

# Degradation of boreal forest soil fertility caused by the invasion of *Kalmia angustifolia* : a forest management problem.

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## 1. Abstract

In Canada's boreal forest, black spruce seedling growth is sometimes kept in check by the invasion of the ericaceous shrub, *Kalmia angustifolia*. A series of experiments were devised to elucidate the mechanisms by which *Kalmia* gains a competitive advantage over spruce. Soil incubations and bioassays revealed that humus formed under *Kalmia* produced very little mineral N. *Kalmia* was able to uptake soil N from this humus whereas black spruce seedlings could not. *Kalmia* foliage produced 5x more tannins than spruce needles, and the addition of purified *Kalmia* tannins to soil resulted in lower mineral N accumulation with no sign of this being provoked by microbial immobilization. Tannin-protein precipitates formed with *Kalmia* tannins contained more N than those produced with spruce tannins. These protein-tannin complexes were more easily metabolized by mycorrhizae associated with *Kalmia* than those associated with spruce. *Kalmia* tannins were shown to inhibit important soil enzymes (acid phosphatase, amidase and  $\beta$ -glucosidase), and these effects were concentration-dependent. Soil enzyme inhibition was higher when *Kalmia* leaves were added to forest humus, and a field study demonstrated a negative relationship between % *Kalmia* ground cover and soil enzyme activity. Silvicultural trials showed that fertilization had a positive effect on black spruce growth, but still better growth and improved soil properties were obtained by the complete eradication of *Kalmia*. Scarification is a promising management option to restore fertility on *Kalmia*-dominated cutovers.

## 2. Introduction

Forestry practices in Canada are principally established in the boreal forest and are said to be extensive, that is, forests are left to regenerate after logging with few silvicultural interventions. Soil quality is, therefore, largely determined by patterns of vegetation change over time. Successional changes in boreal forest species are often cyclical resulting in black-spruce dominated stands. In some areas, however, black spruce growth is kept in check by the invasion of ericaceous shrubs such as *Kalmia angustifolia*. Early studies (Peterson et al., 1965) had demonstrated the potential for *Kalmia* leaf extracts to inhibit the germination of black spruce seedlings, and subsequent studies (Zhu and Mallik, 1994) linked this phenomenon to the production of specific allelochemicals by *Kalmia*. There was, however, evidence that growth-check of black spruce seedlings was not solely related to poor seed germination, as field studies had associated the presence of *Kalmia* to foliar chlorosis of black spruce seedlings on regenerating cutovers (English and Hackett, 1994). The nature of this interference was not well known. On the one hand, it had been noted that *Kalmia* produced an extensive root system which could compete directly with black spruce for soil nutrients. Alternatively, it was hypothesized that *Kalmia* could interfere indirectly for resources by modifying nutrient cycling and energy flow in the soil. Here, I summarize a series of experiments that began in the mid-1990s and are ongoing, where we attempted to assess the extent, and elucidate the mechanisms, by which *Kalmia* gains a nutritional advantage over black spruce seedlings. Laboratory studies were designed to assess the effects of *Kalmia* leaf litter on soil N cycling. Field trials compared biochemical properties of humus under *Kalmia* and black spruce seedlings, measured the effects of *Kalmia* humus on litter decomposition rates and growth performance of black spruce seedlings, and tested various forest management options to restore adequate productivity on *Kalmia*-dominated black spruce cutovers.

## 3. Methods

### 3.1 Assessing N deficiency

A bioassay was performed in which black spruce and *Kalmia* seedlings were grown in humus collected from a *Kalmia*-dominated site. Ten 12 L pots were half-filled with mineral soil, then topped off with 6 L of organic forest floor material. Seedlings of both species were washed free of soil, weighed (20–30 g) and planted into the pots (n=5). Fifteen additional seedlings of each species were washed, weighed, dried, digested in H<sub>2</sub>SO<sub>4</sub> and analyzed for total-N. The initial N content of potted seedlings was estimated from regression equations relating fresh weight to total N-content. The potted seedlings were grown in a greenhouse for 20 wk after which they were harvested, dried, digested and analyzed for total-N. Net N uptake of each seedling was calculated.

