Studies on the effects of growth conditions on taxanes in *Taxus canadensis*

by

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ABSTRACT

This study focused on analyzing the effects of growth site, foliar harvest date, shade coverage, and clone to analyze the three major commercially important taxanes in *T. canadensis* roots, needles and stems: 10-deacetylbaccatin III (10-DAB III), 9-dihydro-13-acetylbaccatin III (9-DHB III), and paclitaxel (PAC). The results indicate that full sun (no shade) was beneficial for needles to accumulate higher 10-DAB III concentrations and stems to accumulate higher PAC concentrations. In contrast high shade coverage (65%) is optimal for needles to accumulate higher PAC concentrations and stems to accumulate higher 10-DAB and 9-DHB III concentrations. For roots, samples with 35% shade coverage had higher 10-DAB III concentration, while shade coverage did not have any effect on 9-DHB III and PAC in the roots. When aiming for total yield only in needles and stems, plants under high shade coverage had higher amount of taxanes. If compared strictly by total yield in needles, stems and roots, then foliage with no shade coverage would be optimal, which would be an important consideration for commercial production. For the same six clones situated within each treatment, the concentrations of taxanes in clones KC was higher than the other clones. The best date for commercial harvesting was September for needles and November for stems. Moreover, tracking chlorophyll variation under different nursery conditions would be a better choice to track the yield of three major taxanes. Since PSII photochemistry (*Fv/Fm*), Ca, Cb, C *(x+c)*, C *(a+b)* and Chl *(a+b)/ (x+c)* have the same variation with three major taxanes, while the ratio of Ca/Cb variation is opposite to three major taxanes.

Keywords: *T. canadensis*, paclitaxel, HPLC, chlorophyll fluorescence, commercialization

Table of Contents

List of Tables

List of Figures

Introduction

In the 1960s when a brand new and entirely natural product with cancer fighting properties was discovered, taxanes were much celebrated and drew much attention from some of the largest pharmaceutical companies, as well as cancer physicians, worldwide (Goodman, Jordan, & Walsh, 2001). Taxane content has been investigated in many *Taxus* species. However, in most studies, only paclitaxel was analyzed (E.L.M., 2000), which still is widely used as an anti-cancer drug to treat breast, ovarian and other cancers (Baebler et al., 2005).

Taxanes were first extracted from the dried bark of Pacific yew, *Taxus brevifolia*. Paclitaxel's sudden high demand soon resulted in overharvesting of Pacific yew. Since the yield of taxanes from yew bark is only about 0.01-0.06% (Zu et al., 2009), the harvesting practice was soon deemed unsustainable. In the US alone, 720,000 pounds of yew bark was required annually (Goodman, Jordan, & Walsh, 2001). However, taxanes are abundant and can be extracted from shoots and needles of Canada yew, *Taxus canadensis*, which is abundant in eastern Canada from the Manitoba/Ontario border to Newfoundland and northeastern US (Thompson, Anderson, & Bartlein, 1999). Thus, considering how important taxanes are for our continuing war against cancer, and keeping in mind the importance of environmental sustainability, it is easy to see why it was important to find an alternative source (other than yew bark) as well as the most optimal conditions in which to cultivate the new source for taxanes as a nursery crop. Early studies mainly focused on the effects age and sex of trees have on the paclitaxel concentration in yew bark. One study found that the concentration of paclitaxel (PAC) in

Himalayan Yew bark is on average 0.0558±0.008% (of dry weight), and is about 64% higher for male plants (averaged across tree age) in comparison to female trees (Nadeem, Rikhari, Kumar, Palni, & Nandi, 2002). The same study also concluded that maximum Paclitaxel content was recorded from trees with age >110 years. Studies on taxanes from stems and needles, however, are limited. From one such study, it was suggested that more research should be carried out to determine why differences in paclitaxel concentrations are not always dependent on different species (Zobel, Furmanowa, Glowniak, & Cragg, 1996). Particularly, it was suggested that environmental factors should be investigated for their effects on Taxol content in Taxus needles and stems. Cameron and Smith (2008) in a season-long study demonstrated that 3 years old shoots (needles and stems) of woodland Canada Yew contained three major taxanes in the concentration range of 350-500 µg/gdwt: 10-deacetylbaccatin III (abbreviated as 10 DAB III); 600-1200 µg/gdwt 9-dihydro-13-acetylbaccatin III (abbreviated as 9-DHB II); and 100-300 µg/gdwt paclitaxel (abbreviated as PAC).

Yews are extremely tolerant of cold and heat, sunny and shady positions, wet and dry soils. They grow in the understory of moist, forested habitats in cool and temperate to subtropical climates (Price, 1990). A previous paper (Gill & Healy, 1974) indicated that Canada yew is well acclimated to growing in deep shade. In open canopy areas, Canada yew has increased root growth in response to the higher light levels (Campbell, Finér, & Messier, 1999). Canada yew stems and needles are mainly commercially harvested in the fall, which is usually from August to late November or early December (Baebler et al., 2005). Only shoot tips are clipped but the roots (which also contain taxanes(Noland, TL and Abou-Zaid, 2008)) could also be harvested if nursery plants were dug up in the fall.

Canada yew contains 10-DAB III, 9-DHB III, and PAC. The values of these three taxanes differ in needles, stems, and roots (Cameron & Smith, 2008). Both 10-DAB III and 9-DHB III are ingredients for the semi-synthesis of Taxol® and Taxotere®, while PAC is the actual active ingredient of Taxol® (Nikolakakis et al., 2000). PAC and 10-DAB III that are extracted from *T.canadensis* are in higher concentration than found for other yew species, and *T.canadensis* is the only species that contains 9-DHB III (E.L.M., 2000; Zamir et al., 1995). Taxanes differ qualitatively and quantitatively by species (van Rozendaal, Lelyveld, & van Beek, 2000), age, season (ElSohly et al., 1995), time of day, and weather (Hook et al., 1999). Shade effects are different in different conifers, because they differ in shade tolerance. A rapid change into fully exposed condition could be detrimental for shaded conifers (Schoonmaker, Hacke, Landhäusser, Lieffers, & Tyree, 2010), so, shade coverage could be an important influence on the concentrations of taxanes. Tracking chlorophyll fluorescence and analyzing chlorophyll content were investigated in this study because they are easier to do and can save a lot of time and money compared with extracting taxanes. As a result, chlorophyll fluorescence and chlorophyll analyses are summarized in the appendix.

The purpose of this study is to analyze the effect of growth site, harvest date, and shade coverage, using six different Canada Yew clones to analyze the three major taxanes in *T. canadensis* roots, needles and stems.: 10-DAB III, 9-DHB III, and PAC. The study objective was to see if optimal conditions could be found in which to grow and harvest the plant to maximize the yields for commercial taxanes harvest.

Methods

Plant Material

A replicated trial to test the effect of different shade levels on *Taxus canadensis* was established at two nursery sites: the Acadia Forest Experiment Station (AFES) and the Canadian Forest Service (CFS) in the province of New Brunswick, Canada in the year 2003. The study was designed with 8 blocks at each site (Figure 3 and Figure 4), with 4 different shade coverages (0%, 35%, 55%, and 65%), and 6 different clones (3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A) in each block. Cuttings were rooted from these clones and one-year old plants were randomly planted under the shade frames and grown for four years. A sample map of a block is shown in Figure 5. The image of needles composite harvested in September with different shade coverages in 6 clones shows in Figure 2. Each year, the frames were put in place in May and taken off in October to November. In the fall of the fourth year two plants from each clone were harvested in each of September, October, and November. The most recent 3 years of growth was used for taxane and other analysis.

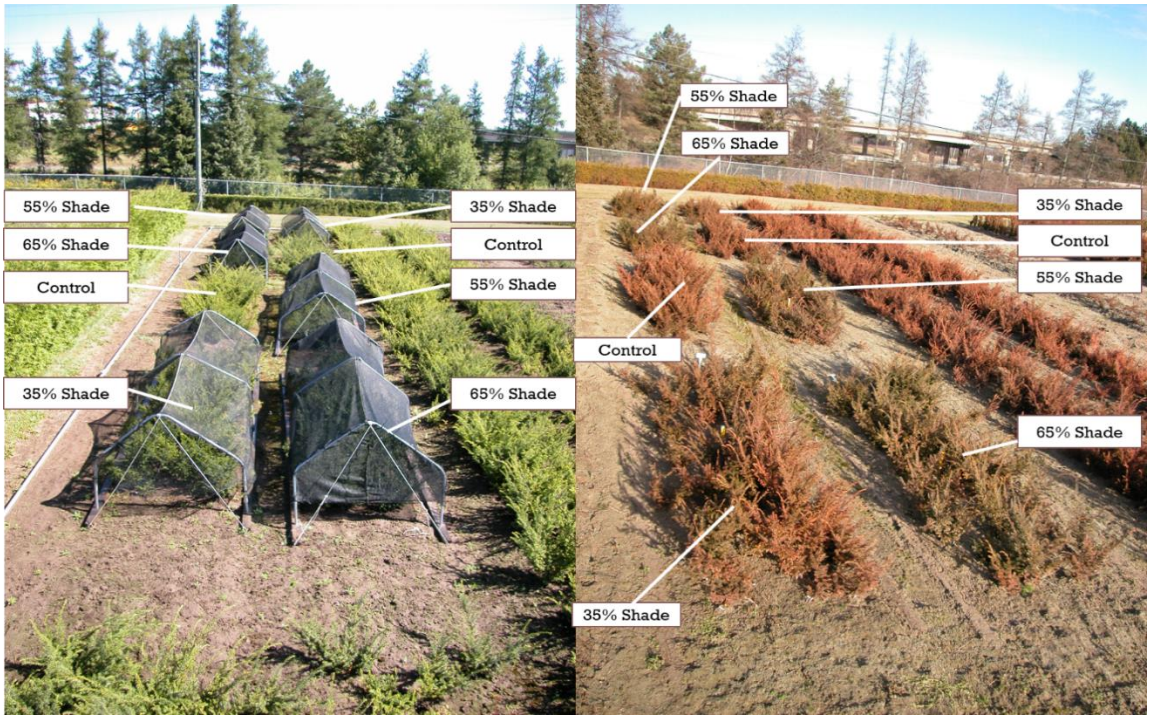


Figure 1. Taxus Shade Experiment Plantation in Canadian Forest Service Nursery in September (left) and December (right)

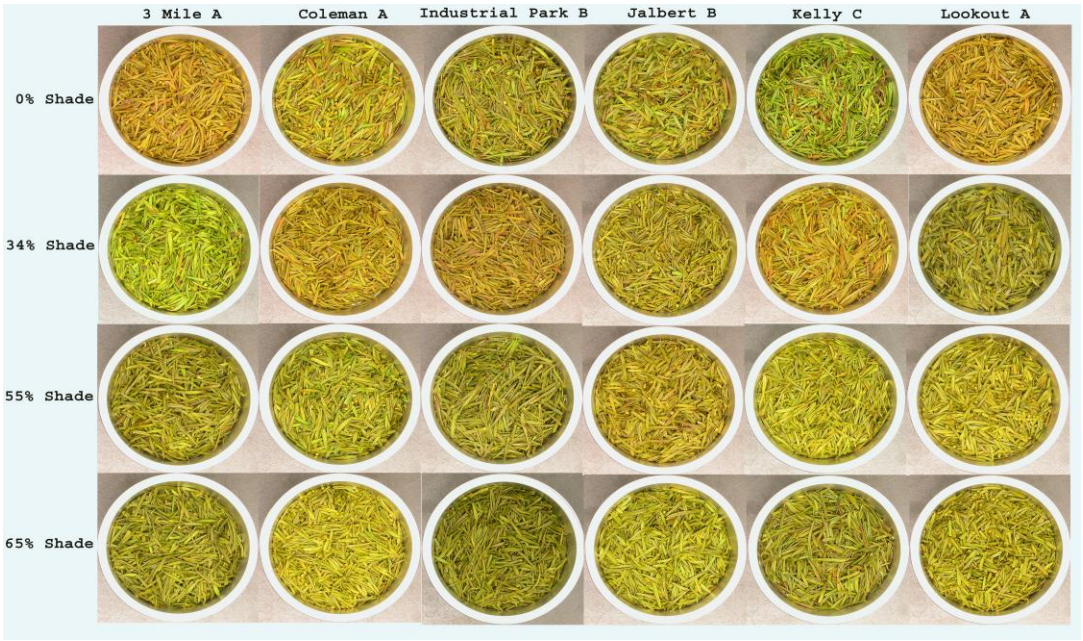


Figure 2. Needles composite harvested in September with different shade coverages in 6 clones (photo by John Letourneau NRCan)

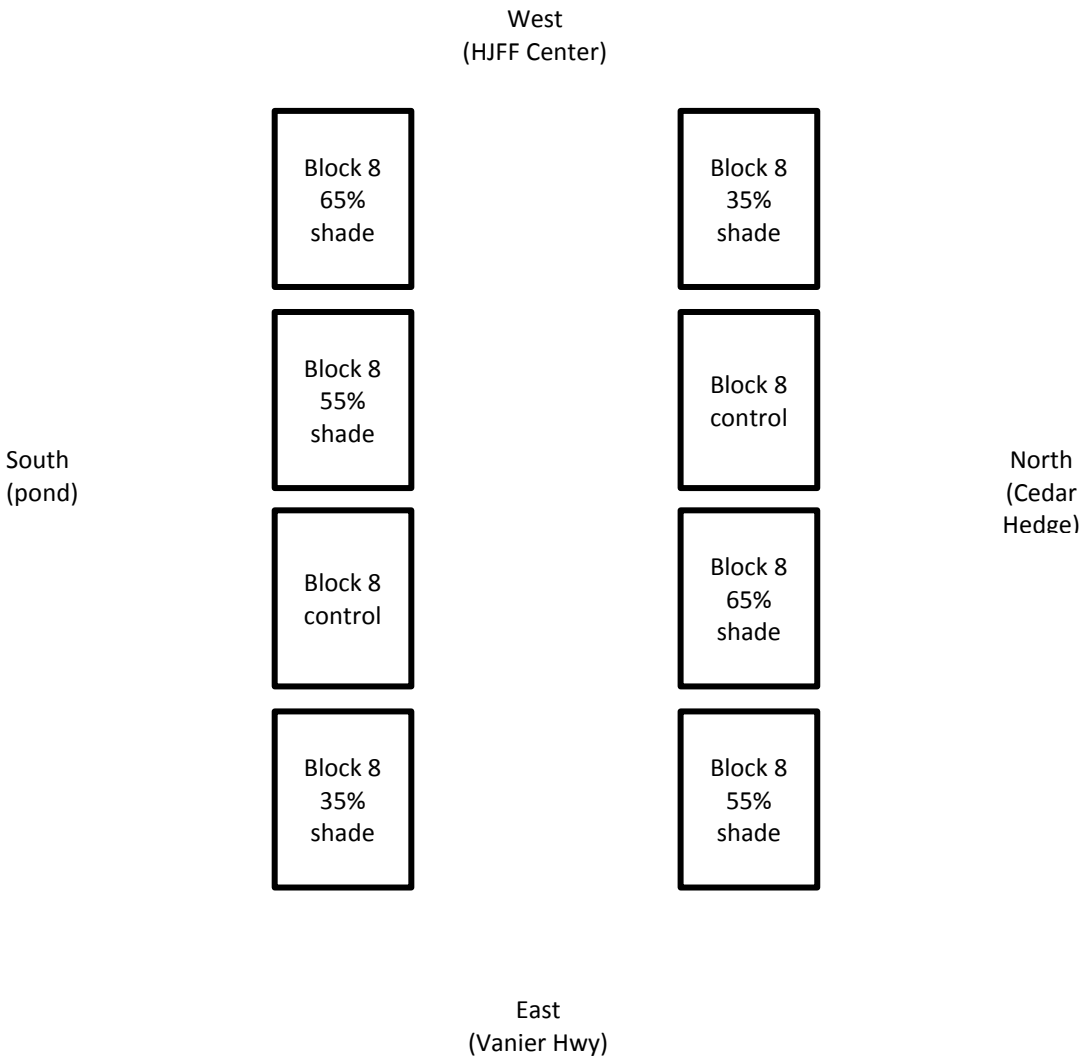


Figure 3. Canadian Forest Service Nursery, Taxus Shade Experiment Plantation Layout (outplanted 2003, map updated 2007)

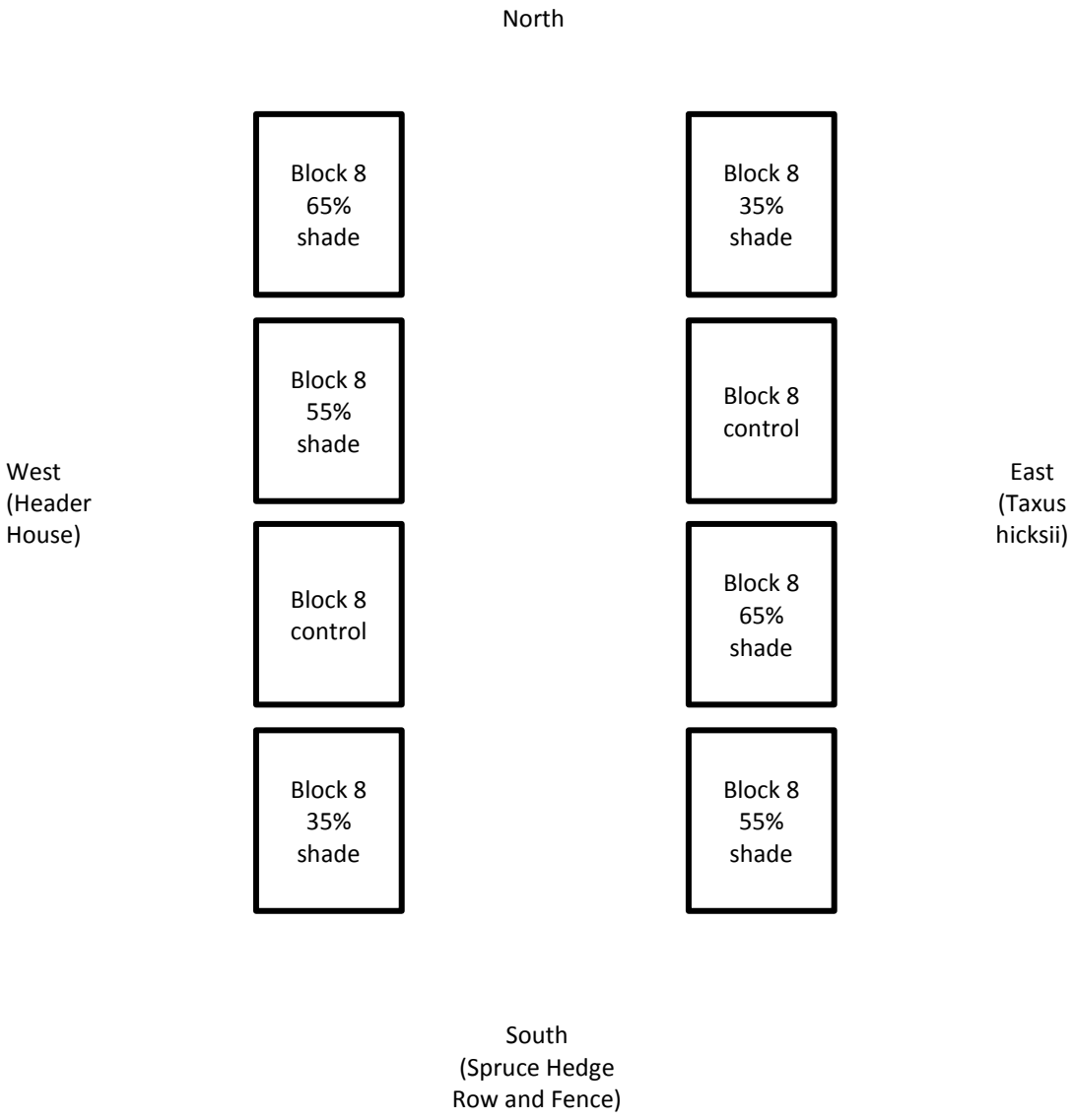


Figure 4. Acadia Forest Experiment Station, Taxus Shade Experiment Plantation Layout (outplanted 2003, map updated 2007)

IB	LA	IB	IB
KC	3A	KC	3A
3A	CA	3A	CA
IB	CA	LA	LA
KC	LA	3A	KC
CA	CA	3A	CA
IB	IB	JB	3A
KC	IB	KC	KC
IB	JB	CA	3A
CA	CA	3A	IB
KC	JB	KC	KC
JB	JB	CA	3A
JB	LA	LA	LA

Figure 5. Canadian Forest Service Nursery, Taxus Shade Experiment Plantation Layout Block 1 55% shade (IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; CA=Coleman A; 3A=3 Mile A; LA=Lookout A)

External Standard Taxanes Samples

10 Dab III, 9DHB III and PAC external standard samples were purchased from ChromaDex, Irvine, CA, USA, and were analyzed on an Agilent 1100 HPLC to establish times at which the device detected peaks for 10-DAB III, 9-DHB III and PAC in collected foliage samples. In order to quantify 10-DAB III, 9-DHB III and PAC

concentrations, dilutions of the standard samples were used. HPLC peaks occurred for 10-DAB III, 9-DHB III, and PAC at 15.45min, 20.31min, and 25.09min respectively.

