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Ethylene, Ethane, Acetaldehyde, and Ethanol Production By Plants under Stress[1]

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ABSTRACT

Red pine (*Pinus resinosa* Ait.) and paper birch (*Betula papyrifera* Marsh.) seedlings exposed to sulfur dioxide produced acetaldehyde and ethanol, and exhibited increased production of ethylene and ethane. Gas chromatographic measurement of head space gas from incubation tubes containing leaves or seedlings was a simple method of simultaneously measuring all four compounds. Increased ethylene production had two phases, a moderate increase from the beginning of the stress period and a large increase just prior to appearance of leaf lesions. Ethane production in SO_2-stressed plants did not increase until lesions appeared. Acetaldehyde and ethanol production began within 6 hours at 0.3 microliter per liter SO_2 and 24 hours at 0.1 microliter per liter SO_2 and continued throughout a 6-day fumigation. Production of acetaldehyde and ethanol continued when plants were removed to clean air for up to 2 days. A higher concentration of SO_2 (0.5 microliter per liter) induced acetaldehyde and ethanol production within 2 hours of the start of fumigation of birch and pine seedlings. A number of other stresses, including water deficit, freezing, and ozone exposure induced production of acetaldehyde and ethanol. Production of these compounds was not due to hypoxia, as the O_2 partial pressure in the incubation vessels did not decline. Increasing the O_2 partial pressure to 300 millimeters Hg did not affect production of these compounds. Production of ethylene, acetaldehyde, and ethanol declined when more than 80% of the leaf area became necrotic, while ethane production was linearly related to the percentage of necrosis. A number of woody and herbaceous plant species produced acetaldehyde and ethanol in response to freezing stress, while others did not. Measurement of these four compounds simultaneously in the gas phase may be a valuable method for monitoring plant stress, particularly air pollution stress.

Production of ethylene by plants increases as a result of environmental stress or wounding (16, 17), and measurement of stress ethylene can be a useful indicator of the onset of stress and/or the degree of stress which a plant is experiencing (14, 16). For example, ethylene evolution by ozone-stressed plants was well correlated with the ozone dose in a large number of plant species (14). There are difficulties with the use of stress ethylene as a diagnostic tool, however. Ethylene is produced by unstressed plants, and the amount varies with age of the tissue and with environmental conditions (16). When stress results in death of cells, ethylene evolution declines. Therefore, the correlation between stress and ethylene evolution may be poor (5).

Plants under stress also produce ethane, and unlike ethylene, the amount produced by unstressed plants is normally quite low. Elstner and Konze (5) found that ethane evolution by freezing-stressed plants was linearly correlated with the amount of leaf necrosis. Other studies indicated that ethane production is a common response to wounding (8, 10), and simultaneous measurement of stress ethylene and ethane may be of considerable use in evaluating plant stress.

Ethane evolution is the result of free-radical-mediated peroxidation of membrane linolenic acids and apparently occurs because free-radical scavenging mechanisms are overcome when cells are decompartmented (6, 7, 10). Bressan *et al.* (2) and Peiser and Yang (12) reported that ethane is evolved from SO_2-stressed plants. The proposed mechanism is that Chl-initiated oxidation of bisulfite by a free-radical mediated process results in co-oxidation of linolenate (12). We investigated the production of ethylene and ethane by woody plants exposed to SO_2 in order to determine whether (a) measurement of ethylene and ethane can be a useful method for objectively evaluating environmental stress; and (b) whether ethane production is the result of a specific SO_2-driven process, as suggested by Peiser and Yang (12), or the result of necrosis and decompartmentation of cells.

During gas-chromatographic measurement of ethylene and ethane production by woody plants, we found that ethanol and acetaldehyde were produced by stressed plants in addition to ethylene and ethane.

Ethanol and acetaldehyde production is usually associated with anaerobic processes such as occur in flooded plants. Under aerobic conditions, little or none of these glycolytic metabolites is normally produced (4). Ethanol and acetaldehyde are also produced by some fruits, such as strawberries, and by deteriorating seeds (11, 15). In all of these cases, reduced O_2 availability or reduced O_2 transport is thought to inhibit TCA electron transport leading ultimately to formation of acetaldehyde and ethanol (4). Our experiments show that ethanol production by plants under stress does not require restricted O_2 availability.

In the present study, we examined production of ethylene, ethane, acetaldehyde, and ethanol by woody plants exposed to SO_2 and water stress. Further experiments examined the production of these compounds as a result of several kinds of stress and wounding in a variety of woody and herbaceous plants.

MATERIALS AND METHODS

Plant Material. Woody plants were grown from commercially available seed in a greenhouse with supplemental lighting to give a 16-h photoperiod. Paper birch (*Betula papyrifera* Marsh.) and red pine (*Pinus resinosa* Ait.) seeds were germinated in 3:2:1 peat:Perlite:vermiculite, and seedlings were transplanted 2 to 4 weeks after germination into the same soil mix in 10-cm pots. Seeds of other woody plants were germinated in peat moss and transplanted into 3:2 loam:sand in 30-cm pots. Plants were watered daily and fertilized weekly with Hyponex. Herbaceous plants were grown in various soil mixes in the greenhouse under continuous lighting.

