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Overcoming *Kalmia*-Induced Growth Inhibition of *Picea mariana* by Mycorrhizal Inoculation[1]

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Picea mariana 生長을 抑制하는 *Kalmia angustifolia*에 대한 外生菌根의 影響[1]

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ABSTRACT

Objective of this study was to select ectomycorrhizal fungi for black spruce(*Picea mariana*) inoculation to overcome the growth inhibitory effects of *Kalmia angustifolia*. Nineteen isolates representing 11 species of ectomycorrhizal fungi were tested for their abilities to grow and form mycorrhizae with black spruce seedlings in the presence of water leachate of leaves of *Kalmia*. Mycelium growth of 9 isolates were inhibited by the leaf leachate. Colony diameter and biomass of the other 10 isolates were either increased or unaffected under the same conditions. Acidic pH of the culture medium(pH 3 and 4) inhibited some of the fungi, but a combination of acidic pH and the leaf leachate was more inhibitory. Thirteen isolates were able to form ectomycorrhizae with black spruce in presence of 25% leaf leachate in pure culture. Four isolates, *Paxillus involutus*(NF4), *Cenococcum geophilum*(GB12), *Laccaria laccata*(GB23), and E-strain(GB45) formed mycorrhizae more successfully than the others in presence of up to 50% *Kalmia* leaf leachate.

Black spruce seedlings pre-inoculated with these fungi were grown with *Kalmia* leaf leachate and live *Kalmia* plants during a four month greenhouse experiment. Abundant mycorrhizae(77 - 91% of root tips) were developed on seedlings pre-inoculated with *P. involutus*, *L. laccata* and E-strain but relatively poor mycorrhization(32% of root tips) resulted with *C. geophilum*. Over 90% of the short root mycorrhizae were attributed to the inoculated fungi although indigenous mycorrhizae also occurred on most seedlings.

Persistence of the mycorrhizae was not affected by living *Kalmia* plants. Over 80% of the mycorrhizae on seedlings inoculated with *P. involutus*, *L. laccata* and E-strain and 53% of the mycorrhizae on seedlings inoculated with *C. geophilum* were attributable to the inoculant fungi. Control seedlings formed about 45% ectomycorrhizal short roots with indigenous fungi. The *L. laccata* and *C. geophilum* inoculated seedlings exhibited enhanced mycorrhizae formation in presence of *Kalmia* leaf leachate. Mycorrhizae formation with inoculant fungi was 4 - 15% lower at pH 4 than at pH 5, with the greatest inhibition occurring for *L. laccata*.

Seedlings inoculated with *P. involutus* had the greatest shoot and root growth followed by *L. laccata* and E-strain inoculated seedlings. The *P. involutus* and *L. laccata* inoculated seedlings were significantly taller with more shoot dry biomass than the uninoculated(control) seedlings. E-strain inoculated seedlings had significantly higher shoot dry biomass and significantly lower number of first order lateral roots compared to the control but other growth parameters such as height, root dry weight

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and number of short root tips were not significantly different from the control. Seedlings inoculated with *C. geophilum* were not significantly different from the uninoculated seedlings in any of the growth parameters except for the number of first order lateral roots which was significantly less than the control seedlings.

Key words : *allelopathy, black spruce,* Kalmia angustifolia, *mycorrhizae, phenolics, regeneration failure*

요 약

Picea mariana 생장을 억제하는 *Kalmia angustifolia*에 영향을 주는 외생근균을 조사 선발하였다. 11개 외생근균중에서 19계통을 선발하여 *Kalmia* 잎침출물이 들어있는 배지에 *P. mariana* 치묘와 함께 근균을 접종하여 자라는 형태와 생장양상을 조사하였다. *Kalmia* 잎추출물을 첨가한 액체배지에서는 균사의 건중량을, 한천배지에서는 코로니의 직경을 측정한 결과 분리된 9개 균주에서는 현저하게 억제되었으나 나머지 10개 균주에서는 반대로 증가되거나 *Kalmia*의 영향이 나타나지 않았다. 배양배지의 pH가 3-4일 때는 생장을 억제 받는 근균도 있었으나 pH와 잎침출물을 조합한 조건하에서는 더욱 강하게 억제되었다. 분리된 13개 계통은 순수배양에서 Kalmia 잎추출물 25%와 함께 배양한 *P. mariana*에서 외생근균이 형성되었다. *Paxillus involutus*(NF4), *Cenococcum geophilum*(GB12), *Lacaria laccata*(GB23), E-strain(GB45)계통에서는 Kalmia 잎추출물 50%에서 배양한 결과 다른 계통보다 많은 외생근균이 형성이 되었다.

이러한 근균을 미리 접종한 *P. mariana*를 *Kalmia* 잎추출물과 같이 배양한 후 온실 안에서 4개월 동안 *Kalmia*와 같이 재배하였다. *P. involutus, L. laccata*와 E-strain을 미리 접종한 치묘에서는 많은 근균(흡수근의 77-91%)이 형성되었으나 *C. geophilum*를 미리 접종한 치묘에서는 근균이 비교적(흡수근의 32%) 적게 형성되었다. 외생균근이 대부분의 치묘에서 생겼음에도 불구하고 근균의 90% 이상이 접종근균에서 생겨났다.

근균의 지속적인 생장은 살아있는 *Kalmia* 식물개체에 영향을 받지 않았다. *P. involutus, L. laccata*와 E-strain과 같이 처리한 치묘 근균의 80% 이상과 *C. geophilum*을 처리한 치묘 근균의 53%가 접종된 균주의 영향을 받았다. 대조구에서는 토착균주에 의해 약 45%의 짧은 뿌리의 외생근균이 형성되었다. *L. laccata*와 *C. geophilum*는 *Kalmia*잎추출물과 같이 배양한 치묘의 근균형성을 촉진하였다. 균주를 접종한 경우 근균형성율은 pH5보다 pH4에서 4-15% 더 낮았으며 *L. laccata*를 접종한 경우 심하게 억제되었다.

*P. involutus*에 접종한 치묘는 *L. laccata*와 E-strain를 접종한 치묘보다 줄기와 뿌리생장이 가장 높게 나타났다. *P. involutus*와 *L. laccata*를 접종한 치묘는 대조구의 치묘보다는 건중량이 많고 키가 훨씬 컸다. E-strain에 접종한 치묘는 대조구와 비교해서 1차 측근 수가 매우 작았으며 줄기 건중량은 매우 높게 나타났으나 다른 형질, 예를 들면 흡수근, 뿌리 건중량, 수고 등은 대조구와 크게 다르지 않았으나 *C. geophilum*에 접종한 치묘는 1차 측근수를 제외한 다른 생장 특징에서는 대조구와 크게 다르지 않았다.

