ASSESSING THE EFFECTS OF MULCH, COMPOST TEA, AND CHEMICAL FERTILIZER ON SOIL MICROORGANISMS, EARLY GROWTH, BIOMASS PARTITIONING, AND TAXANE LEVELS IN FIELD-GROWN ROOTED CUTTINGS OF CANADA YEW (*TAXUS CANADENSIS*).

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ABSTRACT

First-year growth results from two locations of field trials with Canada yew (*Taxus canadensis*) are described. After one year of treatments, inorganic fertilizer and organic fertilizer both significantly increased plant growth compared to the controls. Inorganic fertilizer increased top growth without a concomitant increase in root growth, thereby increasing the shoot:root ratio compared to the controls and compost tea treated plants. In addition to evaluating the observed differences in morphometric measurements, this paper reports on the first-year differences in microorganisms. The relationship between these early differences in plant growth and soil microorganisms are also discussed.

INTRODUCTION

Paclitaxel, also called Taxol®, is a well-established cancer drug that has been sold by Bristol Meyers Squibb (BMS) for clinical use since 1992, and has been called the largest selling anti-cancer drug in the world (Goodman and Walsh 2001). Taxol® and the closely related taxane Taxotere® (docetaxel, produced by Aventis) had sales in 2001 of $2.3 billion USD (Anonymous 2002). Ground hemlock or Canada yew (*Taxus canadensis*) is a woodland understory shrub species occurring in the northeastern USA and throughout eastern Canada from which paclitaxel and other useful taxanes may be extracted. The range of the species is extensive, but populations are not overly abundant in most Canadian provinces and northern states. Over-harvest of woodland plants is a possible threat to both biodiversity and the development of a viable taxane industry based, at least in-part, on this species. A program to domesticate Canada yew was started in 1997 with an objective to identify and mass-propagate, fast-growing, high taxane producing clones.

Developing and optimizing field-culture practices is an integral component of the domestication program. Semi-commercial nursery trials were initiated in 1994 to evaluate the effects of plant spacing, mulch, and organic versus chemical fertilizers on the early growth and establishment of rooted cuttings of Canada yew. Test plantations were established at four locations: Harrington (Charlottetown), PEI; Gaspé, Quebec; Grand Lake, New Brunswick; and Sault Ste Marie, Ontario. The soils and climatic conditions vary considerably among these four locations, so in

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addition to the main goal of developing and optimizing cultural practices, the trials will also be able to elucidate some of the effect(s) and interactions between climate and cultural practices on plant growth and taxane yield. This paper reports on some early data from two of the sites.

METHODS

General Project Description

Nine thousand six hundred plants were used at each location in a common split-plot design (Figure 1). There were a total of 12 treatments: 2 spacings X 3 fertilizers (organic- inorganic-fertilizer, and control) X 2 mulch treatments (with and without mulch). Within a row, there were 200 plants, 100 at each of two spacings (30 X 30 cm and 45 X 45 cm). Treatments were replicated 4 times for a total of 800 plants per fertilizer/mulch treatment combination. Within rows, the trees were planted three-wide, in a staggered pattern.