SO_2 Stress. Plants were transferred to fumigation chambers of the University of Wisconsin Biotron the night before fumigation.

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Environmental conditions in the chambers were: photoperiod, 16 h beginning at 0500 h; quantum flux density, measured with a Lambda Quantameter (Li-Cor, Inc.), 350 ± 25 μE m^{-2} s^{-1} (PAR) at the top of the pots form two 400-w HID[2] lamps and five 25-w incandescent lamps; 25°C day/15°C night temperature; and 70 ± 5% RH. SO_2 was provided from tanks of 3,000 μl l^{-1} SO_2 in N_2, and the SO_2 concentration was monitored with calibrated Thermo Electron (Thermo Electron Corp., Hopkinson, MA) pulsed fluorescent SO_2 analyzers. The fumigation chambers and environmental monitoring methods have been described previously (9).

Other Stresses. Freezing stress was imposed by touching a 6-mm diameter stainless steel rod to the leaves after it was immersed in liquid N_2 (5). The percentage of leaf area killed was determined from the number of times the rod was touched to the leaf and the leaf area, measured with a Li-Cor model 3100 Leaf Area Meter. Wounded leaves were sliced with a razor blade between each lateral vein from the midrib to the margin. Crushing injury was obtained by compression of the leaf mesophyll with the steel rod. Water deficit was induced by H_2O withholding. Water potential (ψ) was measured with a pressure bomb on leaves just above and below those sampled for gas measurement, and the water potential of the sample leaves was taken to be the mean of those measurements. Hypoxia was induced by flushing the flasks with 95% N_2, 5% CO_2.

Incubation of Leaves for Gas Analysis. Leaves of angiospermous plants were removed from the stem and the petiole was cut-off. The leaves were gently rolled and placed in 14-ml test tubes containing 0.1 ml H_2O. The tubes were sealed with silicone rubber serum stoppers which had been wrapped in a single layer of Saran Wrap, and were incubated for 4 or 8 h in a water bath at 25°C under an HID lamp with a quantum flux density (PAR) of 350 μE m^{-2} s^{-1}. In some experiments, leaves were placed in 60-ml modified separatory funnels and incubated in the water bath. The funnels were modified to accept a serum stopper just below the glass stopcock and a Beckman polarographic O_2 electrode at the other end. The electrode was sealed in the funnel with Apiezon Q (American Scientific Products, McGaw Park, IL) and wrapped with several layers of Parafilm. The Apiezon Q was acid washed to remove traces of acetic acid and ethanol. One-ml samples of head space gas in the tubes and funnels were taken by injecting 1 ml air through the stopper and withdrawing 1 ml head space gas with a tuberculin syringe and 26-gauge needle. The needle was pushed into a rubber stopper to prevent leakage during transport to the gas chromatograph. Leakage rates from the tubes, funnels, and syringes were not significant over the time course of our experiments, although low concentrations of ethane may be slightly underestimated.

In experiments with pine seedlings, the entire shoot was incubated. The seedling was excised just above the cotyledons and placed in a 14-ml tube (small seedlings) or a 25-ml tube (larger seedlings). Incubation conditions were as described above.

Gas Chromatography. One-ml samples of head space gas were injected onto a 183 × 0.32 cm stainless steel chromatography column containing 80/100 Porapak Q in a Hewlett-Packard gas chromatograph. The column oven was at 90°C, and the carrier gas (N_2) flow was 38 ml min^{-1}. Peaks were detected by ionization in a H_2 flame, and were identified by coelution with authentic compounds on columns of Porapak N, Porapak Q, and Apiezon L on Chromosorb W-HP (Anspec Co., Ann Arbor, MI). Verification was obtained by GC-MS.

Hydrocarbon production by the plants was calculated as

$$\text{Production} = \frac{\text{GC Response} \times \text{Calibration Factor} \times (\text{Tube Volume} + 1)}{\text{Dry Weight of Plant Material}}$$

where the correction factor was determined from standard curves for each gas. Standard curves were prepared by serial dilution in H_2O of freshly distilled acetaldehyde and ethanol. One-μl aliquots of standards were injected within 15 min of dilution to avoid oxidation of the acetaldehyde. These standard curves were used to determine partition coefficients of acetaldehyde and ethanol between H_2O and air, and subsequent standard curves were prepared by injecting 1-ml samples of head space gas over freshly prepared standards in water. Ethylene and ethane standard curves were prepared by serial dilution of 1,000 μl l^{-1} stock gases using gas-tight syringes.

Details of each experiment are given in the legends to figures and tables.

RESULTS

Production of Volatile Hydrocarbons by SO_2-Stressed Pine. Red pine seedlings exposed to SO_2 produced four major volatile compounds (Fig. 1A, peaks 2, 3, 6, and 7) which were identified by MS as ethylene, ethane, acetaldehyde, and ethanol, respectively. In contrast, SO_2-fumigated peas (*Pisum sativum* L.) produced ethylene and ethane in response to SO_2 stress (Fig. 1C), with only traces of acetaldehyde and ethanol being produced by severely stressed plants.

