td>
</tr>
</tbody>
</table>

Treatment effect  
\( F_{3,30} = 24.41, P < 0.001 \)  
\( F_{3,30} = 55.78, P < 0.001 \)

Day effect  
\( F_{3,30} = 6.33, P < 0.002 \)  
\( F_{3,30} = 9.56, P < 0.001 \)

Block effect  
NS  
\( F_{2,30} = 2.46, P = 0.10 \)  
NS  
\( F_{2,30} = 0.92, P = 0.41 \)

Treatment x day interaction  
\( F_{9,30} = 3.26, P < 0.01 \)  
\( F_{9,30} = 4.16, P < 0.002 \)

The target group of larvae was reduced by between 85% and 89% over the full trial duration, while the non-target group was more heavily affected, with population reductions of between 93% and 100%. The overall analyses produced similar results across the two groups, with day, time and their interaction all being highly significant; however, block effects were not significant in either case. When analysed daily, significant differences were identified on all sampling days and for both larval groups. The exceptions were non-target larvae on day 4 and the target group on day 14, when both populations were at their lowest in the unsprayed control bays. On all other occasions, all treatments tested led to populations significantly (P < 0.05) lower than in the controls (Figure 2).

**Plant establishment**

Plant populations assessed 34 days after sowing are shown in Figure 3. Treatment effects were significant (\( F_{3,6} = 25.08, P = 0.0009 \)), but block effects were not (\( F_{2,6} = 2.11, P = 0.2029 \)). Seedling densities in the control bays averaged 74 plants/m², while mean densities in the treatment bays varied from 374 to 471 plants/m². Although there was an upward trend in plant density with increasing Maestro® application rate, plant densities did not differ significantly between Maestro® treatments – the only significant difference was with the untreated control.

**Fipronil residues in the water column**

Residues of fipronil and fipronil sulphide, the main fipronil breakdown product in anaerobic conditions, are shown in Figure 4. At the lowest (12.5 g Al/ha) rate, the parent compound was below the 0.02 µg/L detection limit on all bays 15 days after application. At this time, fipronil sulphide was undetectable in two of the three replicate bays, and registered at 0.02 µg/L in the third. At the intermediate and high application rates, the parent compound similarly was undetectable in two of the three replicate bays, and just registered at 0.02 µg/L in the third bay. At all rates, the parent
Figure 2 - Response of target and non-target chironomid larval groups to Maestro® treatments. Tan columns represent the target group (subfamilies Chironominae and Orthocladiinae) while blue columns are the non-target Tanypodinae. An asterisk indicates a population significantly (P < 0.05) different to the equivalent control population. The vertical line in (d) indicates the timing of the second 22.5 g Al/ha Maestro® application.

Compound was undetectable in all replicates at 28 days. Fipronil sulphide was undetectable at 15 days and thereafter. Neither the parent compound nor fipronil sulphide were detectable in the water column 28 days after the second fipronil application to the top rate dual application bays. Results from the plant and soil analyses have not yet been finalised.

Discussion

This trial has conclusively demonstrated that fipronil as Maestro® 200SC is an effective and safe treatment for managing bloodworms in establishing rice crops. Applied at 12.5 g Al/ha, the same rate used for the Cosmos® and Emporium® fipronil seed treatments, Maestro® provided 85% control of the target bloodworm group over 21 days. While higher rates led to slight improvements in plant establishment, these improvements were not statistically significant (P > 0.05). Interestingly, higher application rates did not improve percentage control of the target group. The bloodworms recovered from this trial have been slide-mounted to allow more detailed studies to be conducted, and particularly to allow species-level identification of individuals in the target group that survived fipronil treatment.
Figure 3 - Plant establishment, 2018-19 Maestro® bloodworm trial. Bars with different lower case letters are significantly different (ANOVA, P < 0.05).

Figure 4 - Water column concentrations of fipronil and its principal breakdown product, fipronil sulphide, 2018-19 Maestro® bloodworm trial. Detection limit 0.02 µg/L for both compounds. Day 44 samples were taken only from the 22.5 g Al/ha x 2 treatment, and were 28 days after the second of the two chemical applications. bdl, below detection limits in all replicate bays.

Neither fipronil nor its principal breakdown product, fipronil sulphide were detected in surface water 28 days after application at a detection limit of 0.02 µg/L. Neither compound showed average water column concentrations above 0.047 µg/L 10 days after application, even at the maximum rate tested.
demonstrating that these treatments pose no risk to downstream environments if existing 28-day water retention rules are adhered to.

The existing maximum residue limit (MRL) and allowable daily intake (ADI) for fipronil in rice, developed for the registration of the Cosmos® seed treatment, should make registration of the use pattern developed here a relatively simple process. To allow for the variation in water depths that occurs in commercial fields, a minimum rate of 15 g AI/ha should be considered.
Laboratory bioassays of alternative chemicals for bloodworm management

Introduction

Bloodworm communities in establishing rice crops are, in general, quite easily managed with low rates of insecticides. Water chemistry can, however, influence performance, with DOC concentrations in particular known to bind up low solubility insecticides such as pyrethroids. While there are currently no known issues of insecticide resistance in rice field bloodworms in Australia, there is an ongoing need to evaluate newer-generation chemicals for control of bloodworms and other rice field insects. This need stems largely from regulatory pressure on the use of older generic compounds, such as pyrethroids and particularly organophosphates, that have been favoured by growers because of their low cost. At the time of writing, four of the six registered insecticides recommended for use in rice in southern Australia are subject to APVMA regulatory decisions that may restrict or prohibit their future use.

Laboratory screening of newer-generation compounds for bloodworm control was a minor component of this project, which had a stronger focus on evaluating new materials for armyworm control. Two compounds – spirotetramat and spinetoram – were fully evaluated against the rice bloodworm *Chironomus tepperi*.

Materials and methods

**Rearing of Chironomus tepperi larvae and bioassay procedures**

*C. tepperi* larvae were reared using identical procedures to those described in the previous section for the Maestro® fipronil assays. The bioassays followed the same format, with three tubes of 10 larvae at each of six test concentrations, and another three tubes of untreated control larvae per replicate.

Spirotetramat was obtained as Movento® 240SC (Batch # PAIS004044 16/04/14) from Bayer Crop Science Australia, Sydney, while spinetoram was obtained as Success Neo® 120 g/L (Batch # 20 Mar 13 2020971101) from Dow AgroSciences (now Corteva Agriscience), Sydney. Initial dilutions were made in distilled water, with the final dilutions being made in 1x MRS.

Bioassays were each replicated four times, exposing a total of 120 larvae to each concentration of each pesticide. Larvae were assessed for mortality after 24 hours. Data were analysed without replicate pooling using the approach described in the previous section on the Maestro® bioassays.

Results

Bioassay results for spirotetramat (Movento®) and spinetoram (Success Neo®) are shown in Table 5.

Discussion

Spirotetramat (Movento®) was about 70 times less active than spinetoram (Success Neo®) to final instar *C. tepperi* larvae. On this basis, Movento® cannot be considered as a viable candidate for further development for bloodworm control in rice. In contrast, Success Neo® has potential for this use pattern. Although it is less than one-tenth as active as Maestro® 200SC under laboratory conditions, and is substantially less active than most older generation organophosphates and
pyrethroids studied in previous projects, its environmental profile and selectivity may make it more likely to withstand the increased level of regulatory scrutiny now being applied to older chemistries.

Table 5 - Toxicity of spirotetramat (Movento®) and spinetoram (Success Neo®) to final instar Chironomus tepperi larvae in 24-hour laboratory bioassays. Larvae unfed and maintained at 25 ± 1°C, 15L:9D. Figures in brackets are 95% fiducial limits; df = degrees of freedom.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>LC₅₀ µg AI/L</th>
<th>LC₉₀ µg AI/L</th>
<th>Slope (SE)</th>
<th>Χ² (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Movento® 240SC</td>
<td>435 (398 - 466)</td>
<td>681 (625 - 771)</td>
<td>6.6 (0.8)</td>
<td>57.6 (22)</td>
</tr>
<tr>
<td>Success Neo® 120 g/L</td>
<td>6.35 (5.42 - 7.38)</td>
<td>19.5 (15.4 – 27.6)</td>
<td>2.6 (0.3)</td>
<td>52.2 (22)</td>
</tr>
</tbody>
</table>

Two other compounds were examined in the screening program during the course of this project, chlorantraniliprole (Altacor Hort®) and cyantraniliprole (Exirel®), both DuPont legacy products currently being manufactured and distributed by FMC Crop Protection Australia. Inconsistent bioassay results across replicates and samples has prevented us from being able to present definitive data on either of these compounds, despite cyantraniliprole providing promising results in earlier bioassays with an experimental (as opposed to commercial) formulation. The erratic performance of this compound was disappointing, particularly because of its apparently excellent performance in field trials against other bloodworm species in rice fields in the Ebro Delta (personal correspondence, Maria del Mar Català, IRTA, Catalonia, Spain). An different assessment approach, possibly involving longer exposure periods, might be necessary to obtain reliable results for these chemicals.
Development of a diet for rearing common armyworm

Introduction

Artificial diets are often necessary for rearing caterpillars because consistent supplies of fresh food plants are often not available throughout the year. Bioassays involving incorporation of toxicants into foods based on agar or similar matrices might also need to be conducted, as well as more traditional contact bioassays. A standardised diet allows caterpillars of consistent nutritional status to be maintained under controlled conditions throughout the year, providing a steady supply of insects for experimentation across multiple generations.

The scientific literature contains many recipes for caterpillar diets; these have often been developed for individual insect species. Their utility often differs when used with different species, even when they are closely related. As a consequence, we needed to develop an effective artificial diet to facilitate our work with the common armyworm, *Mythimna convecta*.

Our initial work involved a diet published by Griffith and Smith (1977) that was modified from that of Sender (1970). Honey-sugar solution was provided to the adults once they emerged. This larval diet was used to raise neonates of *M. convecta* through to adults, with the authors stating that “most or all of the larvae (of various species) became fertile adults”.

This was, regrettably, not our experience. We found that field-collected late instar larvae could be reared to adults, and that these adults mated and produced viable eggs. Although the F1 larvae could be reared through to adulthood, most eggs produced by the F1 adults were infertile, suggesting some sort of nutritional deficiency in the diet that manifested itself only in the first full generation in laboratory culture.

Materials and methods

We used the Griffith and Smith (1977) larval diet with lucerne supplementation as a starting point, because despite its deficiencies it provided good survival of field-collected larvae through to pupation. Adult nutrition was initially provided as aqueous honey-sugar solution. Initially, we looked at the impact of replacing the maize meal used in the Griffith and Smith diet with chickpea flour, increasing both the protein and fat content of the diet. Then we examined the effects of various supplements mentioned as components of other published caterpillar diets. We concentrated on adding Wesson Salt Mixture and Vanderzant Vitamin Mixture, as assessed by Wang et al. (2014) as components of a diet for *Cnaphalocrocis medinalis*, and used in diets developed for various other species. These supplements were provided to both the larvae and to the adults (added to the honey-sugar solution) during the diet development process.

Sorbic acid, ascorbic acid and Vanderzant Vitamin Mixture were all obtained from Sigma-Aldrich, St Louis, MO. Agar was obtained from AppliChem GmbH, Darmstadt, Germany, while methyl 4-hydroxybenzoate sodium salt was obtained as an Acros Organics product from Thermo Fisher Scientific, Geel, Belgium. Wesson Salts (Salt Mixture W) was purchased from MP Biomedicals LLC, Solon, OH.

Torula yeast was purchased from Lotus Foods Pty Ltd, Braeside, VIC; besan (chickpea) flour from either Lotus Foods or Ward McKenzie Pty Ltd, Altona, VIC; and wheat germ from either Lotus Foods or Select Harvest Food Products Pty Ltd, Thomastown, VIC. Maize flour (polenta) was supplied by
Menora Foods Pty Ltd, Noble Park, VIC, and sucrose (white sugar) was sourced from Metcash Food and Grocery Pty Ltd, Macquarie Park NSW.

The physical rearing conditions used were consistent throughout the diet development process. Groups of pupae were placed in Décor® clear plastic containers (26 x 19 x 10 (height) cm) lined with paper towels and with a fine gauze panel inlaid into the lid to allow air circulation. Adult food (honey-sugar solution with or without additional salts and vitamins) was provided for the emerging adults with cotton dental wicks pushed through the lids of reservoirs made from small plastic sauce cups. Female moths laid eggs directly onto the paper towels. During their early stages, larvae were reared using commercial bioassay trays (C-D international. Inc., Pitman, NJ, Model BIO-BA_128). These trays contained individual wells about 16 mm in diameter at the top and 16 mm in depth. Groups of 3 or 4 larvae were reared in each well through to late third/early fourth instar. The self-adhesive sealing film allowed air flow and prevented escape. In their later developmental stages (about 4th instar onwards through to pupation), larvae were maintained individually in small plastic cups (Solo® Souffles 29.5 mL No. P100 with PL1 snap on lids, Dart Container Corp., Mason, MI), with a small hole (about 2 mm diameter) cut in the lid to allow air flow.

Regardless of the formula being evaluated, larval diets involved combining dry ingredients with a hot agar solution in a commercial blender. For the bioassay trays, the hot mixture was spooned into a plastic squeeze sauce bottle, and a layer about 4 mm in depth was squirted into each well of the tray and allowed to set. For older larvae, the food was poured into a 750 mL capacity rectangular take-away food container to form a layer about 5 mm thick. This was allowed to set, and small cubes were cut away with a scalpel and added to the cups as required. Prepared food and bioassay trays were refrigerated at 4°C and used within 3 weeks.

All armyworm rearing was done in a controlled temperature room at 25 ± 1°C with a 15L:9D photoperiod and at about 65% relative humidity.

**Results**

The diet development was not conducted as a replicated experiment, but rather involved the progressive testing of different formulas and making observations on their effectiveness in terms of the survival of different life stages and, most importantly, the fertility of eggs produced. The formulas tested are summarised below in test order, with their outcomes noted.

1. Field-collected late instar larvae were reared through to pupation on the Griffith and Smith diet with incorporated lucerne. The emergent adults were given with honey-sugar solution, and laid viable eggs. The resultant F1 larvae were reared through to adulthood using the larval diet and adult nutrition. The 129 F1 adults raised in this way laid large numbers of eggs, none of which hatched.