INTRODUCTION

In eastern Canada, sheep laurel(*Kalmia angustifolia* L. var. *angustifolia*, hereafter referred to as *Kalmia*), an ericaceous understory shrub seriously hinders conifer regeneration, especially black spruce(*Picea mariana*) after clear cutting and fire. Regeneration failure of the tree species has been ascribed to *Kalmia* competition(Mallik, 1991, 1992), allelopathy(Mallik, 1987 ; Thompson & Mallik, 1989 ; Mallik & Roberts, 1994 ; Zhu & Mallik, 1994), low availability of nutrients in soil particularly nitrogen(Inderjit & Mallik, 1996) and perhaps low inoculum potential of black spruce mycorrhizae. Our preliminary studies showed

about 50% reduction in mycorrhizae of black spruce in a *Kalmia* dominated black spruce harvested forest soil compared to *Kalmia* free 80 year-old uncut black spruce forest soil(H. Zhu & A.U. Mallik, unpublished data). Such a low inoculum potential observed in *Kalmia* soil may have been the result of antifungal activity of growth inhibitory substances present in *Kalmia*. Allelopathy has been suggested as a potential cause of forest regeneration failure by Fisher (1987). A variety of allelochemicals present in litter and organic matter can affect tree mycorrhizae which in turn bring about changes in structure and dynamics of forest ecosystems (Perry & Choquette, 1987). Zhu & Mallik,(1994) isolated and identified eight phenolic compounds from *Kalmia* leaves. These and other organic acids in ericaceous litter are known to have phyto- and fungi-toxic effects(Jalal & Read, 1983a,b). Allelopathic effects on ectomycorrhizae have been known for more than forty years (Melin, 1946). Since then other studies have shown inhibition of mycorrhizae by water soluble extracts from litter, roots, and leaves of many other plants(Olsen *et al.*, 1971 ; Robinson, 1972 ; Goldner *et al.*, 1986 ; Cote & Thibault, 1988). Regeneration failure of Sitka spruce(*Picea sitchensis*) and Norway spruce(*Picea abies*) was reported in *Calluna* heathlands(Lynton, 1954 ; Handley, 1963). Growth inhibitory factors leaching from live roots of *Calluna vulgaris* were reported by Robinson(1972). Jalal & Read(1983a) isolated and identified several phyto- and fungi-toxic substances particularly certain phenolics and long-chain fatty acids from *Calluna* humus. *Kalmia*, being an ericaceous shrub of similar stature and with similar regeneration response to disturbance as *Calluna*(Mallik, 1994, 1995), is suspected to have similar growth inhibitory effects on black spruce mycorrhizae.

Most tree species in temperate and boreal zones are associated with mycorrhizae(Malloch & Malloch, 1982). Besides improving the survival and growth of tree seedlings by enhanced nutrient uptake, particularly phosphorus, and protecting them against root pathogens, mycorrhizal fungi play key mediative and integrative roles in plant communities(Marx, 1969). Mycorrhizae help host trees to compete successfully with grasses and herbs for resources(Bowen, 1980) and perhaps detoxify allelochemicals produced by competitors (Perry & Choquette, 1987). Mycorrhizae formation depends on many factors, especially mycorrhizae inoculum potential of the soil, site disturbances, such as logging, clearcutting, and forest fire(Parke *et al*, 1984 ; Perry & Rose, 1983).

The problem of tree seedling growth inhibition due to the absence of necessary mycorrhizae inoculum in soil can be overcome by inoculating tree seedlings with appropriate mycorrhizae(Marx & Bryan, 1975 ; Marx, 1980 ; Marx & Cordell, 1987 ; Molina, 1979). Inoculation of seedlings with *Pisolithus tinctorius* has brought about tremendous success in the plantation forests of Georgia, U.S.A.(Marx & Cordell, 1987). In this context it was thought that perhaps the growth inhibition of black spruce in presence of *Kalmia* could be overcome by inoculating black spruce seedlings with appropriate mycorrhizae. However, two criteria must be satisfied in selecting ectomycorrhizae for seedling inoculation : 1)the fungus must be resistant to the water soluble compounds of *Kalmia* leaves and 2)it must be able to form mycorrhizae with black spruce in the presence of *Kalmia*.

The objective of the present study was therefore threefold, first to test the tolerance of a number of mycorrhizal fungi to *Kalmia* leaf leachate, secondly to examine whether the fungi that were able to grow in presence of *Kalmia* leaf leachate can form mycorrhizae with black spruce seedlings and thirdly to test the growth potential of inoculated seedlings in presence of *Kalmia*.

MATERIALS AND METHODS

1. Sources of ectomycorrhizal fungi

Nineteen fungal isolates representing 11 species used in this study were provided by Drs. P. Chakravarty and L. Peterson, University of Guelph(coded as GB) and by the University of Alberta Microfungus Collection(coded as AB). Most of these fungi are known to form ectomycorrhizal association with black spruce in ab-

sence of *Kalmia*. Two mycorrhizal fungi were isolated from a site previously dominated by black spruce in eastern Newfoundland(coded as NF). All the isolates were maintained in modified Melin Norkans(MMN) agar medium(Marx, 1969). Appendix I gives the name of the fungi and the isolate codes.

2. *Kalmia* leaf leachate

Leaf leachate of *Kalmia* was prepared by Zhu and Mallik(1994). The selected concentrations of *Kalmia* leaf leachate were assumed to be comparable to the field conditions since on a dry weight basis annual production of *Kalmia* foliage vary from 48g to 150g/m^2 depending on the site conditions and disturbance regimes(Mallik, 1994). The fresh weight equivalent of the foliage was between 88g and 293g/m^3/year(A.U. Mallik, unpublished data). The moist oceanic climate of eastern Canada, with high annual precipitation (943㎜, 30-year average) keeps the evergreen foliage of *Kalmia* moist for a large part of the growing season. The precipitation runoff from the foliage goes into the site passing through the thick ericaceous litter. Although aqueous extracts of fresh leaves, old leaves, litter and humus of *Kalmia* cause root growth inhibition of black spruce germinants(Mallik, 1987) fresh leaves were used in this study. This was simply because the leaf leachate was chemically less complicated than *Kalmia* humus, easy to prepare and yet has the growth inhibitory effects on black spruce seedlings.

