

RHODODENDROL AND SUSCEPTIBILITY TO THE BRONZE BIRCH BORER¹

by Frank S. Santamour, Jr.

Abstract. Rhododendrol is produced in the inner bark of stressed and dying branches of most white-barked birch species by natural hydrolysis of the glucoside rhododendrin. Rhododendrol was identified by thin-layer chromatography and was synthesized by sodium borohydride reduction of the commercially available ketone. Mated female bronze birch borers (but not males or un-mated females) exhibited a short-range attraction to the synthetic rhododendrol. It is postulated that this compound may be the primary stimulus to oviposition in stressed trees with dying cambial tissue. Interspecific hybrids between rhododendrin-producing species (e.g. *Betula populifolia*) and the borer-resistant *B. nigra*, which does not contain rhododendrin, showed only traces of rhododendrol in hydrolyzed bark but produced another phenolic compound that was not found in either parent species. Studies of this unknown compound and further hybridizations with *B. nigra* to develop whiter-barked, borer-resistant birches are continuing.

Résumé. Le rhododendrol est produit dans l'écorce interne de branches stressées ou mortes de la plupart des espèces de bouleaux à écorce blanche par l'hydrolyse naturelle de glucoside de rhododendrine. Le rhododendrol était identifié par chromatographie en mince couche et était synthétisé par la réduction du commercialement utilisé kétone à l'aide de borohydrure de sodium. Les femelles fécondées de l'agrile du bouleau (mais pas les mâles ou les femelles non fécondées) présentaient une attraction à courte portée au rhododendrol synthétique. Il est considéré comme admis que ce composé peut être le stimulant primaire à l'oviposition dans les arbres stressés avec un tissu cambial mourant. Les hybridations interspécifiques entre les espèces productrices de rhododendrine (ex. *Betula populifolia*) avec l'espèce résistante aux perceurs qu'est *B. nigra* qui ne contient pas de rhododendrine montrait seulement des traces de rhododendrol dans l'écorce hydrolysé mais produisant un autre composé phénolique qui ne se trouvait pas chez l'une ou l'autre des espèces parentes. Les études sur ce composé inconnu en plus des hybridations avec *B. nigra* afin de développer des espèces de bouleaux à écorce blanche résistantes aux perceurs se poursuivent.

The bronze birch borer (*Agrilus anxius*) is the most important limiting factor to the successful long-term cultivation of white-barked birches (*Betula* sp.) in landscape plantings. None of the commonly planted birch species or cultivars, with the exception of *B. nigra* (river birch), is inherently resistant to this pest (6, 7, 8). Neither the underlying

causes of resistance and susceptibility nor the particulars of individual host selection are known at the present time.

Certainly, host selection has little relationship to the feeding preferences of the adult beetles. Slingerland (11) observed feeding on willow and poplar foliage in the field and on elm leaves under cage conditions. Barter (3) noted a distinct preference for leaves of *Populus tremuloides* over birch when both were offered to caged insects. Likewise, the congregation of adult beetles on certain host birches as a result of longdistance pheromonal communication can be ruled out (1).

On the other hand, it has long been recognized that successful borer attacks (oviposition, larval mining in the cambial zone, and subsequent adult emergence) occurred most often in trees with observed or incipient branch dieback brought about by environmental or mechanical stress. In this regard, some of the observations of Barter (3) are especially pertinent. His experiments suggested that in addition to a phototropic response an "olfactory response is also involved in the choice of oviposition site," with preference for girdled or mechanically injured trees rather than uninjured trees. In studies of larval feeding, he noted that "only 36% of the larvae transferred to fresh cambium matured, compared with 100% for those transferred to browning cambium." Further, "if the cambium dies and turns brown too rapidly, development is prolonged considerably and mortality is high." Thus, dying cambial tissue (and inner bark) may be essential for oviposition and larval development.

The search for bark chemicals that might be related to borer resistance led Santamour and Vettel (9) to survey a wide range of birch species for the presence of rhododendrin and rhododendrol. Rhododendrin (bitter) and rhododendrol

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(tasteless) had first been isolated in 1901 (2) in studies of the diuretic and diaphoretic properties of the leaves of *Rhododendron chrysanthum*. Later work (11) on the bark of *Betula alba* (*B. pendula*) revealed similar substances (called "betuloside" and "betuligenol") but the work of Kim (4, 5) on the bark of *B. platyphylla* showed that the compounds from rhododendron and birch were identical. Rhododendrol was determined to be 4-(p-hydroxyphenyl)-butan-2-ol and rhododendrin was the glucoside of this phenolic alcohol.

Our thinking at the inception of the earlier study (9) was that the pharmacologically active rhododendrol might act as a deterrent to cambial mining by larvae of the bronze birch borer. Of 18 species examined, we found rhododendrin (analyzed as rhododendrol in hydrolyzed bark extracts) in all but *B. lenta* and *B. nigra*. In view of our inadequate knowledge of the host range of bronze birch borer in 1978, perhaps the conclusion that "there appears to be no correlation between the presence or absence of rhododendrin and attack by the bronze birch borer" was justified.

