Observations on the physiological mechanisms and chemical constituents of induced oleoresin synthesis in *Pinus resinosa*[1]

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The stimulation of lightwood formation (oleoresin soaking) in *Pinus* resinosa Ait. treated with the bipyridylium salt, paraquat (1,1′-dimethyl-4,4′-bipyridylium dichloride), affected various physiological parameters. Respiration, transpiration, and ethylene synthesis were markedly increased and were correlated with the increased production of rosin and turpentine. Photosynthesis. however, was severely inhibited, and phloem transport was not necessary for oleoresin deposition. These marked changes in function reflect a typical wounding response. Ethylene (supplied as Ethrel (2-chloroethylphosphonic acid)) was capable of replacing the stimulatory effect of paraquat, with a 14-fold increase in rosin over controls and a 25-fold increase in turpentine. This increase in turpentine content was accomplished by a significant enhancement in ß-pinene content. Ethrel-treated trees also showed an increased abietic acid content, with a concomitant decrease in levopimaric acid.

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Pinus resinosa Ait. traité avec du paraquat (sel de bipyridylium) (1 ,l′-diméthyl-4,4′bichlorure de bipyridylium) influeçait divers paramètres physiologiques. La respiration, la transpiration et la synthèse d'éthylène étaient augmentées de façon marquée et correspondaient à la production accrue de colophane et d'essence de térébenthine. La photosynthèse était cependant grandement inhibée et le transport de phloème n'était pas nécessairement pour le dépôt d'oléorésines. Ces changements prononcés de fonction reflètent une réponse typique à une blessure. L'éthylène (fourni comme Ethrel (2-acide chloro-éthylphosphonique)) était capable de remplacer l'effet stimulant du paraquat, avec une augmentation de 14 fois de colophane sur les témoins et une augmentation de 25 fois d'essence de térébenthine. Cette augmentation du contenu d'essence de térébenthine avait été obtenue par une amélioration significative de contenu de ß-pinène. Les arbres traités à I'éthrel ont également montré un contenu élevé d'acide abiétique, avec une diminution concomitante d'acide lévopimarique.

[Traduit par le journal]

Introduction

The deposition of oleoresin[3] in the wood matrix is a universal phenomenon in Pinus species. This oleoresin-laden wood, also known as lightwood or fatwood, is the result of aging (heartwood formation) or the response of the tree to the stress of wounding. An increased synthesis and deposition of oleoresin can also be stimulated by the systemic application of the bipyridylium salts, paraquat and diquat (Roberts 1973), or the ethylene-releasing compound 2-chloroethylphosphonic acid (Wolter 1977).

The objectives of this research were (*i*) to examine the physiological parameters associated with oleoresin induction when paraquat is systemically applied to red pine, specifically, to describe response to treatment of such physiological functions as respiration, transpiration, ethylene production, photosynthesis, and moisture retention, (*ii*) to verify that ethylene *per se* can induce oleoresin synthesis, and (*iii*) to compare the chemical composition of the induced oleoresin with normal oleoresins.

Materials and methods

General

Red pine (*Pinus resinosa* Ait.) was used in all studies. The physiolopical parameters to be investigated necessitated the use of different sizes and ages of material. In experiments on transpiration and solution uptake, 2-year-old terminal branch material from 15-year-old plantation-grown red pine was used. Clonal 3-year-old trees, representative of one genotype, were used in ascertaining the effects of paraquat treatment on respiration, and on ethylene and oleoresin syntheses with respect to time. Nonclonal, 20-year-old plantation-grown trees (located at Crandall Plantation, Wisconsin Dells, WI) were used in translocation studies, oleoresin synthesis, and analysis of chemical constituents.

Method of treatment

A 0.05% stock solution of paraquat was prepared by 1/2000 dilution of commercial paraquat-C1 concentrate. (Commercial paraquat–Clcontains 29.17% (12 mg/100 mL) of paraquat dichloride (1,1′-dimethyl-4,4′-bipyridylium dichloride).) Ethrel stock solutions from 1 to 10% were prepared by a dilution of commercial Ethrel concentrate (commercial Ethrel contains 21.38 2-chloroethylphosphonic acid (CEPHA), 30.8 g/100 mL). Aliquots of the stock solutions were systemically introduced into the vascular system through a frill wound one-third the circumference of the tree, from a reservoir attached on the bole of the tree (Fig. 1). All treatment applications were made in the same manner except those on transpiration studies, in which solution uptake was through the cut branch ends, and extractive analysis studies. In addition to the different treatment concentrations, amounts of a given concentration were varied with the diameter of the tree. These optimal concentrations were determined from preliminary experiments (Wolter and Zinkel 1976; Wolter 1977). Time of treatment and number of replication varied with each experiment (see specific experimental procedure and result section).

