Mechanisms of Douglas-fir resistance to western spruce budworm defoliation: bud burst phenology, photosynthetic compensation and growth rate

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Received January 29, 2001

Summary We compared growth rates among mature interior Douglas-fir (Pseudotsuga menziesii var. glauca (Beissn.) Franco) trees showing resistance or susceptibility to defoliation caused by western spruce budworm (Choristoneura occidentalis Freeman), and among clones and half-sib seedling progeny of these trees in a greenhouse. We also investigated bud burst phenology and photosynthetic responses of clones to budworm defoliation in greenhouse experiments. Resistant mature trees had a higher radial growth rate than susceptible trees, especially during periods of budworm defoliation. Clones from resistant trees grew larger crowns than clones from susceptible trees, whereas stem base diameter at the ground line and height did not differ. Half-sib seedling progeny from resistant trees had larger stem diameter, height, and total biomass than progeny from susceptible trees. Mean 5-year radial growth increment of mature trees was more strongly correlated with growth of seedlings than with growth of clones. Clones from resistant trees that had higher bud burst than clones from susceptible trees, and budworm defoliation of clones depended on the degree of synchrony between bud burst phenology and budworm larval feeding. Clones of resistant and susceptible mature trees showed similar responses of net photosynthetic rate to 2 years of budworm defoliation. We conclude that phenotypic differences in crown condition of Douglas-fir trees following western spruce budworm defoliation are influenced by tree genotype and that high growth rate and late bud burst phenology promote tree resistance to budworm defoliation.

Keywords: Choristoneura occidentalis, genetic variation, insect herbivory, photosynthesis, Pseudotsuga menziesii.

Introduction


For example, variation in tree bud burst phenology can influence the distribution and abundance of insect herbivores on their hosts (Kolb and Teulon 1991, 1992, Hunter 1992, Quiring 1992, 1994, Fox et al. 1997). Variation in the chemical composition of plant tissues, including mineral nutrients, carbohydrates, and allelochemicals, can influence insect fitness by affecting development time and fecundity (Mattson et al. 1982, Mattson and Scriber 1987, Clancy et al. 1995). Likewise, variation in tissue toughness can influence feeding efficiency and digestion by insects and hence regulate their development (Wagner and Zhang 1993, Coley and Barone 1996, McMillin and Wagner 1996). Inherent variations in tree growth rate may influence the rate of tree recovery from herbivory (Barbosa and Wagner 1989). However, certain insect guilds, such as gall-makers and shoot borers, prefer more rapidly growing plants because of the availability of high-quality food sources (Price 1997).

Increased photosynthetic rate in response to defoliation, i.e., photosynthetic compensation, may also promote tree recovery...
from insect herbivory by increasing carbon uptake of remaining leaves (Reich et al. 1993, Kolb et al. 1999, Vanderklein and Reich 1999). Photosynthetic compensation in response to defoliation, in combination with internal reallocation of nutrients and carbohydrates to regrowing tissues (Schoonhoven et al. 1998), can lead to compensatory growth in cases where defoliated plants have higher rates of biomass production than undefoliated plants (Belskey et al. 1993, Reich et al. 1993, Vanderklein and Reich 1999). However, intraspecific genetic variation in photosynthetic compensation has not been widely evaluated as a mechanism of tree resistance to insect herbivory.

Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) is a commercially important tree species in western North America (Silen 1978, Hermann and Lavender 1990, Hardin et al. 2001), and western spruce budworm (Choristoneura occidentalis Freeman) is one of its major defoliators (Brookes et al. 1987), especially of the interior variety (Pseudotsuga menziesii var. glauca (Beissn.) Franco). Western spruce budworm has a univoltine life cycle (one generation per year). Adults begin to emerge in early July. Mating typically occurs within 24 h of eclosion, after which females lay from two to eight clutches of eggs, with each egg mass averaging between 25 and 40 eggs (Brookes et al. 1987). The first instars disperse from the egg mass and spin hibernaculae in sheltered locations on host trees after which they molt into second instars without feeding and overwinter in their hibernaculae. Upon emergence in early May, most second instars passively disperse on silken threads and, landing on a suitable source, begin feeding (Brookes et al. 1987). Suitable food sources include current-year swollen buds and needles of true firs (Abies), Douglas-fir, and spruce (Picea) species. Larvae continue to feed, molting through a total of six instars, and later pupate on foliage. Pupation lasts about 10 days, after which adults emerge to repeat the life cycle (Brookes et al. 1987). Defoliation is caused by larvae feeding on swollen buds and needles, especially current-year needles (Frank and Jenkins 1986, Shepherd 1992, Dodds et al. 1996).


