

The effect of annual ryegrass (*Lolium rigidum*) interference on the growth of four common Australian crop species

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Summary *Lolium rigidum* (annual ryegrass) is a ubiquitous weed of southern Australia infesting the majority of winter crops. This paper considers the role of allelopathy in the interactions between *L. rigidum* and the associated crop species to improve the understanding of why *L. rigidum* is such a successful weed. Four crop species, i.e. lupin, barley, wheat and canola, were exposed to root exudates of *L. rigidum* to determine the extent of variation in growth responses. At high *L. rigidum* densities, root lengths of all crop species were inhibited. Increasing the duration of *L. rigidum* growth in the agar medium before introduction of the crop species further reduced crop growth. However, growth responses differed between crop species. Canola was the most affected by *L. rigidum* interference (67% root inhibition) followed in declining order by wheat (56% root inhibition), barley (37% root inhibition) and lupin (31% root inhibition). Activated carbon and removal of *L. rigidum* plants prior to crop sowing were used to verify the presence of putative inhibitory chemicals.

Keywords *Lolium rigidum*, allelopathy, annual ryegrass, interference.

INTRODUCTION

Annual ryegrass (*L. rigidum*) is known to affect the growth of wheat, barley, canola and lupins. In wheat, yield reductions depend on the relative densities of the weed and crop, relative times of emergence and resources available to each species (Gill 1996). The competitive ability of wheat against *L. rigidum* has been studied extensively (e.g. Reeves 1976, Lemerle *et al.* 1996, Cousens and Mokhtari 1998, Lemerle *et al.* 2001). Although differences in competitive ability between varieties have been reported, discrepancies between varietal rankings in competitiveness are considered to be a function of environment differences (Cousens and Mokhtari 1998, Lemerle *et al.* 2001). Differences in competitive ability to *L. rigidum* between wheat varieties have resulted in yield losses ranging from 0% to 50%, being most severe where *L. rigidum* germination was earlier rather than

simultaneous with wheat. Here, a reduction in crop root length was observed, but could be counteracted by increasing soil nitrogen levels (Rerkasem *et al.* 1980). However, examination of the effect of root separation on root length showed nitrogen was not involved and the authors concluded that there is an 'unseen' aspect of competition occurring.

In Australia, barley is generally considered more competitive against weeds than wheat (Gill and Davidson 2000), although this is largely based on wild oats studies (Bell and Nalewaja 1968). Later studies have shown wheat and barley to be similar in competitive ability against *L. rigidum* (Lemerle *et al.* 1995).

In canola, Harker *et al.* (1999) showed that delaying weed control in the first 2 weeks after crop emergence greatly reduced crop yield depending on variety. Lythgoe *et al.* (2001) found varietal differences in the ability of canola to withstand a density of 450 *L. rigidum* plants m⁻².

Elucidation of the interference mechanism between *L. rigidum* and the crop species it infests may allow farmers to make better decisions regarding cultivar choice and crop rotations. Crop tolerance to the allelochemicals produced by *L. rigidum* may be a trait that can be bred for, or possibly manipulated into cultivars through use of genetic engineering. This paper investigates the mechanisms by which *L. rigidum* inhibits growth of associated crop plants.

MATERIALS AND METHODS

General bioassay method A modified version of the Equal Compartment Agar Method (ECAM) developed by Wu *et al.* (2000) was employed to evaluate the interference interactions between *L. rigidum* and the receiver crop species. Briefly, a 600 mL glass beaker filled with 30 mL of 0.3% water agar was autoclaved. Pre-germinated seeds of *L. rigidum* were uniformly selected (radical length 1–2 mm) and aseptically sown on one half of the agar surface. The beaker was enclosed with parafilm to prevent contamination and evaporation from the agar surface and then positioned in a controlled growth incubator. The receiver

species were included after *L. rigidum* had grown for the prescribed time and the beakers were re-wrapped in parafilm and placed back in the growth incubator for a further 7 days before root measurements were taken. The growth of the receiver species alone was the control. A randomised block design was utilised for each experiment. Wheat and canola experiments had three replicates and barley experiments had four replicates. Lupin experiments had 10 replicates as the lupin seeds were sown into individual 120 mL containers to facilitate unimpeded root growth.

Beakers were sown at each of six different density treatments (0, 5, 10, 15, 20 and 25 *L. rigidum* plants per beaker) except for lupins where the densities were altered to 0, 5, 10 and 20 *L. rigidum* plants per beaker. Beakers were then placed in a growth incubator and grown for prescribed time intervals before introducing the receiver crop species. The Day '0' interval denotes that the crop and weed species were sown simultaneously. Different crop densities were utilised depending on the tested species used (10 plants per beaker for canola, 1 plant per beaker for lupins and 5 plants per beaker for wheat and barley).

Removal experiment The effect of retention or removal of *L. rigidum* prior to crop (canola) sowing was also evaluated. Removal or retention treatments consisted of six densities of *L. rigidum* (0, 5, 10, 15, 20 and 25 plants per beaker). Using the modified ECAM method, *L. rigidum* plants were established for 12 days prior to canola being sown into the agar.