Chemical analysis

Wood samples (treated in May) for chemical analysis were from in the field at time of fall harvest. The frozen samples were subsequently cut into small pieces and coarsely ground while still frozen in a Wiley mill (< No. 20 mesh). The ground wood was thoroughly

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[3]An oleoresin consists of a resin and an essential oil. In pine oleoresins, the volatile essential oil is known as turpentine and the processed resin is called rosin.

mixed to provide a homogenous sample for diethylether extraction (Zinkel 1975). The ether was distilled from the extract in a vacuum evaporator and the nonvolatile extractives dried overnight in a vacuum oven at 105°C for weighing. Turpentine content and composition were obtained by analyzing an aliquot of the ether extract by gas–liquid chromatography (GLC) on a Carbowax 20-m column: diethyl carbitol was used as the internal standard (Anonymous 1971).

Detailed analyses of the nonvolatile extractives were made by methods of Zinkel (1975). The identity and quantitative composition of the resin and fatty acids were determined by GLC on an EGSS-X column (Supelco Inc.); resolution of methyl levopimarate - palustrate was achieved by GLC analysis using a Silar 10C column as per Zinkel and Engler (1977).

Transpiration

Terminal branches (with needles fully formed) from 15-year-old red pine were placed in several concentrations of aqueous paraquat solutions. Uptake of the solutions or distilled water (controls) was monitored over 24 days. Results are reponed on a millilitre uptake per milligram needle dry weight basis. All data were collected under ambient laboratory conditions.

Respiration, ethylene synthesis. and cell viability

Matched 5-year-old clonal red pine (with shoot and needles fully extended) was used for evaluation of these physiological parameters. All trees were treated with the 0.05% stock solution of paraquat delivered in 100-mL aliquots (12 mg/tree of paraquat dichloride) to the second internode above the root collar. Two replicates each of treated and control trees were selected at random and harvested 3, 7, 14, 28, and 56 days after treatment. Extractives content, ethylene synthesis, respiration, and moisture content were measured on samples obtained from treated and control trees at positions one-third (a) and two-thirds (9) (Fig. 1) along the third internode above the root collar (i.e., the internode above the internode that was treated). All determinations were done on the day of harvest except when incubation was necessary.

Samples for measurement of respiration, ethylene synthesis, and nonvolatile extractives content were taken at one-third and two-thirds of the designated internode (Fig. 1). Approximately 50 cross-sectional slices of 0.5 mm thickness were cut from each area. Ten slices were randomly selected for each determination. Respiration rates were measured in a Gilson respirometer at 15-min intervals during a 2-h period. Care was taken to insure that all samples had the same number of exposed surfaces. Oxygen consumption was calibrated on a dry weight basis. Because of the lethal effect of the paraquat OR living tissue (Birchem *et al.* 1979). data were corrected to reflect the proportion of the living cells remaining in the sample. The proportion of viable cells was obtained by using the coloration response of 2,3,5-triphenyltetrazolium chloride (indicating dehydrogenase activity, Feist *et al.* 1971) on the entire radial face of the samples. Radial linear measurements of the zone of remaining living cells across the diameter of these disks (Fig. 1) were converted to a factor representing the proportion of living cells in the entire cross-sectional area $\left(\frac{\pi d^2}{4}\right)$. This correction factor was used to modify oxygen consumption data.

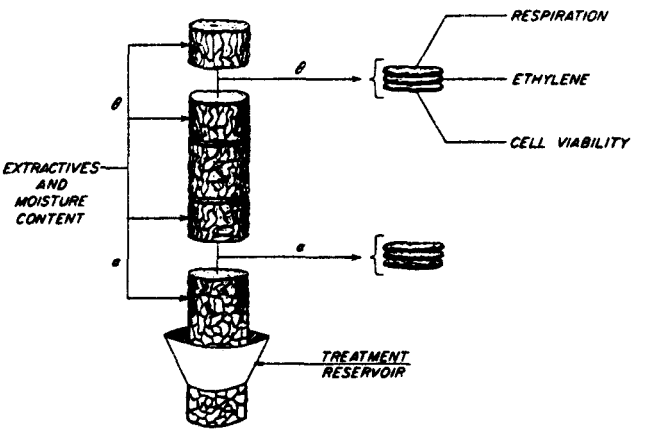

FIG. 1. Schematic sampling procedure. Samples were taken one-third (a-position) and two-thirds (θ-position) along the internode. At each a- and 9-position, samples were analyzed for volatile and nonvolatile extractives, moisture content. respiration, ethylene content. and cell viability.

Ethylene production measurements were by methods adopted from Shain and Hillis (1973) as modified by Nelson (1978). Slices were incubated for 24 h at 31°C and ethylene concentrations analyzed by gas chromatography on activated alumina using standard ethylene - air mixtures for calibration.

Moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight - extractive-free dry weight}}{\text{Extractive-free dry weight}} \times 100$$

Photosynthesis

Net photosynthesis efficiency was tested on a 3-year-old nonclonal red pine (18–24cm in height) rather than larger clonal 5-year-old material because of constraints of the growth chambers. Experimental setup and measurement procedures were those of Gorden and Gatherum (1969). The trees were matched for height, diameter, and crown size, and tests were made after extension of terminal needle growth had ceased in autumn. A gradient of paraquat concentrations was tested (0.0–1.0% in 50-mL aliquots) as well as matched water controls. The lowest levels of paraquat require too long a period for any physiological response and were rejected. The highest concentration of paraquat inhibited photosynthesis completely after 3 h. Therefore, an intermediate level (0.05% per 50 mL, 6 mg/tree) was selected. Constant volume photosynthetic chambers were maintained at a temperature of 25°C and an intensity of 5000 foot candles (53 820 1x). Carbon dioxide uptake was measured with an infrared gas analyzer (Gorden and Gatherum (1969)). Needles were stripped from the individual trees and dried to a constant weight. Two trees were run for each treatment and control.

Transport

To establish if lightwood formation is the result of mobilization of precursors from the entire tree or is a localized phenomenon. the xylem from each of six matched 20-year-old red pine trees was physically separated from the phloem transport system. In May, when the phloem could be separated easily from the mature xylem, multiple incisions parallel to the vertical axis of the trees were made on the internode at breast height. The phloem and cambium were separated from the entire xylem portion of the internode and isolated by a nonpermeable sheet of plastic (Fig. 2). This procedure (Wolter 1977) resulted in segregating the entire xylem portion of the internode from the phloem and cambium. The phloem and cambium were intact at both ends and contiguous with the rest of the phloem transport system. The internode was then reassembled with the intervening plastic sheath and covered with grafting compound to prevent dehydration. Three replicates were either treated with distilled water or paraquat solution (0.05% in 300-mL aliquots, 36 mg/tree) two internodes below the segregated xylem. The trees were harvested 6 months after treatment. The internode at the segregated xylem and the xylem in the

