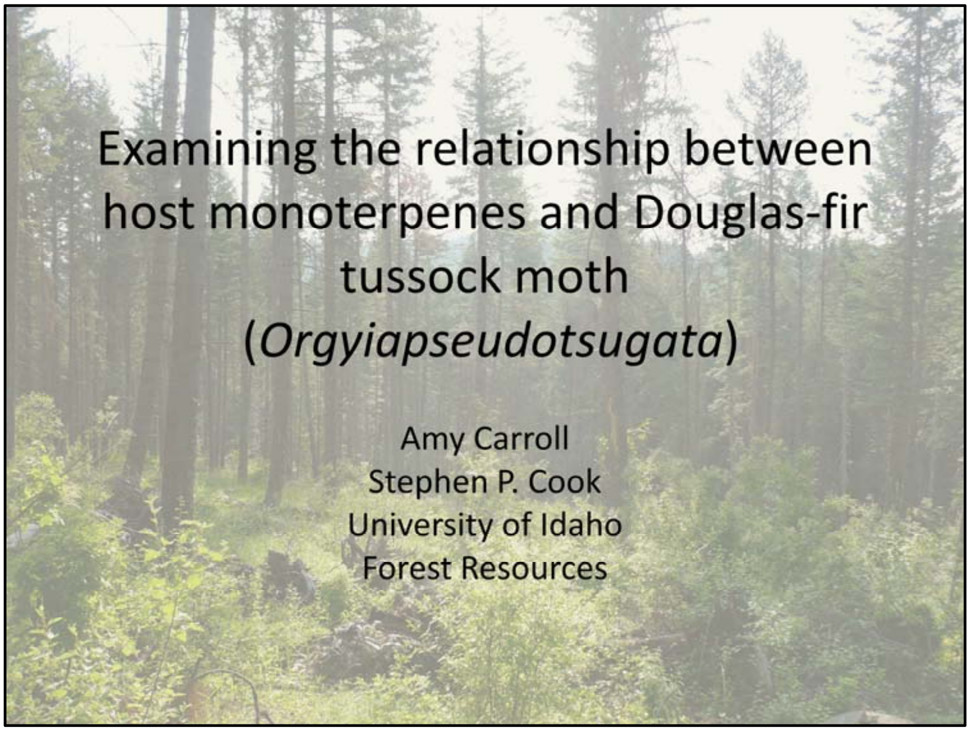
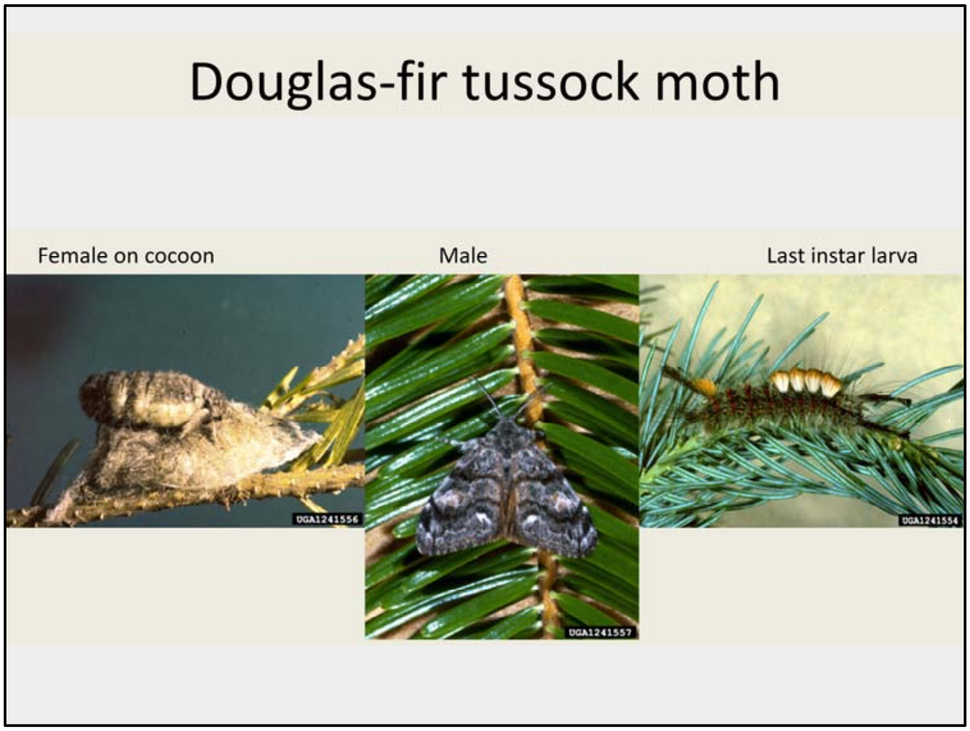


Hello. My name is Amy Carroll and my major professor is Dr. Stephen Cook. My project is titled "Examining the relationship between host monoterpenes and Douglas-fir tussock moth (Orgyia pseudotsugata)".

