Brooks et al.[10] and Chenevert et al.[11] tested the 5-Me, 7-MeO (**34**) and 5,7-diMeO (**35**) derivatives, respectively. Their data are in agreement with the above results and support our conclusion on the role of the substitutents at C-5 in precocene activity.

4. *Trisubstitution at C-5, C-6 and C-7.* The tested compounds were generally inactive, even if the related C-6, C-7 or C-5, C-7 disubstituted derivatives showed precocene activity (cf. e.g. **16, 34** and **43, 46** as well as **20** and **44** in the table). We can conclude that trisubstitution at C-5, C-6 and C-7 decreases or eliminates precocene activity as compared to the disubstituted molecules.

5. *Disubstitution at C-7 and C-8.* The tested molecules (**47, 48**) showed no precocene activity, even if the related C-7-monosubstituted compounds were highly active (cf. **47, 48** and **1** and **2**). As C-8-monosubstitution with MeO did not yield any activity in previous studies[9, 12, 13], the ineffectiveness of the C-7, C-8 disubstituted derivatives is probably due to the C-8 substitution itself.

Toxic effects. As can be seen in the table, the LD50 values did not follow the rules established for precocene activity, hence no clearcut conclusion on the relationship between toxicity and chemical structure can be reached. If we assume that general toxicity is also based on the oxidative activation of these compounds, it is very likely that the target of the toxic effect is much broader than the CA, and includes other peripheral tissues and organs[3].

Conclusions. According to previous studies[9-11] the C-7 substituent has a basic role in precocene activity. In our experiments, only the alkoxy substituents showed activity at C-7. The maximum effect was observed with EtO and nPrO while longer or shorter as well as branching and/or unsaturated alkoxy-substituents decreased the activity.

In the case of C-6, C-7 disubstitution, the C-6 alkoxy-group can seriously modify the effect of the C-7 substituent. This was also observed in precocene experiments carried out on nematodes[20-22]. Notable precocene activity can be observed only if both substituents are alkoxy-groups and the C-7 side-chain is longer than that at C-6. In the reverse case, the precocene effect is invariably lost. In absolute terms, the C-6 alkoxy-group should not be longer than EtO.

In the case of C-5, C-7 disubstitution, C-7 substituents remain active or even gain an enhanced activity if C-5 is occupied by Me. When C-5 bears MeO, the precocene effect is invariably lost.

In the tested C-7, C-8 disubstituted compounds, the C-8 substituent seems to eliminate the precocene activity derived from the C-7 substituted derivative.

Compounds bearing more than two substituents or $-NO_2$ and Cl as substituents, have no appreciable activity.

The LD50 values do not follow the rules established for the precocene effect, hence the toxic effect has to have another target/mechanism.

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Volatiles from clover head *Hypera meles* (Fab.) and alfalfa *H. postica* (Gyllenhal) weevils: search for pheromones

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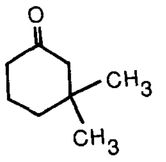
Summary. Volatile fractions of the clover head, *Hypera meles* (Fab.), and alfalfa, *Hypera postica* (Gyllenhal), weevils contained three of four boll weevil, *Anthonomus grandis* (Boh.), pheromone components, (Z)-3,3-dimethylcyclohexane $\Delta^{1,\beta}$-ethanol and (Z)- and (E)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde. Also found were eight oxygenated monoterpenes, previously identified as precursors and intermediates of the boll weevil pheromones.

Key words. Clover head weevil; alfalfa weevil; *Hypera meles* (Fab.); *Hypera postica* (Gyll.); candidate pheromones, Curculionidae.

Volatiles found in clover head and alfalfa weevil extracts by GLC-MS analysis

No.	Elution time (min)	Identity	CHW-F_1 (g/insect)	CHW-OW	AW-F_1
1	5.15	3,3-dimethylcyclohexanone	TR	TR	1.2
2	5.89	3,3-dimethylcyclohex-1-eneacetaldehyde	20.3	30.2	3.8
3	6.48	M^+168	0.7	0.9	0.1
4	6.75	3,3-dimethylcyclohex-1-enecarboxaldehyde	0.6	0.9	0.1
5	7.33	M^+154	–	–	0.2
6	8.28	Methyl-3,3-dimethylcyclohexane carboxylate	2.9	2.4	0.5
7	8.37	Isogeraniol	–	–	0.4
8	8.60	3,3-dimethylcyclohexaneethanol	2.4	3.0	–
9	8.80	(Z)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol	2.5	3.4	–
10	9.13	(Z)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde	7.4	10.5	1.2
11	9.18	(Z)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethyl formate	3.1	4.5	0.8
12	9.25	(E)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde	8.6	11.8	1.4
13	10.35	(Z)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetic acid	14.5	19.8	2.2
14	10.53	(E)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetic acid	12.2	18.1	1.6
15	12.07	Nepetalactone	–	–	2.9
16	17.35	Palmitic acid	9.8	11.2	1.1
17	19.02	Oleic acid	17.6	25.1	3.3
18	19.20	Stearic acid	9.5	12.6	1.1
	Total		112.1	154.4	21.6



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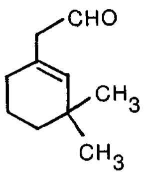

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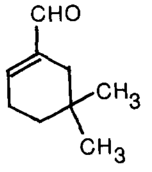

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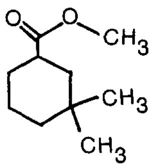

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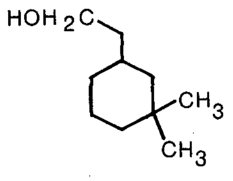

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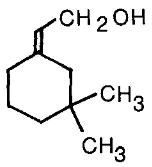

