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# MANDIBULAR GLAND SECRETIONS OF TWO PARASITOID WASPS (HYMENOPTERA: ICHNEUMONIDAE)

N. W. DAVIES[1] and J. L. MADDEN[2]

[1]*Central Science Laboratory*
[2]*Faculty of Agricultural Science*
*University of Tasmania*
*P.O. Box 252C*
*Hobart, Tasmania 7001 Australia*

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**Abstract**—Males of *Rhyssa persuasoria* and *Megarhyssa nortoni nortoni* exhibit marked aggregation behavior prior to and during the emergence of females from host trees, and this has been linked with the secretion of an odorous liquid from the mandibular glands. The volatile components of these secretions were examined by combined gas chromatography–mass spectrometry. While both species contained 6-methylhept-5-en-2-one, *M. nortoni nortoni* was characterized by a series of alkyl spiroacetals and *R. persuasoria* contained 3-hydroxy-3-methylbutan-2-one. The same spiroacetals have previously been isolated from the mandibular glands of other Hymenoptera and have been directly associated with aggregation behavior in some species. The chemical and behavioral aspects of the two species are discussed.

**Key Words**—Mandibular secretions, parasitoids, aggregation pheromones, spiroacetals, 6-methylhept-5-en-2-one, 3-hydroxy-3-methylbutan-2-one, *Rhyssa, Megarhyssa,* Hymenoptera, Ichneumonidae.

## INTRODUCTION

The parasitoids *Megarhyssa nortoni nortoni* Cresson (hereafter referred to as *M. nortoni*) and *Rhyssa persuasoria* L. (Hymenoptera: Ichneumonidae) were introduced into Australia as natural enemies of the wood wasp, *Sirex noctilio* F., an exotic pest of radiata pine (*Pinus radiata* D Don) (Taylor, 1967). Both species attack mature larvae of *S. noctilio* within the wood of infested trees to emerge, in general, 12 months later.

Males of both species aggregate at sites of potential emergence or at sites



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which are otherwise attractive. The aggregation response is initiated by volatiles produced by the symbiotic fungus of *S. noctilio, Amylosterum areolatum* (Madden, 1968). Localized generation of fungal volatiles is presumably stimulated by moisture changes associated with adult eclosion, since injection of water into fine drill holes made in dry, fungus-infested logs will promote aggregation at such sites after 24–48 hr (Madden, unpublished results).

The aggregation behavior of male *M. nortoni* is similar to that described for several other *Megarhyssa* species (Heatwole et al., 1963; Crankshaw and Matthews, 1981), and the group is typically compact with arching of the abdomen so that the distal abdominal segments make contact with the bark substrate at the point of interest discerned by the antennae. Aggregations of *R. persuasoria* are less compact and males are incapable of arching their abdomens, although curvature of the abdomen may be observed.

In laboratory studies with *M. nortoni* males, presentation of the fungus on agar or water extracts of insect frass is sufficient to initiate individual interest followed by, through 4-min exposure periods, accelerated recruitment of males to the sites. This second response is a function of the number of males making contact with each other (Madden, unpublished results). In this situation mutual antagonism occurs between individuals, in which opening of the mandibles is observed. Furthermore, the rapid drumming of the antennae at the sites of emerging insects elicits a like response in the emergent. In the majority of cases, the space between the mandibles is observed to be occupied by a liquid.

Depending on the species of the emergent, a number of outcomes may occur: (1) If the emergent is a nonparasitoid, e.g., *S. noctilio,* the male aggregation of either parasitoid is retained and males will mount the emergent and display precopulatory movements. (2) If the emergent parasitoid species is a conspecific, of either sex, precopulatory activity is enhanced and mounting occurs. (3) If the emergent parasitoid species is dissimilar, the males' precopulatory activity, which may include males mounting males, will subside and the aggregation will disperse.

The odor of each species is characteristically different and, in preliminary experiments, it was found that it could be released if the insects were grasped or shaken. In addition, mandibular opening with the appearance of liquid could be elicited by swift decapitation. The odor source was clearly localized in the head, as crop and stomach contents and thorax and abdomen possessed no such odor.

It would appear that this secretion plays an important role in the complex species recognition, aggregation formation, and defensive behavior of the two species. This paper reports the chemical identity of the major volatile compounds occurring in the mandibular secretion of male and female *R. persuasoria* and *M. nortoni*. Compound classes encountered include spiroacetals, ketones, alcohols, and esters.

