EVALUATING THE MYTH OF ALLELOPATHY IN CALIFORNIA BLUE GUM PLANTATIONS

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by

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ABSTRACT

Evaluating the Myth of Allelopathy in California Blue Gum Plantations

Kristen Marie Nelson

It is widely accepted that allelopathy is not only significant, but more or less singular, in the inhibition of understory vegetation in California *Eucalyptus globulus* (blue gum) plantations. However, there is no published documentation of allelopathy by blue gums against California native species. Here, we present evidence that germination and early seedling growth of five California native species are not inhibited by chemical extracts of blue gum foliage, either at naturally-occurring or artificially concentrated levels. In the greenhouse, seeds were germinated in field-collected soil from mature blue gum plantations and the adjacent native, coastal scrub communities. In petri plates, seeds of native species were germinated in the presence of concentrated volatile and water-soluble compounds from fresh foliage of blue gum, coast live oak (*Quercus agrifolia*) as a negative control, or white sage (*Salvia apiana*) as a positive control, or in a water control. In the greenhouse, blue gum soil supported germination and early seedling growth of native species equal to or better than coastal scrub soil. In the lab, germination of native species was not inhibited when grown in the presence of volatile compounds from blue gum foliage, compared to the native control (coast live oak) or the neutral water control. Germination of three out of five native species tested was not inhibited in the presence of water-soluble compounds from blue gum foliage, compared to coast live oak or the water control. Our results contradict the long-standing paradigm that blue gums are toxic to California natives, which may have significant implications for management and restoration of land historically occupied by blue gum plantations.

Keywords**:** Eucalyptus, blue gum, allelopathy

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INTRODUCTION

One of the aims of ecology is to understand and quantify the impacts that non-native species have on the habitats they invade (Parker et al., 1999; VanKleunen et al., 2010). Allelopathy is a particularly complex and controversial ecological phenomenon that has been studied widely in plants, with mixed findings (DelMoral and Muller, 1969; Chou and Muller, 1972; Heisey and Delwiche, 1984; May and Ash, 1990; Bais et al., 2002, 2003; Halsey, 2004). Allelopathy is a biochemical interaction in which chemicals released into the environment by one plant influence the growth, reproduction, or survival of another plant (Rice, 1984; Rizvi and Rizvi, 1992). Release of allelochemicals can occur via root exudation, volatilization, precipitation-mediated leaching from intact and downed foliage and bark, or decomposition of senesced foliage (Tukey Jr., 1966; Inderjit and Keating, 1999; DeAlbuquerque et al., 2011).

In some natural ecosystems, introduced species have been shown to out-compete native species, thus altering ecological processes and community assemblages (Callaway and Aschehoug, 2000; Kourtev et al., 2002; Dickens et al., 2013; Dickens and Allen, 2014; Li et al., 2014). Understanding the competitive interactions between native and non-native species is fundamental to the successful management of habitats for both anthropogenic and conservation purposes (Parker et al., 1999; Inderjit et al., 2011; Dickens et al., 2013). Allelopathy is one competitive mechanism potentially at play in the invasion of habitats by non-native species (Callaway and Aschehoug, 2000; Inderjit et al., 2011). However, a comprehensive review of the literature on the allelopathic nature of non-natives shows inconsistent, difficult to interpret, and often contradictory results (Baker, 1966; DelMoral and Muller, 1969, 1970; May and Ash, 1990; Willis, 1991; Bais

et al., 2002 [retracted by three authors, with dissent from primary author], Bais et al., 2003 [erratum reported by authors in 2010 indicates results not reproducible]; Halsey, 2004; Bertin et al., 2007; Kaur et al., 2009).

One of the most controversial claims of allelopathy occurs in California, where extensive acres of abandoned *Eucalyptus globulus* Labill. (blue gum) plantations are believed to inhibit understory growth via volatile and water-soluble allelochemicals. Blue gums, which are native to southeastern Australia and Tasmania (Nicolle, 2013), are grown worldwide as a common plantation tree (Willis, 1991; Nicolle, 2013). Many thousands of acres of blue gum plantations were established on public and private land throughout California starting in the late 19th century (Farmer, 2013). However, these plantations were largely abandoned following a 1913 report from the U.S. Department of Agriculture, which concluded that California eucalypts, and blue gums in particular, did not produce commercially viable wood products (Farmer, 2013). Consequently, stands of this unharvested crop currently occupy more than 16,000 hectares (~40,000 acres) in coastal California counties from San Diego to Humboldt, including five of the eight California Channel Islands (Ritter and Yost, 2009; Jepson eFlora, 2016). The typical appearance of a California blue gum plantation is a dense stand of tightly spaced trees in clearly discernable rows, with a thick layer of leaf and bark litter covering the ground, and a very sparse understory (Figure 1).

Allelopathy is credited, both anecdotally and in the literature, as the mechanism by which understory vegetation is inhibited in blue gum stands (Baker, 1966; DelMoral and Muller, 1969; May and Ash, 1990; Watson [unpublished]; California Invasive Plant Council, 2015). However, available data supporting the mechanism of understory inhibition in California blue gum plantations are, in many cases, anecdotal, speculative, and based upon limited experiments (Baker, 1966; DelMoral and Muller, 1969; May and Ash, 1990; Watson, 2000; Halsey, 2004; California Invasive Plant Council, 2015). There is no published documentation of allelopathy by blue gums against any California native species and no assessment of alternative explanations for the lack of understory species. Additionally, there are no published studies that compare the effect of blue gum extracts to anything other than a water control – such as extracts from another plant. An understanding of the ecology of how blue gums interact with California native species and habitats is a critical information gap that needs to be filled in order to properly inform management of these plantations.

Here, we test the effect of blue gum soil, volatile compounds, and water soluble compounds on germination and early seedling growth of five California native species that are common components of the native habitats typically found adjacent to blue gum plantations. We conducted greenhouse experiments using field-collected soil to document the capacity for native species to germinate and establish in the presence of naturally-occurring concentrations of potential allelochemicals; and we conducted laboratory experiments using ecologically relevant controls against which to compare the effect of blue gum leaf extracts.

MATERIALS AND METHODS

Germination in field-collected soil

We collected soil from two field sites (referred to throughout as Site 1 and Site 2) located on the immediate coast in central San Luis Obispo County, where mature blue gum plantations are bordered on at least one side by native coastal scrub. Both blue gum plantations were established in the 1910's and 1930's, and exhibit the typical sparse understory observed in blue gum plantations. The adjacent coastal scrub community was matched with the blue gum grove in aspect and soil type.

We conducted two greenhouse experiments to compare seed germination and early seedling growth of native species in soil collected from the blue gum plantation (blue gum soil) and the native coastal scrub (scrub soil) present at each of our two field sites, for a total of four soil collection locations (one blue gum and one scrub at each site). During soil collection, we removed all duff and leaf litter from the soil surface and collected from the top 8 to 10 inches of soil. The two greenhouse studies were completed using soil collected in different seasons, from December to January (winter experiment) and from April to May (spring experiment), to capture seasonal differences in accumulation of allelochemicals.

In the winter experiment, we compared seed germination of four native species in the two soil types (blue gum and scrub) from both sites (Site 1 and Site 2). We tested seed from *Acmispon glaber* (Vogel) Brouillet, *Eschscholzia californica* Cham., *Lupinus succulentus* Douglas ex K. Koch, and *Stipa pulchra* Hitchc. These species represent common components of the native communities most likely impacted by the establishment of blue gum plantations in California: coastal scrub and coastal prairie/

grassland. Seeds were obtained from S&S Seeds and came from locally-sourced stock on the central coast of California. For each of four native species, a total of 400 seeds were sown in 40 4 x 4-inch pots, with 10 seeds per pot, and 10 pots for each of the four soil collection locations. For all species and treatments. In total, there were a total of 160 pots. Pots were placed in the greenhouse in a blocked design. Each pot received the same amount of water at each watering, approximately 18 to 25 mL every 2 to 3 days. We recorded germination (count out of 10 per pot) 60 days after planting.

During the spring experiment, we collected data on seed germination and biomass. In this experiment, 10 seeds of each of three native species - *L. succulentus*, *S. pulchra*, and *F. microstachys* Nutt.- were sown together in 12 large (15-inch by 15-inch) plastic flats, representing three replicate flats for each of the four soil collection locations. There were a total of 120 seeds per species (10 in each replicate of each treatment). We germinated fewer seeds in these larger flats to allow enough space for seedling establishment, and above-ground biomass quantification. Seeds were planted in a randomized pattern in each flat at equally spaced distances. Each flat received between 200 and 600 mL once or twice daily (enough to maintain saturated conditions) for the duration of the experiment. To ensure uniform greenhouse conditions, we rotated the flats around the greenhouse every other day. We recorded germination and above-ground biomass 69 days after planting. Germinated plants were harvested, placed in individual paper bags, dried for 3 days at 49 C°, and weighed.

