acceptable levels of this and other pests. This research provides a basis for selection and production of *Acacia* species with different levels of resistance to the acacia psyllid to meet varying landscape requirements. In this regard the Saratoga Horticultural Foundation, Saratoga, CA, has identified a horticulturally-desirable *A. iteaphylla* specimen from among plants grown in the trial described above, has perfected means of propagating it vegetatively, and is pursuing a trademark for this accession which notes, among other attributes, its high resistance to the acacia psyllid.

### Significance to the Nursery Industry

This research provides a quantitative rating of the relative resistance of 31 species of *Acacia* to the acacia psyllid. Depending on horticultural desirability and adaptability of these species to a given locale, nurserymen can propagate and market *Acacia* species highly resistant to the psyllid.

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# Root Weevil Feeding on *Rhododendron:* A Review[1]

**Robert P. Doss**[2]
*Horticultural Plants Research Unit*
*USDA-ARS*
*Western Washington Research and Extention Center*
*Puyallup, WA 89371*

## Abstract

Specific chemical compounds present in *Rhododendron* leaves stimulate adult root weevil feeding. The resistance of certain *Rhododendron* species to weevil feeding is due to the presence of volatile terpene constituents of the leaves.

**Index words:** *Sciopithes obscurus* Horn, *Otiorhynchus sulcatus* Fab., black vine weevil, phagostimulant, feeding deterrent, insect repellent, phytosterols, flavonol glycosides, sugars, essential oils

### Introduction

*Rhododendron* is a large genus with about 1000 species and at least 5000 named hybrids (14). Many of these plants are prized as ornamental shrubs and are grown in both northern and southern hemispheres where appropriate climates exist. Some cultivars, particularly from the azalea group, are grown for use as flowering pot plants.

Taxonomists divide the genus into 3 large groups (14). Two groups, the azaleas and elepidotes lack foliar scales and are thereby separated from the lepidotes, which are scale bearing. Lepidotes are subdivided taxonomically by differences in scale morphology (Fig. 4) (4).

Although rhododendrons growing under good conditions are relatively trouble free, some disease and insect problems occur. Coyier (7) recently reviewed some of

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[2] Plant Physiologist.

the diseases, and Nielsen (15) has discussed insect pests. The most serious of the latter, at least in the United States, are the Rhododendron borer (*Synanthedon rhododendri* Beut.) and root weevils. The black wine weevil (*Otiorhynchus sulcatus* Fab.), an old world emigrant, is the only significant root weevil pest of *Rhododendron* in the northeastern United States. The black vine weevil is present in the Pacific Northwest, but is less important there than the obscure root weevil (*Sciopithes obscurus* Horn), an indigenous species (Fig. 2) (5, 13).

**Fig. 1. Cross sections through *Rhododendron* leaves show (top) scales of *Rhododendron trichostomum* and (bottom) a scale of *Rhododendron chryseum*. Scales from these species are morphologically distinct. However, scales of both species contain volatile compounds. Scales average about 30 um in diameter.**

Root weevils damage plants in two ways. Larval feeding on host plant roots can weaken and even kill plants. Particularly susceptible are those grown in containers (1). Adult feeding on leaves probably has little impact on vigor, but does reduce aesthetic appeal and saleability (Fig. 2).

Growers have observed that some species and hybrids exhibit less adult obscure weevil feeding damage than others (5, 15). Resistance of leaves to obscure root weevil feeding has recently been confirmed in both field (1, 5) and laboratory (2, 9) studies. While studying reasons for resistance, much has been learned about the phytochemical factors influencing obscure root weevil feeding on *Rhododendron* leaves. This paper reviews these findings and discusses several interesting aspects of the work.

## The Chemical Determinants

Initiating a study of the phytochemical bases of insect feeding behavior requires the development of a bioassay procedure. With leaf-notching species, it is necessary to select an inert substrate upon which insects will feed when it is treated with host plant extracts or purified feeding stimulants (6). Some commonly used substrates such as filter paper, agar-cellulose discs, or elder pith