2. Adult moths captured in the field in fermentation traps were given with honey-sugar solution in the laboratory, and laid viable eggs. The larvae were reared on the Griffith and Smith diet with incorporated lucerne until they were 12-16 days old. Then larvae were split into two groups: one (102 larvae) continued to be fed with the same diet, while a second group (160 larvae) were fed a modified diet in which the maize flour was replaced with chickpea flour. The development and survival of the two groups is summarised in Figure 5.
Pupation occurred earlier on the chickpea diet, and the overall pupation success was higher (91.9% as opposed to 88.2% on the maize-based diet). Adult emergence initially commenced at a faster rate on the chickpea-based diet, although the final emergence success was higher on the maize-based diet (93.3% compared to 81.6%). Again, however, these adults did not produce viable eggs.

1. Vanderzant Vitamin Mixture was added to the larval food, but did not provide a consistent improvement in the capacity of F1 moths to produce viable F2 eggs.
2. Vanderzant Vitamin Mixture and Wesson Salts were both added to the honey-sugar solution given to adult moths on emergence. This led to moth cultures that produced viable eggs consistently in both the F2 generation and subsequently for multiple generations. Wesson Salts were also subsequently added to the larval food.
3. Subsequent experimentation comparing torula and brewer’s yeasts as components of the larval diet led to no further modifications because they appeared to be equally effective.

The final optimised diet and preparation procedure is outlined below.

**Feeding solutions for adult moths**

1. Add 20 g honey and 20 g sugar to a 1 L autoclavable glass bottle and fill with deionised water. Shake to mix, loosen lid, and autoclave at 121 °C for 15 min. Allow to cool and add 2 g ascorbic acid when the temperature is below 63 °C. Shake to mix and refrigerate until use.
2. **Stock solution**: add 0.84 g Vanderzant Vitamin Mix to a total volume of 45 mL deionised water and refrigerate until use.
3. **Stock solution**: add 0.25 g Wesson Salt to 250 mL deionise water and refrigerate until use.

When establishing containers for adults, provide two dispenser cups of honey-sugar solution. In a third dispenser cup, mix 20 mL of deionised water, 5 mL of Vanderzant Vitamin Mix stock solution and 5 mL of Wesson Salt stock solution (see above). Push a dental wick through the lid of each dispenser.
Artificial larval diet

1. **Part A**: Add 12.5 g agar, 5 g sugar and 400 mL deionised water to a 500 mL autoclavable bottle, and autoclave at 121 °C for 15 min.

2. **Part B**: Place 30 g wheatgerm, 25 g chickpea flour, 25 g torula yeast, 20 g of fresh (or dried) lucerne leaves, 0.75 g methyl-4-hydroxybenzoate sodium salt, 0.75 g sorbic acid, and 4.0 g ascorbic acid in a mixing bowl or disposable plastic container, and mix evenly.

3. **Part C**: Using the stock solutions prepared for adult moths, add 10 mL of Vanderzant Vitamin Mix stock solution and 10 mL of Wesson Salt stock solution to a plastic tube and shake to mix.

When Part A cools to 60–65 °C, pour half into a commercial food blender, add all of Part B, and then add the remainder of Part A. Blend at low to moderate speed until homogeneous. When the temperature falls below 55 °C, add Part C and continue mixing until it is evenly incorporated into the food. The food must then be dispensed into the various trays before it solidifies as the agar cools.

Discussion

Developing a better diet for rearing armyworm in the laboratory was a slow process because each diet variation took about six weeks to assess. Although the final diet is effective, it is unclear which of the experimental changes in the formula were responsible, and to determine that would require considerable effort. The initial change, in which chickpea flour replaced maize flour in the Griffith and Smith diet, led to faster development through to pupation and higher pupation success, but did not overcome the adult infertility issues. They were resolved only by adding vitamins and salts, not only to the larval diet, but most importantly, to the adult liquid diet as well.

While larvae used in bioassays were reared from hatching onwards entirely on the artificial diet described here, larvae conserved for use as brood stock for the next generation appeared to benefit from fresh lucerne and rice leaves as supplements to the artificial diet, particularly when the artificial diet was based on dried rather than fresh lucerne. This observation suggests that, with more research, it might be possible to make more improvements to the agar-based diet.

The diet developed here allowed us to culture common armyworm for up to nine generations in the laboratory, and provided a consistent supply of larvae for use in the insecticide bioassays reported in the following section.
Topical and dietary bioassays of potential armyworm control chemicals

Introduction

Over the last decade, the common armyworm *Mythimna convecta* has become increasingly significant as a pest of rice in southern NSW. While it was once fairly sporadic in occurrence, large sections of the crop now require chemical treatment for armyworm control each season.

The reasons for this change in the pest status of armyworms are not clear, but they could relate to climate change, and also the use of water conservation techniques, such as delayed permanent water and mid-season drying. Armyworms are known to develop in rangeland areas in western NSW, and the adult moths move eastwards on prevailing air currents looking for suitable food sources for the next generation. Chemical signals associated with water-stressed rice plants may well be making the crop more attractive to female moths looking for oviposition sites. However, this is unlikely to be the only factor leading to the consistently high armyworm populations growers have had to deal with in recent years.

The options for chemical control of armyworms in rice are currently limited to organophosphate compounds, such as chlorpyrifos and malathion (maldison) and the pyrethroid alphacypermethrin. Organophosphate compounds are under increased regulatory scrutiny by the APVMA (at the time of writing, registrations for both chlorpyrifos and malathion are under review), and all these compounds have very broad-spectrum activity across different insect groups. This means that while these chemicals may well kill the target caterpillars, they will almost certainly kill the beneficial species – predators and parasitoids – that might regulate low-level infestations and prevent pest resurgence.

While there is a need for more data on the role of parasitoids in regulating armyworms in Australian rice crops before their significance can be fully assessed, there is a clear need to develop new compounds for armyworm control to give the industry some level of security in the face of increased regulatory pressure. These compounds should be chosen on their potential to provide effective intervention during an armyworm outbreak while conserving beneficial species whenever possible. With this goal in mind, we conducted laboratory bioassays against common armyworm caterpillars using four different insecticides from newer and generally more selective insecticide groups. We conducted ‘traditional’ topical assays using pure insecticide actives to determine contact toxicity. We also conducted dietary bioassays using formulated (commercial) products incorporated into the laboratory larval diet at different concentrations. Studies on the noctuid *Helicoverpa armigera* by Bird (2015, 2016) have shown dietary bioassays to be effective methods of assessment for all the chemicals we evaluated, and that they are particularly effective for accurately determining the potential effects of anthranilic diamides, such as chlorantraniliprole and cyantraniliprole, which tend to have high levels of ingestion toxicity not effectively captured in topical bioassays. Additionally, variations in response to chemicals, such as cyantraniliprole, across strains from different locations tend to be less pronounced in dietary assays than in topical ones (Bird, 2016), which we considered an important factor because we were generating baseline data from an armyworm culture originating from a single location.
Materials and methods

Insects and test chemicals

*Mythimna convecta* cultures were established from adult moths collected in fermentation traps at Yanco Agricultural Institute using the techniques and larval and adult foods described in the previous section.

Four active compounds were evaluated, with formulated products being used in the dietary bioassays and technical grade materials in the topical bioassays. Emamectin benzoate was provided by Syngenta Crop Protection Pty Ltd, North Ryde, Australia as Affirm® EC 17 g AI/L, A10325A, Batch No. AAC5G31034, DOM: 08/15 and as technical grade MK244G, Batch No. SNA7K00351, DOM: 11/17 (97.6% purity). The remaining three compounds were all provided by FMC Australasia Ltd, North Ryde, and are products formerly marketed by DuPont Crop Protection Australia. Chlorantraniliprole was provided as Altacor Hort® WG 350 g AI/kg, Batch No. JUN15AC109, DOM: 1/6/15, and as technical grade DPX E2Y45-396 Mfg Batch No. D100855-050 (99.7% purity). Cyantraniliprole was provided as Exirel® SC 100 g AI/L, Batch No. APR14VL322-86, Packed 2/12/14 and as technical grade HGW86-938 Mfg Batch No. MAR17WY042 (96.84% purity). Indoxacarb (S-isomer) was provided as Steward® EC 150 g AI/L, no batch number, DOM: 21/12/2016 and as technical grade KN128-498 (~100% purity).

Dietary bioassay procedure

The dietary bioassay methodology was based on that used by Bird (2015). Bioassays used eight chemical concentrations plus an untreated control. Initial bioassays were used to establish final test concentrations across the full range of response; eight rates plus an untreated control were then assessed for each compound. The formulated compound was initially diluted in deionised water, and the final dilutions were made in a series of Coatasil®-treated 50 mL centrifuge tubes to provide concentrations of toxicant that were 40-fold the desired final concentrations in the dietary media.

Artificial diet was prepared using the protocol described in the previous section, including Parts A and B, but omitting Part C, the vitamin and salt solution, because the larvae had been given this during the rearing process, and it is unnecessary for subsequent short-term larval development. While the diet was hot, aliquots of about 39 g were poured into nine pre-weighed 100 mL glass bottles that were then weighed again and the weights noted. The capped bottles were then placed in a water bath at 60 °C.

The volumes of toxicant needed for each food container were calculated according to the precise weights of the food, and then each bottle was removed from the water bath and the toxicant added by pipette. The food and toxicant were mixed vigorously with a stainless steel spatula, and the mixture was spooned out to form a shallow layer in a 9 cm Petri dish. The dish was covered and labelled, the diet/toxicant mixture was allowed to set, and the dishes stored at 5 °C until use. Deionised water without any toxicant was added to the control food. A new set of nine food/toxicant dishes was prepared for each bioassay replicate.

In each replicate, 15 early third instar *M. convecta* larvae reared from egg hatch on the artificial diet (Parts A, B and C) were tested at each toxicant concentration in individual containers. Small cubes of the dietary media (about 4 to 5 mm a side) were cut from the Petri dish and placed individually in small plastic cups (Solo® Souffles 29.5 mL No. P100), and a single larva was then added. Perforated lids were placed on each cup and all cups were maintained at 25 ± 1 °C with a 15L:9D photoperiod and at about 65% humidity for seven days before final assessment. Cups were checked every 48 hours and the dietary media supplemented or replaced, as necessary. Mortality was assessed using the
protocol of Bird (2015) where larvae are considered dead if one or more of the following criteria are met: unable to perform a coordinated movement when prodded; paralysis of prolegs; larvae very slow to right themselves (time longer than 3 seconds).

Four replicates of each bioassay were conducted on separate occasions using different larval cohorts, giving a total of 540 larvae tested per chemical. In the Exirel® (cyantraniliprole) bioassays, we noted an apparent avoidance response to diet containing higher pesticide concentrations. To document this response, for all larvae surviving at the end of the seven-day test period, we individually weighed on a four-place analytical balance. Frass produced over the full exposure period by the surviving larvae was combined for each test concentration, dried in a desiccator over silica gel at room temperature for four days and weighed. Weights were divided by the number of surviving larvae to determine mean frass production per larva over the post-treatment period.

**Topical bioassay procedure**

The procedure for topical bioassays was similar to that used by Forrester et al. (1993) and by Bird (2016). Bioassays were conducted using seven chemical concentrations and untreated controls. Initial bioassays identified the range of responses. Larvae used in the bioassays were late third instars reared using the same artificial diet and conditions employed for the initial rearing of larvae used in the dietary bioassays. Technical-grade insecticides were prepared as solutions in analytical-grade acetone, and a 1 µL droplet was applied to the dorsal surface of the thorax using a hypodermic needle on a 100 µL glass syringe (SGE, Trajan Scientific Australia Pty Ltd, Ringwood, VIC) attached to a microsyringe applicator fitted with an electronic micrometer (World Precision Instruments Inc., Sarasota, FL). The point of the needle was ground off to remove the possibility of larval injury. After chemical application, the larvae were maintained individually in plastic cups (Solo® Souffles) and each given a small cube of untreated artificial diet. Four replicates of each bioassay were conducted on separate occasions using different larval cohorts, giving a total of 540 larvae tested per chemical. On each occasion, 10 individual larvae were tested at each dose rate, giving a total of 320 larvae tested per chemical. Cups were maintained at 25 ± 1 °C with a 15L:9D photoperiod and at about 65% humidity for four days before final assessment. Mortality was determined by the same protocol used in the dietary bioassays.

**Statistical analysis**

Bioassay data were analysed without replicate pooling using a standalone probit program following the same approach as that developed by Barchia (2001) for use in the Genstat statistical environment. The program applies the method outlined in Finney (1971) including data adjustment for natural mortality using Abbott’s formula (Abbott, 1925). This approach ensured variability between replicates was considered during the analysis. Significant heterogeneity was identified using a χ² test of residual deviance. When heterogeneity was significant (5% level), the variance of the estimated parameters was scaled by the corresponding heterogeneity factor equal to the residual mean deviance (Finney, 1971). Lethal concentration ratios plus the associated 95% confidence intervals, calculated as described by Robertson et al. (2007), were used to determine the significance of differences between LC values.

Data on larval weights (mean weight of survivors in each treatment) and frass production from the larvae that survived in the Exirel® dietary bioassays were analysed using two-way ANOVA to assess treatment and replicate effects. Treatments were separated using post-hoc LSD tests.
Results

The results from probit analysis of the bioassay data are shown in Table 6. There was a reasonable level of consistency in the data when dietary and topical tests were compared. In the dietary test, emamectin benzoate was the most active compound, and was significantly more toxic than any of the other materials. Chlorantraniliprole was around 4.7 times less active than emamectin benzoate on the basis of LC50 estimates, but was still significantly more active than indoxacarb and cyantraniliprole, which were both more than 40 times less active than emamectin benzoate.

In the topical bioassays, emamectin benzoate was again the most active compound, but with a reduced differential to chlorantraniliprole compared to the dietary bioassay results. The toxicities were, however, still significantly different ($P < 0.05$) at both the LC50 and LC90 levels. The relative order of toxicity was reversed for indoxacarb and cyantraniliprole, which were statistically distinct from each other at the LC50 level, but not at the LC90 level due to substantially broader fiducial limits associated with the LC90 estimates.

