HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE PUBLISHED BY THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

Volume 39, Number 6 · May, 1968

Photochemical Oxidant Injury and Bark Beetle (Coleoptera: Scolytidae) Infestation of Ponderosa Pine

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IV. Theory on the Relationships between Oxidant Injury and Bark Beetle Infestation

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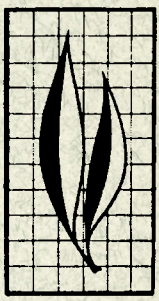

Certain aspects of insect-disease relationships, especially those concerning transmission of pathogens, have been studied extensively and their significance has been well established. However, the role of diseases as factors predisposing coniferous trees to bark beetle infestation has received only minor attention. There has been little effort to determine the extent of the association between disease and bark beetle infestation, the significance of predisposing diseases in the ecology of the beetles, or the effects of disease upon the host that may increase susceptibility to beetle attack.

The series of papers in this issue presents the results of studies to determine (a) the degree of association between photochemical atmospheric pollution injury to ponderosa pine and infestation by bark beetles (paper I), and (b) the changes in the physiology of diseased trees which might influence host susceptibility to bark beetles (papers II and III). The results show that oxidant injury does, in fact, predispose ponderosa pine to beetle infestation, and that the injury leads to physiological changes in the host which may be related to increased bark beetle susceptibility. The significance of these results in relation to the present knowledge on bark beetle ecology and host susceptibility is discussed in paper IV.

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III. Effect of Injury upon Oleoresin Composition, Phloem Carbohydrates, and Phloem pH[1]

INTRODUCTION

THE FIRST PAPER in this Hilgardia has shown that disease caused by chronic exposure to photochemical atmospheric pollution predisposes ponderosa pine, *Pinus ponderosa* Laws., to bark beetle (Scolytidae) infestations. The second paper has shown that the disease affects certain physiological properties of ponderosa pine which may be associated with increased susceptibility to bark beetles. Other possible effects that may be related to increased beetle susceptibility include changes in the chemical composition of oleoresin and/or phloem. These possibilities are strengthened by results showing an increase in resin crystallization with increased severity of disease (see paper II in this issue) and by recent studies indicating that various turpentine constituents differ in their effects upon bark beetles (Smith, 1966). Phloem pH has been suggested as a factor in bark beetle-host relations (Miller and Keen, 1960), and phloem carbohydrates may be important both in beetle nutrition and in host attraction (Person, 1931).

The present study was made to determine the effect of atmospheric pollution injury to ponderosa pine on (a) monoterpene and resin acid composition of oleoresin, (b) sugars and reserve carbohydrates of phloem, and (c) phloem pH.

METHODS

Ponderosa pines were studied in an area of the San Bernardino Mountains of southern California commonly subjected to atmospheric pollution. Samples for oleoresin, phloem carbohydrates and phloem pH analyses were collected in July, 1966. Additional samples were collected in February, 1967 for phloem carbohydrate analyses and in July, 1967, for oleoresin analyses. Trees in three disease classes, healthy, intermediate, and advanced, were included in the study. Descriptions of the disease classes and of the study areas are reported in the two previous papers.

Oleoresin analysis

Oleoresin samples were collected in two 0.5 dram, screw-cap vials positioned on opposite sides of each tree 1 to 1.5 m above the ground. The vials were inserted into 8 mm diameter holes extending into the xylem about 1.5 cm. After four to eight hours, the vials were collected and immediately capped. They were refrigerated during transit and stored in the laboratory at –10°C until analyzed. Monoterpenes and resin acids were analyzed by gas-liquid chromatography. An Aerograph Hy-Fi Model

[1]Submitted for publication June 15, 1967.

600C chromatograph was used in combination with a Sargent Model SR recorder. Quantities of individual compounds were calculated from integrated peaks using a disk chart integrator.

A column 3 m long with an inside diameter of 1.5 mm filled with B, B-oxydipropionitrile (10 per cent) on acid-washed Chromosorb W 60/80 was used for monoterpene analysis. The column was maintained at 65°C (flow rate of 10 ml/min for both H_2 and N_2). About 0.2λ of oleoresin was injected directly after diluting it 1:15 with carbon disulfide. A 15 per cent solution of isopropylbenzene in mineral oil was used as the internal standard to determine the total terpene content of each oleoresin sample (Mirov, *et al.*, 1966). Isopropylbenzene, with a retention volume of 4.4 relative to a-pinene, appeared on the chromatogram a few minutes after β-phellandrene.

