

Allelopathic effect of canola on annual ryegrass

M. Asaduzzaman^{1,2,4}, Min An^{1,4}, James E. Pratley⁴, David J. Lockett^{3,4} and Deirdre Lemerle⁴

¹ Environmental and Analytical Laboratories, Faculty of Science, Charles Sturt University, Wagga Wagga, NSW 2650, Australia

² School of Agricultural and Wine Sciences, Faculty of Science, Charles Sturt University, Wagga Wagga, NSW 2650, Australia

³ NSW Department of Primary Industries, Wagga Wagga, NSW 2650, Australia

⁴ EH Graham Centre for Agricultural Innovation (an alliance between Charles Sturt University and NSW Department of Primary Industries), Wagga Wagga, NSW 2650, Australia
(masaduzzaman@csu.edu.au)

Summary Canola is a leading winter grain crop in Australia but declining yields due to the impacts of weeds and resistances to herbicides is a significant issue for Australian cropping systems. Although weed control herbicide options are available for canola, the prospects of herbicide resistance necessitate considering alternative options such as the allelopathic potential of canola for weed suppression. To assess the allelopathic prospects of canola, a laboratory-based root exudates bioassay was conducted using ECAM (Equal-Compartment-Agar Method). The allelopathic effects of different growth duration (0, 3, 5, 7, 9, 11, 13, and 15 days) and density (15, 30, 45, and 60 seeds/beaker) of canola (var. Ag-Spectrum) against annual ryegrass (as a test species) were investigated. During harvest the inhibited root length and diameter of ryegrass was measured. The experiment was designed as a randomised complete block with three replications and conducted under control conditions. Results showed that canola reduced the root length of ryegrass for 3–9 days, and at a density of 60 seeds/beaker was most inhibitory, suggesting that root exudates of canola are correlated with seedling density. Although, the root surface area was not influenced by density, it was significantly influenced by growth duration. Root surface area of canola was noticeably inhibited during 3–9 days. The effect of retention of canola root exudates prior to ryegrass sowing was also evaluated under the same experimental conditions. Canola was grown for 0, 3, 5, 7, 9, 11, 13 and 15 days with 60 seeds/beaker using ECAM, then canola seedlings were removed from the beakers before 15 pre-germinated ryegrass seeds were transplanted into each beaker. There was a significance difference between ryegrass grown with or without prior canola. Increasing growth duration of canola increased the inhibition effect up to 9 days but with growing time beyond this period there was no further noticeable inhibition.

Keywords Canola, allelopathy, growth duration, density, root length.

INTRODUCTION

Canola is an important rotational break crop in the Australian wheat belt, providing the benefits of a disease break and some improved ability to control weeds (Norton, 2003). Weeds are a major cost to canola production due to reduced yields, lower grain quality, and herbicide inputs. Weed management in canola has improved considerably with the development of a range of herbicide-tolerant (HT) cultivars (Lemerle *et al.* 20011) but Australia faces the prospect of loss of efficacy of the herbicide-tolerant options and so alternative weed management strategies such as crop competition and allelopathy, need to be evaluated.

Allelopathy is the beneficial or harmful effect of one plant on another by the release of bioactive chemicals (Rice 1984). Through this mechanism, plants achieve a competitive advantage by releasing phytotoxins into their surrounds (Pratley *et al.* 1996) and cause the inhibition of weeds. Chon and Kim (2002) showed that in lucerne (alfalfa) crop growth duration affected the release of the allelochemicals.

Research has indicated that canola stubbles (residues) have an allelopathic effect, influencing both the growth of canola itself and weeds (Moyer and Huang 1997, Urens *et al.* 2009). Furthermore, crop density can greatly influence the competitiveness and reduce the negative effect of weeds (Berkowitz 1988). O'Donovan (1994) reported that tartary buckwheat (*Fagopyrum tataricum*) was effectively suppressed with increasing canola density. Therefore, crop growth duration and density of crop may have agronomic potential for non-chemical weed management. A preliminary study was conducted to determine the allelopathic potential of canola, through its density and early growth duration, against test species annual ryegrass.