Figure 2 shows results of a typical fumigation of red pine seedlings with SO_2. At 0.1 μl l^{-1} SO_2, increases in ethylene production were small and nonsignificant, and there was no increase in ethane production. A higher concentration of SO_2 resulted in increased ethylene production within 6 h of the start of the experiment, and this increased production had two phases, a moderate increase from the beginning of the stress period and a marked increase just prior to the appearance of visible leaf lesions. In contrast, there was no increase in ethane production until after

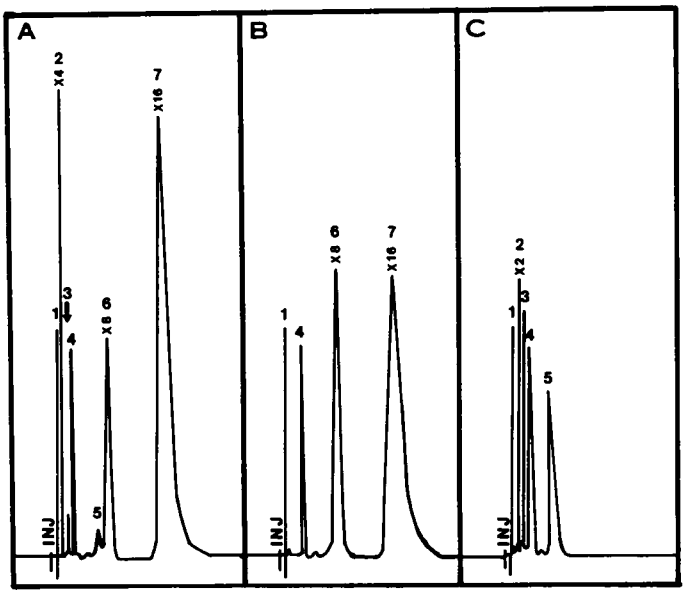

FIG. 1. Gas chromatograms of low-mol-wt hydrocarbons on Porapak Q. A, Head space gas from a 4-h incubation of SO_2-stressed red pine seedling in a 60-ml flask; B, head space gas over a standard solution of acetaldehyde and ethanol in H_2O; C, head space gas from a 8-h incubation of SO_2-stressed peas (*Pisum sativum* L.) in a 60-ml flask. Peaks are: 1, air peak (probably methane); 2, ethylene; 3, ethane; 4, unknown air contaminant; 5, unknown; 6, acetaldehyde; 7, ethanol.

[2] Abbreviations: HID, high-intensity discharge; ADH, alcohol dehydrogenase; pO_2, oxygen partial pressure.

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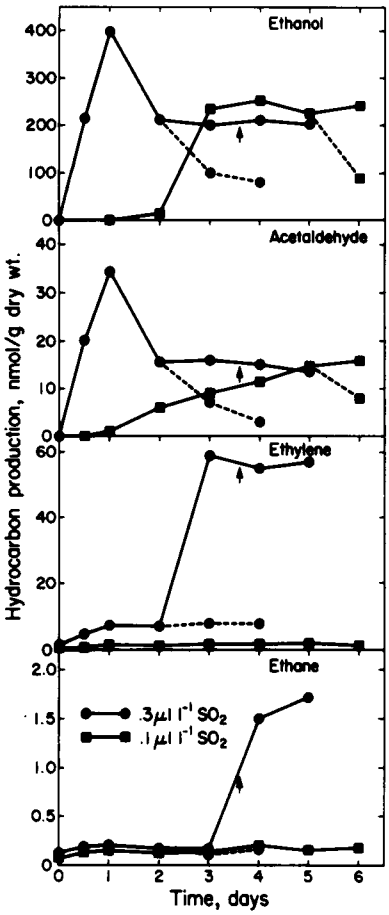

FIG. 2. Effect of exposure to SO_2 on production of ethanol, acetaldehyde, ethylene, and ethane by 6-month-old red pine seedlings. Plants were fumigated continuously from 0900 on day 0 to 1400 on day 6. Plants were harvested at 0900 and 1500 on day 0 and 0900 each subsequent day. Each point is the mean of two assays on three plants in two replicate experiments (n = 12). Arrows, first appearance of visible lesions. Dotted lines show production of gases from plants removed from the fumigation chambers to clean air. Standard errors were ≤10% of the mean, except ethanol and acetaldehyde at 0.3 μl l^{-1} SO_2, where SE was up to 20% of the mean. Unfumigated control plants were harvested at each sampling period. Mean production of gases by control plants was: ethanol, 0; acetaldehyde, 0; ethylene, 0.80; ethane, 0.09 nmol/g dry weight.

