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INHIBITION OF SEEDLING SURVIVAL UNDER *RHODODENDRON MAXIMUM* (ERICACEAE): COULD ALLELOPATHY BE A CAUSE?[1]

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In the southern Appalachian mountains a subcanopy species, *Rhododendron maximum*, inhibits the establishment and survival of canopy tree seedlings. One of the mechanisms by which seedlings could be inhibited is an allelopathic effect of decomposing litter or leachate from the canopy of *R. maximum (R.m.)* on seed germination, root elongation, or mycorrhizal colonization. The potential for allelopathy by *R.m.* was tested with two bioassay species (lettuce and cress), with seeds from four native tree species, and with three ectomycorrhizal fungi. Inhibitory influences of throughfall, fresh litter, and decomposed litter (organic layer) from forest with *R.m.* (+*R.m.* sites) were compared to similar extractions made from forest without *R.m.* (−*R.m.* sites). Throughfall and leachates of the organic layer from both +*R.m.* and −*R.m.* sites stimulated germination of the bioassay species above that of the distilled water control, to a similar extent. There was an inhibitory effect of leachates of litter from +*R.m.* sites on seed germination and root elongation rate of both bioassay species compared with that of litter from −*R.m.* sites. Native tree seed stratified in forest floor material from both forest types had a slightly higher seed germination rate compared with the control. A 2-yr study of seed germination and seedling mortality of two tree species, *Quercus rubra* and *Prunus serotina*, in field plots showed no significant influence of litter or organic layer from either forest type. Incorporating *R.m.* leaf material into the growth medium in vitro depressed growth of one ectomycorrhizal species but did not affect two other species. Leaf material from other deciduous tree species depressed ectomycorrhizal growth to a similar or greater extent as leaf material from *R.m.* In conclusion, *R.m.* litter can have an allelopathic effect on seed germination and root elongation of bioassay species as well as some ectomycorrhizal species. However, this allelopathic affect is not manifest in field sites and is not likely to be an important cause for the inhibition of seedling survival within thickets of *R.m.*

Key words: allelopathy; deciduous forest; ectomycorrhizae; Ericaceae; *Rhododendron*; seedling inhibition; southern Appalachian mountains; subcanopy.

In the southern Appalachian mountains *Rhododendron maximum* L. (*R.m.*) is the dominant subcanopy evergreen species. Approximately 30 million hectares of the southern Appalachian mountains are occupied by *R.m.* This subcanopy evergreen shrub forms extensive thickets, near streams and on north slopes, reaching a height of 3–6 m. On north-facing slopes these thickets form a mosaic of ~40% cover. There is clear evidence that the coverage of *R.m.* thickets has been increasing over the past 30 yr. For example, the area occupied by *R.m.* thickets in the Coweeta basin has increased from 14.8% in 1976 to 31.7% in 1993 (Dobbs, 1995). The increase in abundance of this species is a concern for forest managers because recruitment of canopy tree seedlings under *R.m.* thickets is substantially inhibited. Several studies over the past 50 yr document the extent of seedling inhibition and the potential for inhibition of canopy tree productivity by *R.m.* (Minkler, 1941; Wahlenberg, 1950; Barden, 1979; Day, Phillips, and Monk, 1988). These studies show that recruitment of most dominant canopy tree species are inhibited by *R.m.* However, the mechanisms by which this inhibition occurs are unknown.

The presence of *R. maximum* thickets in the subcanopy of Appalachian forest is important for the basic ecology of the forest and for designing forest management protocols. *Rhododendon maximum* is considered a "keystone species" because of its dominance in stream-side communities. Nutrient and water fluxes between the terrestrial and aquatic ecosystems are filtered and possibly impacted by this shrub (Swank and Crossley, 1988). Furthermore, *R.m.* is most abundant in sites with the highest potential for forest productivity (north-facing slopes and cove positions), which increases the importance of the influence that *R.m.* has on these forests. Consequently, critical adjustments in forest management protocols and forestry practices may be required based on the mechanisms by which *R.m.* inhibits canopy tree seedling recruitment.

Inhibition of canopy tree seedlings by *R.m.* in the subcanopy represents a syndrome in many other forests around the world. In fact, there are subcanopy evergreen layers in many deciduous, tropical, and evergreen forests (such as dwarf bamboo species, tropical palms, and other species in the Ericaceae), and inhibition of canopy tree seedlings is a common trait of these situations (Denslow,

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Newell, and Ellison, 1991; Nakashizuka, 1991; Messier, 1993; Fischer et al., 1994; Mallik, 1995; Widmer, 1998). Therefore, knowing the mechanism by which *R.m.* inhibits its canopy tree recruitment may have implications for similar processes in many other forest locations.

There are several potential allelopathic processes that may limit seedling recruitment under *R.m.* thickets, compared to that in forest without a subcanopy of *R.m.* Among these possibilities are inhibition of seed germination, retardation of root growth, and suppression of ectomycorrhizae by leachates from the *R.m.* canopy or from decomposing *R.m.* litter. The potential significance of allelopathy to seedling inhibition is suggested by several lines of evidence. Leaves of *R.m.* contain secondary compounds that have metabolic effects on humans and insects (Davidian, 1989), and many of the molecules that confer medicinal or antiherbivory properties to plants are the same as those that cause allelopathy (Rice, 1979). The relatively high quantity and the quality of secondary chemicals in *R.m.* leaves may be a reason why there is a paucity of chewing insects that attack *Rhododendron* (Nielson, 1980). Also, other species of *Rhododendron* have been documented to have medicinal properties in China (*R. dauricum*, *R. dabanshanense*), Korea (*R. chrysanthum*), and Russia (*R. luteum*). Field research indicates that light limitation in the understory is not the only factor reducing survivorship of *Acer rubrum* in *R.m.* thickets (Clinton and Vose, 1996). In addition, allelopathy is cited as one of the factors that maintains heath bald communities (dominated by *R. catawbiense* and *R.m.*) of the southern Appalachian mountains (Thomas and Pittillo, 1987). Moreover, species in other genera of the Ericaceae and Empetraceae (*Kalmia*, *Gaultheria*, *Empetrum*, *Ceratiola*) have been shown to have some allelopathic potential (Messier, 1993; Nilsson, 1994; Fischer et al., 1994; Mallik, 1995). Based on these results, the potential exists for allelopathic inhibition of seedling recruitment under *R.m.*