The poor performance of cyantraniliprole in the dietary bioassays (compared to the topical tests) reflects a deterrence effect at higher dose rates. During the bioassays, we noted that while food consumption and larval growth progressed steadily in the controls and at the lowest toxicant concentration tested, at higher rates there was an increasing tendency for less food to be consumed. Consequently, mortality plateaued to some extent, and progressively higher concentrations had little extra impact. This is reflected in the very wide fiducial limits associated with the LC90 estimate of 5.598 µg/g, and also by the weights and frass production of the larvae that survived to the end of the seven-day test period (Figure 6).

Higher cyantraniliprole concentrations in the dietary media significantly affected the weights of surviving larvae ($F_{8,24} = 20.74, P < 0.0001$) and also the dry weight of frass produced ($F_{8,24} = 22.1, P < 0.0001$). Replicate effects were significant for both larval weight ($F_{3,24} = 5.7, P < 0.005$) and frass production ($F_{3,24} = 4.87, P < 0.01$). Specific treatment differences followed an identical pattern for both parameters.

The lowest tested toxicant concentration (0.01 µg/g) did not significantly impede growth or reduce food consumption (as measured by frass production); however, 0.02 µg/g did ($P < 0.05$), and another significant reduction occurred at 0.04 µg/g. While higher rates led to progressive declines in larval weights and frass production, these changes were very small because food consumption had already fallen by 83% relative to control levels at the 0.04 µg/g dose. Consequently, these further reductions were not statistically significant.

Discussion

Our dietary and topical bioassays provided consistent results except for the variation in response of the caterpillars to cyantraniliprole, which we attribute to a repellence effect in the dietary bioassays. The two most active compounds were emamectin benzoate (Affirm®) and chlorantraniliprole (Altacor Hort®); both should be considered for further development. While it may seem that emamectin benzoate should be given priority due to its higher toxicity in our bioassays, more work needs to be done to assess efficacy under field conditions and also the residue profile of these compounds in rice. Due to the high levels of parasitism that occur in *Mythimna convecta* field populations in rice, consideration should also be given to the relative impact of these compounds on parasitoids and other beneficial species.
Table 6 - Dietary and topical toxicity of four insecticides to larvae of the common armyworm Mythimna convecta. Dietary bioassays conducted with formulated products incorporated into an artificial diet; topical bioassays conducted with technical-grade material dissolved in acetone.

<table>
<thead>
<tr>
<th>Active</th>
<th>Formulation</th>
<th>Dietary bioassays</th>
<th>Topical bioassays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LC₅₀ (μg AI/g diet)ᵃ</td>
<td>LC₉₀ (μg AI/g diet)ᵃ</td>
</tr>
<tr>
<td>emamectin benzoate</td>
<td>Affirm® 17EC</td>
<td>0.017 (0.015 – 0.020) a</td>
<td>0.060 (0.049 – 0.079) a</td>
</tr>
<tr>
<td>chlorantraniliprole</td>
<td>Altacor Hort® 350WG</td>
<td>0.080 (0.056 – 0.113) b</td>
<td>0.481 (0.300 – 0.991) b</td>
</tr>
<tr>
<td>indoxacarbᵇ</td>
<td>Steward® 150EC</td>
<td>0.695 (0.589 – 0.812) c</td>
<td>2.230 (1.804 – 2.947) c</td>
</tr>
<tr>
<td>cyantraniliprole</td>
<td>Exirel® 100SC</td>
<td>0.872 (0.556 – 1.841) c</td>
<td>5.598 (2.425 – 32.88) c</td>
</tr>
</tbody>
</table>

ᵃ 95% fiducial limits in brackets.
ᵇ S-isomer
While studies on the chemical control of aquatic species, such as bloodworms and water snails, are complicated by aspects of water chemistry, e.g. dissolved organic carbon levels, and also by physical variables, such as water depth, the development of chemical controls for terrestrial species such as armyworm is far less complex. Our bioassays deliberately used substantially the same technique as Bird (2015, 2016) for studies using the same chemicals against Helicoverpa armigera. The relative order of toxicities across the compounds is reasonably similar for both species, although in general Mythimna convecta seems less susceptible to these insecticides overall. Because these chemicals are now registered for H. armigera control, initial estimates of likely field rates for use against M. convecta can be calculated by adjusting the registered application rates for H. armigera proportionate to the ratio of the LC values for the two species. This will allow efficacy and residue data to be generated under field conditions in a much more targeted manner than would otherwise be possible.
Parasitism of armyworm populations in rice

Introduction

For many years, armyworms were not considered a major problem for rice production in southern Australia. While their pest status was acknowledged, they occurred sporadically and in localised areas each season, and were treated with broad spectrum insecticides when damaging populations were found. The relatively low significance of armyworm meant they were not deemed a priority for research; however, this started to change from about 2012, when it was acknowledged that the incidence and severity of armyworm infestations was increasing.

This project is the first to address the issue of armyworm management in rice in southern Australia, and consequently, this work began with a very limited knowledge base. The strongest illustration of this is that, before this project, armyworm infestations in the crop were attributed entirely to the common armyworm *Mythimna convecta*. While it was indisputable that common armyworm were present in the crop, there was no suggestion that other species may also have been involved despite the fact that other armyworm species (*Leucania separata*, *Spodoptera exempta* and *S. mauritia*) are, along with *M. convecta*, known to attack rice in northern Australia (Stevens, 2007). During this project, we identified a second armyworm species in NSW rice crops, the sugarcane armyworm *Leucania stenographa*, which added considerable complexities to our work. When more than one species is present in the crop, they may occur at different times, cause different forms and levels of damage, and have different levels of susceptibility to parasitoids and to pesticides.

Common armyworm in maize crops in the Riverina are known to be susceptible to attack by parasitoids (Hardwick, 2006), but there was no data available to demonstrate that similar levels of parasitism occur within rice crops, which have a very different physical structure and microclimate. In this study, we collected mid- to late instar armyworm from rice crops near Jerilderie (2016), Gogeldrie (2017) and Leeton (2018), and reared them through to adult moths or to larval or pupal death and the emergence of parasitoids. In 2016, only *M. convecta* adults were obtained, which seemed to confirm the accepted orthodoxy that NSW rice crops contained only a single armyworm species. In 2017 however, adults of both *M. convecta* and *L. stenographa* were reared from caterpillars collected at Gogeldrie, demonstrating not only the presence of a second armyworm species in southern rice, but also that mixed populations can occur. While the adult moths of these species are readily distinguished (Figure 7), we could not reliably separate the caterpillars on morphological characters. This created a substantial problem because the parasitoids that affect armyworms kill their hosts either as larvae or pupae, so when we obtained one or more parasitoids from an armyworm, we could no longer identify the host.

Although the remains of parasitised caterpillars from the 2016 collection had been discarded (because there was no taxonomic uncertainty of their identity at that time), the remains of all parasitised larvae and pupae from 2017 and 2018 were retained. DNA barcoding was used to identify the host species. In summary, this technique involves extracting the DNA from the remains of the caterpillar and then amplifying and sequencing part of the mitochondrial encoded 5’ cytochrome c oxidase subunit I (COI) gene. The sequences are then compared to sequences taken from identified adult moths; in this case, both those reared from our field collections and those available in public DNA sequence repositories, such as BOLD and GenBank, in order to determine the identity of the host caterpillar.
Figure 7 - Adults of a, common armyworm (wingspan ca. 38 mm) and b, sugarcane armyworm (wingspan ca. 32 mm).

Materials and methods

Caterpillar collection locations, dates and techniques
Armyworm caterpillars were collected at RRAPL near Jerilderie, NSW (35°18'26.13"S 145°32'28.61"E) on 20 January 2016; at ‘Bimbil’, Pilkington Road, Gogeldrie, NSW (34°35'8.53"S 146°13'42.59"E) on 8 March 2017; and at ‘Willow Park’, Koonadan Road, near Leeton, NSW (34°29'8.31"S 146°22'32.05"E), on 31 January 2018. In all cases, the larvae were mid- to late instars (head widths 2.5–4.3 mm (2016), 2.3–3.8 mm (2017), 3.0–3.9 mm (2018)). They were collected by sweep net/beating from maturing temperate japonica rice crops under permanent flood. Caterpillars were kept cool and transported to the laboratory where they were reared individually in small plastic cups (Solo® Souffles 29.5 mL No. P100 with PL1 snap on lids, Dart Container Corp., Mason, MI), with small holes (about 2 mm diameter) cut in the lids to allow air flow. Rearing conditions were 25 ± 1 °C with a 15L:9D photoperiod and about 65% humidity. Caterpillars were provided with small cubes of the Griffith and Smith (1977) diet in 2016, and the modified diet developed in this project in 2017 and 2018. Fresh food was provided every two to three days until pupation or larval death. Pupae and dead larvae were retained for another 30 days to allow for either adult moth or parasitoid emergence. Adult moths were identified and the parasitoid types, numbers and emergence patterns were recorded.

In 2016, no further investigations were made because the presence of a second armyworm species in rice was not recognised until 2017. In 2017 and 2018, the same general procedure was followed; however, the remains of larvae and pupae that yielded parasitoids rather than adult moths were retained and stored individually at -20 °C. Parts of each specimen were subsequently isolated using sterile dissecting equipment and stored in molecular grade ethanol (also at -20 °C) until DNA extraction for barcoding.

Barcoding procedure
The identity of parasitised larval and pupal remains was determined genetically using general DNA barcode methods modified from Gopurenko et al. (2013). Dry pinned adults (n = 7) morphologically typed to Leucania stenographa or Mythimna convecta had been reared from larvae found at RRAPL and Gogeldrie during 2016 and 2017, and were included as reference material in the barcoding process. Before DNA extractions, ethanol used as a preservative was removed by evaporation over 48 hours in an incubation chamber (55 °C). Adult and larval specimens were allocated unique alphah-numeric sample IDs for DNA barcode record tracking. A single leg from each adult and larval and pupal samples were non-destructively digested by overnight incubation (55 °C) in aliquots of 270 µl
of buffer ATL and 30 µl of Proteinase K (600 mAU/ml) as provided in the DNeasy® Blood and Tissue Kit (Cat ID: 69506; QIAGEN, Chadstone, VIC). DNA was extracted from 250 µl of each digest using protocols and volume-adjusted reagents supplied in the GenElute® 96 well tissue genomic DNA extraction kit (G1N9604; Sigma-Aldrich, Castle Hill, NSW). Final DNA eluted to 170 µl was used as the template (2 µl) in PCR reactions (15 µl) processed with semi-automated liquid handling protocols for PCR preparation and sequence picking, as described by Gopurenko et al. (2013). PCR targeted the mitochondrial 5’ cytochrome c oxidase subunit I (COI) gene region with PCR reagents modified by use of M13 tailed oligo-nucleotide primers LepF1 (5’-TGTAAAACGACGGCCAGTATTCAACC AATCATAAGATATTGG) and LepR1 (5’-CAGGAAACAGCTATGACCTAAACTTCTGGAT GTCCCCAAAATCA) adapted from Hebert et al. (2004). PCR products were sequenced at AGRF (Brisbane). Bi-directional sequence chromatograms were quality checked, assembled and primer truncated using Lasergene SeqMan Pro ver. 8.1.0 (DNASTAR Inc., Madison, WI, USA) against a reference COI sequence of Leucania stenographa (GenBank accession: HQ951056). DNA barcode haplotype sequences (658 bp) were listed among specimens using FABOX (Villesen, 2007). Haplotypes were queried using online sequence search engines (searched 07.12.2018) for closest matching reported accessions at BOLD (Ratnasingham and Hebert, 2007) and BLASTN (NCBI Resource Coordinators, 2016). Haplotype sequences were conservatively treated as matched if > 99% similar to reported accessions.

Pairwise and unweighted nucleotide distances among aligned sequences were summarised using MEGA6 (Tamura et al., 2013) and excluded unidentified nucleotide sites from pairwise comparisons. Genetic distance relationships among COI haplotypes were inferred by Neighbour-Joining (NJ) tree construction implemented in MEGA6 incorporating a Kimura 2 parameter substitution model to accommodate Ts/Tv nucleotide rate variation, and using bootstrap replication (n = 10,000 replicates) to provide clade significance values. Clade supports of a final optimal NJ tree were determined by bootstrap replication (n = 10,000).

Results

DNA barcoding

High-quality DNA barcode sequences were obtained from 34 of 45 larval and pupal samples. Nine samples failed to amplify, and the sequences from two specimens were affected by the presence of > 5% heterozygous nucleotide sites, possibly incurred by co-amplification of the parasitoid barcodes. Five of the nine amplification failures related to larvae parasitised by wasp #1, an apparently polyembryonic species that effectively consumed practically all soft tissues within the host prior to emergence.

All remaining specimen DNA barcodes (n = 41 for larvae/pupae and reference adults) were free of heterozygous nucleotide sites. Ten DNA barcode haplotypes were identified among specimen sequences, and these were sorted as two genetic groups closest in genetic match (99.68–100% sequence similarity) to representative vouchers of either Leucania stenographa or Mythimna convecta reported to BOLD. Maximum (unweighted) sequence difference among haplotypes in each of the two species was < 0.15%; in contrast, the minimum sequence difference between haplotypes in each group to sequences of the nearest genetic neighbour species in BOLD was > 1.25%.

The NJ distance tree of adult and larval/pupal specimen barcode sequences is shown in Figure 8. It can be seen that sample DNA barcodes fall out as two genetically shallow groups matched to voucher sequences of either Leucania stenographa or Mythimna convecta. Interestingly, larvae sampled in 2017 from Gogeldrie were mostly Leucania stenographa; in contrast, 2018 specimens sampled from
Leeton were all identified to *Mythimna convecta*. Adult specimens typed to species based on their morphologies were verified by their DNA barcode identities.

**Parasitism rates and parasitoid species**

Overall parasitism rates across the armyworm population were high, but variable across sites and seasons, ranging from 27% at the Leeton site in 2018 to 81% at Jerilderie in 2016 (Table 7). All adults reared in 2016 were *M. convecta*. However, the sample size in 2016 was quite small, and DNA barcoding was not applied to the remains of parasitised caterpillars and pupae in 2016, so host-parasitoid identities cannot be confirmed. In 2016, of 26 parasitised armyworm, 17 were parasitised by wasp #1, seven by fly #2, one by fly #3, and one appeared to be parasitised by both wasp #2 and fly #3.

