Effects of pine oil, sugar and covers on germination of serrated tussock and kangaroo grass in a pot trial

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Summary A pot trial investigated the use of an essential oil (pine oil) with or without adjuvants (sugar, Thatch Busta) and covers (plastic, carpet, mulch, biochar) for control of a small seeded C_3 weedy exotic grass serrated tussock (*Nassella trichotoma* (Nees) Hack. ex Arechav.) and the large seeded C_4 indigenous grass, kangaroo grass (*Themeda triandra* Forssk.). Seed bank experiments showed significant pine oil dose response effects on reducing seed germination of both grass species. Covers improved the reduction in seed germination for serrated tussock caused by pine oil but gave little improvement for kangaroo grass. It also showed interactions between pine oil and sugar with serrated tussock seed germination reduced by 98–100%. This is a comparable result to the grass selective herbicide, flupropanate applied as a pre-emergent herbicide and suggests that organic seed bank manipulations with essential oils and carbon could be used as a management tool in appropriate circumstances.

Keywords Pine oil, serrated tussock, kangaroo grass, sugar, covers.

INTRODUCTION

The use of conventional herbicides for weed seed bank control is confined to pre-emergent herbicides or soil fumigants such as methyl bromide (now phased out of use due to environmental concerns) (US Department of Agriculture, 2000). Increasingly, the reliance on use of synthetic herbicides has resulted in environmental (Freeman and Boutin 1995) and human health issues (Cox and Surgan 2006, Weisenberger 1993) and is also leading to increasing incidences of herbicidal resistance among many weed species (Heap 2001). Therefore, efforts to develop alternative means of weed control, which are not only eco-friendly, but also cost effective and biologically robust are required (Duke *et al.* 2002).

Essential oils comprise a complex mix of volatile low-molecular weight isoprenoids, monoterpenes and sequisterpenes and some exhibit herbicidal activity (Angelini *et al.* 2003, Batish *et al.* 2004, Koli *et al.* 1998, Mathews *et al.* 2006). Pine oil has been used with some success to control branched broomrape (*Orobanche ramosa* L.) seed banks in a South Australia weed eradication program (Mathews *et al.* 2006).

The addition of carbon (sugar, wood chips or sawdust) to soil has been shown to increase microbial populations and CO_2 production as a consequence while also reducing soil nitrogen (Eschen *et al.* 2007, Jonasson *et al.* 1996). The addition of sugar has been shown to reduce germination of Chilean needle-grass (*Nassella neesiana* (Trin. & Rupr.) Barkworth) in a grassland seed inundation trial (Faithfull 2012) but the causal mechanism is unknown. This experiment was set up to investigate the effects of the essential oil (pine oil) and carbon (sugar) on the germination of a relatively small seeded C_3 exotic grass (serrated tussock) and a large seeded C_4 indigenous grass (kangaroo grass) and whether covers could affect possible fumigant effects of the pine oil.

MATERIALS AND METHODS

Summary Sixty nine different treatments, that had the potential to reduce the germination or survival of newly germinated seed, were applied to pots in a designed glasshouse experiment. This paper reports the germination of serrated tussock and kangaroo grass from 60 of those treatments that examine the response to different rates of pine oil application, and how the responses are affected by three adjuvant treatments and five soil covering treatments, in a factorial arrangement. For comparison, the survival of germinated seed after herbicide (flupropanate) application is also reported.

Experimental procedure Mature seeds of serrated tussock and kangaroo grass were used for this trial. Five hundred serrated tussock and 50 kangaroo grass seeds were counted and spread evenly onto the top of 175 mm diameter plastic pots containing a commercial top-soil potting mix. The seeds were then covered by 1 cm of the same commercial top-soil and gently rolled to compress the soil and produce a smooth even surface allowing approximately 3 cm at the top of the pot for addition of other treatment materials. To

