Ethylene Production in Pinus radiata in Response to Sirex-Amylostereum Attack

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The authors gratefully acknowledge the technical assistance of A. N. Rozsa and Kerin M. Galbally and the statistical assistance of Nell Ditchburne. The Victorian Forests Commission kindly provided experimental material.

Accepted for publication 15 June 1972.

ABSTRACT

Ethylene production was demonstrated in the sapwood of *Pinus radiata* which was mechanically injured or attacked by the wood wasp *Sirex noctilio*. Ethylene produced by the symbiotic fungus *Amylostereum areolatum*, which is inoculated into the tree at the time of oviposition, was negligible. *Sirex* lesions 1 to 4 weeks old in dominant trees produced significantly more ethylene than mechanically induced lesions or controls (42.3, 13.3, and 2.5 × 10^{-9} liter C_2H_4/g dry wood per hr, respectively). Similar figures for a suppressed tree were 4.6, 9.0, and 2.2 × 10^{-9} liter C_2H_4/g dry wood per hr. Trees which produced large quantities of ethylene in response to attack produced greater quantities of inhibitory phenols at a faster rate. The amount of ethylene produced may be a convenient and rapid means of distinguishing a susceptible from a resistant response. If heritable, the ability to produce large quantities of ethylene in response to attack may be a valuable parameter in breeding for disease resistance.

Phytopathology 62:1407-1409

Additional key words: host resistance.

During oviposition, the wood wasp *Sirex noctilio* F. inoculates *Pinus radiata* D.Don with a mucus secretion (10) and arthrospores of the decay fungus *Amylostereum areolatum* (Fr.) Boldin (6). The response of sapwood of *P. radiata* to *Sirex* attack includes desiccation, resin soaking, and production of phenolic compounds (4). Developed lesions are necrotic and resin-soaked. Phenols usually are concentrated along the edges of the lesion forming a reaction zone (13) which precedes fungal invasion. A dry, metabolically active transition zone usually surrounds the reaction zone while the host is responding to attack (L. Shain & W. E. Hillis, *unpublished data*). Pinosylvin and its monomethyl ether were identified as the major phenolic compounds in *Sirex* lesions in *P. radiata* (7). These phenols were shown to inhibit a variety of decay fungi, including *A. areolatum* (3, 7, 12).

Ethylene is produced by a wide variety of herbaceous angiosperms during flowering and fruit ripening, and in response to injury and infection of succulent plant tissues (1, 11). This gas also has been implicated in playing an important role in the synthesis of phenolic compounds (2, 8).

We are unaware of any report that ethylene is produced by a gymnosperm. Few, if any, studies have been conducted on ethylene production by xylem tissues of trees.

The objectives of this study were to determine (i) if ethylene is produced by *P. radiata* and/or *A. areolatum*; and (ii) if present, how such ethylene production is related to the host response to *Sirex* and mechanically induced lesions.

MATERIALS AND METHODS.—In the summer of 1971, three multiple-stemmed trees of *P. radiata* were subjected to a controlled *Sirex* attack by insects with clipped wings. The trees, two dominant and one suppressed, were 17 years old and growing in the vicinity of Macedon, Victoria, Australia. The lesions which were 1 to 2 m aboveground, were marked immediately after oviposition. To test for differences in host response between *Sirex* attack and mechanical injury, artificial lesions were made with a hypodermic needle of approximately the same dimensions as the wasp's ovipositor. Insect and artificial lesions, as well as adjacent nonaffected tissue (controls), were collected at weekly intervals (usually two of each per week per tree) yielding lesions 1 through 4 weeks old. At the time of harvest, lesion development was rated regarding desiccation, resin soaking, and phenol production. The latter was based on the red coloration developed upon application of diazotized tolidine (13).

Xylem blocks (about 1 g dry wt) with cambial regions scraped off and containing lesions with or without their transition zones, and blocks of adjacent nonaffected tissue (controls), were collected and placed immediately into sealed containers. After 24-hr incubation at 30 (±0.5)C, a 2-ml sample of gas was withdrawn from each container and injected into a Varian 1400 gas chromatograph equipped with a flame ionization detector. The glass column (2 m long, 2 mm inside diam) was packed with 60-80 mesh, fully activated alumina (Coast Engineering Laboratory, Redondo Beach, Calif.). Flow rates of carrier gas (He), H_2, and air were 50, 24, and 250 ml/min, respectively. The oven temperature was 55 C.

A calibration curve for ethylene was prepared based on peak height. The relationship between ethylene concentration and peak height was linear on a $\log_{10}$-$\log_{10}$ scale. Confidence limits (95%) for individual determinations of ethylene concentration

were ±11%. Ethylene production by various tissues was assessed by an analysis of variance and a series of t-tests.

Tests to confirm the identity of ethylene included comparison of retention times with authentic ethylene as well as reactions with mercuric perchlorate and potassium hydroxide (1).

Because some fungi produce ethylene (9), it was necessary to determine whether the ethylene produced by *Sirex*-attacked sapwood was due to the activities of host and/or pathogen. This was done by: (i) comparing ethylene production by lesions with and without their transition zones; and (ii) monitoring the ethylene produced by growing and senescent cultures of *A. areolatum* (four isolates tested). Substrates for fungal growth included malt extract agar with and without milled, autoclaved sapwood of *P. radiata*. Another test was conducted to determine whether the fungus requires precursors in fresh sapwood to produce ethylene. Fresh blocks of sapwood were submerged in liquid nitrogen for 30 min, then thawed at room temperature. A previous test showed that blocks so treated lost their ability to produce ethylene. These blocks then were surface sterilized by a 5-sec dip into boiling distilled water and placed on mats of the fungus growing on malt extract agar in sealed containers. All cultures were incubated at 25 (± 0.5)C.