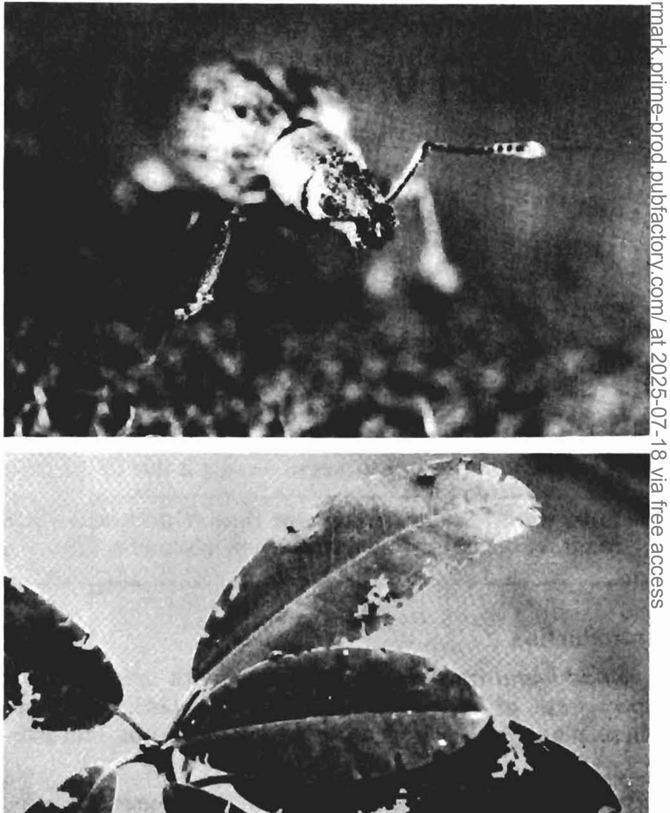

**Fig. 2. (Top) Adult obscure root weevil. Weevils are about 5 mm long. (Bottom) Damage to rhododendron leaves caused by obscure root weevil feeding.**

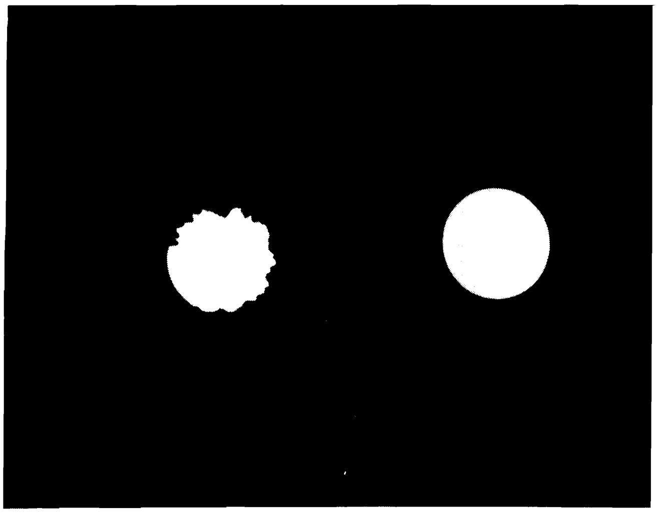

**Fig. 3.** **Feeding on membrane filters bearing hexane extract from *Rhododendron thomsonii*, a susceptible elepidote species (left) and on an untreated filter (right). Filters are 13 mm in diameter.**

fragments were unsatisfactory with obscure root weevil, but cellulose acetate-cellulose nitrate membrane filter discs were an acceptable, experimental substrate (3). Weevils fed extensively upon discs treated with extracts from susceptible *Rhododendron* species, but fed little, if at all, on untreated discs (Fig. 3). Moreover, discs treated with a fairly wide range of solvents did not dissolve or become distorted. Membrane filters were also fed upon by the black vine weevil, and, probably, could be used with other leaf-notching insects (Doss and Shanks, unpublished). Areas eaten from discs by obscure root weevil (3), and black vine weevil (Doss and Shanks, unpublished), were directly proportional to the amount of stimulant applied.

The membrane filter bioassay was indispensable in isolating and identifying obscure root weevil feeding stimulants (3, 8, 12), and more recently, black vine weevil feeding stimulants (Doss and Shanks, unpublished). Chromatography was used to fractionate extracts from *Rhododendron* leaves. Feeding stimulant activity of the fractions was then measured using the membrane filter bioassay. For example, an ethanolic extract from *Rhododendron* 'Cynthia' was subjected to paper chromatography using n-butanol:acetic acid: water (4:1:5, v:v:v, upper layer) solvent system (12). Bioassay of fractions obtained by eluting specific portions of the paper indicated the location of stimulatory bands (Fig. 4), the most active of which was chromatographed on paper in 15% (v:v) acetic acid. This second chromatographic step followed by bioassay (Fig. 4) yielded a band of stimulatory material with the ultraviolet spectral characteristics of quercetin 3-galactoside, a flavonol glycoside. Liquid chromatography of eluate from this band revealed a single major peak corresponding in retention time to quercetin 3-galactoside and a much smaller peak corresponding to the 3-glucoside of quercetin. Synthetic quercetin 3-galactoside and quercetin 3-glucoside, were active as obscure root weevil feeding stimulants (12). Similarly, silicic acid column chromatography and thin-layer chromatography of a lipid extract were used with the membrane filter bioassay technique to show that the common plant sterol, sitosterol, was an obscure root weevil feeding stimulant (12). Sucrose was identified as a stimulant by bioassay of fractions obtained through thin layer chromatography of an ethanolic extract (8). Because sucrose is a nearly universal feeding stimulant for leaf eating insects (16), it would have been reasonable to assume that it would stimulate obscure root weevil feeding. Chromatographic work could have been avoided by first bioassaying authentic sucrose.