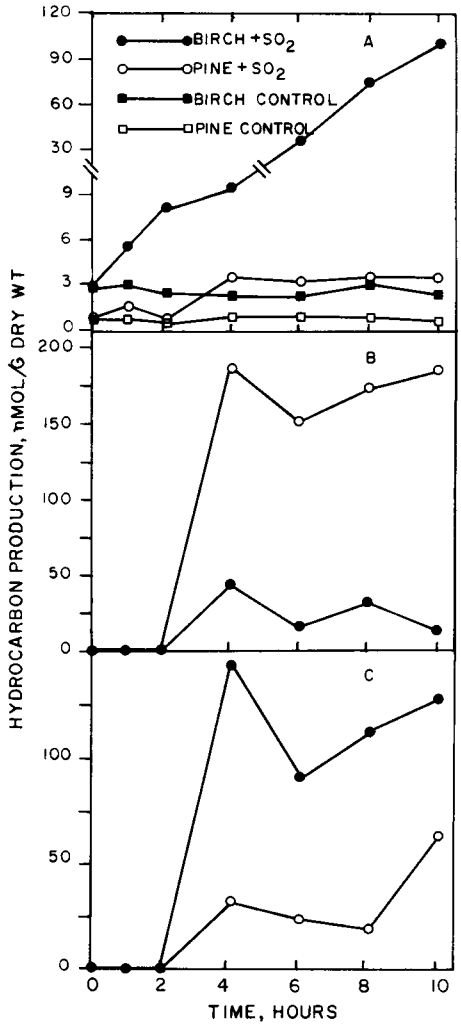

FIG. 3. Effect of exposure to 0.5 μl l^{-1} SO_2 on production of ethanol, acetaldehyde, ethylene, and ethane by 6-month-old red pine and fully expanded leaves of 4-month-old paper birch seedlings. Plants were fumigated beginning at time 0. There were no visible lesions by the end of the experiment. Each point is the mean of two assays on two plants of each species in two replicate experiments (n = 8). Standard errors were ≤10% of the mean. Unfumigated controls produced no acetaldehyde or ethanol. Ethane production was not increased by fumigation above the control means of 0.08 and 0.04 nmol/g dry weight for pine and birch, respectively.

visible injury appeared. Ethanol and acetaldehyde production began in the high-stress plants within 6 h of the start of fumigation and reached a peak within 24 h. At the lower SO_2 concentration, traces of acetaldehyde were detectable by 24 h after the start of fumigation and the amount continued to increase throughout the stress period, while ethanol production peaked on the 3rd d and remained constant thereafter.

Removal of the plants from the fumigation chamber into clean air (Fig. 2, dashed lines) did not eliminate production of acetaldehyde or ethanol, but there was an approximately linear decrease in their production over 2 d. Plants exposed to 0.3 μl l^{-1} SO_2 for 3 d, then removed to clean air, continued to produce elevated levels of ethylene, but there was not a sharp increase, and no lesions formed by the end of the experiment (Fig. 2).

Fumigation of pine and birch seedlings with higher concentrations of SO_2 for shorter periods caused similar changes (Fig. 3), except that: (a) the onset of ethanol and acetaldehyde production was more rapid; (b) there was no increase in ethane and no visible lesions formed during the course of the experiment; and (c) increased ethylene production in birch preceded production of acetaldehyde and ethanol, while in pine they increased simultaneously as in the experiment of Figure 2. Several other differences were observed between the responses of birch and pine seedlings to SO_2. The increase in ethylene production was much greater in birch than in pine, and this may reflect the slightly greater sensitivity of the birch seedlings to SO_2. Production of ethanol by birch seedlings was quite low while acetaldehyde accumulation was much greater in birch than in pine. This may be due to greater ADH activity in pine than in birch (T. W. Kimmerer and T. T. Kozlowski, manuscript in preparation). The amounts of volatile hydrocarbons produced and the response to SO_2 stress varied with age of the seedling and age of the individual leaves (T. W. Kimmerer and T. T. Kozlowski, manuscript in preparation).

Other Stresses. A number of other stresses and injuries induced production of acetaldehyde and ethanol, while others increased ethylene and ethane production without causing production of acetaldehyde and ethanol (Table I). Most of these stresses and injuries resulted in formation of necrotic lesions, and these were accompanied by increases in the amount of ethane produced.

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Table I. *Effects of Various Stresses and Injuries on Volatile Hydrocarbon Production by 4-Month-Old Birch and Pine Seedlings*

Wounding and freezing injury killed 50% of the leaf. There was no visible injury in the ozone-stressed plants or in water-stressed plants at −1.3 MPa. At −2.5 MPa, leaves were severely wilted and slightly necrotic. Data are mean ± SE. Number of replicates is given in parentheses.

Type of Stress or Injury	Species	Ethanol	Acetaldehyde	Ethylene	Ethane
			nmol/g dry wt		
Control	Pine	0	0	0.84 ± 0.27	0.02 ± 0.01 (6)
Control	Birch	0	0	0.80 ± 0.24	0.09 ± 0.01 (9)
Hypoxia, $pO_2 \leq 5$ mm Hg	Pine	275 ± 32	18 ± 1	0.46 ± 0.06	0.38 ± 0.04 (5)
Hypoxia, $pO_2 = 25$ mm Hg	Pine	0	0	0.30 ± 0.02	0.16 ± 0.02 (5)
Water deficit, $\psi = -1.3$ MPa	Birch	0	0	1.10 ± 0.12	0.05 ± 0.01 (6)
Water deficit, $\psi = -2.5$ MPa	Birch	296 ± 136	337 ± 57	10.26 ± 1.31	0.12 ± 0.04 (4)
Wounded by slicing	Birch	0	0	4.36 ± 0.05	0.05 ± 0.01 (4)
Wounded by crushing	Birch	12 ± 6	18 ± 2	4.65 ± 0.19	0.17 ± 0.03 (4)
Freezing	Birch	37 ± 1	64 ± 4	2.37 ± 0.64	0.35 ± 0.01 (4)
Senescence	Birch	0	0	1.21 ± 0.24	0.07 ± 0.01 (6)
Ozone (0.05 $\mu l\ l^{-1} \times 1$ h)	Birch	127 ± 55	586 ± 62	15.46 ± 1.20	0.08 ± 0.01 (8)

