Allelopathic potential of root exudates of annual ryegrass (*Lolium rigidum*)

R. Amini[1], M. An[2,3], J. Pratley[3] and S. Azimi[4]
[1] Department of Agronomy and Plant Breeding, College of Agriculture, University of Tabriz, Tabriz, 51666-16471, Iran
[2] Environmental and Analytical Laboratories, Faculty of Science, Charles Sturt University, Wagga Wagga, NSW 2678, Australia
[3] EH Graham Centre for Agricultural Innovation (Industry and Investment NSW and Charles Sturt University), Charles Sturt University, Wagga Wagga, NSW 2678, Australia
[4] Department of Plant Protection, College of Agriculture, University of Tehran, Karaj, Tehran, Iran
Corresponding author: MAn@csu.edu.au

Summary A laboratory-based root exudates bioassay was conducted to assess the allelopathic potential of annual ryegrass against wheat using the equal-compartment-agar method. The effect of different growth period of ryegrass (2, 4, 6, 8, 11 and 15 days) on the allelopathic response of wheat was investigated. Results indicated that co-growth of annual ryegrass and wheat reduced the root and shoot length of wheat. Ryegrass growth for 6–8 days had the most inhibitive effect on wheat root growth. Increasing ryegrass density beyond 20 plants per beaker had no significant further effect on the allelopathic activity of annual ryegrass. To validate the allelopathic effect between ryegrass and wheat, activated charcoal was added to the growth medium (2% v/v) resulting in alleviation of the inhibition of wheat root and shoot length. Thus, the inhibition effect of annual ryegrass on wheat root and shoot length was chemically directed through its root exudates, with inhibition being greater on wheat roots than shoots.

Keywords Annual ryegrass, root exudates, allelopathy, weeds.

INTRODUCTION

Allelopathy is a process whereby plants provide themselves with a competitive advantage by putting phytotoxins into the adjacent environment. Allelochemicals, organic compounds involved in this phenomenon, can be released through volatilisation, leaching from leaves, degradation of plant residues and root exudation. Root exudates represent one of the largest direct inputs of plant chemicals into the rhizosphere environment (Bertin *et al.* 2003).

Weeds could have an impact on crops through allelopathy. *Chenopodium murale* has been reported to reduce wheat growth. In agar medium containing root exudates, root and shoot length was reduced by nearly 44% and 32%, respectively, whereas seedling weight was reduced by about 52% (Batish *et al.* 2007). Khanh *et al*. (2008) found that dodder (*Cuscuta hygrophilae* H.Pearson) extract was the most inhibitive against the growth of carrot (*Daucus carota* L.), root and shoot length of radish (*Raphanus sativus* L.), and root length of lettuce (*Lactuca sativa* L.).

Annual ryegrass (*Lolium rigidum*) is Australia's worst weed of temperate crop production. San Emeterio *et al*. (2004) evaluated the allelopathic potential of *L. rigidum* on *L. multiflorum* Lam., *Dactylis glomerata* L. and *Medicago sativa* L. Seeds of *L. rigidum* severely affected seedling development and growth of the three species, particularly on *L. multiflorum*. A close relative, fine fescue species (*Festuca* spp.), was reported to produce detectable amounts of m-tyrosine as the major active component in root exudates that had phytotoxic effects (Bertin *et al.* 2007).

The allelopathic potential of root exudates of annual ryegrass were investigated using a laboratory-based root-exudate bioassay, the 'equal-compartment-agar method' (ECAM), developed in our laboratory (Wu *et al.* 2000).

MATERIALS AND METHODS

Seed sterilisation and pre-germination Seeds of wheat (cv. 'ww14192') and annual ryegrass (ARG) were surface sterilised by soaking in 2.5% sodium hypochlorite solution for 15 min followed by five rinses in sterilised distilled water. The surface-sterilised seeds of the wheat and ryegrass were incubated in light at 25°C for 48 and 72 h respectively to germinate. Germinated seeds were selected for bioassay experiments.