Our overall objective was to evaluate growth rate, photosynthetic compensation, and bud burst phenology as resistance mechanisms of interior Douglas-fir to budworm defoliation. We used three experimental systems for this evaluation. First, we compared growth rates of mature trees showing phenotypic resistance with growth rates of trees showing susceptibility to budworm defoliation. Second, we cloned these mature trees and measured growth and photosynthetic responses of the clones to budworm defoliation under controlled greenhouse conditions. Third, we compared growth rate and biomass allocation among half-sib seedling progeny from resistant and susceptible mature trees over 3 years in the greenhouse. We hypothesized that: (1) resistant mature trees and their clones and seedling progeny have higher growth rates compared with susceptible mature trees and their clones and seedling progeny (growth rate hypothesis); (2) bud burst phenology differs between clones of resistant and susceptible trees, and this difference influences the degree of synchrony between bud burst and budworm feeding, thereby regulating the amount of defoliation (bud burst phenology hypothesis); and (3) clones of resistant trees respond to budworm defoliation with a greater increase in net photosynthetic rate than clones of susceptible trees (photosynthetic compensation hypothesis).

Materials and methods

**Mature tree growth rate**

Mature trees were from the Pike National Forest near Deckers, CO (39.23° N, 105.23° W, elevation 2,573 m) and the Kaibab National Forest near Jacob Lake, AZ (36.71° N, 112.22° W, elevation 2,774 m). Both sites contain mixed coniferous species with a history of western spruce budworm defoliation (Clancy et al. 1993). After outbreaks of western spruce budworm in the mid-1980s, 12 pairs of mature trees, each comprising one phenotypically resistant tree and one susceptible tree, were identified based on the amount of crown damage. The resistant trees had a full and healthy crown, whereas the nearby (within 30 m) susceptible trees were severely defoliated. Both trees of each pair were of similar height and diameter at breast height (1.4 m, DBH), and comparable in site aspect and slope (Clancy et al. 1993). Measurements on mature trees included age, height, DBH, and radial growth increment over 5-year intervals from 1966 to 1990, which was determined by measuring yearly xylem production on increment cores sampled at breast height (n = 1 core per tree) with the aid of a binocular microscope equipped with an ocular micrometer (Clancy et al. 1993).

We used multivariate analysis of covariance (MANCOVA) to investigate effects of trait (resistant and susceptible), site (Deckers, CO and Jacob Lake, AZ), the interaction between trait and site, and tree age (covariate variable) on radial growth rate of each 5-year interval between 1966 and 1990. Following a significant (P < 0.05) Wilk’s Lambda test in the MANCOVA, we performed univariate analyses of variance with the same model for each response variable. Mean separations were made by Tukey’s test (Dean and Voss 1999) for all pairwise comparisons.

**Growth rates of half-sib seedlings**

Open-pollinated seeds were collected from 13 pairs of resistant and susceptible mature trees (i.e., 26 families) that were identified as described for mature trees. Seeds were collected from the Pike National Forest near Deckers, CO and the
Collected seeds were sealed in plastic bags and stored in a freezer. In February 1998, 50 seeds per tree were germinated (overall germination rate of over 95%). The resulting half-sib seedlings were grown in a greenhouse (Rocky Mountain Research Station, Flagstaff, AZ) in plastic germination cells (5 × 6 × 20 cm) for the first year (1998). Seedlings were then transplanted to larger plastic pots (15 cm in diameter × 20 cm in height) filled with a mixture of screened peat moss and vermiculite for the second (1999) and third years (2000). Water and a balanced fertilizer were applied frequently to all seedlings. Air relative humidity and temperature in the computer-controlled greenhouse simulated seasonal variations in environmental conditions in natural forests and thus satisfied the chilling requirement of the seedlings. Maximum photosynthetically active radiation (PAR) in the greenhouse was about 1400 µmol m⁻² s⁻¹ as measured with a Li-Cor quantum sensor (Li-Cor, Inc., Lincoln, NE) (about 64% of outdoor PAR) on several bright days throughout the summer.

