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*Acacia holosericea* (Fabaceae) litter has allelopathic and physical effects on mission grass (*Cenchrus pedicellatus* and *C. polystachios*) (Poaceae) seedling establishment

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**Abstract**

Invasion of grass weeds is a major threat for ecosystems. Mission grass (*Cenchrus pedicellatus* and *C. polystachios*) vigorously competes with native vegetation and has become a serious problem in northern Australian savanna. A lower density of mission grass has been observed under the canopy of stands of native *Acacia holosericea*. We used a series of laboratory and shade house experiments to assess the potential for allelopathy and the role of litter on germination, emergence and seedling growth of these two species of mission grass. Different concentrations of aqueous leaf extract of *A. holosericea* were used to assess allelopathic effects on germination. Various depths and types of litter were used to investigate the allelopathic and physical effects of litter on emergence and growth of mission grass seedlings in the shade house. Results indicate that extracts did not affect germination of either species of mission grass but root growth of seedlings was affected. Emergence of seedlings in the shade house was affected by physical litter treatments but not by allelopathy. After emergence no negative effects on seedling growth were detected. Overall we found that there was no allelopathic effect on germination and that the negative effect on emergence was due to the physical properties of the litter. This effect on emergence increased with increasing depth of litter. Allelopathy slightly inhibited root growth but once seedlings emerged, litter tended to facilitate growth. This has implications for the ecological management of mission grass on disturbed lands, using strategies such as manipulation of litter cover through *Acacia* establishment.

**Key words**: allelopathy, germination, emergence, suppression, grass weed

**Introduction**

Leaves of the Australian native species *Acacia holosericea* A.Cunn. ex G.Don may have an allelopathic or physical impact on establishment of two exotic species of mission grass (*Cenchrus pedicellatus* Trin and *C. polystachios* L. Morrone). These two African grass species were introduced to Australia as fodder crops and have become environmental weeds (Douglas *et al.* 2006; Miller 2006). These exotic grass species have the ability to change the ecosystem by altering the fire regimes and nutrient dynamics (Miller 2006; Brooks *et al.* 2010). Woodlands of the Northern Territory are dominated by *Eucalyptus* and *Acacia* trees. Field observations show that mission grass understorey is reduced below stands of *A. holosericea* trees in northern Australia. This may be due to allelopathy or physical effects of the leaf litter underneath these stands. If so then *A. holosericea* could potentially be used to control mission grass establishment on managed disturbed sites, such as on topsoil stockpiles of mine sites.
Plant allelopathy is defined as the effects of the chemical compounds involved in plant-plant interactions (Rice 1984; Kruse et al. 2000). Plants may favourably or adversely affect other plants through allelochemicals, which may be released directly or indirectly from living or dead plants (Putnam and Duke 1978; Rice 1979; Rice 1984; Putnam and Tang 1986; Kruse, Strandberg et al. 2000; Zimdahl 2007). Allelopathy is defined as any process involving secondary metabolites produced by plants, algae, bacteria and fungi which influence the growth and development of agricultural and biological systems (Romeo 2000; Macias et al. 2007). Most plants have the potential to produce chemicals that can inhibit or stimulate other plants. Allelopathy works in three steps. In the first step phytotoxic chemicals are produced, in the second step these chemicals are transported from donor to recipient, and in the last step target plants are exposed to chemicals for a sufficient time and concentration to have an effect (Aldrich 1984; Lovett and Ryuntyu 1992). Many types of allelochemicals are released from plants, affecting germination and growth of target plants. Plant parts that have been implicated in the production of phytotoxic chemicals include roots rhizomes, stems, leaves, flowers, inflorescences and seeds, and their impact varies from species to species. These chemicals are released to the environment through root exudation and leachates from litter (Rice 1984). For example, extracts from leaves of Acacia nilotica and Eucalyptus rostrata reduced the growth and germination of Zea mays and Phaselus vulgaris (El-Khayas and Shehata 2005). Aqueous leachates of Eucalyptus globulus leaves reduce the chlorophyll content in leaves of Eleusine coracana (Padhy et al. 2000). Eucalyptus baxteri releases allelochemicals which suppress understorey vegetation (Lovett 1986). Mousavi et al. (2013) compared extracted solutions from different organs of Melilotus indicus and found that the leaf extract had the highest inhibitory effect on germination and seedling growth of Triticum aestivum. Foliar leachates of many Australian woody plants inhibit the germination of Avena fatua (Hobbs and Atkins 1991). Leaf extracts typically have greater inhibitory properties than root extracts (Chon 2010).