MATERIALS AND METHODS

General bioassay A simple laboratory root exudates bioassay (ECAM-Equal Compartment Agar method) developed by Wu (1999) was selected to evaluate the

allelopathic potential of canola (var. Ag-Spectrum) against annual ryegrass. Treatments comprised eight different canola growth durations (0, 3, 5, 7, 9, 11, 13 and 15 days) and four canola densities (15, 30, 45 and 60 canola seeds/beakers). At each growth time, 15, 30, 45 and 60 pre-germinated and uniform canola seeds were sown on the aseptic agar surface of one-half of a glass beaker (9 cm diameter, 12 cm depth, and 600 mL) which was prefilled with 30 mL of 0.3% water agar. The beakers were sealed with parafilm and kept in a controlled growth chamber (light/dark 12/12 h and 20°C/18°C). After growth of the canola seedlings for 0 (control) 3, 5, 7, 9, 11, 13 and 15 days, 15 pre-germinated seeds of ryegrass were transplanted on the other half of the agar surface. A piece of pre-autoclaved white paperboard was inserted across the centre and down the middle of the beaker with the lower edge of the paperboard kept 1 cm above the agar surface. The beaker was divided into two equal compartments to minimise competition for space and light the between canola and ryegrass seedlings. The roots of canola freely enter the ryegrass compartment so that any allelochemicals produced and released by the canola seedlings can diffuse throughout the entire agar medium to influence ryegrass root growth. After ryegrass sowing, the beakers were again wrapped with parafilm and placed back in the growth chamber for seven days. The root length of the ryegrass seedlings was measured and root surface area was assessed by scanning and image analysis using WinRhizo software (Regent Instruments, Montreal).

Removal experiment The donor species canola was grown for 0 (control), 3, 5, 7, 9, 11, 13 and 15 days with 60 seeds per beaker using the ECAM method, then the canola seedlings were removed prior to 15 pre-germinated ryegrass seeds being sown into the agar surface, grown for a further 7 days growth. The root length of the ryegrass seedlings was then measured.

Experimental design The experiments comprised RCBD (Randomized Complete Block Design) along with three replications under controlled condition. Both experiments were repeated twice under the same condition to check for consistent results. Square root transformation of the raw data was required to normalise and ensure

homogeneity of variance. Data were the two repeats of each experiment analysed separately subjected to an analysis variance (ANOVA) using GenStat version 13.

RESULTS AND DISCUSSION

Effect of growth duration and density The results of analysis of variance showed significant difference between canola growth durations ($P < 0.05$) and between densities ($P < 0.05$). The interaction effects of growth time and density on ryegrass was also significant ($P < 0.05$). At lower densities of canola (15 and 30 canola seeds/beaker), the inhibition of ryegrass root was lower during all canola growth durations (Figure 1). The result suggests that there was no significance difference between densities 15 and 30 in terms of ryegrass root inhibition at each canola growth times except at 3 days. However, the inhibitory activity of canola increased and differences among growing times were significant with the greatest inhibition effect on ryegrass root length being observed at 60 canola seedlings per beaker after 9 days duration; with 5 days duration being next most inhibitive. Increasing the growing duration beyond 9 days had no further effects on annual ryegrass root growth. There were negligible differences between 11, 13 and 15 days duration at any densities. A similar trend with wheat seedling phytotoxicity was reported by Huang *et al.* (2003) with allelochemicals concentrations declining after 6–8 growing days. One explanation could be associated with the limited half-life of these compounds in agar medium. We also observed that after 9 days