Germination in the presence of volatile compounds

We conducted a laboratory experiment in which seeds of native species were germinated in a water control or in the presence of volatile compounds from fresh leaves

of *E. globulus, Salvia apiana* Jeps., or *Quercus agrifolia* Née. Fresh leaf material was cut into ½- to 1-inch-wide pieces, and approximately 2 g of fresh, cut leaf material was placed in a small glass dish. The small dish was then placed in the center of a 90-mm-diamter glass petri dish (large dish) lined with filter paper. Twenty seeds of one species were placed on the filter paper and watered with 7 mL of deionized water. Each large dish was sealed with parafilm and left for two weeks before we recorded germination (yes or no) for each seed. The small petri dish isolated the torn leaf material from interacting with the germinating seeds except through the effect of volatile compounds. There were ten replicate dishes for of each of the four treatments (*E. globulus, S. apiana*, *Q. agrifolia*, water) for each test species (*A. glaber, E. californica, L succulentus, F. microstachys*, and *S. pulchra*), for a total of 40 dishes and 800 seeds total per species. Seeds were considered germinated if the seed coat was broken and a hypocotyl or radicle was visible.

Germination in the presence of water-soluble compounds

In a second laboratory experiment, we isolated the effect of water-soluble compounds extracted from fresh leaves of *E. globulus, S. apiana*, and *Q. agrifolia* on seed germination. Some of the best supporting evidence for existence of allelopathic inhibition has been shown in *S. apiana* (Muller and Muller, 1964; Muller et al., 1964; Muller, 1966), which we used as a positive allelopathic control treatment. As a negative control treatment, we used *Q. agrifolia*, a native tree the forms vast woodlands with robust understory vegetation in California which is not known to have any allelopathic properties. Fresh leaves of these species were collected from individuals grown on the Cal Poly Campus.

Water soluble compounds were extracted by macerating 10 g of fresh leaf material with 250 mL of deionized water for 20 seconds in a blender. The filtrate was strained through four layers of fine-mesh cheesecloth and diluted with enough deionized water to bring each solution up to 500 mL. Twenty native seeds were then placed on filter paper in 90-mm-diamter glass petri dishes and watered with 7 mL of the prepared filtrate, or deionized water in the control. We used five replicate dishes of each treatment (*E. globulus, S. apiana*, *Q. agrifolia*, and water) for each of the five test species (20 dishes per species, 400 seeds total per species). Plates were left for 2 weeks before we recorded germination. We considered a seed germinated if the seed coat was broken and a hypocotyl or radicle was visible.

Field data

We quantified abiotic differences found within both blue gum plantations and adjacent coastal scrub at our two field sites to better understand differences in habitat. At the four soil collection locations used in the greenhouse study, we recorded soil moisture using a moisture probe (HydroSense Soil Water Measurement System), and water drop penetration time (WDPT), a standard test used for quantifying the degree and stability of soil hydrophobicity (DeBano, 1981; Wessel, 1988; Letey et al., 2000; Dekker et al., 2009). Both soil moisture and WDPT were documented in a uniform grid (data points every 100 ft) at fifteen spots within each community where soil was collected. To document WDPT, we placed a single drop of water on the surface of the soil and timed how long it took to infiltrate. WDPTs greater than one to several minutes have been used to classify soils as hydrophobic or non-wettable (Osborn et al., 1967; DeBano, 1981; Doerr et al., 2000). Since infiltration time in blue gum stands was typically very long, we

documented infiltration within only the first 45 minutes. Drops that were still beaded on the soil surface after 45 minutes were recorded as requiring > 45 minutes. These data were collected within adjacent blue gum and coastal scrub stands on the same day and at the same time, but paired communities from the two different field sites were sampled on different days, in mid-December and mid-January.

Data analyses

All statistical analyses were completed using JMP Pro, version 11.2. We conducted our analyses for each test species individually, and also for the pooled response of all species collectively. We analyzed our germination data using a generalized linear model with a binomial distribution and a logit link function. This model was used to run our comparisons in two ways. First, germination in each treatment (*E. globulus, Q. agrifolia, S. apiana*) was compared to germination in the water control. Second, we compared germination in the treatments to germination in the *Q. agrifolia* treatment, where oak instead of water was designated as the baseline. These two comparisons allowed us to compare how the *E. globulus* treatment compared to completely neutral test conditions (water) verses the negative control (*Q. agrifolia*), which represents more ecologically relevant test conditions. The response factor in these tests was a ratio of seeds germinated out of total seeds tested in each dish, with treatment as the predictor. Based on these analyses, we were able to estimate the probability of germination for each species, in each treatment, and construct 95% confidence intervals around each estimate.

We ran a similar model for germination data from the greenhouse studies, where germination in blue gum soil was compared to germination in scrub soil for each species.

We also tested for differences in germination in the same soil type from the two different field locations (e.g., blue gum from Site 1 vs. blue gum from Site 2). Mean dry biomass from the greenhouse study, as well as soil moisture and WDPT were all analyzed using an analysis of variance, comparing blue gum and scrub soil, with site and 'soil nested in site' as explanatory factors.

RESULTS

Germination in field-collected soil

Winter experiment (germination only)

When all species responses (*A. glaber, E. californica, L. succulentus, S. pulchra*) were pooled, there was no difference in seed germination in blue gum soil compared to native scrub soil ($\chi^2(1)=1.59$, p=0.21) (Figure 1). This result remained when we accounted for differences between sites ($\chi^2(3)=4.38$, p=0.22). Individually, *A. glaber*, *E. californica*, and *L. succulentus* were not inhibited by blue gum soil (AG: $\chi^2(3)=0.28$, p=0.96; EC: $\chi^2(3)=5.29$, p=0.15; LS: $\chi^2(3)=1.00$, p=0.80). Germination of *S. pulchra* was significantly inhibited in blue gum soil from Site 1 ($\chi2(1)=7.95$, p=0.005), but significantly enhanced in blue gum soil at Site 2 ($\chi2(1)=10.50$, p=0.001). Individual species responses are presented in Figure A.1 in Appendix A. Estimates given are the mean odds of germination in blue gum soil compared to scrub soil (Table 1), where odds of germination in scrub soil = 1.0; 'lower' and 'upper' values represent the 95% confidence intervals around the estimate (e.g., for AG, the probability of germination in blue gum soil is 106% of the odds of germination in scrub soil [or 6% greater], with germination falling between 76% and 148% [or between 24% lower and 48% greater] compared to scrub, 95% of the time).

Table 1. Odds of germination for individual species in field-collected soil. Mean odds of germination for *A. glaber* (AG), *E. californica* (EC), *L. succulentus* (LS), and *S. pulchra* in soil from both field collection sites. Asterisk indicates estimates that are significantly different from 1.0 at the $\alpha = 0.05$ level.

	Site 1			**Site 2**		
	Estimate	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
AG	**1.06**	0.76	1.48	**1.06**	0.76	1.49
EC	**1.36**	1.00	1.85	**1.12**	0.83	1.49
LS	**1.08**	0.79	1.50	**0.92**	0.65	1.28
SP	**0.66***	0.49	0.88	**1.61***	1.20	2.18

Spring experiment (germination and biomass)

Consistent with the findings from the winter experiment, there was no difference in seed germination of native species collectively (*F. microstachys, L. succulentus, S. pulchra*) in blue gum soil compared to scrub soil ($\chi^2(1)=0.40$, $p=0.53$) (Figure 2). This result remained when we accounted for differences between sites ($\chi^2(3)=2.19$, $p=0.53$). Individually, *F. microstachys* was not inhibited in blue gum soil from Site 1 ($\chi^2(1)=0.62$, $p=0.43$), but it was inhibited in soil from Site 2 ($\chi^2(1)=14.9$, $p<0.001$). *Lupinus succulentus* and *S. pulchra* were not inhibited in blue gum soil from either site (LS: $\chi^2(3)=3.58$, $p=0.31$; SP: $\chi^2(3)=2.29$, $p=0.51$). Individual species

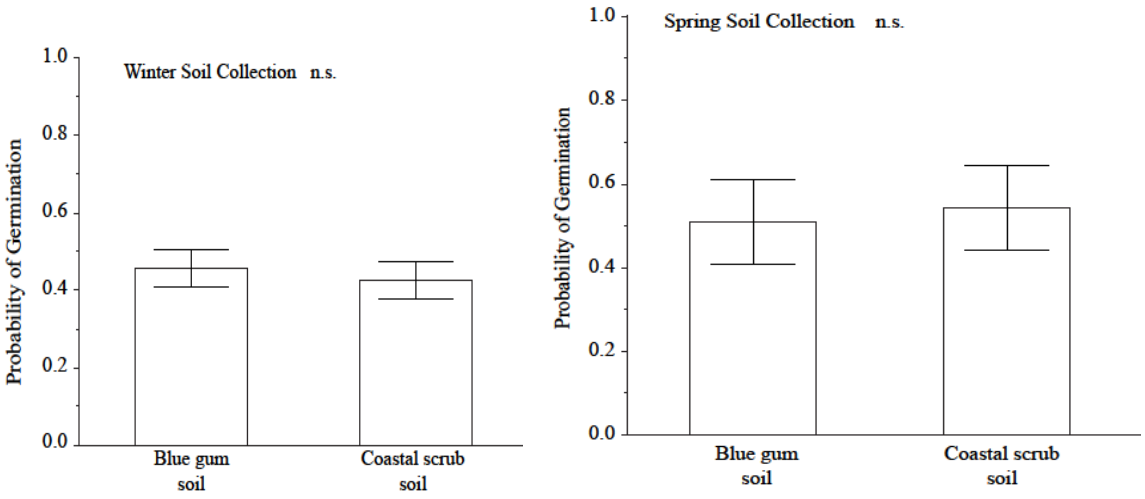


Figure 1. Mean probability of germination in blue gum soil and native coastal scrub soil. Data pooled across all species grown in soil collected in (a) the winter (germination) experiment (n = 40 for each soil type) and, (b) the spring (germination and biomass) experiment (n = 6 for each soil type). Bars represent 95% confidence intervals around the estimated mean.

