Genetic Selection in Coastal Douglas-fir for Tolerance To Swiss Needle Cast (SNC) Disease

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Main Points

- **SNC is a native foliar disease which has become widely expressed on the Oregon Coast over the last 20 years**
  - Peak expression was 2011 with **177,691** ha.
    moderate to severe symptoms

- **Nehalem test series:**
  - Foliage traits indicating tolerance to SNC are heritable, but less heritable than growth traits
  - Age-11 tree size was a better predictor of age-26 DBH than any age-11 foliage trait
  - Usable genetic gains for DBH in the presence of SNC has been demonstrated through age-26

In areas with > 2 years of needle retention gains
Main Points

• **Early Selection Study:**
  – Age-2 selection for foliage traits did not result in any gains in age-10 growth
  – Natural infection is high in appropriately located field tests, no need for artificial inoculation

• **Study of fungal DNA amount and infection:**
  – Variation in impact of SNC seems to be associated with *tolerance*, not *resistance*
    • [Side note: Despite the above, green healthy trees are sometimes observed in badly affected stands]

• **Association of growth gains on Oregon coast with geographic variables of parent trees:**
  – No consistent pattern with latitude or longitude
Main Points

• 2\textsuperscript{nd}-Cycle strategy:
  – 30 2\textsuperscript{nd}-cycle test sites established on OR coast, and 5 test sites on the WA Coast
  – Recurrent selection for GCA remains the strategy
  – Field testing in areas with moderate/severe SNC
  – Needle retention is inexpensive to rate, so we continue to score it even though it does not currently appear to add to selection effectiveness (compared to selection on growth alone)
  – Heritability patterns are similar to those seen in first-cycle
  – Ranks on coastal areas are consistent with ranks in inland areas not subject to SNC
Main Points

• 2\textsuperscript{nd}-Cycle strategy:
  – No marker work done to date or currently planned
  – Cloned breeding population would be expensive
  – No early (e.g. age-2) testing, growth impacts take a while to become evident
  – Despite the “low-tech” approach, tested 2\textsuperscript{nd}-cycle families have shown large realized gains in growth over unimproved DF in the SNC zone (at age-7 and 12)
• **Operational Implementation:**
  
  – Seed orchard blocks have been established for good growth in the SNC zone, and seedlings are being operationally deployed
  
  – Future seed orchards will emphasize the same trait
  
  – Wind-pollinated orchards
  
  – Mixtures of family lots are used
  
  – As DF stands are logged, likely to be replaced by mixed species plantings (moderate SNC) or alternate species (very high SNC)
  
  – Given the strategy, we do not anticipate putting much (if any) selection pressure on the pathogen to become more virulent
Main Points

**Overall context:**
- A low-cost but effective breeding and deployment strategy, for a major temperate conifer to co-exist with a non-lethal native foliar pathogen
  - In contrast to the lethal disease Laminated Root Rot for which no breeding work is attempted in the Pacific PNW, other tree species are planted instead
- The SNC epidemic re-invigorated breeding and testing for western hemlock as an alternate species for the Oregon and Washington coasts
Overview

• Background on SNC and the SNC co-op
• Work in first generation testing programs
  – Genetic control of traits affected by SNC
  – Joint work of breeding co-ops and SNC Co-op
  – Potential genetic gains
  – Early selection work (Fatih Temel)
  – Is it resistance or variation (Fatih Temel)?
• Second-cycle testing programs
  – South Central Coast (jointly with SNCC)
  – Trask
  – Plum Creek T96
• Operational Management of the Disease
Swiss Needle Cast in Oregon
• Swiss needle cast (SNC) of Douglas-fir, caused by the ascomycete fungus *Phaeocryptopus gaeumannii*, is associated with volume growth losses (20-50%+) along the Oregon Coast.

• Although the pathogen is endemic, disease symptoms have intensified in coastal forests of Oregon and Washington since the early 1990s,

  – Reached a peak of 444,228 symptomatic acres (177,691 ha) detected in the 2011 aerial survey of western Oregon.
Area of Douglas-fir forest with Swiss needle cast symptoms detected by aerial surveys, Coast Range, Oregon, 1996-2011
• Climatic conditions near the Oregon coast are often conducive to SNC disease development.
• Mild winter temperatures and spring/summer leaf wetness are key.
• SNC Cooperative has been in operation at OSU since 1997
  – Research: 43
Coastal Douglas-fir is an enormously important timber species for Oregon.

There has been considerable interest and investment in developing seed sources suitable for this zone.

