Laboratory Evaluation of Biologically-Based Compounds as Antifeedants for the Pales Weevil, *Hylobius pales* (Herbst) (Coleoptera: Curculionidae)[1]

S. M. Salom, J. A. Carlson[2], B. N. Ang[3], D. M. Grosman and E. R. Day

Department of Entomology
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0319 U.S.A.

J. Entomol. Sci. 29(3): 407-419 (July 1994)

ABSTRACT Twenty plant-produced compounds or mixtures and one insect-produced semiochemical were evaluated as potential antifeedants for the pales weevil, *Hylobius pales* (Herbst). Initially, a choice laboratory feeding bioassay was conducted to screen the compounds and identify antifeedant activity. This was followed by a no-choice dose-response bioassay to further evaluate the most active compounds from the choice test. In the choice test, nine compounds inhibited feeding by *H. pales* on white pine, *Pinus strobus* L., twigs after 24 h: borneol, bornyl acetate, cucurbitacin, limonin, myrcene, neem extract, *S* (+) and *R* (-) carvone, and verbenone. Five of these compounds remained active after 48 h: borneol, limonin, neem extract, and both carvone isomers. In the no-choice test, 10% concentrations of *S* (+) carvone and limonin were the most active compounds for both males and females after 24 h. Borneol, verbenone, and *R* (-) carvone were active for males only. After 48 h, limonin and *S* (+) carvone remained most active compounds for males, with cucurbitacin and verbenone also showing activity. However, only limonin was active for females. Dose responses (0.1-10% concentrations) were strong for limonin and both carvone isomers. Cucurbitacin, diluted from a 0.3% concentrate, exhibited similar responses at all three doses, indicating that a more concentrated formulation may have potential as an antifeedant for *H. pales*. Limonin, both carvone isomers, and verbenone also show promise and will be further evaluated in future studies.

KEY WORDS Antifeedants, *Hylobius pales*, Coleoptera, Curculionidae, conifer insect pest, choice test, no-choice test, dose-response.

The pales weevil, *Hylobius pales* (Herbst), has long been considered a pest of conifer seedlings from southeastern Canada and northeastern USA (Carter 1916, Peirson 1921) to southeastern USA (Beal and McClintick 1943, Speers 1958, Manwan 1964, Nord et al. 1982). Adult weevils feed on tender bark of pine seedling stems, saplings, and older trees, as well as other conifer species (Carter 1916, Peirson 1921, Lynch 1984). They are attracted by the resinous volatiles produced by dead and dying trees (Peirson 1921, Thomas and Hertel 1969, Hertel 1970, Fox and Hill 1973). Adults then feed and oviposit in the roots of dying trees and stumps where brood will develop until the onset of winter (Doggett et al. 1977). Consequently, newly-emerged adult weevils are present the following spring to feed on newly-planted pine seedlings (Peirson 1921, Beal and McClintick 1943).

[1] Accepted for publication 25 April 1994.

[2] Department of Plant Pathology, Physiology, and Weed Science, VPI and SU, Blacksburg, VA 24061-0331.

[3] Department of Entomology, VPI and SU, Tidewater Research Station, Suffolk, VA 23437.

Hylobius pales is also considered a Christmas tree pest, with reports of damage ranging throughout the eastern and north central USA (Lynch 1984). Direct damage includes flagging of branches (Rennels and Fox 1970) and seedling mortality of the same nature as occurs in forest plantations (Anderson 1980). Indirect damage from *H. pales* feeding on bark and cambial tissue results from their inoculation of Christmas trees with the pathogenic fungus *Leptographium procerum* (Kendr.) Wingf. (Lackner and Alexander 1982, 1983, Lewis and Alexander 1986, Lewis et al. 1987, Nevill and Alexander 1992a). This fungus has been determined to be the causal agent of procerum root disease (PRD) (Lackner and Alexander 1983).

Procerum root disease was first observed in Virginia Christmas tree plantations in the late 1970's (Lackner and Alexander 1982, 1983). Disease severity has increased annually in Virginia, with an estimated loss in 1990 of 800,000 white pine, *Pinus strobus* L., trees valued at $6.4 million (S. A. Alexander, unpublished). Mortality is greatest in trees of harvestable age (Alexander et al. 1988), therefore, the economic impact of the disease is at a maximum on per tree basis. It is our judgement that *H. pales* is the principal vector of *L. procerum.* This is based on the high proportion of weevils found to carry the fungus (Nevill and Alexander 1992b) and the feeding habits of the weevils being consistent with where the fungus colonizes the xylem tissue in the root collar region. Therefore, management of the disease should focus at least partially on minimizing inoculation of trees from *H. pales* feeding.

In forestry situations, successful control of *H. pales* relies on use of both cultural and chemical-based tactics (Nord et al. 1982). However, in Christmas tree plantations, where shearing and yearly harvesting practices lead to continuous production of attractive material, cultural control efforts are less successful (Weidhaas 1989). In Virginia, recommended insecticides include Dursban® (chlorpyrifos) and Lindane® as both seedling and stump sprays, Asana® (esfenvalerate) as a seedling spray only, and Imidan® (phosmet) as a top dip for seedlings (Day et al. 1992). Both Asana and Lindane are restricted-use pesticides, and Imidan is very messy to handle, and as a result, disliked by applicators (T. Tigner, pers. commun.). Despite the current recommendations, seedlings are rarely treated, and Lindane, the most effective of the recommended compounds, is the spray of choice for stumps and older trees (Alexander and Carlson 1992). As environmental concerns increase over the industry's reliance on insecticides for controlling beetle populations, alternative tactics that are less toxic need to be evaluated.