We measured base diameter (1 cm above the soil surface), height, and number of branches (for 1998 and 1999 only) at the end of the 1998, 1999 and 2000 growing seasons. We also harvested 10 randomly selected seedlings per family each year for biomass determination. Each freshly harvested seedling was divided into shoot (stem + leaves) and root, oven dried at 75 °C for 48 h, and weighed. Growth and biomass data were analyzed by one-way analysis of variance (ANOVA). Linear regression was used to analyze the relationship between growth of half-sib seedlings and radial growth rate of their corresponding mature trees.

### Clone growth and response to defoliation

We cloned each of the 24 mature trees by whip-grafting branches collected from the lower third of the crown onto 1-year-old seedling rootstocks in 1991 and 1992. Following grafting, all clones were planted in 15-1 pots containing a mixture of screened peat moss and vermiculite, and watered and fertilized frequently until the start of the experiments in 1998. All clones were raised in a greenhouse, although they were over-wintered in an outdoor shade house from mid-August through January of 1992–1994.

We used 12 ramets (individuals) of each clone in the study. All ramets had characteristics of their respective mature trees (e.g., thick blue-green needles, non-vertical or plagiotropic branch growth, and occasional strobilus production). Ramets averaged 2.1 ± 0.03 cm (mean ± 1 SE) in base stem diameter (about 1 cm above the graft), 9.35 ± 1.6 cm in height, and 45.9 ± 0.7 cm in crown diameter (averaged over two directions) in fall 1997. Six ramets of each clone were randomly selected for the budworm defoliation treatment, and the other six ramets served as undefoliated controls. The experiment had a completely randomized block design comprising six blocks, each containing a factorial combination of 24 clones (12 pairs of resistant and susceptible genotypes) × two defoliation treatments. In total, 288 grafted trees were included in the experiment. However, nine of the grafted trees died during the experiment (eight resistant trees: four defoliated and four undefoliated, and one susceptible tree in the defoliated treatment), resulting in four to six replications per treatment combination for each of the 12 pairs.

We used western spruce budworm larvae to defoliate half of the ramets of each clone over two consecutive years (1998 and 1999). The budworm larvae were from laboratory cultures of diapausing and non-diapausing western spruce budworms, maintained at Rocky Mountain Research Station’s Entomology Laboratory in Flagstaff, AZ. Budworm larvae produced from the non-diapausing culture had growth rates and feeding behavior similar to those of a wild population (Leyva et al. 1995). Both cultures were maintained on a standard budworm artificial diet that is similar to the McMorran (1965) diet (see Clancy 1991a for a description of the variations and rearing conditions, and Clancy 1991b for the nutritional composition). The non-diapausing culture has been maintained since 1985 (Clancy 1991a). The diapausing culture was established from wild material collected in 1996–1998, so it has been in culture for fewer generations than the non-diapausing culture.

In April 1998, third and primarily fourth instar non-diapausing larvae were placed on each grafted tree (one larva per five terminal buds) assigned to the defoliation treatment when about 50% of the buds on the tree were in the fourth (columnar) bud burst developmental stage (Shepherd 1983). We had planned to use second instars in hibernaculae from our diapausing culture to defoliate the grafted trees, but not enough larvae were available from this culture in 1998. The number of buds on each ramet was estimated by a regression equation that predicted the total number of terminal buds from stem base diameter and crown diameter ($r^2 = 0.72, P < 0.001, n = 31$). This method resulted in the placement of larvae on a grafted tree at the bud burst stage when bud and needle tissues were highly palatable. Both defoliated and undefoliated grafted trees were caged with nylon “No-See- um” netting bags (The Rain Shed Corp., Corvallis, OR) that allowed penetration of about 80% of full light in order to contain larvae and maintain all trees in a similar light environment. The bags were not removed until 95% of the larvae had pupated, which required approximately 40 days. The actual amount of defoliation was calculated for each grafted tree based on the number of damaged terminal buds divided by total number of terminal buds. In 1999, we defoliated the same ramets of each clone that were defoliated in 1998, but we used a slightly different method of larval placement. In May 1999, second instar diapausing larvae in hibernaculae (over-wintering structures) were placed on all previously defoliated ramets on the same day (one larva per four terminal buds) when approximately 50% of all ramets were in the second (yellow) bud burst development stage (Shepherd 1983). The initial number of terminal buds on each grafted tree was counted before the defoliation...
treatment. Pieces of gauze containing pre-counted second instar larvae in hibernacula (1–20 larvae per gauze) were pinned to the stem in the upper crown of each defoliated ramet. Thus, in 1999, we did not attempt to place larvae on each tree when its buds were most suitable for feeding as we did in 1998. The actual number of damaged terminal buds was counted immediately after budworm pupation. The cumulative amount of defoliation over both years was calculated as \((1 - (1 - \% \text{ defoliation } 98) (1 - \% \text{ defoliation } 99)) \times 100\). Stem base diameter, height, and crown diameters of clones were measured at the end of the 1998 and 2000 growing seasons.

