

POSITIVE AND NEGATIVE SIGNALS REGULATE GERMINATION IN THE POST-FIRE ANNUAL, *NICOTIANA ATTENUATA*

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Abstract. The litter of many plant species is known to inhibit germination, and this phenomenon is commonly interpreted as allelopathic inhibition of one species by another. However, an alternative interpretation is that seeds may be using environmental signals to inform the timing of their germination and thereby use dormancy as a mechanism of habitat choice. *Nicotiana attenuata* Torr. ex Wats. (Solanaceae) is typically found for less than three years after fire in the sagebrush, blackbrush, and pinyon–juniper forests of the Great Basin desert of North America. A dormant seed bank is established during this ephemeral post-fire period, and pyrolysis products of α -cellulose (containing only C, H, and O) in wood smoke are known to initiate germination in dormant seeds of this species. We demonstrated in a glasshouse experiment that germination into burned soils (as compared to unburned) results in a 12-fold increase in lifetime seed production, which reflects a minimum estimate of the fitness benefit of making accurate germination decisions. With seed bioassays, we examined the distribution of this smoke signal in the A_1 soil layer at several burned areas in southwestern Utah, United States to determine whether the presence of this smoke-derived germination cue predicts the spatial and temporal occurrence of *N. attenuata* populations after fires. Although we found no evidence for the germination signal in areas that had not been burned for 30 yr, the occurrence of the germination signal did not perfectly coincide with the distribution of populations. We found evidence for its transport by wind and water into adjacent unburned areas (from 40 m to 1 km away from a burned site) and its persistence over time (for ≥ 7 yr), making this signal an unreliable indicator of the plant's habitat. To resolve this discrepancy, we examined the effect of unburned A_0 soil horizon on smoke-induced germination. The litter-containing A_0 soil horizon (and aqueous extracts thereof), collected from underneath seven dominant species from later stages of post-fire succession, completely inhibited germination of both dormant and nondormant seeds, even in the presence of a smoke cue in excess of that required to elicit germination. The inhibitory effect was limited to the early stages of germination (48 h after exposure to smoke), and we confirmed these results with natural seed banks. We demonstrated that the A_0 soil horizons and their aqueous extracts are not toxic to *N. attenuata* seeds or growing plants, and they have no effect on lifetime seed production. Moreover, they do not inhibit the germination of the nondormant, conspecific native tobacco, *N. trigonophylla*, which grows in the same area but is not associated with fire. Hence, these negative factors do not function in allelopathically mediated competitive interactions between *N. attenuata* and later successional species. We propose that the occurrence of *N. attenuata* populations after fires can be explained by the combined stimulatory effect of smoke-derived signals on the dormant seed bank and the inhibitory effect of signals from unburned litter, and that both signals are required for *N. attenuata* to identify its germination niche.

Key words: A_0 and A_1 soil horizons; allelopathy; fire; germination signals; litter-inhibited germination; *Nicotiana attenuata*; positive and negative control; seed banks; seed dormancy; smoke-induced germination.

INTRODUCTION

Dormancy functions to distribute organisms in time and, much like its spatial counterpart, dispersal, can be directed or random, allowing plants to synchronize growth with suitable environmental conditions (Bouw-

meester and Karssen 1993). To cope with the lack of reliability of proximate signals, some species may have evolved “bet-hedging” strategies, whereby only a certain fraction of the dormant seed bank germinates under favorable conditions (Brown and Venable 1986, Philippi 1993b). The mechanisms used by seeds to terminate dormancy and initiate growth are expected to allow plants to select favorable habitats. Many seeds use cues as general as temperature, photoperiod, moisture, or seed age (Mirov 1936, Philippi 1993a), but germination signals can be much more habitat specific.

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TABLE 1. Site descriptions for the 10 burned areas sampled in the Great Basin desert of southwestern Utah, United States.

Site no.	Age of burned area (yr)	Site name	Township and range coordinates (section)	BLM Fire no.	Area burned (ha)	Vegetation burned†
1	(1 week)	Ash Creek	T39S R13W (24)	R327	36.8	juniper-sage
2	1	Bracken Pond	T40S R19W (30)	R201	4856.3	juniper-blackbrush-yucca
3	1	Birthday	T43S R18W (36)	R213	1163.9	juniper-sage
4	1	Microwave	T43S R17W (6)	R256	167.5	juniper-sage
5	2	Motoqua	T40S R20W (1,6,7,12)	R332	809.4	juniper-oak
6	3	DI Ranch	T40S R19W (10)	R212	20	blackbrush-bitterbrush-yucca
7	3	Dodge Spring	T40S R19W (18)	R240	80.9	juniper-sage
8	7	Bull Valley	T39S R20W (35)	R330	149.7	juniper-sage
9	>30	Middle Apex	R43S R18W (36)	NA	NA	juniper-sage
10	>30	Nevada	T7S R71E	NA	NA	juniper-oak

Notes: All fires are thought to have originated from lightning strikes occurring toward the end of the growing season. All soil samples were collected between April and July 1996. NA, not available; BLM, Bureau of Land Management.

† Listed in order of dominance.

The most dramatic example of this specificity is *Striga asiatica* (Scrophulariaceae), a holoparasite with stringent requirements for host attachment (Chang and Lynn 1986). Germination is initiated by a root exudate from its host, a hydroquinone that is oxidized to an inactive form as it diffuses through the soil from the host and provides information to the seed about its distance from a suitable host (Lynn and Chang 1990).

The community of annual plants that flourish in post-fire environments contains many species that appear to synchronize their germination with fire-related cues (Keeley and Keeley 1989, Keeley 1991). For these species, the combination of open space, increased availability of resources, and temporary reduction in predators is highly favorable for seedling establishment (Evans 1976, Moreno and Oechel 1991, Simons 1991, Bond and van Wilgen 1996). Fire provides a number of factors that stimulate germination, such as heat, inorganic nutrients, unidentified compounds associated with blackened (charred) wood (Went et al. 1952, Stone and Juhren 1953, Quick and Quick 1961, Christensen and Muller 1975a, b, Keeley et al. 1985, Keeley and Pizzorno 1986, Emery 1992), and components of wood smoke (van de Venter and Esterhuizen 1988, Brown 1993, Baldwin et al. 1994, Baxter et al. 1994, Pierce et al. 1995, Keeley and Fotheringham 1997). However, it is not clear how reliably these cues synchronize seed germination with the post-fire environment in nature. Fire-elevated nutrients can easily be washed out of the burned habitats, and smoke-derived germination cues can waft into neighboring unburned areas, as can anthropogenic emissions containing nitrogen oxides (the components of smoke that germinate *Emmenanthe penduliflora*; Keeley and Fotheringham 1997), and may "trick" seeds into maladaptive seed germination. Here, we ask whether the distribution of a smoke-derived germination cue can account for the distribution of a smoke-germinated plant in nature.

Nicotiana attenuata Torr. ex Wats. (Solanaceae) is a tobacco native to the Great Basin desert of California,

Nevada, Idaho, and Utah, United States (Goodspeed 1954, Wells 1959, Brotherson et al. 1980). It primarily occurs ephemerally (typically less than three growing seasons) after fire in sagebrush and pinyon-juniper ecosystems. Limited populations may occur persistently (for many growing seasons) in isolated washes and as a roadside weed after new construction in a previously undisturbed area (Wells 1959, Barney and Frischknecht 1974, Britton and Ralphs 1978, Young and Evans 1978, Wright et al. 1979, Koniak and Everett 1982, Wright and Bailey 1982, Koniak 1985, Baldwin and Ohnmeiss 1993, Baldwin and Morse 1994, Baldwin et al. 1994; I. T. Baldwin, unpublished data). Close scrutiny (I. T. Baldwin, unpublished data) of a mature juniper habitat in southwestern Utah (site 5; Table 1) found no *N. attenuata* plants for eight consecutive years, and the nearest population was >8 km downslope from this site. A fire on 5 July 1994, originating from a lightning strike, burned 809.4 ha and killed all of the junipers, some of which were >150 yr old. In the growing season following the fire, *N. attenuata* was the dominant plant, with >10 000 individuals growing in this burned area. Because there are no known seed dispersers for this species, it is reasonable to infer that the dramatic post-fire response resulted from the germination of a long-lived dormant seed bank. Based on the age of the junipers, the seed bank may have been more than one century old. Because junipers are easily killed by fires and do not resprout from burned stumps (Wright et al. 1979), their age provides a method of determining the between-fire interval. In pinyon-juniper forests, this interval averages 10–30 yr (Wright et al. 1979), but can be as long as 115 yr (Koniak 1985). The occurrence of plants in nature may be limited by either pre- or post-germination processes, however, post-germination regulation would not be compatible with the maintenance of a long-lived seed bank. We have established that dormant seeds of this species germinate, both in natural seed banks and in laboratory bioassays, in response to water-soluble factors in wood smoke (Bald-

win and Morse 1994, Baldwin et al. 1994). The germination cue(s) is produced after the combustion of pure cellulose (and, hence, is not dependent on the type of biomass burned), contains only C, H, and O (and, hence, is a different mechanism from the NO₂-stimulated germination of *E. penduliflora*; Keeley and Fotheringham 1997), and is active in minute quantities of <1 pg/seed (Baldwin and Morse 1994, Baldwin et al. 1994; I. T. Baldwin and Z. P. Zhang, unpublished data).