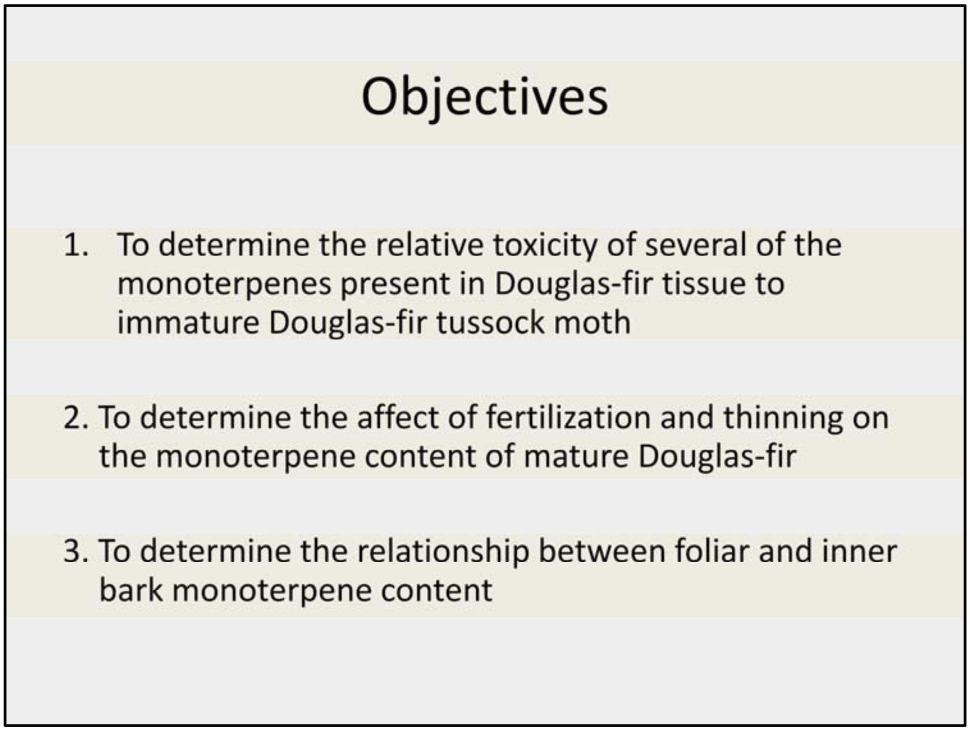


DFTM is a major defoliator of a variety of western conifers. This includes: Douglas-fir, grand fir, concolor fir and subalpine fir. It can be a SERIOUS PEST in our region. Little is known about the relationship between the insect and the host defenses of the tree.

Silvicultural techniques including the use of fertilization and thinning are prescriptions to control DFTM. However, NO ONE has looked to see how these may impact the terpene content of foliage or the ability of the tree to resist attack.

Monoterpenes are common defensive compounds in most coniferous trees. IT IS IMPORTANT TO UNDERSTAND THE DIFFERENTIAL TOXICITY OF INDIVIDUAL COMPOUNDS. We have chosen to analyze 5 different prominent monoterpenes found within Douglas-fir.

The DFTM is destructive ONLY in its larval stage. The female adult cannot fly and usually remains on her cocoon. After mating, the eggs are laid on the cocoon and left to overwinter.



In this project, we attempted to silviculturally modify tree chemistry using fertilization and thinning techniques in a 10 acre mixed conifer forest in the West Hatter Creek unit of the Experimental Forest. There are 3 objectives with this study:

1.To determine the relative toxicity of several of the monoterpenes present in Douglas-fir tissue to immature Douglas-fir tussock moth

2. To determine the affect of fertilization and thinning on the monoterpene content of mature Douglas-fir

3. To determine the relationship between foliar and inner bark monoterpene content…. We understand that looking at the toxicity of monoterpenes against defoliators is not a common practice (because most work has been done with resin acids) - BUT - we are trying to tie together fertilization impacts on both foliage and inner bark tissue, which is why we are doing the monoterpenes.