Forest floor humus material was collected under six patches of *Kalmia* and under six adjacent patches where *Kalmia* had been eradicated. Sub-samples were weighed, dried and reweighed to calculate moisture content. Fresh subsamples from each patch were weighed (~20 g fresh wt.) into 500 mL Mason jars, and extracted in 100 mL of 2M KCL solution. Extracts were filtered and analyzed colorimetrically for NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N using a FIA Quickchem spectrophotometer. A second set of fresh humus subsamples were weighed (~20

g fresh wt.) into 500 mL Mason jars, covered with a polyethylene film and kept in an incubator that provided constant temperature (22°C) and humidity (85%) for 30 d. Humus subsamples were then extracted in KCl and net N mineralization rates were calculated by subtracting initial from final mineral N concentrations.

### 3.2 Field trials

The effects of *Kalmia* removal and spot fertilization on black spruce seedling growth were tested using a split-plot factorial design that covered 0.2 ha. *Kalmia* removal was initiated in August 1999, with the application of glyphosate herbicide. From 2000 to 2006, *Kalmia* re-sprouting on these plots was manually removed. The second main plot treatment was a control, with up to 50% *Kalmia* ground cover. These two main plot treatments were replicated in six complete blocks. In June 2000, we planted 20 containerized black spruce seedlings in each main plot and divided these into two subplots, one fertilized at time of planting, the other left unfertilized. Fertilization consisted of applying 9 g of slow-release N-P-K (26-12-6) contained in a fertilizer bag and buried 5 cm deep and 2 cm to the side of each seedling. In 2006, seedling height was measured in each subplot.

We tested whether litter decomposition rates varied according to vegetation cover by placing four litterbags (10 x 10 cm) in each of three patches dominated by black spruce, and three patches dominated by *Kalmia*. Litterbags, containing 1 g (dry wt) of senescent *Kalmia* leaves, were placed 5 cm deep in the humus, and one litterbag in each patch was retrieved after 3, 5, 12, 25 months. The remaining litter was cleaned, dried and weighed to calculate mass loss.

### 3.3 The role of litter tannins

Plots (50 x 50 m) were established on 20 cutover sites, and forest floor humus was collected under six patches of *Kalmia* and six patches of black spruce. Condensed tannins were measured colorimetrically by butanol-HCl hydrolysis using the proanthocyanidin assay (Preston, 1999). Senescent *Kalmia* leaves and spruce needles were randomly collected in each site and bulked into one main sample per species. After freeze-drying, leaves and needles were ground in a ball mill and analyzed for condensed tannins in the same way.

Purified condensed tannins were prepared from *Kalmia* foliage and black spruce needles, as outlined in Preston *et al.* (1997). Experimental units consisted of 10 plastic Buchner funnels equipped with fritted plastic filter plates. Humus (72 g dry wt equiv.) that had been collected under black spruce was tamped into each funnel, the tops were sealed with polyethylene film to prevent desiccation. After 4 wk, the funnels were assigned one of two treatments (n=5) that were applied bi-weekly for the next 10 weeks. These treatments were (1) a control (50 ml H<sub>2</sub>O), and (2) a *Kalmia* tannin solution (431 mg in 50 ml H<sub>2</sub>O). Prior to each treatment application, humus in each funnel was leached with 200 mL of 10 mM CaCl<sub>2</sub> solution. Leachates were analyzed colorimetrically for NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations using a Technicon auto-analyzer.