However, we now know that many of the birch species once considered resistant to the borer are susceptible, and the widespread planting of *B. nigra* 'Heritage' (8) has re-affirmed the borer resistance of that species. Thus, we have re-studied the rhododendrin-rhododendrol situation with the idea that one or both of these compounds might be the key to borer susceptibility, since the resistant *B. nigra* did not produce either compound.

Materials and Methods

Chemical Studies. At various times during May and June, 1988, branches (2 to 3 cm diameter) were collected from birches in our test plots and analyzed for rhododendrol. Some of the branch samples were from young trees that had not yet been attacked by the borer and some were from trees that had developed as basal sprouts of trees whose tops had been killed by borers. Bark samples were obtained from *B. nigra*, *B. nigra* 'Heritage', *B. papyrifera*, *B. platyphylla* var. *japonica*, 'Whitespire' Japanese birch (which may really be *B. populifolia*), *B. populifolia*, *B. pubescens*, and various hybrids involving *B. papyrifera*, *B. pendula*, *B. platyphylla* var.

japonica, and *B. populifolia*. In addition, one young seedling of *B. populifolia* x *B. nigra* parentage was sacrificed for analysis.

In our standard analytical procedure, as outlined earlier (9), five-gram samples of fresh bark were cut into small pieces and hydrolyzed in boiling 18% hydrochloric acid for 30 min. The hydrolyzate was extracted with ethyl acetate, the extract taken to dryness under vacuum, and the residue dissolved in methanol. The methanolic solutions were banded on commercial silica gel TLC (thin-layer chromatography) sheets and run in benzene-ethyl acetate (55:45V:V). Phenolic compounds were visualized by spraying the sheets with diazotized benzidine. Other analyses were made on "dying" bark of severed branches that had been kept on the lab bench for five or 10 days.

The silica gel from areas of the TLC plates that corresponded to the benzidine-stained zones were scraped from the plates and eluted in ethyl acetate. These eluates were taken to dryness and dissolved in methanol for re-chromatography and ultra-violet spectrophotometric analyses.

Rhododendrol was not available commercially, but we synthesized quantities (ca. one gram at a time) of this compound by sodium borohydride reduction of the ketone 4-(4-hydroxyphenyl)-2-butanone (Aldrich Chem. Co.).

Chemical Attraction Studies. Borer-infested birch logs were obtained from David G. Nielsen (OARDC, Wooster, OH) on 28 February 1989. Adult beetles were reared from bolts placed in emergence cages in the greenhouse at the National Arboretum at various times between 16 March and 16 April 1989. Male and female beetles were collected in individual shell vials, sexed, and tested separately (by sex) in an olfactometer after three different post-emergence times and treatments: (1) within three days after emergence—no feeding, (2) after feeding on willow and birch leaves for two or three days, and (3) after being maintained together (mated?) in a feeding-breeding cage for six to eight days.

Our olfactometer was a pyrex glass double drainline "Y" tube with a mainline diameter of 2 in and arm diameter of 1.5 in. The "top" of the main line was sealed with a layer of parafilm. In a standard choice test, 20 mg of synthetic rhododen-

drol (in methanol) was adsorbed on a 2 x 2 x 0.5 cm pad of cellulose sponge material and air-dried for two hours. The control sponge pad was treated with methanol. The pads were placed at the ends of the arms of the olfactometer between layers of nylon mesh affixed with rubber bands. After placing the beetles (varying numbers per test depending on availability) in the olfactometer, the "bottom" was covered with nylon mesh. Tests were conducted in indirect artificial light at 22 °C. The numbers of beetles located in each arm and in the main barrel were determined after 30 min.

Another test of chemical attraction was devised in which the treated pads were placed in the center of aerated plastic petri dishes (150 x 25mm) and the beetles placed either directly on the pads or in the dish area. The numbers of beetles on the pads were determined after 30 min.

Results

Chemical Studies. Benzidine spraying of TLC's of hydrolyzed fresh bark revealed two major yellow bands in all taxa except *B. nigra* and *B. nigra* 'Heritage'. Although the absolute movement of these two bands varied from test to test, they were always about 35 mm apart (90 mm vs. 55 mm) when the solvent front had run 100 mm. The lower band, rhododendrol, turned yellow with benzidine and had UV absorption maxima at 276 nm and 223 nm. Rhododendrin, the upper band, was more yellow-brown and had UV absorption maxima at 276 nm and 225 nm. Acid hydrolysis of purified rhododendrin produced rhododendrol. The synthetic rhododendrol was identical in chromatographic and spectrophotometric properties to that obtained from birch bark.

Based on colorimetric comparisons, our standard hydrolysis procedures on fresh bark produced extracts in which the ratio of rhododendrin to rhododendrol was approximately 1:1. Extracts prepared from "dying" bark of severed branches kept in the laboratory also produced both compounds, but with rhododendrol predominant at 10:1 to 15:1. Thus, it would appear that rhododendrin is naturally converted (hydrolyzed) to rhododendrol during senescence of bark (cambium and phloem) tissue.

