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Cotton Establishment Challenges: Rotations in the Southern Region



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Cotton Establishment Challenges: Rotations in the Southern Region

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INTRODUCTION

The research project began in October 2014 to examine problems with cotton emergence in southern growing areas. To this end, the work has been focused on the interaction between cotton emergence and crop rotations, particularly that of rice and cotton in the Murrumbidgee and Coleambally Irrigation Areas (MIA and CIA). As the cotton season of 2014/2015 was well underway at the onset of the project the opportunity was taken to have hands on training with DPI scientists, local agronomists and cotton growers in identifying pest, disease and other emergence issues that were specific to cotton grown in the southern growing region. A key area of study was the investigation of arbuscular mycorrhizal fungi (AMF, previously known as VAM), and how they interact with local cropping rotations. Analytical techniques for examining AMF root colonisation were selected and refined early in the project to permit measurement to be made in the 2015/16 season.

Another aspect of the investigation was allelopathy, particularly the impact of rice residues on cotton emergence. This study was undertaken via glasshouse and incubator trials as a high degree of control is needed to determine chemical effects.

Acknowledgement

The authors of this report gratefully acknowledge the financial support given by the Cruiser Research and Development Fund to undertake this work.

EXTENSION ACTIVITIES

- Workshop presentation to cotton growers, May 2015, about beneficial Arbuscular Mycorrhizal Fungi, Griffith, NSW
- Cotton Scientist Conference presentation, August 2015, Toowoomba, Qld.
- Australian Agronomy Conference presentation, September 2015, Hobart, Tasmania (Appendix 1)
- 9th International Root Symposium poster presentation, October 2015, Canberra (Appendix 2)
- Rice weed herbicide trials attendance and Rice Crop Protection Working Group participation, January 2016, to investigate commonalities between rice and cotton production
- Australian Cottongrower article, January 2016, on problems with cotton production after rice and interaction with mycorrhizal fungi (Appendix 3)

CHEMICAL ECOLOGY

Allelopathic potential of rice residues against cotton

Key Words Stubble water extract, cotton seedling emergence, rice residues, southern Australian cotton, allelopathy

INTRODUCTION

Cotton (*Gossypium hirsutum*) is now grown in Australia below latitude 33^{0} S. The challenge this creates is to achieve a commercial yield in a shorter growing season. It is an imperative therefore that cotton seedlings emerge quickly and strongly to ensure that these seasonal limitations are minimised. The colder temperatures experienced in the southern Australian growing zone can intensify seedling diseases due to slower growth but other factors can exacerbate the effects. Where rotations are practised, the carryover effects of one crop on another can be influential. Work in Australia has shown the allelopathic capability of wheat (Wu *et al.*, 2000) and canola (Asaduzzaman *et al.*, 2014) on seedling annual ryegrass and rice on aquatic weeds (Seal *et al.*, 2004) while Leigh *et al.* (1995) showed the impact of phalaris leaf residues on the regeneration of subterranean clover in the autumn. In all cases there was considerable variation in allelopathic capability between varieties and in tolerance to allelochemicals from companion plants.

Studies of inter-crop chemical ecology involving cotton are limited, particularly in Australia. Hulugalle *et al.* (1998) showed that growing leguminous crops, such as chickpea and faba bean, interfered with the growth, development, yield and quality of cotton when crop residues including seed were incorporated prior to cotton establishment. Predisposition to disease was also noted as were differential responses by different cultivars of rice. Cereals were least inhibitive to cotton plants. The allelochemicals involved were not reported.

Interactions between cotton and rice are unique to the southern region and are worthy of more detailed consideration. Studies with other species have confirmed that rice plants and their residues can release compounds with allelopathic activity. Phenolic compounds (e.g. p-salicylic, p-coumaric, vanillic, syringic, ferulic, p-hydroxybenzoic, and mandelic acids) are often considered as the dominant allelochemicals (Seal *et al.* 2004) present in rice residue, although this view is not universally accepted (Olofsdotter *et al.* 2002). An allelochemical isolated from root exudates of rice seedlings was identified as momilactone B (Kato-Noguchi

et al. 2002), although its function has not been finally characterised (Kato-Noguchi and Ino 2003). Additionally, steroidal compounds such as ergosterol peroxide and 7-oxo-stigmasterol have been identified as growth inhibitory agents present in rice (Macías *et al.* 2006). As these compounds are exuded from rice roots during the rice growing season, they are perhaps less relevant for cotton which will be grown as a subsequent crop.

More relevant are the chemicals present in the stubble and root residues. Rice produces a high stubble burden which is likely to carry over to the next growing season with any potency intact due to the cool temperatures. Even after several summer months and several irrigations, incorporated rice residue, including roots, is still present in the cotton root zone and may have some impact on the new crop. Allelopathic effects can be subtle and may not occur every year. The effects can range from root pruning to crop death. In some cases the root damage can predispose the plant to pathogen invasion. In the southern cotton zone the slowing of germination and the reduction in root growth can go unnoticed but have a substantial effect on crop yield from this growth check. Some allelochemicals have been shown to reduce nodulation in legumes so it is possible that AMF in cotton might be also compromised.

The aim of this study was to evaluate whether allelopathic activity of rice residues on the cotton seedling germination and growth was present.

RICE SOIL, ROOT AND STUBBLE RESIDUE GLASSHOUSE EVALUATION

The impact of rice residues on cotton germination and growth were investigated in a series of glasshouse and laboratory experiments. The sensitivities of the cotton varieties Sicot 74BRF and Sicot 71BRF to soil, root and stubble residues of rice were assessed, where research material is available as outlined below. Four pots of each of the six commercial varieties of rice *viz*. Doongara, Sherpa, Kyeema, Langi, Topaz and Reiziq, were grown in a temperature controlled glasshouse as part of the rice breeding program in Yanco Agricultural Institute (Figure 1) and transported to Charles Sturt University for processing. Soil, roots and above ground stubble material (excluding the seed heads) were separated for further use. The soil from each variety was processed to remove root material and was then ground to fit through a 5mm sieve. Soils from the same rice variety were bulked and mixed. The rice stubble, root material and soils were then extracted or directly utilised for evaluating their impact on cotton establishment as described below.



Figure 1 Rice material for the following experiments were grown in a temperature controlled glasshouse at Yanco Agricultural Institute as part of the rice breeding activity

All experiments were initially analysed by Analysis of Variance with a Fischer's lsd *post hoc* test to p < 0.05 level. Data that failed normality and variance tests were re-analysed via non-parametric methods i.e. Kruskill Wallis ANOVA on ranks was used with a Student-Newman-Keuls *post hoc* test to p < 0.05 level.

a. Rice soil on cotton evaluation

Method: The variety-based soils were subsampled and 750 g were then weighed into pots. A complete slow release fertiliser (3.50g Osmocote[®]/pot) was added and incorporated into the top 30mm of the soil. Five cotton seeds from the variety 71BRF were sown into each pot. The experimental control contained virgin soil from the same batch used to generate the original rice experiment. Germination was measured at 7 and 14 days after sowing and final dried biomass was recorded.

Results: The variety of rice grown previously in the soil used for cotton germination studies was highly influential in the early germination of cotton (Figure 2). Whereas Kyeema outcomes were similar to the control all other varieties caused a substantial delay in time to germination with Langi being particularly inhibitive. By day 14, however, variety effects

were less clear (Figure 3). The variability in the performance of cotton under these circumstances seems to have masked any statistically significant outcomes even though Reiziq and Sherpa varieties appear behind the other varieties in performance. There were no significant differences in whole plant biomass at day 14 (Figure 4).



Figure 2 Effect of rice variety soil on cotton germination 3 days after sowing (P = 0.002).



Figure 3 Effect of rice variety soil on cotton germination14 days after sowing. (n.s. vertical bars denote SEM)



Figure 4 Effect of rice variety soil on cotton biomass dry matter 14 days after sowing (n.s. vertical bars denote SEM)

a. Rice soil germination and root inhibition glasshouse

Method: Fifteen seeds were individually sown into separate tray cells (8 x 5, 80 mL capacity) for each of the rice variety soils. Sand was added to the top of each cell, to allow better water infiltration and seedling emergence, and the base of each cell to facilitate drainage. Two cotton varieties, 71BRF and 74BRF, were included to evaluate whether varietal responses were consistent. Seeds were left for 12 days after sowing before biomass was recorded. Cumulative germination percentages were recorded throughout the experiment. The experiment was arranged as a randomised complete block design.

Results: No significant differences were found in cotton biomass from different rice variety soils at 12 days after sowing (Figure 5). Cotton variety 71BRF performed better than 74BRF over all rice variety soils (Figure 7) although there was considerable variation between variety soils which masked any significant cotton variety responses to rice variety soil effects (Figure 6). Over both cotton varieties, however, there were differential rice variety effects (Figure 8) with Langi rice soil being most inhibitive and Sherpa variety soil being least inhibitive. The pattern of cumulative germination was much tighter for 74BRF (Figure 10) than for 71BRF (Figure 9) suggesting that the latter was more reactive to rice variety effects if they exist.



Figure 5 Effect of rice variety soil on cotton seedling biomass 21 days after sowing (n.s. vertical bars denote SEM)





Figure 6 Cotton seedling biomass (g/pot) at **A.**12 days after sowing and **B.** 21 days after sowing. Differences were not significant (n.s.).