The overall objective of this research was to determine whether *R.m.* has the potential for allelopathic inhibition of seed germination, root growth, and ectomycorrhizal growth. We evaluate mycorrhizal growth because in previous research we have shown that seedlings growing under *R.m.* thickets have reduced incidence of mycorrhizal colonization compared to those in forest without *R.m.* (Walker et al., 1999). The experiments were designed to answer five questions. (1) Does rain that passes through the canopy (throughfall) of forest sites with a subcanopy of *R.m.* (+*R.m.* sites) inhibit seed germination more than that from similar forest sites without a subcanopy of *R.m.* (−*R.m.* sites)? (2) Do leachates of forest floor substrate from +*R.m.* sites inhibit seed germination more than that from −*R.m.* sites? (3) Will root growth be retarded for seeds germinating in the presence of leachates of forest floor substrates from +*R.m.* sites compared with that from −*R.m.* sites? (4) Does litter from *R.m.* inhibit the growth of fungi (known to form ectomycorrhizae with canopy tree species) more than litter from other canopy trees? (5) Does the +*R.m.* forest floor substrate inhibit native tree seed germination and seedling survival in situ more than that in −*R.m.* sites?

MATERIALS AND METHODS

Site description—This study site was located in a mature mixed-hardwood forest at Coweeta Hydrologic Laboratory in the Nantahala mountains of western North Carolina (35° 02′ 29″ N, 83° 27′ 16″ W). The site was on a north-facing slope (60%) at an elevation of 1000 m (above sea level) The canopy of this site was dominated by *Acer rubrum* (41%), *Quercus prinus* (22%), *Carya spp.* (7%), *Quercus rubra* (7%), *Nyssa sylvatica* (6%), *Oxydendron arboretum* (6%) and, <6%, *Betula lenta*, *Tsuga canadensis*, *Robinia pseudoacacia*, *Quercus palustre*, *Magnolia fraserii*, and *Acer pensylvanicum* with thickets of *R.m.* in the subcanopy. Thickets formed a mosaic across relatively uniform topography. The *R.m.* canopy was 3–4 m above the ground surface, while the forest canopy was ~20 – 25 m high. Soils were deep and well-drained coarse-loam of the Edneyville series (Thomas, 1996). The mineral soil is covered with a 5 cm thick layer of decomposing litter (organic layer) and a similar thickness of intact leaf litter (litter layer). The regional climate is classified as marine, humid with cool summers, mild winters, and with adequate rainfall (1800 mm annually) during all seasons (Swank and Crossley, 1988).

Leachate sampling design—Throughfall was collected with standard wet-dry collectors throughout the year. Following storm events during each month of the year, the throughfall was collected and immediately frozen (−25°C) until used in bioassays. Forest substrate was collected at five randomly selected locations in both +*R.m.* and −*R.m.* sites. Collections were made in the late spring (the time of most seed germination and seedling establishment). Forest floor substrate was separated into litter and partially decomposed organic layer and pooled by forest type (+*R.m.* or −*R.m.*). Leachates used in bioassays were made by mixing specified masses of fresh litter or organic layer with distilled water in zip-loc bags. The standard ratio of litter or organic layer to water was 1:5, and controls were distilled water alone in the zip-locs. The mixtures were incubated at 25°C for 24 h in a growth chamber and periodically shaken, before filtering through Whatman number 3 filter paper. Leachates were kept in the refrigerator until used within 5 d. The pH and osmolality of leachate solutions were measured before all experiments with a pH meter (Accumet pH meter Model 610, Fisher, Pittsburg, Pennsylvania) and a vapor pressure osmometer (Vapor pressure osmometer Model 5520, Wescor, Logan, Utah), respectively.

Bioassay of solution toxicity—Two bioassay species, lettuce (*Lactuca sativa* L. var. "Black Seeded Simpson" lot number 371, Wyatt-Quarles Seed Co., Garner, North Carolina) and cress (*Lapidum sativum* L. var. "Upland" lot no 1278, Wyatt-Quarles Seed Co., Garner, North Carolina) were used to assay potential inhibition of seed germination and radical elongation by leachates and throughfall. These species were selected because they germinate rapidly and have been used in many bioassay experiments in the past (Rice, 1979). In each bioassay experiment, two layers of Whatman number 1 filter paper were inserted in a sterile petri dish. The filter paper was moistened with 3 mL of leachate or throughfall solution at room temperature. One lot number of lettuce and cress seeds were used for all experiments. In seed germination tests, 30 lettuce or cress seed were randomly placed in the petri dishes, sealed with Parafilm, and placed in a growth chamber at 25°C, 60% relative humidity (RH), and a 12/12 h day/night (photosynthetic photon flux density 90 (μmol photons$\cdot m^{-2}\cdot s^{-1}$). Germination percentage was counted in all petri dishes daily at noon for 7 d (no more germination occurred after this time). The experiment was replicated five times.

Root elongation was studied by placing 20 seeds in two rows down the middle of the petri dish at five replicates per treatment. Each seed was oriented so that the roots grew out toward the edge of the plate. To permit repeated census at the same time each day an image was made of each bioassay plate with the use of an image analysis program on a MAC platform (NIH IMAGE). Length of root for each seedling

was measured using a calibrated scale placed in each petri dish. Mean root length was determined for each plate on each sampling date.

