Journal of Chemical Ecology, Vol. 28, No. 5, May 2002 (© 2002)

THE ROLE OF MONOTERPENES IN RESISTANCE OF DOUGLAS FIR TO WESTERN SPRUCE BUDWORM DEFOLIATION

ZHONG CHEN,[1] THOMAS E. KOLB,[1,*] and KAREN M. CLANCY[2]

[1]*School of Forestry*
Northern Arizona University
Flagstaff, Arizona 86011-5018

[2]*Rocky Mountain Research Station*
2500 S. Pine Knoll Drive
Flagstaff, Arizona 86001-6381

(Received March 30, 2001; accepted January 13, 2002)

Abstract—We conducted defoliation experiments with 7- to 8-year-old clones of Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca*] to assess the role of monoterpenes as a resistance mechanism to western spruce budworm (*Choristoneura occidentalis* Freeman) defoliation. The grafted clones were derived from mature trees that showed resistance or susceptibility to budworm defoliation in the forest. All clones were exposed to either budworm defoliation or nondefoliation treatments in 1998 and 1999 under greenhouse conditions. We found that the total concentration of monoterpenes in current-year foliage varied greatly between two consecutive years in clones in the greenhouse and in their corresponding mature trees in the forest. Fractional composition of different monoterpenes was similar between different years and between clones and mature trees, indicating genetic control of this trait. Two different defoliation experiments were conducted to assess the importance of budburst phenology as a factor determining host plant resistance. In the 1998 experiment, budworm feeding was matched to the budburst of each individual plant. Monoterpene concentration was high in 1998, and budworm potential fitness was greater on clones from the resistant mature trees that had lower concentrations of total monoterpenes. In the 1999 experiment, budworm feeding was matched to budburst of the whole population of plants in order to mimic conditions similar to insects feeding on trees in the field. The concentration of monoterpenes was low in 1999, and budworm fitness was not related to monoterpenes. Total monoterpene concentration was negatively related to foliar nitrogen concentration, suggesting that C/N balance may affect monoterpene synthesis in needles. However, tree growth was not related to total monoterpene concentration. We concluded that

* To whom correspondence should be addressed. E-mail: Tom_Kolb@nau.edu

0098-0331/02/0500-0897/0 © 2002 Plenum Publishing Corporation

expression of differences in budworm resistance among Douglas fir genotypes might be caused by interactions among multiple resistance mechanisms such as needle monoterpenes and tree budburst phenology.

Key Words—Budburst phenology, *Choristoneura occidentalis*, herbivory, monoterpenes, plant-insect interactions, *Pseudotsuga menziesii.*

INTRODUCTION

Terpenes are the largest and most diverse group of plant secondary metabolites. At least 15,000 terpenoids have been described and perhaps thousands more are yet to be discovered (Gershenzon and Croteau, 1991). Monoterpenes (i.e., C_{10} hydrocarbons) are one of the major terpene groups and are widespread in plants, functioning as either deterrents or attractants to herbivores (Hedin et al., 1974; Kogan, 1975). They are also involved in numerous physiological and ecological interactions in forest ecosystems (Harborne, 1991; Gershenzon, 1994; Langenheim, 1994; Lerdau et al., 1995). Monoterpene concentration and composition in coniferous trees are influenced by tree genotype (von Rudloff and Rehfeldt, 1980; Gershenzon and Croteau, 1991; Hanover, 1992; Gershenzon, 1994) and environmental factors such as availability of nitrogen (Lerdau et al., 1995; McKinnon et al., 1998), water, and sunlight (Johnson et al., 1997). Further, monoterpene concentration and composition can change during tissue maturation (Wagner et al., 1989; Gambliel and Cates, 1995; Zou and Cates, 1995) and can vary among different tissues within the same tree (Latta et al., 2000).

The association between terpenes and resistance of host trees to insect herbivores has been investigated in several conifers (Annila et al., 1984; McClure and Hare, 1984; Redak and Cates, 1984; Hanula et al., 1985; Wilkinson, 1985; Cates et al., 1987; Cates and Redak, 1988; Cates and Zou, 1990; Clancy, 1991a; Clancy et al., 1992, 1993; Wagner and Zhang, 1993; Felipe et al., 1994; Tomlin et al., 1997; Manninen et al., 1998; Nault et al., 1999). However, the role of terpenes as a resistance mechanism remains controversial. Several studies have concluded that monoterpenes are an important mechanism of resistance to insect herbivores in conifers (McClure and Hare, 1984; Cates et al., 1987; Cates and Zou, 1990; Felipe et al., 1994; Tomlin et al., 1997), whereas other studies have not (Annila et al., 1984; Wilkinson, 1985; Clancy, 1991a, 1993, 2001; Clancy et al., 1992, 1993; Wagner and Zhang, 1993; Manninen et al., 1998; Nault et al., 1999). This controversy likely arises from the many influences on conifer monoterpene concentration and composition (e.g., tree genotype, ontogeny, environment, and genotype–environment interaction), the evolution of different resistance mechanisms for different host plant–insect systems, and the possible involvement of several mechanisms in host plant resistance.

Evidence for an important role of monoterpenes as a tree resistance mechanism is also mixed for the subjects of our study, Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca*] and western spruce budworm (*Choristoneura occidentalis* Freeman). Some monoterpenes, such as tricyclene, camphene, and bornyl acetate, have been reported to adversely affect western spruce budworm performance on Douglas fir (Cates et al., 1983; Redak and Cates, 1984; Cates and Zou, 1990; Zou and Cates, 1995). In contrast, other studies have found no difference in these monoterpene components between mature Douglas fir trees that showed resistance versus susceptibility to budworm defoliation in the forest (Clancy, 1991a, 2001; Clancy et al., 1993). Further, Clancy et al. (1992) and Clancy (1993) reported little influence of monoterpenes that were microencapsulated in an artificial diet on budworm performance in a three-generation bioassay unless components such as camphene, bornyl acetate, β-citronellol, and linalool were at extremely high concentrations that rarely occurred in forest trees.

The overall objective of this study is to further understand the role of monoterpenes as a resistance mechanism of Douglas fir to budworm defoliation. We used data on total monoterpene concentration and composition from resistant and susceptible mature trees and clones of these trees in the greenhouse to address these questions: (1) Does foliar monoterpene concentration and composition differ between tree genotypes that were resistant to budworm defoliation versus genotypes that were susceptible? (2) Does variation in monoterpene concentration affect budworm performance in a pattern consistent with field observations of tree resistance? (3) Is the influence of foliar monoterpenes on budworm performance mediated by the degree of synchrony between budworm feeding and tree budburst phenology? (4) Does budworm defoliation induce changes in monoterpene concentration and composition? (5) Does carbon allocation to monoterpene synthesis compromise allocation to tree radial growth?

METHODS AND MATERIALS

Douglas Fir Trees and Clones. Our experimental plant material consisted of clones derived from mature Douglas fir trees that differed in western spruce budworm defoliation under field conditions (Clancy et al., 1993). The mature Douglas fir trees were from sites on the Pike National Forest near Deckers, Colorado (105.23°W, 39.23°N, elevation 2573 m) and the Kaibab National Forest near Jacob Lake, Arizona (112.22°W, 36.71°N, elevation 2774 m). At the time the trees were identified (1988 and 1989), most of the trees at the sites had sustained moderate to severe budworm defoliation for at least several years, as determined from their growth form and general condition. We selected seven phenotypically resistant trees at the Pike National Forest site and five phenotypically resistant trees

at the Kaibab National Forest site by identifying trees with full crowns and little other evidence of budworm damage. These trees were visually distinct from other trees in the stand that were characterized as phenotypically susceptible based on their defoliated crowns. Each resistant tree was paired with a nearby (within 30 m) susceptible tree of similar size (height and dbh) and microsite (slope and aspect). In other words, the pairs of resistant and susceptible trees were matched as closely as possible to minimize any size-, age-, or microsite-related effects that could confound effects associated with different levels of herbivory. We deliberately chose pairs at each site that represented a range of size (i.e., age) classes. The age of the 24 trees ranged between 45 and 123 years [79.3 ± 4.1 years (mean ± SE)]; height ranged between 6.4 and 14.9 m (10.4 ± 0.5 m); dbh ranged between 15 and 40 cm (25.3 ± 1.3 cm). We measured radial growth increment on these trees over each 5-year interval between 1966 and 1990 on increment cores sampled at breast height (one sample per tree) with a binocular microscope equipped with an ocular micrometer (Clancy et al., 1993).

