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IN-FLIGHT RESPONSES OF THE PALES WEEVIL, *HYLOBIUS PALES* (COLEOPTERA: CURCULIONIDAE) TO MONOTERPENE CONSTITUENTS OF SOUTHERN PINE GUM TURPENTINE

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ABSTRACT

Relative attraction of the pales weevil, *Hylobius pales* (Herbst), to the six principal monoterpene constituents of gum turpentine, a mixture of these monoterpenes approximating the composition of turpentine and turpentine alone was determined in the field by comparing each of the substances as baits for bounce-column traps. Turpentine attracted significantly more weevils than individual monoterpenes when the dilution rates of all test substances were equal. Traps baited with *alpha*-pinene, a *beta*-phellandrene/limonene mixture, limonene, and *beta*-pinene captured significantly more weevils

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than unbaited traps, and a synthetic mixture of the principal monoterpenes present in gum turpentine was equal to gum turpentine as an attractant for the pales weevil.

RESUMEN

La atracción relatrive de los gorgojos pálidos, *Hylobious pales* (Herbst), hacia las seis principales trementinas constituyentes de la goma de trementina, cuya mezcla de eatas monotrementinas se acerca al compuesto de trementina y solo la trementina se investigó en el campo comparándola con cada una de las substancias como cebo en trampas de rebote-columna (bounce-column). La trementina atrajó significantemente mś gorgojos que monotrementinas individuales cuando el grado de dilución de todas las substancias fue igual. Trampas cebadas con *alpha*-pinene, una mezcla de *beta*-phellanderene/limonene, limonene y *beta*-pinene, capturaron significantemente más gorgojos que trampas sin cebo y mezclas sintéticas de la principal monotrementina presente en la goma de trementina fue igual a la goma de trementina como atrayente de los gorgojos pálidos.

Adults of the pales weevil, *Hylobius pales* (Herbst), are attracted by the odor of freshly cut pine stumps, logs, slash, and lumber (Pierson 1921, 1952, Ciesla and Franklin 1965, Hertel 1970), but the compounds responsible for this attraction have yet to be characterized. Thomas and White (1971) demonstrated that food selection by pales weevils is determined by a synergistic effect of pine phloem extract and sucrose, and Thomas and Hertel (1969) found phloem extracts of loblolly pine (*Pinus taeda* L.) to be more attractive to walking weevils than xylem extracts. In the laboratory bioassays employed by these authors, walking weevils showed a significant response to *alpha*-pinene and other monoterpene hydrocarbons which are secondary plant substances common to the hosts of *H. pales*. Fatzinger (1985) demonstrated a strong flying response of the pales weevil to gum turpentine distilled from the xylem oleoresin of typical slash (*Pinus elliottii* Englem. var *elliottii*) and longleaf (*P. palustris* Mill.) pines. Gum turpentine is composed mainly of monoterpene hydrocarbons (Mirov 1961) further suggesting the importance of these compounds to host-finding mechanisms of the pales weevil.

Field experiments were conducted in North Florida during 1983 to determine the in-flight response of pales weevils to: 1) individual monoterpene constituents of gum turpentine, 2) a synthetic mixture of monoterpenes approximating the composition of gum turpentine, and 3) gum turpentine alone.

METHODS AND MATERIALS

The monoterpene constituents of gum turpentine freshly distilled from the oleoresin of slash and longleaf pines (obtained from Shelton Naval Stores Processing Co., Valdosta, GA) were determined by gas-liquid chromatography using a Hewlett-Packard model 5380A gas chromatograph equipped with an electronic integrator, a 4.5 m stainless steel column with 20% carbowax on 80/100 mesh Chromosorb and a flame ionization detector. Operating temperatures were: injector port 210°C, column 110°C, detector 220°C, and the carrier gas (nitrogen) flow rate was 35 ml/min. Individual monoterpenes were identified by comparing retention times of the sample with those of known standards.