RESULTS.—A gas produced by the xylem tissues studied had the same retention time as authentic ethylene. This gas was adsorbed by mercuric perchlorate and released upon addition of dilute hydrochloric acid. It was not adsorbed by potassium hydroxide. These tests were taken to confirm the identity of ethylene (1).

Ethylene production and the development of *Sirex* and mechanical lesions are summarized in Table 1. Data for the dominant trees were combined as their responses were not significantly different. The influence of lesion age on ethylene production was not apparent in the 1- to 4-week-old lesions. Consequently, ethylene production for the two types of lesions was pooled separately.

Sirex lesions in both dominant and suppressed trees appeared similar after 1 week; both had distinct transition zones and slight resin soaking. The striking difference between crown classes at this time was in the amount of ethylene produced: *Sirex* lesions in the dominant trees produced about 10 times the amount of ethylene as those in suppressed trees, even though ethylene production by nonaffected controls was similar in both crown classes.

Sirex lesions in the suppressed tree after 3 weeks were characterized by intense resin soaking, low ethylene production, and no observable production of phenols. At the same time, lesions in dominant trees were not as resin-soaked, but they had produced substantial quantities of phenols and were still producing large quantities of ethylene (Table 1). After 4 weeks, traces of phenols were present in *Sirex* lesions of the suppressed tree. By this time phenols were rated as intense in the dominant trees.

Mechanical lesions were not as well developed as *Sirex* lesions. Transition zones were present at 1 week, but resin soaking was still slight at the end of the experiment. Slight concentrations of phenols were first observed at 3 weeks in the dominant trees only. Ethylene production was higher than that of the controls in both crown classes, but significantly lower than that of *Sirex* lesions in the dominant trees. No significant difference was detectable in ethylene production by *Sirex* and mechanical lesions in the suppressed tree.

Ethylene production by *Sirex* lesions in dominant trees with transition zones removed was extremely low compared with controls and lesions with transition zones. For example, lesions without transition zones produced from <0.05 to 1×10^{-9} liter C_2H_4/g dry wood/hr as compared to 2.5×10^{-9} for controls and 42.3×10^{-9} for lesions with transition zones. Furthermore, cultures of *A. areolatum* growing on malt extract agar with and without milled or fresh, frozen sapwood produced negligible quantities of ethylene. The higher quantities of ethylene sometimes produced by *Sirex* lesions, therefore, were attributable to host activity in the transition zone.

DISCUSSION.—The production of ethylene by the xylem of a gymnosperm has been established for the first time. It is noteworthy that this xylem tissue of *P. radiata* which contains so little parenchymatous tissue [nitrogen content ca. 0.05% of dry wt (L. Shain, *unpublished data*)] is capable of producing such substantial quantities of ethylene.

Ethylene has been implicated in triggering the synthesis of enzyme systems involved in the

TABLE 1. Ethylene production, resin soaking, and phenol production in *Sirex* and mechanically induced lesions in the sapwood of *Pinus radiata*

Crown class	Control	Tissue: *Sirex* lesions	Mechanical lesions
	10^{-9} liter C_2H_4/g dry wood per hr[a]		
Dominant	2.5 c	42.3 d	13.3 e
Suppressed	2.2 c*	4.6 ce	9.0 e*
	Resin soaking after 3 weeks - relative rating[b]		
Dominant	–	++	+
Suppressed	–	+++	+
	Phenols produced after 3 weeks - relative rating		
Dominant	–	++	+
Suppressed	–	–	–

[a] Ethylene produced after incubation for 24 hr by unaffected sapwood (control) and *Sirex* and mechanical lesions 1-4 weeks old. Means for dominant and suppressed trees are significantly different at the 1% level for *Sirex* lesions only. In dominant trees all means are significantly different from each other at the 1% level. In suppressed trees only control and mechanical lesions differ at the 5% level.

[b] Scale for recording relative rating: – = nil; + = slight; ++ = moderate; +++ = intense.

biosynthesis of phenolic compounds in excised plant tissue (8, 14). The capacity of lesions to produce ethylene clearly preceded phenol, but not resin, accumulation. This is in keeping with the hypothesis that resin and phenol accumulation are triggered by different processes (13).

The two dominant trees produced greater quantities of inhibitory phenols at a faster rate than the suppressed tree in response to injury and/or infection. It follows, therefore, that the dominant trees were displaying a more resistant response than the suppressed tree. These trees also were capable of producing greater quantities of ethylene in response to attack than the suppressed tree. This is in agreement with previous reports suggesting a positive relationship between ethylene production and disease resistance (2, 15). A negative relationship, however, also has been reported (5). The amount of ethylene produced in response to attack, therefore, may be a convenient and rapid means of distinguishing resistant from susceptible responses in some host-pathogen combinations. It is noteworthy that trees displaying the resistant response were stimulated to produce more ethylene by *Sirex-Amylostereum* attack than by mechanical injury.

An intriguing question is whether the suppressed tree produced less ethylene and a less resistant response, largely due to its suppression or to its genetic makeup. We are attempting to resolve this question by comparing responses of clonal material grown on good and poor sites. If heritable, the ability to produce large quantities of ethylene in response to biological attack may prove to be a valuable parameter in breeding for disease resistance. Other studies (L. Shain & W. E. Hillis, *unpublished data*) conducted over the past 18 months further suggest a relationship between ethylene and production of phenols. These include development of kino veins in *Eucalyptus* spp. and the seasonal course of ethylene production in relation to heartwood development.

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