Figure 7 Main effect of rice soils on cumulative germination of cotton varieties 71BRF and 74BRF (p < 0.05; lsd = 3.247)



Figure 8 The effect of rice soil variety on cumulative germination of cotton seedlings (p < 0.05; lsd = 5.133)



Figure 9 Cumulative germination of cotton seedlings from rice variety soils (71BRF).







Figure 11 Effect of rice variety soils on germination of two cotton varieties, 74BRF and 71BRF.

b. Rice stubble and root material on cotton evaluation

Method: Variety-based rice stubbles and rice root material were evaluated for their impact on cotton seedling establishment. A sandy loam soil mixture was prepared (3:1 sand:loam), and

750g were added to each experimental pot. Stubble material and roots and from each of the above rice varieties were ground to pass through a 0.5mm sieve. Each pot received 5g of the rice variety stubble or 2.2g/pot of ground rice variety roots. A complete slow release fertiliser (3.50g Osmocote[®]/pot) was added and incorporated into the top 30mm of the soil. Treatments were arranged in a randomised block design with 5 replicates. Pots were watered and maintained at field capacity for the duration of the experiment. Dried biomass of individual cotton seedlings were recorded for each pot 4 weeks after sowing. Germination was recorded for root material treatments 7 days after sowing.

Results: Rice variety stubbles had no significant effect on 4-week old cotton seedling biomass (Figure 12). Rice variety root treatments were also non- discriminating on cotton seedling biomass (Figure 13) and germination percentage at 7 days although Kyeema was much lower than the other varieties (Figure 14).



Figure 12 Rice stubble variety effects on biomass of cotton seedlings 4 weeks after sowing (n.s. - analysed by non-parametric tests due to non-normality).







Figure 14 Effect of rice variety root treatments on final germination percentage of cotton (n.s.-vertical bars denote SEM)

c. Incubator stubble residue growth cabinet evaluation

Method: A sandy loam soil mixture (7:3 sand:loam) was pasteurised at 70 °C for 1 hour. Subsequently, 130 grams of the soil mix was added to each experimental pot (120mL Sardset[®] container with one drainage hole at the base) and mixed with 1g of the designated rice stubble treatment. The rice stubble treatments consisted of 1g/pot of stubble material from each of the rice varieties Sherpa, Kyeema, Langi, Topaz and Reiziq. The stubble was then mixed evenly with the soil mix. Rice stubble treatments were evaluated against two cotton varieties Sicot 71BRF and Sicot 74BRF. Two controls were included, one with 1g of vermiculite to simulate the mulching effect and one with soil mix only. Pre-germinated cotton seeds from each of the two cotton varieties were uniformly selected and sown into each experimental pot at a depth of 10mm. Pots were watered until field capacity, then covered with their original screw-top lid and placed inside an aluminium tray with a 10mm layer of perlite in the bottom. Water was added to the tray to a height of 5mm to prevent experimental pots from drying out quickly. The growth cabinet was set on a day/night cycle of 12hr/12hr and a temperature regime of 25°C/20°C. Plants were grown for 7 days before root elongation measurements were taken. The experiment was arranged in a randomised block design with 5 replicates except for Sherpa treatments which had 4 replicates.

The above procedure was repeated using Langi variety stubble to evaluate the effects of stubble rates on cotton germination. Treatments comprised control and stubbles at 0.11, 0.33 and 1.0 g per pot. Cotton variety used was 74BRF. Treatments were arranged in a randomised block design with 5 replicates.

Results: All rice variety stubbles significantly restricted root lengths of cotton seedlings (Figure 15), in most cases killing the seedlings (Figure 16). No differences were noted between cotton varieties although the occasional seedling was unaffected. The results suggest that the rate of stubble used was too high and needed to be repeated at lower rates. This was undertaken using variety Langi stubbles.



Figure 15 Effect of rice variety stubble treatments on root length of cotton seedlings 7 days after sowing (p < 0.05).



Figure 16 Photograph of the effect of rice variety stubble treatments on root length of cotton seedlings 7 days after sowing. L to R: CV = Control with vermiculite mixed through, CS = Control-sand only, L = Langi, T = Topaz, K = Kyeema, R = Reiziq, S = Sherpa. Two cotton seed types, right vertical axis: 74 = 74BRF, 71 = 71BRF



Figure 17 Effect of rice (cv. Langi) stubble at different application rates on the root length of cotton seedlings (cv. 74BRF) grown for 7 days at 20°C (L.S.D. = 54.292; P<0.05).

The stubble rate response for the Langi variety stubble is shown in Figure 17. There is a clear impact of stubble rate with the phytotoxicity at the 1g/pot rate affirmed and the inhibition was less severe at the lower rates.

These experiments show that under controlled conditions there is an impact on cotton due to the phytotoxicity of rice residues. The extent to which this occurs in the field needs to be tested.

d. Stubble material extracts

Method: Rice variety stubble material and root material were dried for 72 h at 40 °C, and then ground into a fine chaff to pass through a 0.5mm screen. Ten grams of ground stubble or root material were added to 100mL sterile, distilled water in a 250mL Schott bottle, wrapped in aluminium foil and stored in a Precision Model 818 low temperature incubator set at 20 °C for 72 h. The extract was then filtered through two layers of cheesecloth then filtered through Advantec 5C filter paper under vacuum and finally through a 0.22 μ m millipore syringe filter to remove micro-organisms. The extracts were stored at 4 °C. This extract concentration (i.e. 10 g/100 mL) was considered for future comparisons as the undiluted extract (i.e. 100%). Series dilutions were then undertaken on the root extract using sterile distilled water to obtain extract concentrations of 50, 25, 12.5 and 6.25%. Sterile distilled water was used as the control in the bioassays. All procedures following filtration of the extract were carried out in a cross-flow laminar flow cabinet to minimise contamination. Seeds were surface sterilised with 70% ethanol for 1 min and rinsed several times with sterile distilled water. Seeds were then placed in 9cm petri plates lined with filter paper (Advantec #2) which had been moistened with 10 mL sterile distilled water for germination. The plates were placed in an incubator at 20°C for 48 h.

The rice variety stubble ectracts were evaluated for their effects on two cotton varieties (Sicot 71BRF and Sicot 74BRF) which were supplied by Cotton Seed Distributors. Bioassays were conducted on each cotton variety. Four millilitres of each extract concentration were added to sterile Petri dishes which had been lined with Advantec #2 filter paper. Five pre-germinated seeds (Figure 18) were then added to each Petri dish and the dish sealed with parafilm. Three replicates were arranged in a randomised complete block design under the experimental conditions described. Root elongation was measured on each plant after five days.

A similar procedure was followed for the evaluation of the root extract of rice cultivar Langi. In this case only cotton variety 74BRF was included and 5 replicates were used in a randomised block design. Concentrations assayed were 100, 50, 25, 12.5 and 6.25% of the undiluted extract.



Figure 18 Pre-germinated cotton seedlings were uniformly selected after incubator treatment (20 °C for 48 h) for inclusion in the bioassays.



Figure 19 Water extract of stubble varieties (100% of 100g/L) of selected rice varieties (L to R: Doongara, Sherpa, Reiziq, Topaz, Kyeema, and Langi).

Results: Colour intensities of stubble extracts were noticeably variable despite the parent material being grown and processed under identical conditions (Figure 19).

Rice stubble water extracts showed significant inhibition of cotton root growth compared with the deionised water control (Figure 20). Overall inhibition was greater in the cotton variety 74BRF compared with 71BRF regardless of rice variety with Kyeema seeming to have more inhibition than other rice varieties. . Differences also occurred between extracts of rice varieties.



Figure 20 Effect of rice residues (100% water extracts) on the root length of cotton seedlings (cvs. 71BRF and 74BRF) grown for 5 days at 20°C (lsd =13.956; p = 0.015).

For the impact of Langi root extracts, the appearance of the dilution series is shown in Figure 21 and the effects on cotton variety 74BRF are shown in Figure 22. There was a clear rate response with inhibition increasing directly with concentration of the extract.



Figure 21 Water extract of ground rice roots (cv. Langi) prepared as a serial dilution. Concentrations assayed were 100, 50, 25, 12.5 and 6.25%.



Figure 22 Effect of rice (cv. Langi) root extract concentrations on the root length of cotton seedlings (cv. 74BRF) grown for 5 days at 20°C (lsd = 10.593; p < 0.001).

Comments: This series of experiments has shown that there are chemical responses happening in the early stages of cotton seedling growth when challenged by rice variety backgrounds. At this stage of growth variability can mask effects as a single seedling or an extra day can compromise the trends. It is not surprising that biomass was not particularly useful in determining differences in response as the impact on root development would be balanced to some degree by the dependence on seed reserves in the early stages. The experiments on root development are telling in that in the field any root pruning will have productive penalties as plants become older as their abilities to withstand water insufficiencies and to fossick for nutrients become compromised. We also know that plants with slow root growth also are likely to be more susceptible to root pathogens. We do not know however whether these phytotoxins present in rice residues have any impact on mycorrhizal activity.

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SOIL CONSTRAINTS AND MYCORRHIZAL ASSOCIATIONS

Cotton rotations in south-eastern NSW: A return to Muirhead et al.