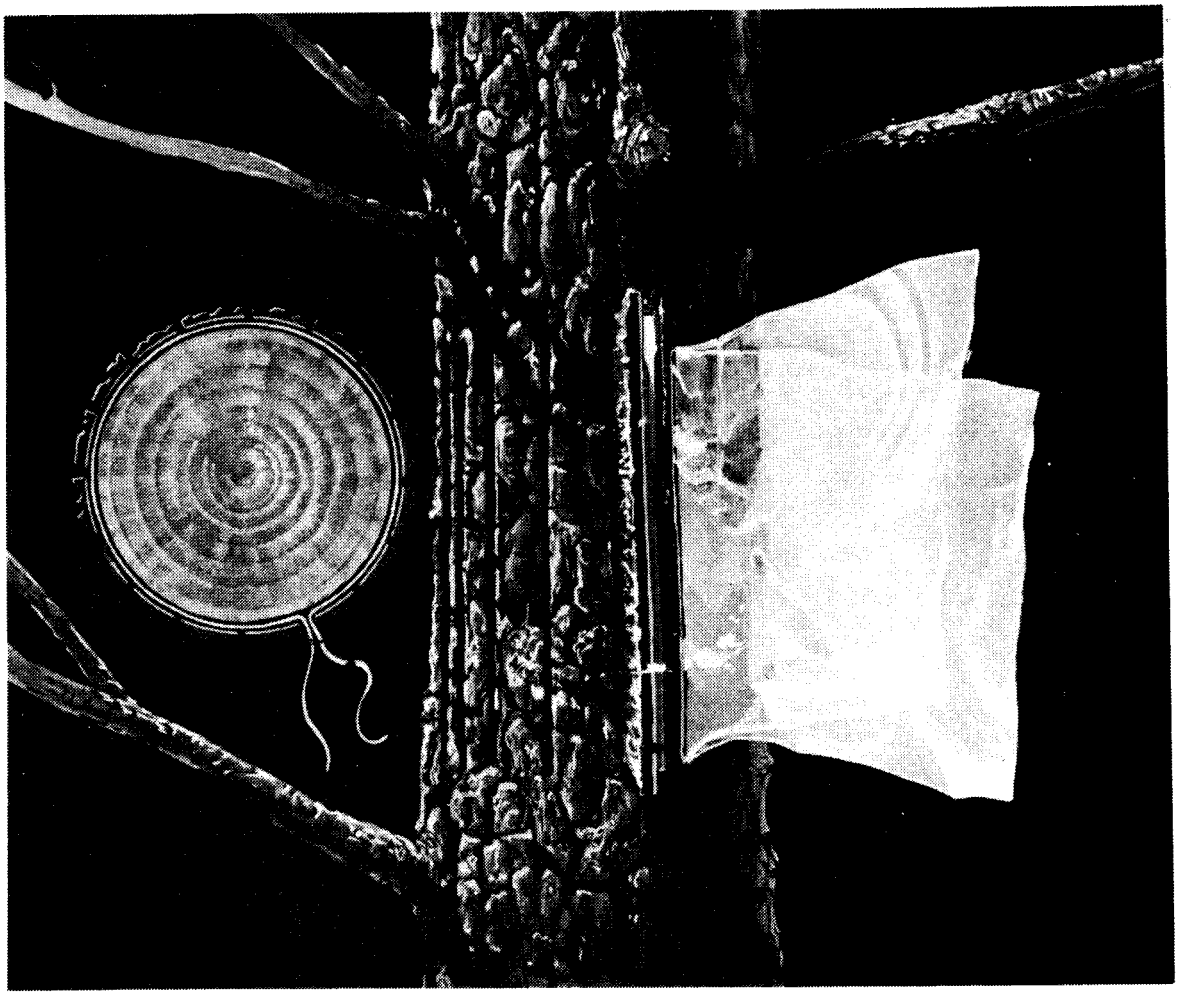

FIG. 2. Schematic diagram showing method of segregating xylem from phloem and cambial tissue with a plastic barrier. The cross section shows that the entire xylem portion of the internode was isolated from the phloem transport system.

internodes directly above and below were analyzed for nonvolatile extractives.

Results and discussion

Physiological parameters

Lightwood, by definition, is oleoresin-impregnated wood. Because oleoresin is about three-quarters resin, and the resin is a major constituent of the nonvolatile extractives, the determination of nonvolatile extractives has sufficed in many lightwood studies as an estimate of treatment response. Thus, the simple nonvolatile extractives measurement has been used in this study to correlate changes in certain physiological factors in lightering induced by paraquat.

In the 5-year-old clonal red pine treated with 0.05% (12 mg/tree) paraquat, an increase in nonvolatile extractives was initially observed at 7 days after treatment (Fig. 3). The increase continued for the duration of the experiment (56 days). As expected, the extractives content was higher at the a-position than at the θ-position. No increase in extractives occurred in control trees.

Evaluation of ethylene synthesis from samples, matched with the extractive data (Fig. 3), indicates a marked elevation in ethylene at the a-position for samples harvested 7–14 days after paraquat treatment followed by a rapid decline (Fig. 4). A slight increase occurred at day 7 for the 8-position but then rapidly declined. The degree of ethylene synthesis is positively correlated with the subsequent increase in oleoresin. Ethylene synthesis in controls showed a moderate decrease over time. The small initial ethylene synthesis may have been due to the wounding that was a common denominator in control trees as well as treated trees.

A similar pattern was observed in the respiration data; i.e., the maximum respiration was at day 7 (Fig. 5). A moderate decrease in respiration took place in control trees for both sample positions. This background decrease of respiration is most likely caused by either the normal tapering off of metabolic activity as spring shoot elongation moderated or by the initial effect of wounding. The data are consistent with the concept that paraquat induces a typical wounding response which is manifested by increased respiration and ethylene synthesis and suggest that ethylene may trigger the stimulation of oleoresin synthesis. This physiological change occurred just prior to and concurrent with the increase of nonvolatile extractives (Fig. 3). The lower amount of extractives in the higher θ-internode position is also reflected in the reduced amount of ethylene synthesis in this tissue. Increased respiration rates were also detected by Birchem *et al.* (1979) in suspension cultures of slash pine exposed to paraquat treatment.

These observations on respiration and ethylene synthesis are consistent with the hypothesis that an increase in nonvolatile

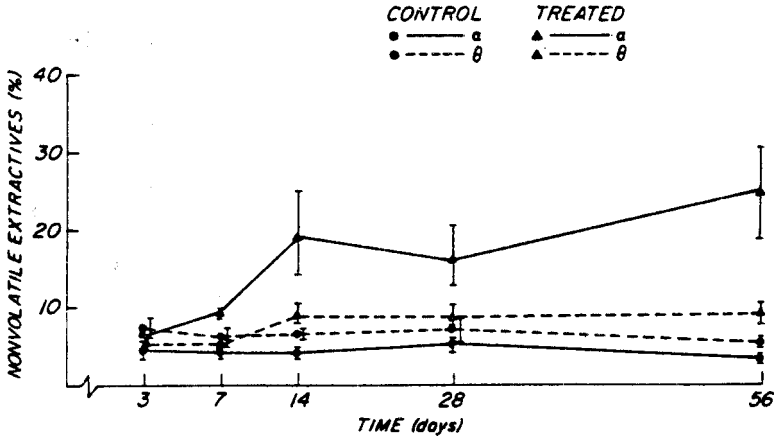

FIG. 3. Effect of paraquat on percentage of nonvolatile extractives in treated and control trees harvested 3, 7, 14, 28, and 56 days after treatment. Each point is an average of two trees; bars represent one standard deviation from the mean.