One area that has received considerable attention is the use of plant-produced compounds as repellents, inhibitors, or antifeedants (Hanover 1975, Schoonhoven 1982, Norris 1986, Morgan and Mandava 1990). When considering natural plant compounds as antifeedants for insects, over 500 were identified between 1976 and 1987 (Warthen 1990, Morgan and Warthen 1990). Of these, 12 compounds came from coniferous tree species. Certain terpene compounds of conifer oleoresin systems have been found to be associated with host resistance to subcortical insects, such as the western pine beetle, *Dendroctonus brevicomis* LeConte (Smith 1975), the southern pine beetle, *D. frontalis* Zimmerman (Gollob 1980), the sixspined engraver, *Ips calligraphus* (Germar) (Cook and Hain 1988), the fir engraver, *Scolytus ventralis* LeConte (Raffa et al. 1985), and the white pine weevil, *Pissodes strobi* Peck (Wilkinson 1980, Alfaro et al. 1980).

One monoterpene, limonene, inhibits attraction of the closely related pine weevil, *H. abietis* (L.), to host volatiles (Nordlander 1990) and susceptible host material (Nordlander 1991).

Insect-produced compounds can also act as repellents. Aggregating bark beetles commonly produce odors which repel new arrival of conspecifics and reduce interspecific competition (Borden 1985). One such compound, verbenone, inhibits aggregation in several tree-killing bark beetle species (Renwick and Vité 1969), and its potential for protecting coniferous forests is being tested in North America (Payne et al. 1992, Salom et al. 1992) and Europe (Vité and Baader 1990).

In this study we evaluated one insect-produced and twenty plant-produced compounds and mixtures in laboratory feeding bioassays. The compounds tested were either extracts or by-products from conifers, extracts from other plants that have shown antifeedant activity with other insect species, or an insect-produced pheromone. The goal of this study was to screen the compounds for antifeedant activity and evaluate their potential for use as a tool against *H. pales* and procerum root disease.

Materials and Methods

Choice Test. A total of twenty compounds and mixtures were evaluated in this experiment (Table 1). All compounds were diluted in ethyl acetate to a concentration of 10%. Therefore, the concentrations of cucurbitacin, Margosan O, and neem extract were 0.03 (300 ppm), 0.03, and 0.3% (3,000 ppm), respectively.

A preliminary choice test was conducted to evaluate the influence of ethyl acetate on weevil feeding, using the procedures described below. No significant differences were found in the amount of feeding by *H. pales* on treated versus untreated twigs after 24 ($t_{(2),\,70} = -0.50$; $P = 0.61$) and 48 h ($t_{(2),\,70} = -1.28$; $P = 0.21$).

White pine twigs, 0.5 - 0.8 cm diam × 4 cm long, were used in the choice test. The twigs were stored in sealed plastic freezer bags in a refrigerator at 4°C for no longer than 2 wks prior to use. At the beginning of each replicate, the twigs were dipped in a test solution and then allowed 1 min to dry. A treated and untreated twig were placed in individual moistened paper sleeves (to prevent contact between twigs) within a 100 × 15 mm petri dish. The sleeves were a strip of paper loosely wrapped around a twig and closed with a staple. One weevil, starved for 18 h, was placed in the dish. All dishes were then placed in an environmental box (light controlled only) held in temperature controlled room at 26°C ± 1°C for 48 h. *Hylobius pales* are known to be nocturnal (Corneil and Wilson 1984). Therefore, no photoperiod was provided during the 48 h test period to maximize feeding activity. Paper towel sleeves were remoistened after 24 h. An area estimate of weevil feeding on cambial tissue was made after 24 and 48 h for each twig. The amount of feeding was measured with a transparent grid of 1.56 mm^2 squares transposed over the feeding sites on the twigs. All treatments were replicated six times for both males and females, on each of five separate testing periods.

No-Choice Dose-Response Test. Assay procedures were similar to those used in the choice test. However, only one twig was placed in a petri dish at a time instead of two. Treatments included the nine most active compounds in the choice test (Table 1), pine oil, and untreated and ethyl acetate-treated twigs, for a total of 12 treatments. The latter two treatments both served as controls. Pine

Table 1. Candidate compounds used in *Hylobius pales* antifeedant bioassays.

Compound	Active Ingredient (If other than compound name)	(%) Active Ingredient	Source*	Experiment**
Abietic Acid		70.0	1	1
alpha-Pinene		98.0	1	1
Borneol		98.0	1	1,2
Bornyl Acetate		97.0	1	1,2
beta-Pinene		99.0	1	1
Camphene		85.0	2	1
Cucurbitacin	Cuc. E, I and respective glycosides	0.3	3	1,2
Limonene		95.0	4	1
Limonin		85.0	5	1,2
Longifolene		98.0	1	1
Menthone		95.0	1	1
Myrcene		77.2	1	1,2
Margosan O	Azadirachtin	0.3	6	1
Neem Extract	Azadirachtin	3.0	6	1,2
Neem Oil	Unknown	–	6	1
Pine Oil	Terpene Alcohols	86.0	7	2
	Terpene Hydrocarbons	13.0		
R (-) Carvone		98.0	1	1,2
S (+) Carvone		96.0	1	1,2
3-Carene		95.0	1	1
Verbenone†		98.0	8	1,2
Wood Rosin	Abietic Acids	58.8	4	1
	Pimaric Acids	17.8		
	Palustric Acid	6.9		

* [1]Aldrich Chem. Co.; [2]Eastman Kodak; [3]MicroFlo Co.; [4]Hercules Inc.; [5]R. Alford, Univ. Maine, [6]W. R. Grace Co.; [7]Busch Boake Allen; [8]Chem. Samp. Co.

