Assessment of Plants with Allelopathy Potential as a Bio-barrier against Undesirable Bio-intrusion on Covers with Capillary Barrier Effects

Evgeniya Smirnova[1], Yves Bergeron[2], Francine Tremblay[2],
Nelson Thiffault[3], Bruno Bussière[1], Gilles Joanisse[4]

[1]Chaire CRSNG-Polytechnique-UQAT en environnement et gestion des rejets miniers, Université du Québec en Abitibi-Témiscamingue (UQAT), 445 boul. de l'Université, Rouyn-Noranda, QC J9X 5E4, tel: (819) 762-0971#2343, fax: (819) 797-6672;
[2] Chaire industrielle CRSNG- UQAT-UQAM en aménagement forestier durable, Université du Québec en Abitibi-Témiscamingue (UQAT), 445 boul. de l'Université, Rouyn-Noranda, QC J9X 5E4;
[3]Direction de la recherche forestière, Ministère des Ressources naturelles et de la Faune du Québec, 2700 Einstein, Québec, QC G1H 2X9;
[4] Centre d'enseignement et de recherche en foresterie de Sainte-Foy inc., 2424 chemin Sainte-Foy, Québec, QC G1V 1T2;
[5] Corporation minière Northern Star, 153 A, ave. Perreault, Val d'Or, QC J9P 2H1
Contact: eugenia.smirnova@uqat.ca

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Abstract

Covers with Capillary Barrier Effects (CCBE) are used to control oxygen migration from atmosphere to the tailings and are constructed to reclaim acid-generating mine sites. It has been reported that natural vegetation succession starts the year of CCBE construction. Trees represent a particular threat for CCBE performance. Five tree species could have a long-term impact on CCBE performance; namely, poplar, aspen, black spruce, alder and willow (target trees). The use of the allelopathy effect (AE), which is the inhibition of germination and growth of certain species of plants by others, can serve as a key mechanism to protect CCBE against undesirable species colonisation. The main objective of this study is to test the capacity of plants with allelopathic effect to reduce tree establishment on an existing CCBE. A field experiment was started in 2008. Three species with well reported AE (sheep laurel, Labrador tea and bluejoint reedgrass) were selected as potential bio-barriers. The selected species were able to grow on the CCBE. Preliminary results suggest that among ericaceous AE, sheep laurel has higher polyphenol content than Larbador tea. Bluejoint reedgrass allelopathic influence on the target trees was the most pronounced compared to the two other AE species.

Introduction

Canada is one of the leading countries in the exploitation of mineral resources. This exploitation produces every year a substantial amount of mine waste that can have negative impacts on the environment. Efficient mine waste remediation implies the integration of knowledge from various disciplines, including mining and mineral processing, ecology and biology, to manage the geochemical, geothechnical and biological issues related to these man-created ecosystems. Here we present results from a multidisciplinary project on mine waste remediation.

Problem description

Acid mine drainage (AMD; also called acid rock drainage ARD) resulting from oxidation of sulphide minerals contained in mine wastes is undoubtedly the most serious environmental problem of the mining industry. Different methods can be used to avoid AMD generation (MEND, 2001). One method consists in limiting oxygen migration (oxygen is the main oxidant of sulphide minerals at near-neutral pH) by the use of multi-layered covers that use capillary barrier effects, also known as Covers with Capillary Barrier Effects (CCBE). The main role of a CCBE in a humid climate is to limit the oxygen diffusion flux reaching reactive waste material. To do so, the cover must maintain a high degree of saturation (typically > 85%; Bussière et al. 2006) in one (or more) of its layers. CCBEs usually contain three to five layers comprising different materials. The first three layers aim to create the oxygen barrier (by using capillary barrier effects) while the upper layers protect the lower ones against erosion and bio-intrusion. More information on CCBE can be found in Bussiere et al. (2003).

Even if performance of CCBEs has been demonstrated in the field over the short term (e.g. Dagenais, 2005; Bussière et al., 2006), the long-term efficiency to control AMD still has to be proven. One of the factors that could affect long-term performance of CCBEs is bio-intrusion. Development of deep-rooted species on a CCBE could reduce the long-term ability of a CCBE to control AMD.

