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The effect of *Kalmia angustifolia* on the growth, nutrition, and ectomycorrhizal symbiont community of black spruce

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Abstract

Kalmia angustifolia L. is an ericaceous shrub that frequently invades black spruce (*Picea mariana* Mill.) clear-cuts in central Newfoundland. Field observations suggest that on many sites where *K. angustifolia* grows, black spruce seedlings become chlorotic and stunted. Previous laboratory research has suggested that allelochemicals of *K. angustifolia* affect the growth and development of black spruce as well as the growth of certain ectomycorrhizal (ECM) fungi associated with black spruce. Black spruce seedlings close to (< 1 m) and far from (> 1 m) *K. angustifolia* were sampled from a clear-cut in central Newfoundland. The ECM community structure, degree of mycorrhizal infection, height, mass, root:shoot ratio, and the foliar concentrations of N, P, K, Ca, and Mg of spruce seedlings growing close to and far from *K. angustifolia* were examined. Seedlings close to *K. angustifolia* had significantly lower foliar concentrations of N and P, had a lower rate of mycorrhizal infection, and were more frequently associated with *Phialocephala dimorphospora* Kendrick, a potential root pathogen of black spruce, than seedlings growing far from *K. angustifolia*. There were positive linear relationships between black spruce foliar N concentration and total seedling height and biomass for seedlings growing away from *K. angustifolia*, but not for seedlings in close proximity to *K. angustifolia*. Hypotheses suggesting possible roles for nutrient competition, allelopathy, and *K. angustifolia*'s ability to increase the occurrence of the pseudomycorrhizal *P. dimorphospora* on black spruce are proposed. © 1998 Elsevier Science B.V.

Keywords: Ericaceae; *Picea mariana*; Ectomycorrhizae; Nutrient competition; Allelopathy

1. Introduction

Kalmia angustifolia L. is a semi-shade tolerant ericaceous shrub that is distributed throughout the Atlantic seaboard of eastern North America, and westward through the provinces of Quebec and Ontario and adjacent American states (Hall et al., 1973; Ebinger, 1974 Ebinger, 1988). In central Newfoundland, *K. angustifolia* is often a component of black spruce (*Picea mariana* Mill.) ecosystems (Meades and Moore, 1989) where it is found scattered beneath

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mature spruce stands, especially where gaps in the canopy permit the entry of light to the forest floor. In a systematic survey of plantations in central Newfoundland, *K. angustifolia* was present in 55% of quadrats sampled (English and Hackett, 1994). *K. angustifolia* can spread very rapidly after spruce stands are lost through harvesting, fire, or severe insect attack. Stocking of *K. angustifolia* can increase from 30% at harvesting to 65% within 5 yr and 85% within 14–15 yr (van Nostrand, 1971). This high rate of site occupancy is attained mainly through vegetative spread from secondary rhizomes (Mallik, 1993; Titus et al., 1995). Having extensively invaded many black spruce plantations, *K. angustifolia* is perceived by forest managers as one of the greatest obstacles to successful reforestation in central Newfoundland.

Field observations suggest that on many sites an increase in *K. angustifolia* cover is associated with chlorosis and decreased height growth of black spruce (English and Hackett, 1994). This condition is similar to the planting check of conifers caused by other ericaceous shrubs (de Montigny and Weetman, 1990) such as heather (*Calluna vulgaris* (L.) Hull) (Weatherell, 1953; McIntosh, 1983; Morgan et al., 1992) and salal (*Gaultheria shallon* Pursh) (Prescott and Weetman, 1994). Litter of *K. angustifolia* (Titus et al., 1995) and extracts of *K. angustifolia* foliage and litter (Peterson, 1965; Mallik, 1987) have been shown to inhibit black spruce seed germination, primary root growth of black spruce (Mallik, 1987), and in vitro growth of certain spruce ectomycorrhizal (ECM) fungi (Mallik and Zhu, 1995; Titus et al., 1995). These mechanisms could account for the reduction in spruce growth observed in the field, but to date have only been tested under laboratory conditions. Although scarification, fertilization, and *K. angustifolia* eradication all enhance early conifer growth (Titus and English, 1997), a better understanding of the mechanisms causing black spruce check in the presence of *K. angustifolia* in the field is needed to refine silvicultural prescriptions to ensure successful reforestation of *K. angustifolia*-invaded sites in central Newfoundland.