** Choice test = 1; no-choice test = 2.

† Enantiomeric ratio of verbenone was 70% (+): 30% (-).

oil was included because it had previously demonstrated repellent activity to several scolytid species (Nijolt 1980, Nijholt et al. 1981, O'Donnell et al. 1986, Berisford et al. 1986) and was not obtained until after the choice test. The ten test compounds were diluted in ethyl acetate to concentrations of 10, 1, and 0.1%, except cucurbitacin (0.03, 0.003 [30 ppm], and 0.0003 % [3 ppm]); and neem extract (0.3, 0.03, and 0.003%). Instead of dipping the twigs in solution, a more effective technique was used where the solutions were applied to the twigs to the point of dripping using disposable pipettes. Due to problems associated with using the same room as in the choice test, the environmental box was placed in a different room where the temperatures were a little cooler at 24°C ± 1°C. All treatments were replicated three times for both males and females, for each of twenty separate testing periods.

Insects. The original source of *H. pales* was from Christmas tree plantations in Montgomery and Floyd counties, VA. A continuous rearing colony was initiated in 1990, using the methods of Speer and Cody (1975), later modified by Salom et al. (1987). The number of weevils in the colony was kept at 400-500, due to space limitations.

Because of the small size of the colony and the number of replicates used in the choice and no-choice tests, it was necessary to use weevils more than once. They were reused once every 4 to 5 testing periods. During the course of our chemical tests, we concurrently carried out a study to determine if there were any differences in weevils that had been exposed to the most active test compounds (experienced) as opposed to weevils that had not previously been tested (naive). No differences in amount of feeding were found between naive and experienced males ($t_{(2),66} = 0.62$; $P = 0.54$) or females ($t_{(2),\,64} = 1.82$; P = 0.07), 24 h following exposure to untreated twigs. Therefore, it was concluded that reuse of weevils within our time frame was acceptable for these feeding studies.

Because the size or weight of weevils tended to vary within the colony (male range ≈ 22 - 53 mg; female range ≈ 32 - 65 mg), we conducted a study to test for correlation between weevil weight and the amount of feeding. After 24 h, no correlation was found for males ($F_{1,30} = 0.02$; $P = 0.89$; $r = 0.03$) or females ($F_{1,32} = 0.01$; $P = 0.94$; $r = 0.01$). As a result, the weevils used in this study were not controlled for size. *Hylobius pales* are capable of living at least one year or up to two growing seasons (Davis and Lund 1966), yet the only control for age used in the study was that all weevils were at least one month old, and therefore, sexually mature (Clark 1975, Hoffman and Raffa 1992).

Statistical Analysis. In the choice test, comparisons were made between treated and untreated twigs for each compound at 24 and 48 h, using the Student's *t*-test (Steel and Torrie 1980). Since all compounds were evaluated within the same experiment, each comparison was not considered independent. Therefore, to achieve an experimental error rate of 0.05, a 0.0025 error rate was used for each comparison (Jones 1984). The no-choice tests were run as randomized complete block designs and analyzed using General Linear Model Procedure (SAS 1989). Each testing period (3 days) served as a block. Differences among treatments at each concentration were determined using the Student-Newman- Keuls (SNK) mean separation test ($P = 0.05$), for measurements at the 24 and 48 h. Differences among concentrations for each treatment at 24 and 48 h were also determined using the SNK test.

Results

Choice Test. After 24 h, significant differences in the amount of cambial tissue fed upon was recorded for 9 of the 20 compounds tested (Table 2). These compounds included bornyl acetate, borneol, cucurbitacin, limonin, myrcene, neem extract, *R* (-) carvone, *S* (+) carvone, and verbenone. After 48 h, significant differences in cambial tissue feeding was still recorded for five of the test compounds: borneol, limonin, neem extract, *R* (-) carvone, and *S* (+) carvone.

No-Choice Dose-Response Test. The amount of feeding by females was significantly greater than males at all three concentrations after 24 and 48 h: 24 h, 10% ($F_{1,956} = 42.1$; $P < 0.0001$); 48 h, 10% ($F_{1,945} = 67.0$; $P < 0.0001$); 24 h, 1%

Table 2. Comparison of the amount of feeding by the pales weevil, *Hylobius pales*, on treated and untreated white pine, *Pinus strobus*, twigs in a laboratory bioassay (n = number of weevils).

