Fungal colonizers and seed shortfall in Lodgepole pine seed orchards

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Lodgepole pine (*Pinus contorta*)

Species distribution:

- Important component of forest ecosystems in BC and AB
- Major commercial species in forestry for BC
- Used for structural lumber and a variety of finished wood products

Credit: BC Tree Atlas

Lodgepole pine in British Columbia

Species status:

- Serious declines have occurred in recent years
- Mountain Pine beetle epidemic in BC and AB
- More than 10 years BC annual harvest lost (~ 750 M m^3)
- *Dothistroma* needle blight in Northern BC
- Increased prevalence of forest fires
- Forest regeneration is a priority in disturbed ecosystems

Credit: Natural Resources Canada

Seed shortfall in Okanagan orchards

The problem for Lodgepole pine:

- Seed production down in Okanagan orchards
- Consistent shortfall in past decade
- *Leptoglossus occidentalis* contributes to losses
- Other causes of shortfall remain undescribed

Credit: BC MOF

Are fungi contributing to seed shortfall?

Objectives of current study:

1. What is the extent of seed shortfall?
2. Do fungal colonizers play a role in this decline?
3. What species of fungi are implicated?
4. Where and when are they detected in the host?

Methods:

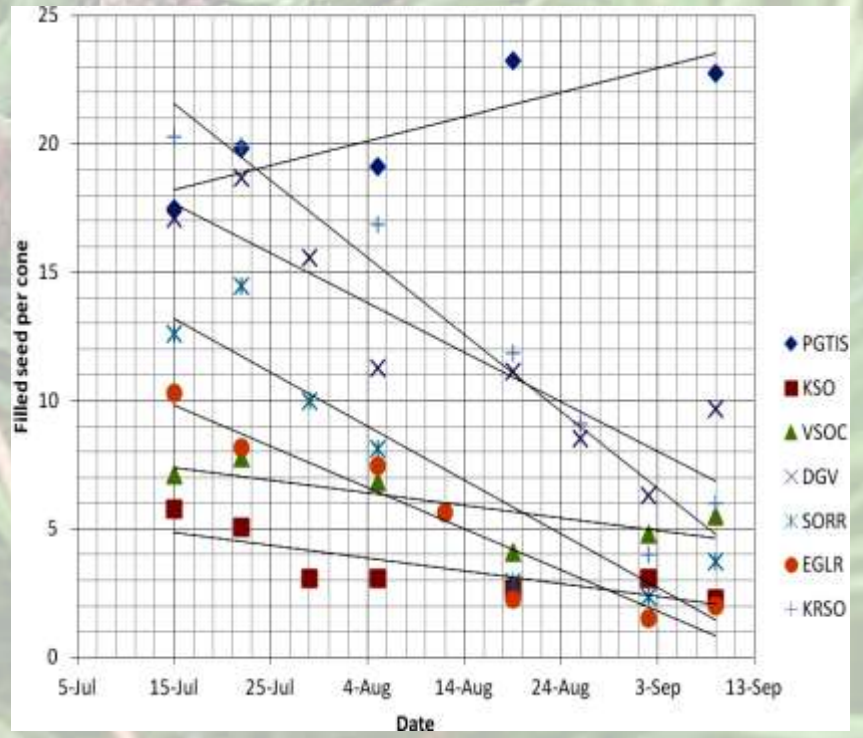
1. Evaluated seed shortfall among orchard sample sites
2. Conducted histological work to describe seed decline
3. Sampled different host tissues and developmental stages
4. Identified fungal isolates obtained from host tissues

Seed shortfall in 2013

Study sites (Lodgepole pine orchards):

Site	Orchard	Location
Eagle Rock (EGLR)	O-339	Armstrong, BC
Kettle River Seed Orchard (KRSO)	O-238	Rock Creek, BC
PRT Grandview (GDV)	O-313	Armstrong, BC
Sorrento Seed Orchard (SORR)	O-241	Sorrento, BC
Vernon Seed Orchard Company (VSOC)	O-218	Vernon, BC
Kalamalka Seed Orchard (KSO)	O-307	Vernon, BC
Prince George Tree Improvement Station (PGTIS)	O-223	Prince George, BC