Overall, dry biomass was significantly higher for plants grown in blue gum soil compared to scrub soil, when all species were pooled ($F(1,34)=6.20$, $p=0.02$). When site was included as a predictor ('soil nested in site'), there was no difference in biomass of plants (all species pooled) grown in blue gum soil and scrub soil from Site 1 ($t(1)=0.77$, $p=0.45$); however, blue gum soil at Site 2 produced significantly higher dry biomass ($t(1)=3.20$, $p=0.003$). Individually, species responses varied in different soil types (blue gum vs. scrub) and in soil from different sites (Site 1 vs. Site 2) (Figure 2). There was no effect of soil type from either site for *F. microstachys* ($F(2,8)=0.14$, $p=0.88$). For *L. succulentus*, blue gum soil from Site 2 resulted in significantly higher dry biomass than scrub soil ($t(1)=5.68$, $p< 0.001$), but there was no effect of soil from Site 1 ($t(1)=0.64$, $p=0.54$). For *S. pulchra*, blue gum soil at both sites resulted in significantly higher dry biomass than scrub soil (Site 1: $t(1)=7.14$, $p< 0.001$, Site 2: $t(1)=6.16$, $p< 0.001$).

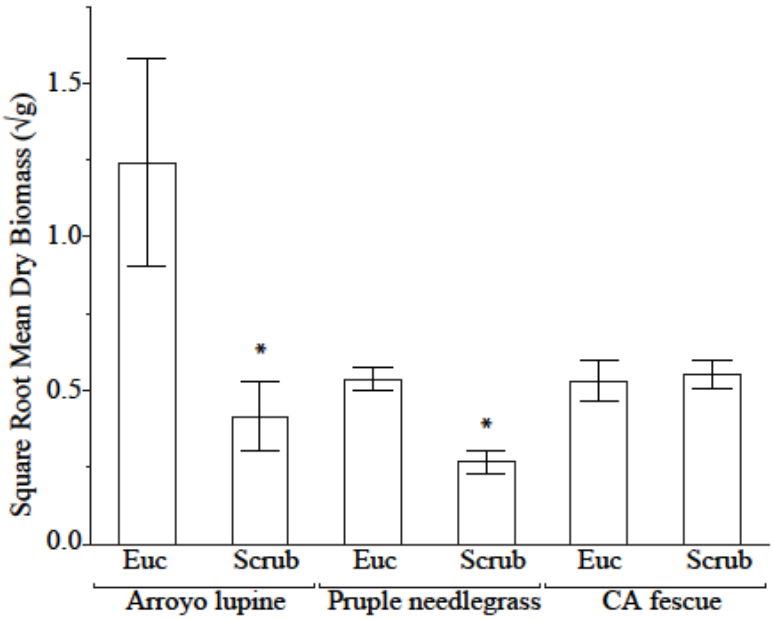


Figure 2. Mean dry biomass of individuals germinated in blue gum soil and native scrub soil. Bars represent ± 1 standard error. Comparisons were made between different soils for each species; asterisk indicates significant difference of soil type within a given species (*L. succulentus*: blue gum, n=18, scrub, n=11; *S. pulchra*: blue gum, n=38, scrub, n= 38; *F. microstachys*: blue gum, n= 35, scrub, n= 49).

Germination in the presence of volatile compounds

When all species responses were pooled (*A. glaber*, *E. Californica, F. microstachys*, *L. succulentus*, and *S. pulchra*) germination was not inhibited by volatile compounds from blue gum ($\chi^2(1)=0.97$, $p=0.33$) or oak ($\chi^2(1)=0.88$, $p=0.35$) compared to the water control, but white sage significantly inhibited germination ($F(1)=43.00$, $p< 0.001$) (Figure 3). When we compared germination to the oak treatment, germination of native species (all pooled) was not inhibited by volatile compounds from blue gum ($\chi2(1)=0.002$, $p=0.96$), but white sage still significantly inhibited germination ($\chi2(1)=31.61$, $p< 0.001$).

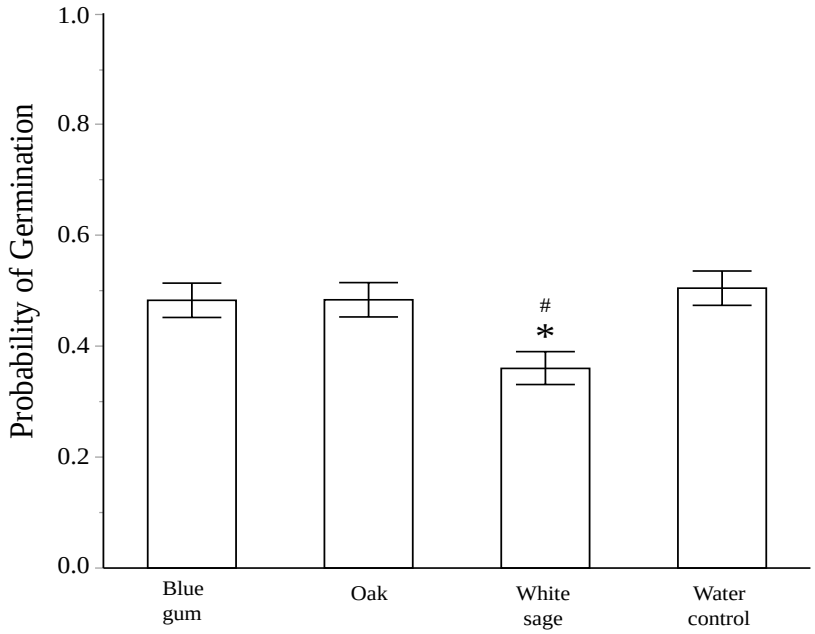


Figure 3. Mean probability of germination in the presence of volatile compounds, pooled across five native species tested. Bars represent 95% confidence intervals around the estimated mean. Asterisk indicates significant difference of treatment compared to the water control and pound sign indicates significant difference of treatment compared to the native oak control; n = 50 for each treatment.

Individually, *A. glaber* and *L. succulentus* germinated equally well across all treatments (AG:$\chi^2(3)=6.39$, p=0.09; LS:$\chi^2(4)=3.28$, p=0.51). *Eschscholzia californica* was significantly inhibited by white sage ($\chi^2(1)=8.80$, p=0.003), but there was no effect of blue gum ($\chi^2(1)=0.01$, p=0.92) or oak ($\chi^2(1)=3.36$, p=0.07). *Festuca microstachys* germination was significantly inhibited by white sage ($\chi^2(1)=61.97$, p< 0.001), but there was no effect of blue gum ($\chi^2(1)=0.67$, p=0.41) or oak ($\chi^2(1)=0.02$, p=0.88). *Stipa pulchra* was significantly inhibited by white sage ($\chi^2(1)=49.34$, p<0.001) and somewhat inhibited by blue gum ($\chi^2(1)=4.42$, p=0.04), but there was no effect of oak ($\chi^2(1)=1.21$, p=0.27), as compared to the water control (individual species responses presented in Figure A.3 in Appendix A). However, when compared to oak, there was no effect of blue gum on germination for *S. pulchra* ($\chi^2(1)=1.01$, p=0.32). The degree of variability

associated with the estimates for odds of germination for each species is summarized in Table A.1 (treatments compared to water) and A.2 (treatments compared to oak) in Appendix A.

Germination in the presence of water-soluble compounds

When all species responses were pooled, germination of native species was significantly inhibited by concentrated water-soluble compounds from blue gum ($\chi^2(1)=6.41$, $p=0.01$) and white sage ($\chi^2(1)=13.96$, $p < 0.001$), compared to the water control (Figure 4). However, when compared to the oak treatment, there was no difference in germination of native species (pooled) in the blue gum treatment ($\chi^2(1)=1.76$, $p=0.18$), but germination in white sage was still significantly inhibited ($\chi^2(1)=6.42$, $p=0.01$).