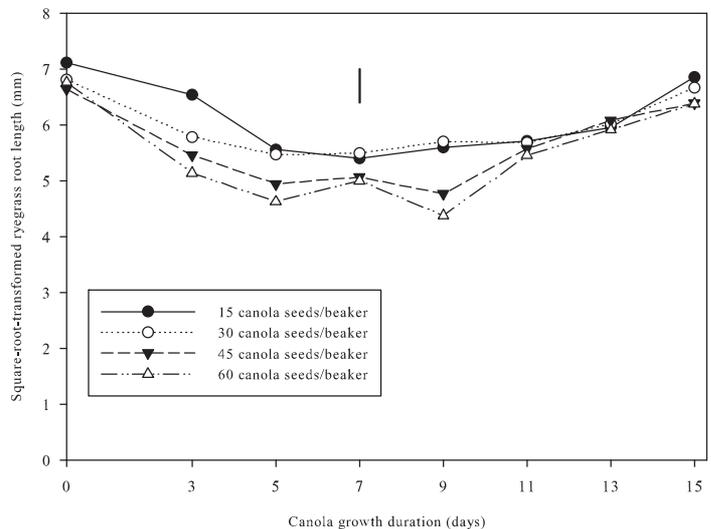


Figure 1. Effect of canola density and growth duration on root length of ryegrass (LSD = 0.56, $P < 0.05$).

of canola growth in the agar medium canola plants became stressed, presumably due to a shortage of nutrients in the agar medium.

The average root surface area of ryegrass was not significantly changed due to interaction effects of canola growing time and density. However, it was significantly ($P < 0.05$) affected by growth duration. The lowest root distribution was observed during 5–7 days (Figure 2). In a similar study with wheat, Li *et al.* (2011) reported that root surface area of ryegrass was significantly affected by both wheat density and genotype.

Removal experiment Root exudates of canola significantly reduced ryegrass root length even after canola was removed from the agar medium ($P < 0.05$). Canola density and growth duration effects on ryegrass growth were greater in the co-growth experiment than those in the ryegrass removal experiment (Figure 3). There was a significant difference between ryegrass grown with and without canola except at 13 days. Increasing growth duration of canola increased the inhibition effect up to 15 days. The root length of ryegrass was noticeably reduced during 0–9 days without canola and beyond this period the inhibition rate was reduced. Increasing canola growing time beyond 9 days produced no further inhibition, possibly due to degradation of the canola root exudates, by the ryegrass metabolism.

CONCLUSION

Growth duration and density of canola played a major role in the suppression of annual ryegrass root growth. The inhibitory activity of canola is growth period related and inhibition in this environment is greater in the early stages of growth (3 to 9 days). The root diameter of ryegrass was also inhibited by growing time. In addition, increasing canola density increased the inhibition, presumably due to increased concentration of root

exudates in the agar medium. This suggests that increased canola competitiveness can be achieved at high density leading to greater suppression of the target weed species. These investigations also suggest that there are opportunities to explore canola genotypes for their abilities to control ryegrass through allelopathy. The root exudates of canola could be used as potential natural herbicides but they must first be collected, purified and characterised.

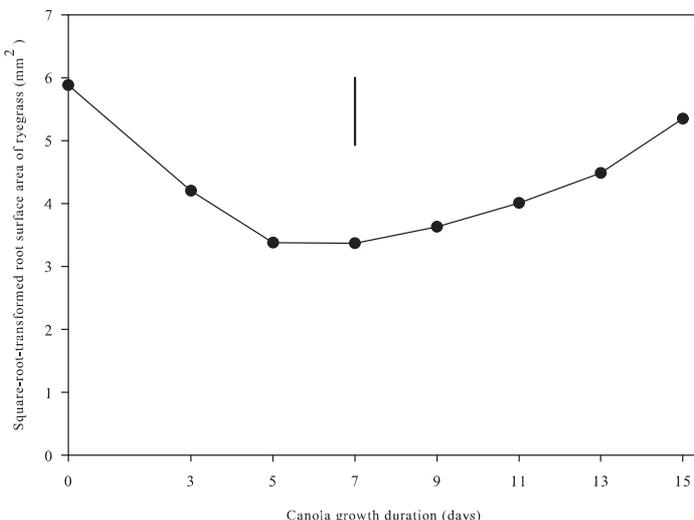


Figure 2. Effect of canola growth duration on average root surface area of ryegrass (LSD = 1.07, $P < 0.05$).

