Plant Physiol. (1993) 101: 1021–1028

Oleoresinosis in Grand Fir (*Abies grandis*) Saplings and Mature Trees[1]

Modulation of this Wound Response by Light and Water Stresses

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The stem content of diterpene resin acids (rosin) increases dramatically following wounding of grand fir (*Abies grandis*) saplings, but the level of monoterpene olefins (turpentine) in the stem decreases following injury, in spite of a significant increase in monoterpene cyclase (synthase) activity. However, this observation was explained when rapid evaporative losses of the volatile monoterpenes from the wound site was demonstrated by trapping experiments, a finding consistent with a role of turpentine as a solvent for the mobilization and deposition of rosin to seal the injury. Mature forest trees responded to stem wounding by the enhancement of monoterpene cyclization capacity in a manner similar to 2-year-old grand fir saplings raised in the greenhouse. Light and water stresses greatly reduced the constitutive level of monoterpene cyclase activity and abolished the wound-induced response. The diminution in monoterpene biosynthetic capacity was correlated with a dramatic decrease in cyclase protein as demonstrated by immunoblotting. Relief of stress conditions resulted in the restoration of cyclase activity (both constitutive and wound induced) to control levels. The results of these experiments indicate that grand fir saplings are a suitable model for studies of the regulation of defensive oleoresinosis in conifers.

Oleoresin (pitch) is a mixture of monoterpene olefins (turpentine) and diterpene resin acids (rosin) that accumulates at sites of injury on conifer stems (Croteau and Johnson, 1985; Johnson and Croteau, 1987). This material is important in the defense against pests and pathogens and in wound sealing (Berryman, 1972; Shrimpton, 1978; Cates and Alexander, 1982; Cheniclet, 1987; Gijzen et al., 1993). A method was developed for assessing the monoterpene biosynthetic capability of conifer stem tissue by measuring monoterpene synthase (cyclase) activity in cell-free extracts, and this method was utilized to establish the relationship among biosynthetic capacity, morphological differentiation of the resin-producing structures, and the level of turpentine present in the oleoresin of a range of conifer species (Lewinsohn et al., 1991b). This technique was also used to examine the influence of stem wounding on monoterpene production, and it was found that 2-year-old grand fir (*Abies grandis*) saplings responded to stem injury by the localized increase in monoterpene cyclase activity (Gijzen et al., 1991; Lewinsohn et al., 1991a), a response that is consistent with the previously observed accumulation of oleoresin at wound sites on mature forest trees (Berryman, 1969, 1972; Wong and Berryman, 1977; Filip et al., 1989). For these reasons, grand fir saplings were adopted as a model system for studying the regulation of wound-induced oleoresinosis (Lewinsohn et al., 1992b).

The wound response in grand fir saplings consists of both the apparent increase of constitutive monoterpene cyclase activities and the appearance of new cyclization activities catalyzing the formation of distinct monoterpene products (Gijzen et al., 1991) (for structures of the principal components of grand fir oleoresin, see Fig. 1). The major wound-inducible monoterpene cyclase was identified as a (−)-pinene synthase (Lewinsohn et al., 1992a) that produced both α- and β-isomers (Wagschal et al., 1991), and the purified enzyme (a 62-kD monomeric protein) was used to generate polyclonal antibodies with which it was shown that the wound-induced increase in monoterpene cyclization capacity was the result of de novo synthesis of cyclase proteins (Gijzen et al., 1992). In further defining the grand fir system as a model for wound-induced resinosis, we now report comparative aspects of oleoresin accumulation and monoterpene cyclase activity in saplings and mature trees, and we also describe the effects of light and water stress as modulators of this defense response.

MATERIALS AND METHODS

Plant Materials, Substrates, and Standards

Two-year-old grand fir (*Abies grandis*) saplings were grown under controlled light conditions (16-h day at 250 μE m^{-2} s^{-1}

[1] This investigation was supported in part by U.S. Department of Agriculture grant 91-37302-6311, Project 0268 from the Washington State University Agricultural Research Center, and by a McKnight Foundation grant.