## METHODS AND MATERIALS

*Insect Material.* Insects were collected as they emerged from infested logs in an insectary, were kept in polystyrene pots, provided with water and honey, and held at 10°C between tests. They were then available for bioassy or chemical examinations as required.

Three main approaches were used in the study of the volatiles. These were the direct collection of the secretions from stimulated individuals, examination of extracts of dissected mandibular glands to confirm the origin of the secretion, and the examination of extracts of excised heads. Males and females were treated separately.

*Collection of Secretions.* The mandibular secretions were collected in the following manner. Individual insects were grasped at the thorax between thumb and forefinger, an action which resulted in the opening of the mandibles and the appearance of the secretion in variable amounts. The tip of a 1-$\mu$l microcapillary tube (Microcaps, Drummond Scientific Co., Broomall, Pa.) was placed between the extended mandibles resulting in the uptake of the liquid. The microcap was either immediately dropped into, or its contents flushed into, a vial of pentane. Maximum yield per collection ranged from 0.25 $\mu$l to 0.80 $\mu$l for *R. persuasoria* and *M. nortoni,* respectively. In general greater quantities were obtained from females and *M. nortoni.* Insects were returned to a new container, stored at 10°C, and could be processed daily for up to 14 days.

*Gland Material.* Mandibles and associated glands, gland reservoirs, and muscles were dissected from parasitoids that had been killed by short exposure to −20°C prior to analysis. Ten to fourteen mandibles for each species and sex were prepared. In another approach, insects were decapitated, antennae removed, and the heads stored in pentane.

*Extraction.* The frozen gland material and heads were crushed and left in pentane overnight. The gland extract was filtered and used without further work-up after evaporation to minimum volume. The head extracts were washed with 5% sodium bicarbonate solution to remove fatty acids, dried over $MgSO_4$, and evaporated to a minimum volume prior to injection.

*Bioassay.* The response of male parasitoids to live females contained in perforated vials, excised female heads, and mandibular secretion was evaluated. In paired comparisons, presentation of a test subject to 25 males of one species was alternated with a similar presentation to 25 males of the second species through 10 presentations for each of the three species treatments. The number of males that visited or revisited and drummed the test site with their antennae during a 2-min exposure was recorded to evaluate the response to each treatment.

*Gas Chromatography–Mass Spectrometry.* A Pye 204 gas chromatograph was employed, directly coupled to a VG 70/70F mass spectrometer with 2035

data system. The mass spectrometer was normally operated in the electron impact mode at 70 eV, 4 KV accelerating volts, and a source temperature of 200°C. Scans from *m/z* 350–20 were stored every 1.6 sec. Chemical ionization analyses were carried out with isobutane as the reagent gas at a pressure of approximately 1 torr in the ion chamber. Accurate masses were determined at a resolution of 1000 by means of perfluorokerosene as an internal reference with peak times relative to the reference peak times being used. The gas chromatography was carried out on 50-m × 0.2-mm fused silica OV-101 or 25-m × 0.2-mm BP1 (bonded-phase equivalent of OV-101, SGE Pty. Ltd) with the column passing through into the ion source. The carrier gas was hydrogen, with a flow rate at 70°C of 1.8 ml atm/min (Davies, 1984). Injections were splitless at 250°C, and a typical column temperature program was 70–250° at 4°/min.

The mandibular secretions inside microcapillaries were sealed inside larger capillaries (2 cm × 1 mm) and injected with a capillary crusher, a modification of a previously described device (Stanley and Kennett, 1973).

Hydrogenations were carried out in sealed Reacti-vials (Pierce) using Adams catalyst. Methylations were carried out with ethereal diazomethane. Trimethylsilylation was carried out with BSTFA (Pierce), and methoxime derivatives were prepared with methoxylamine hydrochloride (2% in pyridine).

Identifications were based on GC retention indices, mass spectra, chemical modification of functional groups and, where possible, by comparison with authentic standards.

## RESULTS

The mandibular gland secretion is presumably produced by the glandular mass of cells that envelop the mandibular gland reservoir (Figure 1). The volumetric capacity per reservoir was approximately 2.0 and 1.4 μl for average-size female and male *M. nortoni,* respectively, and 1.5 and 1.0 μl for average-size female and male *R. persuasoria.* The ducts from the reservoir pass through the mandible to open on the inner margin at the base of the mandible.