The goals of this study were to examine the effects of the proximity of *K. angustifolia* plants on black spruce seedling growth, nutrient status, and root ECM symbiont community under field conditions and to test the hypothesis that seedlings planted close to *K. angustifolia* would have lower growth rates, lower levels of foliar nutrients, lower mycorrhizal infection rates, and a different mycorrhizal community than seedlings planted away from *K. angustifolia* plants. Information on the state of the various seedling parameters examined close to and far from *K. angustifolia* were also used to generate further hypotheses about the mechanisms by which *K. angustifolia* interferes with black spruce seedlings in the field.

2. Materials and methods

2.1. Field site

Black spruce seedlings were collected from a clear-cut in the Northern Arm Brook valley 6 km west of Botwood (49°08′N, 55°26′W) in the north–central subregion of the Central Newfoundland Ecoregion (Damman, 1983). The site lies within the Central Lowlands climatic zone, with 100–120 frost-free days, 1200–1300 degree-days > 5°C, and 950–1050 mm total precipitation per year, including 250–300 cm of snowfall (Banfield, 1983). The sampling area was located on a glacial outwash terrace with till capping and soils were orthic humo-ferric podzols. The previous stand of 65- to 70-yr old black spruce was selectively cut for saw-logs between 1973 and 1974, burned in a wildfire in 1979, and then salvage cut for pulpwood in 1982. The site was planted during the last week of June and the first week of July 1990 with 2-yr old black spruce containerized stock.

2.2. Field sampling

Thirteen points within the plantation were randomly located on a map of the site. At each point a pair of seedlings was selected: the nearest seedling which was less than 1 m from a *K. angustifolia* plant (‘close’ seedlings) and the nearest seedling at a distance greater than 1 m (‘far’ seedlings). Previous research on the same site indicated that *K. angustifolia* plants have a radius of influence of approximately 1 m, within which black spruce seedling growth is suppressed (Titus, unpublished data). Root

systems of black spruce seedlings were excavated carefully so as not to damage roots. Samples were placed in plastic bags and refrigerated at 5°C until processed. Samples were collected on three different dates (June 6 and July 7 and 23, 1992) because of the logistical requirements of the experimental materials, including the maintenance of the viability of ECM on short-roots and the time required to completely process seedlings.

2.3. Evaluation of the degree of infection and identification of ECM type on short-roots

Thirty root segments (each approximately 2 cm long) were cut from the root system of each seedling, from which 10 segments were randomly chosen for evaluation. In all, 4405 short-roots were examined. The degree of infection was determined by direct observation of root segments using light microscopy according to the methods and criteria of Danielson et al. (1984). The numbers of infected and non-infected short-roots of each root segment were counted and recorded. Each ECM type encountered during examination was classified according to colour, root-segment structure and short-root morphology. Short-roots were excised and mantle and emanating hyphae characteristics were observed under a compound microscope. Short-roots were then individually surface-sterilized (70% ethanol for 30 s, 20% solution of a household bleach solution for 1 min, and distilled and deionized water twice) and plated on modified Melin–Norkrans (MMN) agar (Marx, 1969). A total of 460 isolates were prepared in this way. Fine-roots from several *K. angustifolia* plants in the study area were also sampled, surface-sterilized, and plated using the same procedures that were applied to black spruce short-roots.