Risks associated with vegetation on CCBEs

The CCBE is now recognized as an effective oxygen barrier to control AMD generation in humid climates; however, very little is known about vegetation succession on mine sites with CCBEs. To the authors' knowledge, preliminary studies performed at the Lorraine mine site in Québec (Trépanier et al., 2006, Smirnova et al., 2010) were the first attempts to characterize and understand the impact of vegetation on the long-term ability of a CCBE to limit AMD generation.

Vegetation succession results in the improvement of soil condition, which, over time, increases the richness of species and eventually the presence of deep-root species (Cooke and Johnson, 2002; Strong, 2000). The beginning of an ecological succession, such as those observed on CCBEs, is characterized by the absence of organic matter, low soil fertility and low competitive interactions. In such contexts, plant roots explore adjacent area to acquire nutrients, which leads to rapid elongation of roots (Coutts, 1987).The major risks associated with the presence of uncontrolled vegetation on a CCBE are: 1) plant roots can extract water from the fine-grained layer, thereby reducing the degree of saturation, which can reduce the ability of the CCBE to limit oxygen migration; 2) the roots can create macropores that increase water infiltration and oxygen migration through the cover system; and 3) the shallow-rooting trees can be vulnerable to up-rooting, thus increasing the risk of physical damage to the CCBE (DOE, 1990; Hutchings et al., 2001).

Characterization of vegetation on an existing CCBE (different than the one discussed later in this article) has been conducted yearly since 2003. The surveys (Smirnova et al., 2010) showed that some tree-roots have already reached a depth of 60 cm, which is greater than the CCBE top protective layer thickness. At least four of nine tree species found on the site are known to reproduce by vegetative multiplication and are considered as fast-growing species: trembling aspen (*Populus tremuloides* Michx.), balsam poplar (*Populus balsamifera* L.), willows (*Salix spp.*) and black spruce (Picea mariana (Mill.) Britton). Moreover, the roots of *Populus spp.* (balsam poplar, trembling aspen) and *Salix spp.* (willows) are tolerant to anaerobic conditions (Hutchings and al., 2001) and are characterized by intensive water-pumping capacities (Legaré, 2005; Farrar, 1995). These species are commonly used on landfills and contaminated soils with anaerobic conditions for restoration purposes (Carnegie and Ramsay, 2009; Nixon et al., 2001). Therefore, tree roots developing in the fine-grained layer may desaturate the moisture-retaining layer of the CCBE.

Bio-intrusion barriers

Two general strategies are in use to control vegetation on mine sites: fighting it or to work with it. For example: mechanical eradication is commonly used by mining companies technique to eliminate undesirable vegetation. However, mechanical eradication does not provide long-lasting effect because most of the trees found on the mine sites are reproducing abundantly by resprouting from the remained parts (Wan et al., 2006). Different other approaches were used to limit bio-intrusion impacts on cover performance; these include herbicides, asphalt, soil compaction, basaltic gravel, salt water, etc. (Cooke and Johnson, 2002). However, most of them have serious drawbacks, such as high implementation costs, short-term impact on vegetation or severe environmental impacts. Recently it has been suggested that the emulation of the processes observed in the nature *ex.* allelopathic relationship can be an efficient measure to control undesirable species on mine sites (DOE 1990; Vyvyan 2002; Cooke and Johnson, 2002).

Among the advantages of this approach are : it emulates a long-lasting stage of secondary succession in the boreal forest, thus it emulates species composition of the surrounding ecosystems; it emulates interactions among these species; it is cost effective; it does not require utilisation of any herbicides and pesticides.

AE are defined as the inhibition of germination and growth of a certain plant species by another. AE are due to the production of bio-chemical compounds that directly affect plant physiology and growth, or the creation of ecological conditions that exert a negative effect on competitors (Wardle et al., 1998; Siciliano and Germida, 1998). Allelopathic interactions are complex and involve numerous classes of chemicals; namely, phenolic compounds (more than 4000) (Waterman and Mole, 1994), flavonoids, terpenes, steroids, carbohydrates, and amino acids, with mixtures of different compounds (Kruse et al., 2000). Different plant organs, litter, roots, and shoot exudates can exert substantial allelopathic influences on surrounding species (Gallet and Pellissier, 1997; Mallik and Inderjit, 2001, Vivanco et al., 2002).