The secretions showed the presence of several volatile components, with that of *M. nortoni* being considerably more complex than that of *R. persuasoria.* Figure 2 shows the chromatogram obtained from the secretion of a single *M. nortoni* female. Peak 1 was found to be pentan-2-one, and peak 2 to be 6-methylhept-5-en-2-one. This latter compound was also readily identified in the secretion of *R. persuasoria. M. nortoni,* however, also produced a series of compounds (peaks 3–6, Figure 2), the mass spectra of which did not match any compound in the available mass spectral data bases.

Three of these compounds (peaks 3, 4, and 6) were found to have a molecular weight of 184 and prominent fragment ions at *m/z* 112 and 115, while the molecular weight of the other (peak 5) was 168. Peak 5 was not consistently

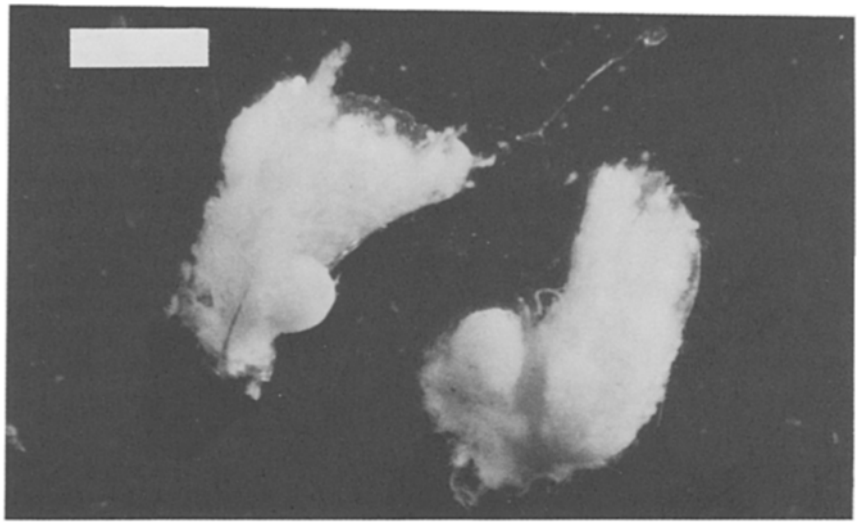

FIG. 1. Excised mandibles and associated glands and tissue from a *M. nortoni* male. Photographed in 70% ethanol. Scale represents 1 mm.

found and appeared to diminish rapidly on storage, indicating an unstable structure. The molecular weight 184 compounds were found from accurate mass determinations to be $C_{11}H_{20}O_2$ (found, 184.149; calculated for $C_{11}H_{20}O_2$, 184.146). Using bulked material from whole heads, compounds 3, 4, and 6 failed to hydrogenate or form any methyl ester, methoxime, or trimethylsilyl deriva-

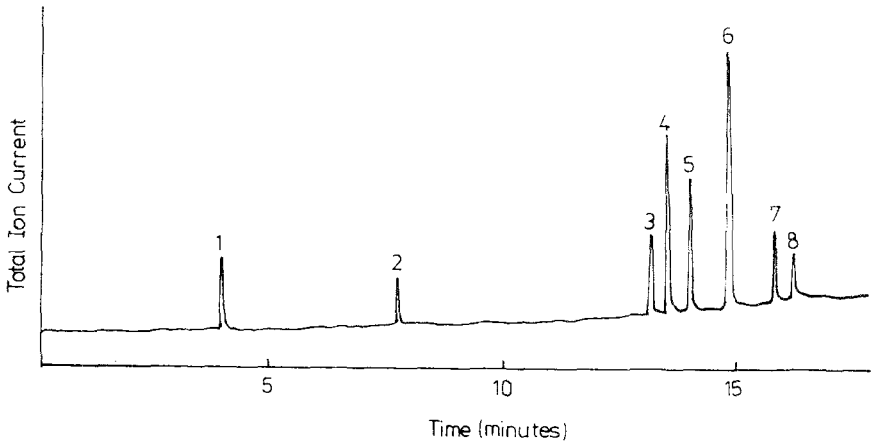


FIG. 2. Chromatogram of the volatile components in the mandibular gland secretion of a single *M. nortoni* female. GC column was 50 m OV-101, fused silica, programed from 70° to 200°C at 4°/min. Peak numbers are referred to in the text and Table 1.

