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Ethylene evolution, radial growth and carbohydrate concentrations in *Abies balsamea* shoots ringed with Ethrel

LEIF EKLUND[1] and C. H. ANTHONY LITTLE[2]

[1] *Department of Engineering and Natural Science, Växjö University, S-351 95 Växjö, Sweden*

[2] *Natural Resources Canada, Canadian Forest Service–Atlantic Forestry Centre, P.O. Box 4000, Fredericton, New Brunswick E3B 5P7, Canada*

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Summary The terminal (1-year-old) shoot of quiescent, 2-year-old *Abies balsamea* (L.) Mill. seedlings was either left untreated or ringed with 0, 1 or 10 mg Ethrel g^{-1} lanolin. After 5 weeks of culture under environmental conditions favorable for growth, the shoots were harvested to measure ethylene evolution and carbohydrate concentrations by gas chromatography, and tracheid number and bark radial width by microscopy. In untreated shoots, the basal rate of ethylene evolution followed the order: cambial region > cortex + periderm = xylem + pith = needles. Wound-induced ethylene production was not detected until at least 4 h after excision, but was evident in all fractions 24 h after excision; the increase in wound-induced ethylene evolution followed the order: cambial region > cortex + periderm > xylem + pith > needles. Compared with untreated controls, the application of plain lanolin, which involved the removal of needles and periderm, increased bark radial width and wound-induced ethylene production by the cambial region and the cortex + periderm, but decreased cambial region concentrations of fructose, glucose and starch at the application point. At the application point, Ethrel concomitantly increased ethylene evolution from the cambial region and the cortex + periderm, tracheid number, bark radial width, and the cambial region concentrations of fructose, glucose, sucrose and starch. No effects of Ethrel treatment were detected above or below the application point, with the exception that the 10 mg g^{-1} Ethrel treatment stimulated ethylene evolution and decreased starch concentration of the cambial region. The results indicate that: (1) the cambial region is the major source of endogenous ethylene in the 1-year-old shoot; (2) the magnitude of the difference in ethylene evolution between particular shoot fractions is different before and after the start of wound-induced ethylene production; (3) the Ethrel-induced increase in tracheid number and bark radial width at the application point is positively related to ethylene evolution from the cambial region and the cortex + periderm, respectively; and (4) ethylene derived from Ethrel applied laterally to a woody stem can mobilize carbohydrates to the application point.

Keywords: balsam fir, bark, cambium, 2-chloroethylphosphonic acid, cortex, fructose, glucose, starch, sucrose, tracheid, xylem.

Introduction

It is well established that shoots of woody species evolve ethylene and that the rate of evolution is increased by stresses such as bending, shaking and flooding (e.g., Brown and Leopold 1973, Robitaille and Leopold 1974, Blake et al. 1980, Yamamoto and Kozlowski 1987*a*, 1987*b*, Telewski 1990, Larson et al. 1993), and by applying the ethylene-generating compound 2-chloroethylphosphonic acid (Ethrel) (Robitaille and Leopold 1974, Yamanaka 1985, Telewski and Jaffe 1986, Yamamoto and Kozlowski 1987*a*, 1987*b*, Eklund and Little 1995, 1996). However, the relative extent to which different parts of the shoot evolve ethylene has rarely been investigated. Stems of *Abies balsamea* (L.) Mill. shoots evolved more ethylene than needles (Eklund and Little 1995). Savidge (1988) reported that cambial tissue dissected from *Abies balsamea* stems evolved ethylene, and Eklund (1991) demonstrated by microdialysis that ethylene evolution occurred from the cambial region of nondissected *Picea abies* (L.) Karst. stems. Drill holes were used to show that the outer sapwood of *Picea abies*, *Quercus robur* L. and *Acer platanoides* L. stems evolved ethylene (Eklund 1990, Eklund et al. 1992, Eklund 1993*b*) and that ethylene evolution was greater from sapwood than from heartwood in *Pinus sylvestris* L. stems (Ingemarsson et al. 1991). In dissection experiments, ethylene evolution from wounded or Ethrel-treated *Chamaecyparis obtusa* (Sieb. and Zucc.) stems was greater from inner phloem + cambial tissue than from outer phloem or xylem, and it decreased in successive fractions from outer sapwood to heartwood (Yamamaka 1985). Similarly, in *Pinus radiata* (D.) Don stems, ethylene evolution was highest from the outer sapwood + cambial tissue and lowest from the heartwood, but it was greater from the transition zone separating sapwood and heartwood than from middle or inner sapwood (Shain and Hillis 1973). Separating the shoot into fractions, however, inevitably results in wounding, which is known to stimulate ethylene evolution over the basal rate. The timing and extent of wound-induced ethylene production vary widely with species, organ, tissue and severity of wounding (Yamanaka 1985, 1986, Telewski and Jaffe 1986, Abeles et al. 1992, Ingemarsson 1994). In contrast, it is not known if the basal rate of ethylene evolution differs among tissue fractions excised from woody shoots.

