In-woods chipping: Possible evidence for allelochemical interaction of leachate generated from trembling aspen (*Populus tremuloides* Michx.) bark and wood waste

by Timothy S. S. Conlin¹

A portable delimber-debarker-chipper, designed for in-woods chipping operations, was used to produce waste bark and wood residue from winter-harvested tree-length trembling aspen logs. The residue was then utilized to create leachate, which was subsequently used to treat white spruce, lodgepole pine, paper birch, aspen and *Calamagrostis canadensis* seedlings grown in sand-filled pots. Treatment with the leachate significantly decreased height growth and shoot and root dry weights of all species relative to the control treatment. Root:shoot ratios of all tree species except lodgepole pine were increased significantly by application of leachate. The root:shoot ratios of treated *C. canadensis* were significantly increased during one season, but significantly reduced in the following season. The data indicated that leachate from aspen bark and wood residue contain allelochemical properties that could affect the regenerative capacity of aspen cutblocks harvested for in-woods chipping operations.

Key words: aspen, leachate, allelochemistry, *Populus tremuloides*, in-woods chipping

Une ébrancheuse-écorceuse-déchiquteuse mobile, conçue pour les opérations de mise en copeaux en forêt, a été utilisée pour produire des résidus d'écorce et de bois provenant de troncs en longueur de peuplier faux-tremble récoltés au cours de l'hiver. Les résidus ont été par la suite utilisés pour créer un médium de percolation qui a été utilisé par la suite pour traiter des semis d'épinette blanche, de pin lodgepole, de bouleau à papier, de peuplier et de *Calamagrostis canadensis* cultivés dans des pots remplis de sable. Le traitement avec le médium a entraîné une diminution significative de la croissance en hauteur et du poids sec de la pousse et des racines de toutes les espèces par rapport au traitement témoin. Les ratios racines/pousse de toutes les espèces d'arbres à l'exception du pin lodgepole ont été augmentés de façon significative suite à l'application du médium. Les ratios racines/pousse des *C. canadensis* traités ont augmenté de façon significative au cours d'une saison, mais étaient significativement réduits la saison suivante. Les données indiquent que le médium obtenu à partir de l'écorce et des résidus de peuplier contient des éléments allélochimiques qui pourraient affecter la capacité régénératrice des parterres de coupe de peuplier récolté pour des opérations de déchiqutage en forêt.

Mots-clés : peuplier, percolation, allélochimie, *Populus tremuloides*, déchiqutage en forêt

Introduction

In British Columbia, the recent expansion in the use of aspen (*Populus tremuloides* Michx.) as a major source of wood for paper, along with the regulated phase-out of beehive burners, has led to increased generation of aspen mill waste. This waste has the potential of creating large amounts of leachate containing solubilized compounds from decaying aspen bark, wood and leaves. Leachate generated from decked aspen logs and waste bark and wood is toxic to aquatic organisms (Goudey and Taylor 1992, Taylor 1994, Taylor *et al.* 1996); aqueous extracts of *P. tremuloides* leaves decreased the growth of several grass species (Younger *et al.* 1980); tissue culture extracts of *P. tremuloides* demonstrated antimicrobial activity (Mathes 1963) while leaf extracts of a closely related species, *P. tremula*, showed anti-fungal properties (Olsen *et al.* 1971).

On-site chipping operations provide an opportunity for substantial reductions in harvesting and processing costs by reducing the amount of waste fibre hauled to the mill and by eliminating costs associated with waste residue disposal and the operation of a mill's wood room (Favreau 1992). However, on-site chipping operations also produce a large amount of bark and wood waste residue (approximately 167 kg of residue per m³ of aspen processed, North Peace Timber Ltd., Taylor, B.C.,

personal communication) which is left at the cutblock. By way of example, this would result in the creation of up to 58.3 tonnes of waste residue per ha⁻¹ based on a high site index aspen stand containing 350 m³ ha⁻¹ in the Peace River area of British Columbia. This material is commonly deposited in a pile a short distance from the delimber-debarker-chipper (DDC) that is used to process trees into wood chips on-site.

Woodland managers interested in incorporating on-site DDCs into silvicultural prescriptions have speculated that redistribution of this bark and wood waste over the block might improve the productivity of the site.