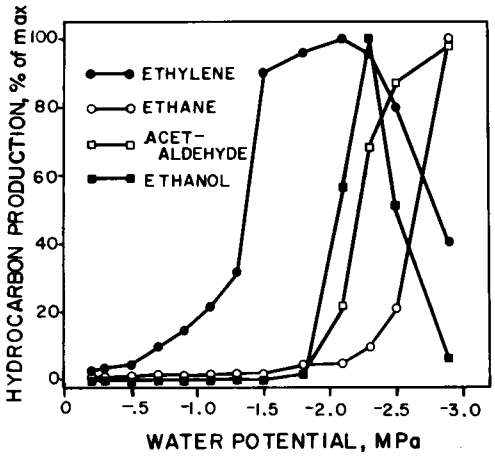

FIG. 4. Hydrocarbon production by leaves of 4-month-old birch seedlings at various water potentials. Plants were drought stressed by withholding water. Each point represents the mean of two gas measurements on two adjacent fully expanded leaves of a single plant. Maximum production for ethylene, ethane, acetaldehyde, and ethanol was 16, 0.42, 307, and 208 nmol/g dry weight, respectively.

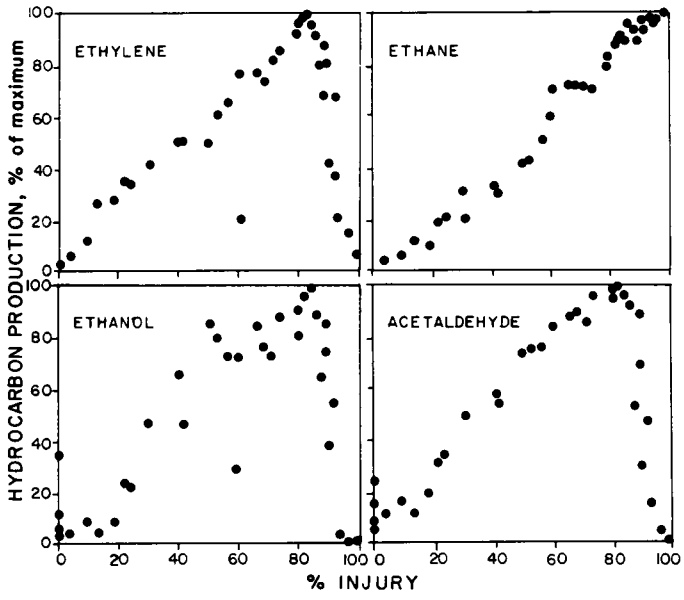

FIG. 5. Relationship between percentage of injury (necrosis) and gas production as percentage of maximum in fully expanded leaves of 4-month-old paper birch seedlings fumigated with an acute dose of SO_2 (0.5 $\mu l\ l^{-1}$ for 8 h). Injury was estimated visually after incubation of tissue for gas analysis. Each point represents the mean of two gas measurements on individual leaves incubated in 14-ml tubes.

Water deficit resulted in production of large amounts of acetaldehyde and ethanol, but not until leaf ψ was quite low, and the leaves were severely wilted (Table I; Fig. 4). With water stress, in contrast to SO_2 stress, acetaldehyde and ethanol production occurred after a large increase in ethylene production and just preceded the increase in ethane production and the onset of visible lesions (Fig. 4). The plants wilted at a leaf ψ of about −1.4 MPa, which is also the point at which the large increase in ethylene production was observed. Note that, although ethanol production declined at very low water potentials, acetaldehyde production continued to increase.

Injury and Volatile Production. In several experiments, ethanol production declined when injury was very severe, while ethane production continued to increase. To examine the relationship between injury and gas production, we compared production of volatiles with percentage of leaf injury in birch seedlings stressed with 0.5 $\mu l\ l^{-1}$ SO_2 (Fig. 5). Production of all four gases was approximately linearly related to the percentage of injury up to about 80%, beyond which production of all gases except ethane declined. In this experiment, only a few leaves without injury were analyzed. As shown in Figure 2, maximal production of acetaldehyde and ethanol may occur in the absence of visible lesions. Thus, at a lower SO_2 concentration than used in the experiment of Figure 5, there would be no linear relationship between injury and the production of these two gases.

Oxygen Concentration Effects. In unstressed pine seedlings, no ethanol or acetaldehyde was produced. When seedlings were exposed to hypoxic conditions, these compounds were produced only when the pO_2 was less than 10 mm Hg (Table I). Analysis of the O_2 tensions in separatory funnels containing stressed seedlings showed that acetaldehyde and ethanol were produced in the presence of normal ambient O_2 tensions. Some representative pO_2 data are shown in Table II. In most cases, the pO_2 changed only slightly with up to 24 h incubation in the light, with a gradual decline in O_2 tension in the dark. Severely injured seedlings showed a net uptake of O_2, with the pO_2 gradually declining. Increasing the O_2 tension to 300 mm Hg by flushing flasks with O_2 did not alter acetaldehyde and ethanol production of SO_2-

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Table II. *Some Representative O_2 Tension Data from Several Experiments*

After 8 h incubation, samples were taken for ethanol and acetaldehyde production. Incubation time is the total time over which the pO_2 was measured. SO_2-stressed plants were fumigated for 8 h with 0.5 $\mu l\ l^{-1}$ SO_2 prior to incubation. Injury was assessed visually at the end of the incubation period.