Shoot radial growth depends on the import of carbohydrates for new cell synthesis and respiratory energy (Kozlowski 1992). In conifer stems, sucrose is the major carbohydrate translocated in the phloem and starch is the most important form of stored carbohydrate (Gholz and Cropper 1991, Fischer and Höll 1992, Kozlowski 1992, Egger et al. 1996). The mechanisms controlling the unloading of the phloem (Sung et al. 1993, 1996), and the strength of growth and storage sinks (Farrar 1993) in the conifer stem are unknown, but endogenous hormones probably play an important role (Kuiper 1993, Little and Pharis 1995, Morris 1996). The involvement of ethylene is indicated by the finding that ringing shoots of various conifers and woody angiosperms with Ethrel increased xylem production and bark radial width at the application point (Brown and Leopold 1973, Robitaille and Leopold 1974, Barker 1979, Telewski and Jaffe 1986, Yamamoto and Kozlowski 1987*a*, 1987*b*, Eklund and Little 1996). Moreover, exogenous ethylene enhanced the transport of ^{14}C-labeled metabolites from leaves to fruit in *Prunus persica* (L.) Batsch (Chalmers et al. 1976) and from scutella to coleoptile in *Oryza sativa* L. (Ishizawa and Esashi 1985, 1988). Accordingly, we hypothesized that ethylene derived from Ethrel applied laterally to a woody stem locally enhances radial growth by promoting the mobilization of carbohydrates to the application point.

The objectives of this study were to determine (1) if the relative capacity of different parts of 1-year-old *Abies balsamea* shoots to evolve ethylene was the same before and after the production of wound-induced ethylene; and (2) if the stimulation of ethylene evolution and radial growth induced by ringing *Abies balsamea* shoots with Ethrel (Eklund and Little 1995, 1996) was accompanied by changes in the concentrations of soluble sugars and starch in the cambial region.

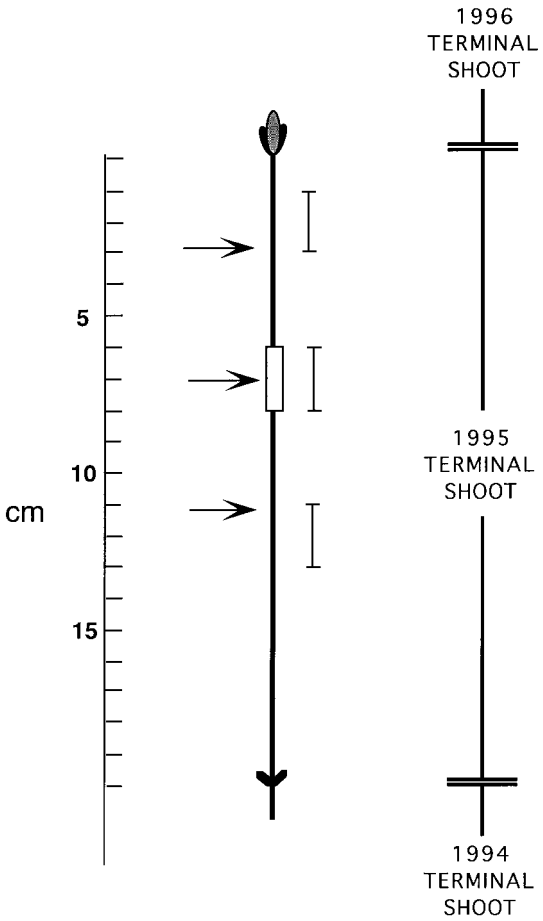

Figure 1. Site of Ethrel application (rectangle) and of segments above and below used to measure ethylene evolution and carbohydrate concentrations (bars) along the length of the 1995 terminal shoot. Leaves are not depicted. Arrows indicate where radial growth was measured.

Materials and methods

Plant material, Ethrel application and shoot sampling

Dormant, 2-year-old balsam fir (*Abies balsamea*) seedlings were lifted in November 1995, from a nursery in Fredericton, New Brunswick, Canada, and stored at –5 °C. In early January 1996, they were thawed, potted and cultured in a greenhouse under favorable growth conditions, as described previously (Eklund and Little 1996). One day after placement in the greenhouse, groups of eight to 10 seedlings were selected such that the average length of the 1995 terminal shoot was the same for each group. One group of seedlings was left untreated. A scalpel was used to remove all axillary buds from the 1995 terminal shoot of the seedlings in the remaining groups, then the needles and periderm were excised from a 2-cm length of stem located 6 to 8 cm below the base of the apical whorl of buds (Figure 1). About 0.8 g of 0, 1 or 10 mg Ethrel g^{-1} lanolin was applied around the circumference of this 2-cm segment, and the application site was covered with aluminum foil. The 1995 terminal shoot was harvested 5 weeks after application of the treatment, when the current-year (1996) terminal shoot had elongated about 4 cm and a band of earlywood tracheids had been produced in the 1995 terminal shoot.

