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DEFENSIVE ODOR EMISSION FROM LARVAE OF TWO SAWFLY SPECIES, *Pristiphora erichsonii* AND *P. wesmaeli*[1]

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Abstract—The emission of species-specific odors by *Pristiphora erichsonii* and *P. wesmaeli* is accompanied by a characteristic defensive behavior called "snap bending." When the larvae are disturbed, blends of volatile compounds are emitted from ventral glands. The odor of *P. erichsonii*, a colonial species, is composed of bornyl acetate, borneol, *trans*-pinocarveol, myrtenol, benzaldehyde, and tetradecyl, hexadecyl, and octadecyl acetates, whereas that of the solitary *P. wesmaeli* is composed of 3-carene-10-al, linalool, myrtenal, and benzaldehyde. The role of these compounds in the defensive behavior of the larvae is discussed.

Key Words—Larch sawfly, *Pristiphora erichsonii*, Hymenoptera, Tenthredinidae, 3-carene-10-al, benzaldehyde, bornyl acetate, larval snap-bending.

INTRODUCTION

Larvae of the larch sawflies, *Pristiphora erichsonii (Hartig)* and *Pristiphora wesmaeli* (Tischbein) (Hymenoptera, Tenthredinidae), produce characteristic scents, easily distinguished by human olfaction. Upon being disturbed, the larvae emit the scents and quickly flex their abdomens to attain a characteristic stiff S-shaped posture, called "snap-bending" ("Schnickender haltung," Nägeli, 1935) (Figure 1).

[1] Hymenoptera: Tenthredinidae.

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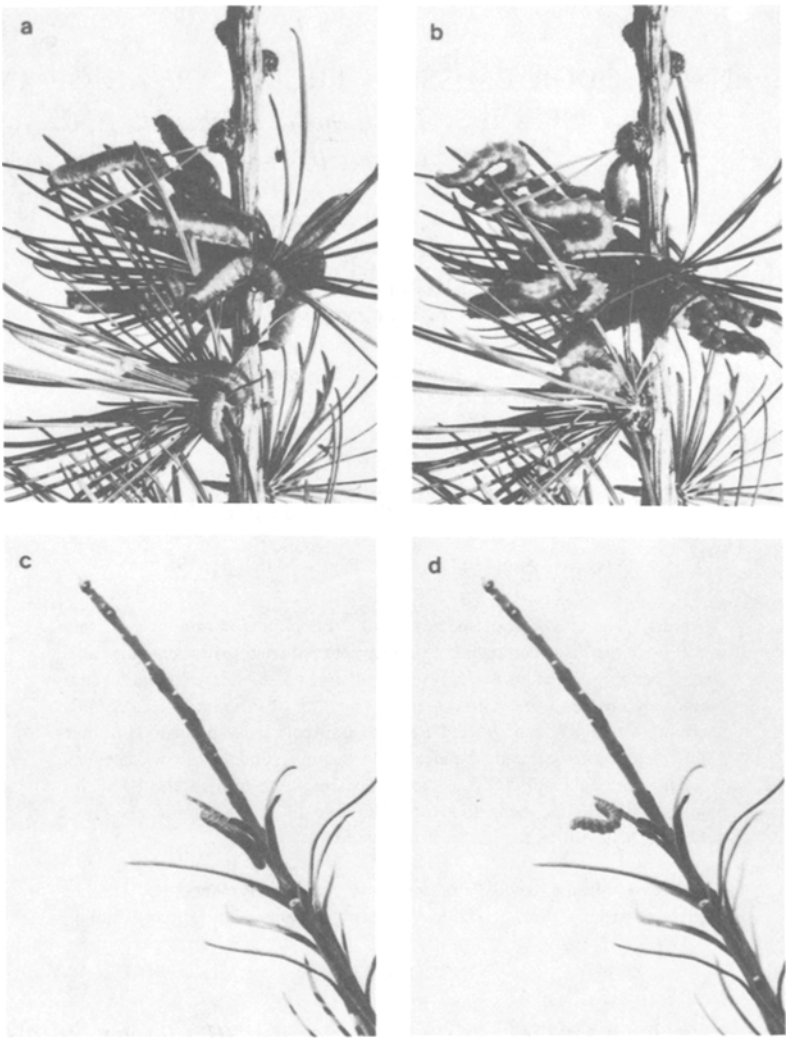