Fungal infection and needle colonization occur passively, through needle stomata and the Pseudothecia of *P. gaeumannii* plug the stomata.
• All Douglas-fir everywhere are naturally infected with *P. gaeumannii*, yet disease only develops in certain geographic settings where pseudothecia are produced on 1 and 2 year-old needles.

• Foliage retention is directly related to tree productivity. Severely infected trees have <1 yr. foliage retention.
Work in First Generation Testing Programs
First-generation of co-operative tree improvement in the PNW was characterized by a large number of small breeding zones.
• As SNC effects became apparent in the 1990s, Randy Johnson (USFS PNWRS) and others focused on Douglas-fir tree improvement programs located on the North Oregon coast where the impact was most severe
  – Nehalem cooperative program
  – US Forest Service Hebo programs
• Some work was also done in the Bureau of Land Management Breeding Unit 12 tests
Coastal Parent Trees

Progeny Test Sites Used

- Nehalem
  - 5 Progeny Test Sites

- USFS
  - 2 Progeny Test Sites

- BLM 12
  - 2 Progeny Test Sites

Slide by: Randy Johnson
• DBH was measured in Nehalem tests at age-11, and again in 5 of the tests at age 17/18
  – Large test series with 400 families, mostly OP

• Following 8-9 more years with severe/moderate SNC, SNCC and the Trask breeding co-op approved in 2010 a 3rd DBH measurement on 5 sites at age 26.

• The 5 sites were all owned by the Oregon Department of Forestry, had estimated NR of 1.0 – 2.0 years based on the Adams / Latta model developed with the support of the SNCC, and had been thinned removing alternate diagonals.

• The most recent measurement was done between July 22 - August 18, 2010 (age 25.5
Nehalem Test Sites and Needle Retention
Some important questions were:

- (1) Are some families falling out with the continuing incidence of SNC, as evidenced by notable changes of ranks for dbh over the years?
- (2) How much diameter growth is occurring on the top families?
- (3) What is the predicted gain for total dbh and dbh increment of the top families?
- (4) What is the correlated gain for dbh at age 25.5 from selection for height/dbh/volume index/crown traits at age-11?
Murphy’s Law # 105:

“Three months after you thin a progeny site and leave the logs on the ground someone will decide to measure it again”
Results in Nehalem First Generation Testing Program
Growth by Color Categories for Nehalem Foliage color
(1=yellow to 3=dark green)

Growth by Crown Density Categories for Nehalem
(1=very sparse to 6=very dense)

Slide by: Randy Johnson
# Narrow-sense heritabilities in 1\textsuperscript{st} cycle Nehalem series:

<table>
<thead>
<tr>
<th>Trait</th>
<th>Across sites</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Individual</td>
<td>Family-</td>
<td>Mean</td>
</tr>
<tr>
<td>Height 11</td>
<td>0.30</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Volume Index 11</td>
<td>0.29</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Dbh 11</td>
<td>0.27</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Dbh 17/18</td>
<td>0.35</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Dbh 26</td>
<td>0.36</td>
<td>0.70</td>
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<tr>
<td>Dbh_Increment_ (age-11 to 17/18)</td>
<td>0.32</td>
<td>0.80</td>
<td></td>
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<tr>
<td>Needle Retention: 1994 primary laterals</td>
<td>0.12</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Needle Retention: 1994 secondary laterals</td>
<td>0.10</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Needle Retention: 1993 secondary laterals</td>
<td>0.23</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Crown Density</td>
<td>0.18</td>
<td>0.71</td>
<td></td>
</tr>
</tbody>
</table>

*Needle retention: 0 = <10\% to 9 = >90\%*
Correlations between predicted gains and z-scores for traits from Nehalem test series
Traits: age-11 **height** ( =HT11) and **DBH** (=DBH11); age-17/18 DBH (=DBH17/18), age-26 **DBH** (=DBH26) **needle retention** on 1994 primary laterals (=NR94p), on 1994 second laterals (=NR94s), and on 1993 second laterals (=NR93s)

