

The Effect of *Eucalyptus* and Oak Leaf Extracts on California Native Plants

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Abstract

Leaf extracts of *Eucalyptus globulus* and *Quercus agrifolia* (oak) were used to investigate their effects on germination and seedling growth of *Achillea millefolium*, *Bromus carinatus*, and *Elymus glaucus*. The percentage of *Achillea* and *Elymus* seeds germinated was significantly less in the *Eucalyptus* treatment than in the control and oak treatments. *Bromus* germination and root length were not affected by the *Eucalyptus* treatment relative to the control. *Eucalyptus* had the greatest germination inhibition of *Achillea* with germination in the *Eucalyptus* treatment 11% of the control and 8% of the oak treatment. In addition, average time of *Achillea* germination was delayed in the *Eucalyptus* treatment, 6.2 days compared to 4.5 in the Oak treatment. The results of this investigation show that water extracts of *Eucalyptus globulus* inhibit the growth of *Achillea* and *Elymus*. Therefore restoration projects intended to replace *Eucalyptus* infested habitats with native plants should consider that the possible effects of allelochemicals persisting in the soil may interfere with recruitment and establishment of native species.

Introduction

Recently there has been an increasing effort to restore native vegetation in areas invaded by exotic plants. The removal of these plants is an objective for wildlife management agencies involved in restoration and conservation of natural habitats. The negative impact by non-native plants on native biota has been well-documented (Elton 1958, D'Antonio and Dudley 1993, Galatowitsch et al. 1999). Invasive plants threaten biodiversity and habitat quality by 1) displacing native plants, 2) forming dense stands that exclude natives, 3) hybridizing with natives thereby changing their genetics, and 4) supporting other non-native plants, animals, and disease (BLM 1998, Randall 1996). Randall (1996) notes that introduced plants can alter ecosystem functions such as the frequency of fires, availability of nutrients or water, and the rate of soil erosion.

Where exotic plants occur they are often able to dominate the landscape and by so doing decrease the biological diversity of that area (Mooney et al. 1986). A dramatic example of this is the neo-tropical tree *Miconia calvescens* introduced to Tahiti in 1937. *M. calvescens* now covers more than two-thirds of the island and has displaced much of Tahiti's native fauna (Meyer and Florence 1996). Characteristic of invasive plants like *M. calvescens* is their ability to reproduce rapidly and disperse readily. The mechanisms often cited to explain the displacement of native plants by invasives include competitive exclusion and changes in nutrient or water availability (Pimm 1989, Vitousek and Walker 1989).

Native to Australia, *Eucalyptus globulus* have been planted throughout California and has displaced native vegetation in coastal grasslands and shrublands (Boyd 1996). Various *Eucalyptus* species have been found to change the composition of native communities and has been associated with reduced plant and insect diversity (Bone et al. 1997, Ferreira and Marques 1998). Around the San Francisco Bay Area, stands of *Eucalyptus* could be replaced by the native oak, *Quercus agrifolia*, in an effort to restore natives. A reduction in community diversity in the presence of some *Eucalyptus* species has been attributed to the tree's allelopathic effects and impact on nutrient cycling (del Moral and Muller 1969, Espinosa-Garcia 1996). *Quercus* might better support native plants if oak litter does not release chemicals allelopathic to native plants.

Allelopathy is direct or indirect harm of one plant by another due to its release of chemicals into the environment (Babu and Kandasamy 1997). *Eucalyptus* have been

reported to contain phenolic acids, tannins, and flavonoids and these chemicals have inhibited the growth of some plants tested (Espinosa-Garcia and Francisco 1996). However, previous investigations of the allelopathic potential of the tree have primarily been concerned with the inhibition of agricultural plants or weeds (Babu and Kandasamy 1997). However, it is important to assess the impact of *Eucalyptus globulus* on California native plant germination and seedling development because of its presence in wildlands and to inform management decisions, regarding the removal and replacement of the trees with native flora.

Eucalyptus is a prolific litter producer, prone to dropping leaves and bark. If allelochemicals are released from *Eucalyptus* litter its accumulation in the soil could result in poor revegetation of native flora even after the tree's removal. Although the compounds isolated from *Eucalyptus* are known to inhibit plant growth or germination it is not known to what extent water extract of the leaves of *Eucalyptus* affect germination and seedling development of California native plants. The purpose of this study was therefore to investigate germination inhibition and seedling growth of selected California natives by *Eucalyptus* leaf extracts relative to the leaf extracts of the native oak.

Methods

Quercus agrifolia (Coast live oak) and *Eucalyptus globulus* (blue gum) leaves were collected from oak and *Eucalyptus* stands on the University of California Berkeley campus. The leaves were allowed to dry for three days at 80.6 C°. The dried leaves were milled using a five millimeter mesh.