INTRODUCTION

Cotton (Gossypium hirustum L.) has been produced in Australia since the 1920s, over a wide geographical area from Queensland through NSW to the Victorian border. Unique agronomic challenges occur in specific areas. In particular, southern areas have a cooler start and this can impede the germination and growth of cotton seedlings, potentially predisposing the plant to pests and diseases. This arrested development can affect plant establishment rate and have impact on the ability of the established plants to develop uniformly. These affect final harvest quality. In northern regions favourable seasonal conditions can alleviate this effect and the plant can compensate in growth such that yield and quality are largely unaffected. Uniform establishment however is an agronomic imperative in cooler southern regions, since an uneven crop or slowly establishing crop has limited scope for compensatory growth and development given the much shorter season. Previous attempts at commercial cotton production in the south ceased in the 1960s due to the lack of availability of short season varieties or adjusted management techniques. Yields attained were not commercially attractive and research investment in the area by government on cotton was withdrawn at that time (Dowling, 2001). The availability of high-yielding full season varieties, suited to the cooler temperature in the south, has allowed the industry to re-establish from the 1998-99 season. These varieties are now managed by planting as early as possible and cutting out in a timely manner. The run of warmer seasons between is an important factor in the success of the southern industry in recent years (Figure 1). For cotton to be viable in the south it needed to be at least as competitive financially as rice (Oryza sativa L.), the main competitor for irrigation water.

In the Riverina, rice growing commenced in 1922 when 2.8 ha of experimental plantings were grown near Leeton from seed introduced out of California. Rice area expanded steadily so that by 1982 it occupied record area of 121 872 ha, bound by latitudes 34 °S and 36 °S and longitudes 144 °E 146.3 °E (Lewin and Hennan, 1987). Rice is usually grown in rotation with winter crops, although it can be grown in sequence with a variety of other irrigated summer crops (Table 1), such as maize, soybeans, sunflowers and sorghum, as well as winter crops such as winter cereals, canola and faba beans. Cotton has become another rotational option with its advancements in the management of cottonvarieties,tolerant gene

technologies, the higher returns per irrigation water input (\$295/ML - \$459/ML in the 2014/15 season) and the availability of processing facilities.



Figure 1 The long term minimum and maximum temperatures as compared with 2014/15 and 2015/16 seasons in Griffith, NSW. The temperatures around planting for these two seasons were well above the long term average, which led to strong cotton germination and establishment.

Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Rice	Wheat	Fallow	Bed conversion	Cotton	Fallow	Cotton	
Rice	Wheat	Fallow	Bed conversion	sunflowers or sorghum	Wheat		
Cotton	Cereal	Fallow	Bed preparation	Cotton	Cereal or fallow	Cotton	
Cotton	Winter preparation	Cotton	Bed preparation	Cotton	Winter preparatio n	Cotton	
Cotton or Maize	Faba beans	Fallow	Canola or Durum	Fallow	Landform	Cotton	
Soybeans	Barley	Soybeans	Winter preparation	Cotton	Winter preparatio n	Cotton	Faba beans

Table 1 Examples of common crop sequences for irrigators in the Murrumbidgee Irrigation Area (MIA). Adapted from DPI/GRDC report (VIC00010).

Cotton area has expanded in response to these factors as well as the high prices achieved and the local availability of cotton (Figure 3). Production of cotton has continued to grow both nationally (Figure 1) and in southern NSW (Figure 3). In the Murrumbidgee Irrigation Area (MIA) and the Coleambally Irrigation Area (CIA) combined, equal proportions of cotton (28%) and rice (26%) were grown in 2014, closely followed by winter grains (23%) (2014 Irrigation Survey, VIC00010).



Figure 2 Cotton lint production in Australia, 2001 to 2014. Source Australian Bureau of Statistics (© Commonwealth of Australia)



Figure 3 Areas planted (ha) of rice and cotton in south-eastern NSW between 2010 to 2015, showing the recent decline in rice and increase in cotton areas.

Irrigators in south-eastern NSW have to make complex decisions about the appropriate crop rotation, often more than a year in advance. Fluctuations in the price gained, yield achieved, water price and availability, and global demand for each crop commodity will likely lead to a cropping system that may include both rice and cotton. New bankless stepped irrigation layouts have allowed this transition to be an option into the future without major layout changes. However, the availability of water and the increased water use efficiency of cotton has limited the number of growers currently using this rotational system. This raises the question about the compatibility of these two crops as farmers move in and out of one crop or the other. It becomes important therefore to examine the interface between cotton and rice crops.

A common problem encountered by irrigators in the southern growing region is the difficulty in transitioning from rice to row crops such as cotton. Cotton crops grown subsequent to rice emerge poorly and have inconsistent growth and thus this particular transition is often abandoned in favour of other crops or a fallow period. The poor emergence of cotton following rice in the rotation is due to interacting biological, physical and chemical factors arising from the contrasting cropping systems. The relative weakness of emerging cotton seedlings compared with other irrigated crops further compounds the challenges when

coming from rice in the rotation. This problem usually presents itself as what is known as Rice Stubble Disorder (RSD) or informally the 'bank and bay' effect (Figure 4), where old rice bank lines can be seen producing more vigorous early cotton growth. Here, we review the literature for what is known about the transition from rice into cotton and offer possible ways to avoid reduced cotton growth following rice.



Figure 4 Cotton crop at Darlington Point following rice in the rotation showing the 'bank and bay' effect of variable emergence and establishment

The resurgence of cotton production in the Murrumbidgee valley has brought with it crop establishment problems for which past research now becomes applicable. The problems associated with a rice-cotton rotation can be broadly viewed in three categories; chemical, biological and physical (Figure 5).



Chemical constraints to growth

The restricted growth seen in cotton seedlings grown after rice is primarily caused by lower level of plant-available phosphorus. A seminal study by Willet et al. (1978) showed that during the ponded phase, where soil is inundated, phosphorus is released from insoluble iron and aluminium compounds. On draining the rice crop, these compounds are oxidised and can adsorb much of the available phosphorus onto soil particles. Specifically, while inundated, Fe³⁺ is reduced to Fe²⁺ and P is released from insoluble Fe and Al compounds. This increase in P availability takes about 30 days after permanent water. Some P is also released from Ca phosphates. After drainage of permanent water during the drying period, Fe and Al compounds react with soil and fertiliser P to form insoluble P compounds. Muirhead (1975) postulated that the organised (crystalline) ferric oxides in the soil act as electron acceptors in the respiration of microorganisms during the ponded phase of rice culture allowing releases of ferrous iron and adsorption of phosphates. On draining the rice crop, the ferrous iron is oxidised and precipitates as an amorphous (non-crystalline) ferric oxide in the soil. This material has a large surface area and can adsorb up to 100 times as much soluble phosphate as the crystalline form of ferric. Growth restrictions due to phosphorus deficiency are particularly evident in emerging cotton crops grown subsequent to rice, even after the application of additional phosphorus. Nevertheless, if the phosphorus is banded in the soil with the seed or at 10 cm below the seed, yield can be enhanced (Willet et al., 1978). Muirhead et al. (1976) reported that 30 kg P/ha mixed in the soil produced a similar maize yield to the unfertilised control. However, when the same amount of fertilizer was banded either with the seed or 10 cm below it, the yield increased by 30%. About one month after emergence, the plants growing on the banded treatment were up to seven times as large as those growing on the mixed treatment.

Much of this research concerning phosphorus immobilisation was undertaken in the 1970s and 1980s. A relevant quote comes from Muirhead, in the proceedings of an international workshop into rice based systems (Muirhead and Humphreys, 1995).

"A new generation of farmers and advisors has appeared in Australia since this research was carried out and who are unaware of it. Many have experienced yield loss through rice stubble disorder in a range of summer cereal, pulse and vegetable crops. The challenge now is to ensure that farmers in the future will not suffer unnecessary crop loss through the *immobilisation of fertilizer phosphorus caused by soil changes occurring during a recent rice crop*".

Allelopathy is another recognised chemical constraint on cotton seedlings (Hicks *et al.*, 1989; Xiuqin, 2001; Kandhro *et al*, 2016). Cotton growth inhibition due to rice is more likely due to leachates from the stubble rather than root exuded compounds carrying over into the next summer season because of the short half lives of the latter. Activated carbon present in the soil post-stubble burning has been shown to reduce the effectiveness of herbicides (Toth *et al.*, 1981; Xu *et al.*, 2008). Therefore in the same way allelopathic effects may be reduced if stubble is burned near time of sowing, a hot complete burn is desirable to reduce the stubble load after rice crops.

Physical constraints to growth

The use of field equipment early in the season when the soil is wet may compact soil, thus reducing the productivity of cotton early in the season (Phillips and Kirkham, 1962; Gameda *et al.*, 1987; Voorhees, 1985). As the soil becomes compacted, particles are rearranged such that the total pore space is decreased and bulk density is increased (Singer and Munns, 1987). In these instances, the larger soil pores (macropores) are eliminated by soil compaction, and this results in reduced content and movement of air, water, heat and nutrients in the soil (Wesley *et al.*, 2001). Soil compaction increases soil strength, thereby increasing the resistance to cotton root penetration (Taylor and Ratliff, 1969). When plant roots cannot explore the entire soil profile, plant nutrients become spatially unavailable, further limiting the early vigour of cotton seedlings.

Soil physical conditions may be improved by the addition of gypsum to improve soil porosity and soil permeability. Strategic tillage may also be used to increase porosity (Loveday *et al.*, 1970). Rice soils are often sodic (Lewin and Heenan, 1987), which also can be improved with the addition of gypsum to increase aggregate stability by displacing sodium ions. Growing a winter cereal, such as wheat or barley will reduce water logging or soil saturation and return the soil to aerobic conditions. However, if traditional cultivation is used after rice in a wet winter, the deleterious effects of water logging may be exacerbated. Tillage practices now utilised partly address this problem by direct drilling rice with disc seeders to minimise soil disturbance.

