

# Short-term effect of defoliation on terpene content in *Thuja plicata*<sup>1</sup>

Gwenaël VOUREC<sup>2</sup>, Centre d'Écologie Fonctionnelle et Évolutive, CNRS UPR 9056, 1919 route de Mende, 34293 Montpellier cedex 5, France.

John RUSSELL, Ministry of Forests, Research Branch, Cowichan Lake Research Station, Box 335, Mesachie Lake, British Columbia V0R 2N0, Canada.

Dominique GILLON & Jean-Louis MARTIN, Centre d'Écologie Fonctionnelle et Évolutive, CNRS UPR 9056, 1919 route de Mende, 34293 Montpellier cedex 5, France.

**Abstract:** Insect herbivory or mechanical wounding in conifers can induce monoterpene biosynthesis. Low risk of herbivory, coupled with low availability of resources, is hypothesized to favour induced responses and to decrease constitutive defences. We studied the response to defoliation in western redcedars (*Thuja plicata*) from two regions: the Haida Gwaii archipelago, where mammalian herbivores were lacking until black-tailed deer were introduced at the end of the 19<sup>th</sup> century and previous work indicated that trees were less well defended, and the north coast mainland (British Columbia, Canada). We predicted that higher induced defences in the island population would compensate for reduced constitutive defences. We used one- and two-year-old nursery-grown seedlings to test *i*) whether defoliation would cause a short-term chemical response in island western redcedar and *ii*) whether mainland western redcedars that have always been exposed to large mammalian herbivores respond differently. The concentration in monoterpene and diterpenes did not vary significantly in response to defoliation over the 5-day period analyzed regardless of the defoliation intensity or the plant's origin.

**Keywords:** defoliation, induction, terpenes, conifers, *Thuja plicata*.

**Résumé :** La biosynthèse des monoterpènes chez les conifères peut être induite par l'herbivorie des insectes ou par des blessures mécaniques. Une faible disponibilité des ressources et un risque peu élevé d'herbivorisme pourraient favoriser des réponses induites et diminuer les défenses des conifères. Nous avons étudié la réponse à la défoliation du Thuya géant (*Thuja plicata*) dans l'archipel de Haida Gwaii, qui n'était peuplé d'aucun mammifère herbivore avant l'introduction du cerf à queue noire à la fin du 19<sup>e</sup> siècle et où des travaux précédents ont montré que les défenses des thuyas étaient peu développées, ainsi que sur la côte nord-ouest du continent nord-américain (Colombie britannique, Canada). Nous avons émis l'hypothèse qu'une plus grande défense induite dans les populations insulaires compenserait pour la réduction des défenses constitutives. Nous avons utilisé des plants issus de pépinières âgés de un et deux ans afin de tester : *i*) si la défoliation provoque à court terme une réponse chimique induite chez les thuyas insulaires et *ii*) si les thuyas du continent, qui ont toujours été exposés aux grands mammifères herbivores, répondent différemment. La concentration en mono- et en diterpènes n'a pas varié significativement en réponse à la défoliation durant la période de cinq jours étudiée, quelle que soit l'intensité de la défoliation ou l'origine de la plante.

**Mots-clés :** défoliation, induction, terpènes, conifères, *Thuja plicata*.

**Nomenclature:** Lambert, 1803; Merriam, 1898; Seton-Thompson, 1900.

## Introduction

Inducible defences have been well documented for several non-woody plants and deciduous trees (Tallamy & Raupp, 1991; Karban & Baldwin, 1997), but less so for coniferous species. Several studies have actually found no induced responses in conifers (Cates & Redak, 1988; Watt, Leather & Forest, 1991; Barnola, Hasegawa & Cedeno, 1994) although insect herbivory or mechanical wounding in conifers can induce monoterpene biosynthesis (Litvak & Monson, 1998; Steele *et al.*, 1998). However, because coniferous species usually store more of their carbon and nutrient reserves in leaves than deciduous trees do (Chapin, 1980), the carbon/nutrient balance hypothesis predicts that severe defoliation would reduce the carbon

available for defence chemicals and thus increase the suitability of conifers to herbivores (Tuomi, Fagerström & Niemelä, 1991; Raffa & Smalley, 1995).

