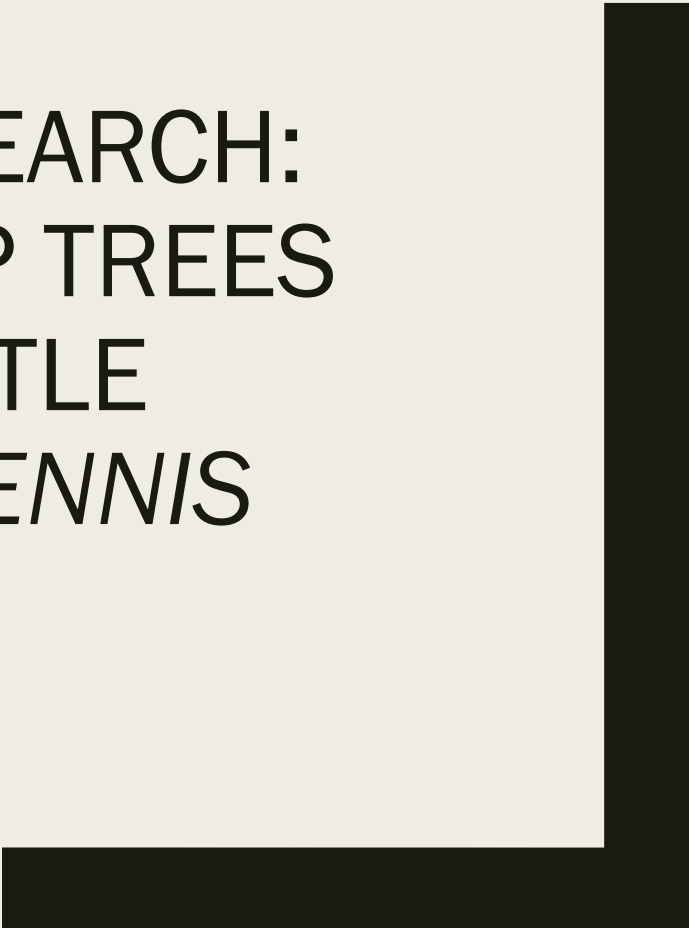


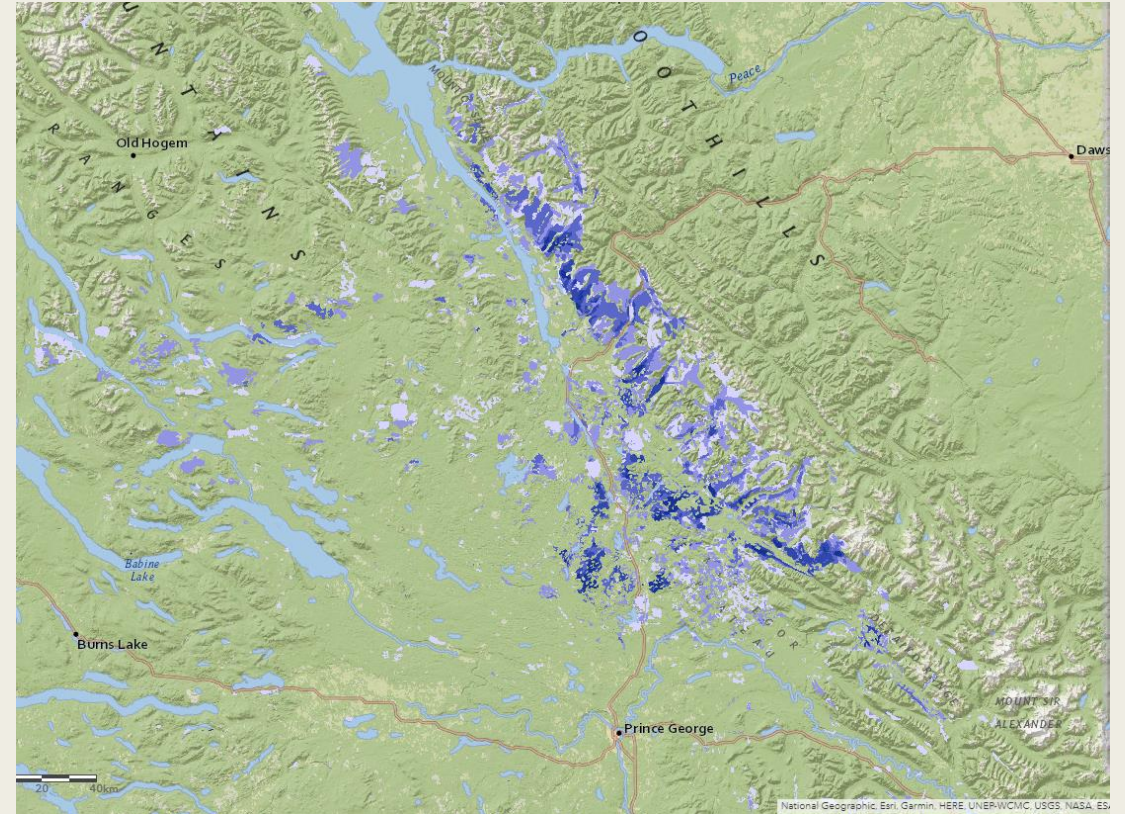
LETHAL TRAP TREES RESEARCH:
DEVELOPING LETHAL TRAP TREES
FOR THE SPRUCE BEETLE
DENDROCTONUS RUFIPENNIS