….. The argument is that they are volatile, and just “blow away”, the insects to look at would be phloem feeders (aphids)

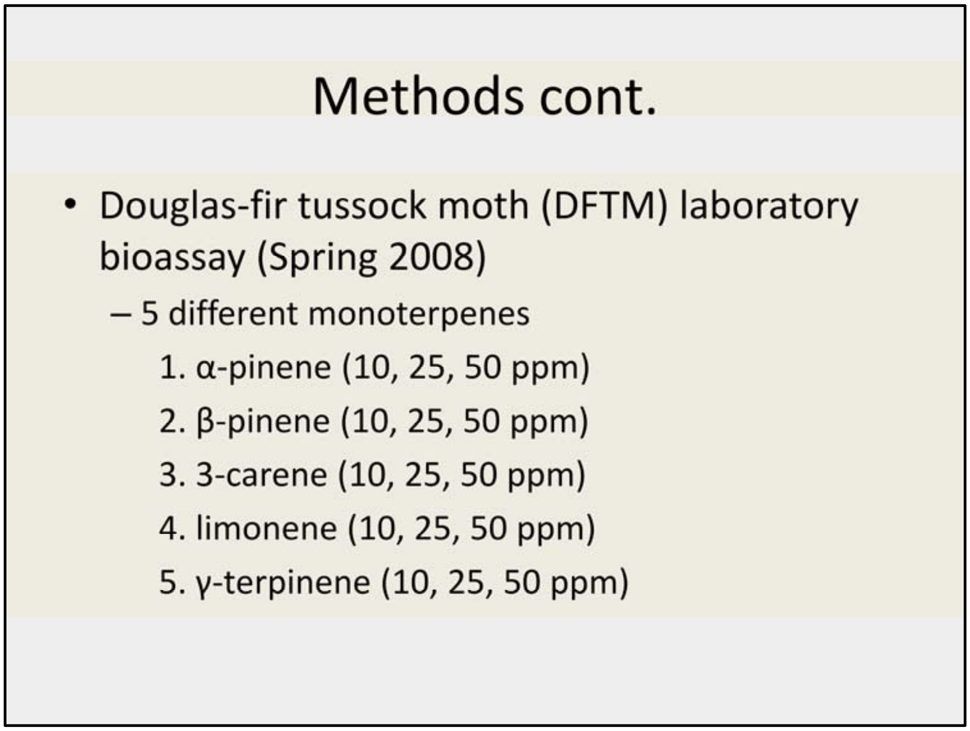
Methods

- Fertilized in Fall 2007
  1. no fertilizer (control)
  2. low N @ 200lbs N/acre
  3. high N @ 400lbs N/ acre,
  4. 'Complete', a blend of N (200lbs/acre), Potassium, Sulfur, and Boron

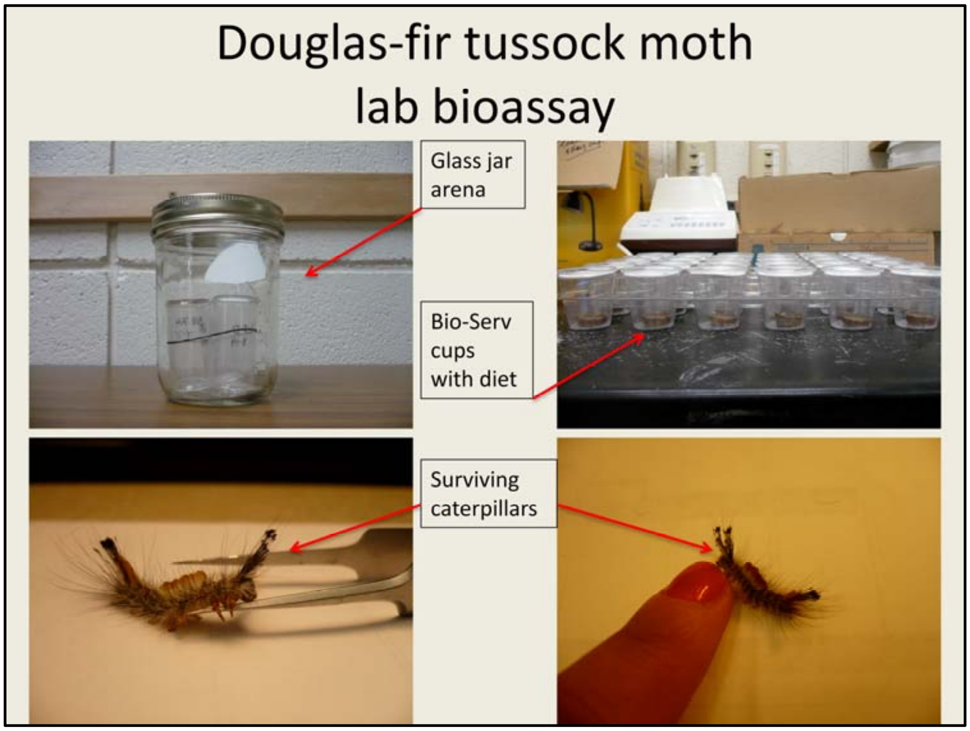
The stand was thinned during July and August of 2007.