The hypothesized value of inducible defences is that a plant incurs the cost only when there is an associated benefit (Tuomi, Fagerström & Niemelä, 1991). Thus, plants that are rarely attacked should rely predominantly or exclusively on induced defences (Zangerl & Bazzaz, 1992), especially if resources available for producing defences are low (Simms, 1992; Agrawal & Karban, 1999). However, plants that have evolved in the presence of herbivores should rely more on constitutive defences to prevent the cost of a delayed response. Beneficial induced defences have been documented in some studies (Karbon & Baldwin, 1997; Baldwin, 1999), but not in others (Fowler & Lawton, 1985; Myers & Bazely, 1991). In practice, however, the existence of costs associated with

<sup>1</sup>Rec. 2001-11-01; acc. 2002-11-29.

<sup>2</sup>Author for correspondence. Present address: Unité d'Epidémiologie Animale, INRA, 63122 Saint-Genès-Champagnelle, France.

plant defences is difficult to demonstrate. Studies have found some association (Mauricio, 1998; Elle, van Dam & Hare, 1999), while other have not detected any (Simms & Rausher, 1989; Gianoli & Niemeyer, 1997). Several reasons have been considered (Bergelson & Purrington, 1996), such as other functions within the plant for the chemicals associated with plant defence (Langenheim, 1994) or a selective advantage for individuals producing cheaper defences (Simms, 1992).

The islands of Haida Gwaii (the Queen Charlotte Islands), a 300-km-long archipelago off the coast of British Columbia, provide a rather unique situation to test some of these hypotheses. These islands are covered by a temperate rain forest that is similar to the coastal mainland forest (Pojar & MacKinnon, 1994). Historically, only one large terrestrial herbivore was native to Haida Gwaii, the extinct Dawson Caribou (*Rangifer tarandus dawsonii*), and it was restricted to parts of the northern island (Cowan, 1989). The forests of Haida Gwaii have thus evolved since the end of the last glaciation (10,000 years BP) without the community of mammalian herbivores typical of the forests on the adjacent mainland (Cowan, 1989). This situation changed at the end of the 19<sup>th</sup> century when Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) were introduced to the archipelago (Carl & Guiguet, 1972). This study samples one of the dominant conifers in these coastal forests, western redcedar (*Thuja plicata*, Cupressaceae) (Pojar & MacKinnon, 1994), to compare the sensitivity to browsing and the amount of chemical defences of island and mainland trees. Haida Gwaii and mainland habitats are similar ecosystems, and although there may be small differences in the nutrients available to trees in these two ecosystems, the differences are not quantifiable. Western redcedar suffers very little damage by insect or pathogens compared to other conifers in British Columbia (Furniss & Carolin, 1977), but it is a preferred food of black-tailed deer (Pojar *et al.*, 1980; Cowan, 1989). Cervids, especially in ecosystems dominated by woody vegetation, avoid feeding on plant tissues with higher concentrations of secondary chemicals even when their nutrient content is high (McArthur *et al.*, 1993; Hanley, 1997). Previous studies have shown that monoterpene concentrations are good markers of western redcedar suitability for deer browsing. Western redcedar monoterpenes are deterrents to Sitka black-tailed deer (Vourc'h *et al.*, 2002a), and deer preferentially eat western redcedar with lower monoterpene concentrations but not necessarily lower diterpenes, or with higher nutritive content (Vourc'h *et al.*, 2001; Vourc'h *et al.*, 2002b). Furthermore, studies on variation in redcedar palatability to deer and terpene composition suggested (1) that western redcedar from Haida Gwaii never exposed to deer were less defended than trees from the mainland (Vourc'h *et al.*, 2001) and (2) that young redcedar from Haida Gwaii that grew while exposed to deer had defence levels comparable to mainland trees (Vourc'h *et al.*, 2001). The latter result may have been caused by the elimination of the least-defended young trees from the island population where deer were present, as suggested by Vourc'h *et al.* (2002b), or by higher induced defences in the island populations that would

compensate for the reduction in constitutive defences. The objective of this study was to explore the validity of the latter hypothesis by looking for induced responses in young island and mainland western redcedars after experimental defoliation. More specifically, our objective was to determine whether (1) moderate to severe defoliation triggered short-term changes in the concentration of monoterpenes, (2) such a response to defoliation was stronger in plants from Haida Gwaii than in plants from the mainland, and (3) the response was age dependant, as Vourc'h *et al.* (2001) showed for constitutive defences.