Extraction method development

The external standard taxane samples also were used for developing a taxane extraction method. A series of 0.5g bulk standard samples with 5 ml 75% methanol were put in test tubes in a sonicator for different lengths of time (30 min, 60 min, 90 min, 120 min, 180 min, and 240 min). For each peak, the y-axis of the chromatogram is a measure of the intensity of absorbance (in milli-Absorbance Units, or units of mAu*s). The x-axis is in units of time (typically minutes) and is used to determine the retention time (tR) for each peak, the peak was calculated to be height from baseline*1/2 width of the peak at half height. To calculate the peak area, which represents total absorbance, we used the unit mAu*s in this experiment. Higher peak area indicates higher taxane concentration. Four replicates were done for each time period (Figure 6).

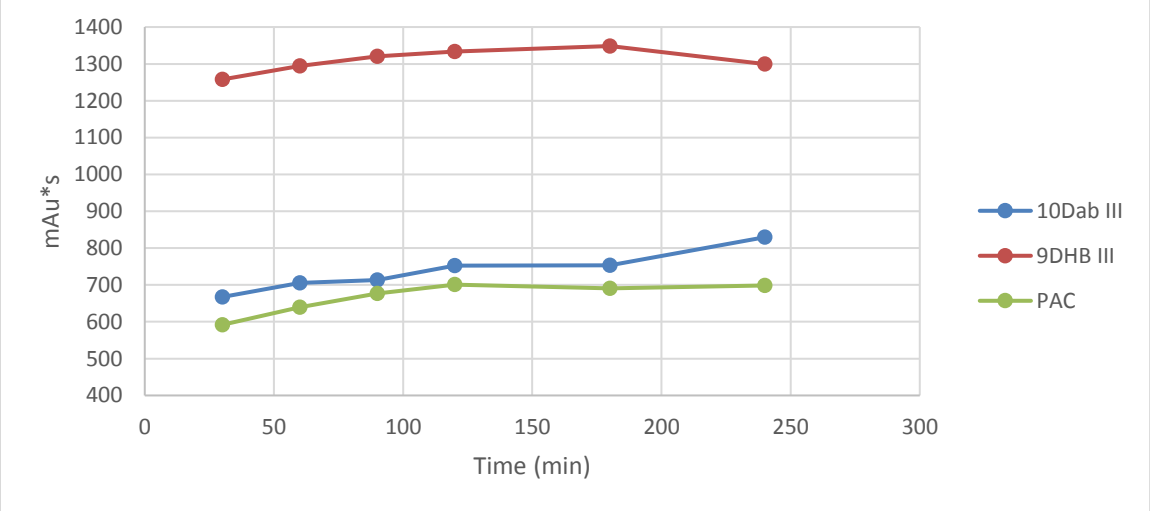


Figure 6. Average of mAu*s of taxanes of bulk standard sample which were sonicated for each of 6 different time periods

From Figure 6, the optimal sonication time was determined to be 180 min. Longer sonication time did not result in any increase in the amount of taxanes which were extracted and the mAu*s of 9-DHB III even went down.

Next, new 0.5g bulk standard samples with 5 ml 75% methanol were put in test tubes in an Omni Bead-Ruptor extractor (VWR Canada) with 5 different cycles (1, 2, 3, 5, 7) of 30 seconds per cycle at a speed of 6.00 m/s. Four replicates were done for each time period (Figure 7).

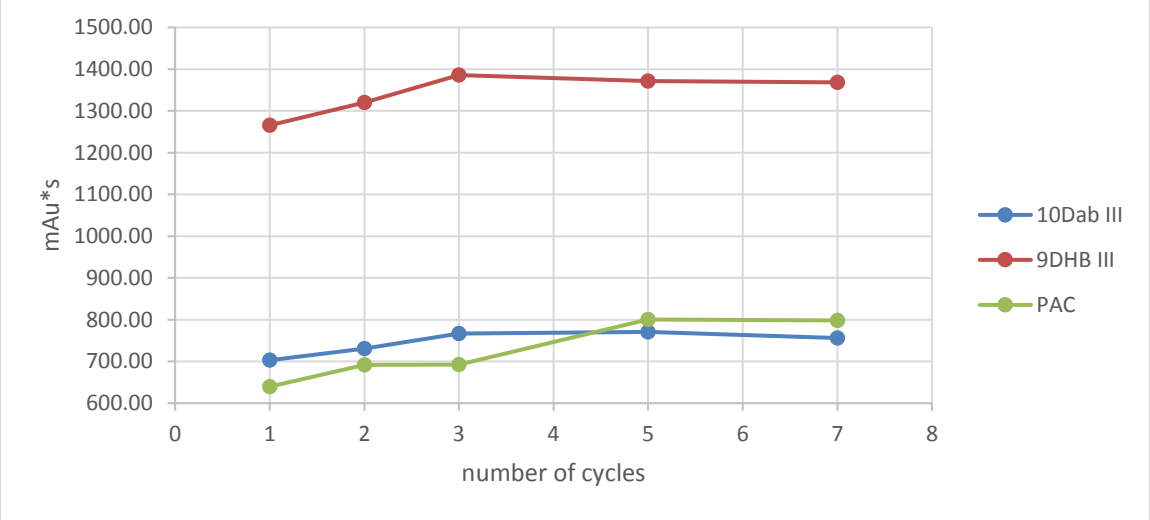


Figure 7. Average of mAu*s of taxanes of bulk standard sample which were sonicated for each of 5 cycles in Omni extractor

From Figure 7, the optimal extraction cycle was 5, beyond which no further taxanes were extracted and the mAu*s of taxanes even went down. The mAu*s values of taxanes in the Omni extractor were comparable (slightly higher) to the results obtained using the sonicator, so it was determined that extraction using the Omni unit was the preferred method.

Taxanes Extraction

The foliage samples were dried at 60°C for 24-30 h and divided into different plastic bags in 2007 according to their age and part (needles and stems; roots also were included, but only for the samples dug up at the end of November). The age classes were: current year's growth (C), 1-year-growth (C+1), 2-year-old growth (C+2), and more than 2-year-old growth (C+>2).

Dried ground needles from each age class were stored separately, the weight of each class was known. Therefore, to make the composite sample, samples of each class could be mixed together based on their ratio of abundance. A composite needle sample was mixed in 2018 based on combining aliquots from each of the years based on their weights in the original foliar samples. And this was likewise done with stem and root samples. For each plant, 0.5 g of each of needles, stems, or roots composite samples were placed into separate 7 ml test tubes (designed to fit in the Omni unit) containing agate beads, with 5 ml 75% methanol (Fisher OPTIMA grade) added and shaken well. Then the test tubes were put into the automated sample extractor for 5 cycles of 30 seconds per cycle at a speed of 6.00 m/s, and dwell time for 20 seconds between each cycle.

After extracting, all test tubes were placed into an Eppendorf Benchtop Centrifuge (Fisher) for 35 minutes at a speed of 3500 r/min. A 1-2 ml portion of the supernatant in the test tube was then extracted using a pipette and put into a mini-uniprep HPLC filter vial (Whatman).

HPLC Analysis

Each sample was analyzed by HPLC on an Agilent 1100 HPLC (Agilent Canada Ltd., Mississauga, ON, Canada) at 230 nm equipped with a Kinetex Polar C18 column (Phenomenex Corp., Torrance, CA, USA) at 40°C at 0.5 ml/min flow rate with injection of 10 µL using (A)- distilled water with 0.2% acetic acid; and (B)-acetonitrile with 0.2% acetic acid in a linear gradient programme as detailed in Table 1.

Table 1. Linear gradient programme for HPLC analysis (A- distilled water with 0.2% acetic acid; B- acetonitrile with 0.2% acetic acid)

Time (min)	Solvent A (% volume)	Solvent B (% volume)
0	90	10
3	90	10
33	20	80
35	5	95
42	90	10
45	90	10

Statistical Analysis

All data were analyzed using R studio. Any statistical significance between site, shade, and clone was determined using a repeated measure mixed effects ANOVA. For the taxanes in needles and stems, the repeated measures were fitted as random effects with date nested within Site. The full fixed effects model included Clone, Shade, Date, and all two-way and three-way interactions. Interaction terms were backward eliminated based on significance of associated p-values. For the taxanes in roots, statistical significance

between Clone and Shade was determined by two-way ANOVA. A p-value<0.05 was statistically significant. If the provided treatment df ≥3, any F≥3 is significant at p-value<0 .05 was considered to be statistically significant. When the treatment effect interactions in ANOVA were found to be insignificant, the ANOVAs were rerun and reduced models presented in the results. However, the full model ANOVA tables are presented in the Appendix.

Results

HPLC analysis of taxane concentrations

Needle taxane concentrations

10-DAB III concentration

Two conclusions can be drawn from the figures below: (1)10-DAB III concentration decreases with increasing shade levels within a date, with a gradual decrease with each successive collection date; (2) 10-DAB III concentrations of clones CA and KC are much higher than other clones, although a bigger variance can be found in CA than in KC, thus making clone KC a better choice overall.

The interaction between Date and Shade is shown by beanplots in Figure 8. 10-DAB III concentrations in needles decreased over time (Figure 8). Increasing shade also reduced the concentrations but only in September (Figure 8). Minor reductions were observed in October and no differences by November (Figure 8), and there was not much variability shown between different shade with in dates. In terms of clone, the 10-DAB III concentrations for clones CA and KC were much higher than other clones (Figure 9), Clone CA tended to have higher variation than all of the other clones (Figure 9).

The ANOVA table (Table 30) for the full repeated measures mixed effects ANOVA for 10-DAB III in needles is shown in appendix, and the reduced model is shown in Table 2. The only interaction that was significant was the Shade*Date interaction. In terms of random effects Date nested within Site accounted for a significant amount of variation

with September versus October accounting for most of that variation, while September versus November accounted for very little additional variation.

Table 2. ANOVA table for 10-DAB III in needles (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	15495269	3099054	205.3947	3.51e-124*
Shade	3	327466	109155	7.4659	0.000125*
Date	2	195118	97559	6.4659	0.001636*
Shade*date	6	343852	57309	3.7982	0.002464*
Random Effects			Variance		
Site	1		122.3		0.03495 *
Date Sep vs Oct / site	5		302.5		0.09581
Date Sep vs Nov / site			3.245		
Residual	566		437000		

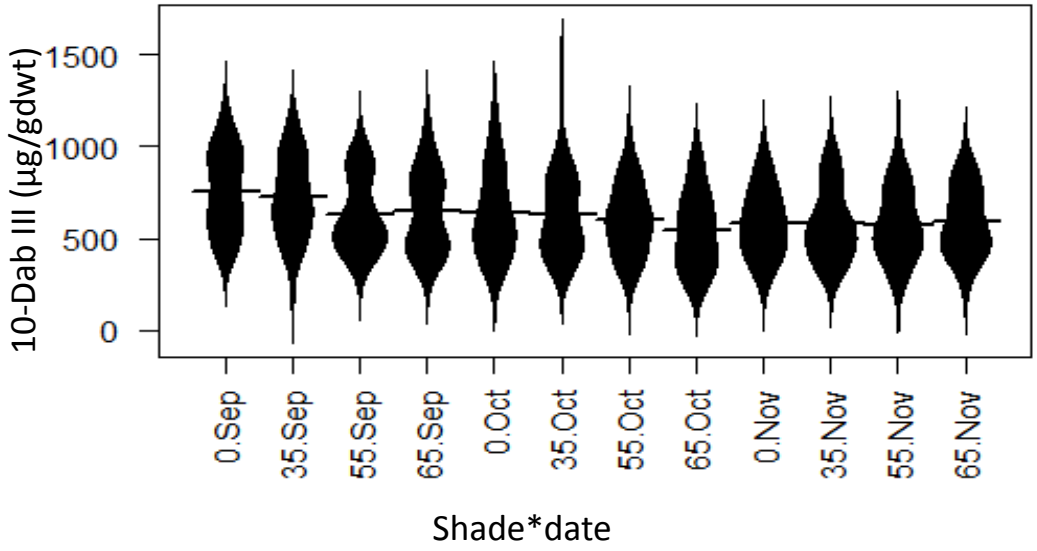


Figure 8. Bean plot for 10-DAB III concentration in needles with shade and date as interaction (Shade-0%, 35%, 55%, 65%; Date-September, October, November).

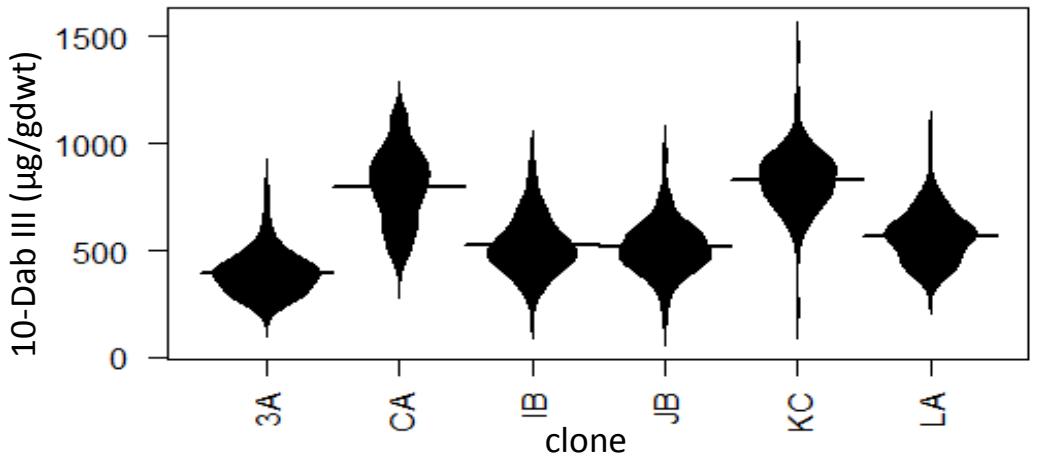


Figure 9. Bean plot for 10-DAB III concentration of different clones in needles (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

9-DHB III concentration

Two conclusions can be drawn from the figures below: (1) 9-DHB III concentration decreases over time; (2) 9-DHB III concentrations of clones KC is much higher than other clones.

The 9-DHB III decreased in concentration over time, the concentration in November is much lower than September and October (Figure 11). In terms of clone, 9-DHB III concentrations of clones KC are much higher than other clones, the concentration of 9-DHB III in clone KC is 542.6 µg/gdwt higher than clone CA on average (Figure 10). The ANOVA table Table 31 for the full repeated measures mixed effects ANOVA for 9-DHB III in needles is shown in appendix, and the reduced model is shown in Table 3. There are no significant interactions between shade, date and clone. In terms of random effects Date nested within Site accounted for a significant amount of variation with September versus October accounting for most of that variation, while September versus November accounted for very little additional variation.

Table 3. ANOVA table for 9-DHB III in needles (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	335137294	67027459	153.3914	5.11e-103*
Shade	3	3666924	1222308	2.7972	0.052732
Date	2	23652768	11826384	27.0645	5.39e-12*
Random Effects			Variance		
Site	1		122		1
Date Sep vs Oct / site	5		303		1
Date Sep vs Nov / site			3.25		
Residual	566		437000		

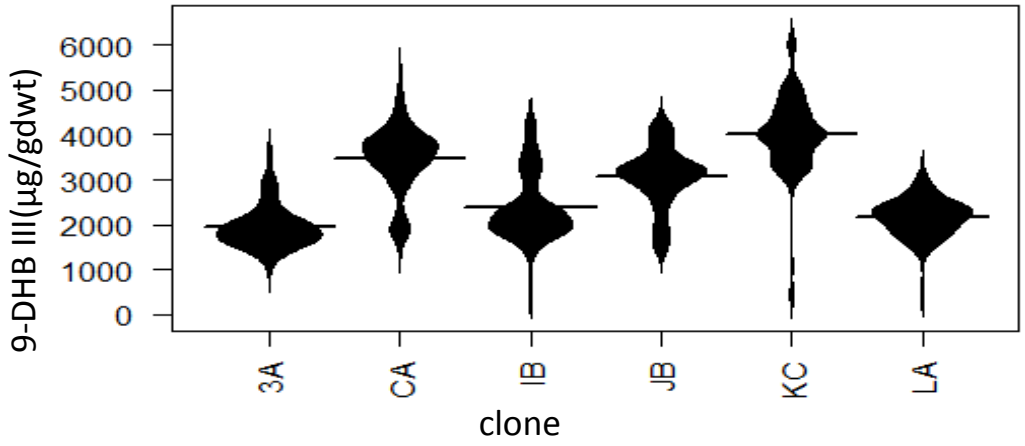


Figure 10. Bean plot for 9-DHB III concentration of different clones in needles (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

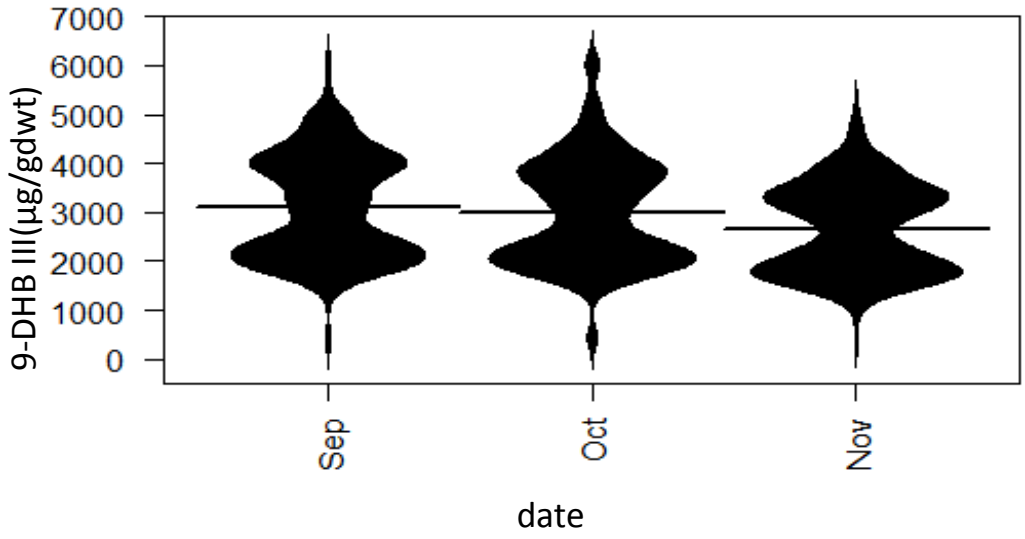


Figure 11. Bean plot for 9-DHB III concentration of different dates in needles (Date-September, October, November).

PAC concentration

Two conclusions can be drawn from the figures below: (1) PAC concentration increases with increasing shade levels within a date, with a gradual decrease with each successive collection date; (2) PAC concentrations of clones KC is higher than other clones and has lower variance than other clones.

The interaction between Date and Shade is shown by beanplots in Figure 13. PAC concentrations in needles decreased over time (Figure 13). Increasing shade also increased the concentrations in October and November (Figure 13). A little decrease was observed under 55% shade coverage in September but increased overall Figure 13. Minor reductions were observed in October and no differences by November (Figure 13). In terms of clone, the 9-DHB III concentrations for clones CA, IB and KC were higher than other clones (Figure 12), Clone KC tended to have lower variation than clones CA and IB (Figure 12).

The ANOVA table (Table 32) for the full repeated measures mixed effects ANOVA for PAC in needles is shown in appendix, and the reduced model is shown in Table 4. The only interaction that was significant was the Shade*Date interaction. In terms of random effects Date nested within Site accounted for a significant amount of variation with Site accounting for most of that variation, while September versus November accounted for very little additional variation.

