Role of Oleoresin Flow in Initial Colonization of Loblolly Pine by Southern Pine Beetle (Coleoptera: Scolytidae)[1]

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J. Entomol. Sci. 38(4): 576-582 (October 2003)

Abstract The influence of total resin flow in loblolly pine, *Pinus taeda* L., on initial colonization by the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), was investigated. Resin flow of trees was manipulated mechanically so that it followed the same pattern of decrease and near cessation as seen in trees successfully attacked by southern pine beetle. There were also intermediate treatments where resin flow was allowed to recover after near cessation. Beetles were introduced by means of mesh cages attached to the mid-bole of trees and left until the end of the longest wounding treatment. In trees where resin flow was reduced, significant increases in number of attacks, total gallery length, and length of gallery free of resin occurred.

Key Words Southern pine beetle, *Dendroctonus frontalis, Pinus taeda,* loblolly pine, oleoresin, defenses

It has been assumed from the earliest work on southern pine beetle, *Dendroctonus frontalis* Zimmermann, that the oleoresin system of southern pines is responsible for or is a major contributor to tree resistance. Levels of resistance are thought to be related to differences in chemical properties (Hodges and Lorio 1973, Hodges and Lorio 1975, Coyne and Lott 1976, Hodges et al. 1985) and physical properties (Anderson and Anderson 1968, Lorio and Hodges 1968a, Lorio and Hodges 1968b, Lorio and Hodges 1974, 1977, Hodges et al. 1977, 1979, 1985) of the oleoresin. Physical properties, such as oleoresin exudation pressure, total flow, rate of flow, viscosity, and rate of crystallization (resin hardening) are more important in conferring initial resistance than chemical properties (Hodges et al. 1977, 1979). Of the physical properties of loblolly pine oleoresin, total flow is related most closely to resistance to southern pine beetle. Although total resin flow in loblolly pine has been shown to be the most important factor in determining resistance to southern pine beetle, there is a complex interaction of factors including physical and chemical properties of oleoresin, nutritional status of the phloem, and associated microorganisms that changes during the growing season that affects resistance (Lorio 1993, Raffa et al. 1993, Nebeker et al. 1993).

In order for southern pine beetle to successfully invade a tree, the flow of oleoresin

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must be significantly reduced. Southern pine beetle attacks typically occur en masse with an ensuing decrease in oleoresin exudation pressure and flow (Hodges et al. 1979). The resulting decrease of resin flow following mass attack has been attributed to water stress due to girdling of the tree by the beetles and tracheal blockage by associated fungi (Lorio and Hodges 1974, 1977, Fares et al. 1980, Hodges et al. 1985). However, it has been demonstrated that water potential may not change for as many as 10 to 30 days after the decrease in oleoresin pressure and flow (Hodges et al. 1979). Thus, it appears that depletion of the oleoresin is due simply to the mass wounding of the tree by the beetles during the initial attack.

Several studies have been conducted to investigate the role of resin flow in conferring resistance to attack by southern pine beetle (Lorio and Hodges 1977, Raffa and Berryman 1982, Cook and Hain 1987, Nebeker et al. 1988, 1991, 1992, 1993). However, a direct relationship between resin flow and tree resistance was not demonstrated because it has not been possible to directly regulate total resin flow in individual trees. We have developed a method of variably decreasing resin flow from temporary cessation to that which follows the same pattern as occurs in a successful southern pine beetle attack (i.e., within 3 days after mass attack by southern pine beetle resin flow ceases almost completely [Hodges et al. 1989]). The results of using this methodology to measure the effect of resin flow in loblolly pine, *Pinus taeda* L., on initial colonization of southern pine beetle is reported herein. We hypothesized that with a decrease in resin flow from pretreatment levels, i.e., mass attack, more southern pine beetle attacks would occur, longer egg galleries would be constructed, and there would be less resin in the egg galleries that were constructed.