## Methods

### DURATION OF EXPERIMENTS AND DEFOLIATION PROTOCOL

A preliminary study involving the defoliation of 20 one-year-old mainland western redcedar seedlings (Vourc'h, unpubl. data) resulted in (1) an increase in monoterpene concentration three days after 50% of the leaves had been removed, suggesting the potential for induced responses to browsing in western redcedar, and (2) a decrease in terpene concentration after a 75% defoliation, suggesting a reduction in the production of secondary compounds as a consequence of reduced foliage nutrient content caused by severe defoliation. Based on these observations, a study was designed to monitor the variation in monoterpene concentration over a five-day period after increasing intensities of experimental defoliation.

### EXPERIMENT 1: ONE-YEAR-OLD SEEDLINGS

One-year-old seedlings were grown in nurseries on southern Vancouver Island from seeds that had been collected from trees older than 100 years in two populations, one on Haida Gwaii (247 m elevation and 52° 43' N, 131° 40' W) and one on the mainland (northern British Columbia at 200 m elevation and 54° 50' N, 128° 45' W). In the case of Haida Gwaii, this ensured that the seed was from trees that had grown before deer were introduced. On February 17, 2001, the seedlings were transplanted into 11-litre pots. At this time, the Haida Gwaii seedlings had a root plug diameter of 4.10 cm and the mainland seedlings, 4.15 cm. Seedlings were fertilized with a balanced soluble fertilizer (N, P, K in equal amounts) with micronutrients until May 2. Initial defoliation treatments were applied on May 17, when the seedlings averaged a height of ca 40 cm. Nursery growth of western redcedar was just entering the stage of rapid continuous growth, typical of late spring field growth. This stage in the field corresponds with high deer herbivory pressure, since growth of understory forage is just beginning. Monitoring of the chemical response to defoliation requires the removal of a small sample of leaf tissue each day from the same individual. This additional sampling may influence the chemical response of the plant to the initial defoliation. To minimize this bias, the experiment was designed so that a different individual was sampled each day for each treatment and origin. The experiment was based on an 8-block split-plot design with 192 Haida Gwaii and 192 mainland seedlings. Treatments (25%, 50%, and 75% initial defoliation) and control (0% initial defoliation) were randomized within block, sampling days

(0, 1, 2, 3, 4, and 5) within treatment, and seedling origin (Haida Gwaii or mainland) within sampling day. Thus, eight seedlings were sampled per origin per treatment at each date. The proper amount of leaf material to be initially cut by scissors to mimic herbivory was estimated from the total number of leaves per seedling at day 0.

#### EXPERIMENT 2: TWO-YEAR-OLD SEEDLINGS

Two-year-old western redcedar seedlings were grown in a common greenhouse from seeds originating from two Haida Gwaii populations (45 trees) and three mainland populations (45 trees) (Table I). The seedlings were fertilized as in experiment one during the first growing season, but no fertilizer was applied during the second year. The seedlings averaged 1 to 1.50 m when the experiment started on May 17. Two levels of initial defoliation were applied: 25% and 50%. Repeated sampling for chemical analysis occurred daily on each individual since the trees were larger than in the first experiment. However, to verify that this sampling scheme did not affect the production of secondary compounds, two control treatments (0% defoliation) were initiated: 1) 0%-same tree control, which consisted of trees that were sampled for chemical analysis each of the 6 days, and 2) 0%-different tree control, which consisted of trees that were sampled only once, on one of the 6 days; thus, different trees were sampled each day. The experiment was designed as a 5-block split-plot where treatments (two controls and two initial defoliation intensities) were randomized within block, and seedling origin (Haida Gwaii or mainland) within treatment. On each date, the five same seedlings were sampled per origin for 0%-same tree control, and five different seedlings were sampled per origin for 0%-different tree control (*i.e.*, in total, 30 seedlings per origin were sampled for 0%-different tree control throughout the experiment).