Native seed germination experiments—Individual lots of seed for seven species native to the research site were obtained from seed suppliers: eastern hemlock – *Tsuga canadensis* (L.) Carr, northern red oak –*Quercus rubra* L., and red maple –*Acer rubrum* L. seeds from Sheffield's Seed Co. Inc., Locke, New York; yellow poplar –*Liriodendron tulipifera* L. from F. W. Schumacher Co. Inc., Sandwich, Massachusetts; white pine –*Pinus strobus* L., black tupelo –*Nyssa sylvatica* Marshall, black birch –*Betula lenta* L. from Herbst Tree Seed, Inc., Fairview, North Carolina. Initial germination trials indicated that germination rates of three species (*Liriodendron tulipifera*, *Acer rubrum*, and *Betula lenta*) were too low (5-10%) to be useful in these experiments. Therefore, only four species, *Quercus rubra*, *Pinus strobus*, *Nyssa sylvatica*, and *Tsuga canadensis*, were analyzed at the end of the experiment. In each zip-loc bag, 50 seeds of each species were placed in 250 g of substrate brought to field capacity with distilled water. Three substrate types were used: forest floor substrate (litter and organic layer) from +*R.m.* sites, forest floor substrate from −*R.m.* sites, and vermiculite. The experiment was replicated three times for each species. Seed were imbibed for 3 mo at 3°C and then transferred into a growth chamber at 25°C, 60% RH, and a 12/12 h day/night. Germinating seed were counted each week for the next 5 mo. Moisture lost from bags was replaced as needed on a weekly basis.

Field substrate transplant experiments—A total of six 0.25-ha main plots were randomly located within ($N = 3$) and outside ($N = 3$) of the *R.m.* thicket. In each main plot, 15 2 × 2 m subplots were established resulting in a total of 90 plots, 45 within the *R.m.* thickets (+*R.m.*) and 45 in forest free of *R.m.* (−*R.m.*). The 15 subplots were randomly assigned to five substrate manipulation treatments: four combinations of −*R.m.* and +*R.m.* litter, and −*R.m.* and +*R.m.* organic layer, plus a control with no disturbance to the substrate (Fig. 1). In the fall of 1995, litter and the organic layer were scraped separately from the subplots, bulked, and equally redistributed back to appropriate assigned subplots. Each of the five treatment types was represented by three replicate subplots per main plot. The aim of the substrate manipulation was to evaluate the possible inhibitory effects of the *R. maximum* litter and organic layers. The experimental plots were allowed to settle over the winter before seeds were planted in the following spring.

Two species, *Quercus rubra* and *Prunus serotina*, abundant in the canopy of the region were selected. Acorns were collected for northern red oak (*Quercus rubra*) in the vicinity of the experimental plots in March and April 1996 and planted in mid-April 1996. The naturally stratified acorns were planted into the plots at 16 acorns per subplot. Black cherry (*Prunus serotina*) seeds of local provenance were soaked in concentrated H_2SO_4 for 30 min, stratified in two separate lots of −*R.m.* and +*R.m.* organic substrate in 4°C for 4 mo, and planted at 16 seeds per subplot. Seed germination and seedling mortality were measured for the next 2 yr. A split-plot design analysis of variance was used to test for significant main plot (the six 0.25-ha plots), and substrate type (treatment) on seed germination and seedling mortality (SAS, 1998). The data were partitioned according to main plot (MPLOT), forest type (FTYPE), and substrate treatment (TREAT). The degrees of freedom for FTYPE was 1, for MPLOT, TREAT, and their interaction was 4, and for the interaction of MPLOT and TREAT was 16. Significance of 0.05 was used in all cases. The two main forest types (+*R.m.* and −*R.m.*) are not strictly treatments, but rather represent naturally occurring heterogeneity in the forest. Therefore, the main effect of canopy type on germination and survivorship was determined using the following sums of square (SS of Type III ANOVA, proc ANOVA; SAS, 1998) ratio to calculate the F statistic with df 1,4;

$$F = \frac{\text{SS forest type } /1}{\text{SS main plot (forest type)}/4.}$$

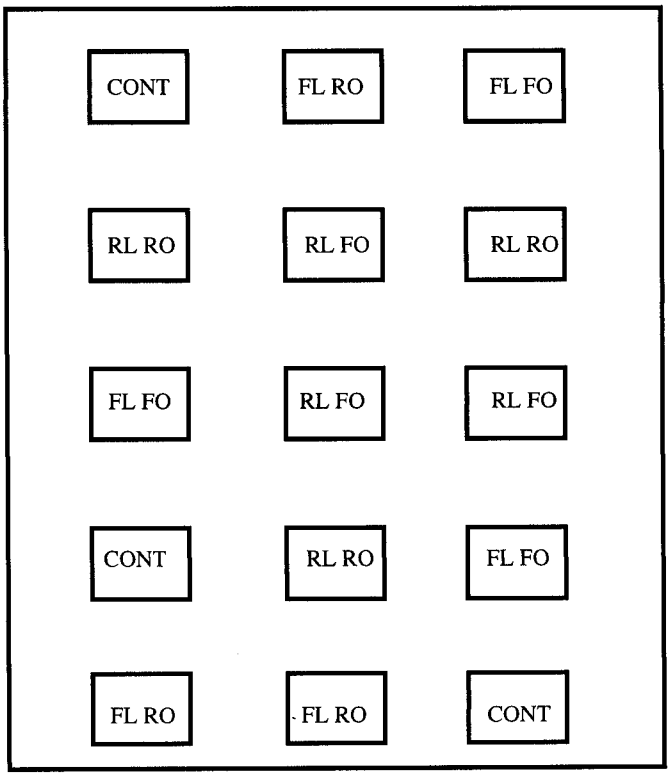


Fig. 1. Plot design for the forest substrate translocation study. Three such plots were randomly placed in forest with a thicket of *R. maximum* present in the subcanopy and three were in forest without a thicket of *R. maximum*. Main plot number 5 is shown here. Control no substrate manipulation, RL litter from under *R. maximum* thickets, RO organic layer from substrate under *R. maximum* thickets. FL litter from forest without *R. maximum* present, FO organic layer from substrate in forest without *R. maximum* present.

Bioassay of litter toxicity to ectomycorrhizae—Decoction experiments were conducted using solid Hagem's media modified by Cripps and Miller (1995) with and without the addition of 16 g/L of chopped leaf materials. All leaf litter was collected during July and stored frozen until used. The leaves were cut into fine pieces and incorporated into the media, which was then autoclaved for 18 min at 250°C. The media was poured on petri plates while warm with constant agitation to evenly distribute the leaf materials among the plates. After cooling, the decoction media plates and controls were inoculated with the mycelium of ectomycorrhizal fungi prepared as follows.