	Mean ± SE Feeding on Twigs (mm^2)								
	24 Hours					48 Hours			
Treatment	n	Treated	n	Unreated		n	Treated	n	Unreated	
Abietic Acid	30	33.1 ± 7.3	30	33.4 ± 7.8		29	57.9 ± 12.2	29	63.3 ± 14.3	
alpha-Pinene	30	33.9 ± 6.1	30	42.9 ± 9.1		30	65.3 ± 9.2	30	77.4 ± 13.2	
Bornyl Acetate	28	16.3 ± 4.1	28	47.9 ± 7.0	*	28	54.7 ± 11.0	28	78.5 ± 10.2	
Borneol	30	9.3 ± 3.4	30	48.7 ± 6.7	*	30	27.4 ± 5.6	30	80.4 ± 11.3	*
beta-Pinene	30	48.8 ± 6.9	30	26.6 ± 6.7		28	84.3 ± 12.0	28	48.9 ± 12.1	
Camphene	30	26.8 ± 7.0	30	29.3 ± 5.8		29	63.9 ± 10.1	29	50.8 ± 7.4	
Cucurbitacin	30	9.7 ± 2.5	30	43.7 ± 8.6	*	30	26.2 ± 4.4	30	64.0 ± 11.7	
Limonene	30	28.9 ± 6.6	30	35.8 ± 6.1		30	68.1 ± 11.1	30	62.4 ± 9.0	
Limonin	29	12.8 ± 3.1	29	50.0 ± 9.8	*	29	23.4 ± 5.2	29	81.7 ± 14.3	*
Longifolene	29	24.3 ± 4.9	29	35.6 ± 6.3		29	62.5 ± 11.5	29	67.8 ± 11.5	
Menthone	30	26.0 ± 5.5	30	40.7 ± 7.8		30	57.8 ± 10.2	30	79.6 ± 12.1	
Margosan O	30	19.9 ± 4.8	30	36.6 ± 6.8		30	33.2 ± 6.0	30	57.3 ± 9.4	
Myrcene	30	23.0 ± 5.0	30	54.1 ± 8.4	*	30	41.3 ± 7.2	30	85.0 ± 11.5	
Neem Extract	30	10.3 ± 3.0	30	49.3 ± 6.6	*	30	21.2 ± 4.6	30	84.3 ± 11.2	*
Neem Oil	30	30.4 ± 6.2	30	43.3 ± 7.9		30	49.8 ± 8.8	30	70.5 ± 11.8	
R(-) Carvone	30	10.9 ± 5.1	30	52.3 ± 6.8	*	30	27.4 ± 9.1	30	97.2 ± 9.3	*
S(+) Carvone	28	10.5 ± 4.1	28	57.3 ± 8.0	*	28	26.6 ± 9.2	28	97.8 ± 14.3	*
3-Carene	30	19.6 ± 5.4	30	43.0 ± 6.8		30	45.0 ± 9.7	30	73.9 ± 10.4	
Verbenone	18	12.3 ± 4.5	18	51.6 ± 11.0	*	18	42.9 ± 12.6	18	90.3 ± 17.6	
Wood Rosin	30	31.9 ± 5.7	30	27.8 ± 6.0		30	58.6 ± 8.6	30	46.3 ± 7.0	

* represents significant differences at $P \leq 0.05$ level for the experiment and at $P \leq 0.0025$ level for individual comparisons.

($F_{1,950}$ = 54.2; $P < 0.0001$); 48 h, 1% ($F_{1,942}$ = 71.9; $P < 0.0001$); 24 h, 0.1% ($F_{1,955}$ = 36.4; $P < 0.0001$); and 48 h, 0.1% ($F_{1,942}$ = 44.4; $P < 0.0001$). Therefore, all further analyses were separated by sex.

Cambial tissue feeding by *H. pales,* on twigs treated with 10% concentration of test compounds, differed significantly among treatments (Fig. 1). After 24 h, males fed less on twigs treated with *S* (+) carvone, limonin, borneol, verbenone, and *R* (-) carvone than on ethyl acetate-treated twigs, and less on twigs treated with limonin and *S* (+) carvone than on untreated twigs (Fig. 1A). Females fed less on twigs treated with limonin and *S* (+) carvone than on ethyl acetate-treated twigs, and less on only *S* (+) carvone than on untreated twigs (Fig. 1B). After 48 h, males fed less on twigs treated with verbenone, cucurbitacin, *S* (+) carvone, and limonin than on ethyl acetate-treated twigs, and less on only limonin than on untreated twigs (Fig. 1C). Females fed less on only limonin than on ethyl acetate- and untreated twigs (Fig. 1D).

No differences in feeding occurred among treatments at 1% concentration after 24 h: male ($F_{11,476}$ = 1.59; $P > 0.1$) and female ($F_{11,473}$ = 0.67; $P > 0.77$) and 48 h: male ($F_{11,473}$ = 1.54; $P > 0.11$) and female ($F_{11,468}$ = 0.97; $P > 0.48$). No differences were observed among treatments at 0.1% concentration after 24 h: male ($F_{11,479}$ = 1.33; $P > 0.21$) and female ($F_{11,475}$ = 1.40; $P > 0.17$). After 48 h, differences in feeding by males were observed among treatments, yet none were different from the ethyl acetate- and untreated-controls (SNK test; $P > 0.05$). No differences were observed among treatments for females ($F_{11,468}$ = 1.25; $P > 0.25$).

Dose-responses were evaluated for compounds showing significant antifeedant activity at the 10% concentration (ie *S* (+) carvone, *R* (-) carvone, limonin, borneol, verbenone, and cucurbitacin) (Fig. 1). Feeding on twigs treated with limonin and *S* (+) carvone, the two most active compounds after 24 h, decreased dramatically with increasing concentration (Fig. 2A-D). A strong dose-response was also exhibited on twigs treated with *R* (-) carvone (Fig. 2E,F). Dose-response for verbenone occurred with males but not females, was generally weak for borneol, and was non-existent for curcurbitacin (Fig. 2G-L). However, as noted earlier, curcurbitacin concentrate began by containing only 0.3% (3,000 ppm) of the active ingredient, therefore, the concentrations that we observed ranged from 3 to 300 ppm.