We cloned each of the 24 mature trees by whip-grafting branches collected from the lower third of the crown onto 1-year seedling rootstocks in 1991 and 1992. This is a common and widespread technique for reproducing mature tree characteristics in a smaller plant (Hartmann and Kester, 1983; Zobel and Talbert, 1984). Such cloning resulted in the fixation of the genotype and tissue developmental stage of mature trees but not tree environment. All cloned trees were grown in plastic pots (15-liter volume) containing a mixture of screened peat moss and vermiculite in the greenhouse; they were regularly watered and fertilized during the growing season. Cloned trees averaged 2.11 ± 0.03 cm (mean ± 1 SE, here and throughout) in base stem diameter (approximately 1 cm above the graft), 93.5 ± 1.6 cm in height, and 45.9 ± 0.7 cm in crown diameter (averaged over two directions) in spring 1998 before the start of the budworm defoliation experiments.

Experimental Design and Budworm Defoliation. The experiment had a completely randomized block design composed of six blocks, each containing 48 clonally propagated trees [i.e., 2 treatments (budworm defoliation versus control) × 2 traits (resistant versus susceptible)/pair × 12 pairs]. In total, 288 cloned trees were included in the experiments. However, 11 trees died before the experiment started; therefore, there were actually four to six replications of each treatment combination for each of the 12 pairs. In order to test the role of budburst phenology as an influence on budworm performance, we conducted the budworm defoliation experiment differently in 1998 and 1999. The budworm larvae used in our study were from our laboratory cultures of diapausing and nondiapausing western spruce budworms, maintained in the Entomology Laboratory at Rocky Mountain Research Station, Flagstaff, Arizona. The nondiapausing colony has growth rates and feeding behavior similar to a wild population (Leyva et al., 1995).

In the 1998 experiment, nondiapausing third- and primary fourth-instar budworm larvae (one larva to five terminal buds) were used to defoliate each cloned

tree when approximately 50% of its buds were in the fourth (i.e., columnar) budburst development stage. This stage is highly suitable for budworm feeding (Shepherd, 1983). Because budworm larval feeding was purposely matched to the fourth budburst stage of each clone, the effect of genetic variation in budburst phenology among trees on budworm feeding was minimized. In the 1999 experiment, diapausing second-instar larvae in hibernacula (one larva to four terminal buds) were introduced to the same clones that were defoliated by budworms in 1998. However, larvae were placed on all trees at the same date when approximately 50% of all terminal buds in the population were in the second (i.e., yellow) budburst stage (Shepherd, 1983). This schedule of larval introduction allowed genetic differences in budburst phenology among trees to influence the developmental stage of buds available for budworm feeding, as can occur in Douglas fir forests (Clancy et al., 1993). Our measurements of budburst phenology of nondefoliated clones in the greenhouse in 1998 revealed that clones of susceptible trees broke bud earlier than clones of resistant trees (Chen et al., 2001a), and the same pattern occurred for the mature trees in the forest (Clancy et al., 1993).

Other experimental procedures were the same in both years. Both defoliated and nondefoliated tree clones were caged with nylon "No-See-um" netting bags (The Rain Shed Corp., Corvallis, Oregon) that allowed approximately 80% of full light to penetrate in order to contain larvae and create similar growing conditions for all clones. The bags were not removed until 95% of the larvae pupated, which required approximately five weeks.

Budworm Bioassay. Most of the pupae were removed from defoliated tree clones within 24–48 hours of pupation. Male and female pupae were separately weighed (to the nearest 0.1 mg), and sorted into trays based on clone genotype. The pupae were refrigerated at 10°C for up to seven days until an adequate number of male and female pupae were collected from the same clone genotype for mating, which occurred in brown paper mating bags at room temperature (20°C). Female pupal weight was used to estimate the number of oocytes per female or potential fecundity (Wagner et al., 1987).

When about 10% of the moths had emerged in the mating bags, freshly clipped Douglas fir foliage was added for adult oviposition substrate. Once the foliage was added, the moths were allowed to mate and oviposit for 7–10 days. Next, the foliage was removed, and the number of next-generation (noted as F_1) egg masses larger than 4 mm was counted. Then, mating bags were frozen to kill the adult moths, and the unemerged pupae (i.e., dead) in each bag were counted and sexed to determine percent survival through the pupal stage and the number of female moths that had emerged. The collected F_1 egg masses were placed in Petri dishes, sealed inside plastic bags, and incubated in the laboratory (20°C) for 7–10 days to determine if the egg masses were viable (i.e., if $\geq$50% of the eggs in the mass hatched).

The measurement of budworm potential fitness was based on the following population growth model modified from Clancy (1991b):

$$
\begin{aligned}
\text{Number of } F_1 \text{ larvae } (N) = &\ [\text{number of parent generation (noted as } P_1\text{) larvae}] \\
&* (P_1 \text{ proportion cohort survival to the adult stage}) \\
&* (P_1 \text{ average potential fecundity per female}) \\
&* (F_1 \text{ proportion of egg masses with viable eggs})
\end{aligned}
$$

This model estimated the number of first instars alive at the beginning of the F_1 generation, assuming that clones from the resistant and susceptible trees had equal population sizes at the beginning of the P_1 generation (i.e., we assumed that all populations started with a single P_1 larva). Cohort survival to the adult stage was based on survival through the larval and pupal stage (multiplied together) when we used data from the 1998 experiment. For the 1999 experiment, this value was based on survival through the pupal stage alone because the data on larval survival were unavailable due to the survival of some residual F_1 larvae on some trees from the 1998 experiment.

We acknowledge that our estimations of budworm potential fitness were inflated in 1998 (because of the lack of data on survival of first-instar larvae to the third or fourth instars) and in 1999 (because of the lack of reliable data on survival of second-instar larvae to the pupal stage). However, the lack of these data did not influence our comparisons between the potential fitness of budworms after feeding on clones of resistant and susceptible trees within each experimental year (i.e., the biases were consistent between the resistant and susceptible trees in each year).

Foliage Collection and Terpene Analysis. Current-year foliage was sampled from mature trees in late June of the 1989 and 1990 growing seasons, corresponding to the late-instar feeding period of the western spruce budworm (foliage was at the feather duster, or seventh developmental stage) (Clancy et al., 1993). One branch was clipped at random from the mid-crown area of the north, south, east, and west quadrants of each tree; the needles collected from these quadrants were pooled for each tree prior to chemical analysis. Although we have previously reported the foliar monoterpene data for the mature trees (Clancy et al., 1993; Clancy, 2001), this study is the first comparison of foliar monoterpenes in the parent trees and their corresponding clones.

Current-year foliage from the clonal trees in the greenhouse was sampled in 1998 and 1999 when late instars of the budworm were actively feeding on the defoliated trees, and the foliage was at the seventh (or feather duster) developmental stage (Shepherd, 1983). Two clusters of current-year foliage were clipped at random from the upper third of the crown. The foliage sampled from defoliated trees was not directly damaged by budworms. The foliage sampled in each year was pooled over the four to six trees from the same clone and treatment (i.e., budworm defoliation versus control). Therefore, no block effect was included in

the analysis of terpene data. A total of 48 pooled foliage samples were used for chemical analysis [i.e., 2 treatments (defoliated versus control) × 2 traits (resistant versus susceptible clones) × 12 pairs].

Foliage samples were sealed in plastic bags and temporarily stored in a freezer before being transferred to an ultralow freezer for long-term storage (up to seven months) at −60°C before being analyzed. Approximately 250 mg of needle tissue was extracted from each sample in 5 ml pentane. The extracts were analyzed with a Hewlett Packard 5890 series II gas chromatograph containing an HP-1 capillary column (30 m × 0.25 mm). Monoterpene concentrations were calculated on a fresh mass basis (Clancy et al., 1993) because foliage samples collected from the clonal trees were at the same phenological stage and were grown under similar environments; thus, they had little variation in moisture content. All chemical analyses were conducted by the Analytical Services Laboratory at Northern Arizona University.

Statistical Analysis. Since the sampled Douglas fir population from the Pike National Forest site (Colorado) was not genetically differentiated from the sampled population from the Kaibab National Forest site (Arizona) based on an isoenzyme study (Chen et al., 2001b), we treated the 12 pairs of trees from these two sites as one population for statistical analysis. We used a multivariate analysis of variance (MANOVA) to investigate the overall effect of tree pair (i.e., 12 pairs of resistant and susceptible mature trees from which clones were derived), trait (resistant versus susceptible), treatment (defoliation versus non-defoliation), and their interactions on variations in total monoterpene concentration and fractional composition (i.e., percentage) of different monoterpenes. After that, we performed a univariate analysis of variance (ANOVA) test with the three-way interaction term as the error term for each monoterpene component using the same model as described for the MANOVA. Since we measured multiple response variables (e.g., 11 different terpenes) from the same set of experimental units, we used a Bonferroni correction to the P value we accepted as indicating significant differences in the univariate ANOVAs in order to maintain the correct P value of 0.05 for the type I error rate over the whole set of univariate ANOVAs. For this study, we focused on the effects of trait, defoliation treatment, and their interaction because they were most relevant to our research questions. Spearman's rank correlation coefficient (SAS Institute, 1995) was used to compare the rank order of percent composition of different monoterpene components between years and between clones and mature trees.