Six principal monoterpenes present in gum turpentine were tested individually and compared to whole gum turpentine (undiluted) and 95% ethanol as trap baits for the pales weevil. All individual monoterpenes were dispensed as 10% solutions in 95% ethanol. Bait dispensers were those described by Fatzinger (1985) except that a 50 ml bottle was used to hold bait formulations. Traps were similar to the bounce-column model described by Clements and Williams (1981) and unbaited traps served as controls during all experiments. *Alpha*-pinene (98% purity) and *beta*-pinene (98% purity) were obtained from Aldrich Chemical Co., Milwaukee, WI. Camphene (84% purity), myrcene (92% purity), and a *beta*-phellandrene/limonene mixture (34% *beta*-phellandrene/62% limone) were obtained from SCM Organics, Jacksonville, FL. Purified *beta*-phellandrene was unavailable in quantities necessary to equal the dilution rates of other monoterpenes. Limonene (98% purity) was obtained from K&K Laboratories, Plainville, NY. The experiment initially was conducted over a period of 27 days during August and September 1983 (Rep. 1). It was repeated during September and October 1983 with the exception that turpentine also was dispensed as a 10% solution in 95% ethanol so that its release rate was comparable to that of the individual monoterpene baits (Rep. 2).

Studies were conducted in mixed stands of slash and longleaf pine in Baker County near Olustee, FL. Traps were spaced 30 m apart in a straight line (north to south) and different baits were assigned to the traps in a modified Latin square experimental design described by Billings et al. (1976) to account for variations in captures due to trap locations. Baits were rotated among traps so that each bait occupied a different trap location for a 3-day period (total of 9 treatments x 3 days/trap location = 27 trapping days and 9 collections). Numbers of pales weevils captured were recorded for each 3-day period. The significance of differences among treatment means were determined at the 0.05 probability level by an analysis of variance procedure (ANOVA) of the Statistical Analysis System (SAS version 1982.3) in which trap baits, trap collection dates and trap locations were analyzed as main effects. Differences between treatment means were tested for significance at the 0.05 probability level using Duncan's (1955) multiple range test.

A synthetic bait of six principal monoterpenes mixed in the same proportions as they occurred in gum turpentine was also compared to whole gum turpentine as an attractant for the pales weevil. The percentage composition of monoterpenes in the synthetic bait did not vary from that of gum turpentine by more than 1.2% (Table 1). Both baits were individually diluted to concentrations of 10% in 95% ethanol and were alternated among six traps at 3-day intervals over an 18-day period during October 1983 (2 treatments x 2 replications/treatment x 3 days/trap location = 18 trapping days or 6 collections/trap). Differences between the mean numbers of weevils attracted to the baits per 3-day interval were tested for significance by a t-test comparison at the 0.05 probability level.

TABLE 1. COMPOSITIONS OF THE SIX PRINCIPAL MONOTERPENES IN GUM TURPENTINE AND A SYNTHETIC MIXTURE OF MONOTERPENES USED AS BAITS FOR PALES WEEVILS.

	% Composition[a]	
Monoterpene	Gum Turpentine	Synthetic Mixture
Alpha-pinene	65.0	65.4
Camphene	0.8	1.2
Beta-pinene	31.0	29.8
Myrcene	0.5	0.5
Limonene	1.0	1.4
Beta-phellandrene	1.5	1.6

[a]Percent compositions determined by gas chromatography and calculated by the area percent of total monoterpenes.

RESULTS

The analysis of variance in which trap bait, trap location and trap collection dates were analyzed as main effects showed that significant differences existed between trap baits in both Rep. 1 and Rep. 2. No differences existed between trap locations in Rep. 1 or Rep. 2. There were significant differences among trapping dates only in Rep. 2, but it is expected that the number of flying weevils will vary from day to day due to such factors as temperature, precipitation, and intensity of emergence. Differences in date of collection do not appear to affect the relative attraction of the various trap baits, although interactions between trap bait and date of collection could not be tested due to insufficient replication.

Alpha-pinene attracted significantly more pales weevils than whole gum turpentine, but when turpentine was dispensed as a 10% solution in ethanol, it was significantly more attractive than any of the individual monoterpenes (Table 2). *Alpha*-pinene was significantly more attractive than the other monoterpenes tested in Rep. 1, but numbers of weevils attracted to *alpha*-pinene in Rep. 2 were not significantly different from those responding to the *beta*-phellandrene/ limonene mixture and *beta*-pinene. *Alpha*-pinene, the *beta*-phellandrene/limonene mixture, limonene, and *beta*-pinene were all significantly more attractive than unbaited traps, whereas myrcene, camphene, and ethanol showed no significant attraction of pales weevils. It is not clear if the significant increase in attraction of the *beta*-phellandrene/limonene mixture over limonene alone in Rep. 2 was due to a response to the presence of *beta*-phellandrene or a synergistic effect between the two monoterpenes.

