Phytochemistry, 1980, Vol. 19, pp. 2379–2380. Pergamon Press Ltd. Printed in England. 0031-9422/80/1101-2379 $02.00/0

GERMACRONE, A SESQUITERPENE REPELLENT TO OBSCURE ROOT WEEVIL FROM *RHODODENDRON EDGEWORTHII*

ROBERT P. DOSS,* RUTH LUTHI† and BJORN F. HRUTFIORD†

*USDA–SEA/AR, Ornamental Plants Research Unit, Western Washington Research and Extension Center, Puyallup, WA 98371; † College of Forest Resources, University of Washington, Seattle, WA 98195, U.S.A.

(*Received* 12 *March* 1980)

Key Word Index—*Rhododendron edgeworthii*; Ericaceae; *Sciopithes obscurus*; Curculionidae; obscure root weevil; insect repellent; sesquiterpene; germacrone.

Abstract—A steam volatile fraction from *Rhododendron edgeworthii* leaves inhibited obscure root weevil (*Sciopithes obscurus*) feeding on sucrose-treated membrane filters. The most prominent component in this fraction was the sesquiterpene germacrone. Germacrone was found to be an obscure root weevil repellent.

INTRODUCTION

A number of lepidote (scaled) *Rhododendron* species are resistant to foliar feeding by the obscure root weevil (*Sciopithes obscurus* Horn.) [1, 2], a species endemic to the Pacific Coast of North America and an important pest of *Rhododendron* in that region. Hexane extracts from resistant lepidote species often inhibit obscure root weevil feeding on sucrose-treated membrane filters whereas extracts from susceptible elepidote species typically promote feeding [2, 3]. Because glandular scales of the type that characterize the lepidote *Rhododendrons* often secrete volatile terpenoids, a steam-volatile fraction from leaves of the resistant lepidote *R. edgeworthii* [1, 2] was tested for its ability to influence obscure root weevil feeding. A steam-volatile fraction from *R.* cv Cynthia, a susceptible elepidote cultivar (*R. catawbiense* Michaux × *R. griffithianum* Wight.) [2, 3], was examined to provide a basis for comparison.

RESULTS

Leaves from *R. edgeworthii* yielded 4.4 mg steam-volatile materials per g fresh weight, whereas *R.* cv Cynthia yielded only 0.64 mg per g. GC–MS indicated that the majority of the materials extracted from both *R. edgeworthii* and *R.* cv Cynthia had molecular weights and retention times characteristic of sesquiterpenes (Fig. 1). The steam-volatile fraction from *R. edgeworthii* completely inhibited obscure root weevil feeding on sucrose-treated membrane filters, whereas feeding on filters treated with an equivalent amount of extract from *R.* cv Cynthia was not different from that with filters bearing sucrose alone.

PLC of the extract from *R. edgeworthii* yielded two well-resolved fractions. Fraction 1, at $R_f \simeq 0.54$, consisted of the single most prominent component of the extract (peak labelled with 218 in Fig. 1) and represented 36.1% of the steam-volatile materials. Fraction 2 included several cochromatographing materials ($R_f \simeq 0.75$) (as determined by GLC) that together comprised 46.6% of the extract. Several minor components representing 17.3% of total steam-volatile mixture had R_fs less than 0.50.

Feeding on sucrose-treated membrane filters (150 μg sucrose/filter, mean area eaten for five filters bearing only sucrose = 15.4 mm^2) was significantly inhibited by application of either fraction 1 (amount applied = 28.5 μg/filter, mean area eaten for five filters = 0.1 mm^2) or fraction 2 (amount applied = 54.0 μg/filter, mean area eaten for five filters = 1.4 mm^2). An olfactometer study indicated that fraction 1 was an obscure root weevil repellent. Weevils exhibited a clear avoidance reaction to a filter paper disc bearing 57 μg of the purified compound and in four trials the average number of weevils ($\pm$ s.e.) approaching to within 15 mm of a treated filter paper disc was 3.3 ($\pm$ 1.7) vs 26.3 ($\pm$ 7.0) weevils approaching to within 15 mm of an untreated disc. The mean nearest approach to the treated disc made by obscure root weevils was 14.5 mm vs 8.2 mm for the untreated disc. In the four olfactometer runs, no weevils came into contact with the treated disc whereas the untreated disc was contacted 25 times.