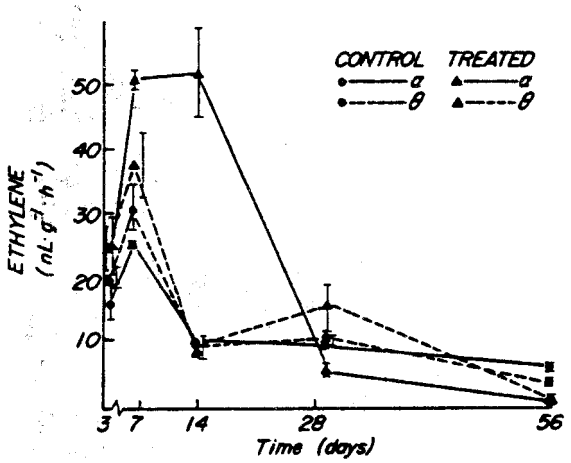

FIG 4 The effect of paraquat on ethylene content at two internode positions for control and paraquat-treated trees harvested 3, 7, 14, 28, and 56 days after treatment Each point is the average of two trees, bars represent one standard deviation from the mean

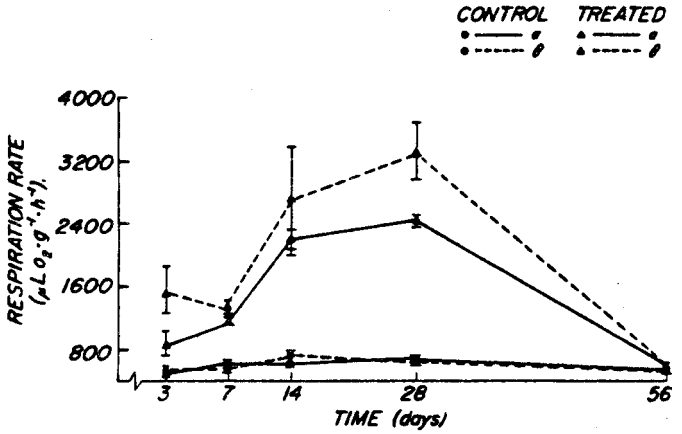

FIG. 5. Effect of paraquat on respiration at two positions for control and paraquat-treated trees harvested 3, 7, 14, 28, and 56 days after treatment. Respiration rates were corrected for percentage of living cells. Each point is the average of two trees; bars represent one standard deviation from the mean.

extractives in paraquat-treated trees is closely correlated with stimulation of ethylene synthesis and heightened respiration rates. Both physiological functions are typically associated with cell-wounding response and stress (Abeles 1973). In *Pinus* species, this response results in an increase in oleoresin under natural stress conditions. Tests using an ethylene-releasing compound (Ethrel) instead of paraquat indicated that this growth hormone was indeed able to stimulate oleoresin synthesis in more mature trees (Wolter 1977). Further tests on 20-year-old red pine treated with 1, 3, 5, and 10% solutions of Ethrel confirmed these results (Wolter 1977). In general, maximum lightering was obtained with 5% Ethrel at 15 cm above the point of application. Concentrations lower than 3% did not affect lightwood formation. Higher concentrations (10%) stimulated only below the point of treatment and caused crown necrosis as well. Verification of these results (Table 1) confirms the large 14-fold increase in nonvolatile extractives and 25-fold increase in turpentine as a result of 5% Ethrel treatment for three control and four treated trees.

Photosynthesis

Prolonged increase in photosynthetic efficiency with paraquat was not detectable; instead, paraquat inhibited photosynthesis completely even at the 0.05% (6.0 mg/tree of active ingredient) level within the 250-h experimental period (Fig. 6). At higher concentrations, inhibition occurred after 3 h. It should be noted that the external appearance of the crown was still normal after 250 h at the 0.05% level. Therefore, external necrosis observed on paraquat treatment occurred long after photosynthesis had ceased. This work confirmed the observation of photosynthetic shutdown made by Ryan and Schwarz (1 979) who reported that photosynthetic capacity (as measured by detached needle assay) ceased after 30 days in paraquat-treated 3-year-old Virginia pine.

