Seed Stratification, Maturation and other Seedy Tidbits

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Cone Scales Break - Do Not Flex

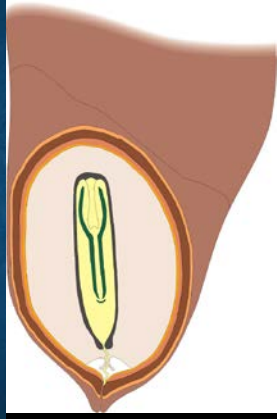
- Whitebark pine cones have evolved for bird dispersal
  - Whitebark scales have a thin fracture zone allowing ‘easy’ scale removal to expose seeds
  - Course-fibre tracheids are NOT present = no differential shrinkage of on top and bottom of cone scale
  - Thickened scale may have a heat protection function?

Whitebark pine has evolved attractive seeds

CROP PROTECTION is critical !

Fatty seeds are attractive
Ensure adequate ventilation

Seed Adaptations

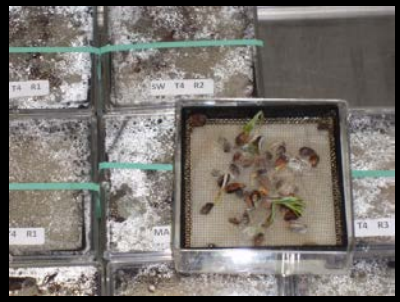


- *Pinus albicaulis* has some significant differences compared to most pines:
  - Wingless seeds (other dispersers)
  - Seed 'Immaturity' relative to other conifers "variability"
    - Embryos not fully elongated on most seeds in most years
    - Embryos will mature given proper 'conditioning'
  - Thick 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 ? More radicle emergence vs. moisture uptake
  - 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 (related to maturity)

Germination

Germination is not a population characteristic

- Varies by individual year (some may always be low)
- Seed abortion factors compound :
  - Pollination success (late frosts – wet weather challenges)
  - Fertilization success (pollination – pollen germination + growth)
  - Developmental success (pollination, fertilization, GDD free of pests)
- Varies by type and extent of processing
  - 2 kg seed @ 40% germination
  - 1 Kg seed @ 80% germination
- Varies by type of pretreatment
  - Duration of warm and cold stratification
  - Clipping?
- Varies by germination criteria
  - Radicle emergence vs. seed coat shedding vs. seedling production
  - Duration of assessment - 14 days vs. 28 days vs. ++ (staggered)
  - Sample size (4 x100) vs. (4 X 25) = highly variable



X-ray Based Seed Viability Estimates

- Seed **Value** , quantity, time, # individual tree collections make germination tests 'unrealistic'
- Operational seedlots have germination estimated based on x-rays
- Viability (alive=potential) vs. germinability (overcome dormancy ++)
- Range in seedlot quality below – PROCESSING EFFICIENCY