Table 7 - Parasitism rates for armyworm populations sampled during 2016, 2017 and 2018, with DNA barcoding results for host larval and pupal remains, and identified host-parasitoid associations.

<table>
<thead>
<tr>
<th>site</th>
<th>Jerilderie 2016</th>
<th>Gogeldrie 2017</th>
<th>Leeton 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (initial)</td>
<td>36</td>
<td>75</td>
<td>84</td>
</tr>
<tr>
<td>n (completed)</td>
<td>32</td>
<td>50</td>
<td>77</td>
</tr>
<tr>
<td>adult <em>M. convecta</em></td>
<td>6</td>
<td>25</td>
<td>55</td>
</tr>
<tr>
<td>adult <em>L. stenographa</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>parasitised</td>
<td>26</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>% parasitised*</td>
<td>81%</td>
<td>48%</td>
<td>27%</td>
</tr>
</tbody>
</table>

DNA barcoding results – host identity

| parasitised *M. convecta* |  - | 1 | 12 |
| parasitised *L. stenographa* | - | 22 | 0 |
| host undetermined | - | 1 | 9 |

Associations confirmed by barcoding (# of individuals)

*Mythimna convecta*

| wasp #1 | - | 0 | 5 |
| wasp #2 | - | 0 | 5 |
| wasp #3 | - | 0 | 0 |
| wasp #4 | - | 0 | 1 |
| fly #1 | - | 1 | 0 |
| fly #2 | - | 0 | 1 |
| fly #3 | - | 0 | 0 |

*Leucania stenographa*

| wasp #1 | - | 0 | 0 |
| wasp #2 | - | 1 | 0 |
| wasp #3 | - | 1 | 0 |
| wasp #4 | - | 0 | 0 |
| fly #1 | - | 14 | 0 |
| fly #2 | - | 5 | 0 |
| fly #3 | - | 1 | 0 |

The Gogeldrie sample had higher caterpillar mortality (33%) from unknown causes than in either 2016 (11%) or 2018 (8%). Superficially, it seemed that *M. convecta* dominated the population (96% of successful rearings to adult moths). However, barcoding showed 22 of the 23 parasitised armyworms
Figure 8 - Neighbour-Joining (NJ) distance tree of armyworm DNA barcode sequences. Scale bar = 1% K2P distance. NJ node supports estimated by bootstrap replication (N =10,000) and indicated where > 60%. Reference sequences of Leucania stenographa [accession: HQ951056] and Mythimna convecta [accession: HQ951045] included as available at GenBank and BOLD. Samples from Gogeldrie in 2017 and Leeton in 2018 indicated by shaded and unshaded triangles, respectively.

that could be identified were actually *L. stenographa* and that, therefore, the larval populations were quite evenly distributed (47% *L. stenographa*, 53% *M. convecta*). Additionally, barcoding showed that parasitism rates varied strongly across the two species (96% for *L. stenographa* and only 4% for *M. convecta*. This was caused by a very high incidence of parasitism in *L. stenographa* by fly #1.
In 2018, *M. convecta* strongly dominated successful host rearings (98%) and in contrast to Gogeldrie in 2017, this species was also the only one identified from the larval/pupal remnants of parasitised larvae and pupae. Over 98% of individuals identified through either adult morphology or DNA barcoding were *M. convecta*.

The barcoding process has allowed us to identify direct associations between the two armyworm species and the six parasitoid species recognised in our study during the 2017 and 2018 seasons:

- *M. convecta* – wasp #1, wasp #2, wasp #4, fly #1, fly #2
- *L. stenographa* – wasp #2, wasp #3, fly #1, fly #2, fly #3.

The species-level identities of the parasitoids are still being determined by specialists; however, they are illustrated in Figure 9, and observations on their biology are given below.

**Wasp #1.** Body length ~ 1.0–1.3 mm. This wasp appears to be polyembryonic and causes premature larval death, after which time the caterpillar gradually blackens and the host body fills with large numbers of parasitoid pupae. Of 71 instances of parasitism, this wasp was responsible for 27 (38%), despite not being found at the Gogeldrie site. The average number of wasps emerging per caterpillar was exactly 1,000 (range 536–1397) and 966 (range 536–1,332) where the host was unambiguously identified as *M. convecta*. This parasitoid was not found at Gogeldrie in 2017, and has not been recovered from *L. stenographa*.

**Wasp #2.** Body length ~ 2.0 mm. Larvae of wasp #2 tunnel out of the living host caterpillar and pupate externally in fluffy cocoons. On average, 45 (range 9–71) individuals were recovered from *M. convecta* larvae, while 36 were recovered from the single specimen of *L. stenographa* parasitised by this species. This species was responsible for 14% of the parasitism across all armyworm communities.

**Wasp #3.** Body length ~ 12 mm. Only one individual was recorded, which emerged from an *L. stenographa* larva.

**Wasp #4.** Body length ~ 11 mm. Only one individual was recorded, which emerged from an *M. convecta* pupa.

**Fly #1.** Body length ~ 10–12 mm. This species was recorded only at Gogeldrie in 2017 and was responsible for 14 of the 24 instances of parasitism at that site. Single flies emerge from the host pupae after being parasitised during the larval stage. It has been recorded from both armyworm species.

**Fly #2.** Body length ~ 9–11 mm. Also known from both host species and also with single flies emerging from the host pupae, this species was responsible for 21% of parasitism over all three seasons.

**Fly #3.** Body length ~ 3.5–4 mm. This species was recorded only twice: once from RRAPL in 2016 and again from Gogeldrie (host *L. stenographa*) in 2017. Multiple larvae (4–11 recorded, 11 from the *L. stenographa* specimen) emerge from the host larvae and pupate externally.
Discussion

This study has provided important baseline information on the armyworm populations associated with maturing rice crops in southern NSW. Initially, we were starting from a very low base, with no confirmed method of rearing field-collected armyworms through to adulthood, no understanding of the level of parasitism occurring in field populations, and an untested assumption that armyworm populations in rice consisted entirely of the common armyworm *Mythimna convecta*. As a consequence of this work (and on artificial diets outlined in previous sections), we now have proven techniques for assessing parasitism, knowledge on the diversity of parasitoids involved, and have recognised that a second and much less well-studied armyworm species, the sugarcane armyworm *Leucaena stenographa*, is also found in NSW rice crops. Because of the similarity between the larvae of the two armyworm species, we used DNA barcoding to identify parasitised hosts from larval and
pupal remains, and successfully identified the majority of hosts. This provided useful extra information on the armyworm communities; the base data suggested that *L. stenographa* is only a minor component of the overall community. However, the 2017 barcoding showed that the two species were more or less equally abundant. Identification of the parasitoids to species level is likely to reveal previously unknown host-parasitoid associations, particularly involving *L. stenographa*.

The situation differs from that in maize in southern NSW, where Hardwick (2006) reared only *M. convecta* from field-collected armyworm caterpillars. He found a parasitism rate of 96%, much higher than we found in rice (27–81% across mixed species communities). As in rice, dipteran species tended to dominate the parasitoid community in maize, where wasp #1, #2, and #4 were not recorded, despite their known association with *M. convecta* (Table 7).

In conventional crops such as wheat, the value of natural parasitism in controlling armyworms is undisputed. Armyworm species pupate in the soil near the base of plants, and parasitism affecting the egg, larval and pupal stages directly reduces the number of individuals produced in subsequent generations. In rice, however, the value of parasitism has yet to be fully understood. At least in regard to *M. convecta*, adult moths are known to develop in rangeland environments further west. Adults carried eastwards on prevailing winds initiate populations in rice and other crops. In a flooded rice crop, the mature caterpillars cannot pupate in the soil, and although a small number of larvae may successfully pupate in the leaf sheaths, it is probable that most mature larvae simply fall off the plants and drown. Any internal parasitoids would also be killed – the crop effectively becomes a dead-end for both pest and parasitoid. In this scenario, only parasitism that restricts food consumption by killing the host well before the completion of larval development will be of direct value.

While the conservation of beneficial species through the limited use of selective insecticides should always be considered good policy, more data is needed on the nature and extent of armyworm parasitism. Specifically, work should be undertaken on the incidence and effect of parasitism at the egg and early larval stages to determine its role in regulating overall crop damage by armyworm populations. The discovery of a second armyworm species in rice also leads to questions about insecticide susceptibility because there is no data available to indicate whether the sugarcane armyworm *L. stenographa* has the same level of susceptibility to insecticides as the common armyworm *M. convecta*. 
Using fermentation traps to monitor adult armyworm activity

Introduction

Despite the discovery of a second armyworm species in NSW rice crops, the common armyworm *Mythimna convecta* is still likely to be the most abundant species in rice and be responsible for most crop damage. In contrast to the sugarcane armyworm, the common armyworm has been studied extensively in relation to its distribution (McDonald et al., 1995) and techniques for its monitoring (McDonald, 1990; McDonald and Farrow, 1990). A design for a fermentation trap for monitoring adult moth activity was published by McDonald (1990). This approach to trapping was shown to have a higher level of specificity for common armyworm than light traps and lure pots, which both caught high numbers of *Agrotis infusa* (bogong moths) (McDonald and Farrow, 1990).

In this study, we modified two emergence traps previously used for rice bloodworm research and used them to monitor adults of the common armyworm at Yanco Agricultural Institute over a two-year period. The objective was to determine their effectiveness and obtain a qualitative comparison of moth activity adjacent to rice fields and in an adjacent dryland area. An effective trapping method for adult moths could be used in an early warning system for growers and encourage timely crop monitoring, avoiding some of the losses of recent seasons.

Materials and methods

Two fermentation traps were constructed by modifying bloodworm emergence traps to conform to the structure described by McDonald (1990). The main frame (Figure 10) was constructed from 10 and 15 mm diameter steel rod with a lower hoop 83 cm in diameter and an upper ring for mounting the collection jar 12 cm in external diameter with a 6 cm diameter central hole. The six steel rods joining the upper and lower rings were each 78 cm long. The body of the trap was covered with standard nylon flyscreen mesh. A ring 65 cm in diameter was formed from heavy gauge fencing wire and suspended inside the lower ring of the frame by drawing the excess lower mesh inwards and sowing it over the wire. This discouraged moths that fly into the trap from escaping by walking downwards on the mesh. The original short legs on the trap were extended by constructing legs from steel tubing that raised the trap so that the bottom of the main frame was 1 m above the ground. This increased height led to some instability, so three single links of heavy chain were welded to alternate arms of the frame to facilitate the attachment of guy ropes. A corresponding set of chain links were welded directly inside the frame for the attachment of nylon cords to hold the fermentation bait within the body of the trap. The inverted lid of a 2 L plastic jar was attached to the upper ring of the trap, and an inverted plastic funnel made from a clear disposable drink cup was taped to the inside of the collection jar that was then screwed to the lid (Figure 10). A removable gauzed tube lid was fitted to the side of the collection jar to prevent condensation and to allow easy removal of captured moths.

The attractant solution in each trap consisted of 50 mL of port wine and 75 g of brown sugar made up to a total volume of 500 mL with deionised water. The solution was placed in a 2 L white plastic bucket suspended within the main body of the trap.

The two traps were deployed at Yanco Agricultural Institute (34°37’S, 146°26’E). One was placed between irrigation bays used for rice production adjacent to the institute driveway and the main canal. The other was placed in a dryland area used for winter crop production about 1 km to the east and 900 m from the nearest rice crop. The traps were established in June 2017 and were operated until early July 2019. Traps were emptied at least twice weekly and generally three times per week during
warmer periods, when the fermentation lure needed to be either topped up with water or renewed more frequently. Adult common armyworm moths were separated from any bycatch and were not sexed because after identification they were used to supplement the laboratory breeding cultures. Other moth species were not recorded because the trap showed a high level of specificity for common armyworm.

Captures were recorded in a spreadsheet and aggregated for each calendar week. The structure of the data set precluded any detailed statistical analysis.

![Fermentation trap used to monitor flight activity of common armyworm moths at Yanco Agricultural Institute.](image)

**Results**

Results of the trapping program are shown in Figure 11, with both data sets portrayed at the same scale. Only 60 moths were captured at the dryland site over a two-year period; however, 345 were caught during the same period at the rice site. There appeared to be three periods of adult moth activity at the rice bay site, centred on week 4 (late January), week 22 (early June) and week 47 (late November). Comparisons are difficult to draw with data from the dryland site because of the small number of moths collected overall; however, there are indications of increased moth activity that correspond to both the June and November peaks at the rice bay site. There is, however, no indication of substantial adult armyworm activity in the dryland area that coincides with the January peak.
Discussion

The November and January peaks of activity at the rice site correspond with periods of active rice production. However, the June peak does not. It is unclear why so many more armyworms were caught at the rice site during June-July 2019, when rice was absent but young winter cereal crops were available near the dryland site. The two peaks of activity at the rice site, if correlated to oviposition activity in the rice crop, would lead to a November generation that would primarily cause foliar damage, and a January generation that would reach maturity as the crop approached harvest, leading to the severing of panicles and much more significant yield loss.

This study has demonstrated that fermentation traps can be used to monitor adult common armyworm activity. A network of traps could be used effectively as an early warning system, indicating to growers if and when they need to start crop monitoring. For this to be effective, however, the traps need to be adjacent to rice crops because traps positioned in dryland areas (to monitor armyworm activity in wheat crops, for example) are unlikely to detect any activity peak near rice crops during the critical period from January through to harvest.

![Graph showing total catches of adult common armyworm collected in fermentation traps over a two-year period at Yanco Agricultural Institute.](image)

Figure 11 - Total catches of adult common armyworm collected in fermentation traps over a two-year period at Yanco Agricultural Institute.

A complicating factor, however, relates to the potential of sugarcane armyworm to become more dominant in rice systems. We did not catch any sugarcane armyworm in our fermentation traps, and this corresponds well with the results of McDonald and Farrow (1990) who found *L. stenographe*
(referred to in that paper as *Mythimna loreyimina*) was only rarely caught in fermentation traps and lure pots; it was captured more often in light traps and vehicle nets. If the sugarcane armyworm is confirmed as a significant pest of rice in NSW, then an alternative to the fermentation trap will need to be developed for monitoring purposes.
Impact of simulated armyworm damage on rice yield and quality

Introduction

Armyworm damage to rice crops appears to occur in two distinct phases, with different impacts on plant yield. When infestations occur relatively early in the season (late November and through December), feeding damage is confined to the leaves because the panicles have not yet developed. Anecdotally, this damage appears to have little impact because of the rice plant’s ability to compensate for the loss of vegetative tissue as growth continues. In the second phase of crop damage, which arises from infestations initiated from around January onwards, the larger caterpillars often bite through the spikelets on the panicles, causing the developing grain to fall into the water. This is both the most severe and most obvious form of armyworm damage.