Bud burst phenology and shoot development were monitored on randomly selected, labeled terminal buds (8–20 buds depending on tree size; 20 buds were assessed on most trees) from the upper two-thirds of the crown on each undefoliated grafted tree in the 1998 experiment. From March 19 to May 7, we recorded the developmental stage of each labeled bud/shoot weekly based on Shepherd’s (1983) bud burst scale. In total, 2620 current-year buds and shoots were monitored (1284 on resistant clones, and 1336 on susceptible clones). Monitoring date was used as a repeated factor in analysis of variance (ANOVA) on bud burst stage.

We measured net photosynthetic rate \((P_n)\) and stomatal conductance \((g_s)\) on fully developed, undamaged foliage from the terminal portion of mid-crown lateral branches of all grafted trees at the end of July in both 1998 and 1999. Both first- (current) and second-year needles were measured in 1998 (one measurement per grafted tree for each needle age class), but only first-year needles were measured in 1999 (two measurements on different twigs on each tree). We performed all measurements over a 30-s period in a 0.25-l cuvette with a closed-circuit portable photosynthesis system (LI-6200, Li-Cor, Inc.) between 1000 and 1400 h under high light conditions (mean PAR 1141.3 ± 8.4 (1 SE) µmol m\(^{-2}\) s\(^{-1}\)) created by a combination of sunlight and artificial light from a metal halide lamp (MH-250W; Hubbell Lightning, Inc., Christiansburg, VA). Air temperature and relative humidity during these measurements averaged 28.2 ± 0.2 (1 SE) °C and 41.5 ± 0.5%, respectively. Both \(P_n\) and \(g_s\) were expressed on a projected leaf area basis (one side). We measured nitrogen (N) concentration of all foliage used for the gas exchange measurements in 1998 and 1999, and also measured stable carbon isotope ratio (\(\delta^{13}C\)) on foliage sampled in 1999. These measurements were made with a continuous-flow mass spectrometer (C Instruments NC2100 and Delta Plus XL, Finnigan, MAT, San Jose, CA). Finally, base stem diameter, height, and mean crown diameter of all ramets were measured at the end of the 1998 and 2000 growing seasons. We used a mixed ANOVA statistical model (Table 1) to analyze the responses of growth, \(P_n\), \(g_s\), N concentration, and \(\delta^{13}C\) of clones to budworm defoliation. The model was based on four assumptions: (1) the two clones from paired mature trees at a site were considered the basic sampling unit; (2) each pair included trees that differed in resistance trait (resistant versus susceptible); (3) each trait within a pair was further split to incorporate the treatment in the greenhouse (defoliated versus undefoliated); and (4) tree pairs were random factors and other factors were fixed. We focused on the effects of trait, budworm defoliation treatment, and their interaction (Table 1) because these sources of variation are most relevant to our hypotheses. If the F-tests of these factors were significant in the mixed model ANOVA, all pair-wise comparisons were made with Tukey’s test to separate the means of these effects at \(P = 0.05\). All statistical analyses were performed with SAS JMP software (SAS Institute, Inc., Cary, NC). The relationship between growth of clones and that of their corresponding mature trees was evaluated by linear regression analysis.

Results

Mature tree growth rate

Overall, 5-year radial growth rate differed significantly between phenotypically resistant and susceptible mature trees (MANCOVA, \(P = 0.004\)). Site, interaction between site and trait, and tree age (as a covariate variable) did not affect radial growth rates (MANCOVA, \(P \geq 0.177\)). Thus, mature trees at the Deckers, CO and Jacob Lake, AZ sites had similar growth rates, and differences in radial growth rate between resistant and susceptible trees were similar at each site. Resistant trees had a greater growth rate than susceptible trees at every measurement interval from 1966 to 1990 (Table 2). However, these differences were significant only for the interval from 1986 to 1990 (\(P < 0.001\)) (Table 2) when the western spruce budworm population outbreak occurred.