Biological constraints to growth

Weeds can invade rice growing areas after harvest during winter and capitalise on wet conditions. These winter weeds, such as annual ryegrass can carry over into the subsequent cotton crops. Seedling cotton is relatively uncompetitive, and uncontrolled weeds can result in no harvestable cotton (Keeley et al. 1986). Weeds may also interfere with water flow through channels and fields, reducing irrigation efficiency, increasing waterlogging, and may reduce harvest efficiency. Weeds harbour insect pests and cotton disease pathogens and are common vectors for new infestations and outbreaks. The effect of weed competition on cotton yield has not been closely examined in Australia, although results are published by researchers elsewhere. Snipes et al. (1987) found that Xanthium strumarium (cocklebur) that emerged with cotton and was removed after only 2 weeks still reduced cotton yield. Buchanan and Burns (1970) found that weeds which emerged with cotton caused yield reductions if not controlled within 4-6 weeks, but weeds emerging after more than 8 weeks did not reduce yield. However the weed spectrum has changed since that work was done and the current weed relationships need to be revisited. Weeds of cotton have also evolved resistance to herbicides – these include awnless barnyard grass, liverseed grass, sow thistle, feathertop Rhodes grass, sweet summer grass, windmill grass, and regrass, and fleabane. Careful management is needed to inhibit the build-up of resistant weeds and preserve the efficacy of available herbicides. In particular the risks relating to glyphosate resistance are highly important given that conservation farming systems rely on its effectiveness. Glyphosate tolerant cotton has been rapidly adopted by the Australian cotton industry since its introduction 14 years ago and currently accounts for nearly all of the crops sown. Herbicide tolerant cotton varieties have greatly reduced the utilisation of pre emergent residual herbicides. These changes have resulted in a shift in the weed species found across cotton growing regions. Increasingly, the broadleaf weeds flax leaf fleabane and sow thistle dominate weed spectrums in cotton crops with increasing weed burdens in the non-cotton component of the rotation. Risks are heightened because of the use of 'Roundup Ready' varieties now grown. Experience in North America with evolved glyphosate resistance provides a lesson for Australian growers. Glyphosate is a once in a century herbicide that underpins cotton production in Australia and thus requires responsible stewardship to preserve it for future use. The implementation of a rigourous intergrated weed management program will underpin the successful control of weeds in cotton production areas in the south. Furthermore, the introduction of the extended herbicide tolerance package (XtendFlexTM) (roundup ready cotton stacked with dicamba and glufosinate tolerant genes) in the near future

will mean the industry will have to remain vigilant and adaptable to the rise of altered weed spectrums into the future.

Arbuscular mycorrhizal fungi (AMF) are known to contribute significantly to the ability of cotton seedlings to access nutrients and promote early growth. Cotton's association with mycorrhizal fungi enhances the plant's ability to acquire nutrients and moisture particularly in early growth when access to phosphorus is essential. However, mycorrhizal fungi are reduced by the flooded conditions of rice production. Cotton roots begin to be colonised by AMF within five days of germination, infection rapidly increases between 5 and 25 days, eventually infecting approximately 70 % of the total root length (Rich and Bird, 1974). The relative field mycorrhizal dependency (RFMD) is a measure of the increase in plant dry matter between infected and non-infected plants. RFMD varies with climatic and soil conditions and the growth and development of the plant. The potential benefit of AMF is affected by soil phosphorus levels, such that there is a decreasing benefit with increasing available soil phosphorus concentration. However, phosphorus lock-up caused by inundated soils increases the need for AMF. Therefore, the ability to increase and maintain AMF soil inoculums levels would maximise the resilience of cotton seedlings and may alleviate early growth disorders found in emerging cotton.

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The potential of commercial mycorrhizal inoculants to enhance cotton seedling establishment

Key Words Mycorrhizal inoculants, cotton seedling emergence

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) grown in southern New South Wales needs to emerge strongly to ensure that yields in the shorter summer season are maximised. Mycorrhizal associations assist emerging cotton seedlings to access phosphorus and zinc more efficiently (Nehl *et al.*, 1996) and are essential for normal cotton growth (Allen and Nehl, 1999). If phosphorus and zinc are limiting, the developing cotton seedling become more susceptible to diseases, competition from weeds and temperature fluctuations which can impede their early vigour. Arbuscular mycorrhizal fungi (AMF) are prevalent in Australian cropping soils and form associations with the majority of species present (> 60 % of species). However, root colonisation by mycorrhizal fungi is impeded by cooler temperatures (Tibbett and Cairney, 2007) and higher soil phosphorus levels (Mosse, 1973; Abbot and Robson, 1977). The question therefore is whether inoculation of the soils enhances colonisation of mycorrhizal fungi to compensate for the slower establishment conditions in southern NSW.

Commercial AMF products are not regulated under the APVMA and there seems to be no clear consensus as to their effectiveness for boosting crop production. AMF inoculants are not widely used and their effects are often difficult to measure. However, resilient propagules of AMF can be produced, such as spores, mycorrhizal root pieces and organic matter containing hyphae (Brundrett et al., 1996) and the science is clear that advantages may be gained by boosting mycorrhizal presence in the soil. There is potential for these inoculants to be utilised on-farm if properly produced and adequately stored. Dry inoculum can remain viable for many years while at room temperature (Jarstfer and Sylvia, 1994), whereas refrigerated moist inoculums have a shorter life-span of approximately two years (Morton et al., 1993). Corkidi et al, (2004) notes that difficulty in measuring the effectiveness of these commercial products may also be confounded by the addition of non-mycorhizzal agents such as fertilisers and organic matter. Therefore utmost care must be taken whilst producing and supplying these products as to not misrepresent the potential yield gains achieved and to maintain mycorrhizal viability through until the time of application of such products. However the widespread occurrence of these fungi in nearly all agricultural soils may negate their usage completely. The feasibility of using commercially available

inoculants for broadacre cotton to enhance seedling nutrient acquisition, and thus improve speed of establishment, is the primary aim of this research.

MATERIALS AND METHODS

Glasshouse, laboratory and field testing was employed to evaluate the effectiveness of commercial mycorrhizal inoculants including the verification or otherwise of label claims for such products. Different rates of inoculants and their interaction with high and low soil nutrients levels were evaluated with respect to their mycorrhizal root colonisation. Subsamples of inoculants were also evaluated for AMF content as described below. All root samples were washed then processed as follows. Briefly, 0.3-0.5g fresh roots were transferred to custom staining tubes and submerged in 10% KOH and then placed in a 90° C water bath for one hour. Roots were subsequently acidified with 2% HCL for 5 minutes before the staining solution (acidified glycerol, water and 0.05% trypan blue) was added and placed back in a 90° C water bath for 20 minutes. Stained roots were then placed for 30 minutes in a 90° C water bath with acidic glycerol to remove excess stain. The staining method is adapted from Koske and Gemma (1989). Colonisation of cotton roots (as percent of root length infected) were then assessed using the line intersect method described by Giovannetti and Mosse (1980). All statistical analyses were performed in R statistical package (R Development Core Team 2008) utilising multiple one-way ANOVAs tested against the measured parameters.

Microscopy of commercial inoculants products

Aliquots (0.1g or 0.1mL) of each of the four products (Table 1) were thoroughly mixed, sampled and added to 3mL of staining solution (acidified glycerol, water and 0.05% trypan blue). Products were boiled in solution for 20 minutes, prepared onto a glass slide with a cover-slip and examined under a compound microscope for the presence of fungal propagules. Micrographs were taken of fungal material identified as potentially mycorrhizal with a Canon 60D digital single-lens reflex camera fitted with a microscope camera adapter.

Product	High rate	Low rate
Advance rootzone powder TM	2.5mg/pot	50mg/pot
Advance rootzone liquid TM	2.5µL/pot	50 μL/pot
VAM Microbesmart start-up TM	2.5mg/pot	50mg/pot
VAM grow plus liquid [™]	2.5µL/pot	50 µL/pot

Table 1 Four commercial inoculants used and concentrations used for high and low treatments

Glasshouse evaluation of mycorrhizal inoculants

The impact of AMF inoculants on cotton germination and growth were evaluated in a series of field and glasshouse experiments. All products were utilised within one year of acquisition, sealed and kept at 4 °C until used. Four inoculants were used (Table 1), viz. Advance rootzone powder[™] (2.5mg/pot and 50mg/pot), Advance rootzone liquid[™] (2.5µL/pot and 50µL/pot), VAM Microbesmart start-up[™] (2.5mg/pot and 50mg/pot) and VAM grow plus liquid[™] (2.5µL and 50µL). These rates were applied inside a folded filter paper (Advantec No. 2) containing two cotton seeds then placed in the soil at a depth of 20 mm. This method was to ensure contact between the germinating cotton seed (variety Sicot 71BRF) and the specific inoculum. The soil mix comprised sand:loam at 7:3 to facilitate steam penetration during the pasteurisation process using a soil steamer with temperature maintained at 70 °C for 1 hour. Cotton seedlings were later thinned to the strongest seedling per pot after germination to reduce variation. Each inoculant treatment was exposed to two nutrient regimes relating to phosphorus and trace elements. Phosphorus and micronutrients were applied according to Tables 2 and 3. Soluble nitrogen in the form of urea (Richgro[™] urea, 46 % w/w nitrogen) was applied at a rate of 0.15g per pot every 2 weeks from the start of the experiment for the duration of the experiment. Soluble sulphate of potash (RichgroTM, Potassium as sulphate 41.5% w/w and sulphur as sulphate 17 % w/w) was applied at the start of the experiment to each experimental pot at the rate of 0.11g per pot.

