

Allelopathic effects of *Eucalyptus dundasii* on germination and growth of ryegrass and barley grass

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ABSTRACT

To overcome the rapid development of herbicide resistance in weeds, there is an increasing demand for alternative weed control. There is limited understory vegetation within the dripline of *Eucalyptus dundasii* Maiden in fields. We studied the allelopathic potential of *E. dundasii* on two important Australian weeds species: annual ryegrass (*Lolium rigidum* Gaudin) and barley grass (*Hordeum glaucum* Steud.). Distillation of eucalyptus leaves yielded three bioactive fractions: essential oil fraction and the aqueous fractions A and B. These 3- fractions inhibited the germination and seedling growth of annual ryegrass and barley grass. Essential oils proved most inhibitory to germination and shoot growth of both test weeds, followed by the aqueous fraction B and fraction A. Both weeds had similar responses, when exposed to essential oils. However, the germination and seedling growth of barley grass were more sensitive than annual ryegrass to aqueous fraction A and the germination of annual ryegrass was more sensitive than barley grass when exposed to fraction B. The phytotoxicity was concentration-dependent. The phytotoxic effects identified in the aqueous fraction A indicated that significant rainfall could be washed off some of compounds of essential oils into the ground to provide natural chemical defense against understory vegetation. Further study on other *Eucalyptus* species, followed by the identification of bioactive compounds, might provide chemical leads for the development of new herbicides with new modes of action.

Keywords: Allelopathy, annual ryegrass, barley grass, *Eucalyptus dundasii*, *Hordeum* spp., *Lolium rigidum*, weeds.

INTRODUCTION

Weeds are major agricultural pest causing the causing maximum loss (32%) in crop yield as compared to losses caused by insects (18%) and diseases (15%) (23). In Australia alone, weeds cause \$A4 billion per annum loss in production, poor produce quality and in control measures (26). Modern agriculture relies heavily on synthetic

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chemicals in improving the crop productivity against weeds. However the over-reliance on herbicides has resulted in the development of resistance to herbicides in weeds and the increasing public concerns about herbicide residues in the environment. In Australia, there are 35 weed species resistant to herbicides (9), including annual ryegrass (*Lolium rigidum* Gaudin) and barley grass (*Hordeum glaucum* Steud.). Annual ryegrass is the worst weed of Australian broadacre farming systems. Barley grass has become an emerging weed in Australian cropping systems (15,21) and estimated to cost \$A96 million per annum to Australia sheep industry alone (29). Annual ryegrass has developed resistance to seven major herbicide groups, including glyphosate and paraquat (16). There is a rapid increase in herbicide resistance in annual ryegrass (7). Even though to a lesser extent, barley grass has also developed resistance to ACCase inhibitors (both aryloxyphenoxypropionates – ‘fop’ and cyclohexanediones – ‘dim’ herbicides) and bipyridiliums (16). The development of herbicide resistance in these weeds has limited grower’s control options to effectively and economically manage the weeds. Alternative control options are increasingly needed to address this ever-evolving herbicide resistance issue (12,31).

In past three decades, allelopathy is intense area of research due to its prospect in integrated weed management (13,22,31,14). Allelopathy has long been recognised as one of the main mechanisms for the exclusion of understorey vegetation under some eucalyptus species (20). Del Moral *et al.* (11) demonstrated that the allelopathic potential of *Eucalyptus baxteri* suppressed the understorey growth of *Casuarina pusilla* and *Leptospermum myrsinoides*. Eucalyptus is a rich source of bioactive constituents, possessing secondary metabolites with herbicidal, fungicidal and insecticidal activities (33). The bioactive compounds derived from eucalyptus caused phytotoxicity to a range of field crops, such as wheat (*Triticum aestivum*), maize (*Zea mays*), radish (*Raphanus sativus*) and rice (*Oryza sativa*) (3,4,18).

The phytotoxic effects of eucalyptus allelochemicals have also been evaluated on many 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.) (8). The eucalyptus oil is herbicidal against *Parthenium hysterophorus*, *Cassia occidentalis*, *Echinochloa crus-galli* and *A. viridis* (3,4,28). These results suggest that bioactive compounds in eucalyptus oils have potential commercial value for further exploitation as natural herbicides (33).