Here, we ask whether the stimulation of dormant seed banks by the smoke-derived germination cue can account for the distribution of *N. attenuata* populations in nature. Because this smoke-derived germination cue is water soluble, the distribution of the cue after a fire may not coincide with the areas that were actually burned, for wind- and water-based transport could move the cue into adjacent unburned habitats. We collected soil samples to test three predictions regarding the distribution of the germination signal in nature. First, the amount of cue in the soils should be greatest immediately following a fire and should decrease rapidly, disappearing after a maximum of three years, reflecting the limited persistence of *N. attenuata* populations after a fire. Second, because the cue is water soluble (Baldwin et al. 1994), and persistent populations can be found in washes, we expect to find the cue leached downslope in washes leading away from recently burned areas. Third, when wind moves smoke into the unburned areas adjacent to a fire, it may cause seeds to germinate in these areas. However, as we do not find *N. attenuata* plants growing outside of the burned area, we predict that the concentration of the cue is not sufficient to initiate germination. The results did not fit many of our predictions and prompted us to consider other controls on the germination of these long-lived seed banks.

The inhibition of germination by plant-derived factors is more commonly reported than the stimulation of germination (Bewley and Black 1994), but the interpretation of their functional significance depends on the origin of these negative regulators of germination. When these negative factors are derived from the same taxa that produced the seeds, they are interpreted as examples of adaptive regulation of germination, e.g., leachable germination inhibitors found in seed coats functioning to gauge rainfall (Went 1949), or to signal successful dispersal from the parent plant (Evenari 1949, Harper 1977, Bewley and Black 1994). However, when these negative regulators arise from potential competitors in the plant community, their occurrence is commonly interpreted as competition mediated by allelopathic factors (Muller 1968, Rice 1984, Wardle et al. 1996). Allelopathic compounds can inhibit pre- and/or post-germination growth (Muller 1968, Rüdiger and Lohaus 1987), and xeric habitats may favor the accumulation of these allelochemicals due to their low rates of leaching and reduced soil microflora activity (Friedman 1987). Allelopathy has long been invoked

as a mechanism explaining the dramatic post-fire flush of annuals, because fire may destroy allelopathic compounds that inhibit germination (McPherson and Muller 1969, Chou and Muller 1972, Chou 1973, Christensen and Muller 1975b, Christensen 1977), but the evidence for this explanation is thought to be weak (Keeley and Keeley 1989). For some species, therefore, fire may provide signals that activate the germination process and remove allelopathic compounds, suggesting that synchronized growth within the post-fire environment involves both positive and negative controls over germination.

We report evidence for the existence of water-soluble factors in the A₀ horizons collected under seven species of dominant vegetation in the habitat of *N. attenuata*. These factors dramatically inhibit the initiation of germination of *N. attenuata* seeds, but have no deleterious effects on subsequent growth or reproductive performance. Moreover, these negative regulators of germination do not inhibit the germination of another native species of tobacco that occurs in the same area as *N. attenuata*, but is neither dormant nor associated with fires (Wells 1959). Thus, these inhibitors of germination are not the type envisioned by many researchers studying allelopathic interactions in fire-prone ecosystems, namely that of chemical suppression of one species growth by another. Rather, the inhibitors provide information and are used as chemical cues to time the germination of dormant seeds into appropriate habitats. *Nicotiana attenuata* clearly requires both positive and negative germination cues to accurately locate its germination niche. We propose that chemical "eavesdropping" by dormant seeds on products from established plants may be a better explanation of the frequently observed phenomenon of germination inhibition by the litter of many plant species.

MATERIALS AND METHODS

Site descriptions.—Ten sites were sampled from April to July 1996 (Table 1). Data on fire dates and areas burned were from Bureau of Land Management (BLM) records at Cedar City, Utah. For the two oldest burns, fire dates were estimated from conversations with retired, local firefighters and from the age of trees (determined by coring and counting rings) that had grown since the fires. This cold desert is characterized by winter precipitation, with 42% of the annual total occurring from January through March, and only 12% from May through July. Annual precipitation at the Gunlock Powerhouse, Gunlock, Utah (T40S R17W; section 28), as recorded by the National Climatic data center, Asheville, North Carolina, averaged 36.06 ± 4.33 cm per year (mean ± 1 SE) from 1985 to 1995.

Soil collection.—The A₁ soil horizon is poorly developed in this arid climate and, at unburned sites, lies below a thick, litter-containing A₀ horizon. Soil from the A₁ horizon was sieved through wide- and fine-mesh sieves (U.S. Standard Sieve Series; No. 4: 4.75-mm

opening, No. 18: 1.0-mm opening, respectively; W. S. Tyler Company, Cleveland, Ohio, USA) and was stored in plastic bags for germination cue extraction and bioassays. All samples were collected adjacent to burned juniper stumps to control for the effects that different vegetation types may have on the surrounding soil. To determine the cue content in burns of different ages (Table 1), we sampled locations where ash or charcoal was still discernible and collected control samples in adjacent unburned areas, on separate slopes with the same vegetation history (except for the two oldest burned areas, sites 9 and 10, for which we could not determine the fires' distribution). To determine how the germination cue is distributed within a burned area, we collected five samples at site 3 along a 20-m transect leading away from a burned juniper stump in the center of the burn. The A_1 soil horizons were also collected from two washes, leading ~ 1 km downslope from sites 4 (16 samples) and 5 (19 samples). Two upwind (20-m, 10 samples each) and one downwind (40-m, six samples) transects of samples were established from the edge of a 1-wk-old burn (site 1) into the adjoining unburned habitat. Prevailing wind directions at the time of the fire were obtained from the firefighters who helped to extinguish the fire. All samples were collected from April to July 1996.

A₀ collection.—The litter-containing A_0 horizon underneath the following species was collected from the unburned area adjacent to site 4: mormon tea (*Ephedra* sp.), blackbrush (*Coleogyne ramosissima* Torr.), bitterbrush (*Purshia tridentata* (Pursh) DC), big sage (*Salvia* sp.), holly oak (*Quercus* sp.), and pinyon pine (*Pinus monophylla* (Torr. and Frem.)). The A_0 horizon underneath juniper (*Juniperus osteosperma* (Torr.) Little) was collected in unburned areas adjacent to site 5. Juniper berries were removed from this collection. All samples were stored in large plastic bags at room temperature.

A₀ and A₁ extracts.—Soil horizons were ground in a Wiley Mill (Thomas Scientific, Swedesboro, New Jersey, USA) to standardize texture and grain size (850- μ m mesh). Equal volumes (10 cm³) of the soil and hot distilled water were combined in a 25-mL glass scintillation vial and agitated on a shaking table for 8–12 h. To avoid inhibiting germination by the lack of nutrients, 1 mM KNO₃ was used instead of distilled water to make the extract of the A_0 horizon for the germination experiment with *N. trigonophylla*. Extracts were centrifuged (233 rps, rotations per second) and the supernatant was used immediately or refrigerated at 4°C.

Germination bioassays.—The ability of soils (and extracts thereof) to stimulate germination of dormant seeds was determined using a previously described seed germination bioassay (Baldwin and Morse 1994, Baldwin et al. 1994). Three replicate bioassays, each consisting of 20 *N. attenuata* seeds from a dormant genotype (germinating in 9 d only when exposed to extracts of wood smoke) were used for each treatment

in all experiments. Although some genotypes of *N. attenuata* show the highest germination in response to combinations of smoke and nitrate (Baldwin et al. 1994; C. A. Preston and I. T. Baldwin, unpublished data), the genotypes used here responded only to smoke cues. In order to determine the percentages of viable and nondormant seeds, each experiment included three replicate smoke-control bioassays, in which seeds were exposed to a 1:300 dilution of liquid smoke (House of Herbs, Passaic, New Jersey, USA), and three replicate water-control bioassays, in which seeds were treated only with distilled water. After a 1-h exposure to the test solution, seeds were transferred to soufflé cups (Solo 1 oz (29.6 mL), P100, Urbana, Illinois, USA) containing ~ 5.5 g sterile sand saturated with their respective treatment solutions. Cups were sealed with transparent lids (Solo PL 1 lids, Urbana, Illinois, USA) and were placed in a growth chamber (Percival, Boone, Iowa, USA; model E-54U) with a 16L:8D photoperiod of 200 μ mol·m⁻²·s⁻¹ PAR and a 32°C day–27°C night temperature cycle. Seeds were examined after 72 h, and were monitored daily for 7–28 d (depending on the experiment) for radicle emergence and cotyledon development.