Ethylene evolution and carbohydrate concentrations were measured in fractions excised from 2-cm-long segments located along the 1995 terminal shoot, corresponding to above, at and below the Ethrel application point (Figure 1). Each segment was subdivided into the following fractions: (1) cambial region, (2) xylem + pith, (3) cortex + periderm and (4) needles, except for the segment obtained at the application point for the 0, 1 or 10 mg Ethrel treatment, in which case the fractions were: (1) cambial region, (2) xylem + pith and (3) cortex, because the needles and periderm had been removed. The cambial region was obtained by removing the needles (if present), peeling the bark, scraping the exposed surface of the xylem side with a scalpel, stripping the surface tissues on the bark side with fine-nosed forceps, and combining the scrapings and strippings for each segment. Thus, the cambial region sample contained differentiating xylem, the cambium, and current-year and previous-year phloem. The remainder of the bark peeling was denoted the cortex + periderm, whether or not the periderm was present, and the stem piece left after scraping the differentiating xylem was denoted the xylem + pith.

Measurement of ethylene evolution

Each fraction was placed in a 3.5-ml glass vial containing a 2 × 0.5 cm piece of Whatman No. 1 filter paper moistened with distilled water. Each vial was sealed with a rubber stopper that did not release ethylene, and the vials were incubated at room temperature for either one 30-min period starting immediately after excision or five periods of 30 min spaced over 24 h, namely, 0 to 0.5 h, 0.5 to 1 h, 2 to 2.5 h, 3.5 to 4 h or 23.5 to 24 h after excision. For those fractions measured five times, the vial was opened immediately before the start of each incubation period except for the first one, and ethylene was removed by flushing with air. At the end of every incubation period, a 1-ml air sample was drawn from the head space with a gas-tight syringe and injected into a Varian 3400 gas chromatograph (Varian Canada, Mississauga, Ontario, Canada). The chromatographic conditions were as described previously (Eklund and Little 1996). Ethylene concentration was calculated on a dry weight basis by reference to a 1 ppm ethylene standard (Canadian Liquid Air, Fredericton, Canada), measuring and subtracting the volume of the fraction from the vial volume, and determining the fraction's dry weight after 24 h at 70 °C.

Measurement of carbohydrates

The concentrations of fructose, glucose, sucrose and starch in the cambial region were measured in pooled samples obtained from two seedlings per segment location and treatment. After homogenizing in liquid nitrogen, the sample was weighed (about 200 mg) and extracted at room temperature in 3 ml of 12:5:3 (v/v) methanol:chloroform:water (MCW; Dickson 1979). After centrifugation at 1200 *g* for 3 min, the supernatant was retained and the pellet was extracted twice more in 2 ml of MCW and recentrifuged. The combined supernatants were used to measure fructose, glucose and sucrose. Starch was measured in the pellet.

To measure the sugars, 1 ml of water and 1.4 ml of chloroform were added to the combined supernatants to partition pigments and lipids into the chloroform phase (Dickson 1979). A 500-µl subsample of the water phase, containing 250 µg of phenyl β-D-glucoside as internal standard, was evaporated to dryness in a Savant Speed Vac concentrator (Fisher Scientific, Ottawa, Canada). The oximes of fructose and glucose (Li et al. 1982, Biermann 1989) were prepared by dissolving the dried samples in 100 µl of pyridine containing 2.5 mg hydroxylamine hydrochloride and heating the mixture for 20 min at 70 °C with 1 min of mixing on a vortex mixer at intervals. The sugars were derivatized by adding 50 µl of *N*-trimethylsilylimidazole (TMSI) and heating for 20 min at 70 °C.

Starch was measured following a procedure based on Rose et al. (1991). After drying the pellet at 55 °C and taking its weight, a subsample (15 mg) was incubated for 1 h at 55 °C in 1.2 ml of 0.1 *N* sodium hydroxide to solubilize the starch. The pH was adjusted to 5.1 with 1.5 ml 0.1 *N* acetic acid, and the starch was hydrolyzed to glucose by adding 1 mg amyloglucosidase (Sigma A-3514) and incubating the mixture overnight at 55 °C. After centrifugation at 1200 *g* for 3 min, 250 µg of internal standard was added to a 500-µl subsample, and glucose oximes were prepared as described above. Samples were dried in a Savant Speed Vac concentrator, dissolved in 100 µl of pyridine, and then silylated with TMSI as described above.