Table 4. ANOVA table for PAC in needles (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	2270166	454033	99.1922	1.13e-74*
Shade	3	88013	29338	6.4094	0.000392*
Date	2	112654	56327	12.3057	5.64e-06*
Shade*date	6	69397	11566	2.5269	0.045380*
Random Effects			Variance		
Site	1		1973.93		< 2.2e-16*
Date Sep vs Oct / site	5		68.34		0.07143
Date Sep vs Nov / site			101.95		
Residual	560		4577.31		

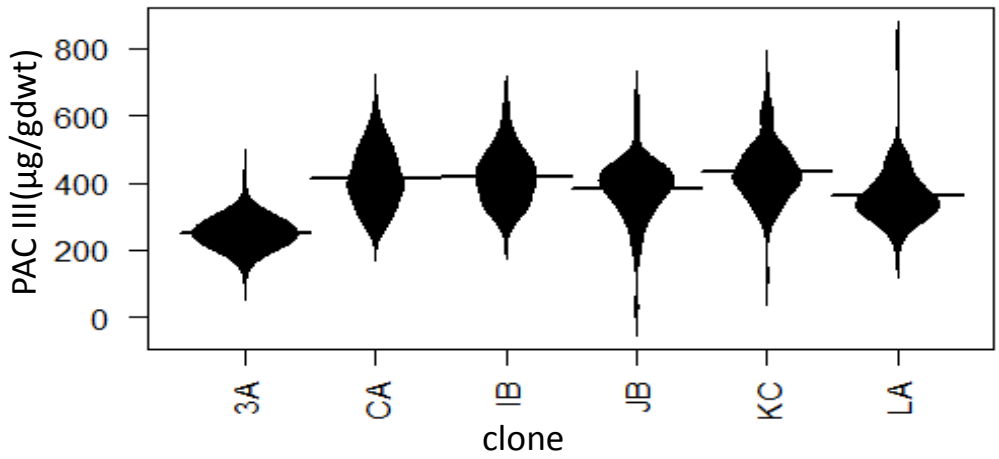


Figure 12. Bean plot for PAC concentration of different clones in needles (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

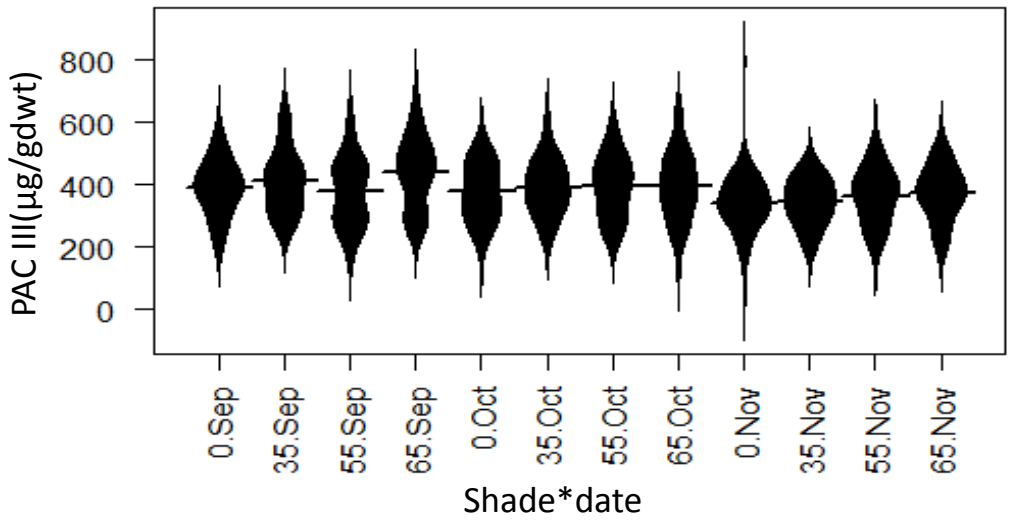


Figure 13. Bean plot for PAC concentration in needles with shade and date as interaction (Shade-0%, 35%, 55%, 65%; Date-September, October, November).

Stems taxane concentrations

10-DAB III concentration

Three conclusions can be drawn from the figures below: (1) 10-DAB III concentration increases over time; (2) 10-DAB III concentration increases with increasing shade coverage; (3) 10-DAB III concentrations of clones JB and KC are much higher than other clones.

The 10-DAB III increase in concentration with increasing shade (Figure 15) and also increased over time (Figure 16). In terms of clone, the 10-DAB III concentrations for clones JB and KC were much higher than other clones (Figure 14).

The ANOVA table (Table 33) for the full repeated measures mixed effects ANOVA for 10-DAB III in stems is shown in appendix, and the reduced model is shown in Table 5. There are no significant interactions between shade, date and clone. In terms of random effects Date nested within Site did not accounted for a significant amount of variation.

Table 5. ANOVA table for 10-DAB III in stems (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	1601209	320242	189.9158	1.46e-118*
Shade	3	43361	14454	8.5715	1.97e-05*
Date	2	35128	17564	10.416	3.48e-05*
Random Effects			Variance		
Site	1		1.25e-11		1
Date Sep vs Oct / site	5		2.29e-13		1
Date Sep vs Nov / site			6.48e-12		
Residual	566		1.69e+03		

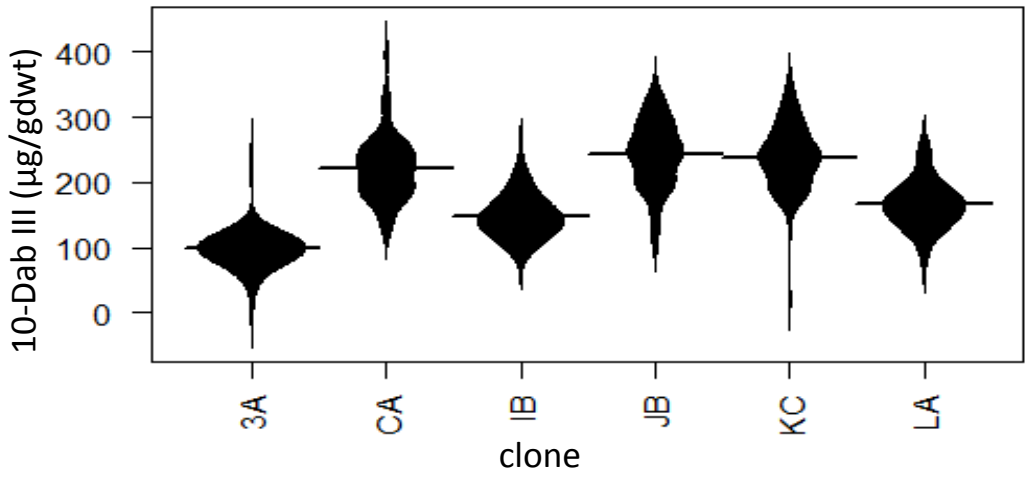


Figure 14. Bean plot for 10-DAB III concentration of different clones in stems (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

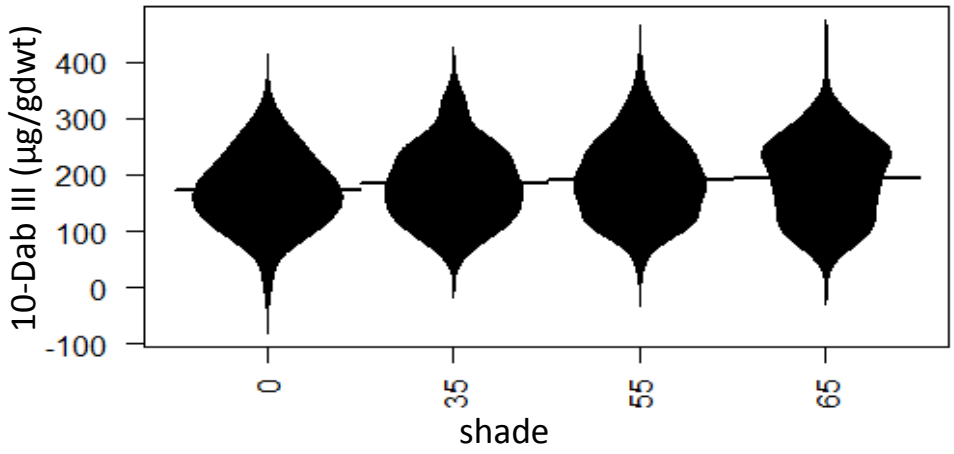


Figure 15. Bean plot for 10-DAB III concentration of different shade coverages in stems (Shade-0%, 35%, 55%, 65%).

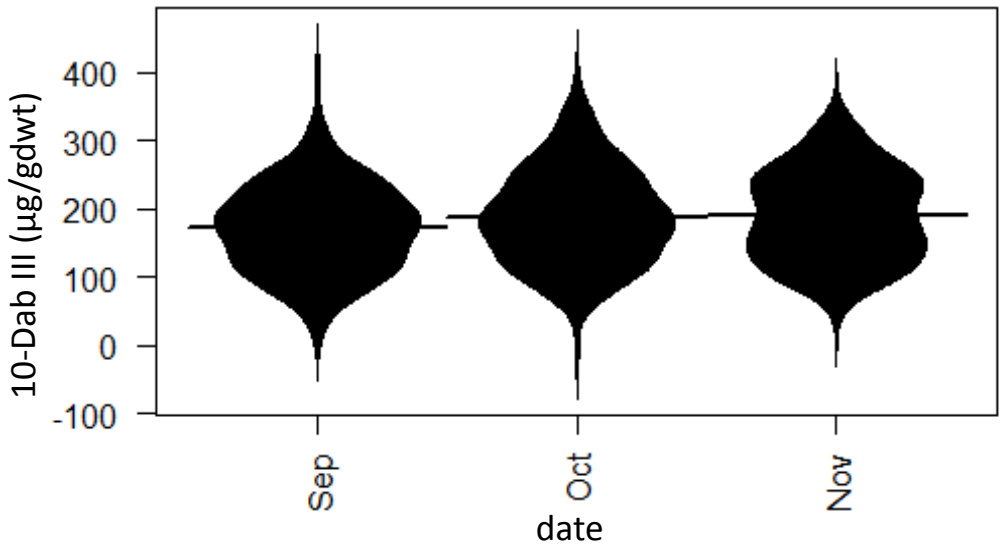


Figure 16. Bean plot for 10-DAB III concentration of different dates in stems (Date-September, October, November).

9-DHB III concentration

Two conclusions can be drawn from the figures below: (1) 9-DHB III concentration increases over time; (2) 9-DHB III concentrations of clones CA and JB are much higher than other clones.

The 9-DHB III increase in concentration with increasing shade (Figure 18). In terms of clone, the 9-DHB III concentrations for clones CA and JB were much higher than other clones (Figure 17), but clones CA and JB tended to have higher variation than the other clones.

The ANOVA table (Table 34) for the full repeated measures mixed effects ANOVA for 9-DHB III in stems is shown in appendix, and the reduced model is shown in Table 6. There are no significant differences between dates, and no significant interactions between shade, date and clone. In terms of random effects Date nested within Site accounted for a significant amount of variation with Site accounting for most of that variation, while September versus October accounted for very little additional variation.

Table 6. ANOVA table for 9-DHB III in stems (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	4996709	999342	34.8937	7.24e-31*
Shade	3	260105	86702	3.0273	0.038980*
Random Effects			Variance		
Site	1		4709		1.36e-08*
Date Sep vs Oct / site	5		25.2		0.2781
Date Sep vs Nov / site			552		
Residual	568		28639.6		

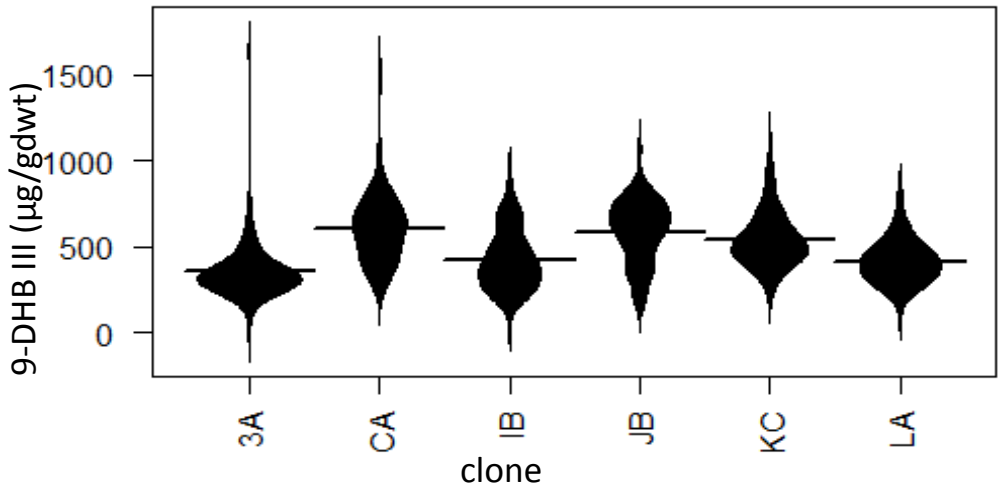


Figure 17. Bean plot for 9-DHB III concentration of different clones in stems (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A

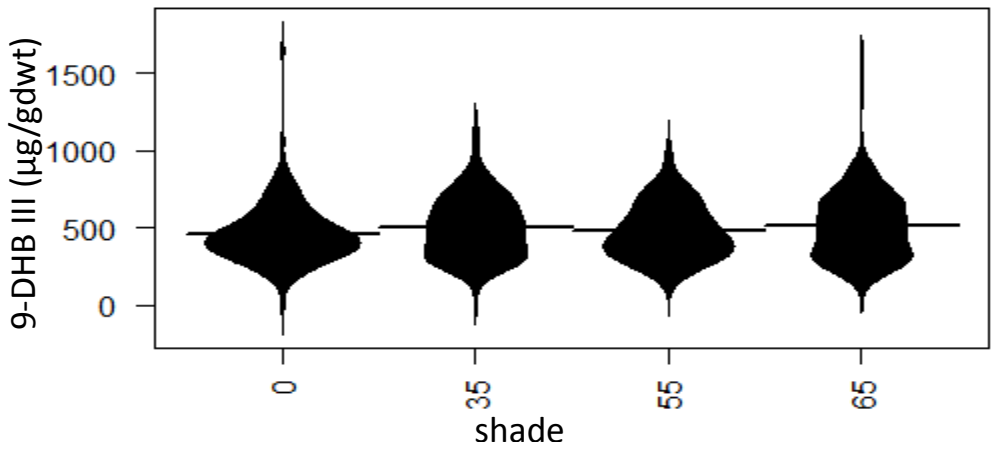


Figure 18. Bean plot for 9-DHB III concentration of different shade coverages in stems (Shade-0%, 35%, 55%, 65%).

PAC concentration

Two conclusions can be drawn from the figures below: (1) PAC concentration increases with increasing shade levels within a date, with a gradual decrease with each successive collection date; (2) PAC concentrations of clones JB and KC are much higher than other clone.

The interaction between Date and Shade is shown by beanplots in Figure 20. PAC concentrations in needles increased over time (Figure 20). Increasing shade also increased the concentrations but only in November but decreased with 65% shade coverage (Figure 20). Minor reductions were observed in September and October (Figure 20). In terms of clone, the PAC concentrations for clones JB and KC were higher than other clones (Figure 19).

The ANOVA table (Table 35) for the full repeated measures mixed effects ANOVA for PAC in stems is shown in appendix, and the reduced model is shown in Table 7.

The only interaction that was significant was the Shade*Date interaction. In terms of random effects Date nested within Site accounted for a significant amount of variation with September versus November accounting for most of that variation, while Site accounted for very little additional variation.

Table 7. ANOVA table for PAC in stems (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	90770	18154	28.4057	2.01e-25*
Shade	3	23181	7727	12.0905	1.53e-07*
Shade*date	8	18912	2364	3.6989	0.000983*
Random Effects			Variance		
Site	1		10.804		0.8884
Date Sep vs Oct / site	5		0.138		0.007296*
Date Sep vs Nov / site			58.576		
Residual	560		639.099		

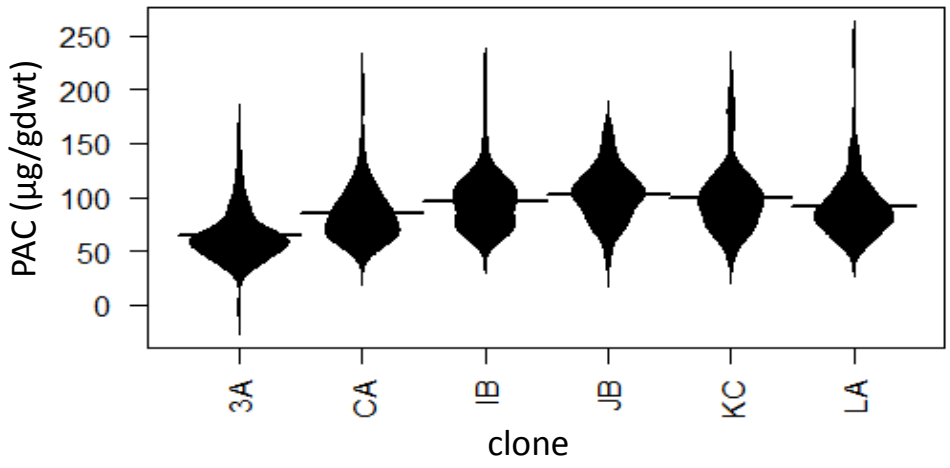


Figure 19. Bean plot for PAC concentration of different clones in stems (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

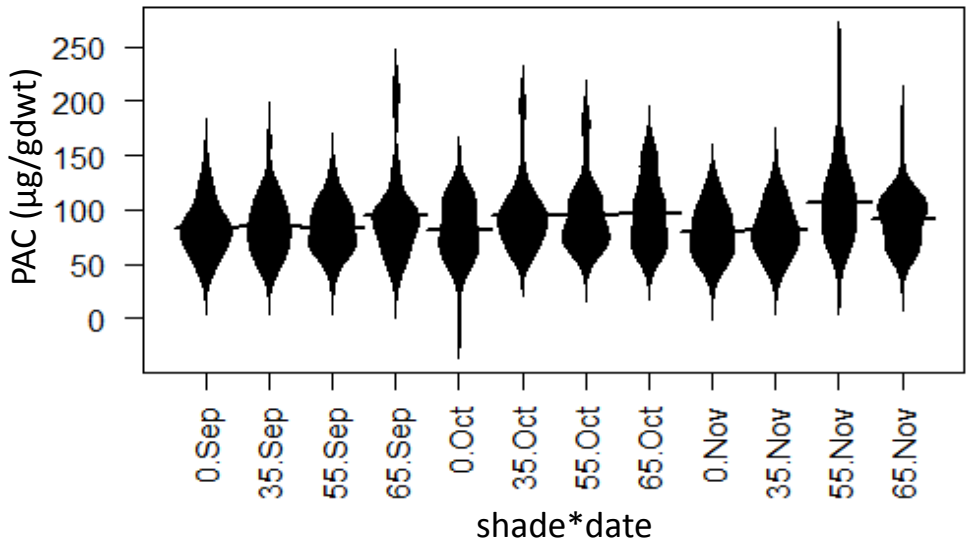


Figure 20. Bean plot for PAC concentration in stems with shade and date as interaction (Shade-0%, 35%, 55%, 65%; Date-September, October, November).

Roots taxane concentrations

10-DAB III concentration

Two conclusions can be drawn from the figures below: (1) 10-DAB III concentration decreases over time; (2) 10-DAB III concentrations of clones JB and KC are much higher than other clones.

The beanplot in Figure 22 shows the 10-DAB III concentration decreased over time, 10-DAB III concentration with 0% and 35% shade coverage had lower variance than 55% and 65% shade coverage. In terms of clone, the 10-DAB III concentrations for clones JB and KC were much higher than other clones (Figure 21), clone IB and JB had lower variance than other clones.

The ANOVA table (Table 36) for the full two-way ANOVA for 10-DAB III in roots is shown in appendix, and the reduced model is shown in Table 8. There is no significant interaction between shade and clone.

Table 8. ANOVA table for 10-DAB III in roots (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Clone	5	766878	153376	4.0603	0.002188*
Shade	3	272392	90797	2.4037	0.072255
Residuals	96	3626368	37775		

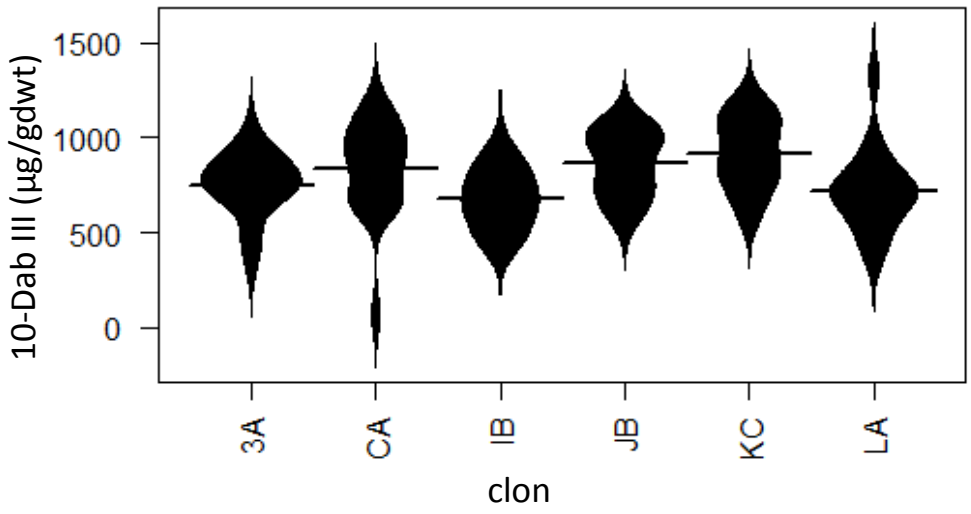


Figure 21. Bean plot for 10-DAB III concentration of different clones in roots (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

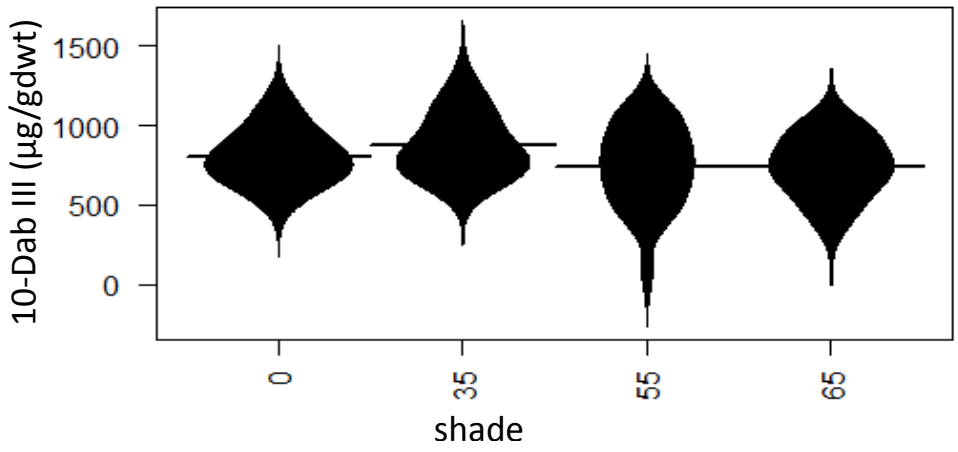


Figure 22. Bean plot for 10-DAB III concentration of different shade coverages in roots (Shade-0%, 35%, 55%, 65%).