The bioassay protocol was modified in some experiments. The A_1 horizon extracts were used in the germination bioassays at full strength and the A_0 horizon extracts were used at full strength or diluted with distilled water. In one experiment, the sterile sand was replaced with burned A_1 soil horizon. The same volume of soil was used, and 20 seeds were placed on top of the soil, which was then saturated with distilled water. To determine if the inhibitory effect of the A_0 horizon from under juniper (JA_0) on seed germination required direct contact with the horizon (perhaps due to unfavorable germination microsites), or if there is a leachable inhibitory component, 0.5 cm³ of intact or ground JA_0 , was placed above and below the sand layer in the bioassay chamber containing seeds that had been previously exposed to liquid smoke. In this experiment, seeds were either in direct contact with JA_0 or were separated by a layer of sand. In another experiment, ground JA_0 was placed in increasing volumes below a layer of burned A_1 horizon on which seeds were placed, to examine the quantitative interaction between inhibitory cues leached from the A_0 horizon and stimulatory cues leached from burned A_1 horizon. Cups contained 2 cm³ of burned A_1 horizon collected at site 5, placed on top of 0–3 cm³ of ground JA_0 , increasing in five increments. Twenty seeds were placed on top of the A_1 horizon and distilled water was added to saturate the horizons.

To determine when positive and negative germination signals influence the germination process, we moved seeds from bioassay containers with smoke extracts to containers with A_0 horizon extracts after different lengths of time. Under our bioassay conditions, radicles emerge from seeds beginning 3 d after expo-

sure to smoke extracts (Day 0). To determine when the negative cue (–) can inhibit radicle emergence, seeds were exposed to 1:300 liquid smoke on Day 0, and were transferred to new containers with extracts made from the A₀ horizon under unburned juniper trees (JA₀Ex) separately on Days 1–6 (+ to – transfer; see Fig. 6A). To determine when the positive cue (+) can initiate radicle emergence after seeds have been exposed to the negative cue, seeds were exposed to JA₀Ex on Day 0 and were transferred to new containers with 1:300 liquid smoke separately on Days 1–6 (– to + transfer; see Fig. 6B). Three replicate seed cups were used for each treatment and transfer day. Hence, we used 51 germination bioassays for this experiment (21 + to – transfer; 21 – to + transfer; six smoke and JA₀Ex controls; and three water controls). Because most of the seeds in the – to + transfer treatment had not germinated 9 d after their transfer to smoke extracts, we treated these seeds with a 5% hypochlorite solution for 2 min to determine whether these nongerminating seeds were still viable (Baldwin et al. 1994). Bleached seeds were examined for radicle development for 19 additional days.

Seed bank experiment.—To examine the interaction of + and – germination signals under more natural conditions, we constructed 86 replicate seed banks, treated them with different combinations of smoke and JA₀Ex, and monitored seed germination for 24 d, the approximate length of time each spring during which seeds germinate from natural seed banks in Utah (C. A. Preston and I. T. Baldwin, unpublished data). Seed banks consisted of soil collected from an unburned area at site 2, enriched with seed dehiscence from mature plants growing at site 3 and, hence, contained both dormant and nondormant seeds. The use of seed banks with both dormant and nondormant seeds allowed us to determine whether JA₀Ex also inhibited the germination of nondormant seeds. Both soil and seed were collected in July 1996. Two centimeters of the seed-enriched soil were placed on top of 7.5-cm pots containing sterile sand. Seed banks were randomly assigned to the following treatments: (1) a 1:100 dilution of liquid smoke, (2) JA₀Ex, (3) a 1:1 mixture of 1:50 liquid smoke and JA₀Ex, (4) distilled water, or (5) 1:100 liquid smoke applied at Day 0 and JA₀Ex applied on Day 2. All seed banks were retreated every 10 d (treatment 5 received only JA₀Ex). All treatment volumes were 20 mL, which for the JA₀Ex treatment was equivalent to a 0.3 cm thick juniper litter (A₀) layer. Seed banks were placed under a sodium vapor light delivering a minimum PAR level of 300 mmol·m⁻²·s⁻¹ at the soil surface, and their positions on the bench were rotated every 5 d. Seed banks were kept moist by bottom-watering into individual 8.5-cm saucers with distilled water. The number of seedlings germinating was counted every 4–6 d and seedlings were removed.

Effects of JA₀Ex on plant growth.—Having established that JA₀Ex dramatically inhibits germination, we

sought to determine if JA₀Ex is allelopathic to *N. attenuata*, inhibiting its vegetative and reproductive growth. Chiapusio et al. (1997), in their recent review of bioassays in allelopathic research, concluded that seed germination bioassays were inferior to growth bioassays in predicting allelopathic behavior in the field. We assigned 20-d-old seedlings to the following four treatments, with eight replicates each: (1) unburned A₁ horizon, (2) burned A₁ horizon, (3) burned A₀ horizon with a 1-cm layer of ground JA₀ on the soil surface, and (4) burned A₁ horizon watered with 30 mL JA₀Ex, equivalent to a 0.3 cm thick A₀ horizon. We filled 1-L pots, one-third full with sterile sand and two-thirds full with either burned or unburned soil collected from site 2 (Table 1). Pots on the glasshouse bench were rotated every other day. Pots in treatments 3 and 4 were top-watered with 30 mL of distilled water or JA₀Ex, respectively, every other day. To keep the soil moist, pots were bottom-watered with distilled water when necessary. The JA₀Ex was prepared in larger quantities than in the seed germination experiments (100 cm³ of ground A₀ horizon from under juniper in 100 mL of distilled water), and was refrigerated until use. Plants were grown until senescence to determine lifetime seed production. Numbers of mature capsules were counted and two capsules were collected from branch positions 2–8, which have uniform capsule sizes (Baldwin et al. 1997), to determine estimates of the average seed mass per capsule and mass per seed for each plant. Lifetime seed production was estimated as the product of capsule number and average seed mass per capsule divided by the average mass per seed.

Germination effects of JA₀Ex on a nondormant tobacco species.—To determine if the inhibitory effect of JA₀Ex could be extended to other species growing in the same area, we exposed seeds of a nondormant tobacco species, *Nicotiana trigonophylla*, as well as seeds of the dormant tobacco species, *N. attenuata*, to undiluted JA₀Ex. *Nicotiana trigonophylla* is commonly found in desert habitats of southern California and northern Mexico (Goodspeed 1954), but occurs sporadically in washes in Utah and Arizona. It is not associated with the post-fire environment (Wells 1959). Seeds originated from a single plant collection in 1987 in southwestern Utah (T41S R17W; section 29), and were propagated in the greenhouse for one generation of selfing. Germination bioassays were performed as previously described, with the exception that JA₀Ex was produced using 1 mM KNO₃ instead of distilled water, and control replicates received only 1 mM KNO₃. The use of KNO₃ prevents reduced germination responses that can be attributed to a lack of nutrients and not the presence of JA₀Ex.

Statistical analysis.—The percentage of all seeds germinating in each bioassay container at each observation was arcsine-transformed for normality. Factorial ANOVAs on the transformed percentages were used to analyze main effects. Post hoc tests were performed