However, given the evidence that aspen tissue can release toxic or allelochemical agents, it is possible that redistribution of wood and bark waste from on-site aspen chipping operations have the potential of producing large quantities of leachate which may delay or inhibit the subsequent regeneration of tree seedlings on an aspen cutblock. The purpose of this experiment was to determine if aspen leachate originating from residue generated by mobile chippers had a growth inhibiting effect upon white spruce (*Picea glauca*), lodgepole pine (*Pinus contorta*), paper birch (*Betula papyrifera*) or trembling aspen (*Populus tremuloides*) seedlings. Seedlings

Timothy Conlin

¹Research Scientist, British Columbia Ministry of Forests Research Branch, PO Box 9519, Stn. Prov. Govt., Victoria, B.C. V8W 9C2. E-mail: Timothy.Conlin@gems9.gov.bc.ca

of *Calamagrostis canadensis* were also tested to examine the potential for using leachate as a biological control for this species.

Methods

Plant Seedlings and Experimental Design

Aspen seeds (Quality Seed Collections Ltd. – seedlots 9503 – Fort Nelson and 9504 – Chetwynd) were sown into sand-filled pots, allowed to germinate, and later thinned to one plant per pot. Paper birch (collected from the Fort Nelson Forest District courtesy of Dr. M. Carlson, Kalamka Research Station, B.C. Forest Service) and *C. canadensis* (collected from km 524, Alaska highway, courtesy of Dr. R. Winder, Canadian Forest Service, Pacific Forestry Centre) seeds were pricked into sand-filled pots one week after germination. White spruce and lodgepole pine seeds (Surrey Seed Centre, seedlot numbers 33269 and 08992 respectively) were germinated in flats over a two-week period and then transplanted to sand-filled pots. Thawed 415B 2+0 white spruce plugs (courtesy of Dave Cheyne, hardwood specialist, Fibreco Pulp Ltd.) were also planted in pots filled with sand.

Untreated sand from a local gardening supplier was used as a rooting medium to ameliorate uncontrollable stochastic responses that might have occurred if natural mineral soils were used. Two-gallon pots were used in all experiments. Although all plants were of the same age, there was some small variability in individual sizes. In order to ensure an equitable distribution of plant sizes and to ameliorate sampling bias, each plant was matched with another plant of similar size until two groups of potted plants were formed. The sample to be treated with leachate was then randomly selected using a coin toss. Seedlings within each treatment were watered twice daily (250 ml + 250 ml) using tap water or aspen leachate applied directly to the soil. A 1% solution of 20-20-20 fertilizer was substituted for one regular watering every second day. The watering schedule was occasionally supplemented with 20-minute misting periods on dry, hot days.

All plants were grown in a greenhouse environment during the summer growing season. With the exception of the white spruce plugs, each experiment with each species was repeated at least once in a subsequent year with a different batch of aspen residue. This was to ensure that seedling responses to the leachate treatments were not specific to aspen from a particular stand. Although the experiments were repeated with each species, environmental conditions could not be completely replicated and the first hot and dry summer of experimentation was followed by a cool and humid summer. As a consequence, aspen from the Chetwynd and Fort Nelson seed sources were merged into one group during the first year because of a drought-induced mortality event prior to treatment. This group was subsequently split into the control and leachate treatments. In the second year, the cool humid weather appeared to have contributed to the mortality rate observed in the seedlings during treatment.

Following harvest, each individual plant was divided into shoot and roots, dried at 80°C for 48 hours, and weighed. Spruce plug roots were further subdivided into the old plug root mass and new root growth, the latter based on succulent white roots growing outside the parameters of the plug root mass composed of peat and fibrous brown roots. The old plug roots were washed free of peat rooting media, and dried along with excised new roots and shoot material. Dry weights

and root:shoot ratios of the control and treatment samples were compared using a two-sample Student's t-test.

Production and Application of Leachate

Leachate was produced by sprinkling 54 litres of tap water over a hopper filled with approximately 300 kg of aspen residue collected from North Peace Timber Ltd. at Taylor, B.C. The hopper was constructed from plywood and lined with a large plastic tarpaulin to prevent leakage at the seams. The residue originated from recent winter-logged aspen stems, which had their crowns removed prior to processing with a Peterson Portable Flail Delimber-Debarker-Chipper™. Prior to experimentation in each year, the old lot of residue was replaced with a load of new, fresh residue, which was then used to generate all batches of leachate during a growing season. The extract was collected in a carboy through a spigot at the bottom of the hopper within minutes of adding water. A fresh carboy was produced every three to four days, which was used for leachate treatment application to seedlings. Watering the treated plants with leachate commenced at the four- and two-leaf stages for aspen and birch respectively; after approximately three weeks for *C. canadensis* and the white spruce plugs; and after nine weeks for pine and spruce seedlings. Leachate was applied to all treated species over a six-week period for broadleaf seedlings and over eight weeks for conifer seedlings. Harvest occurred immediately after the cessation of leachate application for each species.