Stress	L/D[a]	Ethanol	Acetaldehyde	Time of Incubation	Initial pO_2	Final pO_2	Injury
		nmol/g dry wt		*h*	*mm Hg*		
None	L	0	0	24	160	158	None
None	D	0	0	24	155	148	None
SO_2	L	245	32	24	160	160	None
SO_2	L	146	19	24	158	160	Slight
SO_2	L	130	10	24	160	152	Moderate
SO_2	L	102	5	24	160	140	Severe
SO_2	D	0	0	24	160	120	Severe
Freezing	L	112	28	12	162	135	Severe
Freezing	D	5	0	12	158	97	Severe
None	L	0	0	12	300	260	None
SO_2	L	267	38	12	310	240	None
SO_2	L	210	42	12	295	210	Moderate
SO_2	D	0	0	12	305	196	Moderate

[a] Incubated in light (L) or dark (D).

stressed pine seedlings. Measurement of O_2 tensions in the 14-ml incubation tubes confirmed these results: production of acetaldehyde and ethanol by stressed plants is not the result of anaerobiosis due to O_2 consumption in the incubation tubes. Localized anaerobiosis due to water-soaked lesions is also unlikely, as acetaldehyde and ethanol were produced by plants that did not develop visible lesions. Microscopic examination of leaves of stressed birch seedlings which were producing acetaldehyde and ethanol failed to reveal any small water-soaked or necrotic areas.

Production Rates and Gas/Liquid Relationships. We examined the time course of accumulation of these volatile products in 60-ml separatory funnels by taking periodic 1-ml head space samples during 24 h incubation of stressed pine seedlings in the light and dark. Figure 6 shows a representative experiment. Production of ethylene, ethanol, and acetaldehyde was much more rapid in the light than in the dark, with acetaldehyde and ethanol in the dark declining after the first 5 h incubation. There was no lag in the onset of production of these compounds, with ethylene, ethanol, and acetaldehyde detectable within 10 min of the start of the incubation. Ethane production was very low in these experiments as the tissue was not necrotic. Ethane accumulation was linear over time, though not detectable until about 2 h after the start of fumigation. Ethane accumulation was not affected by light (data not shown).

Ethylene accumulation in the light appeared to be autocatalytic, with the slope of accumulation rate increasing over time. In contrast, acetaldehyde and ethanol production reached a constant within 12 h of incubation, with acetaldehyde sometimes, but not always, showing a sharp peak within 5 h of incubation.

Production of volatile hydrocarbons by stressed birch leaves showed similar kinetics, with two important differences: ethanol accumulation was considerably less than that of acetaldehyde, and light did not affect the rate of accumulation (T. W. Kimmerer and T. T. Kozlowski, manuscript in preparation).

In the 14-ml tubes, saturation of acetaldehyde and ethanol appeared to occur within 4 h of incubation. However, because the sample volume withdrawn for chromatography (1 ml) was large relative to the tube volume, kinetics of accumulation could not be examined in detail. We found that 4 h was an adequate incubation time for ethanol and acetaldehyde accumulation to be measured, but at least 8 h were required for accurate estimation of ethane production.

The data presented thus far are for gas-phase hydrocarbons from head space samples. While ethylene and ethane can be expected to be entirely in the gas phase under the conditions of our experiments, the same cannot be said for acetaldehyde and ethanol. Pure acetaldehyde boils at 21°C, while ethanol boils at 78.5°C. At 25°C, the temperature of our fumigations and incubations, most of the acetaldehyde could be expected to boil off, and little ethanol would accumulate unless diffusion of acetaldehyde away from the leaf was inhibited. At lower temperatures, much greater accumulation of both metabolites could be expected. Table III shows the results of extraction of pine needles in comparison with the gas-phase production of ethanol and acetaldehyde. While acetaldehyde and ethanol were produced by the stressed plants prior to enclosure in the incubation tubes, there was relatively little accumulation of the products due to volatilization of the acetaldehyde. At lower temperatures, however, both compounds do accumulate to an appreciable degree (Table III).

Responses of Other Species to Stress. We surveyed a number of crop and woody plants to determine whether acetaldehyde and ethanol accumulation are common responses to stress in plants by using the point freezing assay of Elstner and Konze (5). As shown in Table IV, a number of species and cultivars produced these compounds under freezing stress. There was no clear correlation between taxonomic relationships and production of the stress metabolites, nor did the plants that produced them under one kind of stress always produce them in response to other stresses (data not shown).

DISCUSSION

SO_2 stress, as well as a number of other stresses and injuries, induced production of acetaldehyde and ethanol in addition to increasing production of ethylene and ethane. Acetaldehyde and ethanol are not normal products of plants in aerobic conditions, and our results suggest a substantial alteration of respiratory metabolism in stressed plants. Measurement of these two compounds in the gas phase is relatively easy and allows simultaneous determination of these compounds as well as two other important stress metabolites, ethylene and ethane. Gas chromatographic measurement of these four compounds may be a useful, sensitive method for evaluating plant stress, particularly SO_2 stress. Unlike ethylene and ethane, acetaldehyde and ethanol appear to be produced only under stress conditions; we have never detected ethanol or acetaldehyde production by unstressed plants in any of

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our experiments.