#### TERPENE ANALYSIS

In both experiments, 0.5 g of fresh leaf material was sampled from each plant, and leaf monoterpenes were extracted by soaking the whole leaf material in 5 mL of methanol for 48 h. Approximately 1 g of additional leaf material was also collected from each plant and dried at 55°C for 24 h to calculate dry matter content.

After filtration, the methanol extracts were stored at -20°C until analyzed for terpene content by gas chromatography. Extracts were analyzed on a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector, and monoterpene peaks were identified using the same method as described in Vourc'h *et al.* (2001).

TABLE I. Western redcedar populations used in the experiment with two-year-old seedlings.

Origin	Number of seedlings	Location	Elevation	Latitude	Longitude
Haida Gwaii	24	Sewell Inlet	10 m	52° 63'	131° 59'
	21	Kumois	20 m	53° 45'	132° 06'
Mainland	15	Chute Lake	100 m	53° 20'	129° 03'
	15	Data River	50 m	53° 52'	128° 31'
	15	Big Falls Creek	150 m	53° 58'	129° 35'

#### STATISTICAL ANALYSIS

All data were analyzed by generalized linear models. For the first experiment, a multivariate analysis of variance (MANOVA) was run using a split-plot model, with total mono- and diterpenes as response variables (SAS, 1999). Block and treatment effects (0, 25, 50, and 75% initial defoliation) were tested for significance with block\*treatment as the error term, the effects of origin (Haida Gwaii and mainland) and origin\*treatment with origin\*treatment\*block as the error term, and finally the effects of time, time\*treatment, time\*origin, and time\*treatment\*origin with the residual as the error term. Significant effects for each response variable were identified by univariate ANOVA corrected for multiple tests by the improved Bonferroni method proposed by Simes (1986).

In the second experiment, the two sets of control individuals (0%-same tree control and 0%-different tree control) were compared at each day for each response variable using least-squares means (LSMEANS, SAS, 1999). The repetitively sampled plants (0%-same tree control, 25%, and 50% initial defoliation) were analyzed for each response variable by repeated-measure time analyses with block, treatment, origin, block\*treatment, block\*origin, and treatment\*origin as explanatory variables (SAS, 1999). Corrections for multiple tests were applied as in the first experiment.

## Results

#### EXPERIMENT 1 : ONE-YEAR-OLD SEEDLINGS

Mono- and diterpene concentrations showed a significant effect of time, origin, and time\*origin interaction, but no treatment effect with MANOVA (Table II). In addition, the univariate ANOVA on monoterpene concentration showed significant effects of time ( $F_{5,279} = 15.96$ ,  $P_c < 0.001$ ), origin ( $F_{1,28} = 8.42$ ,  $P_c = 0.014$ ), and time\*origin ( $F_{5,279} = 3.27$ ,  $P_c = 0.007$ ) (Figure 1). Differences between origins were significant at day 0, 2, and 3, where island seedlings had less monoterpene than mainland ones ( $P < 0.05$ ) (Figure 1a). This difference disappeared at the end of the experiment because monoterpene of island trees increased steadily.

TABLE II. Results of the multivariate analysis of variance on the total concentration of mono- and diterpene in one-year-old western redcedars during five days after an initial defoliation of 0%, 25%, 50%, or 75%. Trt = treatment, Num DF = numerator degree of freedom, Den DF = denominator degree of freedom, e = error term used for the above effects. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

Effect	Pillai's Trace	F	Num DF	Den DF	P
Block	0.57	1.19	14	42	0.321
Trt	0.18	0.70	6	42	0.648
e=trt*block					
Origin	0.41	9.35	2	27	< 0.001***
Origin*trt	0.13	0.66	6	56	0.680
e=ori*trt*block					
Time	0.31	10.41	10	558	< 0.001***
Time*trt	0.12	1.24	30	558	0.177
Time*origin	0.19	2.74	10	558	0.003**
Time*trt*origin	0.07	0.72	30	558	0.861
e=residual					