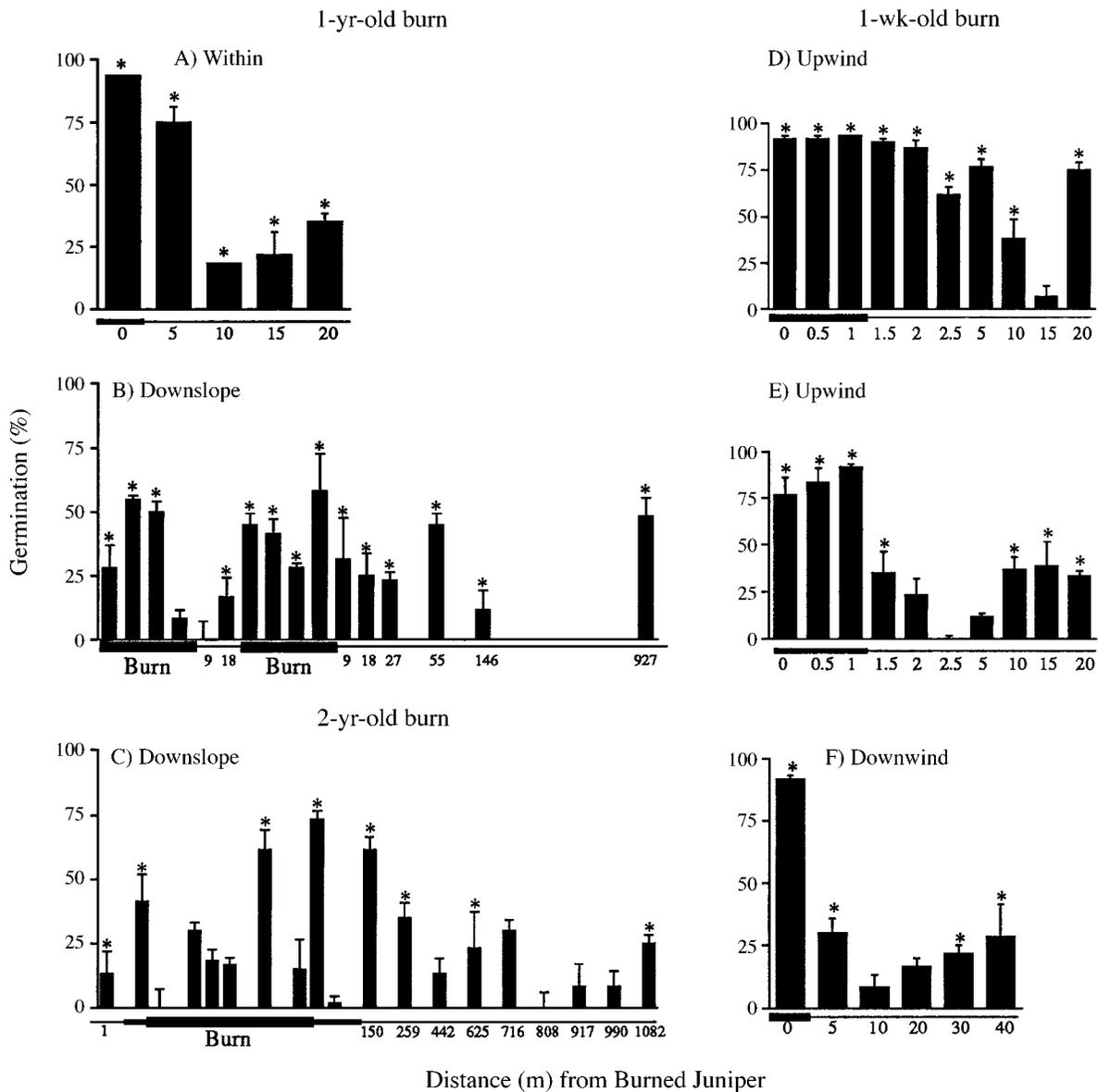


FIG. 1. Mean (± 1 SE) differences in percentage germination of *Nicotiana attenuata* seeds treated with A₁ soil extracts vs. controls (seeds treated with water) 9 d after initial treatment in seed cup bioassays; asterisks indicate significant differences (see Results: Germination potential of A₁ horizon extracts for P levels). Each treatment and control had three replicate bioassays, each with 20 *N. attenuata* seeds. Extracts were prepared from soils collected at different distances: (A) from a burned juniper stump within the burned area of site 3; (B) downslope in a wash from site 4; (C) downslope in a wash from site 5; (D) and (E) along two upwind transects; and (F) along one downwind transect leading from burned juniper stumps at the edge of the burned area into adjacent unburned areas of site 1. Separate controls were performed for each experiment and had germination values of 6.7–16.7%. See Table 1 for site descriptions. Heavy bars along the x-axis depict the location of the burned area, and numbers describe the distance from the nearest burned juniper stump within the burned area.

with the Bonferroni adjustments of significance levels to compare individual treatments. Seed capsule number, seed mass per capsule, and mass per seed were analyzed with one-way ANOVAs. Analyses were performed with the STATVIEW 4.5 statistical package (Abacus Concepts, Berkeley, California, USA).

RESULTS

Germination potential of A₁ horizon extracts.—Figures 1A–F and 2 depict the mean differences in percentage germination between seeds treated with A₁ soil horizon extracts and water-treated seeds. In all experiments, some extracts resulted in 100% germination,

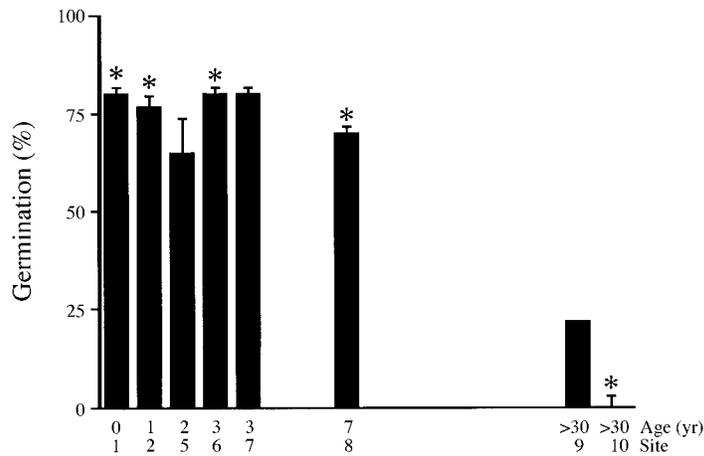


FIG. 2. Mean (+1 SE) differences in percentage germination of *N. attenuata* seeds in seed cup bioassays treated with extracts of soils collected at burned areas of different ages vs. control bioassays treated with water 9 d after initial treatment; asterisks indicate significant differences. Each treatment and control had three replicate bioassays with 20 *N. attenuata* seeds. The age of each burned area is listed above the site number along the x-axis. See Table 1 for site descriptions. Note that the burned soil extract from site 10 significantly suppressed germination. For each soil collection at a burned area, a separate unburned soil collection was made within 20 m of each burned soil collection. With one exception, none of the extracts from the unburned soils significantly influenced germination. (See Results: Germination potential of A₁ horizon extracts for statistics.)

but the background germination of water-treated seeds reduced the differences in mean germination responses to values <100%. All statistical analyses were performed on the arcsine-transformed germination percentage from each bioassay, and not on the difference data presented in Figs. 1A–F and 2.

All samples collected from within a 1-yr-old burned area showed significantly increased germination above water controls (Fig. 1A; $F_{5,12} = 10.33, P < 0.0001$). The two samples nearest to the burned juniper stump elicited significantly greater germination responses than did A₁ soil horizon samples collected at greater distances (all $P < 0.0001$). We conclude that the germination potential of A₁ horizon extracts can vary within a burned area and appears to be strongest closest to the fuel for fires.

Eighty-eight percent (14/16) and 42% (8/19) of the extracts from A₁ horizon samples collected from washes leading downslope from 1- and 2-yr-old burned areas at sites 4 and 5, respectively, significantly stimulated germination (all $F > 6.9, P < 0.0001$; Fig. 1B, C). All samples collected in the wash leading from the 1-yr-old burn (Fig. 1B), had significantly increased germination ($P < 0.001$), except for one collection from within the burn ($P = 0.084$) and one outside the burn ($P = 0.115$). The germination potential of A₁ soil horizons collected from the wash leading from the 2-yr-old burn was more variable. Of the eight collections that had significantly increased germination, three were from within and five were from outside the burned area. Interestingly, the samples collected 1 km downslope from the burned area elicited a significant germination response. Site differences in slope and fire history make comparisons between washes difficult; however, these results demonstrate the long-distance transport of smoke-derived germination signals at great distances downslope into unburned areas, most likely by water.

Eighty percent (16/20) of the A₁ horizon samples collected along the upwind transects at the 1-yr-old burn significantly stimulated germination more than

did water, including the two samples collected ~20 m into the adjacent unburned area ($F_{20,42} = 28.37, P < 0.0001$; Fig. 1D, E). Sixty-seven percent (4/6) of the samples collected along the downwind transect significantly stimulated germination ($F_{6,14} = 16.035, P < 0.0004$; Fig. 1F). The two samples collected at the greatest distances (30 and 40 m) elicited significant germination responses. Because no rain had fallen during the intervening week between the fire and the soil collections, we conclude that the smoke germination cue can be transported by wind over large distances from burned areas into adjacent unburned areas.

Extracts of A₁ horizon samples collected from burned areas ≤7 yr old resulted in significant germination responses ($F_{8,18} = 15.807, P < 0.0004$; Fig. 2). Collections from 2- and 3-yr-old burns (sites 5 and 7; Fig. 2) had marginally significant responses after the Bonferroni adjustments of significance levels (0.0064 and 0.0038, respectively; $P_{crit} = 0.0014$). Bioassays treated with extract of the A₁ horizon collected at site 10, believed to be at least a 30-yr-old burn, had fewer seeds germinating than did the water-treated control bioassays ($P = 0.0002$; Fig. 2). All extracts from A₁ soil horizons collected from unburned areas adjacent to the different-aged burned areas elicited germination at levels that were not significantly different than that elicited by water ($F_{6,14} = 4.789, P > 0.1055$), with the exception of a collection adjacent to a 1-yr-old burn at site 2 ($P < 0.0001$). This sample may have been contaminated by the cue leaching through runoff from a small burned area located upslope of the collection site. We conclude that the smoke-derived germination cue can be transported by wind and water out of the burned area, but is largely contained within the burned area. However, the cue can exist in soils of burned areas for substantially longer than the 3-yr post-fire time period when *N. attenuata* plants are commonly found (see Introduction).

