

Herbicidal potential of *Eucalyptus dundasii* on *Lolium rigidum* Gaud. and *Hordeum* spp.

Hanwen Wu¹, Jinbiao Zhang^{1,3}, Rex Stanton², Min An², De Li Liu¹ and Deirdre Lemerle²

¹EH Graham Centre for Agricultural Innovation (Industry and Investment NSW and Charles Sturt University), Wagga Wagga Agricultural Institute, PMB, Wagga Wagga, NSW 2650, Australia.

²EH Graham Centre for Agricultural Innovation (Industry and Investment NSW and Charles Sturt University), Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia

³Test Centre, Fujian Agricultural and Forestry University, Fuzhou, Fujian 350002, PR China

Corresponding author: hanwen.wu@industry.nsw.gov.au

Summary Field observation has found that there is limited understory vegetation within the dripline of *Eucalyptus dundasii*. Research was conducted to assess the allelopathic potential of *E. dundasii* on two important weeds species, annual ryegrass and barley grass. The eucalyptus leaves were collected and subjected to a distillation process. The distillation process yielded three bioactive fractions, essential oil fraction, water fraction A and water fraction B. These three fractions were phytotoxic to the germination and seedling growth of the annual ryegrass and barley grass. Essential oils caused the most inhibitory effects on germination and shoot growth of both weeds, followed by water fraction B and fraction A. Both weeds had similar responses when exposed to the essential oils. However, the germination and seedling growth of barley grass were more sensitive than annual ryegrass to water fraction A and the germination of annual ryegrass was more sensitive than barley grass when exposed to water fraction B. The phytotoxicity was concentration-dependent. The research has found that although essential oils are highly volatile, these compounds can partly dissolve in water, resulting in the phytotoxic activity identified in the water fraction A. These results indicate that any significant rainfall events will wash off the essential oils into the ground to perform natural chemical defense against understory vegetation, including weeds. Further study on the identification of bioactive compounds might provide chemical leads for the development of new herbicides with new modes of action.

Keywords *Eucalyptus dundasii*, allelopathy, weeds, annual ryegrass, barley grass.

INTRODUCTION

Eucalyptus is a member of Myrtaceae family and a native to Australia. It has long been recognised that some eucalyptus species are capable of suppressing understory vegetation growth via allelopathy (May and Ash 1990). Del Moral *et al.* (1978) demonstrated that *Eucalyptus baxteri* was capable of allelopathically suppressing the understory growth of *Casuarina pusilla*

and *Leptospermum myrsinoides*. Eucalyptus contains a rich source of bioactive constituents, possessing fungicidal, insecticidal and herbicidal activities (Zhang *et al.* 2010). The bioactive compounds derived from eucalyptus have been reported to cause phytotoxicity to a range of field crops, such as wheat (*Triticum aestivum*), maize (*Zea mays*), radish (*Raphanus sativus*) and rice (*Oryza sativa*) (Batish *et al.* 2004, Batish *et al.* 2006, Khan *et al.* 2008).

The phytotoxic effects of eucalyptus allelochemicals have also been evaluated on a number of weed species. Aqueous leaf leachate of *Eucalyptus globulus* suppressed the establishment of vegetative propagule and early seedling growth of purple nutsedge (*Cyperus rotundus* L.) and Bermuda grass (*Cynodon dactylon* L.) (Chandra Babu and Kandasamy 1997). The herbicidal activity of eucalyptus oils against *Parthenium hysterophorus*, *Cassia occidentalis*, *Echinochloa crus-galli* and *A. viridis* has also been documented (Batish *et al.* 2004, Batish *et al.* 2006, Singh *et al.* 2005). These results suggest that bioactive compounds in eucalyptus oils have potential commercial value for further exploitation as natural herbicides (Zhang *et al.* 2010).

Field observation has found that there is limited understory vegetation within the dripline of Dundas blackbutt (*Eucalyptus dundasii*). This research was conducted to assess the allelopathic potential of the eucalyptus on two important weeds, annual ryegrass (*Lolium rigidum* Gaud.) and barley grass (*Hordeum* spp.).

MATERIALS AND METHODS

Plant material Fresh leaves of *E. dundasii* were collected from fields in Ungarie, NSW, Australia. Seeds of annual ryegrass were purchased commercially and barley grass seeds were collected locally from Wagga Wagga Agriculture Institute research station in 2008.