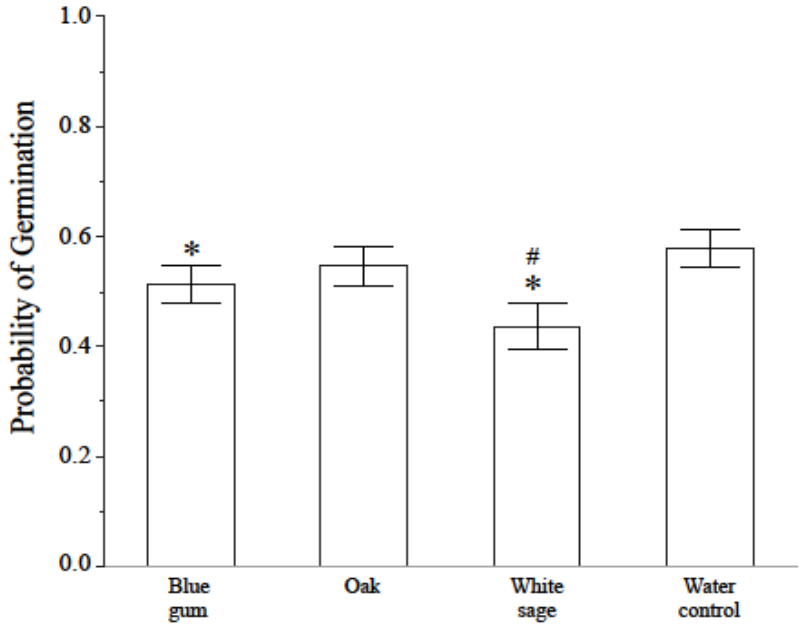


Figure 4. Mean probability of germination in the presence of water-soluble compounds, pooled across five native species tested. Bars represent 95% confidence intervals around the estimated mean. Asterisk indicates significant difference of treatment compared to the water control and pound sign indicates significant difference of treatment compared to the native oak control; n = 25 for each treatment.

When we analyzed the data individually for each species and compared germination in each treatment to germination in water, A. *glaber* and *F. microstachys* germinated equally well across all treatments (AG: $\chi^2(3)=0.33$, $p=0.95$; FM: $\chi^2(3)=1.80$, $p=0.61$). *Eschscholzia californica* was significantly inhibited by white sage ($\chi^2(1)=7.51$, $p=0.006$), but there was no effect of blue gum or oak ($\chi^2(1)=0.49$, $p=0.48$ for both). Both *L. succulentus* and *S. pulchra* were significantly inhibited by blue gum and white sage, compared to the water control (LS: $\chi^2(1)=4.69$, $p=0.03$ for both; SP: blue gum, $\chi^2(1)=8.91$, $p=0.003$; white sage, $\chi^2(1)=20.03$, $p<0.001$) (individual species responses presented in Figure A.2 in Appendix A). However, when compared to oak, there was no effect of blue gum on germination for *L. succulentus* ($\chi^2(1)=1.58$, $p=0.21$), but *S. pulchra* was still inhibited in blue gum relative to oak ($\chi^2(1)=4.57$, $p=0.03$). The degree of variability associated with the estimates for odds of germination for each species is summarized in Table A.3 (treatments compared to water) and A.4 (treatments compared to oak) in Appendix B.

Field data

Soil from both blue gum plantations had significantly higher percent soil moisture than the adjacent, native scrub communities ($F(1,58)=26.6$, $p < 0.001$). This difference remained even when differences due to site were accounted for ($F(2,56)= 13.97$, $p < 0.001$) (Figure 5).

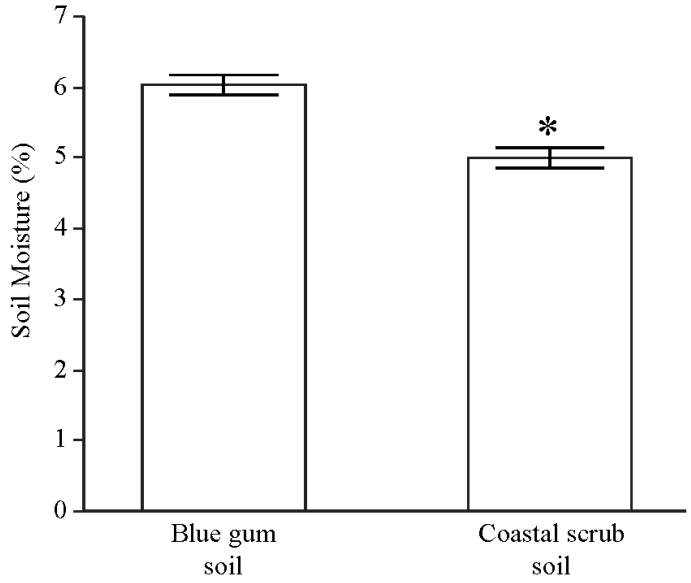


Figure 5. **Mean percent soil moisture**, averaged across both field sites (n = 30). 'Euc' mean = 6.0 ± 0.14; 'Scrub' mean = 5.0 ± 0.14.

In the field, blue gum plantations had significantly longer WDPT compared to the adjacent, native scrub soil ($F(1,58)=495.2$, $p < 0.001$). This difference remained even when differences due to site were accounted for ($F(2,56)= 392.6$, $p < 0.001$) (Figure 6).

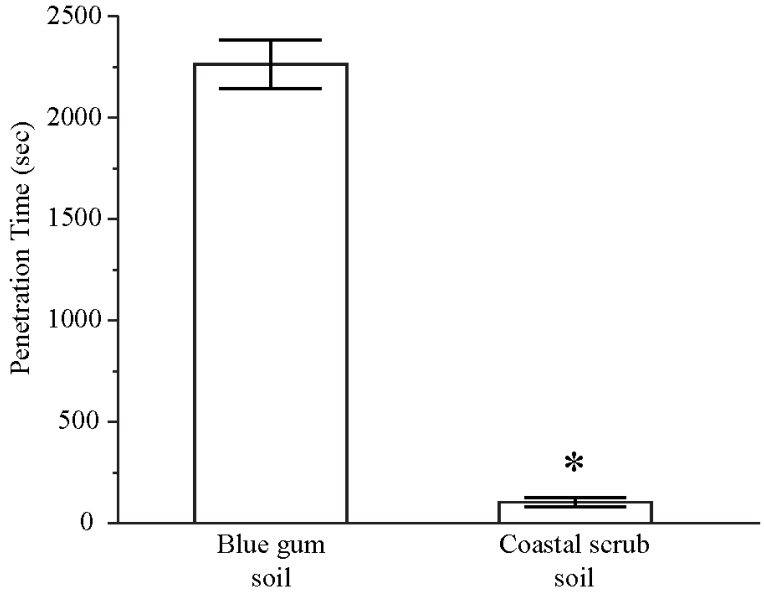


Figure 6. **Mean penetration time**. Data averaged across both field sites (n = 30 for each soil type). Blue gum ('Euc') mean penetration time = 2264 ± 120; Scrub: Mean = 103.7 ± 23.7.

DISCUSSION

It is widely accepted that allelopathy is not only significant, but more or less singular, in the inhibition of understory vegetation in California blue gum plantations. However, the results of this study failed to support this long-standing paradigm about the ecology of blue gums in California. In our greenhouse studies, we showed that soil from a blue gum plantation is equally capable of supporting native seed germination as soil from a native coastal scrub community. Further, once seeds were germinated, early seedling growth was significantly more robust in blue gum soil than native scrub soil. In the laboratory, we demonstrated that isolated volatile compounds from fresh blue gum foliage do not inhibit germination, even at artificially concentrated levels, and that concentrated water-soluble compounds were mostly not inhibitory to native seed germination. For only one species tested, *S. pulchra*, did water-soluble compounds from blue gum inhibit seed germination compared to the water control and native oak treatment. Our results failed to support allelopathy as the mechanism of understory inhibition often observed in blue gum stands.

These results are significant because they are the first to test an allelopathic effect of blue gum against ecologically relevant species. We selected native test species whose seeds are likely or potentially in the local seed bank near blue gum stands, and thus represent species of direct concern to ecologists and restoration managers. In allelopathy research, there is a common tendency for experimenters to extrapolate results of limited laboratory bioassays to inferring allelopathy in the field, without consideration of alternative or interacting factors, and without addressing ecologically relevant test conditions (Willis, 1991). We addressed these common shortcomings by testing

naturally-occurring concentrations of potential allelochemicals in field-collected soil, using native test species and ecologically relevant controls. The failure to detect an allelopathic effect in our laboratory experiments is notable, as we intentionally tested circumstances that represented more extreme exposure to allelochemicals (in terms of concentration and time) than would be expected under natural conditions.