Finally, we used linear regression to examine the association between: monoterpenes and budworm potential fitness in the 1998 and 1999 greenhouse experiments with clones; the most recent 5-year radial growth (1986–1990) of mature trees in the forest and total monoterpene concentration in 1989 and 1990; and total monoterpene concentration of clones and their corresponding mature trees.

RESULTS

Monoterpene Composition and Structure. Eleven monoterpenes were detected in the needles of cloned trees in greenhouse experiments. The rank order of monoterpene concentration in 1998 was bornyl acetate, camphene, limonene, β-pinene, α-pinene, unknown A, unknown C, myrcene, unknown B, tricyclene, and terpinolene (Table 1). Five monoterpenes, α-pinene, camphene, β-pinene, limonene, and bornyl acetate, accounted for approximately 78% of total monoterpenes in 1998 and 86% in 1999 (Table 1). Fractions of bornyl acetate and limonene increased from 1998 to 1999 ($P \leq 0.017$), fractions of camphene, β-pinene, and α-pinene did not change ($P \geq 0.239$), and unknown monoterpenes A, B, C, and terpinolene declined ($P \leq 0.002$) (Table 1). Based on Spearman's rank correlation coefficient, the rank order in concentration of monoterpenes in 1998 and 1999 was highly correlated ($\rho = 0.955$, $P < 0.001$), indicating high consistency of fractional composition over years.

The same 11 monoterpenes present in clones were also present in mature tree foliage (Table 1). The rank order of fractional composition of monoterpenes in mature trees was consistent between 1989 and 1990 (Spearman's $\rho = 0.734$, $P = 0.010$). Moreover, monoterpene fractional composition was similar between mature trees and clones ($\rho = 0.682$–0.902, $P = 0.021$–0.001, depending on years).

Total monoterpene concentration of clones was greater in 1998 than 1999 by approximately 245% ($P < 0.001$), whereas the total monoterpene concentration in mature trees was approximately 315% greater in 1990 than in 1989 ($P < 0.001$) (Table 1). Further, variation in total monoterpene concentration among individual ramets ($r = 0.164$, $P = 0.269$, $N = 48$) and mature trees ($r = 0.339$, $P = 0.104$, $N = 24$) was not significantly correlated between consecutive sampling years. Moreover, there was no clear relationship in total monoterpene concentration between mature trees and clones. For example, total foliar monoterpene concentrations were positively, but weakly, correlated between mature trees in 1989 and clones in both 1998 and 1999 ($P \leq 0.067$; Figure 1). However, this was not the case for mature trees in 1990 ($P > 0.338$) (data not shown).

Effect of Tree Resistance Trait and Treatment on Monoterpenes. Monoterpene concentration and fractional composition in foliage of clones differed among levels of the experimental factors in the 1998 and 1999 greenhouse experiments (MANOVA, $P < 0.001$) (Table 2). Tree pair and the interaction between pair and trait (resistant versus susceptible) were significant (MANOVA, $P \leq 0.002$) in both years, indicating that both concentration and fraction of monoterpenes differed among the clones from 12 pairs of trees and that the difference between the resistant and susceptible trees within each pair was not consistent.

In the 1998 experiment, trait (resistant versus susceptible) had a marginally significant overall effect on monoterpene concentrations (MANOVA, $P = 0.059$) (Table 2). Further univariate ANOVA for each monoterpene component

TABLE 1. COMPARISON OF MONOTERPENE FRACTIONAL COMPOSITION (% OF TOTAL MONOTERPENE CONCENTRATION) AND TOTAL MONOTERPENE CONCENTRATION IN CURRENT-YEAR FOLIAGE FROM DOUGLAS FIR CLONES AND MATURE TREES OVER TWO CONSECUTIVE SAMPLING YEARS[a]

Monoterpene (abbreviation)	Clones ($N = 48$)					Mature trees ($N = 24$)				
	1998		1999			1989		1990		
	Mean	SE	Mean	SE	P^*	Mean	SE	Mean	SE	P
Bornyl acetate (bc), %	17.68	0.81	20.73	0.96	0.017	27.14	1.67	14.62	0.96	0.001
Camphene (ca), %	16.75	0.73	17.81	0.76	0.239	19.32	1.22	15.98	0.96	0.037
Limonene (lm), %	15.49	0.81	19.62	1.09	0.003	12.71	1.14	11.7	0.51	0.422
β-Pinene (bp), %	15.01	0.94	15.54	1.07	0.712	16.68	1.44	11.06	0.91	0.002
α-Pinene (ap), %	12.88	0.36	12.66	0.37	0.672	13.56	0.58	12.21	0.59	0.110
Unknown A (ua), %	8.09	0.81	2.75	0.49	0.001	3.74	0.77	18.11	1.73	0.001
Unknown C (uc), %	4.51	0.54	2.42	0.37	0.002	1.12	0.43	7.35	1.05	0.001
Myrcene (my), %	3.63	0.19	4.29	0.28	0.051	1.69	0.25	2.67	0.13	0.001
Unknown B (ub), %	3.01	0.24	1.58	0.25	0.001	0.45	0.06	3.09	0.32	0.001
Tricyclene (tr), %	2.21	0.11	2.03	0.09	0.234	3.14	0.29	2.3	0.13	0.012
Terpinolene (tp), %	0.92	0.06	0.57	0.09	0.002	0.45	0.06	0.91	0.05	0.001
Total concentration, ppm	1489.69	105.83	432.00	34.46	0.001	622.08	83.99	2579.33	196.46	0.001

[a] The P value indicates whether there were differences between two sampling years.

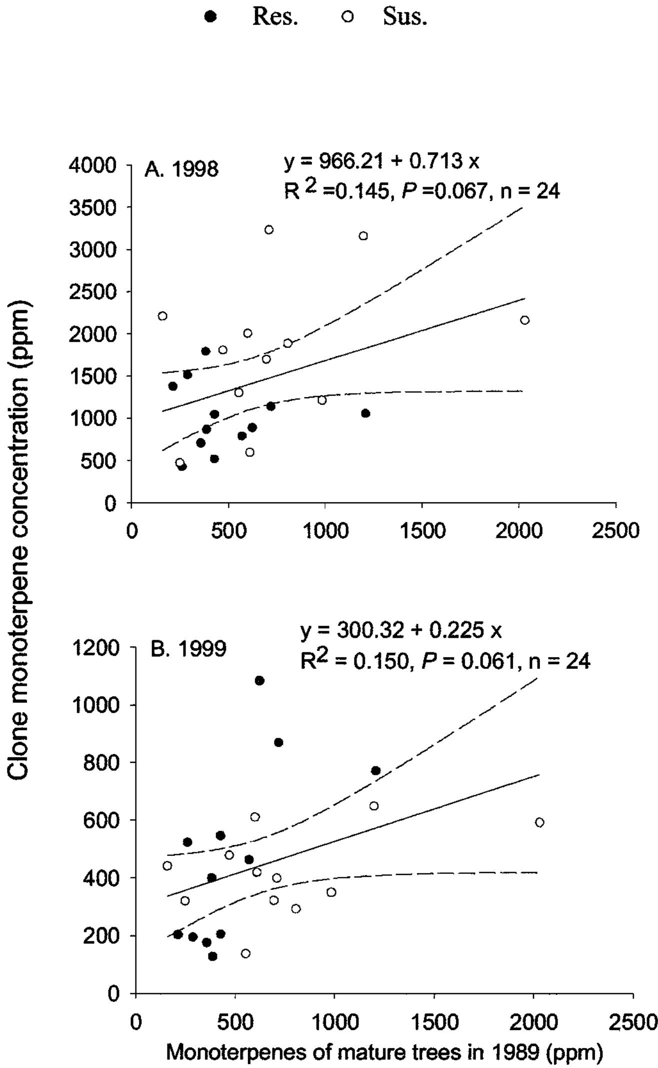

FIG. 1. Regression of foliar concentration (ppm fresh weight) of total monoterpenes in current-year foliage of clones derived from mature Douglas fir trees against concentrations in the original parent trees. All needles were at the seventh (i.e., feather duster) developmental stage when they were sampled. Clonal data is shown for 1998 (A) and 1999 (B) and is plotted against data from the parent trees for 1989. Data are shown for mature trees that appeared to be resistant (●) versus susceptible (○) to western spruce budworm defoliation. Dashed lines around the regression line indicate the 95% confidence limits for the mean response, and the equations for the regression lines and their associated statistics are shown on the graphs.