The initial increase in photosynthetic efficiency shown by both treated and control trees is attributed to the normal adjustment in photosynthetic rate, reflecting transfer of the trees to the chambers with new lighting intensities and CO_2 concentrations. From the parameters tested here and other data, paraquat (at these levels) apparently does not stimulate photosynthetic efficiency, and it appears unlikely that increases in photosynthates are responsible for oleoresin synthesis.

Transpiration

Observations on the uptake of solutions from the treatment

TABLE 1. Composition (milligrams per gram ovendry extractive-free wood) of extractives and turpentines in 5% Ethrel-treated red pine[a]

	Nonvolatile extractives								
			Fatty acids						
Sample[b]	Total	Resin acids	Total	Free	Esterified	Nonsaponifiables	Turpentine[c]	Oleoresin[d]	Resin acids: turpentine ratio
C	30.5	6.4	19.3	0.3	19.0	3. I	1.6	8.0	4.0
C	35.2	10.7	18.7	1.0	17.7	1.7	3.5	14.2	3.1
C	25.0	6.2	15.9	0.4	15.5	2.0	1.8	8.0	3.4
Average	30.2	7.7	18.0	0.6	17.4	2.3	2.3	10.1	3.5
E	122.3	87.1	21.0	5.4	15.6	6.8	42.8	139.8	2.1
E	154.5	116.9	19.0	2.3	16.7	9.6	73.5	190.4	1.6
E	120.8	89.4	21.6	2.5	19.1	7.7	54. I	143.5	1.7
E	141.2	108.7	22.4	1.5	20.9	9.5	63.4	172.1	1.7
Average	144.7	100.5	21.0	2.9	18.1	8. I	58.5	159	1.8

[a]Whole disk samples taken at 15 cm above treatment of 20-year-old trees.
[b]C, control; E, Ethrel treated. Ethrel treatment was 5% in 300-mL aliquots (4.62 g CEPHA/tree) in May; trees were harvested in November.
[c]*a*- and ß-pinene composition is given in Table 3.
[d]Sum of resin acids plus turpentine (does not include the neutral diterpenes that are pan of the nonsaponifiable fraction).

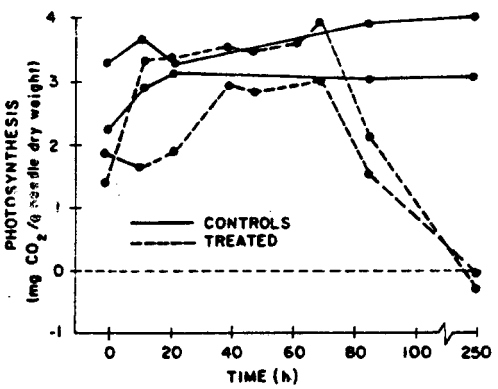

FIG. 6. Effect of paraquat on the net photosynthesis of each 3-year-old red pine treated with 0.05% (6 mg/tree) paraquat and monitored for 250 h. Matched control trees were also monitored for the duration of the experiment.

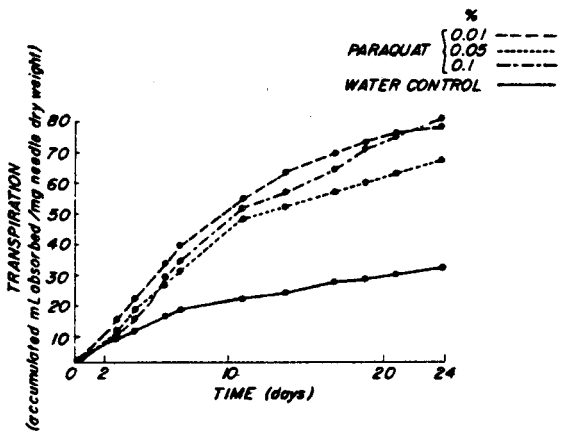

FIG. 7. Effect of paraquat on transpiration over 24 days following treatment with 0.01, 0.05, and 0.1% paraquat solutions and water. Each point is the accumulative average uptake for two branches; branches were harvested in August.

reservoir in all these studies indicated that paraquat solutions were always more rapidly absorbed into the xylem than either Ethrel or water. This was true for all sizes of specimens that were treated. This observation was verified in uptake studies with cut branches taken at the end of the growing season in August. The branches absorbed paraquat solutions much more rapidly over the 24-day experimental period in comparison to water controls (Fig. 7). In addition, there was a reduction in moisture content in the paraquat-treated branches, indicating the loss of water had to occur via transpiration. Preliminary investigation suggested that paraquat seems to affect stomatal apertures, permitting uncontrolled transpiration from the needle surface.

Transport

Carbohydrates, as a precursor for oleoresin synthesis, can either be provided by locally stored carbohydrates or transported via the phloem system from current photosynthates or other storage reserves. Experiments with paraquat-treated xylem isolated from the phloem transport system provided data regarding the potential source of these carbohydrates.

