Impact of fertilization and thinning on tree resistance to Mountain Pine Beetle (*Dendroctonus ponderosae*) and associated fungi

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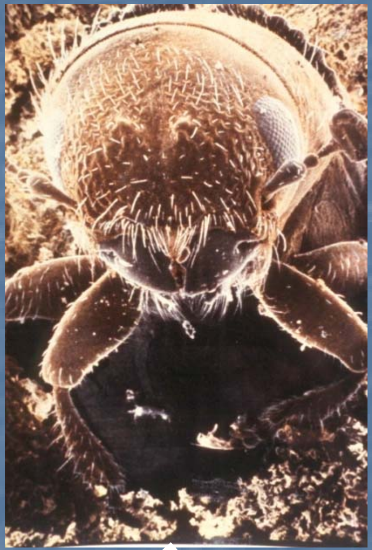
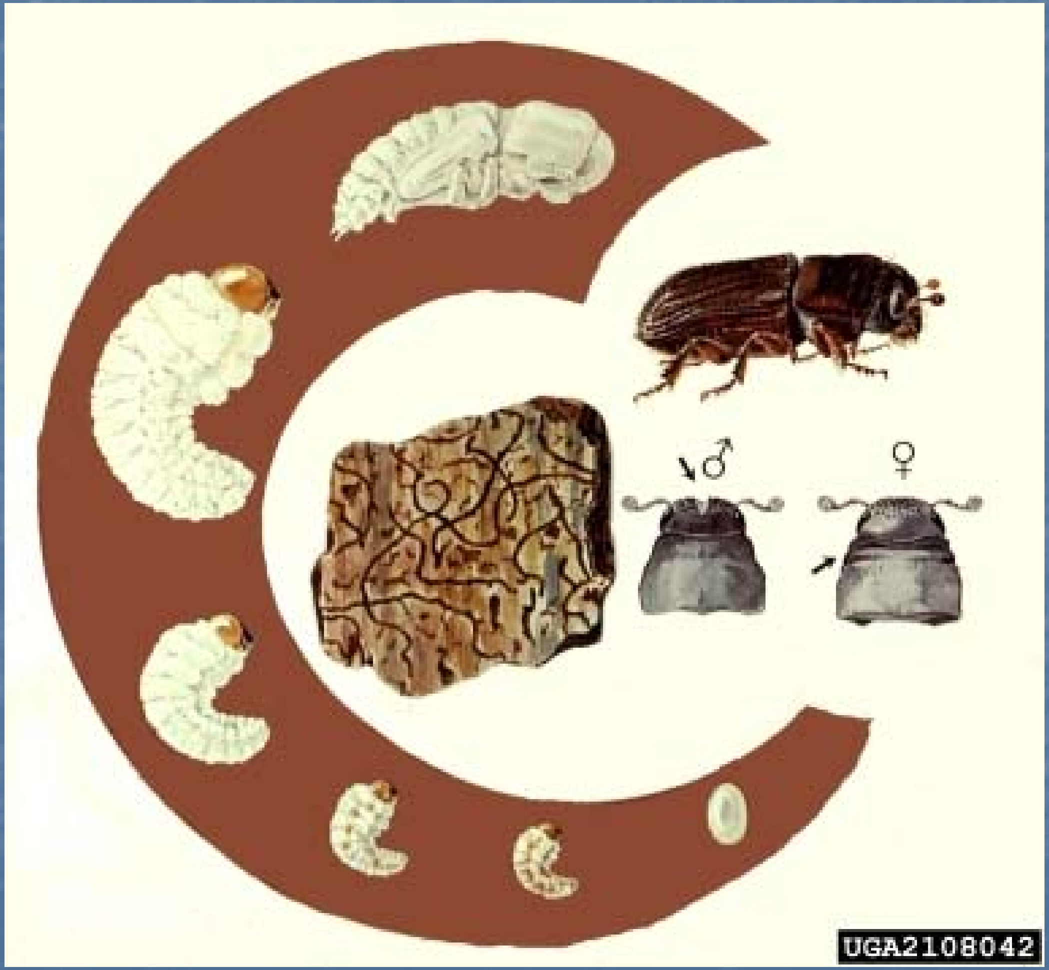
Bark beetles can have rapid infestation growth:



Bark beetle life cycle



From : Richard Kliefoth, Boyce Thompson Institute

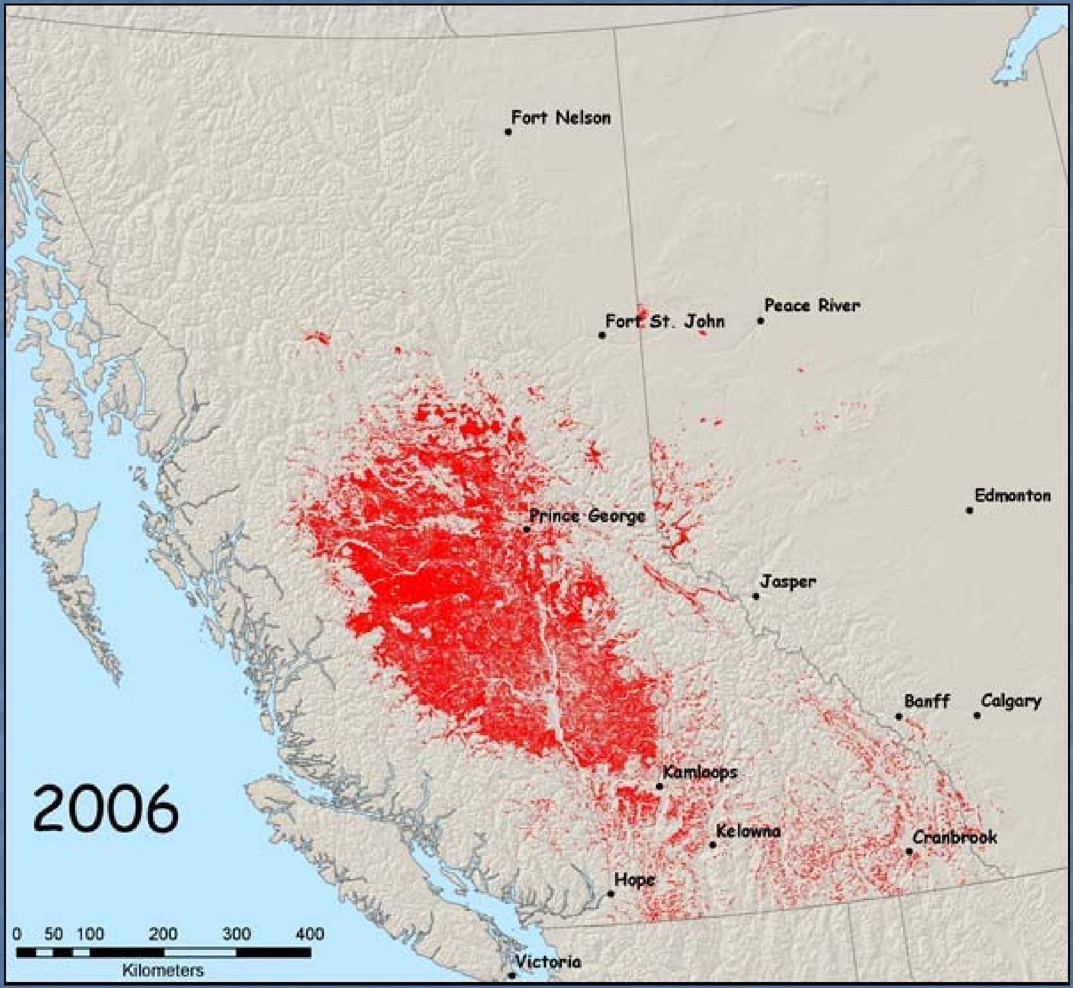
Can we modify the relationship between the beetle and its associated fungi by altering some basic tree chemistry?

Where does the developing larva get its N?

How involved are the fungi with larval nutrition?

Does tree nutrition play a role?