There is a widespread perception among growers and agronomists that armyworm outbreaks are starting to become more common early in the growing season, leading to a potentially greater risk of foliage loss directly reducing eventual yield and quality. In this study, we tested the theory that rice plants can recover from extensive foliar damage early in the season, without significant losses of yield or changes in quality. Two trials involved the artificial defoliation of rice plants shortly after panicle initiation, and the assessments of yield and other parameters at harvest.

Materials and methods

Two defoliation trials were conducted at Yanco Agricultural Institute (34°37’S, 146°26’E), one during each of the 2016-17 and 2017-18 rice seasons, and both following the same format.

The crops were both cv. Reiziq drill-sown at 150 kg seed/ha with 25 cm row spacings. Urea was applied at 120 kg N/ha immediately before permanent water, and no additional N was applied at panicle initiation in either season. Key dates for the two trials are given in Table 8.

Table 8 - Key dates for the 2016-17 and 2017-18 artificial defoliation trials.

<table>
<thead>
<tr>
<th></th>
<th>2016-17 season</th>
<th>2017-18 season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sowing</td>
<td>27 October 2016</td>
<td>27 October 2017</td>
</tr>
<tr>
<td>Permanent water</td>
<td>6 December 2016</td>
<td>1 December 2017</td>
</tr>
<tr>
<td>Panicle initiation</td>
<td>5 January 2017</td>
<td>4 January 2018</td>
</tr>
<tr>
<td>Experimental defoliation</td>
<td>18–25 January 2017</td>
<td>15-23 January 2018</td>
</tr>
<tr>
<td>Plot harvest</td>
<td>3-7 April 2017</td>
<td>12-13 April 2018</td>
</tr>
</tbody>
</table>

In each trial, 12 plots, each 1 m², were used with four plots randomly allocated to each of three treatments: 25% defoliation by leaf length; 50% defoliation by leaf length; and an undefoliated control. The corners of each plot were marked with colour-coded fibreglass poles, and all plant leaves within the defoliation plots were individually trimmed to length with scissors (Figure 12). The plant material removed was retained. With a leaf area meter, a subsample was assessed before being weighed after dehydration overnight at 80 °C to estimate the relationship between leaf area and plant dry weight. The remaining plant material from each plot was then dehydrated and weighed.
The plots were monitored weekly to ensure no natural infestations of armyworms or other foliar pests damaged the plants. When the plants were ready for harvest, the lateral rows and the ends of the central rows were cut away. The central 60 cm of the three middle rows were harvested by cutting the complete plants at a uniform height of 11 cm above ground level. The bagged plants were taken to the laboratory and allowed to dry for two days before further processing.

The number of panicles in each sample was counted. The paddy was then manually stripped, weighed, and moisture was assessed with a CropScan 3000B near-infrared transmission analyser (Next Instruments, Condell Park, NSW). Grain weights were then adjusted to 10% moisture.

Grain samples were processed with a mechanical aspirator to separate full and sterile grains, with the separation checked manually to confirm machine settings. Full grains from each sample were counted with a Contador seed counter (Pfeuffer GmbH, Kitzingen, Germany). The number of steriles was calculated on the total weight of steriles in the sample and the weight of 200 manually counted steriles per sample after dehydration. Percentage sterility was then calculated for each sample.

The grain samples were then dehulled and taken to the cereal chemistry section at Yanco Agricultural Institute to analyse sulphur and protein content. This content was measured in rice flour using an MPA FT-NIR spectrometer (Bruker, Billerica, MA, USA) calibrated against commercially tested N standards using the Dumas method (AOAC, 1995), and with LECO instrumentation (LECO, St. Joseph, MI, USA). Protein content is reported with a N:protein conversion factor of 5.95.
**Statistical analysis**

Data from each trial was analysed separately. The quantities of foliage initially removed from the 25% and 50% defoliation plots were compared using unpaired t-tests. Post-harvest data assessed (per plot sample) included foliage dry weight, number of panicles, paddy weight at 10% moisture, and full grain weight at 10% moisture. Percentage sterility, grains per panicle, filled grains per panicle, single filled grain weight, % sulphur and % protein were also analysed.

Percentage data was inverse-sine transformed, then all data sets were tested for normality using the Shapiro-Wilks test, and for variance homogeneity using Levene’s test. Data sets that conformed to the assumptions of analysis of variance were analysed using one-way ANOVA and LSD tests to separate means. In 2016-17, square root transformation was applied to the panicle data before analysis to reduce variance heterogeneity.

Data sets that could not be transformed to fit the assumptions of ANOVA (2016-17: grains per panicle, % protein; 2017-18: % sulphur) were analysed using the non-parametric Kruskal-Wallis test.

**Results**

Comparison of dry weights and leaf area removed indicated that 1 g of leaf tissue (dry weight) removed at around panicle initiation corresponded to 164.8 cm² of leaf area. The mean total leaf areas removed from the 1 m² plots were 8,853 (25%) and 17,910 (50%) cm² in 2016-17, and 11,532 (25%) and 25,260 (50%) in 2017-18. In each year, the mean areas of leaf tissue removed from the two defoliation treatments were significantly different (t-test, $P = 0.0009$ (2016-17), $P < 0.0001$ (2017-18)).

Results for the harvest characteristics, other than sulphur and protein, are shown in Figures 13 and 14. Some parameters showed consistent and significant trends across both years, while others were more variable. Foliage dry weight and paddy yield (both total and full grains only) showed identical patterns in both years, where 50% defoliation produced a significant yield loss (14–17% of total paddy) relative to the controls, but 25% defoliation did not. Both defoliation treatments produced small but significant declines in individual filled grain weights.

Other parameters were less consistent across seasons, and in general, produced declines at 50% defoliation in 2017-18 (but not at 25% defoliation), while in 2016-17, no significant effects on these parameters were recorded. These parameters were % sterility and grains per panicle (both total and full grains). The number of panicles per plot sample was not significant in 2017-18. While it was significant in 2016-17, it did not follow a clear trend with increasing plant damage.

Results for grain sulphur and protein are shown in Figure 15. While the differences between treatments were not statistically significant, there was a strong downward trend in both parameters with increasing levels of defoliation. Differences in protein concentrations approached the nominal 5% level of significance in both trials.

**Discussion**

The results of these two trials generally confirm the belief that rice can withstand substantial damage during vegetative growth, and can compensate for it later in the growing season. The only parameter significantly negatively affected by 25% defoliation shortly after panicle initiation was single grain weight, which declined by 1.1 to 3.1%. However, at 50% defoliation, a range of other parameters...
Figure 13 - Results from the artificial defoliation trials. Years refer to harvest years. Graphs with red bars have significant differences between treatments while those with grey bars do not. In each graph with red bars, columns with different letters are significantly different (ANOVA, LSD test, $P < 0.05$). Data not transformed except where indicated otherwise.
Figure 14 - Results from the artificial defoliation trials. Years refer to harvest years. Graphs with red bars have significant differences between treatments while those with grey bars do not. In each graph with red bars, columns with different letters are significantly different (ANOVA, LSD test, $P < 0.05$, or Kruskal-Wallis test, $P < 0.05$). Data not transformed except where indicated otherwise.
were significantly affected, notably overall yield and vegetative biomass. After 50% defoliation, the total paddy yield was reduced by 14 to 17%.

Comparisons with other studies on artificial defoliation are difficult because they invariably involve different cultivars defoliated to different levels at varying times during the growing season, which is further complicated by agronomic and climatic variations. Taylor (1972) found that upland varieties of rice not only tolerated 50% defoliation by leaf length, but yield actually increased, provided defoliation occurred before flowering, and large yield penalties were not incurred until more than 67% of leaf length was removed. Profound effects on yield resulting from defoliation after tillering and before flowering occurred only when the plant was completely defoliated at ground level, with shorter season varieties being most strongly affected. In contrast, Bowling (1978) found that 25% and 50% defoliation by mower at either the seedling or tillering stage produced significant yield loss that approached 12% in some instances. Rice et al. (1982) used a trial structure with variable levels of defoliation of directly adjacent plants, with defoliation at 81–83 days, which was similar to the timing used in our study (80–90 days). In common with our study, they found no significant effect on yield at 25% defoliation, but significant reductions at 50% and 100% foliage removal. Greater sterility (‘blanking’) was identified at 50% defoliation, which we also observed in our 2017-18 trial.

Rice et al. (1982) also compared the consequences of artificial defoliation to that caused directly by the armyworm *Pseudaletia unipuncta* (Haworth), and found that 25–30% larval damage led to a much greater level of yield loss than 25% artificial defoliation. This was attributed to the different nature of genuine armyworm damage, where leaves have lateral sections removed on either side of the midrib, and damage can be ongoing rather than arising from a single event. Another factor that might increase the relative impact of genuine insect defoliation is the salivary secretions of insects, which can influence how plants respond to attack by herbivores. These secretions on the margins of leaf injuries can trigger plants to synthesize and release compounds that are involved in plant-plant signaling. They might be involved in tritrophic interactions, attracting beneficial predators and parasitoids that attack the herbivores. Such elicited responses, which are generally not triggered by artificial damage, could come at considerable metabolic cost to the plants, leading to greater yield losses. Further studies are

![Figure 15 - Sulphur and protein content of rice from the artificial defoliation trials. Inverse-sine transformed data analysed by ANOVA, LSD test, P < 0.05 (where F value provided), or the Kruskal-Wallis test, P < 0.05 (where KW value provided)).](image)
needed into direct armyworm feeding to determine whether and to what extent the data from artificial defoliation studies is underestimating the true impact of leaf area loss.
Stem rot evaluations in the Finley district

Introduction

Stem rot caused by the fungus *Sclerotium oryzae* was first recorded in the Riverina in the 1994-95 rice season (Watson and Priest, 1998). Stem rot has also been referred to in the literature as *Magnaporthe salvinii* or *Nakataea oryzae*.

The fungus attacks the rice plant at the water line, causing rotting of the leaf sheath and stem (Figure 16). The rotting of the stem subsequently affects both grain yield (Figure 17) and quality (increase in chalk) and, in severe cases, causes plants to lodge. Stem rot produces tiny infective structures called sclerotia that are formed on and inside the infected plant tissue. After harvest, they remain in the stubble, and at flooding in the following season, they float to the surface of the water and reinfect the new crop. In some overseas regions, stem rot causes serious issues; however, yield loss was minimal when observed in the Riverina during surveys in 1995. Stem rot found in a rice crop in northern Victoria in 2014 caused lodging and substantial yield loss (estimated at 2 t/ha). This was the first record of yield loss caused by this pathogen in Australia. In the following year, a rice crop was sown on the same blocks that had stem rot the previous year. As a precaution against potential yield loss, an APVMA permit was obtained for a product containing azoxytrobin (Amistar®) as a two-time fungicide application.

In 2016, a request was issued for any stem rot samples to be submitted to the plant pathology laboratory at Yanco Agricultural Institute. Only a small number were submitted, and surveillance continued into the next season.

Figure 16 - The disease cycle of stem rot of rice showing stem symptoms and sclerotia found on and inside the stem.
In 2017, some crops were showing symptoms of stem rot, mainly in the Finley area of southern NSW. Stem rot had not previously been seen in that area. Some farms also had some crop yellowing noticeable as large patches, which was initially believed to be caused by stem rot. Plant material close to harvest was collected to determine whether yield loss may have occurred as a result of stem rot infection. Some questions that could be answered included whether there was any disease difference between farms, and whether the lowest part of the bay would be worse for stem rot, as had been reported previously (Watson and Priest, 1998). There was also a need to collect information on the effect of stem rot on yield, notably for grain number and whether levels of sterility are affected. The frequency of unfilled grains resulting from stem rot infection is not known because empty grains are part of the trash that is expelled from the header in the harvesting process.

**Materials and methods**

**Extension**

In early February 2017, visits to affected farms with a local agronomist confirmed that stem rot was the causal organism; others were informed about disease identification.

**Disease severity and incidence**

Close to harvest, four farms (CB, JP, MB and TM) were selected in the region where stem rot had been observed, all with the cultivar Sherpa. Only one farm (CB) had any yellowing. On another farm, one sample only was collected (AJ). Quadrat (1 m²) samples of rice were taken at five positions about 30 m apart, progressively moving up a bay from the lowest point to the highest (Figure 18). GPS coordinates were recorded at each sampling point. The rice was cut at ground level, placed inside large white polypropylene bags and returned to the laboratory.

Leaf sheaths on each stem were pulled apart to expose the stem that was rated for the level of stem rot damage. The stems were examined for three factors. First, the level of stem rot damage to the main stem as a visual rating: 0 for no stem rot; 5 for severe stem rot; and 1, 2, 3 and 4 as intermediate scores. The rating of 5 was allocated to stem rot where the stem was either totally dead due to severe infection, or the stem had severe damage with the fungus rotting it away. The second factor recorded was the presence or absence of aggregate sheath spot, and the third was whether or not the stem was totally dead.
Yield loss
Panicles from the samples were removed from the stems and put into paper bags based on their stem rot disease rating. Ratings of 1+2 and 3+4 were bagged together, and therefore there were four sample categories: 0, 1+2, 3+4, and 5. To examine any effect of stem rot on yield, 20 panicles from each of the sample 1 plots were pulled apart by hand, and grains were divided into full and empty, counted and weighed, as per Figure 19. Additionally, a more complete analysis was undertaken where panicles from all the assessment categories for all sample plots were assessed from the CB and JP farms only. Data was analysed using ANOVA and LSD intervals in Genstat 18th edition.

Results
Extension
First reports of stem rot came from a Finley agronomist in early February 2017 when samples submitted to Yanco Agricultural Institute confirmed the presence of the disease. This was the first significant outbreak since 1995; awareness of the disease was minimal, although it had been publicised at field days and industry conferences since 2014 when stem rot was detected on one farm in VIC.