The isolates obtained from surface sterilized short-roots of spruce and fine-roots of *K. angustifolia* were classified according to culture characteristics (hyphal mat characteristics, presence of sclerotia, and pigmentation) on MMN agar. DNA was extracted (Zolan and Pukkila, 1986) from agar plugs taken from the edges of cultures. Sections of ribosomal DNA were amplified by the polymerase chain reaction (PCR) (Saiki et al., 1988). Reaction conditions were the same as used by Stoyke et al. (1992) except that the primers used were ITSI (5′-TCC GTA GGT GAA CCT GCG G-3′) and NL8mun (5′-TTG GTC CGT GTT TCA AGA CG-3′). The location of the former is given in White et al. (1990) and the latter is located approximately 600 bp downstream of the 5′ end of the 28S rRNA gene (Egger, 1995). Samples were amplified using a Perkin–Elmer-Cetus DNA thermocycler for 30 cycles as follows: 94°C for 1 min, 42°C for 1 min, and an extension cycle of 72°C for 2 min (with an extension of 1 s per cycle), and with the initial denaturation cycle increased to 5 min.

Restriction fragment length polymorphism (RFLP) analysis (with restriction enzymes AvaII, DdeI, HaeIII, MspI, and NdeII) was applied to the products of PCR (Stoyke et al., 1992). Gel electrophoresis was on 2% agarose gel (1.5% NuSieve, 0.5% Seakem GTG agarose; FMC Bioproducts). A data matrix was generated from RFLP fragment patterns and an unweighed-pair-group-method-using-arithmetic-averages (UPGMA) cluster analysis (Sneath and Sokal, 1973) was performed (Stoyke et al., 1992) in order to confirm the grouping of isolates by culture characteristics and compare isolates obtained from field sampling with known isolates.

Grouping of isolates by culture characteristics and by RFLP fragment pattern analysis corresponded closely. The only exception was that two of the culture groups were shown to be identical following restriction analysis. Five types of spruce root endophytes were recorded: *Cenococcum geophilum* Fr., *Phialocephala fortinii* Wang and Wilcox, *Phialocephala dimorphospora* Kendrick (formerly known as *Mycelium radicis atrovirens* Melin (Richard and Fortin, 1973)) and two undetermined types designated FW and SW for the purposes of this study. Named isolates were identified by comparison of restriction patterns and culture morphology of isolates with descriptions in Stoyke et al. (1992). However, these identifications are tentative because the fragments selected for amplification differed in the two studies, the fragment amplified in this study being a sub-section of the fragment in Stoyke et al. (1992). Furthermore, only two of the restriction enzymes were common between the two studies. Correspondence of *P. fortinii* was strong but the isolates identified as *P. dimorphospora* and *C. geophilum* differed slightly in RFLP patterns from those of Stoyke et al. (1992). Classification of ECM types by

short-root characteristics did not correspond to classification by culture type based on culture characteristics and confirmed by UPGMA grouping of RFLP patterns. Since morphological characterization appeared insufficient to distinguish ECM types, culture type resulting from the plating of surface sterilized short-roots was considered to be a better representation of the type of intercellular fungal symbiont associated with each short-root. Culture type was therefore used as the indicator of ECM type associated with each short-root and ericoid mycorrhizae type for *K. angustifolia* fine-root segments. The various ECM types were noted as being present or absent on each spruce seedling. Three types of root-endophytic fungi were isolated from *K. angustifolia*. One of these closely resembled *P. dimorphospora* in culture characteristics and RFLP fragment patterns, suggesting either a close phylogenetic relationship or conspecificity with *P. dimorphospora*.

2.4. Seedling analysis

Stem height and the mass of foliage, roots, twigs, and stems were determined for each spruce seedling. A sub-sample of the foliage of each seedling was digested using standard Kjeldhal methods and colorimetrically analyzed for N (Bremmer, 1960) and P (Twine and Williams, 1971). A second set of sub-samples of spruce foliage was ashed and then dissolved in 'aqua regia' for the determination of metallic cation concentrations (K, Ca, and Mg) using an atomic absorption spectrophotometer (Desjardins, 1978).

2.5. Statistical analysis

Foliar metallic cation concentrations could not be determined for five of the 26 samples because of the small size of certain foliar samples. Least-square means of K, Ca, and Mg concentrations of seedlings growing close to and far from *K. angustifolia* were estimated using the LSMEANS step in the GLM procedure of SAS (SAS Institute, 1985) and were included in the analysis as substitutes for the missing values of foliar metallic cation concentrations.