One of four fertilizer treatments were applied on an individual tree basis: 1) no fertilizer (control), 2) low nitrogen at 200 lbs of nitrogen per acre, 3) high nitrogen at 300 lbs of nitrogen per acre, or 4) Complete, a blend of nitrogen, potassium, sulfur, and boron.

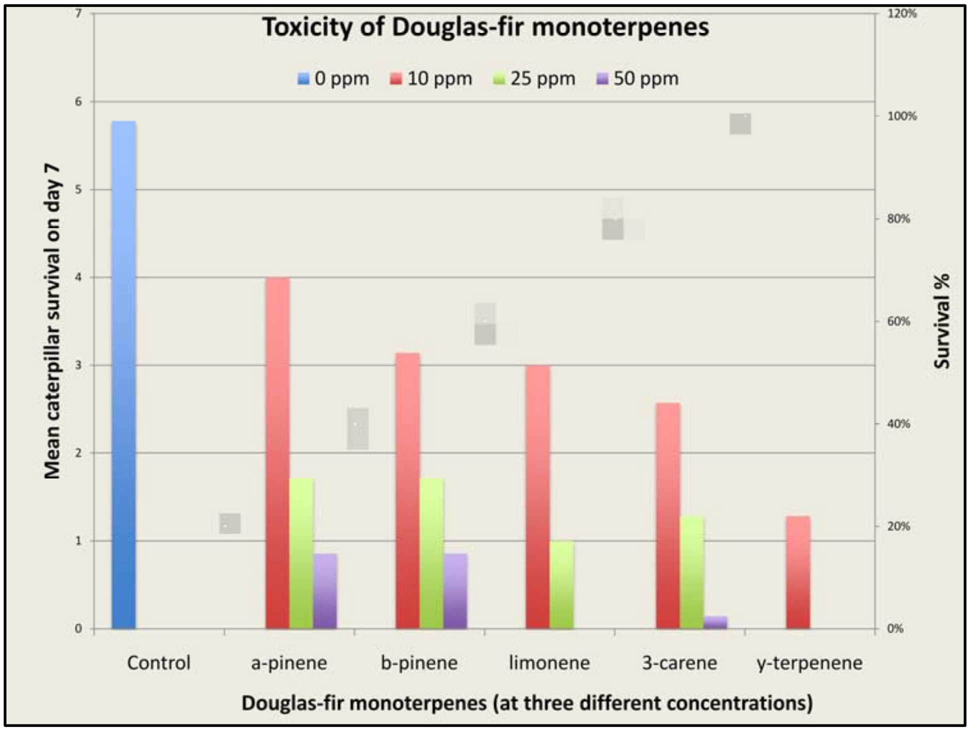
All of the fertilizers were pre-measured before application in the woods. Application was completed using a hand spreader.



Laboratory bioassays were conducted in Spring 2008. DFTM egg masses from Colville, Washington were reared in the lab on artificial diet. 2^{nd} instar tussock moth caterpillars were used to determine lethal time and concentration for the following five monoterpenes present in Douglas-fir: 1) alpha-pinene, 2) beta-pinene, 3) 3-carene, 4) limonene, and 5) gamma-terpinene. ….. We recognize that this is a subset of the many known monoterpenes in Douglas-fir….



Six 2nd instar caterpillars were placed into glass jar arenas with one of the five monoterpenes at either 10, 25, or 50 ppm concentration. Nine repetitions were completed and survival rates were recorded for seven consecutive days. Each surviving caterpillar was placed into an individual Bio-Serv cup with diet and allowed to complete their life cycle.