9-DHB III concentration

The conclusion can be drawn from the figures below: 9-DHB III concentration of clones JB is much higher than other clones.

The beanplot in Figure 23 shows the 9-DHB III concentration for clones JB was much higher than other clones (Figure 21). Though clone JB tended to have higher variation than all of the other clones, the root samples in clone JB had vary high 9-DHB III concentration.

The ANOVA table (Table 37) for the full two-way ANOVA for 9-DHB III in roots is shown in appendix, and the reduced model is shown in Table 9. There are no significant differences between shade and no significant interactions between shade and clone.

Table 9. ANOVA table for 9-DHB III in roots (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Clone	5	605390	121078	4.463	0.001041*
Residuals	99	2685804	27129		

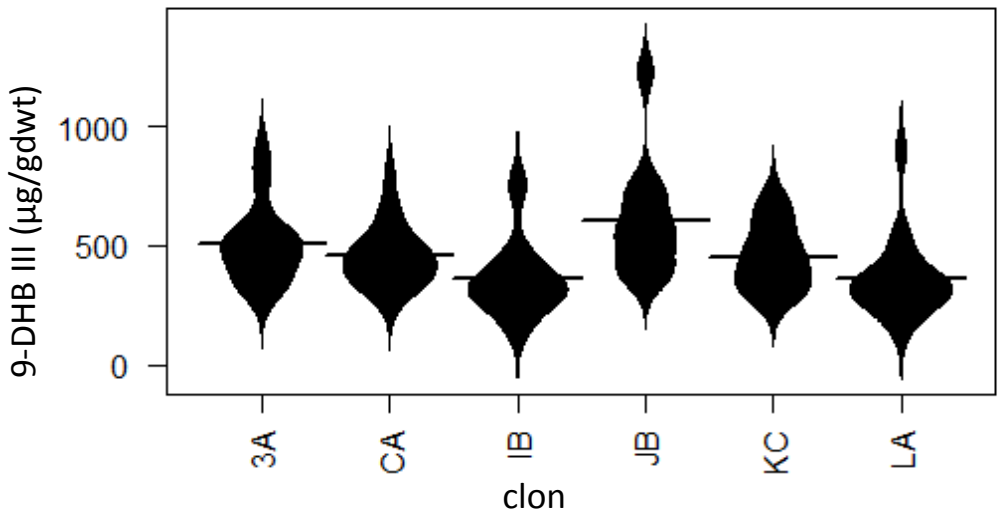


Figure 23. Bean plot for 9-DHB III concentration of different clones in roots (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

PAC concentration

The conclusion can be drawn from the figures below: PAC concentration of clones JB is higher than other clones.

The beanplot in Figure 24 shows the PAC concentration for clones IB and JB was much higher than other clones (Figure 21), clone IB tended to have higher variation than all of the other clones. As a result, clone JB tended to be the optimal choice.

The ANOVA table (Table 38) for the full two-way ANOVA for PAC in roots is shown in appendix, and the reduced model is shown in Table 10. There are no significant differences between shade and no significant interactions between shade and clone.

Table 10. ANOVA table for PAC in roots (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Clone	5	438696	87739	9.6768	1.52E-07*
Residuals	99	897631	9067		

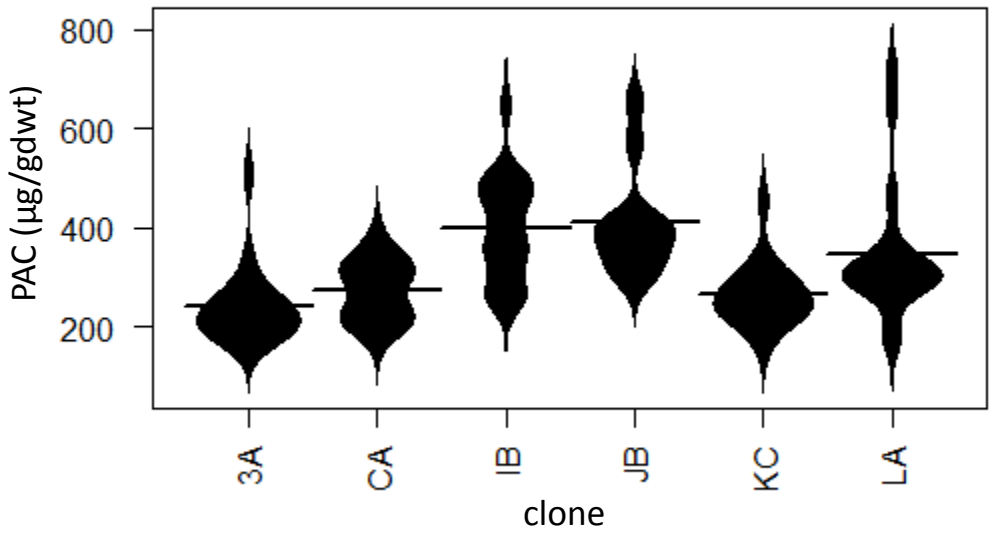


Figure 24. Bean plot for PAC concentration of different clones in roots (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

Taxanes Yield Analysis

Taxanes Yield from needles and stems

10 DAB III yield

Two conclusions about 10-DAB III yield from needles and stems can be drawn from the figures below: (1) 10-DAB III yield decreases over time; (2) 10-DAB III yield of clone KC is higher than other clones.

The 10-DAB III yield from needles and stems decreased over time (Figure 26). In terms of clone, the 10-DAB III yield for clones CA and KC are much higher than other clones (Figure 25) and clones KC has lower variation than the other clones.

The ANOVA table (Table 40) for the full repeated measures mixed effects ANOVA for 10-DAB III in needles and stems is shown in appendix, and the reduced model is shown in Table 11. There are no significant differences between shade, and no significant interactions between shade, date and clone. In terms of random effects Date nested within Site accounted for a significant amount of variation with Site accounting for most of that variation, while September versus November accounted for very little additional variation.

Table 11. ANOVA table for 10-DAB III in needles and stems (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	5348182	1069636	209.3906	1.79e-126*
Date	2	47572	23786	4.6563	0.004352*
Random Effects			Variance		
Site	1		71.882		0.8516
Date Sep vs Oct / site	5		5.918		0.2667
Date Sep vs Nov / site			249.323		
Residual	569		5108.332		

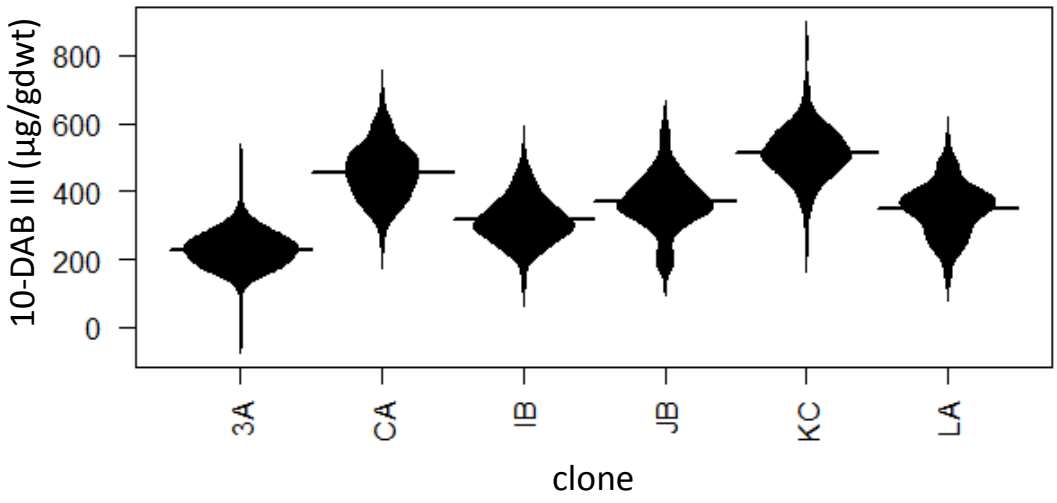


Figure 25. Bean plot for 10-DAB III yield of different clones in needles and stems (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

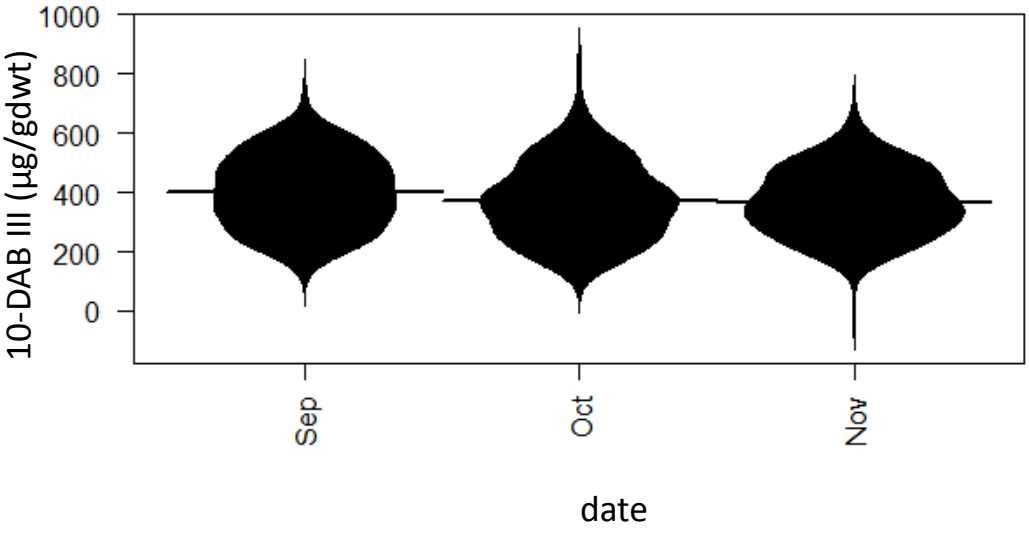


Figure 26. Bean plot for 10-DAB III yield of different date in needles and stems (Date-September, October, November).

9-DHB III yield

Two conclusions about 9-DHB III yield from needles and stems can be drawn from the figures below: (1) 9-DHB III yield decreases over time but increase with increasing shade coverage; (2) 9-DHB III concentrations of clone KC is higher than other clones but clone KC has high variability.

The 9-DHB III yield decreased over time (Figure 29), which has same trend with 10-DAB III yield. Increasing shade increased the 9-DHB III yield (Figure 28). A little decrease was observed under 55% shade coverage in September but increased overall (Figure 28). In terms of clone, the 9-DHB III concentrations for clone KC was much higher than other clones (Figure 27), but clones KC also tended to have higher variation. The ANOVA table (Table 40) for the full repeated measures mixed effects ANOVA for 9-DHB III in needles and stems is shown in appendix, and the reduced model is shown in Table 12. There are no significant interactions between shade, date and clone. In terms of random effects Date nested within Site accounted for a significant amount of variation with Site accounting for most of that variation, while September versus October accounted for very little additional variation.

Table 12. ANOVA table for 9-DHB III in needles and stems (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	88048931	17609786	112.6497	2.04e-82*
Shade	3	1572090	524030	3.3522	0.025338*
Date	2	3945531	1972765	12.6198	4.16e-06*
Random Effects			Variance		
Site	1		18815.4		2.546e-11*
Date Sep vs Oct / site	5		1182.5		0.963
Date Sep vs Nov / site			279.5		
Residual	566		156323.5		

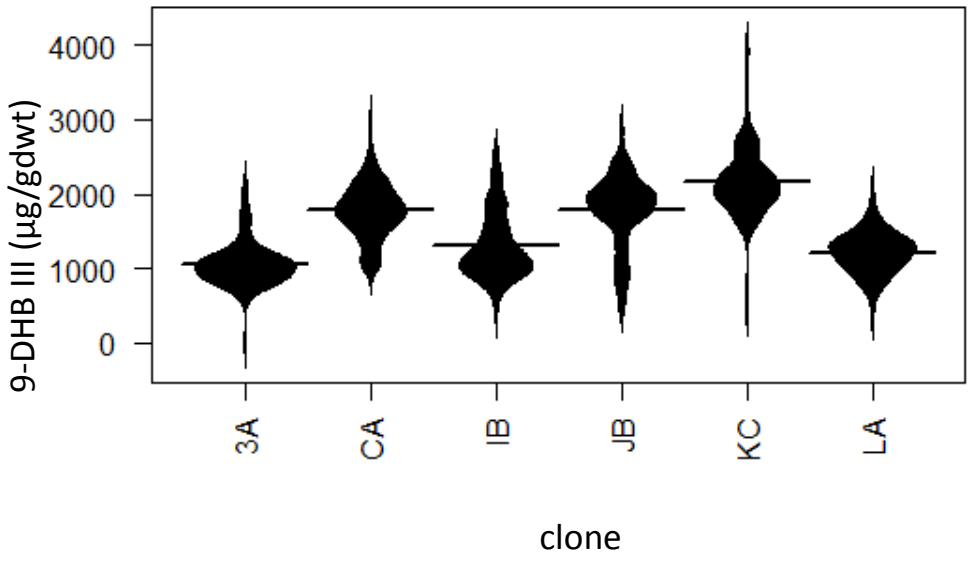


Figure 27. Bean plot for 9-DHB III yield of different clones in needles and stems (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

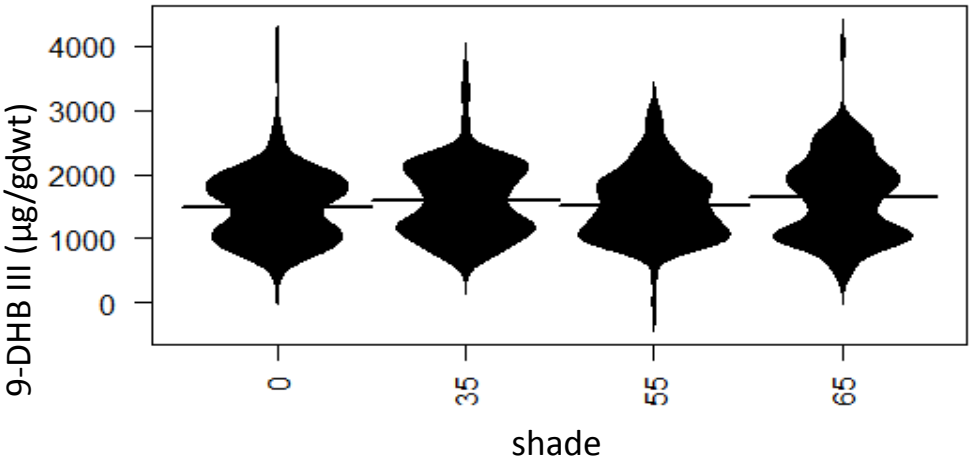


Figure 28. Bean plot for 9-DHB III yield of different shade coverage in needles and stems (Shade-0%, 35%, 55%, 65%).

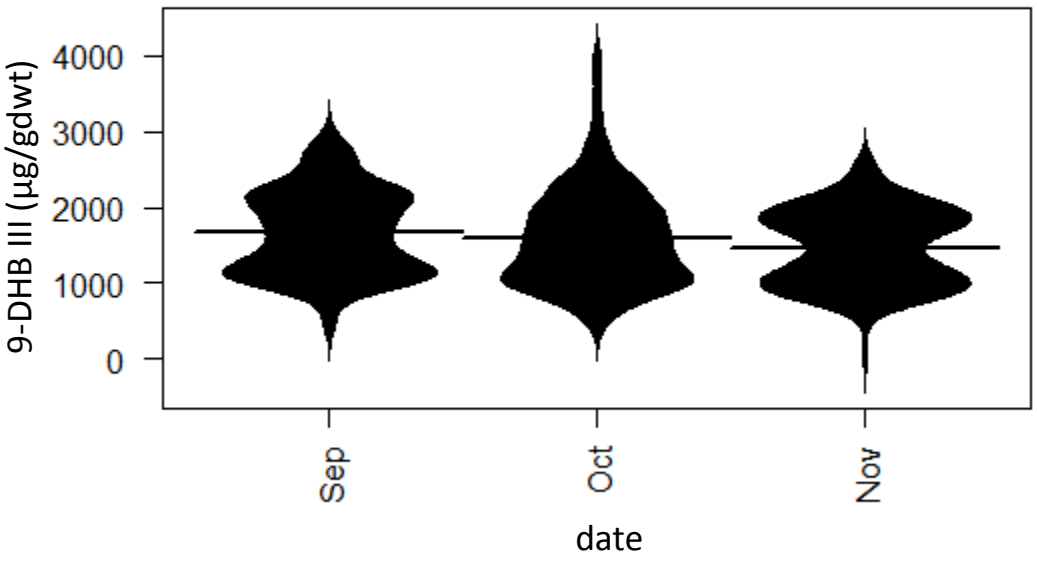


Figure 29. Bean plot for 9-DHB III yield of different date in needles and stems (Date-September, October, November).

PAC yield

Two conclusions about PAC yield from needles and stems can be drawn from the figures below: (1) PAC yield decreases over time but increase with increasing shade coverage; (2) 9-DHB III concentrations of clone KC is higher than other clones.

The PAC increased in yield with increasing shade (Figure 31) and decreased over time (Figure 32). The variances of PAC yield in September and October are similar, November tended to have lower variance than September and October (Figure 32). In terms of clone, the PAC yield for clone KC was much higher than other clones (Figure 30) and clone KC not has high variance compared with other clones.

The ANOVA table (Table 41) for the full repeated measures mixed effects ANOVA for PAC in needles and stems is shown in appendix, and the reduced model is shown in Table 13. There are no significant interactions between shade, date and clone. In terms of random effects Date nested within Site accounted for a significant amount of variation with Site accounting for most of that variation, while September versus October accounted for very little additional variation.

Table 13. ANOVA table for PAC in needles and stems (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	700211	140042	71.1884	1.44e-57*
Shade	3	45803	15268	7.7611	6.04e-05*
Date	2	17503	8752	4.4488	0.011917*
Random Effects			Variance		
Site	1		99.86		0.00633*
Date Sep vs Oct / site	5		50.06		0.1114
Date Sep vs Nov / site			23.34		
Residual	566		1967.2		

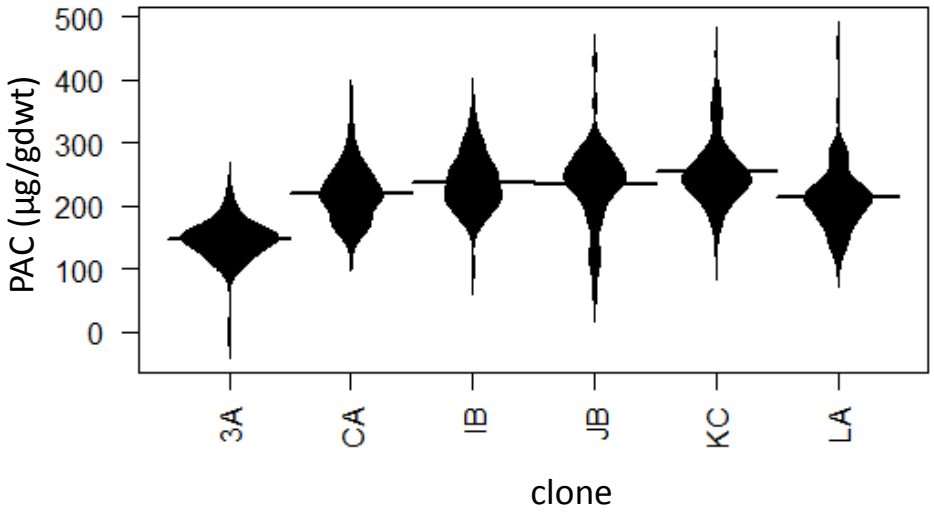


Figure 30. Bean plot for PAC yield of different clones in needles and stems (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

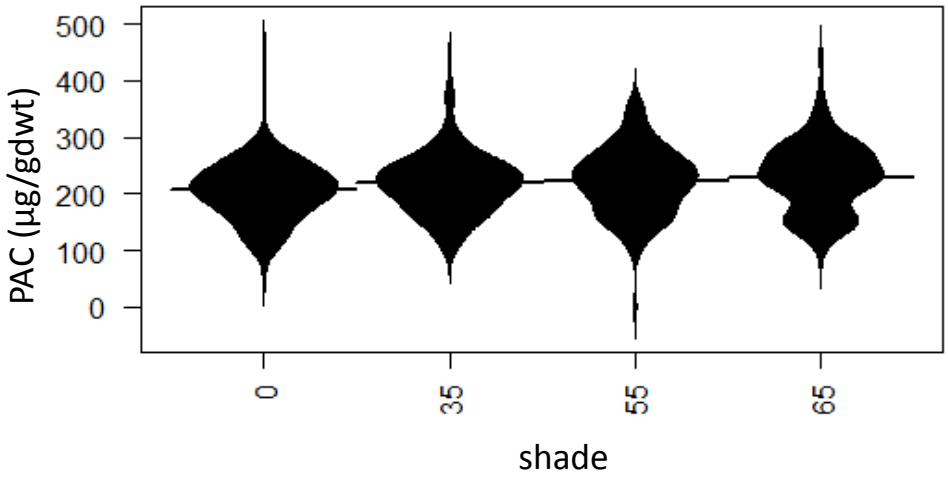


Figure 31. Bean plot for PAC yield of different shade coverages in needles and stems (Shade-0%, 35%, 55%, 65%).