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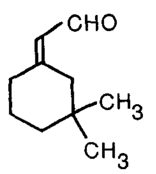

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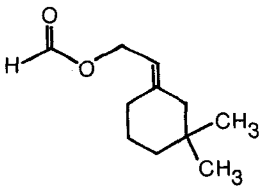

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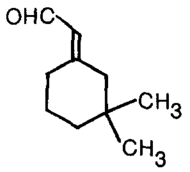

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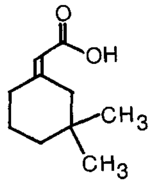

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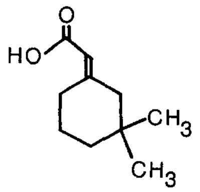

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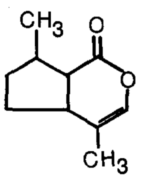

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Structures of the constituents isolated from clover head and alfalfa weevil volatiles. (Numbering follow's table).

The alfalfa weevil, *Hypera postica* (Gyllenhal), and the clover head weevil, *H. meles* (Fab.), are important pests of alfalfa, *Medicago sativa* (L.), and clovers, *Trifolium* spp., respectively. In spite of their economic importance, pheromones have not been isolated or identified for either insect. Aggregative behavior has been documented for alfalfa weevils by Hamlin et al.[2] and Simpson and Welborn[3], and have been observed in clover head weevils (Ellsbury, unpublished). We report the identification by GLC-MS of a number of volatile constituents of these insects as part of a search for pheromones.

Methods and materials. Adult clover head weevils (1200 F_1 and 485 overwintered, mixed sexes), and 330 adult alfalfa weevils (mixed sexes) were covered with 500–1000 ml hexane, and stored at 4 °C until they were poured without concentration on to a 5×14 cm silicic acid column (Bio-Sil A, 200–400 mesh Bio-Rad Lab., Richmond, CA) that had been slurried in hexane. The column was sequentially eluted with 500 ml hexane, 500 ml methylene chloride/hexane: 1/9, 500 ml methylene chloride/hexane: 1/1, and 500 ml methylene chloride. The eluates were concentrated under reduced pressure to ca 20 ml, examined by TLC, and combined to give 3–5 composited fractions. Each was examined by GLC-MS, on a DB-1 fused silica column ($15 \text{ m} \times 0.322$ mm) that was interfaced to a Hewlett Packard 5985-B^R quadrupole mass spectrometer. An approximation of relative concentrations of components was obtained by comparing the MS data system total abundance count of the ion chromatogram with that of the appropriate standards available from our previous work[4,5].

Results and discussion. Found in the clover head weevil (both F_1 and overwintered) were 11 oxygenated monoterpenes and 3 fatty acids. Most prominent were 3 of the 4 boll weevil, *Anthonomus grandis* (Boh.), pheromone components previously identified by us[4], (Z)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol **9**, and (Z)-and (E)-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde **10** and **12** (table, fig.). The other 8 oxygenated monoterpenes have recently been identified by us as boll weevil pheromone intermediates or by-products[5] (table, fig.).

Identified from the alfalfa weevil (F_1) were the 2 boll weevil pheromone aldehydes ((Z)- and (E)-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde), **10** and **12**, six of the oxygenated monoterpenes found in the clover head weevil, isogeraniol, nepetalactone, and 3 fatty acids (table, fig.). The yield of volatiles from the alfalfa weevil was lower (about 20%) than from the clover head weevil (table).

The identities of the compounds were confirmed by comparison of the GLC retention volumes and mass spectral fragmentation patterns to those of the standards that had been synthesized in previous work[4,5].

It would not be unexpected for the clover head and alfalfa weevils to biosynthesize components similar or identical to the sex pheromones of the boll weevil, since all three are members of the family Curculionidae. Several other curculionids have sex pheromone components in common with the boll weevil[6]. However, the identification of the complete pheromones of these two insects awaits additional studies and associated chemical work[7].

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[1] Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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[7] Because of program changes, rearing of these two insects has been discontinued here. Hence, no further work by us is possible.

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The contents of the pygidial gland of the primitive ant *Nothomyrmecia macrops* (Hymenoptera: Formicidae)

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Summary. The principal constituent of the pygidial gland of *Nothomyrmecia macrops* is 3,7-dimethyloct-6-en-2-one, a substance not previously identified in insects. Also identified were 2,6-dimethylhept-5-enal, 2-nonanone, indole, γ-dodecalactone, and the hydrocarbons pentadecane, heptadecane, heptadecene and heptadecadiene, all in low nanogram quantities.

Key words. Ant; pygidial gland; *Nothomyrmecia;* dimethyloctenone; γ-dodecalactone.

We have recently collected specimens of the extremely elusive and primitive ant *Nothomyrmecia macrops* Clark at Poochera, South Australia. This is considered the most primitive living ant, and because of some peculiar anatomical features, has been placed by itself in the subfamily Nothomyrmeciinae[1]. It has therefore been the subject of several recent studies of its anatomy and phylogenetic position[2], its behaviour[3], genetics[4], and sting morphology[5]. We have now undertaken a combined study of the ultrastructure[6] and chemical contents of its exocrine glands. We have already described the large number of substances identified in its Dufour gland[7].

We describe here the contents of the pygidial gland and our attempt to study the mandibular gland. The pygidial gland (fig. 1), is associated with the intersegmental membrane between the 6th and 7th abdominal tergites of ants. Large pygidial glands are found in most species of the subfamily Dolichoderinae (where they were erroneously called 'anal glands') and in a number of species scattered throughout other subfamilies[8]. Excepting the dolichoderines, they have