3. Effect of leaf leachate on fungal growth

Bioassay was conducted using MMN agar medium prepared with *Kalmia* leaf leachate at the final concentrations of 0, 10, 25, and 50%. In each Petri-plate(15×10㎜) 25ml of medium was poured. The plates were then inoculated with a 6㎜ diameter mycelium disc cut from the margins of colonies growing on MMN agar. Each plate received one mycelium disc and 5 replicate plates were used for each fungal treatment. The inoculated plates were incubated in the dark at room temperature(22℃). Diameters of mycelial colonies on agar plates were measured weekly for up to 5 weeks.

4. Effect of leaf leachate on fungal growth at different pH

The method of preparing the leachate-containing medium was the same as described above except no agar was added. The pH of the media was adjusted to 3, 4, or 5 with either 0.1 N HCl or NaOH before autoclaving. The final concentration of the leachate in the media was 50%. Twenty-five ml of the medium was dispensed into each Petri-plate followed by inoculation with a mycelium disc. Each plate received one mycelium disc and 5 replicate plates were used for each fungal treatment. The inoculated plates were incubated at 22℃ in the dark for 25 days. Mycelium was harvested by filtration through Whatman No.1 filter paper and mycelium dry weight was determined after oven drying at 75℃ for 24h.

5. Study of mycorrhizae in presence of *Kalmia* leaf leachate

Seeds of black spruce were surface sterilized in 30% H_2O_2 for 15 min, and germination was carried out on sterile 1% water agar at 20 - 22℃ under fluorescent light. For the synthesis of mycorrhizae, plastic Petri-plates(15×100㎜) were aseptically filled with 30ml autoclaved vermiculite and peat moss(4 : 1) and 20ml of MMN liquid medium(pH5.5). The plastic plates were inoculated with three mycelium disks and incubated at 22℃ for 7 - 14 days before adding another 10ml MMN medium containing 0, 25 and 50% leaf leachate. An individual seedling(2 - 4㎝) was planted into the slit of each plate so that only the root would remain in aseptic conditions inside the plate. The Petri-plates holding the seedlings were placed in a growth chamber at 18℃, 60 - 80% relative humidity, and 16h photoperiod(220μE m^{-2} s^{-1}). The seedlings were examined for the degree of mycorrhizae formation(by cleaning, staining and counting % mycorrhizal short roots) and for root and shoot development 45 days after fungal inoculation and leaf leachate treatments.

6. Preparation of mycorrhizal inocula

Inocula of the fungi were prepared by using vermiculite and peat moss(28 : 1) wetted with liquid MMN medium in 1L glass jars as described in Marx & Kenney(1982). Mycelium discs(6㎜ in diameter) from 15 to 20-day-old fungal colonies on agar plates were used for inoculating the substrate. Ten mycelial discs were put into each jar and fungus-free discs were used for the control jars. The cultures were then grown in darkness at 20 - 22℃ for 35 days for *P. involutus*, *C. geophilum*, *L. laccata* and E-strain. After incubation, inoculum of each fungus from various jars was wrapped in four layers of cheesecloth, irrigated for 5 min under cool tap water to remove nonassimilated nutrients and excess water was removed by squeezing the inoculum in the cheesecloth by hand. The leached inocula were used for seedling inoculation.

7. Inoculation of black spruce seedlings

A growth substrate containing equal volumes of vermiculite and peat moss was autoclaved at 125℃ for 20 min to eliminate resident fungi, and then the substrate was thoroughly mixed with mycorrhizal inoculum at the ratio 10 : 1. Tree nursery multipot containers(120ml/cell) were filled with the inoculum. Two or three seeds of black spruce(surface sterilized by 30% H_2O_2 for 20min.) were sown on the surface of each compartment of the multipot container. The seeded containers were then covered with a 5 - 10mm layer of sterilized vermiculite, watered and then placed on greenhouse benches where the germinants were thinned to one per container. All seedlings were grown in the greenhouse at Mount Pearl Tree Nursery, St. John's, Newfoundland using a growth regime of 24/16℃(day/night), 60 - 80% humidity, and light of approximately 240μE/m^2/s for 16h. Seedlings were watered with tap water as needed. Three weeks after germination and monthly thereafter, 20 - 20 - 20 NPK soluble fertilizer was applied. After four months, ten seedlings were randomly selected from each inoculation treatment to determine mycorrhizae formation and seedling root-shoot length, collar diameter and oven dry biomass.

8. Growth of inoculated seedlings with *Kalmia* leaf leachate

Soil used in this study was collected from an open site approximately 20km west of Terra Nova National Park, Newfoundland in Septembar, 1989. The vegetation of the site was predominantly *Kalmia angustifolia* with some *Rhododendron groenlandicum* and *Vaccinium angustifolium*. The site was previously occupied by black spruce forest with some *Kalmia* in the understorey. After harvesting, the site was replanted with black spruce. Ten years after harvesting, the planted black spruce seedlings showed poor growth(less than 70cm in height). The soil appeared to be well drained within the top 30cm and had a litter layer about 10cm thick with a mean pH 4.6.

The soil of the top 25cm was collected from ten random sampling points within a 10m×10m plot. Soil samples were transported to the greenhouse and processed within twenty four hours of collection. The samples were pooled, mixed thoroughly and put in 18cm diameter, 20cm deep plastic pots. One 4-month-old black spruce seedling inoculated with one of the four mycorrhizal fungi was transplanted into each of 100pots. The inoculated plants were divided into four groups of 25 seedlings each and each group received the following treatments : (1)*Kalmia* leaf leachate at pH5 ; (2)leaf leachate at pH4 ; (3)water at pH5 ; (4)and water at pH4. The leaf leachate was prepared by soaking 100g fresh leaves of *Kalmia* in 1L distilled water for 24h followed by filtration through Whatman No 1 filter paper. The resultant filtrate was designated as 100% leaf leachate and stored at -18℃ until used. Further dilutions of the leachate were prepared by adding appropriate amounts of distilled water(Zhu & Mallik, 1994). The pH of the leaf leachate was adjusted to 4 and 5 before being applied. Three hundred ml of 10% leaf leachate were applied once a week to the pots containing the inoculated black spruce seedlings. The pots containing the black spruce seedlings were placed randomly on greenhouse benches and rearranged monthly to reduce the local environmental differences in the greenhouse. Two weeks after trans-

planting 50ml of 100ppm 20 - 20 - 20, NPK fertilizer was applied in each pot.

The seedlings were grown in the greenhouse for 4 months, following which they were removed from pots and their roots gently washed with tap water. From each treatment combination, 20 seedlings were randomly selected to determine shoot length and the number of first order lateral roots longer than 5cm. Five first order lateral roots were selected randomly from each seedling and their total length, number of total short roots, mycorrhizal short roots, and non-mycorrhizal short roots were determined. Root and shoot dry weights were determined after ovendrying at 70℃ for 48 hrs.