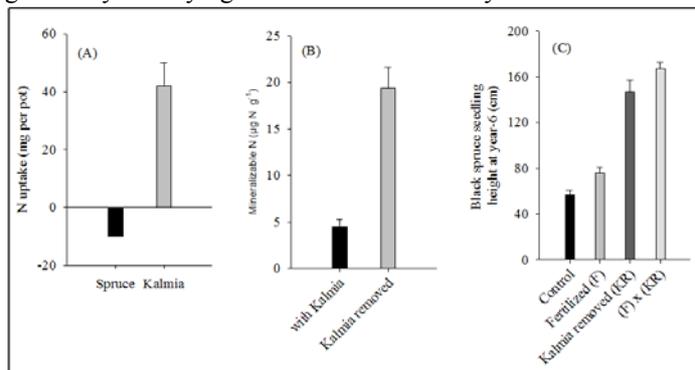
The protein precipitation capacities of *Kalmia* and black spruce condensed tannins were compared. Three grams of each tannin type were dissolved in 500 mL H<sub>2</sub>O, and filter-sterilized. A solution containing 2.5 g L<sup>-1</sup> of bovine serum albumin (BSA) in sodium acetate buffer (10 μM, pH 4.5) was prepared and filter sterilized. We transferred 2, 5, 10 and 15 mL of each tannin solution in duplicate sterile centrifuge tubes, and raised the volume in each tube to 20 mL using the BSA solution. Mixtures were mixed with a vortex, kept at 4 °C for 24 h, centrifuged (4000 rpm) and supernatants discarded. Precipitates were freeze-dried, weighed and analyzed colorimetrically for total-N following acid digestion.

We tested the ability of different mycorrhizal strains associated to *Kalmia* (*Rhizoscyphus ericae* and *Phialocephala fortinii*) or black spruce (*Hebeloma crustuliniforme* and *Cenococcum geophilum*) to grow on medium amended with *Kalmia* tannin-protein complexes as the principal N source. For the tannin-protein precipitate treatment, we added 500 mg of precipitate per litre of autoclaved Modified Melin Norkrans (MMN) nutrient agar medium. Mineral N was added (250 mg NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> L<sup>-1</sup> solution) to the control treatment. We poured 25 mL of each agar growth medium into twenty 9 cm dia. Petri dishes. Five Petri dishes of each growth medium were inoculated with each mycorrhizal strain, sealed with parafilm and incubated 60 d in the dark at 20°C. These were then heated (50 °C) to melt the agar, and to recover and weigh the mycelia after freeze-drying.

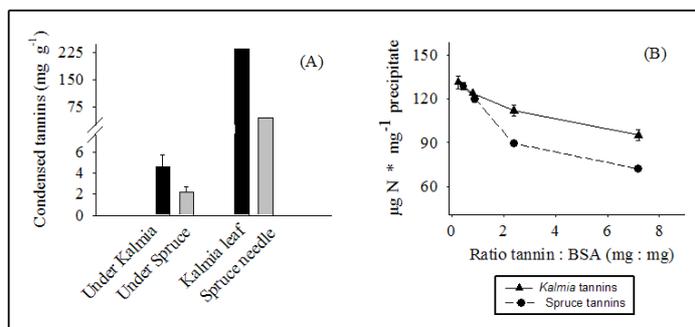
We tested the effects of incremental additions of *Kalmia* and spruce tannins on β-glucosidase activity in soil solutions from 24 field sites. Enzyme activity was measured using microplate-based fluorimetric assays. The substrate used was 4-MUB-β-d-glucoside, which fluoresces once the enzyme has cleaved the substrate. One g (dry wt equiv.) subsamples of forest floor material were mixed with 50 mL distilled water and 1.5 mL toluene to inhibit microbial activity. Following the addition of 20 μL of soil extract to each well, we added 20 μL of MES (2-[N-Morpholino]ethanesulfonic acid) solution carrying *Kalmia* or black spruce tannins so as to yield final tannin concentrations of 0, 0.025, 0.05, 0.10 and 0.20 mg mL<sup>-1</sup>. Plates were incubated (30°C, 10 min), shaken and fluorescence measurements taken after 60 min using a microplate fluorimeter. In a second study, we amended forest floor humus with *Kalmia*:spruce litter mixtures of varying proportions (0:100, 25:75, 50:50, 75:25 and 100:0%). Fifteen g of humus were weighed into sixty 500 mL Mason jars, and 3.0 g of each *Kalmia*:spruce litter mixtures were added to 12 jars. The jars were sealed and incubated in the dark at 20°C. After 2, 6, 13, and 46 wk, three jars from each treatment were destructively sampled and β-glucosidase activity measured. In a third study, we tested the relationship between *Kalmia* cover, estimated in 20 quadrats (0.25 m<sup>2</sup>) on each field site, and β-glucosidase activity in the forest floor from each quadrat.

