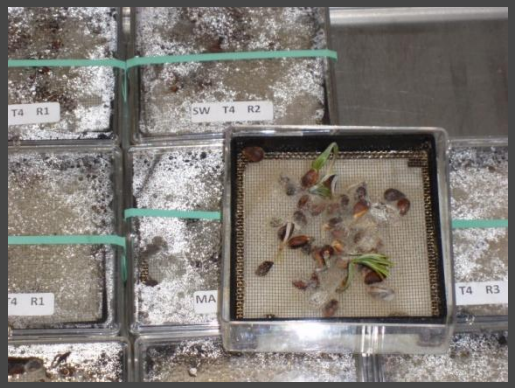
Whitebark Pine: Seeds and Germination



Dave Kolotelo

July 14, 2011

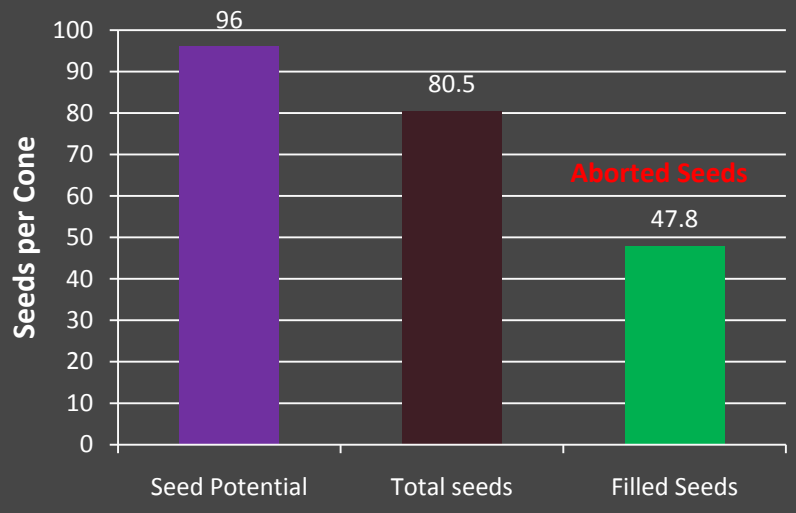
Whitebark Pine Ecosystem Foundation of Canada Meeting

Overview

perspective relative to planting seedlings

- Background – Seeds and Germination
- Whitebark pine pretreatment review
  1. Soak
  2. Warm stratification
  3. Sanitation
  4. Cold Stratification
  5. Clipping
- Dibbling Germinants
- Ex situ Collection Testing

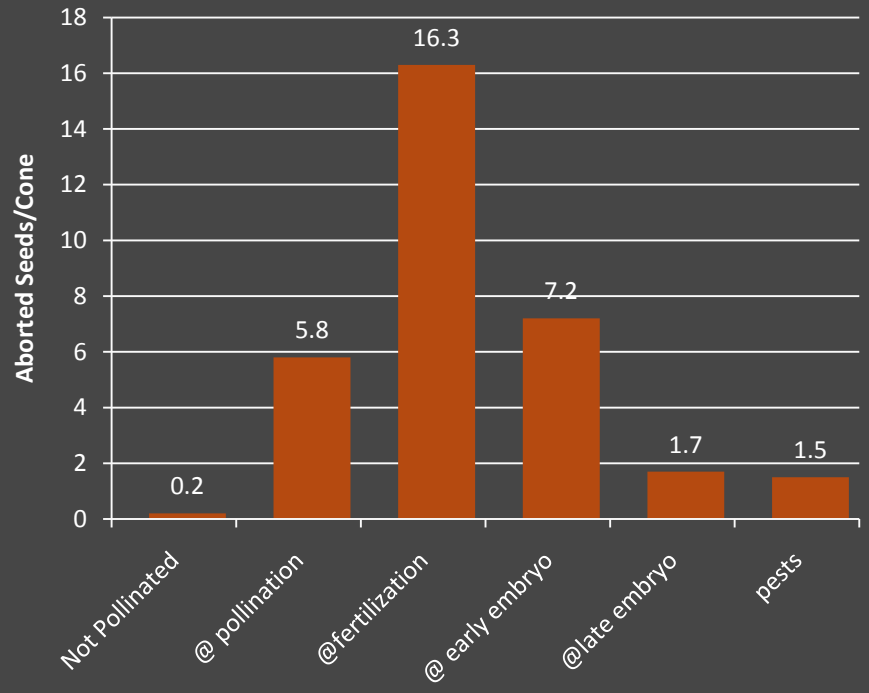
• Seed Production (Owens et al 2008)



• 77% of scales are fertile in whitebark – contrast this with the 20-25% observed in lodgepole pine !