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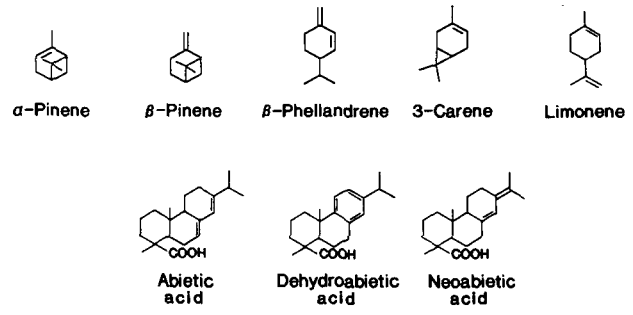

Figure 1. Principal monoterpene olefins and diterpene resin acids of grand fir oleoresin.

cool-white and incandescent light) and were watered daily (−0.7 MPa xylem water potential with 36% soil moisture). Saplings were wounded by razor cuts as described previously (Gijzen et al., 1991; Lewinsohn et al., 1991a, 1991b). The fungal pathogens *Ceratocystis clavigera* (American Type Culture Collection No. 24286) and *Trichosporium symbioticum* (isolated from bark beetle-infested grand fir, courtesy of Greg Filip, U.S. Forest Service, La Grande, OR) were grown at room temperature on malt extract agar. [1-^{3}H]Geranyl PPi (150 Ci/mol) was synthesized by established procedures (Croteau and Cane, 1985). Monoterpene standards were from our collection, hendecane was from Alltech (Deerfield, IL), and resin acid standards were from Helix Biotech (Vancouver, BC, Canada). All other biochemicals and reagents were from Sigma or Aldrich.

A stand of mature grand fir trees (approximate average age 80 years, diameter at breast height 35–50 cm, free of insect and pathogen damage) in the Umatilla National Forest near La Grande, OR (T1S, R36E, Sec. 22), was chosen to test the wound response in a natural setting. At 14 and 7 d before sampling, a 2-mm diameter nail was driven to a depth of about 3 cm into the tree trunk at breast height. The wounded trees, 15 individuals per time point, were selected at random and were distributed throughout the site, as were the 15 nonwounded controls sampled at the completion of the study on August 29, 1990. Tissue was obtained by first removing the outer phelloderm with a chisel, and a circular section surrounding the wound was taken with a 21-mm diameter cork borer. The sections were approximately 0.75 cm thick and contained phloem, cambium, and xylem tissues. Samples were immediately frozen in liquid N_2 and kept frozen until extraction and enzyme assay (see below).

Light and Water Stress Regimens

Two-year-old saplings were kept well watered in darkness (<0.05 μE m^{-2} s^{-1}) or were maintained in full light using the water regimen described below for 2 weeks before wounding and then were kept for an additional 2 weeks using the same regimen before the determination of monoterpene cyclase activity. For stress alleviation, the plants were returned to normal watering and light conditions for 2 weeks before wounding and then were kept for an additional 2 weeks under normal light and watering conditions until determination of enzyme activity.

To attain different levels of water stress, the normal watering interval of 1 to 2 d (36% soil moisture; −0.7 MPa xylem water potential) was extended to 4 d for moderate water stress (18% soil moisture; −2.1 MPa xylem water potential) and to 6 d for severe water stress (8% soil moisture; −3.0 MPa xylem water potential); any longer interval between watering resulted in tree death.

Oleoresin Analysis

A microscale method was developed for the simultaneous analysis of monoterpene olefins and diterpene resin acids and it will be described in detail elsewhere. In brief, two midstem sections (2.5 cm long, approximately 2 g fresh weight) of a 2-year-old sapling were extracted for 2 d by immersion in 1.5 mL of methyl-*tert*-butyl ether containing 100 μg of hendecane and 200 μg of nonadecanoic acid as internal standards. The extract was washed with 0.2 mL of 0.1 M $(NH_4)_2CO_3$ (pH 8.0), and the methyl-*tert*-butyl ether was separated into two fractions, one of which was treated with diazomethane to methylate the resin acids. The methylated sample was then evaporated to dryness to remove residual diazomethane, a procedure that also removed the volatile monoterpenes. This fraction was combined with the other original fraction (containing the monoterpenes) and treated with silicic acid and Na_2SO_4 to remove polar materials (e.g. free acids) and dry the sample before analysis of an aliquot by split injection GLC on a capillary column coated with RSL-150. The monoterpenes and diterpene resin acid methyl esters were quantitated by electronic integration of the flame ionization detector signals, relative to the internal standards, and the identities of the products were confirmed by GLC-MS.