Three boreal plant species that are recognized for their AE were tested in this study as a potential bio-barrier: bluejoint reedgrass (*Calamagrostis canadensis* Michx. Deauv.) inhibits trembling aspen and white spruce growth and establishment (Landhäusser et al., 2007; Landhäusser and Lieffers, 1998; Collet et al., 2006); sheep laurel (*Kalmia angustifolia* L.) has negative effects on seedling establishment and growth of black spruce, balsam fir and red pine (Mallik and Inderjit, 2001; Thiffault et al. 2004); and bog Labrador tea *(Rhododendron groenlandicum* (Oeder) K&J, formerly *Ledum groenlandicum* Oeder) inhibits reproduction and growth of black spruce (Lavoie et al., 2006; Hébert and Thiffault, 2011). These species have never been tested as a barrier to bio-intrusion on CCBE. There is also a lack of knowledge about their inhibiting effects on the undesired tree species identified earlier, and about the inhibition mechanisms involved.

Different approaches have been used to identify AE and the significance of direct resource competition. Thus, AE could be revealed through the study of morphological and bio-chemical characteristics of neighbouring plants (Wardle et al., 1998).

Study objectives and hypothesis

The main objective of the project is to evaluate the capacity of bio-intrusion barriers to limit long-term deterioration of CCBEs (in the Quebec context) caused by tree-root invasion. Our specific objectives are: 1) to introduce the selected bio-barrier species with known AE (bluejoint reedgrass, sheep laurel and bog Labrador tea) to an existing CCBE; and 2) to evaluate the production of phyto-toxins and resource limitation of bio-barrier species on the target tree species.

The main hypothesis is that direct resource competition and allelopathic interference of potential bio-barrier species will inhibit tree establishment and growth.

Study area

The studied tailings impoundment (called LTA site hereinafter) covers an area of approximately 60 ha and was rehabilitated using a CCBE (Bussière et al., 2006). The cover design configuration consists of three layers. From bottom to top they are (Ricard et al. 1997): a 0.5 m layer of sand used as support and a capillary break layer; a moisture-retaining layer with a thickness of 0.8 m (this layer is made of a non-plastic silty material); and finally a 0.3 m sand and gravel layer on top. Vegetation was not established on the top (nearly flat) area of the site, which represents nearly 80 to 85% of the total area. Nevertheless, because the site is surrounded by forest, some trees and shrubs were observed on the site shortly after the end of cover construction; this observation was reported in Trépanier (2005).

Monitoring of the CCBE has shown that two different zones are present on the site (see Bussière et al. 2006 for details). In the wet zone the water table is high (in or near the CCBE) and the water content in the layers are relatively high (near saturation in the bottom capillary break layer and in the moisture-retaining layer, and usually greater than 12% in the top sand and gravel layer). In the dry zone the water table is more than a meter below the bottom of the CCBE (the water content in the layers is lower than in the wet zone). Study areas have been delimited in both zones (dry and wet) because vegetation growth could differ depending on moisture conditions of the area.

Methods

Sampling design and plant introduction in the experimental plots

One hundred and twenty (120) experimental plots (1 m x 1 m), grouped in six experimental replicate blocks (3 in the dry zone, 3 in the wet zone; see Figure 2a were delimited and prepared in May 2008 on the CCBE. The plots were weeded and big cobbles were removed. In June 2008, an organic substrate was deposited and spread out on the plots with a mechanical shovel. In July 2008, sheep laurel and Labrador tea plants were excavated from the adjacent forest and their rhizomes transplanted on the site; 80 specimens of each of these species were planted systematically in each experimental plot (see Figure 1b).