Effects of A₀ extracts on germination.—Aqueous extracts of all litter-containing A₀ soil horizons (with and

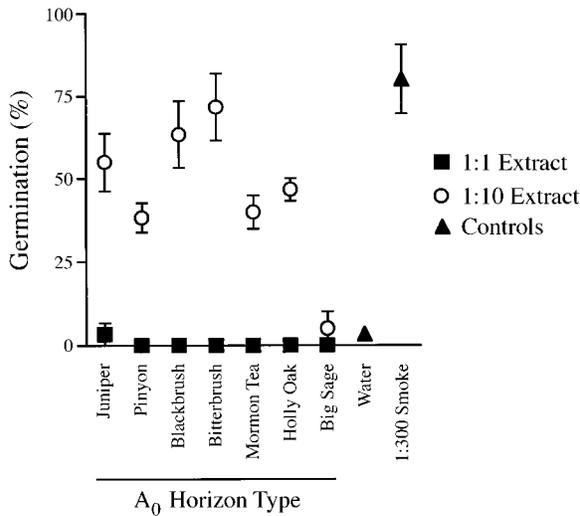


FIG. 3. Mean (± 1 SE) germination percentages of *N. attenuata* seeds in bioassays treated with extracts of A_0 soil horizons collected from under seven species that represent the majority of the aboveground biomass found in unburned areas. Each treatment had three replicate bioassays of 20 *N. attenuata* seeds. All seeds, except those used in the water-treated control bioassays, were soaked for 1 h in 1:300 liquid smoke before being placed in bioassay containers containing A_0 extracts without dilution (■), or diluted 1:10 with distilled water (○). Without dilution, bioassays received an amount of extract approximately equivalent to 1 cm³ of A_0 soil horizon. Germination percentages for water- and smoke-treated control bioassays are also depicted (▲).

without a 10-fold dilution) significantly reduced the germination of seeds previously treated with smoke cue ($F_{15,32} = 41.966$, $P < 0.0001$; Fig. 3), with the single exception of the 1:10 dilution of bitterbrush litter extract (Fig. 3). Germination responses from all A_0 horizon extracts, when applied without dilution, were not statistically different from the background germination rate observed in water-treated seeds ($P > 0.1924$). We conclude that extracts of the A_0 horizon from unburned vegetation will prevent the germination of *N. attenuata* seeds exposed to a smoke cue, even at a 10-fold dilution.

Effects of A_0 horizon on germination.—Because seeds in natural seed banks may or may not be in direct contact with leaf litter of the A_0 horizon, we compared the effects of direct and indirect contact with JA_0 on the germination of seeds previously exposed to wood smoke. All treatments significantly reduced germination responses below those of the smoke controls ($F_{13,28} = 104.606$, $P < 0.0001$; Fig. 4). Ground JA_0 was more effective than unground JA_0 in inhibiting germination; only the treatment with unground JA_0 horizon located below the sand layer exhibited germination rates significantly greater than those found in the water-treated controls ($P = 0.0002$). We conclude that direct contact with the A_0 horizon is not required to inhibit smoke-

induced germination, and that a water-leachable signal from the A_0 horizon is as effective.

In a separate experiment with burned soil (instead of smoke-treated seeds in sand), we added ground JA_0 horizon in increasing increments below the burned A_1 horizon to determine when the negative effect of the A_0 horizon would override the positive effect of the A_1 horizon (Fig. 5). Germination was reduced to 20% with the presence of 0.5 cm³ of A_0 horizon underneath a 2 cm³ burned A_1 layer, and to nearly 0% with greater volumes of JA_0 . Germination in the presence of the A_0 horizon was significantly lower than that with soil and water alone ($F_{6,14} = 72.910$, $P < 0.0001$), and with ≥ 1 cm³ of A_0 horizon, germination was lower than that observed in the water controls ($P < 0.0005$). We conclude that the presence of an A_0 horizon from species commonly found in the latter stages of post-fire succession can inhibit the germination of *N. attenuata* seeds even if the positive smoke-derived germination cue is in the soil.

Precedence of + and - germination cues.—Fig. 6A depicts the germination percentages of seeds treated with smoke extract (+) for different lengths of time before being transferred to containers with JA_0 Ex (-). Seeds exposed to (+) for ≤ 48 h did not germinate when moved to an environment with (-), and had significantly fewer seeds germinating than did water controls

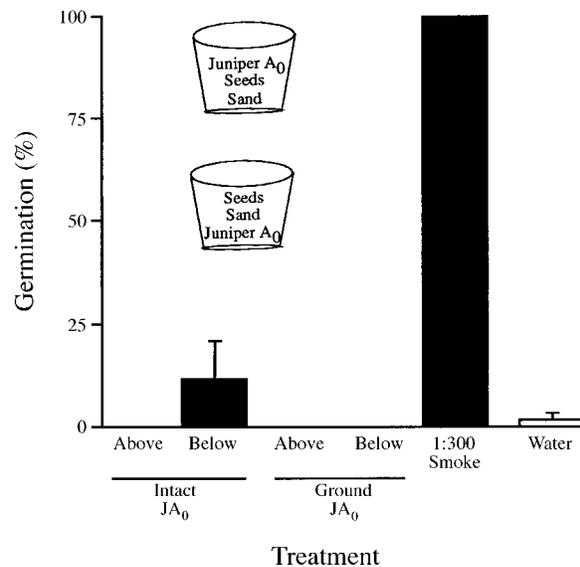


FIG. 4. Mean (± 1 SE) germination percentages at Day 7 of three replicates of 20 *N. attenuata* seeds in each of four spatial arrangements with the A_0 horizon collected from underneath unburned juniper trees. Seeds were placed either above or below the A_0 horizon, which was either left intact or ground in a Wiley mill, as depicted in the diagram. In all treatments, seeds were soaked in 1:300 liquid smoke for 1 h before being placed on a layer of sand. The seed cups were moistened with additional 1:300 liquid smoke until the sand was saturated. Treatments with ground A_0 soil horizon completely inhibited germination.

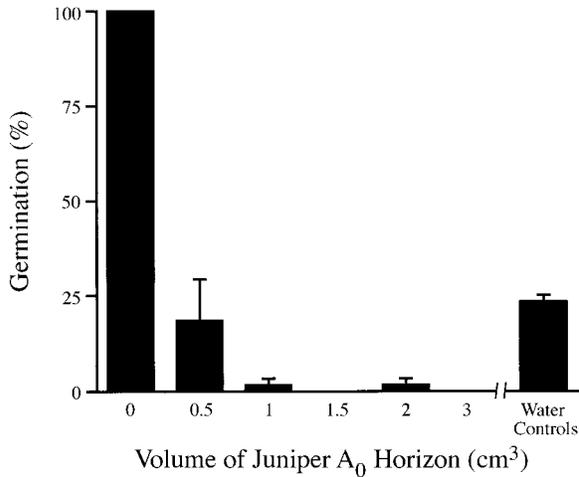


FIG. 5. Mean (+1 SE) germination percentages at Day 14 for seeds placed on 2 cm³ of burned soil (collected from site 2; Table 1), above different volumes (0–3 cm³) of ground A₀ horizon collected from underneath juniper. Thus, seeds were not in direct contact with the A₀ horizon. Water controls contained only 2 cm³ of sterile sand to provide background germination values. All treatments were saturated with distilled water, and each treatment had three replicate bioassays, each with 20 *N. attenuata* seeds.

($P < 0.0001$). However, after a 48-h exposure to (+), germination was not inhibited by (-) (Fig. 6A). Seeds initially exposed to (-) and transferred to containers with (+) germinated at levels significantly lower than water controls ($P < 0.0001$; Fig. 6B). The (-) did not kill seeds; when these seeds were scarified with a short exposure to hypochlorite, 54–87% of the dormant seeds germinated after 19 d (Fig. 6B, inset). We conclude that the inhibitory effect of the A₀ horizon on germination functions at the initial stages of seedling development before the radicle emerges from the seed coat. Once the seed has been exposed to the smoke cue for >48 h, A₀ horizon components can no longer prevent germination. However, if the seeds are first exposed to extracts of the A₀ horizon, they do not germinate when moved to an environment with only the smoke cue, possibly indicating that the A₀ horizon extracts prevent the binding of the (+) cues to the seed. The A₀ horizon extracts are not toxic to the seeds, but rather enforce their dormancy.

Seed bank experiment.—The cumulative number of seedlings germinating from seed banks treated with smoke extract was significantly higher than all other treatments, whereas seed banks treated only with A₀ horizon extracts had significantly fewer seedlings germinating than those treated with water ($F_{4,85} = 15.749$, $P < 0.0001$; Fig. 7). When seed banks were treated with either a 1:1 combination of smoke extract (+) and JA₀Ex (-) or (+) two days before (-), to produce environments with both signals, the cumulative numbers of seedlings germinating were not significantly different from seed banks treated with water ($P >$

0.1456). Additionally, seed banks treated with the 1:1 combination of (+) and (-) cues had significantly more seedlings germinating than did seed banks treated with only the (-) cue ($P = 0.0005$), but not more than the seed banks receiving (+) before the (-) ($P = 0.1038$). We conclude that the extracts of the A₀ horizon inhibit nondormant individuals in a natural seed bank, suppressing germination below that of water-treated controls when no smoke cue is present. Moreover, when a smoke cue is added to the soil, either before or concurrent with A₀ horizon extracts, the negative effects of the A₀ horizon extract override the positive effects of the smoke cue.