#### 4. Results and Discussion

When grown in a *Kalmia* soil, spruce seedlings lost an average of 10 mg N over the course of a 20 wk bioassay (Fig. 1A). This indicates that the loss of plant-N, most likely through rhizodeposition, was not compensated by N-uptake by roots. In contrast, when individuals from the same population of spruce seedlings were grown in soil from a birch-dominated site, seedlings absorbed 48 mg N over a 20 wk trial (data not shown). In contrast, *Kalmia* seedlings with the same initial weight absorbed about 40 mg N from the *Kalmia* soil (Fig. 1A) and 65 mg N from a birch soil (data not shown). Net N mineralization rates in forest floor humus from plots where *Kalmia* had been eradicated were > 4x greater than in humus from *Kalmia*-dominated plots (Fig. 1B). Our field study confirmed that both *Kalmia* eradication and fertilization increased spruce seedling growth, and that these effects were additive (Fig. 1C). In the litter bag experiment, the remaining mass of *Kalmia* litter was significantly lower under *Kalmia* shrubs (ca. 50%) than under spruce seedlings (ca. 70%) (data not shown). Taken collectively, these four studies provide strong evidence that *Kalmia* interferes indirectly with spruce growth by modifying soil nutrient availability.



**Figure 1 Results from three independent studies suggesting low soil N availability on *Kalmia*-dominated sites : (A) results from a bioassay evaluating N uptake from *Kalmia* humus; (B) N mineralization rates in plots with and without *Kalmia*; (C) effects of fertilization and *Kalmia* removal on spruce seedling growth.**

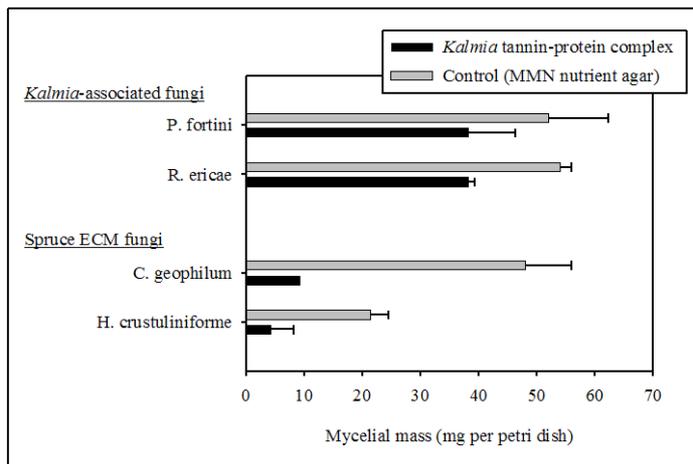


**Figure 2 (A) Condensed tannin concentrations in humus under *Kalmia* and spruce, and in *Kalmia* and spruce foliage; (B) N concentration of tannin-protein precipitates formed with *Kalmia* and spruce tannins.**

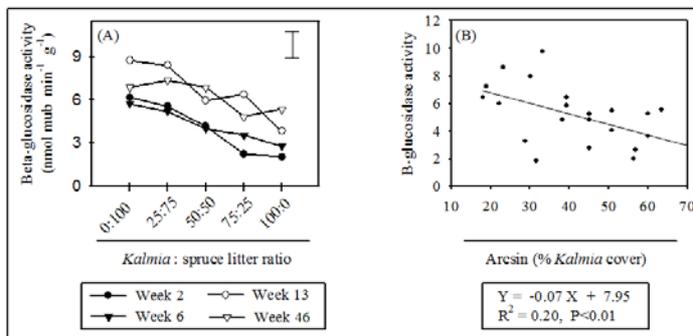
fungi associated to *Kalmia* than those associated to black spruce (Fig. 3). Taken collectively, these studies suggest that litter tannins produced by *Kalmia* may be an important component of indirect interference with spruce seedling growth, and that plants such as *Kalmia* that have evolved in tannin-rich environments have co-evolved with mycorrhizal symbionts efficiently capable of metabolizing tannin-protein precipitates and, perhaps, shunting this acquired N to the host plant.

