Impacts of pasture legume phase on the seed bank of barnyard grass in drill sown rice

by Jhoana Opena, James Pratley, Deirdre Lemerle, Hanwen Wu and Jeffrey McCormick
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Foreword

Australian rice growers endeavour to increase water productivity by adopting water-saving systems, such as direct drill sowing of rice (DSR) in dry soil and delaying the application of permanent water (DPW) until late tillering. These water-saving methods, however, provide an opportunity for barnyard grass (*Echinochloa* spp.) to proliferate.

Barnyard grass is an annual summer grass weed that has been reported across 61 countries and grows as a weed in 36 crops. In Australia, a 5 t ha\(^{-1}\) rice yield loss due to competition from barnyard grass has been reported, while it has also caused rice seed contamination during harvest and removed up to 80% of soil mineral nitrogen. Barnyard grass was also reported to have evolved herbicide resistance to nine herbicide modes of action in 20 countries, and in four crops, including rice. Growers can spend from $400-870 ha\(^{-1}\) for herbicide control in DSR. In order to sustain water productivity gains with these water-saving strategies in rice production, it is imperative that this weed is managed effectively.

This research aimed to provide rice growers with an alternative option to manage barnyard grass in drill sown rice to ensure productivity is not compromised using these water-saving strategies. The objectives of the research in the context of the Australian DSR system were to determine: the impact of pasture legume residues on the emergence and growth of barnyard grass; the influence of pasture legumes on the seed bank dynamics of barnyard grass; and the effects of environmental factors on the germination/emergence and early growth of barnyard grass.

The report recommends that more than two years of pasture legume rotation be employed against barnyard grass, and that this strategy be combined with other weed management tools, such as no-till, drill sowing implements with discs, delayed rice sowing, the stale seedbed technique, and the use of competitive rice cultivars with early vigour.

This report for the AgriFutures Rice Program is an addition to AgriFutures Australia’s diverse range of research publications. It forms part of our Growing Profitability Arena, which aims to enhance the profitability and sustainability of our levied rural industries. Most of AgriFutures Australia’s publications are available for viewing, free download or purchase online at www.agrifutures.com.au.

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Jhoana Opena is a PhD research student in the School of Agricultural and Wine Sciences and a member of the Graham Centre for Agricultural Innovation, Charles Sturt University at Wagga Wagga NSW. This research was submitted in fulfilment of the requirements for the Doctor of Philosophy to Charles Sturt University.

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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BYG</td>
<td>Barnyard grass</td>
</tr>
<tr>
<td>DAS</td>
<td>Days after sowing</td>
</tr>
<tr>
<td>DAL</td>
<td>Days after exposure to light/dark</td>
</tr>
<tr>
<td>DSR</td>
<td>Drill sown rice</td>
</tr>
<tr>
<td>DPW</td>
<td>Delayed permanent water</td>
</tr>
<tr>
<td>MAH</td>
<td>Months after harvest</td>
</tr>
<tr>
<td>RB</td>
<td>Root biomass</td>
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Executive summary

This research highlights the key findings of a study on the impact of winter pasture legumes in the rotation in managing the seed bank of barnyard grass (*Echinochloa* spp.) in south-east Australian drill sown rice systems. Barnyard grass is considered a major constraint in achieving water productivity gains from drill sown rice and delayed permanent water. This research is directed at achieving productivity gains via mitigating barnyard grass risks in drill sown rice with delayed permanent water systems in temperate south-east Australia.

Background

Australian rice growers endeavour to increase water productivity by adopting water-saving systems, such as direct drill sowing of rice (DSR) in dry soil and delaying the application of permanent water (DPW) until late tillering. These water-saving methods, however, provide an opportunity for barnyard grass to proliferate.

Barnyard grass is an annual summer grass weed. It has been reported across 61 countries and grows as a weed in 36 crops. In Australia, a 5 t ha$^{-1}$ rice yield loss due to competition from barnyard grass has been reported, while it has also caused rice seed contamination during harvest and removed up to 80% of soil mineral nitrogen. Barnyard grass was also reported to have evolved herbicide resistance to nine herbicide modes of action in 20 countries, and in four crops, including rice. Growers can spend from $400-870 ha$^{-1}$ for herbicide control in DSR. In order to sustain water productivity gains with these water-saving strategies in rice production, it is imperative that this weed is managed effectively.

Farmer anecdotes have suggested that the resultant barnyard grass population is determined to some extent by the lead-in crop or pasture. However, very little research has been done to support this observation, and the mechanisms of suppression involved are unknown. An understanding of the seed bank dynamics as they relate to tools to manage barnyard grass effectively, and information on the factors affecting germination of barnyard grass associated with DSR in Australia, are also needed.

Aims/objectives

This research aimed to provide rice growers with an alternative option to manage barnyard grass in drill sown rice to ensure productivity is not compromised using these water-saving strategies. The objectives of the research in the context of the Australian DSR system are:

1. Determine the impact of pasture legume residues in relation to phytotoxic and allelopathic inhibitory effects on the emergence and growth of barnyard grass.
2. Determine the influence of pasture legumes on the seed bank dynamics of barnyard grass.
3. Determine the effects of environmental factors on the germination/emergence and early growth of barnyard grass.

Methods used

This research has been designed to investigate the different suppressive mechanisms of winter pasture legumes on the seed bank dynamics, establishment and early growth of barnyard grass. Suppressive mechanisms such as inhibition/delay of establishment, restriction of growth and reduction of seed longevity were evaluated through laboratory assays, glasshouse pot trials and field experiments. The effects of the environmental factors temperature, light and burial depth on germination and early growth of barnyard grass, as well as dormancy, were investigated to understand the seed bank dynamics involved.
Results/key findings

Allelopathic potential of pasture legume species against barnyard grass

There is a scope to use pasture legumes to limit barnyard grass establishment, growth and seed production. Pasture legume cultivar residues were found to have differential inhibitory effects on barnyard grass growth. This study also showed that there were potent phytotoxic compounds involved in some pasture legume species/cultivars. Identification of such allelochemicals may lead to the development of effective bioherbicides for commercial use against barnyard grass.

Seed bank dynamics of barnyard grass

This study of the seed bank dynamics of barnyard grass showed that of the rotation species in the rice-based system, the winter pasture legume phase had the greatest negative impact on the barnyard grass seed bank. Pasture legumes in the rotation depleted the barnyard grass seed bank by reducing seed rain through minimising grass seed set prior to sowing rice, reduced emergence with mulched residues, and suppressed growth by allelopathy. The seed bank was also reduced over time by increasing barnyard grass seed withdrawal through decay during at least a two-year pasture legume phase under no-till and through stale seedbed actions.

Barnyard grass ecotype response and implication for integrated weed management

The barnyard grass seed ecotype (from the DSR area in south-east Australia) used in this study showed distinctly different germination responses compared with previous studies of *Echinochloa* species from Australia and overseas. The ecotypic response of barnyard grass in this study highlighted the potential benefits of integrating different weed management tools, including no-till, the stale seedbed technique, sowing implements with minimum soil disturbance and the use of competitive DSR cultivars.

Recommendations

This research recommends that more than two years of pasture legume rotation, using allelopathic pasture legume species/cultivars, be employed against barnyard grass, and that this strategy be combined with other weed management tools, such as no-till, drill sowing implements with discs, delayed rice sowing, the stale seedbed technique, and the use of competitive rice cultivars with early vigour.
Introduction

The Australian rice industry is focused on increasing water productivity (Clarke, 2018), particularly because of the limited availability of irrigation water. One water-saving strategy is the direct drilling of rice into dry soil in order to delay the application of permanent water (DPW) until late tillering. Over four years, Dunn (2018) showed that drill sown rice (DSR) with DPW produced yields comparable to aerially sown rice, but with significantly less water use (4.5 ML ha⁻¹), translating to higher water productivity (0.9 and 1.1 t ML⁻¹) relative to aerially sown rice (0.8 t ML⁻¹).

Consequently, there is a trend towards using DSR in the Australian rice industry. A survey conducted by the Rice Extension Team (Yanco) in three rice seasons from 2015-16 to 2017-18 showed adoption of the practice had increased from 32.7 to 45.1%, respectively, driven by declining water availability for rice production. As water becomes limiting, growers clearly opt for more water saving measures.

A major constraint for rice productivity in these water-saving strategies, however, is weed infestation, particularly barnyard grass (*Echinochloa crus-galli* (L.) Beauv.). Drill sowing of rice results in the concurrent establishment of rice and weeds. In the event that no control is exercised, weeds commonly out-compete the crop, resulting in poor economic and environmental outcomes from the resources invested (Taylor, 2004). Weed densities have been reported to be higher in dry seeded rice than in wet seeded rice (Bajwa and Chauhan, 2017) due to the absence of inundation to control the non-aquatic weeds that cannot survive under submerged conditions. Researchers have recognised that grass weeds are a particular problem in drill sown rice, necessitating early control (Ampong-Nyarko and De Datta, 1991; Dunn and Ford, 2018). In dry soil conditions, the competitiveness of grass weeds such as barnyard grass against rice increases. Experience shows that barnyard grass favours DSR and the biggest risk involved with DPW is its control. Growers usually apply a mixture of paraquat, pendimethalin and clomazone after the first flush of irrigation to remove early established weeds and provide residual grass control. But with the delay of permanent water, there may not be enough residual herbicide control for ongoing barnyard grass (Dunn and Ford, 2018).

Barnyard grass is an annual summer grass weed. It has been reported in 61 countries, including as a major weed in Australia, and grows as a weed in at least 36 crops (Holm et al, 1991). In rice, it has been reported to cause 30-100% rice yield reduction (Chauhan and Johnson, 2011), as well as rice seed contamination during harvest (Pratley et al, 2008). Furthermore, it has been reported to have evolved herbicide resistance to 10 herbicide modes of action, in 23 countries and in four crops, including rice (Heap, 2020). In Australia, growers can spend in the range of $255-665 ha⁻¹ on herbicide application for weed management in DSR (Troldahl and Stevens, 2019).

The competitiveness of barnyard grass against rice is attributed to its features, such as its C4 pathway of carbon fixation, prolific seed production, mimicry to rice, dormancy and possibly allelopathic capability. Its C4 pathway of carbon fixation helps it compete for nutrients, light and other resources for plant uptake against the C3 rice crop (Bajwa et al, 2015). Its seed production is prolific, wherein one plant can produce 39,000 seeds if it emerges along with rice (Bagavathiannan et al, 2011). It mimics rice, particularly at the seedling stage, and hence can easily escape manual hand weeding. Seeds of barnyard grass do not germinate simultaneously but undergo a dormancy period, only germinating when conditions are most suitable for growth (Vleeshouwers and Bouwmeester, 2001). Freshly produced seeds are innately dormant and can remain dormant for 8-9 years, according to Chin (2001). Germination of freshly produced seeds has been reported as low as 0.3-1.4%, whereas storage for up to 8 months increased germination to 50% (Van Acker, 2009). Barnyard grass also seems to have a strong allelopathic capability; researchers have identified 37 allelochemicals affecting rice and other weeds (Bajwa et al, 2015). These features make barnyard grass a threat to rice production and particularly to DSR with DPW. In order to sustain water productivity gains with these water-saving strategies in rice production, it is imperative that this weed is controlled effectively.
Crop rotation has long been used as a component of weed management programs. Rotating crops enables growers to rotate other aspects of the crop management system, such as timing and type of tillage operation, seeding date, timing and type of herbicide application, and type and amount of fertiliser application. The rotation breaks the crop-weed association, thereby reducing weed adaptation and the chances of it becoming a problematic weed. As a rule, a crop rotation that takes advantage of multiple opportunities to suppress and remove weeds from the field will improve weed management in the field (Liebman et al, 2001). Rotations limit the need to use herbicides with the same modes of action over a long period of time, thereby limiting the evolution of herbicide resistance. Thus far, there have been no reports of barnyard grass herbicide resistance in Australia (Heap, 2020). A close relative, *Echinochloa colona* (L.) Link, however, has been reported resistant to glyphosate in rice, cotton and watermelon crops in Western Australia (Goh et al, 2018; Heap, 2020). Crop rotation, therefore, with its continuous suppression and removal of weeds, can limit the build-up of the seed bank and mitigate herbicide resistance concerns.