Mountain pine beetle in Canada



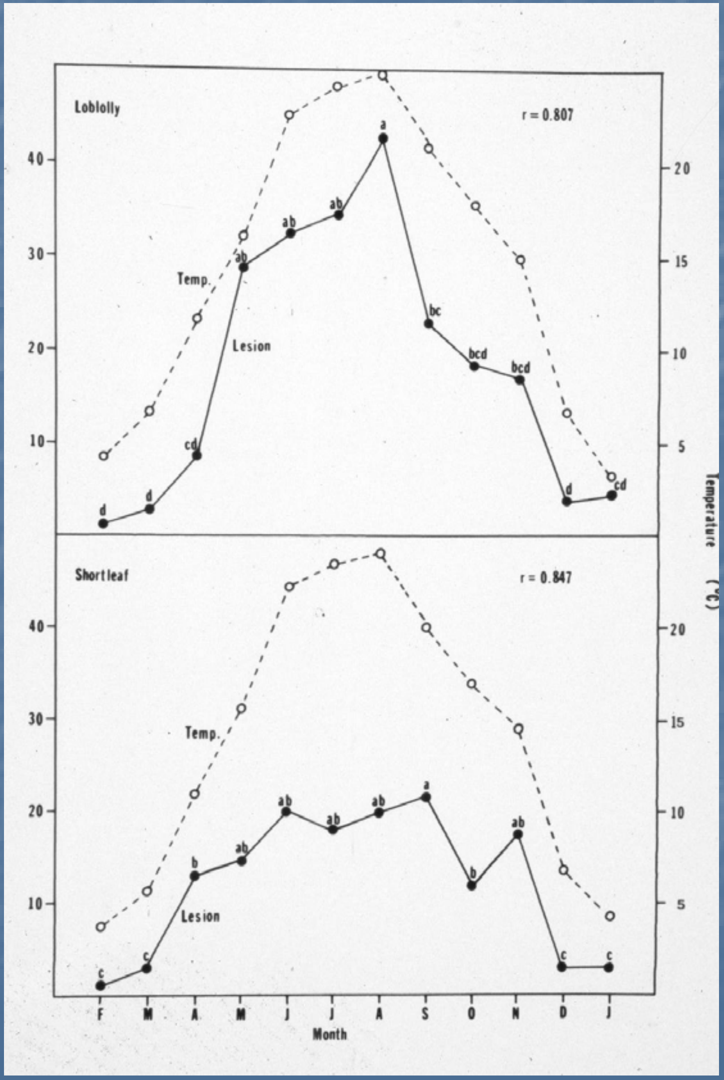
From: Natural Resources Canada

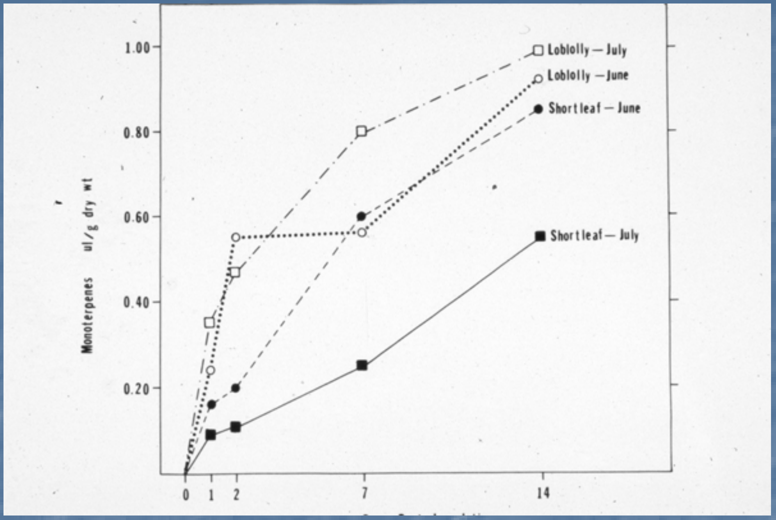
Review of tree resistance to bole invasion

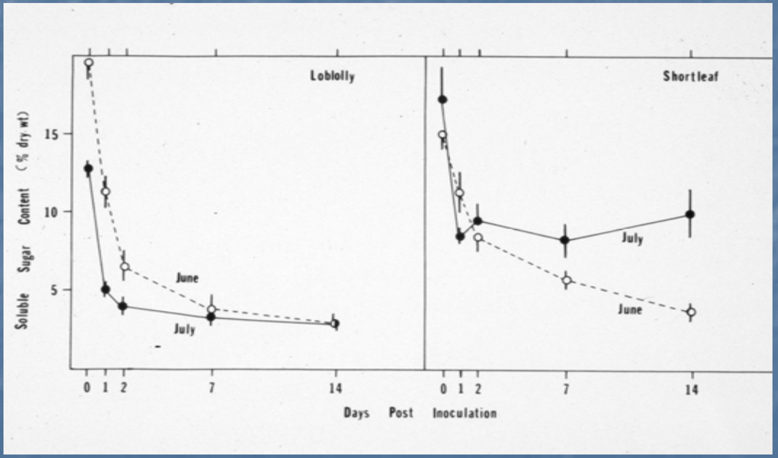
- Wound cleansing
  - Constitutive mechanism (pre-formed)
  - Resin flow (rate, quantity and quality / viscosity)
- Infection Containment
  - Induced mechanism
  - Lesion formation
  - Biochemical changes (monoterpenes and soluble sugars)
- Wound healing
  - Sealing off of the infected area

Review of tree resistance mechanisms

Review of tree resistance mechanisms







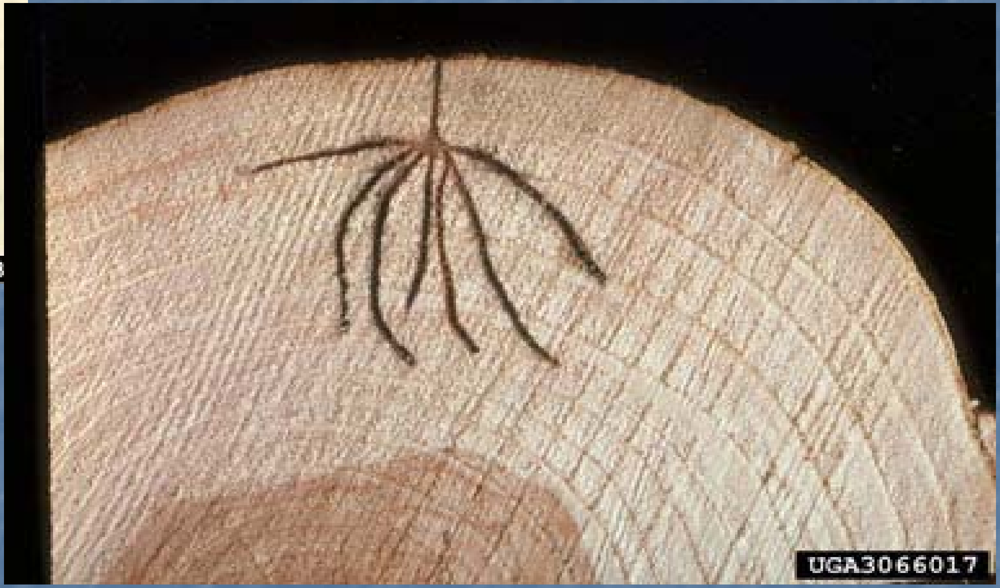
From: Cook & Hain 1985; Cook et al. 1986

But there is a problem with this scenario – what if we chose to examine the wrong relationship?