Materials and Methods

The loblolly pine trees used in this study were on International Paper Company land located near McCool, MS. In 1992, the mean height of trees was 19.05 m, mean diameter breast height (dbh) was 19.81 cm, and mean height to first live limb was 10.36 m. In 1993, the mean height of trees was 19.20 m, mean dbh was 22.35 cm, and mean height to first live limb was 10.52 m.

Wounding trees with a radial increment hammer to the xylem surface simulated southern pine beetle attack. This produced a wound slightly larger than that inflicted by an adult southern pine beetle. Wounding was calculated at a rate of 2 wounds per 100 cm^2 of total bole area, from the base to the first live limb. For the first 3 d, one-third of the calculated wounds were made each day. After the third day, additional wounds were inflicted at the rate of 15% of the number calculated to simulate boring activity after mass attack. The wounds were unevenly dispersed along the bole with the majority of wounds around mid bole and below. Our preliminary experiments demonstrated that this wounding schedule produces a pattern of resin flow comparable to that observed during a natural southern pine beetle attack.

Resin flow measurements were taken the day before wounding treatments began and then every 3 d after initiation of wounding up to and including the last day of wounding for each treatment. Measurements were performed by punching a 2.54-cm diam hole through the corky bark and phloem to the xylem surface. Care was taken not to score the xylem surface. After punching the hole, a triangular-shaped piece of aluminum with edges folded upward (P. L. Lorio, Jr., unpubl.) was placed under the hole in a groove cut into the bark. This directed the flow of resin into a disposable scintillation vial for collection and measurement. The scintillation vial was attached to the tree using a fencing staple. The amount of resin was measured in grams. Two samples were taken on opposite sides of each tree at each collection interval. The first samples were taken at 1.22 m, and each successive sample was taken 5 cm to the right and 5 cm above the previous sample.

Beetles were introduced using mesh cages attached to trees on day 3 of the wounding schedule. Each cage was 60 × 91 cm and was attached at mid-bole with the 60 cm side vertical. Each cage was wrapped around the tree and closed by means of a velcro strip, facilitating introduction of beetles in the cage. Duct tape was used at the top and bottom of each cage so that it fit snugly in the crevices of the bark.

Beetles were obtained from natural infestations on the Noxubee National Wildlife Refuge in Winston Co., MS. Bark containing late-instar larvae and pupae was stripped from infested trees, transported to our laboratory, and the beetles reared to the adult stage. After emergence, adult beetles were placed in cold storage (no more than 3 d at 4°C) until sufficient numbers were collected for the experiment. In 1992, beetles were sexed using the pronotal mycangia to identify females (Wood 1982). In 1993, we simply assumed a 1:1 sex ratio and did not sex the beetles.

Wounding treatments and numbers of beetles introduced differed between years. In 1992, there were 2 wounding treatment durations (3-, and 6-day with a control); 50 pairs of beetles were introduced to each tree. In 1993, there were 3 wounding treatment durations (3-, 6-, and 12-day with a control); 200 pairs of beetles were introduced to each tree. In 1992, treatments began on 21 July and in 1993 treatments began on 8 June. A sample size of 5 trees was used for all treatments in both years.

All trees were felled the day following termination of the longest wounding treatment. The section of bole with the cage was removed and placed in cold storage (4°C) until measurements could be taken. Total number of attacks was determined by counting the number of entrance holes that reached the inner bark. Total gallery length and length of gallery free of resin were determined by locating an entrance hole and scraping the bark away until the entire length of the gallery was exposed. Total length and length of clear gallery were then measured.

Total gallery length and length of gallery free of resin data were analyzed by analysis of variance using SPSS ANOVA command (SPSS 1990). Time-series analysis was used to analyze resin flow data using SPSS multivariate analysis of variance (MANOVA) command. Where ANOVA or MANOVA indicated significant differences, the least significance difference procedure (LSD) was used to separate means. Data for total number of attacks were analyzed using chi-square. For all analyses, $P = 0.05$.