On a field trip to the region, a microscope was used to show characteristics of the disease to various agronomists. Awareness of the possibility of stem rot in a rice crop is important for its early detection and future management. Information collected and given to industry in various forms culminated in the preparation of a NSW DPI Primefact (Watson, 2018).
Figure 19 – Separating method for grains from each panicle.

The Finley area weather data (Table 9) shows that 2016 had regular rainfall, and generally more than the long-term mean. Rainfall common from May onwards could have made burning stubble more difficult. This supported accounts by growers and agronomists that difficulty in burning stubble during 2016 would have contributed to the higher stem rot incidence in the subsequent crop.

**Disease severity and incidence**

Each farm and paddock had different levels of stem rot severity (Table 10), where the highest was at JP at 2.8 ($P < 0.001$, LSD = 0.1). Disease incidence was also significantly different between farms ($P < 0.001$, LSD 5% = 16.2). The number of stems with symptoms out of the total number of stems examined was very high on some farms, up to 93% at AJ and JP (Table 10). The mean number of dead stems was also variable across the farms, and significant differences were detected, although the significance level ($P < 0.075$) was just outside the nominally accepted 5% level. Plots from the lowest part of the bay (sample 1) had significantly higher disease levels than the other samples (Table 10). An example of the proportion of stems in each category is represented in Figure 20, where there are a high proportion of stems with the rating of 5 in sample 1 from farm JP.

**Yield loss**

Full and empty grains were pulled from panicles, weighed and counted. Stems with the ratings of 5 (1.1 g) were significantly lower for grain weight than the other ratings (mean across ratings 1+2, 3+4 and 0 = 1.6 g) ($P = 0.002$, LSD = 0.22, Figure 21). This indicated that infected stems of rating 5 had a yield loss of about 27%.
Table 9 – Rainfall data (Bureau of Meteorology) for the Finley area, 2016.

<table>
<thead>
<tr>
<th>Month</th>
<th>2016 rainfall (mm)</th>
<th>long-term average (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>86.7</td>
<td>33.0</td>
</tr>
<tr>
<td>February</td>
<td>12.6</td>
<td>29.1</td>
</tr>
<tr>
<td>March</td>
<td>22.0</td>
<td>34.5</td>
</tr>
<tr>
<td>April</td>
<td>11.6</td>
<td>32.2</td>
</tr>
<tr>
<td>May</td>
<td>80.5</td>
<td>41.0</td>
</tr>
<tr>
<td>June</td>
<td>84.7</td>
<td>44.1</td>
</tr>
<tr>
<td>July</td>
<td>53.8</td>
<td>42.2</td>
</tr>
<tr>
<td>August</td>
<td>76.8</td>
<td>43.2</td>
</tr>
<tr>
<td>September</td>
<td>121.5</td>
<td>39.3</td>
</tr>
<tr>
<td>October</td>
<td>37.4</td>
<td>43.0</td>
</tr>
<tr>
<td>November</td>
<td>48.9</td>
<td>34.9</td>
</tr>
<tr>
<td>December</td>
<td>13.8</td>
<td>34.5</td>
</tr>
</tbody>
</table>

Table 10 - Disease severity and incidence across the five farms, and ratings across all farms and all stems in relation to sample position.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Overall disease severity*</th>
<th>Overall disease incidence %**</th>
<th>Dead stems per sample</th>
<th>Sample</th>
<th>Mean rating for all stems assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM</td>
<td>0.5 a</td>
<td>26.0 a</td>
<td>0.3</td>
<td>1 (drain end)</td>
<td>2.7 a</td>
</tr>
<tr>
<td>CB</td>
<td>1.6 b</td>
<td>72.2 b</td>
<td>2.2</td>
<td>2</td>
<td>1.6 b</td>
</tr>
<tr>
<td>MB</td>
<td>1.7 b</td>
<td>56.5 b</td>
<td>7.4</td>
<td>3</td>
<td>1.3 c</td>
</tr>
<tr>
<td>JP</td>
<td>2.8 c</td>
<td>93.0 c</td>
<td>8.0</td>
<td>4</td>
<td>1.4 c</td>
</tr>
<tr>
<td>AJ***</td>
<td>2.3 c</td>
<td>93.0 c</td>
<td>1.0</td>
<td>5</td>
<td>1.3 c</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.075</td>
<td>P</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>0.1</td>
<td>16.2</td>
<td>NSD</td>
<td>LSD 5%</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Disease severity over the 5 quadrats taken from each farm. Stem rot rating when rated from 0 to 5, with 0 no stem rot and 5 the most severe, for each farm.

** Disease incidence measured as a percentage of the total number of stems, with symptoms out of the total number of stems assessed.

*** AJ was not included in the analysis because it was unreplicated; however, it is included in the graph for reference.

When the grain weights and numbers for all the plots for farms JP and CB (the worst affected farms with replicate samples) were analysed, full grain weight was higher in all categories than the rating of 5, weight of empty grain was higher in the ratings of 5 than in the other categories (Figures 22 to 25). Full grain numbers were higher for all categories compared to the rating of 5; empty grain numbers were higher in rating 5 than the other ratings. The disease ratings of 1+2 and 3+4 had minimal effect on the yield factors.
Figure 20 - An example of the frequency of stem rot severity ratings as a % of the stems assessed. Sample 1 (drainage end) JP farm on the left, and sample 5 on the right. A much larger proportion of stems with a 5 rating (39%) were found near the lower end of the bay.

Figure 21 - Yield (as grain weight per panicle) for sample 1 (drainage end) when measured across all the farms for each rating. Panicles from rating 5 stems have significantly less yield (27%, P = 0.002, and LSD = 0.22) than those with other ratings.
Figure 22 - Weight of full grain per panicle for each of the four disease categories for each of the sample categories 1–5. Sample 1 (i.e. JP1 and CB1) was at the drainage end of the bay, JP data at the top; and CB below. Samples in category 5 of CB were in one of two shorter yellow patches in the crop and, consequently, had lower yield. JP – significant across sample categories P < 0.002 and across damage levels within categories P < 0.001, LSD 5% 0.39. CB – not significant across sample categories, but P < 0.001, LSD 5% 0.19 across damage levels within categories.
Figure 23 - Weight of empty grain per panicle for each of the four disease categories for each of the sample categories 1–5. Sample 1 (i.e. JP1 and CB1) was at the drainage end of the bay, JP at the top; and CB below. Samples in category 5 of CB were in one of two shorter yellow patches in the field. JP – not significance across sample categories, but significant across damage levels within categories P < 0.001, LSD 5% 0.06. CB – significant across sample categories P < 0.001 and across damage levels within categories P < 0.002, LSD 5% 0.028.
Figure 24 - Number of filled grains per panicle for each of the four disease categories for each of the sample categories 1–5. Sample 1 (i.e. JP1 and CB1) was at the drainage end of the bay. JP at the top; and CB below. Samples in category 5 of CB were in one of two shorter yellow patches in the field, and consequently had lower yield. JP – not significant across sample categories but significant across damage levels within categories $P < 0.001$, LSD 5% 0.06. CB – significant across sample categories $P < 0.001$ and across damage levels within categories $P < 0.002$, LSD 5% 0.028.
Grain number: empty

Figure 25 - Number of empty grains per panicle for each of the four disease categories for each of the sample categories 1–5. Sample 1 (i.e. JP1 and CB1) was at the drainage end of the bay. JP at the top; and CB below. Plants with damage rating 5 had significantly more empty grains than plants with lower damage ratings. JP – not significant \( P = 0.085 \) across sample categories but significant across damage levels within categories \( P < 0.001 \), LSD 5% 5.8. CB – significant across sample categories \( P < 0.001 \) and across damage levels within categories \( P < 0.001 \), LSD 5% 9.3.

When assessing stems for disease levels, it was noted that those in the rating class ‘nil’ might have lacked disease symptoms because they were more immature than those that were diseased, suggesting they were later-developing stems with less exposure to sclerotia and, consequently, slower symptom development. Actual yield loss due to stem rot for the plots can be calculated by using the number of stems and the proportions of each category of rating (Figure 20) combined with the mean weight of full grains from each category, as in Figure 21. This has been represented in Table 11, using a mean
number of 600 stems, collected per quadrat cut, i.e. 1m². Yield loss was calculated to be 12.5%, but this figure will be variable across a block.

Table 11 - Yield loss for sample plots at the bottom ends of bays on a farm severely affected by stem rot. The yield loss in these plots was 12.5%.

| Number of stems | 240 | 360 | 12.5% |
| Weight of full grain | 1.1 | 1.6 |     |
| Grain weight | 264 | 576 | 840 |
| If all healthy | 0 | 600 | 960 |

**Discussion**

Even though the 2016-2017 season involved many reports of stem rot, there were far fewer reports in the following season. The 2016-17 season was characterised by a wet period between harvesting the 2015-16 crop in April-May 2016 and sowing in October-November 2016. There were many reports that burning crop residues in 2016 was extremely difficult due to the wet conditions. Therefore, any sclerotia present in unburnt plant material would remain viable and could infect the subsequent crop. Stem rot must have been unnoticed in the region before 2016. There was one record in 2014, so it might have been there for a while and slowly built up.

Based on the information collected in 2017 and 2018, we have a much better understanding of the effects of stem rot on rice in the region. Stem rot is in all growing areas, but current management practices are controlling the disease. On occasions when some factor interrupts normal management practices, stem rot can build up, as in 2016-17, when prior inadequate crop residue burning was a contributing factor. When combined with regular rice cropping and minimal or zero crop rotations on some fields, poor stubble destruction provides ideal conditions for the disease to proliferate. From observations and experiments conducted when stem rot appears severe, yield loss is generally minimal and invariably worse at the lower end of the bays, which is where the greatest number of free-floating sclerotia would accumulate as bays are dried down before harvest in any given year.

Stem rot is a potentially serious disease that causes damage to stems. However, it is slow to progress in the plant, so damage, although obvious, appears to have minimal effect on yield. Shorter season varieties would be expected to better tolerate stem rot, as would drill-sown crops in which permanent water is delayed, reducing the period floating sclerotia have to infect the stem. Water around the plant is a major requirement for infection.

The yield loss caused by stem rot was previously estimated at 10%; however, the grower seriously affected in 2014 had a yield loss of 2 t/ha. In the samples we collected from the worst infected areas, the yield loss was 12.5% or 1.2 t/ha. A grower in the Finley area reportedly lost 0.5 t/ha due to stem rot in 2018 (Greg Sefton, personal communication), which was after four crops of rice over five years. While the current practice of repeat cropping rice to use residual water in the soil profile from the previous crop clearly saves water, the available evidence indicates that it will foster snail problems and stem rot outbreaks.

While farms with a second consecutive rice crop often had high levels of stem rot, other farms that were not growing a second consecutive crop also had the disease present. Sclerotia can survive for a
number of years. It is important to note that even though there may be a year’s break between rice crops, if standing rice stubble remains then that extra year’s fallow will not fully resolve the problem.

Stubble slashing might also be counterproductive because it could increase the dispersal of sclerotia. Those that fall into cracks on the ground may be protected from heat needed to destroy them during a subsequent burn.

Where drainage water goes after the end of a rice crop contributes to future disease levels. When water was more readily available, the deliberate draining of crops may have moved a proportion of sclerotia off-farm, while the current practice of locking up bays and allowing the remaining water to be used by the crop effectively means that stem rot sclerotia are retained in the field where they have developed, potentially providing a source of inoculum for the next rice crop.

Careful consideration should be given to optimal methods of burning stubble. It was shown that with similar organisms to stem rot, aggregate sheath spot *Rhizoctonia oryzae-sativae* and sheath spot *R. oryzae* (or *Waitea circinata*), the fungi could survive as mycelia in rice debris, whether or not that debris was buried or remained on the surface (Lanoiselet et al., 2005). The same effect would occur with stem rot – the fungus could keep growing on any plants left in or on the ground during a fallow period before burning. The plant material with the fungus would also continually produce sclerotia. Lanoiselet et al. (2005) also considered that stubble burning did not affect fungal sclerotia.

**Other diseases**

During assessments for stem rot in the crops from Finley, aggregate sheath spot *Rhizoctonia oryzae-sativae* (Figure 26) was also seen in many samples, but its impact was not as severe as that caused by stem rot. Sheath spot *R. oryzae* (*Waitea circinata*) was also recorded. The effects of both diseases appeared to be minimal on plant health.

![Figure 26 - Aggregate sheath spot (left) and sheath spot (right).](image)
Susceptibility of Australian rice cultivars to stem rot

Introduction

Resistance to stem rot has not been used as a trait for selecting new rice varieties in Australia, and as a consequence it is likely that all current commercial varieties and those under development are vulnerable to the disease to some extent. Nevertheless, differences might occur. The extent of these differences needs to be understood because choosing a variety with some tolerance to the disease might minimise its impact if circumstances dictate that growers need to plant rice in fields with a recent history of stem rot, and where stubble burning has been problematic.

Studies in a previous project (Stevens and Watson, 2016) were confined to two of the current varieties, Reiziq and Sherpa, along with a group of older cultivars. In this project, we looked at a much broader set of current varieties, using both tank and single-stem inoculation techniques, with the tank inoculation work reported in this section.

Two experiments were conducted, the first of which looked at both varietal susceptibility and the capacity of Amistar Top®, a product containing azoxystrobin and difenoconazole, to control stem rot. This is the first time this product has been used in an experimental situation in association with stem rot management in Australia.

In the second experiment, with Sherpa, we sought to quantify any yield loss caused by stem rot using the same experimental conditions as in the cultivar experiment. Earlier data had shown that Sherpa is particularly vulnerable to stem rot infection.

Materials and methods

Cultivar experiment

Ten seeds of each of the cultivars Sherpa, Reiziq, Koshihikari, Opus, Illabong, Langi, Doongara, Topaz, YRM70 and YRK5 were planted into soil in 200 mm pots. Two pots per cultivar were randomly placed in each of six fibreglass tanks (1.1 m x 1.1 m x 0.25 m deep). Each tank was fitted with a float valve to maintain the water level. The tubs were placed in a greenhouse with a temperature range of 20–30 ºC.

Stem rot sclerotia were extracted from the base of cut samples collected from the Finley area by crushing them within a polypropylene bag and then sieving through a 250 µm sieve. About 1,000 sclerotia (0.1 g) were placed in each pot eight weeks after planting, and just before permanent water. One day later, the tubs were fertilised with urea to the equivalent rate of 150 kg N/ha.