Accumulation of litter is an important factor affecting the establishment of plants (Facelli and Pickett 1991b). Litter of different depths can create different micro sites for plant establishment (Molofsky and Augspurger 1992), which affects the structure and composition of plant communities (Facelli and Pickett 1991b; Baker and Murray 2010). The effect of leaf litter on plant recruitment can be positive, negative or neutral. Negative effects of leaf litter other than allelopathy can be due to a mechanical barrier limiting radicle or shoot growth, or due to modification of the quality and quantity of light reaching seeds. Negative effects of litter on germination and seedling growth often increase with increased depth of litter (Xiong and Nilsson 1999). Leaf litter modifies the soil micro climate by changing the radiation reaching the soil surface, as well as moisture, temperature and nutrient levels in the soil (Facelli and Pickett 1991b). Litter can increase seedling establishment by improving soil moisture (Facelli and Pickett 1991b) and by protecting the seeds from predators (Cintra 1997). The impact of litter on seedling establishment is more likely to be positive when it is present in small quantities (Loydi et al. 2013). The effect of litter also varies with species and seasons (Facelli and Pickett 1991a; Ruprecht et al. 2010).

We evaluate the impact of the leaf litter of A. holosericea on C. pedicellatus and C. polystachios germination in the laboratory and on seedling emergence and growth in a shade house. We address the hypothesis that leaves of A. holosericea suppress the germination, emergence and growth of mission grass via allelopathy and physical mechanisms. The laboratory study investigated the allelopathic potential of A. holosericea on seed germination and seedling growth of two species of invasive mission grass. The shade house study investigated the effect of different types and depths of litter on seedling emergence and
growth. Implications are discussed for using *A. holosericea* for the ecological control of mission grass in disturbed sites to assist in rehabilitation. Bioassays are the most common and widely accepted technique used to investigate the effect of allelopathy in the laboratory (Lovett and Ryuntyu 1992). Laboratory bioassays need to be supplemented with nursery trials, as laboratory trials may create chemical conditions which do not occur in field.

**Material and methods**

*Plant sampling and preparation of aqueous extract*

Mature seeds of annual *C. pedicellatus* and perennial *C. polystachios* were collected in May 2013, seven months before the start of this study. Seeds were stored at a constant air temperature of 18°C in paper bags. Germinability immediately before the experiment was 99%.

Mature leaves of *A. holosericea* were picked from plants growing on a disturbed site at the Charles Darwin University, Darwin, Australia (12°22'S, 130°52'E). These leaves were rinsed with distilled water to remove dust particles (Sarkar *et al.* 2012) and then placed on a 0.5 cm layer of soil in a dry shaded area. They were kept there for four weeks to let them dry thoroughly and to allow interactions with soil bacteria and fungi. After drying, the leaves were crushed gently by hand to allow leaching of compounds from the leaf. Leaves were not ground, as grinding of plant material can disrupt cellular integrity which impacts on the release of allelochemicals (Putnam and Duke 1978; Inderjit and Dakshini 1995).

Leaf material was soaked in distilled water at the rate of 10, 20, 40 and 80 g of dry leaf L\(^{-1}\), at 29 ± 1°C for three days as described by Al-Humaid and Warrag (1998) and Warrag (1995). This mixture was then filtered through Whatman filter paper no. 4 (Heisey 1990). The pH of these solutions was measured by using a TPS digital pH meter (LC80A). The electrical conductivity was measured using a Hanna HI-8733 conductivity meter. The pH ranged from 5.46 to 5.27 and the EC ranged from 0.00 mS cm\(^{-1}\) to 1.19 mS cm\(^{-1}\) (40 g leaf L\(^{-1}\)) and 2.4 mS cm\(^{-1}\) (80 g leaf L\(^{-1}\)). Solutions were then refrigerated in plastic bottles at 5°C.