9. Growth of inoculated seedlings with live *Kalmia* plants

Kalmia plants(6 - 11 stems) including the top 30cm soil were excavated from the same site where the soil was collected and transplanted into 30×30cm plastic pots. In the greenhouse three mycorrhiza-inoculated black spruce seedlings were transplanted to each pot. Five pots were used for each inoculation treatment. Another five pots, each containing similar *Kalmia* plants were planted with five noninoculated black spruce seedlings, and kept as controls. The seedlings were maintained under greenhouse conditions as described above, but no leaf leachate was added. After 4 months, the seedlings were harvested by soaking the pots in cold water for 15 min and the roots of seedlings were carefully separated from the *Kalmia* plants. Growth and mycorrhizae formation on black spruce seedlings were determined as described above.

10. Data analysis

The data were subjected to analysis of variance(ANOVA). The experiment on leaf leachate effects at different pH levels was a completely randomized factorial design and the data were analyzed using a three-way-ANOVA model. The experiment with living *Kalmia* plants was analyzed using a one-way-ANOVA model. The significance of mean differences was determined using Tukey's test at $P=0.05$.

RESULTS

1. Effects of *Kalmia* leaf leachate on fungal growth

The effect of *Kalmia* leaf leachate on diameter growth of fungal colonies varied depending on the fungal isolate and the leachate concentration applied(Appendix Ⅰ). *Hebeloma cylindrosporum* (GB6), *Paxillus involutus*(NF4 and GB41) colony diameters of mycelium was increased by the leaf leachate. Diameter growth remained unaffected in *P. involutus*(GB40 and GB24), *Leccinium scabrum*(NF1), E-strain(GB45), *Thelephora terrestris*(GB50), *Cenococcum geophilum*(GB12), and *Laccaria laccata*(GB8) and was inhibited in *Hebeloma crustuliniforme*(AB2), *Pisolithus tinctorius*(GB14 and GB4), *C. geophilum*(AB1), *L. laccata*(AB5 and GB20). Colony diameter of *Laccaria laccata*(GB23) was severely inhibited at all the three concentrations(10, 25, and 50%) of *Kalmia* leaf leachate. Colony diameter growth of *Pisolithus tinctorius*(GB25) and *Lycoperdon perlatum*(GB56) was inhibited by the 50% leachate concentration.

When the fungi were grown in liquid medium containing 50% leaf leachate at pH3, 4 and 5, dry biomass of all the fungi except *Cenococcum geophilum* was inhibited(Appendix Ⅱ). *Hebeloma crustuliniforme*(AB2), *Leccinium scabrum*(NF1) and *Lycoperdon perlatum*(GB56) were unable to grow at pH3 even in the absence of *Kalmia* leaf leachate. *Hebeloma cylindrosporum*(GB6), *Pisolithus tinctorius*(GB25), *Laccaria laccata*(AB5 and GB20) failed to grow at pH3 in presence of *Kalmia* leaf leachate. At pH4, mycelial dry biomass of *Paxillus involutus*(NF4 and GB40), *Thelephora terrestris*(GB50), E-strain(GB45), *Cenococcum geophilum*(GB12) and *Laccaria laccata*(GB23) was either increased or remained unaffected by the leaf leachate and dry biomass of the other 13 isolates was reduced by 10 - 70% in the presence of the leaf leachate. At pH5, mycelial dry biomass of *Hebeloma cylindrosporum*(GB6), *Paxillus involutus*(NF4, GB40 and GB24), *Leccinum scabrum* (NF1), E-strain(GB45), *Thelephora terrestris* (GB50), *Cenococcum geophilum*(GB12) and *Lac-*

caria laccata(GB23) was not inhibited by *Kalmia* leaf leachate, and the dry biomass of the other 10 fungi was reduced by 10 - 55% in presence of the leaf leachate.

2. Effects of *Kalmia* leaf leachate on mycorrhizae formation

Mycorrhizae formation on the seedlings varied among the 19 fungi. *Lycoperdon perlatum*(GB56), *Thelephora terrestris*(GB50) and *Pisolithus tinctorius*(GB25) failed to form ectomycorrhizae in any of the three leaf leachate treatments. *Paxillus involutus*(NF1), *Cenococcum geophilum*(AB1), and *Laccaria laccata*(GB2) were able to form mycorrhizae only in the absence of *Kalmia* leaf leachate. The rest of the 13 fungi formed mycorrhizae with black spruce seedlings in the presence of leaf leachate(Fig. 1). The seedlings inoculated with *Paxillus involutus*(NF4), E-strain(GB45), *Laccaria laccata*(GB23) and *Cenococcum geophilum*(GB12) formed 50% or more mycorrhizal short roots in black spruce that was not affected by *Kalmia* leaf leachate.

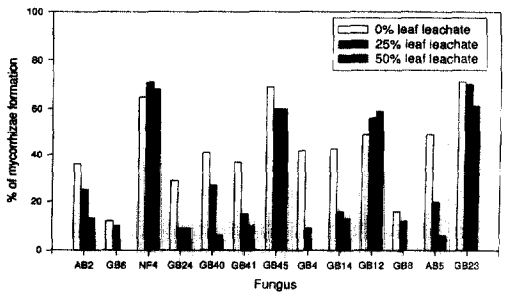

Fig. 1. Effect of *Kalmia* leaf leachate on mycorrhizae formation in black spruce. Appendix I give the full names of the fungi with the isolate codes.

3. Mycorrhizae formation and growth of seedlings in *Kalmia* soil

Ectomycorrhizae were abundant on seedlings inoculated with *Paxillus involutus*(NF4), *Laccaria laccata*(GB23) and E-strain(GB45)(Table 1). Although indigenous mycorrhizae also were found on most of these seedlings, over 90% of the mycorrhizae resulted from the inoculated fungi. Seedlings inoculated with *Cenococcum geophilum* (GB12) formed an average 73% mycorrhizae in their root systems ; however, only 34% of the mycorrhizae were attributable to the inoculant. Uninoculated(control) seedlings formed about 45% ectomycorrhizae with unidentified indigenous fungi. Among the four fungal treatments, seedlings inoculated with *P. involutus* had the greatest shoot height and dry weight, root dry weight and number of short and lateral roots. Seedlings inoculated with *C. geophilum* had the lowest values for those parameters except for number of short roots(Table 1). The seedlings inoculated with *P. involutus* were significantly taller with higher root-shoot dry weight, number of short root tips and first order lateral roots than those of control(Table 1). The *L. laccata* inoculated seedlings had significantly lower height but significantly higher root dry weight, number of short root tips and first order lateral roots compared to those of control. E-strain inoculated seedlings had significantly higher root dry weight

Table 1. Mycorrhizal formation and growth of black spruce seedlings inoculated with four mycorrizal fungi and growth in *Kaimia* soil[a].