Three of the six tubs were sprayed with Amstar Top® at 2.5 L/ha, which is twice the recommended rate. The product was applied with a backpack sprayer twice, before panicle initiation and then two weeks later. The boom was held above the plants as if they were being sprayed from a tractor-mounted boom.

At plant maturity, each pot was removed and each stem was assessed for disease severity by measuring the stem rot lesion length on the outer leaf sheath and stem, and also by rating the lesion appearance on a scale of 0–5, as in previous experiments. Panicles were removed, and fresh and dry weights were recorded.
Sherpa experiment
Four more pots of Sherpa were placed in the same tubs to use as an extra comparison between treated and untreated tubs. Plants, stems and panicles per pot were counted, and the whole plants were weighed. Full and empty grains were counted and weighed. Data was analysed using ANOVA and LSD intervals in Genstat 18th edition.

Results

Cultivar experiment
Symptom development was good, with early symptoms obvious (Figure 27). Disease levels were significantly different across the cultivars (Table 12), with Sherpa showing a high level of vulnerability and Reiziq being one of the more tolerant varieties. These results were similar to those from previous experiments (Stevens and Watson, 2016). The ratings for leaf sheaths were close across all cultivars, but did not correlate closely with the ratings for stems. The Amistar Top® treatment was very successful at controlling stem rot, as shown in Table 13. In the cultivar experiment, there were slightly more panicles in the treated tubs, but no significance difference in panicle dry weights (Table 14).

Figure 27 - Early stem rot symptoms in the greenhouse experiment.

Sherpa experiment
Similar results were obtained in the Sherpa experiment. Even though total dry plant weight was significantly higher in Amistar Top® treatment (Table 15), there was no statistically significant difference in grain yield. The plants in the treated tubs were greener and were free of totally black lower stems, as seen in Figures 28 and 29.
Table 12 - Disease ratings for stem rot in the greenhouse experiment with 10 cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Lesion length: stem (mm)</th>
<th>Lesion score: stem**</th>
<th>Lesion length: leaf sheath (mm)</th>
<th>Lesion score: leaf sheath**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doongara</td>
<td>104.5 a*</td>
<td>1.6 a</td>
<td>129.5 cd</td>
<td>3.9 bcde</td>
</tr>
<tr>
<td>Reiziq</td>
<td>120.0 ab</td>
<td>2.3 b</td>
<td>124.8 cd</td>
<td>3.5 ab</td>
</tr>
<tr>
<td>Topaz</td>
<td>130.6 b</td>
<td>2.4 bc</td>
<td>125.4 cd</td>
<td>3.2 a</td>
</tr>
<tr>
<td>Langi</td>
<td>137.7 bc</td>
<td>2.9 d</td>
<td>113.9 a</td>
<td>3.8 bcde</td>
</tr>
<tr>
<td>Illabong</td>
<td>139.8 bcd</td>
<td>2.7 cd</td>
<td>140.8 e</td>
<td>4.1 ef</td>
</tr>
<tr>
<td>Koshihikari</td>
<td>156.0 cde</td>
<td>3.4 e</td>
<td>115.7 ab</td>
<td>3.6 bc</td>
</tr>
<tr>
<td>YRK5</td>
<td>159.6 de</td>
<td>2.9 d</td>
<td>131.2 d</td>
<td>4.1 ef</td>
</tr>
<tr>
<td>Opus</td>
<td>166.9 ef</td>
<td>3.8 ef</td>
<td>122.6 abc</td>
<td>3.7 bcde</td>
</tr>
<tr>
<td>YRM70</td>
<td>181.6 ef</td>
<td>4.0 f</td>
<td>130.4 b</td>
<td>4.0 def</td>
</tr>
<tr>
<td>Sherpa</td>
<td>186.0 f</td>
<td>3.9 ef</td>
<td>140.1 e</td>
<td>4.3 f</td>
</tr>
</tbody>
</table>

*Values that do not share the same letter are significantly different. ** 0–5 score.

Table 13 - Comparison of disease levels between fungicide-treated pots and untreated infected pots.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lesion length: stem (mm)</th>
<th>Lesion score: stem**</th>
<th>Lesion length: leaf sheath (mm)</th>
<th>Lesion score: leaf sheath**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungicide</td>
<td>7 a</td>
<td>0.2 a</td>
<td>82.6 a</td>
<td>1.1 a</td>
</tr>
<tr>
<td>Untreated</td>
<td>153 b</td>
<td>3.2 b</td>
<td>127.1 b</td>
<td>3.8 b</td>
</tr>
</tbody>
</table>

*Values that do not share the same letter are significantly different. ** 0–5 score.

Table 14 - Panicle number and dry weight at harvest for the cultivar experiment comparing fungicide-treated pots to untreated pots.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of panicles</th>
<th>Mean panicle dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungicide</td>
<td>4.6 a*</td>
<td>2.3</td>
</tr>
<tr>
<td>Untreated</td>
<td>4.1 b</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*Values that do not share the same letter are significantly different.

Table 15 - Results from the Sherpa experiment showing the effect of fungicide treatment on dry weight, panicle number, weight of empty grain and dry plant weight.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dry weight grain (g/pot)</th>
<th>Panicle number</th>
<th>Dry weight grain per panicle (g)</th>
<th>Weight empty grain (g)</th>
<th>Dry plant weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungicide</td>
<td>41.6</td>
<td>21.5</td>
<td>1.88</td>
<td>0.44 a*</td>
<td>65.0 a</td>
</tr>
<tr>
<td>Untreated</td>
<td>37.8</td>
<td>20.1</td>
<td>1.88</td>
<td>0.99 b</td>
<td>43.7 b</td>
</tr>
</tbody>
</table>

*Values that do not share the same letter are significantly different.
The method of using sclerotia in tubs was successful, with disease symptoms observed across all cultivars. This method most closely mimics the infection process under field conditions. Yield loss was not confirmed in these experiments; however, this could have been due to the numbers of sclerotia used being too low to maximise the level of damage. The numbers of sclerotia influence disease severity, therefore on-farm yield losses associated with any of the tested cultivars cannot be ruled out on the basis of this experiment. The symptoms looked serious but did not cause enough damage to reduce yield; however, the significant increase in plant dry weight and the decline in the dry weight of sterile grains in response to fungicide treatment in the Sherpa experiment strongly suggests yield loss would have occurred had the plants been exposed to greater infection pressure. It must be noted that the controls in this study were infected plants treated with Amistar Top®, rather than plants that were not infected at all. In future studies, uninfected control plants should also be included as a third treatment for each cultivar.
The Amistar Top® treatment at a higher rate than recommended worked remarkably well, reducing disease symptoms and maintaining crop greenness, which is the first time this has been demonstrated with Australian rice. Precautions are necessary when using this product because the azoxystrobin component has serious effects on fish, aquatic invertebrates and algae, and it is also quite persistent. According to Regulation (EC) No. 1907/2006, the half-life of azoxystrobin is 214 days; the fate of any drainage water requires careful consideration. Environmental concerns associated with the product were not considered in some recent work into the use of azoxystrobin for controlling rice blast (Pak et al., 2017)
Using detached rice stems for assessing responses to stem rot

Introduction

The previous section demonstrated that pot inoculation in large tubs is an effective way of assessing rice cultivar responses to stem rot under conditions that mimic how infections are initiated in the field. The flooded pot approach, however, is difficult to apply routinely because of the space and infrastructure required, particularly if it is necessary to assess multiple varieties with untreated controls, and exposed plants with and without different fungicide regimes. If different fungal strains are also being assessed, the scale of the experiments can become unmanageable.

In this section, we assess the potential of a laboratory technique where detached rice stems are exposed to the stem rot pathogen cultivated on agar plugs. This technique was assessed using individual cultivars exposed to different stem rot strains (assessing variations in pathogenicity), and also multiple cultivars exposed to the same fungal strain in order to provide more data on cultivar tolerance to the disease.

Materials and methods

Twelve experiments were conducted to determine whether this method could be a useful option for assessing cultivar susceptibility to stem rot and any variation in the virulence of the isolates so far collected from southeast Australia. The method with detached rice stems was developed from that used by Chaijuckam and Davis (2010). The isolates included one collected and stored at the NSW herbarium since 1995 (DAR 71531), one from 2014 (DAR 83466), and the 2016-2017 isolate. The fungal isolates were grown on one-quarter strength potato dextrose agar, and used after two weeks growth at 25 ºC.

Four experiments were with the cultivar Sherpa grown in pots in a greenhouse. Nine weeks after sowing, 20 cm long stem pieces were cut. The stems were washed in sterile water, dipped in a 10% solution of sodium hypochlorite, and then dried with tissues. The stem pieces were placed in food storage containers (22 x 22 cm). A piece of agar (cut with a 5 mm cork borer) with the 2-week-old fungal isolate growing on it was placed on each stem and then covered with moist cotton wool. The pieces were left for 7 days, and then any lesions were rated and measured. Another six experiments were conducted in exactly the same way; this time they involved Reiziq and Langi, and the stems were collected from buffer crops in rice experimental paddocks.

In the last two experiments, 10 cultivars were used: Koshihikari, Opus, Illabong, Doongara, Topaz, YRM70, YRK5, Sherpa, Reiziq and Langi. They were compared against each other after exposure to the 2016-17 isolate only.

Results

This method was successful in producing disease symptoms in a short time, but symptoms were variable in expression (Figure 30). Results are presented in Tables 16 and 17. In common with the results from other methods used to measure susceptibility, Sherpa was more susceptible than the other varieties, particularly Reiziq. Although some experiments indicated some isolates were more pathogenic than others, the results were not consistent.

This method was successful in producing symptoms, but careful preparation is needed to ensure stems are at the most appropriate age.
Discussion

Interestingly, the positions of Sherpa and Reiziq were similar in both Tables 12 and 16, with different methods of inoculation, i.e. with sclerotia in the greenhouse trial and with the detached stem method. The other cultivars were variable in both tables.

More work is needed to find the best method of artificially infecting rice plants in greenhouse experiments, especially if a standard method for newly introduced cultivars and their reaction to stem rot is to be developed. Various phenotyping methods are described in Rosas et al. (2016). More work should also be done on Australian stem rot isolates to compare their virulence, but results so far have been variable; experiments need to be repeated. The use of detached stems appears to be useful and it is also quick, but the sclerotial method used in the greenhouse experiment more closely mimicked the field situation, as well as providing some information on the effects on yield.
Table 16 - Disease severity ratings using detached stems of different cultivars with different isolates of stem rot.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sherpa</td>
<td>5.7 a</td>
<td>3.18 a</td>
<td>13.7 b</td>
<td>2.90 x</td>
<td>1.16 y</td>
<td>2.96 x</td>
<td>&lt;0.001</td>
<td>1.71</td>
</tr>
<tr>
<td>2</td>
<td>Langi</td>
<td>8.2 a</td>
<td>21.4 b</td>
<td>24.6 b</td>
<td>0.68 x</td>
<td>0.83 x</td>
<td>1.58 y</td>
<td>&lt;0.001</td>
<td>7.19</td>
</tr>
<tr>
<td>3</td>
<td>Reiziq</td>
<td>32.5</td>
<td>26.6</td>
<td>22.5</td>
<td>2.03</td>
<td>2.28</td>
<td>1.98</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>4</td>
<td>Langi</td>
<td>22.2</td>
<td>22.4</td>
<td>15.6</td>
<td>1.05</td>
<td>0.85</td>
<td>0.98</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>5</td>
<td>Reiziq</td>
<td>17.6 a</td>
<td>16.0 a</td>
<td>7.3 b</td>
<td>0.95</td>
<td>1.08</td>
<td>0.88</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>Langi</td>
<td>10.1 a</td>
<td>4.9 b</td>
<td>15.7 a</td>
<td>0.98 x</td>
<td>0.55 y</td>
<td>1.35 x</td>
<td>&lt;0.001</td>
<td>3.64</td>
</tr>
<tr>
<td>7</td>
<td>Langi</td>
<td>10.7</td>
<td>8.7</td>
<td>11.0</td>
<td>0.82</td>
<td>0.68</td>
<td>0.98</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>8</td>
<td>Sherpa</td>
<td>16.0</td>
<td>14.5</td>
<td>19.5</td>
<td>1.10 x</td>
<td>1.50 x</td>
<td>3.00 y</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>9</td>
<td>Sherpa</td>
<td>15.5</td>
<td>10.0</td>
<td>14.5</td>
<td>1.90</td>
<td>1.50</td>
<td>1.70</td>
<td>NS</td>
<td>N/A</td>
</tr>
<tr>
<td>10</td>
<td>Sherpa</td>
<td>19.5</td>
<td>13.0</td>
<td>20.5</td>
<td>4.30 x</td>
<td>1.40 y</td>
<td>2.80 y</td>
<td>NS</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*L* = length of the lesion in mm; and R a severity rating based on a visual score of 0–5, where 0 is where stems are free of infection, and 5 those with severe infection. Values with the same letter are not significantly different in the same row for either length or rating only.
Table 17 - Response of detached stems of different cultivars infected with stem rot. All measurements in mm.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Experiment 11</th>
<th>Experiment 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion length</td>
<td>Lesion score</td>
</tr>
<tr>
<td>Koshihikari</td>
<td>10.0 a*</td>
<td>1.0 a</td>
</tr>
<tr>
<td>Reiziq</td>
<td>10.5 a</td>
<td>1.3 ab</td>
</tr>
<tr>
<td>Opus</td>
<td>12.0 a</td>
<td>1.8 abc</td>
</tr>
<tr>
<td>YRM70</td>
<td>12.5 a</td>
<td>2.1 bcd</td>
</tr>
<tr>
<td>Doongara</td>
<td>14.5 ab</td>
<td>1.7 abc</td>
</tr>
<tr>
<td>Langi</td>
<td>15.0 ab</td>
<td>1.8 abc</td>
</tr>
<tr>
<td>Topaz</td>
<td>15.5 ab</td>
<td>2.9 de</td>
</tr>
<tr>
<td>YRK5</td>
<td>16.0 ab</td>
<td>2.1 bcd</td>
</tr>
<tr>
<td>Sherpa</td>
<td>21.0 b</td>
<td>2.3 cd</td>
</tr>
<tr>
<td>Illabong</td>
<td>22.0 b</td>
<td>3.3 e</td>
</tr>
<tr>
<td>$P$</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>7.90</td>
<td>0.96</td>
</tr>
</tbody>
</table>

*Values with the same letter in the same column are not significantly different.
An analysis of yellowing crops in Finley in 2017

Introduction

Coinciding with the observation of stem rot on some properties, there was also some leaf yellowing and stunted plant growth. To clarify the relationship between the yellowing and stem rot, five farms were examined by walking through the crops and collecting plant material for nutrient testing.