Early researchers have already recognised the value of pasture legumes in rotation with rice. Pastures in the rotation help alleviate high reliance on pesticides and inorganic fertilisers, high input cost, environmental concerns and weed management (Lattimore, 1994). An organic rice grower (B. Barnhill, pers. comm., 2018), described controlling rice weeds through the rotation with pasture legumes. However, while the role of a pasture legume for weed management in rice rotations in Australia has been recognised to some extent, the nature of this weed suppression strategy in the particular DSR and DPW system is not well-understood.

The knowledge of seed bank dynamics is very important as it reflects past weed population and management practices. The weed seed bank comprises the net outcome of seed deposits and seed withdrawals. Many weed management methods focus on reducing the seed deposits (such as the use of herbicides and other mechanical/cultural control methods), but they do not address the option of increasing seed mortality through increasing seed decay and fatal germination. The winter pasture legume phase potentially has an important role in depleting the seed bank through, among other things, promoting seed decay and fatal germination of barnyard grass.

Allelochemicals exuded by plants into the environment are toxic to other plants. These phytotoxins can suppress the growth of receiver plants, thereby affecting their seed fecundity and replenishment of the seed bank. Phytotoxicity studies on a pasture legume lucerne (*Medicago sativa* L.) showed that increasing concentrations of lucerne leaf extracts reduced root lengths of barnyard grass (Chon et al, 2002). Similar studies with white clover (*Trifolium repens*) showed inhibition on germination of five legumes and five grass species (Macfarlane et al, 1982a) due at least in part to phenolics. Information on the possible allelopathic/phytotoxic effect of pasture legumes, specifically on barnyard grass, is limited.

An understanding of the seed bank dynamics also requires knowledge of factors that affect the germination of barnyard grass, and this will enable the development of effective cultural management practices to control this problematic weed, either through inhibiting its germination or encouraging germination when weed seedlings can be easily controlled. Such factors affecting weed seed germination include temperature, light and burial depth as affected by tillage and flooding (Chauhan and Johnson, 2010). Many studies have shown that temperature is the most important factor determining weed emergence time (Guan et al, 2009). Seeds of barnyard grass were found to germinate under a range of temperatures, from 13 to 40 °C, with an optimum fluctuating day/night temperature of 30/20 °C (Roche and Muzik, 1964). Studies have also shown that continuous exposure to light resulted in germination in 60% of barnyard grass seeds, whereas only 6% germinated in continuous dark. Seedling emergence was also found to be higher in shallow depths of 1-2 cm (Maun and Barrett, 1986). Under field conditions, light effects on the germination of barnyard grass seeds will be determined by the vertical distribution of seeds in the soil, as light will be absent when the seeds are buried at depth. Optimal germination at shallow depths suggests that barnyard grass plants are likely to be more prevalent in reduced tillage systems, but this may depend on whether there is ecotype development in the weed towards this farming system (Chauhan and Johnson, 2010; Lee et al,
There are no studies on the biology of, or the factors that affect, germination of barnyard grass associated with drill sown rice in Australia, nor on ecotype responses.

The management of barnyard grass is critical in order to minimise rice yield loss resulting from water-saving practices in rice-based cropping systems. Pasture legumes in the rotation have long been used by Australian farmers and have the potential to assist in the management of this weed in these systems. However, there is limited literature on understanding the mechanisms of weed suppression by pasture legumes in the rotation and the factors that signal germination of barnyard grass. Water for irrigation is limiting in Australia, and the adoption of water-saving strategies needs to be supported by the development of new tools and practices for weed management to maintain productivity. This research aims to provide rice growers with an option to manage problem weeds, particularly barnyard grass, in drill sown rice to ensure productivity is not compromised using these water-saving strategies.
Objectives

The main hypothesis of the thesis was that “pasture legume species in rotation with rice deplete the seed bank of barnyard grass and thereby reduce its competitive impact on the rice crop in that rotation”.

The objectives of the research in the context of the Australian DSR system are:

4. Determine the impact of pasture legume residues in relation to phytotoxic and allelopathic inhibitory effects on the emergence and growth of barnyard grass.
5. Determine the influence of pasture legumes on the seed bank dynamics of barnyard grass.
6. Determine the effects of environmental factors on the germination/emergence and early growth of barnyard grass.
Chapter 1 Allelopathic potential of winter pasture legumes/crops against barnyard grass and rice

Methodology

Phytotoxicity of the full-strength aqueous extracts on barnyard grass and rice

Preliminary screening for potential phytotoxic effects of the pasture legume species and selected winter crops on barnyard grass and rice was conducted in the Charles Sturt University Agronomy laboratory and Phytotron from March to May 2018. The same protocol described in the barnyard grass and rice seed germination was used in this experiment, except for placing 5 mL of each plant extract in each Petri dish instead of water only. Germinated seeds with >1 mm radicle were recorded at 2-day (d) intervals until 7 days, and root and shoot lengths (mm) were measured after 7 days of incubation or when no further germination was observed in rice.

Phytotoxicities of aqueous extracts at various concentrations on barnyard grass

The effect of different concentrations of extracts on the germination and growth of barnyard grass was investigated from July to August 2018. Each plant extract was prepared at a concentration series of 100% (full strength), 50%, 25%, 12.5% and 0% (deionised water control). The same protocol described in the preliminary screening was used in this study. Germinated seeds with >1 mm radicle were recorded, and root and shoot lengths were measured after 10 days of incubation. The termination day was changed from 7 (preliminary screening) to 10 days due to observed barnyard grass germination until 10 days. Rice seed fungal contamination was observed in the preliminary screening, despite seed surface sterilisation. This experiment was conducted 8-9 months later after collection of the pasture legume and crop residues due to waiting for newly harvested rice seeds to hopefully avoid the fungal contamination in rice due to storage. However, even after using the newly harvested rice seeds and seed surface sterilisation, the fungal contamination in rice was still observed. Hence, rice seeds were not included in this experiment. This experiment was conducted twice.

Shaftal Persian clover residue effects on barnyard grass and rice

A pot bioassay was conducted to determine the allelopathic effects of pasture legumes on barnyard grass and rice seed emergence and growth. Previous extract phytotoxicity screening suggested that Persian (cv Shaftal) clover aqueous extract (100 g L⁻¹) reduced germination, root growth, and shoot growth of barnyard grass by 96, 100, and 97%, respectively. However, Persian clover extract also reduced the germination of rice (cv YRM70) by 38%. YRM70 (Viand) is a recently released short-season rice variety suitable for DSR in conjunction with DPW.

In this bioassay, barnyard grass and rice seeds (cv YRM70) were sown and grown in separate pots in association with Persian clover plant residues. The temperatures inside the glasshouse were set at 30/20 °C fluctuating day and night, and records for 2019 are provided in Appendix 1.1. The experiment was conducted in the Charles Sturt University Wagga Wagga glasshouse complex.

Matured Shaftal Persian clover grown during the 2018 winter season (May-November) was harvested from the CSU Horticulture field bay, Wagga Wagga NSW (35° 7’ 2.1900” S 147° 21’ 23.4792” E). Collected plant samples were oven-dried at 40 °C for 72 hours, placed inside paper bags and stored at room temperature. The plant samples were then chopped into 1 cm pieces and weighed.

The soil was collected from the drill-sown rice fields in Jerilderie NSW (35° 19’ S 145° 09’ E). The soil is described as a brown-grey heavy clay with a pH(1:5 H₂O) of 6.1. The top ~10 cm of soil was
collected by front-end loader. The soil was then passed through a 10 mm sieve. This soil was used for all pot experiments in this study.

Barnyard grass and rice seeds were sown and grown in separate pots in association with Shaftal Persian clover residues on April 1 and May 13, 2019. Sieved soil (2.5 kg) was placed inside the pots (16 cm height, 16 cm diameter). Paper was placed at the bottom of each pot to cover the holes and prevent the soil from being lost. Barnyard grass (100 seeds pot⁻¹) was sown on the surface and covered lightly with sieved soil. Rice seeds were sown on separate pots at 1-2 cm depth. The Shaftal Persian clover residues were either mulched or incorporated at the equivalent of 3 and 6 t ha⁻¹ in each pot. Control pots (without residues) sown with barnyard grass and rice were also included. Pots were initially sprinkler-irrigated and then sub-irrigated for 42 days after sowing (DAS).

The number of emerged barnyard grass seeds was recorded at 3-day (d) intervals until 21 DAS. Barnyard grass and rice seedlings were then thinned to 10 seedlings per pot at 21 DAS (no further emergence was observed). The height (cm), number of tillers and number of leaves were recorded at 10-day intervals until 40 DAS. At harvest (42 DAS), the shoot (leaf and stem) were oven-dried at 60 °C for 72 hours and biomasses were recorded. The roots were carefully retrieved by placing the potting soil over a 2 mm sieve plate and thoroughly washing with water until all soil particles were removed. The retrieved roots were also oven-dried, and the biomass was recorded. This experiment was conducted twice.

**Different pasture legume cultivar residue effect on barnyard grass**

An experiment was conducted to evaluate the cultivar effect of residues of pasture legumes on the emergence and early growth of barnyard grass in the glasshouse from October to December 2019 in pots (20 cm diameter x 20 cm height). Glasshouse temperatures in 2019 are provided in Appendix 1.2.

Pasture legume species balansa (cvs Border, Paradana, Bolta, and Frontier), Persian (Shaftal, Morbulk, Nitro, and Prolific) and subterranean (cvs Antas, Trikkala, Dalkeith, and Seaton Park) clovers were sown in April 2019 in pots, including a control (no crop). Emerged seedlings were thinned into 12 plants pot⁻¹ at 14 DAS. Legume inoculant (Nodule NTM Peat, Strain C) was used at sowing. Basal application of single super phosphate (45% P₂O₅) at 200 kg ha⁻¹ was used in all pots. Pots were initially sprinkler-irrigated and then sub-irrigated during the experimental period. All crops were harvested by cutting the above-ground parts at 6 months after sowing (October 2019).

The shoots (leaves, stems and flowers) were oven-dried at 60 °C for 72 hours. The oven-dried shoot residues of the pasture legume cultivars were cut into ~1 cm lengths and placed into the pots as a mulch at the equivalent of 1 t ha⁻¹ (2.5 g shoot residues pot⁻¹). In an actual field situation after the winter pasture legume phase, a certain amount of above-ground residue will be left on the soil surface after grazing; hence we placed 1 t ha⁻¹ mulch on the soil surface. A lesser amount was added compared with the previous experiment to avoid the competition for the light effect of the mulch on barnyard grass seed.