Two ectomycorrhizal bioassay experiments were performed. In the first experiment, one litter type (*R.m.*) and three ectomycorrhizae species, *Cenococcum geophilum* Fr. (VTCC 1409), *Pisolithus tinctorius* Pers. (VTCC 3303), and *Suillus pictus* (Pk.) Smith and Theirs (VTCC 3326), were used. Voucher cultures for all three strains of the fungal species are being maintained in the Virginia Tech Culture Collection. In the second experiment, five litter types (*Rhododendron maximum*, *Tsuga canadensis*, *Quercus rubra*, *Betula lenta*, and *Prunus serotina*) and two mycorrhizae species (*Cenococcum geophilum* and *Pisolithus tinctorius*) were used. For both experiments, the fungi were initially grown on solid Hagem's media as modified by Cripps and Miller (1995), for 1 mo at 20°C. Uniform squares of mycelium and agar were cut from these plates, near the actively growing margin of the fungal mycelium, and used to center inoculate the bioassay plates. Immediately after inoculation, two perpendicular lines were drawn on the lid of the plate. Measurements of radial growth of the fungi were taken along the predetermined lines from the edge of the inoculum block. In the first experiment, measurements were taken over a period from 28 to 37 d at

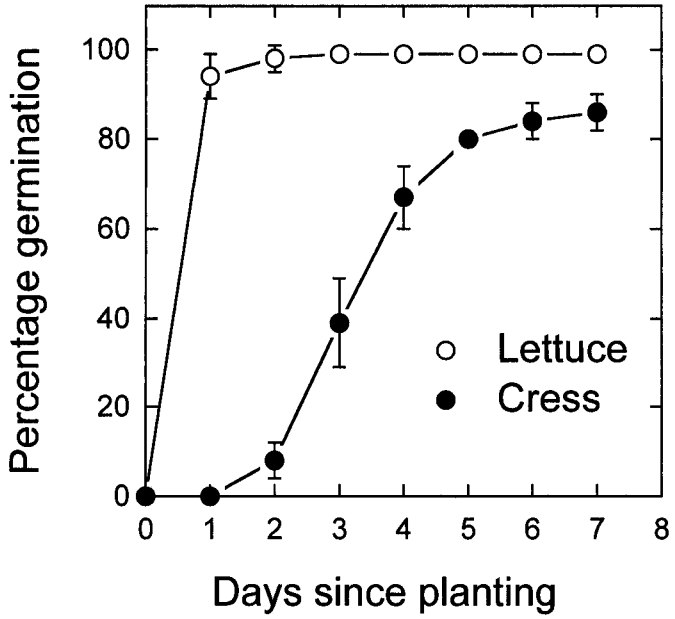


Fig. 2. Seed germination of lettuce (*Lactuca sativa* L.) var. Black Seeded Simpson and cress (*Lapidum sativum* L) var. Upland imbibed with distilled water in a growth chamber at 25°C, 60% RH, and a 12/12 h day/night (photosynthetic photon flux density = 90 μmol photons·m^{-2}·s^{-1}). Each point on the plot is a mean of five plates containing 30 seeds each (150 seeds total). Error bars represent ±1 SE of the mean.

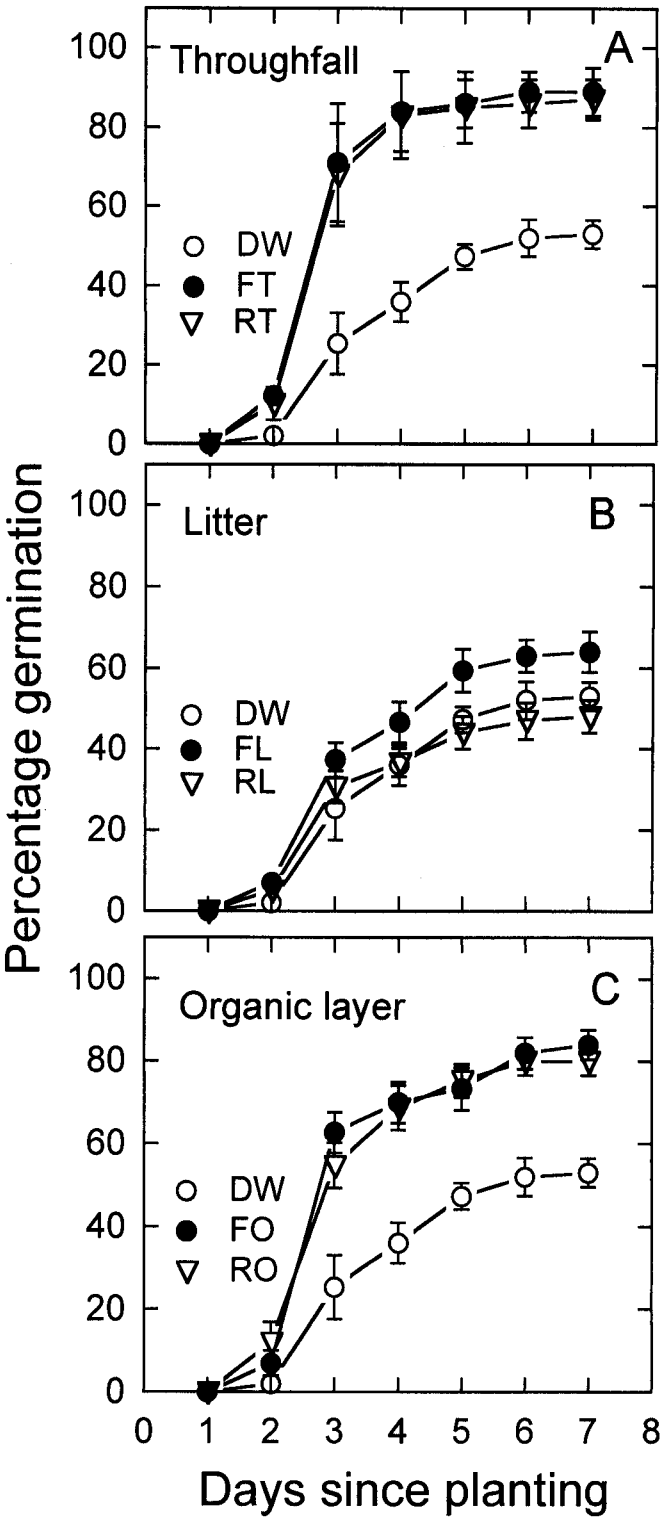
~1-wk intervals. The second experiment was only measured once, 6 wk after inoculation.