In pine seedlings stressed with SO_2, acetaldehyde and ethanol production began very soon after the start of fumigation, in the absence of any visible injury. Removal of the plants from the SO_2 atmosphrere into clean air did not result in immediate cessation of production of these compounds (Fig. 2). SO_2 stress evidently causes lasting metabolic changes in leaf cells even at fumigant concentrations which do not cause visible injury. Production of these compounds may have serious consequences for the plant: while it is unlikely that toxic levels of these metabolites accumulate in stressed plants at temperatures above the boiling point of acetaldehyde, the loss of acetaldehyde and ethanol vapors may be a significant loss of fixed carbon from the plant. Since the products volatilize, it is unlikely that product inhibition could occur, and biosynthesis of these compounds might then be unregulated. Moreover, glycolysis may be competing with the TCA cycle and reducing the rate of ATP synthesis per mol glucose consumed. At lower leaf temperatures, it is possible that toxic amounts of either acetaldehyde or ethanol could accumulate.

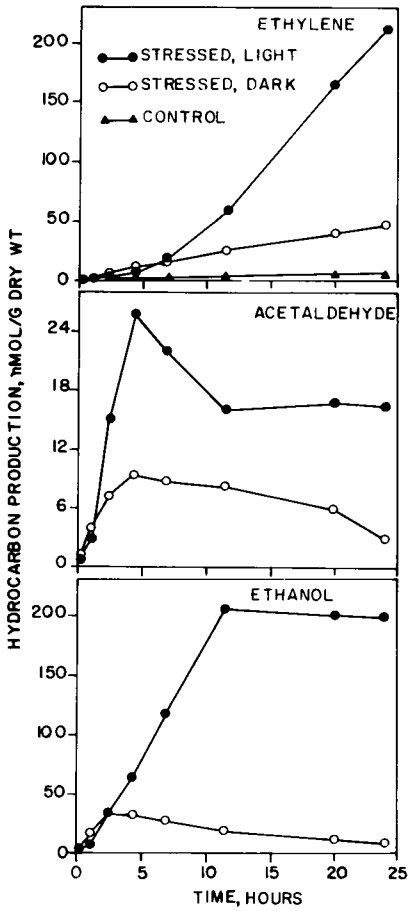

FIG. 6. Rate of accumulation of ethanol, acetaldehyde, and ethylene in 60-ml separatory funnels containing 8-month-old red pine seedlings which had been fumigated with 0.2 $\mu l\ l^{-1}$ SO_2 for 8 h. Seedlings were incubated in the dark or under a HID light providing 350 $\mu E\ m^{-2}\ s^{-1}$ (PAR) at 25°C. At each sampling time, 1 ml air was injected into the flask and 1 ml head space gas was withdrawn for GC. Control seedlings produced no ethanol or acetaldehyde. Ethane accumulated at a linear rate for the entire incubation, and the rate was not affected by light.

The production of ethanol evidently requires living cells, as does ethylene biosynthesis (1). Figure 5 shows that as necrosis increased above 80%, the production of these compounds declined. This is similar to the results of Elstner and Konze (5) who found that ethylene production by freezing-stressed plants declined when more than 50% of the leaf was necrotic. The reason for the higher threshold for declining production of the gases in our experiments may be the timing of the injury assessment; we measured necrosis after the incubation and measurement of gas production. There may have been more living cells at the beginning of the incubation, producing ethylene and the other gases, and becoming necrotic during incubation as a consequence of the initial stress. The production of acetaldehyde is presumed to require living cells, but the evidence is somewhat contradictory. In SO_2-stressed birch seedlings, severe necrosis was accompanied by a decline in acetaldehyde production (Fig. 5). However, in water-stressed birch seedlings, severe water deficits which resulted in a decline in the production of ethylene and ethanol did not cause a concomitant decrease in acetaldehyde production (Fig. 4).

We have assumed that the source of both acetaldehyde and ethanol in stressed plants is glycolysis, with stress somehow affecting either the uptake of O_2 or promotion of pyruvic decarboxylase activity at the expense of pyruvic dehydrogenase activity. It is possible that there is another source of acetaldehyde in plant cells. Lipid peroxidation *in vitro* can lead to production of several aldehydes, including acetaldehyde (13). If SO_2 causes extensive lipid peroxidation, as Peiser and Yang (12) claimed, then acetaldehyde may be evolved. Since ADH is a constitutive or inducible enzyme in the cytoplasm of plant cells (4), the production of acetaldehyde by lipid peroxidation would lead to ethanol synthe-

Table III. *Acetaldehyde and Ethanol in Gas and Liquid Phases following 4 Hours Fumigation of Red Pine Seedlings with 1.0 $\mu l\ l^{-1}$ SO_2*

Half the seedlings (10 plants) were incubated for 8 h in 14-ml tubes, the head space gas was assayed, and the shoots were frozen in liquid N_2, ground to a fine powder, and extracted with H_2O at 15°C. After filtering, 5 μl of the extract were injected on the GC. The remaining seedlings were left for 4 h at 15°C (five plants) or 25°C (five plants) in clean air, then frozen and extracted as above without incubation in the tubes.