Plastic Petri dishes (90 mm diameter) were lined with two sheets of Whatman No. 1 filter paper. Twenty five seeds were placed onto the filter papers in each Petri dish, and then moistened with 5 ml of one of the four aqueous solutions of *A. holosericea* leaf extract along with a control moistened with distilled water (Rejila and Vijayakumar 2011). Four replicate Petri dishes were arranged in a randomized block design for the bioassay for both grass species. The Petri dishes were sealed in plastic bags to reduce evaporation and placed in growth chambers at 25-32°C and 12/12 hours dark/light photoperiods.

Seeds were considered germinated where the radicle of the hypocotyl exceeded 1 mm. The number of germinated seeds was counted daily and the root and shoot length of all seeds that had germinated were measured with a ruler at five days after sowing. Germination rate was calculated as the average time until germination occurred using the following equation.

\[
\text{Germination rate} = \frac{G_1/t_1 + G_2/t_2 + G_3/t_3 + G_4/t_4 + G_5/t_5}{n}
\]

Where G was the percentage of seeds that germinated on that day and t was the number of days into the germination period.
Shade house study of leaf litter

The effect of the different litter types on seedling emergence and growth of *C. pedicellatus* and *C. polystachios* was investigated in germination trays in a shade house at Charles Darwin University. Litter treatments comprised fresh *A. holosericea* leaves, dried *A. holosericea* leaves and synthetic litter (Detpak brown paper bags A2317).

To select the appropriate materials to create synthetic litter, a pilot study was carried out to evaluate a selection of materials similar to those used in other studies (Barritt and Facelli 2001; Harris et al. 2003; Rotundo and Aguiar 2005). Packing strips (Signode polypropylene strapping), paper folders (cardboard file folder), shade cloth (90% universal shade cloth), polypropylene bags (woven polypropylene bags) and paper bags (Detpak brown paper bags A2317) were tested. Paper bags showed similar results to natural litter in colour, volume, packing structure and physical response to wetting and drying.

Fresh leaves were obtained by cutting mature leaves from trees the day before the seeds were sown. Leaves were rinsed with deionised water before being placed on the trays. Dry leaves were prepared by collecting fresh leaves, which were then rinsed and placed as a 0.5 cm layer on soil for four weeks in a dry shaded area, before being placed on the trays. Synthetic litter was made by cutting the paper bags into strips the same size as a typical *A. holosericea* leaf, 3.5 cm wide by 12.5 cm long.

Treatments were: 1 cm depth of fresh leaves, 1 cm and 3 cm depths of dried leaves, 1 cm and 3 cm depths of synthetic litter, two concentrations of *A. holosericea* leaf extract aqueous solutions (40 g leaf L\(^{-1}\) and 80 g leaf L\(^{-1}\)) and a control with no litter or extract treatment. Leaf litter depths of 1 cm and 3 cm were chosen as they reflect the depth of litter typically found under field conditions (unpublished data). Field measurements of mass per unit area of dry litter were used to calibrate the amount of litter used to create 1 cm and 3 cm treatments.

To estimate the amount of paper leaves required for the synthetic litter treatment, we used the equivalent area of leaves used in the dry litter treatment. Leaf area was measured with a leaf scanner (Epson Perfection V33) and Image J software (developed by Wayne Rasband National Institutes of Health, Maryland). On the basis of this measurement, the weight of synthetic litter required to create the two depth treatments was calculated.

Topsoil was collected from the field away from the *Acacia* plants and was sieved through a 10 mm sieve to remove gravel from the shallow lateritic soil. This sieved soil was then mixed with 20% coco peat for better drainage to avoid water logging and placed in 34 x 28 x 5 cm seedling trays. Each tray was divided into halves using a plastic barrier; one half sown with 50 seeds of *C. pedicellatus* and the other sown with 50 seeds of *C. polystachios*. Four replicate trays were used per treatment. Germination trays were arranged randomly in a shade house.