Differences between mean radial growth on control plates vs. decoction media plates were analyzed using Students *t* tests for each fungal strain on each measurement date for the first experiment. Analysis of variance was used for the second experiment to examine the main effects of inoculum species and the leaf material added (SAS, 1998). Ten replicates were used per treatment in the first experiment and seven per treatment for the second experiment.

RESULTS

Seed germination—Both bioassay species of cress and lettuce attained their highest germination rates in all experiments within 1 wk (Fig. 2). Lettuce seed germinated to 90% within 24 h and to 95% within 2 d of imbibing. Cress seed germinated at a slower rate and to a lower percentage reaching a maximum of 80% after 7 d. Due to relatively fast germination rates there was no contamination of any plates of either seed type by fungi or bacteria. Lettuce seed germination was not influenced by any treatments (data not shown), probably because of the very rapid germination.

There was no difference in the germination percentage of cress when exposed to throughfall collected from *+R.m.* or *−R.m.* sites (Fig. 3A) on all dates (only one date for cress is shown in Fig. 3A). However, germination rates of cress in the presence of distilled water (reaching



thicket of *Rhododendron maximum*. DW distilled water control. (B) The response of seed germination of cress (*Lapidum sativum* L) var. Upland to leachates made from forest floor litter, FL = litter from forest sites without *R. maximum*, RL = litter from forest sites with *R. maximum*. (C) The response of seed germination of cress (*Lapidum sativum* L) var. Upland to leachates made from forest floor organic layer. RO = organic layer from forest sites with *R. maximum*, FO = organic layer from forest sites without *R. maximum*. Each point on the plot is a mean of five plates containing 30 seeds each (150 seeds total). Error bars represent 1 SE of the mean.

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Fig. 3. (A) The response of seed germination of cress (*Lapidum sativum* L) var. Upland to throughfall collections. Throughfall was collected on 15 October 1996 in forest without (FT) and with (RT) a

Table 1. Mean germination percentages for four species native to the southern Appalachian mountains when imbibed in forest floor substrate from sites without (−*R.m.*) or with (+*R.m.*) a thicket of *Rhododendron maximum*. *Q.r.* = *Quercus rubra*, *P.s.* = *Pinus strobus*, *N.s.* = *Nyssa sylvatica*, *T.c.* = *Tsuga canadensis*. The control treatment was pure vermiculite. Numbers in parentheses are standard errors of the mean.

Treatment	Species			
	Q.r.	*N.s.*	*P.s.*	*T.c.*
−R.m.	42.0 (4.1)	22.7 (1.9)	44.3 (3.7)	15.2 (5.4)
+*R.m.*	45.7 (1.3)	22.0 (1.6)	46.3 (2.1)	16.6 (3.1)
Control	36.5 (1.5)	16.3 (1.3)	49.3 (1.2)	28.0 (1.6)

only 55%) were less than those of both throughfall treatments. Cress seed attained ~85% germination when exposed to leachate made with the organic layer from either forest type (Fig. 3C). However, cress seed imbibed in leachate made with −*R.m.* litter reached only 68% and that imbibed with +*R.m.* leachate attained 52% germination (Fig. 3B).

Germination studies of native seeds are more difficult than studies with bioassay species because the seed of native species in temperate regions often require vernalization, the germination percentage is low, and the germination rate is slow. This was taken into account by vernalizing seed of native species in forest substrate from +*R.m.* and −*R.m.* sites, and retaining the seeds in the same substrate for the germination test. The *Q. rubra* seed germinated most quickly followed by *P. strobus*, *Nyssa sylvatica*, and *T. canadensis*. There was no significant difference in final germination percentages between the two forest substrate types (Table 1). However, vernalization in forest substrate resulted in higher germination of *Q. rubra* and *N. sylvatica* than in the control substrate and lower than the control for *P. strubus* and *T. canadensis*. Total germination was below 50% for all native species.

Root elongation—Data on root length are presented for lettuce only, although results for cress were similar. Root lengths of lettuce seedlings germinated in throughfall taken from the two forest types were not different (Fig. 4A). Similar results were attained when studying throughfall from other collection dates (data not presented). Root lengths at the end of the experiment were slightly greater for throughfall treatments compared to the distilled water control.

Root lengths for lettuce seedlings were the longest among all experiments when tested with leachates of organic layer from either forest type (Fig. 4C). There was no significant difference in root length of the bioassay species when exposed to leachates of organic layer from the two forest types, but root lengths of both species were at least double that of the distilled water control.

Root lengths for lettuce seedlings were longer when tested with leachates of litter from −*R.m.* sites in comparison to that from +*R.m.* sites (Fig. 4B). It is important to note that when leachates of litter from +*R.m.* sites were used as a bioassay solution, the resultant root elongation rate was not significantly different from that of distilled water. These initial root length experiments showed that root elongation was stimulated compared with the dis-

