Response of Young Peach Trees to *Ambrosiella sulphurea*, a Symbiotic Fungus of *Xyleborinus saxeseni*

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ABSTRACT

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Young peach trees were infected with *Ambrosiella sulphurea*, a primary ambrosia fungus of the ambrosia beetle *Xyleborinus saxeseni*, using two different inoculation techniques. Primary and secondary growth, flower bud set, and yield of peach trees were monitored. Percent discoloration in woody tissue was calculated and nutrient composition of peach leaves observed between treatments. Trees infected with *A. sulphurea* had 22% more secondary growth and 138% more discolored wood than control trees. Primary growth varied according to inoculation technique used. Yield and flower bud set were not affected by *A. sulphurea*. Nutrient composition of leaves from control and infected trees was similar for most minerals analyzed except Ca, Fe, K, Mg, and Mn, which were higher in leaves from infected trees than in those from control trees. These levels were within sufficiency ranges for normal peach tree development, however. Results from this study indicate that peach tree decline observed in association with *X. saxeseni* is not due to *A. sulphurea*.

Additional key words: peach diseases, peach insects, plant injury, wounds, *Xyleborus*

In 1982, numerous second-leaf peach trees (*Prunus persica* (L.) Batsch) located in commercial orchards in Florence County, South Carolina, were found to be in a weak or rapidly dying state. After examination, several species of ambrosia beetles were found infesting these trees and were suspected of causing the decline (12).

Ambrosia beetles obtain their nourishment from ambrosia fungi that are carried in specialized structures called mycetangia. During gallery formation, fungal spores are spread from this structure onto tunnel walls, infecting host plants (8). Ambrosia beetles carry two types of ambrosia fungi, primary and auxiliary (4). Primary ambrosia fungi are essential for growth and development of adult beetles and larvae. They are always present at the time of gallery excavation and are generally species-specific. *Ambrosiella* is an important genus of primary ambrosia fungi of ambrosia beetles found in the United States (3,9,14,18–20). *Ambrosiella sulphurea* Batra is the primary ambrosia fungus of *Xyleborinus saxeseni* Ratz. (3), the most common ambrosia beetle found in South Carolina peach orchards (12). Auxiliary ambrosia fungi may or may not be present in the mycetangium at the time of tunneling and may serve as secondary food sources for the beetles (2,15). Some auxiliary ambrosia fungi, such as *Ophiostoma ulmi* (Buism.) Nannf. (5,7,15), *Fusarium solani* (Mart.) Appel & Wr., *F. moniliforme* Sheld., and *F. lateritium* Nees (1,11,13,15,20), may cause wilt or canker disease in plants.

The purpose of this investigation was to study the response of young, healthy peach trees to *A. sulphurea* by observing the effect of this infection on peach tree growth, yield, nutrient level in leaves, and percent discoloration of woody tissue.

MATERIALS AND METHODS

Experiments, using two inoculation techniques, were used to study the effects of *A. sulphurea* on young peach tree growth and yield. *A. sulphurea* (ATCC 18095, American Type Culture Collection, Rockville, MD) was maintained on malt extract agar enriched with 1% yeast extract (3) and was the source of inoculum for both experiments.

In the first experiment, 100 2-mm-diameter holes were randomly drilled to the center of the trunk (about 50 cm high) of 40 healthy third-leaf peach trees (cv. Blake) to simulate tunneling activity of *X. saxeseni*. Before each hole was drilled, the bark and the drill bit were surface-sterilized with 75% ethanol. Holes were drilled in the spring (14 April 1984) to simulate mass attacks by emerging populations of ambrosia beetles. Twenty of the drilled trees were inoculated with *A. sulphurea* by inserting a toothpick containing a small block of fungus-covered agar into each hole. Toothpicks were twisted during insertion into the holes to mimic the ambrosia beetle behavior of smearing fungal spores onto gallery walls. Toothpicks containing agar alone were inserted into uninoculated trees in the same manner. Twenty trees were left uninjured to serve as controls.