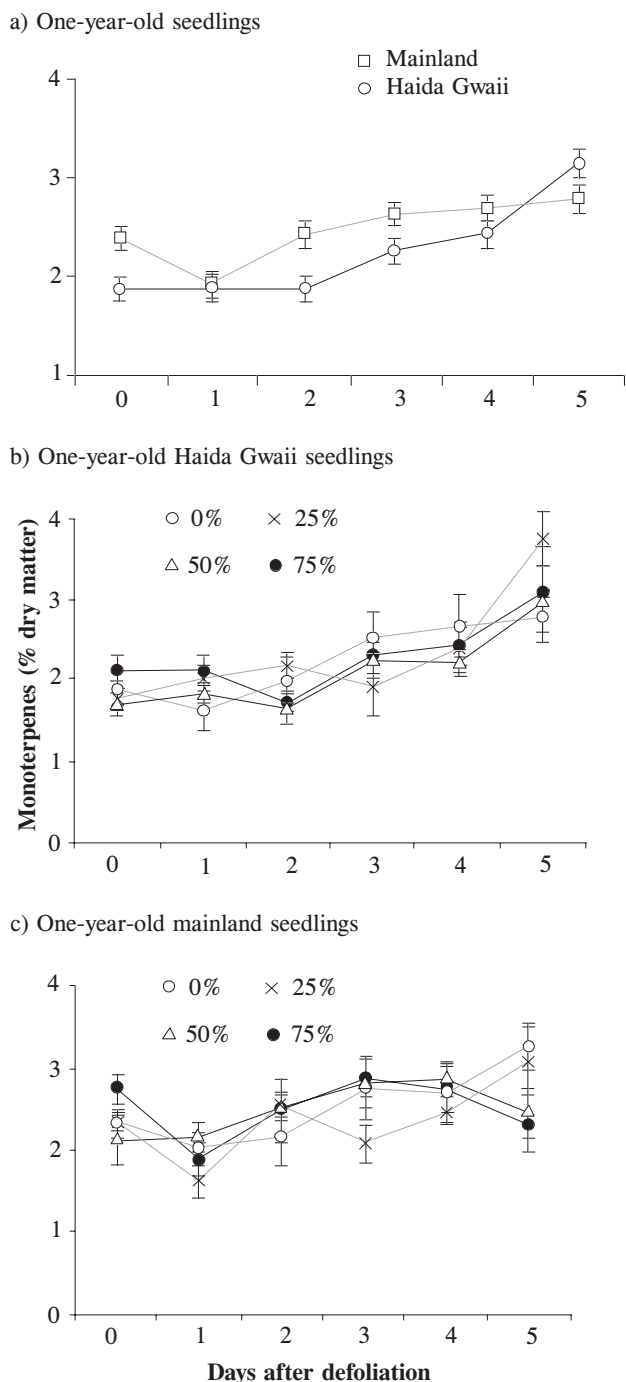


FIGURE 1. Variation in total monoterpene concentrations of one-year-old western redcedar seedlings from a) Haida Gwaii and the mainland pooled over all initial defoliation, b) Haida Gwaii according to the initial defoliation, and c) the mainland according to the initial defoliation. Vertical bars are standard errors. \*  $P < 0.05$ .

This increase occurred for every treatment (including 0% defoliation), but seemed especially marked for defoliated Haida Gwaii trees at day 5 (Figure 1b). For diterpene concentration, there was a small overall significant increase with time ( $F_{5,279}=9.54$ ,  $P_c < 0.001$ ) and a significant effect of time\*origin ( $F_{5,279}=5.38$ ,  $P_c < 0.001$ ), but with no significant differences observed at any dates (Figure 2).

EXPERIMENT 2: TWO-YEAR-OLD SEEDLINGS

For total monoterpene concentrations, there were no differences between the two sets of controls (0%-same tree control, *i.e.*, trees that were repetitively sampled, and 0%-different tree control, *i.e.*, different trees sampled at each date) at any sampling dates, except for day 1. At this day, monoterpene concentration in the 0%-different tree control was significantly higher ( $P=0.015$ ). There were no significant effects of time, block, origin, treatment, or their interactions in the repeated-time analysis done on the plants that were sampled each of the five days (Table III, Figure 3). The 0%-different tree control, however, had lower total concentration of diterpenes than 0%-same tree control at day 0 ( $P=0.032$ ), but then had significantly more at days 1, 2, 3, and 4 (all  $P < 0.022$ ). The only significant effect in the repeated-time analysis with diterpenes was the time effect (Table III, Figure 4).