Oak and *Eucalyptus* solutions were prepared by soaking 20 grams of dried leaves in 400 ml of distilled water in 500 ml containers. Each container was shaken for 10 seconds and kept unsealed and in the dark for 30 hours. The solutions were passed through a sieve to remove the organic material. Fifty ml of each leaf solution was then diluted with 150 milliliters of distilled water. Thirty seeds of each plant were placed in 100x15 mm glass petri dishes filled with 72 grams of sand. Each treatment received 17 ml of solution and was replicated three times. The control treatments used 17 ml of distilled water in each petri dish and were otherwise identical to the other treatments.

The seeds of two grasses, *Bromus carinatus* (California brome), *Elymus glaucus* (blue wildrye), and a perennial forb, *Achillea millefolium lanulosa* (yarrow), were purchased from

Larner Seed Company, Bolinas, California. These seeds were germinated in the dark between 60-70 F in a growth chamber at U.C. Berkeley's Oxford Tract greenhouse. Root and shoot measurements of *Elymus* and *Bromus* were taken six days after planting and after seven days for *Achillea*.

Poor germination of *Achillea* in the *Eucalyptus* solution in the initial experiment was investigated by repeating the procedures with the addition of treatments of 50 ml leaf solution diluted in 350 ml of distilled water and are denoted 1/8 oak and 1/8 euc treatments. Soil samples were also taken under oak and *Eucalyptus* litter. Soil solutions were prepared and applied following the same procedures as were used for the 50ml:150ml leaf solutions. The soil solutions were used to observe if the same effects from the leaf treatments would occur from the associated soil. Seeds were considered germinated after the seed coat was broken and one millimeter of the root was exposed. Each day the number of germinated seeds were recorded and removed from the petri dishes.

Statistical analyses An average time of germination (ATG) was calculated as follows: $(n^{\circ} \text{ germinated seeds} \times \text{days}) / \sum \text{ germinated seeds}$ (Marchiol et al. 1999). Root and shoot lengths were \log_{10} transformed prior to statistical testing. All data were subjected to standard analysis of variance procedures using Systat 8.0 (SPSS Inc. Chicago, IL). Differences between means were identified using a Tukey test at $P < 0.05$.

Results

The average root and shoot lengths in response to the control, oak, and *Eucalyptus* treatments are shown in Fig 1. *Eucalyptus* treatments resulted in shorter roots and shoots for all species with the exception of *Bromus*. *Bromus* developed longer roots in the *Eucalyptus* treatment than in the control, however the oak treatment had longer roots than the *Eucalyptus* treatment. The *Eucalyptus* treatment inhibited the growth of the roots in all plants relative to the control more so than shoots. *Achillea* root and shoot lengths, as a percentage of the control, were more inhibited by the *Eucalyptus* treatment than were *Bromus* or *Elymus*.

The percent germination of *Achillea*, *Bromus*, and *Elymus* in response to the three treatments is shown in Fig. 2. *Achillea* and *Elymus* had significantly ($P < 0.05$) fewer seeds germinate in the *Eucalyptus* treatment compared to both the control and oak treatments.

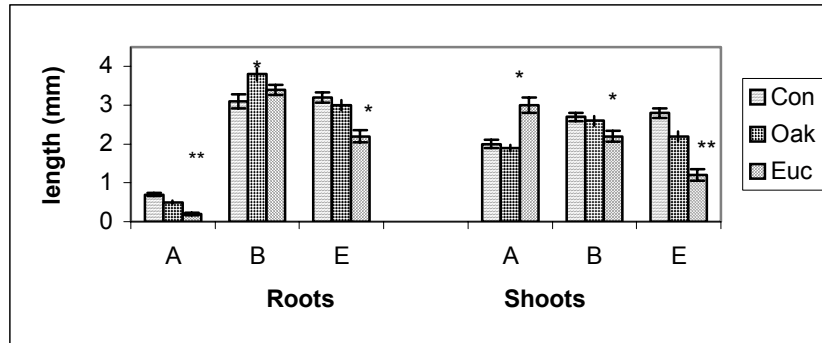


Figure 1. Effect of treatments on roots and shoots. Notice the root and shoot lengths of *Achillea* and *Elymus* in the *Eucalyptus* treatment are shorter than roots in the control and oak treatments. Significant difference from control or control and oak denoted by * or ** at 0.05 level according to a post hoc Tukey test. Calculated F-ratio and P-value determined by 1-way ANOVA for *Achillea* (13.7, <0.001), *Bromus* (4.4, 0.01), and *Elymus* (13.3, <0.001). Key con = control, oak =oak, and euc = *Eucalyptus* A=*Achillea*, B=*Bromus*, E=*Elymus*. Error bars represent the standard error.