Interactive lab exercise

Hi-lite 100 seeds

- Green = viable
- Pink = non-viable
- Yellow = questionable

Compare assessments between technicians

Discuss → Consensus

Final seedlot determination at left

Tree Variability on same Site

Elizabeth mine

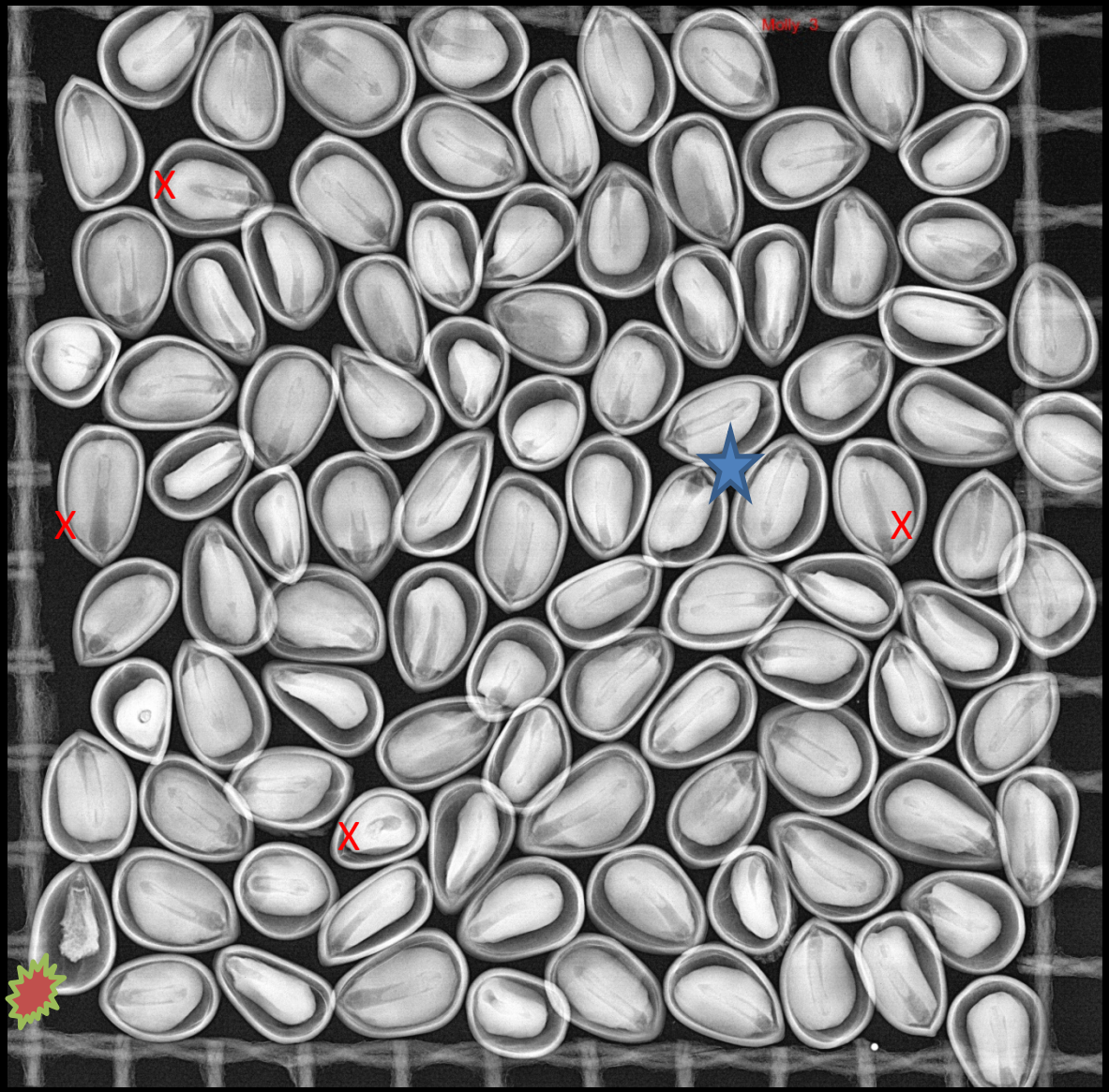
- Tree variability in seed quality can be high in the same year at the same site?
- Variability in Pollination, Fertilization , Development

EM Tree #4

EM Tree #11

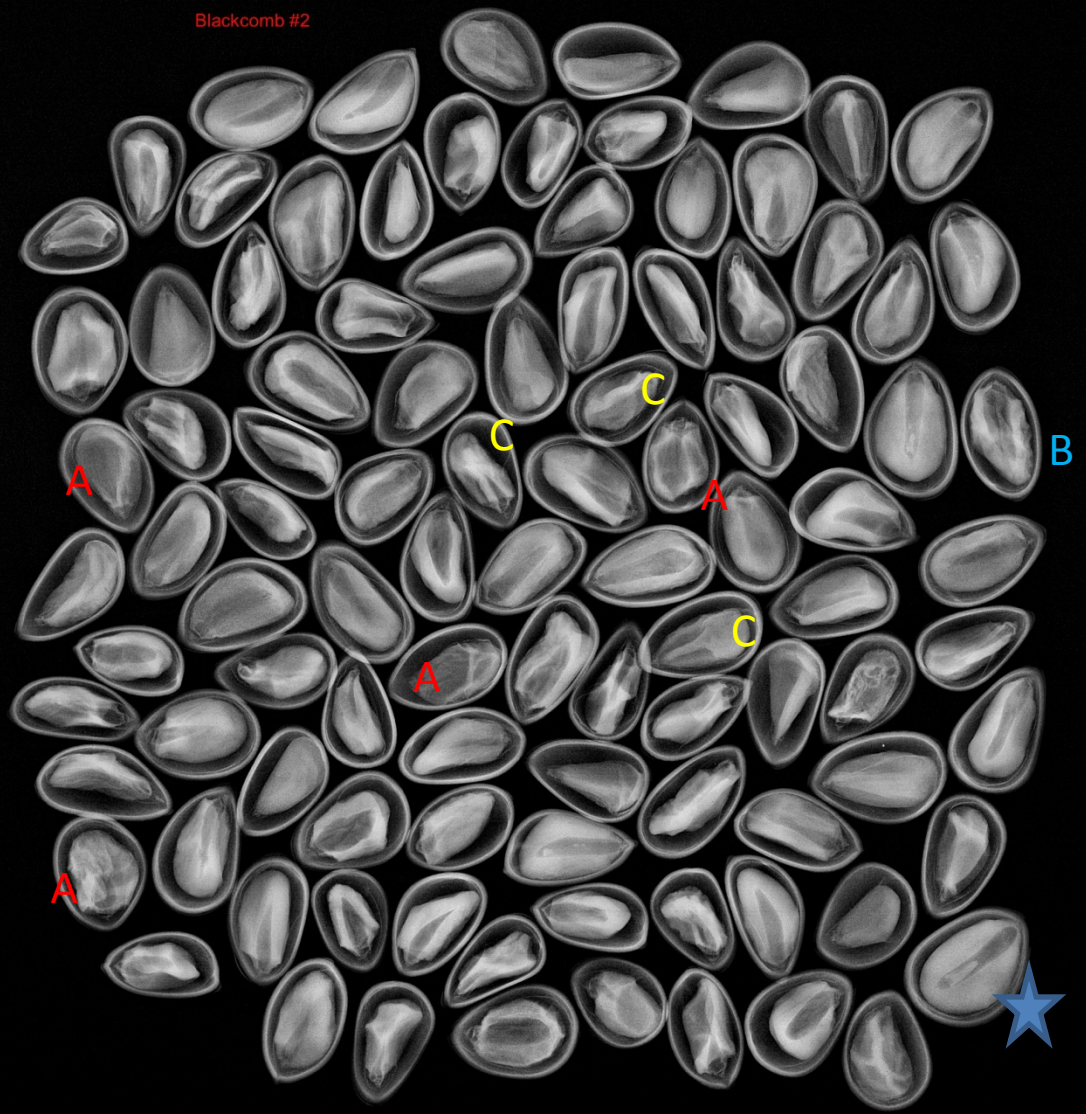
Good Seed Quality example Molybdenite Tree #3