$\beta$ -glucosidase activity in soil solutions was reduced equally by the addition of *Kalmia* and spruce tannins (data not shown). Given that this effect increased linearly over the range of tannin concentrations that we tested, and given that tannin concentrations in *Kalmia* litter are 5x greater than in spruce litter, we conclude that soil enzyme activities are likely to be lower in *Kalmia*-dominated sites. The second study in this series consisted of testing this hypothesis by simulating the litter effects associated with *Kalmia* invasion on spruce cutovers. We

Condensed tannin concentrations in humus under *Kalmia* were about twice as high than under spruce seedlings (Fig. 2A). This difference was likely due to differences in litter quality between the two species, as the concentration of condensed tannins were nearly 5x greater in *Kalmia* leaves than in spruce needles (Fig. 2A). Although we do not know the fate of litter tannins, the fact that humus concentrations were 1–2 orders of magnitude lower than the corresponding foliar concentrations, suggests a large and rapid decline in tannins during the first year of decomposition. In our microcosm study, the chronic addition of *Kalmia* tannins to spruce humus resulted in significantly lower extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations (data not shown). Following on this study, we tested the protein (BSA) precipitation capacities of both spruce and *Kalmia* tannins in aqueous solution. Both tannin types formed similar amounts of precipitate along a gradient of tannin:BSA ratios (data not shown). However, precipitates formed with *Kalmia* tannins had higher N concentrations than those formed with spruce tannins (Fig. 2B), suggesting that a given unit weight of *Kalmia* tannins will sequester significantly more protein than spruce tannins. Precipitates formed with *Kalmia* tannins were more easily metabolized by symbiotic



**Figure 3** Mycelial mass of mycorrhizae associated with *Kalmia* and spruce when grown on medium containing  $\text{NH}_4^+$  or tannin-protein complexes as sole N source.



**Figure 4** (A)  $\beta$ -glucosidase activity in humus amended with various ratios of *Kalmia* and spruce litter; (B)  $\beta$ -glucosidase activity in forest floor humus as a function of *Kalmia* cover.

found significant negative relationships between the *Kalmia* : spruce litter ratio and  $\beta$ -glucosidase activity in the amended soils (Fig. 4A). The third study in this series revealed a significant negative relationship between % *Kalmia* cover in the field and  $\beta$ -glucosidase activity in the soil (Fig. 4B). This enzyme is involved in the final step of cellulose degradation that provides simple sugars for soil microorganisms. In parallel studies, we found similar results, albeit not as consistent, with other soil enzymes such as amidase, which catalyses the deamination of aliphatic amides to produce ammonia, and acid phosphatase, which catalyses the hydrolysis of phosphoric acid (data not shown).

In summary, we have shown that *Kalmia* interferes indirectly with black spruce growth by modifying humus quality and nutrient cycling. The nutritional deficiencies of black spruce can be related, in part, to elevated concentrations of tannins found in *Kalmia* litter. These tannins efficiently bind soil proteins in forms that are difficult to access by black spruce, but are more easily metabolized by mycorrhizal symbionts associated to *Kalmia*. Furthermore, *Kalmia* litter tannins interfere with the activity of important soil enzymes that are involved in the degradation of soil organic matter. The fact that genetically based plant traits such as leaf litter tannin quality and concentration leads to inter-specific plant interactions, supports the notion that litter tannin

production is an evolutionary trait in *Kalmia* driving ecosystem processes and structure so as to improve its competitive ability. In a practical sense, our data suggest that black spruce growth could be improved by the removal of *Kalmia* from regenerating cutovers. While complete eradication would be difficult and expensive to achieve, ongoing studies have shown that scarification, which consists of creating deep furrows and exposing mineral soil horizons, alleviates the negative influence of *Kalmia* and allows black spruce seedlings to gain about 50% growth over seedlings on non-scarified cutover sites (Thiffault et al., 2004). Whether this gain is temporary or permanent remains to be seen, but scarification appears, for the moment, to be the most economical and efficient silvicultural option to manage *Kalmia*-dominated cutovers.

## 5. References

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