Analysis		% w/w
Phosphorus		
	P as water soluble	7.3
	P as citrate soluble	1.3
	P as water insoluble	0.5
	Total phosphorus	9.1
Sulphur as s	ulphate	11.5
Calcium as p	phosphate	20.0

Table 2 ManutecTM super phosphate used in experiment, used at a high rate of 0.4g/pot and a low rate of 0.1g/pot

Analysis	% w/w
Sulphur as sulphate	6.29
Calcium as carbonate	10.00
Magnesium as sulphate	3.62
Manganese as sulphate	2.88
Iron as chelate	2.73
Copper as sulphate	1.25
Zinc as sulphate	1.00
Boron as borate	0.09
Molybdenum as molybdate	0.0038

Table 3 ManutecTM trace nutrients used in experiment, used at a high rate of 15g/L and a low rate of 3.75g/L watered into each pot until field capacity.

Experimental design comprised a randomised complete block design with four replicates in a temperature controlled glasshouse at 30/20 °C for 12h/12h, day/night cycle. Pots were maintained at field capacity for the duration of the experiment. The experimental control contained pasteurised sandy loam soil without the incorporation of inoculants. Germination was recorded each day. Growth and root colonisation by AMF were evaluated at time of harvest, 6 weeks after sowing.

RESULTS AND DISCUSSION

Microscopy of commercial inoculants products

Three of the four products had a mixture of mycorrhizal propagules present (Figures 1-4), including hyphae, root fragments and spores. Microbesmart start-up[™] had no detectable propagules present. The detection of available propagules did not indicate the viability or infectivity of the products tested. Only one batch of each product was examined and so no conclusions can be drawn on variability between batches of product. However, there was large variability between the same inoculant products that were prepared onto microscope slides, making it difficult to determine the number of propagules in each product. Further work is needed to determine the variability between batches of commercial mycorrhizal inoculants.



Figure 1 Micrograph showing 40x magnification of Advance rootzone liquid®.



Figure 2 Micrograph showing root segment in Advance rootzone liquid® with hyphal and spore propagules present (**A.** 400x and **B.** 1000x).



Figure 3 Micrograph showing fungal spores present in Advance rootzone powderTM (1000x magnification)



Figure 4 Micrograph showing free floating fungal hyphae in VAM grow plus liquid[™].

Glasshouse inoculation experiment

All four products failed to produce mycorrhizal colonies in both nutrient regimes tested. This may be an indication of an absence of viable propagules or an insufficient number of propagules to cause infection. The inclusion of an inoculant rate approximately 20 times that of the recommended rate produced no response. This outcome provides further support to the view that the inoculants tested were either grossly under the concentration of propagules needed or were non-viable. A nutrient growth response occurred (Figure 5) suggesting that mycorrhiza could have colonised to reduce the difference in growth obtained. In other studies, commercial inoculants that were properly stored and applied have also failed to show responses under varying conditions (Corkidi *et al.*, 2004).



Figure 5 The effect of fertiliser rate on the dried whole plant biomass (P = 0.001; L.S.D = 0.0685)

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Observation	P value
Dry weight	0.285
Shoot length	0.844
Root length	0.441
Number of nodes	0.958
Fertiliser rate	(P < 0.001; L.S.D = 0.0685)

Table 4 List of observations and the P value attained for each parameter in multiple one-way

 ANOVAs.

Germination, plant growth responses and root colonisation by AMF were taken at time of harvest but none of these measured parameters showed significant improvement in relation to any mycorrhizal product used (Table 4).

These results therefore question the claims made by suppliers of the various products. The high costs of the products and their lack of effect suggest that they do not represent value for producers. Also raised is the lack of protection for producers in regard to biological products as they are not subject to the approval processes that chemical products require through the APVMA. Such an approval process would provide protection to farmers that the claims made have been authenticated.

Our assessment of the four commercial AMF products tested is that they are largely ineffectual. Producers instead should use appropriate crop species to build up mycorrhizal inoculum for better, more assured outcomes.

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Bank vs bay II – From soil cores to field scale measurements

Anecdotal evidence suggests a yield penalty exists for cotton crops grown after rice in crop sequence. Old bank-lines tend to be areas of unrestricted plant growth, which is in contrast to the old bay areas, where stunted cotton seedlings predominate. A considerable amount of literature has been published on the effects of the immobilisation of phosphorus after rice production. These studies examined the effect of reducible iron on phosphate sequestration and the slow return to available phosphorus after rice bays are converted into row crop formats. Recent evidence suggests a depression in mycorrhizal levels subsequent to rice (Moore *et al.*, 2015), which may further limit cotton seedling growth by restricting nutrient acquisition. Furthermore, inhibitory chemical residues from rice stubble and impeding soil physical properties may also limit cotton seedling establishment and growth.

To further evaluate the effect of rice production on subsequent cotton growth a field was selected and sampled prior to conversion to row crop format. However, the determination of causative factors for growth differences on a small scale is technically challenging. This study gives an account of our research from the soil core point scale up to the field scale.

Materials and Methods

1. Glasshouse experiment utilising field collected soil cores

Paired soil cores were taken in December 2015 from a field in the Coleambally Irrigation Area (CIA) previously used for rice production (2014/15 season). Samples were taken along the top of the bank line and 10m into the adjacent bay area to form a series of paired samples. Five soil cores of each were taken in PVC tubes (100mm diameter, 250mm depth). Each location also had a soil sample (0 – 25 cm) taken for chemical analyses. Subsequently, the PVC tubes were sown with three cotton seeds per pot and thinned two weeks later to one cotton plant per pot. Cotton plants were grown with no nutrients applied in a temperature controlled glasshouse (day/night cycle 30° C/ 20° C) before being harvested after 6 weeks growth. At harvest, the soil cores were placed in a large watertight container and each container filled with a 2g L⁻¹ sodium hexametaphosphate solution and allowed to soak for one hour to facilitate the separation of roots and soil. Soil was then agitated vigorously with a jet of water. After allowing the sample to settle for several seconds, the supernatant was poured onto a fine sieve (250µm) and washed down with copious amounts of water. Roots were then recovered and stored in 70% ethanol until the staining process for mycorrhiza detection was performed. The staining method is adapted from Koske and Gemma (1989). Briefly, 0.3-0.5g fresh roots were transferred to custom staining tubes and submerged in 10% KOH and placed in a 90° C water bath for one hour. Roots were subsequently acidified with 2% HCL for 5 minutes before the staining solution (acidified glycerol, water and 0.05% trypan blue) was added and placed back in a 90° C water bath for 20 minutes. Stained roots were then destained for 30 minutes in a 90° C water bath with acidic glycerol to remove excess stain. Colonisation of cotton roots (as percent of root length infected) were then assessed using the line intersect method described by Giovannetti and Mosse (1980). Additionally, shoot weight, shoot height, root length and number of nodes per plant were measured. Soils used in the glasshouse experiment was subjected to a suite of analyses to determine plant nutrient levels for identification of any potential impediments to growth.

The paired soil cores were arranged in a split-plot randomised block design. Effects of location in the field (bank or bay) were tested using multiple ANOVA and soil analysis results were evaluated using multiple paired t-tests. All statistical analyses were performed in R statistical package (R Development Core Team 2008).

2. Soil samples 1st and 2nd summer out from rice

A separate site in the CIA that displayed growth differences between bank and bay areas was visited over two summer seasons (2014/15 and 2015/16). Results from a glasshouse growth experiment utilising soil cores from these areas is published in Moore *et al.* (2015 - see appendix 1). Using GPS coordinates of original sampling locations these areas were sampled in the first and second cotton crop after rice in the sequence to compare soil chemistry changes between cotton seasons. Soil was subjected to a suite of analyses to determine plant nutrient levels and to try and identify any potential impediments to growth (Table 2).

3. Using yield maps to examine field scale effects

Yield maps were acquired from an irrigation property to provide a means to better illustrate the bank-line effect at a field scale. This effect was first observed by local agronomists in the 2013/14 summer season. Maps available were from multiple fields and times but provide an understanding of the temporal and spatial variability over time for year 1 and year 2 cotton row crops after fallow following rice.

Results and discussion

1. Glasshouse experiment utilising field collected soil cores

Contrary to expectations, there were no significant differences in growth and development of plants between bank and bay soil cores. Germination rate was not recorded. Further research is needed to measure this, as a slower rate of germination and final establishment rate may reflect what is observed on a field scale. It is also possible that the soil cores were not from locations where the effect was prevalent or likely to occur. Another possible explanation is that sufficient time had elapsed between rice crops and time of sampling such that the effect was not likely to occur.

The mean results from the soil analyses are presented in Table 1. There was some evidence to support the hypothesis that soil chemistry differences existed between the bank and bay area. In particular, pH, extractable iron, total nitrogen, extractable phosphorus and total P were significantly different between bank and bay (p < 0.05). However, these nutrients seem not to be in the range limiting to growth. Extractable manganese levels were concerning and suggest likely toxicity in the bay areas where soil pH is acidic.