Means of seedlings by sampling date were compared using the Student–Newman–Keuls test (SAS Institute, 1985). Seedlings from the second sampling date (July 7) had significantly lower total seedling height, foliar and total mass, root:shoot ratio, and foliar N concentrations (Table 1). These results suggest that although care was taken to randomly select seedlings, the seedlings selected on the second sampling date were significantly more nutrient stressed than seedlings sampled on the other two sampling dates. Therefore, the seedlings collected on the second sampling date were omitted from the analyses determining the significance of differences between seedlings growing close to and far from *K. angustifolia*.

Paired *t*-tests were conducted on seedling height; foliage, stem, twig, root, and total mass; root:shoot ratio; foliar N, P, K, Ca, and Mg concentrations; and the percentage of non-mycorrhizal short-roots to determine the significance of differences between seedlings close to and far from *K. angustifolia*. Differences for each parameter were tested for normality to ensure the validity of reported probabilities.

The occurrence of mycorrhizal types on seedlings was analyzed using a chi-square test on frequency of occurrence data.

The magnitude of the differences between seedlings growing close to and far from *K. angustifolia* of sampling dates 1 and 3 were compared to the differences between seedlings growing close to and far from *K. angustifolia* of the second sampling

Table 1
Comparison of seedling characteristics from three sampling dates

Date of sampling	Total seedling height (cm)	Foliar N (% dry weight)	Mass (g)		Root:shoot ratio
			Foliage	Total	
06/06/92	22.56 a	1.0935 a	1.46 a	3.30 a	0.320 a
07/07/92	15.80 b	0.6415 b	0.70 b	1.86 b	0.469 b
23/07/92	24.90 a	0.9713 a	1.98 a	4.44 a	0.333 a

Note: means followed by the same letter are not significantly different at $\alpha = 0.05$.

date. *T*-tests for unequal sample sizes were carried out to determine if seedlings of sampling date 2 were affected by *K. angustifolia* as were seedlings of sampling dates 1 and 3. These tests were modified for unequal variances when indicated by an *F*-test for homoscedasticity (Sokal and Rohlf, 1981).

The nitrogen concentration of spruce foliage was used to predict the total height of seedlings and total

Table 2
Results of paired *t*-tests comparing spruce seedlings growing far from and close to *K. angustifolia*

Proximity	Total seedling height (cm)	Mass (g)					Root:shoot ratio
		Foliage	Stem	Twigs	Root	Total	
Far	25.00	2.28	0.96	0.72	0.97	4.93	0.28
Close	22.71	1.14	0.64	0.28	0.80	2.86	0.39
Difference (far − close)	2.29	1.14	0.32	0.44	0.17	2.07	−0.11
p-Value	0.110	0.018	0.003	0.008	0.229	0.004	0.162

p-Values indicate the probability that values close and far are the same (difference = 0).

Table 3
Foliar nutrient concentrations for spruce seedlings growing far from and close to *K. angustifolia* plants

Proximity	Foliar nutrient concentration (% dry weight)				
	N	P	K	Ca	Mg
Far	1.2440	0.2095	0.5889	0.3732	0.0805
Close	0.8399	0.1504	0.5604	0.3811	0.0900
Difference (far − close)	0.4041	0.0591	0.0285	−0.0079	−0.0095
p-Value	0.043	0.021	0.493	0.897	0.240

p-Values indicate the probability that values close and far are the same.

Table 4
Percentage of non-mycorrhizal short-roots and the frequency of occurrence of ECM types on roots of spruce seedlings growing far from and close to *K. angustifolia* plants, with the corresponding probability that values are the same

Proximity	Non-mycorrhizal short-roots (%)	Occurrence of ECM (frequency of occurrence)				
		P. dimorphospora	*P. fortinii*	*C. geophilum*	SW[a]	FW[a]
Far	3.93	0.333	0.583	0.167	0.750	0.167
Close	43.94	0.700	0.700	0.400	0.800	0.200
p-Value	0.011	0.087	0.571	0.221	0.780	0.840

[a]Unidentified ECM fungi.