This graph illustrates the mean caterpillar survival rates after seven days of monoterpene toxicity treatment. The experiment demonstrates that the toxicity is dependent upon both the individual terpene and the concentration; The higher the monoterpene concentration, the lower the survival rates of the caterpillars. Alpha-pinene was the least toxic of all the terpenes tested, and gamma-terpinene was the most toxic with no surviving caterpillars on day seven at 25 and 50 ppm. …. This is exciting because y-terp has not been studied much…

Terpene	Mean (ppm)	LCI	UCI
control	45.66 a	36.06	65.39
α-pinene	45.7 a	35.49	68.02
β-pinene	30.43 a	25.79	36.20
3-carene	39.52 a	31.61	52.82
limonene	19.54 b	16.90	22.71
γ-terpinene	19.65 b	16.87	22.78

Table 1: Estimated concentration of each monoterpene to kill 50% of the exposed insects at day two with upper and lower confidence intervals.

Using a probit analysis in SAS 9.2, 50% lethal concentration was determined for each tested monoterpene at day 2 and day 4. Table 1 shows 50% lethal concentration in ppm at day 2. The lower and upper confidence intervals show that limonene and gamma-terpinene are significantly different in toxicity at day 2 from the control, a-pinene, beta-pinene, and 3-carene. It's interesting to note that we're seeing significant differences QUICKLY, at just day 2. and here the concentration required to produce 50% mortality is roughly half for limonene and gamma-terpinene.

Terpene	Mean (ppm)	LCI	UCI
Control	24.78 a	18.42	32.98
α-pinene	25.3 a	16.64	36.63
β-pinene	21.73	17.25	27.52
3-carene	29.38 a	22.63	38.31
limonene	10.1 b	8.51	13.99
γ-terpinene	9.67 b	7.99	11.67

Table 2: Estimated concentration of each monoterpene to kill 50% of the exposed insects at day four with upper and lower confidence intervals.

Table 2 shows 50% lethal concentration in ppm at day 4. The lower and upper confidence intervals show that limonene and gamma-terpinene are significantly different in toxicity at day 4 from the control, a-pinene, beta-pinene, and 3 carene. Here the concentration required to produce 50% mortality is a third of the other monoterpenes …. At day four, we are still seeing significant differences.

For objective 2, To determine the affect of fertilization and thinning on the monoterpene content of mature Douglas-fir, we needed to collect foliage samples.The study site is approximately 10 acres of mixed conifer forest on the University of Idaho Experimental forest. Foliage samples were successfully collected in August using a 12-gage shotgun. Each sample was placed into a scintillation vial for gas chromatography analysis.

Total monoterpene concentrations in foliage of Douglas-fir

Treatment	Total monoterpeneconcentration (mg/g) mean ± SEM
control	7.01 ± 0.88 a
low nitrogen	8.55 ± 1.18 a
high nitrogen	6.87 ± 0.62 a
complete	6.83 ± 1.45 a

ANOVA results
F = 0.53
P>F = 0.6673

Using gas chromatography, quantitative and qualitative monoterpene content was determined for each sample with the different fertilization treatments. Using Statistix and running an ANOVA test, there is no significant difference among the fertilization treatments and the quantitative monoterpene concentrations. Qualitative analysis has yet to be run to determine any differences among monoterpene content and will be done later in this project. It’s worth mentioning that the low nitrogen treatment has a higher monoterpene concentration, even though it is not significantly higher.

Concentrations of individual tested monoterpenes in foliage of Douglas-fir

Treatment	α-pinene	β-pinene	3-carene	limonene	γ-terpinene
control	1.64±0.19	0.74±0.10	0.0015±0.0010	0.33±0.05	0.006±0.001
low nitrogen	1.77±0.37	0.91±0.25	0.0006±0.0006	0.33±0.05	0.0048±0.001
high nitrogen	1.62±0.14	0.69±0.16	0.02±0.02	0.34±0.03	0.0051±0.0006
complete	1.83±0.30	0.78±0.17	0.005±0.003	0.39±0.07	0.005±0.001

This table shows the concentrations of individual tested monoterpenes in the Douglas-fir foliage samples.

We didn't have any significant differences among the treatments, but these are the five monoterpenes we conducted the analysis on. The qualitative analysis will be done on all terpenes present in the foliage. One of our goals in this project is to be able to compare foliage with inner bark monoterpene content. We also have nutrient analysis from the foliage and will be looking at associations between monoterpene and nutrient contents.