<table>
<thead>
<tr>
<th>Trait</th>
<th>VOL 11</th>
<th>DBH 11</th>
<th>DBH 26</th>
<th>NR 94p</th>
<th>NR 94s</th>
<th>NR 93s</th>
<th>crown density</th>
<th>foliag color</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT5</td>
<td>0.82</td>
<td>0.74</td>
<td>0.56</td>
<td>-0.20</td>
<td>-0.13</td>
<td>-0.11</td>
<td>0.15</td>
<td>0.03</td>
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<tr>
<td>HT 11</td>
<td>0.88</td>
<td>0.80</td>
<td>0.67</td>
<td>-0.04</td>
<td>0.03</td>
<td>0.05</td>
<td>0.15</td>
<td>0.23</td>
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<tr>
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<td>0.97</td>
<td>0.72</td>
<td>0.00</td>
<td>0.06</td>
<td>0.03</td>
<td>0.34</td>
<td>0.30</td>
</tr>
<tr>
<td>DBH 11</td>
<td></td>
<td></td>
<td>0.66</td>
<td>0.03</td>
<td>0.08</td>
<td>0.03</td>
<td>0.39</td>
<td>0.35</td>
</tr>
<tr>
<td>DBH 17/18</td>
<td></td>
<td></td>
<td>0.84</td>
<td>0.17</td>
<td>0.21</td>
<td>0.17</td>
<td>0.51</td>
<td>0.51</td>
</tr>
<tr>
<td>DBH _Increment (age-11 to 18)</td>
<td></td>
<td></td>
<td>0.84</td>
<td>0.25</td>
<td>0.29</td>
<td>0.26</td>
<td>0.50</td>
<td>0.55</td>
</tr>
<tr>
<td>DBH26</td>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
<td>0.20</td>
<td>0.16</td>
<td>0.48</td>
<td>0.32</td>
</tr>
<tr>
<td>NR94p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.83</td>
<td>0.87</td>
<td>0.23</td>
<td>0.41</td>
</tr>
<tr>
<td>NR94s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.74</td>
<td>0.23</td>
<td>0.41</td>
</tr>
<tr>
<td>NR93s</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.16</td>
<td>0.38</td>
</tr>
<tr>
<td>crown density</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
</tbody>
</table>
Potential Genetic Gains

- Nehalem: When the top 10% parents were selected for a given trait, the average predicted gain ($\Delta G$) for age-26 DBH was:
  - age-26 DBH $\Delta G = 18.2\%$
  - age-11 DBH $\Delta G = 13.4\%$
  - age-11 HT $\Delta G = 11.0\%$
  - age-5 HT $\Delta G = 9.3\%$
  - NR on 1993 secondary laterals $\Delta G = 1.3\%$
Conclusions from 1\textsuperscript{st}-gen programs

- Foliage traits are heritable ($h^2 \approx 0.1$ to $0.2$), but less heritable than growth traits ($h^2 \approx 0.3$)
  - Could be a result of foliage traits being subjectively scored
  - **Crown density** was seen as a better indicator of tolerance than NR (Johnson 2002)

- Growth after age-11 was more strongly associated with age-11 tree size (type B correlations $\approx 0.7$) than with foliage traits (type B correlations $\approx 0.2$ to $0.5$)
  - Still rank changes have taken place for DBH
Conclusions from 1st-gen programs

• Usable gains for DBH were seen even at age-26
  – The top 10% of parents for DBH-26 had predicted gains of 18.2% over population mean for DBH-26
  – The top 10% of parents for DBH-26 had predicted gains of 23.7% over population mean for DBH 18 to 26 increment
  – In areas with > 2 years of foliage, gains from selection for DBH should be adequate to offset volume growth losses from SNC, and keep Douglas-fir as a viable plantation species

• Selecting on an index of age-11 crown density + age-11 DBH (≈13.8% gain) was
Early Selection – Will it help DF breeding programs?
Coastal Parent Trees

- 2-yr-old tests
- USFS 10-yr-old Progeny Test Sites

Slide by: Randy Johnson
Field Traits Measured in 2-yr-old Trials

- Foliage Color (whole plant)
- Needle Color (selected branch)
- Retention
- Crown density

Foliage assessed in the lab for:

<table>
<thead>
<tr>
<th>$P. \text{gaeumanni}$ infection</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of fungal DNA / needle</td>
<td>Needle color</td>
</tr>
<tr>
<td>% stomata occluded with pseudothesia</td>
<td>Needle retention</td>
</tr>
<tr>
<td></td>
<td>Needle wt</td>
</tr>
<tr>
<td></td>
<td>Needle specific area</td>
</tr>
<tr>
<td></td>
<td>Needle length</td>
</tr>
</tbody>
</table>

Slide by: Randy Johnson
• Better $h^2$ observed in the field than in the lab, so will focus only on field traits symptoms
• Heritabilities were similar at both ages for the field traits
  All moderately low, 0.11 to 0.37
### Genetic correlations among age-2 and age-10/13 traits

<table>
<thead>
<tr>
<th></th>
<th>Age-2 traits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Needle color</td>
</tr>
<tr>
<td>Needle retention (age10)</td>
<td>0.75</td>
</tr>
<tr>
<td>Crown density (age-10)</td>
<td>0.19</td>
</tr>
<tr>
<td>Foliage color (age-10)</td>
<td>0.53</td>
</tr>
<tr>
<td>Ht 10</td>
<td>-0.05</td>
</tr>
<tr>
<td>DBH 13</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Slide by: Randy Johnson
Is it resistance or tolerance - Why do we see variation?