The UV and IR spectra of the purified repellent compound were virtually identical to published spectra for the sesquiterpene germacrone [4, 5]. The ^{1}H NMR spectrum was consistent with published data for germacrone [6, 7] and was identical to a ^{1}H NMR spectrum of authentic germacrone provided by Dr. P. A. Grieco, Professor of Chemistry, University of Pittsburg, U.S.A.

DISCUSSION

Germacrone has been isolated from leaves of at least three other lepidote *Rhododendron* species: *R. dauricum* L. [8], *R. primulaeflorum* Bur. et Franch (= adamsii) [4] and *R. thymifolium* Maxim. [9]. Along with *R. edgeworthii*, these species represent four distinct series [10] (or subsections [11]), indicating the probable widespread occurrence of germacrone in the lepidote species of this genus.

The fact that not only germacrone, but also a combination of several less polar steam-volatile materials not separated by PLC inhibited obscure root weevil

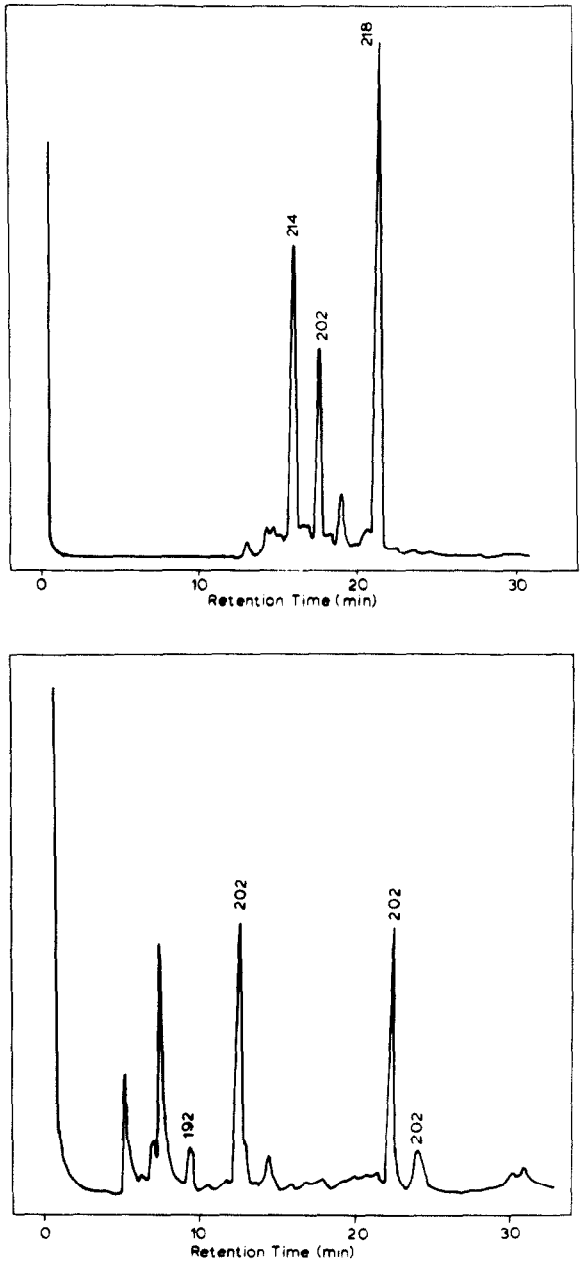

Fig. 1. GLC traces of steam volatile fractions from *R. edgeworthii* (top) and *R.* cv Cynthia (bottom). Approximate molecular weights, if ascertained, are indicated above peaks.

feeding on membrane filters suggests that the repellent nature of germacrone is not specific. Rather, it is likely that other volatile terpenes have similar properties. The general resistance of the lepidote *Rhododendrons* to obscure root weevil foliar feeding is probably due to the production and secretion of germacrone and related compounds by the glandular scales.