Monoterpene Emissions

Following stem wounding, seven grand fir saplings were placed in a 23- × 31- × 60-cm plexiglass chamber through which was passed a constant flow of air at 150 mL min^{-1}. Both incoming and outgoing air were filtered through separate Sep-pak cartridges (Waters). Following trapping intervals of 1 to 2 h, the outlet Sep-pak cartridge was eluted with 2 mL of pentane (containing 10 μL mL^{-1} of hendecane as internal standard) to remove adsorbed volatile organic products. This pentane extract was treated with silicic acid to remove traces of moisture and polar organics, and the monoterpene olefins were then analyzed by capillary GLC (split injection on a 30-m column coated with a 1-μm film of RSL-150; initial column temperature 100°C [5 min] and then 10°C min^{-1} to 220°C; detector temperature 250°C; injector temperature, 200°C; inlet pressure at 10 p.s.i. of He). Quantitation was by electronic integration of output signal. Identical trapping experiments were carried out with nonwounded, control saplings.

Enzyme Assays and Other Measurements

Frozen woody core samples from mature trees or sapling stem sections (1–5 g) were pulverized and extracted (Lewinsohn et al., 1991b) with 5 mL per g fresh weight of tissue of

Table I. *Increases in monoterpene cyclase activity in trunks of mature grand fir trees following wounding*

Nails were hammered into the bark and wood of 80-year-old trees as described in "Materials and Methods." Tissues surrounding the wound sites were analyzed at the time specified. Means and SE of 15 samples, each from a different tree, are shown. Letters depict grouping levels by the Duncan test at $\alpha = 0.05$.

Treatment	Monoterpene Cyclase Activity	Fold Increase
	pmol h^{-1} μg^{-1} *of protein*	
Nonwounded	0.33 ± 0.13^{a}	
7 d after wounding	0.64 ± 0.20^{ab}	1.9
14 d after wounding	1.49 ± 0.48^{b}	4.5

a buffer consisting of 50 mM Hepes (pH 7.8), 5 mM sodium ascorbate, 5 mM $Na_2S_2O_5$, 5 mM DTT, 5 mM $MnCl_2$, 20 mM $MgCl_2$, and 10% (v/v) glycerol and containing 1% PVP (M_r approximately 40,000) and 1% polyvinylpolypyrrolidone (both w/v) to protect proteins against phenolic damage. Monoterpene cyclase activity in the extracts was determined by measuring the conversion of [^{3}H]geranyl PPi to olefinic products as before (Lewinsohn et al., 1991b). Boiled controls were included in each experiment, and protein was determined by a dye-binding method (Bradford, 1976).

Procedures for western immunoblotting with anti-(pinene cyclase) polyclonal antibodies have been described (Gijzen et al., 1992). Goat anti-rabbit immunoglobulin G-linked alkaline phosphatase was from Pierce. Prestained mol wt markers were from Diversified Biotech. Soil water content was determined gravimetrically, and xylem water potential was measured with a Scholander pressure chamber (PMS Instruments, Corvallis, OR).

RESULTS AND DISCUSSION

Oleoresin Biosynthesis in Mature Trees

Grand fir saplings have been developed as a convenient model system for the study of induced oleoresinosis (Lewinsohn et al., 1992b). Although oleoresin accumulation has been demonstrated in mature grand fir trees following trunk injury (Berryman, 1969; Wong and Berryman, 1977; Raffa and Berryman, 1982a), no enzyme level studies of oleoresin biosynthesis have been carried out with adult trees in the forest setting. When mature trees were injured by trunk spiking, increased levels of monoterpene cyclase activity were readily observed in cell-free extracts of tissue surrounding the wound site, as compared to nonwounded controls (Table I). Thus, mature trees in a natural setting respond to injury by increasing the capacity for turpentine biosynthesis in a similar way to saplings maintained under greenhouse conditions (Lewinsohn et al., 1992b), indicating that this model system for evaluating the defense response is relevant to the forest setting. Adult forest trees, however, are slower to respond than saplings (cf. Table I to findings by Lewinsohn et al., 1991a), and they display greater variation in this measure of the wound-healing process, which is likely due to genotypic, developmental, and environmental differences between individuals in the field.