- Mostly filled seeds, but this is just as much a function of processing (extract seeds vs. remove 'empties") as biology**!**
- Filled Seeds show good embryo growth and solid megagametophyte
  - Species exhibits large variation in embryo length (What is enough? X)
  - Nutritive tissue shrinkage away from seed coat related to moisture content – dry for storage



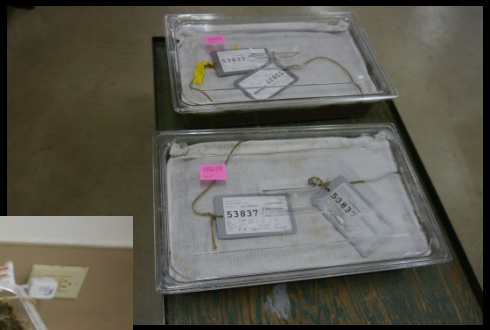
Bad Seed quality example Blackcomb Tree #2

- Seed coat development occurs prior to fertilization
- Externally, filled and empty seeds are no different
- Deterioration progression
- A-early : pre-fertilization
- B-development : fertilization
- C- late embryo
- Categorization to time of ‘abortion’ not easy



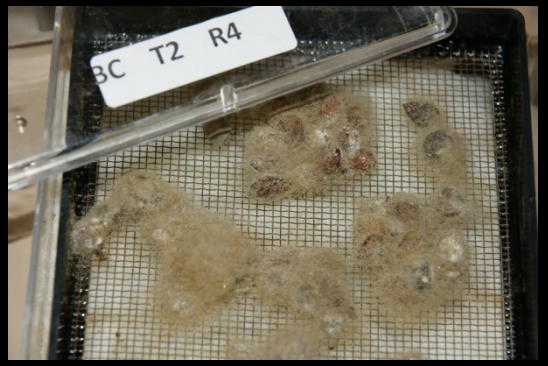
TSC Stratification Practices

- 2016 - seedling requests -builds on experience of many others **(3 day soak – 28 day Warm – 77 day cold)**
- TSC will focus on whitebark pine seedling requests
- Imbibed seed placed in mesh bag, covered in sand and then bar towel on top
- Seed weighed/adjusted weekly – critical in warm stratification