If allelopathy cannot explain the sparse understory characteristic of California blue gum plantations, then what alternative hypotheses can explain this pattern? Light and water competition, soil hydrophobicity, and dense leaf and bark litter (often > 1 foot deep) are some of the factors potentially at play in the interaction between blue gums and potential understory species. None of these alternatives have been addressed in the literature. In California, blue gums typically occur in coastal areas where the native vegetation is dominated by open shrub and grasslands. It is not surprising that grasses, herbs, and shrubs present in these habitats, and adjacent to mature blue gum stands, are not capable of establishing in the understory of the dense tree canopy, where light infiltration is patchy and typically indirect. Thus, light competition may feasibly be influencing the pattern of sparse understory vegetation present in California blue gum plantations.

Blue gums are well known for their copious leaf litter and deciduous bark. One study found fuel loads (total downed vegetative material) in some blue gum stands as high as 31 tons/acre, which is significantly higher than a typical California bay forest (19 tons/acre) or coast live oak woodland (12 tons/acre) (California Invasive Plant Council, 2015). Using an average fresh weight of 2 g per mature leaf, which we observed in the lab, a fuel load of 31 tons/acre translates to about 3,400 leaves per square meter and even

more for dry, senesced leaves. Even if this represents a high value, it seems likely that such a high volume of falling leaf and bark litter would be inhibitory to seed germination and seedling establishment as a result of smothering and excessive shading.

Competition for water is another factor that may be influencing the observed vegetation pattern in blue gum understories. The establishment of a high-density plantation of fast-growing hardwoods in place of a grass or shrub land will undoubtedly cause a shift in the water balance of the local community and watershed. A series of studies conducted in southern India in the late 1980's assessed the annual and monthly water balance of a watershed before and after conversion from natural grassland to blue gum plantation (Samraj et al., 1988; Sharda et al., 1988). These studies found a significant reduction in water yield (runoff) following conversion to blue gum plantation and showed that blue gums extract moisture mostly from the upper soil layers, where germinating seeds and establishing seedlings of potential understory species would need it most (Samraj et al., 1988; Sharda et al., 1988).

In addition to direct water competition, other ecological changes in the local environment under a blue gum canopy may be affecting the water balance and soil chemistry of the community. Hydrophobic soils have been shown to reduce germination and vegetative establishment by reducing the moisture available to seeds and seedlings (Osborn et al., 1967). The results from our WDPT tests were dramatic, with blue gum soils taking greater than 45 minutes to penetrate (and potentially much longer) compared to scrub soil taking less than one minute to penetrate (7 out of 30 points took between 5 and 10 minutes). The extreme degree and stability of hydrophobicity of soils within blue gum stands documented here is consistent with findings from a study in Portuguese blue

gum plantations (Doerr et al., 1996), and represents an alternative explanation for the lack of understory vegetation typically observed in California blue gum plantations.

Allelopathy remains one of the most intractable areas of ecological investigation (Willis, 1991; Inderjit et al., 2006). The data presented in this study provides an important layer of information about the mechanism of understory inhibition in California blue gum plantations. It seems clear that a great deal more work is necessary to characterize the nature of the interactions between blue gums and native vegetation, or to begin to understand the mechanism of understory inhibition in California blue gum stands. Future research in this system should focus on long-term field studies, investing the effects of light competition, water competition, physical assault from copious leaf and bark litter, and soil hydrophobicity on germination and establishment of potential understory species. Since blue gums occupy such a large geographic range in California (Humboldt to San Diego County), site and region-specific studies would add valuable information to the current body of literature (Wolf and DiTomaso, 2016). In order to begin to move this field forward, we must first accept that our current understanding about allelopathic blue gums is extremely limited.

REFERENCES

BAIS, H.P., R. VEPACHEDU, S. GILROY, R.M. CALLAWAY, and J.M. VIVANCO. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science (New York, N.Y.)* 301: 1377–80. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12958360.

BAIS, H.P., T.S. WALKER, F.R. STERMITZ, R.A. HUFBAUER, and J.M. VIVANCO. 2002. Enantiomeric-dependent phytotoxic and antimicrobial activity of (+-)-catechin. A rhizosecreted racemic mixture from spotted knapweed. *Plant Physiology* 128: 1173–1179.

BAKER, H.G. 1966. Volatile growth inhibitors produced by Euucalyptus globulus. *Madrono* 18: 207–210. Available at: http://www.jstor.org/stable/41423223.

BERTIN, C., L.A. WESTON, T. HUANG, G. JANDER, T. OWENS, J. MEINWALD, and F.C. SCHROEDER. 2007. Grass roots chemistry: meta-tyrosine, an herbicidal nonprotein amino acid. *Proceedings of the National Academy of Sciences of the United States of America* 104: 16964–16969.

CALIFORNIA INVASIVE PLANT COUNCIL. 2015. Assessment of Tasmanian blue gum (Eucalyptus globulus). Available at: http://www.cal-ipc.org/ip/inventory/pdf/Eucalyptus_globulus_PAF_2015March.pdf.

CALLAWAY, R.M., and E.T. ASCHEHOUG. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* 290: 521–523. Available at: http://www.sciencemag.org/content/290/5491/521.abstract%5Cnhttp://www.sciencemag.org/content/290/5491/521.short.

CHOU, C., and C.H. MULLER. 1972. Allelopathic mechanisms of Arctostaphylos

glandulosa var . zacaensis. *The American Midland Naturalist* 88: 324–347. Available at: http://www.jstor.org/stable/2424358.

DEALBUQUERQUE, M.B., R.C. DOS SANTOS, L.M. LIMA, P. DE A. MELO FILHO, R.J.M.C. NOGUEIRA, C.A.G. DE CÂMARA, and A. DE R. RAMOS. 2011. Allelopathy, an alternative tool to improve cropping systems: a review. *Agronomy for Sustainable Development* 31: 379–395.

DEBANO, L.F. 1981. Water repellent soils: a state-of-the-art.

DEKKER, L.W., C.J. RITSEMA, K. OOSTINDIE, D. MOORE, and J.G. WESSELING. 2009. Methods for determining soil water repellency on field-moist samples. *Water Resources Research* 45: 1–6. Available at: doi:10.1029/2008WR007070.

DELMORAL, R., and C.H. MULLER. 1969. Fog drip: A mechanism of toxin transport from Eucalyptus globulus. *Bulletin of the Torrey Botanical Club* 96: 467–475.

DELMORAL, R., and C.H. MULLER. 1970. The allelopathic effects of Eucalyptus camaldulensis. *American Midland Naturalist* 83: 254–282.

DICKENS, S.J.M., and E.B. ALLEN. 2014. Exotic plant invasion alters chaparral ecosystem resistance and resilience pre- and post-wildfire. *Biological Invasions* 16: 1119–1130.

DICKENS, S.J.M., E.B. ALLEN, L.S. SANTIAGO, and D. CROWLEY. 2013. Exotic annuals reduce soil heterogeneity in coastal sage scrub soil chemical and biological characteristics. *Soil Biology and Biochemistry* 58: 70–81. Available at: http://dx.doi.org/10.1016/j.soilbio.2012.09.028.

DOERR, S.H., R.A. SHAKESBY, and R.P.D. WALSH. 1996. Soil hydrophobicity variations with depth and particle size fraction in burned and unburned Eucalyptus globulus and Pinus pinaster forest terrain in the Agueda Basin, Portugal. *Catena* 27: 25–47.

DOERR, S.H., R.A. SHAKESBY, and R.P.D. WALSH. 2000. Soil water repellency: its causes, characteristics and hydro-geomorphological significance. *Earth Science Reviews* 51: 33–65.

FARMER, J. 2013. Trees in Paradise: A California History. W.W. Norton & Company, New York.

HALSEY, R.W. 2004. In search of allelopathy: an eco-historical view of the investigation of chemical inhibition in California coastal sage scrub and chamise chaparral. *Journal of the Torrey Botanical Society* 131: 343–367. Available at: http://courses.ttu.edu/biol3309-dschwilk/resources/Halsey_2004.pdf%5Cnhttp://workspace.bananahill.net/library/Pest Control/2004_Halsey_RW_Allelpathy_history.pdf.

HEISEY, R.M.., and C.. C.. DELWICHE. 1984. Phytotoxic volatiles from Trichostema lanceolatum (Labiatae). *American Journal of Botany* 71: 821–828. Available at: http://www.jstor.org/stable/2443473.

INDERJIT, R.M. CALLAWAY, and J.M. VIVANCO. 2006. Can plant biochemistry contribute to understanding of invasion ecology? *Trends in Plant Science* 11: 574–580.

INDERJIT, and K.I. KEATING. 1999. Allelopathy: principles, procedures, processes, and promises for biological control. *Advances in Agronomy* 67: 141–231. Available at: http://www.sciencedirect.com/science/article/pii/S0065211308605155.