TABLE 2. RESULTS OF MULTIVARIATE ANALYSIS OF VARIANCE WITH WILKS' LAMBDA TEST FOR EFFECTS OF TREE PAIR, TRAIT, AND TREATMENT ON CONCENTRATION (FRESH-WEIGHT-BASED) AND FRACTIONAL COMPOSITION (% OF TOTAL MONOTERPENE CONCENTRATION) OF 11 MONOTERPENES FROM 1998 AND 1999 GREENHOUSE EXPERIMENTS WITH DOUGLAS FIR CLONES[a]

Source of variation	Wilks' lambda test (Num DF, Den DF)	*P* 1998 Concentration	*P* 1998 Composition	*P* 1999 Concentration	*P* 1999 Composition
Pair	121, 23	<0.001	<0.001	<0.001	<0.001
Trait	11, 1	0.059	0.233	0.491	0.608
Treatment	11, 1	0.550	0.201	0.518	0.595
Pair × trait	121, 23	<0.001	<0.001	<0.001	<0.001
Pair × treatment	121, 23	0.281	0.294	0.423	0.892
Trait × treatment	11, 1	0.533	0.521	0.219	0.429

[a] *P* value represents the probability for the null hypothesis of no overall effect from each source of variation.

indicated that clones from resistant trees generally had a lower concentration of monoterpenes than clones from susceptible trees (ANOVA, $P \leq 0.021$) (Table 3; Figure 2A). Moreover, clones of resistant trees had a lower average total monoterpene concentration (1181 ± 75 ppm) than clones from susceptible trees in 1998 (1798 ± 75 ppm) ($P < 0.001$; Table 3). In contrast, overall monoterpene fractional composition did not differ between clones from resistant and susceptible trees in 1998 (MANOVA, $P = 0.233$; Table 2). However, univariate ANOVA suggested that clones from resistant trees had a higher fraction of camphene and bornyl acetate and a lower fraction of β-pinene and unknown C than clones from susceptible trees ($P \leq 0.004$; Table 3) (Figure 2C). Budworm defoliation treatment in 1998 did not influence the overall concentration or fractional composition of monoterpenes (MANOVA, $P \geq 0.201$; Table 2). However, the univariate ANOVAs suggested a reduction in the fraction of myrcene and limonene in defoliated versus nondefoliated tree clones ($P \leq 0.005$; Table 3; Figure 3C).

Clones from susceptible trees had a greater ratio of total monoterpene concentration to nitrogen concentration (i.e., monoterpene/N ratio; 0.083 ± 0.005) than clones from resistant trees (0.054 ± 0.005) in the 1998 experiment ($P = 0.001$). Furthermore, budworm defoliation caused a marginal increase in the monoterpene/N ratio (0.075 ± 0.005) compared to the control (0.062 ± 0.005) in 1998 ($P = 0.088$). The interaction between tree trait and treatment did not influence the concentration or fractional composition of monoterpenes in 1998 (MANOVA, $P \leq 0.533$; Table 2) or the monoterpene/N ratio, indicating similar effects of defoliation on clones from resistant and susceptible trees. Univariate ANOVA also supported the absence of any significant trait × treatment interactions ($P \geq 0.033$; Table 3).

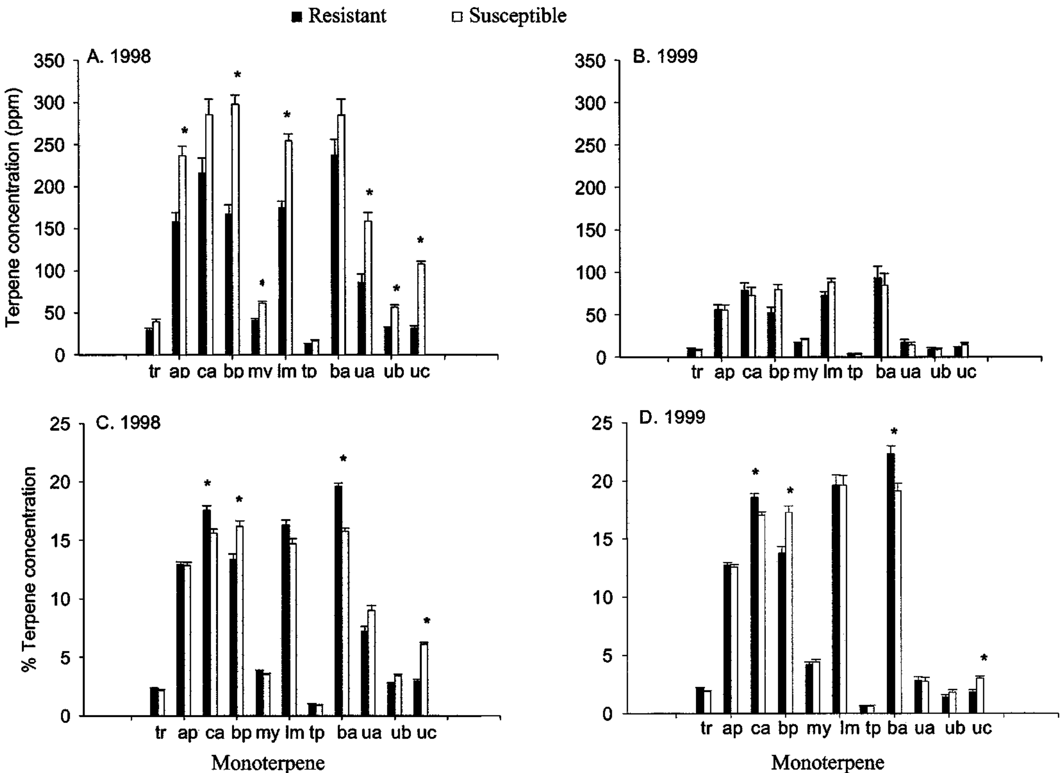

FIG. 2. Mean (+1 SE) concentration (A, B) and fractional composition (C, D) of 11 monoterpene components in Douglas fir needles sampled in the 1998 (A, C) and 1999 (B, D) greenhouse experiments for clones from mature Douglas fir trees that appeared to be resistant (■) versus susceptible (□) to western spruce budworm defoliation. Abbreviations represent 11 monoterpenes defined in Table 1. Asterisks (∗) indicate there was a significant difference between the resistant and susceptible clones (ANOVA, Bonferroni adjusted $\alpha \leq 0.005$).

TABLE 3. RESULTS FROM UNIVARIATE ANALYSIS OF VARIANCE TESTS (F TEST AND PROBABILITY VALUES) FOR EFFECTS OF TRAIT, TREATMENT, AND THEIR INTERACTION ON CONCENTRATION (FRESH-WEIGHT-BASED) AND FRACTIONAL COMPOSITION (% OF TOTAL MONOTERPENE CONCENTRATION) OF EACH MONOTERPENE COMPONENT OF DOUGLAS FIR CLONES IN 1998 EXPERIMENT WHEN BUDWORM FEEDING WAS MATCHED TO BUDBURST OF EACH INDIVIDUAL PLANT[a]

	Trait (resistant vs. susceptible)				Treatment (defoliation vs. nondefoliation)				Trait × treatment			
	Fresh wt		%		Fresh wt		%		Fresh wt		%	
1998 data	$F_{1,11}$	P	$F_{1,11}$	P	$F_{1,11}$	P	$F_{1,11}$	P	$F_{1,11}$	P	$F_{1,11}$	P
Tricyclene	7.74	0.018	1.96	0.189	3.66	0.082	3.95	0.072	2.38	0.151	1.72	0.216
α-Pinene	24.34	0.001	0.05	0.821	3.13	0.104	1.36	0.268	2.57	0.137	0.01	0.943
Camphene	7.27	0.021	12.99	0.004	3.91	0.074	7.05	0.022	2.78	0.124	2.13	0.172
β-Pinene	69.84	0.001	13.35	0.004	0.11	0.748	3.06	0.108	0.98	0.343	1.94	0.192
Myrcene	51.38	0.001	3.77	0.078	0.01	0.977	11.96	0.005	2.37	0.152	2.09	0.176
Limonene	51.81	0.001	7.27	0.021	0.01	0.980	13.71	0.004	2.62	0.134	2.54	0.139
Terpinolene	9.31	0.011	2.29	0.158	8.23	0.015	10.17	0.009	5.96	0.033	5.59	0.038
Bornyl acetate	3.06	0.108	94.13	0.001	2.85	0.119	9.61	0.010	3.71	0.081	5.34	0.041
Unknown A	24.73	0.001	8.59	0.014	0.74	0.409	0.25	0.628	0.76	0.403	0.01	0.919
Unknown B	56.88	0.001	10.14	0.009	0.84	0.381	0.12	0.742	0.56	0.472	0.10	0.751
Unknown C	274.74	0.001	210.25	0.001	0.28	0.610	0.18	0.678	0.00	0.980	0.00	0.947
Total	33.65	0.001			2.27	0.161			2.97	0.113		

[a] The Bonferroni adjusted P value of $\alpha = 0.005$ (i.e., 0.05 divided by 11) was used to determine significance, in order to maintain the correct P value of 0.05 for the type I error rate over the whole set of univariate ANOVAs for the 11 individual terpene compounds.