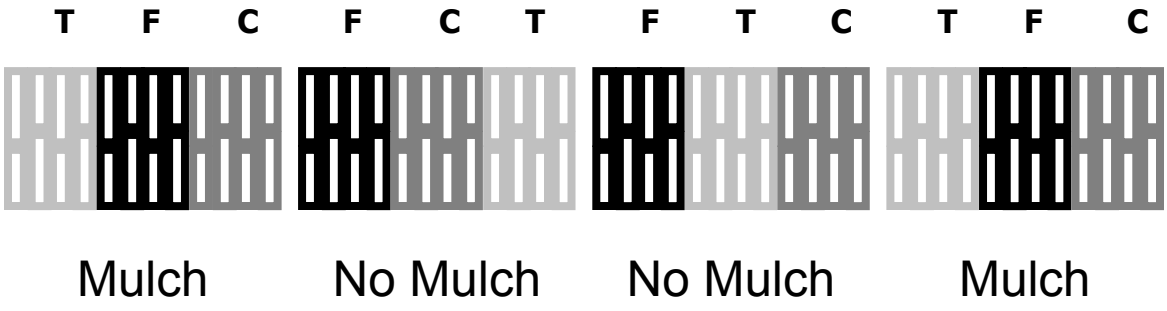

Figure 1. Diagram illustrating the field layout and offset pattern of the sub-blocks. The long and short blocks represent 45 and 30 cm spacing within rows respectively. Each sub-block contains 100 trees. T = compost tea, F = inorganic fertilizer, C = control.

Plant production and Test Establishment

Rooted cuttings used in this experiment were produced under contract with Chatham Biotec Ltd. (CBL) by the Nova Scotia Department of Natural Resources at their Strathlorne Forest Nursery in Cape Breton Nova Scotia. Original cutting material was collected by CBL staff, shipped to Strathlorne in the fall of 2002, and rooted. Plants were shipped to the Acadia Research Forest of CFS in the fall of 2003, overwintered under shelter, and held at Acadia prior to shipping and planting in June 2004. Following planting, mulch was applied to half of the blocks (Figure 1). At the PEI site, six to eight month old composted bark (spruce and fir) was used and applied at a depth of 5 cm whereas cedar shavings were used at the NB site. Manual weed control and general site maintenance were conducted as required in 2004. No plant harvesting or assessments were made in 2004. Fertilizer treatments began in the spring of 2005. Inorganic fertilizer treatments comprised 3 applications of 15-15-15 at 150 kg N/ha (June 2, July 28, and Sept 7). The organic fertilizer treatment was a compost tea mixture (proprietary mixture of aerobic compost tea, fish, and a liquid humate/seaweed solution) applied 4 times (May 16, June 22, July 27, and Aug 23). Plots were drip irrigated as required in 2004 and 2005.

Harvesting and Measurements

In October 2005, 200 plants from each of the 12 treatments (1 row containing 200 plants; 100 at each spacing) were harvested from 2 of the 4 sites (Grand Lake and Harrington). Total height, root collar diameter, and root and top (needles plus stems) dry weights (48 hrs at 60° C) were measured for forty, randomly selected healthy plants (intact leader and minimal needle loss) from each block. The remaining harvested plants from each block were combined into bulk top and root samples and total dry weight measured.

Taxane Analyses

Levels of paclitaxel, 10-deacetylbaccatin III, and 13-acetyl-9-DHB III were measured on bulk needle samples from the 40 harvested plants. Dried, needle samples ground to 20 mesh were extracted using methanol containing 0.1% (v/v) acetic acid in a Dionex ASE 200 extraction unit (Dionex Corp., Sunnyvale CA, USA). The extract (23 ml) was dried to approximately 5 ml, then reconstituted with acidified methanol to 10 ml. Aliquots were filtered through 0.02µ Anotop filters (Whatman Inc., Florham Park NJ, USA). Taxane concentrations were measured in duplicate on two Agilent 1100 HPLCs (Agilent Inc., Santa Clara CA, USA) at 230 nm using a 0.3 x 250 mm Synergi Hydro column (Phenomenex Corp., Torrence CA, USA) and a 74:26 to 48:52 water: acetonitrile gradient over 23 min with 2 and 5 min isocratic segments at the beginning and end, respectively

Soil Microorganisms

Soil samples were collected in the fall of 2004, each comprised of 6 to 8, 15 cm soil plugs taken at random from each treatment subplot of 4 rows (Figure 1). A preliminary assessment of soil microorganisms (bacteria, fungi and protozoa) was conducted for both sites. A detailed analysis (biomass of bacteria, fungi and protozoa) was conducted for the PEI site in the fall of 2005.