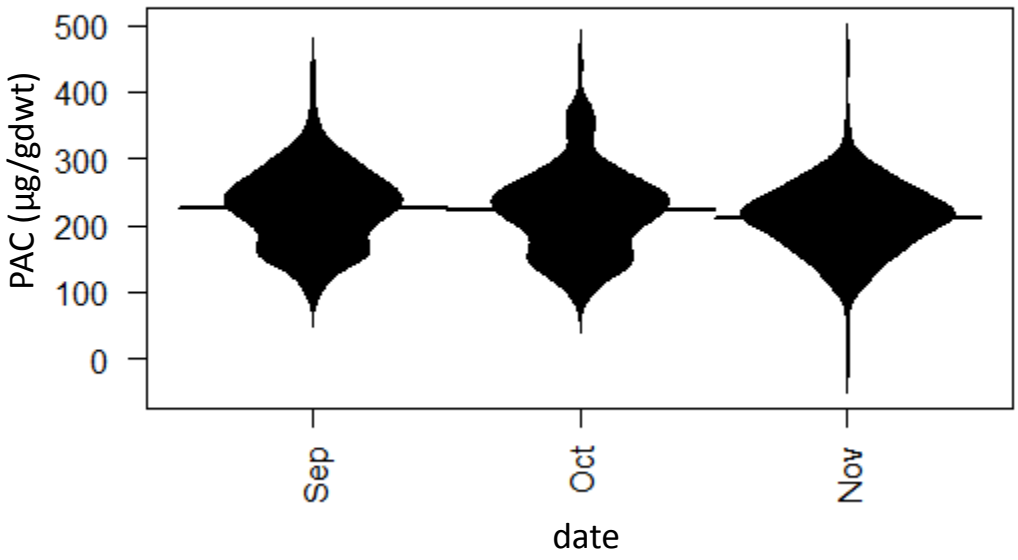


Figure 32. Bean plot for PAC yield of different date in needles and stems (Date-September, October, November).

Taxanes yield from needles, stems and roots

10-DAB III yield

Two conclusions about 10-DAB III yield from needles, stems and roots can be drawn from the figures below: (1) 10-DAB III yield decreases with increasing shade coverage; (2) 10-DAB III yield of clone KC is higher than other clones.

The beanplot in Figure 34 shows increasing shade reduced the 10-DAB III yield. A little increase was observed under 35% shade coverage but increased overall (Figure 34). In

terms of clone, the 10-DAB III concentrations for clone KC was higher than other clones and does not have high variance compared with other clones (Figure 33).

The ANOVA table for the full two-way ANOVA for 10-DAB III in needles, stems and roots is shown in Table 14. There is significant interaction between shade and clone.

Table 14. ANOVA table for 10-DAB III in needles, stems and roots

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Clone	5	957476	191495	25.7836	3.41e-15*
Shade	3	98998	32999	4.4432	0.006211*
Clone*Shade	15	109644	7310	0.9842	0.479852
Residuals	77	571880	7427		

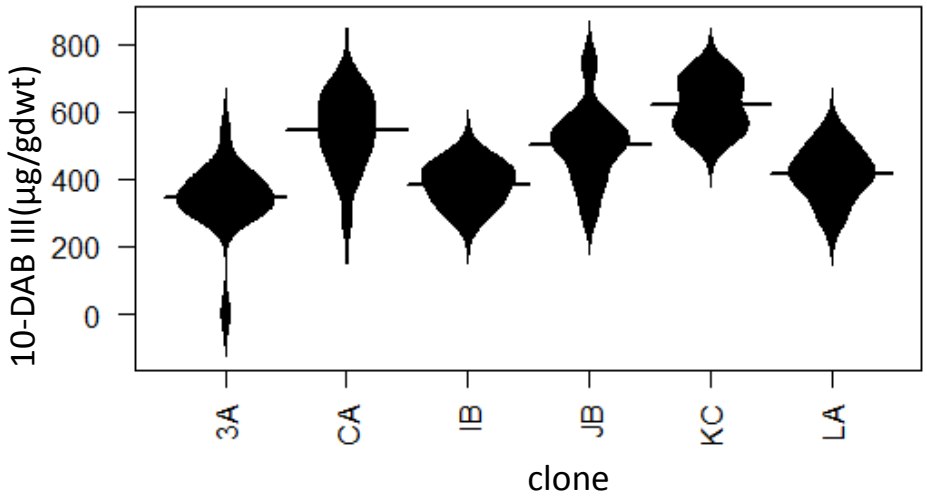


Figure 33. Bean plot for 10-DAB III total yield of different clone in needles, stems and roots (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

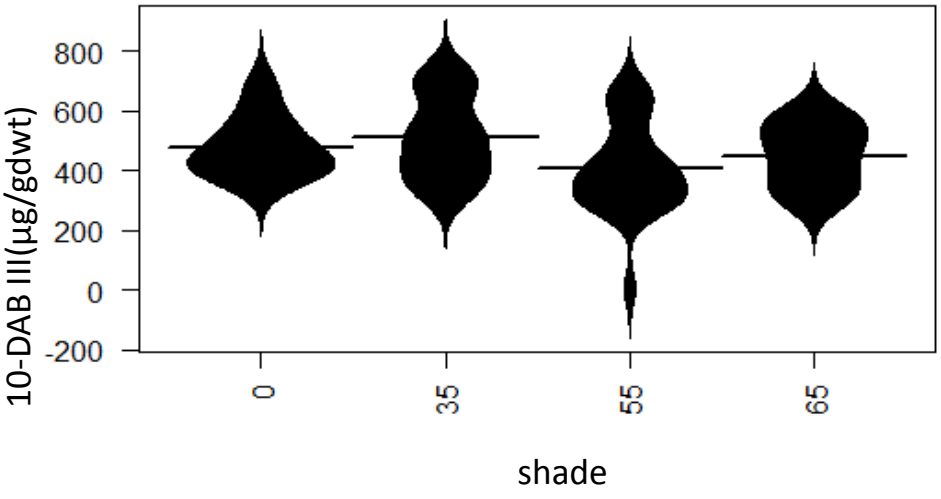


Figure 34. Bean plot for 10-DAB III total yield of different shade coverage in needles, stems and roots (Shade-0%, 35%, 55%, 65%).

9-DHB III yield

Two conclusions about 9-DHB III yield from needles, stems and roots can be drawn from the figures below: (1) 9-DHB III yield decreases with increasing shade coverage; (2) 9-DHB III yield of clone KC is higher than other clones.

The beanplot in Figure 36 shows increasing shade reduced the 10-DAB III yield. A little increase was observed under 35% shade coverage but increased overall (Figure 36). In terms of clone, the 10-DAB III concentrations for clone KC was higher than other clones and does not have high variance compared with other clones (Figure 35).

The ANOVA table for the full two-way ANOVA for 9-DHB III in needles, stems and roots is shown in Table 15. There is no significant interaction between shade and clone.

Table 15. ANOVA table for 9-DHB III in needles, stems and roots

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Clone	5	10212669	2042534	33.9635	< 2e-16*
Shade	3	593598	197866	3.2901	0.02502*
Clone*Shade	15	822827	54855	0.9121	0.5542
Residuals	77	4630707	60139		

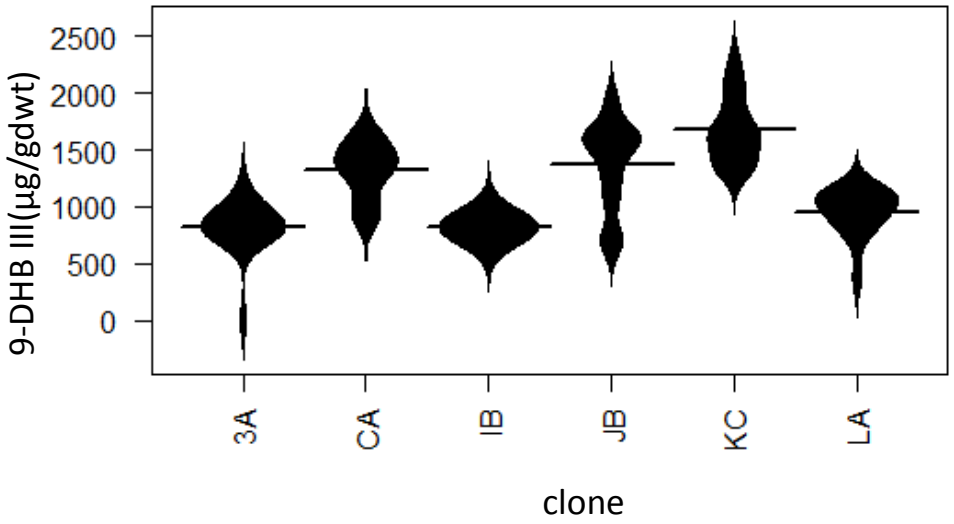


Figure 35. Bean plot for 9-DHB III total yield of different clone in needles, stems and roots (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

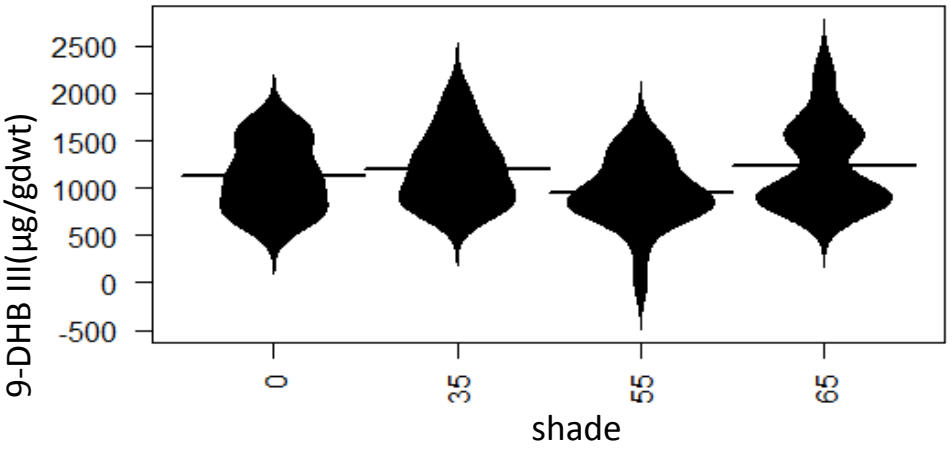


Figure 36. Bean plot for 9-DHB III total yield of different shade coverage in needles, stems and roots (Shade-0%, 35%, 55%, 65%).

PAC yield

The conclusion about PAC yield from needles, stems and roots can be drawn from the figures below: PAC yield of clone JB is higher than other clones, but clone JB has higher variance than other clones.

The beanplot in Figure 37 shows the PAC concentrations for clone JB was higher than other clones, but clone JB has higher variance than other clones. Though clone KC is not the highest, the level of clone KC is not much lower than clone JB and has low variance compared with other clones.

The ANOVA table for the full two-way ANOVA for PAC in needles, stems and roots is shown in Table 16. There is significant interaction between shade and clone.

Table 16. ANOVA table for PAC in needles, stems and roots

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Clone	5	189689	37938	19.8273	1.20e-12*
Shade	3	5603	1868	0.976	0.4085
Clone*Shade	15	23598	1573	0.8222	0.6503
Residuals	77	147332	1913		

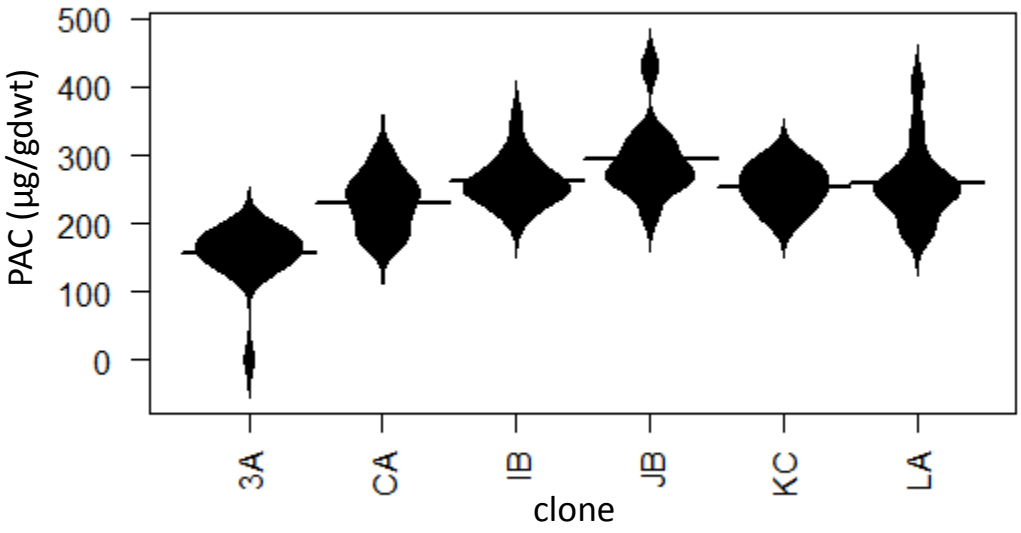


Figure 37. Bean plot for PAC total yield of different clone in needles, stems and roots (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

Discussion

Taxane concentrations varied with season

All three taxane concentrations decreased over time in needle samples, and September tended to have higher variation than October, November tended to have the highest variation. 10-DAB III and PAC concentrations in stem samples increased over time, which different from needles, and October tended to have higher variation than September and November. 9-DHB III in stems did not have significant differences between three months. The taxane concentrations variation results agree with the study of taxanes in *Taxus canadensis* needles (Cameron & Smith, 2008), the maximum taxane concentrations were observed in September, and decline over winter. In the study of *Taxus mairei* needles (Ho, Chang, & Tsai, 2000), 10-DAB III concentration maintained similar in the fall, and significantly increased 3-4 times in concentration in early winter, but dropped down to the lowest point in late winter. However, all three major taxane concentrations were much higher in the foliage than in the woody stem tissue based on the results on concentration of taxanes (Cameron & Smith, 2008). Though taxane concentrations in stems were lower in September than November, September would be a better choice to harvest shoots since high concentrations were observed in September. When looking at the yields from only needles and stems, taxane yield decrease over time, meanwhile, the taxane yield from needles, stems, and roots, no significant differences from the differing dates can be concluded from this study. This is due to the Fresh-dry weight and nutrition fluctuations in different dates influence the taxane concentrations. Nutrient contents of the conifer needles are most constant in late autumn or winter,

which is the best season to harvest (Tamm, 1955), and there were very little Fresh-dry weight fluctuations after October (Gary, 1971).

Taxane concentrations varied with shade

Increasing shade reduced the 10-DAB III concentrations in needles and PAC in stems. In contrast PAC concentrations in needles and 10-DAB III and 9-DHB III in stems increased with increasing shade coverage. 10-DAB III concentration was increased slightly at 35% shade coverage in roots, but there were no significant differences observed with 9-DHB III and PAC concentrations. Since Canada yew is a shade-tolerant evergreen shrub, high soil moisture and low solar radiation (e.g., concave and/or north-facing slopes) are suitable for Canada yew growth (Stachowicz & Allison, 1995). High shade coverage can help needles accumulate PAC, and help stems accumulate 10-DAB III and PAC.

When looking at the taxane yield from only needles and stems, increasing shade coverage increase the 9-DHB III and PAC yield, and shade had no significant effect on 10-DAB III yield. For the yield from needles, stems, and roots, increasing shade reduced 10-DAB III and 9-DHB III yield. As a result, if nursery growers of *Taxus canadensis* intending to harvest taxanes only from needles and stems, high shade coverage would be optimal. If nursery growers of *Taxus canadensis* intending to harvest taxanes from needles, stems and roots, the plants should be cultivated under full sun, thus providing the highest taxane content while avoiding the cost of expensive shade structures.

Taxane concentrations varied with clone

The taxane concentrations for clones CA and KC were much higher than other clones in needles and stems, and clone KC tended to have lower variation than CA. Higher taxane concentrations were found in clone JB and CA in roots, but clone JB has high variation compared with other clones. In terms of comparing between yields from only needles and stems and from needles, stems, and roots, clone KC has higher yield than other clones. In other studies about genetic effects on taxane content (Wheeler et al., 1992), significant variation in taxane content exists among and within populations and species. Thus, choosing a clone that can accumulate higher yield of taxanes is helpful for commercial use and clone KC is a better choice. However, needles and stems could be clipped at the hedge, allowing the remainder of the plant to stay in the ground. This could be done, for example, every 2-5 years, whereas the root could only be collected once per plant. Increasing the amount harvested per plant or branch would typically result in the opposite trend in the total taxane yield per unit of dry weight due to the low concentration of taxanes in the woody portions. As a consequence, biomass with low taxane concentration requiring high volume of harvesting will be less valuable to pharmaceutical companies because it increases extraction and purification costs (Cameron, Smith, & Kierstead, 2005). If production aims solely at 9-DHB III (the most abundant of the three taxanes), then in the interest of sustainability it might be better to collect only needles and stems for taxane production as opposed to the whole plant including the roots (which provides only 9.39% higher yield from whole plant). But if all three taxanes are desired, or if 10-DAB III and/or PAC are the main interest, then due to the significant boost in yield from

whole plants, it would prove beneficial to harvest whole plants as opposed to only from needles and stems.

Chlorophyll compared with taxanes

The amount of PSII photochemistry (*Fv/Fm*), Ca, Cb, C *(x+c),* C *(a+b)* and Chl *(a+b)/ (x+c)* increased over time, but in contrast, the ratio of Ca/Cb decreased. As shade coverage increased as well, the amount of PSII photochemistry (*Fv/Fm*), Ca, Cb, C *(x+c),* C *(a+b)* and Chl *(a+b)/ (x+c)* increased, but in contrast, the ratio of Ca/Cb also decreased. Clones IB and 3A had the highest levels of PSII photochemistry (*Fv/Fm*), Ca, Cb, C *(x+c),* C *(a+b),* and Chl *(a+b)/ (x+c).* However, Clone IB and 3A also had lowest Ca/Cb ratio. The Ca, Cb, C *(x+c),* C *(a+b),* and Chl *(a+b)/ (x+c)* had similar trend, the Ca/Cb had opposite trend compared to others.

Chlorophyll b differs from chlorophyll a only in one of the functional groups bonded to the porphyrin (Britton, 1983), and acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll a (Vicaş, Laslo, Pantea, & Bandici, 2010). In our studies, under 35% shade coverage, *T. canadensis* foliage had a higher Chlorophyll *a/b* ratio, because the trend of chlorophyll a concentration increased faster than chlorophyll b, which indicated that leaves had a better developed photosynthetic apparatus. The variation in chlorophyll content is related to leaf development and senescence (Carter & Knapp, 2001). The ratio of chlorophylls to carotenoids *(a + b)*/ (x + c) in this study indicated that none-to-moderate coverage was better for *T. canadensis* growth. At the same time, the maximal efficiency of PSII photochemistry (*Fv/Fm*) can be influenced by any type pf stress (Long & Humphries, 1994), or the induction of sustained quenching

(Demmig‐Adams & Adams, 2006). The results in our study showed that *Fv/Fm* ratio in September was higher, which means there was less stress in September for the plant growth than later in the fall.

Furthermore, the trend of Chlorophyll *a*, Chlorophyll *b*, Chlorophyll (*a+b),* chlorophylls to carotenoids *(a + b)*/ (x + c), and PSII photochemistry (*Fv/Fm*) is proportional to the yield in needles and stems, and opposite to the total yield of all three major taxanes in needles, stems and roots. Therefore, tracking the trend of Chlorophyll *a+b,* chlorophylls to carotenoids *(a + b)*/ (x + c), and PSII photochemistry (*Fv/Fm*) might be helpful tool by which to select a date to harvest where one can extract the greatest amount of taxanes .