Effects of A₀ horizon on growth and lifetime seed production.—Fig. 8 depicts the consequences for lifetime seed production of growing in unburned soil, burned soil, or burned soil amended with either JA₀Ex or ground JA₀. Seedlings grown in burned soil, with or

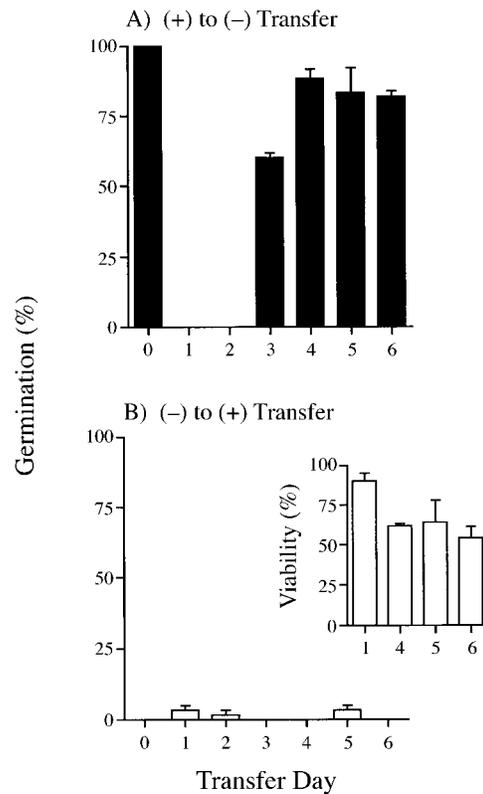


FIG. 6. Mean (+1 SE) germination percentages for seeds exposed to positive-to-negative vs. negative-to-positive transfers. (A) Seeds treated on Day 0 with 1:300 liquid smoke (+) were transferred to new containers with extract of the A₀ horizon collected under juniper (-) on Days 1–6. (B) Seeds treated on Day 0 with extract of the A₀ horizon collected under juniper (-) were transferred to new containers with 1:300 liquid smoke (+) on Days 1–6. The inset in (B) depicts viability (mean germination percentage + 1 SE) of seeds that had not germinated for 9 d after (-) to (+) transfer. Viability was defined as germination within 19 d after the seeds had been soaked in a 5% hypochlorite solution for 2 min to scarify the seed coat.

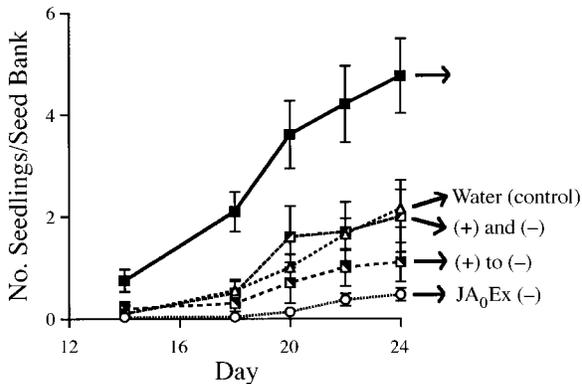


FIG. 7. Germination response in replicate seed banks under different treatments: (+), 1:100 smoke, 20 replicates; (-), extract of the A_0 horizon collected under juniper (JA_0Ex), 30 replicates; (control) distilled water, 20 replicates; (+) and (-), a 1:1 v/v mixture of 1:50 liquid smoke and JA_0Ex , 10 replicates; and (+) to (-), 1:100 liquid smoke followed 2 d later by JA_0Ex , 10 replicates. Values are the cumulative mean numbers of *N. attenuata* seedlings germinating after 24 d for each treatment (± 1 SE).

without A_0 horizon amendments, produced more capsules, heavier capsules, and heavier seeds ($F_{3,24} = 17.26, 8.770, \text{ and } 11.975$, respectively; $P < 0.0002$) than did those seedlings grown in unburned soil. From these parameters, the estimated lifetime seed production of plants growing in burned soil (5181 ± 916 seeds per plant, mean ± 1 SE) was 12-fold higher than that of plants growing in unburned soil (385 ± 146 seeds per plant). We conclude that JA_0 or JA_0Ex in concentrations sufficient to completely inhibit germination do not have any fitness consequences for plants once they have germinated. The accumulation of an A_0 soil horizon provides signals that influence germination, but does not inhibit the growth of plants after germination. The fitness cost to *N. attenuata* of germinating in an unburned environment is clearly very high.

Germination effects of JA_0Ex on a nondormant tobacco species.—On Day 8, germination of seeds of a nondormant tobacco species, *N. trigonophylla*, was not significantly different in JA_0Ex as compared with nutrient controls ($F_{1,4} = 1.323$; $P = 0.3141$), whereas JA_0Ex significantly reduced germination of *N. attenuata* ($F_{1,4} = 31.890$; $P = 0.0048$; Fig. 9). We conclude that the extracts produced from the A_0 horizon affect only the germination response of the dormant tobacco, *N. attenuata*, and not the conspecific, nondormant tobacco, *N. trigonophylla*.

DISCUSSION

When *Nicotiana attenuata* seedlings germinate and grow in burned soil in the glasshouse, their lifetime seed production is 12 times greater than it is if they germinate in unburned soil (Fig. 8), reflecting the 40-fold differences in available nitrogen in these soils (Lynds and Baldwin 1998). Moreover, when potted *N.*

attenuata plants are placed in freshly burned juniper habitats, the amount of leaf area consumed by herbivores is 1–20 times less than that of plants placed in adjacent unburned habitats (I. T. Baldwin and L. Morse, unpublished data). Hence, this species clearly gains a large fitness benefit by synchronizing its germination and growth in recently burned areas (Baldwin 1998). The distribution of *N. attenuata* populations in nature is consistent with its germination and growth being regulated by habitat-specific signals, because it is found

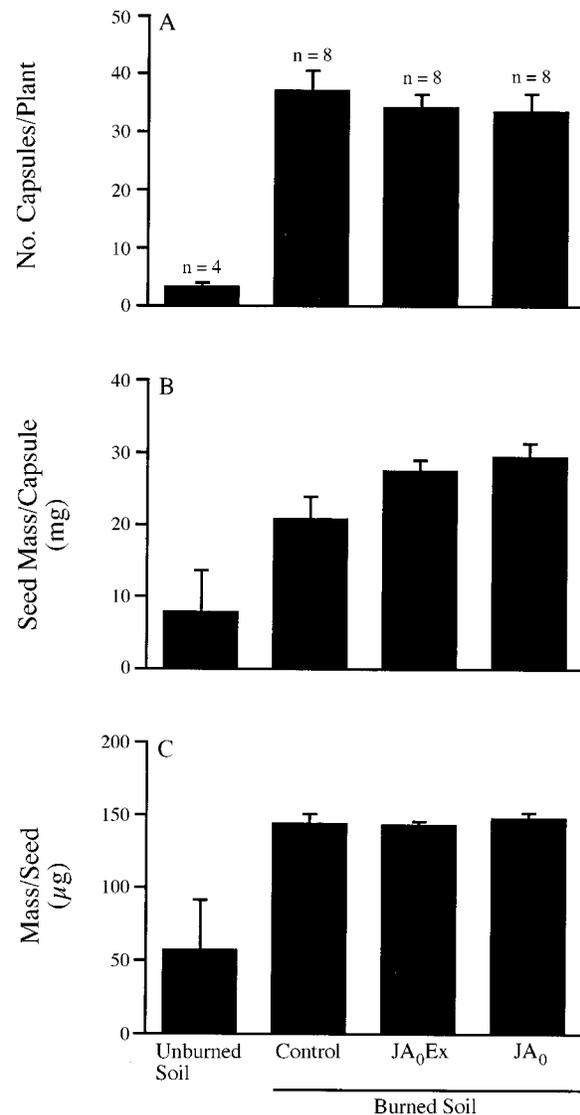


FIG. 8. Mean (± 1 SE) cumulative seed production, indicated by (A) no. capsules/plant; (B) seed mass/capsule; and (C) mass/seed of plants grown in unburned soil, burned soil, burned soil amended with extract from the A_0 horizon under juniper (JA_0Ex), and burned soil covered with 1 cm of ground A_0 horizon under juniper (JA_0). Values above the bars in (A) indicate the number of replicates in each treatment. Half of the plants grown in unburned soil died before seed production. Soils were collected from site 2 (see Table 1).

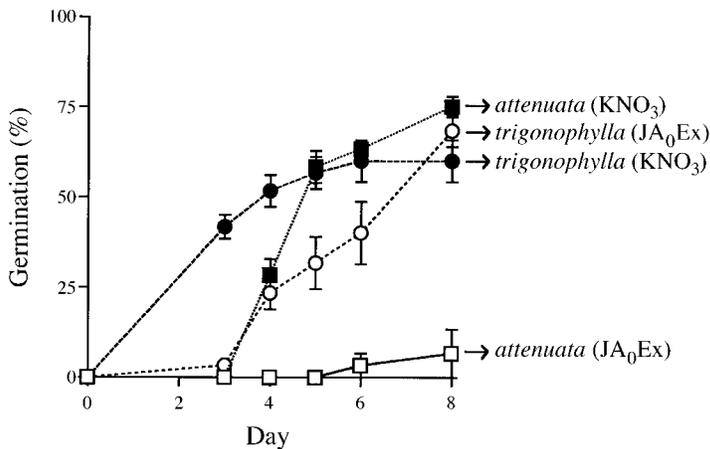


FIG. 9. Mean (± 1 SE) germination percentages for *N. trigonophylla* seeds (circles) and *N. attenuata* seeds (squares) for Days 0–8 after treatment with either undiluted JA₀Ex (open symbols) or 1 mM KNO₃ (solid symbols). Extracts of the litter-containing A₀ horizon collected under juniper were produced using KNO₃, so that all replicates received nutrients. Each treatment had three replicate bioassays of 20 seeds.

primarily in the immediate post-fire environment (Young and Evans 1978). Pierce et al. (1995) found that, in the Mesembryanthemaceae family, many succulents germinated in response to smoke, irrespective of whether they were found in fire- or non-fire-prone habitats of South Africa, and questioned the ecological significance of smoke as a germination signal. This is not the case for tobacco, as other species of *Nicotiana* do not exhibit an enhanced germination response when exposed to the smoke-derived cue (Baldwin et al. 1994). However, from our analysis of the location of the smoke-derived germination cue in the field, seeds cannot reliably identify the post-fire environment by the presence of the cue alone. We found the cue (1) to be heterogeneously distributed within a burned area, with quantities in the soil varying with their proximity to burned vegetation (Fig. 1A); (2) to be transported (probably by both wind and water) into adjacent unburned areas in quantities sufficient to germinate dormant seeds (Fig. 1B–F); and (3) to reside in soils of burned areas for ≥ 7 yr (Fig. 2), which is 4 yr longer than the realized niche for this species (see *Introduction*). We did not find evidence of the germination signal in any of the eight sites that had not had a fire in the past 30 yr, indicating that the smoke cue does deteriorate with age and is not found everywhere. However, the cellulose combustion products that signal germination in *N. attenuata* seed banks are distributed in nature far more broadly than the plant's germination niche, indicating that other factors must regulate germination from these long-lived seed banks.