STATISTICAL ANALYSES

All data were tested for normality using PROC UNIVARIATE in SAS with no transformations being required. Analyses of variance and mean separation tests were done using PROC GLM in SAS, specifying block (mulch), spacing and fertilizer treatments as fixed effects. Only data from the Harrington and Grand Lakes sites will be discussed.

RESULTS AND DISCUSSION

Survival

Mean survival was 85.4 % and 67.8 % for the PEI and Grand Lake sites, respectively (based on 2400 plants per site). The higher mortality at the Grand Lake site was attributed to frost heaving in the spring of 2005. Frost heaving appeared random across blocks and treatments. Survival for the controls, inorganic and compost tea treatments was 62.3, 65.0 and 66.2% respectively but these differences were not statistically significant. Weed competition, especially in the non-mulched rows receiving inorganic fertilizer, resulted in some localized mortality within blocks.

Although not quantified, the effects of competition within blocks likely affected growth through shading and competition for water and nutrients.

Growth Measurements

Although survival at Harrington was higher than at Grand Lake, there was no difference in plant height between the two sites. Conversely, plants had greater mass at Grand Lake than at Harrington (Table 1). While the differences between the two sites in other growth measurements were often statistically significant, they were, in practical terms, quite small i.e., the difference in mean total plant dry weight was only 0.5 g between the two sites (Table 1). Even with initially modest differences, should the growth trend generally observed in Taxus (doubling in dry weight each year for 3 to 5 years, data on-file) hold true here, then the magnitude of the increases in yield can be expected to be higher in subsequent years.

At both sites, both the inorganic fertilizer and the compost tea treatments produced significantly larger plants than the controls whereas mulch alone did not have an effect on any of the growth parameters. There was a significant mulch by fertilizer interaction observed at both sites (c.f. Table 2). Mulch reduced the response (effectiveness) of the compost tea applications but not that of the inorganic fertilizer.

Table 1. Means (SE) of growth parameters for the Grand Lake and Harrington sites. Means (n = 960 per site) followed by different letters are significant at $\alpha = 0.05$.

Variable	Grand lake	PEI
Height (cm)	30.0 (0.25) a	30.4 (0.23) a
Top dry weight (g)	5.33 (0.10) a	5.08 (0.08) b
Root dry weight (g)	2.63 (0.05) a	2.35 (0.04) b
Total plant dry weight (g)	7.96 (0.15) a	7.42 (0.12) b
Shoot:root ratio	2.15 (0.03) a	2.29 (0.02) b
Root collar diameter (mm)	5.0 (0.05) a	4.7 (0.03) b

Table 2. Summary of ANOVAs for total plant dry weight and shoot to root ratios.

		Dry Weight			Shoot to Root Ratio		
Factor	df	SS	F	p	SS	F	p
Site	1	129.36	8.41	0.0038	8.96	19.05	0.0001
Mulch(M)	1	3.16	0.21	0.6507	0.02	0.05	0.8227
Fert(F)	2	171.89	5.59	0.0038	10.22	21.72	0.0001
Spacing (S)	1	44.58	2.90	0.0889	0.59	1.25	0.2645
Site*M	1	61.89	4.02	0.0451	8.39	9.24	0.0001
Site*F	2	1169.33	38.00	0.0001	7.13	7.58	0.0005
M*F	2	473.15	15.37	0.0001	18.48	19.65	0.0001
Site*S	1	7.42	0.48	0.4874	7.60	16.16	0.0001
M*S	1	107.21	6.97	0.0084	6.93	14.73	0.0001
F*S	2	26.66	0.87	0.4206	1.29	1.37	0.2541
Error	1820	28005.26	15.39		856.05	0.47	
Total	1834	30199.92			935.87		

Taxane Levels

Levels of paclitaxel, were highest for plants receiving the compost tea treatments (Figure 2). As for the growth data, increase in taxane levels for plants receiving compost tea were generally less in the mulch than non-mulch plots (Figure 2). Levels of 9-DHB and baccatin generally followed those of paclitaxel (data not shown).

