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HOST FINDING BY *RHYSSA PERSUASORIA* (L.), AN ICHNEUMONID PARASITE OF SIRICID WOODWASPS

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In Europe, siricid woodwasps attack dead, dying or weakened coniferous trees in a variety of ecological situations. Female siricids drill into trees, making characteristic punctures in the bark and wood, and oviposition shafts in which no eggs or one or more eggs are deposited. Arthrospores of a symbiotic fungus, which are stored in paired mycangia at the base of the oviduct, are injected into the wood by the drilling female. After introduction, the fungus develops in the wood. The burrowing larvae feed on the wood and fungus, and produce cylindrical tunnels packed with frass, which contain the exuviae.

One of the most common and widely distributed insect parasites of woodwasp larvae and pupae is *Rhyssa persuasoria* (L.) (Hymenoptera: Ichneumonidae), which drills into the wood during its search for hosts. The present paper describes the activity of searching *R. persuasoria* females in relation to the infested log environment, and analyses the factors involved in the detection of woodwasp hosts.

Behaviour on Infested Logs

Methods

R. persuasoria females from a variety of host tree species collected in many European countries were used. They were mated after emergence and studied at 25°C, or stored at 5°C, in gauze-covered observation cages (30 cm^3). The adults were supplied with water and with honey containing 1 per cent protein hydrolysate.

Culture logs of Scots pine (*Pinus sylvestris* L., 19 to 25 cm long; 10 to 16 cm diameter; bark 2 to 7 mm thick) containing larvae of *Sirex noctilio* F., *S. juvencus* L. or *S. cyaneus* F. were used, the cut surfaces being sealed with beeswax to protect against secondary fungal infection and desiccation. With few exceptions, the tunnels and hosts occupied the peripheral 2 cm. Logs which had not been exposed to siricids, or with oviposition punctures but no larval development were also used. Observations were made at 25°C and 70 per cent relative humidity, using fluorescent lighting. In many experiments, the logs were dissected and the positions of hosts and galleries in relation to parasite activities graphically recorded.

To determine the behaviour of females on logs containing inactive host larvae, culture logs were placed at —20°C for 6 days and controls maintained at 25°C. Dissection of treated logs confirmed that the larvae had been killed. Exploratory and drilling activity on treated and control logs was recorded on 5 successive days (total observation time was 10 hr), and afterwards the logs were dissected to determine the number of host and parasite larvae.

Results

Exploratory behaviour. The searching behaviour of the female on an infested log comprises three major behavioural components. Firstly, there is 'surveying', a preliminary exploration in which the female walks fairly rapidly over the log with the tips of the antennae tapping the surface and the head moving from side to side so that a narrow field about 3 cm wide is examined. Secondly, in some areas the female stops and examines the surface with the tips of the antennae more thoroughly by rapid tapping, an activity termed 'palpating'. Finally, the parasite draws up the ovipositor, manoeuvres it into position near the antennae and begins drilling. Exploratory drills of less than 2 min duration and having a depth of less than 3 mm in the wood are termed 'probes', and the more prolonged insertions to a greater depth are 'drills'.

The behaviour of one female exploring an infested log during 1 hr is illustrated in Fig. 1. The exploratory routes in areas which stimulated little or no palpating and drilling were typically less convoluted than in areas where oviposition activity was more intense. Assuming a survey width of 3 cm, an average of 42 per cent of the total surface area (750 cm^2) was surveyed per female per hr (range 14 to 62 per cent, $n=6$). During 5 hr of continous exploratory activity one female surveyed 85 per cent of a log. Uninfested logs elicited no oviposition behaviour although surveying and occasional palpating were observed. During 12 hr of observations on

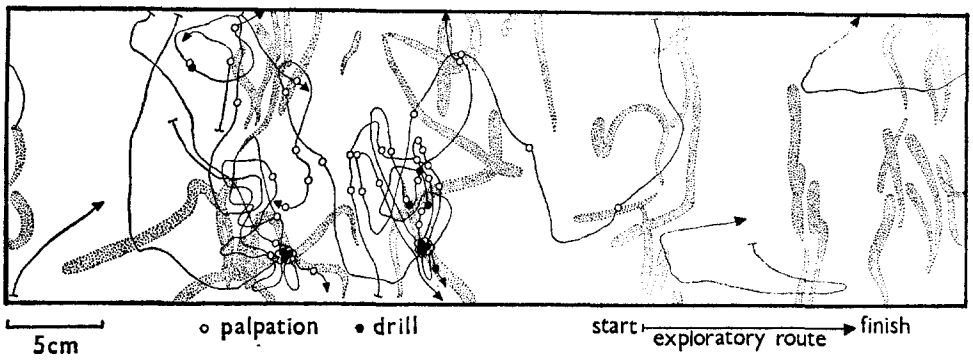

Fig. 1. Exploratory activity during 1 hr of one *R. persuasoria* female on an infested log. (Area occupied by hosts and tunnels indicated by stippling.)

four females on infested logs, 20·5 per cent of the time was spent surveying, 11·7 per cent palpating, 58·2 per cent drilling and 9·6 per cent resting or not on the logs, with a mean of 30·2 palpations, 8·3 probes and 1·3 drills per female per hr. When drilling occurs, it takes up most of the time. The mean durations of a palpation, probe and drill were 13 (2 to 42) s, 1·7 (1 to 3) min and 13·9 (5 to 33) min respectively (n=50).