Chemical Analysis

Each time a 54-litre carboy of leachate was produced, a sample was frozen and stored until it could be chemically analyzed at the end of each growing season. Samples were analyzed using a Fisher Accumet 915 pH meter with automatic temperature compensation and a Broadley James pH electrode and a Horiba DS-15 Conductivity Meter with automatic temperature compensation and a Horiba micro flow-through cell. A Waters High Pressure Liquid Chromatography system was utilized to determine the elemental concentration of NH₄, Cl, NO₃, PO₄, SO₄, Na, Mg, and Ca ions while an ARL 3560 Inductively Coupled Plasma was used to determine the concentration of all other chemical ions. Aluminum concentrations in the leachate were not measured in the 1996 and 1997 treatment years. Mean pH and conductivity of the tap water before creation of leachate were 6.3 (s.e. 0.4) and 0.70 (s.e. 0.07) mS cm⁻¹ respectively.

Results

The data for these experiments are summarized in Tables 1, 2 and 3. Some mortality of tree species occurred during 1996 and 1997 and this is expressed by the final sample sizes. In every situation, shoot height was significantly reduced by the application of leachate. Root and shoot dry weights were also significantly reduced in all seedlings grown from seed. The leachate treatment did not effect the root:shoot ratios of *P. contorta*, while the root:shoot ratios of *P. tremuloides* and *B. papyrifera* were significantly increased by the leachate treatments. Mean root:shoot ratios of spruce were significantly higher than the control treatments only during the 1998 season. Root:shoot ratio responses of *C. canadensis* were less conclusive than those of the other species, with the ratio significantly higher in response to leachate in 1996, but significantly lower to

Table 1. Means and standard errors for shoot height, root and shoot dry weight and root:shoot ratio for seedlings treated with aspen leachate during the summer of 1996; n = sample size at end of treatment, (N) = sample size at start of treatment. All treatment means are significantly different from controls using two-tailed t-test to determine the probability of a type I error.

Species and treatment	n (N)	shoot height (cm)	p-value	root dry weight (mg)	p-value	shoot dry weight (mg)	p-value	root:shoot	p-value
<i>Populus tremuloides</i>									
leachate	16 (20)	1.7 (0.1)	<0.01	37 (4)	<0.01	35 (4)	<0.01	0.96 (0.07)	<0.01
no leachate	14 (20)	8.3 (0.7)		205 (28)		354 (35)		0.57 (0.05)	
<i>Calamagrostis canadensis</i>									
leachate	12 (12)	8.3 (0.5)	<0.01	23 (3)	<0.01	29 (6)	<0.01	0.85 (0.08)	<0.01
no leachate	10 (12)	26.8 (1.7)		430 (60)		970 (149)		0.47 (0.03)	

Table 2. Means and standard errors for shoot height, root and shoot dry weight and root:shoot ratio for seedlings treated with aspen leachate during the summer of 1997; n = sample size at end of treatment, (N) = sample size at start of treatment. All treatment means are significantly different from controls using two-tailed t-test to determine the probability of a type I error.

Species and treatment	n (N)	shoot height (cm)	p-value	root dry weight (mg)	p-value	shoot dry weight (mg)	p-value	root:shoot	p-value
<i>Populus tremuloides</i> (Fort Nelson seed)									
leachate	10 (12)	2.5 (0.3)	<0.01	37 (4)	<0.01	76 (8)	<0.01	0.51 (0.04)	<0.01
no leachate	12 (12)	9.1 (1.4)		103 (15)		305 (49)		0.36 (0.02)	
<i>Populus tremuloides</i> (Chetwynd seed)									
leachate	7 (12)	2.0 (2.9)	<0.01	24 (4)	<0.01	48 (6)	<0.01	0.49 (0.06)	<0.01
no leachate	10 (12)	10.8 (1.8)		102 (18)		354 (63)		0.28 (0.03)	
<i>Calamagrostis canadensis</i>									
leachate	11 (11)	28.5 (1.0)	<0.01	278 (41)	<0.01	469 (60)	<0.01	0.59 (0.03)	<0.01
no leachate	11 (11)	36.8 (2.0)		1,280 (135)		1,607 (154)		0.78 (0.04)	
<i>Betula papyrifera</i>									
leachate	0 (11)	Dead		Dead		Dead		N/A	
no leachate	9 (11)	10.5 (1.4)		187 (24)		430 (70)		0.45 (0.02)	
<i>Pinus contorta</i>									
leachate	7 (13)	4.3 (0.3)	0.048	61 (13)	<0.01	106 (43)	0.017	0.69 (0.14)	0.79
no leachate	8 (13)	6.3 (0.7)		199 (31)		322 (56)		0.64 (0.04)	
<i>Picea glauca</i>									
leachate	6 (13)	2.8 (0.4)	<0.01	34 (6)	<0.01	71 (17)	0.01	0.72 (0.28)	0.52
no leachate	7 (13)	6.2 (0.7)		130 (24)		255 (147)		0.52 (0.16)	

Table 3. Means and standard errors for shoot height, root and shoot dry weight and root:shoot ratio for seedlings treated with aspen leachate during the summer of 1998; n = sample size at end of treatment, (N) = sample size at start of treatment. All treatment means are significantly different from controls using two-tailed t-test to determine the probability of a type I error.