In the comparison of gum turpentine and a synthetic mixture of the principal monoterpenes, no significant differences existed in the number of weevils responding to the two baits. Average weevil captures ± S.E. per 3-day trapping periods were 26.7 ± 3.2 for turpentine and 32.3 ± 4.8 for the synthetic monoterpene mixture.

TABLE 2. MEAN NUMBER OF PALES WEEVILS CAPTURED IN TRAPS BAITED WITH INDIVIDUAL MONOTERPENES AND GUM TURPENTINE IN REPLICATION 1, CONDUCTED IN AUGUST AND SEPTEMBER 1983, AND REPLICATION 2, CONDUCTED IN SEPTEMBER AND OCTOBER 1983, IN BAKER COUNTY, FL.

	$\bar{x}$ Number of Pales Weevils Captured/3-day Trapping Period	
Trap Bait[a]	Rep. 1	Rep. 2
Alpha-pinene	34.0 a	42.5 b[b]
Beta-phellandrene/limonene	21.2 b	36.2 b
Limonene	15.7 bc	18.2 cd
Whole Turpentine	14.0 bcd	—[c]
10% Turpentine	—[c]	57.2 a
Beta-pinene	12.9 bcd	26.4 bc
Myrcene	6.9 cde	9.6 de
Ethanol	3.5 de	2.0 de
Camphene	2.9 de	3.3 de
Control	2.1 e	0.3 e

[a]All baits were dispensed as 10% solutions in 95% ethanol, except for whole turpentine in Replication 1.
[b]Means within a column followed by the same letter are not significantly different at the P<0.05 level [Duncan's (1955) multiple range test].
[c]Not tested.

Discussion

No single monoterpene constituent of gum turpentine appears entirely responsible for its attractancy to the pales weevil since turpentine was significantly more attractive than individual monoterpenes when the dilution rates of all test substances were equal. The combination of all monoterpenes, however, was equally attractive to turpentine, suggesting that more than one monoterpene contributes to its attractiveness. It is not clear which of the monoterpenes are involved, or if the proportion of monoterpenes is important to the overall attraction. These results do imply that the host-finding mechanism of the pales weevil is dependent upon the monoterpene hydrocarbons of its hosts.

Thomas and Hertel (1969) reported that the (+) enantiomer of *alpha*-pinene was more attractive than (-) *alpha*-pinene. In this study, however, (-) *alpha*-pinene was tested since it is the form present in slash pine oleoresin (Mirov 1961), which is the major source of gum turpentine. The pales weevil has a wide range of hosts including trees from 11 genera and 29 species (Lynch 1984) which differ both qualitatively and quantitatively in monoterpene composition. It is possible that a more attractive combination of monoterpenes may exist from the oleoresin of a preferred host, but the ability of pales weevils to exploit a wide host range may be related to the insect's ability to respond to a variety of different monoterpenes.

It appears from this study that ethanol is synergistic to the attractive constituents of gum turpentine for the pales weevil although a direct comparison of whole turpentine and ethanol-diluted turpentine was not possible since the two baits were not present in the same experiment. Similar results have been previously reported for the pales weevil (Fatzinger 1985), for two species of ambrosia beetles (Family Scolytidae), *Trypodendron lineatum* (Oliv.) (Moeck, 1970, 1971) and *Gnathotrichus sulcatus* (LeConte) (Cade et al. 1970, Moeck 1971), and for three species of pine sawyer beetles (Family Cerambycidae), *Monochamus alternatus* Hope (Kobayashi et al.), *M. carolinensis* (Oliv.), and *M. titillator* (F.) (Fatzinger 1985). It is not apparent, however, whether pales weevils are responding to ethanol as a naturally occurring, primary attractant or if it is simply acting as a dispersal agent for the attractive constituents of gum turpentine.

Acknowledgements

Appreciation is expressed to Carl F. Fatzinger, Christopher A. Mullin, James L. Nation, and Robert C. Wilkinson, Jr. for their critical reviews of this manuscript. This research was supported through a cooperative agreement with the USDA Forest Service, Southeastern Forest Experimental Station, with funds provided by the Integrated Pest Management Program, Southern Forest Experiment Station. Florida Agricultural Experiment Station Journal Series No. 7341.

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