Oleoresin Accumulation in Wounded Saplings

To further validate the model system, an analytical evaluation of oleoresin accumulation in wounded sapling stems was undertaken. Wounding caused a significant increase in the level of stem resin acids 11 d after injury, reaching a maximum resin acid content at day 14 (Fig. 2A). Subsequently, there was a decline in extractable resin acids, which was probably the result of oxidative polymerization of these materials at the wound site (Croteau and Johnson, 1985; Johnson and Croteau, 1987). The resin acid fraction of grand fir oleoresin consists primarily of abietic acid and dehydroabietic acid (Fig. 1) (in approximately equal amounts and accounting for up to 97% of the resin acid fraction in some trees), with lesser amounts of other types including neoabietic acid and pimaric acid isomers. The composition of the resin acid fraction does not change significantly upon wounding.

Increases in resin acids at wound and fungal infection sites have been previously reported in pines (*Pinus* spp.) (Gref and Ericsson, 1985; Cheniclet, 1987; Croteau et al., 1987; Walter et al., 1989) but not in grand fir. Unlike true firs, pines possess an extensive, interconnected system of resin ducts throughout the stem (Penhallow, 1907; Savage and Croteau, 1991; Lewinsohn et al., 1992b), enabling mobilization of preformed resin from distal sites of production (Shrimpton, 1972). Because grand fir possesses only isolated resin blisters (Penhallow, 1907) and is unable to translocate resin from these scattered production sites, we conclude that the accumulation of diterpene resin acids that follows stem injury is the result of de novo synthesis. This conclusion is consistent with the finding that the localized capacity for de novo monoterpene biosynthesis increases dramatically at stem wound sites (Gijzen et al., 1991; Lewinsohn et al., 1991a, 1992b) and suggests a common regulatory mechanism for the production of monoterpenes and diterpenes in response to wounding.

The production of resin acids in stem tissue was accompanied by red-brown staining of the areas adjacent to wound sites, an observation previously noted in wounded mature

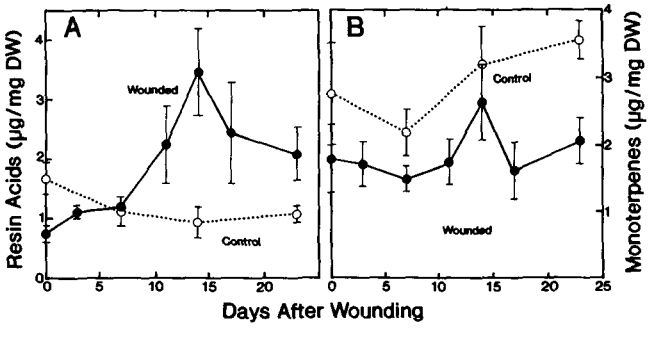

Figure 2. Wound-induced changes in diterpene resin acids (A) and monoterpene olefins (B) of the oleoresin of grand fir sapling stems. Control saplings were not wounded. Following extraction, the monoterpenes and resin acids (as methyl esters) were analyzed by GLC as described in "Materials and Methods." Means and SE of five replicates are given, each replicate consisting of 2 g fresh weight stem tissue from an individual sapling. DW, Dry weight.

grand fir trees (Berryman, 1972; Wong and Berryman, 1977). Formation of visible pigment was paralleled by an increase in A_{280} of ether extracts of the injured sapling stems, suggesting that phenolic materials also accumulate at wound sites in response to injury and account for the discoloration noted in these damaged tissues.

In contrast to the significant accumulation of rosin diterpenoids (Fig. 2A), and our consistent observation of wound induction of monoterpene cyclase activity (Gijzen et al., 1991; Lewinsohn et al., 1991a, 1992b), a concomitant increase in the monoterpene content of sapling stems upon wounding could not be demonstrated (Fig. 2B). The volatility of monoterpene olefins, coupled with the pronounced turpentine odor of the extruded pitch, provided a possible explanation for this apparent discrepancy. The loss by evaporation of the monoterpene (turpentine) fraction of the oleoresin upon wounding was verified by measuring the emission of monoterpenes from saplings placed in a sealed plexiglass chamber (via polymer adsorption and GLC analysis of the extract).