Fungi found on Whitebark pine

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

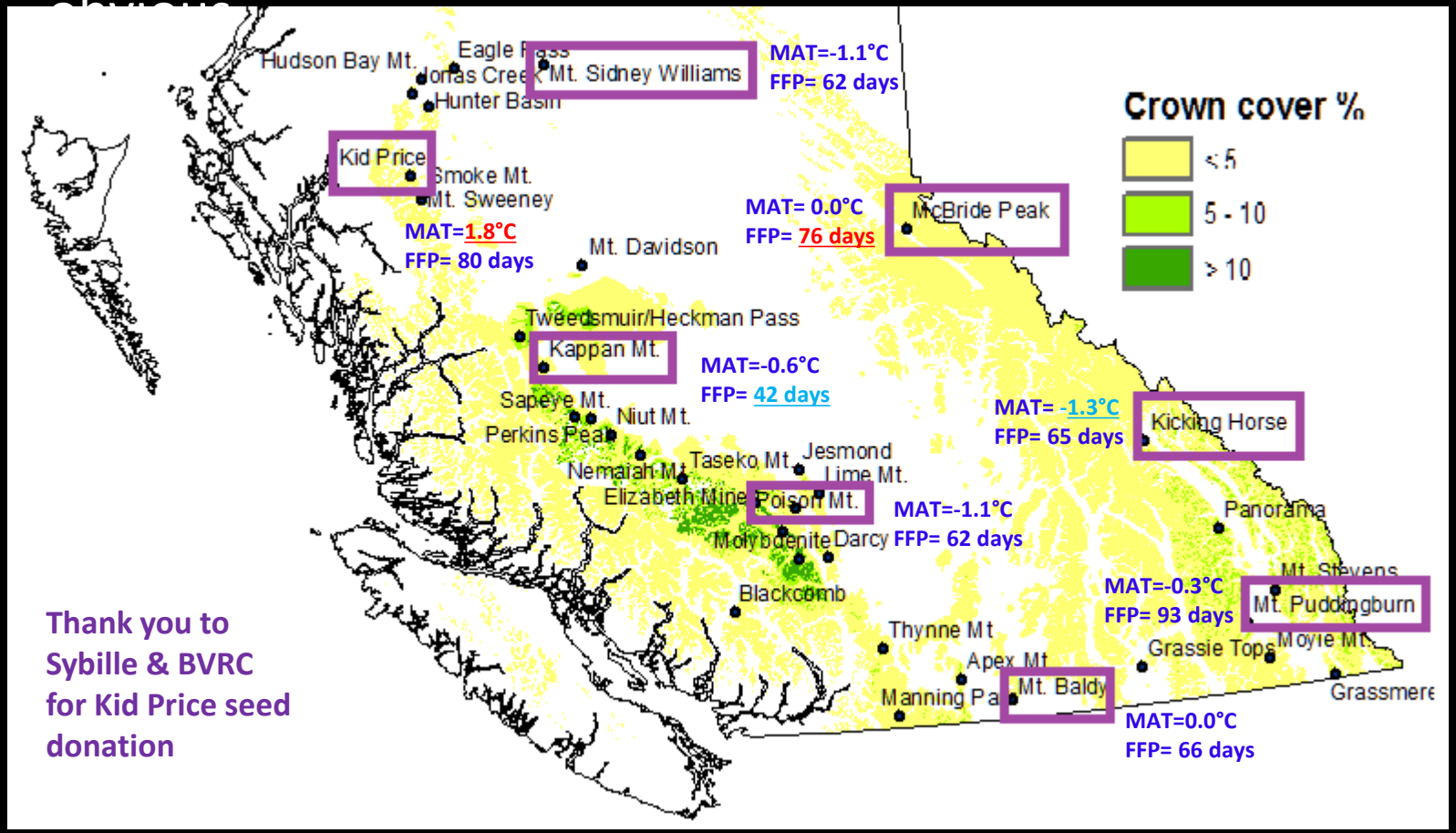


- Mainly saprophytic fungi
- Probably comes in after initiation of

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

BC Stratification Trial

- 8 individual trees from widely dispersed populations
- Minimize within seed source variability – seed availability
- MAT and FPP (time to produce seed) not always intuitively obvious