Photo from: Joe O’Brien US-Forest Service

- There are tree-killing *Ophiostoma* spp. (i.e. Dutch elm disease)
- But what about that other relationship with bark beetles – fungi as food.

Ambrosia beetles:
The extreme case



Photos from: insectimages.org

Mountain Pine Beetle

- Two field sites:
  - Craig Mountain
  - University of Idaho Experimental Forest
- Fertilizer applied to individual trees (fall or winter)
  - Measure inner bark N content
  - Measure resin flow
  - Measure inner bark monoterpene content (in progress)
- Controlled laboratory studies focused on:
  - *Grosmannia clavigera*
  - *Ophiostoma montium*

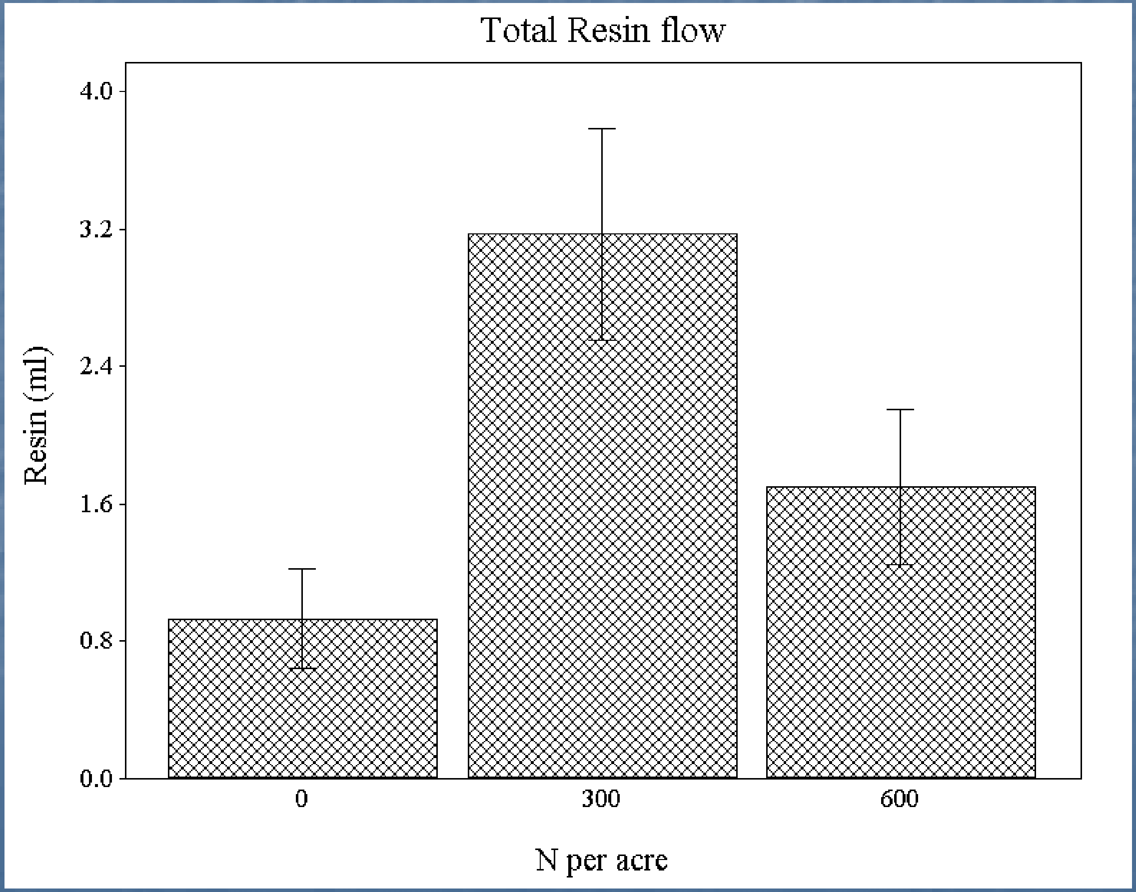
Square Death

- One of the more typical fertilization treatments is Nitrogen
- Intermountain Forest Tree Nutrition Co-op started seeing mortality associated with specific fertilization treatments (N) often associated with specific rock
  - Mortality was associated with bark beetles
  - Mortality typically occurred beginning at approximately year 4 following treatment and continuing through 10 years
  - Roots cannot keep up
  - We have hypothesized that the fertilization benefits the beetle through fungal-mediated nutritional gains

Photo provided by: T. Shaw

Methods and Results
Nitrogen fertilization of individual
Trees at 3 concentrations (0, 300 and 600 lbs/ac)

Fertilizer applied October 2007
Resin flow measured in July, 2008



Monoteprene Defenses: A double-edged sword

- Toxic to the beetle
  - Different compounds have different toxicities
  - Compounds can have differential toxicity to different beetles

- Act as attractants
  - Directly (primary attraction – pine engraver)
  - Indirectly (pre-cursor to beetle pheromones
  - Indirectly (synergize pheromone activity – mountain pine beetle)

Conophthorus – attraction tests
What do we use in the monitoring traps?