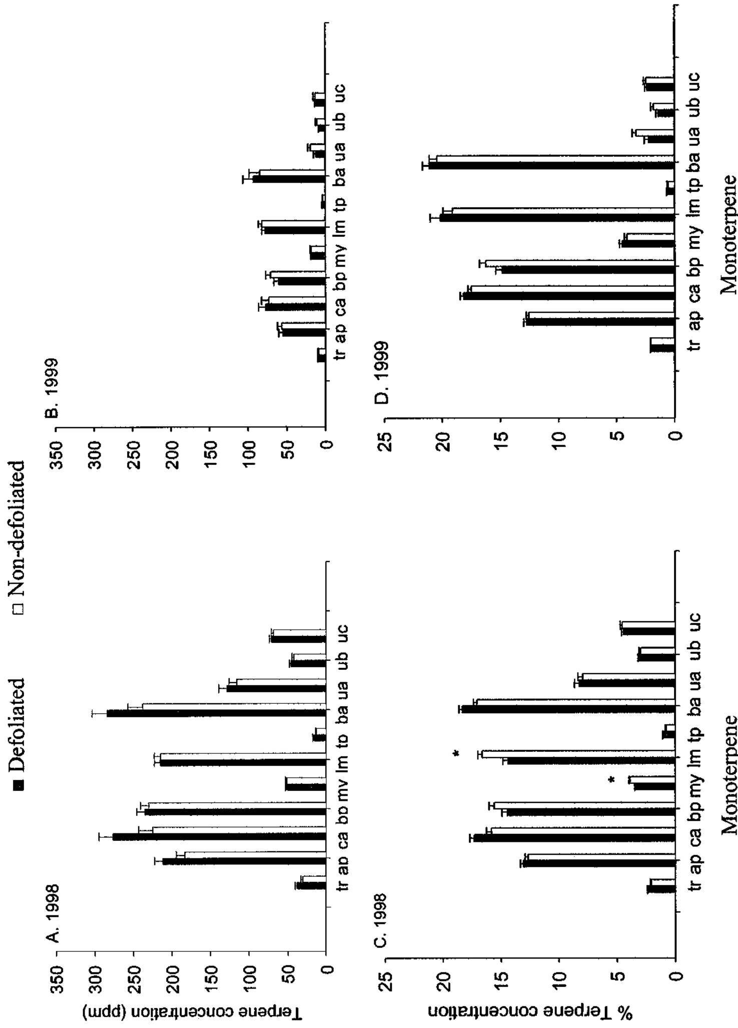

FIG. 3. Mean (+1 SE) concentration (A, B) and fractional composition (C, D) of 11 monoterpene components in Douglas fir needles sampled in the 1998 (A, C) and 1999 (B, D) greenhouse experiments for defoliated (■) versus nondefoliated (□) clones from mature Douglas fir trees (data pooled for resistant and susceptible clones). Abbreviations represented 11 monoterpenes defined in Table 1. Asterisks (∗) indicate there was a significant difference between the defoliated and non-defoliated clones (ANOVA, Bonferroni adjusted $\alpha \leq 0.005$).

In the 1999 experiment, when total monoterpene concentration was 71% lower than in 1998, tree trait, defoliation treatment, and their interaction did not influence the concentration or composition of monoterpenes (MANOVA, $P \geq 0.219$; Table 2). Univariate ANOVAs also failed to detect significant effects on concentrations of individual or total monoterpenes ($P \geq 0.011$; Table 4; Figures 2B and 3B) or on the monoterpene/N ratio ($P > 0.509$). Likewise, the fractional composition of monoterpenes was unaffected by defoliation in 1999 ($P \geq 0.058$; Table 4; Figure 3D) and there were no detectable trait × treatment interactions ($P \geq 0.036$; Table 4). However, the univariate ANOVAs indicated there were significant differences between resistant and susceptible trees in the proportions of camphene, β-pinene, bornyl acetate, and unknown terpene C ($P \leq 0.005$; Table 4). Interestingly, the patterns were identical to those observed in the 1998 experiment (Figure 2C); resistant trees had higher percentages of camphene and bornyl acetate, whereas susceptible trees had higher fractions of β-pinene and unknown C (Figure 2D).

Effect of Monoterpenes and Terpene/N Ratio on Budworm Performance. In the 1998 experiment, when total monoterpene concentration was high and budworm feeding was matched to the budburst of each individual plant, budworm potential fitness declined as total monoterpene concentration increased ($P = 0.027$); approximately 20% of the variation in potential fitness was explained by total monoterpene concentration (Figure 4A). Individual terpenes that were negatively correlated with budworm fitness included myrcene ($P = 0.046$), limonene ($P = 0.029$), and unknown C ($P = 0.029$). Further, the monoterpene/N ratio was negatively related to budworm fitness in 1998 ($r = -0.481$, $P = 0.017$; Figure 4B).

In contrast, in the 1999 experiment, monoterpene concentrations were low and genetic differences among the clones in budburst phenology affected the quantity and quality of the buds and needles available for larval feeding. Under these conditions, budworm fitness was not related to total monoterpene concentration ($P = 0.567$), the fractional composition of monoterpene components (P values ranged from 0.124 to 0.954), or the monoterpene/N ratio ($P = 0.531$).

The monoterpene/N ratio was significantly higher in 1998 (0.069 ± 0.004) than in 1999 (0.011 ± 0.004) ($P < 0.001$). Although nitrogen concentration was not related to monoterpene concentration in each year, total monoterpene concentration declined exponentially as foliar N concentration increased for data pooled over both years (Figure 5). The higher monoterpene/N ratio in 1998 was caused by both higher total monoterpene concentration and lower nitrogen concentration compared to the data in 1999 (Figure 5).

Relationship between Monoterpene Concentration and Growth. Total monoterpene concentration was not correlated with the relative growth rates of the base diameter, crown, or height of the clones between the start of the experiments and the end of the 1998 growing season ($P \geq 0.814$; $N = 24$). Moreover, the five-year

TABLE 4. RESULTS FROM UNIVARIATE ANALYSIS OF VARIANCE TESTS (F TEST AND PROBABILITY VALUES) FOR EFFECTS OF TRAIT, TREATMENT, AND THEIR INTERACTION ON CONCENTRATION (FRESH-WEIGHT-BASED) AND FRACTIONAL COMPOSITION (% OF TOTAL MONOTERPENE CONCENTRATION) OF EACH MONOTERPENE COMPONENT OF DOUGLAS FIR CLONES IN 1999 EXPERIMENT WHEN BUDWORM FEEDING WAS MATCHED TO BUDBURST OF ENTIRE POPULATION OF PLANTS TO MIMIC CONDITIONS *similar to those* OCCURRING IN FOREST[a]

	Trait				Treatment (defoliation vs. nondefoliation)				Trait × treatment			
	Fresh wt		%		Fresh wt		%		Fresh wt		%	
1999 data	$F_{1,11}$	P	$F_{1,11}$	P	$F_{1,11}$	P	$F_{1,11}$	P	$F_{1,11}$	P	$F_{1,11}$	P
Tricyclene	0.62	0.449	5.78	0.035	0.00	1.000	0.04	0.839	0.70	0.421	0.42	0.529
α-Pinene	0.01	0.958	0.47	0.508	0.05	0.821	0.41	0.538	1.24	0.290	0.10	0.761
Camphene	0.17	0.691	13.57	0.004	0.08	0.788	2.54	0.139	0.48	0.502	2.92	0.116
β-Pinene	9.69	0.011	20.17	0.001	1.34	0.271	3.26	0.099	2.12	0.173	2.36	0.153
Myrcene	7.97	0.017	0.62	0.449	0.37	0.556	1.53	0.242	2.11	0.175	0.35	0.564
Limonene	6.00	0.032	0.01	0.957	0.41	0.533	0.81	0.388	4.35	0.061	0.01	0.920
Terpinolene	0.29	0.598	0.07	0.792	0.58	0.463	0.26	0.621	0.58	0.463	5.72	0.036
Bornyl acetate	0.19	0.674	12.07	0.005	0.18	0.681	0.40	0.539	0.31	0.588	0.81	0.388
Unknown A	0.41	0.536	0.02	0.888	2.38	0.151	4.46	0.058	4.43	0.059	1.05	0.327
Unknown B	0.01	0.965	1.60	0.232	1.31	0.279	1.78	0.210	2.19	0.167	0.82	0.385
Unknown C	2.56	0.138	243.32	0.001	0.24	0.631	0.17	0.691	3.57	0.086	0.10	0.756
Total	0.26	0.623			0.07	0.798			1.51	0.247		

[a] The Bonferroni adjusted P-value of $\alpha = 0.005$ (i.e., 0.05 divided by 11) was used to determine significance, in order to maintain the correct P-value of 0.05 for the type I error rate over the whole set of univariate ANOVAs for the 11 individual terpene compounds.