Fifty seeds of barnyard grass collected from 2017-18 drill-sown rice crops (98% germination rate at the time of sowing) were surface broadcast on each pot previously sown with different pasture legume species. Fewer barnyard grass seeds compared with the previous pot experiment were used to minimise barnyard grass seedling crowding and a competition effect. Pots were initially sprinkler-irrigated and then sub-irrigated until termination (42 DAS).

The number of barnyard grass seeds that emerged was recorded at 3-day (d) intervals until no further emergence or at 21 DAS. At harvest (42 DAS) in December 2019, the shoots (leaf and stem) were oven-dried at 60 °C for 72 hours, and the biomass was recorded. The roots were carefully retrieved by placing the potting soil over a 2 mm sieve plate and thoroughly washing with water until all soil particles were removed. The retrieved roots were also oven-dried, and biomass was recorded. This experiment was conducted once.
Data analyses

All experiments were arranged in a randomised complete block design (RCBD) with three replications. Data were analysed using ANOVA (GenStat 18th edition) to evaluate differences between treatments, and the means were separated using the least significant differences (LSD). In the plant concentration series experiment, the two trials were analysed separately due to their significant interaction with the treatments plant extracts x concentrations for germination and root length parameters (P<0.01 and P<0.001, respectively). The two trials were combined for analyses of shoot length data due to non-significant interaction with the treatments plant extracts x concentrations.

The two trials of the pot experiment (effect of Shaftal Persian clover residues) were analysed separately for the emergence data due to significant interaction (P=0.02) with the treatment placement x amount. However, the two trials were combined for analyses of the parameters height, number of leaves plant\(^{-1}\), number of tillers plant\(^{-1}\), root biomass and shoot biomass, due to non-significant interaction between placement and amount.

Results

Phytotoxicity of the full-strength aqueous extracts on the germination and early growth of barnyard grass and rice

The germination of barnyard grass and rice cultivars were influenced by the plant extracts (P<0.001). Canola (cv NUSEED 314TT Monola), barley (cv Cape), and balansa (cv Border) clover extracts delayed barnyard grass germination (Figure 1.1). In control, 50% barnyard grass germination was achieved at ~4 DAS, but the addition of canola, barley and balansa extracts delayed germination (50%) until ~6 DAS. Subterranean (cv Antas) and Persian (cv Shaftal) clover extracts severely inhibited barnyard grass germination by 50 and 96%, respectively, at 7 DAS (Figure 1.1, Table 1.1). The germination of all rice cultivars (Opus, Sherpa, YRK5 and YRM70) was also inhibited 14-38% by the addition of the subterranean and Persian clover extracts (Figure 1.1). Despite seed surface sterilisation, rice seeds fungal contamination with the addition of plant extracts except for the control (water) was observed. Barnyard grass did not have any fungal contamination in any of the treatments.

Figure 1.1 Cumulative % germination of barnyard grass at 2-day intervals from 1 until 7 days after sowing (DAS). Line intersection is at 50% germination. Error bars indicate standard deviation.
Table 1.1 Effect of shoot extracts of pasture legumes (balansa, Persian and subterranean clovers) and winter crops (canola and barley) on germination of barnyard grass (BYG) and rice (Opus, Sherpa, YRK5 and YRM70) seeds at 7 days after sowing.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>BYG</th>
<th>Opus</th>
<th>Sherpa</th>
<th>YRK5</th>
<th>YRM70</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (water)</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>74</td>
<td>79</td>
</tr>
<tr>
<td>canola (cv NUSEED 314TT Monola)</td>
<td>73</td>
<td>80</td>
<td>79</td>
<td>75</td>
<td>77</td>
</tr>
<tr>
<td>barley (cv Cape)</td>
<td>76</td>
<td>79</td>
<td>80</td>
<td>78</td>
<td>77</td>
</tr>
<tr>
<td>balansa (cv Border)</td>
<td>74</td>
<td>79</td>
<td>79</td>
<td>71</td>
<td>79</td>
</tr>
<tr>
<td>subterranean (cv Antas)</td>
<td>40</td>
<td>61</td>
<td>64</td>
<td>64</td>
<td>51</td>
</tr>
<tr>
<td>Persian (cv Shaftal)</td>
<td>3</td>
<td>65</td>
<td>56</td>
<td>61</td>
<td>49</td>
</tr>
</tbody>
</table>

LSD extracts x seeds (0.05) 17.61

The shoot (P<0.001) and root (P<0.05) lengths of barnyard grass and rice cultivars were influenced by the plant extracts. Barnyard grass had an 85-100% root and 31-97% shoot length reduction due to extracts of pasture legume species (balansa, subterranean and Persian), canola and barley (Table 1.2). Compared with the control, barnyard grass seedlings had the highest shoot (97%) and root (100%) length reduction when treated with Shaftal Persian clover extracts. However, all rice cultivars (Opus, Sherpa, YRK5 and YRM70) were also inhibited, with 86-99% root length and 42-82% shoot length reduction with all plant extracts. We observed that all rice seed cultivars had fungal contamination when treated with all the plant extracts, but none with the control (water).

Table 1.2 Effect of shoot extracts of pasture legumes (balansa, Persian and subterranean clovers) and winter crops (canola and barley) on root and shoot lengths (mm) of barnyard grass (BYG) and rice cultivars (Opus, Sherpa, YRK5 and YRM70) at 7 days after sowing.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>BYG</th>
<th>Opus</th>
<th>Sherpa</th>
<th>YRK5</th>
<th>YRM70</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (water)</td>
<td>41.15</td>
<td>51.75</td>
<td>49.65</td>
<td>46.75</td>
<td>43.75</td>
</tr>
<tr>
<td>canola (cv NUSEED 314TT Monola)</td>
<td>1.30</td>
<td>2.05</td>
<td>3.50</td>
<td>2.05</td>
<td>3.95</td>
</tr>
<tr>
<td>barley (cv Cape)</td>
<td>6.15</td>
<td>7.25</td>
<td>6.70</td>
<td>3.90</td>
<td>4.20</td>
</tr>
<tr>
<td>balansa (cv Border)</td>
<td>0.60</td>
<td>1.80</td>
<td>2.45</td>
<td>1.55</td>
<td>2.80</td>
</tr>
<tr>
<td>subterranean (cv Antas)</td>
<td>2.00</td>
<td>1.55</td>
<td>2.70</td>
<td>3.50</td>
<td>2.05</td>
</tr>
<tr>
<td>Persian (cv Shaftal)</td>
<td>0.00</td>
<td>0.35</td>
<td>0.35</td>
<td>0.80</td>
<td>0.25</td>
</tr>
</tbody>
</table>

LSD extracts x seeds (0.05) 3.98

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>BYG</th>
<th>Opus</th>
<th>Sherpa</th>
<th>YRK5</th>
<th>YRM70</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (water)</td>
<td>21.60</td>
<td>28.35</td>
<td>23.00</td>
<td>31.95</td>
<td>29.35</td>
</tr>
<tr>
<td>canola (cv NUSEED 314TT Monola)</td>
<td>14.15</td>
<td>11.50</td>
<td>10.35</td>
<td>12.65</td>
<td>9.70</td>
</tr>
<tr>
<td>barley (cv Cape)</td>
<td>14.80</td>
<td>13.50</td>
<td>13.30</td>
<td>16.55</td>
<td>11.15</td>
</tr>
<tr>
<td>balansa (cv Border)</td>
<td>13.25</td>
<td>10.00</td>
<td>9.20</td>
<td>12.45</td>
<td>8.90</td>
</tr>
<tr>
<td>subterranean (cv Antas)</td>
<td>10.05</td>
<td>5.75</td>
<td>7.00</td>
<td>7.50</td>
<td>5.35</td>
</tr>
<tr>
<td>Persian (cv Shaftal)</td>
<td>0.60</td>
<td>8.05</td>
<td>7.30</td>
<td>9.70</td>
<td>6.40</td>
</tr>
</tbody>
</table>

LSD extracts x seeds (0.05) 2.07
Phytotoxicities of aqueous extracts at various concentrations on germination and early growth of barnyard grass

The plant extracts and different concentrations influenced the germination of barnyard grass only at the early stage (4-6 DAS). There was a significant interaction (P<0.01) between trials and the plant extract x concentration interaction, hence the trials were analysed separately. A plant extract x concentration interaction occurred in barnyard grass germination at 4 and 6 DAS (P=0.02; P=0.002) in the first trial and at 6 DAS in the second trial (P=0.002). Barnyard grass seeds had the highest reduction in germination, 80 and 90% at 4 and 6 DAS, respectively, with the addition of Persian clover (cv Shaftal) extracts at full concentration in trial 1 (Figure 1.2). In trial 2, at 4 DAS, all plant extracts (canola, barley, and balansa, subterranean and Persian clovers) at full concentration reduced barnyard grass germination by 94-100%. In this experiment, the germination of barnyard grass was not influenced by the extracts at a later stage from 8-10 DAS (trial 1) and from 6-10 DAS (trial 2). In the previous phytotoxicity screening on the full concentration of the plant extracts, there was a significant delay and reduction in the germination of barnyard grass with the addition of subterranean and Persian clover. This experiment was conducted 4-5 months later than the first phytotoxicity trial.

Barnyard grass root (P<0.001 and P<0.01) and shoot (P<0.001) lengths (mm) were influenced by the species extract x concentration interaction. The trials had significant interaction to plant extracts x concentrations for root length (P<0.001), whereas the trials had non-significant interaction for shoot lengths. The root lengths of barnyard grass measured at 10 DAS were reduced by all extracts from 25 to 100% concentration in both trials, by 30-93% (Table 1.3). All plant extracts had higher barnyard grass root lengths at 25% concentration (trial 1) and at 12.5% concentration (trial 2) compared with root lengths of barnyard grass at 100% concentration of the extract. Shoot length of barnyard grass was reduced by 14-22% when treated with all pasture legume species extracts at 100% concentration (Table 1.4).