There is limited understorey vegetation within the dripline of Dundas blackbutt (*Eucalyptus dundasii* Maiden) in the field. This research was conducted to assess the allelopathic potential of the eucalyptus on two important weeds, annual ryegrass (*L. rigidum*) and barley grass (*H. glaucum*).

MATERIALS AND METHODS

Fresh leaves of *E. dundasii* Maiden were collected from the fields in Ungarie, NSW, Australia. Seeds of annual ryegrass were purchased commercially and barley grass seeds were collected from our 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 1,200 mL distilled water to generate steam. The distillation process produced three fractions: essential oils and aqueous fractions A and 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 the aqueous 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 due to the reflux of vapour through the eucalyptus leaves contained in the upper distillation flask. This residual water was filtered, collected and designated as the aqueous fraction B (full strength, 100%). The collected aqueous 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 (3) 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 randomized complete block design with three replicates was used. Germinated seeds with > 1 mm radicle were recorded and shoot lengths measured after 10 d of incubation.

Bioassays of aqueous fractions A and B on weed germination and growth

The bioassay protocol developed by Wu *et al.* (32) was adopted. A concentration series [0 (water control), 25, 50, 75, 100%] was made up from the full strength (100%) solutions of the aqueous fractions A or B. The 50 seeds of annual ryegrass or 25 seeds of barley grass were sown in 9-cm dia Petri dishes lined with one layer of Whatman No.1 filter paper. An aliquot (5 mL) of each concentration of fractions A or B was added to each Petri dish. The management of Petri dishes and measurements were as previously described.

Data analysis

All the laboratory bioassays were repeated twice overtime. Combined data were used due to the insignificance differences between bioassays at two different times. The dose-response data were subjected to the analysis of whole-range assessment proposed by An *et al.* (1). 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 (19):

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

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

The germination of both annual ryegrass and barley grasses were inhibited by the essential oils (Figure 1a) and by the two aqueous fractions A and B (Figure 1b). The germination of 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 drastically inhibited (> 90%) at 50 μL dish⁻¹ essential oil.

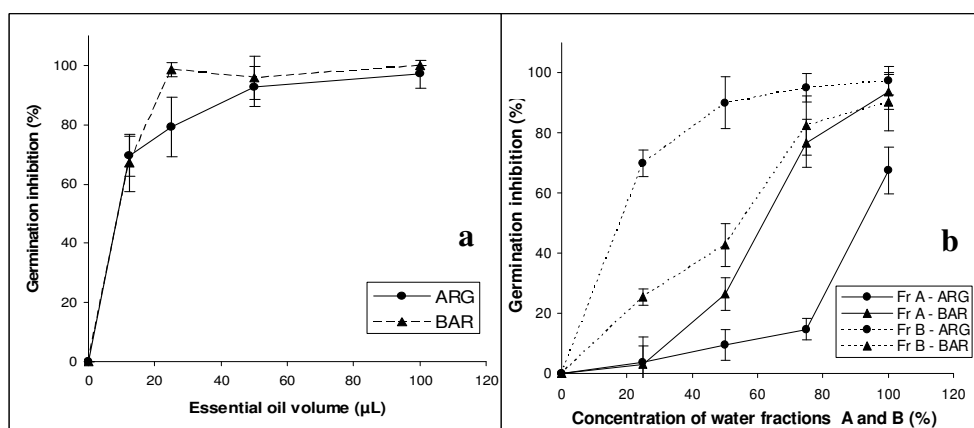


Figure 1. Germination inhibition (%) of annual ryegrass (ARG) and barley grass (BAR) by (a) eucalyptus essential oils and (b) aqueous fractions A and B. Bars represent standard error of the mean. Percentage inhibition was calculated as (control – treated)/control*100.

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

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

Table 1. Inhibition index (%) for germination and shoot growth of annual ryegrass (ARG) and barley grass (BAR) treated with *E. dundasii* distillation fractions. The overall allelopathic potential is indicated.