Derivatized samples (1 µl) were injected into a Varian 3400 gas chromatograph equipped with a flame ionization detector and a 15 m × 0.53 mm i.d. fused silica capillary column with a 50 µm crossbonded 5% diphenyl-95% dimethyl polysiloxane stationary phase (Restek, Rtx-5). Column temperature was programed to increase from 100 to 280 °C at 20 °C min^{-1}, and the injector and detector had a temperature of 280 and 300 °C, respectively. The helium carrier gas flowed at 8 ml min^{-1} with a makeup of 22 ml min^{-1}, and the flow rates of hydrogen and air were 30 and 300 ml min^{-1}, respectively. Sugar concentrations were expressed on the basis of dry weight, and were calculated from the ratio of the peak areas of the internal standard and the endogenous sugar, relative to a standard curve based on the ratio of internal standard and varying amounts of authentic sugar. Both oximes of fructose and glucose were included in the peak area measurement. Starch concentration was calculated from the amount of glucose, based on a hydrolysis factor of 0.9 (Li et al. 1982, Omi and Rose 1991).

Fructose, glucose and sucrose were identified by cochromatography with authentic sugars and by mass spectra obtained with an HP 5890 gas chromatograph linked by a direct inlet to an HP 5770 mass selective detector controlled by a 9133 data system (Hewlett Packard, Kirkland, Ontario, Canada). Chromatography was done on a 25 m × 0.25 mm i.d. SE-30 column with a film thickness of 0.25 µm, under the chromatographic conditions described above.

Measurement of radial growth

Transverse, hand-cut sections were obtained at three locations, corresponding to above, at and below the Ethrel application point (Figure 1). After the sections were stained in a saturated aqueous solution of phloroglucinol in 20% hydrochloric acid and mounted in glycerol, the radial width of bark and the number of tracheids per radial file produced during the 5-week experimental period were determined as described previously (Eklund and Little 1996).

Statistical analysis

Analysis of variance was applied to each data set, and the significance of the difference between means was determined by the Duncan New Multiple Range test as performed by the SuperANOVA statistics package (Abacus Concepts, Inc., Berkley, CA).

Results

Ethylene evolution

Ethylene evolution from untreated shoots (i.e., shoots that were not treated with 0, 1 or 10 mg Ethrel g^{-1} lanolin) varied with time of measurement after excision and with fraction, but not with segment location (Figure 2). During the first 4 h after excision, the evolution of ethylene from each fraction was constant, except for a small increase measured at 4 h in the cambial region above the application point. Throughout this period, the rate of ethylene evolution (denoted the basal rate)

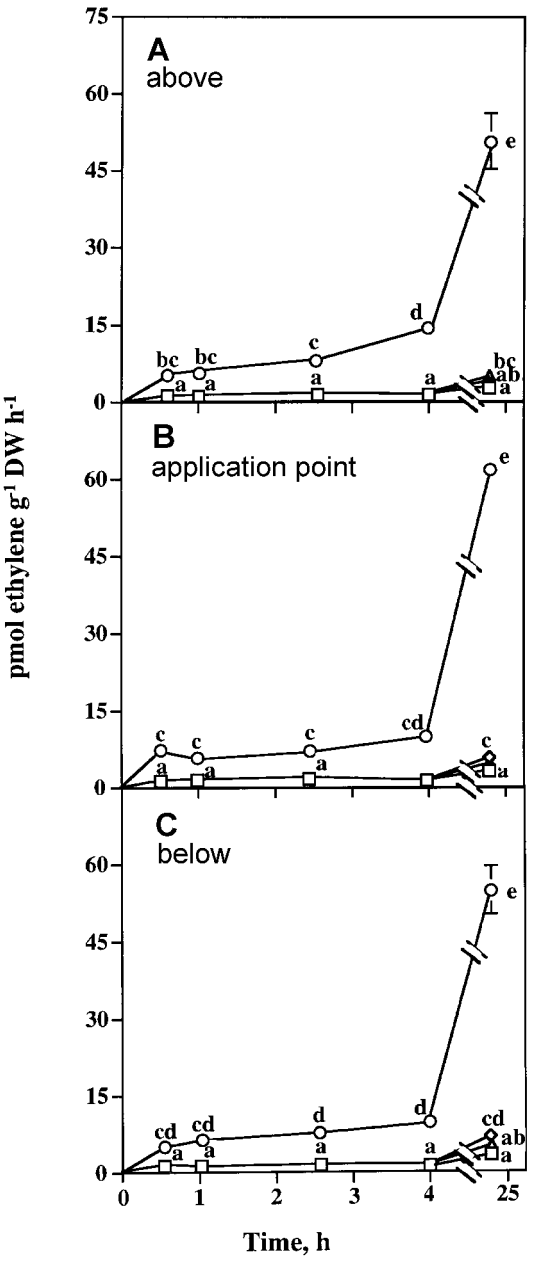

Figure 2. Effect of time since excision on ethylene evolution from the cambial region (○), cortex + periderm (◇), xylem + pith (△) and needles (□) of segments obtained from untreated shoots at locations corresponding to above (A), at (B) and below (C) the Ethrel application point in treated shoots. Mean ± SE, $n = 8$, measured after 5 weeks of culture. Means accompanied by the same letter are not significantly different at $P \leq 0.05$.

was about 5-fold higher in the cambial region than in the other fractions, whose rates were similar. After 24 h, however, ethylene evolution from all fractions had increased, reflecting the production of wound-induced ethylene. The increase was about 7-, 4-, 2- and 0.5-fold from the cambial region, cortex + periderm, xylem + pith and needles, respectively. Because the basal rate of ethylene evolution and the production of wound-induced ethylene from the needles and the xylem + pith were relatively minor, only the cambial region and the cortex + periderm were used to measure ethylene evolution in subsequent experiments.