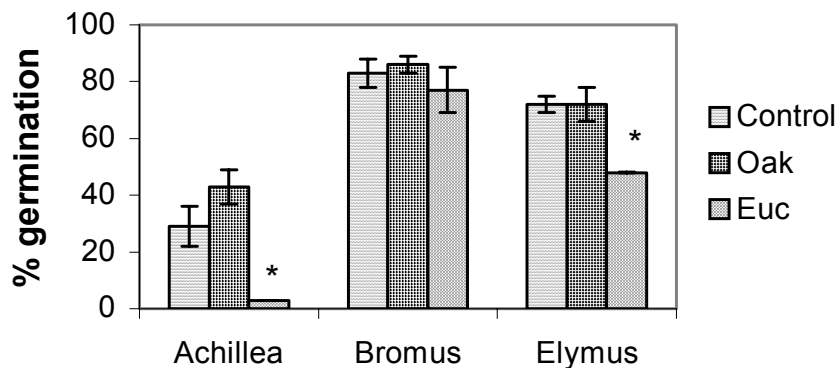


Figure 2. Percentage germination of *Achillea*, *Bromus*, and *Elymus*. Asterisks indicate values significantly different, according to a Tukey post hoc test at the 0.05 level, than the control and oak treatments. The error bars represent the standard error. Calculated F-ratio and P-value determined by 1-way ANOVA for *Achillea* (12.62, <0.01) *Bromus* (0.87, 0.47), and *Elymus* (10.08, 0.01).

Eucalyptus inhibited *Achillea* germination the most and did not inhibit *Bromus* germination. Percent germination of *Achillea* in the second experiment with the addition of two soil solutions and two concentrations of leaf solutions is shown in Fig. 3. Only the oak soil treatment and euc 1/4 were significantly ($P < 0.05$) different from each other. However, each *Eucalyptus* treatment resulted in fewer seeds germinated than its oak counterpart. The euc 1/4 treatment was most different ($P = 0.086$) from the control. Although no significant ($P = 0.71$) difference was found between the two *Eucalyptus* leaf treatments, the less concentrated

treatment had 33% higher germination. In comparison the oak 1/8 treatment had a 12% higher seed germination than the oak 1/4.

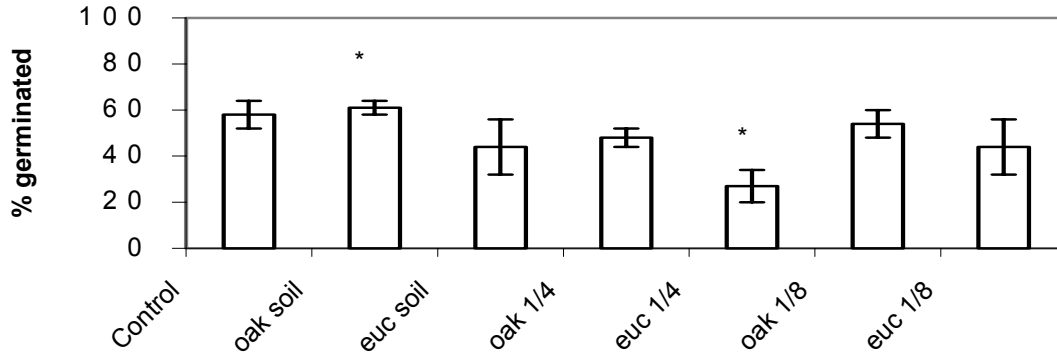


Figure 3. The percent germination of *Achillea* in the second experiment. Notice each *Eucalyptus* treatment resulted in fewer seeds germinated than its oak counterpart. Only the oak soil and euc 1/4 treatment were statistically different according to a Tukey post hoc test at the 0.05 level (1-way ANOVA, F-ratio=2.685, P=0.06). Significant differences indicated by asterisks.

The average time of *Achillea* germination was recorded in the second experiment and is shown in Fig. 4. Seeds in the euc 1/4 treatment were slower to germinate than seeds in all other treatments and was the only treatment that had a significantly ($P < 0.001$) longer ATG than the control. The more concentrated *Eucalyptus* treatment resulted in a longer ATG ($P < 0.001$) than the more dilute euc 1/8 treatment. However, concentration had no effect ($P = 0.5$) on ATG in the oak treatments.