- Filled seed in maturing cones from July to September
- Prince George and Okanagan orchards (n=20)



Degeneration of seed tissues

Dissected megagametophytes:

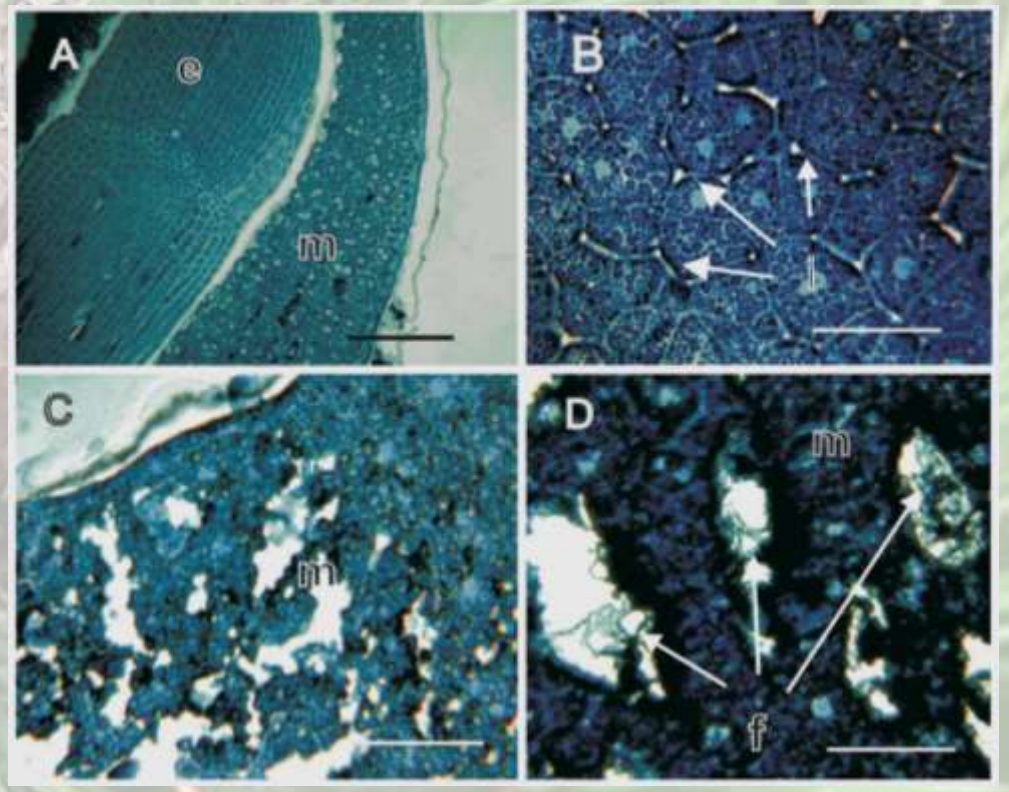
- Normal (A) and degenerated (B) megagametophytes

- Seed tissues were examined weekly from mid-July onwards
- Increasing levels of tissue degeneration were observed in all orchards
- Fungal colonization of seeds was a common occurrence

Histology of seed degeneration

Fungal colonization of host tissues:

- Healthy megagametophyte tissue (A)
- Early stage tissue damage with intercellular spaces (B)
- Advanced degeneration of host tissue (C)
- Fungal hyphae within intercellular spaces (D)



Scale bar = 50 µm

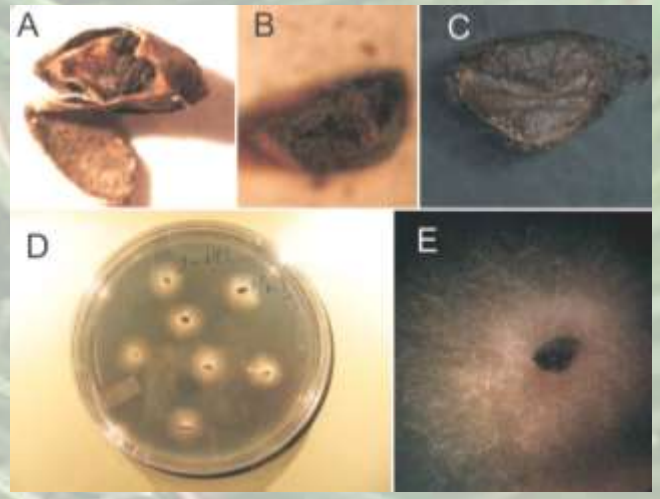
Isolation of fungi from colonized seeds

Survey of seeds for living fungal isolates:

- Fungal isolation work focused on four orchards
- Okanagan (SORR, KRSO, 2x KSO) and Prince George (PGTIS)

- Preliminary survey of the fungal species present
- Examined different host tissues as well

- Symptoms of fungal infection in dissected seeds (A – C)
- Fungal growth from surface-sterilized seeds (D and E)



Isolation of fungi from other sources

Tissues and material tested for fungi:

- Conelets (first year cones)
- Healthy needles
- Pollen samples
- Air column in orchard

Fungal species detected

Fungal species in orchard samples:

- Isolates identified by culture morphology and DNA sequence analysis (ITS-rDNA region)
- Common species:
    - *Sydowia polyspora*
    - *Alternaria* sp.
    - *Cladosporium* sp.
    - *Fusarium* sp.
- Similar fungi detected in all tissues and in all orchards
- Filled seeds per cone much lower in Okanagan orchards

Source	Fungal species
Seed	*Sydowia polyspora*
	Alternaria sp.
	Cladosporium sp.
	Penicillium sp.
Conelets	*Sydowia polyspora*
	Alternaria sp.
	Cladosporium sp.
	Fusarium sp.
Needles	*Sydowia polyspora*
	Alternaria sp.
	Cladosporium sp.
	Fusarium sp.
Pollen	*Sydowia polyspora*
	Unidentified fungi
Air column	*Sydowia polyspora*
	Alternaria sp.
	Cladosporium sp.
	Fusarium sp.
	Unidentified fungi

Sydowia polyspora

Features of this fungal species:

- Ascomycete (anamorph *Hormonema dematioides,* order Dothideales)
- Described as foliar endophyte and opportunistic pathogen of conifers on needles and seeds

- Causal agent of a needle cast disease (Current season needle necrosis)
- In Europe on Nordmann fir (*Abies nordmanniana*), noble fir (*Abies procera*), grand fir (*Abies grandis*)
- In BC and PNW on Douglas fir (*Pseudotsuga menziesii*), noble fir (*A. procera*), grand fir (*A. grandis*)

- In Finland as a foliar endophyte of Scots Pine (*Pinus sylvestris)* and Norway spruce (*Picea abies)*

Other fungal colonizers of Lodgepole pine

Generalist species of fungi:

- *Alternaria* sp., *Fusarium* sp. And *Cladosporium* sp.
- Species level identification more difficult (requires sequence data from other gene loci)
- Have also been described as endophytes of woody and herbaceous plants

Examples:

- *Fusarium oyxsporum* – soil saprophyte, endophyte and plant pathogen
- *Alternaria alternata* – soil saprophyte, endophyte (*Pinus* spp.) and plant pathogen
- Include a wide range of lifestyles and hosts