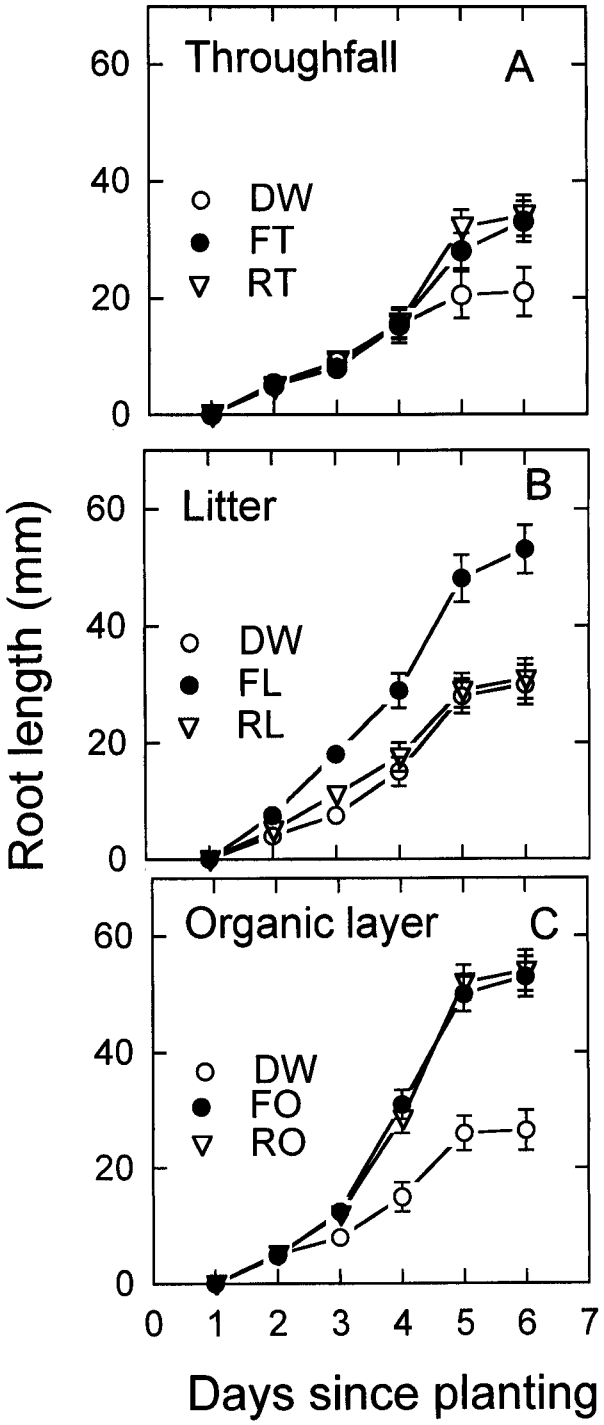

Fig. 4. (A) The response of root length for seedlings of lettuce (*Lactuca sativa* L.) var. Black Seeded Simpson lot number 371 (1997) to throughfall collected on 15 October 1996 in forest without (FT) and with (RT) a thicket of *Rhododendron maximum* in the subcanopy. (B) The response of root length for seedlings of lettuce to leachates from fresh litter collections. Litter was collected in August 1996 from forest without (FL) and with (RL) a thicket of *Rhododendron maximum*. (C) The response of root length for seedlings of lettuce to leachates from decomposed organic layer collections. Organic layer was collected in August 1996 in forest without (FO) and with (RO) a thicket of *Rhododendron maximum*. Each point on the plot is a mean of five plates containing 20 seeds each (100 seeds total). Error bars represent ±1 SE of the mean.

Table 2. The pH and osmolality of rain passing through the canopy (throughfall) and leachates from forest floor substrates used in a test of allelopathic potential of *Rhododendron maximum* in the southern Appalachian mountains. DW = distilled water control, RT = throughfall from forest with a stand of *R. maximum*, FT = throughfall from forest without a stand of *R. maximum*, RO = leachate of organic layer from forest with a stand of *R. maximum*, FO = leachate of organic layer from forest without a stand of *R. maximum*, RL = leachate of litter from forest with a stand of *R. maximum*, FL = leachate of litter from forest without a stand of *R. maximum*.

Measurement	Extraction (g/g) (dry mass/water mass)	DDW	RT	FT	RO	FO	RL	FL
pH	none	7.04	6.75	7.05	—	—	—	—
	1:5	7.04	—	—	4.67	5.19	5.94	5.05
Osmolality (mmol/kg)	none	5	7	8	—	—	—	—
	1:5	5	—	—	6	8	7	7

tilled water control by most leachates and that stimulation increased from throughfall, to litter, to organic layer.

Measurement of solution pH indicated that the distilled water control had a pH close to neutrality (Table 2). The pH of the *+R.m.* site organic layer was below 5, while that of the *−R.m.* site organic layer was slightly higher. The pH of the *R.m.* litter was the highest of all leachates (~6). Osmotic potential of all solutions was <10 mmol/kg (Table 2) with no discernible differences among leachates. Neither pH nor osmotic potential was a likely factor associated with reduced seed germination and root elongation in the *+R.m.* litter leachates because there was very little variation in these factors among all treatments.

Ectomycorrhizal growth—Growth rates of *Suillus pictus* (*S.p.*) and *Cenococcum geophilum* (*C.g.*) were not affected by *R.m.* leaf litter. In contrast, growth of *Pisolithus tincorius* (*P.t.*) was significantly reduced ($P < 0.01$) by *R.m.* litter in the medium by more than 65% in the first experiment (Fig. 5) and by 25% ($P>0.05$) in the second experiment (Fig. 6.). However, growth of *P.t.* also was significantly inhibited by the litter of all other species tested (Fig. 6). In fact, the inhibition of *P.t.* growth by litter from *Betula lenta*, *Quercus rubra*, and *Prunus serotina* was 50% greater than that by *R.m.* The growth of *C.g.* was not inhibited by litter from *R.m.* as in the previous experiment, but was significantly inhibited by all other litter types (Fig. 6). Litter from *Prunus serotina* caused the greatest inhibition of *C.g.* growth.

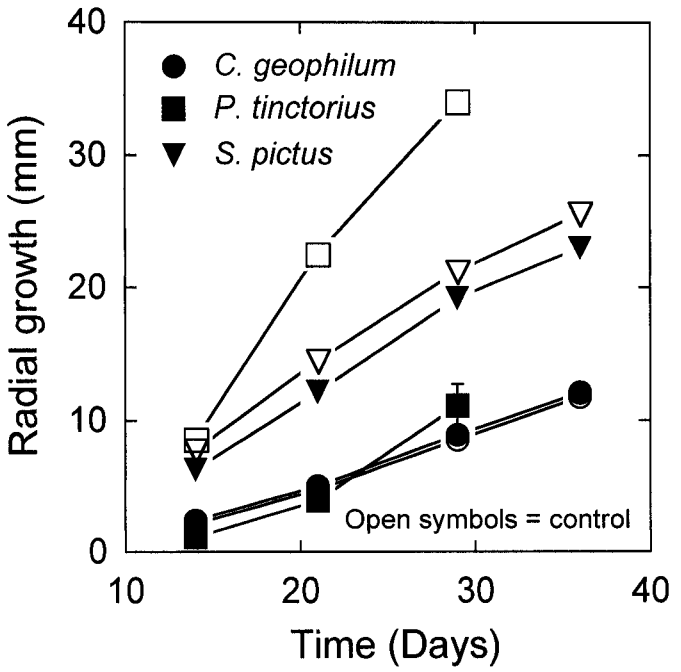

Fig. 5. Radial growth of three species of ectomycorrhizae on medium with (decoction) or without (control) the incorporation of litter from *R. maximum*. Each point is a mean of ten plates per treatment. Error bars represent ±1 SE of the mean.