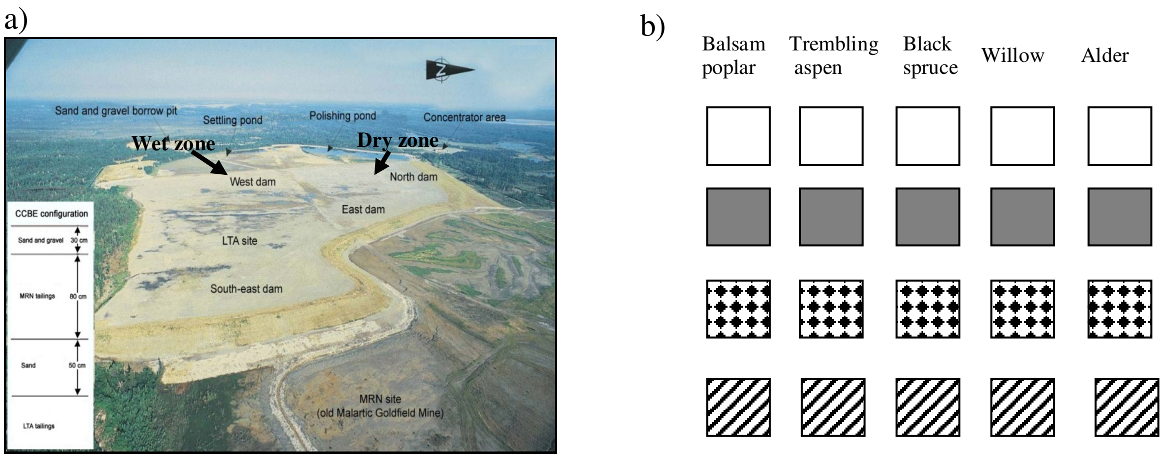


Figure 1. (a) Study site (modified from Bussière et al. 2006), experimental blocks are located in the dry (North down) and wet zones (West down); (b) schematic of one experimental block with 20 plots: control (only target trees), target tree + bog Labrador tea, target trees + sheep laurel, target trees + blue joint reedgrass.

After the first growing season, the mortality of AE plants exceeded 80%. The dead AE plants were replaced during the summer 2009; however, mortality of the AE grafts remained high. Following analyses of the potential causes of such high mortality rates, it was decided to improve the quality of the planting substrate. The substrate was a loamy sand with low organic matter content (50 to 54 g/kg) and low macro-nutrient concentrations (N 10-16 mg/kg; P 38-41 mg/kg; K 31-41 mg/kg; Ca 468-779 mg/kg; Mg 35-48 mg/kg).

In early summer 2009, a new substrate (1 m^3/m^2) with a high organic matter concentration (506-514 g/kg) and nutrient content (N 30-47 mg/kg; P 43-56 mg/kg; K 42-48 mg/kg; Mg 235-368 mg/kg; Ca 1560-2420 mg/kg) was added on each plot. Dead plants were replaced, and bluejoint reedgrass was sieved. Ericaceous AE plants were clipped to stimulate stem-base sprouting. Shade cloths were installed to protect the plants from excessive solar radiation and were kept until July to facilitate establishment. The experimental plots were watered and weeded regularly.

Preliminary germination tests showed that bluejoint reedgrass seeds have a very low germination percentage. Bluejoint grass plants were thus purchased from a local nursery and transplanted in the experimental plots in May 2010.

In August 2008, target trees were introduced in the experimental plots. Seedlings of black spruce and speckled alder were obtained from a local tree nursery; trembling aspen, willow and balsam poplar seedlings were excavated directly on the site. A total of four seedlings were planted systematically in each plot between the AE species. Prior to planting, the height, basal diameter, and diameter of coarse roots of each seedling were measured, and fine roots abundance was estimated.

Sampling and measurements

In order to assess the influence of the edaphic factors on the AE species, temperature of the substrate was measured on a continuous basis during three growing seasons (2009, 2010 and 2011). Thermocouples were installed at two depths (5 and 15 cm) in the control plots and in plots with sheep laurel and Labrador tea in one block per zone.

To assess the potential of AE species to produce and secrete bio-chemical compounds with potential allelopathic influence, shoot and root exudates were collected monthly during the growing season. Live and dead leaves and soils were collected at the end of 2009 (end of September) growing season. Total polyphenols and condensed tannins were measured in the leaf according to the methods described in Joanisse et al. (2007, 2009).

Impacts of AE species on tree seedling growth were assessed by annual measurements of height, basal diameter, and number of branches. Measurements were performed in September and October.