Measurement	Control	Fungal treatment[b]			
		P. involutus	*L. laccata*	E-strain	*C. geophilum*
Shoot height(cm)	19.2±5.4a	23.8±3.9a	17.0±3.9c	19.3±3.8b	15.0±5.8b
Shoot dry weight(mg/plant)	454±166b	753±215a	405±167b	446±129b	280±125c
Root dry weight(mg/plant)	177±39c	216±69a	159±49b	157±32b	103±43c
Shoot root tip(#/cm lateral root)	4.3±1.3b	6.6±1.8a	6.1±1.4a	6.5±1.7a	4.8±1.7b
First order lateral roots(#/plant)	10.3±3.2b	15.9±5.1a	14.1±3.8a	11.0±2.8b	7.6±3.2c
% mycorrhizal roots due to : Inoculant	0	89±10a	82.3±10.5a	84.8±8.5a	33.7±16.1b
Unknown	44.5±27.3a	5.5±2.0b	7.0±4.7b	7.8±3.8b	39.5±16.5a

[a] Data are presented as means and their standard deviations of 20 replicates.
[b] Reading across, mean values followed by the same letter were not significantly different at $p=0.05$ acco- ding to Tukey's test.

Table 2. Mycorrhizal formation and growth parameters of black spruce seedlings inoculated with mycorrhizal fungi and grown in *Kalmia* soil with *Kalmia* leaf leachate treatments[a].

Fungus	Treat[b]	% mycorrhizal roots colonized by		Shoot height (cm)	Shoot dry weight (mg/plant)	Root dry weight (mg/plant)	Short root (#/cm)	1st order root (#/plant)
		Inoculant	Unknown fungi					
Control	C	0	47.5±a	20.2±4.9	493±195	121±45	4.8±1.5a	10.5±3.5
	K	0	41.3±b	18.3±5.7	415±124	114±33	3.5±1.1b	8.1±3.1
P. involutus	C	91.1±9.9	2.3±2.1	23.7±3.2	778±189	231±76	7.1±1.9a	16.4±4.1
	K	88.4±9.4	3.1±2.9	23.7±4.6	729±241	202±60	5.9±1.3b	15.4±6.1
L. laccata	C	77.0±8.7b	11.1±6.3a	17.7±4.6	451±187	171±54	5.6±1.3	14.1±4.2
	K	87.5±9.1a	4.6±2.8b	16.3±3.1	359±133	147±43	6.5±1.5	13.9±3.5
E-strain	C	83.7±8.4	7.9±5.2	21.0±4.3	386±107	156±25	6.2±1.5	10.2±2.8
	K	84.7±7.6	6.3±5.4	17.9±2.7	345±123	159±39	6.8±1.9	11.8±2.5
C. geophilum	C	31.6±14.8	42.3±17.6	16.6±4.7	303±102	105±42	4.8±1.5	7.7±3.2
	K	36.5±14.9	37.3±14.7	13.5±6.5	257±143	101±46	4.8±1.8	7.6±3.4

[a] Data are presented as means and their standard deviations of 20 replicates. Mean values followed by the same or no letter between the control (C) and *Kalmia* leaf leachate (K) treatment were not significantly different at $p=0.05$ according to Tukey's test.
[b] Treatments are: water (C) and *Kalmia* leaf leachate (K).

and number of short root tips than control but height and shoot dry weights did not differ significantly from the control. The *C. geophilum* inoculated seedlings responded either negatively or with no significant difference in the above parameters compared to control(Table 1).

Over all the effects of the mycorrhizal fungi were highly significant in terms of all the root-shoot parameters of black spruce. The effect of pH was also significantly related to seedling height and shoot dry weight. Significant interaction effects were found between pH and the mycorrhizal fungi in terms of seedling height, root dry weight and first order lateral roots. The mycorrhizae and *Kalmia* interactions were significant for shoot height and dry weight and the number of short roots. A three way interaction (pH×mycorrhizae×*Kalmia*) was significant only for the number of first order lateral roots.

Based on their morphology, two types of indigenous mycorrhizae could be distinguished : (i) one that occurred mostly in the upper portion of the root system which was dark brown, strikingly narrow and cylindrical, without a well developed mantle and(ii) a second type occurred as a cluster in the middle one third of the root system which was pale white, slightly swollen and had a smooth mantle.

4. Effects of *Kalmia* leaf leachate

Generally speaking, the addition of *Kalmia* leaf leachate caused a reduction in mycorrhizae formation but this reduction was significant only in *L. laccata* inoculated seedlings(Table 2). The leaf leachate had no significant effect on mycorrhizae formation in *P. involutus*, *L. laccata* and E-strain. *Kalmia* leaf leachate, as the main factor, did not significantly affect seedling height, but the leachate-fungus treatment had a significant interaction effect on seedling shoot height, shoot dry weight and short root number(Table 2). Seedlings receiving leaf leachate produced lower shoot height and dry weight compared with seedlings receiving no leaf leachate in all the fungal treatments. However, significant inhibition was only found in seedlings inoculated with *L. laccata*. In general, the seedlings inoculated with *P. involutus*, *L. laccata*, and E-strain grew better than the control and *C. geophilum* inoculated seedlings.

5. Effects of pH

Mycorrhizae of inoculant fungi was 4 to 15% lower at pH4 than pH5 with the greatest inhibition occuring in *L. laccata* inoculated seedlings(Table 3). Mycorrhizae colonization by indigenous fungi was greatly reduced at pH4 on uninoculated seedlings than those inoculated with

Table 3. Mycorrhizal formation and growth parameters of black spruce seedlings inoculated with mycorrhizal fungi and grown in *Kalmia* soil with *Kalmia* leaf leachate and water (control) at pH4 and 5[a].