Nicole de Blois



Spruce Beetle in BC

- Currently experiencing an unprecedented outbreak.
 - Over 500,000 hectares infested as of 2019, 350,000 in the Omineca region.¹
 - Largest since the 1980s.
 - Rapid expansion of the infestation is expected to continue.



Representation of the Omineca Spruce beetle outbreak. Darker blue shading indicates more severe infestation.

Source: Government of BC

(<https://governmentofbc.maps.arcgis.com/home/webmap/viewer.html?webmap=27707c3e6d29477386b0a55aec96fb49>)

MSMA Lethal Trap Trees

- Previous trap trees were treated with an arsenic-based pesticide called monosodium methanearsenate (MSMA).²
- MSMA is highly toxic and is no longer licensed for use in Canada.
- Since MSMA trap trees are no longer in use, lethal trap trees are not currently in use.³
- We are working towards a new targeted and non-toxic lethal trap tree.

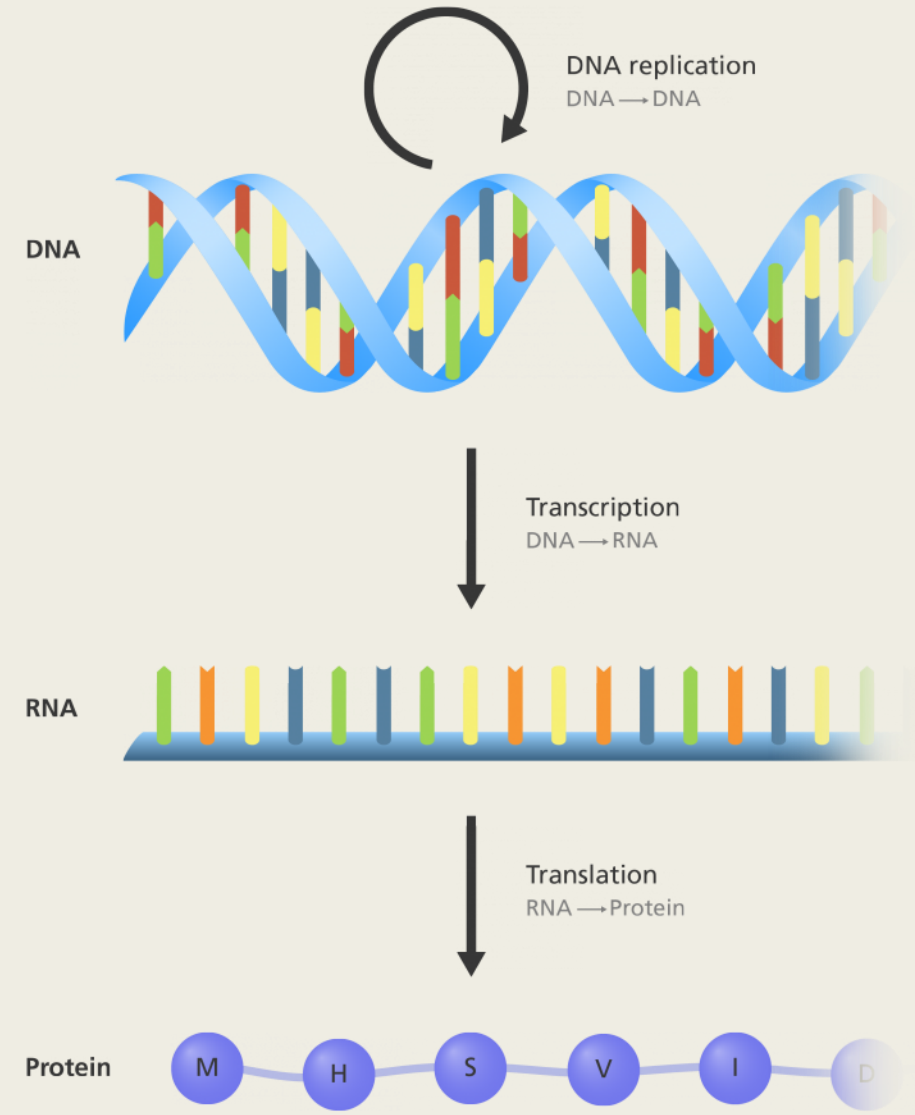


MSMA Trap Trees.

Photo retrieved from <http://www.bcforestsafe.org/node/701>

RNA basics

- RNA is the middle step in going from DNA to protein.
- RNA is generally single stranded.
- RNA is our target: Destroy the RNA to prevent protein production



New Lethal Trap Trees?

- Our research will focus on using a natural cell pathway – RNA interference (RNAi) against the spruce beetle.
- The RNAi pathway is thought to be a rudimentary defense against viruses, and is triggered by the presence of viral RNA.⁴
 - Most RNA is single stranded, but viral RNA is double stranded (dsRNA).
 - This pathway leads to the destruction of any RNA that is complementary to the dsRNA.
- We can exploit this pathway by introducing synthetic dsRNA that corresponds to a target sequence.

Advantages and Challenges of RNAi

Advantages	Challenges
<ul style="list-style-type: none">• Highly specific.⁴<ul style="list-style-type: none">• Unlikely to affect non-target organisms.• Low chance of environmental toxicity.<ul style="list-style-type: none">• Unlike MSMA• RNA is unstable and degrades rapidly in the environment.• Already in use in the agricultural sector.• Coleopterans are generally susceptible to ingested dsRNA.	<ul style="list-style-type: none">• Sequencing of the spruce beetle genome is still in progress.