•Seed Efficiency =filled seeds/total seeds = 59.3%

•32.7 aborted seeds per cone



Not pollinated small flat ovules
@ pollination small rounded ovules
@ fertilization full size seeds, contents aborted/dry
@ early embryo full size seeds, contents collapsed
@ late embryo full seeds, larger collapsed embryo
Damage by insects or disease

Ability to determine stages of seed abortion post-collection with non-processed seed

Cone Scales Break -Do Not Flex

- Whitebark pine lacks the anatomy for flexing
  - Course-fibre tracheids not present = no differential shrinkage of on top and bottom of cone scale
  - Whitebark scales have a thin fracture zone
  - Thickened scale may have a heat diffusion function?

Whitebark pine has evolved attractive seeds

- CROP PROTECTION is critical !

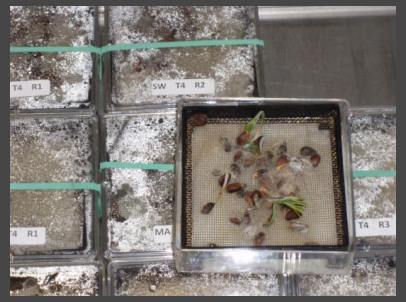
Seeds

- *Pinus albicaulis* has some significant differences compared to most pines:
  - Wingless seeds (other dispersers)
  - Seed 'Immaturity' relative to other conifers "variability"
    - Embryos not fully elongated on most seeds in most years
    - Embryos will mature given proper 'conditioning'
  - Thick seed coat – reduces rate of deterioration and allows for short-term 'seed bank' to persist
  - Deep embryo dormancy "variable"
  - Evidence for seed coat dormancy based on the success of clipping treatments
  - Fungal problems can be significant, especially during 'warm conditioning' to allow embryo maturation
  - Lipids account for over 50% of seed dry weight, but a notable proportion of unstable storage reserves is noted

Germination

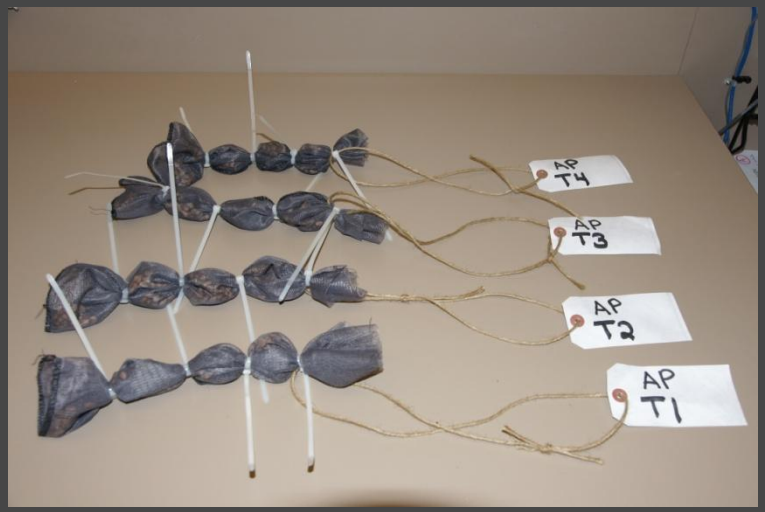
Germination is not a population characteristic

- Varies by individual year (some may always be low)
  - Seed abortion factors compound – starts with pollination success
- Varies by type and extent of processing
  - 2 kg seed @ 40% germination
  - 1 Kg seed @ 80% germination
  - X-ray empty seed removal (physiology vs. operational)
- Varies by type of pretreatment
  - Duration of warm and cold stratification
  - Clipping?
- Varies by germination criteria
  - Radicle emergence vs. seed coat shedding vs. seedling
  - Duration of assessment - 14 days vs. 28 days vs. ++ (staggered)
  - Sample size (4 x100) vs. (4 X 25) = highly variable



Whitebark Pine Treatment

1. Soak – imbibe seed – nothing happens until
2. Warm stratification – embryo maturation
3. Sanitation – required especially In warm treatment
4. Cold stratification – overcome embryo dormancy
5. Clipping – overcome seed coat dormancy