Fungus	pH	% mycorrhizal roots due to		Shoot height diameter(cm)	Shoot dry weight (mg/plant)	Root dry weight (mg/plant)	Short root (#/cm)	1st order root (#/plant)
		Inoculant	Unknown					
Control	5	0	54.0±9.9	20.7±6.0	476±197	126±45	4.5±1.1	11.5±3.4a
	4	0	40.0±8.8	17.8±4.4	431±130	109±32	4.2±1.1	9.1±2.8b
P. involutus	5	90.5±7.7	3.5±2.6	23.7±4.9	720±219	205±72	6.7±1.9	18.9±5.0a
	4	88.9±9.9	1.8±1.3	23.8±2.5	787±212	227±65	6.5±1.3	13.8±3.3b
L. laccata	5	87.3±5.6a	6.0±3.4b	18.6±4.1a	468±170a	155±42	5.8±1.0	14.4±4.2
	4	77.3±5.2b	9.8±2.1a	15.4±4.4b	342±140b	163±57	6.3±1.7	13.8±3.4
E-strain	5	86.0±6.9	6.0±4.3	20.8±3.9a	468±147	156±37	6.7±1.5	10.3±1.7
	4	82.3±7.8	8.3±5.8	18.2±3.3b	424±107	159±29	6.2±1.8	11.8±2.5
C. geophilum	5	35.5±9.7	47.6±9.5	18.1±5.9a	333±132a	123±48a	5.4±1.7a	7.7±2.8
	4	32.0±6.0	32.1±9.9	12.1±4.0b	227±93b	83±27b	4.2±1.4b	7.6±3.7

[a] Data are presented as means and their standard deviations of 20 replicates. Mean values followed by the same or no letter between the pH4 and 5 were not significantly different at $p=0.05$ accord-ing to Tukey's test.

Table 4. Mycorrhizal formation and growth of black spruce seedlings inoculated with different fungi and grown with *Kaimia* plants[a].

Measurement	Control	Mycorrhizae[b]			
		P. involutus	*L. laccata*	E-strain	*C. geophilum*
Shoot height(cm)	10.4±2.2bc	14.2±2.0a	12.2±1.8b	11.8±1.9bc	10.4±1.9c
Shoot dry weight(mg/plant)	154±42c	356±37a	256±64b	250±53b	171±51c
Root dry weight(mg/plant)	43±1.0c	124±28a	83±26b	74±21bc	54±21c
Shoot root tip(#/cm lateral root)	4.3±1.0b	6.2±1.7a	4.8±1.1bc	5.2±1.0b	3.3±0.7d
First order lateral roots(#/plant)	7.1±1.5b	11.2±1.8a	7.8±1.9b	5.8±1.2c	4.4±0.7c
% mycorrhizal roots due to : Inoculant	0	81.3±6.4b	80.9±8b	88.7±3.8b	53.6±8.8c
Unknown	54.5±2.7a	3.1±2.0c	2.9±1.6c	10.2±5.7c	23.0±13.5b

[a] Data are presented as means and their standard deviations of 15 replicates.
[b] Mean values followed by the same letter within a row were not significantly different at $p=0.05$ ac-cording to Tukey's test.

C. geophilum. The *P. involutus* inoculated seedlings receiving *Kalmia* leachate of pH4 and 5 showed no significant difference in height, root-shoot dry weights and the number of short roots but the first order lateral roots were significantly less at pH4. Significantly less height was obtained in seedlings inoculated with *L. laccata*, E-strain and *C. geophilium* compared to control. The pH of the leaf leachate alone or in cojunction with the fungal treatments, had a significant effect on shoot height and shoot dry weight. Root growth of uninoculated and *C. geophilum* inoculated seedlings was less at pH4 than that at pH5.

6. Mycorrhizae formation and growth of seedlings in presence of live *Kalmia*

Persistence of inoculated mycorrhizae was not affected by the living *Kalmia* plants ; over 80% of mycorrhizae on seedlings inoculated with *Paxillus involutus*, *Laccaria laccata* and E-strain and that of 54% on seedlings with *Cenococcum geophilum* were attributable to the inoculant fungi(Table 4). Indigenous mycorrhizae occurred on all the uninoculated seedlings and on most of the inoculated seedlings. The highest indigenous mycorrhizae formation was 54% on the root systems of uninoculated seedlings. Much less indigenous mycorrhizae(4 - 13%) were found on the seedlings inoculated with *P. involutus*, *L. laccata*, and E-strain as compared to the uninoculated ones. Seedlings inoculated with *C. geophilum* formed 23% of indigenous mycorrhizae.

Seedlings inoculated with *P. involutus* had the greatest shoot and root growth compared with the other three fungal treatments and the control

seedlings(Tables 1 and 4). *L. laccata* and E-strain inoculated seedlings were also greater in both shoot and root growth than the uninoculated seedlings, although they were only significantly different in shoot and root dry weights. Seedlings inoculated with *C. geophilum* were not significantly different from the uninoculated seedlings in any of the growth parameters except for short root number.

DISCUSSION

Influence of *Kalmia* leaf leachate on the growth of ectomycorrhizal fungi varied greatly among the isolates. When they were grown in the presence of *Kalmia* leachate, nine out of 19 fungal isolates exhibited more than 20% reduction in mycelium growth, while the growth of the other ten isolates were either stimulated or remained unaffected. Similar inhibition and stimulation of growth of mycorrhizal fungi by plant residues, leaf leachates, and soil extracts have been reported by others. For example, Olsen *et al.* (1971) found that water extract of aspen leaves had a strong inhibitory effect on the growth of different *Boletus* species ; Cote & Thibault(1988) tested seven species of ectomycorrhizae associated with black spruce in water extract of raspberry (*Rubus idaeus*) leaves and found that the growth of five fungi was significantly inhibited and the other two were stimulated ; Goldner, *et al.*(1986) studied four ectomycorrhizal fungi common to bituminous stripmine spoils and found that three of these fungi were completely inhibited or killed by acetonetoluene extracts of a lichen, *Cladina cristatella*, while the fourth one was unaffected ; Rose, *et al.*(1983), studying the influence of watersoluble leachates from various types of litter on growth of four ectomycorrhizal fungi, also found a highly significant interaction between fungal species, litter type, and leachate concentration.

From our experiments, we suggest that the growth inhibition effect of *Kalmia* leaf leachate on ectomycorrhizal fungi was due to the toxicity effects of the leaf leachate. Wilson & Griffin (1979) studied higher basidiomycetes growing on agar medium using various solute potentials(0 to -5.5 bar) and found that their colony radial growth was not significantly affected by osmotic action. The 50% leachate of *Kalmia* leaves used in the present study had a solute potential of -0.7 bar. At this concentration the osmotic effect on the fungal growth would be minimum. Furthermore, lower concentrations(10 and 25%) of the leaf leachate also had growth inhibitory effects on some mycorhizae.

Influence of pH on fungal growth is well known. Since the pH of the cultures for both the control and the treatments was adjusted at the same value, the pH effect was presumably removed from our experiments. Therefore, the growth inhibition of some mycorrhizae observed in this study was mainly due to the growth inhibitory compounds present in the leaf leachate rather than to its pH. A variety of chemical compounds can inhibit the mycelium growth of mycorrhizal fungi ; for example, benzoic acid and catechol from aspen leaves(Olsen *et al.*, 1971), D-usnic acid from *Cladina cristatella*(Goldner, 1986). Eight phenolic acids have been identified from the water leachate of *Kalmia* leaves(Zhu & Mallik, 1994).