2002 Results

Treatment	Beetles/trap	
Control	0.1 ± 0.6	a
Pityol + myrcene	1.0 ± 1.3	a
Pityol	9.7 ± 2.8	b
Pityol + (a)-pinene	10.6 ± 2.6	b
Pityol + b-pinene	12.8 ± 2.5	b
Pityol + 3-carene	27.8 ± 4.2	c

2003 Results

Treatment	Beetles/trap	
Control	0.0 ± 0.0	a
3-carene	0.3 ± 0.8	a
(a)-pinene	0.3 ± 0.8	a
Pityol	20.7 ± 3.8	b
Pityol + 3-carene	26.8 ± 4.7	bc
Pityol + (a)-pinene	37.4 ± 4.9	c

Conophthorus ponderosae: 2004 Toxicity trials of myrcene and a-pinene

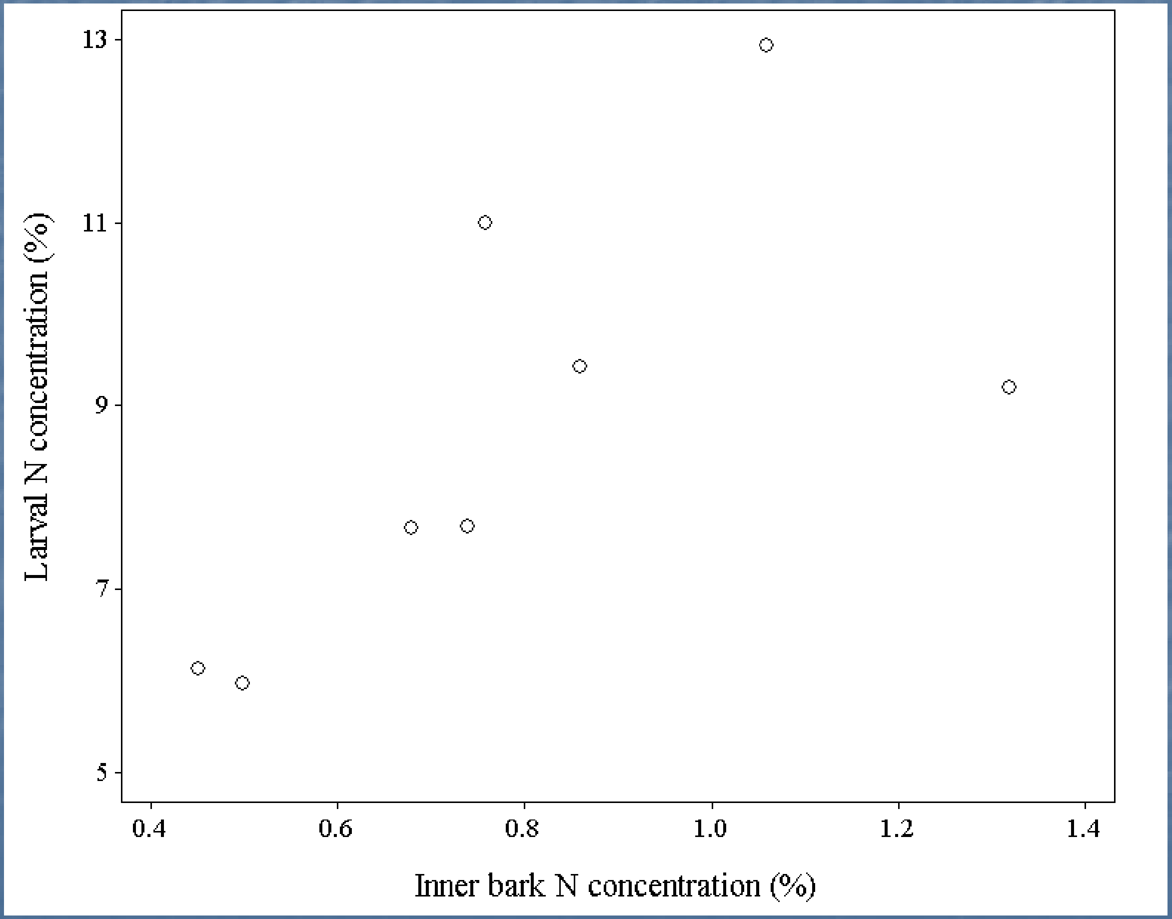
Day	Terpene	LD_{50} (ppm)	95% C.I.
2	Control	no mortality	
	myrcene	52.6	43.3 – 67.0
	a-pinene	61.3	55.6 – 68.8
3	Control	no mortality	
	myrcene	45.1	37.3 – 56.1
	a-pinene	51.4	47.5 – 55.9