Conclusion

T. canadensis nursed with no shade coverage was optimal for needles to accumulate higher 10-DAB III concentrations and stems to accumulate higher PAC concentration. In contrast high shade coverage (65%) is optimal for needles to accumulate higher PAC concentrations and stems to accumulate higher 10-DAB and 9-DHB III concentrations. The best harvest dates are September for needles, and November for stems if a way could be found to collect them separately (as opposed to clipping a whole shoot). 10-DAB III in roots is higher with 35% shade coverage, but 9-DHB III and PAC do not vary significantly as shade coverage changes. When aiming for total yield only in needles and stems, plants under high shade coverage had higher taxane yields, meanwhile, foliage with no shade coverage is optimal if harvest the whole plant (needles, stems and roots). For commercial use, clone KC is the empirical better choice when compared with other clones for nursery cropping. Tracking PSII photochemistry (*Fv/Fm*), Ca, Cb, C *(x+c)*, C *(a+b)*, Chl *(a+b)/ (x+c)*, Ca/Cb variation under different nursed condition may be a better choice to track the yield of three major taxanes rather than analyzing the foliage using HPLC. Since PSII photochemistry (*Fv/Fm*), Ca, Cb, C *(x+c)*, C *(a+b)* and Chl *(a+b)/ (x+c)* have the same variation with three major taxanes, while the ratio of Ca/Cb variation is opposite to three major taxanes, using one or more of these indicators may be be an inexpensive and rapid way to monitor fall taxane development.

Appendix I

Introduction (chlorophyll fluorescence and chlorophyll)

In 1960, the changes in the yield of chlorophyll fluorescence were firstly observed (Kautsky & Appel , 1960). Chlorophyll fluorescence is extensively used as a rapid, efficient and non-invasive tool for identifying functional changes of photosynthetic apparatus under abiotic or biotic stress (Dai, Zhou, & Zhang, 2007). The ratio of F_v (variable fluorescence) and F_m (maximum fluorescence) represents the estimate of maximum quantum efficiency of PSII photochemistry (Kitajima & Butler, 1975), and the ratio is also used to detect stress-induced perturbations in the photosynthetic apparatus (Bertin, Bouharmont, & Kinet, 1997; Bilger & Schreiber, 1986; Dai et al., 2007).

The flow of electrons through PSII indicates the overall rate of photosynthesis in plants (Maxwell & Johnson, 2000), with healthy samples typically achieving a maximum Fv/Fm value of approximately 0.79-0.85, and lower values indicating plant are under stress (Maxwell & Johnson, 2000) in response to drought, fall dormancy or cool temperature.

The plants possess a higher photosynthetic capacity per unit leaf area and chlorophyll (Chl), exhibit higher values of light-harvesting Chl a/b (H K Lichtenthaler, Prenzel, & Kuhn, 1982), and a much lower level of light-harvesting Chl a/b proteins (LHCII) (Hartmut K. Lichtenthaler, Ač, Marek, Kalina, & Urban, 2007). The values for the ratio Chl a/b in sun (range:from 3.0 to 3.4) and shade leaves (range: from 2.4 to 2.7) as well as the values for the ratio chlorophylls to carotenoids $(a + b)/ (x + c)$ (sun leaves 3.8 to 4.4, shade leaves 4.8 to 5.7) are found in the normal range of physiologically active leaves

(Hartmut K Lichtenthaler & Babani, 2004). Physiological and morphological adjustments of yew foliage allows growth under both high and low light conditions, which allows the trees to thrive in a wide range of light environments and indicates that yew is suited to nursery cultivation and regeneration of open sites (Mitchell, 1998).

Scanning chlorophyll fluorescence and analyzing the concentration of chlorophyll are two potential methods of measuring taxanes concentrations that also offer great economy of effort. The purpose of this study is to analyze the trend that chlorophyll fluorescence and chlorophyll change in different date under different shade coverage and compare with the three major taxanes.

Method (chlorophyll fluorescence and chlorophyll)

Chlorophyll Fluorescence

For the chlorophyll fluorescence measurements, two shoots from all test plant were clipped and placed in water (a rose holder) and in sealed bags to prevent them from drying out and were kept between 2-4°C until measured overnight. Before being scanned, shoots were warmed to room temperature in the dark. All preparatory work on shoots was done under a dim green safelight. A FluoroCam imager (Photon System Instruments, Drasov, Czech Republic) was used to measure chlorophyll fluorescence. Before running the Fluorocam experiment, turn on the green safelight and turn off the room lights. The experiment provided the shoots image and the FluoroCam software provide the chlorophyll fluorescence data analysis (e.g. F_0, F_v, F_m, etc.).

The PSII photochemistry operating efficiency was taken on the imager. PSII was measured as F_v/F_m which equals $(F_m-F_0)/F_m$, and does not require a dark-adapted plant measurement (Long & Humphries, 1994; Maxwell & Johnson, 2000).

Chlorophyll Analysis

Following the chlorophyll fluorescence measurement, the two shoots from each plant were placed in 1 or 2 small plastic bags with their bases inserted into two rose holders. Each shoot was processed in an identical manner. The shoots were immediately stored in a refrigerator at 4-5°C until extraction. All needles were removed from each shoot into a small container and mixed. 200 mg of needles were placed in a 15 ml heavy-wall test tube (Pyrex no. 9860), and 2-3 ml of liquid N2 was added to the test tube. The needles were then broken up into small pieces and 10 ml of spectral grade N, N dimethyl

formamide was added to the tube. The test tubes were then placed in a rack and placed in a refrigerator at 4-5°C for 2-4h. The fresh samples were sonicated overnight. Finally, the liquid was scanned from 400-750 nm in a Varian/Cary 1 UV/Vis spectrophotometer in double beam mode.

For the determination of chlorophyll a, chlorophyll b, and total carotenoids, the following formulas were used (Wellburn, 1994):

Chlorophyll a: $Ca = 12.00 * Abs_{663.8} - 3.11 * Abs_{646.8}$ (µg / ml solution)

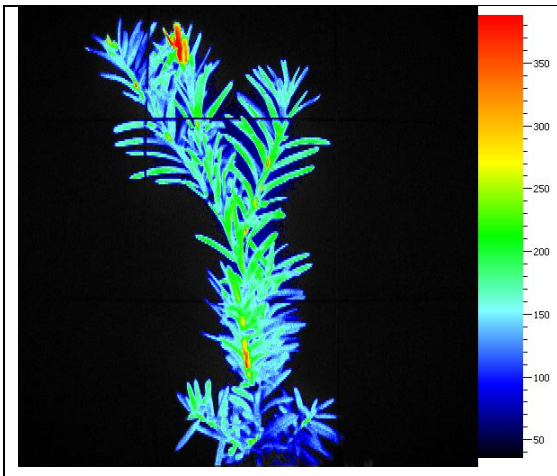
Chlorophyll b: $Cb = 20.78 * Abs_{646.8} - 4.88 * Abs_{663.8}$ (µg per ml solution)

Total carotenoids: $Cx+c = (1000 * Abs_{480.00} - 1.12 * Ca - 34.07 * Cb) / 245$ (µg per ml solution)

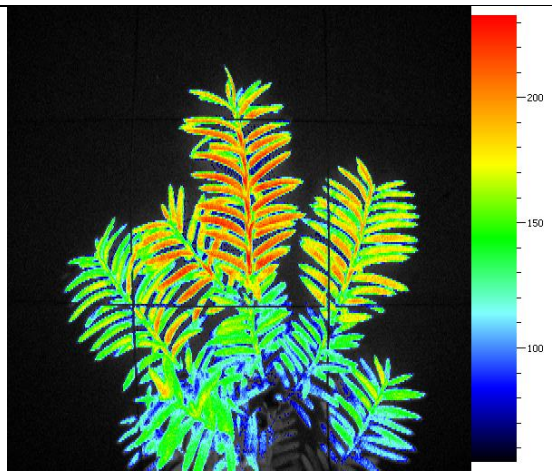
Results (chlorophyll fluorescence and chlorophyll)

Chlorophyll Fluorescence Analysis

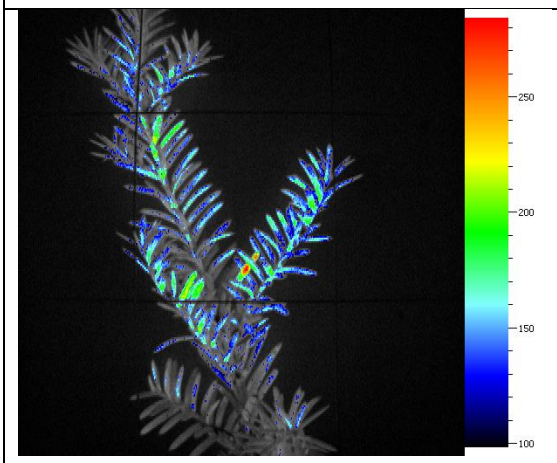
Chlorophyll fluorescence images are chosen from CFS of clone KC under 35% shade coverage and 65% shade coverage September and November, shows in Figure 38.



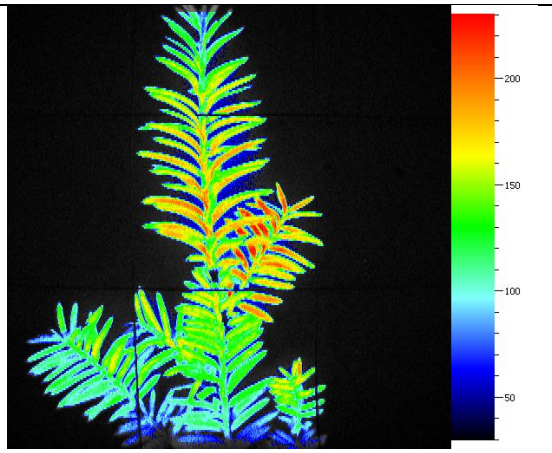
09-19-2007-CFS-B3-KC (35% shade coverage)



09-19-2007-CFS-B2-KC (65% shade coverage)



11-19-2007-CFS-B3-KC (35% shade coverage)



11-19-2007-CFS-B2 (65% shade coverage)

Figure 38. Chlorophyll fluorescence images from CFS of clone KC under 35% shade coverage and 65% shade coverage September and November

Two conclusions can be drawn from the figures below: (1) PSII photochemistry (*Fv/Fm*) increases with increasing shade levels within a date, with a gradual decrease with each successive collection date; (2) PSII photochemistry (*Fv/Fm*) of clone IB is higher than other clones.

The interaction between Date and Shade is shown by beanplots in Figure 40. PSII photochemistry (*Fv/Fm*) in needles increased over time (Figure 40). Increasing shade also increased the concentrations except November (Figure 40). A little decrease was observed under 55% shade coverage in November but increased overall (Figure 40). Minor increases were observed in October than September (Figure 40). PSII photochemistry (*Fv/Fm*) in November tended to have higher variation than other dates, September tended to have the lowest variation (Figure 40). In terms of clone, the PSII photochemistry (*Fv/Fm*) for clones IB was higher than other clones (Figure 39), Clone JB tended to have higher variation than all of the other clones (Figure 39).

The ANOVA table for the full repeated measures mixed effects ANOVA for PSII photochemistry (*Fv/Fm*) in needles is shown in Table 17, and the reduced model is shown in Table 18. The only interaction that was significant was the Shade*Date interaction. In terms of random effects Date nested within Site accounted for a slightly variation, while September versus October accounted for very little additional variation.

Table 17. ANOVA table for PSII photochemistry (*Fv/Fm*) in needles (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	0.1413	0.02825	6.6276	9.72e-06*
shade	3	0.5461	0.18202	42.7025	6.79e-26*
date	2	3.5316	1.7658	414.2612	4.37e-133*
Clone*shade	15	0.0979	0.00653	1.531	0.323435
Clone*date	10	0.0862	0.00862	2.0223	0.090234
Shade*date	6	0.1002	0.0167	3.9188	0.001743*
Clone*shade*date	30	0.1226	0.00409	0.9588	1.559794
Random Effects			Variance		
Site	1		0.0001148		0.0005783*
Date Sep vs Oct / site	5		1.925e-07		0.9693
Date Sep vs Nov / site			0.00001154		
Residual	1033		0.004263		

Table 18. ANOVA table for PSII photochemistry (*Fv/Fm*) in needles (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	0.1459	0.02918	6.7335	7.62e-06 *
shade	3	0.535	0.17833	41.1469	4.61e-25 *
date	2	4.5763	2.28814	527.9594	2.84e-161 *
Shade*date	6	0.0941	0.01569	3.6203	0.003582*
Random Effects			Variance		
Site	1		1.08e-02		0.00105*
Date Sep vs Oct / site	5		1.35e-05		0.9934
Date Sep vs Nov / site			2.39e-03		
Residual	1088		6.58e-02		

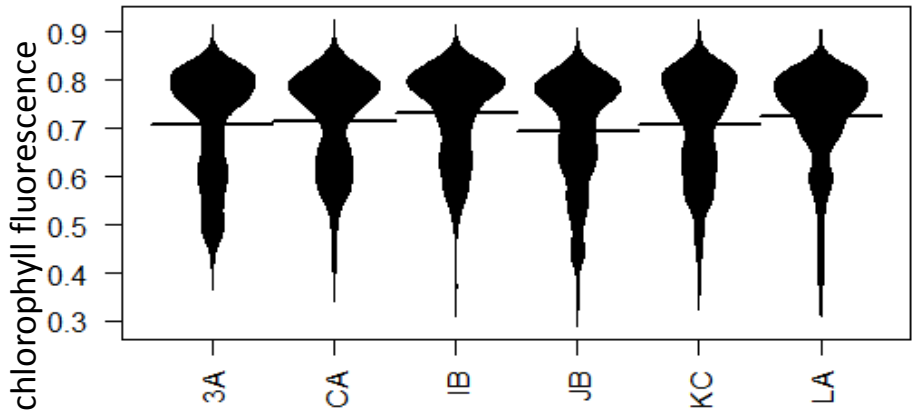


Figure 39. Bean plot for PSII photochemistry (*Fv/Fm*) of different clones in needles (Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

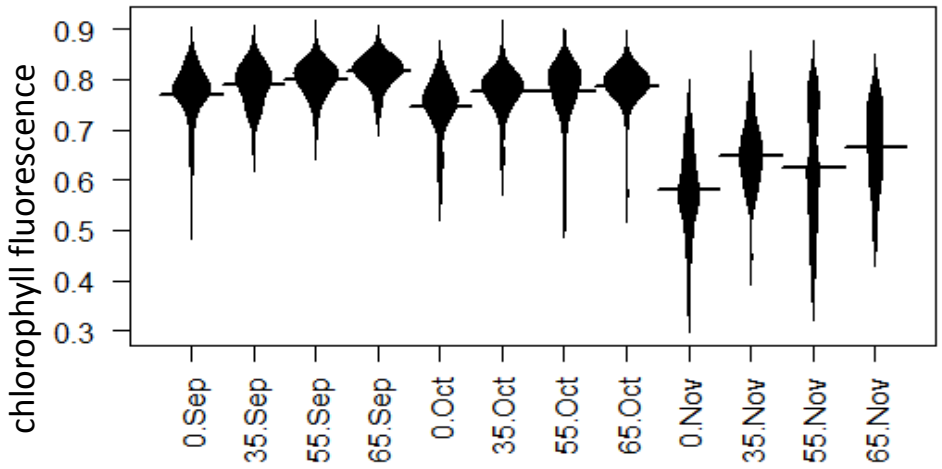


Figure 40. Bean plot for PSII photochemistry (*Fv/Fm*) in needles with shade and date as interaction (Shade-0%, 35%, 55%, 65%; Date-September, October, November).

Chlorophyll Analysis

Chlorophyll a concentration

Two conclusions can be drawn from the figures below: (1) Chlorophyll a concentration increases with increasing shade levels within a date, with a gradual decrease with each successive collection date; (2) Chlorophyll a concentration for clones IB is higher than other clones under 35%, 55% and 65% shade coverage, clone 3A is higher than other clones with no shade coverage.

The interaction between Date and Shade is shown by beanplots in Figure 41, and the interaction between Clone and Shade is shown by beanplots in Figure 42. Chlorophyll a concentration in needles increased over time (Figure 41). Increasing shade also increased the concentrations (Figure 41, and Figure 42) in November (Figure 41). A little decrease was observed under 55% shade coverage in September and October but increased overall (Figure 41). Minor increases were observed in October than September (Figure 41). Chlorophyll a concentration in October tended to have higher variation than other dates (Figure 41). In terms of clone, the Chlorophyll a concentration for clones IB was higher than other clones under 35%, 55% and 65% shade coverage, clone 3A was higher than other clones under 0% shade coverage (Figure 42).

The ANOVA table for the full repeated measures mixed effects ANOVA for chlorophyll a concentration in needles is shown in Table 19, and the reduced model is shown in Table 20. The interactions that were significant were the Shade*Date and Clone*Shade interactions. In terms of random effects Date nested within Site accounted for a

significant amount of variation with Site accounting for most of that variation, while September versus November accounted for very little additional variation.

Table 19. ANOVA table of table for chlorophyll a in needles (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	5980464	1196093	21.3663	4.87e-20*
shade	3	6661276	2220425	39.6643	3.91e-24*
date	2	2184005	1092002	19.5069	4.66e-09*
Clone*shade	15	2941113	196074	3.5025	3.64e-05*
Clone*date	10	1055853	105585	1.8861	0.134071
Shade*date	6	1558976	259829	4.6414	0.000291*
Clone*shade*date	30	2917868	97262	1.7374	0.060900
Random Effects			Variance		
Site	1		3962.9		1.297e-07*
Date Sep vs Oct / site	5		1373.5		0.1406
Date Sep vs Nov / site			986.2		
Residual	1030		55980.4		

Table 20. ANOVA table of table for chlorophyll a in needles (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	5986309	1197262	20.7736	1.67e-19*
Shade	3	6658043	2219348	38.5077	1.66e-23*
Date	2	1970806	985403	17.0976	4.75e-08*
Shade*date	6	1497443	249574	4.3303	0.000631*
Clone*shade	15	2955012	197001	3.4181	5.66e-05*
Random Effects			Variance		
Site	1		4263		1.231e-07*
Date Sep vs Oct / site	5		1434		0.1173
Date Sep vs Nov / site			1209		
Residual	1070		57634		

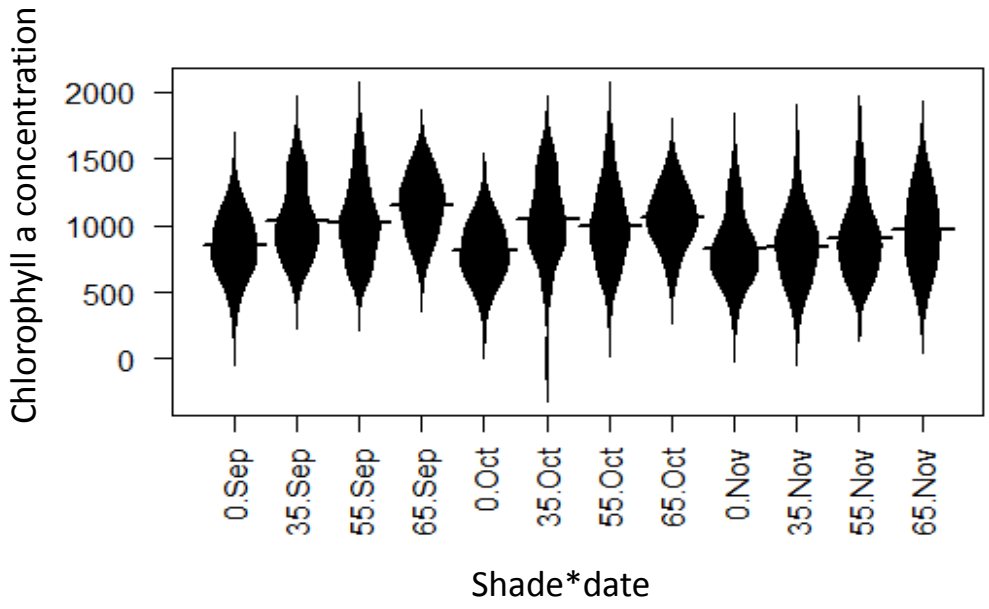


Figure 41. Bean plot for chlorophyll a in needles with shade and date as interaction (Shade-0%, 35%, 55%, 65%; Date-September, October, November).

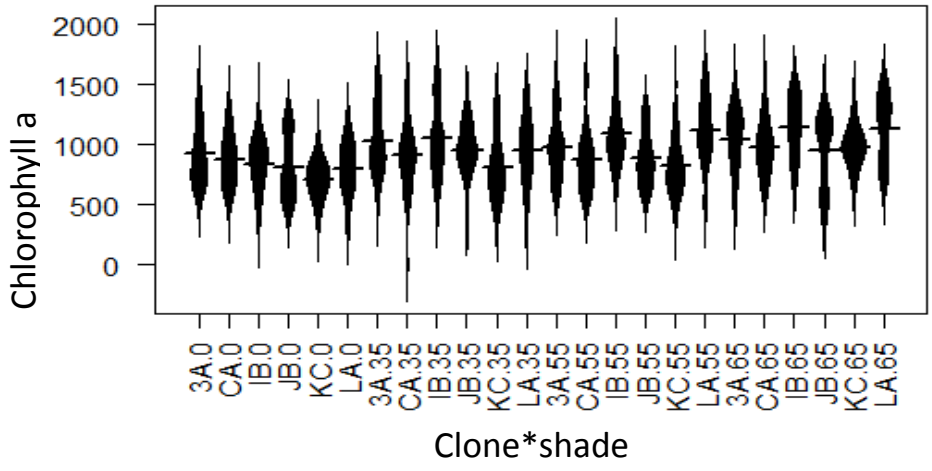


Figure 42. Bean plot for chlorophyll a in needles with clone and shade as interaction (Shade-0%, 35%, 55%, 65%; Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

Chlorophyll b concentration

Two conclusions can be drawn from the figures below: (1) Chlorophyll b concentration increases with increasing shade levels within a date, with a gradual decrease with each successive collection date; (2) Chlorophyll b concentration for clones IB is higher than other clones under 35%, 55% and 65% shade coverage, clone 3A is higher than other clones with no shade coverage.