Discussion

DEFOLIATION AND INDUCED TERPENES?

In neither experiment did we detect a significant effect of defoliation on total mono- or diterpene concentrations, except for a temporary increase in monoterpene concentration between day 4 and 5 in defoliated one-year-old seedlings from Haida Gwaii. This result does not confirm the induced responses found in the preliminary experiment, which was done with a small number of

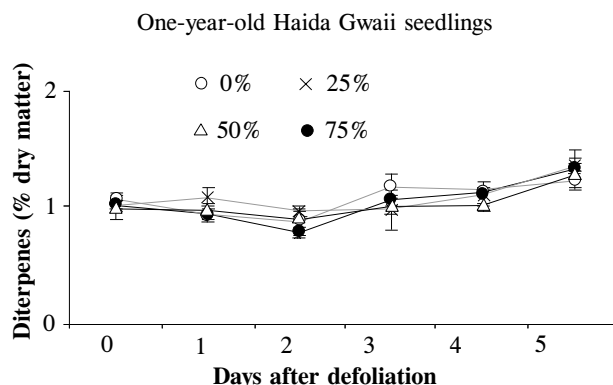
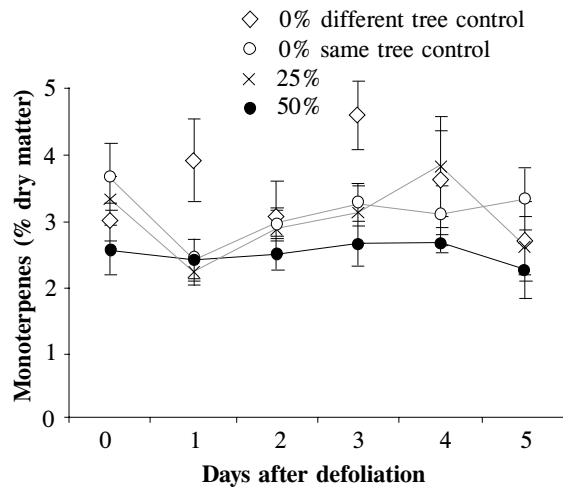


FIGURE 2. Variation in total diterpene concentrations of one-year-old western redcedar seedlings from Haida Gwaii according to the initial defoliation treatments. Vertical bars are standard errors.

TABLE III.  $P$ -values of the multivariate repeated-time analysis with the Pillai's trace statistic on the total concentration of monoterpene and diterpene in leaves of two-year-old western redcedars during five days after an initial defoliation of 0%, 25%, and 50%. Trt = treatment, ori = origin. \*  $P < 0.05$ .

Effect	Num DF	Den DF	$F$	
			Monoterpene	Diterpene
Time	5	4	1.97	9.52*
Time*block	20	28	0.92	0.79
Time*trt	10	10	1.03	1.39
Time*ori	5	4	1.03	0.95
Time*trt*block	40	40	0.96	1.06
Time*ori*block	20	28	1.08	0.83
Time*ori*trt	10	10	0.51	0.70

## a) Two-year-old Haida Gwaii seedlings



## b) Two-year-old mainland seedlings

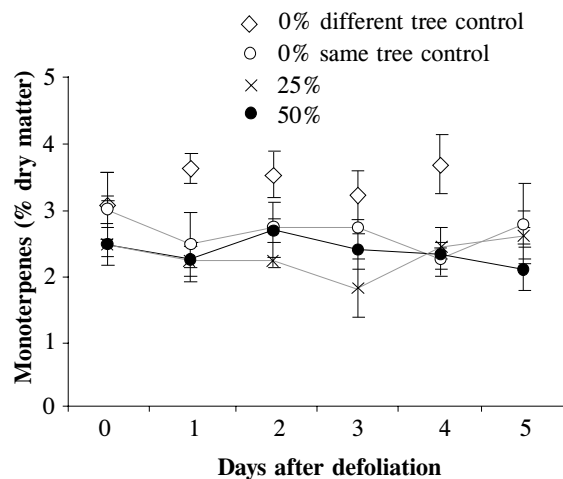


FIGURE 3. Variation in total monoterpene concentrations of two-year-old western redcedar seedlings from a) Haida Gwaii and b) the mainland according to the initial defoliation treatments. (0%- same tree control are trees that were 0% initially defoliated and repetitively sampled each of the 6 days. 0%-different tree control correspond to trees that were 0% initially defoliated but sampled only once at one of the 6 days.) Vertical bars are standard errors.