Table 5
Comparison of differences between spruce seedlings growing close to and far from *K. angustifolia* (difference = far − close) from the second sampling date to differences between seedlings growing close to and far from *K. angustifolia* from the first and third sampling dates

Sampling dates	Foliar P (% dry weight)	Total seedling height (cm)	Mass (g)				
			Foliage	Stem	Twigs	Shoot	Total
07/07/92	−0.0046	−2.72	−0.27	−0.11	−0.03	−0.41	−0.58
06/06/92 and 23/07/92	0.0591	2.29	1.14	0.32	0.44	1.90	2.07
p-Value	0.015	0.032	0.006	0.001	0.005	0.003	0.001

p-Value is for absence of difference between two values (difference = 0).

seedling biomass using simple linear regression. Separate regressions were run for seedlings close to and far from *K. angustifolia*. Adjusted r^2 values (Daniel, 1987) are reported due to the small sample size.

3. Results

Differences between values of parameters for seedlings growing close to and far from *K. angustifolia* were normally distributed for all parameters tested. Spruce seedlings growing close to *K. angustifolia* (<1 m) had significantly lower masses of foliage, stems, and twigs and lower total biomass than seedlings growing farther from *K. angustifolia* (>1 m), but did not differ significantly in total seedling height, root mass, or root:shoot ratio (Table 2). Concentrations of foliar N and P were significantly lower in seedlings close to *K. angustifolia* but concentrations of foliar K, Ca, and Mg did not vary with proximity to *K. angustifolia* (Table 3).

The proportion of short-roots that were non-mycorrhizal was higher on seedlings growing close to *K. angustifolia* than on seedlings far from *K. angustifolia* (Table 4). The occurrence of all endophytes was similar on seedlings growing close to and far from *K. angustifolia* with the exception of *P. dimorphospora* which occurred twice as often on seedlings growing close to *K. angustifolia* (Table 4).

The magnitude of the differences between seedlings growing close to and far from *K. angustifolia* for all variables except Mg were consistently lower in seedling pairs harvested at the second date of sampling. These differences were significantly

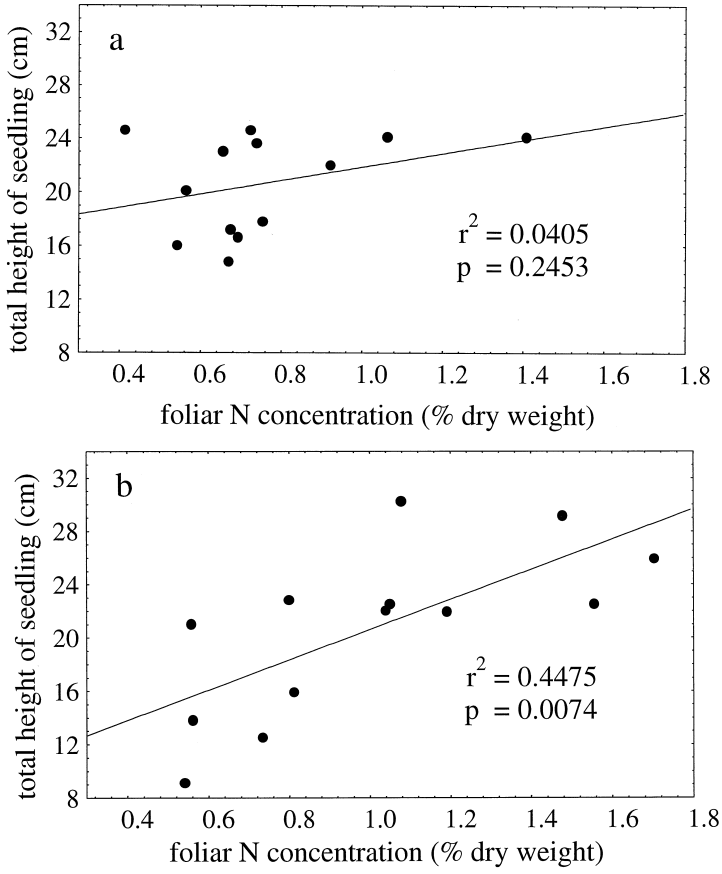

Fig. 1. Spruce seedling total height as a function of spruce seedling foliar nitrogen concentration using simple linear regression for seedlings close to (a) and far from (b) *K. angustifolia* plants.

lower than the differences between seedlings close to and far from *K. angustifolia* observed at the first and third sampling dates for foliar P concentration, total seedling height, and for the masses of foliage, stems, twigs, and shoots as well as for total seedling mass (Table 5).