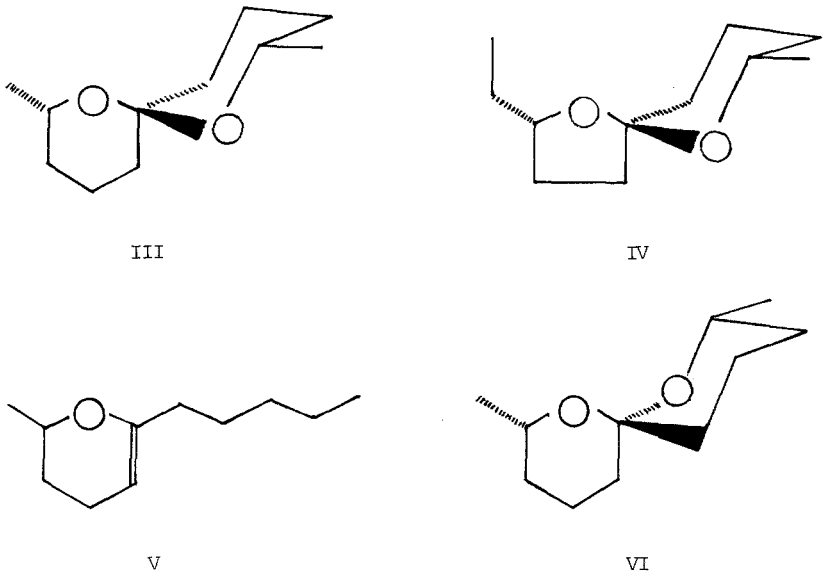


FIG. 3. Structures of characteristic *M. nortoni* compounds, with numerals corresponding to peak numbers in Figure 2. III: *E*, *E*-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane; IV: *E*,*E*-2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane; V: 2-methyl-6-pentyl-3,4-dihydro-2H-pyran; VI: *Z*,*E*-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane.

tive, indicating that there were no double bonds present and that the oxygens were not present as acid, ketone, or alcohol groups. Saponification with ethanolic potassium hydroxide also effected no change, indicating that no ester function was present.

The two degrees of unsaturation, therefore, had to be accounted for by ring systems, with the oxygens in the ring, suggesting a cyclic acetal could account for the structure. An examination of the literature for known acetals of the same formula showed that the mass spectra of compounds 3, 4 and 6 did in fact match closely a series of previously reported spiroacetals (Francke et al., 1978, 1979, 1980a) from the mandibular glands of bees and wasps. Close comparison of mass spectra and gas chromatographic retention indices with those of authentic samples run under identical conditions confirmed the identities, and enabled stereoisomers to be assigned. Peak 3 was shown to be *E*,*E*-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, peak 4 to be *E*,*E*-2-ethyl-7-methyl-1,6-dioxaspiro-[4.5]decane, and peak 6 to be *Z*,*E*-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane. (Figure 3)

The two stereoisomers of the synthetic dimethyl-1,7-dioxaspiro[5.5]-undecanes are well separated on nonpolar stationary phases and can be distinguished as well by their mass spectra which show significant differences (Francke

et al., 1980a). The two synthetic dimethyl-1,6-dioxaspiro[4.5] decanes (*E,E* and *Z,E* isomers) were much closer in relative retention time and had virtually identical mass spectra. Only the *E,E* and *Z,E* steroisomers of the 1,7-dioxaspiro[5.5]undecanes would appear to be thermodynamically stable (Francke et al., 1980b; Mori and Tanida, 1981). The chirality of the spiroacetals was not determined.

Peak 5 was shown to correspond to $C_{11}H_{20}O$ (found, 168.147; calculated for $C_{11}H_{20}O$, 168.151), and it appeared that it could be related to an unknown previously reported in association with the same spiroacetals (Bergström et al., 1982) which was subsequently shown to be the biogenetically related 2-methyl-6-pentyl-3,4-dihydro-2H-pyran (Francke, 1984; Francke et al., 1985). Peak 5 had a mass spectrum with *m/z* 168 (23%), 125 (42), 112 (100), 97 (30), 84 (16), 83 (31), 70 (25), 58 (15), 55 (61), and 41 (36). Comparison of this spectrum with that of the above dihydropyran (Francke, personal communication) confirmed that the spectra were very similar, and an authentic sample run under the same conditions confirmed the assignment based on an identical retention index and mass spectrum.