Discussion

Choice tests were conducted to screen the candidate compounds for further dose-response no-choice tests. In field settings, especially Christmas tree plantations, *H. pales* will have alternative host sources to feed on (e.g., stumps and roots of harvested trees), approximating the choice test scenario. However, we wanted to identify compounds that are active under the most severe conditions provided by the no-choice scenario.

It is surprising that some compounds identified as active in the choice test were not active in the no-choice test. In fact, of the nine most active compounds identified in the choice test after 24 h (Table 1), only five showed significant activity in the no-choice test after 24 h (Fig. 1): *S* (+) and *R* (-) carvone, limonin, verbenone, and borneol. Neem extract (one of three neem tree products tested that also included neem oil and Margosan O) was active through 48 h in the choice test, yet was not active at either time in the no-choice test.

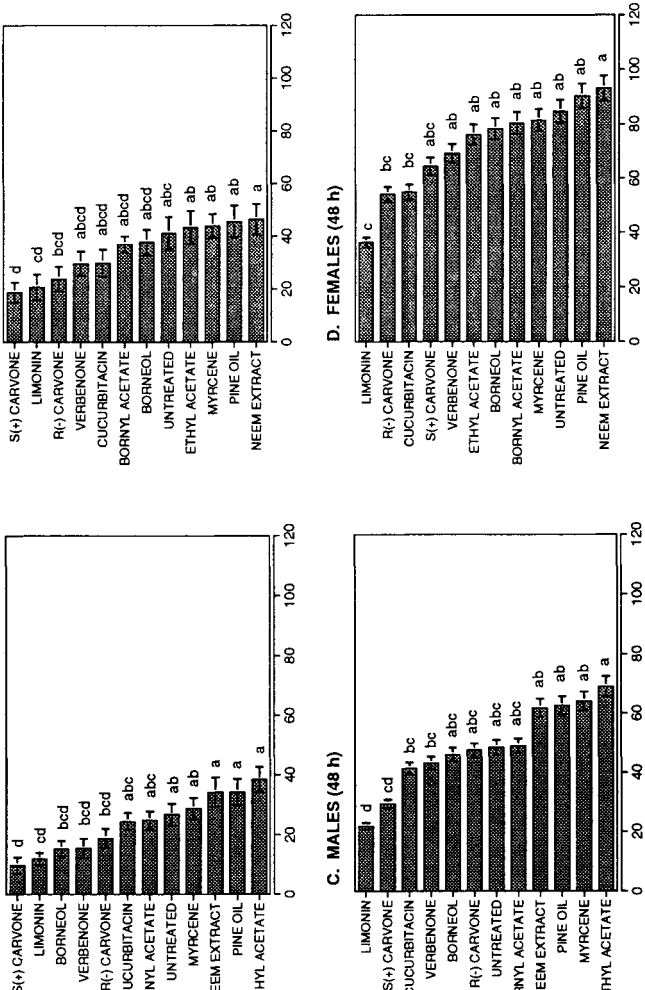

Fig. 1. Mean feeding by *Hylobius pales* on cambial tissue of *Pinus strobus* twigs treated with test compounds dissolved in ethyl acetate at a 10% concentration. Sample size for both sexes at each time period are based on two weevils tested in each of 20 replicates. Symbols at the end of each bar indicate the standard error. Significant differences among compounds are indicated by different lower case letters (SNK; $P < 0.05$).

After 48 h in the no-choice test, limonin and *S* (+) carvone retained relatively strong activity for males, while limonin, was the only active compound for females. Borneol lost effectiveness by 48 h, yet cucurbitacin moved up in the rankings and was considered active for males. The potency of cucurbitacin is much greater than other active compounds, given that its actual concentration was 300 ppm as opposed to ≈ 100,000 ppm for the other compounds showing activity. In addition, similar response to cucurbitacin-treated twigs at concentrations as low as 3 ppm active ingredient (Fig. 2K, L) have to make this one of the principal compounds targeted for future evaluation.

Oxygenated tetracyclic triterpenoid cucurbitacins are bitter and toxic substances of plants in the family Cucurbitaceae, and serve as protective semiochemicals for this and allied plant families against herbivore attack (Metcalf 1986). Although they are naturally produced compounds, the toxicity of cucurbitacins E and I, with an LD_{50} in mice of 40 mg/kg orally (Stroesand et al. 1985), is a human safety concern. In our study, however, we found no evidence of weevil mortality induced by exposure to cucurbitacin.

Limonin, another triterpenoid, consistently provided strong antifeedant activity at all levels of our study. Limonin is a member of one of the most potent classes of insect antifeedants, liminoids, and has potential for use in pest management because it can be easily isolated in large quantities from seeds available as a by-product of the citrus industry (Klocke and Kubo 1982). It has been shown to deter feeding on corn earworm, *Heliocoverpa zea* (Boddie), the fall armyworm, *Spodoptera frugiperda* (Smith) (Klocke and Kubo 1982), the tobacco caterpillar, *S. litura* (F.) (Koul 1983), and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Alford et al. 1987).

Both carvone isomers showed strong antifeedant activity in the choice and no-choice tests. *S* (+) carvone is a terpenoid found in dill (Umbelliferae) plants and has the odor of carraway (Chapman et al. 1981). In contrast, *R* (-) carvone has the odor of spearmint and is found in various plant species such as *Mentha crispa* L. While both isomers have been shown to serve as attractants to aphids (Chapman et al. 1981), *R* (-) carvone has been shown to act as an antifeedant to *S. littoralis* Boisduval (Meisner et al. 1982).