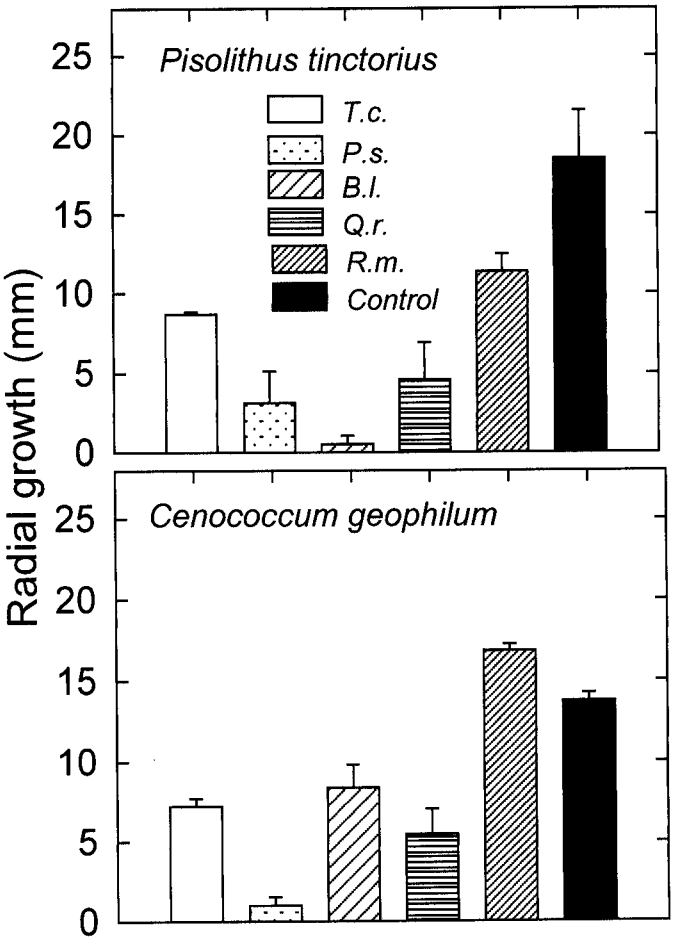

Fig. 6. Radial growth of the ectomycorrhizae *Pisolithus tinctorius* and *Cenococcum geophilum* in modified Hagem's media without (control) or with leaf litter (decoction). Each bar refers to the mean of seven plates containing a decoction media with the litter of one species. Error bars represent ±1 SE of the mean. *B.l.* = *Betula lenta*, *Q.r.* = *Quercus rubra*, *P.s.* = *Prunus serotina*, *R.m.* = *Rhododendron maximum*, *T.c.* = *Tsuga canadensis*.

Table 3. Results of ANOVA on seedling survivorship for *Quercus rubra* (oak) and *Prunus serotina* (cherry) in a substrate manipulation experiment (TREAT) after 2 yr of growth in forest with and without the presence of *R. maximum* (FTYPE).The experiment had six plots (MPLOT; three of each FTYPE) five treatments (unmanipulated control and all combinations of litter and organic layer manipulation), and three replicates of each treatment per main plot.

ANOVA	Oak			Cherry		
	df	*F*	Pr > *F*	df	*F*	Pr > *F*
FTYPE	1	65.01	0.01	1	59.18	0.01
FTYPE (MPLOT)	4	1.90	0.12	4	2.16	0.09
TREAT	4	1.20	0.08	4	2.14	0.09
FTYPE × TREAT	4	0.20	0.94	4	2.18	0.08
MPLOT × TREAT × FTYPE	16	0.62	0.86	16	1.78	0.06

Field experiment—Seed of *Q. rubra* germinated the first year after planting, while that of *P. serotina* did not germinate until the spring of the second year. There was no significant effect of main plot or treatment on the germination of either species. The mean (±1 SE) germination rate for *Q. rubra* was 10.8 ± 0.8 in −*R.m.* plots and 9.0 ± 0.6 in +*R.m.* plots and for *P. serotina* was 3.0 ± 0.8 in −*R.m.* plots and 3.5 ± 0.7 in +*R.m.* plots. Our ANOVA results on survivorship of both *Q. rubra* (2 yr old) and *P. serotina* (1 yr old) seedlings indicated no significant effects of treatment, but a significant effect of forest type (Table 3). The mean number of *Q. rubra* seedlings remaining after 2 yr was 6.25 ± 0.5 in −*R.m.* plots and 2.0 ± 0.3 in +*R.m.* plots. The mean number of *P. serotina* seedlings remaining after 1 yr was 1.9 ± 0.3 in −*R.m.* plots and 0.5 ± 0.1 in +*R.m.* plots.

DISCUSSION

There are many studies in the literature that suggest various members of the Ericaceae and Empetraceae have allelopathic potential (Rice, 1979; Putnam and Tang, 1986; Fischer et al., 1994; Nilsson, 1994; Mallik, 1995) including *Rhododendron* (Yang and Wang, 1978; Nielson, 1980; Pittillo, 1980; Wang and Yang, 1981). Even though several *Rhododendron* species can have negative influences on other species, there have been no studies of the allelopathic potential of these taxa native to North America and Europe. This is surprising because several members of the genus can become invasive or suppressive in these regions (Fuller and Boorman, 1977; Monk, McGinty and Day, 1985). To infer that *Rhododendron maximum* exerts dominance over competing species through allelopathy, we must first demonstrate its potential toxicity.