Surface features that possibly influence the point of ovipositor insertion include debarked areas, cracks in the bark, fissures in the wood, siricid oviposition punctures and exit holes. The results of observations on logs with one or more of these features are given in Table I and demonstrate that cracks in the wood and bark act as a focus for drilling.

Two or more females on an infested log frequently become antagonistic. This behaviour is released when two females come into close proximity when surveying or when one is drilling. During threat display, the females face each other, raise the body, lift the prothoracic legs, and 'fence' with their antennae. Threat postures generally result in a brief conflict, when the females attempt and occasionally succeed in biting each other, after which one of the combatants moves to a different part of the log. In this way, individual females acquire loosely defined territories that tend to persist for several days. Observations showed that females drilled readily in areas previously drilled by other females.

Drilling in relation to hosts and tunnels. The parasite ovipositor is normally inserted into the wood at an angle of 75° to 90° to the surface, and does not always pass through the wood in a straight line. Therefore, insertions made at some

Table I. The Influence of Surface Features of Logs on the Drilling Response

Feature	Per cent of total surface area occupied by feature	Number of replicates	logs examined	females per replicate	Total drills recorded	Per cent of total drills into feature	Significance *P*
Fissures in wood	1·7	9	2	1	89	61	<0·001
Cracks in bark	3·3	3	1	1	39	41	<0·001
De-barked areas	4·7	6	1	1	50	12	ns
Siricid exit-holes	0·6	5	5	3	107	9	<0·001
Siricid oviposition punctures (galleries present)	0·3 (498 punctures)	4	4	3	231	0·4	ns*
Siricid oviposition punctures (galleries absent)	0·03 (11 punctures)	3	1	1	13	0	ns

*If a puncture is assumed to occupy 1 mm^2 or 10 mm^2 the results were still not significant.

distance from the host (in surface view) can result in its detection. For example, in Fig. 2a, eleven drills resulted in host detection of which only two were directly over hosts. The maximum distance from the point of insertion to a point overlying a 2-cm deep parasitized host was 12 mm, giving an insertion angle of 31°. Consequently, in the study of drilling activity in relation to the position of hosts and tunnels, the proportions of drills within 5 mm and 10 mm boundaries around hosts and tunnels was computed.

Table II shows the proportion of the total surface area immediately above hosts or above