The effect of applying 0 mg (plain lanolin), 1 mg or 10 mg g^{-1} Ethrel on ethylene evolution varied with segment location, time of measurement after excision and Ethrel concentration. Comparing the temporal pattern of ethylene evolution from shoots treated with plain lanolin (Figure 3) with that from

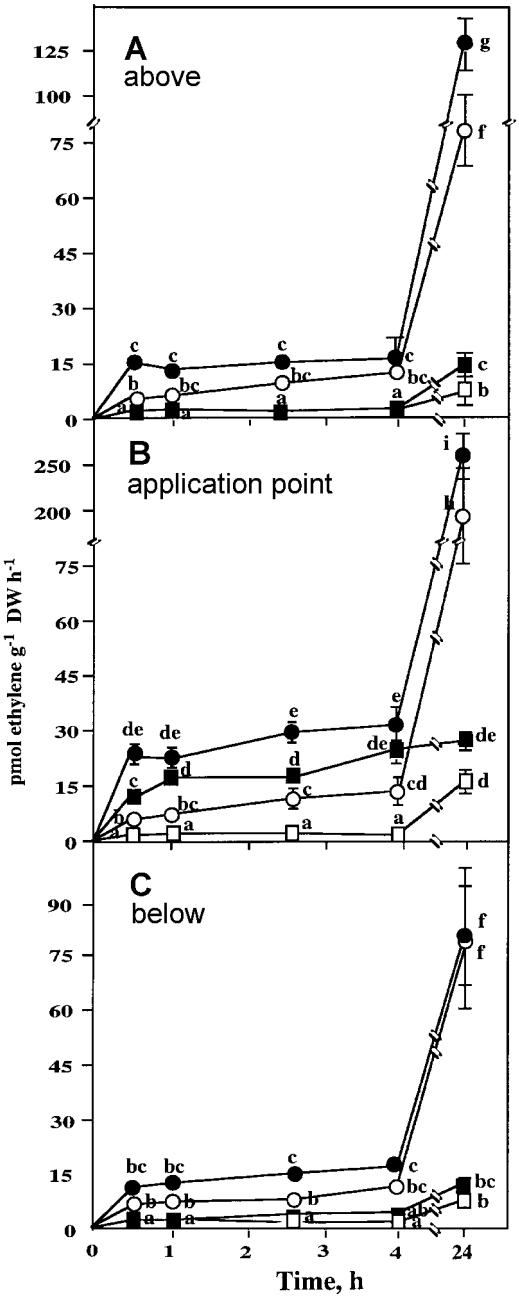

Figure 3. Effect of time since excision on ethylene evolution from the cambial region (circles) and cortex + periderm (squares) of segments located above (A), at (B) and below (C) the point of applying 0 (open symbols) or 1 (closed symbols) mg Ethrel g^{-1} lanolin for 5 weeks. Mean ± SE, $n = 8$. Means accompanied by the same letter are not significantly different at $P \leq 0.05$.

untreated shoots (Figure 2), revealed that the lanolin treatment affected the production of wound-induced ethylene by the cambial region at the application point (effect significant at 2.5 h after excision), and increased wound-induced ethylene production by the cambial region and the cortex + periderm at and above the application point at 24 h. The 1 mg g^{-1} Ethrel treatment did not alter the temporal pattern of ethylene evolution observed in shoots treated with plain lanolin, but it increased the evolution of ethylene from the cambial region and the cortex + periderm at the application point throughout the 24-h measurement period, as well as above the application point at 24 h (Figure 3). The Ethrel-induced stimulation of ethylene evolution from the cambial region and the cortex + periderm at the application point, measured 30 min after excision, increased with increasing Ethrel concentration (Figure 4). At the application point, the 10 mg g^{-1} Ethrel treatment stimulated ethylene evolution from the cortex + periderm more than from the cambial region, and thus reversed the relative capacity to evolve ethylene that was found in shoots treated with plain lanolin or 1 mg Ethrel g^{-1}. Above and below the application point, neither the 1 nor 10 mg g^{-1} Ethrel treatment altered ethylene evolution from the cortex + periderm, and the 1 mg g^{-1} Ethrel treatment also did not affect the evolution of ethylene from the cambial region. In contrast, the 10 mg g^{-1} Ethrel treatment stimulated ethylene evolution from the cambial region both above and below the site of application, particularly the former location.