The difference in radial growth rate between resistant and susceptible mature trees was greatest between 1986 and 1990 (about 120%), intermediate during 1966–1970 (29%) and 1981–1985 (25%), and lowest during 1971–1975 (8%) and 1976–1980 (2%). Radial growth rate was about 28% higher in resistant trees than in susceptible trees (\(P = 0.088\)) averaged over all 1966–1990 measurements (Table 2).

Growth rates of half-sib seedlings

In the absence of western spruce budworm defoliation, height and base diameter of half-sib seedlings from resistant trees were significantly greater compared with seedlings from susceptible trees in all 3 years of growth in the greenhouse (\(P < 0.001\)) (Table 3). Seedlings from susceptible trees had significantly more lateral branches than seedlings from resistant trees during the first year (1998) (\(P < 0.001\)), but not in the second year (1999) (Table 3). Total biomass was similar for seedlings from resistant and susceptible trees for the first year (\(P = 0.469\)) (Table 3). However, seedlings from resistant trees had significantly (\(P < 0.001\)) greater total biomass than seedlings from susceptible trees in the second and third years. Shoot/root biomass ratio was similar for seedlings from resistant and susceptible trees in all years (\(P \geq 0.128\)) (Table 3).

Clone bud phenology and defoliation

Clones of susceptible trees had earlier bud burst than clones of resistant trees (\(P < 0.001\)) (Figure 1). Resistant clones required about 90–110 more degree-days to reach the same bud burst stage as susceptible clones.
In the 1998 experiment, when larvae were placed on each grafted tree when buds were highly suitable for feeding, between 9 and 84% of the terminal buds were damaged and defoliation averaged 37%. Resistant clones were defoliated more (41.7 ± 1.70%) than susceptible clones (34.0 ± 1.65%) (P = 0.002) (Figure 2). In the 1999 experiment, when larvae were placed on all trees on the same date regardless of burst stage, defoliation ranged between 5 and 85%, and averaged 30%. Resistant clones were defoliated less (26.3 ± 2.17%) than susceptible clones (33.0 ± 2.10%) in 1999 (P = 0.032) (Figure 2). Cumulative defoliation over 2 years was similar for resistant (56.4 ± 1.89%) and susceptible clones (56.1 ± 1.83%) (P = 0.90) (Figure 2).

Clone growth and response to defoliation

Before the budworm defoliation experiment (1997), resistant and susceptible clones had similar base diameters and heights (P > 0.22). However, resistant clones had greater crown diameter (48.3 ± 0.95 cm) than susceptible clones (43.8 ± 0.93 cm) (P < 0.001). After the 1998 defoliation, base diameter and height did not differ between resistant and susceptible clones (P = 0.542), but crown diameter was still greater in resistant clones (62.2 ± 1.13 cm) than in susceptible clones (56.7 ± 1.08 cm) (P < 0.001). The 1998 budworm defoliation did not influence mean base diameter, height, or crown diameter (P > 0.367) (Figures 3A, 3C, and 3E) or their relative growth rates between 1997 and 1998 (P > 0.108). Although the trait × defoliation treatment interaction was not significant (P = 0.629) for 1998 base stem diameter and height (Figures 3A and 3C), this interaction was significant for crown diameter (P = 0.033). Defoliation in 1998 decreased crown diameter in resistant clones and caused a small increase in crown diameter in susceptible clones (Figure 3E).

After two consecutive years of defoliation (2000), resistant clones still had a greater mean crown diameter (70.2 ± 2.71 cm) than susceptible clones (66.2 ± 2.73 cm) (P = 0.005) (Figure 3F), whereas base stem diameter and height did not
differ ($P > 0.499$) (Figures 3B and 3D). However, 2 years of defoliation reduced mean base stem diameter ($P = 0.058$) and its relative growth rate between 1997 and 2000 ($P = 0.010$; data not shown). In 2000, defoliated clones had similar mean height to undefoliated clones ($P = 0.972$, Figure 3D), but defoliation reduced relative growth rate of height (1997–2000) by 17% ($P = 0.013$; data not shown). In contrast, 2 years of defoliation did not influence mean crown diameter (Figure 3F) or its relative growth rate over 3 years ($P \geq 0.178$). The trait × defoliation treatment interaction did not have a significant influence on base diameter, height, and crown diameter ($P \geq 0.129$) after two consecutive years of defoliation (2000) (Figures 3B, 3D and 3F). Overall, cumulative defoliation over both years was negatively correlated with relative growth rate (1997–2000) of base stem diameter ($r = -0.304$, $P = 0.006$), and height ($r = -0.236$, $P = 0.036$), but it was unrelated to relative growth rate of crown diameter ($r = -0.091$, $P = 0.428$, $n = 138$).