Steam distillation Fresh leaves (300 g) of *E. dundasii* were subjected to steam-distillation for 2.5 h using a Pyrex oil distillation apparatus with a flat bottom flask (2 L) containing 1200 mL distilled water

to generate steam. The distillation process produced three fractions: essential oils, water fraction A and water fraction B. The volatile components from leaves were condensed through cooling tubes. The essential oil afloat on top of the condensed water was collected through a separation funnel. The corresponding condensed water was also collected and designated as water fraction A (full strength, 100%). The collected essential oils were stored in a sealed vial at 5°C before use.

After steam distillation, the residual water remaining in the flat bottom flask turned brown as a result of the reflux of vapour through the eucalyptus leaves contained in the upper distillation flask. This residual water was filtered, collected and designated as water fraction B (full strength, 100%). The collected water fractions A and B were stored in a freezer prior to use.

Bioassays of essential oils on weed germination and growth

A previous bioassay protocol (Azirak and Karaman 2008) was adopted with slight modifications. Fifty seeds of annual ryegrass or twenty-five seeds of barley grass were separately sown onto 9 cm Petri dishes lined with one layer of Whatman No.1 filter paper. Distilled water (5 mL) was initially delivered to each Petri dish. An aliquot of 0, 12.5, 25, 50 or 100 µL of *E. dundasii* essential oil was then added directly onto the filter paper to test the inhibitory effect of the essential oil. Immediately after the treatment, each Petri dish with its cover was sealed with a piece of parafilm to reduce evaporation. All Petri dishes were maintained in a growth incubator with a diurnal cycle of 30°C with light and 15°C with dark and a photoperiod of 12 h. A randomised complete block design with three replicates was used. Germinated seeds with >1 mm radicle were recorded and shoot lengths measured after 10 days of incubation.

Bioassays of water fraction A and B on weed germination and growth

The bioassay protocol developed by Wu *et al.* (2003) was adopted. A concentration series was made up from the full strength (100%) solutions of water fraction A or B into 100%, 75%, 50%, 25% and 0% (water control). Fifty seeds of annual ryegrass or twenty-five seeds of barley grass were sown onto 9 cm Petri dishes lined with one layer of Whatman No.1 filter paper. An aliquot (5 mL) of each concentration of water fraction A or B was delivered to each Petri dish. The management of Petri dishes and measurements were as previously described.

Data analysis The dose-response data were subjected to the analysis of whole-range assessment

proposed by An *et al.* (2005). The whole-range assessment considers overall effect/response across the whole range of application rates, instead of assessing the effect of each individual rate on test species. The program WESIA (Whole-range Evaluation of the Strength of Inhibition in Allelopathic-bioassay) was used to calculate the inhibition index based on the following equation (Liu *et al.* 2007):

$$I = \frac{\int_{D_c}^{D_n} [R(0) - f(D)] dD}{\int_0^{D_n} R(0) dD} = 1 - \left[\frac{D_c}{D_n} + \frac{1}{R(0)D_n} \int_{D_c}^{D_n} f(D) dD \right]$$

where the $0, D_1, D_2, \dots, D_n$ are the dose-concentrations tested and the $R(0), R(D_1), R(D_2), \dots, R(D_n)$ are the corresponding responses, respectively. The D_c is the threshold dose at which response equals the value of control and above which the responses are inhibitory. $f(D)$ represents the response function.

RESULTS

Germination Results revealed that germination of both annual ryegrass and barley grasses were inhibited by the essential oils (Figure 1a) and by the two water fractions A and B (Figure 1b). The germination of the two weeds had similar responses to *E. dundasii* essential oils. The phytotoxicity of essential oils was enhanced by increased amount of essential oils applied. The germination of both weeds was inhibited by more than 90% at the essential oil quantity of 50 µL dish⁻¹.

The germination of the two weeds responded differentially when exposed to water fractions A or B. Water fraction B was more inhibitory than fraction A (Figure 1b). For water fraction A, barley grass germination was more sensitive than ryegrass germination. However, ryegrass seed germination was more sensitive to water fraction B than barley grass.

The analysis of whole-range assessment showed that phytotoxicity of the three fractions ranked in decreasing order as essential oils, water fraction B and water fraction A (Table 1).

Seedling growth Annual ryegrass and barley grass had similar responses when exposed to essential oils or the water fraction B (Figure 2), while the weeds responded differentially to the water fraction A. Water fraction A was more inhibitory to the seedling growth of barley grass than annual ryegrass.