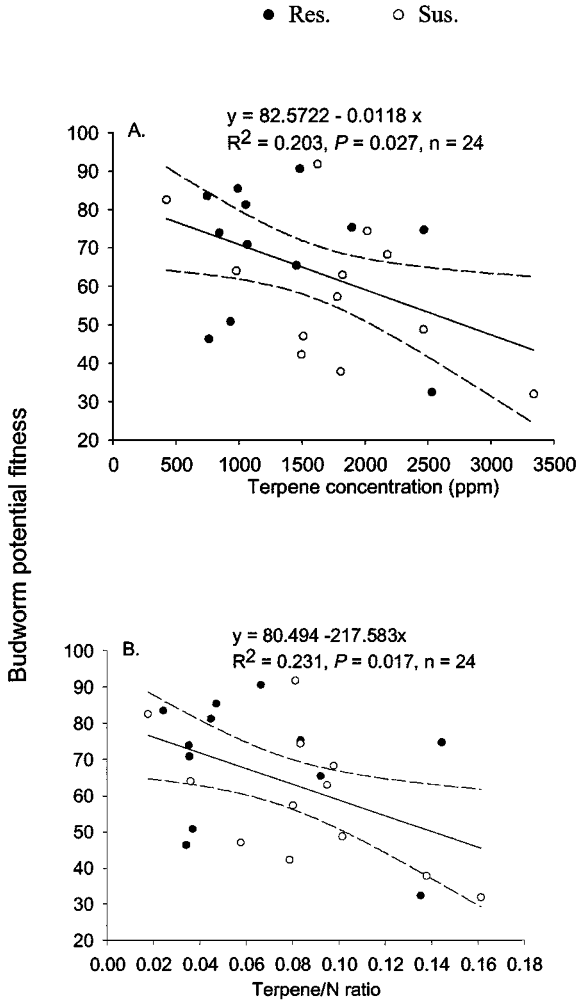

FIG. 4. Regression of western spruce budworm potential fitness against foliar concentration (ppm fresh weight) of total monoterpenes (A) and the ratio of total monoterpene concentration to foliar nitrogen (B) in the current-year foliage of clones derived from trees that appeared to be resistant (●) versus susceptible (○) to western spruce budworm defoliation. Data are from the 1998 experiment; needles were at the feather duster (seventh) developmental stage when they were sampled. Dashed lines around the regression line indicate the 95% confidence limits for the mean response, and the equations for the regression lines and their associated statistics are shown on the graphs.

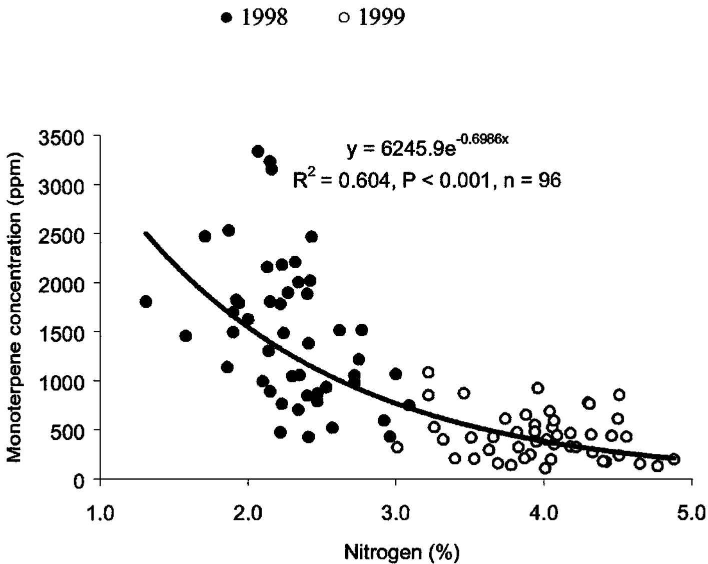

FIG. 5. Regression of foliar concentrations (ppm fresh weight) of total monoterpenes against nitrogen (% dry weight) in current-year foliage of Douglas fir clones. All needles were at the feather duster (seventh) developmental stage when they were sampled. Data are shown for the 1998 and 1999 experiments, pooled for the resistant and susceptible clones. The equation for the regression line and the associated statistics are shown on the graph.

radial growth rate of mature trees between 1986 and 1990 also was not related to the total monoterpene concentration of the mature trees in 1989 ($P = 0.902$) or 1990 ($P = 0.498$; $N = 24$).

DISCUSSION

Eleven monoterpenes were detected in current-year needles of Douglas fir clones sampled at Shepherd's (1983) feather duster (or seventh) developmental stage. They included the most common monoterpenes reported in other studies of Douglas fir, but did not include several monoterpenes, such as α-phellandrene, *cis*-β-ocimene, and 3-carene, that occurred at low concentrations in other studies (von Rudloff and Rehfeldt, 1980; Wagner et al., 1989; Zou and Cates, 1995). Compared with the relative percentage of major leaf monoterpenes of Douglas fir trees in the northern Rocky Mountains (von Rudloff and Rehfeldt, 1980), trees from southern Colorado and northern Arizona in our study had a much higher relative percentage of limonene, β-pinene, and myrcene, but a lower percentage of

tricyclene, camphene, bornyl acetate, and α-pinene. The rank order of concentration for different monoterpenes was highly correlated between the sampling years and between mature trees and their clones (Table 1), despite the variations in total monoterpene concentration over years (Figures 2A,B and 3A,B). These findings indicated remarkable consistency in fractional composition of monoterpenes.

We hypothesized that clones from resistant trees would have a higher monoterpene concentration than clones from susceptible trees based on the assumption that high monoterpene concentration would limit budworm defoliation. Testing this hypothesis with clones grown in a uniform greenhouse environment allowed us to eliminate environmental differences as a causal factor. Contrary to our hypothesis, clones from resistant trees had a lower total monoterpene concentration than clones from susceptible trees in the 1998 experiment (Figure 2A), and budworm potential fitness was greater after feeding on clones from resistant trees (Figure 4A). Thus, results from the 1998 experiment were not consistent with our field observations of resistance. This suggests that the ability of resistant mature trees to escape heavy budworm defoliation cannot be attributed to a greater genetic capacity for monoterpene biosynthesis in foliage. A difference in budburst phenology is a more likely explanation for the difference in budworm defoliation between the resistant and susceptible trees. For example, clones from resistant trees required about 90–110 more degree-days to reach the same budburst developmental stage than clones from susceptible trees (Chen et al., 2001a). This difference in budburst phenology among clones is consistent with differences in budburst phenology for mature trees in the forest (Clancy et al., 1993). Resistant trees are genetically predisposed towards later budburst that could cause poor food quality for the budworm if late budburst forces them to feed on old foliage (Clancy et al., 1993; Dodds et al., 1996).

The different results from the 1998 and 1999 experiments on the clones suggest a role of budburst phenology in regulating the influence of foliar monoterpenes on budworm performance. In 1998, we allowed budworms to feed on each clone when buds offered the best suitable food source. In this situation, genetic differences in total monoterpene concentration among clones influenced budworm fitness (Figure 4A). In contrast, in the 1999 experiment, we introduced larvae to all clones on one date when approximately 50% of the buds in the whole population were in Shepherd's (1983) second budburst stage that was just accessible for larval mining. With this approach, suitable buds available for larval feeding varied widely among clones because of genetic differences in budburst phenology (Chen et al., 2001a); the potential influence of genetic differences in monoterpenes among clones on budworm performance may have been overshadowed by differences in foliage quality associated with budburst and shoot development (Dodds et al., 1996).