INDERJIT, D.A. WARDLE, R. KARBAN, and R.M. CALLAWAY. 2011. The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology and Evolution* 26: 655–662.

JEPSON EFLORA. 2016. The Jepson Herbarium: Jepson eFlora. *Regents of the University of California*. Available at: http://ucjeps.berkeley.edu/eflora/.

KAUR, H., R. KAUR, S. KAUR, I.T. BALDWIN, and INDERJIT. 2009. Taking ecological function seriously: soil microbial communities can obviate allelopathic effects of released metabolites. *PLoS ONE* 4: 1–6.

KOURTEV, P.S., J.G. EHRENFELD, and M. HÄGGBLOM. 2002. Exotic plant species alter the microbial community structure and function in the soil. *Ecology* 83: 3152–3166.

LETEY, J., M.L.K. CARRILLO, and X.P. PANG. 2000. Approaches to characterize the degree of water repellency. *Journal of Hydrology* 231–232: 61–65.

LI, H., B. XIAO, W. LIU, and F. WAN. 2014. Changes in soil biota resulting from growth of the invasive weed, Ambrosia artemisiifolia L. (Compositae), enhance its success and reduce growth of co-occurring plants. *Journal of Integrative Agriculture* 13: 1962–1971. Available at: http://dx.doi.org/10.1016/S2095-3119(13)60569-9.

MAY, F.E., and J.E. ASH. 1990. An Assessment of the allelopathic potential of Eucalyptus. *Australian Journal of Botany* 38: 245–254.

MULLER, C.H. 1966. The role of chemical inhibition (allelopathy) in vegetational composition. *Bulletin of the Torrey Botanical Club* 93: 332–351. Available at: http://www.jstor.org/stable/2483447.

MULLER, C.H., W.H. MULLER, and B.L. HAINES. 1964. Volatile growth inhibitors produced by aromatic shrubs. *Science* 143: 471–473.

MULLER, W.H., and C.H. MULLER. 1964. Volatile growth inhibitors produced by Salvia species. *Bulletin of the Torrey Botanical Club* 91: 327–330.

NICOLLE, D. 2013. Native Eucalypts of South Australia. Adelaide.

OSBORN, J., J. LETEY, F. DEBANO, and E. TERRY. 1967. Seed Germination and establishment as affected by non-wettable soils and wetting agents. *Ecology* 48:

494–497. Available at: http://www.jstor.org/stable/1932685.

PARKER, I.M., D. SIMBERLOFF, W.M. LONSDALE, K. GOODELL, M. WONHAM, P.M. KAREIVA, M.H. WILLIAMSON, ET AL. 1999. Impact: toward a framework for understanding the ecological effects of invader. *Biological Invasions* 1: 3–19.

RICE, E.L. 1984. Allelopathy. 2nd ed. Academic Press, Inc., Orlando, FL.

RITTER, M., and J. YOST. 2009. Diversity, reproduction, and potential for invasiveness of Eucalyptus in California. *Madrono* 56: 155–167.

RIZVI, S.J.H., and V. RIZVI. 1992. Allelopathy: basic and applied aspects. 1st ed. Chapman & Hall, London.

SAMRAJ, P., V.N. SHARDA, S. CHINNAMANI, V. LAKSHMANAN, and B. HALDORAI. 1988. Hydrological behaviour of the Nilgiri sub-w a t ersheds as affected by bluegum plantations, part I. the annual water balance. *Journal of Hydrology* 103: 335–345.

SHARDA, V.N., P. SAMRAJ, S. CHINNAMANI, and V. LAKSHMANAN. 1988. Hydrological behaviour of the Nilgiri sub-watersheds as affected by bluegum plantations, part II. monthly water balances at different rainfall and runoff probabilities. *Journal of Hydrology* 103: 347–355.

TUKEY JR., H.B. 1966. Leaching of metabolites from above-ground plant parts and its implications. *Bulletin of the Torrey Botanical Club* 93: 385–401.

VANKLEUNEN, M., E. WEBER, and M. FISCHER. 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* 13: 235–245.

WATSON, K. 2000. The effect of Eucalyptus and oak leaf extracts on California native plants.

WESSEL, A.T. 1988. On using the effective contact angle and the water drop penetration

time for classification of water repellency in dune soils. *Earth Surface Processes and Landforms* 13: 555–561. Available at: http://dx.doi.org/10.1002/esp.3290130609.

WILLIS, R.J. 1991. Research on allelopathy on Eucalyptus in India and Pakistan. *The Commonwealth Forestry Review* 70: 279–289.

WOLF, K., and J. DITOMASO. 2016. Management of blue gum eucalyptus in California requires region-specific consideration. *California Agriculture*39–47.

APPENDIX A. SUPPLEMENTARY TABLES AND FIGURES

Figure A.1. Individual species responses for germination in winter greenhouse experiment. Individual responses for: (a) *Acmispon glaber,* (b) *Eschscholzia californica,* (c) *Lupinus succulentus*, and (d) *Stipa pulchra.*

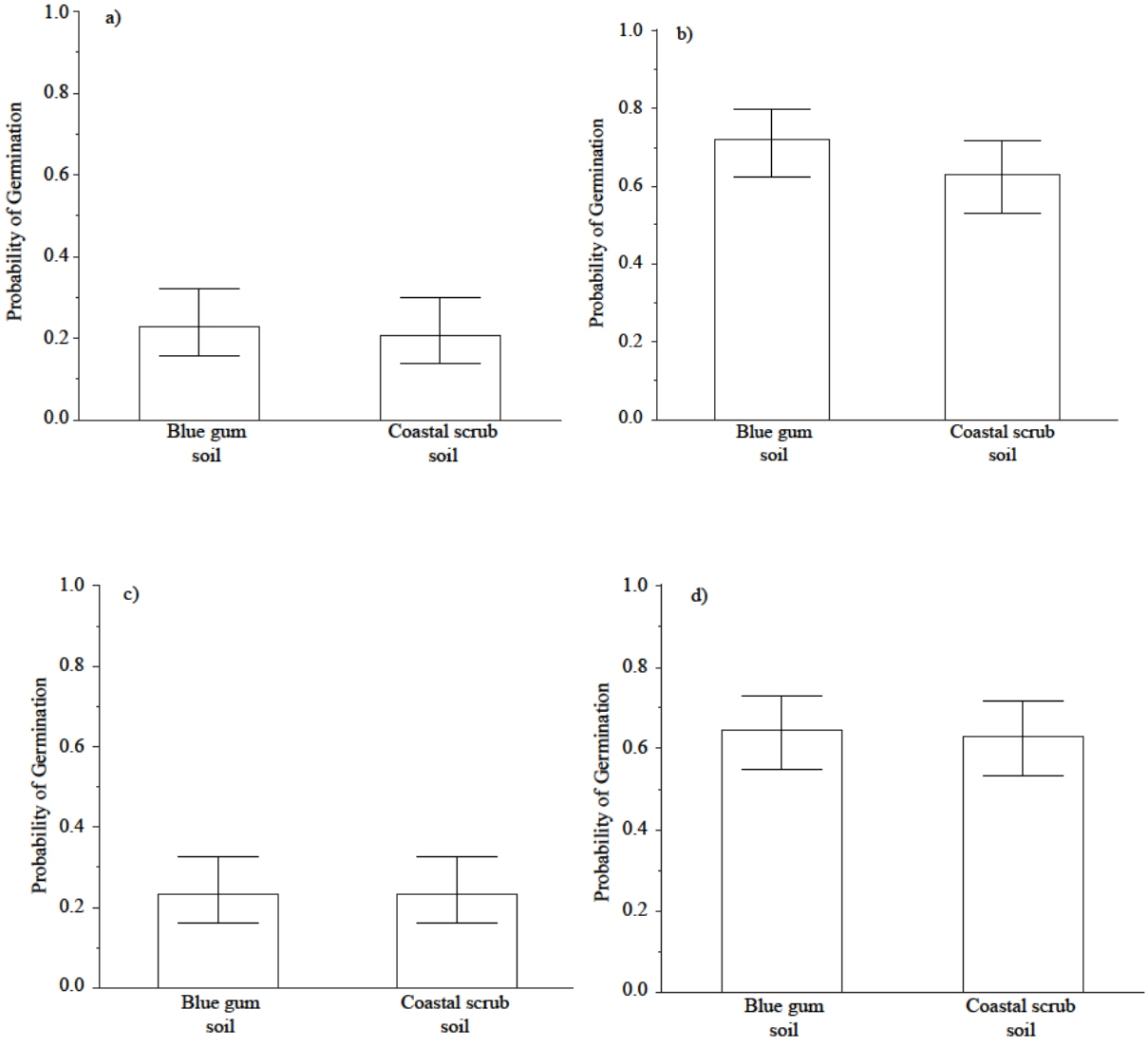
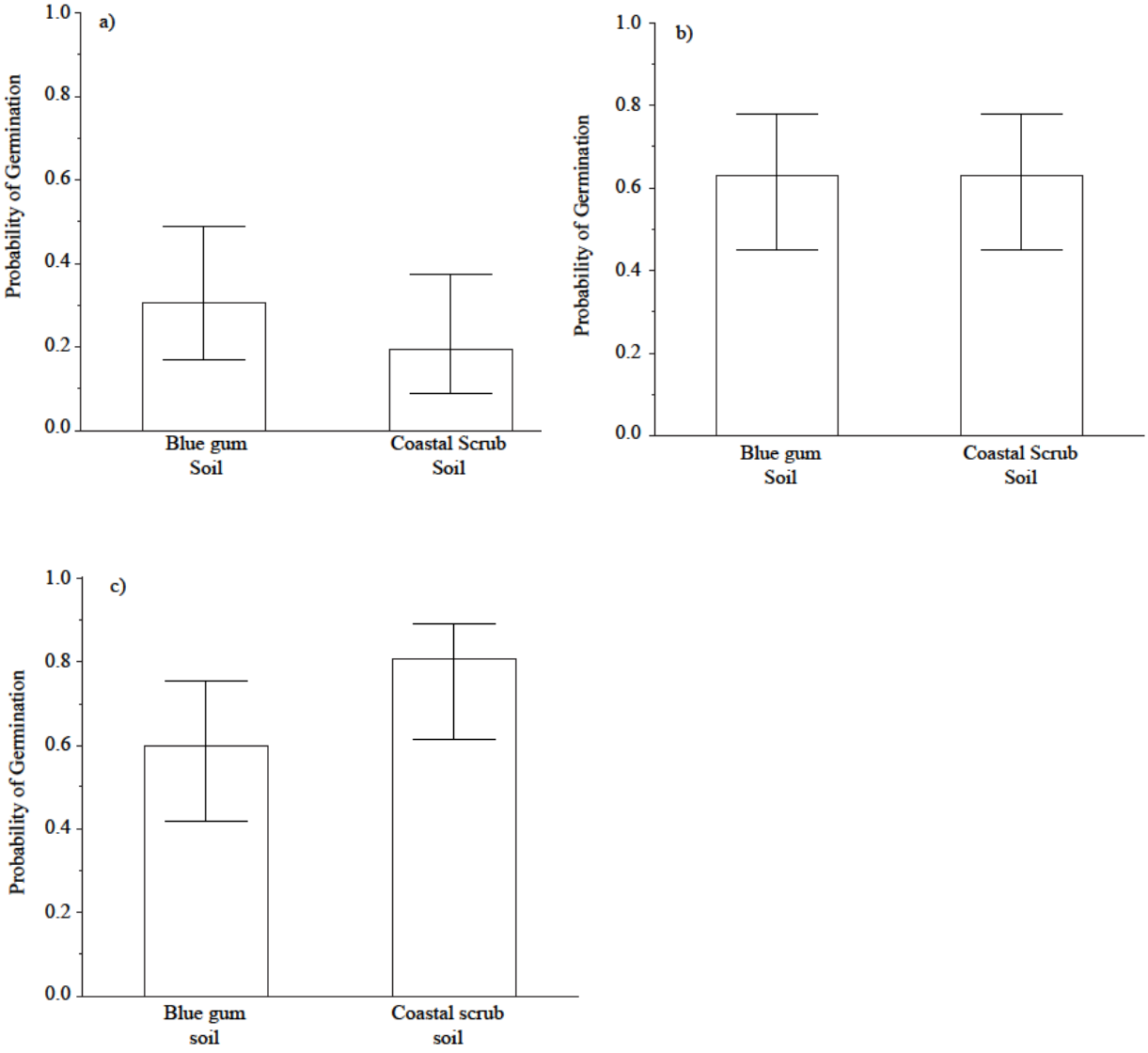