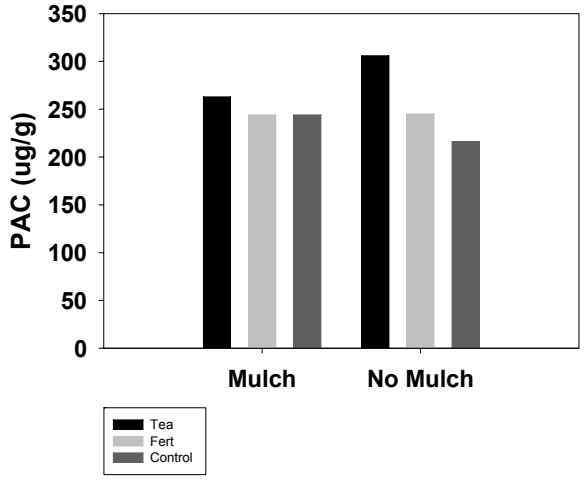

Figure 2. Paclitaxel (PAC) levels in needles of two-year-old plants from the Grand Lake site. Bars represent means of 4 needle samples.

Soil Microorganisms

A preliminary assessment of soils from the two sites indicated that bacteria and fungi were more balanced and levels more uniform at the Harrington than the Grand Lake site. The soil texture at Harrington was a sandy loam, compared to the finer texture soil at Grand Lake (soils at Grand Lake varied from a silt to clay loam). The bacteria counts were higher in the heavier soil; it is likely that the soil is still too bacterial for Taxus, but a full test would be necessary to confirm that generalization. A more detailed follow-up assessment of the soils at the Harrington site showed significantly higher levels of fungi in response to the compost tea applications (Figure 3). The increase in fungal hyphae was greater in the mulch than in the non-mulch rows.

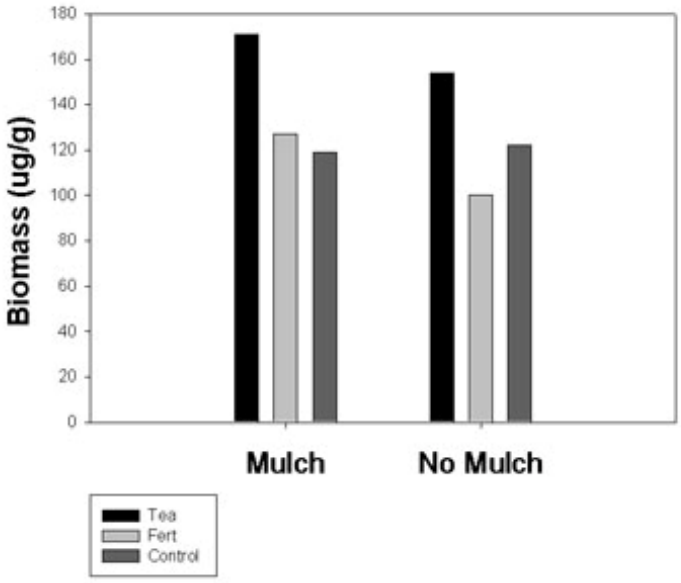

Figure 3. Plot of total fungal biomass (ug/g) by treatment for the Harrington site.

CONCLUSIONS

First-year results from these trials indicate that both organic and inorganic fertilizer applications increased growth relative to control plants. Although significantly more nutrients were applied with the inorganic fertilizer treatments compared to the organic tea applications, total increases in plant biomass were comparable, but the patterns of biomass allocation between shoots and roots was different between the two. Inorganic fertilizer increased top growth without a concomitant increase in root growth. Detailed analyses of soil and foliar nutrients and taxane levels are underway to try to elucidate possible physiochemical causes of the observed differences in growth.

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