Radial growth

In untreated shoots, bark width and tracheid number tended to be greater above than at or below the application point (Table 1). Compared with untreated shoots (Table 1), the plain lanolin treatment (Figure 5) accentuated the positional effect on tracheid number and increased the bark width at the application point. Ethrel application increased both bark width and tracheid number at the application point, but not above or below the application point, the extent of the increase being positively related with Ethrel concentration (Figure 5). The Ethrel-induced promotion of bark width at the application point was associated with an increase in cortex width.

Carbohydrate concentrations in the cambial region

In untreated shoots, concentrations of fructose, glucose and sucrose declined basipetally, whereas starch concentration tended to be higher below than at or above the application point (Table 1). Compared with untreated shoots (Table 1), application of plain lanolin (Figure 6) decreased the concentrations of fructose and glucose at and above the application point, as well as the starch concentration at the application site. Applying Ethrel increased the concentrations of all three sugars at the application point, but not above or below the application point (Figure 6). At the application point, the 1 and 10 mg g^{-1} Ethrel treatments had a similar stimulatory effect on the concentrations of fructose and glucose, whereas only the 10 mg g^{-1} Ethrel treatment raised the sucrose concentration. Ethrel application also increased starch concentration at the application point, but decreased it above and below the application point.

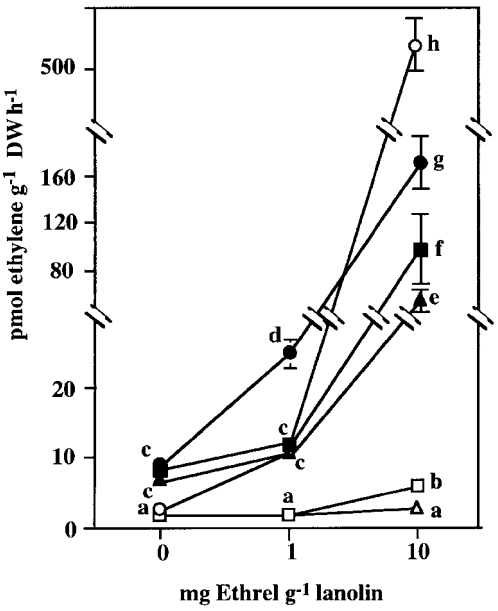

Figure 4. Ethylene evolution from the cambial region (closed symbols) and cortex + periderm (open symbols) of segments located above (squares), at (circles) and below (triangles) the point of applying 0, 1 or 10 mg Ethrel g^{-1} lanolin for 5 weeks. Measurement made 30 min after excision. Mean ± SE, $n = 8$. Means accompanied by the same letter are not significantly different at $P \leq 0.05$.

Discussion

The initial, basal rate of ethylene evolution and the subsequent production of wound-induced ethylene varied among fractions in the untreated shoots. The basal rate of ethylene production of each fraction, which followed the order: cambial region > cortex + periderm = xylem + pith = needles, was maintained for at least 4 h after excision (Figure 2). Twenty-four hours after excision, however, the production of wound-induced ethylene was evident. Wound-induced ethylene production was also delayed for several hours after wounding in experiments with nondissected stem segments of *Pinus taeda* L. (Telewski and Jaffe 1986) and *Picea abies* (Ingemarsson 1994), stem fractions of *Chamaecyparis obtusa* (Yamanaka 1985), and an inner phloem + cambial tissue fraction excised from the stem of various conifers (Yamanaka 1986). We found that the increase in ethylene evolution at 24 h after excision followed the order: cambial region > cortex + periderm > xylem + pith > needles. Thus, the relative capacities of the shoot fractions to evolve ethylene were similar, whereas the extent of the difference between particular fractions varied before and after the production of wound-induced ethylene occurred. Our finding that the cambial region had the highest basal rate of ethylene evolution, and produced the most wound-induced ethylene, extends results obtained by Yamanaka (1985), who showed that inner phloem + cambial tissue had the greatest wound-induced ethylene production of the tissues of *Chamaecyparis obtusa* stems. Why ethylene evolution was greater from the cambial region than from other shoot fractions is unknown, but

Table 1. Radial growth (mm) and carbohydrate concentrations (mg g_{DW}^{-1}) in the cambial region of segments obtained from untreated shoots at locations corresponding to above, at and below the Ethrel application point in treated shoots. Mean ± SE, $n = 10$ for radial growth and $n = 5$ for carbohydrate concentrations, measured at the end of the 5-week experimental period. Means accompanied by the same letter are not significantly different at $P \leq 0.05$.

Location	Radial growth		Sugars			Starch
	Tracheid number	Bark width	Fructose	Glucose	Sucrose	
Above	7.9 ± 0.7 a	0.98 ± 0.05 a	8.4 ± 0.9 a	4.1 ± 0.3 a	154 ± 16 a	10.2 ± 3.0 a
At	6.5 ± 0.5 a	0.91 ± 0.03 a	7.0 ± 0.8 ab	3.1 ± 0.3 b	113 ± 5 ab	10.1 ± 0.9 a
Below	6.7 ± 0.6 a	0.93 ± 0.04 a	5.3 ± 0.2 b	2.0 ± 0.2 c	100 ± 15 b	13.2 ± 2.1 a

could reflect a greater capacity to: (1) synthesize the immediate precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC), (2) convert ACC to ethylene, (3) import ACC from the xylem (Bradford and Yang 1980, Eklund 1993*a*) and perhaps the phloem (Morris and Larcombe 1995), or (4) decrease the conjugation of ACC to *N*-malonyl-ACC (Abeles et al. 1992).