Results and discussion

Objective I: introduction of AE species on the experimental plots

The first objective, which was to introduce the AE species on the experimental plots, was partly attained in 2009 for the Labrador tea and sheep laurel. At the end of the 2009 growing season, approximately 10% of the sheep laurel and Labrador tea plants produced seeds; approximately 60% of the plants produced shoots. The addition of the organic amendment in 2009 substantially reduced AE mortality. The mortality of AE species at the end of the 2010 vegetation season was less than 2% in the dry zone and approximately 5% in the wet zone for sheep laurel. Labrador tea mortality was approximately 15% in the dry zone and less than 5% in the wet zone. Furthermore, approximately 80% of the sheep laurel and Labrador tea transplants have produced new shoots in 2010. An average of 5–10 and 3–8 shoots per graft were observed for sheep laurel and Labrador tea, respectively. The number of shoots on Labrador tea grafts varied greatly depending on the zone; shoots were more abundant in the wet zone contrary to sheep laurel, which did not seem to be influenced by humidity conditions.

Planted bluejoint reedgrass was surveyed during the 2010-2011 vegetation seasons. Mortality was approximately 15% at the end of the season. In most of the experimental plots, the plants have expanded their root systems and have produced new shoots; mortality was similar in the dry and wet zones. Taken together, these results suggest that the three AE species have successfully established on the study site.

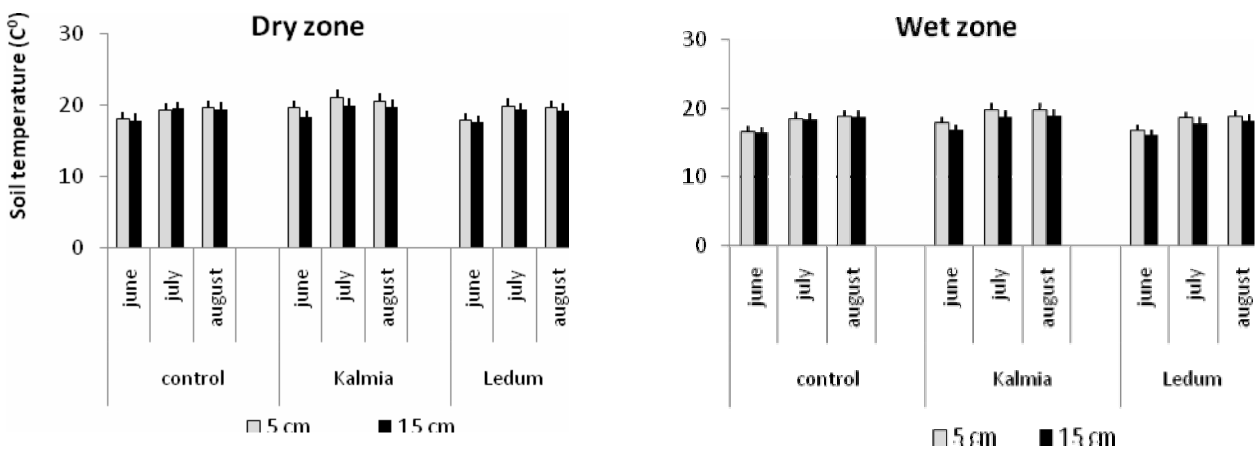


Figure 2. Mean soil temperature (n=1900) and SD measured during the summer 2010 on the CCBE. Thermocouples were buried at two depths (5 and 15 cm) in the planting substrate of the plots (control and plots with sheep laurel and the Labrador tea in two blocks located in the dry and wet zone).

Although sheep laurel and Labrador tea were successfully established in the plots in 2009, it is believed they were growing slowly because of the harsh environmental conditions of the CCBE. The environmental conditions on the CCBE differ considerably from those of forest ecosystems; namely, high solar radiation due to the absence of mature trees (leading to important temperature oscillations), strong winds, low air humidity, poor nutrient concentrations in the rooting material, and high water saturation levels of the silt layer. Soil temperature measurements conducted annually on the experimental plots revealed that soil temperatures varied from 16 to 19.5 (^{o}C) (Fig. 2). As a comparison, Fréchette et al. (2011) reported an average temperature of 13.4 (±1.5) (^{o}C) for the spruce forest soil of western Quebec during the summer. Moreover, although this parameter was not quantified, we observed that the upper layer of the planting substrate (first 5 cm) was very dry during the summer.

Regardless that the planting substrate was similar to the natural forest, it was observed that AE plant phenological phases (i.e. periodicity of plant life cycles) were desynchronized and shortened on the CCBE compared to phases in adjacent natural conditions. Foliar senescence and litterfall of all sheep laurel and Labrador tea plants started two to three weeks earlier on the study site compared to adjacent forest ecosystems.