Foliar nitrogen concentration was a statistically significant predictor of total seedling height (Fig. 1a) and total seedling biomass (not shown) for seedlings growing far from *K. angustifolia* ($p = 0.0074$ and $p = 0.0038$, respectively). Over 44% of the variability of the total height and 50% of the variability of the total biomass of seedlings growing far from *K. angustifolia* could be explained by variation in spruce foliar nitrogen concentration. The residuals from both these regressions were normally distributed. Similar regressions for seedlings growing close to *K. angustifolia* explained only 4% of the variability in total seedling height (Fig. 1b) and 16% of the variability in total seedling biomass (not shown); the regression coefficients for these regressions were not statistically significant ($p = 0.2453$ and $p = 0.0946$, respectively).

4. Discussion

The results of this study suggest that many characteristics of black spruce seedlings, including height growth, biomass, foliar nutrient concentrations, and mycorrhizal infection rate, are influenced by proximity to *K. angustifolia* in the field. These results are consistent with observed reductions in conifer germinant and seedling growth in the presence of *K. angustifolia* extracts in greenhouse and laboratory studies (Peterson, 1965; Mallik, 1987; Thompson and Mallik, 1989; Titus et al., 1995) and in the field (English and Hackett, 1994). Seedling growth, nutrition, and mycorrhizal infection are intimately related because, while mycorrhizal fungi depend on carbon fixed by the host through photosynthesis, carbon fixation depends largely on foliar nutrients, particularly N (Evans, 1989). Nitrogen in the foliage is in turn controlled by nutrient uptake by mycorrhizae.

The following four hypothetical mechanisms could account for the differences observed between seedlings growing close to and far from *K. angustifolia*: (1) *K. angustifolia* affects the availability of soil nutrients; (2) *K. angustifolia* produces allelochemicals that affect black spruce seedlings directly; (3) *K. angustifolia* specifically affects the activity of mycorrhizal fungi, decreasing seedling nutrient uptake; and (4) *K. angustifolia* acts as an alternate host for a pathogen of spruce.

4.1. Hypothesis 1: K. angustifolia affects the availability of soil nutrients

The lower concentrations of N and P in the foliage of black spruce seedlings growing close to *K. angustifolia* could be attributed to mechanisms controlling soil nutrient availability, either: i) *K. angustifolia* decreases soil nutrient availability by competitive nutrient uptake or ii) *K. angustifolia* reduces the capacity of the soil or humus to mineralize nutrients, as proposed by Damman (1971). *K. angustifolia* plants develop an extensive system of fine fibrous roots that proliferate in the organic layer of the soil. This species, like many ericaceous plants, is well adapted to acidic impoverished soils (Titus et al., 1995) and may be capable of outcompeting black spruce seedlings for N and P under conditions such as those encountered at the study site. That the significantly more nutrient-stressed seedlings from sampling date 2 were significantly less affected by *K. angustifolia* than the other seedlings examined in this study seems consistent with the hypothesis that *K. angustifolia* outcompetes spruce seedlings for nutrients; at microsites where there were fewer resources for which to compete, the presence of *K. angustifolia* did not seem to affect the nutrient status or growth of black spruce seedlings. Since N and P concentrations were lower in the foliage of seedlings growing close to *K. angustifolia* and since nutrient-stressed seedlings tend to allocate more resources to roots, seedlings growing close to *K. angustifolia* would have been expected to allocate more resources to nutrient acquisition, including the development of greater rates of mycorrhization. The results of this study, however, suggest that these seedlings allocated less resources to the development of ECM than seedlings growing farther from *K. angustifolia*.