Soil analyses	Units	Bank	Bay
Boron, extractable	mg/kg	0.4	0.6
Calcium, soluble/exchangeable	cmol/kg	10.6	6.4
Copper, extractable	mg/kg	3.8	5.2
Iron, extractable	cmol/kg	7.5	91.4
Manganese, extractable	mg/kg	42.3	55.1
Zinc, extractable	mg/kg	1.7	2.1
pH (1:5 CaCl2)	pH units	6.4	5.1
Effective Cation Exchange Capacity	cmol/kg	18.5	13.6
Extractable Phosphorus (Colwell)	mg/kg	37.0	52.2
Phosphorus, Total	mg/kg	282.4	346.8
Phosphorus Buffer Index	mg/kg	96.8	95.6
Magnesium, soluble/exchangeable	mg/kg	5.8	5.7
Nitrate/Nitrite as N	mg/kg	38.4	28.2
Nitrogen, total	L/kg	454.6	610.2
Exchangeable Sodium Percentage	%	3.3	2.8
Sodium Adsorption Ratio	ratio	<1	<1
Sodium, soluble/exchangeable	cmol/kg	0.6	0.4
Potassium, soluble/exchangeable	cmol/kg	1.6	1.3

Table 1 Mean data from soil analyses of bank and bay areas taken from field area 1 (prior to row conversion).

2. Soil samples 1st and 2nd summer out from rice

Soil analyses between years revealed an increase in many of the essential plant nutrients, especially available phosphorus (P) and nitrogen (N). Colwell P increased from 28.3 and 21.7 to 49.0 and 43.0 in bank and bay areas respectively. Soil N also increased substantially and may explain the lack of observable bank-line effects in the second cotton crop.

Table 2 Mean data from soil analyses of ex-rice bank and bay areas from soil cores taken from the same field area from the 2014/15 and 2015/16 summer seasons.

	Season			
	2014/15		20)15/16
Soil Analyses	Bank	Bay	Bank	Bay
Boron, extractable (mg/kg)	0.3	0.3	0.1	0.2
Calcium, soluble/exchangeable (cmol/kg)	15.1	13.3	5.89	8.14
Copper, extractable (mg/kg)	4.8	4.6	4.79	5.51
Iron, extractable (cmol/kg)	29.1	21.3	231	80.1
Manganese, extractable (mg/kg)	37.1	43.4	79.4	61.9
Zinc, extractable (mg/kg)	1.9	1.7	2.09	4.19
pH (1:5 CaCl2) (pH units)	6.1	6.1	4.3	5.8
Cation Exchange Capacity cmol/kg	26.7	24.7	11	15
Extractable Phosphorus (Colwell) (mg/kg)	28.3	21.7	49	43
Phosphorus, Total (mg/kg)	174.3	164	239	213
Phosphorus Buffer Index	196.7	190	236	164
Magnesium, soluble/exchangeable (cmol/kg)	10.1	9.9	4.58	5.51
Nitrate/Nitrite as N (mg/kg)	18.9	16.7	296	185
Nitrogen, total (mg/kg)	412.3	343.3	2540	1120
Exchangeable Sodium Percentage (%)	1.9	2	2.2	2.4
Sodium, soluble/exchangeable (cmol/kg)	0.5	0.5	0.25	0.36
Sodium Adsorption Ratio (ratio)	<1	<1	<1	<1
Potassium, soluble/exchangeable (cmol/kg)	1.1	0.9	0.64	0.89

3. Using yield maps to examine field scale effects



Figure 1 First year cotton yield (bales/ha) map (A.) and yield (bales/ha) distribution (B.) of a field converted from rice bays into bed layout for row crop production.



Figure 2 Cotton yield (bales/ha) map and yield distribution of four adjacent fields from the 2015/16 season converted from rice bays into bed layout for row crop production.

Bank-line effects can be clearly observed utilising yield maps from ex-rice areas. Figure 1 shows that cotton yield averaged at 12.79 bales/ha, although the average differential between bank and bay areas was approximately 2 bales/ha. This difference constitutes a profit loss of around \$1100/ha. Figure 2 demonstrates the effect over multiple fields as well as showing that extreme variation can occur depending on the cut-and-fill regime of a particular field. Yield was not as severely affected toward the tail drain end of the paddock (Bottom section of Figure 2).

Figure 3 demonstrates the bank-line effect over two years. Figure 3A shows the yield map for a first year cotton crop in the 2014/15 season following one maize crop after rice bay conversion. Old rice bank and bay areas can be observed more clearly on the right side of the image. Figure 3B shows the 2015/16 season yield map from the second cotton crop. The demarcation of bank and bay is no longer evident.



Figure 3 Cotton yield (bales/ha) map and yield distribution of field converted from rice bays into bed layout for row crop production A. 2014/15 cotton season and B. 2015/16 cottonseason

Conclusion

This research was limited by the availability of appropriate sites that were being converted from rice into cotton. Nevertheless, we have shown that bay and bank-line effects are evident in cotton crops following rice in the cropping sequence. This can cause inconsistent crop growth and a significant yield differential at time of harvest. Effects were clearly demonstrated through soil testing over multiple years and by examining yield maps at a field scale. Although these findings are based on a small sample of cotton fields, they do suggest that at least three years of row crops are needed to negate the effects of rice crops on row crop production.

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FUTURE RESEARCH

The Future

The research reported here poses more questions than answers. They relate to the two sections presented in the previous pages.

Soil Challenges

The research suggests that the transition from rice to cotton presents many challenges. Clearly, as rice is currently grown, soil compaction and chemical reduction processes resulting from several months of inundation during rice production provide a disincentive to cotton production. Some farms and fields with heavy soil types that enable lower rice water use are set up for rice systems whereas other farms and fields on lighter soils are directed towards row crops as growers look to grow more hectares for less water, thus making the farm unit more productive. To this extent rice and cotton appear somewhat complementary.

Nevertheless the process is evolving and there will always be some fields in transition from rice to row crop. In the event that water allocations reduce over time, or that water becomes more expensive, there could be around 50,000 ha of rice country in the MIA and Murray Valley available to other crops such as cotton, as has happened in the 2015-16 season. In the event that a producer wishes to grow cotton on an area previously supporting rice then there are important issues to be considered. Experience suggests that cotton in the succeeding summer after rice is not a commercial proposition. Land forming will take the first summer anyway after rice but it will need a cereal or other crop(s) as an intermediary between rice and cotton.

Even then the bank and bay effect is evident, as described in the report. The crop on the former bay area is slower to establish and shows for most of the season a lower dry matter than that in the bank area, resulting in a substantial yield differential. It appears the delay in flowering in cotton does not allow time to compensate for yield, particularly in a cool season. Closing that yield gap remains a challenge. Although bankless layouts may alter crop sequences without layout changes, the risk of negative impacts on any row crop remains. The question is what crop will facilitate and hasten the transition. Maize, for example, does show

the bank and bay effect after rice but there seems to less effect on maize yield compared with cotton - maize is around a month shorter in maturity so there is some compensatory time. A suggested crop sequence for transition could be rice, winter cereal, short fallow and bed formation, maize, short fallow then cotton. However there is a need for a long term rotational trial to elucidate the best options.

The questions that follow relate to changes that are taking place, or might take place, in rice production. For decades 'long season rice' has been grown – the seed is pre-germinated and sown into permanent water. This permanent water remains on the crop at varying depth for the duration of the crop particularly to buffer temperature and is drained around the end of March. The challenge for the rice industry is to produce the same yield of rice for significantly less water use and new shorter season varieties are now available. Concurrently there has been a trend away from aerial sowing into permanent water in favour of drill sowing and flushing such that permanent water is not applied until around panicle initiation. At the end of the rice crop, prospects exist for early drainage but this incurs a yield penalty. So permanent water for a shorter period, up to 20% (4-6 weeks) shorter, raises the questions around whether this shorter period of inundation has the same or less effect on the soil compaction and nutrient availability, including whether the bank and bay effect is as pronounced and the transition phase duration is changed. This needs to be investigated.

Mycorrhizal and other microbes

We know that inundation in the rice crop affects mycorrhizal levels for a subsequent cotton crop. It might be expected that other aerobic microbes would be likewise affected. In suitable soils then rice may play a useful role in reducing cotton soil pathogens. The question is however whether the mycorrhiza absence results in a yield penalty or whether its build-up is sufficient to provide the nutritional advantage in time. The availability of commercial products raises the question of whether these provide a useful addition to the system. At this stage their role is unclear and preliminary investigations have not provided the evidence to suggest that their use is beneficial.

In the transition from rice to cotton, the need for an intermediary crop seems to be a valuable step and so the prospects exist for that intermediary crop to provide scope for the build-up in AMF without the need for inoculation. While it is known which plant species host AMF, there is little information about the best crop or pasture options for rapid build-up of inoculum such that the transition from rice to cotton is hastened, thereby allowing cotton development to be uninhibited.

Canola and related crops are known for not requiring AMF and so inoculum is low after such crops. Whether the absence of AMF is because canola is not a host or whether there is active chemical control through root exudates in not understood. Canola and mustard are known for their biofumigation capabilities and whilst this may be a negative towards a following cotton crop, the removal of other pathogens may well be a useful outcome provided that AMF can be built quickly. It is not known whether the biofumigation capability of canola and mustard could be used for pupae control. It is recognised that *Heliothis* pupae form a water-tight chamber and thus may be protected but it is worthy of some investigation just in case there is some opportunity.