FIG. 1. A colony of *Pristiphora erichsonii* larvae (a, b) and a single *P. wesmaeli* larva (c, d) on needles of *Larix decidua* in the field. In a and c the larvae are feeding undisturbed, while in b and d the same larvae are shown in the snap-bending posture.

Glands producing volatile secretions are situated ventrally on the first seven abdominal segments in several Tenthredinidae (Yuasa, 1923; Maxwell, 1955; Boevé and Pasteels, 1985). The glands on the second to seventh segments are protrusile, those on the first segment are not. It has been suggested that the latter produce the odor emitted by snap-bending larvae (Benson, 1950, p. 76).

The sympatric *P. erichsonii* and *P. wesmaeli* differ in several respects, including feeding and oviposition behavior. *P. erichsonii* attack needles growing from tufts produced the preceding summer, whereas *P. wesmaeli* feed only on the long shoot needles initiated the same summer. Larvae of *P. erichsonii* live colonially with the most pronounced aggregation during the early instars, while *P. wesmaeli* larvae are solitary (Pschorn-Walcher and Zinnert, 1971). *P. wesmaeli* larvae are light green with two somewhat lighter bands along the body, and they exhibit homochromy in that they resemble larch needles. *P. erichsonii* larvae are greyish-green with a black head and are easier to discover at distance.

We have isolated compounds from the secretions which emanate from gland cells of the first abdominal segment of each species of sawfly larvae. The volatile components have been identified in an attempt to understand the role of these volatiles in the behavior related to the different life histories of these two species.

METHODS AND MATERIALS

Feeding larvae of *P. erichsonii* and *P. wesmaeli* were collected from *Larix* spp. near Uppsala in eastern Sweden or near Vårgårda in southwestern Sweden during the years 1975, 1979–1981, and 1985. In the laboratory, the larvae were fed with fresh foliage of *Larix decidua* (Miller).

To collect the volatiles emitted from the first abdominal segment, the larvae were induced to snap-bend and the glandular openings were wiped with 3 × 12-mm pieces of filter paper held with forceps. This procedure was repeated four to six times for each larva, with at least 12 h of undisturbed feeding between collections. The filter papers were immediately extracted in either redistilled pentane or pentane:ethyl acetate (95 : 5 v/v). The extracts were stored at −20°C and concentrated by evaporation at 42°C. Analyses were made on a coupled gas chromatograph–mass spectrometer (GC-MS), Finnigan 4021, San Jose, California. Glass capillary columns coated with either FFAP/OV-17 (6 : 5) (0.3 mm × 33 m) or Superox FA (0.25 mm × 56 m) or a fused silica column coated with OV-351 (0.2 mm × 25 m) were used. All compounds were identified by comparing their retention characteristics and mass spectra with those of synthetic reference compounds. The mass spectra were scanned from *m/e* 31 to

300, and, for the quantitative determinations, the area under each peak in the reconstructed ion chromatogram was measured. A response factor of 1.0 was used for all compounds.

The gas chromatographic phases were purchased from Alltec Assoc. Bellefonte, Pennsylvania and Supelco Inc. Deerfield, Illinois. The 3-carene-10-al was derived was derived by oxidation of 3-carene-10-ol synthesized as described in Lanne et al. (1987).

In a series of experiments at the laboratory, groups of the bug *Anthocoris nemorum L.* (Heteroptera, Anthocoridae) were placed one by one in Petri dishes (diameter 70 mm) containing a piece of filter paper with synthetic compounds (0.5–1 μg of each compound). As control, insects were studied in the same situation but without odors added to the filter paper. Three replicates were made of each test.