From: Shirley & Cook (2007)

What happens when you apply fertilizer:
Fertilizer applied in March, Measurements in July
Change in inner bark N content (dry weight)

N treatment	Pre-fertilization	Post-fertilization	Difference
Control, 0 lbs/ac	0.50 ± 0.03 a	0.51 ± 0.02	0.04 ± 0.03 a
Low, 300 lbs/ac	0.52 ± 0.02 a	0.78 ± 0.07	0.31 ± 0.07 b
High, 600 lbs/ac	0.55 ± 0.03 a	0.75 ± 0.08	0.19 ± 0.11 a

Methods and Results
Correlation between tree inner bark and larval N contents

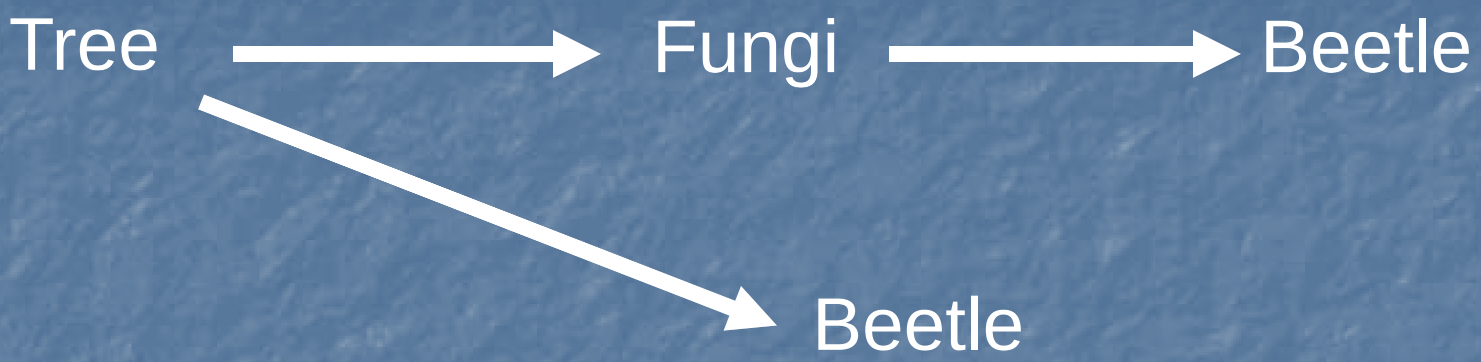


Pre-attack tree N
Larvae collected the following year

r = 0.6727

P = 0.0675

Trophic movement of N:



There will be a different shift in isotopic N content depending on the source of the N acquired by the developing beetle.

Where does the N in the beetle come from: Change in isotopic ratios gives a clue. Still need to complete fungal trials.

	δ15N air	
Treatment (N / acre)	Tree	Larvae
0	-1.59 $\pm$ 0.30	
300	-0.96 $\pm$ 0.31	
600	-1.68 $\pm$ 0.39	
combined	-1.39 + 0.19	0.92 + 0.36

Nutritional Benefits to within-tree larvae

Ayers et al. 2000
Positive relationship between tree and SPB N concentrations

Bentz and Six 2006
Ergosterol content of larvae

Do all of the fungi perform as well?

Fungal Isolations

- We have isolated and keep in culture two of the fungi associated with MPB:
  - *Ophiostoma montium*
  - *Ophiostoma clavigerum*
- We are growing the fungi on media with differing N content
- We are 'feeding' these fungi to beetles to determine impact on adult longevity

Media % N	Temp.	*G. clavigera*	*O. montium*
Experiment 1			
2.05	20	5.80 ± 0.06	5.46 ± 0.06
2.05	25	5.78 ± 0.06	4.85 ± 0.20
Experiment 2			
2.05	20	4.41 ± 0.12	3.75 ± 0.13
2.19	20	5.78 ± 0.07	5.35 ± 0.06
4.01	20	6.84 ± 0.15	5.92 ± 0.13
4.60	20	6.88 ± 0.09	5.27 ± 0.13