Primary growth was measured as shoot length. Three shoots per tree, chosen before bud break, were measured by counting the number of leaves per shoot (10). The same three shoots per tree were monitored throughout the growing season. Secondary growth was measured as trunk circumference 15 cm above the soil. All measurements were taken twice a month from March through September during 1984 and 1985. Yield was measured as mean fruit weight at harvest.

Thirty leaves per tree were sampled in mid-July and dried to determine nutrient composition. Leaves were analyzed for K, Ca, Mg, Zn, Cu, Mn, and Fe by atomic absorption, for S terpidometrically, for P spectrophotometrically, and for N by Kjeldahl digestion.

To study the effect and response of younger peach trees to *A. sulphurea*, a second experiment was designed using a proven method of inoculation similar to that described by Paine (16). *A. sulphurea* was cultured in a 1% malt-yeast nutrient broth containing peach wood plugs 6.5 mm in diameter. The nutrient medium containing the wood plugs was sterilized in an autoclave before inoculation. The fungus was grown in shake culture in the dark at room temperature for 7 days. Wood plugs were then transferred to petri dishes containing *A. sulphurea* growing on 1% yeast-malt extract agar. These were cultured for an additional 7

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Table 1. Effect of *Ambrosiella sulphurea* on growth and yield of third-leaf peach trees after one growing season (measurements taken 11 September) using toothpick method of inoculation to simulate ambrosia beetle tunneling behavior

Treatment	Primary growth (leaves/shoot)	Secondary growth (circumference, cm)	Yield (mean fruit weight, g)
Control	43.7 a[z]	4.97 a	139.8 a
100 Holes without *A. sulphurea*	38.1 b	4.60 a	128.1 a
100 Holes with *A. sulphurea*	40.2 c	6.05 b	136.1 a
Standard error	0.75	0.22	6.39

[z] Means in each column followed by the same letter are not significantly different ($P \leqslant 0.05$), LSD.

Table 2. Percent discoloration in peach trees infected with *Ambrosiella sulphurea* using wood plug method of inoculation

Treatment	Area discolored (%)	Length of discoloration (cm)
Wounded trees without *A. sulphurea*	14.7 a[z]	4.2 a
Trees with *A. sulphurea*	35.0 b	10.8 b
Standard error	2.7	0.7

[z] Means in each column followed by the same letter are not significantly different ($P \leqslant 0.05$), LSD.

days. Control plugs were treated in the same manner except they were not inoculated with the fungus.

Three 7-mm-diameter holes were drilled about 7 mm deep into the trunk of 20 randomly selected second-leaf peach trees. Holes were drilled at the base of the tree near the soil line in the spring (24 April 1985). The bark was surface-sterilized with 75% ethanol, and inoculated or control plugs were inserted into the holes. Growth variables and nutrient content were determined as in experiment 1 except that flower bud set the following year (flower buds per centimeter of shoot) was recorded instead of yield, since second-leaf peach trees produce few fruit. Growth variables of 10 unwounded trees were also measured.

To assess the extent of discoloration of the young peach trees to *A. sulphurea* infection, the trees in experiment 2 were cut and sectioned at the end of the growing season and discolored areas were measured. Cross sections were made at the site of inoculation. Longitudinal sections were made perpendicular to growth rings when wood plugs were inserted. Discolored areas of cross-sectioned wood were measured planimetrically using a Zidas digitized computer board and percent discoloration was calculated. Discolored areas of longitudinal sections were measured from the center of the inoculation plugs to determine the length of discoloration. Leaf nutrient levels were measured as in experiment 1. All data were analyzed statistically and means separated using least square analysis ($P \leqslant 0.05$).