Objective II: production of phyto-toxins and resource limitation of bio-barrier species on the target tree species assessment

Preliminary and partial results (29 samples of foliar tissues) of total polyphenols and condensed tannins produced by AE plants are presented below. The results indicate that foliar polyphenol concentrations were almost twice higher in sheep laurel than in Labrador tea, and green leaves had almost three times higher concentrations than dead foliar tissues. The measured concentrations were lower than those reported by Joanisse et al. (2009). Lower polyphenol concentrations for AE plants compared to forest conditions may reflect the influence of environmental stress to which the plants are subjected to on the CCBE.

It has also been observed (Fig. 3a) that polyphenol concentrations varied with the zone hydrological type; polyphenol concentrations of both AE species were more than twice higher in the wet zone than in dry conditions. Polyphenol concentrations differed among years (Fig. 3b). In 2010, polyphenol concentrations in leaves were almost twice lower in the wet zone than in the year 2009; foliar polyphenol concentrations in the dry zone did not change during these two years. This result suggests that the efficiency of AE species as bio-barriers might be sensitive to environmental conditions. The analyses of polyphenol concentrations in bluejoint reedgrass foliar tissue are being processed.

a) b)

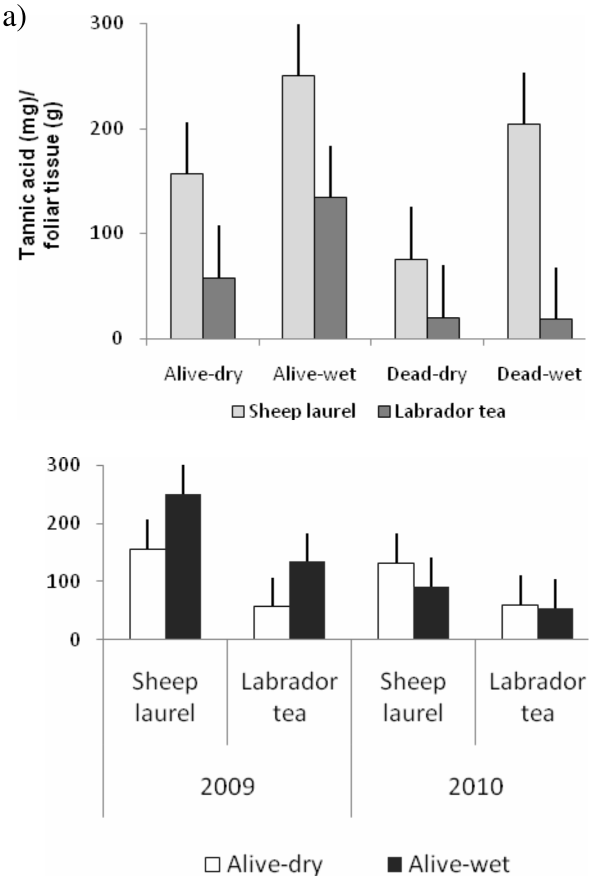
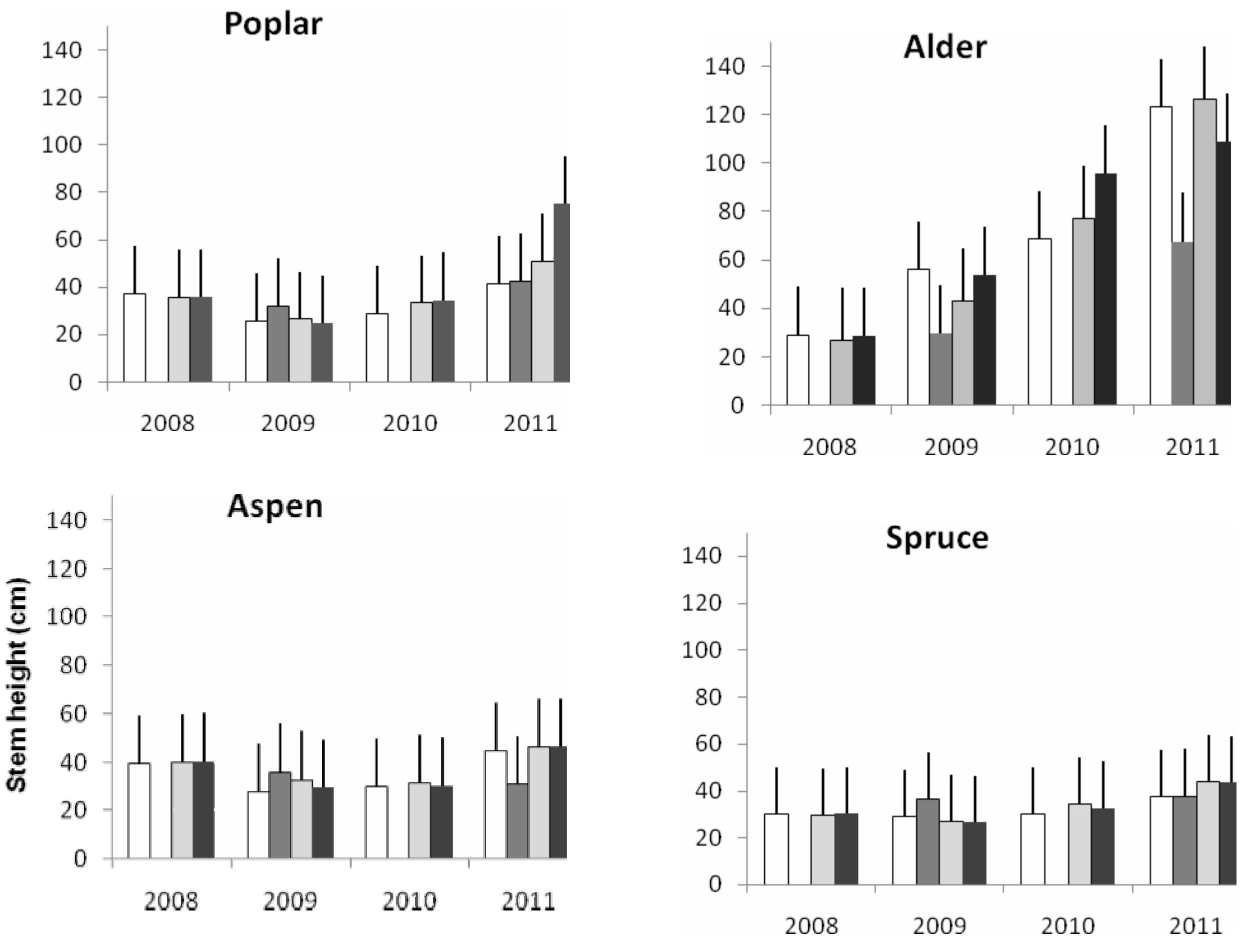