Quercetin 3-galactoside, sitosterol, and sucrose were shown to be obscure root weevil feeding stimulants at levels present in the plant (10). However, feeding stimulated by any individual compound was far less than the amount of feeding that occurred when combinations of these materials were applied to membrane filter discs

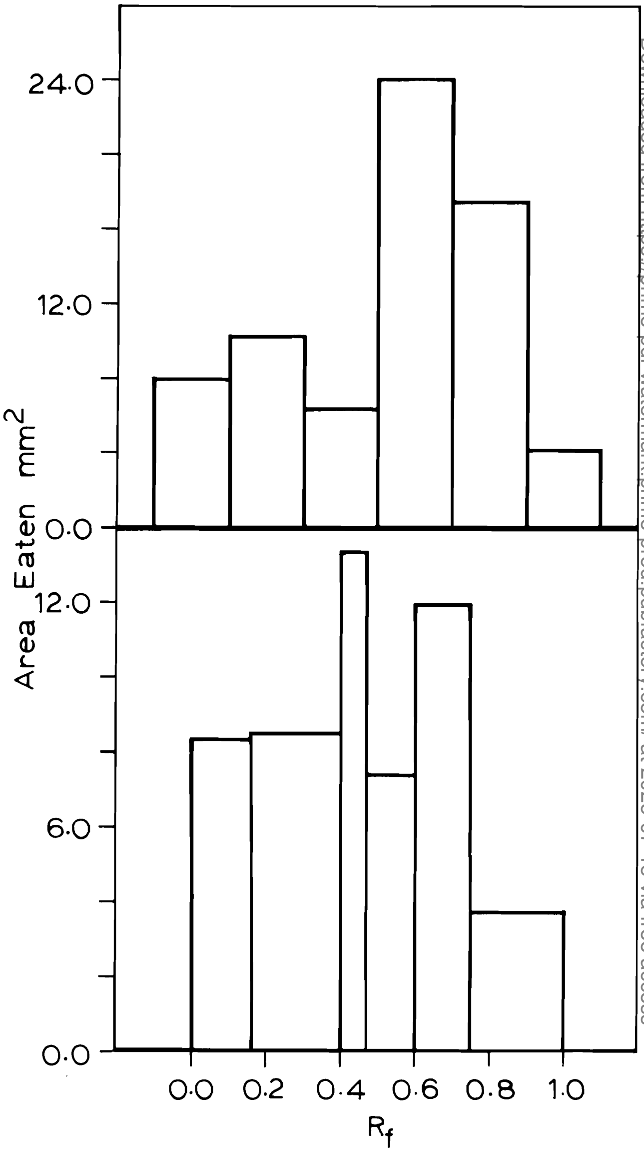

**Fig. 4.** **Areas eaten by obscure root weevils from membrane filters (Top) bearing fractions obtained through paper chromatography of an ethanolic extract from *Rhododendron* 'Cynthia' leaves using a n-butanol:acetic acid:water (4:1:5, upper layer) solvent system, and (bottom) bearing fractions obtained through paper chromatography of the fraction obtained from $R_f$ region 0.5 to 0.7 (see above) using a 15% acetic acid solvent system. Quercetin 3-galactoside was the principal component of the narrow band at Rf = 0.43. From 12, with permission.**

(Fig. 5) (12). This dramatic synergism suggested that the compounds were active in the plant, as well as on inert substrate. It also explained the failure to observe large amounts of feeding on discs treated with any purified fraction. For example, in fractionating an ethanolic extract, sucrose was separated from the flavonol glycosides. These compounds were strongly synergistic in stimulation of feeding (Fig. 5).