Seeds were sown into each germination tray prior to the placement of the leaf litter treatments on top of the soil. Germination trays were watered manually daily and emergence was recorded on a daily basis for three weeks. At the end of the experiment, three weeks after sowing, the number of tillers were counted and root and shoot length were measured using a ruler. Root and shoot dry weights were also recorded after harvest. Soil was carefully washed from the roots and the seedling samples were dried in an oven at 65°C for 24 hours.
The effect of leaf extracts and litter treatments on germination, emergence and growth was analysed separately for each species using one way ANOVA. Analyses were performed using STATISTICA 11 (StatSoft, Tulsa, USA). Data were transformed where necessary and Tukey’s test was performed for post hoc comparison of means.

Results

Seed germination and seedling growth bioassay
For the laboratory bioassay using the aqueous leaf extracts, seed germination was high in all treatments. There were no significant effects of the aqueous solutions of A. holosericea leaf extract on germination of C. pedicellatus or C. polystachios in the laboratory experiment (P>0.05; Fig. 1). Germination for C. pedicellatus ranged from 95-99% and C. polystachios from 87-100%.

Seedling root growth in the laboratory was significantly affected by the treatments for both species (P<0.05; Fig. 2a and b). The shortest mean root length for C. pedicellatus occurred in the 80 g leaf L⁻¹ solution treatment (15.2 ± 0.4 mm) and the longest mean root length in the control (33.6 ± 0.5 mm). C. polystachios also had longest mean root length in the control (25.7 ± 1.7 mm) and shortest (4.3 ± 0.2 mm) in the highest extract concentration (80 g leaf L⁻¹). Differences in shoot length were significant for C. pedicellatus (P<0.05) but not C. polystachios (P>0.05). Shoot length of C. pedicellatus was smallest when exposed to the most concentrated extract solution (80 g leaf L⁻¹) and this was significantly different to that in the 20 g leaf L⁻¹ and 40 g leaf L⁻¹ treatments.

Seedling emergence through leaf litter
In the shade house trial there was a slight allelopathic effect on one species. Leaf extracts of A. holosericea significantly affected emergence of C. polystachios (P<0.05) at the highest concentration but there was no effect on the emergence of C. pedicellatus (P>0.05), which had 80 – 90% emergence in the extract treatments and control (Fig. 3a). Only 74% emergence of C. polystachios occurred when exposed to the most concentrated extract solution (80 g leaf L⁻¹) and this was significantly different to the 90% germination occurring in the control (Fig. 3b).

Different litter treatments affected the emergence of C. pedicellatus and C. polystachios seedlings and the effects were greater than the allelopathic responses to treatments involving application of aqueous leaf extracts. Increases in litter depth significantly decreased emergence (P<0.05). Emergence of C. pedicellatus was 88% in the control, 38% in trays with 1 cm of dry leaf litter and only 10% in trays with 3 cm of dry leaf litter (Fig. 3a). Emergence in trays with 1 cm of fresh leaf litter was significantly lower as compared to control and significantly higher than the dry litter treatments. The effects of the synthetic litter treatments were very similar to those of the equivalent depths of dry leaf litter.

Effects of the litter treatments on emergence of C. polystachios seedlings were similar to C. pedicellatus (P<0.05; Fig. 3b). However, the degree of suppression of C. polystachios emergence was greater. The suppression of emergence by fresh leaves was significantly different to that under dry and synthetic litter. There were no significant differences in emergence between the dry leaf litter and the same depth of synthetic litter. Mean emergence in the 3 cm litter treatments was about half that of the 1 cm litter treatments but the
differences were not significant. Emergence was reduced from 90% in the control to just 4% in the treatment with 3 cm depth of dry leaf litter.

**Allelopathic effect on growth of seedlings in the shade house**

The aqueous leaf extracts of *A. holosericea* had no effect on shoot length of *C. pedicellatus* or *C. polystachios* or on root length of *C. pedicellatus* (*P* > 0.05; Fig. 4a and b). Root length of *C. polystachios* was significantly shorter (*P* < 0.05) in the highest extract concentration of 80 g leaf L⁻¹ as compared to the control, although the 40 g leaf L⁻¹ treatment was not significantly different to the control or 80 g leaf L⁻¹ extract treatment (Fig. 4b).