The average nonvolatile extractive contents of each of the three internodes from the six replicate-treated and control trees with physically isolated phloem and xylem are shown in Table 2. In both treated and control trees, the results showed that the isolated xylem portion was very high in extractive content in comparison to the internodes above and below. The paraquat-treated trees also showed an increase in extractives in the adjacent internodes, whereas the control trees contained the normal range of 3–4% extractives (Wolter 1977; Wroblewska *et al.* 1977). The very high extractive content in the segregated internodes in both treated and control trees probably was due to the effect of wounding of the xylem–phloem interface and the subsequent production of ethylene. Resealing the wounded area probably decreased the diffusion of endogenous ethylene from the wound site. The increased extractives content in this internode in control trees in excess of that in the treated ones implies that the endogenous wound response system (resulting in ethylene release) may be more efficacious than paraquat in this system in stimulating local synthesis of oleoresin. Since it is known that paraquat at certain concentrations can cause necrosis (Birchem *et al.* 1979), there may be fewer cells capable of oleoresin synthesis in the treated isolated internode.

The main objective of this experiment, namely to see if the phloem transport system is essential to oleoresin soaking (lightering), has been made clear. Oleoresin precursors do not

TABLE 2. Nonvolatile extractives content (percent, extracted dry weight basis) of xylem tissue physically separated from the phloem transport system after treatment with paraquat and water controls[a]

Relation to segregated xylem	Paraquat (0.05%)[b]		Control[c]	
	Nonvolatile extractives	Xylem moisture content	Nonvolatile extractives	Xylem moisture content
Below internode	6.9	25.7	3.9	46.4
At internode	12.8 (8.8)[d]	23.9	21.0 (16.4)[d]	37.7
Above internode	5.2	23.0	3.2	55.3

[a]Trees treated on May 6 and harvested October 10. Values are the average of six trees for treatment and controls.
[b]Paraquat was fed in 300-mL aliquots (36 mg/tree).
[c]Nonvolatile extractive content measured in similar nontreated trees ranged from 2.7 to 3.3% (Wolter 1977).
[d]Values in parentheses are after surface resin was removed.

TABLE 3. Turpentine composition (percent) of control and in 5% Ethrel-treated red pine[a]

Sample[b]	α-pinene	β-pinene	α:β
C	74.7	17.8	4.2
C	77.4	14.6	5.2
C	64.4	15.0	4.3
Average	72.2	15.8	4.6
E	60.4	30.7	2.0
E	58.7	31.4	1.9
E	54.6	41.2	1.3
Average	58.8	33.6	1.8

[a]Trees of Table 1.
[b]C, control; E, Ethrel treated.

apparently move via the phloem in this system nor is there a reduction of oleoresin content (from normal levels) in the adjoining internodes. This suggests that there is no mobilization of oleoresin from other immediate portions of the tree and that lightwood induction in this case is most likely dependent on in situ synthesis from carbohydrate sources. The lack of "vertical parenchyma" in this species (Kukachka 1960) also precludes movement of oleoresin within these cell types in the xylem.

Although there may be some increase in resin duct formation in the vertical direction (Wolter and Zinkel 1976) as a result of treatment, this apparently cannot account for the large amount of oleoresin deposition in cells not associated with resin canals. Miniutti (1977) and Sachs (1978) have described the mechanism of the physical movement of oleoresin into the wood matrix. They make it clear that the oleoresin in lightwood originates in the ray cells and moves via unaspirated pits into the tracheid lumens.

An understanding, as represented by the current literature, of the source of carbohydrates for oleoresin synthesis in lightering is incomplete. For example, Ryan and Schwarz (1979) found that there was an increase in starch levels above the point of treatment after 15 days and suggested that the starch reflected the localized accumulation of current photosynthate. Brown *et al.* (1979), however, found that blocking the downward translocation of current photosynthate does not prevent resin soaking of the xylem above the treatment site when blockage occurs 1.0 and 0.25 m above treatment. Blockage closer to the treatment site probably reduces resin soaking because of a reduction in the available local carbohydrates. Defoliation of the trees reduced the amount of oleoresin deposited; however,

TABLE 4. Comparison of turpentine content (milligrams per gram ovendry extractive-Free wood) and composition above and below the point of application in three Ethrel-treated red pine[a]

Distance from application (cm)	Tree 1	Tree 2	Tree 3	$\bar{x}$	α:β[b]
−15	6.1	7.9	7.5	7.2	1.6
+15	60.2	48.5	67.5	58.8	1.4
+25	22.8	40.5	54.1	39.2	1.4
+50	12.1	24.1	30.3	24.8	1.4
+100	6.2	5.9	8.1	6.7	2.2
+150	5.3	3.1	3.2	3.9	1.9
+15[c]					
Lightered portion			109.3		1.4
Nonlightered portion			4.1		2.9

[a]Treated in May and harvested October (300 mL of 5% Ethrel (11.25 g CEPHA) per tree). Data are for whole disk samples.
[b]Examples of α- and β-pinene determinations are for tree 3.
[c]Portions of adjacent cross section were analyzed separately.

the defoliated trees still produced 50% more oleoresin than wounded control trees that were not defoliated. These conflicting data can only be resolved by a complete carbohydrate balance study.