Ethrel application stimulated the evolution of ethylene from the cambial region and the cortex + periderm at the application point, and the 10 mg g^{-1} Ethrel treatment also increased ethylene evolution from the cambial region above and, to a lesser extent, below the application site (Figures 3 and 4), indicating that Ethrel, and perhaps also derived ethylene, moved radially from the lanolin through the cortex into the cambial region and then longitudinally in the vascular transport system, particularly acropetally. This interpretation is consistent with results obtained in translocation studies with ^{14}C-Ethrel, ^{14}C-ethylene and ethylene gas (Jackson and Campbell 1975, Foster et al. 1992, Eklund 1993*a*). An Ethrel-induced stimulation of ethylene evolution at the application point has also been demonstrated in nondissected stem segments of woody shoots (Robitaille and Leopold 1974, Yamamoto and Kozlowski 1987*a*, Eklund and Little 1996). The stimulation is attributed to the release of ethylene from Ethrel (Biddle et al. 1976), combined with a minor production of ethylene induced by the wounding associated with applying the Ethrel, i.e., excising the needles and periderm (Figures 2 and 3; also Eklund and Little 1996). It is also possible that Ethrel-derived ethylene promoted the synthesis of endogenous ethylene (Sitrit et al. 1986, Schierle et al. 1989, Ievinsh et al. 1990, Foster et al. 1992). Ethylene evolution was higher from the cambial region than from the cortex + periderm when 1 mg Ethrel g^{-1} was applied, but lower when 10 mg Ethrel g^{-1} was used (Figure 4), presumably because the equilibrium within the two fractions among the rates of Ethrel uptake, ethylene release, and radial movement of Ethrel and derived ethylene varied with concentration.

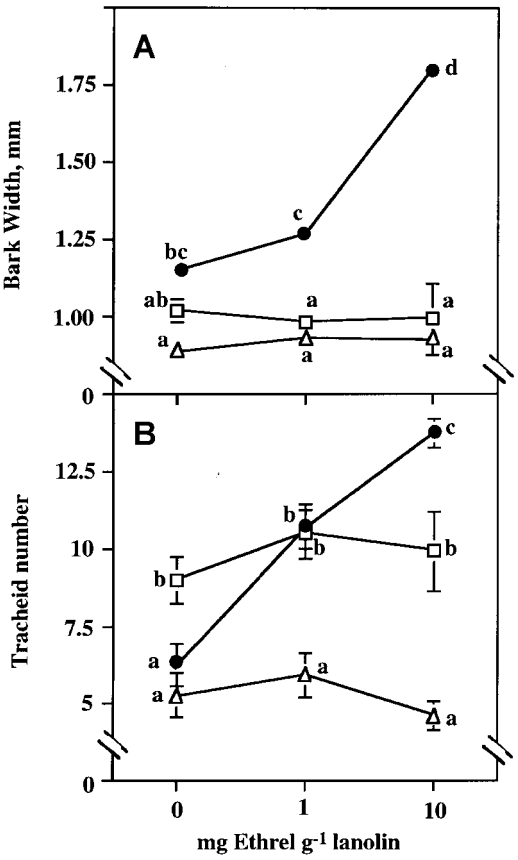

Figure 5. Bark radial width (A) and tracheid number (B) measured above (□), at (●) and below (△) the point of applying 0, 1 or 10 mg Ethrel g^{-1} lanolin for 5 weeks. Mean ± SE, $n = 10$. Means accompanied by the same letter are not significantly different at $P \leq 0.05$.

Our results provide additional evidence that ethylene plays a role in the control of cortex hypertrophy (Yamamoto and Kozlowski 1987*a*, 1987*b*, Abeles et al. 1992) and cambial growth (Little and Pharis 1995). The stimulation of ethylene evolution induced by the Ethrel treatment (Figures 2 and 3) was associated with an increase in bark radial width at the application point (Table 1 and Figure 5). Moreover, the Ethrel-induced increase in ethylene evolution from the cortex + periderm was restricted to the application point, which was the only segment location where bark width was increased (Figures 4 and 5). Similarly, the Ethrel-mediated stimulation of ethylene evolution from the cambial region was positively related to tracheid number at the application point (Figures 4 and 5). However, the stimulation of ethylene evolution from the cambial region above and below the 10 mg Ethrel g^{-1} application site was not associated with an increase in tracheid number. A positive relationship between ethylene evolution, measured in nondissected stem segments, and tracheid number was also detected at, but not above or below, the site of