The search for phytochemicals from resistant *Rhododendron* species that could inhibit feeding was aided by knowing materials that could stimulate feeding on membrane filters, aided Filters treated with a stimulatory amount of sucrose were used to examine the feeding deterrent qualities of extracts from *Rhododendron* species classified as resistant by workers at Oregon State University (2). Leaf-choice tests were carried out to ensure that these species were resistant (9). These tests were conducted by placing a leaf from a supposedly resistant plant into an arena along with a leaf from a susceptible variety. Resistance was confirmed if feeding on the test leaf was markedly less than feeding on the leaf from the susceptible plant (Fig. 6). Many, but not all, of the plants examined were confirmed as resistant in leaf choice-tests (9). All but one of the resistant plants were lepidote or scale bearing rhododendrons. The exception was the elepidote *R. williamsianum,* a species with a tightly curled leaf edge that apparently presents a physical barrier to feeding (9).

Hexane extracts from resistant lepidote species inhibited feeding on sucrose-treated membrane filters. Similar extracts from *R. williamsianum* and several other elepidote species promoted feeding (Fig. 7) (9). The most distinguishing feature of the lepidote rhododendrons is the foliar scales. Such scales are often sources of nonpolar volatile components that are soluble in hexane. Thus, steam distillation, a method used for extraction of such materials, was carried out using leaves from *R. edgeworthii,* a very resistant species (11). The steam distillate strongly inhibited weevil feeding on membrane filters; steam distillate from a susceptible elepidote (cv. Cynthia) did not. Gas chromatography/mass spectrometry showed that the steam volatile materials were principally sesquiterpenes (Fig. 8) (11). Several fractions from the steam distillate that inhibited obscure root weevil feeding were obtained using thin-layer chromatography. One of these fractions contained a sesquiterpene called germacrone. Germacrone inhibited obscure root weevil feeding. Black vine weevil feeding was also inhibited by extracts of volatile components from resistant lepidote rhododendrons (Doss, unpublished). It has been confirmed that the scales are the source of these materials (Doss, unpublished).

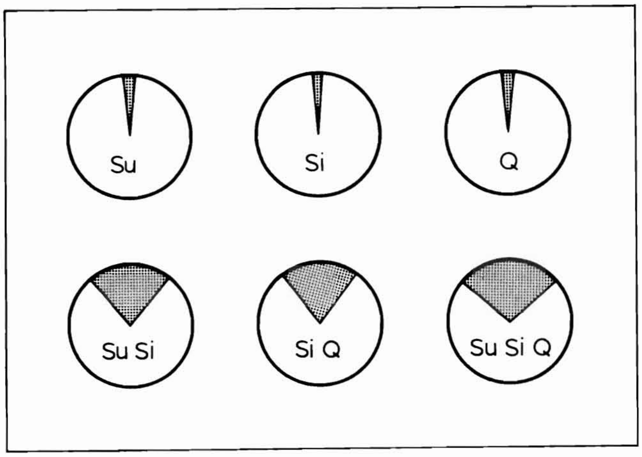

**Fig. 5. The areas eaten from membrane filters by obscure root weevils are represented by shaded areas. Much more feeding occurred when membrane filters bore combinations of phagostimulants. Abbreviations are as follows: Su—sucrose, 1 mg; Si—sitosterol, 5 g; Q—quercetin 3-galactoside, 400 mg. From 10, with permission.**

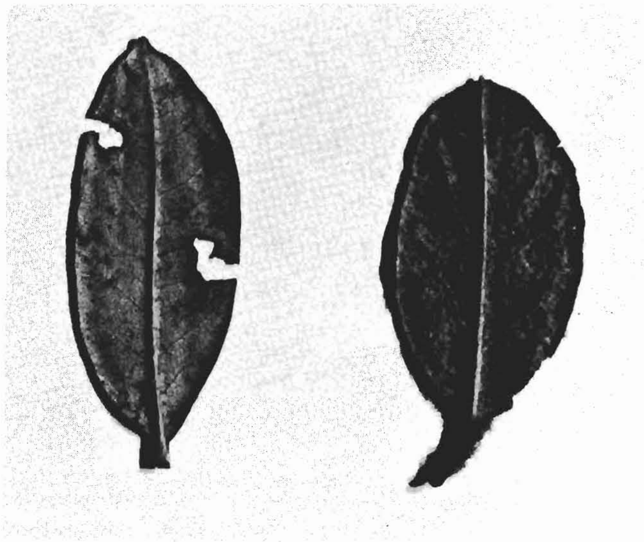

**Fig. 6. Feeding on *Rhododendron* 'Cynthia' (left) and *R. edgeworthii* leaves used in a leaf choice test. From *Ornamentals Northwest Newsletter* 5(5):6-8.**