The different litter treatments had no significant effect on shoot or root growth of *C. pedicellatus* or *C. polystachios* (*P* > 0.05) but results were very variable between plants. Mean shoot length of *C. pedicellatus* was 104 ± 11 mm in the control and a mean maximum of 293 ± 24 mm occurred in the 1 cm dry leaf litter treatment (Fig. 4a). Root lengths varied consistently with shoot lengths. Similarly the *C. polystachios* treatment with the longest mean shoot length (171 ± 38 mm) was the dry leaf litter to 1 cm depth and mean shoot growth in the control was 109 ± 29 mm (Fig. 4b).
Discussion

Leaf extracts of A. holosericea had some limited effects on C. pedicellatus and C. polystachios in the laboratory. The leaf extracts did not affect the proportion of seeds that germinated. However seedlings had reduced root growth. Chon et al. (2002), Olson and Wallander (2002) and Kelsey and Locken (1987) also found that root growth was more sensitive to toxicity as compared to germination percentage or shoot length. This effect was not due to pH differences as there was little difference in pH between the strongest solution and the control. EC differences between the extracts were also small but the EC of the strongest solution may just be sufficient to affect the growth of sensitive species (Landon 1991). However, bioassay trials are used only to determine that plant to plant interactions occur. Whether this is ecologically important needs to be confirmed in more natural conditions (Inderjit and Moral 1997; Inderjit and Nilsen 2003) and so the findings of the shade house experiment must also be considered.

When the A. holosericea leaf extracts were applied to soil the effect on mission grass was minimal. Only a minor effect on C. polystachios emergence at the highest extract concentration was observed and there was no significant effect on emergence of the annual C. pedicellatus. This inhibition effect could be due to the osmotic concentration of the extract rather than due to particular toxicity effects. For example, Chou et al. (1998) found for Acacia confusa, that inhibition can occur both due to osmotic concentration of the extract and phytotoxicity. The osmotic concentration of their 5% extracts ranged from 40 to 50 mosmol. Normally when osmotic concentration exceeds 50 mosmol, it may cause inhibition of emergence. Regardless, there is little evidence of allelopathy.

Both the laboratory and the shade house results in our study suggested that at most there is minimal influence of allelopathy on germination and emergence. Allelopathic effects can vary with time, as allelochemicals can be toxified or detoxified in soil by microorganisms (Inderjit 2001; Bhadoria 2011). Gonzalez et al. (1995) suggested that continuous presence of Acacia leaves on soil might be responsible for toxicity and that they are more toxic during the early period of decomposition (Souto et al. 1994). If this were so for A. holosericea we would have expected it to occur in the fresh leaf treatment.

Litter can affect seedling emergence due to physical, chemical or biological factors or a combination of these factors (Facelli and Pickett 1991b; Cavieres et al. 2006). Litter accumulation alters the physical environment by changing light conditions and soil temperature. Light quality, light quantity or temperature conditions received by seeds can inhibit seed germination and emergence. Chemical factors affecting emergence could be through the release of nutrients, chemicals stimulating germination or toxic chemicals (Facelli and Pickett 1991b). Biological impacts of litter can be through changes to the soil biology influencing fungal and non-fungal diseases killing seeds and seedlings (Facelli and Pickett 1991b; Rotundo and Aguiar 2005). However, our study determined the major effect on mission grass was physical with a minor chemical impact.

Seedling emergence of both species of mission grass decreased considerably with increased depth of litter. This effect was not via allelopathy, as the effect of synthetic litter was similar to that of Acacia leaf litter. Barritt and Facelli (2001) documented that litter reduced seedling emergence and that natural and artificial litter can have the same physical effect on seedling emergence. They assessed the effects of Casuarina pauper litter on the emergence and growth of an introduced annual forb, Carrichtera annua and a native grass Danthonia caespitosa. They concluded that litter had strong and consistent negative effects on the
emergence of the seedlings of both species due to the physical barrier provided by litter. Baker and Murray (2010) also found that increased leaf litter depth reduced emergence and establishment. Hamrick and Lee (1987) observed that hypocotyl length of Carduus nutans was longer under high litter conditions as compared to less or no litter and suggested that mortality was higher due to the use of more stored energy used to penetrate the litter layers. This extra use of energy weakened the seedlings and many died before reaching the surface or soon afterwards. Our study concurs with these previous findings.