<ul style="list-style-type: none">• Will be using mountain pine beetle for initial probe development.• It is difficult to screen for non-target effects because most insects do not have a sequenced genome.<ul style="list-style-type: none">• However we will be testing probes on other insects such as <i>Triboleum spp</i> and <i>Dendroctonos ponderosae</i>.• Effective delivery in the field can be challenging.

Research Questions

- Can the spruce beetle produce an RNAi response to injected dsRNA that results in a measurable decrease in the target mRNA sequence?
- Will ingested dsRNA cause a systemic RNAi response and knockdown of target genes in *D. rufipennis*?
- What target or combination of gene targets will result in the rapid death or sterilization when they are silenced?
- Can a dsRNA in solution be transported up through a tree's vascular system resulting in sufficient concentrations of dsRNA throughout the phloem of the tree to silence the target gene(s).

Methods Overview

Gene Targets

- Housekeeping genes
- Vitellogenin
 - Results in a “yolkless” egg in *Blatulla germanica*.⁵
- Chitin Synthases
 - Caused death at all life stages in *Tribolium castaneum*.⁶
- Cytochrome p450s
 - Can be involved in a variety of processes, including detoxifying plant compounds and the synthesis and breakdown of juvenile hormones.⁷
- Juvenile hormone binding proteins and receptors.
 - Important in regulating molting and development.⁷

Selection of Target Sequences

- Because the *D. rufipennis* genome is not yet publicly available, initial sequences will be developed using the mountain pine beetle genome.
 - Once targets are identified, they will be compared to other species to prevent off-target effects.
 - Insects such as the European spruce bark beetle, *Ips typographus*, the red flour beetle *Tribolium castaneum* and less related species such as *Drosophila spp.* can be used as comparisons.
 - We will confirm the presence of the target genes in spruce beetle and ensure the specificity of the dsRNA probe.
- The probes can then be screened through injection.
 - The probes with the best results can then be tested through ingestion.