Species and treatment	n (N)	shoot height (cm)	p-value	root dry weight (mg)	p-value	shoot dry weight (mg)	p-value	root:shoot	p-value
<i>Betula papyrifera</i>									
leachate	18 (18)	1.9 (0.2)	<0.01	55 (8)	<0.01	76 (13)	<0.01	0.78 (0.05)	<0.01
no leachate	17 (17)	20.4 (1.4)		422 (49)		1351 (155)		0.32 (0.01)	
<i>Pinus contorta</i>									
leachate	10 (10)	3.3 (0.2)	<0.01	20 (3)	<0.01	31 (3)	<0.01	0.65 (0.09)	0.83
no leachate	10 (10)	6.0 (0.6)		54 (4)		86 (10)		0.67 (0.05)	
<i>Picea glauca</i>									
leachate	10 (10)	1.9 (0.1)	<0.01	9 (1)	<0.01	12 (1)	<0.01	0.74 (0.06)	<0.01
no leachate	10 (10)	4.8 (0.4)		19 (2)		60 (7)		0.33 (0.02)	

this treatment in 1997. All of the *Betula papyrifera* treated with leachate in 1997 died before the end of treatment application.

Application of leachate to spruce seedling plugs during 1998 resulted in a significant reduction in the weight of new, white

roots (0.035 g [s.e. 0.005] compared to the control treatment (0.115 g [s.e. 0.017]). Shoot and brown root weights and root:shoot ratios were not significantly influenced by the leachate treatments. The spruce plugs did not flush during the

Fig. 1. Conductivity (bar graphs) and pH (plotted points) of each batch of leachate generated from aspen waste bark and wood residue in 1997 (—●—), 1998 (—▼—) and 1999 (—■—). Each number between the X-axis and the year represents a 54-litre tank of leachate that was subsequently applied to the treated seedlings.

Table 4. Mean (s.e.) elemental concentrations of tap water used to create leachate and of the resulting leachate during 1998.

Element	Tap Water (n=10)	Leachate (n=10)
B ($\mu\text{g l}^{-1}$)	< 0.01	0.37 (0.08)
Ca ($\mu\text{g l}^{-1}$)	5.45 (0.23)	75.00 (10.11)
Cl ($\mu\text{g l}^{-1}$)	8.69 (0.95)	17.37 (2.40)
K ($\mu\text{g l}^{-1}$)	1.52 (0.78)	227.10 (29.64)
Mg ($\mu\text{g l}^{-1}$)	1.37 (0.08)	39.19 (6.13)
NH ₄ ($\mu\text{g l}^{-1}$)	0.46 (0.08)	0.22 (0.11)
NO ₃ ($\mu\text{g l}^{-1}$)	0.14 (0.04)	< 0.001
P ($\mu\text{g l}^{-1}$)	0.04 (0.01)	28.71 (6.77)
PO ₄ ($\mu\text{g l}^{-1}$)	0.20 (0.0)	76.17 (18.29)
SO ₄ ($\mu\text{g l}^{-1}$)	1.40 (0.07)	11.35 (3.33)
Zn ($\mu\text{g l}^{-1}$)	0.13 (0.02)	1.69 (0.33)
Al ($\mu\text{g l}^{-1}$)	0.69 (0.20)	1.28 (0.29)
Na ($\mu\text{g l}^{-1}$)	1.32 (0.06)	3.30 (0.23)
Mn (ng l ⁻¹)	22.00 (4.44)	474.30 (87.55)
Fe (ng l ⁻¹)	99.20 (7.39)	1198.40 (146.44)
Cu (ng l ⁻¹)	4.22 (0.70)	31.10 (14.68)

experiment and it was therefore impossible to determine if the leachate had an influence on shoot growth.

The effect of leachate application was especially noticeable in the broadleaf tree species, with growth reduction making itself apparent within two weeks of leachate application. A noticeable reaction to leachate application occurred in the leaves of paper birch, which became dark green and took on a purple hue soon after treatments had begun. *Calamagrostis canadensis* plants treated with leachate appeared more yellow in colour than did grass in the control treatments.