Essential oils caused the most inhibitory effects on shoot growth of both weeds, followed by water fraction B and fraction A (Table 1).

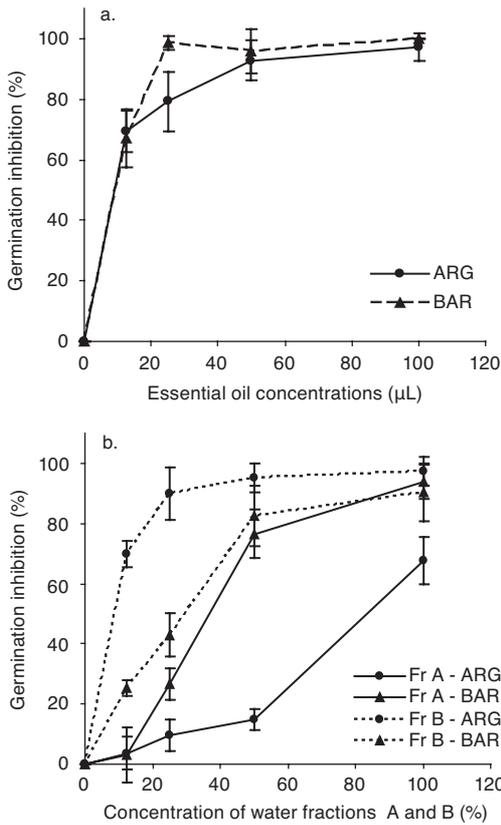


Figure 1. Germination inhibition (%) of annual ryegrass (ARG) and barley grass (BAR) by (a) eucalyptus essential oils and (b) water fractions A and B. Bars represent standard error of the mean. Percentage inhibition was calculated as (control – treated)/control*100.

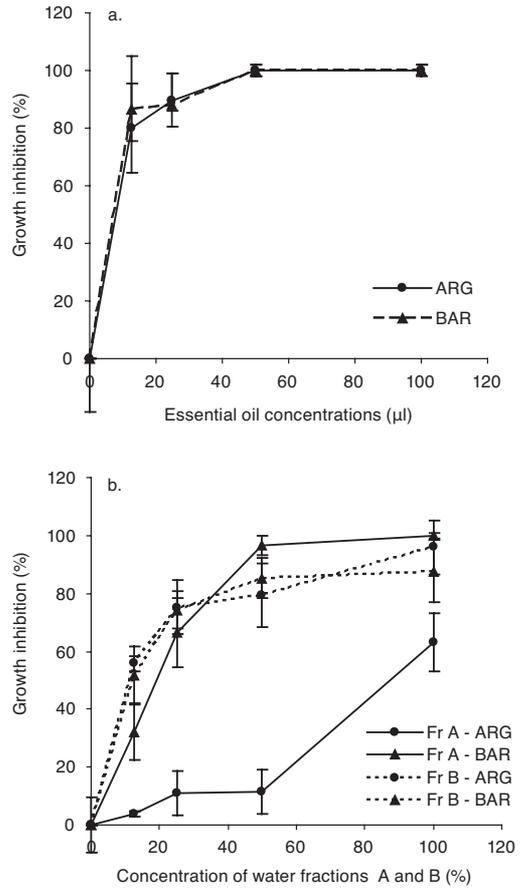


Figure 2. Growth inhibition (%) by (a) eucalyptus essential oils and (b) water fractions A and B. Bars represent standard error of the mean. Percentage of the inhibition was calculated as (control – treated)/control*100.

Table 1. Inhibition index (%) for germination and shoot growth of weeds treated with *E. dundasii* distillation fractions. The overall allelopathic potential is indicated.

Fractions	Weed species ^A	Inhibition index		Allelopathic potential
		Germination	Shoot growth	
Essential oil	ARG	85.6	86.7	Strong ↓ Weak
Essential oil	BAR	80.5	86.7	
Fraction B	ARG	75.8	65.7	
Fraction B	BAR	48.8	62.2	
Fraction A	BAR	38.8	58.7	
Fraction A	ARG	15.0	14.0	

^AARG = annual ryegrass and BAR = barley grass.