Effect of Ethrel on the content and composition of extractives

The lightwood response in the 20-year-old red pine to 5% Ethrel treatment, supplied in 300-mL aliquots per tree, is expressed in Table 1 as total nonvolatile extractives, resin acids, turpentine, and as reconstructed oleoresin (here the sum of resin acids and turpentine). The content of resin acids increased on an average of 14-fold over that of controls with turpentine increasing 25-fold. The relative increase in turpentine content of the oleoresins (decreased ratio of resin acids to turpentine) on lightwood formation is greater in red pine than has been observed in southern pine (Zinkel and McKibben 1978). .

In contrast with the role of oleoresins in stress response, fatty acids (free and esterified as triglycerides) are part of metabolic reserves of the tree. Although there have been conflicting repons on the fate of fatty acids in lightwood, the most reliable data indicated that little if any change occurs in total fatty acids, but that hydrolysis of the triglycerides increases with the degree of paraquat-induced lightering (Zinkel and McKibben 1978). The data of Table 1 suggest a small increase in total fatty acids accompanied by a small amount of triglyceride hydrolysis.

Accompanying the increase in turpentine to Ethrel treatment was a significant enhancement of $-pinene content in newly synthesized turpentine (Table 3). In another series of experiments with 20-year-old trees, the lightering response to 5% Ethrel treatments was evaluated by determining the content of turpentine (Table 4). Again, significant enhancement in ß-pinene content was obtained. These observations are in contrast to the findings of Wroblewska *et al.* (1977), who found a small increase in the relative composition of ß-pinene but little change for *a*-pinene in the turpentine from paraquat-treated red pine. The apparent lack of increase in ß-pinene in our previous work with red pine (Wolter and Zinkel 1976) is not necessarily significant owing to the short time following treatment and to the juvenile nature (5-year-old clonal material) of the trees.

Information on the composition of resin acids in lightwood often has been obscured by inadequate analytical methods. The

TABLE 5. Composition of resin acids (percent of total) in ethyl ether extractives of control and Ethrel-treated red pine[a]

	Resin acids							
Sample[b]	Pimaric	Sandaracopimaric	Levopimaric	Palustric	Isopimaric	Abietic	Dehydroabietic	Neoabietic
C	8.0	2.2	27.0	6.6	6.0	18.6	15.2	15.3
C	7.7	1.8	34.0	17.5	4.1	12.1	6.5	15.8
C	5.9	1.7	24.7	17.2	5.5	20.8	7.0	16.1
Average	7.3	1.9	28.6	13.8	5.2	17.2	9.6	15.7
E	5.6	1.5	10.6	17.1	5.6	33.0	2.7	22.5
E	5.2	1.6	11.6	15.5	6.2	34.5	2.4	22.4
E	4.7	1.6	7.3	15.9	7.1	36.6	1.4	24.8
E	4.2	1.6	10.8	14.8	6.4	33.3	1.7	26.3
Average	4.9	1.6	10.1	15.8	6.3	34.3	2.0	24.0

[a]Samples as in Table 1. Control resin acids contained trace amounts of communic acid (GLC identification); samples from Ethrel treatment contained an average of 0.5%. Small amounts of other minor components (total less than 1%) were not identified.
[b]C, control; E, Ethrel treated.

limited reliable evidence indicates that little change in resin acid composition occurs with paraquat-treated southern pines (Zinkel and McKibben 1978). Recalculation of the resin acid composition data (normalized to 100% for red pine of Wroblewska *et al.* (1977) reveals an apparent increase in levopimaric acid on paraquat-induced lightering and a decrease in abietic and most of the other resin acids. However, analysis of the resin acid composition in the extractives of control and Ethrel-treated trees here (trees of Table 1) shows a decided decrease in levopimaric acid with a large increase in abietic and neoabietic acids (Table 5). This observation has been supported in random analyses of other samples from Ethrel treatments. Although this pattern could be the result of partial acid-catalyzed isomerization of levopimaric acid, the effect is more likely a result of specificity in ethylene-induced synthesis of resin acids, analogous to the above observations for the a- and ß-pinenes.

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