Results and Discussion

Our results demonstrate that total resin flow in loblolly pine is a major factor in deterring attack and conferring resistance to southern pine beetle. In 1992, the pattern of resin flow after wounding was similar to that observed during southern pine beetle attack. Wounded trees had significantly reduced resin flow (Hotellings $F = 2.73$; df = 6, 34; $P = 0.028$) throughout the treatment period (Table 1). In 1993, the pattern of resin flow after wounding was also similar to that observed during southern pine beetle attack. There were significant differences in resin flow (Hotellings $F = 2.73$; df = 6, 34; $P = 0.028$) among treatments throughout the treatment period. The trees wounded for 3 days had recovered to control levels by day-6 (3 d after wounding was stopped). The trees wounded for 6 d did not recover to control levels even 6 d after wounding had stopped; however, the resin flow was near pretreatment levels. The

Table 1. Mean ± (SEM) weight (gms) of resin collected from wounded loblolly pine trees at varying days after wounding

Days after initiation of treatments	Treatment			
	Control	3 Day	6 Day	12 Day
		1992		
−1	2.76 ± 0.90a	1.97 ± 0.49a	2.17 ± 0.52a	
3	1.54 ± 0.89a	0.58 ± 0.18b	0.12 ± 0.06b	
6	2.08 ± 0.64a	0.49 ± 0.28b	0.54 ± 0.18b	
		1993		
−1	1.19 ± 0.46a	0.65 ± 0.13a	1.40 ± 0.26a	0.68 ± 0.13a
3	0.91 ± 0.26a	0.19 ± 0.09b	0.21 ± 0.08b	0.08 ± 0.06b
6	0.79 ± 0.20a	0.70 ± 0.24a	0.01 ± 0.00b	0.23 ± 0.12b
9	1.10 ± 0.26a	1.10 ± 0.35a	0.25 ± 0.14b	0.02 ± 0.00c
12	1.43 ± 0.25a	1.22 ± 0.26a	0.78 ± 0.26b	0.05 ± 0.05c

Means within rows followed by the same letters are not significantly different at the $P = 0.05$ level.

slight increase in resin flow of the control treatment may have been due to daily variation. However, the results for the intermediate wounding treatments (3-day in 1992; 3- and 6-day in 1993) are not as clear and are opposite of our stated hypothesis. This may be explained in part by the large variation associated with resin flow measurements (Tisdale and Nebeker 1992).

Resin flow is important in imparting resistance or lack of resistance to attack. An example of lack of resistance to attack is in lightning-struck trees that are frequently attacked by southern pine beetle. Lorio and Bennett (1974) found that in August 1965 alone, 77% of the infestations included lightning-struck trees. Blanche et al. (1985) described the changes in bark beetle susceptibility indicators in lightning-struck loblolly pine. They reported that no oleoresin exudation could be detected in the lightning-struck tree for at least 3 d following strike. Eleven days after the strike, a small amount of exudate was obtained. The flow was about one-tenth of that before the strike on the eleventh day, but after 21 d flow had increased and was about 2.3 times as high as before the strike. Thus, we can see that trees colonized by bark beetles just after being struck, in essence, do not have to deal with the physical and chemical properties of the resin to successfully colonize the tree.

Tree resin flow varies in time. In 1992, resin flow was about 3 times greater than in 1993, yet gallery lengths were also greater in that year. This can best be explained in terms of growth-differentiation theory (Lorio 1986). The 1992 trial was performed in late-July when growth was beginning to slow, vertical resin duct formation was beginning (i.e., more oleoresin), and photosynthates were in surplus because of the reduced growth. The 1993 trial was performed in early June when growth was greater, there was less oleoresin, and less photosynthates were available in the inner bark. The differences in resin flow reported here are within the normal range for the times of year they were measured (Lorio and Sommers 1986) and does little to change the relative resistance of the trees to attack by southern pine beetle (Lorio et al. 1990).