DISCUSSION

The herbicidal activity of *E. dundasii* was detected in all the three fractions tested, with essential oils being the most phytotoxic, followed by the water fraction B and fraction A. The germination of annual ryegrass and barley grass responded similarly when exposed to the essential oils. Growth of both weeds also had similar responses to the essential oils and the water fraction B. However, the germination and seedling growth of barley grass were more sensitive than annual ryegrass to water fraction A and the germination of annual ryegrass was more sensitive than barley grass when exposed to water fraction B. These results indicated that the herbicidal activities of *E. dundasii* might be selective depending on the fractions.

These results suggest that eucalyptus should be further explored as a bioherbicide for weed management. This control option could be an alternative to arrest the rapid evolution of herbicide resistance in annual ryegrass and barley grass (Owen *et al.* 2007, Heap 2010). However, it should be borne in mind that eucalyptus oils can also cause injuries to crops (Batish *et al.* 2004, Batish *et al.* 2006). It is therefore critical to maximise the herbicidal activity of eucalyptus against weeds but at the same time to minimise the negative impact on crop growth.

Further research is underway to identify bioactive compounds derived from eucalyptus. The identification of these compounds might provide chemical leads for the development of new herbicides with new modes of action.

ACKNOWLEDGMENTS

The authors are grateful for the financial support from Meat and Livestock Australia.

REFERENCES

- An, M., Pratley, J.E., Haig, T. and Liu, D.L. (2005). Whole-range assessment: a simple method for analysing allelopathic dose-response data. *Nonlinearity in Biology, Toxicology and Medicine* 3, 245-60.
- Azirak, S. and Karaman, S. (2008). Allelopathic effect of some essential oils and components on germination of weed species. *Acta Agriculturae Scandinavica, Section B – Plant Soil Science* 58, 88-92.
- Batish, D.R., Setia, N., Singh, H.P. and Kohli, R.K. (2004). Phytotoxicity of lemon-scented eucalypt oil and its potential use as a bioherbicide. *Crop Protection* 23, 1209-14.
- Batish, D.R., Singh, H.P., Nidhi, S., Shalinder, K. and Kohli, R.K. (2006). Chemical composition and phytotoxicity of volatile essential oil from intact and fallen leaves of *Eucalyptus citriodora*. *Zeitschrift für Naturforschung* 61, 465-71.
- Chandra Babu, R. and Kandasamy, O.S. (1997). Allelopathic effect of *Eucalyptus globulus* Lahill. on *Cyperus rotundus* L. and *Cynodon dactylon* L. Pers. *Journal of Agronomy and Crop Science* 179, 123-6.
- Del Moral, R., Willis, R.J. and Ashton, D.H. (1978). Suppression of coastal heath vegetation by *eucalyptus baxteri*. *Australian Journal of Botany* 26, 203-19.
- Heap, I.M. (2010). International survey of herbicide resistant weeds. www.weedscience.org (accessed 06 June 2010).
- Khan, M.A., Hussain, I. and Khan, E. (2008). Allelopathic effects of eucalyptus (*Eucalyptus camaldulensis* L.) on germination and seedling growth of wheat (*Triticum aestivum* L.). *Pakistan Journal of Weed Science and Research* 14, 9-18.
- Liu, D.L., An, M. and Wu, H. (2007). Implementation of WESIA: whole-range evaluation of the strength of inhibition in allelopathic-bioassay. *Allelopathy Journal* 19, 203-14.
- May, F.E. and Ash, J.E. (1990). An assessment of the allelopathic potential of *Eucalyptus*. *Australian Journal of Botany* 38, 245-54.
- Owen, M.J., Walsh, M.J., Llewellyn, R.S. and Powles, S.B. (2007). Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum*) populations. *Australian Journal of Agricultural Research* 58, 711-18.
- Singh, H.P., Batish, D.R., Setia, N. and Kohli, R.K. (2005). Herbicidal activity of volatile oils from *Eucalyptus citriodora* against *Parthenium hysterophorus*. *Annals of Applied Biology* 146, 189-94.
- Wu, H., Pratley, J. and Haig, T. (2003). Phytotoxic effects of wheat extracts on a herbicide-resistant biotype of annual ryegrass (*Lolium rigidum*). *Journal of Agriculture and Food Chemistry* 51, 4610-16.
- Zhang, J.B., An, M., Wu, H., Stanton, R. and Lemerle, D. (2010). Eucalyptus essential oils: chemistry and bioactivity. *Allelopathy Journal* 25, 313-30.