Experiment 1: growth period of annual ryegrass The ECAM developed by Wu *et al.* (2000) was employed to evaluate the allelopathic potential of ARG to wheat. Twelve pre-germinated ARG seeds were sown on the aseptic agar surface with the embryo upwards, in three rows on one-half of a glass beaker (500 mL) prefilled with 30 mL of 0.3% water agar. The beakers were sealed with parafilm and placed in a controlled growth cabinet with a daily light:dark cycle of 13:11 h and a temperature cycle of 25:13°C. The fluorescent light intensity in the cabinet was 3.56 ±

0.16×10^3 lux. After the growth of ryegrass seedlings for 2, 4, 6, 8, 11 or 15 days, 12 pre-germinated seeds of wheat were transplanted on the other half of the agar surface in three rows. 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 entire beaker was thereby divided into two equal compartments that were occupied separately by wheat and ryegrass seedlings. Competition above the agar surface between wheat and ryegrass was thus avoided by confining plants within their own compartments. However, the ryegrass roots could freely enter the wheat compartment. After sowing wheat, the beakers were again wrapped with parafilm and placed back in the growth cabinet for a further 10 days. The growth of wheat alone was included as the control.

Another separate experiment was conducted as per the above conditions except that ARG seedlings were removed. ARG was grown for 2, 4, 6, 8, 11 or 15 days and then removed from the beakers prior to the 12 pre-germinated wheat seeds being transplanted into the beakers and grown for 10 days alone without annual ryegrass. Wheat seedlings without ryegrass pre-treatment were used as the control.

Experiment 2: activated charcoal effects To determine if there is an allelopathic effect between ARG and wheat, 2% (v/v) activated charcoal (0.140 g per beaker) was added to the agar in the beakers. Activated charcoal absorbs organic compounds such as allelochemicals and therefore any allelopathic effect, if present, will be reduced. The ryegrass densities used were 4, 8, 12, 16, 20, 24, 35, 50, 70 or 100 plants per beaker. The germinated seeds were transplanted into the beakers and incubated as previously described. Treatments without activated charcoal were used for comparison. After 7 days of incubation, 12 pre-germinated wheat seeds were added to each beaker and incubated for another 10 days.

Experimental design and measurements The experiments were arranged in a randomised complete block design with three replicates. After 10 days of co-growth of ryegrass with wheat in the growth cabinet, the longest root and shoot length of the wheat seedlings were measured.

Statistical analysis Experimental data were subjected to analysis of variance using MSTATC and Minitab and the treatment means were tested separately using standard error. The data that were used in ANOVA met the assumptions of normality and homogeneity of variance and did not require transformation.

RESULTS AND DISCUSSION

Experiment 1: wheat root length ARG grown for 2 days significantly decreased wheat root length. Even after annual ryegrass (ARG) was removed from the agar medium, wheat root length was still significantly reduced, indicating chemicals are released from and remain present in the agar (Figure 1). Inhibition increased with increased ARG growth period and reached a maximum after 11 days. Increasing ARG growth period beyond 15 days produced no further significant inhibition. Those results indicate that the allelopathic activity of root exudates of ARG was growth period related and the maximum inhibition was caused by ARG growth between 8 to 15 days.

In the ARG removal experiment, root exudates of ARG also significantly reduced wheat root length but this effect was smaller than that when ARG was co-grown with the wheat. However, the allelopathic pattern remained similar. There was no significant difference between 2 and 4 days of ARG growth period, and ARG growth for 6 and 8 days had greatest inhibition on wheat root growth. Increasing ARG growing time up to 15 days gave no further significant