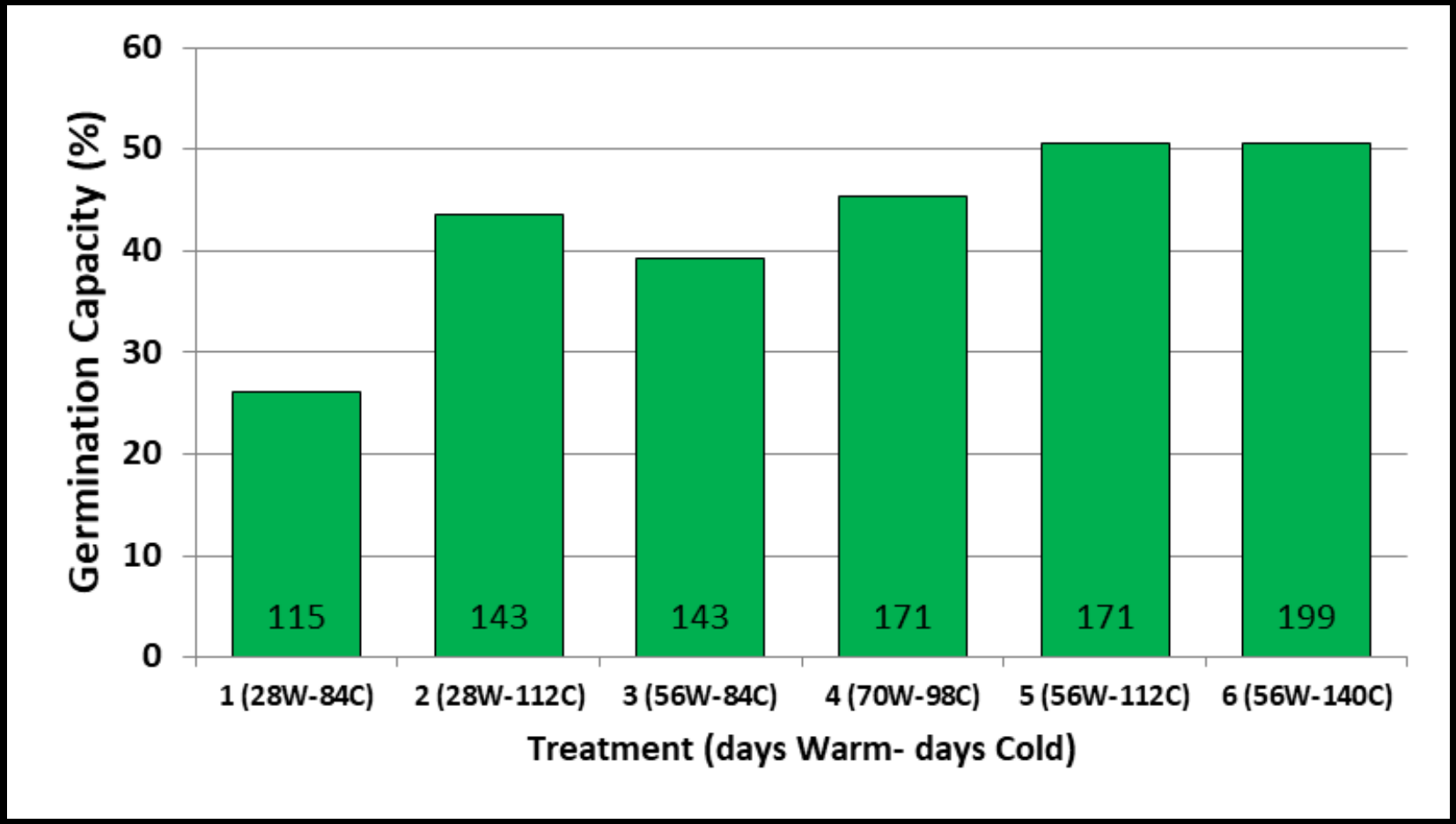


Stratification Treatments

Treatment	Soak - days	Warm (20 C) days	Cold (2-5 C) days	Total days
1	3	28	84	115
2	3	28	112	**143**
3	3	56	84	**143**
4	3	70	98	**171**
5	3	56	112	**171**
6	3	56	140	199

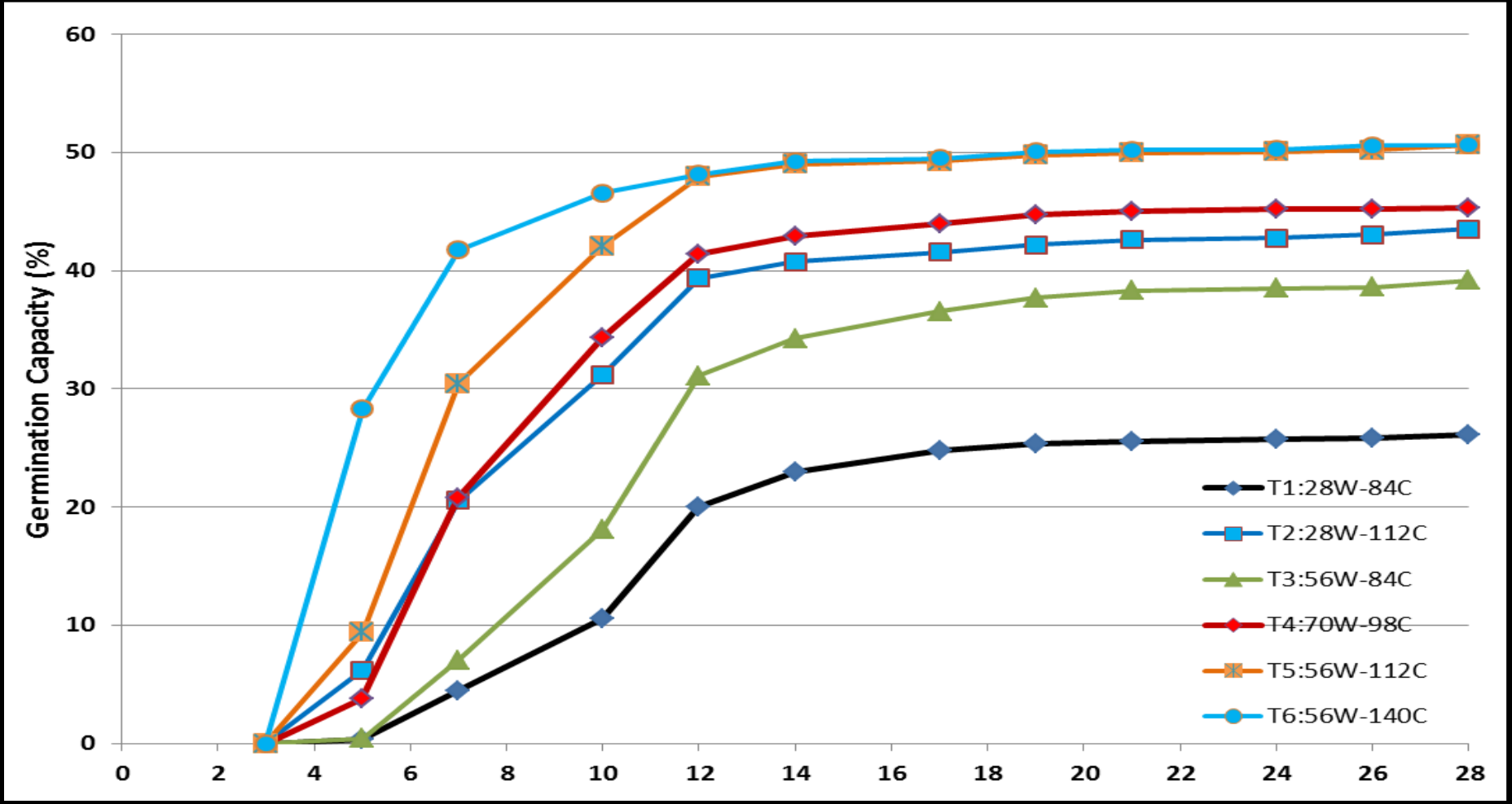
- Extended 3–day running water soaks used based on work performed by Bob Karrfalt
- Warm and cold stratification performed on sand
- No seed sanitation performed *(wouldn't disregard that step again)*
- 4 replicates of 50 seeds = 200 seeds per treatment (9600 seeds)
- Seeds germinated under 25° C (8 hours) / 15 °C (16 hours) conditions
- Germination criteria – radicle = length of seedcoat
- Germinants salvaged – shipped to nurseries