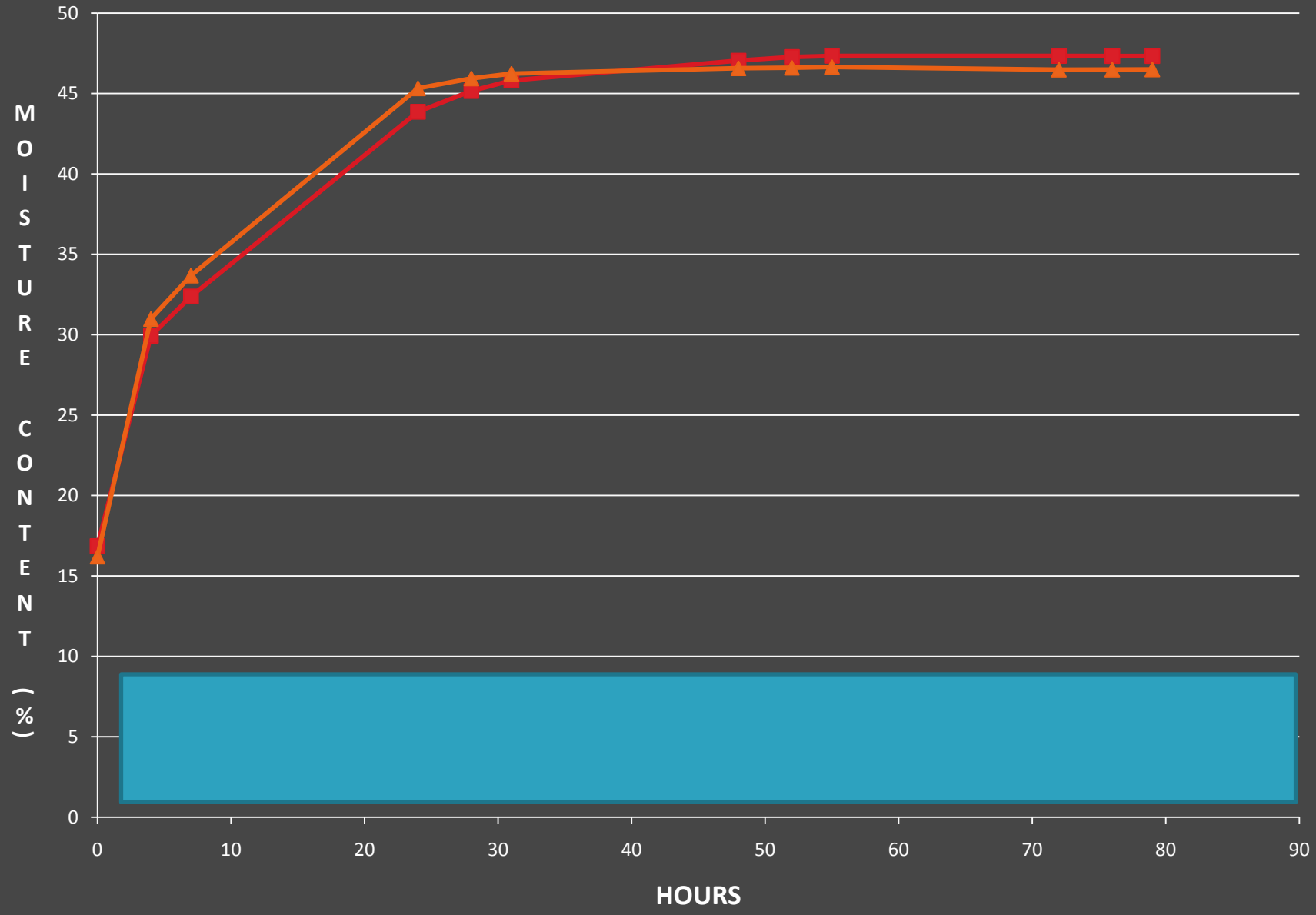


Soak

Metabolic Activitaion

- **We currently use a 48-hour running water soak (RWS)**
  - Imbibe the seed
  - Reduce the level of contaminants (i.e. *Fusarium*)
  - Some sanitize seed before initial soak
- Hard, but permeable seed coat – specific tissues offer protection from drying, predators and microbes
- Water uptake was initially quite rapid reaching 30% within one day, but uptake continued to 96 hours (Leadem 1985)
- "Burr" methodology also introduces a weekly RWS !!