Figure 1.2 Cumulative % germination of barnyard grass as affected by different plant extracts (barley, canola, balansa, subterranean and Persian) at different concentrations (0, 12.5, 25, 50, 75 and 100) at 2-day intervals starting at day 2 until 10 days after sowing. Error bars indicate standard deviation.
Table 1.3 Effect of shoot extracts of winter crops (canola and barley) and pasture legumes (balansa, subterranean and Persian clovers) at different concentrations (0, 12.5, 25, 50, 75 and 100) on root lengths (mm) of barnyard grass at day 10 after sowing.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Root length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td></td>
<td>0 12.5 25 50 75 100</td>
</tr>
<tr>
<td>canola (cv NUSEED 314TT Monola)</td>
<td>5.03 1.08 1.24 3.54 2.58 1.60</td>
</tr>
<tr>
<td>barley (Cape)</td>
<td>4.61 4.49 3.25 0.46 2.35 1.55</td>
</tr>
<tr>
<td>balansa (cv border)</td>
<td>4.82 3.75 2.28 2.19 2.13 0.82</td>
</tr>
<tr>
<td>subterranean (cv Antas)</td>
<td>4.68 1.23 1.63 1.61 1.27 0.35</td>
</tr>
<tr>
<td>Persian (cv Shaftal)</td>
<td>4.35 3.62 2.23 1.77 1.05 0.53</td>
</tr>
</tbody>
</table>

LSD plant extract by conc (0.05) 1.28

Table 1.4 Effect of shoot extracts of winter crops (canola and barley) and pasture legumes (balansa, subterranean and Persian clovers) at different concentrations (0, 12.5, 25, 50, 75 and 100) on shoot lengths (mm) of barnyard grass at day 10 after sowing; means of combined trials 1 and 2.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Shoot length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 12.5 25 50 75 100</td>
</tr>
<tr>
<td>canola (cv NUSEED 314TT Monola)</td>
<td>2.51 2.82 2.98 2.56 2.38 2.46</td>
</tr>
<tr>
<td>barley (Cape)</td>
<td>2.51 2.74 2.82 2.54 2.68 2.39</td>
</tr>
<tr>
<td>balansa (cv border)</td>
<td>2.48 2.73 2.78 2.41 2.30 2.13</td>
</tr>
<tr>
<td>subterranean (cv Antas)</td>
<td>2.46 2.82 2.59 2.33 2.25 1.91</td>
</tr>
<tr>
<td>Persian (cv Shaftal)</td>
<td>2.50 2.71 2.98 3.05 2.50 2.05</td>
</tr>
</tbody>
</table>

LSD plant extract by conc (0.05) 0.24

Shaftal Persian clover residue effects on the emergence and growth of barnyard grass

The emergence of barnyard grass and rice was influenced by the plant x amount (P<0.001) and placement x amount interactions (P<0.001) in trial 1, and a significant plant x placement x amount interaction (P<0.001) in trial 2. Barnyard grass emergence was enhanced by 20% (trial 1) with the addition of 3 tonnes ha⁻¹ and reduced by 25 and 29% (trial 1 and 2 respectively) with the addition of 6 tonnes ha⁻¹ of Persian clover (cv Shaftal) shoot residues, regardless of placement. In contrast, rice emergence was reduced by 13 and 14% (trial 1 and 2 respectively) with the addition of 3 tonnes ha⁻¹ and 15 and 16% (trial 1 and 2 respectively) with the addition of 6 tonnes ha⁻¹ Persian clover (cv Shaftal) shoot residues. The incorporation of 3 and 6 tonnes ha⁻¹ Persian clover (cv Shaftal) shoot residues led to 6 and 11% reduced emergence of both barnyard grass and rice. Whereas mulching with 3 tonnes ha⁻¹ Persian clover (cv Shaftal) shoot residues had no effect, there was a 25% reduced emergence with 6 tonnes ha⁻¹ in both crops (trial 1). Barnyard grass emergence was reduced by 41 and 43% (trial 1 and 2 respectively) when 6 tonnes ha⁻¹ of Persian clover (cv Shaftal) shoot residues were
AppliD as mulch (Figure 1.3). Rice (cv YRM70) emergence was also reduced by 24 and 30% with the incorporation of 3 and 6 tonnes ha\(^{-1}\) of Shaftal Persian shoot residues, respectively (trial 2).

![Figure 1.3 Emergence (%) of barnyard grass (BYG) and rice (cv YRM70) at 21 days after sowing as affected by different Persian clover shoot residue placements: incorporated (INC) and mulch, and amounts: 0, 3 and 6 tonnes ha\(^{-1}\). Error bars indicate standard deviation.](image)

Barnyard grass and rice growth were influenced by the number of Persian clover residues (0, 3 and 6 tonnes ha\(^{-1}\)), placement (mulch or incorporated) and their interaction. Barnyard grass growth in terms of height (cm) was influenced by the amount the barnyard grass root (\(P=0.007\)) and shoot (\(P=0.002\)) biomass were influenced by the number of residues, while rice root (\(P=0.001\)) and shoot (\(P=0.002\)) biomass were influenced by the placement (mulch or incorporated) \(x\) amount interaction and placement, respectively. Barnyard grass height was enhanced by the addition of 3 tonnes ha\(^{-1}\) Persian clover residues at 10 DAS (Table 1.5). Rice growth, in terms of height at 10-40 DAS, the number of leaves plant\(^{-1}\) at 20-40 DAS and root biomass at 42 DAS, was enhanced with the addition of Persian clover residues at the rate of 3 and 6 tonnes ha\(^{-1}\) mulch (Table 1.5). The incorporation of 3 and 6 tonnes ha\(^{-1}\) of Persian clover residues, however, reduced rice height at 10-40 DAS.
Table 1.5 Effect of Persian clover (cv Shaftal) shoot residue placements (incorporated and mulch) and quantities (0, 3 and 6 tonnes ha\(^{-1}\)) on barnyard grass and rice (cv YRM70) height (cm), number of leaves plant\(^{-1}\), and number of tillers plant\(^{-1}\) at 10-day intervals starting 10 days after sowing (DAS) until 40 DAS and shoot and root biomass (g 10plants\(^{-1}\)) at 42 DAS.

<table>
<thead>
<tr>
<th>Plant Placement/amount (tonnes ha(^{-1}))</th>
<th>BYG</th>
<th>Decorated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incorporated</td>
<td>Mulch</td>
</tr>
<tr>
<td></td>
<td>10 DAS</td>
<td>20 DAS</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.89</td>
<td>18.64</td>
</tr>
<tr>
<td>6</td>
<td>3.53</td>
<td>17.16</td>
</tr>
<tr>
<td>Mulch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.86</td>
<td>17.85</td>
</tr>
<tr>
<td>3</td>
<td>4.15</td>
<td>20.20</td>
</tr>
<tr>
<td>6</td>
<td>3.70</td>
<td>19.50</td>
</tr>
</tbody>
</table>

LSD placement x amount (0.05) ns 2.04 ns ns ns ns l ns ns ns ns ns ns ns

**Rice**

<table>
<thead>
<tr>
<th>Plant Placement/amount (tonnes ha(^{-1}))</th>
<th>Incorporated</th>
<th>Mulch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 DAS</td>
<td>20 DAS</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.89</td>
<td>24.21</td>
</tr>
<tr>
<td>6</td>
<td>3.03</td>
<td>16.99</td>
</tr>
<tr>
<td>Mulch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.85</td>
<td>22.68</td>
</tr>
<tr>
<td>3</td>
<td>4.55</td>
<td>26.15</td>
</tr>
<tr>
<td>6</td>
<td>4.46</td>
<td>28.06</td>
</tr>
</tbody>
</table>

LSD placement x amount (0.05) 0.99 1.87 5.36 5.93 ns l l l ns ns ns ns ns 0.09

ns – not significant
Different pasture legume cultivars residue effect on the emergence and growth of barnyard grass

Barnyard grass emergence was influenced (P<0.001) by the residues of the different pasture legume cultivars. Barnyard grass grown in pots with balansa (cv Frontier, Paradana, Border and Nitro), subterranean (cv Trikkala and Antas) and Persian clover residues (cv Bolta and Morbulk) had significant increase in emergence by 11-29% compared with the control (Figure 1.4). The highest barnyard grass emergence (52%) was observed in pots with Frontier cultivars. This was followed by balansa clover cultivars Paradana, Border and Nitro (45-46%), subterranean clover cultivars Trikkala and Antas (41-43%) and Persian clover cultivars Bolta and Morbulk (45-46%). Barnyard grass emergence in pots with subterranean clover cultivars Dalkeith and Seaton Park (39-40%) and Persian clover cultivars Prolific and Shaftal residues were similar to that of the control (37%).

Figure 1.4 Barnyard grass emergence (%) at 21 days after sowing as affected by the residues of different pasture legume balansa (cv Frontier, Paradana, Border and Nitro), subterranean (cv Trikkala and Antas) and Persian clover (cv Bolta and Morbulk) cultivars. Error bars indicate standard deviation.

Barnyard grass shoot biomass was influenced (P<0.001) by the different pasture legume cultivars. Root biomass results for barnyard grass grown with different pasture legume cultivars were not significantly different. Barnyard grass grown in pots with balansa (cvs Frontier, Paradana, Border and Nitro), subterranean (cvs Dalkeith, Seaton Park, Trikkala and Antas), and Persian (cv Prolific and Bolta) clover residues had reduced shoot biomass (g plant⁻¹) by 19-56% relative to the control pots (Figure 1.5). Persian clover cv Morbulk had similar shoot biomass to the control, whereas cv Shaftal had increased shoot biomass by 27% compared with the control.
Figure 1.5 Shoot biomass (g plant⁻¹) of barnyard grass grown in pots with different pasture legume balansa (cvs Frontier, Paradana, Border and Nitro), subterranean (cvs Dalkeith, Seaton Park, Trikkala and Antas) and Persian clover (cvs Prolific, Bolta, Morbulk and Shaftal) cultivars. Error bars indicate standard deviation.
Chapter 2 Barnyard grass seed bank dynamics

Methodology

General details and description of the experiments

The choice of pasture legume and crop species in this study was based on the responses of rice growers during the scoping study conducted in 2018. Pasture legume species included were balansa clover (cv Border), Persian clover (cv Shaftal) and subterranean clover (cv Antas). Winter crops were canola (cv AV Garnet) and barley (cv Hindmarsh). Barnyard grass seeds used in all experiments were collected in February 2018 from a continuous drill-sown rice area in Cobram, Victoria (35° 51’ 43" S 145° 34’ 30" E).

Newly harvested barnyard grass seeds were tested for initial germination prior to burial by placing 25 seeds of barnyard grass onto 9 cm Petri dishes lined with two layers of Whatman No.1 filter paper, and 5 mL of sterilised deionised water was placed in each Petri dish. Afterwards, each Petri dish was sealed with Parafilm to reduce evaporation. All the dishes were placed in growth chambers/incubators set at 30/20 °C fluctuating day and night temperatures and 12 hours photoperiod (light and dark conditions). The 30/20 °C temperature was identified as the optimum temperature for germination of barnyard grass (Roche and Muzik, 1964). Germinated seeds with >1 mm radicle length were recorded until there was no further germination or at 15 days after sowing (DAS).

Effect of crop/pasture species, depth and duration of burial on barnyard grass seed mortality in a) the field and b) pots in the glasshouse over two winter seasons from May-December of 2018 and 2019

In both field and pot experiments, the pasture legume species balansa, Persian and subterranean clovers, and winter crops canola and barley, were sown at 10 kg ha⁻¹, 4 kg ha⁻¹ and 110 kg ha⁻¹ seeding rates, respectively, and adjusted for variation in % germination. Legume inoculant (Nodule NTM Peat, Strain C) was applied with the pasture legumes, and then fertilisers were applied as follows: basal application of single super phosphate (45% P₂O₅) at 200 kg ha⁻¹ in all plots/pots, and a follow-up application of urea (46%N) at 100 kg ha⁻¹ in control (no crop), canola and barley plots/pots at 21 days after sowing (DAS).

The freshly harvested barnyard grass seeds were placed in packets (100 seeds per packet) made of organza fabric woven into small bags (10 cm x 7.5 cm). Seed packets were buried in the field or pots at the specified depths (surface-0, 5, and 10 cm). The germination rate of barnyard grass seeds at the time of burial was 0% due to dormancy.

Barnyard grass seed longevity field experiment

Treatments were arranged in a split-split plot design, with plant species (five plant species and nil crop as control) as the main plots, two burial durations (6 and 18 months) as the sub-plots and three depths (surface-0, 5 and 10 cm) as the sub-sub plots for two winter cropping seasons from May 2018 to December 2019.