Charcoal experiment Activated carbon was evaluated for its ability to reduce the inhibition of *L. rigidum* exudates. Treatments, with five replicates, consisted of three densities of *L. rigidum* (0, 10 and 20 plants per beaker) with and without the addition of activated carbon at 0.5% (w/v). Activated carbon was included before the beakers were autoclaved. Beakers containing the carbon-agar mix were gently swirled to suspend the activated carbon before the mix was allowed to cool and set.

Using the modified ECAM method, *L. rigidum* plants were established for 9 days prior to canola being sown into the agar. Canola was selected and used as the receiver species in these experiments because of its sensitivity to *L. rigidum* interference.

Statistical analysis Raw data were converted from length (mm) to percent growth relative to the control treatment. All treatment means were subjected to an analysis of variance (ANOVA) using GenStat version 11. Data fulfilled the requirements of normality and equal variance for this analysis.

RESULTS

Overall, wheat root length was most inhibited at the higher densities of *L. rigidum* with inhibition ranging from 6.6% to 56.6% (Figure 1) ($P < 0.05$).

The range of inhibition observed for barley root length was -3.9% to 37%. Densities greater than 10 *L. rigidum* plants per beaker inhibited growth compared to the control (Figure 2) ($P < 0.001$). However, there was no further inhibition seen in the higher density treatments. The day 0 treatment was less inhibited than the day 2 and day 6 treatments ($P < 0.001$). There were no further inhibition effects seen between day 2, day 4 and day 6 (Figure 3).

There was some stimulation of lupin root growth in the shorter duration treatments. The Day 0 treatment showed more stimulation of growth compared to the other duration treatments ($P < 0.05$), although there were no differences between day 3, day 6 and day 9 (Figure 4). There was an interaction between the density and day treatments ($P < 0.05$) largely due to stimulation of growth in the short duration treatments

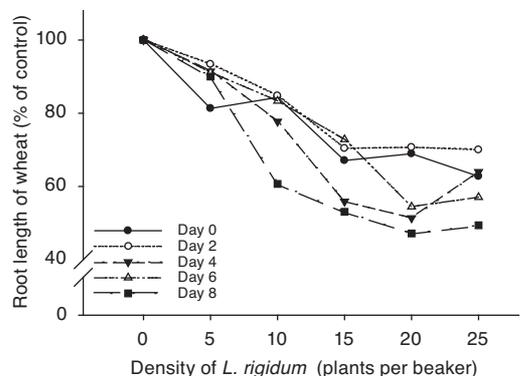


Figure 1. Effect on wheat root length (% of control) of *L. rigidum* densities and growing times before wheat was introduced (LSD = 16.0, $P < 0.05$).

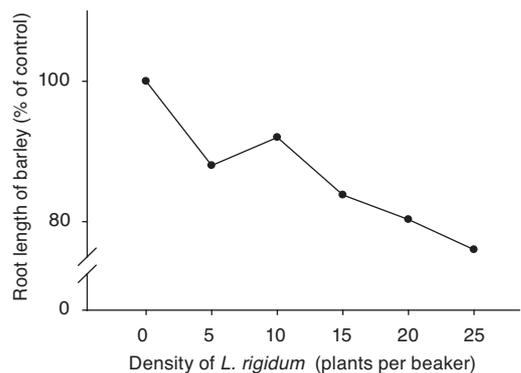


Figure 2. Effect on barley root length (% of control) of *L. rigidum* density (LSD = 13.4, $P < 0.001$).

relative to inhibition of growth in the longer duration treatments (Figure 4).

Canola root elongation was severely affected by the presence of *L. rigidum*. Inhibition ranged from 4.5% to 83.6% (Figure 5) ($P < 0.05$). All density treatments were inhibited compared to the control, but there were no differences between the higher *L. rigidum* densities ($P < 0.05$).

Removal experiment Increased inhibition of root length was observed up to the density of 10 *L. rigidum* plants per beaker ($P < 0.001$), although there were no differences between the higher density treatments of 10, 15, 20 and 25 *L. rigidum* plants per beaker. There was no effect of removing the *L. rigidum* from the agar relative to retaining the *L. rigidum* seedlings (Figure 6).

Charcoal experiment All density treatment means were different from each other ($P < 0.001$). As the density of *L. rigidum* increased, so did the inhibition

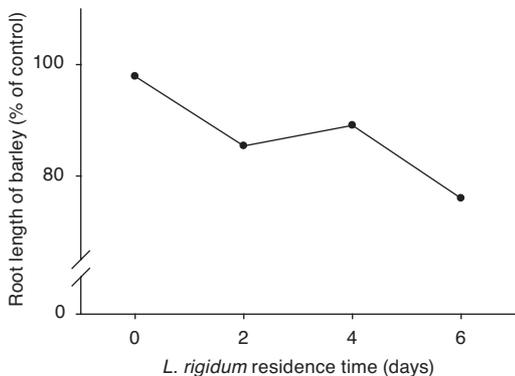


Figure 3. Effect on barley root length (% of control) of *L. rigidum* residence times before barley was introduced to the system (LSD = 10.9, $P < 0.001$).