Figure A.2. Individual species responses for germination in spring greenhouse experiment. Individual responses for: (a) *Lupinus succulentus,* (b) *Stipa pulchra*, and (c) *Festuca microstachys.*



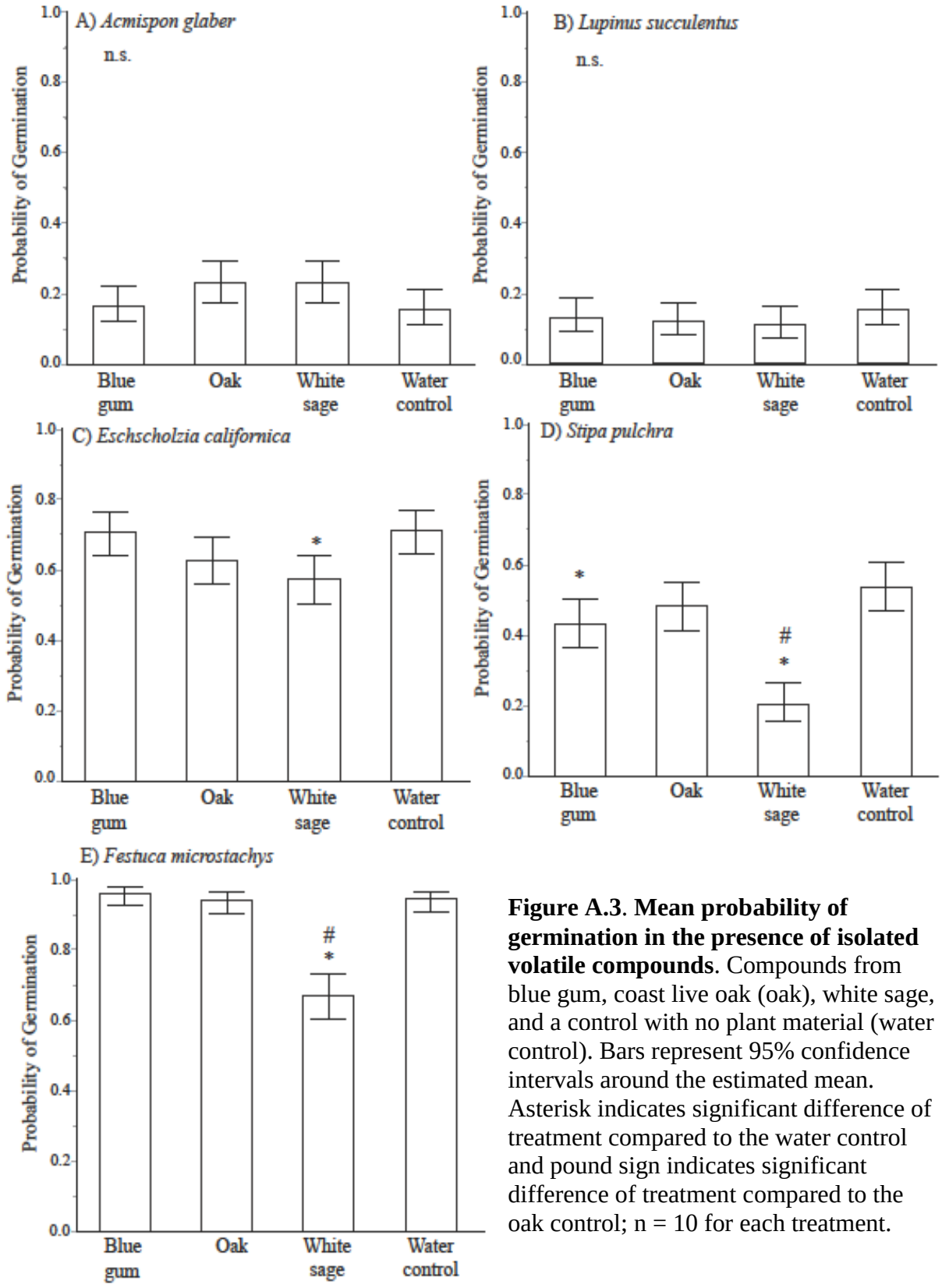


Figure A.3. Mean probability of germination in the presence of isolated volatile compounds. Compounds from blue gum, coast live oak (oak), white sage, and a control with no plant material (water control). Bars represent 95% confidence intervals around the estimated mean. Asterisk indicates significant difference of treatment compared to the water control and pound sign indicates significant difference of treatment compared to the oak control; n = 10 for each treatment.

Table A.1. Germination in all volatile treatments compared to water control. Mean odds of germination for A. glaber (AG), E. californica (EC), F. microstachys (FM), L. succulentus (LS), and S. pulchra in the presence of volatile compounds from fresh leaves of blue gum, coast live oak, and white sage. 'Estimate' is the mean odds of germination in each treatment compared to the water control, where odds of germination in the water control = 1.0; 'lower' and 'upper' values represent the 95% confidence interval around the estimate (e.g., for AG, the odds of germination in blue gum is 108% of the odds of germination in water [or 8% greater], with germination falling between 63% and 184% [or between 37% lower and 84% greater] compared to water, 95% of the time). Asterisk indicates estimates that are significantly different from 1.0 at the $\alpha = 0.05$ level.