The Relationship between SNC symptom severity and level of *Phaeocryptopus gaeumannii* Colonization

More of Fatih Temel’s Ph.D. Thesis
Trial #1 – The early selection study
2-year-old progeny tests – Lab results

<table>
<thead>
<tr>
<th>Trait</th>
<th>Family-mean heritabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needle color</td>
<td>0.26</td>
</tr>
<tr>
<td>Needle retention</td>
<td>0.57</td>
</tr>
<tr>
<td>Dry weight</td>
<td>0.40</td>
</tr>
<tr>
<td>Needle length</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Fungal DNA</strong></td>
<td>Non Significant</td>
</tr>
<tr>
<td>Pseudothecia counts</td>
<td>0.43</td>
</tr>
<tr>
<td>Needle specific area</td>
<td>0.41</td>
</tr>
</tbody>
</table>

No family differences in amount of fungal DNA in needles

Slide by: Randy Johnson
Trial #2

- 2 Nehalem progeny test sites
- 6 families
  - 2 severely diseased, 2 average, 2 lightly diseased
- Total of 108 15-year old trees
- Sampled branch from 4th whorl down
- Looked at relationships between symptom severity and level of *P. gaeumannii* colonization

Slide by: Randy Johnson
Symptoms differed between the 3 disease severity groups 

(no surprise, since they were selected for differences in growth and foliage symptoms)
Infection did NOT show statistical differences between the 3 disease severity groups

Two separate studies showed no differences in the amount of fungus in needles of different families

Concluded that this must be *tolerance* rather than *resistance*: while all trees were infected and had similar amounts of fungus in their needles the family groups displayed different levels of symptom severity

Slide by: Randy Johnson
Potential mechanism: *(Temel):*

Tolerant families produce photosynthetically efficient “inexpensive” needles and drop them as they get occluded before they become a photosynthetic sink.
Conclusions

• Early selection could improve the efficiency (gain/yr) of selection for foliage traits
  – But we didn’t see any gains in growth traits at age-10 resulting from age-2 selection for foliage traits

• We can breed for tolerance to SNC (not resistance)

• The best trait to select upon is growth in the presence of the disease
  – Foliage traits (at age-11) can help a little

• Deploy tolerant stock in areas of moderate SNC impact

• In areas with higher disease pressure and ≤ 2 years foliage retention, can use alternate timber
Implementation of Testing for Needle Cast Tolerance in 2nd-Generation Programs
2nd-Cycle Programs

• With insights from 1st-gen data and previous experience, the following steps were taken:
  – Established 2nd-cycle programs on the Oregon Coast
    • South Central Coast in the South, with the mainline in the core Douglas-fir area, and a smaller satellite program in the zone most affected by SNC
    • Trask in the North, with a Coast program (moderately affected by SNC), and an Inland program (little affected by SNC)
    • Plum Creek’s Toledo program in the same area as Trask Coast
  – Established 2nd-cycle program on the Washington Coast
2nd-Cycle Programs

– We have selected, and will continue to select, parents and trees with a track record of growing well in areas subject to SNC
  • 1st-gen selections used for crossing
  • Selections from 2nd-cycle tests

– Needle retention is being assessed at age-7 and age-12
  • One age-6 assessment in SCC was funded by SNCC

– DBH is being assessed at age-7 and age-12, so that an age-7 to age-12 DBH increment can be calculated
  • Possibly additional (later) DBH assessments to confirm continued growth in DBH
2\textsuperscript{nd} Cycle South Central Coast

- **Phase I**
  - 3 sites established along the southern Oregon coast, in areas specifically selected for obvious SNC symptoms. Each site had 50 full-sib families selected for high 1\textsuperscript{st}-cycle gains in growth.
  - Another 6 mainline test sites using 284 full-sib families (including the 50 in the SNC sites) in areas with moderate SNC.
  - 4 checklots in each of the 9 sites, including 2 woodsrun lots from the north and south ends of the SCC testing zone

- **Phase II**
  - 6 more sites established along the southern Oregon coast in areas with moderate SNC. Each
Geographic locations of testing sites in SCC

red flags – 2\textsuperscript{nd}-cycle mainline sites;

blue flags – 2\textsuperscript{nd}-cycle SNC sites;

red dots – 1\textsuperscript{st}-cycle sites
Miller Flyway (between Reedsport & Florence)