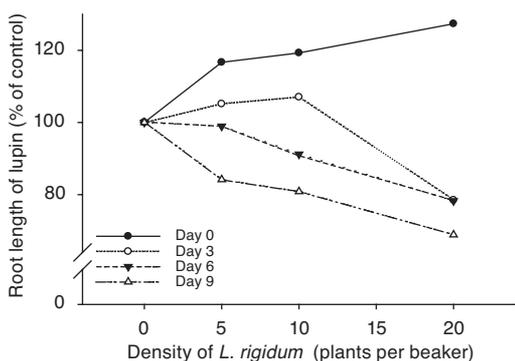


Figure 4. Effect on lupin root length (% of control) of *L. rigidum* densities and residence times before lupins were introduced to the system (LSD = 28.8, $P < 0.05$).

response on root elongation. The activated carbon treatments reduced the inhibition compared to the no charcoal treatments ($P < 0.001$) (Figure 7).

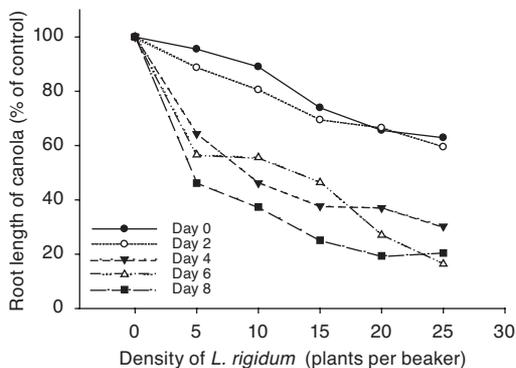


Figure 5. Effect on canola root length (% of control) of *L. rigidum* densities and residence times before canola was introduced (LSD = 24.0, $P < 0.05$).

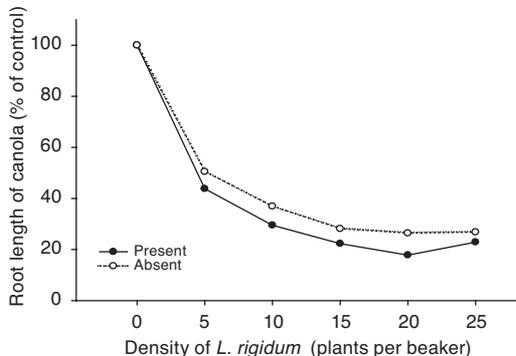


Figure 6. The effect on canola root length (% of control) of *L. rigidum* densities. *L. rigidum* was either present or had been removed (absent).

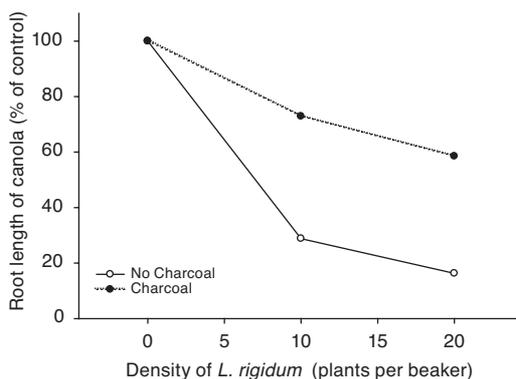


Figure 7. The effect on canola root length (% of control) of activated carbon and different *L. rigidum* densities (LSD = 17.2, $P < 0.001$).

DISCUSSION

In this study, the ECAM bioassay was employed to examine the phytotoxicity of *L. rigidum* root exudates. The bioassay was successful in showing that *L. rigidum* interfered with crop growth, presumably due to toxic root exudates. Similar results were also obtained by Amini *et al.* (2009) when testing the effects of *L. rigidum* root exudates on wheat. The ECAM bioassay was successful at showing the density dependant nature of the interference. The results from the initial crop-weed screenings showed that at high *L. rigidum* densities, crop root length was significantly reduced. Increasing the duration of *L. rigidum* in the agar, before introduction of the crop species, significantly reduced crop growth. However, the growth responses differed for each crop species.

Activated carbon was used to verify the presence of putative inhibitory chemicals. Its use can indicate the presence of organic chemicals (Nilsson 1994). The interference observed between *L. rigidum* and canola was significantly reduced when activated carbon was added to the system suggesting that the crop growth responses observed were due to the presence of inhibitory organic chemicals. The removal experiment also provided confirmation that the observed inhibition was likely due to the effect of allelopathic compounds rather than the resource competition mechanisms alone as *L. rigidum* plants were removed prior to inclusion of the crop plants.

This study indicates that chemical interactions represent at least part of the interference taking place between *L. rigidum* and the associated crop species, although the extent of the effect in the field requires further study.

Identification of the compounds responsible through bioassay guided fractionation is the first step in elucidating the chemical basis of such *L. rigidum* allelopathy (Rimando *et al.* 2001). If there are differences between crop cultivars in their abilities to withstand *L. rigidum* then it may be an option to breed varieties for increased weed tolerance.

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