Nonwounded control saplings released very low levels of "constitutive" monoterpenes (0.5–3 ng h^{-1}) (Fig. 3A), whereas saplings that had been wounded 1 to 2 h before the trapping experiments released as much as 200 to 335 ng of monoterpenes h^{-1} (Fig. 3B). Such a dramatic, sustained increase in the rate of monoterpene emission from wound sites would more than account for the lack of turpentine accumulation in the injured tissues. Moreover, this phenomenon is entirely consistent with a proposed role of the monoterpenes (turpentine) as a solvent for the mobilization and ultimate deposition of resin acids at the open wound site to seal the injury (Hodges et al., 1979; Croteau and Johnson, 1985; Johnson and Croteau, 1987).The composition of the emitted monoterpenes in both wounded and control plants was similar to the corresponding turpentine extracted from the stem (Lewinsohn et al., 1991b). Although monoterpene emission from conifers (pines) has been documented previously (Tingey et al., 1991), this analysis of grand fir appears to represent the first comparison of the process in wounded and nonwounded stems. That monoterpene accumulation has been observed at wound sites of mature grand fir trees (Berryman, 1969; Wong and Berryman, 1977; Raffa and Berryman, 1982a) can likely be attributed to the fact that monoterpene biosynthesis is of a much larger scale in adults, such that turpentine production exceeds the rate of evaporation during the initial phases of wound healing. In addition, the relative damage in spiking an adult tree is much less severe than multiple razor wounds on the stem of a sapling as used in this work.

Influence of Stresses on Oleoresinosis

The accumulation of monoterpenes at fungal infection sites has been examined in adult trees of several conifer species (Shrimpton, 1978; Cheniclet, 1987), including grand fir (Berryman, 1969, 1972; Wong and Berryman, 1977). The alteration in monoterpene cyclase activity following fungal inoculation of grand fir sapling stems was therefore assessed. The fungal pathogen *T. symbioticum* is carried by the fir engraver beetle (*Scolytus ventralis*) that attacks and often kills grand fir trees (Berryman, 1969, 1972; Filip et al., 1989). Similarly, *C. clavigera*, a so-called blue stain fungus, is a symbiont of the highly destructive mountain pine beetle (*Dendroctonus ponderosa*) (Cates and Alexander, 1982). Inoculation of grand fir saplings with live mycelia (500 μg in 2 μL of water injected into a small wound produced by a 22-gauge sterile needle prick approximately 3 mm into the stem) of either *T. symbioticum* or *C. clavigera* resulted in a 4.5- to 5-fold increase in the level of monoterpene cyclase activity per g of tissue compared to controls at 7 d following treatment (data not