Laboratory Study
Paired comparison of N dry weight

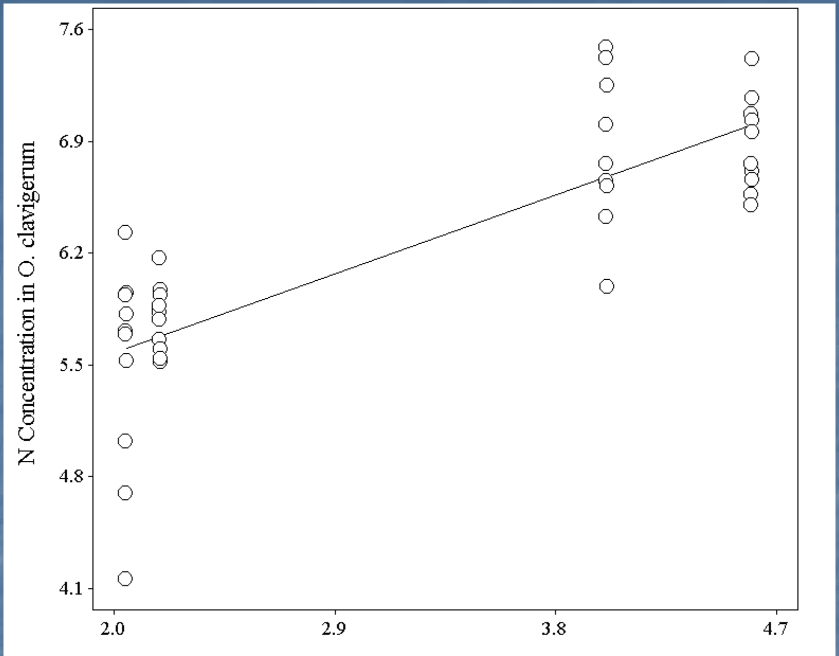
$t = 4.35$

$[P>t] = 0.0074$

G. clavigera consistently had a higher N concentration

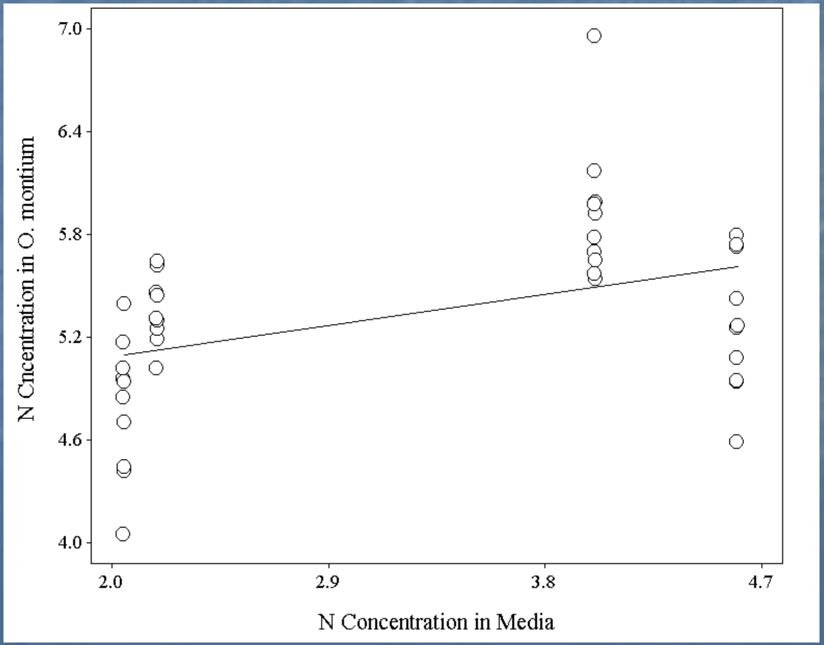
Strong linear relationship between N concentration in the growth media and *G. clavigera*

$R2 = 0.8116$



Weaker relationship when we Examine the *O. montium*

$R2 = 0.1801$



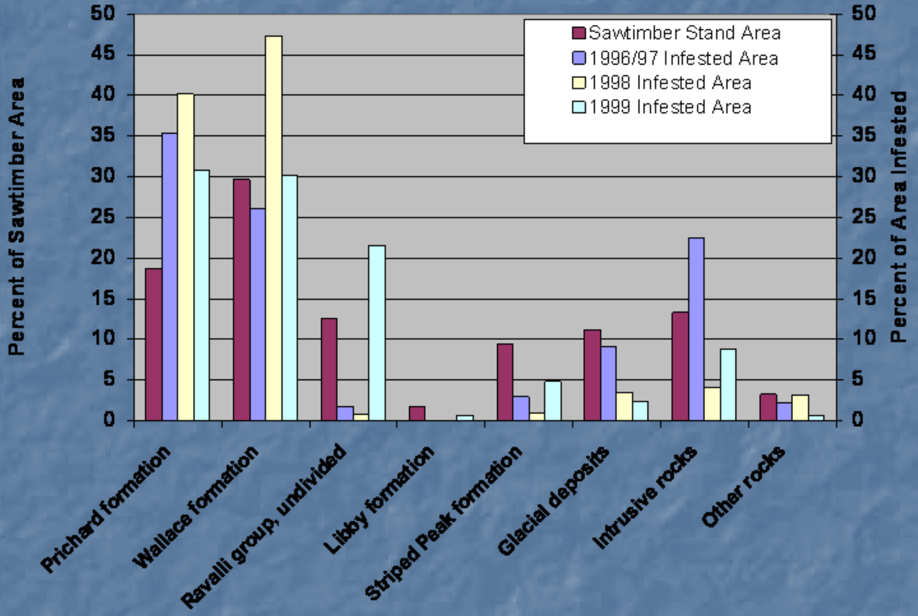
Relationship between N concentration and temperature

Temp	*G. clavigera*	*O. montium*
15	----	5.29 ± 0.10
20	5.80 ± 0.06	5.46 ± 0.06
25	5.78 ± 0.06	4.85 ± 0.20
30	----	----

G. clavigera had a consistent N content but we were not able to grow it at the high or low temperatures

We were able to grow *O. montium* at all of our temperatures but there was a decreasing N concentration

Tree Nutritional Considerations – or – What about the real world?