Figure 3. Average concentrations (n=29) and SD of polyphenols: (a) in live (green) and dead foliar tissue of sheep laurel and Labrador tea and (b) concentration changes with time since establishment in experimental plots located in the dry and wet zones of the study site.

The overall inhibitory effects of the AE species on trees include competition for environmental resources (light, water, nutrients) and the direct and indirect influences of the phytotoxins. We anticipate that the allelopathic effects of sheep laurel, Labrador tea and bluejoint reedgrass will alter target tree growth characteristics (stem height, basal diameter, branching, root architecture), and eventually cause tree mortality. Growth characteristics of the aboveground part of trees and tree mortality were measured each year since 2008 (Fig. 4). Speckled alder exhibited the highest growth rates among the five target tree species planted in the experimental plots. Alder height has increased almost six times since planting on the experimental plots (2008). Stem height in the wet zone was always superior than in the dry zone (results not shown) for all target species.

Among the three allelopathic species, bluejoint reedgrass had the strongest influence on the target trees. The highest tree mortality and growth suppression were observed on the experimental plots colonized with bluejoint reedgrass, while no mortality caused by environmental conditions was documented on the control plots (Fig. 4). It took only one year for this species to inhibit tree growth and provoke significant tree mortality. Poplar and aspen were the most affected by bluejoint reedgrass. This effect of bluejoint reedgrass on poplar is consistent with results reported in the western boreal forest (Landhäusser and Lieffers 1998). On the experimental plots with bluejoint reedgrass, alder and willow stem height was visibly smaller than the control, sheep laurel and Labrador tea plots.