Table 3. Height, base stem diameter, number of branches, total biomass, and shoot/root dry mass ratio ($\pm 1$ SE) over three growing seasons (1998–2000) of half-sib seedlings from Douglas-fir trees showing phenotypic resistance ($n = 13$ trees) or susceptibility ($n = 13$ trees) to western spruce budworm defoliation.

<table>
<thead>
<tr>
<th>Year/trait</th>
<th>Height (cm)</th>
<th>Diameter (mm)</th>
<th>No. branches</th>
<th>Total biomass (g)</th>
<th>Shoot/root ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>25.82 ± 0.34</td>
<td>3.26 ± 0.04</td>
<td>4.43 ± 0.14</td>
<td>4.95 ± 0.21</td>
<td>1.84 ± 0.28</td>
</tr>
<tr>
<td>Susceptible</td>
<td>22.95 ± 0.33</td>
<td>2.96 ± 0.04</td>
<td>5.78 ± 0.15</td>
<td>4.73 ± 0.21</td>
<td>1.92 ± 0.28</td>
</tr>
<tr>
<td>$P$-Value</td>
<td>$&lt; 0.001$</td>
<td>$&lt; 0.001$</td>
<td>$&lt; 0.001$</td>
<td>0.469</td>
<td>0.831</td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>44.00 ± 1.09</td>
<td>7.26 ± 0.15</td>
<td>15.15 ± 0.57</td>
<td>29.94 ± 0.80</td>
<td>1.56 ± 0.04</td>
</tr>
<tr>
<td>Susceptible</td>
<td>38.24 ± 1.09</td>
<td>6.57 ± 0.15</td>
<td>16.42 ± 0.57</td>
<td>20.83 ± 0.80</td>
<td>1.52 ± 0.04</td>
</tr>
<tr>
<td>$P$-Value</td>
<td>$&lt; 0.001$</td>
<td>$&lt; 0.001$</td>
<td>0.113</td>
<td>$&lt; 0.001$</td>
<td>0.383</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>66.10 ± 1.40</td>
<td>13.27 ± 0.24</td>
<td>–</td>
<td>58.71 ± 1.81</td>
<td>1.84 ± 0.04</td>
</tr>
<tr>
<td>Susceptible</td>
<td>58.57 ± 1.40</td>
<td>11.83 ± 0.24</td>
<td>–</td>
<td>49.82 ± 1.81</td>
<td>1.75 ± 0.04</td>
</tr>
<tr>
<td>$P$-Value</td>
<td>$&lt; 0.001$</td>
<td>$&lt; 0.001$</td>
<td>–</td>
<td>$&lt; 0.001$</td>
<td>0.128</td>
</tr>
</tbody>
</table>

1 Height, diameter and number of branches were based on the measurement of 1,014 seedlings; 34–40 seedlings per family ($n = 26$). Among them, 509 seedlings were from resistant families ($n = 13$), and 505 from susceptible families ($n = 13$). Total biomass and shoot/root ratio dry mass values were based on a subsample of 10 seedlings per family.

2 Height, diameter and number of branches were based on the measurement of 520 seedlings; 20 seedlings per family ($n = 26$). Total biomass and shoot/root dry mass ratio values were based on a subsample of 10 seedlings per family.

3 The number of branches was not counted in 2000. All other measurements were based on a subsample of 10 seedlings per family ($n = 26$).
Net photosynthetic rate ($P_n$) of both first- and second-year needles did not differ significantly between resistant and susceptible clones or between defoliated and undefoliated clones after the first year of defoliation (1998) ($P = 0.297$) (Figures 4A and 4B). However, the trait × defoliation treatment interaction was significant for $P_n$ of first-year needles in 1998 ($P = 0.049$). Defoliation increased $P_n$ of first-year needles of resistant clones, but decreased $P_n$ of susceptible clones (Figure 4A). This pattern also occurred in second-year needles in 1998 ($P = 0.148$). In 1998, $P_n$ was 20–25% greater in first-year needles than in second-year needles. The $P_n$ of first-year needles was negatively correlated with the amount of defoliation in 1998 ($r = -0.169$, $P = 0.053$), whereas $P_n$ of second-year needles was not correlated with defoliation in 1998 ($r = -0.117$, $P = 0.176$, $n = 138$).