	Blue Gum			**Coast Live Oak**			**White Sage**		
	Estimate	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
AG	**1.08**	0.63	1.84	**1.63**	0.99	2.71	**1.63**	0.99	2.71
EC	**0.98**	0.63	1.51	**0.67**	0.44	1.03	**0.53***	0.35	0.81
FM	**1.58**	0.54	5.04	**0.89**	0.33	2.32	**0.08***	0.04	0.17
LS	**0.82**	0.46	1.43	**0.75**	0.42	1.32	**0.68**	0.38	1.21
SP	**0.66***	0.44	0.97	**0.80**	0.54	1.19	**0.22***	0.14	0.34

Table A.2. Germination in volatile blue gum and white sage treatments compared to coast live oak treatment. Mean odds of germination for *A. glaber* (AG), *E. californica* (EC), *F. microstachys* (FM), *L. succulentus* (LS), and *S. pulchra* in the presence of volatile compounds from fresh leaves of blue gum and white sage. 'Estimate' is the mean odds of germination in each treatment compared to the coast live oak, where odds of germination in oak = 1.0; 'lower' and 'upper' values represent the 95% confidence interval around the estimate (e.g., for AG, the odds of germination in blue gum is 66% of the odds of germination in oak [or 34% lower], with germination falling between 40% and 109% [or between 60% lower and 9% greater] compared to water, 95% of the time). Asterisk indicates estimates that are significantly different from 1.0 at the $\alpha = 0.05$ level.

	Blue Gum			**White Sage**		
	Estimate	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
AG	**0.66**	0.40	1.09	**1.00**	0.63	1.59
EC	**1.43**	0.95	2.18	**0.80**	0.53	1.19
FM	**1.76**	0.62	5.52	**0.10***	0.05	0.21
LS	**1.09**	0.61	1.98	**0.91**	0.49	1.67
SP	**0.82**	0.55	1.21	**0.28***	0.18	0.43

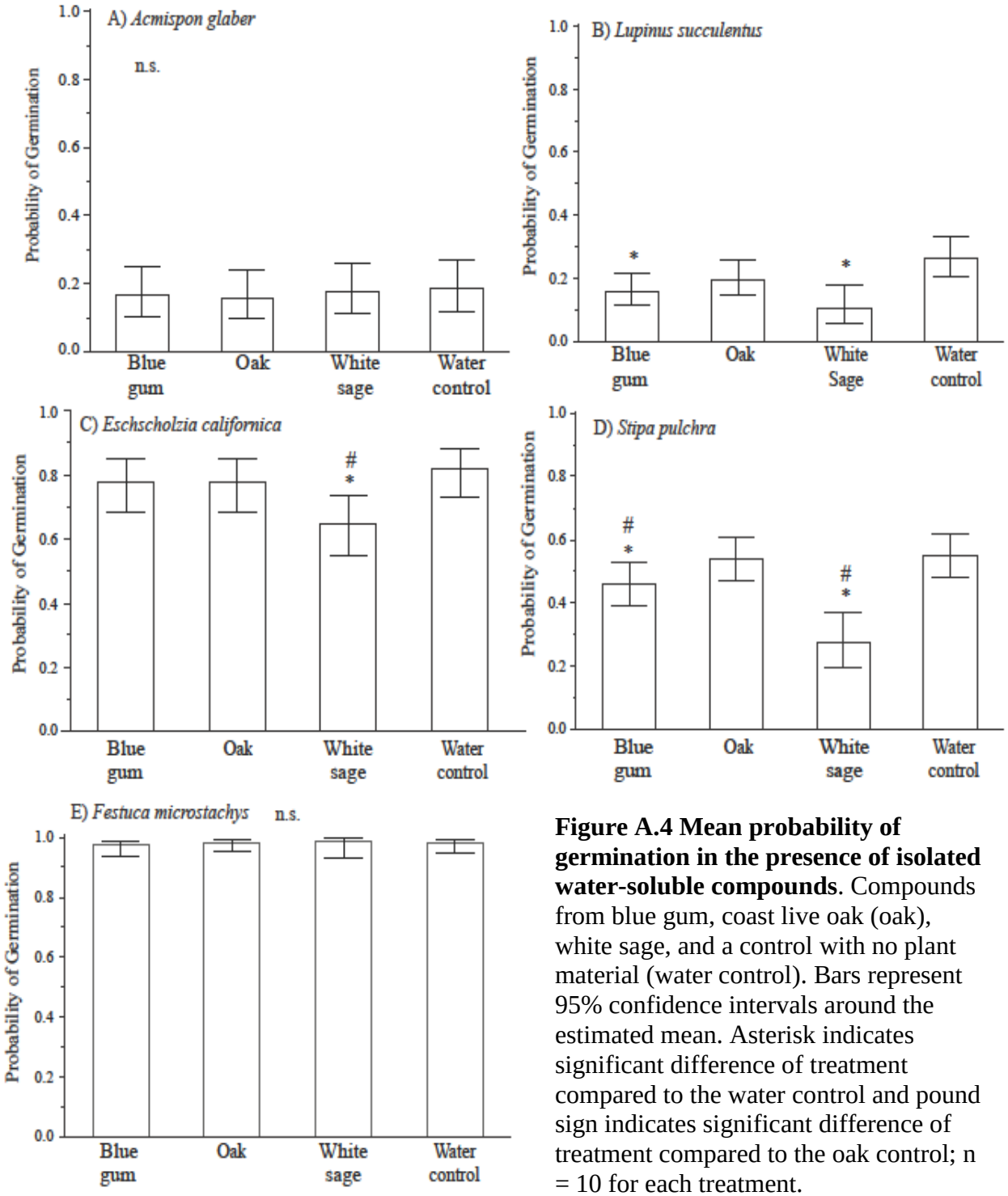


Figure A.4 Mean probability of germination in the presence of isolated water-soluble compounds. Compounds from blue gum, coast live oak (oak), white sage, and a control with no plant material (water control). Bars represent 95% confidence intervals around the estimated mean. Asterisk indicates significant difference of treatment compared to the water control and pound sign indicates significant difference of treatment compared to the oak control; n = 10 for each treatment.

Table A.3. Germination in all water-soluble treatments compared to water control. Mean odds of germination for A. glaber (AG), E. californica (EC), F. microstachys (FM), L. succulentus (LS), and S. pulchra in the presence of water-soluble compounds from fresh leaves of blue gum, coast live oak, and white sage. 'Estimate' is the mean odds of germination in each treatment compared to the water control, where odds of germination in the water control = 1.0; 'lower' and 'upper' values represent the 95% confidence interval around the estimate (e.g., for AG, the odds of germination in blue gum is 87% of the odds of germination in water [or 13% lower], with germination falling between 42% and 181% [or between 58% lower and 81% greater] compared to water, 95% of the time). Asterisk indicates estimates that are significantly different from 1.0 at the $\alpha = 0.05$ level.

	Blue Gum			**Coast Live Oak**			**White Sage**		
	Estimate	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
AG	**0.87**	0.42	1.81	**0.81**	0.38	1.69	**0.93**	0.45	1.93
EC	**0.78**	0.39	1.55	**0.78**	0.39	1.55	**0.41***	0.21	0.78
FM	**0.54**	0.09	2.52	**0.71**	0.12	3.71	**1.68**	0.22	18.64
LS	**0.43***	0.19	0.93	**0.72**	0.35	1.46	**0.43***	0.19	0.93
SP	**0.43***	0.24	0.75	**0.79**	0.45	1.37	**0.27***	0.15	0.49

Table A.4. Germination in water-soluble blue gum and white sage treatments compared to coast live oak treatment. Mean odds of germination for *A. glaber* (AG), *E. californica* (EC), *F. microstachys* (FM), *L. succulentus* (LS), and *S. pulchra* in the presence of volatile compounds from fresh leaves of blue gum and white sage. 'Estimate' is the mean odds of germination in each treatment compared to the coast live oak, where odds of germination in oak = 1.0; 'lower' and 'upper' values represent the 95% confidence interval around the estimate (e.g., for AG, the odds of germination in blue gum is 66% of the odds of germination in oak [or 34% lower], with germination falling between 40% and 109% [or between 60% lower and 9% greater] compared to water, 95% of the time). Asterisk indicates estimates that are significantly different from 1.0 at the $\alpha = 0.05$ level.

	Blue Gum			**White Sage**		
	Estimate	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
AG	**1.08**	0.50	2.31	**1.16**	0.55	2.46
EC	**1.00**	0.51	1.95	**0.53***	0.88	0.98
FM	**0.77**	0.17	3.24	**2.38**	0.38	24.89
LS	**0.59**	0.25	1.35	**0.59**	0.25	1.35
SP	**0.55***	0.31	0.95	**0.35***	0.19	0.62