Mycorrhization and plant nutrition are closely linked. Most conifers depend on mycorrhizae to

improve their ability to acquire nutrients, while the supply of photosynthates to a fungal symbiont is controlled in part by the ability of a plant to fix atmospheric carbon (Marx et al., 1977; Dixon et al., 1981; Reid et al., 1983). In turn, carbon fixation is dependent on the supply of nutrients required for the synthesis of photosynthetic enzymes (Amundson et al., 1992; Reich and Schottle, 1988; Macdonald and Lieffers, 1990). Marx et al. (1977) demonstrated that soil-applied nitrogen fertilizer can reduce the rate of mycorrhizal infection. These findings could be interpreted to mean that a nutrient deficient seedling will allocate more resources to nutrient acquisition through mycorrhizal symbiosis than a seedling that is nutrient sufficient. However, Reid et al. (1983) have shown that *Pinus taeda* L. seedlings with very low and very high rates of nutrient supply had lower rates of mycorrhization than seedlings given an intermediate amount of fertilization. It seems that when nutrients are deficient, an improvement in N nutrition through fertilization can lead to an increase in the export of carbohydrates to the short-roots. Consequently, the reduced rates of mycorrhization on seedlings close to *K. angustifolia* observed in this study, though perhaps accentuating nutrient deficiencies in seedlings, could have been caused by nitrogen and phosphorus deficiencies in black spruce seedlings resulting from a reduction in nutrient availability by *K. angustifolia*.

With regards to the hypothesis that *K. angustifolia* reduces mineralization rates, it is worth noting that the concentrations of foliar nutrients dependent on microbial or biochemical mineralization (N and P) were decreased in the presence of *K. angustifolia* while concentrations of K, Ca, and Mg, which depend more on uptake from soil exchangeable pools, were not.

Though the results presented here suggest that *K. angustifolia* influences nutrient availability, the presence of a linear relationship between foliar nitrogen content and height or total biomass of spruce in seedlings far from *K. angustifolia* and the lack of such a relationship in seedlings close to *K. angustifolia* would suggest that *K. angustifolia* limits the growth of nearby spruce in a manner that is independent of direct nutrient competition. If nutrient competition between *K. angustifolia* and black spruce was solely responsible for the differences between seedlings growing close to and far from *K. angustifolia*, we would have expected N to be a good predictor of seedling growth close to *K. angustifolia*.

4.2. Hypothesis 2: K. angustifolia allelochemicals affect spruce directly

The differences in spruce foliage N and P concentrations between seedlings close to and far from *K. angustifolia* suggest that *K. angustifolia* decreases soil nutrient availability to or nutrient uptake by nearby spruce. However, these nutritional effects may not necessarily be mediated by direct nutrient competition alone. Zhu and Mallik (1994) have identified 8 phenolic acids present in the foliage of *K. angustifolia* which reduced the rate of development of black spruce root tissue. Though their study was carried out under greenhouse conditions and their conclusions may therefore not be directly applicable to seedlings growing under field conditions, they have shown that *K. angustifolia* can have an inhibitory effect on the development of black spruce tissue. Any allelopathic effect on spruce seedling tissue, and root tissue in particular, could conceivably result in nutritional and mycorrhizal effects on spruce seedlings such as those observed in this study.

4.3. Hypothesis 3: K. angustifolia allelochemicals affect the mycorrhizae of spruce

The decreased rate of mycorrhizal infection of spruce seedlings growing close to *K. angustifolia* may have resulted from suppression of the formation or maintenance of ECM on the short-roots of black spruce by allelopathic compounds produced by *K. angustifolia*. Titus et al. (1995) reported that *K. angustifolia* leachate can reduce the growth of a *Suillus* sp. mycorrhizal fungus grown in vitro and Mallik and Zhu (1995) found that, of 51 mycorrhizal fungi tested on MMN agar, the growth of 41 fungal isolates was reduced in the presence of *K. angustifolia* extracts; it is therefore conceivable that compounds produced by *K. angustifolia* could affect the survival of either inoculum in the soil or the fungal symbiont on the roots of black spruce seedlings in the field. A similar explanation has been proposed by Robinson (1972) for the dominance of *C. vulgaris* (L.) Hull over Sitka spruce seedlings in the United