In 1999 after 2 years of defoliation, $P_n$ of first-year needles did not differ significantly between resistant and susceptible clones or between defoliated and undefoliated clones ($P = 0.587$) (Figure 5A). The trait × defoliation treatment interaction was not significant for $P_n$ in 1999 ($P = 0.883$). In first-year needles, $P_n$ was similar in 1998 and 1999 and was positively correlated for individual trees ($r = 0.167$, $P = 0.054$, $n = 279$). Linear regressions pooled over all clones showed that $P_n$ and stomatal conductance ($g_w$) of first-year needles in 1999 were positively correlated with cumulative defoliation over both years ($P_n$: $r = 0.218$, $P = 0.012$, $n = 138$; $g_w$: $r = 0.171$, $P = 0.048$, $n = 138$) (Figure 5C).
Mean N concentration in first-year needles was significantly higher in 1999 (2.02 ± 0.03%) than in 1998 (1.52 ± 0.02%) (P < 0.001), but trait, defoliation treatment, and their interaction were not significant (P ≥ 0.074) (Figure 5D). Although there were no significant treatment effects on δ13C of first-year needles in 1999 (Figure 5B), δ13C was significantly correlated with $P_n$ ($r = -0.195$, $P = 0.007$) and $g_w$ ($r = -0.361$, $P = 0.016$, $n = 236$).

Relationships among growth rates of mature trees, seedlings, and clones

Mean 5-year radial growth rate of mature trees was not significantly correlated with mean stem base diameter, height, or crown diameter of their greenhouse-grown clones ($r ≤ 0.311$, $P ≥ 0.653$, $n = 24$). There was a positive but weak correlation between mean 5-year radial growth rate of mature trees and stem base diameter ($r = 0.363$, $P = 0.096$) and height ($r = 0.342$, $P = 0.120$) of their greenhouse-grown seedling progeny ($n = 26$).

Discussion

We obtained evidence to support our first hypothesis that resistant mature trees, their clones, and half-sib seedling progeny have higher growth rates than their susceptible counterparts (growth rate hypothesis). Growth rates were higher in resistant mature trees than in susceptible trees in the forest. This growth difference was largest between 1986 and 1990 when the stands were defoliated by budworm, suggesting that the greater growth rate of resistant mature trees compared with susceptible mature trees was largely a consequence of a differential effect in defoliation intensity rather than genetic variation in growth rate. However, in the absence of budworm defoliation in a uniform greenhouse environment, clones and half-sib seedling progeny of resistant trees also had higher growth rates than those of susceptible trees. Thus, we conclude that resistant trees were genetically predisposed to grow faster than susceptible trees. Inherently higher growth rate may promote tree resistance or recovery from budworm defoliation by rapid regrowth.

Radial growth rate of resistant mature trees increased 42% between the 1981–85 and 1986–90 periods, whereas radial growth rate of susceptible mature trees decreased 20% over the same period. These results suggest less budworm defoliation during 1986–90 on resistant trees than on susceptible trees in the forest. The bud burst phenology of susceptible trees may have favored budworm feeding. That is, defoliation may have been greater on trees whose bud burst occurred when larvae were starting to feed because larvae feed preferentially on swollen buds and young needles (Shepherd 1992, Dodds et al. 1996). Consistent with this idea, bud burst was earlier for susceptible mature trees than for resistant mature trees at both the Arizona and Colorado study sites (Clancy et al. 1993), and this difference also occurred in the greenhouse-grown clones. Thus, there is evidence that the difference in bud burst phenology reported by Clancy et al. (1993) for these mature trees growing in the same stand resulted from genotypic differences. This is consistent with the finding of high heritability for bud burst phenotype in Douglas-fir (Li and Adams 1993).