Conductivity and pH of the samples drawn from the leachate are shown in Fig. 1. Elemental analysis of the leachate, along with its neutral to slightly acidic pH range, is shown in Table 4. Large increases in the concentrations of K, Ca, P, PO₄, Mn and Fe were observed in the leachate over that of the tap water, while decreases were observed in NH₄⁺ levels and NO₃⁻, which was almost completely removed from the leachate. Smaller increases in the concentrations of other elements were observed.

Discussion

The results demonstrated that leachate generated from aspen bark and wood waste significantly reduced growth rates of seedlings of several different tree species and *C. canadensis*. Also, during the 1997 experiment, leachate treatment of the birch resulted in the death of all seedlings in the sample population. However, it is unclear whether this one mortality event was a direct consequence of leachate application or due to a combination of stresses, including leachate application, which predisposed birch seedlings to mortality.

Populus deltoides was shown to exert an allelopathic influence on surrounding vegetation, which was attributed to the presence of large quantities of leachable phenolic acids found in the leaves and surrounding soil (Kohli 1998). Phenolic compounds can compose up to 10% of the bark and cambial tissue of aspen (Taylor *et al.* 1996). This suggests that aspen leachate had allelochemical properties (Jobidon and Thibault 1982) and that the potential impact of this allelochemistry should be considered when developing silvicultural plans for harvesting methods using in-woods chipping of aspen logs.

Chemical analysis of the aspen leachate indicated the presence of elements that, under the right conditions, may also induce a reduction in plant growth. Aluminum is known to have a toxic, growth-inhibiting effect upon plants, especially at lower pH concentrations (Mengel and Kirkby 1982). However, the high threshold concentration levels for Al toxicity in the species used in this experiment, along with pH values in the neutral to slightly acidic range, make it unlikely that Al toxicity contributed to the reduced growth of seedlings treated with leachate (Schaedle *et al.* 1989).

Boron, which was also found in the leachate, may promote micronutrient toxicity symptoms in trees and other plant species (Stone 1990). Neary *et al.* (1975) reported on red pine plantations that experienced boron toxicity when irrigated with waste water containing 0.51 to 0.91 mg l⁻¹ boron. Although this concentration range was considered quite small, the authors discovered that the waste water was loading the irrigated soil with boron, resulting in a toxic accumulation of this

element. Thus, there was the possibility that a steady application of aspen leachate may have loaded potted soils with toxic levels of boron. Boron can also be associated with saline soils, with many crop species showing a range of toxicity threshold values from 0.5 to 60 $\mu\text{g l}^{-1}$ (Maas 1986).

Concentrations of other micronutrients in the leachate did not approach the levels required to induce toxic symptoms in white spruce (van den Driessche 1989) and were within the range of optimal concentrations observed in fertilizer solutions used for conifer seedling nursery stock (Donald 1991).

Another explanation for the reduced growth of seedlings treated with leachate was the possibility that they were experiencing a saline-induced response. Conductivity of the leachate was comparable to those found in irrigation water associated with soil salinity (Ulery and Ernst 1997) over the three growing seasons. However, the elemental composition of the leachate, with the exception of boron, is not characteristic of solutions associated with saline soils. Both Na and Mg concentrations were relatively low and Ca and K levels, while observed to be high, are not associated with salinity-related problems (Mengel and Kirkby 1982).

The dark green leaves observed in birch seedlings watered with leachate suggests phosphorus deficiency symptoms (Erdmann *et al.* 1979). Mengel and Kirkby (1982) also remarked on the general foliar symptoms of phosphorus deficiency that included darker green leaves with a red tinge. This suggests that aspen leachate might have interfered with the uptake of phosphorus by birch. Van den Driessche (1989) also showed that white spruce responded to low phosphorus by producing bright purple foliage. However, these symptoms were not observed in needles of white spruce exposed to leachate during the course of the experiments described in this manuscript, nor were visible foliar symptoms of phosphorus deficiency seen in the other species.

Although the data presented here suggested the possibility that leachate generated from aspen wood and bark waste had an allelochemical effect upon the species used in this experiment, the exact nature of this effect is uncertain. If the effect is related to organic compounds such as phenols, quantitative information is required about how these compounds affect plant growth, the microbiota responsible for their degradation, and their allelochemical threshold levels and residence time in forest soils. If, however, the allelochemistry is due to other factors, such as a micronutrient toxicity or salinity problems brought about by excessive amounts of a particular element in leachate, then foresters and managers must know of these properties before they can make informed decisions regarding in-woods chipping of aspen stands.

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