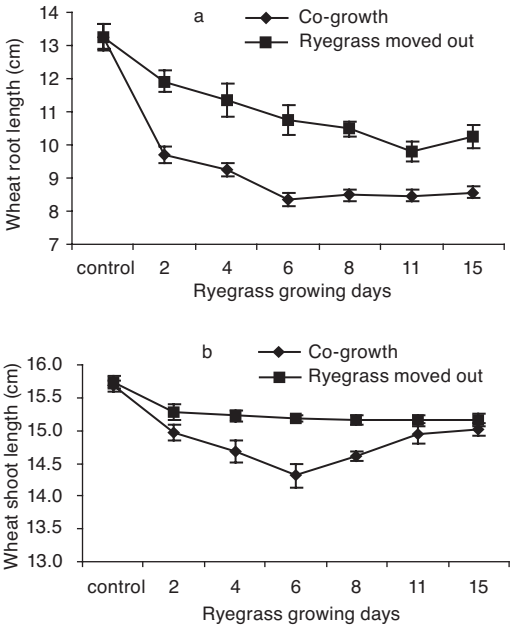

Figure 1. Length (cm) of (a) wheat seedling roots and (b) wheat seedling shoots after growth in media where annual ryegrass had grown for various periods. Data are presented for both co-growth and ryegrass removal experiments. Bars indicate the standard error of observations for each treatment.

inhibition. ARG peak inhibition was reached after an 11 day growth period. Increasing the growth period beyond 11 days had no further significant effect on ARG inhibitory activity, possibly due to degradation of the ryegrass root exudates. Huang *et al*. (2003) found that concentration of allelopathic compounds, such as phenolics and BOAs, was greatest between 6–8 growing days and after this time their concentration declined. Macias *et al*. (2002) reported that DIMBOA had a half-life of 5.3 h at 28°C at pH 6.75 and decomposed to the more stable MBOA that also had inhibitory activity, but to a lesser degree. The decline in allelopathic compounds toward the end of the experimental period could also be due to resorption by the growing ARG plants as has been observed in wheat (Kobayashi *et al.* 1996) and *Agropyron repens* (Friebe *et al.* 1995).

Experiment 1: wheat shoot length ARG root exudates significantly reduced wheat shoot length in the co-growth experiment but not in the ARG removal experiment (Figure 1b). In the co-growth experiment increasing the ARG growth period up to 6 days reduced wheat shoot length. By increasing the ARG growth period beyond 6 days, the inhibitory effect of ARG on wheat shoot length decreased. There was no significant difference in wheat shoot length when ARG grew for 2, 11 or 15 days (Figure 1b). Other researchers also reported that root exudates have a lesser inhibitory effect on shoot growth than on root growth (Batish et al. 2007, Bruckner 1998, Mandal 2001).

Experiment 2: the effect of activated charcoal In the absence of activated charcoal, ARG inhibited wheat root growth and this effect increased significantly with increasing ARG density up to 20 plants per beaker. Increasing ARG density beyond 20 plants had no further significant inhibitory effect on wheat root length (Figure 2). Addition of activated charcoal to the growth medium substantially alleviated the inhibition effect of annual ryegrass on wheat root growth. Numerous researchers have used activated charcoal as an adsorbent of allelopathic compounds to demonstrate the presence of allelopathic activity (Callaway and Aschehoug 2000, Kulmatiski and Beard 2006, Nilsson 1994).

The results presented here indicate that ARG roots have released some allelopathic compounds that inhibit wheat root growth and activated charcoal has absorbed these compounds thereby reducing the allelopathic effects of ARG on wheat. The seedling establishment stage is critical in the growing season and allelopathic activity of ARG root exudates can severely suppress growth of crop seedlings.

ACKNOWLEDGMENTS

The project was financially supported by the Australian Research Council Discovery Grant (DP0773508ARC) and CSU Competitive Grant.

REFERENCES

Batish, D.R., Lavanya, K., Pal Singh, H. and Kohli, R.K. (2007). Root-mediated allelopathic interference of nettle-leaved goosefoot (*Chenopodium murale*) on wheat (*Triticum aestivum*). *Journal of Agronomy and Crop Science* 193, 37- 44.