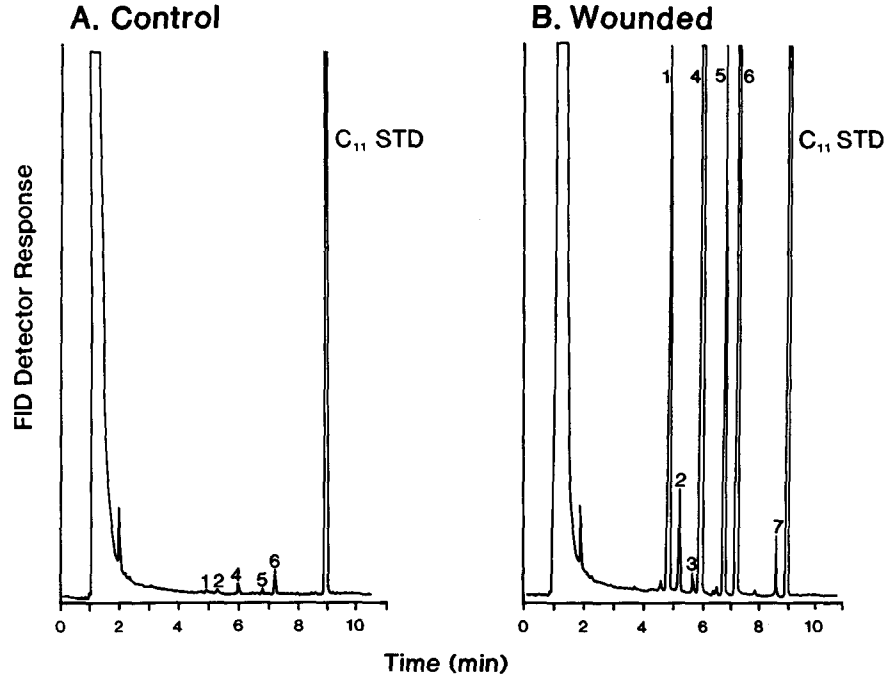

Figure 3. Monoterpene emission from control (nonwounded) (A) and wounded (B) grand fir saplings. Volatile monoterpenes were trapped on adsorbent polymer, extracted, and analyzed by GLC as described in "Materials and Methods." The components indicated are: 1, α-pinene; 2, camphene; 3, sabinene; 4, β-pinene; 5, 3-carene; 6, β-phellandrene; and 7, terpinolene. C_{11}STD indicates the hendecane internal standard. FID, Flame-ionization detector.

shown). The small wound alone produced a 2.5-fold increase in cyclase activity at day 7, whereas a heavier localized wound (produced by 20 needle pricks) or a localized burn (of 10 mm in diameter) caused a 5-fold increase in cyclization capacity over the control stems under the conditions of the experiments.

It has been established previously that the extent of induction of monoterpene cyclase activity in grand fir stems is proportional to the intensity of the injury applied (Lewinsohn et al., 1991a, 1992b). Similarly, the resin acid levels in *T. symbioticum*-inoculated wounds were 6- to 10-fold higher on a dry weight of tissue basis than were those levels found in similar noninoculated wounds 16 d after wounding (not shown). The enhanced defense response observed in the presence of fungus may be due to either increased wounding by fungal intrusion into the cambial tissue or a specific fungal elicitor(s) that is recognized by grand fir, as has been postulated (Lieutier and Berryman, 1988). Treatment of a razor wound with 2 μg of crab chitosan, a well-known elicitor (Hadwiger and Beckman, 1980; Bioshell Inc., Albany, OR, 80–90% deacetylated) administered in 3 μL of 175 mM potassium acetate (pH 5.8), was without effect on cyclase activity as compared to similarly wounded controls.

Whether fungal elicitors are effective in inducing turpentine production in pines is controversial (Croteau et al., 1987; Lieutier and Berryman, 1988), in part because *Pinus* species contain an interconnected system of resin ducts through which oleoresin can be translocated (Shrimpton, 1978), and it is difficult to determine whether the resin accumulated at wounds is simply transported from distal sites or is biosynthesized de novo on location (Gijzen et al., 1991; Lewinsohn et al., 1991a, 1992b). In true firs that contain only disperse, multicellular resin blisters in the trunk (Penhallow, 1907), such long-range translocation of resin is not possible. In this case, analytical measurements and determinations of cyclization activity are better indicators of induced resin production.

To assess the influence of other stresses on constitutive and wound-induced oleoresinosis in grand fir, saplings were exposed to periods of limiting light and water stress. When saplings were wounded and placed in near darkness (<0.05 μE m^{-2} s^{-1}) for 2 weeks along with the appropriate controls, it was shown that constitutive oleoresinosis was severely compromised and that the wound response was abolished (Fig. 4). When saplings were first placed in near darkness for 2 weeks and then returned to normal light (250 μE m^{-2} s^{-1}), wounded, and maintained in the light, both constitutive cyclase activity levels and wound-induced activity levels were similar to those of the corresponding full-light controls at the 2-week test interval (Fig. 4).

Previous studies have shown that light is necessary for the biosynthesis of monoterpenes and resin acids in pine needles (Gleizes et al., 1980; Gref and Tenow, 1987), and several lines of evidence (Wright et al., 1979; Lorio, 1988) indicate that oleoresinosis in pine and fir stems is restricted by substrate and/or energy availability, a limitation expected under low-light conditions. The present results, however, implicate a more direct effect of light stress on biosynthetic capacity at the enzyme level.