Treatments Mean Results



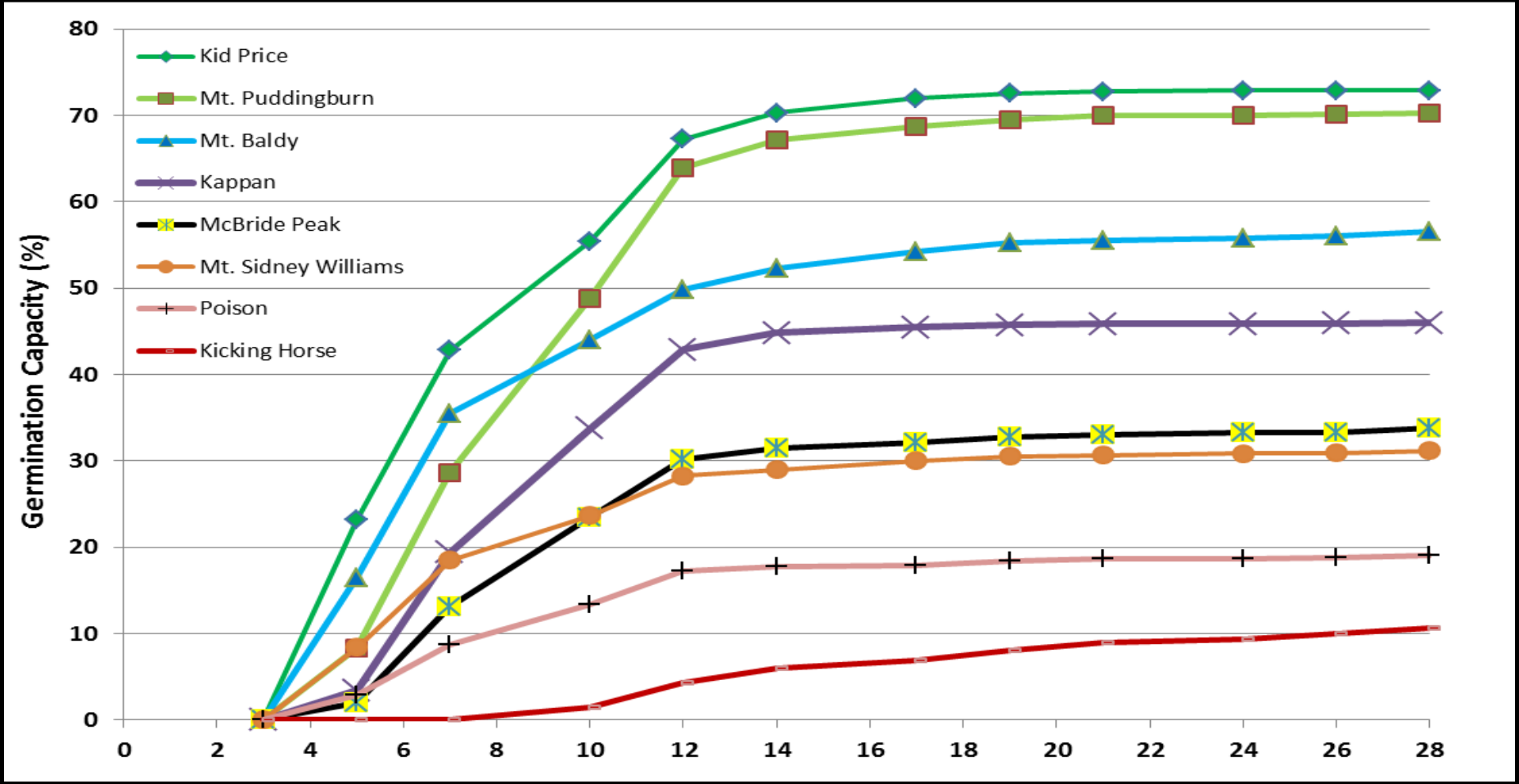
- Increased cold stratification is the best use of time
- Extending warm stratification beyond 28 days is beneficial
- Increasing cold beyond 112 days had minimal benefit on germination under "optimal conditions" – increased stratification beneficial under suboptimal conditions