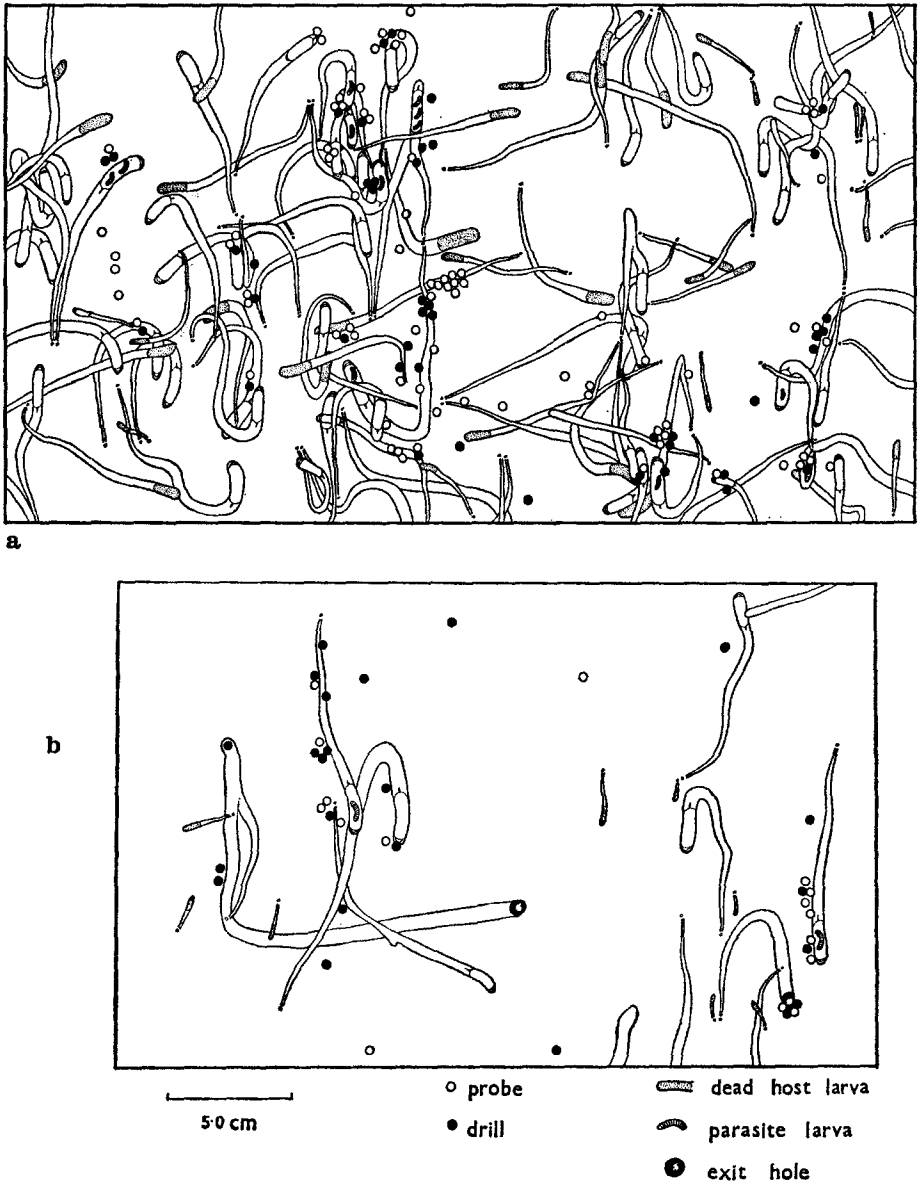

Fig. 2. Drilling activity of *R. persuasoria* females on siricid infested logs. a, Heavily infested log no. 5. b, Lightly infested log no. 1. N.B. Two-dimensional drawings of the logs in which galleries and larvae were 0 to 2 cm deep in the wood.

Table II. Drilling activity in Relation to Hosts and Tunnels in Infested Logs

Log no.	Surface area sq. cm (excluding cut ends)	Number of host larvae	Number of drills by parasite	Number of hosts parasitized	Per cent of drills near tunnels and hosts: 0	0–5	5–10	>10	Per cent of drills near hosts only: 0	0–5	5–10	>10
					distance from host and/or tunnels (mm)							
1	570	5	23	2	30(5)	44(23)	17(16)	9(56)	4(0·7)	9(1·7)	9(3·6)	78(94)
2	615	5	32	3	28(7)	35(27)	3(24)	34(42)	13(1·4)	12(4·6)	9(5·0)	66(89)
3	656	1	20	0	20(7)	25(17)	5(15)	40(61)	0(1·7)	5(4·3)	25(4·0)	70(90)
4	656	21	47	2	57(8)	34(30)	3(42)	6(20)	9(0·7)	23(7·3)	23(7·0)	45(85)
5	720	48	40	8	75(20)	18(23)	7(32)	0(25)	11(3·5)	30(11·5)	16(11)	43(74)
Mean	643	16	32	3	42(9)	31(24)	9(26)	18(41)	7(1·6)	16(5·9)	16(6)	60(86)
		Significance P			<0·001	>0·05	<0·001	<0·001	<0·001	<0·001	<0·001	<0·01

The percentages of the surface area occupied and tunnels and/or hosts are given in parentheses.

hosts and their tunnels, and the percentage of the areas within 5 mm and 5 to 10 mm of their boundaries. If drilling was random, it would be expected that the proportion of drills within a particular area would be equal to the proportion of the total area occupied. There were 4·8 (range 3 to 7) and 5·8 (4 to 13) times more drills over tunnels and hosts respectively than would be expected if drilling was random. Comparison of drilling activity in relation to tunnels showed a small difference in the 0 to 5 mm boundary, but significantly fewer drills were recorded into the 5 to 10 mm and beyond the 10 mm boundary. Significantly more drills were made near host material alone than if drilling were random, even into the 5 to 10 mm boundary. In log 3, there was only one living larva, the remainder having been invaded by secondary fungus, which probably accounts for the low proportion of drills near the larvae.

The relationship between probes and drills, and the geography of hosts and tunnels in the wood is illustrated in Fig. 2. In the heavily infested log 5 (Fig. 2a), 95 per cent of the probes and all the drills were within 10 mm of host material. Non-random drilling was emphasized by log 1 (Fig. 2b) in which 88 per cent of the probes and 91 per cent of the drills were within 10 mm of host material which occupied only 5 per cent of the total surface area.

The overall parasitism was 23 per cent, but 89 per cent of the drills did not result in host detection.

Drilling by the parasite does not follow any well defined sequence in relation to a particular tunnel suggestive of following a concentration gradient, although some areas stimulate repeated drilling.