Did throughfall from +*R.m.* sites inhibit seed germination more than that from −*R.m.* sites? No, throughfall from neither forest type inhibited germination of any species at any time of the year. Compared to the distilled water control, throughfall from both forest types actually stimulated seed germination. Stimulation of germination could have been the result of nutrients in the throughfall or the slightly acidic pH compared to the control.

Did the forest floor substrate (litter or organic layer) from +*R.m.* sites inhibit seed germination more than that from −*R.m.* sites? We found contrasting results. Seed germination of cress was not inhibited by leachates of the organic layer from either forest type when compared to the distilled water control. In contrast, seed germination of cress was stimulated to a lesser degree by leachates from fresh litter compared to that from organic layer or throughfall. Furthermore, the only difference in germination percentage of cress between leachates of the two forest types was attributable to litter. Leachates of litter from +*R.m.* sites may have been more toxic than that from −*R.m.* sites because other potential effects on germination, such as pH or osmolality, were not different between litter leachates. Alternatively, the nutritional quality of leachate from litter in +*R.m.* sites could have been deficient enough to limit germination (*R.m.* leaves have relatively low nutrient content; Monk, McGinty, and Day, 1985; personal observation).

To determine whether results from cress seed (which germinates in 7 d) were relevant to natural conditions, similar experiments were performed with seed of native tree species. The lack of significant difference between +*R.m.* and −*R.m.* sites in this experiment indicates that the forest floor substrate from locations with a *R.m.* thicket does not inhibit germination of native seeds any more than that from locations without a *R.m.* thicket. These results were supported by germination trials with northern red oak in field plots. However, the two deciduous species (northern red oak, black gum) had higher germination in either forest substrate treatment than in the vermiculite control. In comparison, both evergreen species (white pine, eastern hemlock) had lower germination in either substrate treatment than in the vermiculite. This difference was unrelated to forest substrate type and thus must be related to seed germination requirements and the differential structure or chemistry of substrate vs. vermiculite.

Was root length shorter in the presence of forest floor leachates from +*R.m.* sites compared with that from −*R.m.* sites? The answer to this question was different depending on which substrate was used to make the leachate. Root growth of lettuce seedlings was enhanced compared to the distilled water control in all treatments except litter leachate from +*R.m.* sites. No differences in root length of lettuce seeds were found in −*R.m.* sites for throughfall or organic layer leachates. However, litter leachates from +*R.m.* sites resulted in a significantly reduced root length compared to that from −*R.m.* sites.

Does leaf material from *R.m.* inhibit ectomycorrhizal growth? Yes, in one of the ectomycorrhizal species studied, *Pisolithus tinctorius*, growth was inhibited by *R.m.* leaf material. However, inhibition was less than that from leaves of other canopy tree species. Therefore, it is not likely that leaf litter on the forest floor under a thicket of *R.m.* will be any more inhibitory to ectomycorrhizal growth than leaf litter from forest without *R.m.*.

Our experiments with native seedling survivorship in situ also showed that there was no effect of substrate type in either forest type. If substrate from +*R.m.* sites was toxic by itself, then there should have been a clear treatment effect on seedling survival in both forest types. Furthermore, if toxicity were due to the combination of substrate and long-term throughfall effects, there should have been a treatment effect in +*R.m.* sites, but we did not detect any significant interaction of treatment with forest type.

It is important to note that seedling growth (not germination) in the field was significantly lower for *+R.m.* plots compared with *−Rm.* plots (data not presented). If this difference was due to allelopathy it could not be the result of litter, organic matter, or short term throughfall components of the system. The reduced growth rate of seedlings growing in *+R.m.* sites is most likely due to reduced resource (light) availability. However, a long-term continual influx of toxin from root exudates and throughfall may be an additional factor reducing seedling growth rate.

Although our study did not discern a significant allelopathic influence in the field, there are examples in other forest situations where allelopathy may be an important process for shrub inhibition of canopy tree seedlings. For example, there is strong evidence that allelopathy is an important mechanism by which black spruce seedlings are inhibited by *Kalmia angustifolia* in Newfoundland (Mallik, 1995). Moreover, toxicity of *Kalmia angustifolia* may be manifest through an inhibition of ectomycorrhizae (Yamanashi et al., 1998). More studies on the allelopathic potential of subcanopy evergreen shrubs are needed before a generalized model can be developed.

CONCLUSIONS

When the findings from these experiments are considered together, there is some support for allelopathic interference of seed germination and root growth by litter from *+R.m.* sites. However, this is not likely to be relevant to natural conditions because germination and root growth occur mostly in the organic layer where toxicity was not demonstrated. The unlikely presence of allelopathic effect in situ is further supported by the absence of inhibition of native seed germination and the absence of a detectable substrate effect in field trials of seedling survivorship.

Data collected in this study clearly indicate that for those species tested, direct allelopathic influences of *R.m.* on seed germination, initial root growth, or ectomycorrhizal growth cannot be considered an important factor associated with the inhibition of seedling survival under *R.m.* Our results with ectomycorrhizae do not apply to species that are colonized by vesicular-arbuscular (VA) mycorrhizae such as *Prunus serotina* or *Liriodendron tulipifera*. Further studies on toxicity to VA mycorrhizae is important to completely rule out allelopathy as a significant factor determining low seedling recruitment under *R.m.* thickets. Our data also do not rule out indirect allelopathic effects. For example, litter leachates and throughfall may not be inhibitory to the growth of mycorrhizal mycelia, but may be inhibitory to mycorrhizal colonization of tree seedlings under *R.m.* thickets. Since a majority of native species require mycorrhizal synthesis for survival in the subcanopy, allelopathic inhibition of this colonization could reduce seedling survival. Also, the release of allelochemicals in *R.m.* litter may inhibit the normal action of bacteria and invertebrates resulting in reduced soil nutrient availability. Lower soil nutrient availability is a characteristic of forests where *R.m.* is present and may be an important process for seedling survival. In fact, the combination of resource limitation (nutrients and light) and inhibition of mycorrhizal synthesis may be the predominant process regulating canopy tree seedling survival in the southern Appalachian mountains.

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