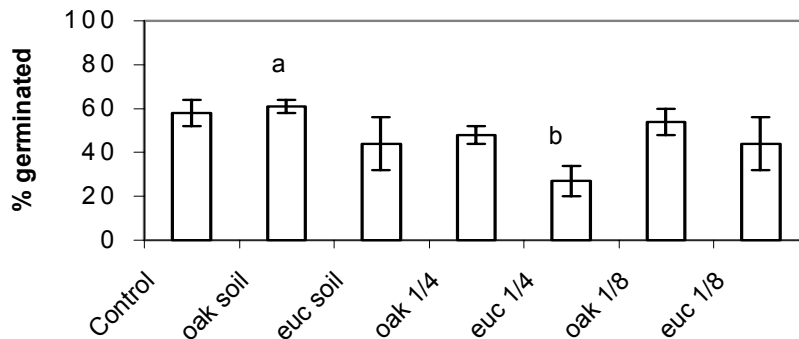


Figure 4. Average time of germination of *Achillea* in the second experiment. Notice Euc 1/4 treatment significantly delayed germination longer than any other treatment. Significant differences at 0.05 level according to a Tukey post hoc test indicated by different letters. Calculated F-ratio and P-value determined by 1-way ANOVA 28.96, < 0.001 .

Discussion

There was no significant difference between the average number of *Elymus* and *Bromus* seeds that germinated in the control, however, fewer *Elymus* seeds germinated in the *Eucalyptus* treatment. *Eucalyptus* had an inhibitory effect on *Elymus* resulting in shorter roots and shoots and fewer seeds germinated relative to the control and oak treatment. *Bromus* germination and root length were not affected by the *Eucalyptus* treatment relative to the control. *Bromus* root length was significantly greater in the oak treatment compared to the control. It is possible that oak tree leaves provide some benefit to the growth of *Bromus* and *Eucalyptus* leaves do not. The differing results for the two grasses suggests that the extent to which *Eucalyptus* inhibits native grasses might be species specific. Testing more species of grasses would provide a broader assessment of the inhibitory effects of *Eucalyptus* on native grasses.

Fewer *Achillea* seeds germinated in all of the treatments compared to *Bromus* and *Elymus* which suggests that *Achillea* may have lower germination success than the two grasses under most conditions. *Achillea* was the most inhibited plant with respect to germination in the *Eucalyptus* treatment. Weidenhamer et al. (1987) found that the amount of an allelochemical available per seed affected inhibition. *Achillea* seeds were smaller than the grass seeds which may explain greater sensitivity to allelochemicals.

Only three of 90 *Achillea* seeds germinated in the *Eucalyptus* treatment and their roots were shorter than the seeds in the oak and control treatments. The differences were statistically significant, however, more measurements should be taken from seedlings in the Euc 1/4 treatment. The shoot and germination results show inhibition, however, the shoots were longer in the *Eucalyptus* treatment than in the oak and control.

Restoration implications Using grass seed to revegetate is probably more successful than using *Achillea* seed under any circumstance in terms of the percentage of seeds that germinate. *Bromus* might be a good selection for the initial planting of a former *Eucalyptus* site because it was particularly successful at germinating in the *Eucalyptus* treatment. *Achillea* and other species, perhaps species with equally small seeds, that might be more sensitive to the presence of allelochemicals from *Eucalyptus* would best be planted after the amount of allelochemicals in the soil has been reduced. If an equal distribution of *Achillea*,

Bromus, and *Elymus* was desired initially, it would be appropriate to use less *Bromus* seed, more *Achillea* seed and an intermediate amount of *Elymus*. In replanting a former *Eucalyptus* site it would make sense to weight the number of seeds used, by their expected germination success. Because of the low germination success of *Achillea* seeds in all the treatments transplanting *Achillea* from nursery stock would likely yield better revegetation success per seed planted.

Experiment II In the second experiment *Achillea* germination in the Euc 1/4 treatment improved, however, germination was counted for two additional two days. The germination of *Achillea* in the *Eucalyptus* treatments always tended to be poorer than in the oak counterpart, although the differences were not significant. Root length has been shown to be a more sensitive measure of inhibition and root measurements might have been able to detect differences between the treatments (Haugland and Brandsaeter 1996). Because seedlings in this experiment were removed after daily germination counts those measurements were not made.

The *Eucalyptus* soil treatment did not result in germination inhibition relative to the control which suggests that allelochemicals present in the leaves are reduced or absent in the soil. Allelochemicals may have been leached out of the soil from winter rains. Soil samples taken in different seasons might have different concentrations of allelochemical compounds. It is possible that the accumulation of allelochemicals accumulate in the soil until the first rains after which rainfall leaches the toxic compounds from the soil. Soil samples taken from *Eucalyptus* sites before and after the rainy season would be valuable to test if the amount of allelochemicals in the soil can cause inhibition and if the amount varies seasonally. The ATG results show that the *Eucalyptus* 1/4 treatment delayed germination but the *Eucalyptus* soil treatments did not. This also suggests that allelochemicals might not have been in sufficient quantities to cause inhibition.

Conclusion

Test species responded negatively in a variety of ways to the water extracts of *Eucalyptus* leaves but seeds in the oak treatments did not. The results of this investigation support the hypothesis that one or more compounds in the water extract of *Eucalyptus globulus* leaves

inhibits plant growth of two California natives. It is important to investigate the significance of these results with the consideration of field conditions.

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