A column 4.6 m long × 3.2 mm inside diameter, maintained at 220°C (flow rate of 40 ml/min for H_2 and N_2) and filled with 10 per cent DEGS (diethyleneglycol succinate) on acid-washed, dimethylchlorosilane-treated Chromosorb W 60/80, was used to determine the resin acid composition. The oleoresin was first methylated with an excess of diazomethane for 5 minutes at 0°C in a solution of 10 per cent methanol in ether. The resulting mixture was dehydrated with potassium hydroxide and decanted; the unreacted diazomethane and solvent were then removed by evaporation. The residue was dissolved in 2 ml ether, and 0.2λ was injected into the chromatograph. Resin acid composition was then determined by the method used for monoterpenes.

Carbohydrate analysis

Four phloem samples were collected from each tree in early evening by the method described in paper II for phloem moisture samples. Within three hours after collection, the samples were weighed to determine fresh weight, immediately placed in bottles containing 80 per cent ethanol and boiled for 20 minutes. The bottles were then sealed until analyses were made. Additional phloem samples were taken adjacent to those above for determination of percentage moisture content. These moisture contents were used to calculate the dry weight of the carbohydrate samples.

The samples, preserved in 80 per cent ethanol, were thoroughly macerated in a VIRTIS homogenizer. Samples collected in July were then transferred quantitatively to the cellulose cups of a Soxhlet extractor. Extraction with 80 per cent ethanol was completed after 24 hours. In February, samples were macerated as before but placed in a 200 ml centrifuge bottle and held for 20 minutes in a water bath at 100°C. The samples were then centrifuged, and the supernatant decanted and retained. The procedure was repeated three times to collect all sugars from the residue. The extracts were diluted to a constant volume with 80 per cent ethanol. Each extract was further diluted to obtain a quantity of sugars within an optical density range facilitating measurement with the anthrone test (Adams and Emerson, 1961).

The quantity of reserve carbohydrates, including both starch and nonstarch polysaccharides, was determined by extraction of the sugar-free residue with 52 per cent perchloric acid (Adams and Emerson, 1961; Hassid and Neufeld, 1964). The perchloric acid extracts were diluted with 80 per cent ethanol and reacted with anthrone reagent. Fresh anthrone reagent was prepared daily and standarized with known glucose concentrations.

An attempt was made to separate starch from nonstarch polysaccharides with the method described by Hassid and Neufeld (1964). However, inter-

ference from other materials in the samples precluded accurate analyses of the separate components.

The sample from each tree was analyzed three times. The results were expressed as average glucose equivalents for 80 per cent ethanol soluble sugars and 52 per cent perchloric acid extractable materials.

Phloem pH determinations

Four samples of phloem were taken from each tree at the time samples were collected for carbohydrate analysis. These samples were placed in containers with 25 ml distilled water immediately upon removal from the tree. All substances exuding from the phloem were thus collected in the water. After allowing the samples to stand in water for eight to 12 hours, the pH of the resultant solution was determined with a Beckman pH meter.

RESULTS AND DISCUSSION

Oleoresin analysis

Oleoresin samples from only healthy and advanced-diseased trees were analyzed. In 1966, the number of trees from which samples were analyzed for monoterpene constituents was limited to seven in each disease class because several of the diseased trees that were tapped failed to yield enough oleoresin for analysis. Oleoresin from 20 additional pairs of trees (one healthy and one advanced-diseased) was analyzed in 1967.

The combined results of the 1966 and 1967 analyses showed no significant differences between disease classes in any of the terpene components (table 1). Thus, we must conclude that atmospheric pollution injury does not lead to qualitative changes in the major terpene components of ponderosa pine sapwood oleoresin.

The monoterpene analyses did show a marked difference in the relative amounts of limonene and Δ^3-carene in all but one of the sampled trees compared to those reported for ponderosa pine from other areas (Mirov, 1961). This difference may indicate a unique popu-

TABLE 1

RELATIVE AMOUNTS OF MONOTERPENES IN THE XYLEM OLEORESIN OF HEALTHY PONDEROSA PINES AND PINES INJURED BY ATMOSPHERIC POLLUTION

Disease class	Monoterpene constituents of xylem oleoresin					
	α-pinene	β-pinene	3-carene*	myrcene	limonene	B-phellandrene
	Percentage of total terpenes†					
Healthy	10.3	50.3	Trace	11.2	27.4	0.6
(range)	(7–19)	(37–61)	....	(2–19)	(20–39)	(0.1–2.0)
Diseased	9.5	49.4	Trace	10.0	29.3	0.9
(range)	(7–16)	(33–65)	....	(2–18)	(13–44)	(0.1–2.0)

* Although the turpentine of ponderosa pine in other areas of the geographic range are composed of large amounts of 3-carene, only trace amounts of this terpene were detected in most of the trees included in our analyses. One tree, in which 22 per cent of the turpentine fraction was 3-carene, is not included in the table.

† Based on the average of 27 trees in each disease class. None of the differences between healthy and diseased trees were statistically significant.