Transit Hill (near Hauser)
Coast I – 215 families
-Sown 2003, 6 sites, 27M trees

Coast II – 91 families
-Sown 2006, 5 sites, 10M trees

Inland I – 149 families
-Sown 2004, 5 sites, 15M trees

Inland II – 91 families
-Sown 2006, 5 sites, 10M trees

Slide: Jim Smith
Radio Flyer (north of Newport)

Neskowin (near Neskowin)
Hidden Flats - Coast Phase II

Slide: Jim Smith
Narrow-sense heritabilities from 2\textsuperscript{nd} cycle tests (age-7)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Individual-tree</th>
<th>Family-mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC: Mainline sites</td>
<td>HT</td>
<td>0.348</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>0.224</td>
</tr>
<tr>
<td></td>
<td>VOL</td>
<td>0.276</td>
</tr>
<tr>
<td></td>
<td>Needle Retention</td>
<td>0.121</td>
</tr>
<tr>
<td>SCC: SNC sites</td>
<td>HT</td>
<td>0.477</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>0.364</td>
</tr>
<tr>
<td></td>
<td>VOL</td>
<td>0.402</td>
</tr>
<tr>
<td></td>
<td>Needle Retention</td>
<td>0.269</td>
</tr>
<tr>
<td>Trask Coast Phase I</td>
<td>HT</td>
<td>0.216</td>
</tr>
<tr>
<td></td>
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<td>0.184</td>
</tr>
<tr>
<td></td>
<td>VOL</td>
<td>0.204</td>
</tr>
<tr>
<td></td>
<td>Needle Retention</td>
<td>0.121</td>
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### Genetic correlations between traits from 2nd cycle tests (age-7)

<table>
<thead>
<tr>
<th></th>
<th>HT7</th>
<th>DBH7</th>
<th>VOL7</th>
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<tbody>
<tr>
<td>SCC Mainline NR7</td>
<td>0.44</td>
<td>0.76</td>
<td>0.69</td>
</tr>
<tr>
<td>SCC Needlecast sites NR7</td>
<td>-0.09</td>
<td>-0.05</td>
<td>-0.04</td>
</tr>
<tr>
<td>Trask Coast I NR7</td>
<td>0.20</td>
<td>0.25</td>
<td>0.27</td>
</tr>
</tbody>
</table>

- **SCC:**
- Type B genetic correlations for NR between the SNC sites (0.65~0.90) were much higher than between the mainline sites (0.21~0.39).
- Gains for growth on mainline sites and SNC sites were strongly correlated (0.91 at the family level for Vol7)
Other Findings From 2nd-Cycle Programs

- **SCC**: the mainline group had higher NR (1.72) than the SNC group (1.45) on average.

- **Trask**: NR varied from 1.4 to 2.1 with an average of 1.81.

- **SCC**: tested families had high growth gains over woodsrun (39.9\% age-7 Volume index on SNC sites) and slightly higher NR (2.8\% gain over woodsrun).

- **TRASK**: Top 50 families had high growth gains over woodsrun (48.2\% age-7 Volume index) and slightly higher NR (5.7\% gain over woodsrun).
Summary for 2\textsuperscript{nd}-cycle programs

- A strong resource of 30 test sites have been established in areas of the Oregon coast with strong to moderate SNC incidence, and 5 test sites established on the WA Coast
  - Needle retention is being scored operationally in these tests
  - Strength of genetic control (heritability) for NR is similar to those seen in 1\textsuperscript{st}-gen tests
  - Tested families showed high genetic gains over woodsrun controls for growth traits, and small genetic gains for NR
Operational Implementation
SNC Tolerant Seed Orchards

• Oregon Department of Forestry Tillamook and Astoria Districts had a strong commitment to growing a proportion of D-fir on the coast, plus large acreages.

• Saw tree improvement as a viable tool to counteract SNC.

• Took the lead in developing an orchard block around 2002.
  – Associated with the Nehalem orchard, in place since the mid 1990s at the Schroeder orchard complex near St Paul Oregon.
  – Incorporated selections from Nehalem, Hebo, BLM Breeding unit 12.
  – 6 acres in extent.
  – Other cooperators have subsequently joined.
SNC tolerant block at Schroeder in 2002-2003

Nehalem

Nehalem selections

USFS Hebo

USFS Hebo selections

BLM 12

BLM 12 selections
Seed Production and Use

- Tree-form wind-pollinated orchards are likely to be the predominant engine for producing tolerant seed.
- Mixtures of family lots are used.
- As pure Douglas-fir stands are logged along the Oregon coast, they likely to be replaced by mixed species plantings (moderate SNC areas) or alternate species such as