Of course, an alternative explanation for the different results between the 1998 and 1999 experiments is that monoterpene concentration in 1999 was too

low to influence budworm performance (Figures 2B and 3B). This explanation implies that budworm larval performance may not be affected by total monoterpene concentration less than 1000 ppm. Nonetheless, our results raise an interesting possibility that mature trees classified as "resistant" to budworm defoliation in the forest could actually be "susceptible" in situations where the onset of budworm larval feeding coincides with their budburst. Thus, the mixed conclusions about the role of monoterpenes as a resistance mechanism against budworm defoliation in Douglas fir (Cates et al., 1983; Redak and Cates, 1984; Cates and Zou, 1990; Clancy, 1991a, 1993, 2001; Clancy et al., 1993) are perhaps not surprising, as factors such as budburst phenology may mediate the expression of this mechanism.

It is also noteworthy that there were consistent differences in 1998 and 1999 between the resistant and susceptible clones in the proportions of camphene, β-pinene, bornyl acetate, and unknown terpene C ($P \leq 0.005$; Tables 3 and 4). The resistant trees had higher percentage of camphene and bornyl acetate, whereas the susceptible trees had higher fractions of β-pinene and unknown terpene C (Figures 2C and D). This finding, to some extent, also agrees with the results of previous studies that camphene and bornyl acetate were highly toxic to several populations of budworm in Idaho, Montana, and New Mexico (Cates et al., 1983; Cates and Redak, 1988; Cates and Zou, 1990).

One or two years of budworm defoliation under our study conditions did not influence the total concentration or fractional composition of monoterpenes in either the 1998 or 1999 experiments with the clones (Figure 3). Furthermore, the large differences in total monoterpene concentration of clones (Figures 2A,B and 3A,B) and mature trees (Clancy et al., 1993) between two consecutive sample years could not be clearly explained by effects of defoliation. Moreover, defoliation had a similar effect on monoterpene concentrations of clones from resistant and susceptible trees. The lower monoterpene concentration of clones in 1999 compared to 1998 was probably caused by a decrease in tree carbon/nitrogen balance in 1999 (Figure 5) that reduced synthesis of monoterpenes and other carbon-based secondary metabolites (Bryant et al., 1983; Larsson et al., 1983; Mattson and Haack, 1987). The strong negative relationship between total foliar monoterpene concentration and nitrogen concentration for data pooled over two years of experiments support this view (Figure 5) and suggest that the carbon/nitrogen balance is important in regulating carbon allocation to monoterpenes in Douglas fir trees.

Many other traits of tree tissues can vary with budburst phenology and seasonal cycles of growth and dormancy (Mattson and Scriber, 1987; Clancy et al., 1988a,b, 1995). For example, positive correlations of growth and reproduction of insect herbivores with variation in one chemical constituent of their food do not prove cause and effect, because changes in the level of the chemical constituent in a plant may be accompanied by changes in the levels of nutrients, water, fiber, and numerous allelochemicals (Clancy, 1992). We recognize that the strong intercorrelations among many different characteristics in host plants make

it difficult to determine an herbivore's response to one specific trait, such as foliar monoterpenes. Moreover, it is impossible to maintain a constant level of monoterpenes (or other potentially confounding nutrients, etc.) throughout the duration of a greenhouse experiment because a plant's biochemical composition is continuously changing (Clancy, 1992). Thus, differences in resistance to budworm defoliation among Douglas fir genotypes are most likely caused by interactions or linkages among multiple resistance mechanisms such as monoterpenes, nutrients, and tree budburst phenology.

Finally, despite the high cost of monoterpene synthesis (Gershenzon, 1994), we found no evidence for a tradeoff between growth and monoterpene concentration in the clones and mature trees. The proportion of energy allocated to monoterpene synthesis was perhaps too small to compromise tree growth. On the other hand, perhaps high carbon allocation to monoterpenes reduced allocation to other tree uses of carbon (e.g., roots, leaves, mycorrhizae) that could not be detected by measuring stem radial growth at breast height (mature trees) or canopy size (clones).

In summary, our results suggest remarkable consistency in the percent composition of different monoterpenes in Douglas fir trees, in spite of much year-to-year variation in concentrations of these compounds. Moreover, genetic variation in budburst phenology among trees may mediate the influence of inherent differences in total monoterpene concentration on budworm fitness.

REFERENCES

ANNILA, E., HILTUNEN, R., and LOYTTYNIEMI, R. 1984. Defoliation by *Neodiprion sertifer* (Hymenoptera, Diprionidae) in relation to the terpene composition of *Pinus contorta*. *Ann. Entomol. Fenn.* 50:33–36.

BRYANT, J. P., CHAPIN, F. S., and KLEIN, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40:357–368.

CATES, R. G. and REDAK, R. A. 1988. Variation in the terpene chemistry of Douglas-fir and its relationship to western spruce budworm success, pp. 317–341, *in* K. C. Spencer (ed.). Chemical Mediation of Coevolution. Academic Press, San Diego, California.

CATES, R. G. and ZOU, J. 1990. Douglas-fir (*Pseudotsuga menziesii*) terpene chemistry and its role in budworm (*Choristoneura occidentalis*) dynamics, pp. 69–182, *in* A. Watt, S. Leatyer, M. Honter, and N. Kidd (eds.). Population Dynamics of Forest Insects. Intercept Ltd., London.

CATES, R. G., REDAK, R., and HENDERSON, C. 1983. Pattern in defensive natural product chemistry: Douglas-fir and western spruce budworm interaction. *Am. Chem. Soc. Symp. Ser.* 208:3–19.

CATES, R. G., HENDERSON, C., and REDAK, R. 1987. Responses of western spruce budworm to varying levels of nitrogen and terpenes. *Oecologia* 73:372–316.

CHEN, Z., KOLB, T. E., and CLANCY, K. M. 2001a. Mechanisms of Douglas-fir resistance to western spruce budworm defoliation: Budburst phenology, photosynthetic compensation, and growth rate. *Tree Physiol.* 21:1159–1169.

CHEN, Z., KOLB, T. E., CLANCY, K. M., HIPKINS, V. D., and DEWALD, L. E. 2001b. Allozyme variation in interior Douglas-fir: Association with growth and resistance to western spruce budworm herbivory. *Can. J. For. Res.* 31:1691–1700.

CLANCY, K. M. 1991a. Douglas-fir nutrients and terpenes as potential factors influencing western spruce budworm defoliation, pp. 124–134, *in* Y. N. Baranchikov, W. J. Mattson, F. Pain, and T. L. Payne (eds.). Forest Insect Guilds: Patterns of Interaction with Host Trees. USDA Forest Service General Technical Paper NE-153.

CLANCY, K. M. 1991b. Multiple-generation bioassay for investigating western spruce budworm (Lepidoptera: Tortricidae) nutritional ecology. *Environ. Entomol.* 20:1363–1374.

CLANCY, K. M. 1992. Response of western spruce budworm (Lepidoptera: Tortricidae) to increased nitrogen in artificial diets. *Environ. Entomol.* 21:331–344.

CLANCY, K. M. 1993. Are terpenes defensive compounds for the western spruce budworm? *Suppl. Bull. Ecol. Soc. Am.* 74:193.

CLANCY, K. M. 2001. Biochemical characteristics of Douglas-fir trees resistant to damage from the western spruce budworm: patterns from three populations, pp. 115–125, *in* R. I. Alfaro, K. Day, S. Salom, A. Liebhold, H. Evans, F. Lieutier, M. Wagner, K. Futai, K. Suzuki, and K. S. S. Nair (eds.). Protection of World Forests: Advances in Research. IUFRO World Series No. IUFRO Secretariat, Vienna, Austria.

CLANCY, K. M., WAGNER, M. R., and TINUS, R. W. 1988a. Variations in nutrient levels as a defense: identifying key nutritional traits of host plants of the western spruce budworm, pp. 203–213, *in* W. J. Mattson, J. Levieux, and C. Bernard-Dagan (eds.). Mechanisms of Woody Plant Defenses Against Insects: Search for Pattern. Springer, New York.

CLANCY, K. M., WAGNER, M. R., and TINUS, R. W. 1988b. Variation in host foliage nutrient concentrations in relation to western spruce budworm herbivory. *Can. J. For. Res.* 18:530–539.

CLANCY, K. M., FOUST, R. D., HUNTSBERGER, T. G., WHITAKER, J. G., and WHITAKER, D. M. 1992. Technique for using microencapsulated terpenes in lepidopteran artificial diets. *J. Chem. Ecol.* 18:543–560.