Whitebark Pine Imbibition Pattern



Warm Stratification

Maturation

embryos 30-50% cavity still capable of germinating

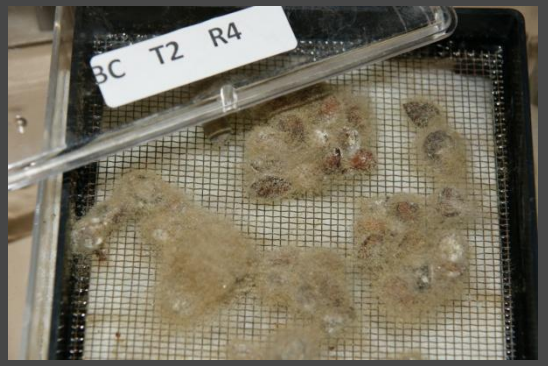
Implications for processing! Immaturity → Variability

- **We currently use 20° C for 28 days**
  - Swelling of embryo and megagametophyte
    - cutinization of hypocotyl, stomata and resin ducts
    - Vascular tissue differentiation
    - Expansion of glandular trichomes in cotyledons and hypocotyl
  - Tillman-Sutela et. al 2007

    Dormancy overcome by removal of inhibitors through repeated soak and changes in growth regulators during stratification rather than actual embryo growth
  - Warm stratification required and potentially large benefits exist by introducing a soak during the warm stratification phase – possibly also the cold stratification phase. **We'll adjust our procedures**
  - **Fungal problems prevalent in warm stratification**

Fungi found on Whitebark pine

- *Fusarium*
  - *Phoma*
  - *Cephalosporium*
  - *Gliocladium*
  - *Penicillium*
  - *Chromolosporium*
  - *Rhizopus*
  - *Cladosporium*
  - *Trichoderma*



- Mainly saprophytic fungi
- Probably comes in after initiation of

'cone dissection' by Clark's nutcracker – <u>via air or beak</u>

Sanitation

- Conditions good for fungal growth during warm stratification
  - Elevated temperatures, high humidity
- **We sanitize with H_2O_2 as required (can be > once /week)**

  To sanitize before and/or after soaking? WHEN?

  1) Initial sanitation may result in uptake of non-desirable compounds?
  2) Use of hydrogen peroxide may result in oxygenation benefit once H_2O_2 breaks down?

  Bleach vs. Hydrogen Peroxide ? Cinnamon! WHAT?

  Some have used ethanol (70%) to spot-sanitize

Be prepared to employ some form of sanitation treatment

Cold Stratification

Overcome embryo dormancy

We have used 2 months as a minimum - try and increase

- "All" evidence points to longer cold stratification resulting in increased, more uniform germination
  - *Some results have shown increases after additional warm (germination) followed by increased cold stratification* (i.e. low germination –put back in cooler)
- Sanitation not as critical – introduction of soaks at this stage may also removes inhibitors (**YC**) ?

Clipping

Remove Barrier (Physical Dormancy)

- Most studies indicate performing this at treatment end (*emergence barrier)* vs. pre-soak (*water uptake barrier*)
- Pre-soak clipping may also allow fungi to enter seed and deteriorate contents
- Some studies show this has a large benefit to germination, others not so – probably seedlot and treatment related
- Labour intensive step
- Re-hydration may assist clipping

Dibbling Germinants

- In our germination trials we have attempted to dibble germinants directly into styroblocks
- Information was the product – seedlings the byproduct
- Dave Enns (Landing nursery) has done a great job for us
  - Dibble as early as possible (don't wait until radicle is 4X seed coat)
  - Remove fungal infested seeds to reduce further losses

Ex Situ Collection Testing

- GCTAC of the Forest Genetics Council has invested in whitebark pine collections and received seedlot donations
- To have representative sample from populations in BC (10+ individuals/site)
- Single tree collections primarily
  - Genetic sampling / conservation
  - Studies of genetic architecture
  - Potential for blister rust/ other trait evaluation
- Some bulk collections (seedlots) donated
- Some collections obtained separately with Research Branch funding

GCTAC Collections

- 29 Collections (26 Sites – Apex/Lime/Blackcomb Mt X2)
  - Almost 50 Kg of seed
  - 317 individual tree collections
  - 5 bulk collections

- Will test a 'representative sample' of families within each collection
- Obtain a provenance estimate of seed quality
- Where do we have extra?
  - National Seed Bank
  - Restoration work
- Starting in roughly October for May sowing