TABLE 2

RESIN ACID CONSTITUENTS OF PONDEROSA PINE OLEORESIN FROM TWO HEALTHY TREES AND TWO TREES SEVERELY AFFECTED BY ATMOSPHERIC POLLUTION

Resin acid	Disease class				Relative retention volume*
	Healthy		Advanced-Diseased		
	Tree #1	Tree #2	Tree #1	Tree #2	
	Per cent				
Dihydropimaric	0.5	1.0	0.5	Trace	0.94
Pimaric	8.0	6.0	7.5	9.0	1.00
Tetrahydroabietic	5.0	4.5	6.0	4.0	1.11
Laevopimaric + Palustric	29.5	31.5	29.5	35.5	1.24
Isopimaric	16.0	22.0	20.5	15.5	1.38
Abietic	14.5	14.5	13.0	10.0	2.00
Dehydroabietic	4.5	3.4	3.5	6.0	2.07
Neoabietic	17.5	14.5	13.5	16.5	2.29
X†	3.5	3.0	4.5	2.5	1.45
Y†	1.0	Trace	1.5	1.0	1.82

* Retention volume relative to pimaric acid (= 30.5 minutes).
† Unidentified.

lation of ponderosa pines in southern California (also observed by R. H. Smith, personal communication).

Studies reported in paper II have shown that oleoresin crystallization apparently increases with increase in disease severity. Since resin acids may be important in determining rate and amount of crystallization, an analysis of these constituents was made (table 2). Samples from only two trees in each disease class were analyzed. The relatively small differences between disease classes cannot be considered significant. Except for the higher percentage of laevoprimaric + palustric acid and the lower percentage of abietic acid, the relative amounts of resin acids from the sapwood of ponderosa pine in southern California were generally similar to those from ponderosa pine heartwood (Riffer and Anderson, 1966).

Phloem carbohydrates

Samples were collected from nine healthy, ten intermediate- and ten advanced-diseased trees in July, 1966, and from 16, 18, and 25 trees in each disease class, respectively, in February, 1967. The results (table 3) indicate that chronic exposure to photochemical atmospheric pollutants may cause a reduction in the quantity of both soluble

TABLE 3

THE CARBOHYDRATE CONTENT OF PONDEROSA PINE PHLOEM IN RELATION TO SEVERITY OF SYMPTOMS CAUSED BY CHRONIC EXPOSURE TO PHOTOCHEMICAL AIR POLLUTANTS

Time of sampling and disease class	Carbohydrate content expressed as glucose equivalents	
	Soluble sugars	Reserve polysaccharides
	Mg/g dry weight	
July, 1966:		
Healthy	409 ± 12*	230 ± 20
Intermediate	364 ± 16	232 ± 13
Advanced	367 ± 14	174 ± 13
February, 1967:		
Healthy	331 ± 8	70 ± 5
Intermediate	305 ± 7	104 ± 7
Advanced	309 ± 3	83 ± 3

* 95 per cent confidence limits of the mean.

sugars and reserve polysaccharides. The difference between healthy and diseased trees appeared to be greater in summer than in winter, and the effect on soluble sugars appeared to occur earlier during symptom development than that on reserve polysaccharides. However, only the difference in reserve polysaccharides between intermediate- and advanced-diseased trees in summer was statistically significant (0.05 level of probability).

Results of analyses of the samples collected in February indicate a lower concentration of sugars and polysaccharides, especially the latter, than in July. These results do not agree with those of Hepting (1945) who, by using the takadiastase test, found that reserve carbohydrates (principally starch) in the "stem bark" of healthy shortleaf pine were higher in the winter. Possibly, the difference may be a reflection of the stress under which the trees in our study were growing.

Phloem pH

The pH of phloem samples from 12 ponderosa pines in each of the three disease classes was measured. The average pH and pH range were as follows:

Healthy
5.49 (5.30–5.70)

Intermediate-Diseased
5.59 (5.28–5:80)

Advanced-Diseased
5.51 (5.33–5.80)

Thus, there was no apparent effect of disease caused by photochemical atmospheric pollution on the pH of phloem exudates, nor any relation between pH and susceptibility of ponderosa pine to bark beetle attack. However, it should be emphasized that the phloem was not macerated and the pH, as measured, may not reflect possible changes in cell pH.

SUMMARY

Results of oleoresin analyses indicate that changes in relative proportions of monoterpenes do not occur in diseased trees; nor is there evidence of change in resin acids. Both soluble sugars and reserve polysaccharides were less in diseased trees, and lower in both healthy and diseased trees in winter than in summer. Reduction in quantity of sugars apparently occurred at an earlier stage of disease development than that of polysaccharides. Phloem pH did not appear to be affected. The significance of these results is discussed in paper IV of this Hilgardia.

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