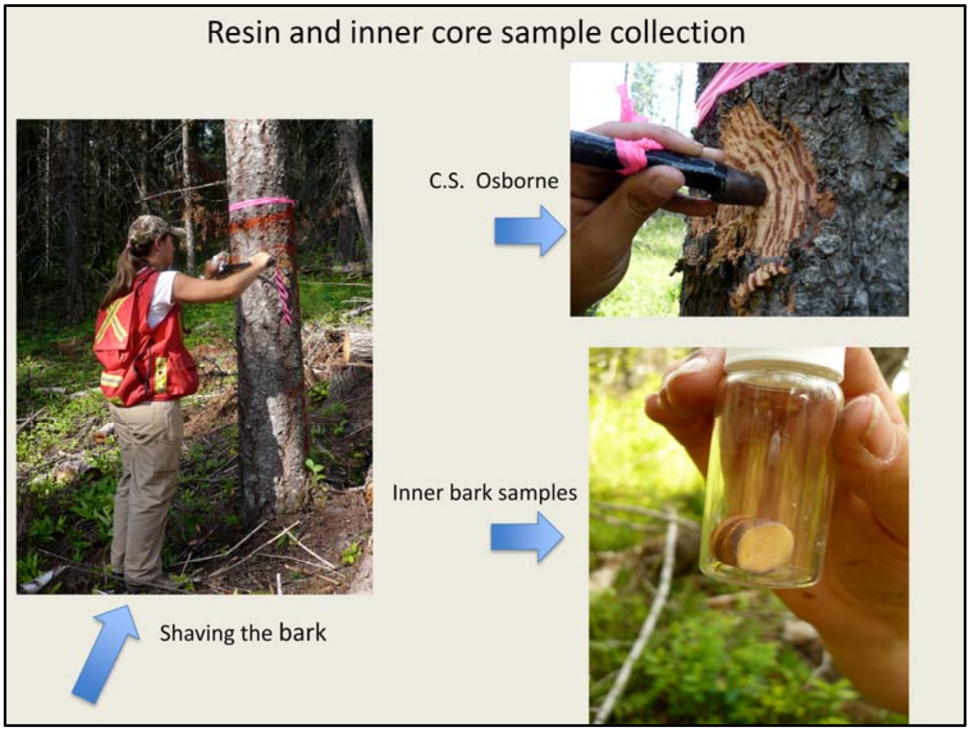
….. Is higher monoterpene content correlated with higher nitrogen content?….

Preliminary results

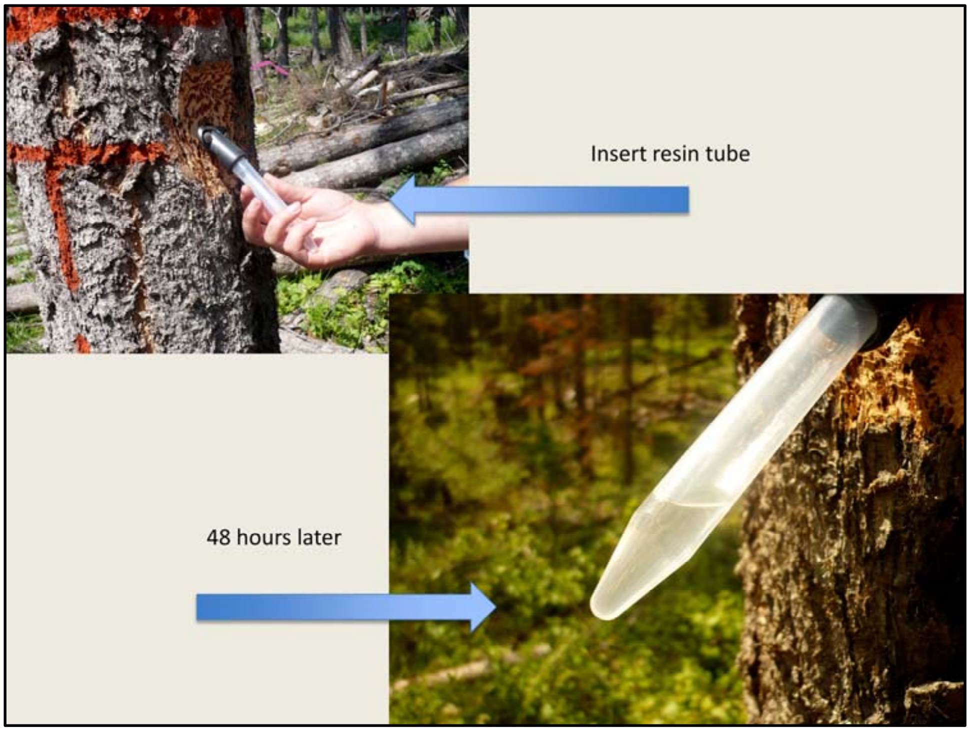
- There are differences in toxicity among the 5 tested monoterpenes
  - γ-terpinene and limonene were more toxic than α-pinene, β-pinene and 3-carene
- There were no significant differences among fertilization treatments in foliar monoterpene concentration