The only insect-produced compound tested, verbenone, provided sufficient antifeedant activity in the choice and no-choice tests to require further evaluation. Verbenone is an oxygenated terpene derived from *alpha*-pinene (Hughes 1975), a common component of conifer resin systems (Mirov 1961). As mentioned earlier, verbenone serves as an antiaggregation pheromone for some bark beetle species. More recently, it has been identified as an antifeedant to *H. abietis* in Europe (L. R. Kirkendall, pers. comm.).

This study has provided us with initial data concerning activity of potential antifeedants for use against *H. pales*. Further studies are needed to evaluate whether antifeedant activity of these compounds can be maintained on live pine seedlings, and if so, for what length of time.

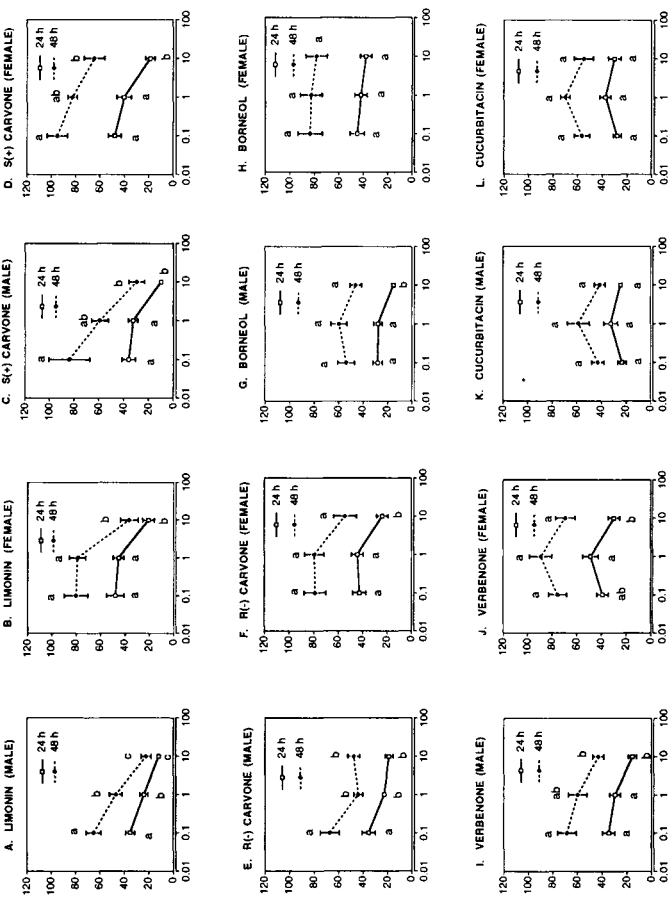

Fig. 2. Mean feeding by *Hylobius pales* on cambial tissue of *Pinus strobus* twigs treated with 10, 1, and 0.1% dilutions of the most active test compounds. Sample size for both sexes at each concentration is based on two weevils tested in each of 20 replicates. Bars at each data point indicate the standard error. Significant differences among concentrations for each compound, sex, and time period are indicated by different lower case letters (SNK; $P < 0.05$).

Acknowledgments

The authors would like to thank A. R. Alford and G. Nordlander for reviewing earlier versions of the manuscript. This project was supported in part by Virginia Agriculture Council Grant No. 256 to S.M.S. and USDA/CSRS grant No. 230-11-110A-007-333-593-1 to S.M.S. and J.A.C.

References Cited

Alexander, S. A. and J. A. Carlson. 1992. Christmas tree diseases, Pp. 189-190. *In* Pest Management Guide for Horticultural and Forest Crops. Va. Coop. Ext. Public. 456-017.

Alexander, S. A., and W. E. Horner and K. J. Lewis. 1988. *Leptographium procerum* as a pathogen of pines, Pp. 97-112. *In* T. C. Harrington and F. W. Cobb, Jr. (eds.), *Leptographium* root diseases on conifers. APS Press, St. Paul, MN.

Alfaro, R. I., H. D. Pierce Jr., J. H. Borden and A. C. Oehlschlager. 1980. Role of volatile and nonvolatile components of Sitka spruce bark as feeding stimulants for *Pissodes strobi* Peck (Coleoptera: Curculionidae). Can. J. Zoo. 58: 626-636.

Alford, A. R., J. A. Cullen, R. H. Starch, and M. D. Bentley. 1987. Antifeedant activity of limonin against the Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 80: 575-578.

Anderson, G. W. 1980. Pine reproduction weevils. (Coleoptera: Curculionidae) infesting Christmas trees plantations in southwest Virginia. M. S. thesis. VPI and SU, Blacksburg, VA. 65 pp.

Beal, J. A. and K. B. McClintick. 1943. The pales weevil in southern pine. J. Econ. Entomol. 36: 792-794.

Berisford, C. W., O. E. Brady, C. W. Fatzinger and B. H. Ebel. 1986. Evaluation of a repellent for prevention of attacks by three species of southern pine bark beetles. J. Entomol. Sci. 21: 316-318.

Borden, J. H. 1985. Aggregation pheromones, Pp. 257-285. *In* Kerkut, G. A. (ed.). Behavior, Vol. 9. *In* Kerkut, G. A. and L. I. Gilbert (eds.), Comprehensive insect physiology, biochemistry, and pharamacology. Pergamon Press, Oxford.