The interaction between Clone and Shade is shown by beanplots in Figure 43, the interaction between Clone and Date is shown by beanplots in Figure 44, and the interaction between Date and Shade is shown by beanplots in Figure 45. Chlorophyll b concentration in needles decreased over time (Figure 44 and Figure 45). Increasing shade increased the concentrations (Figure 43, and Figure 45) in September (Figure 45). A little decrease was observed under 55% shade coverage in October, and under 35% shade coverage in November but increased overall (Figure 45). Chlorophyll b concentration in October tended to have higher variation than other dates (Figure 45). In terms of clone, the Chlorophyll b concentration for clone IB was higher than other clones (Figure 43 and Figure 44) under 35%, 55% and 65% shade coverage (Figure 43), clone 3A was higher than other clones under 0% shade coverage (Figure 43).

The ANOVA table for the full repeated measures mixed effects ANOVA for chlorophyll b concentration in needles is shown in Table 21. All the fixed effects interactions were significant. In terms of random effects Date nested within Site accounted for a significant amount of variation with September versus November accounting for most of that variation, while September versus October and September versus November accounted for less variation.

Table 21. ANOVA table of table for chlorophyll b in needles (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	906405	181281	18.2959	4.39e-17*
shade	3	943259	314420	31.7329	1.73e-19*
date	2	436810	218405	22.0426	4.06e-10*
Clone*shade	15	463879	30925	3.1211	0.000274*
Clone*date	10	285357	28536	2.88	0.005404*
Shade*date	6	377544	62924	6.3506	3.68e-06*
Clone*shade*date	30	566268	18876	1.905	0.018946*
Random Effects			Variance		
Site	1		1164		1.435e-09*
Date Sep vs Oct / site	5		1149		1.8e-10*
Date Sep vs Nov / site			1505		
Residual	1030		9908		

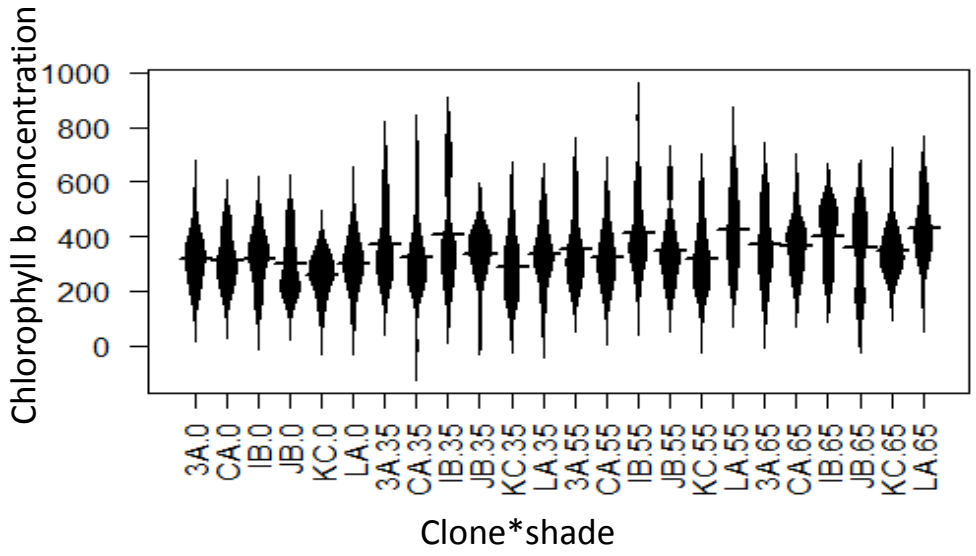


Figure 43. Bean plot for chlorophyll b in needles with clone and shade as interaction (Shade-0%, 35%, 55%, 65%; Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

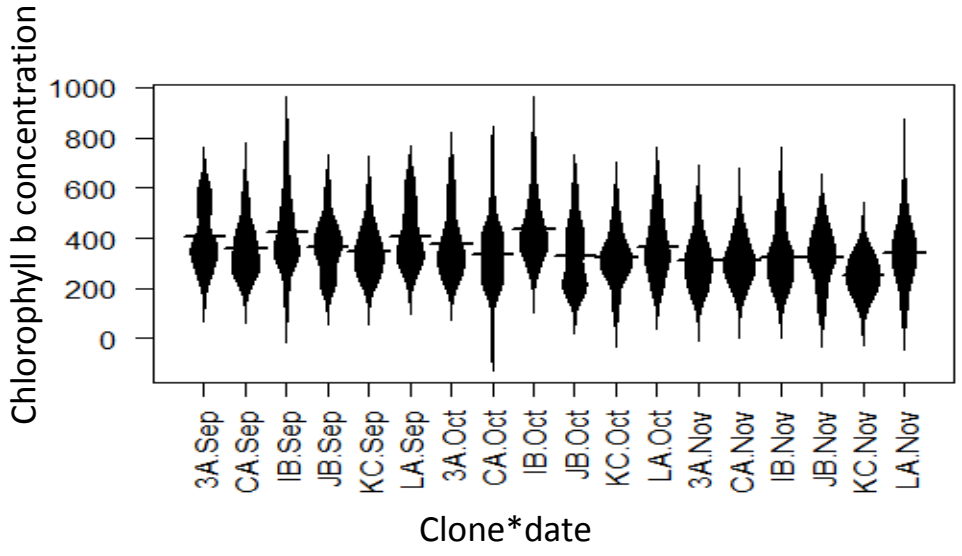


Figure 44. Bean plot for chlorophyll b in needles with clone and date as interaction (Date-September, October, November; Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

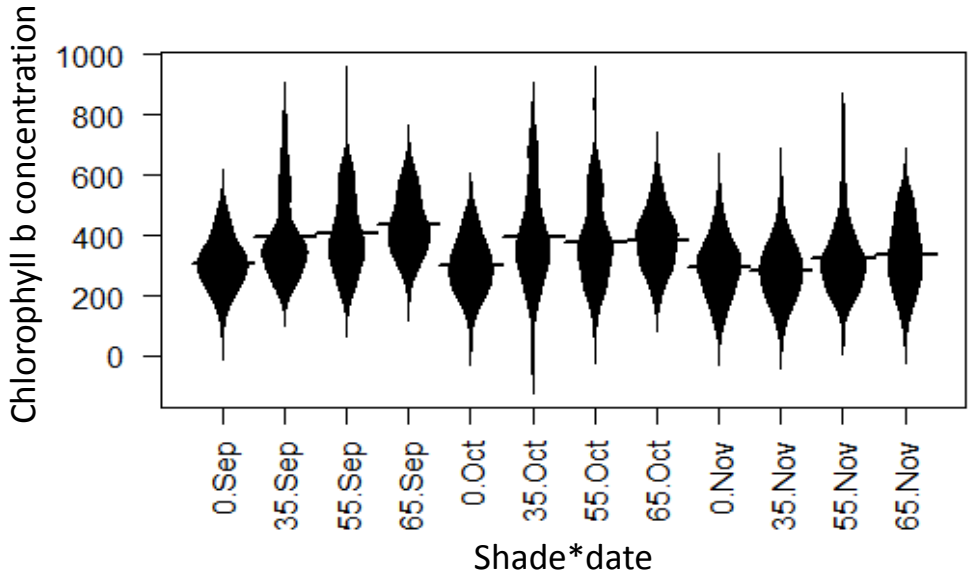


Figure 45. Bean plot for chlorophyll b in needles with shade and date as interaction (Shade-0%, 35%, 55%, 65%; Date-September, October, November).

Carotenoid concentration

Two conclusions can be drawn from the figures below: (1) Carotenoid concentration increases with increasing shade levels within a date and increases with each successive collection date; (2) Carotenoid concentration for clones 3A and IB are higher than other clones.

The interaction between Clone and Shade is shown by beanplots in Figure 46, the interaction between Clone and Date is shown by beanplots in Figure 47, and the interaction between Date and Shade is shown by beanplots in Figure 48. Carotenoid concentration in needles increased over time (Figure 47and Figure 48). Increasing shade increased the concentrations (Figure 46, and Figure 48). A little decrease was observed under 55% shade coverage in September and October, and under 35% shade coverage in

November but increased overall (Figure 48). In terms of clone, the Carotenoid concentration for clones 3A and IB were higher than other clones (Figure 46 and Figure 47).

The ANOVA table for the full repeated measures mixed effects ANOVA for Carotenoid concentration in needles is shown in Table 22, and the reduced model is shown in Table 23. All the fixed effects interactions were significant, except Clone*Shade*Date. In terms of random effects Date nested within Site accounted for a significant amount of variation with Site accounting for most of that variation, while September versus October accounted for very little additional variation.

Table 22. ANOVA table of table for Carotenoid concentration in needles (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	325503	65101	24.2343	8.97e-23*
shade	3	127636	42545	15.8379	6.32e-10*
date	2	35109	17555	6.5349	0.001494*
Clone*shade	15	107973	7198	2.6796	0.002553*
Clone*date	10	63280	6328	2.3557	0.031967*
Shade*date	6	100408	16735	6.2296	5.04e-06*
Clone*shade*date	30	137134	4571	1.7017	0.076966
Random Effects			Variance		
Site	1		276.29		7.901e-11*
Date Sep vs Oct / site	5		2.04		0.1841
Date Sep vs Nov / site			44.15		
Residual	1030		2686.3		

Table 23. ANOVA table of table for Carotenoid concentration in needles (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	325378	65076	23.7572	2.36e-22*
Shade	3	127650	42550	15.5338	9.58e-10*
Date	2	31909	15954	5.8245	0.003016*
Clone*shade	15	108058	7204	2.6299	0.003241*
Clone*date	10	63332	6333	2.3121	0.036753*
Shade*date	6	100352	16725	6.106	6.90e-06*
Random Effects			Variance		
Site	1		283.173		1.414e-10*
Date Sep vs Oct / site	5		1.776		0.1393
Date Sep vs Nov / site			52.072		
Residual	1060		2739.189		

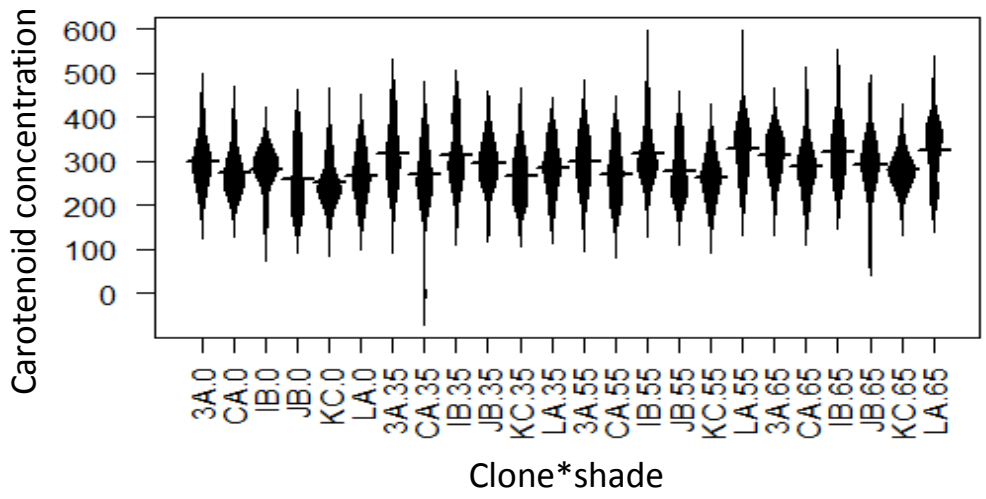


Figure 46. Bean plot for Carotenoid concentration in needles with clone and shade as interaction (Shade-0%, 35%, 55%, 65%; Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

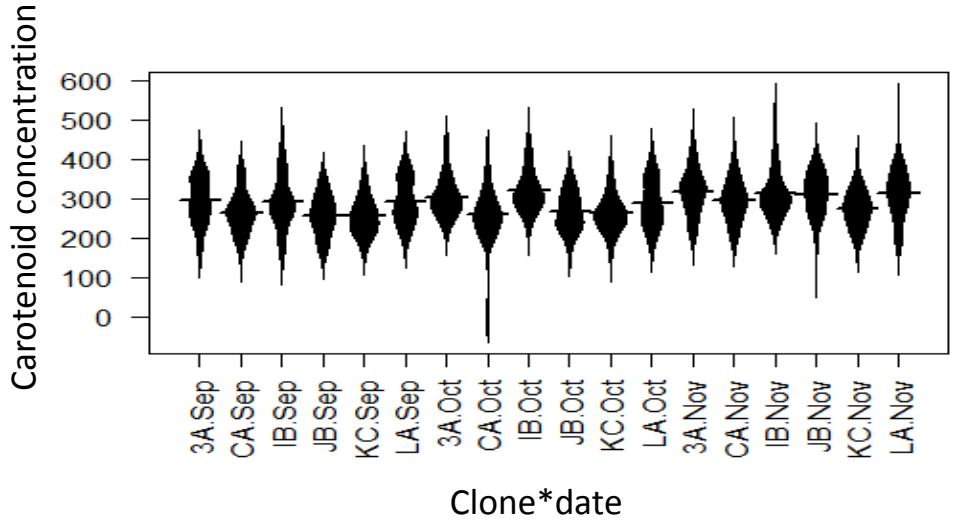


Figure 47. Bean plot for Carotenoid concentration in needles with clone and date as interaction (Date-September, October, November; Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

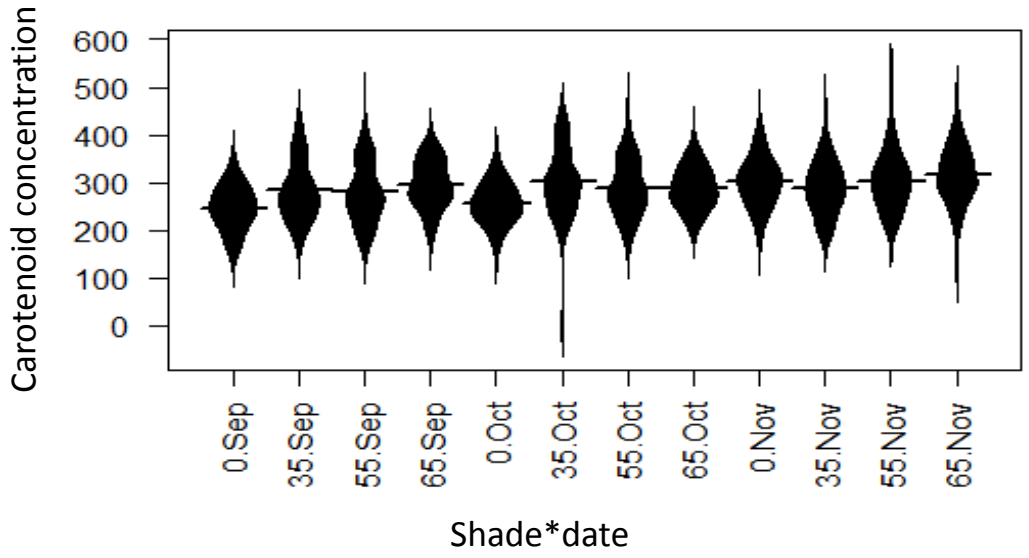


Figure 48. Bean plot for Carotenoid concentration in needles with shade and date as interaction (Shade-0%, 35%, 55%, 65%; Date-September, October, November).

Chlorophyll a/b ratio

Three conclusions can be drawn from the figures below: (1) Chlorophyll a/b ratio increases with each successive collection date; (2) Chlorophyll a/b ratio for clones 3A is higher than other clones; (3) Chlorophyll a/b ratio with 35% shade coverage was higher than other shade coverages.

The interaction between Clone and Date is shown by beanplots in Figure 50. Chlorophyll a/b ratio in needles increased over time (Figure 50). The Chlorophyll a/b ratio for clone 3A was much higher than other clones (Figure 50). In terms of shade, the Chlorophyll a/b ratio increased from 0% to 35% shade coverage, decreased from 35% to 55% shade coverage, and raised from 55% to 65% shade coverage (Figure 49). Overall, the

Chlorophyll a/b ratio under 35% shade coverage was higher than other shade coverages (Figure 49).

The ANOVA table for the full repeated measures mixed effects ANOVA for chlorophyll a concentration in needles is shown in Table 24, and the reduced model is shown in Table 25. The interactions that were significant were the Clone*Date and Clone*Shade*Date interactions. In terms of random effects Date nested within Site accounted for a slightly variation with September versus November accounting for most of that variation, while Site accounted for very little additional variation.

Table 24. ANOVA table of table for chlorophyll a/b ratio in needles (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	2.711	0.5422	3.8562	0.003835*
shade	3	2.5561	0.85205	6.0598	0.000608*
date	2	3.9482	1.97408	14.0397	9.39e-07*
Clone*shade	15	3.6648	0.24432	1.7376	0.158586
Clone*date	10	4.295	0.4295	3.0546	0.002903*
Shade*date	6	1.5026	0.25044	1.7811	0.205200
Clone*shade*date	30	10.3423	0.34474	2.4518	0.000232*
Random Effects			Variance		
Site	1		0.003927		0.3443
Date Sep vs Oct / site	5		0.024551		< 2.2e-16*
Date Sep vs Nov / site			0.037639		
Residual	1030		0.140607		

Table 25. ANOVA table of table for chlorophyll a/b ratio in needles (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	2.711	0.5422	3.8562	0.003835*
Shade	3	2.5561	0.85205	6.0598	0.000608*
Date	2	3.9482	1.97408	14.0397	9.39e-07*
Clone*date	10	4.1715	0.41715	2.9668	0.003974*
Clone*Shade*Date	51	15.6333	0.30653	2.1801	7.36e-05*
Random Effects			Variance		
Site	1		0.003927		0.3443
Date Sep vs Oct / site	5		0.024551		< 2.2e-16*
Date Sep vs Nov / site			0.037639		
Residual	1030		0.140607		

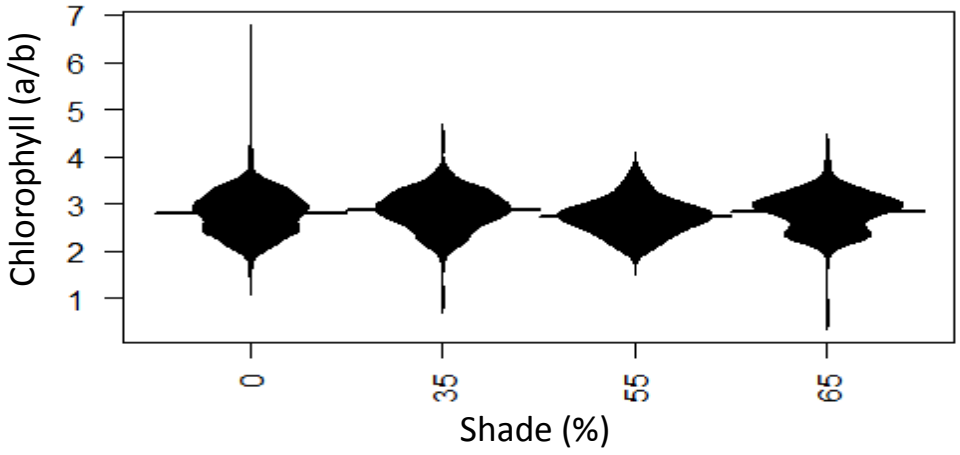


Figure 49. Bean plot for chlorophyll a/b ratio of different shade in needles (Shade-0%, 35%, 55%, 65%).