Treatment Germination Curves



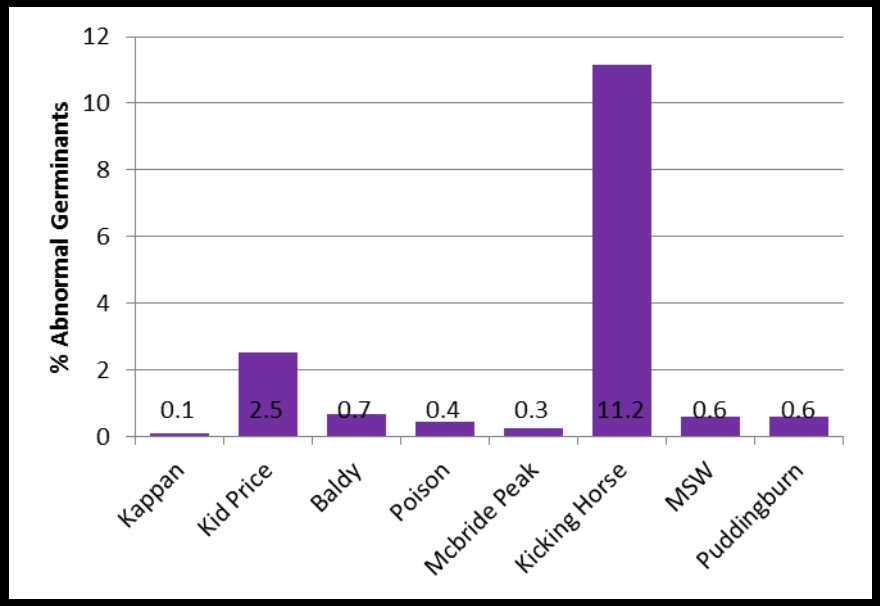
- Increasing cold stratification beyond 112 days increased germination rate
- T1 (115 days) achieved about half the germination one can get with 171 days

Individual Family Results



- Very large difference in family lot quality (11 to 73%)
- Germination is related to processing efficiency vs. geography (Germ vs. Yield)
- Kid Price did receive secondary processing (YPP)

Abnormals Germinants by Family



- Abnormal germinants are ‘viable’ seeds that will not produce a seedling
- Reversed and stunted radicle most common
- Kicking Horse has a very high %

Stratification Recommendations

- Allot plenty of time (6 months!)

3-day running water soak

H_2O_2 treatment = 3% for between 2-4 hours

other options may also work

56 days warm stratification

This is the wildcard element – probably maturation dependant

112 days of cold stratification

More isn't detrimental in most seedlots

- Monitoring is important

Other Recommendations

- **Alberta (Lindsay Robb)**
  - 48 hour soak
  - sand medium recommended
  - 12 weeks warm stratification (20°C)
  - , 16 weeks cold stratification
- **Coeur d'Alene Idaho (2016)**
  - 48 hour running water soak
  - 30-days warm stratification (30 °C/ 20 °C) 12 hours each
  - 90 days cold stratification
    - Seeds rinsed for 1 hour every week
    - Every month plastic bag changed

- **Dorena – 2014**
  - 24 hour hydrogen peroxide soak, rinsed then 24-hour water soak
  - 30 days warm stratification (10 °C)
  - 110 days cold stratification
  - Each seed hand scarified with sanding machine
  - Sowing germinants

- **2015 Trials**

- 1) **48-hour soak in aerated water – sand – no scarification**
  2) 24 hour GA3 +24 hr H20 – scarified
- 3) 24 hour H2O2 + 24 hr H20 – mesh in peatmoss 140 days (poor)

Maturation Help ?