The influence of water stress was next evaluated by withholding water until the xylem water potential had reached moderate (−2.1 MPa; 18% soil moisture) and severe (−3.0 MPa; 8% soil moisture) levels. Water potentials of −2 MPa or less are commonly experienced by trees under dry summer conditions (stated in Puritch and Mullick, 1975). Under moderate stress, constitutive (nonwounded control) levels of monoterpene cyclase activity were unaltered; however, the ability to respond to wounding (at the level of geranyl PPi cyclization capacity) was reduced by approximately half compared to wounded but unstressed (−0.7 MPa; 36% soil moisture) controls (Fig. 5). Severe water stress resulted in significant reduction in both constitutive and wound-induced cyclase activity relative to the corresponding controls, and alleviation of this stress condition by a 2-week period of normal watering (to −0.7 MPa) restored both cyclization activities to their former levels (Fig. 5). Western blotting of protein extracts from stressed and control tissues revealed that lowered levels of cyclase activity were the result of the reduction in levels of cyclase protein (Fig. 6), suggesting an intricate mechanism for regulation of wound-inducible oleoresinosis that can be moderated by other physiological stress conditions.

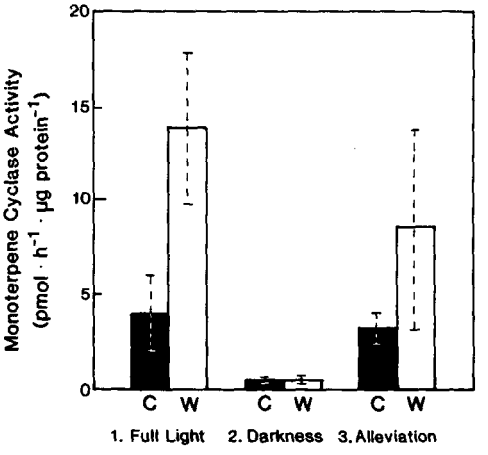

Figure 4. Effect of light deprivation on the constitutive and wound-inducible monoterpene cyclase activity in grand fir saplings. Trees were kept at full light (1) or in virtual darkness (2) for 2 weeks, wounded, and kept under the same light conditions for 2 more weeks until the monoterpene cyclase activity was determined. For the alleviation experiments (3), the saplings were kept in darkness for 2 weeks, then returned to full light conditions for 2 weeks, wounded, and kept for 2 subsequent weeks under normal light conditions, when monoterpene cyclase activity was determined. C, Nonwounded controls; W, wounded saplings. Means and SE of three replicates are given, each replicate consisting of 2 g fresh weight stem tissue originating from three different saplings.

Water stress greatly inhibits net photosynthesis in grand fir (Puritch, 1973) and also retards the formation of nonsuberized impervious tissues in mechanically injured grand fir stem (Puritch and Mullick, 1975), an important step in wound healing. Additionally, a normal rate of nonsuberized impervious tissue formation was restored upon alleviation of the water-stress conditions (Puritch and Mullick, 1975).

A considerable body of evidence indicates that environ-

mental stress (primarily water stress) renders pines, spruces, and firs more susceptible to bark beetle invasion by a general reduction in oleoresin flow (Vité, 1961; Lorio and Hodges, 1977; Ferrel, 1978; Shrimpton, 1978; Raffa and Berryman, 1982b; Larsson et al., 1983; Mulock and Christiansen, 1986), and, at least in some conifer species, the induced response to bark beetle attack is diminished under stress conditions (Christiansen et al., 1987; Paine and Stephen, 1987). A threshold of −2 MPa xylem water potential was found to cause susceptibility to fir engraver attacks in water-stressed white fir stands. This water-stress-induced susceptibility was accompanied by diminished monoterpene oleoresin content at attack sites (Ferrel, 1978).

The present results with grand fir saplings also show a threshold of about −2 MPa for the abolition of monoterpene cyclase induction, and they confirm earlier reports (Filip et al., 1989) that the potential for oleoresin production is dependent on the physiological condition of the tree. They also indicate that light and water stresses can compromise the ability to produce constitutive chemical defenses and to respond to wounding (and subsequent invasion by pests and pathogens) by direct reduction in the level of key oleoresin biosynthetic enzymes. There is precedent for the down-regulation of gene expression under water stress and limiting light conditions (Bartholomew et al., 1991), and this mechanism for the stress depression of oleoresinosis in grand fir is now under investigation.