All the crop/pasture seeds were sown using an 8-row drill seeder with 0.2 m distance between rows on 21 May 2018, and 24 May 2019, respectively. Each plot was 11.2 m² (7.0 m x 1.6 m), with a 0.5 m gap between plot row lengths and plot widths. In the 2019 winter season cropping, the same pasture legume species were sown in the same plots as in 2018, except that the canola plots in 2018 were sown with barley, and vice versa. The rotation between canola and barley is a common practice to minimise crop-associated soil-borne diseases, and is farmers’ usual practice. The field plots were
initially flood irrigated after sowing and then sprinkler-irrigated as needed. The Wagga Wagga field temperature and rainfall growing conditions in 2018 and 2019 are in Appendix 2.1-2.3.

Seed packets were buried immediately after sowing. Two sets of a packet of seeds for each burial duration were buried in each plot between the second and third rows at 1 m from the start of the plot and sixth and seventh rows at 1 m from the end of each plot. Packets were retrieved 6 months after burial (MAB) following harvest of winter crops/pastures on 16-17 November 2018 and 18 MAB following harvest of the second winter cropping on 4-5 December 2019.

In the 2018 winter season, the field plots were damaged by ducks, requiring a fence to be set up to prevent further damage. The herbicides MCPA 2 ethylhexyl ester + Diflufenican at 0.75g ai ha⁻¹ and Clopyralid at 300g ai ha⁻¹ were applied to all plots on 13 July 2018, for capeweed (*Arctotheca calendula*) and wireweed (*Polygonum erectum*). No herbicides were applied in 2019 due to poor plant emergence of the pasture legumes and canola. Plots were hand-weeded in both years (3-4 September 2018, 24-25 August 2019). After the 2018 winter crop harvest, all field plots were sprayed with glyphosate at 570g ai ha⁻¹ tank-mixed with dicamba at 500g ai ha⁻¹ to remove summer weeds.

During harvest, the crop/pasture above-ground biomass was measured in each plot. The above-ground biomass was collected within two quadrats (0.4 x 0.4 m) placed in the fourth and fifth inner rows, avoiding the 1 m border from both ends of each plot. The collected shoots were oven-dried at 70 °C until constant weights, and plant biomass was recorded as kg m⁻².

After retrieval of seed packets at 6 and 18 MAB, the total mortality and total remaining viable barnyard grass seeds (dormant and non-dormant) in the packet at different burial depths were recorded. The retrieved seeds were checked by gently pressing with fingers. Seeds that were weak and broke under little pressure using fingers were recorded as decayed. The intact and firm seeds were subjected to germination and viability tests. Seeds that germinated inside the packets were added to the mortality. The other retrieved weed seeds were tested for germination. All seeds that did not germinate were tested for viability using tetrizolium chloride (TZ) at 1% (1 mg TZ per 100 mL distilled water). The total mortality, viable non-dormant (germinated) and viable dormant (viable in TZ test) numbers were recorded.

**Barnyard grass seed longevity pot experiment**

The treatments were arranged in a two-factorial randomised complete block design with five plant species and three burial depths (surface-0, 5 and 10 cm) with four replicates. The experiment was first undertaken in 2018 and repeated in 2019.

As described in the field experiment, barnyard grass seeds (100 seeds) in packets were buried at different depths (0, 5, and 10 cm), this time in pots with a surface area of 0.05 m² (25 cm height x 24 cm diameter), prior to sowing. The barnyard grass seed germination rate at the time of burial was 1%.

Pasture legumes and crops were sown in pots on 9 May 2018 and 31 May 2019 at the equivalent seed rates, treatment and fertiliser application as the field experiment. All seeds in the pot experiment were hand-sown and were buried at 1 to 2 cm depth in each pot (0.05 m² area). The seedlings were thinned to six plants per pot at 14 DAS.

Pots were initially sprinkler-irrigated and then sub-irrigated during the entire growing period. The soil used in the 2018 trial was collected from the CSU Horticulture field bay, but in the 2019 trial, the soil used was from a rice field area in Jerilderie NSW (35° 19' S 145° 09' E). The CSU soil had been under fallow from the previous year; it was brown in colour, clay texture, and had a pH(1:5 H₂O) of 5.3. The Jerilderie soil had been under DSR from the previous year; it was brown-grey in colour, heavy clay, and with pH(1:5 H₂O) of 6.1. Each pot contained ~8.6 kg of soil. Other weeds were removed. The glasshouse temperatures in 2018 and 2019 are provided in Appendix 2.4.
During crop and pasture harvest (1 November 2018, and 25 November 2019), the actual plant number was recorded (plants m⁻²), then shoots (leaves, stems and inflorescence) were cut, oven-dried at 70 °C until constant weight and dry matter biomass (kg m⁻²) was recorded. During harvest, the barnyard grass seed packets were carefully retrieved from each pot to minimise soil disturbance during crop harvest, and total mortality, viable non-dormant (germinated) and viable dormant (viable in TZ test) numbers were recorded. The pots were left with pasture legume species/crop root residues in the soil. These pots were used in the succeeding experiments.

Effect of crop/pasture legume on barnyard grass emergence and growth grown in pots

An experiment to determine the effects of the different crop/pasture species on the emergence and growth of barnyard grass was undertaken in pots in the glasshouse over two winter seasons, from May-December 2018 and 2019. The glasshouse temperatures are provided in Appendix 2.4. The pots were arranged in a randomised complete block design, with six winter pasture legume species/crops as treatments and four replicates, and the experiment repeated over two years (2018 and 2019).

One hundred barnyard grass seeds were mixed with 5 g of sieved soil and broadcasted on the soil surface of all pots 21 days after sowing (9 May 2018 and 31 May 2019) of the three pasture legumes (balansa, Persian and subterranean clovers), two winter crops (canola and barley) and the control (no crop). Pots were initially sprinkler-irrigated and then sub-irrigated during the entire growing period. Other weeds were removed.

During harvest (1 November 2018 and 25 November 2019), barnyard grass emergence per pot (n m⁻²) was recorded, after which barnyard grass shoots were harvested, oven-dried at 70 °C until constant weight and biomass dry matter (g m⁻²) was recorded.

Effect of crop/pasture legume root residues and soil disturbance on barnyard grass emergence and early growth grown in pots

This pot experiment was conducted from December-January in both 2018 and 2019. The aim was to determine the carry-over effects of pasture legume species (Persian, balansa and subterranean clovers) and winter crops (canola and barley) and soil disturbance (not disturbed and 10 cm disturbance) on barnyard grass emergence and growth. A hand trowel marked at 10 cm was used for soil disturbance. The experiment was arranged in a randomised complete block design with four replicates, with the plant root residues and soil disturbance the first and second factors.

One hundred seeds of barnyard grass (96% germination rate at the time of sowing) were surface broadcasted on each pot. Pots were initially sprinkler-irrigated and then sub-irrigated during the entire growing period. Other weeds were removed. The glasshouse temperatures from 2018 to 2019 are recorded in Appendix 2.4. The barnyard grass emerged seedlings were thinned to 10 plants per pot until no further emergence was observed or at 21 DAS.

The number of barnyard grass seeds that emerged was recorded at 7-day intervals until 21 DAS. At 48 DAS, the height (cm), number of tillers (n plant⁻¹) and number of leaves (n plant⁻¹) were recorded as an average of three barnyard grass plants in each pot. Then shoots (leaves and stems) were oven-dried at 70 °C for 72 hours, and the biomass (g plant⁻¹) was recorded. Barnyard grass roots were carefully retrieved by placing them over a 2 mm sieve plate and thoroughly washing with water until free from soil and other particles. The retrieved roots were oven-dried at 70 °C for 72 hours, and the biomass (g plant⁻¹) was recorded. The barnyard grass roots were easily distinguished from crop and pasture legume root residues because the barnyard grass root is intact, interconnected and light brown in colour. The crop/pasture legume root residues, on the other hand, were dark brown to black in colour and mostly disintegrated.
Data analyses

All experiments were arranged in a randomised complete block design with four replicates. All data were analysed using ANOVA (GenStat 18th edition) to evaluate differences between treatments, and the means were separated using the least significant differences (LSD). The glasshouse experiments in 2018 and 2019 were analysed separately due to their significant interaction with the treatment plant species.

Results

Barnyard grass seed mortality and viability in the field

Barnyard grass seed mortality, dormant viability and non-dormant viability rates in the 2018-19 field experiments were influenced by the depth of burial, with P<0.001. Results showed a higher barnyard grass seed mortality rate (31%) on the soil surface (0 cm) than in seeds buried at 5 cm (22%) and 10 cm (19%) soil depth (Figure 2.1). Barnyard grass seeds buried at 5 and 10 cm depths had higher viability rates at 78 and 81%, respectively, compared with the barnyard grass seeds on the soil surface (0 cm) at 69%. The barnyard grass seed mortality and viability (dormant and non-dormant) were not influenced by winter crop/pasture but were influenced by the duration and depth of burial interaction (P<0.01). The mortality rates at 18 MAB were 54, 39 and 40% at the burial depth of 0, 5 and 10 cm, while they were only 8, 5 and 4% at 6 MAB, respectively (Figure 2.2). Barnyard grass seed viability across depths after retrieval remained high (94%) after 6 MAB or 1 winter cropping, but this was significantly reduced to 58% after 18 MAB or after 2 winter cropping.

![Figure 2.1 Barnyard grass seeds mortality, non-dormant viable and dormant viable (%) at 0, 5 and 10 cm burial depths in 2018 and 2019, combined. Error bars indicate standard deviation.](image-url)
Barnyard grass seed mortality and viability, emergence and growth in pots

Barnyard grass seed mortality rates were not influenced by the pasture legume species and winter crops but were influenced by the interaction of plant and depth of burial, with $P<0.04$ and $P<0.001$ in the 2018 and 2019 glasshouse experiments, respectively (Figure 2.3). In the 2018 glasshouse experiment, barnyard grass seed mortality rates in the following treatments were similar: control-0 cm (12%), canola-0 and 5 cm (11 and 8%), barley-0 cm (10%), balansa-0 cm (7%), Persian-0 and 5 cm (8 and 9%), and subterranean-10 cm (7%). In 2019, the barnyard grass seed mortality rate was highest in control-0 cm (45%), followed by barley-0 cm (35%) and canola-0 cm (35%), and balansa-0 cm (19%), while all other treatments had similar mortality rates (1-6%).