Small seeds are more susceptible to negative effects compared to large seeds. Mission grass has a small seed and seed size can influence whether litter has a negative or positive effect on emergence. In contrast to our findings, Molofsky and Augspurger (1992) trialled different litter depths and found that Gustavia seedling emergence was greater under litter than on bare ground. This positive effect on Gustavia emergence was due to the higher moisture and humidity in soil with litter cover and the seedlings of Gustavia are shade tolerant. However they also found that emergence of small seeded Luehea, Ochroma and Ceiba were negatively affected by litter and this effect increased with increases in litter depths.

Subsequent to emergence, the positive effects of litter treatments on growth might be due to moisture conservation (Xiong and Nilsson 1999). Favourable growth could be due to benefits of reduced evaporation and increased water holding capacity. Litter may serve as a source of nutrients and soil insulation from high temperatures (Cheplick and Quinn 1987; Facelli and Pickett 1991c; Facelli and Brenton 1996).

While allelopathic effects of A. holosericea did not affect the proportion of seedlings that emerged in the shade house, it inhibited the seedling growth of C. polystachios at the highest concentration, affecting root growth. These findings are consistent with other studies. For example, percent germination, shoot length and root length of rice and cow peas have been shown to decrease due to Acacia auriculiformis leaf leachates, and root and shoot length were affected more than germination (Hoque et al. 2003; Oyun 2006). A. nilotica and E. rostrata released allelochemicals which reduced the growth of Z. mays and P. vulgaris (El-Khawas and Shehata 2005; Bargali and Bargali 2009). Lorenzo et al. (2011) reported the inhibitory effects of Acacia dealbata on understory Dactylis glomerata, and suggested that allelopathic interference seems to contribute to this process. Many other Australian trees, and especially Eucalyptus species, produce allelochemicals which affect the understory vegetation (Bowman and Kirkpatrick 1986; May and Ash 1990). While not completely suppressing establishment of seedlings, reduced root development in seedlings may contribute to a reduction in the development of understory.

Field observations show that mission grass understory is reduced below A. holosericea trees in northern Australia. From this study it is concluded that there is no effect of allelopathy on germination, but that litter has a negative physical effect on emergence and this is greater for thicker litter layers. Allelopathy may have a slight inhibitory effect on seedling root growth but after emergence thin litter layers could later have a facilitative effect. The physical impact of litter is more important than allelopathy on the establishment and growth. A. holosericea has relatively thick robust leaves which provide more of a physical barrier than small and thin leaved species. The slight effect of allelopathy in reducing grass seedling root length may increase water stress which would become more critical in the field when combined with competition with trees. Thus the findings of this study point to the control of mission grass establishment in the field, by the physical impact of dense A. holosericea leaf litter, combined with a mild allelopathic effect on seedling root growth and with tree-grass competition.
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Fig. 1. Effects of leaf extract of *A. holosericea* on germination of *C. pedicellatus* (■) and *C. polystachios* (□). Values are mean ± s.e. (n=4).
Fig. 2. Effects of leaf extracts of *A. holosericea* on shoot lengths and root lengths of (a) *C. pedicellatus* (b) *C. polystachios*. Values are mean ± s.e. (n=4). Different letters indicate significant differences determined by Tukey’s HSD within root or shoot values only. Significant differences of shoot length are denoted by lower case letters whereas significant differences of root length are denoted by upper case letters ($P<0.05$).
Fig. 3. Effects of different litter treatments and leaf extracts of *A. holosericea* on emergence of (a) *C. pedicellatus* (b) *C. polystachios*. Values are mean ± s.e. (n=4). Different letters indicate significant differences determined by Tukey’s HSD ($P < 0.05$).
Fig. 4. Effects of different litter treatments and leaf extracts of *A. holosericea* on shoot length and root length of (a) *C. pedicellatus* (b) *C. polystachios*. Values are mean ± s.e. (n=4). Within either root or shoot values, bars that do not share the same letter are significantly different as determined by Tukey’s HSD. Significant differences in shoot length are denoted by lower case letters and significant differences in root length are denoted by upper case letters (*P* < 0.05).