Peaks 7 and 8 in Figure 2 were found to be undecan-2-one and undecan-2-ol, respectively. Methyl oleate was observed as a major component in the *M. nortoni* secretion. A major peak in the *R. persuasoria* secretion gave a mass spectrum with *m/z* 31 (42%), 39 (17), 41 (28), 43 (49), 59 (100) and 87 (5). After chemical ionization mass spectrometry to obtain a molecular weight, and subsequent comparison of GC and MS data with an authentic sample, this was found to be 3-hydroxy-3-methylbutan-2-one.

The components identified in the two secretions are summarized in Table 1. Analysis of excised mandibular gland extracts confirmed that the volatiles in the secretion did in fact originate from this gland. In addition to the compounds listed, several other peaks were observed at longer retention times in *M. nortoni* which have yet to be identified. The gland and head extracts of *M. nortoni* were also found to contain normal fatty acids such as palmitoleic, palmitic, oleic, linoleic, and stearic acids and a range of cuticle wax hydrocarbons including *n*-heneicosane, *n*-tricosane, *n*-pentacosane, *n*-heptacosane, as well as a C-25 alkene, while *R. persuasoria* contained the same range of fatty acids with a number of branched-chain alkanes in addition to the *n*-alkanes.

Whole females, excised female heads, and female secretions were used in bioassays to determine the degree of attraction to conspecific males and the degree of interspecific attraction. Table 2 summarizes these results. In each comparison, a significant preference for males to respond to females of the same species, their excised heads, and in particular their mandibular secretions was apparent. Excised heads were the least effective in eliciting activity by males, followed by live females, and then mandibular secretion which was the most active and specific.

TABLE 1. PRINCIPAL COMPONENTS FOUND IN MANDIBULAR SECRETIONS[a]

| Peak no. | Compound | *M. nortoni* | | *R. persuasoria* | |
|---|---|---|---|---|---|
| | | Male | Female | Male | Female |
| 1 | Pentan-2-one | + | + | | |
| | 3-Hydroxy-3-methylbutan-2-one | | | ++ | ++ |
| 2 | 6-Methylhept-5-en-2-one | + | + | ++ | ++ |
| 3 | *E*, *E*-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane | + | + | | |
| 4 | *E*, *E*-2-Ethyl-7-methyl-1,6-dioxaspiro[4.5]decane | ++ | ++ | | |
| 5 | 2-Methyl-6-pentyl-3,4-dihydro-2H-pyran | | ++ | | |
| 6 | *Z*, *E*-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane | +++ | +++ | | |
| 7 | Undecan-2-one | + | + | | |
| 8 | Undecan-2-ol | + | + | | |
| | Methyl oleate | +++ | +++ | | |

[a]Key: + = minor component, ++ = medium component, +++ = major component.

TABLE 2. RESPONSE OF MALE PARASITOIDS TO LIVE FEMALES, EXCISED HEADS, AND MANDIBULAR SECRETIONS OF FEMALE PARASITOIDS[a]

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Excised heads | | Live females | | Mandibular secretion | |
| | *R. persuasoria* | *M. nortoni* | *R. persuasoria* | *M. nortoni* | *R. persuasoria* | *M. nortoni* |
| Male *R. persuasoria* | 14 | 1 | 39 | 8 | 78 | 16 |
| Male *M. nortoni* | 1 | 9 | 25 | 59 | 50 | 156 |
| $\chi^2$ | 15.4 | | 34.16 | | 89.77 | |
| Significance | $P < 0.001$ | | $P < 0.001$ | | $P < 0.001$ | |

[a]Key response was the number of visits made by males, from 25 of each species, to the presentation point over two minutes.

## DISCUSSION

Males of both parasitoid species aggregate at potential emergence sites by a common stimulus of fungal origin (Madden, 1968; Madden and Coutts, 1979). Male *M. nortoni*, the larger of the two species, excludes *R. persuasoria* where they compete for a common site, and this exclusion is complemented by the release of repellent mandibular secretions from a compact aggregation. *R. persuasoria* males will then stand outside the aggregation and may reform if the emerging insect results in the dispersal of *M. nortoni*.