Our single analysis on the hydrolyzed bark of a

hybrid between *B. populifolia* and *B. nigra* showed no rhododendrin, only a trace of rhododendrol, and a strong yellow band that migrated about 17 mm beyond rhododendrol. The UV absorption maxima of this unknown compound, which was not found in either parent species, were very similar to those of rhododendrol and suggested that these compounds are closely related.

Chemical Attraction Studies. There was no directed response to rhododendrol in our standard olfactometer tests by freshly emerged male or female beetles. Likewise, after feeding, neither males nor females were attracted to rhododendrol. Only female beetles that had been paired with males for six to eight days exhibited a positive response. Of the total of 74 supposedly mated female beetles tested in six different tests, 25 were attracted to rhododendrol, one to methanol, and the remainder either stayed at one or the other end of the main olfactometer barrel or simply wandered about. Males showed no response. These data are, obviously, not as conclusive as one might wish, but they do indicate a differential response between the sexes and between unmated and possibly-pregnant females.

Tests in petri dishes were not as extensive, but gave results similar to those in the olfactometer in that only "mated" females exhibited any response. When these females were simply placed in the dish containing a pad soaked in either rhododendrol or methanol, they simply wandered about, making incidental and momentary contact with the pad. However, when mated females were placed directly on the pads, those put in contact with the rhododendrol-treated pad tended to remain on the pad, and many exhibited oviposition behavior. Of 20 females (two trials of 10 each) on rhododendrol, 17 stayed on the pad during the 30-min test, but only two of 20 stayed on the methanol pad. Again, the data are suggestive, but not conclusive.

Discussion

The following facts and hypotheses have emerged from these studies.

1. All white-barked birches, except *B. nigra*, may produce rhododendrol in stressed and dying bark (cambial and phloem) tissue.
2. All white-barked birches, except *B. nigra*, are

probably susceptible to bronze birch borer attack.

3. It is likely that rhododendrol is a major factor in stimulating oviposition by female beetles and in the survival and maturation of beetle

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ABSTRACT

DAY, STEVEN J. 1989. **Much ado about freeze injury.** Colorado Green 5(2):4-5.

Plants are "cold-blooded"; that is, they assume the temperature of their environment. Temperature is a major uncontrollable climatic factor, and can dramatically affect the health and growth of most plants. Research is now being conducted on developing inherent characteristics for temperature stress resistance. How does injury occur when plants or plant parts freeze? First, water in the leaves and stems supercools. Ice forms outside the cell first, because the water there contains less solutes. Then, since there is no contact of ice with water inside the cells, a vapor pressure gradient is formed from inside the cell to the outside, and water evaporates out of the cells to the point where the cells dehydrate and die. When freezing injury of roots and stems occurs, many physiological processes are affected. Uptake of water and elements is decreased; tissues dry out; leaves on evergreens turn red-brown; and flower and leaf buds die. Don't be too quick to pull out the pruners and saws. Sometimes the freezing symptoms are worse than the injury. It's virtually impossible to second-guess the tree as to which stems will live and support the tree, and which will die. Timely, professional pest control can encourage recovery from freeze injury and conserve the tree's energy systems. Sometimes the "cure" for freeze injuries is worse than the "disease".

Santamour: (Continued from page 262)

probably susceptible to bronze birch borer attack.

3. It is likely that rhododendrol is a major factor in stimulating oviposition by female beetles and in the survival and maturation of beetle larvae.
4. The development of birches with superior white bark and borer resistance may require hybridization between *B. nigra* and borer-susceptible species.

Inasmuch as we had only produced a few *bona fide* hybrids with *B. nigra*, we increased our efforts in hybridization. In 1986, we pollinated 107 female catkins of *B. nigra* 'Heritage' with pollen of 13 different birches representing a wide range of species and hybrids and several levels of polyploidy. All of the 279 seedlings raised were apomictic *B. nigra*. In 1988, we made 127 crosses of *B. nigra* 'Heritage' on selected plants of *B. populifolia*, *B. populifolia* x *B. pendula*, and true *B. platyphylla* var. *japonica*. Of the 447 seedlings that germinated, only about 50 could be considered as putative hybrids and the rest were apomictic. Hybridity was judged on the basis of leaf isozymes.

There may be some question as to why other birch species, notably *B. papyrifera* and *B. pubescens*, were not utilized more extensively in our hybridization work. Both of these species are polyploid, with *B. pubescens* being tetraploid ($2n=56$ chromosomes) and *B. papyrifera* having chromosome numbers ranging from tetraploid to hexaploid ($2n=84$). We believe that the use of these species would increase the possibility of borer-susceptibility in hybrids with *B. nigra* and that more progress could be made by confining our work to species and hybrids with the same (diploid, $2n=28$) chromosome number.

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