RESULTS AND DISCUSSION

Results of experiment 1 (toothpick method of inoculation) are shown in Table 1. Primary growth was reduced 13% by drilling 100 holes into the third-leaf trees. Secondary growth measurements from trees with holes but no fungus were similar to those from control trees. Trees inoculated with *A. sulphurea* showed only an 8% reduction in primary growth when compared with control trees. Infected trees produced 22% more secondary growth than control trees. This increase may be an overcompensatory response by the tree to outgrow damage caused by the fungus. This agrees with the observation of Raffa and Berryman (17) that the response of fir trees to mechanical injury was less pronounced than that of trees inoculated with the fungal symbiont, *Trichosporium symbioticum* Wright, of the bark beetle *Scolytus ventralis* LeConte. Drilling holes or infecting young peach trees with *A. sulphurea* had no effect on yield or return bloom.

No difference in primary growth or secondary growth could be observed between treatments during the second season after inoculation. Trees that were larger after one season of growth remained larger after the second season. Trees infected with *A. sulphurea* increased circumference by 10.20 cm over the 2-yr period, while control trees increased circumference by 8.76 cm. Most of this difference in secondary growth occurred during the first year after inoculation. Second-season yield data could not be obtained because of killing spring frosts in 1985.

Most nutrient levels from leaves of trees in experiment 1 were not affected by mechanical injury or fungal infection. Mineral differences did occur for Cu, Mn, K, and Mg. Mechanical injury to the trunks of peach trees reduced Cu levels 33%. Levels of Mn in leaves from mechanically injured as well as inoculated trees were 19% higher than in leaves from control trees. Inoculation of trees with *A. sulphurea* increased leaf nutrient levels of K by 9% and of Mg by 6%. Most of these mineral differences between treatments are considered insignificant, since all nutrient levels were within the sufficiency range for normal peach tree development (6).

Results of experiment 2 (wood plug method of inoculation) showed no differences in primary growth, secondary growth, or flower bud set. Even inoculating the three (7-mm-diameter) holes with *A. sulphurea* did not affect growth over one growing season.

Nutrient composition of leaves, from experiment 2 trees, for most of the elements analyzed showed no difference between treatments. Exceptions included P, which was 16% lower in leaves from mechanically injured trees than in those from control trees, and Mg, which was 16% higher in leaves from mechanically injured trees than in those from control trees. Leaves from trees infected with *A. sulphurea* contained higher levels of Ca (24% more), Mg (29% more), and Fe (34% more) than leaves from control trees. These differences in nutrient levels were greater than those from leaves of control trees but, again, probably had little effect on growth, since these levels were within the sufficiency range for normal peach tree development and were considered nontoxic.

Although no measurable change in growth was observed between treated and control trees in experiment 2, dissection of trees showed an increase in discoloration in trees inoculated with *A. sulphurea* (Table 2). Cross sections revealed 138% more discolored wood in trees infected with *A. sulphurea* and longitudinal sections revealed that this staining was 6.6 cm longer in infected trees than in uninoculated trees. This increase in discoloration may explain why infected trees had 22% more secondary growth. Peach trees may respond to *A. sulphurea* by plugging xylem tissue at the time of inoculation, resulting in more discolored wood. As the demand for water and minerals increases during the growing season, the demand for new xylem tissue also increases and the tree responds by increasing secondary growth. This response of the tree to increase discoloration does not seem to have any adverse effects on mineral uptake or primary growth over one season.

In summary, young peach trees respond to *A. sulphurea* infections by increasing wood discoloration and secondary growth without significantly reducing primary growth or mineral uptake. Leaf analysis from inoculated trees showed increases in Ca, Fe, K, Mg, and Mn, but none of these increases was large enough to have any great effect on normal peach tree growth or physiology. From the results of these experiments, it appears that any immediate decline observed in young peach trees infested with *X. saxeseni* is not due to its primary ambrosia fungus, *A. sulphurea*, but may be due to auxiliary ambrosia fungi associated with the beetles or to other environmental factors.

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