RESULTS

The gland on the ventral side of the first abdominal segment of *P. erichsonii* larvae was studied by light microscopy and was found to consist of a thin layer of glandular cells in a pocket. The gland is not protrusible, but the glandular pocket was observed to open and close regularly while the larvae were snap-bending. The secretions from these glands of *P. erichsonii* and *P. wesmaeli* were analyzed.

Gas chromatograms of the volatiles from the two species (Fig. 2) show that they are made up of several different compounds (Table 1). Benzaldehyde was the only compound common to both species, although present in different relative amounts. In *P. erichsonii*, the four oxygenated monoterpenes found in appreciable amounts were bornyl acetate, borneol, myrtenol, and *trans*-pinocarveol. Of these, bornyl acetate was the major component. The *P. wesmaeli* secretion contained three other oxygenated monoterpenes: linalool, myrtenal, and 3-carene-10-al. The mass spectrum of 3-carene-10-al, the major compound in *P. wesmaeli*, was compared with that of the synthetic reference (Figure 3). The *P. erichsonii* secretion also contained straight-chain tetradecyl, hexadecyl, and octadecyl acetates. Individual larvae of *P. erichsonii* and *P. wesmaeli* contained on the average 50 ng bornyl acetate and 1 ng 3-carene-10-al, respectively. Different instars of *P. erichsonii* and *P. wesmaeli* were analyzed. Third and fifth instars were compared for *P. erichsonii*. These analyses, based on 150 larvae of each instar, showed that none of the components in the secretion differed more than a few percent relative to the main component, bornyl acetate. Three groups of *P. wesmaeli* were analyzed, the first (120 larvae) consisted of a mixture of second- and third-instar larvae, the second group (75 larvae) consisted of third-instar larvae, and the third group (41 larvae) a mixture of fourth- and fifth-instar larvae. For *P. wesmaeli*, the amount of linalool and myrtenal did not differ more than 3% relative to 3-carene-10-al.

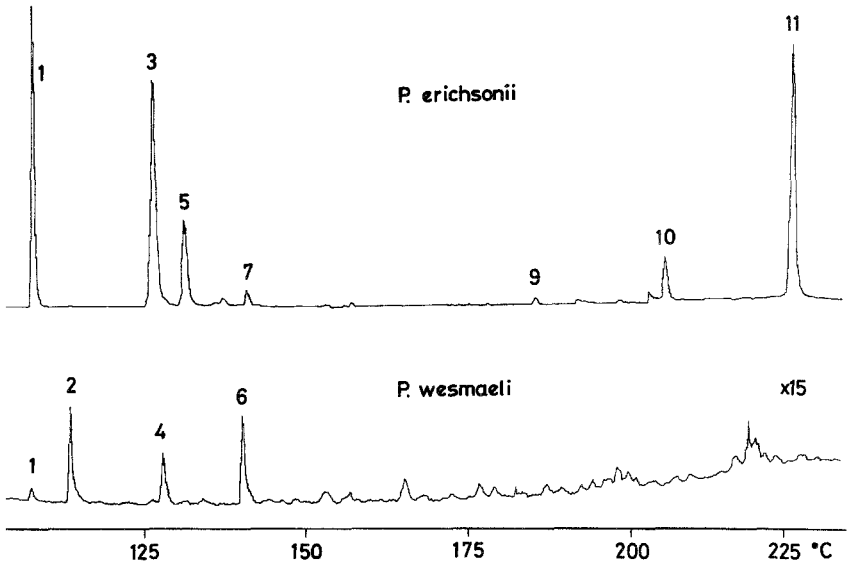

FIG. 2. Examples of capillary gas chromatograms of volatile secretions from *P. erichsonii* and *P. wesmaeli*. The GC column was coated with FFAP/OV-17 (see Methods and Materials). Compound 8 was not evident in this analysis as it coeluted with bornyl acetate on this particular column.