imbibe seeds, all pot treatments were saturated with water for 24 hours before treatment application. Pots were maintained in a glasshouse at 20–25°C receiving automatic overhead watering of three minutes duration per day for the duration of the experiment. Pine oil (BioWeed™) (Certified Organics Pty Ltd) is an essential oil extracted from *Pinus radiata* D.Don. Pine oil treatments were applied using a watering can applying individual rates of 0, 2.5%, 5%, 10% applied at 2 L m^{-2} to individual pots. Sugar treatments were applied directly to the soil surface directly after pine oil application at a rate of 0.31 kg C as weighed granules. Thatch Busta™ (BioStart Pty Ltd) is a naturally fermented product containing signal metabolites that stimulate saprophytic fungi to start the decomposing process to turn dead organic matter (thatch) into soil humus. Thatch Busta was applied at the recommended 3 L ha^{-1} rate using a watering can. Cover treatments (nil, plastic (100 µm thick), carpet (8 mm synthetic cut pile), biochar (chicken waste – applied at 37 g C kg^{-1} (10 tons ha^{-1})) and mulch (oversize green mulch – applied at 90 g per pot)) were applied directly to soil surface of individual pot treatments after application of essential oils, sugar and Thatch Busta. Plastic and carpet covers were cut into disks made to fit inside the 175 mm plastic pots and were then removed one week after treatment to enable seed germination. The herbicide, flupropanate was included as a single extra treatment. Pots containing the buried seeds were sprayed with Taskforce® (745 g a.i. L ha^{-1} flupropanate) using a mechanical track sprayer in a spray cabinet with a flat nozzle (AI11002), to deliver a spray volume of 150 L ha^{-1} at 280 kPa at the recommended field rate (1.49 kg a.i. L ha^{-1}). Eight other treatments were included in the experimental set-up, but are not reported here.

Monitoring seed dormancy Attempts were made to use tetrazolium to assess seed survival at the end of the trial. This was confounded by some seeds staining pink all over compared to control seeds that just stained bright red within the seed embryo. We believe this was soil microbe contamination. Seed squash tests showed that most treated seeds were brown and discoloured suggesting they were dead. Two pots treated with 5% pine oil were monitored 1.5 years after treatment. Only one serrated tussock and no kangaroo grass seedlings had emerged suggesting seed was dead.

Experimental design The experiment was a 69 treatment, three replicate randomised complete block design with single pots as the experimental unit and blocks consisting of 69 pots on a single bench. The 69 pots in each block were rotated around the bench, in an ad hoc manner, on a weekly basis and blocks of pots were rotated between benches about every five weeks. For each pot, seedlings were harvested and counted on 25/01/2012, 75 days after treatment applications.

Statistical analysis The square root of the number of adjuvants (sugar and Thatch Busta) is divided into two orthogonal comparisons, namely (i) the effect of sugar versus the average of no adjuvant and Thatch Busta adjuvant and (ii) the effect of Thatch Busta versus no adjuvant. All 69 treatments were used in calculating the residual variation used in the analysis of variance. One pot, with a large observed germination for a pot with 5% pine oil was deleted as an outlier. Germination responses are presented graphically as back transformed means on a square root scale so that standard errors of difference can easily be presented.

RESULTS

There was no evidence ($P > 0.05$) that germination of either serrated tussock or kangaroo grass in the presence of Thatch Busta adjuvant differed to the germination when no adjuvant was present. Consequently, all results presented without sugar are for the no adjuvant and Thatch Busta adjuvant were combined.

There was no evidence ($P > 0.05$) of a pine oil concentration by sugar application by cover type interaction for percentage germination of kangaroo grass. There was statistical evidence ($P = 0.005$) of a pine oil concentration by sugar application by cover type interaction for percentage germination of serrated tussock, but the effect was both hard to explain and small compared to other effects observed. As a consequence, it was decided to present results for the effect of pine oil with or without sugar only.

Increasing pine oil concentration decreased germination of both serrated tussock ($P = 1.8 \times 10^{-94}$) and kangaroo grass ($P = 1.9 \times 10^{-55}$), to the extent that, at 10% pine oil, germination was similar or lower to seedlings surviving the flupropanate application (Figure 1). The addition of sugar improved the effectiveness of the pine oil to the extent that, at 2.5% pine oil, germination was similar or lower to seedlings remaining with the flupropanate application (Figure 1). All cover treatments except biochar at 5% pine oil, reduced seed germination for serrated tussock ($P = 0.015$). All cover treatments reduced seed germination for kangaroo grass at the 2.5% pine oil rate but this trend was not consistent at the 5% and 10% pine oil rates ($P = 0.10$).