Ingestion

- Phloem sandwiches
 - Consist of phloem sandwiched between two pieces of clear material (such as plexiglass).⁸
 - Can be saturated with dsRNA in solution, and the survival, fecundity, and hatching of beetles can be observed.
 - Test different concentrations and combinations of probes against controls.
- Force Feeding
 - Using PCR tubes, beetles can be force-fed with dsRNA solution or a mixture with phloem.
 - This may be an alternative to phloem sandwiches.



Example of a phloem sandwich with visible *D. ponderosae* galleries.⁹

Delivery

- Frilling²
 - The traditional method for producing lethal trap trees, used on MSMA trap trees.
 - The act of frilling kills the tree, which is advantageous for trapping spruce beetle.
- Trunk injection⁹
 - Allows for the injection of aqueous solutions without killing the tree.
- The ability of the tree to transport dsRNA can be assessed using nonsense dsRNA in solution or using green food colouring in solution.





Bringing it all Together

- Creating a new lethal trap tree using similar protocol to that used for MSMA trap trees.
- dsRNA in solution can be applied to the frilled trunk.
 - After a sufficient amount of time the tree can be felled.
 - We need to determine how long it takes to translocate in the tree.
- Survival within these lethal trees can be compared with untreated controls.
- Inexpensive
- Targeted
- Non-toxic

1. BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development. (2019). 2019 Aerial Overview Summary for British Columbia. https://www2.gov.bc.ca/assets/gov/environment/research-monitoring-and-reporting/monitoring/aerial-overview-survey-documents/2019_aerial_overview_summary_for_british_columbia.pdf Ministry of Forests, Lands, and Natural Resource Operations. (2020) Spruce Beetle Management in B.C. Retrieved from: https://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/forestry/forest-health/bark-beetles/spruce_beetle_guidebook.pdf
2. Hodgkinson, R.S. (1985). Use of Trap Trees for Spruce Beetle Management in British Columbia, 1979-1984, A review with recommendations. Ministry of Forests, Prince George, BC.
3. Omineca Region Spruce Beetle Beneficial Management Practices. March 2016. Prince George, BC. <https://www.for.gov.bc.ca/ftp/DMK/external/!publish/SpruceBeetle/Documents/Omineca%20Region%20Spruce%20Beetle%20Document%20Series/v3%20SB%20Document%20Series%20Omineca%20Region%20June%202017.pdf>
4. Zotti, M.J., & Smagghe, G. (2015). RNAi Technology for Insect Management and Protection of Beneficial Insects from Diseases: Lessons, Challenges and Risk Assessments. *Neotrop Entomol* 44(3), 197-219. doi:10.1007/s13744-015-0291-8.
5. Ciudad, L., Piulachs, M.-D., & Bellés, X. (2006). Systemic RNAi of the cockroach vitellogenin receptor results in a phenotype similar to that of the *Drosophila* *yolkless* mutant. *The FEBS Journal*, 273(2), 325–335.
6. Arakane, Y., Specht, C. A., Kramer, K. J., Muthukrishnan, S., & Beeman, R. W. (2008). Chitin synthases are required for survival, fecundity and egg hatch in the red flour beetle, *Tribolium castaneum*. *Insect Biochemistry and Molecular Biology*, 38(10), 959–962. <https://doi.org/10.1016/j.ibmb.2008.07.006>
7. Vijaya Sudhakara Rao, K., Renuka, P., Sheshu Madhav, M., & Mangrauthia, S. K. (2015). Key enzymes and proteins of crop insects as candidate for RNAi based gene silencing. *Frontiers in Physiology*, 6(MAR). <https://doi.org/10.3389/fphys.2015.00119>
8. BC Ministry of Forests. (1985). Use of Trap Trees for Spruce Beetle Management in British Columbia, 1979-1984.
9. Delivery of Hairpin RNAs and Small RNAs Into Woody and Herbaceous Plants by Trunk Injection and Petiole Absorption. 2018. Dalakouras, A., Jarausch, W., Buchholz, G., Bassler, A., Braun, M., Manthey, T., Krczal, G., and Wassenegger, M.