Methodology

Healthy plants and those that showed yellowing symptoms were collected from five farms. The farms were coded as CB, AG, RH, DC, and TP. The growth stage at the time of collection would have been about six to eight weeks before harvest. Samples were sent to Phosyn Analytical, Burleigh, QLD, where the elements Fe, Mn, B, Cu, Mo, Zn, Ca, Mg, Na, K, P, S and N were analysed in the tissue. Grain samples were also sent away for arsenic analysis from five quadrat cuts from farm CB. Whole grain millout was also assessed. An important aim was to confirm that the yellowing was not due to stem rot infection, the initial claim made by local observers.

Results

Drone footage of a farm with yellow patches (CB) throughout the field was provided, with the cause initially being proposed as stem rot. An example of the pattern and intensity of the yellowing and some of the leaf symptoms are shown in Figure 31. All crops examined for yellowing had inconsistent yellowing symptoms; one crop was yellow across the entire bay, while others were patchy.

Healthy plants and the shorter plants with yellowing symptoms generally had less N, P, K and S (Table 18). Information from previous soil tests on one of the farms (CB) indicated low pH and a low Ca:Mg ratio, both factors suggesting that K would not be available to the plant. Sulphur was low in the soil tests and also low in the yellow patches. This indicated a nutrient deficiency in the yellowing plants. Additionally, stubble that was not fully burnt from the previous crop could be responsible for tying up nutrients, particularly nitrogen.

The results from arsenic analysis are given in Table 19. Plot 1 (0.31 µg/g) had unusually high levels of inorganic arsenic as AS5, or arsenate, with the average for all samples about 0.08 µg/g.

Discussion

Poor plant nutrition in relation to some essential elements was the cause of the patchy yellowing, which could reflect either nutrient levels being inadequate at sowing, or being adequate but not available to the plant due to low soil pH.

Soil samples had been collected in the previous season by David Troldahl on farm CB, where crops had also shown some yellowing. Manganese and aluminium were at high levels in the soil samples. The optimum pH for rice is 5.5 to 6. Low pH could be reducing the availability of some nutrients, such as potassium, magnesium and sulphur, but particularly magnesium when soil levels are low to begin with. While the submergence process increases pH in rice crops, low soil pH could still influence nutrient availability.

In the leaf tissue tests, the potassium, nitrogen and magnesium were all low. Magnesium was low in the soil test as well as the plant tissue tests. Low potassium in the plant is known to make rice more
susceptible to stem rot. Therefore, while stem rot and patchy crop yellowing may occur in the one field, the yellowing is not directly caused by stem rot infection.

Figure 31 - Upper photo: symptoms typical of the yellowing, with the patches clearly shown, as well as closer symptoms on the right (farm CB). Lower photo: aerial view from drone footage.
Table 18 - Leaf sample analysis of samples from farms in Finley in 2017. The affected plants are the “yellow” samples, i.e. CB yellow; the healthy plants “green” i.e. CB green. Values identified as low are in red; high values are in blue, according to the laboratory reports.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fe</th>
<th>Mn</th>
<th>B</th>
<th>Cu</th>
<th>Mo</th>
<th>Zn</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
<th>P</th>
<th>S</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB green 21/2/2017</td>
<td>72</td>
<td>365</td>
<td>4</td>
<td>1.9</td>
<td>3.4</td>
<td>20</td>
<td>2900</td>
<td>1400</td>
<td>500</td>
<td>10600</td>
<td>2600</td>
<td>1200</td>
<td>19500</td>
</tr>
<tr>
<td>CB yellow 21/2/2017</td>
<td>97</td>
<td>386</td>
<td>4</td>
<td>2.2</td>
<td>4.2</td>
<td>19</td>
<td>2500</td>
<td>1200</td>
<td>500</td>
<td>12000</td>
<td>2100</td>
<td>900</td>
<td>15900</td>
</tr>
<tr>
<td>CB green 2/3/2017</td>
<td>77</td>
<td>274</td>
<td>4</td>
<td>2</td>
<td>3.9</td>
<td>16</td>
<td>2100</td>
<td>1200</td>
<td>500</td>
<td>9000</td>
<td>2400</td>
<td>1000</td>
<td>15900</td>
</tr>
<tr>
<td>CB yellow 2/3/2017</td>
<td>72</td>
<td>327</td>
<td>4</td>
<td>2.5</td>
<td>4.9</td>
<td>17</td>
<td>2400</td>
<td>1100</td>
<td>500</td>
<td>9100</td>
<td>1900</td>
<td>500</td>
<td>11400</td>
</tr>
<tr>
<td>TP green</td>
<td>70</td>
<td>268</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>18</td>
<td>2900</td>
<td>1300</td>
<td>500</td>
<td>11800</td>
<td>2000</td>
<td>800</td>
<td>13000</td>
</tr>
<tr>
<td>TP yellow</td>
<td>118</td>
<td>248</td>
<td>6</td>
<td>2.3</td>
<td>6.4</td>
<td>18</td>
<td>3000</td>
<td>1400</td>
<td>500</td>
<td>8300</td>
<td>2500</td>
<td>600</td>
<td>11200</td>
</tr>
<tr>
<td>RH green</td>
<td>259</td>
<td>281</td>
<td>5</td>
<td>1.9</td>
<td>6.3</td>
<td>25</td>
<td>2700</td>
<td>1700</td>
<td>500</td>
<td>13600</td>
<td>2600</td>
<td>1100</td>
<td>16900</td>
</tr>
<tr>
<td>RH yellow</td>
<td>206</td>
<td>142</td>
<td>4</td>
<td>1.5</td>
<td>4.9</td>
<td>20</td>
<td>2100</td>
<td>1200</td>
<td>500</td>
<td>8300</td>
<td>2200</td>
<td>600</td>
<td>11000</td>
</tr>
<tr>
<td>DC green</td>
<td>61</td>
<td>593</td>
<td>5</td>
<td>1.6</td>
<td>2.2</td>
<td>20</td>
<td>2400</td>
<td>1400</td>
<td>500</td>
<td>12400</td>
<td>1900</td>
<td>1100</td>
<td>13300</td>
</tr>
<tr>
<td>DC yellow</td>
<td>121</td>
<td>1171</td>
<td>7</td>
<td>2.3</td>
<td>3.5</td>
<td>16</td>
<td>3400</td>
<td>1400</td>
<td>500</td>
<td>17200</td>
<td>1720</td>
<td>1300</td>
<td>15600</td>
</tr>
<tr>
<td>AG green</td>
<td>75</td>
<td>485</td>
<td>9</td>
<td>4.7</td>
<td>1.7</td>
<td>29</td>
<td>2800</td>
<td>2100</td>
<td>500</td>
<td>18600</td>
<td>2500</td>
<td>1600</td>
<td>19700</td>
</tr>
<tr>
<td>AG yellow</td>
<td>282</td>
<td>76</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>26</td>
<td>2700</td>
<td>1400</td>
<td>500</td>
<td>10900</td>
<td>2100</td>
<td>1200</td>
<td>16900</td>
</tr>
</tbody>
</table>

*As recommended by Phosyn Analytical.  **Samples were collected at CB twice as per the date.
Table 19 - Levels of arsenic in grain from one of the farms. Values for CB1 are high for arsenic

<table>
<thead>
<tr>
<th>Plot</th>
<th>DMA* µg/g</th>
<th>MMA** µg/g</th>
<th>AS5*** µg/g</th>
<th>% whole grain millout</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB1</td>
<td>0.15</td>
<td>&lt;0.01</td>
<td>0.31</td>
<td>62.33</td>
</tr>
<tr>
<td>CB2</td>
<td>0.10</td>
<td>0.06</td>
<td>0.05</td>
<td>69.06</td>
</tr>
<tr>
<td>CB3</td>
<td>0.11</td>
<td>&lt;0.01</td>
<td>0.09</td>
<td>70.15</td>
</tr>
<tr>
<td>CB4</td>
<td>0.06</td>
<td>&lt;0.01</td>
<td>0.07</td>
<td>69.28</td>
</tr>
<tr>
<td>CB5</td>
<td>0.10</td>
<td>0.03</td>
<td>0.12</td>
<td>52.49</td>
</tr>
<tr>
<td>Sherpa (appraisal samples)</td>
<td>0.14</td>
<td>0.06</td>
<td>0.08</td>
<td>57.40</td>
</tr>
<tr>
<td>Sherpa (appraisal samples)</td>
<td>0.17</td>
<td>0.04</td>
<td>0.07</td>
<td>68.82</td>
</tr>
</tbody>
</table>

*DMA Organic arsenic as monomethylarsonic acid
** MMA Organic arsenic represented as dimethylarsinic acid
*** AS5 Inorganic arsenic represented as arsenate
Implications

- While the work on niclosamide for snail control cannot be presented in detail here for commercial reasons, the data we have obtained in this project suggests there should be no major technical obstacles to its future registration.

- Six insecticides are registered and recommended for use in southern rice crops; four of them—chlorpyrifos, diazinon, malathion and fipronil—are all currently under regulatory review by the APVMA. In the future, growers are likely to need access to newer classes of insecticides as older categories are withdrawn. The registration process for new chemical use patterns is becoming progressively more expensive, prejudicing the business case for registering new materials for use in smaller crops. There is a clear need to develop new chemistries for bloodworm control; the fastest pathway to bring a new treatment to market is to register fipronil (a currently registered seed treatment) for direct spray application. The data generated in this project will support an application for commercial fipronil spray trials; however, given that fipronil itself is now under review, it is unclear whether the best option would be to follow through with commercial trials on this compound, or look at newer materials not under immediate regulatory threat, such as spinetoram or abamectin.

- Armyworms are likely to continue to increase in importance as pests of rice in southern Australia. As with bloodworm control, it is imperative that new chemical treatments are developed to provide insurance against loss of the organophosphates. The work done in this project has identified emamectin benzoate and particularly chloraclantraniliprole as having potential as more selective compounds for armyworm control. Because our bioassay work used a protocol based on that used for Helicoverpa, it should be possible to use the laboratory toxicity data to calculate a multiplication factor that can be applied to the registered Helicoverpa application rates for these compounds. This would give an estimate of the application rate that may be necessary for common armyworm control in the field, and provide a starting point for small-scale residue trials to be conducted as a lead-up to commercial trial permit applications. It should be noted, however, that currently there is no evidence that the sugarcane armyworm has the same susceptibility to these compounds as the common armyworm.

- We have demonstrated that fermentation traps are effective for common armyworm, and have the potential to provide an early warning system for growers, alerting them to populations of adult moths becoming active around their crops. These traps are not effective for monitoring sugarcane armyworm, a species not previously known from rice in southern Australia, and apparently common in at least a proportion of crops each season. If future studies confirm it has a significant role in rice systems, then an alternative monitoring system will be needed.

- Both species of armyworms found in rice crops are heavily parasitised by flies and wasps during the latter part of their life cycle. Further studies are needed to determine whether parasitism of eggs and younger larvae is also significant, and to confirm the supposition that very few armyworms can reach adulthood in a flooded rice crop. This will help determine the extent to which beneficial insects contribute to minimising crop losses, and the degree of emphasis that needs to be placed on their conservation.

- Defoliation studies indicate that plants can compensate for significant foliage losses before yield loss occurs; however, more work needs to be done to determine whether actual armyworm damage, or artificial damage combined with potential salivary elicitor compounds produces the same result. Most yield loss associated with armyworm damage appears linked
to panicle damage close to harvest, rather than foliar damage associated with earlier infestations.

- Studies on stem rot have demonstrated that pot and single stem inoculation are both effective techniques for assessing the interactions between rice varieties and fungal isolates. While there is no true resistance to stem rot in commercial cultivars, Reiziq appears to be somewhat tolerant while Sherpa is particularly vulnerable to this disease. Growers with a history of stem rot outbreaks should look towards growing a Reiziq crop if they have no option other than to plant rice in a field with potential disease carryover from previous seasons.

- Repeat cropping encourages outbreaks of snails and stem rot; however, stem rot issues can be minimised or even eliminated by ensuring complete stubble burning. Patchy yellowing in a crop at Finley was caused by nutrient deficiencies rather than stem rot, and patches of unburnt stubble from the previous season may have been responsible for reducing nutrient availability.
Recommendations

- The findings on stem rot from this project should be promoted further among growers and agronomists, either at preseason meetings or field days.
- While stem rot resistance is not a selection criterion for the current rice breeding program, screening for stem rot vulnerability should be conducted on all new rice varieties to identify those with the best tolerance to the disease. On-farm cultivar testing under field conditions should be carried out, now that a farm is known to have enough stem rot inoculum because natural infection may provide more uniform results than greenhouse experiments.
- Legal arrangements between the NSW Department of Primary Industries, AgriFutures, and Conquest Crop Protection need to be finalised to facilitate the registration of niclosamide for snail control.
- Greater regulatory pressure on older generic chemicals, particularly the organophosphates, puts pest management in southern rice at considerable risk. The industry should support residue studies and commercial trials with emamectin benzoate and chlorantraniliprole for common armyworm, and small plot trials of newer generation chemicals for bloodworm control. These bloodworm trials should be conducted regardless of whether commercial trials of fipronil spray applications lead to registration, because fipronil itself is now under APVMA review.
- More studies on armyworm ecology should be conducted to clarify the importance of sugarcane armyworm in NSW rice crops. If it occurs as a significant proportion of the armyworm community, then screening experiments should be conducted to determine whether it differs in pesticide susceptibility relative to the common armyworm.


Prevalence and survival, with emphasis on stubble burning, of Rhizoctonia spp., causal agents of sheath diseases of rice in Australia. Australasian Plant Pathology 34, 135-142.


NCBI Resource Coordinators, 2016. Database Resources of the National Center for Biotechnology Information. Nucleic Acids Research, 45(D1), D12-D17.


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and diseases

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