Carter, E. E. 1916. *Hylobius pales* as a factor in the reproduction of conifers in New England. Proc. Soc. Amer. For. 11: 297-307.

Chapman, R. F., E. A. Bernays and S. J. Simpson. 1981. Attraction and repulsion of the aphid, *Cavariella aegopodii,* by plant odors. J. Chem. Ecol. 7: 881-888.

Clark, E. W. 1975. Reproductive diapause in *Hylobius pales.* Ann. Entomol. Soc. Am. 68: 349-352.

Cook, S. P. and F. P. Hain. 1988. Toxicity of host monoterpenes to *Dendroctonus frontalis* and *Ips calligraphus* (Coleoptera: Scolytidae). J. Entomol. Sci. 23: 287-292.

Corneil, J. A. and L. F. Wilson. 1984. Some light and temperature effects on the behavior of adult pales weevil, *Hylobius pales* (Coleoptera: Curculionidae). Great Lakes Entomol. 17: 225-228.

Davis, R. and H. O. Lund. 1966. Some studies of *Hylobius pales* (Herbst) and *Pachylobius picivorous* (Germar). J. G. Ent. Soc. 1: 25-28.

Day, E. R., S. M. Salom, P. B. Schultz and T. C. Tigner. 1992. Christmas tree insects, Pp. 185-188. *In* Pest management guide for horticultural and forest crops. Va. Coop. Ext. Public. 456-017.

Doggett, C. A., C. R. Grady, H. J. Green, M. B. Kunselman, H. Layman and S. Taylor. 1977. Seedling debarking weevils in North Carolina. N. C. Forest Service, Forestry Note 31. 13 pp.

Fox, R. C. and T. M. Hill. 1973. The relative attraction of burned and cutover pine areas to the pine seedling weevils *Hylobius pales* and *Pachylobius picivorus.* Ann. Entomol. Soc. Am. 66: 52-54.

Gollob, L. 1980. Monoterpene composition in bark beetle-resistant loblolly pine. Naturwissen. 67: 409-410.

Hanover, J. W. 1975. Physiology of tree resistance to insects. Ann. Rev. Entomol. 20: 75-95.

Hertel, G. D. 1970. Response of the pales weevil to loblolly pine seedlings and cut stems. J. Econ. Entomol. 63: 995-997.

Hoffman, G. D. and K. F. Raffa. 1992. Maturation of the males pales weevil (Coleoptera: Curculionidae) reproductive system and its effect on male response to females. Ann. Entomol. Soc. Am. 85: 571-577.

Hughes, P. R. 1975. Pheromones of *Dendroctonus:* origin of *alpha*-pinene oxidation products present in emergent adults. J. Insect Physiol. 21: 687-691.

Jones, D. 1984. Use, misuse, and role of multiple comparison procedures in ecological and agricultural entomology. Environ. Entomol. 13: 635-649.

Klocke, J. A. and I. Kubo. 1982. Citrus liminoid by-products as insect control agents. Entomol. exp. appl. 32: 299-301.

Koul, O. 1983. Feeding deterrence induced by plant liminoids in the larvae of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). J. Appl. Entomol. 95: 161-171.

Lackner, A. L. and S. A. Alexander. 1982. Occurrence and pathogenicity of *Verticicladiella procera* in Christmas tree plantations of Virginia. Plant Disease. 66: 211-212.

1983. Root disease and insect infestations on air-pollution-sensitive *Pinus strobus* and studies of pathogenicity of *Verticicladiella procera*. Plant Disease 67: 679:681.

Lewis, K. A., and S. A. Alexander. 1986. Insects associated with the transmission of *Verticiladiella procera*. Can. J. For Res. 16: 1330-1333.

Lewis, K. A. S. A. Alexander and W. E. Horner. 1987. Distribution and efficacy of propagules of *Verticiladiella procera* in soil. Phytopathology. 77: 552-556.

Lynch, A. M. 1984. The pales weevil, *Hylobius pales* (Herbst): a synthesis of the literature. J. Ga. Entomol. Soc. 19: 1-34.

Manwan, I. 1964. The biology of pales weevil, *Hylobius pales* (Herbst) in Arkansas. M. S. Thesis, Univ. of Arkansas, Fayetteville, AR. 74 pp.

Meisner, J., A. Fleischer and C. Eizick. 1982. Phagodeterrency induced by (-)- carvone in the larva of *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. Econ. Entomol. 75: 462-466.

Metcalf, R. L. 1986. Coevolutionary adaptations of rootworm beetles (Coleoptera: Chrysomelidae) to cucurbitacins. J. Chem. Ecol. 12: 1109-1124.

Mirov, N. T. 1961. Composition of gum turpentines of pines. USDA, For. Serv. Tech. Bull. 1239.

Morgan, E. D. and N. B. Mandava. 1990. CRC handbook of natural pesticides. Vol. VI. insect attractants and repellents. CRC Press, Inc. Boca Raton, FL. 249 pp.

Morgan, E. D. and J. D. Warthen, Jr. 1990. Part B. Insect feeding deterrents (1980-1987), Pp. 83-104. *In.* Morgan, E. D. and N. B. Mandova (eds.), CRC Handbook of natural pesticides. Vol. VI. insects attractants and repellents. CRC press, Inc. Boca Raton, FL.