We demonstrate the existence of negative, germination-inhibiting factors in the A₀ horizon of soils from unburned areas. Our experiments show that these negative signals: (1) are found in the litter-containing A₀ horizon of seven species that dominate the vegetation growing in these habitats later in succession after fires (Fig. 3); (2) are water soluble and seeds do not require direct contact with the litter-containing A₀ layer for seeds not to germinate (Fig. 4); (3) can completely

inhibit the germination of seeds as long as the seeds have not been exposed to the smoke cue for >48 h and the radicle has not emerged from the seed coat (Fig. 6A); (4) function to inhibit germination at concentrations that are neither toxic to seeds (Fig. 6B) nor inhibit the growth of mature plants or their reproductive output (Fig. 8); (5) suppress germination of nondormant *N. attenuata* seeds (seeds that do not require exposure to smoke to initiate germination) (Fig. 7); and (6) do not inhibit the germination of a nondormant species of tobacco, *N. trigonophylla* (Fig. 9), which is not associated with fires. Although these germination inhibitors profoundly enforce dormancy in *N. attenuata*, they have no toxic effects on seed viability and plant growth and are unlikely to be allelopathic agents mediating competitive interactions among plants. We conclude that these negative regulators of germination are likely to function solely to temper the germination responses to the smoke factors and to focus germination to the post-fire habitat during the 2–3 yr phenological window before successional regrowth produces a new A₀ soil horizon.

We propose that the distribution of populations of *N. attenuata* in nature can best be understood as being partly a consequence of both positive and negative control over the germination from long-lived seed banks. Fire pyrolyzes the litter-containing A₀ horizon and the constituents that inhibit germination, leaving the positive germination signals in the smoke to stimulate germination within the burned area. Germination of *N. attenuata* requires the smoke signal and is not controlled solely by the removal of litter by a fire (i.e., it is not merely the presence or absence of the negative cue that signals germination), as shown by the negligible germination in the water-treated control bioassays, where seeds have never been exposed to litter (Figs. 3–5). The majority of the input of seeds into the seed bank occurs during the first 3 yr after a fire, as seeds accumulate in the exposed A₁ layer of the burned soil and come in contact with the smoke signal, which

is present in the soil for ≥ 7 yr after a fire. However, germination does not continue after the 3 yr post-fire window. This is due, in part, to the negative factors leaching from the newly developing, litter-containing A_0 horizon above the seed-containing A_1 horizon. Wind and water transport of the smoke cue does not stimulate germination in adjacent unburned areas, because the litter-derived factors are able to override the positive effects of the smoke cue and thereby preserve the dormancy of these seed banks. Other disturbances that remove the A_0 horizon and its suppression of the seed banks, such as road construction, can result in a germination response from the seed bank and the appearance of ephemeral populations. Areas where the A_0 horizon does not accumulate, as in washes, will allow for persistent populations of nondormant individuals or dormant individuals responding to smoke signals leached from burned areas upslope.

Although fire-associated release of dormancy involves physical scarification of the seed coat for some species (Atwater et al. 1980, Brits et al. 1993, Qi et al. 1993), or the oxidation of seed coats (Keeley and Fotheringham 1997), the response of dormant seeds may be much more complicated than these physical mechanisms imply. For example, exposure of *Emmenanthe penduliflora* to oxidizing gases does not increase the water permeability of the seed and, therefore, is not a simple scarification of the seed coat (Keeley and Fotheringham 1997). Similarly, the smoke cue that stimulates germination in *N. attenuata* appears to be a specific germination signal (Baldwin et al. 1994). We are presently identifying the component(s) of wood smoke that stimulate germination in dormant *N. attenuata* populations. Keeley and Fotheringham (1997) have recently suggested that nitrogen oxides are the components of smoke that are responsible for initiating germination in dormant seeds of the California chaparral species, *E. penduliflora*. However, our laboratory's investigations utilizing GC/MS (gas chromatography/mass spectrometry) to separate smoke fractions and NMR (nuclear magnetic resonance) techniques on an active peak have elucidated a structure in the active fraction that is composed of carbons, hydrogens, and oxygens (I. T. Baldwin and Z. P. Zhang, unpublished data). This ecological investigation provides valuable information about the nature of the smoke-derived germination signal, against which a synthesized structure can be tested.

The inhibitory factors are also likely to be specific signals. Although the litter of several species has been found to inhibit germination (Mayer and Poljakoff-Mayber 1989), and many phenolics and terpenoids found in litter are known to inhibit germination (Koves and Varga 1958, Inderjit and Dakshini 1995), the chemical basis of this process is not understood. Substances identified as germination inhibitors in some species may function as germination promoters in other species, or in the same species at different concentrations.

Jasmonic acid, a ubiquitous wound signal in plants (Karban and Baldwin 1997), can function as both an inhibitor (Gross and Parthier 1994) and a stimulator (Berestetzky et al. 1991). Similarly, some allelochemicals are generally accepted as stimulators at low concentrations and as inhibitors at high concentrations (An et al. 1993).

Keeley and Fotheringham (1997) speculated that dormant seed banks of *E. penduliflora* may be "tricked" by inputs of nitrogen oxides from anthropogenic pollution, and may germinate in unburned habitats. Although such a potentially maladaptive germination response may occur in *E. penduliflora*, *N. attenuata*, with its combined positive and negative control over germination, is clearly assessing its environment in a more sophisticated fashion and would not be "tricked" into germinating. Dormant seeds are very likely utilizing a diverse suite of chemical signals provided by their environment to signal germination. We propose that many species use both positive and negative controls over their germination responses, and that the ubiquitous negative effects of litter and plant exudates on germination, but with little negative effects on subsequent growth, argue forcefully for the interpretation that these negative effects are due to signal perception rather than allelopathically mediated competition between plants.

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LITERATURE CITED

- An, M., I. R. Johnson, and J. V. Lovett. 1993. Mathematical modeling of allelopathy: biological response to allelochemicals and its interpretation. *Journal of Chemical Ecology* **19**:2379–2388.
- Atwater, B. R. 1980. Germination, dormancy and morphology of the seeds of herbaceous ornamental plants. *Seed Science and Technology* **8**:523–573.
- Baldwin, I. T. 1998. Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences (USA)* **95**:8113–8118.
- Baldwin, I. T., and L. Morse. 1994. Up in smoke: II. Germination of *Nicotiana attenuata* in response to smoke-derived cues and nutrients in burned and unburned soils. *Journal of Chemical Ecology* **20**:2373–2391.
- Baldwin, I. T., and T. E. Ohnmeiss. 1993. Alkaloidal responses to damage in *Nicotiana* native to North America. *Journal of Chemical Ecology* **19**:1143–1153.
- Baldwin, I. T., C. A. Preston, M. Euler, and D. Gorham. 1997. Patterns and consequences of benzyl acetone floral emissions from *Nicotiana attenuata* plants. *Journal of Chemical Ecology* **23**:2327–2343.
- Baldwin, I. T., L. Staszak-Kozinski, and R. Davidson. 1994. Up in smoke: I. Smoke-derived germination cues for post-