Bertin, C., Weston, L.A., Huang, T., Jander, G., Owens, T., Meinwald, J. and Schroeder, F.C. (2007). Grass roots chemistry: meta-tyrosine, an herbicidal nonprotein amino acid. *Proceedings of the National Academy of Sciences* 104, 16964-9.

Bertin, C., Yang, X. and Weston, L.A. (2003). The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil* 256, 67-83.

Bruckner, D.J. (1998). The allelopathic effect of ragweed (*Ambrosia artemisiifolia* L.) on the germination of cultivated plants. *Novenytermeles* 47, 635-44.

Callaway, R.M. and Aschehoug, E.T. (2000). Invasive plants versus their new and old neighbours: a mechanism for exotic invasion. *Science* 290, 521-3.

Friebe, A., Schulz, M., Kuck, P. and Schnabl, H. (1995). Phytotoxins from shoot extracts and root exudates of *Agropyron repens* seedlings. *Phytochemistry* 38, 1157-9.

Huang, Z., Haig, T., Wu, H., An, M and Pratley, J. (2003). Correlation between phytotoxicity on annual ryegrass (*Lolium rigidum*) and production

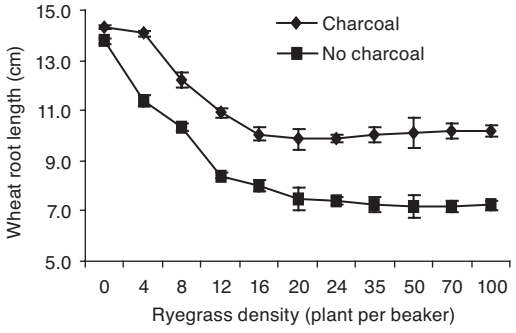

Figure 2. Wheat root length (cm) after growth in media with different densities of annual ryegrass with and without activated charcoal. Bars indicate the standard error of observations for each treatment.

dynamics of allelochemicals within root exudates of an allelopathic wheat. *Journal of Chemical Ecology* 29, 2263-79.

Khanh, T.D., Cong, L.C., Xuan, T.D., Lee, S.J., Kong, D.S. and Chung, I.M. (2008). Weed-suppressing potential of dodder (*Cuscuta hygrophilae*) and its phytotoxic constituents. *Weed Science* 56, 119-27.

Kobayashi, A., Kim, M.J. and Kawazu, K. (1996). Uptake and exudation of phenolic compounds by wheat and antimicrobial components of the root exudate. *Zeitschrift für Naturforschung* 51c, 527-33.

Kulmatiski, A. and Beard, K.H. (2006). Activated carbon as a restoration tool: potential for control of invasive plants in abandoned agricultural fields. *Restoration Ecology* 14, 251-7.

Macias, F.A., Marin, D., Castellano, D., Velasco, R.F., Oliveros-Bastidas, A., Chinchilla, D. and Molinill, M.G. (2002). Synthesis and bioactivity evaluation of hydroxamic acids. Proceedings, 3rd World Congress on Allelopathy, p. 107. Tsukuba, Japan.

Mandal, S. (2001). Allelopathic activity of root exudates from *Leonurus sibiricus* L. (Raktodrone). *Weed Biology and Management* 1, 170-5.

Nilsson, M.C. (1994). Separation of allelopathy and resource competition by the boreal dwarf shrub *Empetrum hermaphroditum* Hagerup. *Oecologia* 98, 1-7.

San Emeterio, L., Arroyo, A. and Canals, R.M. (2004). Allelopathic potential of *Lolium rigidum* Gaud. on the early growth of three associated pasture species. *Grass and Forage Science* 59, 107-12.

Wu, H., Pratley, J., Lemerle, D. and Haig, T. (2000). Laboratory screening for allelopathic potential of wheat (*Triticum aestivum*) accessions against annual ryegrass (*Lolium rigidum*). *Australian Journal of Agricultural Research* 51, 259-66.