Chemical ecology

This project showed that differential responses by cotton occurred depending on the rice variety previously grown in the soil or on residues added to the soil. There also seemed to be a differential response depending on the cotton variety. This potentially opens up a major line of investigation relating to chemical ecology. We know that herbicide resistance is a big challenge. We can expect it to intensify, particularly to glyphosate, as more and more dependence is placed on herbicidal weed control. Other crops and pastures have shown that there is a wide varietal range of potency towards allelochemical control of weeds and other companion species. We know that this can be used to advantage for companion plantings, in rice production in other countries and for better management. We also know there is a wide range of tolerances within crop species to the allelochemicals from other, usually weed, species. We need to look for other tools to alleviate the pressure we are placing on herbicides both to reduce incidence buildup and to preserve for longer the efficacy of herbicides we have. Figure 1 shows the potential for allelopathic varieties in canola while the residual effect on weed growth subsequently is in Figure 2. Whether these capabilities exist in cotton varieties remains to be evaluated.



Figure 1. Allelochemical canola variety (LHS) and non-chemical canola variety (RHS)



Figure 2. Noticeable residual effect of allelochemical canola varieties (foreground) and nonallelochemical varieties (Background).

There is thus a major study of the ability of cotton germplasm to control a range of weed species through allelopathy. One issue here is the very narrow germplasm base on which the commercial crop is currently delivered. There is also a need to look at the impact of weeds on cotton varieties. This would then allow the development of varieties that were both potent to

the weeds and tolerant of the weeds. The first step needed is consultation with the breeders in CSIRO to consider the range of germplasm in breeding lines that could be evaluated for allelopathic capability and their potential to control emerging problem weeds such as fleabane and awnless baryard grass.

There is also the opportunity to explore the relationships of crop rotations. We do not know whether there are positive or negative effects of cotton and its residues on other crop species or other crop effects on cotton. We know this aspect of the researchneeds to be done at the variety level rather than at the species level.

APPENDICES

1. Mycorrhizal status in the rotation: the importance to subsequent cotton establishment

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Abstract

Early vigor in cotton is related to its ability to access nutrients present in the soil. Mycorrhizal associations are known to benefit cotton establishment by enhancing nutrient and moisture acquisition, particularly phosphorus and zinc. If the colonisation of arbuscular mycorrhizal fungi (AMF) is incomplete, the cotton crop may be restricted in its establishment and growth. The need for vigorous germination, strong emergence and establishment at appropriate densities becomes paramount to subsequent crop development and ultimate yield. Commercial mycorrhizal testing of 'pre-plant' soil samples showed a wide range of spore counts (4 -100 spores/g), dependant on the crop rotational circumstances. Additionally, a field site previously used for rice that displayed growth differences between old bank lines and the adjacent rice bay area was utilised for soil sampling. Paired soil core samples from these areas were taken in PVC tubes (100mm diameter, 250mm depth). Plant height and establishment rates were measured in the field at time of sampling. Plant heights at time of soil core sampling between bank and bay areas were significantly different (p < 0.001). Subsequently,

PVC tubes were then sown with cotton and utilised in a pot experiment. Cotton plants were grown for six weeks in a temperature controlled glasshouse (day/night cycle 30°C/20°C). Roots were then washed, stained and mycorrhizal colonisation was determined in each sample. Mycorrhizal colonisation was greater in soil cores sampled from old bank areas

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compared with the adjacent bay (p < 0.05).

This paper discusses the relationship between crop rotations and their effect on colonisation of mycorrhiza in cotton and the implications of converting old rice bays into raised beds for cotton production.

Key words

Crop rotations, cotton emergence, soil biology

Introduction

Cotton is one of the leading plant fibre crops worldwide and is grown commercially in the temperate and tropical regions of more than 50 countries (Smith 1999). In Australia, the bulk of the cotton industry is concentrated in northern New South Wales and southern Queensland. However the industry has made steady inroads to expanding the southern NSW area planted. Cotton is now grown commercially from the Victorian border to Emerald in central Queensland, and as far west as Bourke and Lake Tandou in New South Wales. Cotton is grown either as a dryland crop, relying on rainfall, or as an irrigated crop where a reliable water supply is available. The total area planted to cotton in Australia was about 583,000 hectares in 2011/12 season. Cotton in southern regions is grown in rotation with rice and winter crops, thus experiences a different set of agronomic challenges specific to the south. The shorter season associated with southern cotton production necessitates strong germination, emergence and establishment as there is little time for compensatory growth resulting in lowered yields. The switch in crop from rice to cotton also presents unique growth and development problems to cotton in southern Australia, which are not yet elucidated.

As mycorrhizal associations are known to benefit cotton establishment, determining their colonisation subsequent to rice in the rotation was investigated. Mycorrhiza are ubiquitous plant symbionts which colonise the root systems of most terrestrial plants (Nehl *et al.* 1996). The fungus relies on the plant to provide carbohydrates and in exchange provides the plant with an underground hyphal network that extends the root system and allows increased uptake of nutrients and moisture (Ho and Trappe 1973). The predominant type of

mycorrhiza found associated with 60-70% of plant species are arbuscular mycorrhizal fungi (AMF) and these are associated with the roots of cotton. The two objectives of this study were-

- 1. To measure the AMF content in soil to determine the level and variability of colonisation following different crop rotations in the southern NSW region.
- 2. Investigate mycorrhizal colonisation in soil cores taken from an area which exhibited poor cotton growth after rice bay conversion to raised beds.

Method

Pre-plant field collection

Field soil and cotton roots for pre-plant testing and in crop testing were collected from two farms in the Coleambally and Darlington Point areas. Pre-plant soil was sampled according to Forecasta pre-plant® requirements and commercially tested by Microbiology Laboratories Australia. Briefly, supplied containers were filled with field collected soil and sampled at a rate of one sub sample per hectare using a diagonal transect through each cotton field. Each sample was bulked into the final sample and subsequently sealed and immediately sent for commercial testing.

Glasshouse experiment

Paired soil cores utilised in the pot experiment were taken from a cotton field in the Coleambally irrigation area where the old bank lines were and adjacent rice bay areas were apparent. Core samples were taken in

PVC tubes (100mm diameter, 250mm depth). Subsequently, the PVC tubes were sown with cotton as a pot experiment. Cotton plants were grown for four, five and six weeks in a temperature controlled glasshouse b(day/night cycle 30° C/ 20° C) before being sampled. Soil cores were held in individual plastic bags and placed in a large watertight container and each bag filled with a 2g L⁻¹ sodium hexametaphosphate solution and allowed to soak for one hour to facilitate the separation of roots and soil. Soil was then transferred to a bucket and agitated vigorously with a jet of water. After allowing the sample to settle for several seconds, the supernatant was poured onto a fine sieve (250µm). Roots were then

recovered and stored in 70% ethanol until the staining process was performed. The staining method is adapted from Koske and Gemma (1989). Briefly, 0.3-0.5g fresh roots were transferred to custom staining tubes and submerged in 10% KOH and placed in a 90° C water bath for one hour. Roots were subsequently acidified with 2% HCL for 5 minutes before the staining solution (acidified glycerol, water and 0.05% trypan blue) was added and placed back in a 90° C water bath for 20 minutes. Stained roots were then de-stained for 30 minutes in a 90° C water bath with acidic glycerol to remove excess stain. Colonisation of cotton roots (as percent of root length infected) were then assessed using the line intersect method described by Giovannetti and Mosse (1980).

Experimental design and statistical analysis

Paired soil cores were arranged in a randomised block design with three sampling times. Effects of sampling time and location in the field (bank or bay) were tested using a two way analysis of variance. For the field measurements, the effect of location in the field was tested using a one way analysis of variance. Correlation between shoot dry matter and mycorrhizal colonisation was tested using a Pearson product moment correlation. All statistical analyses were performed in R statistical package (R Development Core Team 2008).

Results and discussion

Pre-plant field collection

Canola crops had the lowest mycorrhizal spore and colonisation levels followed by rice (Table 1). Canola does not associate with mycorrhizal fungi. Canola crops are known to produce a biofumigation effect on the soil with toxic chemicals such as glucosinolates and their breakdown products isothiocyanates likely to be responsible for decreased mycorrhiza in the soil (Glenn *et al.* 1988; Vierheilig *et al.* 2000). Variation in spore counts are likely to have been affected by different management practices between sampling locations.

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centage.	
Spore count (spores g ⁻¹)	Colonisation (%)
4	12.8
5	16
6	19.2
7	22.4

28.8

41.6

64

100

100

100

100

Table 1 Results from commercial (VAMwise) 'Forecasta pre-plant' tests of soil collected from cotton fields in 2014/15 season for mycorrhizal spore content (spores/g) and predicted colonisation percentage.

Glasshouse experiment

9

13

20

35

40

51

70

2013/14 crop

Canola Canola Canola

Canola

Canola

Fallow

Wheat

Cotton

Cotton

Rice

At the time of soil core sampling plant heights were significantly different (p < 0.001; Table 2), Bank areas sampled exhibited higher growth as compared with old rice bay areas. There was a clear effect seen in the field at the time of sampling and the old bank line was easily distinguished from the adjacent bay. However there was no effect on establishment rate per metre of row (Table 2) or the mean number of nodes found per plant per metre row when the soil cores were taken. There was a significant main effect between combined sampling time means of bank and bay shoot matter (p < 0.05). There were also differences in mycorrhizal colonisation (p < 0.05) between combined means of bank and bay soil cores. Mycorrhiza have been reported to always be associated with the roots of cotton and found to increase the growth and development of cotton plants, as well as cause earlier flowering and boll formation (Rich and Bird, 1974; Price *et al.* 1989; Nehl *et al.* 1996). There was a strong positive correlation between combined sampling time means of shoot dry matter and AMF colonisation (Pearson correlation coefficient =0.781; $p \le 0.01$).