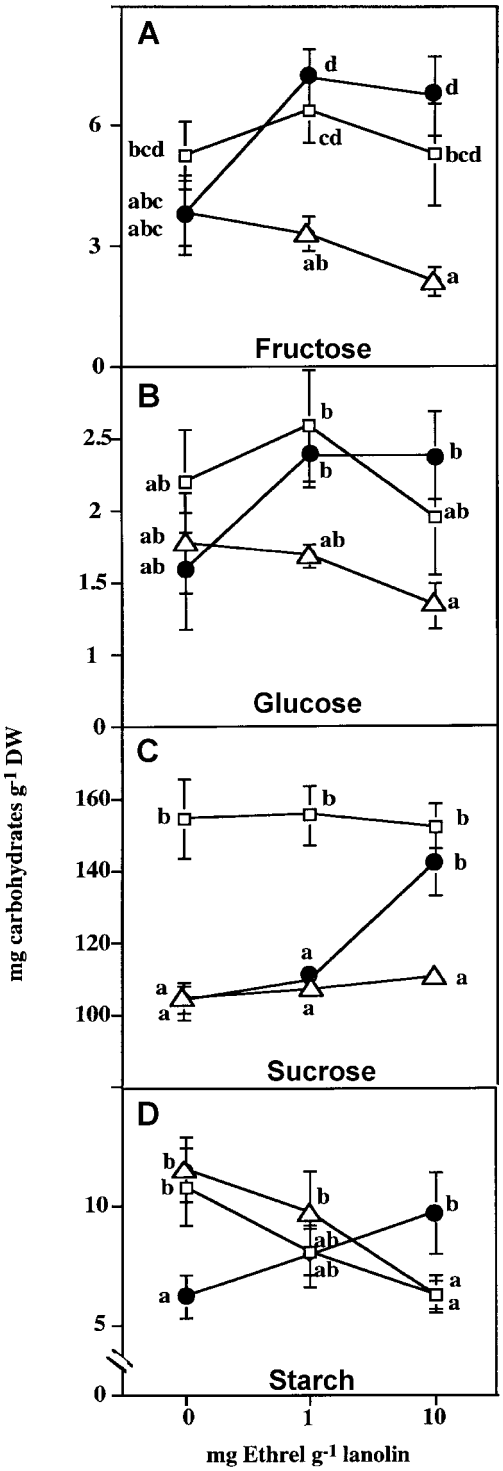

Figure 6. Concentrations of fructose (A), glucose (B), sucrose (C) and starch (D) in the cambial region of segments located above (□), at (●) and below (△) the point of applying 0, 1 or 10 mg Ethrel g^{-1} lanolin for 5 weeks. Mean ± SE, $n = 5$. Means accompanied by the same letter are not significantly different at $P \leq 0.05$.

application of the 10 mg g^{-1} Ethrel treatment to shoots of *Abies balsamea* seedlings (Eklund and Little 1996). Moreover, in *Abies balsamea* cuttings, feeding Ethrel apically or basally increased ethylene evolution from nondissected stem segments without affecting tracheid number, and applying Co^{2+} basally inhibited ethylene evolution but not tracheid number (Eklund and Little 1995). Considered together, these results indicate that the Ethrel-mediated increases in ethylene evolution and tracheid production are positively related only at the site of Ethrel application.

The finding that the stimulation of ethylene evolution at the Ethrel application point was associated with an increase in the cambial region concentrations of fructose, glucose, sucrose and starch (Figures 4 and 6) provides the first evidence that Ethrel-derived ethylene can induce the mobilization of carbohydrates in the stem of a woody species. We conclude that the wounding associated with the Ethrel application method did not contribute to the carbohydrate increase, because the plain lanolin treatment decreased the cambial region concentrations of fructose, glucose and starch at the application point (Table 1 and Figure 6). We speculate that the primary action of Ethrel-derived ethylene on carbohydrate mobilization in our experimental system was to stimulate phloem unloading rather than sink activity, because the carbohydrate concentrations, particularly of starch, were increased concomitantly with radial growth (Figures 5 and 6). This speculation is supported by evidence that application of ethylene, as well as other hormones, promotes the activity of sucrose-cleaving enzymes (Abeles et al. 1992, Morris 1996). Additional research is required to confirm the ability of exogenously applied ethylene to mobilize carbohydrates in woody shoots and to establish its mechanism of action. It will also be necessary to determine if applied ethylene acts directly or indirectly by altering the concentration of other hormones such as indole-3-acetic acid (IAA), a promoter of cambial growth (Little and Pharis 1995). A role for IAA in the stimulation of radial growth induced by Ethrel at the application point is supported by the findings that laterally applied Ethrel locally increased the IAA concentration in the cambial region of *Abies balsamea* shoots (Eklund and Little 1996) and that applying IAA to the apical cut surface of debudded + distally defoliated *Pinus sylvestris* cuttings promoted the acropetal movement of ^{14}C-photosynthate (Little et al. 1990).

Acknowledgments

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