Kingdom, who demonstrated that compounds produced by the roots of *C. vulgaris* inhibited the growth of certain ECM of spruce. The observed reduction of mycorrhization rate in the field by *K. angustifolia* could account for the decreased rates of spruce growth under greenhouse conditions (Mallik, 1987; Titus et al., 1995) and in central Newfoundland plantations (English and Hackett, 1994; Titus and English, 1997) as well as the lower foliar N and P concentrations of seedlings growing close to *K. angustifolia* observed in this study.

4.4. Hypothesis 4: K. angustifolia increases the occurrence of P. dimorphospora on spruce roots

Richard and Fortin (1970) found that black spruce growth was depressed when seedlings were inoculated with *P. dimorphospora*. Seedlings became chlorotic and stunted following infection by this pseudomycorrhizal fungus, a condition similar to that which has been attributed to *K. angustifolia* induced check of spruce seedlings. This fungus occasionally invades the stele of red spruce roots and becomes pathogenic under certain environmental conditions (Wilcox and Wang, 1987). A fourth set of mechanisms may therefore be suggested by the increased occurrence of *P. dimorphospora* on seedlings growing close to *K. angustifolia*: either i) *K. angustifolia* renders spruce seedlings more susceptible to *P. dimorphospora* or ii) *K. angustifolia* increases the amount of inoculum of this fungus in the vicinity of spruce roots by harbouring *P. dimorphospora* on its own roots. With regards to the former hypothesis, Richard et al. (1971) observed that black spruce seedlings protected by true mycorrhizal fungi were less likely to suffer the pathogenic effects of *P. dimorphospora* when inoculated with this fungus. Therefore, *K. angustifolia* may be increasing the susceptibility of black spruce seedlings to *P. dimorphospora* by reducing rates of true mycorrhizal infection on seedling roots as outlined in the third hypothesis. The observation reported by Titus et al. (1995) that extracts of *K. angustifolia* inhibited the growth of a *Suillus* sp. but not of *Phialocephala* sp. (possibly *P. dimorphospora*) is consistent with this hypothesis. Since Richard et al. (1971) studied seedlings grown under laboratory conditions, the effect of other fungi competing for the surface area of short-roots could not be taken into account. Similarly, it also cannot be determined from the present study whether an increase in the occurrence *P. dimorphospora* on seedlings growing close to *K. angustifolia* was caused by a decreased rate of mycorrhizal infection or by the direct effect of the presence of *K. angustifolia*.

5. Conclusion

This study has shown that the presence of *K. angustifolia* can reduce the growth of certain seedling components and foliar concentrations of nitrogen and phosphorus, and can modify the structure of the ECM community of black spruce seedlings growing in the field. Although we were not able to elucidate mechanisms precisely, four hypothetical modes of influence through which *K. angustifolia* may be affecting black spruce seedlings have been suggested. Though no hypothesis can be completely discounted, the results suggest that the effect of *K. angustifolia* on spruce seedling nutrition alone cannot account for the responses observed. Hence, fertilization with N and P may correct nutrient deficiencies but may not compensate for all of the influences of *K. angustifolia* on black spruce. If *K. angustifolia* affects spruce by allelopathic inhibition of ECM, spruce growth may be improved by inoculating seedlings with resistant ECM strains. If direct allelopathy is responsible for kalmia-check then solutions may lie in managing *K. angustifolia* to confer an advantage to spruce during stand establishment. If the pathogen *P. dimorphospora* is responsible for mediating the effect of *K. angustifolia* on black spruce then inoculation of seedlings with protective ECM before planting may be a solution. Given that these approaches to management of the kalmia-check problem are very different, there is a need to determine the relative importance of the mechanisms involved before effective preventative or remedial measures can be developed.

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