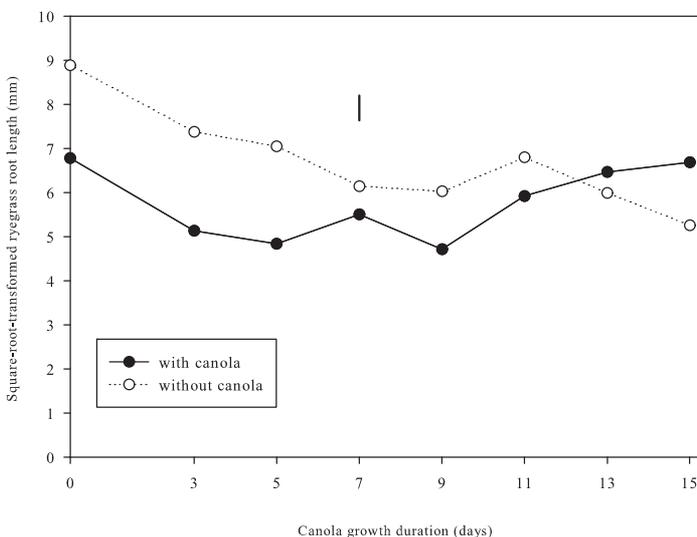


Figure 3. Root length of ryegrass as influenced by presence or absence of canola seedlings in the growth medium (LSD = 0.56, $P < 0.05$).

REFERENCES

- Berkowitz, A.R. (1988). Competition for resources in weed-crop mixtures. In M. Altieri and M. Lieb-Man (Eds). 'Weed management in agroecosystems: ecological approach'. Boca Raton, Florida: CRC press, p 89-119.
- Chon, S.U. and Kim, J.D. (2002). Biological activity and quantification of suspected allelochemicals from alfalfa plant parts. *Journal of Agronomic crop Science* 188, 281-285.
- Huang, Z.Q., Haig, T., Wu, H.W., An, M. and Pratley, J. (2003). Correlation between phytotoxicity on annual ryegrass (*Lolium rigidum*) and production dynamics of allelochemicals within root exudates of allelopathic wheat. *Journal of Chemical Ecology* 29, 2263-2279.
- Lemerle, D., Lockley, P., Koetz, E., Lockett, D. and Wu, H. (2011). Manipulating canola agronomy for weed suppression. 17th Australian Research Assembly on Brassicas, 15-17 August, Wagga Wagga, NSW, Australia.
- Li, C.J., An, M., Saeed, M., Li, L. and Pratley, J. (2011). Effects of wheat crop density on growth of ryegrass. *Allelopathy Journal* 27 (1), 43-54.
- Moyer, J.R. and Huang, H.C. (1997). Effect of aqueous extracts of crop residues on germination and seedling growth of ten weed species. *Botanical Bulletin of Academia Sinica* 38.
- Norton, R.M. (2003). 'Conservation Farming Systems and Canola'. The University of Melbourne, Melbourne.
- O'Donovan, J.T. (1994). Canola (*Brassica napus*) plant density influences tartary buckwheat (*Fagopyrum tataricum*) interference, biomass, and seed yield. *Weed Science* 42 (3), 385-389.
- Pratley, J.E. (1996). Allelopathy in annual grasses. *Plant Protection Quarterly* 11, 213-214.
- Rice, E.L. (1984). 'Allelopathy'. Second Edition. Academic Press, New York, pp 422.
- Urems, I., Arslan, M., Sangun, M. K., Uygur, V. and Isler, N. (2009). Allelopathic potential of rapeseed cultivars on germination and seedling growth of weeds. *Asian Journal of Chemistry* 21, 2170-2184.
- Wu, H., Pratley, J.E., Lemerle, D. and Haig, T. (1999). Crop cultivars with allelopathic capability. *Weed Research* 39, 171-180.