Sample	Gas Phase		Liquid Phase	
	Acetaldehyde	Ethanol	Acetaldehyde	Ethanol
	nmol/g fresh wt		*μmol/g fresh wt*	
A. After 8 h incubation				
Control, 25°C	0	0	Trace	0
Stressed, 25°C	3.4 ± 0.8	46.3 ± 3.6	0.7 ± 0.1	14.2 ± 4.6
B. Prior to incubation				
Control, 25°C			0	0
Control, 15°C				
Stressed, 25°C			0.6 ± 0.1	0.3 ± 0.1
Stressed, 15°C			7.8 ± 0.1	118.3 ± 13.4

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Table IV. *Production of Volatile Hydrocarbons by Herbaceous and Woody Plants*

Plants were stressed by freezing 30% of the leaf area. Data are given in nmol/cm^2 leaf area, except for *Pinus halepensis*, for which nmol/g dry weight is presented. Each value is the mean of two determinations on two replicate samples. Standard errors were less than 10% of the mean for controls and up to 30% of the mean for stressed plants.

Species	Treatment	Ethylene	Ethane	Acetaldehyde	Ethanol
Zea mays	Control	1.37	3.12	0	0
	Stressed	1.92	21.85	0	0
Cucurbita maxima	Control	12.48	0	0	0
	Stressed	4.12	1.23	0	0
Pisum sativum	Control	0.98	0.07	0	0
	Stressed	1.76	2.67	3.25	1.33
Brassica oleracea	Control	21.06	0.78	0	0
	Stressed	5.73	3.45	Trace	Trace
Tagetes erecta	Control	5.07	0.19	0	0
	Stressed	4.12	19.53	40.64	107.73
Haemanthus katherinae	Control	44.72	0.13	0	0
	Stressed	187.64	2.42	44.32	120.37
Platanus occidentalis	Control	2.21	0.13	0	0
	Stressed	5.23	9.62	Trace	Trace
Ulmus americana	Control	9.63	0.12	0	0
	Stressed	20.96	1.34	Trace	0
Eucalyptus camaldulensis	Control	5.12	0.48	0	0
	Stressed	7.93	0.95	36.72	194.68
Quercus macrocarpa	Control	0.62	0.07	0	0
	Stressed	6.52	0.21	9.26	18.74
Pinus halepensis	Control	0.53	0.06	0	0
	Stressed	5.42	0.74	42.32	186.24

sis. We consider this less likely than an origin of both compounds from glycolysis for two reasons: (a) the quantities of both acetaldehyde and ethanol produced are quite large, and it seems unlikely that a minor product of lipid peroxidation could provide this amount of either compound; and (b) ethane is known to be derived from peroxidized membrane fatty acids, and if acetaldehyde is derived from the same source, the time of peak production of both compounds should coincide. This was clearly not the case (Figs. 2–4). This evidence is somewhat circumstantial and needs to be confirmed by more direct methods.

In plants under anaerobic conditions, the production of acetaldehyde and ethanol may be a result of a decline in cytoplasmic pH due to accumulation of organic acids leading to activation of pyruvic decarboxylase (4). This requires that the control of cytoplasmic pH be overriden by a sufficiently large accumulation of acid species. This may also happen in SO_2-stressed plants, since dissolution of SO_2 in H_2O produces acid products (3). If this is the case, acetaldehyde production will begin as soon as pyruvic decarboxylase is activated by reduced pH, and ethanol production will begin immediately if ADH is present, or when ADH is induced or activated by acetaldehyde. However, it could be expected that cytoplasmic pH would recover soon after SO_2 was removed from the air, since the acid products of SO_2 are highly reactive and could be expected to disappear rapidly from the cytoplasm (3). This was not the case, as shown by the persistent production of both acetaldehyde and ethanol in clean air (Fig. 2). Moreover, there is no compelling evidence that the other stresses which induced acetaldehyde and ethanol production would lower cytoplasmic pH.

Ethane production was increased by SO_2 in our experiments, but not until lesions appeared (Fig. 2). This occurred considerably later than other signs of stress, including increased ethylene production and the onset of acetaldehyde and ethanol production. If ethane synthesis were closely linked to the toxic effects of SO_2 by the peroxidation of lipid membranes, as suggested by Peiser and Yang (12) and by Bressan *et al.* (2), ethane production could reasonably be expected to increase well before the appearance of lesions, perhaps even before the increase in stress ethylene production. The disruption of membranes by peroxidation of constituent lipids could then increase membrane permeability, lead to loss of metabolic control, and ultimately cause cellular necrosis. This clearly was not the case with the woody plants we studied. Rather, ethane production accompanied the formation of necrotic lesions resulting from a variety of stresses (Table I; Fig. 2). In woody plants, then, ethane appears to be a product of the death of cells regardless of the cause of death and is not an important product of stress metabolism. Monitoring of ethane in woody plants under stress may only be useful as a simple, objective measure of necrosis, which is not always easy to assay visually, especially when the extent of injury is small.

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