Table 2 Mean observations comparing old bank lines and the adjacent rice bay area

Observations	Bank	Bay	
In-crop heights (mm)	733	499	<i>p</i> < 0.001
Establishment (plants m ⁻¹)	12.2	13.1	NS
Nodes (nodes plant ⁻¹ m ⁻¹)	13	11	NS
Colonisation (%)	43.88	21.80	p < 0.05
Shoot dry matter (g plant ⁻¹)	0.460	0.374	<i>p</i> < 0.05

Where rice has been the prior crop, rice bay area's had a lower mycorrhizal colonisation rate compared with bank areas. The inundation of rice crops changes the soil biology and chemistry, previous research having shown that phosphorus and zinc are immobilised in the soil post-rice (Willet *et al.* 1978) leading to slower early growth.

Conclusions

AMF spores were lower in soil of cotton fields that had previously canola. This may be due to the biofumigation effect canola produces. The lower spore counts found in cotton fields previously sown with rice are likely due to the inundation of the soil whereby the anaerobic conditions may have inhibited mycorrhizal spore production. AMF are essential for normal cotton growth and yield. If the number of AMF propugales in the soil is low, colonisation of the roots is delayed and plant growth may be depressed, with subsequent delays in maturity and reductions in yield. The importance of mycorrhizal associations in southern NSW, and their potential for enhancing cotton establishment, requires further investigation.

Acknowledgements

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The importance of mycorrhizal associations for early emergence in cotton in southern NSW

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Introduction

Early vigour in cotton is related to its ability to access nutrients present in the soil. Mycorrhizal associations are known to benefit cotton establishment by enhancing nutrient and moisture acquisition. In the Riverina region of southern NSW cotton is commonly grown in rotation with rice. A field site previously used for rice displayed growth differences between old bank lines and the adjacent rice bay areas.

Methods

 Paired soil core samples were taken from old bank and adjacent rice bay areas.

Soil nutrient analysis performed

Each core was then located in a glasshouse and planted with a single cotton seedling.
Roots were stained using trypan blue and mycorrhizal colonization was determined for each

plant using the grid line intersect method.



Figure 1. Variable cotton growth following rice in the rotation

Results

Variable measured	Bank	Bay	
In crop height (mm)	733	499	p < 0.001
Establishment (plants/m)	12	13	NS
Nodes (nodes/plant)	13	11	NS
Colonisation (%)	43.9	21.8	p < 0.05
Shoot dry matter (g/plant)	0.460	0.374	p < 0.05



Conclusion

Cotton following rice is a problem Nutrient uptake likely a factor (P an Zn) AMF colonisation lower in the rice bay



An alliance between Charles Sturt University and NSW Department of Primary Industries

W Charles Sturt



Low mycorrhizae levels cause problems in cotton following rice

By Joe Moore – Charles Sturt University

THE expansion of the cotton industry into southern NSW is likely to result in new challenges for growers as the crop is grown in rotations involving rice and winter crops. A research project, funded by the Cruiser Fund (CSD and Syngenta),



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is evaluating these challenges, with a focus on enhancing crop. emergence and establishment.

The need for strong germination, emergence and establishment is paramount as the short southern season allows little opportunity for compensatory growth of the crop. Any delays will result in lower yields and increase the prospects of greater weed invasion. Problems specifically associated with cotton emergence in southern Australian conditions will require altered management practices if productivity is to be maximised.

A unique southern problem is the establishment of cotton following a rice crop in the rotation. Yields of cotton crops can be as low as four bales per hectare in areas previously used for rice. Rice is grown under flooded conditions where soil remains anaerobic for extended periods, modifying soil physical, chemical and biological properties which have impacts on following



Plants grown in soil cores collected from newly formed raised beds after rice in the rotation. Old rice bay bank area (right) and old rice bay area (left). (MOTO: Joa Moons)

December 2015–January 2016

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pionisation. Cotton can overcome early nutrient deficiency they are infected plants with mycorrhizae.

cotton crops. These impacts include the phenomenon commonly referred to as 'phosphorus tie-up'.

Our research considers how mycorrhizae might help address this problem, including identifying crops which best maintain soil inoculum levels for subsequent cotton crops. Mycorrhizal associations benefit cotton establishment by enhancing nutrient and moisture acquisition. If the colonisation of mycorrhizae is incomplete the cotton crop is likely to have restricted establishment and growth.

Rice in the rotation has been found to reduce mycorrhizal infection in subsequent cotton crops, thereby limiting early growth. This then is a two-fold problem:



Variable growth in cotton following rice in the rotation. (PHOTO: Joe Means)

- Plants have a limited supply of phosphorus due to the tie-up; and,
- A reduced ability to overcome this due to the lack of the beneficial mycorrhizal association.

Often soil levels can be reclaimed through the use of other crops such as wheat in the rotation between rice and cotton. The feasibility of using commercially available mycorrhizal inoculants as biological soil ameliorants is also being investigated.

The research team is led by Professor Jim Pratiey, CSU and comprises early career researcher Joe Moore, CSU, Cotton agronomist Richard Malone, and Kieran O'Keefe (Cottoninfo).

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December 2015–January 2016
Cotton and rice rotations in south-eastern Australia

JR Moore, JE Pratley, K O'Keeffe, R Malone and KA Kirkby

Key points

Cotton performs poorly following rice due to:

- Iow soil porosity and permeability
- Iow soil P
- allelopathy

Iow mycorrhizal fungi

- The transition can be assisted by:
 - fallow periods
 - growing a winter cereal break crop
 - applications of banded gypsum

Introduction

The Murrumbidgee and Coleambally Irrigation Areas (MIA and CIA) produce some of the highest quality rice and cotton in the world. The climate in the MIA and CIA is ideal for both crops, although each has specific management requirements. The transition from rice to cotton in rotation needs to be carefully managed to ensure cotton crops emerge and establish successfully. Physical, chemical and biological soil constraints need to be considered.

Case study- Darlington Point

An irrigation field in Darlington Point was monitored over the 2014/15 and the 2015/16 seasons. Rice had been grown the previous summer and variable cotton growth was observed. This coincided with the bank lines present before conversion to row crop layouts (Figure 1). Plants that were inside the previously flooded bay area were stunted and had lower levels of mycorrhizal fungi, which are known to assist in nutrient uptake. Soil phosphorus (P) levels were similar between the bank and bay sites, although nutrients were more available to cotton plants on the bank line. Subsoil structure was investigated in a recently harvested rice crop adjacent to this area, revealing low porosity and permeability, which limited drainage and root penetration.



Figure 1 Cotton grown in the summer following rice. Bank lines can be clearly seen as areas with better cotton growth. Photo Joe Moore.

Cotton sampled on old bank lines showed better growth and their roots were highly colonised with beneficial mycorrhizal fungi compared with old rice bay areas. Cotton is

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known to have improved access to nutrients when infected by these fungi and this is likely to be a key factor indifferences observed between bank and bay areas.

Cotton was grown back to back after rice. When the site was revisited the in the second cotton year (2015/16 season), no differences were found in cotton seedling growth between old bank areas and old rice bay areas. Extractable P levels (Colwell) increased from 20 mg/kg in the first season out of rice up to 40mg/kg in the second season. These results indicate that P tie-up was a significant factor in reducing cotton vigour. Higher soil P is also known to reduce mycorrhizal dependency.

Allelopathy

Rice residues may also be present when rotating between rice and cotton. Chemical inhibition may occur from rice residues decaying and releasing herbicide-like compounds into the soil, and these carrying over to the next crop in sequence. These and other bioactive compounds may also be deposited into the soil directly from rice roots. Such compounds can cause germination and growth inhibition for subsequent crops grown in that soil for a short period. There are many factors that affect the severity of these allelopathic effects, such as UV light exposure, rainfall, frequency of wetting-drying cycles, soil biology and the specific characteristics of each compound.

Soil constraints

Soil physical constraints are common in crops following rice in the rotation. When a soil is flooded for rice production its structure collapses. The reduced number of macropores in turn leads to lower permeability, poor soil drainage and reduced heat and air exchange. Soils that are compacted limit root growth and affect the ability of seedling roots to access nutrients and moisture. Gypsum has been shown to be effective in ameliorating soil structure for better cotton growth when applied in bands with the seed or 10cm below seed level. Strategic tillage can also be used to increase the porosity of the soil. However, this option should be used sparingly to avoid permanent structural decline.



Figure 2 Agronomic problems are often due to many small effects adding to a significant problem. The likely contributing factors in reduced cotton growth following rice in the rotation interact depending on seasonal conditions, soil type and crop sequence.

Conclusion

Limited success has been experienced in having cotton immediately follow rice. Key factors identified include low available soil P, lower levels of beneficial mycorrhizal fungi, and

allelopathic compounds leached from rice residues. Currently, best management practice is to follow rice with a winter cereal crop beforegrowing cotton. Application of gypsum and strategic tillage can be employed to remediate soil structure.

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