To summarize our preliminary results, there are significant differences in toxicity among the five tested monoterpenes (specifically, gamma-terpinene and limonene were more toxic than alpha-pinene, beta-pinene, and 3-carene to the immature tussock moths.

There were no significant differences among the fertilization treatments in foliar monoterpene concentration.



In addition to the lab bioassay and the foliage analysis, we collected resin and inner core samples. The inner core samples will be used to determine the affect of fertilization and thinning on the monoterpene content of mature Douglas-fir, and will compare it with the foliar monoterpene content.

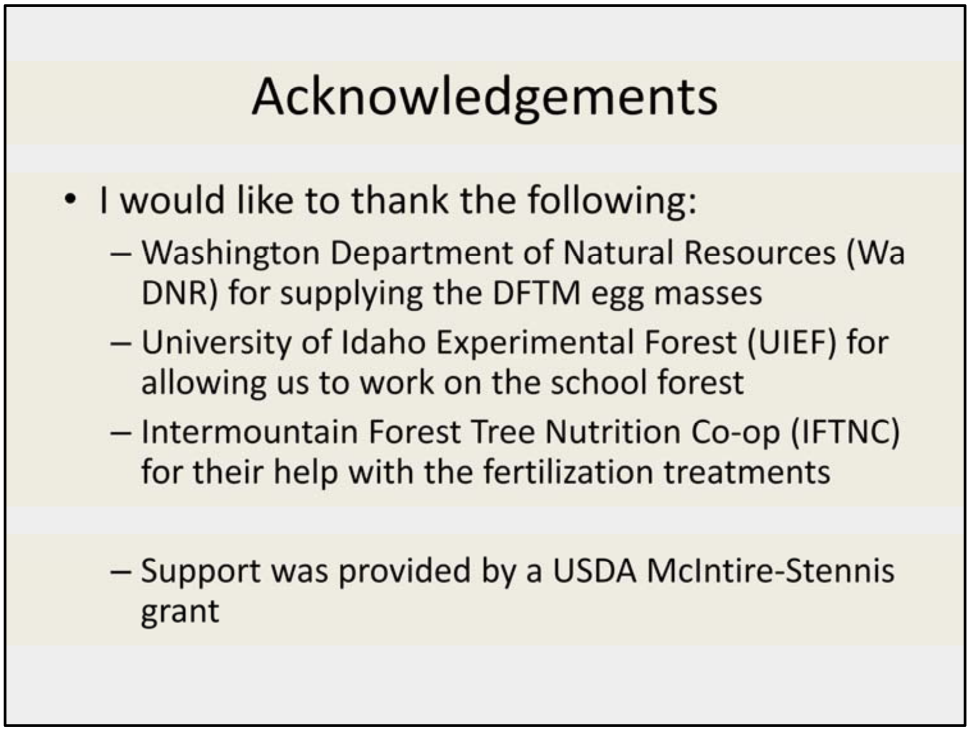


Resin samples were collected in summer 2008. After the bark was shaved on the North and South sides of each DF at breast height, the resin tubes were inserted into each hole where inner core samples were removed. The resin tubes were removed 48 hours later and resin-flow volumes were recorded.

This leads me to our final step in this project, which is to complete Objective 3: to determine the relationship between foliar and inner bark monoterpene content

We can then look for differences among fertilizationtreatments in resin flow.

And finally, once completed, the project will expand our knowledge about the relationship between DFTM and the host defenses of DF. From there, recommendations to incorporate fertilizations and/or thinning to improve tree resistance can be made.



Thank you all for listening. I believe I have 5 minutes for any questions.