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

Seedling growth

Annual ryegrass and barley grass had similar responses when exposed to essential oils or the aqueous fraction B (Figure 2), while the weeds responded differentially to the fraction A. Aqueous fraction A was more inhibitory to the seedling growth of barley grass than to annual ryegrass. Essential oils caused the most inhibition in shoot growth of both weeds, followed by aqueous fractions B and A (Table 1).

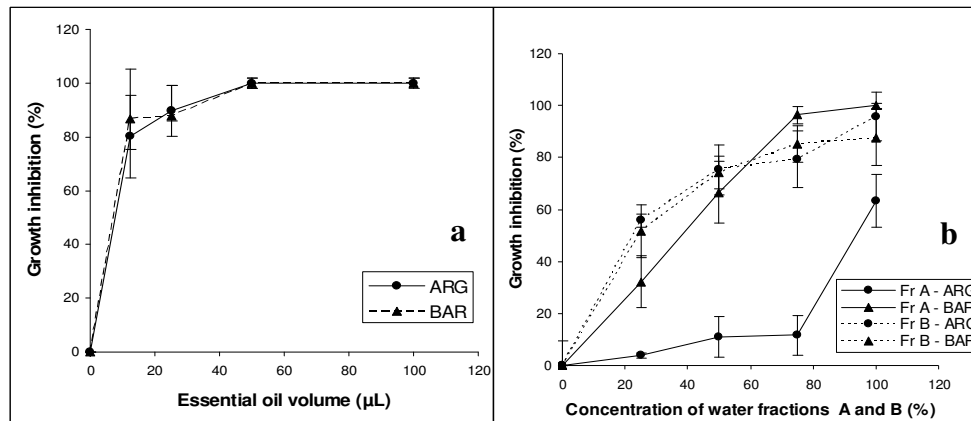


Figure 2. Growth inhibition (%) by (a) eucalyptus essential oils and (b) aqueous fractions A and B. Bars represent standard error of the mean. Percentage of the inhibition was calculated as (control - treated)/control*100.

DISCUSSION

The herbicidal activity of *E. dundasii* was detected in all three fractions tested, with essential oils being the most phytotoxic, followed by the aqueous fractions B and A, respectively. 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 aqueous fraction B. However, the germination and seedling growth of barley grass were more sensitive than annual ryegrass to the aqueous fraction A and the

germination of annual ryegrass was more sensitive than barley grass when exposed to fraction B. These results indicated that the herbicidal activities of *E. dundasii* might be selective depending on the fractions.

Zhang *et al.* (34) reported that essential oil of *E. dundasii* also inhibited the germination and seedling growth of silverleaf nightshade (*Solanum elaeagnifolium*). A total of 55 compounds were identified in the essential oil extracted from the leaves of *E. dundasii*, with 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo [2.2.2]octane) being the dominant component (66%). The herbicidal activity of 1,8-cineole has been tested on a wide range of weed species, including annual ryegrass (2,25,27). 1,4-Cineole, an analogue of 1,8-cineole, has been successfully used as a lead compound in the development of a pre-emergence herbicide cinmethylin (*O*-methylbenzyl ether of racemic 2-exo-hydroxy-1,4-cineole) for use in broadleaf crops such as soybeans [*Glycine max* (L.) Merr.] (5). Although essential oils are highly volatile, these compounds can partly dissolve in water (30), resulting in the phytotoxic activity identified in the aqueous fraction A which was co-condensed with the essential oil during distillation process.

These results suggest that eucalyptus essential oil should be further explored as a bioherbicide for weed management (10). This control option could be an alternative to arrest the rapid development of herbicide resistance in annual ryegrass and barley grass (16,24). However, it should be borne in mind that eucalyptus oils can also cause injuries to crops (3,4). 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.

It has been estimated that there are over 800 eucalyptus species in 13 subgenera (6) widely spread in Australia (17). The diverse range of Eucalyptus species provides a unique opportunity to screen and evaluate their allelopathic activity against some of the most important weeds in Australian broadacre production systems. Identification of bioactive compounds derived from eucalyptus might provide chemical leads for the development of new herbicides with new modes of action.

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