Plants in their natural environment are continuously subjected to diverse stresses, and it is important for the survival of the species that a plant properly respond to each stimulus. It has been documented that responses to many environmental stresses are preferentially expressed in plants following a discrete hierarchy. Thus, a strong stimulus overrides a weak, presumably less important, one. For example, heat-shock responses override responses to elicitors and UV light in parsley cells (Walter, 1989) and block the defense response to *Fusarium solani* in normally resistant peas (Hadwiger and Wagoner, 1983) by preventing phytoalexin biosynthesis. Environmental stresses have profound effects on the levels of many defensive compounds in numerous plants, including conifers (Gershenzon, 1984). We document here that both light and water stresses diminish the ability of grand fir trees to produce oleoresin, thereby suppressing the efficacy of this line of defense.

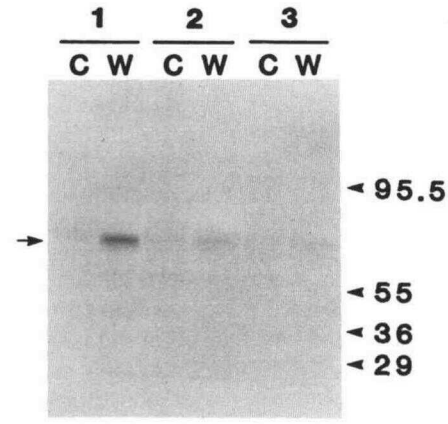

Figure 6. Diminution of wound-inducible cyclase proteins due to water-stress conditions as determined by immunoblotting. Cell-free extracts from wounded plants (W) and the respective nonwounded controls (C), which were subjected to several levels of water-stress treatments, were purified by anion exchange chromatography and immunoblotted as described in "Materials and Methods" (18 μg of protein per lane). Lane 1, Normal watering conditions; lane 2, moderately water-stressed plants; lane 3, severely water-stressed plants. The positions of migration of the prestained molecular mass markers are indicated at the right. The arrow on the left indicates the position of the 62.5-kD wound-inducible pinene cyclase from grand fir used as the immunogen.

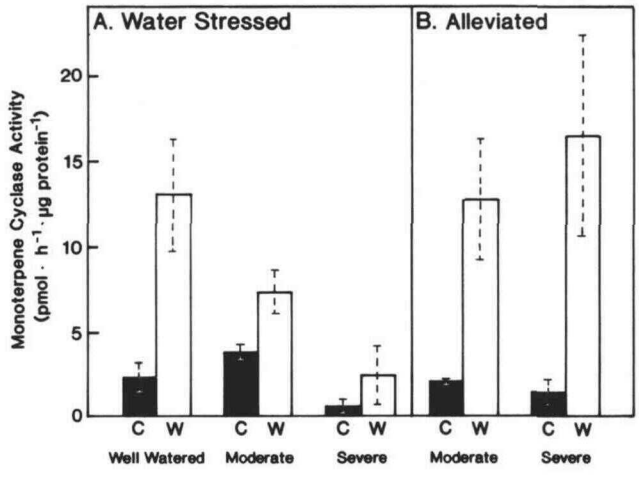

Figure 5. Suppression of wound-inducible monoterpene cyclase activity by water-stress (A) and reversal upon stress alleviation (B). A, Moderate and severe water stress conditions were attained as described in "Materials and Methods." Saplings were kept under water stress regimens for 2 weeks before and after wounding. Monoterpene cyclase activity was measured 2 weeks after wounding. B, Plants subjected for 2 weeks to the moderate and severe water-stress conditions described above were returned to normal watering conditions for 2 weeks. They were then wounded and kept under normal watering conditions for 2 additional weeks, when monoterpene cyclase activity was determined. Means and SE of three replicates are given, each replicate consisting of 2 g fresh weight stem tissue originating from three different saplings. C, Nonwounded controls; W, wounded saplings.

ACKNOWLEDGMENTS

We thank Greg Wichelns for raising the plants; Al Koepp for technical assistance; Lee Hadwiger of the Plant Pathology Department, Washington State University, for a sample of crab chitosan; Greg Filip of the U.S. Forest Service, La Grande, OR, for supplying *Trichosporium symbioticum*; John Marshall, Department of Forestry, University of Idaho, Moscow, Idaho, for the use of the Scholander pressure chamber; Kevin Hosman and John Owen for assistance with the field work; Christopher Steele for helpful discussion; and Joyce Tamura-Brown for typing the manuscript.

Received October 1, 1992; accepted November 30, 1992.

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