- fire annual *Nicotiana attenuata* Torr. Ex. Watson. *Journal of Chemical Ecology* **20**:2345–2371.
- Barney, M. A., and N. C. Frischknecht. 1974. Vegetation changes following fires in the pinyon–juniper type of west-central Utah. *Journal of Range Management* **27**:91–96.
- Baxter, B. J. M., J. Van Staden, J. E. Granger, and N. A. C. Brown. 1994. Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra*. *Environmental and Experimental Botany* **34**:217–223.
- Berestetzky, V., W. Dathe, T. Daletskaya, L. Musatenko, and G. Sembdner. 1991. Jasmonic acid in seed dormancy of *Acer tataricum*. *Biochemical Physiology Pflanzen* **187**:13–19.
- Bewley, J. D., and M. Black. 1994. *Seeds: physiology of development and germination*. Second edition. Plenum Press, New York, New York, USA.
- Bond, W. J., and B. W. van Wilgen. 1996. *Fire and plants*. Chapman and Hall, New York, New York, USA.
- Bouwmeester, H. J., and C. M. Karssen. 1993. Annual changes in dormancy and germination in seeds of *Sisymbrium officinale* (L.) Scop. *New Phytologist* **124**:179–194.
- Brits, G. J., F. J. Calitz, N. A. C. Brown, and J. C. Manning. 1993. Desiccation as the active principle in heat-simulated seed germination of *Leucospermum* R. Br. (Proteaceae) in fynbos. *New Phytologist* **125**:397–403.
- Britton, C. M., and M. H. Ralphs. 1978. Use of fire as a management tool in sagebrush ecosystems. *The Sageland Ecosystem: A Symposium*. Utah State University, College of Natural Resources, Logan, Utah, USA.
- Brotherson, J. D., L. A. Szyska, and W. E. Evenson. 1980. Poisonous plants of Utah. *Great Basin Naturalist* **40**:229–253.
- Brown, J. S., and D. L. Venable. 1986. Evolutionary ecology of seed-bank annuals in temporally varying environments. *American Naturalist* **127**:31–47.
- Brown, N. A. C. 1993. Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* **123**:575–583.
- Chang, M., and D. G. Lynn. 1986. The haustorium and the chemistry of host recognition in parasitic angiosperms. *Journal of Chemical Ecology* **12**:561–579.
- Chiapusio, G., A. M. Sánchez, M. J. Reigosa, L. González, and F. Pellissier. 1997. Do germination indices adequately reflect allelochemical effects on the germination process? *Journal of Chemical Ecology* **23**:2445–2453.
- Chou, C. H. 1973. The effect of fire on the California chaparral vegetation. *Botanical Bulletin Academia Sinica* **14**:23–24.
- Chou, C. H., and C. H. Muller. 1972. Allelopathic mechanisms of *Arctostaphylos glandulosa* var. *zacaensis*. *American Midland Naturalist* **88**:324–347.
- Christensen, N. L. 1977. Fire and soil–plant nutrient relations in a pine wiregrass savanna on the Coastal Plain of North Carolina. *Oecologia* **31**:27–44.
- Christensen, N. L., and C. H. Muller. 1975a. Effects of fire on factors controlling plant growth in *Adenostoma* chaparral. *Ecological Monographs* **45**:29–55.
- Christensen, N. L., and C. H. Muller. 1975b. Relative importance of factors controlling germination and seedling survival in *Adenostoma* chaparral. *American Midland Naturalist* **93**:71–78.
- Emery, D. E. 1992. *Seed propagation of native California plants*. Santa Barbara Botanic Garden, Santa Barbara, California, USA.
- Evans, G. C. 1976. A sack of uncut diamonds: the study of ecosystems and the future resources of mankind. *Journal of Ecology* **64**:1–9.
- Evenari, M. 1949. Germination inhibitors. *Botanical Review* **15**:153–194.
- Friedman, J. 1987. Allelopathy in desert ecosystems. Pages 118–128 in G. Waller, editor. *Allelochemicals: role in agriculture and forestry*. American Chemical Society, Washington, D.C., USA.
- Goodspeed, T. H. 1954. *The genus Nicotiana*. Chronica Botanica Company, Waltham, Massachusetts, USA.
- Gross, D., and B. Parthier. 1994. Novel natural substances acting in plant growth regulation. *Journal of Plant Growth Regulation* **13**:93–114.
- Harper, J. L. 1977. *Population biology of plants*. Academic Press, London, UK.
- Inderjit, and K. M. M. Dakshini. 1995. On laboratory bioassays in allelopathy. *Botanical Review* **61**:28–44.
- Karban, R., and I. T. Baldwin. 1997. *Induced responses to herbivory*. University of Chicago Press, Chicago, Illinois, USA.
- Keeley, J. E. 1991. Seed germination and life history syndromes in the California chaparral. *Botanical Review* **57**:81–116.
- Keeley, J. E., and C. J. Fotheringham. 1997. Trace gas emissions and smoke-induced seed germination. *Science* **276**:1248–1250.
- Keeley, J. E., and S. E. Keeley. 1989. Allelopathy and the fire-induced herb cycle. *Natural History Museum of the Los Angeles County Science Service* **34**:65–72.
- Keeley, J. E., B. A. Morton, A. Pedrosa, and P. Trotter. 1985. Role of allelopathy, heat and charred wood in the germination of chaparral herbs and suffrutescents. *Journal of Ecology* **73**:445–458.
- Keeley, S. C., and M. Pizzorno. 1986. Charred wood stimulated germination of two fire-following herbs of the California chaparral and the role of hemicellulose. *American Journal of Botany* **73**:1389–1397.
- Koniak, S. 1985. Succession in pinyon–juniper woodlands following wildfire in the great basin. *Great Basin Naturalist* **45**:556–566.
- Koniak, S., and R. L. Everett. 1982. Seed reserves in soils of successional stages of piñon woodlands. *American Midland Naturalist* **108**:295–303.
- Koves, E., and M. Varga. 1958. Growth-inhibiting substances in rice-straw. *Acta Biologica Szeged* **4**:13–16.
- Lynds, G. Y., and I. T. Baldwin. 1998. Fire, nitrogen and defensive plasticity in *Nicotiana attenuata*. *Oecologia* **115**:531–540.
- Lynn, D. G., and M. Chang. 1990. Phenolic signals in cohabitation: implications for plant development. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**:497–526.
- Mayer, A. M., and A. Poljakoff-Mayber. 1989. *The germination of seeds*. Pergamon, New York, New York, USA.
- McPherson, J. K., and C. H. Muller. 1969. Allelopathic effects of *Adenostoma fasciculatum* ‘chamise,’ in the California chaparral. *Ecological Monographs* **39**:177–198.
- Mirov, N. T. 1936. Germination behavior of some California plants. *Ecology* **17**:667–672.
- Moreno, J. M., and W. C. Oechel. 1991. Fire intensity and herbivory effects on post-fire resprouting of *Adenostoma fasciculatum* in southern California chaparral. *Oecologia* **85**:429–433.
- Muller, C. H. 1968. The role of chemical inhibition (allelopathy) in vegetational composition. *Bulletin of the Torrey Botanical Club* **93**:332–351.
- Philippi, T. 1993a. Bet-hedging germination of desert annuals: beyond the first year. *American Naturalist* **142**:474–487.
- . 1993b. Bet-hedging germination of desert annuals: variation among populations and maternal effects in *Lepidium lasiocarpum*. *American Naturalist* **142**:488–507.
- Pierce, S. M., K. Esler, and R. M. Cowling. 1995. Smoke-induced germination of succulents (*Mesembryanthema*

- ceae) from fire-prone and fire-free habitats in South Africa. *Oecologia* **102**:520–522.
- Qi, M. Q., M. K. Upadhyaya, N. H. Furness, and B. E. Ellis. 1993. Mechanism of seed dormancy in *Cynoglossum officinale* L. *Journal of Plant Physiology* **142**:325–330.
- Quick, C. R., and A. S. Quick. 1961. Germination of ceanothus seeds. *Madroño* **16**:23–30.
- Rice, E. L. 1984. *Allelopathy*. Second edition. Academic Press, New York, New York, USA.
- Rüdiger, W., and Lohaus, E. 1987. Germination and growth inhibitors as allelochemicals. Pages 118–128 in G. Waller, editor. *Allelochemicals: role in agriculture and forestry*. American Chemical Society, Washington, D.C., USA.
- Simons, L. H. 1991. Rodent dynamics in relation to fire in the sonoran desert. *Journal of Mammalogy* **72**:518–524.
- Stone, E. C., and G. Juhren. 1953. Fire stimulated germination: effect of burning on germination of brush seed investigated in physiological study of chamise. *California Agriculture* **7**:13–14.
- van de Venter, H. A., and A. D. Esterhuizen. 1988. The effect of factors associated with fire on seed germination of *Erica sessiliflora* and *E. hebecalyx* (Ericaceae). *South African Journal of Botany* **54**:301–304.
- Wardle, D. A., K. S. Nicholson, and A. Rahman. 1996. Use of a comparative approach to identify allelopathic potential and relationship between allelopathy bioassays and “competition” experiments for ten grassland and plant species. *Journal of Chemical Ecology* **22**:933–948.
- Wells, P. V. 1959. An ecological investigation on two desert tobaccos. *Ecology* **40**:626–644.
- Went, F. W. 1949. Ecology of desert plants II. *Ecology* **30**:1–13.
- Went, F. W., G. Juhren, and M. C. Juhren. 1952. Fire and biotic factors affecting germination. *Ecology* **33**:351–364.
- Wright, H. A., and A. W. Bailey. 1982. *Fire ecology*. John Wiley, New York, New York, USA.
- Wright, H. A., L. F. Neuenschwander, and C. M. Britton. 1979. The role and use of fire in sagebrush–grass and piñon–juniper plant communities. U.S. Forest Service General Technical Report **INT-58**.
- Young, A., and A. Evans. 1978. Population dynamics after wildfires in sagebrush grasslands. *Journal of Range Management* **31**:283–289.