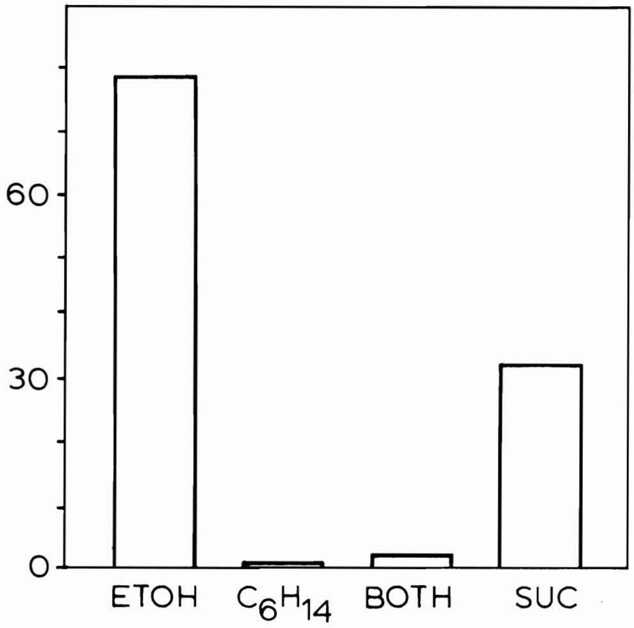

**Fig. 7. Feeding activity is influenced by extracts. Each membrane filter was treated with 150 g sucrose. Feeding was inhibited by application of hexane ($C_6H_{14}$) extracts from *Rhododendron chryseum* to filters, and stimulated by ethanolic (ETOH) extracts. Feeding on filters bearing sucrose only is indicated by bar labelled SUC. From 9, with permission.**

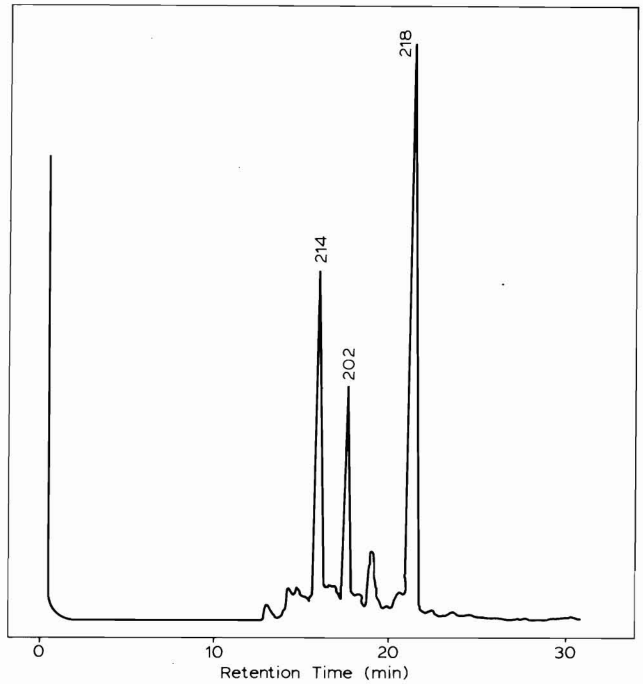

**Fig. 8. Gas liquid chromatograph trace of steam distillate fraction from *Rhododendron edgeworthii.* Numbers above peak indicate molecular weights. Largest peak represents germacrone. From 11, with permission.**

A fairly good understanding of the phytochemical factors that influence adult obscure root weevil feeding on *Rhododendron* leaves is now available. Feeding is stimulated by quercetin 3-galactoside or related flavonol glycosides (12), by sucrose and other sugars (8), and by phytosterols such as sitosterol (12). These feeding stimulants are constituents of virtually all higher plants. This explains the wide host range exhibited by obscure root weevil.

Resistant lepidote rhododendrons possess these feeding stimulants. Ethanolic extracts from some resistant lepidotes often stimulate considerable weevil feeding (Fig. 7) (9), because such extracts contain sucrose and flavonol glycosides but do not contain the terpene constituents that inhibit weevil feeding. Hexane extracts contain the inhibitory compounds present in the scales (Fig. 7).

## Significance to the Nursery Industry

Knowledge of root weevil feeding stimulants and repellents could result in the development of better control measures. Experimental baits formulated using combinations of root weevil stimulants are fed on avidly and incorporation of an insecticide into the baits results in weevil death (Dr. C.H. Shanks, Jr., personal communication). Germacrone and certain other volatile terpenes can repel root weevile and prevent feeding. Methods may be found to use such materials to protect plants.

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