Fungal colonizers of Lodgepole pine

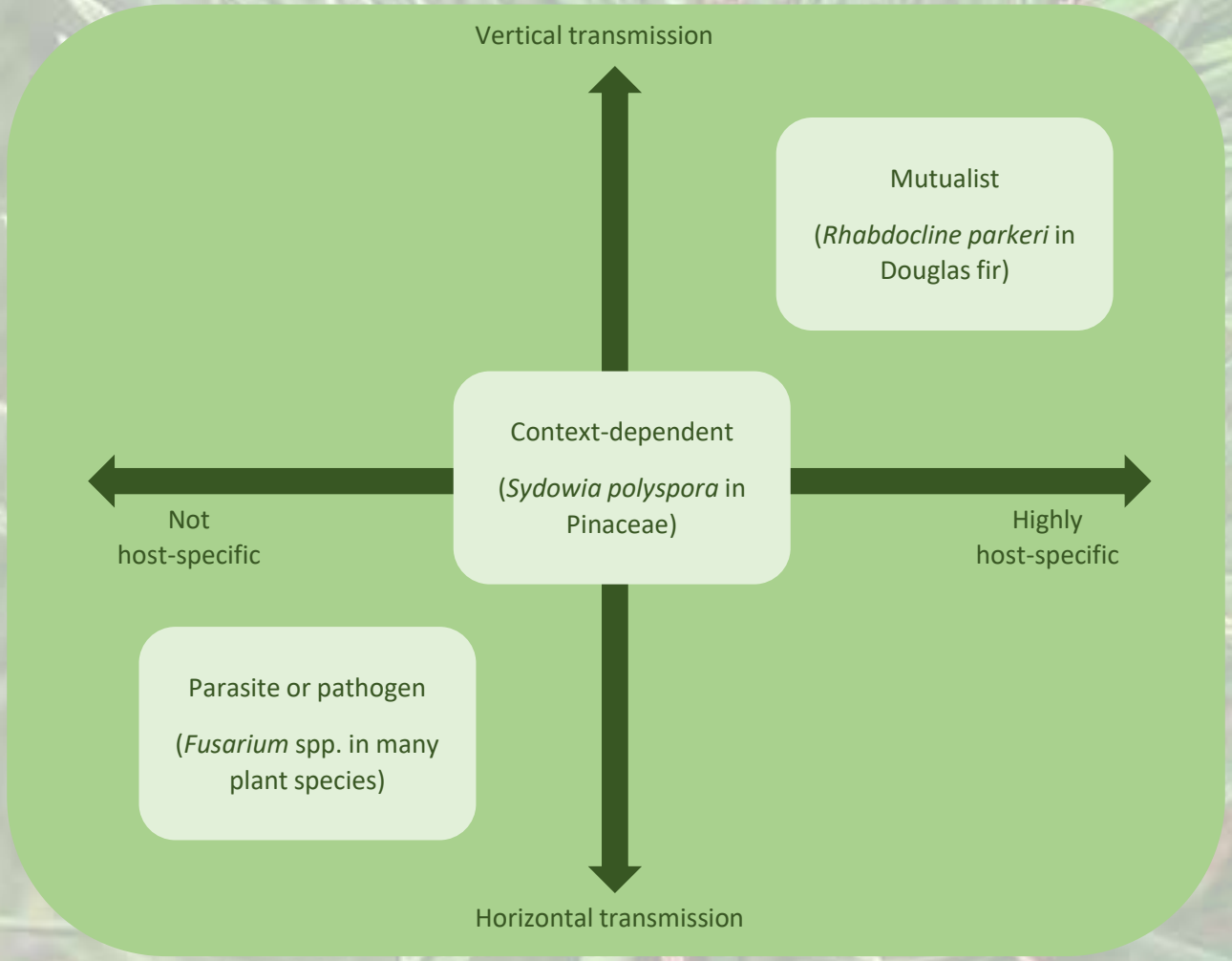
Observations to date:

1. Our results suggest one or more species could be opportunistic seed pathogens
2. Fungi likely contribute to seed mortality in Okanagan orchards compared to Prince George orchard

3. Fungi are present in various plant tissues during seed cone development
4. Both vertical and horizontal transmission appear possible in Lodgepole pine

5. Fungal biotrophic status is not clear (pathogen – parasite - endophyte?)
6. Status may be context-dependent (influenced by host and fungal genotypes, growing conditions)

Biotrophic status of conifer-associated fungi



Future directions - Research priorities

What questions remain?

1. What is the role of host tree genotype, host physiology and geographical location in seed loss?
2. Is fungal biotrophic status influenced by the above variables?
3. Are these fungal species introduced or native to this region (Okanagan)?

Research priorities include:

1. Evaluate host genotype and physiology as it relates to orchard site (Okanagan versus Prince George)
2. Examine changes in host tree secondary metabolite production (terpenes, tannins, phenolics)
3. Evaluate the genetic diversity of fungal species detected among orchards (metagenomics approach)

Outcomes?

1. Determine importance of fungal microbiome in seed loss
2. Determine role of host genotype, host physiology and growing conditions in seed loss
3. Identify targets for tree improvement in a changing climate

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