There were significant differences in total gallery lengths (F = 8.62; df = 2, 190; P = 0.0003) and lengths of gallery free of resin (F = 26.45; df = 2, 190; P < 0.001) among wounding treatments in 1992. Total gallery lengths were significantly longer (P = 0.05) in the 6-day wounding treatment (18.84 ± 1.67 cm), than the control and 3-day treatments (9.12 ± 0.94 and 11.92 ± 0.83 cm, respectively). The total gallery length for the 3-day treatment was also significantly greater (P = 0.05) than the control. The 6-day wounding treatment had the greatest amount of clear gallery (9.49 ± 1.58 cm) and was significantly different (P = 0.05) from the 3-day (1.88 ± 0.18 cm) and the control (0.36 ± 0.18 cm) treatments. The 3-day and control treatments were not significantly different in clear gallery.

There were also significant differences in total gallery lengths (F = 22.09; df = 3, 550; P < 0.001) and lengths of gallery free of resin (F = 24.36; df = 3, 550; P < 0.001) among wounding treatments in 1993. The 12- and 6-day wounding treatments had the longest mean (±SEM) total gallery lengths (9.46 ± 0.66 and 8.83 ± 0.61 cm, respectively), while the control treatment had the shortest (1.0 ± 0.14 cm). The mean total gallery lengths of trees in the 3-day treatment were intermediate (4.49 ± 0.36 cm). The 12- and 6-day wounding treatments had the longest mean (±SEM) lengths of gallery free of resin (8.84 ± 0.67 and 7.94 ± 0.62 cm, respectively), while the control treatment had the shortest (0.1 ± 0.04 cm). Mean length of gallery free of resin for trees in the 3-day treatment were intermediate (3.34 ± 0.36 cm). In all cases the gallery length and resin free area of the gallery were significantly (P = 0.05) longer in the 6- and 12-day treatments, they were not significantly different from each other. There was also a significant difference (P = 0.05) between the controls and the 3-day treatment for total gallery length and length of gallery free of resin. The longer galleries constructed in 1992 were probably the result of the higher levels of photosynthate available as an energy source for the beetles (Hodges and Lorio 1969).

Table 2. Number of southern pine beetle attacks in response to resin flow treatments

	Attack	
Treatment	Yes	No
	1992	
Control	42	208
3 Day	72	178
6 Day	79	171
	1993	
Control	24	976
3 Day	170	830
6 Day	193	807
12 Day	167	833

There was a significant differences in the number of attacks in both 1992 and 1993 (χ^2 = 16.17, df = 2, n = 250, P < 0.01, χ^2 = 149.89, df = 3, P < 0.001, n = 1000, respectively) among treatments (Table 2). The least number of attacks occurred in the control trees in both years. This would be expected because the southern pine beetle had to overcome the resin flow. Mass attack by southern pine beetle and subsequent reduction in resin flow is necessary for rapid successful colonization of southern pine beetle (Nebeker et al. 1993). The constitutive defensive (resin) system is important in deterring and pitching out adult southern pine beetle, and, although not tested here, we would suggest it is also likely involved in antibiosis of eggs and larvae. These results further corroborate our earlier suggestion (Nebeker et al. 1992) that tree improvement programs consider the resin production capabilities of families selected for planting to reduce the impact of southern pine beetle during the rotation period.

Acknowledgments

We thank Richard Brown, Sonny Ramaswamy, and John Schneider for helpful comments on this manuscript. Funds for this project were provided in part through the Mississippi Forestry and Agricultural Experiment Station, USDA Competitive Research Grants Program Grant No. 91-37302-6767 and USDA Forest Service Cooperative Grant No. 19-92-039. Approved for publication as Journal Article No. J-10133 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University. With regret Dr. Tisdale passed away while completing the revisions of this manuscript and represents some of his last works to be published.

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