The barnyard grass seed non-dormant viability and dormant viability rates were not influenced by the plant, depth, and plant x depth interaction in 2018, but in 2019, barnyard grass seed non-dormant viability was influenced by the species x depth interaction ($P=0.001$, Figure 2.3). The barnyard grass seed non-dormant viability was lowest in pots with Persian clover at 0 and 5 cm (15 and 16% respectively) and with balansa clover at 5 and 10 cm depths (18%). In 2019, the proportion of dormant viable barnyard grass seeds was influenced by species ($P<0.001$) and depth ($P<0.001$). There were more dormant viable barnyard grass seeds in the pots with balansa, subterranean and Persian clovers (68, 67 and 69% respectively) than in the pots with canola and barley or control (52, 42 and 52% respectively). There were more dormant viable barnyard grass seeds at 5 cm (70%) and 10 cm (64%) depths than for seeds retrieved from the surface (42%).
Barnyard grass emergence and growth during the winter pasture legume phase

Barnyard grass emergence and growth were influenced by all plant species in both 2018 and 2019 (Table 2.1). Barnyard grass sown with the pasture legumes (balansa, subterranean and Persian clovers) had emergence reduced by 80-100% in 2018 and by 75-100% in 2019, and growth reduced by 99-100% in 2018 and by 90-100% in 2019 relative to the control (no crop). The winter crops barley and canola reduced barnyard grass growth by 99% in 2018 and 98% in 2019 compared with the control (no plant). In the control pots, barnyard grass was observed to be at the inflorescence stage but not in any other treatments during harvest, with their growth significantly suppressed (Figure 2.4).
Table 2.1 Pasture legume species (balansa, subterranean and Persian clovers) and winter crops (canola and barley) effects on barnyard grass density (n m⁻²) and shoot biomass (g m⁻²) in 2018 and 2019.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>2018</th>
<th>2019</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Density (n m⁻²)</td>
<td>Shoot biomass (g m⁻²)</td>
</tr>
<tr>
<td>control (no plant)</td>
<td>97a</td>
<td>34.20a</td>
</tr>
<tr>
<td>canola (cv AV Garnet)</td>
<td>78a</td>
<td>0.40b</td>
</tr>
<tr>
<td>barley (cv Hindmarsh)</td>
<td>70bc</td>
<td>0.50b</td>
</tr>
<tr>
<td>balansa (cv Border)</td>
<td>2c</td>
<td>0.00b</td>
</tr>
<tr>
<td>subterranean (cv Antas)</td>
<td>7c</td>
<td>0.40b</td>
</tr>
<tr>
<td>Persian (cv Shaftal)</td>
<td>20bc</td>
<td>0.10b</td>
</tr>
</tbody>
</table>

Means with the same letter are non-significantly different (LSD₀.₀₅).

Figure 2.4 Barnyard grass plants after the winter pasture legume/crop phase (May-December 2018 and 2019) in pots with no plant, canola, barley, and balansa, subterranean and Shaftal Persian clovers.

Barnyard grass emergence and growth as affected by pasture legume and winter crop soil rhizosphere and soil disturbance

Barnyard grass emergence was influenced by pasture legume soil rhizosphere and soil disturbance interaction. In 2018 there was lower emergence of barnyard grass by 19-32% (14 DAS) and 24-39% (21 DAS) in pots with Persian soil rhizosphere (Table 2.2). In 2019, however, no significant differences were recorded for barnyard grass emergence by pasture legume soil rhizosphere and soil disturbance interaction.

The growth of barnyard grass was also influenced by some pasture legume soil rhizosphere and soil disturbance. In 2018, barnyard grass sown with Persian clover had a reduction of 43-49% in height, 67% in tiller number, 42-56% in leaf number, 74-88% shoot biomass and 63-80% root biomass compared with other plant treatments (Table 2.3). There was also a greater reduction (52%) in the shoot biomass (1.40 g plant⁻¹) of barnyard grass in pots with balansa soil rhizosphere relative to the
pots without plants (2.94 g plant⁻¹). Also, in 2018, regardless of the plant sown, barnyard grass height, root biomass and shoot biomass were lower with soil disturbance than in undisturbed pots (Table 2.4). There was a 7% reduction in height, a 22% reduction in shoot biomass, and a 40% reduction in root biomass when the soil was disturbed.

In 2019, the findings differed from those in 2018 (Table 2.3). In 2019, barnyard grass sown with Persian clover had an increase of 18-28% in height, 35-56% shoot biomass and 70-127% root biomass compared with all other treatments. Regardless of the pasture legume species/crop sown, barnyard grass shoot and root biomass were greater when the soil was disturbed than in undisturbed pots (Table 2.4), resulting in a 20% increase in shoot biomass and a 35% increase in root biomass.

<table>
<thead>
<tr>
<th>Plant treatment</th>
<th>GH 2018</th>
<th>7 DAS</th>
<th></th>
<th>14 DAS</th>
<th></th>
<th>21 DAS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td>control (no crop)</td>
<td>36</td>
<td>28</td>
<td>57</td>
<td>45</td>
<td>66</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>canola (cv AV Garnet)</td>
<td>49</td>
<td>48</td>
<td>62</td>
<td>58</td>
<td>70</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>barley (cv Hindmarsh)</td>
<td>35</td>
<td>45</td>
<td>58</td>
<td>70</td>
<td>66</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>balansa (cv Border)</td>
<td>47</td>
<td>29</td>
<td>73</td>
<td>48</td>
<td>78</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>subterranean (cv Antas)</td>
<td>38</td>
<td>40</td>
<td>64</td>
<td>55</td>
<td>75</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Persian (cv Shaftal)</td>
<td>28</td>
<td>14</td>
<td>42</td>
<td>22</td>
<td>43</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

LSD plant x disturbance (P=0.05) ns 16 17

<table>
<thead>
<tr>
<th>Plant treatment</th>
<th>GH 2019</th>
<th>7 DAS</th>
<th></th>
<th>14 DAS</th>
<th></th>
<th>21 DAS</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td>control (no crop)</td>
<td>41</td>
<td>32</td>
<td>54</td>
<td>45</td>
<td>58</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>canola (cv AV Garnet)</td>
<td>41</td>
<td>33</td>
<td>56</td>
<td>32</td>
<td>57</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>barley (cv Hindmarsh)</td>
<td>43</td>
<td>48</td>
<td>57</td>
<td>58</td>
<td>60</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>balansa (cv Border)</td>
<td>51</td>
<td>48</td>
<td>58</td>
<td>63</td>
<td>58</td>
<td>62</td>
<td></td>
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<tr>
<td>subterranean (cv Antas)</td>
<td>54</td>
<td>43</td>
<td>64</td>
<td>57</td>
<td>67</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Persian (cv Shaftal)</td>
<td>50</td>
<td>54</td>
<td>62</td>
<td>64</td>
<td>67</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

LSD plant x disturbance (P=0.05) ns ns ns

ns – not significant
### Table 2.3 Effect of soil rhizosphere on barnyard grass height, tiller number, leaf number, shoot biomass (SB) and root biomass (RB) at 48 days after sowing in 2018 and 2019. Combined undisturbed and 10 cm depth disturbed soil treatment.

<table>
<thead>
<tr>
<th>Plant treatment</th>
<th>Height (cm)</th>
<th>Tiller (n plant(^{-1}))</th>
<th>Leaves (n plant(^{-1}))</th>
<th>SB (g plant(^{-1}))</th>
<th>RB (g plant(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (no crop)</td>
<td>115</td>
<td>3</td>
<td>16</td>
<td>2.94</td>
<td>0.37</td>
</tr>
<tr>
<td>canola (cv AV Garnet)</td>
<td>108</td>
<td>3</td>
<td>13</td>
<td>2.00</td>
<td>0.40</td>
</tr>
<tr>
<td>barley (cv Hindmarsh)</td>
<td>112</td>
<td>3</td>
<td>13</td>
<td>2.33</td>
<td>0.37</td>
</tr>
<tr>
<td>balansa (cv Border)</td>
<td>104</td>
<td>3</td>
<td>12</td>
<td>1.40</td>
<td>0.27</td>
</tr>
<tr>
<td>subterranean (cv Antas)</td>
<td>113</td>
<td>3</td>
<td>14</td>
<td>2.59</td>
<td>0.49</td>
</tr>
<tr>
<td>Persian (cv Shaftal)</td>
<td>59</td>
<td>1</td>
<td>7</td>
<td>0.36</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>LSD (P=0.05)</strong></td>
<td>13</td>
<td>1</td>
<td>2</td>
<td>0.78</td>
<td>0.20</td>
</tr>
</tbody>
</table>

### Table 2.4 Effect of soil disturbance on barnyard grass plant height, tiller number, leaf number, shoot biomass (SB) and root biomass (RB) at 48 days after sowing in 2018 and 2019.

<table>
<thead>
<tr>
<th>Tillage treatment</th>
<th>Height (cm)</th>
<th>Tiller (n plant(^{-1}))</th>
<th>Leaves (n plant(^{-1}))</th>
<th>SB (g plant(^{-1}))</th>
<th>RB (g plant(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tillage</td>
<td>105.4</td>
<td>2</td>
<td>12</td>
<td>2.17</td>
<td>0.42</td>
</tr>
<tr>
<td>Tillage (10 cm)</td>
<td>98.0</td>
<td>3</td>
<td>13</td>
<td>1.70</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>LSD (P=0.05)</strong></td>
<td>7.50</td>
<td>ns</td>
<td>ns</td>
<td>0.45</td>
<td>0.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tillage treatment</th>
<th>Height (cm)</th>
<th>Tiller (n plant(^{-1}))</th>
<th>Leaves (n plant(^{-1}))</th>
<th>SB (g plant(^{-1}))</th>
<th>RB (g plant(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tillage</td>
<td>64.0</td>
<td>1</td>
<td>5</td>
<td>0.59</td>
<td>0.17</td>
</tr>
<tr>
<td>Tillage (10 cm)</td>
<td>66.5</td>
<td>1</td>
<td>6</td>
<td>0.71</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>LSD (P=0.05)</strong></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.12</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*ns – not significant*
Chapter 3 Dormancy and germination response of barnyard grass seed ecotype in drill sown rice

Methodology

Barnyard grass seed collection

Seeds of mature barnyard grass were harvested in February 2018 from drill-sown rice fields at Cobram, Victoria (35° 51’ 43" S 145° 34’ 30" E). Another set of mature barnyard grass seeds was collected from the same area in February 2019. This second set of seeds was used in the experiment on dormancy. The collected seeds each year were bulked, cleaned and stored at room temperature.

Germination and viability test

The freshly harvested seeds (2018 seed lot) were tested for initial germination and viability. Twenty-five seeds of barnyard grass were placed in a 9 cm diameter Petri dish lined with two pieces of Whatman No.1 filter paper and 5 mL deionized water. The Petri dishes were sealed with parafilm and placed inside incubators at fluctuating day/night temperatures of 30/20 °C in light/dark condition. The 30/20 °C temperature was identified by Roche and Muzik (1964) as the optimum temperature for germination of barnyard grass. The photoperiod was set at 12 hours to coincide with the higher temperature interval. Seeds with a visible protrusion of the radicle (>1 mm) were considered to have germinated. The number of germinated seeds was counted progressively until no further germination was observed. All seeds that did not germinate were tested for viability using the tetrazolium chloride (TZ) at 1% (1 mg TZ per 100 mL distilled water). From the date of harvest, the rate of germination and viability was tested using the above procedure and recorded monthly for nine consecutive months. The experiment was conducted after the seeds of barnyard grass had achieved relatively high germination (≥70%).

Effect of temperature and light on barnyard grass seed germination

To determine the effect of temperature and light on seed germination of barnyard grass, a laboratory experiment was conducted from February to April 2018. Following the germination test as described above, the Petri dishes containing barnyard grass seeds were placed in growth chambers under different fluctuating day/night temperature regimes (25/10, 30/20, and 40/25 °C) in both 12-hour light/dark (L/D) and continuous dark (D) conditions. The temperature regimes were selected to reflect temperature variations during spring and summer from October to March at Cobram, Victoria (Bureau of Meteorology, 2017). The actual recorded growth chamber temperatures are provided in Appendix 3.1. For the continuous dark treatment, the dishes were wrapped with three layers of aluminium foil to prevent light penetration.