TABLE 1. VOLATILE COMPOUNDS IDENTIFIED IN GLANDULAR SECRETION OF *Pristiphora erichsonii* AND *P. wesmaeli* LARVAE

No.	Compounds	Relative amount[a]	
		P. erichsonii[b]	*P. wesmaeli*[c]
1.	Benzaldehyde	60	0.5
2.	Linalool		30
3.	Bornyl acetate	100	
4.	Myrtenal		27
5.	Borneol	14	
6.	3-Carene-10-al		100
7.	Myrtenol	2	
8.	*trans*-Pinocarveol	7	
9.	Tetradecyl acetate	1	
10.	Hexadecyl acetate	3	
11.	Octadecyl acetate	36	

[a] Expressed as percentage of the major compound in the respective secretion.
[b] Mean value of five extracts of instars 3, 4, and 5. More than 400 larvae were analyzed.
[c] Mean value of three extracts of instars 1–5. Seventy-six larvae were analyzed.

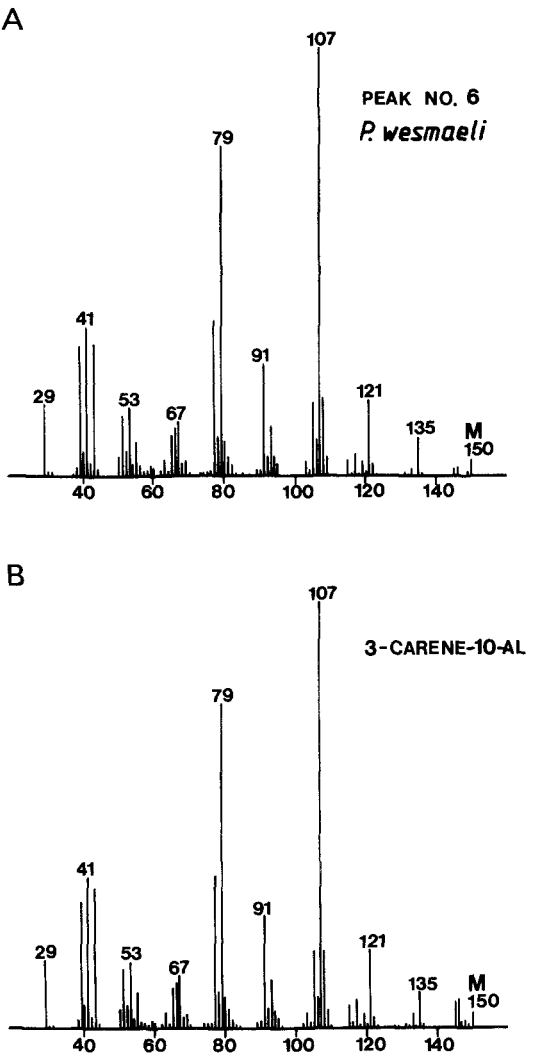

FIG. 3. Mass spectra of (A) compound 6 in *P. wesmaeli*, and (B) synthetic 3-carene-10-al.

Needles of *Larix sibirica* (Ledebour) and *L. decidua* were extracted and analyzed. Bornyl acetate, the only detectable volatile compound common to both *P. erichsonii* larvae and needles, was present in trace amounts in *L. sibirica*.

Mechanical or visual stimuli were used to evoke snap-bending and odor

emission under experimental conditions in the field and in the laboratory. Under natural conditions in the field, attacks by heteropteran predators such as *Orius* sp., *Anthocoris nemorum*, and *Ligyrocoris silvestris* L., were observed to release snap-bending behavior and odor emission. The attacking insects normally reacted to the behavior and excretions of the larch sawfly larvae by retreating and then grooming their antennae. When the predator *A. nemorum* was exposed to compounds found in the secretion of *P. erichsonii* in the laboratory, the insects were alerted and increased the intensity of their activities. They groomed their antennae, twitched, ran around, and attempted to fly. A mixture of borneol, bornyl acetate, myrtenol, and tetradecyl and hexadecyl acetate evoked a strong response, and when the compounds were tested one by one; hexadecyl acetate gave the strongest response. Benzaldehyde also stimulated the insects but only for a short while.