trees. It either reflects a lack of production of induced terpenes or an inability to increase terpene within 5 days.

Several other studies failed to find an induced increase in terpene concentration in conifers (Barnola, Hasegawa & Cedeno, 1994). Gershenson and Croteau (1990) suggested that, in mature needles, the lack of induction could result from developmental constraints on terpenoid synthesis. The depletion of starch reserves during defoliation (Ericsson *et al.*, 1985) may also decrease the potential for the synthesis of secondary metabolites in needles (Bryant, Tuomi & Niemelä, 1988). Furthermore, simulated herbivory may elicit a weaker induced response because some potential stimuli provided by the herbivore are missing (Haukioja, 1990; Litvak & Monson, 1998).

In contrast to the above, Litvak and Monson (1998) reported that real and/or simulated herbivory increased

## Two-year-old Haida Gwaii seedlings

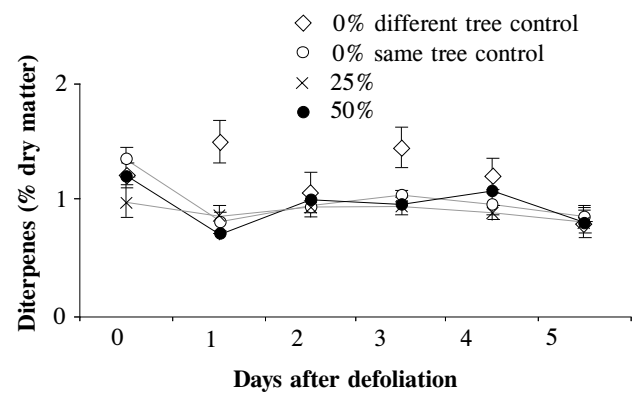


FIGURE 4. Variation in total diterpene concentrations of two-year-old seedlings from Haida Gwaii according to the initial defoliation treatments. (0%- same tree control are trees that were 0% initially defoliated and repetitively sampled each of the 6 days. 0%-different tree control correspond to trees that were 0% initially defoliated but sampled only once at one of the 6 days.) Vertical bars are standard errors.

the activity of the monoterpene cyclase in needles of several conifer species (*Pinus ponderosa*, *Pinus contorta*, and *Abies concolor*) within 4-8 days after defoliation. Actual monoterpene concentrations in the wounded needles, however, were still significantly lower than in controls because of volatilization losses. In such a context an absence of decrease in monoterpene concentrations in defoliated trees when compared to control trees could actually reflect an increase in terpene production.

## ISLAND-MAINLAND DIFFERENCES

At day 0 of the experiment, one-year-old seedlings from Haida Gwaii had significantly lower monoterpene concentrations than mainland plants, suggesting a reduction in constitutive defences for island trees. Diterpene concentrations showed little variation. These results are consistent with the findings of Vourc'h *et al.* (2001) and with other studies showing that island plants that evolved under low herbivory pressure tend to have less-capable defences (Janzen, 1973; Bowen & Van Vuren, 1997). On the other hand, this difference in constitutive terpene levels was not found for the samples of two-year-old seedlings, which could partly result from differences in the populations sampled and/or from the small sample size (20 individuals per origin).

The absence of short-term response to defoliation on Haida Gwaii and on the mainland is consistent with the interpretation of Vourc'h *et al.* (2001; 2002b) that the lack of chemical difference between island and mainland young redcedars exposed to deer results from the selective elimination by deer of the trees with low terpene levels from the island population rather than from a stronger induced response in the island trees that would compensate for the reduction in constitutive terpene levels.

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