Nevill, R. J. and S. A. Alexander. 1992a. Transmission of *Leptographium procerum* to eastern white pine by *Hylobius pales* and *Pissodes nemorensis* (Coleoptera: Curculionidae). Plant Dis. 76: 307-310.

1992b. Distribution of *Hylobius pales* and *Pissodes nemorensis* (Coleoptera: Curculionidae) within Christmas tree plantations with procerum root disease. Environ. Entomol. 21: 1077-1085.

Nijholt, W. W. 1980, Pine oil and oleic acid delay and reduce attack on logs by ambrosia beetles (Coleoptera: Scolytidae). Can. Ent. 112: 199-204.

Nijholt, W. W., L. H. McMullen and L. Safranyik. 1981. Pine oil protects living trees from attack by three bark beetle species, *Dendroctonus* spp. (Coleoptera: Scolytidae). Can. Ent. 113: 337-340.

Nord, J. C., J. H. Ghent, H. A. Thomas and C. A. Doggett. 1982. Control of pales and pitch-eating weevils in the South. USDA Forest Service, Forestry Report SA-FR-21. 24 pp.

Nordlander, G. 1990. Limonene inhibits attraction to *alpha*-pinene in the pine weevils *Hylobius abietis* and *H. pinastri*. J. Chem. Ecol. 16: 1307-1319.

1991. Host finding in the pine weevil *Hylobius abietis:* effects of conifer volatiles and added limonene. Entomol. exp. appl. 59: 229-237.

Norris, D. M. 1986. Anti-feeding compounds. Chem. Plant Prot. 1: 98-146.

O'Donnell, B. P., T. L. Payne and K. D. Walsh. 1986. Effect of pine oil on landing and attack by the southern pine beetle (Coleoptera: Scolytidae). J. Entomol. Sci. 21: 319-321.

Payne, T. L., R. F. Billings, C. W. Berisford, S. M. Salom, D. M. Grosman. M. J. Dalusky and W. W. Upton. 1992. Disruption of *Dendrotonus frontalis* (Col., Scolytidae) infestations with an inhibitor pheromone. J. Appl. Ent. 114: 341-347.

Peirson, H. B. 1921. The life history and control of the pales weevil (*Hylobius pales*). Harvard Forest. Bull. 3. Harvard Forest, Petersham, MA. 33 pp.

Raffa, K. F., A. A. Berryman, J. Simasko, W. Teal and B. L. Wong. 1985. Effects of grand fir monoterpenes on the fir engraver, *Scolytus ventralis* (Coleoptera: Scolytidae), and its symbiotic fungus. Environ. Entomol. 14: 552-556.

Rennels, R. G. and H. W. Fox. 1970. The pales weevil - a serious pest in Illinois Christmas tree plantations. Amer. Christ. Tree J. 14: 17-18.

Renwick, J. A. A. and J. P. Vité. 1969. Bark beetle attractants: mechanism of colonization by *Dendroctonus frontalis*. Nature 224: 1222-1223.

Salom, S. M., F. M. Stephen and L. C. Thompson. 1987. Development rates and temperature-dependent model of pales weevil, *Hylobius pales* (Herbst), development. Environ. Entomol. 16: 956-962.

Salom, S. M., R. F. Billings, W. W. Upton, M. J. Dalusky, D. M. Grosman, T. L. Payne, C. W. Berisford and T. N. Shaver. 1992. Effect of verbenone enantiomers and racemic *endo*-brevicomin on response of *Dendroctonus frontalis* (Coleoptera: Scolytidae) to attractant-baited traps. Can. J. For. Res. 22: 925-931.

SAS. 1989. user's guide: statistics, verions 6. SAS Institute Inc., Cary, NC.

Schoonhoven, L. M. 1982. Biological aspects of antifeedants. Entomol. exp. appl. 31: 57-69.

Smith, R. H. 1975. Formula for describing effect of insect and host tree factors on resistance to western pine beetle attacks. J. Econ. Entomol. 68: 841-844.

Speers, C. F. 1958. Pales weevil rapidly becoming serious pest of pine reproduction in the South. J. For. 56: 723-726.

Speers, C. F. and M. G. Cody. 1975. Technique for rearing the pales weevil, *Hylobius pales* (Coleoptera: Curculionidae). J. Ga. Entomol. Soc. 10: 251-254.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill Book Co., New York, 633 pp.

Stroesand, G. S., A. Jaworski, S. Shannon and R. W. Robinson. 1985. Toxicologic response in mice fed *Cucurbita* fruit. J. Food Prot. 48: 50-51.

Thomas, H. A. and G. D. Hertel. 1969. Response of pales weevil to natural and synthetic host attractants. J. Econ. Entomol. 62: 383-386.

Vité, J. P. and E. Baader. 1990. Present and future use of semiochemicals in pest management of bark beetles. J. Chem. Ecol. 16: 3031-3041.

Warthen, J. D., Jr. 1990. Insect feeding deterrents. Part A: Insect feeding deterrents (1976-1980), Pp. 23-82. *In* E. D. Morgan and N. B. Mandava (eds.), CRC handbook of natural pesticides. Vol. VI. insect attractants and repellents. CRC Press, Inc. Boca Raton, FL.

Weidhaas, J. A. 1989. Insects, Pp. 123-136. *In* J. E. Johnson (ed.), Christmas tree production manual Va. Coop. Ext. Public. 420-075.

Wilkinson, R. C. 1980. Relationship between cortical monoterpenes and susceptibility of eastern white pine to white pine weevil attack. For. Sci. 26: 581-589.