The budworm defoliation experiments on the clones provided additional support for a role of genetic variation in bud burst phenology as an influence on budworm defoliation (bud burst phenology hypothesis). In the 1998 experiment, when clones of resistant trees were defoliated more than clones of susceptible trees, the influence of bud burst phenology on the amount of defoliation was minimized because we introduced larvae to each tree at the same bud burst stage. In contrast, genetic differences in bud burst phenology influenced defoliation in the 1999 experiment when we introduced larvae to all trees on the date when 50% of all buds in the pooled population were expanding. Under this scenario, clones of resistant trees were defoliated less than clones of susceptible trees because more of their buds were tightly closed and thus not available for larval feeding. Thus, our second hypothesis, that bud burst phenology differs genetically between resistant and susceptible trees and that this difference influences defoliation, was supported. Our findings are consistent with other studies highlighting the importance of intraspecific variation in bud burst phenology as a mechanism of tree resistance to insects that feed on swollen buds and young leaves in both broad-leaved and coniferous trees (Kolb and Teulon 1991, Hunter 1992, Kolb et al. 1992, Lawrence et al. 1997, Teulon et al. 1998, Ostaff and Quiring 2000). Moreover, because foliar nutrients (Clancy et al. 1995) and allelochemicals (e.g., monoterpenes) (Wagner et al. 1989, Gambriel and Cates 1995, Zou and Cates 1995) often change during tissue maturation, which...
is related to bud burst phenology, they also likely influenced budworm defoliation in our study.

Our third hypothesis that clones of resistant trees respond to budworm defoliation by a greater increase in $P_a$ than clones of susceptible trees (photosynthetic compensation hypothesis) was not supported. Although we found evidence of a stronger compensatory photosynthetic response to defoliation in resistant clones than in susceptible clones after the first defoliation in 1998, this difference was probably caused by greater defoliation of resistant clones, rather than a genetic difference in compensatory capacity. For example, the degree to which $P_a$ was stimulated by budworm feeding on 3-year-old Douglas-fir seedlings was positively related to the amount of defoliation (Kolb et al. 1999). Increased $P_a$ in 1998 in defoliated resistant clones likely resulted from increased $g_w$ caused by a more favorable balance between leaf and root area (Kruger and Reich 1993, Reich et al. 1993), and not an increase in leaf N concentration as can occur following heavy defoliation (e.g., Kolb et al. 1999). The similar responses of $P_a$, $g_w$, N concentration, and $\Delta^{13}C$ of the clones to defoliation over 2 years, when cumulative defoliation was similar for resistant and susceptible clones, also suggest a similar capacity for compensatory photosynthesis in resistant and susceptible trees. Because late bud burst phenology apparently enabled resistant trees to avoid heavy budworm defoliation, there is probably little selection for mechanisms leading to compensatory photosynthesis in resistant genotypes.

We acknowledge that our mature-tree clones growing in the greenhouse do not behave exactly as mature trees of the same genotype growing in the forest. Resource availability, water relations, and growing environment obviously differ between small, container-grown trees given ample water and nutrients in the greenhouse and mature forest trees that are subject to the rigors of nature. Nevertheless, the clones allowed us to study the relationship between bud burst phenology, budworm defoliation, and leaf gas exchange using mature tissues that could be easily manipulated in greenhouse experiments and provided a useful model system for phenological studies. However, they were less useful for genetic studies of growth rate, because the correlation with radial growth of mature trees was stronger for half-sib seedling progeny than for clones in the greenhouse.

In summary, we obtained evidence that two mechanisms, growth rate and bud burst phenology, are important in explaining variation in crown condition of mature Douglas-fir trees following western spruce budworm outbreaks. In contrast, we found little support for the idea that resistant trees have a greater inherent capacity for photosynthetic compensation after budworm defoliation than susceptible trees. Further, the pattern of differences in growth rate and bud burst phenology between resistant and susceptible trees was similar between mature trees in the forest and clones or half-sib seedling progeny in the greenhouse, indicating genetic control of these traits, which might be useful in Douglas-fir genetic improvement programs that include resistance to western spruce budworm.

Acknowledgments

Sincere thanks to Jan Huntsberger and Jerry Snow, USDA Rocky Mountain Research Station, Flagstaff, for help in maintaining clones and half-sib seedlings in the greenhouse, and rearing western spruce budworm in the laboratory, respectively. Rudy King, Biometrician at USDA Rocky Mountain Research Station, and Dr. Ian Harris, Northern Arizona University, provided substantial advice on statistical models. Dr. George Koch, Northern Arizona University, helped with analysis of nitrogen, carbon, and $^{13}C$ data, and had important comments on the organization of the manuscript. This research was supported by Research Joint Venture Agreement No. 28-JV7-963 between the USDA Rocky Mountain Research Station and Northern Arizona University, and the Mission Research Program, School of Forestry, Northern Arizona University.

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