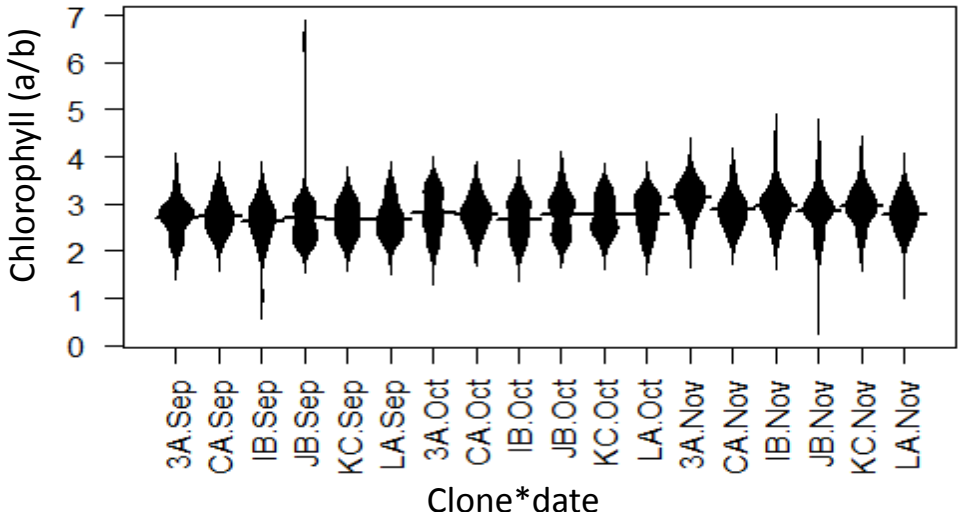


Figure 50. Bean plot for chlorophyll a/b ratio in needles with clone and date as interaction (Date-September, October, November; Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

Chlorophyll a+b

Two conclusions can be drawn from the figures below: (1) Chlorophyll (a+b) concentration increases with increasing shade levels within a date, with a gradual decrease with each successive collection date; (2) Chlorophyll (a+b) concentration for clones IB is higher than other clones under 35%, 55% and 65% shade coverage, clone 3A is higher than other clones with no shade coverage.

The interaction between Clone and Shade is shown by beanplots in Figure 51, and the interaction between Date and Shade is shown by beanplots in Figure 52. Chlorophyll (a+b) concentration in needles decreased over time (Figure 52). Increasing shade also increased the concentrations (Figure 51, and Figure 52) except October (Figure 52). A little decrease was observed under 55% shade coverage in October but increased overall (Figure 52). Minor increases were observed in September than November (Figure 41). In terms of clone, the Chlorophyll (a+b) concentration for clones IB was higher than other clones under 35%, 55% and 65% shade coverage, clone 3A was higher than other clones under 0% shade coverage (Figure 51).

The ANOVA table for the full repeated measures mixed effects ANOVA for chlorophyll (a+b) concentration in needles is shown in Table 26, and the reduced model is shown in Table 27. The interactions that were significant were the Clone*Shade and Shade*Date interactions. In terms of random effects Date nested within Site accounted for a significant amount of variation with Site accounting for most of that variation, while September versus October and September versus November accounted for similar additional variation.

Table 26. ANOVA table of table for chlorophyll (a+b) in needles ANOVA (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	11436104	2287221	21.3663	4.87e-20*
shade	3	12522877	4174292	38.9946	9.58e-24*
date	2	4278360	2139180	19.9834	2.94e-09*
Clone*shade	15	5474626	364975	3.4095	5.99e-05*
Clone*date	10	2277795	227780	2.1278	0.065613
Shade*date	6	3419108	569851	5.3233	5.19e-05*
Clone*shade*date	30	5590016	186334	1.7407	0.059579
Random Effects			Variance		
Site	1		9436		1.197e-08*
Date Sep vs Oct / site	5		5034		0.002331*
Date Sep vs Nov / site			4921		
Residual	1030		107048		

Table 27. ANOVA table of table for chlorophyll (a+b) in needles ANOVA (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	11441397	2288279	20.715	1.90e-19*
Shade	3	12519385	4173128	37.7779	4.45e-23*
Date	2	3992035	1996018	18.0692	1.85e-08*
Clone*Shade	15	5479814	365321	3.3071	0.000102*
Shade*Date	6	3319090	553182	5.0078	0.000115*
Random Effects			Variance		
Site	1		9910		1.68e-08*
Date Sep vs Oct / site	5		5194		0.002027*
Date Sep vs Nov / site			5519		
Residual	1070		110465		

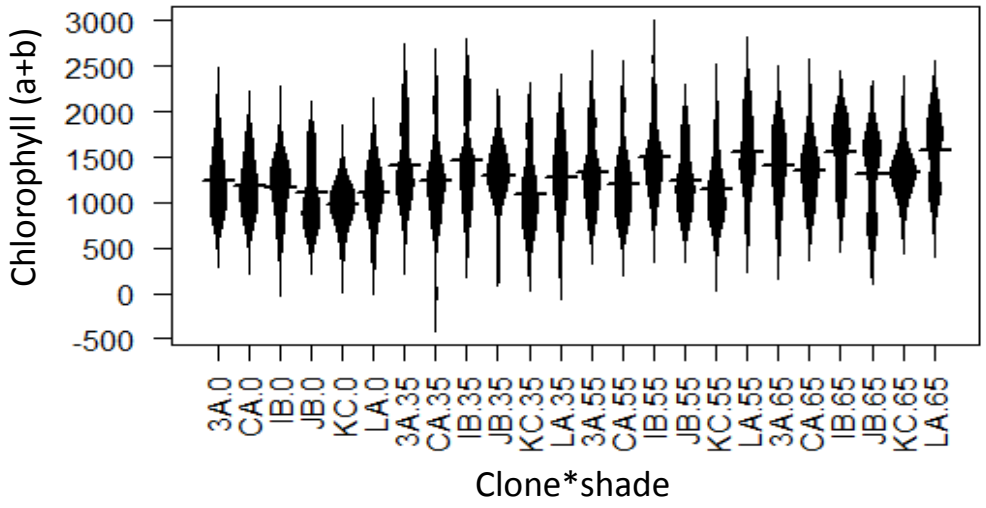


Figure 51. Bean plot for chlorophyll (a+b) in needles with clone and shade as interaction (Shade-0%, 35%, 55%, 65%; Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

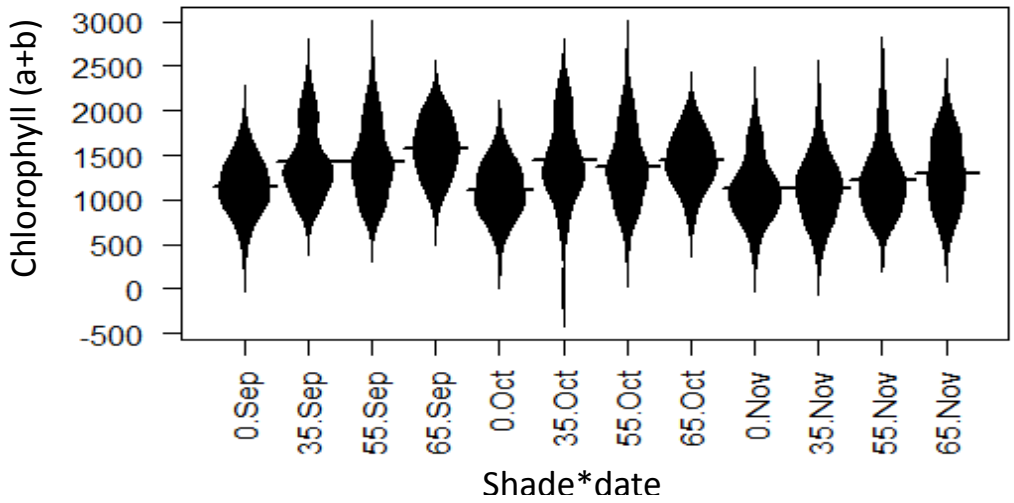


Figure 52. Bean plot for chlorophyll (a+b) in needles with shade and date as interaction (Shade-0%, 35%, 55%, 65%; Date-September, October, November).

Chlorophyll (a+b) / Carotenoid (x+c)

Two conclusions can be drawn from the figures below: (1) Chlorophyll (a+b) / Carotenoid (x+c) concentration increases with increasing shade levels within a date, with a gradual decrease with each successive collection date; (2) Chlorophyll (a+b) / Carotenoid (x+c) concentration for clones IB is higher than other clones under 35%, 55% and 65% shade coverage, clone 3A is higher than other clones with no shade coverage. The interaction between Clone and Shade is shown by beanplots in Figure 53, and the interaction between Date and Shade is shown by beanplots in Figure 54. Chlorophyll (a+b) / Carotenoid (x+c) concentration in needles decreased over time (Figure 54). Increasing shade also increased the concentrations (Figure 53). In terms of clone, the Chlorophyll (a+b) / Carotenoid (x+c) for clones IB was higher than other clones under 35%, 55% and 65% shade coverage, clone 3A was higher than other clones under 0% shade coverage (Figure 53). The Chlorophyll (a+b) / Carotenoid (x+c) for clone IB was higher in September and October than other clones, and clone CA was higher in November than other clones (Figure 53).

The ANOVA table for the full repeated measures mixed effects ANOVA for chlorophyll (a+b) / Carotenoid (x+c) concentration in needles is shown in Table 28, and the reduced model is shown in Table 29. The interactions that were significant were the Clone*Shade and Clone*Date interactions. In terms of random effects Date nested within Site accounted for a slightly variation with September versus October accounting for most of that variation, while Site accounted for very little additional variation.

Table 28. ANOVA table of table for chlorophyll (a+b)/(x+c) in needles (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	12.902	2.58	9.5912	1.31e-08*
shade	3	47.5	15.833	58.8527	5.16e-35*
date	2	246.019	123.01	457.231	4.01e-143*
Clone*shade	15	15.074	1.005	3.7353	5.99e-05*
Clone*date	10	6.644	0.664	2.4697	0.022008*
Shade*date	6	3.842	0.64	2.3801	0.061435
Clone*shade*date	30	14.43	0.481	1.7879	0.043330*
Random Effects			Variance		
Site	1		0.004543		0.1591
Date Sep vs Oct / site	5		0.086509		1.886e-15*
Date Sep vs Nov / site			0.014468		
Residual	1030		0.269032		

Table 29. ANOVA table of table for chlorophyll (a+b)/(x+c) in needles (reduced model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
Clone	5	12.902	2.58	9.5912	1.31e-08*
Shade	3	47.5	15.833	58.8527	5.16e-35*
Date	2	246.019	123.01	457.231	4.01e-143*
Clone*Shade	15	15.074	1.005	3.7353	1.03e-05*
Clone*Date	10	6.644	0.664	2.4697	0.022008*
Clone*shade*date	36	18.272	0.508	1.8866	0.011927
Random Effects			Variance		
Site	1		0.00523		0.1591
Date Sep vs Oct / site	5		0.08788		1.886e-15*
Date Sep vs Nov / site			0.016		
Residual	1030		0.27497		

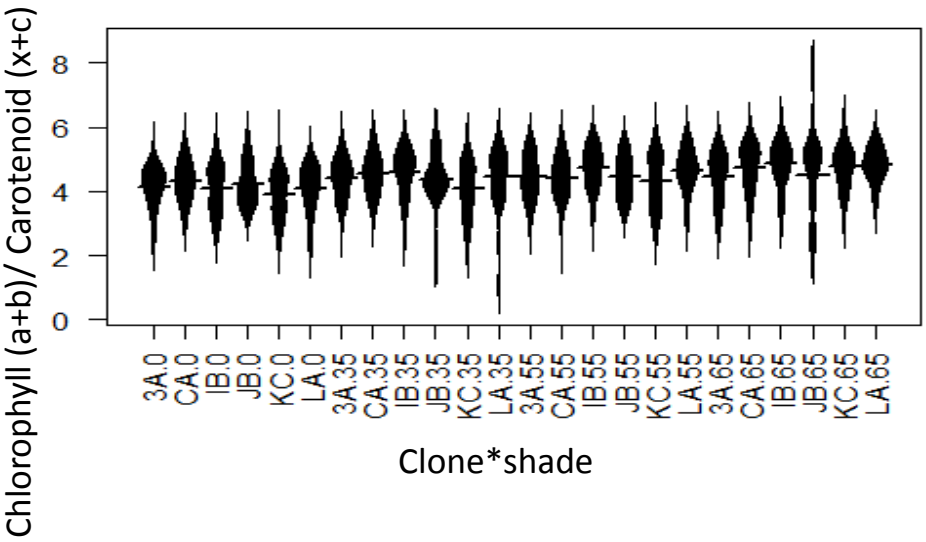


Figure 53. Bean plot for Chlorophyll (a+b)/Carotenoid (x+c) in needles with clone and shade as interaction (Shade-0%, 35%, 55%, 65%; Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

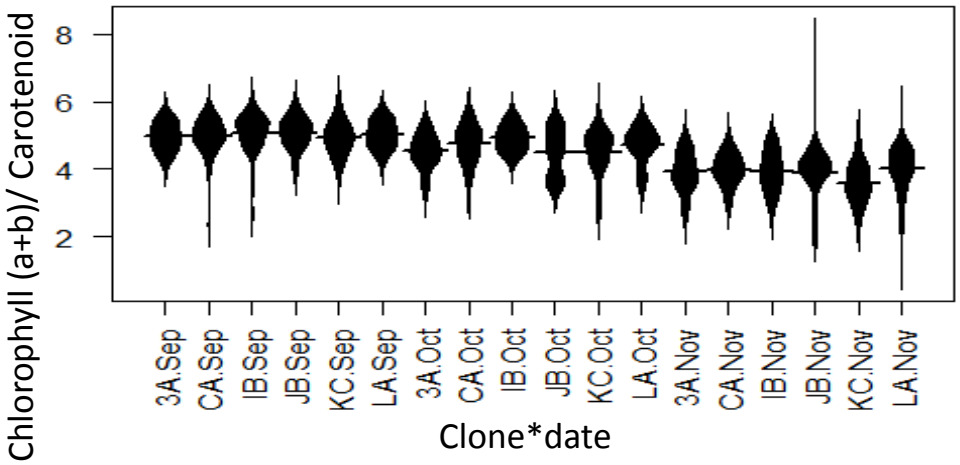


Figure 54. Bean plot for Chlorophyll (a+b)/Carotenoid (x+c) in needles with clone and date as interaction (Date-September, October, November; Clone-3A=3 Mile A; CA=Coleman A; IB=Industrial Park B; JB=Jalbert B; KC=Kelly C; LA=Lookout A).

HPLC Analysis full model tables

Needles

Table 30. ANOVA table of table for 10-DAB III in needles (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	15497274	3099455	205.8673	1.88e-119*
shade	3	327234	109078	7.245	0.000126*
date	2	189958	94979	6.3085	0.001920*
Clone*shade	15	298026	19868	1.3197	0.594004
Clone*date	10	133124	13312	0.8842	0.948085
Shade*date	6	343144	57191	3.7986	0.002492*
Clone*shade*date	30	417179	13906	0.9236	1.547211
Random Effects			Variance		
Site	1		334.037		0.02245*
Date Sep vs Oct / site	5		9.375		0.09383
Date Sep vs Nov / site			613.212		
Residual	505		15055.595		

Table 31. ANOVA table of table for 9-DHB III in needles (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	335054450	67010890	155.936	5.76e-100*
shade	3	3666903	1222301	2.8443	0.049647*
date	2	23396610	11698305	27.2222	5.35e-12*
Clone*shade	15	10546146	703076	1.6361	0.231148
Clone*date	10	2897176	289718	0.6742	0.910970
Shade*date	6	2935331	489222	1.1384	0.571875
Clone*shade*date	30	13938118	464604	1.0811	1.321310
Random Effects			Variance		
Site	1		168		1
Date Sep vs Oct / site	5		506		1
Date Sep vs Nov / site			1.08		
Residual	505		430000		

Table 32. ANOVA table of table for PAC in needles (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	2269608	453922	98.0712	5,21e-72*
shade	3	88032	29344	6.3399	0.000437*
date	2	112388	56194	12.1409	6.76e-06*
Clone*shade	15	45467	3031	0.6549	0.916411
Clone*date	10	31846	3185	0.688	0.930721
Shade*date	6	69415	11569	2.4996	0.048286*
Clone*shade*date	30	148333	4944	1.0683	1.354018
Random Effects			Variance		
Site	1		1981.41		< 2.2e-16*
Date Sep vs Oct / site	5		74.48		0.03709*
Date Sep vs Nov / site			109.06		
Residual	505		4628.49		

Stem

Table 33. ANOVA table of table for 10-DAB III in stems (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	1601209	320242	182.4593	9.62e-111*
shade	3	43361	14454	8.235	3.21e-05*
date	2	35128	17564	10.0071	5.26e-05*
Clone*shade	15	22842	1523	0.8676	1.143971
Clone*date	10	17106	1711	0.9746	0.889980
Shade*date	6	1674	279	0.159	0.213230
Clone*shade*date	30	25956	865	0.493	0.167698
Random Effects			Variance		
Site	1		1.36e-10		1
Date Sep vs Oct / site	5		4.84e-12		1
Date Sep vs Nov / site			7.56e-11		
Residual	505		1.76e+03		

Table 34. ANOVA table of table for 9-DHB III in stems (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	4993492	998698	35.1517	1.32e-30*
shade	3	260429	86810	3.0555	0.037621*
date	2	105000	52500	1.8479	0.157479
Clone*shade	15	502991	33533	1.1803	0.811883
Clone*date	10	272260	27226	0.9583	0.902607
Shade*date	6	34740	5790	0.2038	0.305863
Clone*shade*date	30	1053032	35101	1.2355	0.86238
Random Effects			Variance		
Site	1		3493.9		2.64e-09*
Date Sep vs Oct / site	5		199.2		0.7291
Date Sep vs Nov / site			440.3		
Residual	505		28411.1		

Table 35. ANOVA table of table for PAC in stems (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	90875	18175	28.6062	2.76e-25*
shade	3	23163	7720.8	12.1521	1.48e-07*
date	2	1907	953.6	1.5009	0.222599
Clone*shade	15	13427	895.1	1.4089	0.468706
Clone*date	10	7107	710.7	1.1186	0.752254
Shade*date	6	16574	2762.4	4.3478	0.000666*
Clone*shade*date	30	17046	568.2	0.8943	1.532470
Random Effects			Variance		
Site	1		11.92296		0.6961
Date Sep vs Oct / site	5		0.01569		0.003333*
Date Sep vs Nov / site			58.91652		
Residual	505		635.35316		

Roots

Table 36. ANOVA table for 10-DAB III in roots (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Clone	5	766878	153376	4.0315	0.002565
Shade	3	272392	90797	2.3866	0.075063
Clone*Shade	15	544781	36319	0.9546	0.50949
Residuals	81	3081587	38044		

Table 37. ANOVA table of table for 9-DHB III in roots (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Clone	5	605390	121078	4.4179	0.001313
Shade	3	156366	52122	1.9018	0.135845
Clone*Shade	15	309527	20635	0.7529	0.723876
Residuals	81	2219912	27406		

Table 38. ANOVA table of table for PAC in roots (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Clone	5	438696	87739	9.196	5.83E-07
Shade	3	11714	3905	0.4093	0.7468
Clone*Shade	15	113091	7539	0.7902	0.6847
Residuals	81	772825	9541		

Taxanes Yield full model tables

Taxanes Yield from needles and stems

Table 39. ANOVA table of table for 10-DAB III in needles and stems (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	5376312	1075262	204.1974	7.47e-119*
shade	3	18777	6259	1.1886	0.378955
date	2	47220	23610	4.4837	0.011540*
Clone*shade	15	30241	2016	0.3829	0.221535
Clone*date	10	37359	3736	0.7095	0.944743
Shade*date	6	72445	12074	2.2929	0.074785
Clone*shade*date	30	110369	3679	0.6987	0.941106
Random Effects			Variance		
Site	1		0		0.7914
Date Sep vs Oct / site	5		1.462		0.7026
Date Sep vs Nov / site			161.806		
Residual	505		5265.799		

Table 40. ANOVA table of table for 9-DHB III in needles and stems (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	88036911	17607382	113.1091	5.55e-80*
shade	3	1573850	524617	3.3701	0.024798*
date	2	4035581	2017790	12.9622	3.08e-06*
Clone*shade	15	3057543	203836	1.3094	0.609422
Clone*date	10	1785841	178584	1.1472	0.721568
Shade*date	6	764364	127394	0.8184	0.772096
Clone*shade*date	30	4267045	142235	0.9137	1.544592
Random Effects			Variance		
Site	1		18518		5.191e-08*
Date Sep vs Oct / site	5		1320		0.9427
Date Sep vs Nov / site			208		
Residual	505		155667		

Table 41. ANOVA table of table for PAC in needles and stems (full model)

Source of Variation	Df	Sums of Square (SS)	Mean Square (MS)	F-value	p-value
Fixed Effects					
clone	5	700032	140006	70.5531	9.50e-56*
shade	3	45813	15271	7.6955	6.75e-05*
date	2	17374	8687	4.3776	0.012813*
Clone*shade	15	24178	1612	0.8123	1.130978
Clone*date	10	17802	1780	0.8971	0.941753
Shade*date	6	29563	4927	2.483	0.050037
Clone*shade*date	30	39593	1320	0.6651	0.788579
Random Effects			Variance		
Site	1		101.97		0.003106*
Date Sep vs Oct / site	5		53.7		0.05784
Date Sep vs Nov / site			25.33		
Residual	505		1984.41		

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Appendix II

CURRICULUM VITAE

Candidate's full name: Jiaman Su

Universities attended:

Northeast Forestry University, 2013-2017, Bachelor of Science in Agriculture

Publications:

Shuai Yang, Lili Sun, Shilin Deng, Biyu Yuan, Jiaman Su,Zhiying Wang, et al. “Effects of Trichoderma asperellum Fermentation Broth on Antioxidant System and Lipid Peroxidation of Alternaria solani Sorauer[J]”. Chinese Agricultural Science Bulletin, 2017,33(5):90-94

Conference Presentations: None.