It has been demonstrated that the mandibular glands produce distinctly different compounds which confer species identification. In *M. nortoni* these compounds include a number of closely related spiroacetals, while in *R. persuasoria* the species-specific compound would appear to be 3-hydroxy-3-methylbutan-2-one. 6-Methylhept-5-en-2-one may act as a repellent and alarm pheromone. After the first report of this compound in ants (Cavill and Ford, 1953), it has been confirmed to be associated with alarm and defense in numerous species of ants (Regnier and Law, 1968; Blum, 1978), as well as in secretions of *Andrena* bees (Tengo and Bergstrom, 1976), other parasitic bees (Hefetz et al., 1982) and some beetles, such as the Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopkins) (Ryker et al., 1979). The species-specific compounds may act to synergize the repellent effect of the secretion while masking or potentiating its effect on the precopulatory activity of conspecifics.

Compound 4 was first found together with the *Z,E* isomer in three vespine wasp species (Francke et al., 1978), while compounds 3 and 6 were first reported in the mandibular secretions of *Andrena* bees (Francke et al., 1980a) together with the two former spiroacetals. The spiroacetals represent a class of compounds increasingly being identified as having pheromone activity. The first isolation of them from insects would appear to be 'chalcogran' (2-ethyl-1,6-dioxaspiro[4.4]nonane), an aggregation pheromone of the beetle, *Pityogenes chalcographus* (Francke et al., 1977). Cyclic acetals such as brevicomin (Silverstein et al., 1968) have, of course, been known for some time as insect pheromones, again principally as attraction or aggregation pheromones. Since the first reported spiroacetal, a range of these compounds has been found in bees (Francke et al., 1981; Tengö et al., 1982; Bergström et al., 1982), wasps (Francke et al., 1979) and the olive fly (Baker et al., 1980). The ring systems include dioxaspiro[4.4]nonanes, -[4.5]decanes, -[5.5]undecanes, -[4.6]undecanes, and -[5.6]dodecanes. The alkyl substituents range from methyl to butyl, with over 20 skeletons identified, excluding stereoisomers. These are almost always based on an odd carbon number and unbranched skeletons, as are the three found in *M. nortoni*. In these reports and the other references to spiroacetals, the function assigned has generally been that of attraction to members of the same species.

Undecan-2-one and undecan-2-ol, which are biosynthetically related to the spiroacetals found in *M. nortoni* were also found in association with the spiroacetals in *Andrena* bees (Bergström et al., 1982). These two compounds are widespread among the Hymenoptera. There appear to be few reports of normal fatty acid methyl esters appearing in insects generally. Methyl oleate was found along with the methyl esters of other fatty acids in extracts of the Argentine ant, *Iridomyrmex humilis* (Cavill et al., 1980). 3-Hydroxy-3-methylbutan-2-one has only been isolated once before from an insect source (Francke et al., 1974), as an attractant for the ambrosia beetle, *Xyloterus domesticus*. Pentan-2-one has not been commonly found in insects, but has been detected in cockroach secretions (Brossut, 1978), beetles (Moore and Brown, 1979), and bumblebees (Cederberg, 1977), again in mandibular secretions.

Although no mixed species aggregations were observed in the present study, the phenomenon is common among *Megarhyssa* species (Heatwole et al., 1963; Crankshaw and Matthews, 1981) and could be due to the presence of similar spiroacetals in other members of the genus, with final recognition only occurring when the emergent insect releases its secretion. In contrast, *R. persuasoria* is predominantly allopatric with respect to other *Rhyssa* species (Kirk, 1975).

Male aggregations have also been observed in the ichneumonid *Certonotus tasmaniensis* (Turner) which is an indigenous species which has adapted to attack *S. noctilio* in radiata pine. This species also has a characteristic odor. Cephalic secretions are not confined solely to the Ichneumonidae, as myrtenol and methyl oleate were found to be the major volatiles in the mandibular secretion of *Schletterarius cinctipes* (Cresson) of the family Stephanidae. (Davies and Madden, unpublished results).

It is, therefore, apparent that mandibular secretions occur within the parasitic Hymenoptera, acting to facilitate species recognition in mating behavior and the possible exclusion of competing species during oviposition.

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