Despite its inhibitory effect, the water leachate of *Kalmia* leaves stimulated the mycelial growth of some ectomycorrhizal fungi tested in this study. Presumably these fungi have the ability to degrade the toxic compounds in the leachate and use them as additional carbon and nitrogen source(Leake & Read, 1991). Harley & Smith (1983) suggested that the differential susceptibility to allelochemicals that occur among ectomycorrhizal fungi is related to their ability to produce phenol oxidases. Giltrap(1982) studied the production of polyphenol oxidases by 104 isolates of 67 species of mycorrhizae, and he found that most of these isolates produced little or no polyphenol oxidase except 7 out of 8 *Lactarius* species, which produced the enzyme vigorously. In another study by Ramstedt & Soderhall(1983), significant amounts of phenol oxidase were found in all the four ectomycorrhizae tested.

Application of *Kalmia* leaf leachate did not significantly affect the shoot or root biomass of

the inoculated or uninoculated seedlings. The reason for such a result is not known. It is possible that the phytotoxic compounds in the *Kalmia* soil was already present in a concentration which was high enough to produce maximum inhibition of the root growth, and an addition of leaf leachate to the soil would not result in a significant difference.

When grown with living *Kalmia* plants, biomass production of inoculated black spruce seedlings decreased in the order *P. involutus*>*L. laccata*>E-strain>*C. geophilum*>control. The shoot and root dry mass was two to three times higher in *P. involutus* inoculated seedlings compared to control. The remarkable increase in biomass of seedlings inoculated with *P. involutus* was likely due to the direct effect of mycorrhizae formation, and the ability of the fungus to with stand *Kalmia* toxicity. This, in turn, may have increased the competitive ability of the inoculated black spruce seedlings. Results from our study have shown that several mycorrhizae, including *P. involutus*(NF4), *L. laccata*(GB23), E-strain (GB45), and *C. geophilum*(GB12), were able to grow in presence of leaf leachate of *Kalmia*. Increase in compatibility of host plants by mycorrhizal fungi have been well documented(Perry & Choquette, 1987). The difference in biomass increase of seedlings inoculated with the four fungi may perhaps be explained by the difference in nutrient uptake and carbon demands as the result of mycorrhizal association and their ability to grow in presence of *Kalmia* toxicity as Nilsson *et al.* (1992) reported that nitrogen uptake was three times faster in mycorrhizae inoculated *Pinus sylvestris* than the non-inoculated plants. Inoculated plants were able to overcome the toxic effects of water extract of leaves of *Empetrum hermaphroditum*, another boreal understory species in northern Sweden known to affect tree regeneration by allelopathy(Zackrisson & Nilsson, 1992).

Implications for forest regeneration

The effect of the water leachate of *Kalmia* leaves on ectomycorrhizae formation among selected fungal isolates and black spruce seedlings in pure culture has important silvicultural implications. Failure and reduction in mycorrhizae formation by some isolates could be due to the toxic effect of the leaf leachate affecting mycorrhizae colonization and root elongation of the seedlings (Mallik, 1987, 1992). It is noteworthy that the fungi that were more resistant to *Kalmia* leaf leachate generally formed well developed ectomycorrhizae in black spruce seedlings. However, *Laccaria laccata*(GB23) was an exception. This fungus formed well developed mycorrhizae with black spruce but exhibited poor growth in presence of all the three concentrations of *Kalmia* leaf leachate.

From our results, we conclude that leaf leachate of *Kalmia* has growth inhibitory effects on some ectomycorrhizae associated with black spruce. This growth inhibitory effect may be responsible for the low mycorrhizae inoculum potential for black spruce in *Kalmia* dominated sites. Allelopathic growth inhibition of ectomycorrhizal fungi may produce dramatic changes in plant communities. For instance, failure of Sitka spruce plantations in Scotland was attributed to inhibition of spruce mycorrhizae by substances leaching from heather(*Calluna vulgaris*) roots (Robinson, 1972) and/or it's raw humus(Jalal & Read, 1983a, b). In Finland, unidentified substances leached from reindeer lichen inhibited ectomycorrhizae formation on various tree species (Brown & Mikola, 1974). In other cases the effects are more subtle, with allelopathy altering the composition of ectomycorrhizal fungi rather than causing complete failure of mycorrhizae formation(Schoenberger & Perry, 1982 ; Cote & Thiabult, 1988). Apparently, there is a great variability among ectomycorrhizae in terms of their responses to allelopathic effects. This variability can provide an opportunity to select certain ectomycorrhizal fungi for seedling inoculation in achieving silvicultural success(Kropp & Langlois, 1990) by overcoming the growth inhibitory effects of certain plants such as *Kalmia* (Mallik & Zhu, 1995).

Allelopathic effects on mycorrhizae have been known for several decades(Melin & Nilsson, 1953). Ectomycorrhizae are often sensitive to substances exuded and leached from plant mate-

rial(Rice, 1984). Olsen *et al.*(1971) found that at high concentration, aqueous extracts of aspen (*Populus tremuloides*) leaves strongly inhibited growth of several species of mycorrhizae, but at low concentration(5%) fungal growth was stimulated. Our studies have shown that the responses of mycorrhizal fungi to *Kalmia* toxicity is difficult to generalize, since there was a considerable variation in colony diameter and mycelial biomass among the fungal species and among isolates within species. Such variation can be used to our advantage in selecting fungal species or isolates that may be useful in overcoming allelopathic growth inhibition. Three out of the four fungi in our greenhouse experiment continued to form abundant ectomycorrhizae with black spruce seedlings in presence of *Kalmia*. Persistence of inoculated mycorrhizae was not inhibited by the *Kalmia* soil, *Kalmia* leaf leachate and living *Kalmia* plant. In the cases of *Laccaria laccata* and *Cenococcum geophilum*, the mycorrhizae formation was slightly stimulated by *Kalmia* leaf leachate.

Seedlings of black spruce inoculated with *Paxillus involutus* had the greatest root and shoot growth which were 15 to 30% greater in shoot dry weight, root dry weight and short and lateral root numbers compared to the uninoculated seedlings or the ones that were inoculated with the other fungi. Increase in seedling height of Douglas fir(*Pseudotsuga menziessii*) inoculated with *Rhizopogon vinicolor* was reported by Berch & Roth(1993). Among the four fungi, *P. involutus* appeared to be the most effective for mycorrhizae inoculation on black spruce seedlings. This particular fungal isolate was collected from a *Kalmia* site in Grand Falls, Newfoundland. This suggests the importance of isolation of local mycorrhizae and including them during screening in order to select an inoculant fungus best-suited to soil and host plant. Trappe(1977) also pointed out the need for selecting mycorrhizal strain suitable for a particular host/soil/climate combination.