The number of germinated seeds under the 12-hour L/D treatment were recorded at 3-day intervals from 3 to 15 days after sowing (DAS). However, for those grown in the dark, the germinated seeds were only counted at 15 DAS. Seeds that did not germinate in the continuous dark treatment were exposed to light and provided with additional 5 ml deionized water as needed and seed germination recorded at 3 (18 DAS) and 6 (21 DAS) days after exposure to light. Data are expressed as % germination.

Effect of burial depth on barnyard grass seedling emergence

A pot experiment was conducted to determine the effect of burial depth on seed emergence of barnyard grass in the glasshouse from September to October 2020. The glasshouse temperature was
set at 30/20 °C day/night temperature. The actual recorded glasshouse temperatures are in Appendix 3.2. Paper was placed in the base of the pots (16 cm diameter x 17.5 cm height) to prevent soil leakage through the drainage holes. The soil used for this experiment was collected from Jerilderie NSW (35° 22’ S 145° 28’ E) from a field with DSR the previous summer. It was a brown-grey, heavy clay with a pH(1:5 H2O) of 6.1. The soil was passed through a 5 mm sieve prior to potting. Fifty seeds of barnyard grass (2018 seed lot) were sown on the soil surface and covered to the same depths of 0, 1, 3, 6, 9, 12 and 15 cm. Pots were irrigated continuously from the bottom by filling up saucers with water and allowing water to move upward by capillary rise. This was done to avoid barnyard grass seed disturbance. Overhead sprinkler irrigation by mist was also done as needed to avoid the soil drying or crusting.

The number of shoots that emerged was recorded at three-day intervals until no further emergence was observed, at 21 DAS. The visibility of the coleoptile on the soil surface indicated emergence. At termination, the emerged barnyard grass seedlings from depths were verified by carefully retrieving, washing and measuring the seedling root length.

Effect of soil burial in the field and existing crop on barnyard grass dormancy break

A field experiment was conducted to determine the effect of burial and existing crop on the dormancy break of barnyard grass seeds at Charles Sturt University Wagga Wagga field bay from March to December 2019. The newly harvested barnyard grass seeds were tested for initial germination and viability. Barnyard grass seeds (2019 seed lot) were placed inside packets (100 seeds per packet) made of organza fabric woven into small bags (10 cm x 7.5 cm). Seed packets were buried in the field in March 2019 at 0.5 cm depth in plots with no crop and in plots that had been sown with Shaftal Persian clover for two consecutive years (2018 and 2019). Packets were retrieved at monthly intervals for nine months (April to December 2019). Barnyard grass seeds from the same seed lot were kept at room temperature (23 °C) in the dark. During the retrieval, the buried and room-stored barnyard grass seeds were tested for germination and viability. Below and above-ground temperatures in the field were recorded using a soil temperature logger and are shown in Appendix 3.3.

Data analyses

All experiments were arranged in a randomised complete block design with four replicates. The temperature and light experiment was conducted three times, and the temperature regime experiment was rotated in three different growth chambers. The temperature and light experiment data were combined for analysis and were analysed using ANOVA (GenStat 18th edition) to evaluate differences between treatments, and the means were separated using the least significant differences (LSD). Data were also analysed using regression analysis to determine the relationships among different temperature regimes and were fitted to a functional three-parameter sigmoid model using SigmaPlot 14.0. The model fitted to the fluctuating day/night temperature was:

\[ G = \frac{G_{\text{max}}}{1 + \frac{e^{[-(T - T_{50})]}}{Grate}} \]

where \( G \) is the mean germination (%) at time \( T \), \( G_{\text{max}} \) is the maximum germination (%), \( T_{50} \) is the time required for 50% of the maximum germination, and \( Grate \) indicates the slope. Parameter estimates were compared using their standard errors. The burial experiment was conducted twice, and data were combined due to non-significant interaction with treatment burial depth.
Results

Effect of temperature and light

Barnyard grass germination was influenced by the interaction between temperature and light ($P<0.04$). It was the lowest (23%) at 25/10 °C day/night temperature regime under continuous dark conditions (Figure 3.1). While light is not an absolute requirement, it stimulated barnyard grass germination by 67%. In this study, barnyard grass seeds germinated in all temperature regimes; 25/10, 30/20 and 40/25 °C.

![Figure 3.1 Barnyard grass seed germination (%) at 15 days after sowing at alternating day/night temperatures (25/10, 30/20, 40/25 °C) and light (light/dark and continuous dark). Error bars indicate standard deviation.](image)

The barnyard grass seeds exposed to light/dark at the lowest temperature regime (25/10 °C) took longer to reach 50% germination ($T_{50}$) (Figure 3.2). The time it took for 50% of maximum germination at 25/10 °C was $7.22\pm4.05$ days after sowing, whereas at 30/20 and 40/25 °C it was $3.14\pm3.32$ and $2.57\pm3.75$ days after sowing, respectively. Cumulative germination at 12-15 days was similar among the tested temperatures.

Barnyard grass seeds that were subjected to the continuous dark condition germinated after exposure to light (Figure 3.3). Within 6 days after exposure to light, barnyard grass germination rates increased to 89-95%, suggesting that barnyard grass seeds can remain under dark or buried conditions and increase germination when they receive light or during tillage operation.
Figure 3.2 Barnyard grass seed cumulative germination (%) at alternating day/night temperatures (25/10, 30/20, 40/25 °C) and light (light/dark). The lines represent a three-parameter sigmoid model fitted to the data.

25/10 °C; G% = 96.03/[1 + e\(^{-(-x-7.22)/0.65}}\)]; R\(^2=0.99\)
30/20 °C; G% = 96.62/[1 + e\(^{-(-x-3.14)/0.80}}\)]; R\(^2=0.99\)
40/25 °C; G% = 96.18/[1 + e\(^{-(-x-2.57)/0.43}}\)]; R\(^2=0.99\)

Figure 3.3 Barnyard grass seed germination (%) at alternating day/night temperatures (25/10, 30/20, 40/25 °C) in continuous dark for 15 days and 3 and 6 days after exposure to light/dark (DAL). Error bars indicate standard deviation.

Effect of burial depth on barnyard grass emergence

The emergence of barnyard grass seedlings was influenced by the burial depth (P<0.001). The cumulative seedling emergence declined with increasing burial depth (Figure 3.4). The maximum emergence of 70% (21 DAS) was observed at the soil surface, but no emergence was observed at 15
cm burial depth. The emergence of barnyard grass at the soil surface and 1 cm burial depths were similar until 6 DAS. However, from 9 DAS onwards, surface-sown barnyard grass emergence was greater than that at 1 cm burial depth. At 21 DAS, the cumulative seedling emergence declined with increasing burial depth. At 1, 3, 6, 9, 12 and 15 cm burial depth, seedling emergence decreased by 50, 62, 72, 84, 94 and 100% respectively compared with 0 cm-surface. No additional barnyard grass emergence was observed at 18 and 21 DAS.

Figure 3.4 Barnyard grass seed cumulative emergence (%) at 0, 1, 3, 6, 9, 12 and 15 cm burial depths observed at 3-day intervals starting at 3 until 21 days after sowing (DAS) grown in glasshouse conditions. Error bars indicate standard deviation.

Barnyard grass dormancy break

Barnyard grass dormancy was influenced by the interaction of seed burial/storage and time after burial (P<0.001). Greater than 80% of newly harvested dormant barnyard grass germinated after two months of burial in the field both with no crops and sown with a winter pasture legume. Faster breaking of dormancy was observed when it was buried in the field both with no crops and sown with Shaftal Persian clover than when barnyard grass seeds were kept at storage (Figure 3.5). There was a slightly induced dormancy in October 2019 or at 7 MAB (after the winter months).
Figure 3.5 Monthly germination of newly harvested barnyard grass seeds stored at room temperature (23 °C) and buried (0.5 cm depth) under fallow and clover sown plots from 0 to 9 months after burial.
Discussion

Allelopathic potential of pasture legume species against barnyard grass

The allelopathic impacts of winter pasture legume species/cultivars observed in this study during the pasture legume phase on barnyard grass seed longevity (plant to seed) and emergence and growth (plant to plant), and after the pasture legume phase on barnyard grass germination/emergence and growth (residue to plant), are summarized in Figure 4.1. These impacts suggest that there is a scope to use pasture legumes to limit barnyard grass establishment, growth and seed production. However, results of this study showed considerable variation in the potency of the pasture legume species through exudates, leachates and competition.

![Figure 4.1 Allelochemical impact of pasture legume species against barnyard grass (BYG) during and after the pasture legume phase.](image)

**Pasture legume species/cultivars**

Winter pasture legumes species (Persian, balansa and subterranean clovers) and crops (canola and barley) are commonly rotated with rice. Among these species, Shaftal Persian clover extracts/residues were found to have the strongest suppression against barnyard grass. However, its potency diminished
as the residues aged and the results varied with soil type. A delay (4-5 months) in the conduct of the phytotoxicity experiment (different concentrations) resulted in a reduced potency of Shaftal Persian clover extracts against barnyard grass. Opposite results (inhibition and enhancement) were observed when a different soil type was used. These variations are influenced by many factors such as soil physical and chemical property, soil mobility, microorganisms degradation, environmental (e.g. UV radiation, temperature and environmental stress), and chemical property (e.g. volatility), and a combination of these processes may have influenced the expression of the phytotoxic effects (Kobayashi, 2004; Kong et al, 2007; Teasdale et al, 2012; Li et al, 2013; Trezzi et al, 2016).

Other pasture legume cultivar residues were found to have differential inhibitory effects on barnyard grass growth. A planned parallel extract bioassay was not able to be conducted to support these observations as the onset of COVID-19 restrictions prevented laboratory access. Numerous studies have screened for and identified rice cultivars with allelopathic capability against barnyard grass (Gealy et al, 2003; Ahn et al, 2005; Cheema et al, 2012), but little progress has been made with respect to pasture species impacts on that weed. This study has shown that considerable capability exists but further study is needed to identify pasture legume species/cultivars with the most potency against barnyard grass but that are beneficial or harmless to rice. The management of this dynamic also needs further research.

In the field, the expression of allelopathy is influenced by many factors, including stages of growth, weather conditions and cultural operations, which influence the accumulation and then decomposition of the bioactive compounds (Rice, 1984; Cheema et al, 2012). In Australian rice crop rotations, pasture legume species are grown as mixed swards of varying duration, and seedbed preparation for rice may or may not include tillage. These aspects in combination need to be explored under field conditions to ascertain the key management practices that provide maximum effect. In the study reported here, the incidence of an extremely severe drought causing a lack of water for rice production did not allow field work to continue during the candidature period, and so commercial testing of the principles was not possible. It is suggested that field refinement of these aspects could be used to reduce the barnyard grass seed bank gradually in rice, thereby providing savings due to reduced chemical usage and potentially less harmful effects on the environment (Duke et al, 2001).

This study showed that there were potent phytotoxic compounds involved in some pasture legumes. Identification of such allelochemicals may lead to the development of effective bioherbicides for commercial use against barnyard grass.