EXPERIMENTAL

Plant material. Fresh leaves of *Rhododendron edgeworthii* Hook. f. were obtained from clone No. 65-383 maintained at the Rhododendron Species Foundation, Federal Way, WA, U.S.A. Fresh leaves from *R.* cv Cynthia were obtained from Western Washington Research and Extension Center, Puyallup, WA, U.S.A.

Extraction, isolation and identification. Steam-volatile materials were extracted into hexane using an oil extraction apparatus. GC was done using a 1.8 m × 3.2 mm packed 3% SE-30 column programmed from 70 to 190° at 4°/min. Carrier gas was N_2 at 30 ml/min. Injector and FID were set at 250°. The MS coupled to this unit was a magnetic sector instrument operated at 70 eV ionization voltage. Fraction 1 was isolated from the steam distillate fraction from *R. edgeworthii* using PLC on Si gel GF-254 Merck (layer thickness = 0.5 mm) with C_6H_6–$CHCl_3$ (1:1). Bands were detected under fluorescent light and eluted using CH_2Cl_2. UV (EtOH) [4, 5], IR (glassy film) [4, 5], and ^{1}H NMR ($CDCl_3$) [6, 7] spectra of this material were consistent with published data for the sesquiterpene germacrone.

Feeding bioassays and olfactometer studies. Weevil feeding bioassays utilized Millipore membrane filters (cat No. HAWP 01300) as an inert substrate, and were conducted as described [2, 3, 12]. Fractions were applied in hexane. Olfactometer studies were carried out using a rectangular glass and Plexiglass chamber (90 mm wide × 150 mm long × 5 mm deep). Purified germacrone (57 μg), in CCl_4, was placed on a 6 mm dia filter paper disc centered in either the right or the left half of the rectangular chamber. A similar disc treated with solvent alone was placed in the center of the other half of the chamber. Adult obscure root weevils (30) were then introduced into the chamber which was maintained under 108 lx of fluorescent light at 10–15°. After a 0.5–1 hr conditioning period, weevil movement was monitored for 0.5 hr. Weevils approaching to within 15 mm of either the treated or untreated disc were counted and the closest approach to the disc made by any weevil entering the 15 mm radius was measured.

REFERENCES

1. Bell, H. T. and Clark, R. G. (1978) *J. Econ. Entomol.* **71**, 869.
2. Doss, R. P. (1980) *Environ. Entomol.* (in press).
3. Doss, R. P. (1980) *Environ. Entomol.* **9**, 155.
4. Pizulevskii, G. V. and Belova, N. V. (1964) *Zh. Obeshch. Khim.* **34**, 1345.
5. Ohloff, G., Farnow, W., Phillip, W. and Schade, G. (1969) *Justus Liebigs Ann. Chem.* **625**, 206.
6. Bates, R. B. and Gale, D. M. (1960) *J. Am. Chem. Soc.* **82**, 5749.
7. Sathe, R. N., Kulkarni, G. H. and Kelkar, G. R. (1968) *Chem. Ind.* **14**, 448.
8. Hsu, C.-C. and Yu, T.-C. (1976) *Hua Hsueh Hsueh Pao* **34**, 275. [(1978) (see *Chem. Abstr.* **88**, 60139d,].
9. Lab. Resources, Qinghai Sheng Inst. Biol. Lab. Phytochem., Inst. Bot., Acad. Sinica (1978) *Acta Bot. Sinica* **20**, 135. (English abstract).
10. Synge, P. M. (1967) *The Rhododendron Handbook. Part One. Rhododendron Species in Cultivation.* Royal Hort. Soc. London.
11. Cullen, J. and Chamberlain, D. F. (1978) *Notes, R. Bot. Garden Edinburgh* **36**, 105.
12. Bristow, P. R., Doss, R. P. and Cambell, R. L. (1979) *Ann. Entomol. Soc. Am.* **72**, 16.