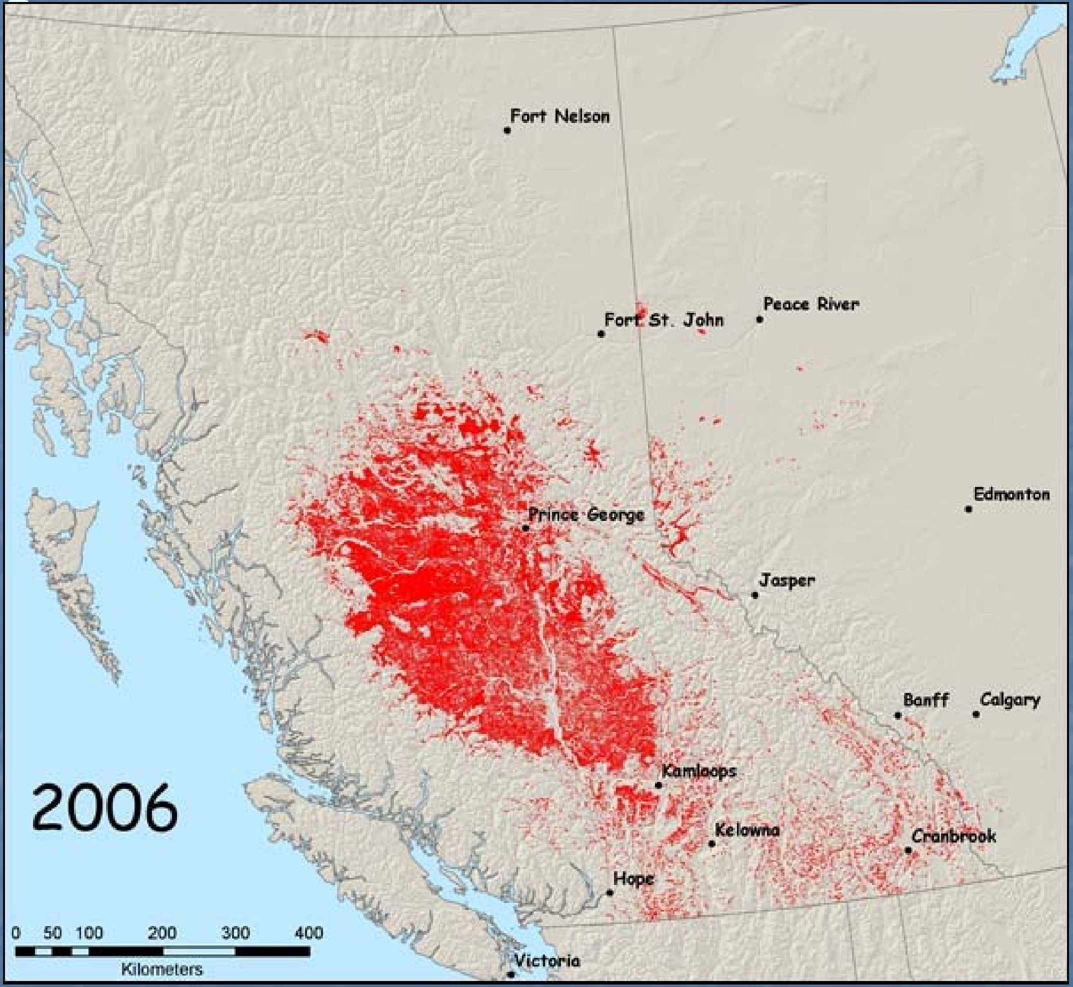


From: Garrison-Johnson et al. (2003)

- I talked about square death but we also observe a phenomena called:
  - Good Rock-Bad Rock
- Why is rock type important?
  - Different nutrient concentrations.
- Metasedimentary rocks are poor nutrient producers.
  - Nitrogen, Potassium, Sulfur and Boron

Mountain pine beetle in Canada

What are the implications for bark beetle range expansion given that the fungi behave differently under different temperature and nutritional regimens?



From: Natural Resources Canada

Summary

•We can modify the resin flow and N concentration of inner bar within the tree through fertilization.

•The increased N concentration within the tree results in increased N concentration within beetle larvae

- Is the increase due to tree N or fungal N concentration?

•Increases in N concentration of the growth media result in increased fungal N concentration <u>and</u> Changing temperatures also results in changed N concentration for *O. montium*

- Obviously there are implications for the impact of climate change on beetle-fungus relationships
- Our next step is to continue to use isotopic ratios to determine where (proportionally) the developing larvae are acquiring their N

The end – any questions?