**Pasture legume amount and placement**

The amount and placement of the pasture legume residues were shown to have an effect on the growth of both barnyard grass and rice. High amounts (equivalent of 6 tonnes ha⁻¹) of Shaftal Persian clover shoot residues as mulch were needed to reduce barnyard grass emergence. This suppression could be the result of a physical barrier by the residues or chemical inhibition (Buhler et.al, 1996; Chauhan and Johnson, 2011). A previous study showed that barnyard grass growth and seed production was significantly reduced by shading (Chauhan, 2013). This study did not distinguish if this was due to an inhibitory phytotoxic effect or competition, or a combination of both. The high amount of residues needed for barnyard grass suppression, however, may not be feasible in legume-based pastures as, commonly, not much residue is left on the field after animal grazing, hay collection, burning and/or knockdown herbicide application.

These pasture legume species and the residue placement, though, had a negative impact on rice. Rice growth was inhibited by all the pasture legume extracts (Persian, balansa and subterranean clovers) and by incorporation of Shaftal Persian clover residues. Direct drill sowing of rice (without tillage) after the pasture legume phase may prevent this negative impact from occurring under field conditions and may enhance rice growth with residues left on the surface as mulch.
Seed bank dynamics of barnyard grass

Rice growers in south-east Australia generally follow one to three years of winter pasture legumes (Persian, balansa and subterranean clovers), or one season of winter crops (canola, barley), and/or a winter fallow period, with rice. This study of the seed bank dynamics of barnyard grass showed that of the rotation species in the rice-based system, the winter pasture legume phase had the greatest negative impact on barnyard grass (Figure 4.2).

In DSR, there is a continuous build-up of the barnyard grass seed bank due to: the crop-weed association; the absence of water to control barnyard grass during the early stage of rice growth; and insufficient herbicide residual control (Troldahl et al, 2017; Dunn and Ford, 2018; Troldahl and Stevens, 2019). Previous studies have shown that grass weed species, including barnyard grass, are favoured in DSR systems (Bajwa and Chauhan, 2017). The current weed management for DSR in south-east Australia relies heavily on continued use of herbicides with a limited range of modes of action. This poses a high risk of the evolution of resistance to these herbicides (Troldahl and Stevens, 2019). As such, the system is not sustainable and requires the inclusion of other barnyard grass management options.

Figure 4.2 Barnyard grass seed bank dynamics under winter fallow/crop/pasture legume and DSR rotations.
Winter fallow – DSR rotation

During the winter fallow, this study showed that barnyard grass can establish and reach the inflorescence stage. If left uncontrolled, barnyard grass then may have the opportunity to set seeds and replenish its seed bank prior to the start of DSR. In the rice-based rotation system, there is a longer window for barnyard grass seed set, particularly with the use of short-season rice varieties for DSR (sown as late as December). During the short fallow before sowing, barnyard grass can emerge, grow and set seed (late September to December). Barnyard grass grows fast and can respond to a range of photoperiods, flowering on photoperiods ranging from 8 to 16 hours of day length (Holm et al, 1991). It is able to produce inflorescence and reached seed maturity within 28 and 45 days respectively (Peerzada et al, 2016). Effective control measures are absolutely necessary to prevent barnyard grass weed replenishment. No-till during this fallow period, however, can promote barnyard grass seed decay, thereby reducing the seed bank during the rice phase.

Winter crop – DSR rotation

Winter crops such as canola and barley suppressed barnyard grass growth and thus may prevent barnyard grass seed set prior to rice. Results of this study, however, suggest that no-till is required to keep most barnyard grass seeds on the soil surface for enhanced decay. During the winter crop sowing event, even minimal soil disturbance may bury barnyard grass seeds under the top soil. Additionally, the duration of the winter crop is likely only one year, whereas at least two years of winter crop/pasture is needed for reduced viability of barnyard grass seed.

Winter pasture legume – DSR rotation system

Winter pasture legumes significantly suppressed seed viability and reduced the seed bank of barnyard grass. This was achieved with at least two years of winter rotation with pasture legumes, enhanced seed decay if left on the soil surface, reduced density and growth of barnyard grass with winter pasture legumes, and a potential differential allelopathic effect on barnyard grass depending on pasture legume species and variety (Figure 4.2). These findings suggest that there are practical options to manage the barnyard grass seed bank in DSR temperate rice systems in Australia.

The limited time frame of this study enabled only a two-year duration of winter cropping/legume pasture. Barnyard grass is a known prolific seed producer, with about 40,000 seeds plant\(^{-1}\) (Holm et al, 1991; Bagavathiannan et al, 2011). The remaining viable barnyard grass seeds after two years may still contribute to seed bank increases if left uncontrolled prior to seed production. The greater-than-two-year pasture legume-rice rotation may provide more benefit in terms of barnyard grass seed depletion, and there is a need to quantify barnyard grass persistence under longer pasture legume phase durations (three to five years).

During the pasture legume phase, barnyard grass seed bank depletion can also be achieved through continuous animal grazing and chemical/mechanical control that can reduce additions to the existing seed bank. A previous study by Hendrickson et al (2014) showed that barnyard grass density was significantly reduced by no-till and animal grazing in annual crops. However, they did not measure the degree of impact on weeds. Destroying the emerged barnyard grass seeds with selective grass herbicides or by mechanical methods before the rice phase is also necessary (Cloutier et al, 2007; Chauhan and Johnson, 2011; Bàrberi, 2019). Harvest weed seed control offers potential opportunities to reduce barnyard grass seed inputs into the weed seed bank (Broster et al, 2016).

It should be noted that this study of variable factors was under controlled conditions. It is recognised that, in the field, such influences are much more variable, but circumstances limited the opportunity for extending the investigations to the field. The responses achieved under controlled conditions, if repeated in the field, would provide options for producers to address the barnyard grass challenge, and it thus remains important that further research be undertaken in the field to determine the scope for action.
Barnyard grass ecotype response and implication for integrated weed management

The barnyard grass seed ecotype (from the DSR area in south-east Australia) used in this study was shown to be more persistent compared with previous studies of *Echinochloa* species from Australia and overseas. This ecotype from DSR had 58% viability after 18 months of burial, whereas previous studies on *E. colona* (a close relative of barnyard grass) in Australia showed 1-2% viability at 0 cm depth and 20% viability at 10 cm depth after two years of burial (Wu et al, 2004). Barnyard grass, in a study in Vietnam, also had shorter persistence, with only 14% viability after one year of burial (Chin, 2001). This variability indicates the likelihood of the need for modified management options for different *Echinochloa* populations, e.g. from different regions or management practices. The studied ecotype was from southern New South Wales in a naturally dry summer and cold winter climate and this may explain its longer persistence than was reported in the other studies by Wu et al. (2004) in a summer rainfall environment, and in Vietnam with its monsoonal season.

Researchers have emphasised the need to combine a variety of weed management tools for more sustainable and effective control of barnyard grass in DSR (Pratley et al, 2008; Chauhan et al, 2015; Shekhawat et al, 2020). The ecotypic response of barnyard grass in this study highlighted the potential benefits of integrating different weed management tools, including no-till, the stale seedbed technique, sowing implements with minimum soil disturbance and the use of competitive DSR cultivars.

**No-till and sowing implements with discs**

The outcomes of this research emphasised that no-till prior to rice enhances barnyard grass seed decay and fatal germination, avoids germination of previously buried dormant seeds, reduces seed viability over time (>2 years) and minimises the negative impact of pasture legume residues on rice. The continuous no-till system in DSR, however, has been shown to promote abundance of other weed species in rice e.g. *E. colona* and *Cyperus iria* (Shekhawat et al, 2020). The pasture legume stubbles may also impede rice establishment. No-till also relates to the sowing operation, with use of sowing implements that deliver minimal soil disturbance. Thus, drill sowing implements with discs were being used to limit soil disturbance compared with tynes (Chauhan, 2012).

**Stale seedbed technique**

Early management is necessary to achieve a more effective and maximum control of barnyard grass and reduce its seed bank accumulation in the rice field. The stale seedbed technique is one system that could fit in the Australian DSR environment. This stale seedbed technique employs delayed sowing of rice until December in order to stimulate barnyard grass emergence during October/November through irrigation. Emerged seedlings of barnyard grass and other weeds are killed with a knockdown herbicide prior to sowing of rice. The enhancement of germination and killing of barnyard grass may be done several times before sowing to achieve maximum exhaustion of the seed bank, thereby limiting the competition with the establishing rice seedlings. The use of short-season rice varieties allows for later planting and fits with the stale seedbed technique.

**Competitive DSR rice cultivars**

The reduced germination in the absence of light and under burial indicates barnyard grass sensitivity to shading. A previous study by Chauhan (2013) also showed reduced barnyard grass growth and seed production under shade. This observation is also consistent with the observed suppression of barnyard grass emergence and growth with pasture legume species. The dense canopy and horizontal leaf angle of the pasture legume swards intercepted more light, limiting emergence and growth of barnyard grass. The use of competitive rice cultivars with early vigour and early canopy closure, developed for the DSR system, can therefore provide an advantage against the shade-sensitive barnyard grass ecotype.
Recommendations

This research recommends that more than two years of pasture legume rotation, using allelopathic pasture legume species/cultivars, be employed against barnyard grass, and that this strategy be combined with other weed management tools, such as no-till, drill sowing implements with discs, delayed rice sowing, the stale seedbed technique, and the use of competitive rice cultivars with early vigour.

The results of this study showed the need for further research to refine the management applications of the findings. It is suggested that the research needs include:

1. Screening and identification of highly phytotoxic pasture legume species and cultivars, identification of the potent phytotoxic compound associated with the highly phytotoxic pasture legumes through chemical analysis, and verification of results under field conditions.

2. Field investigation on the impacts of different phytotoxic pasture legume species, longer durations of the pasture legume phase tillage and no-till effects combined with the stale seedbed technique, animal grazing and chemical/mechanical weed management during the pasture legume phase on the barnyard grass seed bank and rice productivity in DSR.

3. Field investigation on the impacts of rice sowing time (early vs late), sowing implements (disc vs tynes), and highly competitive rice cultivars (with early vigour) on the barnyard grass seed bank and rice productivity in DSR.
Appendix 1.1. Glasshouse actual temperatures (°C) from April 2019 to June 2019 during the conduct of the pot bioassay in Chapter 1.
Appendix 1.2. Glasshouse actual temperatures (°C) from April 2019 to January 2020 during the conduct of the pot experiments in Chapter 1.
Appendix 2.1. Wagga Wagga 2018 minimum and maximum temperature (°C) during the conduct of the field experiment in Chapter 2.

Wagga Wagga AMO (072150) 2018 minimum temperature

Wagga Wagga AMO (072150) 2018 maximum temperature

Note: Data may not have completed quality control
Observations made before 1910 may have used non-standard equipment

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Appendix 2.2. Wagga Wagga 2019 minimum and maximum temperature (°C) during the conduct of the field experiment in Chapter 2.
Appendix 2.3. Wagga Wagga 2018 and 2019 rainfall (mm) during the conduct of the field experiment in Chapter 2.
Appendix 2.4 Glasshouse temperatures (°C) in 2018 and 2019 during the conduct of the pot experiments in Chapter 2.
Appendix 3.1. Actual temperature (°C) inside the three growth chambers during the conduct of the temperature and light experiments in Chapter 3.
Appendix 3.2. Actual temperature (°C) inside the glasshouse during the conduct of the burial experiments in Chapter 3.
Appendix 3.3. The actual field above (lighter grey) and below (darker grey) soil temperature (°C) from April to December 2019 during the conduct of the dormancy monitoring experiment in Chapter 5.
References


Impacts of pasture legume phase on the seed bank of barnyard grass in drill sown rice

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