CLANCY, K. M., ITAMI, J. K., and HUEBNER, D. P. 1993. Douglas-fir nutrients and terpenes: potential resistance factors to western spruce budworm defoliation. *For. Sci.* 39:78–94.

CLANCY, K. M., WAGNER, M. R., and REICH, P. B. 1995. Ecophysiology and insect herbivory, pp. 125–180, *in* W. K. Smith and T. M. Hinckley (eds.). Ecophysiology of Coniferous Forests. Academic Press, San Diego, California.

DODDS, K. A., CLANCY, K. M., LEYVA, K. J., GREENBERG, D., and PRICE, P. W. 1996. Effects of Douglas-fir foliage age class on western spruce budworm oviposition choice and larval performance. *Great Basin Nat.* 56:135–141.

FELIPE, B. L., HASEGAWA, M., and CEDENO, A. 1994. Mono- and sequisterpene variation in *Pinus caribaea* needles and its relationship to *Atta laevigata* herbivory. *Biochem. Syst. Ecol.* 22:437–445.

GAMBLIEL, H. and CATES, R. 1995. Terpene changes due to maturation and canopy level in Douglas-fir (*Pseudotsuga menziesii*) flush needle oil. *Biochem. Syst. Ecol.* 23:469–476.

GERSHENZON, J. 1994. The cost of plant chemical defense against herbivory: a biochemical perspective, pp. 105–173, *in* E. A. Bernays (ed.). Insect–Plant Interactions, Vol. 5. CRC Press, Boca Roton, Florida.

GERSHENZON, J. and CROTEAU, R. 1991. Terpenoids, pp. 165–219, *in* G. A. Rosenthal and M. R. Berenbaum (eds.). Herbivores, Their Interactions with Secondary Metabolites, the Chemical Participants. Academic Press, New York.

HANOVER, J. W. 1992. Applications of terpene analysis in forest genetics. *New For.* 6:159–178.

HANULA, J. L., BERISFORD, C. W., and DEBARR, G. L. 1985. Monoterpene oviposition stimulants of *Dioryctria amatella* in volatiles from fusiform rust galls and second-year loblolly pine cones. *J. Chem. Ecol.* 11:943–952.

HARBORNE, J. B. 1991. Recent advances in the ecological chemistry of plant terpenoids, pp. 399–426, *in* J. B. Harborne and F. A. Tomes-Barberan (eds.). Ecological Chemistry and Biochemistry of Plant Terpenoids. Clarenden Press, Oxford.

HARTMANN, H. T. and KESTER, D. E. (eds.). 1983. Plant Propagation: Principles and Practices. Prentice-Hall, Inc. Englewood, Cliffs, New Jersey.

HEDIN, P. A., MAXWELL, F. G., and JEKINS, J. N. 1974. Insect plant attractants, feeding stimulants, repellents, deterrents, and other related factors affecting insect behavior, pp. 497–557, *in* F. G. Maxwell and F. A. Harris (eds.). Proc. Summer Insect Biological Control of Plant Insects and Diseases. University of Mississippi Press, Jackson, Mississippi.

JOHNSON, R. H., YOUNG, B. L., and ALSTAD, D. N. 1997. Responses of ponderosa pine growth and volatile terpene concentrations to manipulation of soil water and sunlight availability. *Can. J. For. Res.* 27:1794–1804.

KOGAN, M. 1975. Plant resistance in pest management. pp. 103–146, *in* R. L. Metcalf and W. H. Luckman (eds.). Introduction to Insect Pest Management. Wiley, New York.

LANGENHEIM, J. H. 1994. Higher plant terpenoids: A phytocentric overview of their ecological roles. *J. Chem. Ecol.* 20:1223–1280.

LARSSON, S., OREN, R., WARING, R. H., and BARRETT, J. W. 1983. Attacks of mountain pine beetle as related to tree vigor of ponderosa pine. *For. Sci.* 29:395–402.

LATTA, R. G., LINHART, Y. B., LUNDQUIST, L., and SNYDER, M. A. 2000. Patterns of monoterpene variation within individual trees in ponderosa pine. *J. Chem. Ecol.* 26:1341–1357.

LERDAU, M., MATSON, P., FALL, R., and MONSON, R. 1995. Ecological controls over monoterpene emissions from Douglas-fir (*Pseudotsuga menziesii*). *Ecology* 76:2640–2647.

LEYVA, K. J., CLANCY, K. M., and PRICE, P. W. 1995. Performance of wild versus laboratory populations of western spruce budworm (Lepidoptera: Tortricidae) feeding on Douglas-fir foliage. *Environ. Entomol.* 24:80–87.

MANNINEN, A. M., VUORINEN, M., and HOLOPAINEN, J. K. 1998. Variation in growth, chemical defense, and herbivore resistance in Scots pine provenances. *J. Chem. Ecol.* 24:1315–1331.

MATTSON, W. J. and HAACK, R. A. 1987. The role of drought stress in provoking outbreaks of phytophagus insects, pp. 365–407, *in* P. Barbosa and Schultz, J. C. (eds.). Insect Outbreaks. Academic Press, Toronto.

MATTSON, W. J. and SCRIBER, J. M. 1987. Nutritional ecology of insect folivores of woody plants: water, nitrogen, fiber, and mineral considerations, pp. 105–146, *in* F. Slansky, Jr., and J. Rodriguez (eds.). Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. Wiley, New York.

MCCLURE, M. S. and HARE, J. D. 1984. Foliar terpenoids in *Tsuga* species and the fecundity of scale insects. *Oecologia* 63:185–193.

MCKINNON, M. L., QUIRING, D. T., and BAUCE, E. 1998. Influence of resource availability on growth and foliar chemistry within and among young white spruce trees. *EcoScience* 5:295–305.

NAULT, J. R., MANVILLE, J. F., and SAHOTA, T. S. 1999. Spruce terpenes: Expression and weevil resistance. *Can. J. For. Res.* 29:761–767.

REDAK, R. and CATES, R. G. 1984. Douglas-fir (*Pseudotsuga menziesii*)–spruce budworm (*Choristoneura occidentalis*) interactions: The effect of nutrition, chemical defenses, tissue phenology, and tree physical parameters on budworm success. *Oecologia* 62:61–67.

SAS Institute. 1995. JMP Statistics and Graphics Guide, Version 3. SAS Institute, Cary, North Carolina.

SHEPHERD, R. F. 1983. A technique to study phenological interactions between Douglas-fir buds and emerging second-instar western spruce budworm, pp. 17–20, *in* R. L. Talerico, and M. Montgomery (eds.) Forest Defoliator–Host Interactions: A Comparison between Gypsy Moth and Spruce Budworms. USDA Forest Service General Technical Report NE-85.

TOMLIN, E. S., BORDEN, J. H., and PIERCE, H. D., JR. 1997. Relationship between volatile foliar terpenes and resistance of Sitka spruce to the white pine weevil. *For. Sci.* 43:501–508.

VON RUDLOFF, E. and REHFELDT, G. E. 1980. Chemosystematics studies in the genus *Pseudotsuga.* IV. Inheritance and geographic variation in the leaf oil terpenes of Douglas-fir for the Pacific Northwest. *Can. J. For. Res.* 58:546–556.

WAGNER, M. R. and ZHANG, Z. 1993. Host plant traits associated with resistance of ponderosa pine to the sawfly, *Neodiprion fulviceps*. *Can. J. For. Res.* 23:839–845.

WAGNER, M. R., CLANCY, K. M., and KIRKBRIDE, D. M. 1987. Predicting number of oocytes in adult western spruce budworm, *Choristoneura occidentalis* (Lepidoptera: Tortricidae). *Environ. Entomol*. 16:551–555.

WAGNER, M. R., CLANCY, K. M., and TINUS, R. W. 1989. Maturational variation in needle essential oils from *Pseudotsuga menziesii, Abies concolor*, and *Picea engelmannii. Phytochemistry* 28:765–770.

WILKINSON, R. C. 1985. Comparative white-pine weevil attack susceptibility and cortical monoterpene composition of western and eastern white pines. *For. Sci*. 31:39–42.

ZOBEL, B. and TALBERT, J. (eds.). 1984. Applied Forest Tree Improvement. Waveland Press, Prospect Heights, Illinois.

ZOU. J. and CATES, R. G. 1995. Foliage constituents of Douglas-fir (*Pseudotsuga menziesii*): Their seasonal variation and potential role in Douglas-fir resistance and silviculture management. *J. Chem. Ecol.* 21:387–402.