- Based on some work done with Limber pine from two sources (Windy Point & Prairie Bluff)
- Opportunities for ex-situ cone maturation
- **20° C at 90% RH** – scary conditions for many of us
- Cones were kept this way for 0, 2, 4 and 6 weeks for cones collected between mid-August to October
- The 2 earliest harvests resulted in increased embryo length and germination
- Using this with the latest harvests resulted in 'signs of degradation' **SCOPE**
- Potential for very early harvests or sites with very short growing seasons (Radiata pine in NZ)


HC01

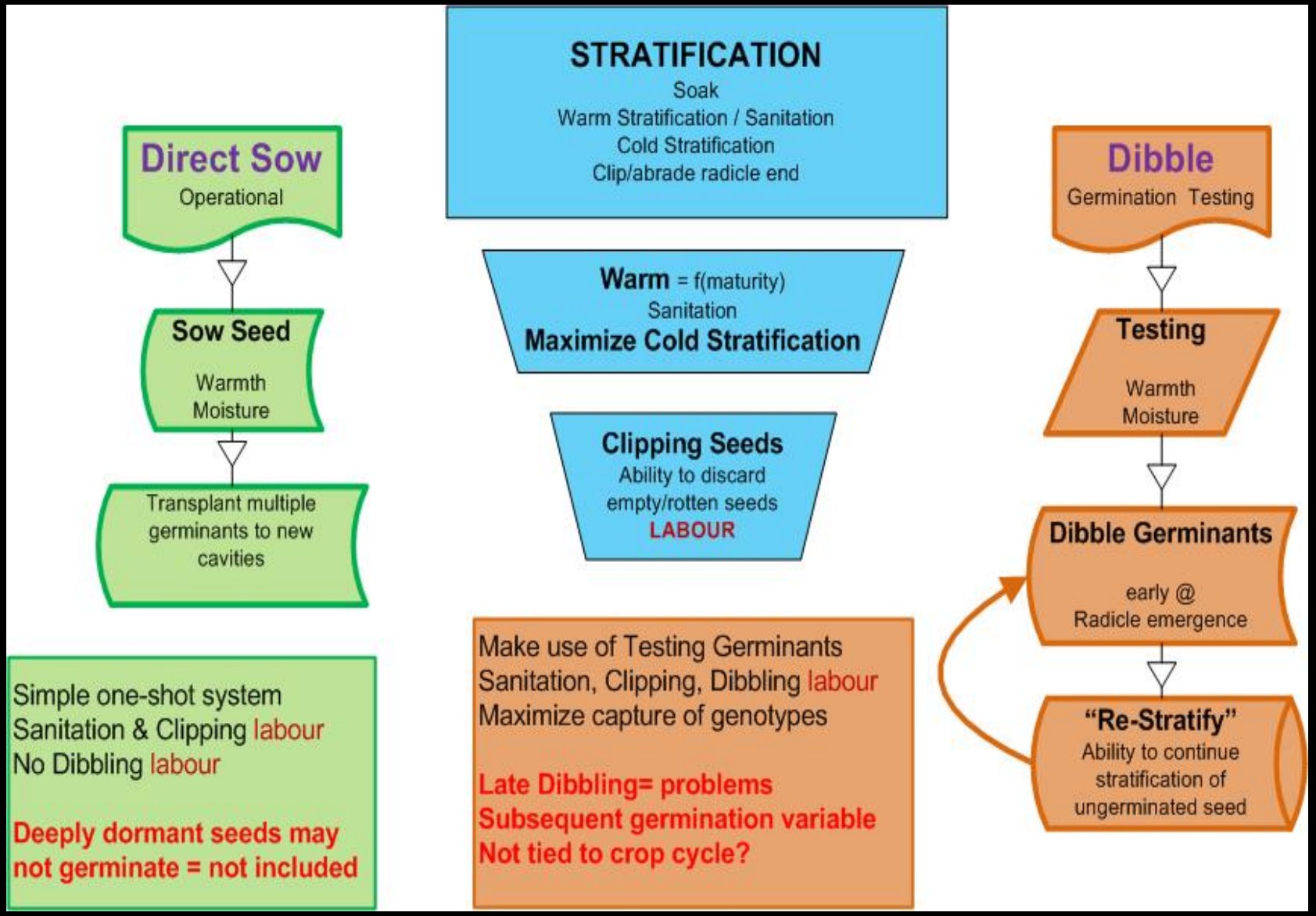

WE03
WE03
HC01

Processing Efficiency!

- **Removal of non-viable seeds and deteriorated seeds**
- **That's the best sanitation method**

- Immaturity causes problems = variability in specific gravity – uncertainty whether we can get a seedling ?
- Probably worthwhile being conservative – almost all embryos have a chance (alternative is to accept losses)
- We want maximum maturity, but that probably never happens on the tree at some sites
- Embryo maturation probably happens over several seasons
- The rational for the warm stratification
- Leaving caged cones on trees is RISKY

Propagation Options (Sow vs. Dibble?)



A Bit of Heresy?

Clonal Forestry

Somatic Embryogenesis is possible!
AB has about 40 lines developed by CFS

How will the blister rust selection program produce enough propagules to make a difference ?

How many resistant individuals will need to be put on the landscape to make a difference - regardless of propagule type?

What are the current thoughts regarding individual tree vs. population resistance levels?