DISCUSSION

This trial showed a significant dose response effect of pine oil reducing seed germination of both serrated tussock and kangaroo grass. Previous studies have

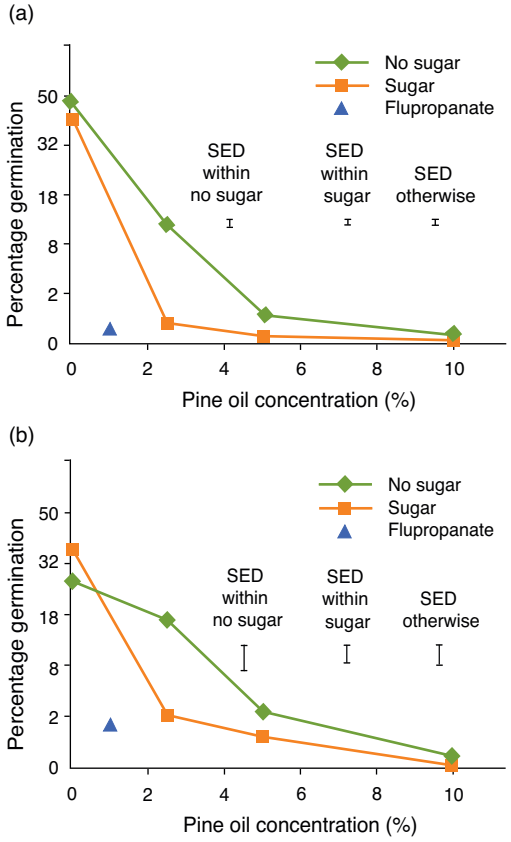

Figure 1. Effect of pine oil concentration, with or without addition of sugar, on percentage of (a) serrated tussock and (b) kangaroo grass germinating for pots excluding the flupropanate treatment. No sugar includes Thatch Busta adjuvant treatments. For comparison the flupropanate back transformed mean is also presented. Note that SED values are only for non-flupropanate treatments.

shown that essential oils can reduce seed germination (Angelini *et al.* 2003, Kohli *et al.* 1998, Mathews *et al.* 2006, Batish *et al.* 2004, Mathews *et al.* 2006). Being typical lipophiles, essential oil cytotoxicity is usually caused by the essential oil molecules passing through the cell wall and the cytoplasmic membrane, disrupting the structure of the different layers of polysaccharides, fatty acids and phospholipids and permeabilising them (Bakkali *et al.* 2008). It is believed that high application rates of pine oil can affect the waxy seed coat of the seed lemma within the seed bank, particularly if the seed is imbibed before application (John Mathews personal communication). Kremer and Spencer showed that mechanical damage to seeds especially in the seed coat, makes seeds more susceptible to invasion by microbes. We propose that microbial attack was partly responsible for the observed reduced seed bank germination observed for the pine oil treatments in this trial.

Previous trials have shown that vapours of certain essential oils can be phytotoxic to a number of other plant species (Koli *et al.* 1998, Dudai *et al.* 1999) and that pine oil vapour can have a fumigant effect and will reduce serrated tussock seed germination (McLaren unpublished data). This trial also investigated whether the efficacy of pine oil could be improved by capturing the pine oil fumes using covers. Covers did result in decreased germination of serrated tussock seeds with the carpet treatment being the most effective. Plastic covers were not sealed to the ground edges which may have reduced performance. Covers did not affect kangaroo grass seed banks as much as serrated tussock and may reflect that the larger seeds of kangaroo grass are more resilient to this treatment.

Addition of sugar in this trial provided a strong synergistic interaction with pine oil resulting in significantly less germination of both grass species (Figure 1). Sokoloff (1951) showed that addition of molasses (carbon) to compacted soil reduced seed germination of a range of grass and weed species. He postulated that this was due to the anaerobic growth of bacteria that deprived oxygen to seeds in the seed bank and that the bacteria would also have some direct pathogenic impacts to seeds. Nottingham *et al.* 2006 showed that addition of sugar at a rate of 3 g C pot^{-1} (6 mg C g^{-1} soil) resulted in a 169% loss of C as CO_2 from the soil caused by increased microbial activity. *Pinus radiata* essentials oils such as those used in this trial are primarily made up of the α-pinene, β-pinene, camphere and germacrene (Petrakis *et al.* 2001 and Sacchetti *et al.* 2005). A number of monoterpenes such as *α*-pinene have been reported to act as uncouplers of oxidative phosphorylation and suppress respiration as a consequence (Penuelas *et al.* 1996, Abrahim *et al.*, 2000). Pine oil has been used successfully to control branched broomrape seed banks in a weed eradication program in South Australia. Field trials showed that 5% concentrations of Interceptor (BioSeed™) (Certified Organics Pty Ltd) could reduce seed numbers by as much as 95% (Mathews *et al.* 2006). We propose that the combined actions of the pine oil and carbon are causing some physical damage to the seed lemma from the pine oil and the sugar is creating a pulse of microbial activity that is both directly impacting the seeds but is also depleting O_2 from the soil and essentially asphyxiating the soil seed bank. Such a mechanism could be managed to provide a useful weed seed bank management tool.

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