In summary, our results suggest that the selected fungi *Paxillus involutus*, *Laccaria laccata*, and E-strain can form abundant ectomycorrhizae with black spruce seedlings in *Kalmia* soil with *Kalmia* leaf leachate under experimental conditions. These fungi, particularly *P. involutus*, can stimulate the biomass growth of the seedlings and increase the compatibility of the host plant with *Kalmia*. However, these results should be interpreted cautiously and they must be verified under field conditions by outplanting the inoculated black spruce seedlings in *Kalmia* dominated sites. Because the inoculated seedlings not only have to overcome the growth inhibitory property of *Kalmia*, they also have to out compete the naturally occurring mycobionts of the habitat in order to flourish as black spruce symbionts. In an outplanting experiment with mycorrhizal and nonmycorrhizal jack pine(*Pinus banksiana*) seedlings, McAfee & Fortin(1986) demonstrated that competitive interactions between inoculated ectomycorrhizae and the naturally occurring mycobionts depend upon the fungal species as well as habitat conditions. As Leake & Read(1991) pointed out, the relevency of experiments with mycorrhizae to the real world must be tested by performing field experiments to take into account the circumstances prevailing in nature.

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Appendix I. Effect of *Kalmia* leaf leachate on clony diameter of mycorrhizal fungi. Mean value of five replicates ±SD.

Fungus	Code	Colony diameter(㎜) on plate with leachate			
		0%	10%	25%	50%
Hebeloma crustuliniforme	AB2	41±1a	27±3b	24±3b	24±3b
Hebeloma cylindrosporum	GB6	13±1c	24±6ab	25±2a	19±1b
Paxillus involutus	NF4	62±3c	74±2b	78±3b	85±2a
Paxillus involutus	GB41	49±3b	51±9b	80±2a	85±3a
Paxillus involutus	GB40	22±1	22±1	22±2	24±3
Paxillus involutus	GB24	81±4	74±3	75±3	72±6
Leccinum scabrum	NF1	21±1	17±3	18±1	20±2
E-strain	GB45	27±2	23±3	30±4	28±2
Thelephora terrestris	GB50	39±5	36±1	35±2	35±2
Pisolithus tinctorius	GB14	44±2a	26±2b	24±3b	22±3b
Pisolithus tinctorius	GB25	71±3a	68±6ab	71±1a	55±7b
Pisolithus tinctorius	GB4	42±5a	26±2b	23±1b	19±1b
Cenococcum geophilum	GB12	37±3	33±1	33±3	32±4
Cenococcum geophilum	AB1	40±1a	27±2b	25±2b	28±1b
Laccaria bicolor	GB8	39±4	35±3	38±2	31±4
Laccaria laccata	AB5	58±3a	49±5b	43±2b	34±1c
Laccaria laccata	GB20	41±1a	34±4b	33±1b	27±2c
Laccaria laccata	GB23	54±3a	36±4b	41±2b	38±2b
Lycoperdon perlatum	GB56	22±2a	23±1a	26±5a	16±3b

NOTE : Mean values followed by the same or no letter do not differ significantly according to Tukey's multiple range test at $p=0.05$.

Appendix II. Effect of culture pH and *Kalmia* leaf leachate on mycelial dry weight of ectomycorrhizal fungi. Mean value of five replicates ±SD.

Fungus	Code	Treatment[a]	Mycelial dry weight(mg/plate)[a]		
			pH5	pH4	pH3
Hebeloma crustuliniforme	AB2	C	6.7±0.3	0	0
		L	2.9±0.5	0	0
Hebeloma cylindrosporum	GB6	C	5.5±0.5	3.3±0.4	3.3±0.4
		L	5.9±0.5	0	0
Paxillus involutus	NF4	C	12.0±1.8	10.3±0.8	10.3±0.8
		L	15.0±0.7	8.5±0.5	8.5±0.5
Paxillus involutus	GB41	C	14.3±1.0	5.5±0.6	5.5±0.6
		L	12.6±1.1	3.1±0.5	3.1±0.5
Paxillus involutus	GB40	C	8.5±0.4	2.4±1.0	2.4±1.0
		L	8.0±0.5	1.2±0.2	1.2±0.2
Paxillus involutus	GB24	C	16.2±1.0	4.2±0.3	4.2±0.3
		L	15.1±0.5	2.5±0.7	2.5±0.7
Leccinum scabrum	NF1	C	7.5±0.7	0	0
		L	7.8±0.7	0	0
E-stain	GB45	C	8.8±0.8	5.0±0.5	5.0±0.5
		L	8.1±0.7	4.2±0.8	4.2±0.8
Thelephora terrestries	GB50	C	7.0±1.0	4.8±0.4	4.8±0.4
		L	7.3±0.4	2.5±0.5	2.5±0.5
Pisolithus tinctorius	GB14	C	10.5±1.1	15.0±1.4	15.0±1.4
		L	5.0±0.7	8.0±1.2	8.0±1.2
Pisolithus tinctorius	GB25	C	18.5±1.5	2.5±0.5	2.5±0.5
		L	13.7±0.8	0	0
Pisolithus tinctorius	GB4	C	7.3±0.4	7.3±2.4	7.3±2.4
		L	5.8±0.8	4.8±0.4	4.8±0.4
Cenococcum geophilum	GB12	C	7.8±0.4	9.5±1.1	9.5±1.1
		L	21.0±1.2	20.3±1.8	20.3±1.8
Cenococcum geophilum	AB1	C	20.0±1.5	5.5±1.2	5.5±1.2
		L	15.7±0.5	1.0±0.5	1.0±0.5
Laccaria bicolor	GB8	C	13.0±0.7	9.3±0.4	9.3±0.4
		L	6.5±1.1	3.5±0.5	3.5±0.5
Laccaria laccata	AB5	C	10.7±0.5	2.6±0.2	2.6±0.2
		L	7.5±0.6	0	0
Laccaria laccata	GB20	C	9.5±1.1	6.8±1.1	6.8±1.1
		L	2.5±0.5	0	0
Laccaria laccata	GB23	C	5.3±0.4	3.8±0.4	3.8±0.4
		L	5.0±0.7	2.8±0.4	2.8±0.4
Lycoperdon perlatum	GB56	C	7.9±0.1	0	0
		L	5.6±0.6	0	0

[a] Treatment C was distilled water as control and L was the water leachate of *Kalmia* leaves.