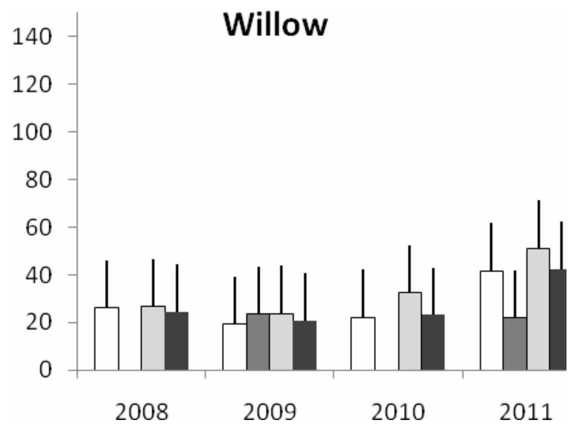


Figure 4. Target tree height (average values for wet and dry zones) on the experimental plots, measured during four vegetation seasons on the control plot □, and plots with three allelopathic species: bluejoint reedgrass ■, sheep laurel □, Labrador tea ■

Mortality of target trees was also the highest with bluejoint reedgrass, compared to the other treatments. Spruce was apparently less affected than the other tree species, particularly in the dry conditions (Fig. 5). Black spruce resistance to the influence of bluejoint reedgrass could be related to its rooting habits. Indeed, it has been reported that bluejoint reedgrass can impact tree growth through modifications of the environmental conditions; the gramineae produces a thick mat of old roots and litter. This mat alters the soil water regime and decreases soil temperature, which affects deep rooted trees (*e.g.* poplar and aspen; Landhäusser et al., 2007). On the other hands, black spruce grows superficial roots in the organic soil layers (Krause and Morin, 2005). Therefore, black spruce would be adapted to the ecological conditions created by bluejoint reedgrass. On the dry plots, we observed higher survival of black spruce seedlings on the plots with bluejoint reedgrass, which supports this interpretation. Others factors, such as biochemical interactions among plants and adapted mycorrhizal communities for black spruce might have influenced these results. Further monitoring of these plots will be necessary to verify these hypotheses.

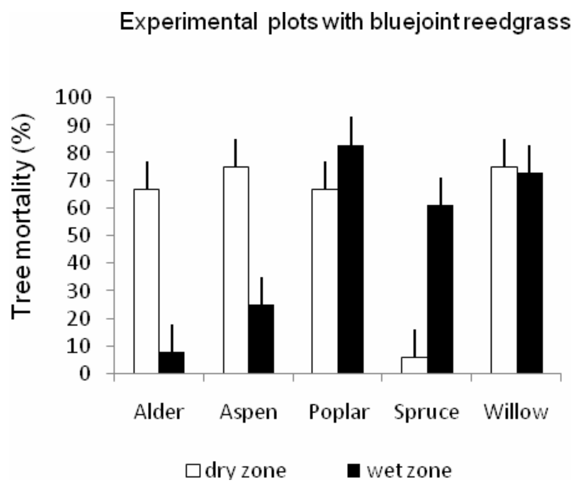


Figure 5. Tree mortality on the plots with bluejoint reedgrass in 2011.

Summary and Preliminary Conclusions

This field experiment, initiated in 2008, confirms that it is possible to introduce allelopathic species (sheep laurel, Labrador tea and bluejoint reedgrass) on a CCBE located in the boreal zone of Quebec. Preliminary results suggest that concentrations of polyphenols synthesized in the foliar tissue of sheep laurel and Labrador tea growing on the LTA CCBE are lower than those reported in natural ecosystems (Joanisse 2007, 2009). The concentrations were higher in sheep laurel than in Labrador tea; live foliar tissue contained higher concentrations of polyphenols than dead plant parts. For a given AE species, polyphenol concentrations in foliar tissue differed among CCBE zones and years. Results also suggest that among the AE species tested, bluejoint reedgrass has the strongest inhibitory effects on the target trees, particularly on balsam poplar and trembling aspen.

Based on these preliminary results, the authors believe that the use of allelopathic species to inhibit undesirable tree growth on CCBEs is a promising approach. However, for this vegetation management tool to be effective, it is essential to apply appropriate agronomic approaches to facilitate AE species establishment and growth in the relatively harsh environmental conditions associated with a CCBE.

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