DISCUSSION

The spectrum of defense mechanisms known for larvae of Tenthredinidae include: (1) rapid changes in position (Figure 1b and d); (2) hiding (Figure 1a and c); (3) camoflage (homochromy) (Figure 1a and c); (4) display, which may be aposematic (Figure 1b and d); and (5) deterrence. Coupling between these modes of defensive behavior is common and augmentative (Benson, 1950). Glandular ejections of ill-smelling or protective liquids by sawfly are effective against spiders, which are chemotactically sensitive (Bristowe, 1941). Recently, Boevé and Pasteels (1985) have shown that the secretions from Nematinae sawfly larvae with well-developed ventral glands in the abdominal region protect the larvae from attacks by ants. Our field observations of *A. nemorum* and other heteropteran predators also show that the sawfly larval secretions protect the larvae from predators. Furthermore, our laboratory experiments indicate that the predators are affected by the glandular compounds of *P. erichsonii*.

The patterns of oxygenated monoterpenes found in the larch sawfly larvae show great similarity to the patterns of such compounds found in the hindguts of many bark beetles. In bark beetles, many of the oxygenated monoterpenes were originally suggested to result from detoxification of host terpenes (White et al., 1980), but the compounds are known now, in some cases, to be used as aggregation pheromones (Wood, 1982; Borden, 1982). The ability to oxygenate host monoterpene hydrocarbons is widespread in several insect orders. Two plausible precursors of *trans*-pinocarveol, myrtenol, and myrtenal are α-pinene and β-pinene, while 3-carene is probably the precursor of 3-carene-10-al. These three monoterpene hydrocarbons are present in many conifers (Karrer, 1976). It is also possible that the insects selectively sequestered some of the trace oxygenated monoterpenes that can be present in a conifer host (Heemann and

Francke, 1977). If this is the case for the larch sawflies, the different amounts of monoterpenes in *P. erichsonii* and *P. wesmaeli* should reflect their different choices of food.

Most studies of larval odors have dealt with defensive secretions (for a review see Blum, 1981). The Tenthredinidae *Neodiprion sertifer* (Geoffrey) larvae, upon being disturbed, perform a type of snap-bending similar to that of larch sawfly larvae, but their heads are lifted rather than their abdomens. In this disturbed situation, they discharge the content of diventricular pouches in their foreguts. These pouches contain sequestered host-plant terpenoic resins (Eisner et al., 1974) that deter predators.

Boevé et al. (1984) and Boevé and Pasteels (1985) have investigated eight species of Tenthredinidae, subfamily Nematinae, all of which have glands that produce secretions repellent to ants. These glands are situated ventrally on the second to seventh abdominal segments and are protrusible. The authors have, as have we, isolated the secretions by wiping the glands with pieces of filter paper. Seven compounds were identified by analysis of these secretions: *trans*-2-hexenol, *trans*-2-hexenal, *trans*-4-oxo-2-hexenal, geranial, neral, *cis*- and *trans*-dolichodial, and benzaldehyde. Since the openings of the gland we have studied and the protrusible glands that Boevé and coworkers investigated are closely situated on the larvae, it cannot be ruled out that the benzaldehyde we found could have been produced in the eversible glands on the midabdominal region rather than in the glands of the first abdominal segment.

Several authors have reported varying compositions of the secretions of papilionids from the different larval instars (Seligman and Doy, 1972; Burger et al., 1978; Honda, 1980). We did not observe this phenomenon. However, we did not try to determine whether there are differences in the amounts of volatiles in the different instars.

In contrast to *P. wesmaeli*, the aposematic *P. erichsonii* larvae are colonial, and simultaneous snap-bending can occur. It may be possible, therefore, that the volatiles not only have a deterring effect on predators but also may have an effect in alarming the whole colony. If all the larvae in a colony snap-bend simultaneously, very large amounts of deterring compounds would be released, and the visual scaring-off effect would be complemented.

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