Behaviour on logs containing inactive host larvae. The results of parasite host-searching behaviour on logs containing dead and live host larvae are given in Table III. Exploratory behaviour (number of palpations and probes) was similar in both groups but more drills were made in the control logs with live larvae ($P<0·01$), although the total number of ovipositor insertions (probes + drills) was not significantly different between the two groups. Dead host larvae, including those in early stages of decomposition, were successfully located and parasitized. The percentages of drills resulting in parasitism were 13·4 in the treated group and 10·5 in the controls, and were not significantly different, but parasitism was 17 per cent in the treated logs and 51 per cent in the controls.

Experimental Data

Procedure

To study the different components of the infested log environment in relation to host detection, a bioassay procedure was developed whereby females were stimulated to host-searching activity, permitting a quantitative study of the factors which elicit the drilling response.

The apparatus consisted of a sheet of Perspex (30 cm × 15 cm × 1 cm thick) which had in it

Table III. Activity of Females on Logs Containing Dead and Live Host Larvae

Replicate	No. ♀♀	Logs with dead larvae					Logs with live larvae				
		Number of					Number of				
		palpations	probes	drills	host larvae	parasitized hosts	palpations	probes	drills	host larvae	parasitized hosts
1	3	58	23	22	20	2	73	31	56	38	23
2	3	*	*	20	4	0	*	*	33	2	1
3	4	88	63	12	14	7	54	43	12	3	1
4	3	39	12	1	11	0	48	21	4	2	0
5	4	72	48	11	5	0	54	46	4	4	0
Total		257	146	66	54	9	229	141	109	49	25

*No observations made.

forty cavities (20 mm × 7 mm²). Test materials were put into the cavities which were then covered with paper fixed with cellulose acetate tape. The same quantities of test materials were each put into 3 to 6 cavities per sheet, the same number of cavities being used for each substance. The test materials in cavities were arranged at random. The sheet was put on the top of an observation cage with the cavities facing downwards thus making them accessible to the female. Except where indicated, three females were used in each cage. Generally young females were offered siricid-infested logs to initiate drilling before being used in bioassay studies. Activity was recorded by observing the palpations and drills, or by counting the number of drills made in the paper. The durations of experiments was 1, 16 and 64 hr. Except where indicated, frass samples used in bioassay studies were 7 mg samples taken from the 3 cm of tunnel directly behind developing siricid larvae. Fungal cultures were maintained at 24°C on potato dextrose agar medium, and samples were obtained with a 5 mm diameter cork borer, using 3 to 4 discs of fungus-impregnated agar per cavity.

Experiments were made at approximately 25°C and 70 per cent relative humidity with fluorescent lighting or total darkness.

Materials and Results

Analysis of log components. To evaluate the relative attractiveness of the gross components of the infested log, the following were compared: sawdust from infested logs (but not including larval frass); sawdust from clean logs; siricid larvae; frass from siricid tunnels; the fungal symbiont of *S. juvencus*; and larvae and frass of the melandryid beetle, *Serropalpus barbatus* Schaller, whose larvae are frequently found in the same log with siricid larvae. The larvae were not washed before testing. The results of nine replicates (each of 1 hr duration) are summarized in Table IV and clearly show the dominant attractiveness of siricid frass and, at a much lower level, of wood from infested timber.

To compare the relative attractiveness of siricid frass and the beetle frass from the same log, samples of each were bioassayed, demonstrating (Table V, test 1) that compared to siricid frass, the beetle frass elicits little host-searching activity.

To determine whether the presence of siricid larvae stimulates the drilling response, samples of siricid frass with and without larvae in the cavities, were bioassayed. The results (Table V, test 2) did not demonstrate any difference in drilling response, although significantly more palpations were made over cavities containing frass and larvae ($P<0{\cdot}001$). When washed siricid larvae were compared with frass, drilling was only elicited by the frass (Table V, test 3).

The effects of frass on host-searching behaviour. Frass was bioassayed to determine whether there was a gradient in response to different parts of the tunnel extending from the siricid larva to the oviposition shaft. Frass samples from each 1-cm length of 5-cm tunnels were tested, using three cavities per 4 mg sample in each of five replicates. The results are summarized in Table VI. The differences in attract-

Table IV. Host-searching Behaviour of Females when Exposed to Different Materials Associated with Timber

Materials tested	No. palpations	Per cent of total palpations	No. drills	Per cent of total drills
Siricid frass	142	58	93	84
Sawdust from infested log	38	16	10	8
Symbiotic fungus (*S. juvencus*)	31	13	2	2
S. barbatus frass	8	3	2	2
Sawdust from uninfested log	2	1	2	2
Siricid larvae	17	7	1	1
S. barbatus larvae	5	2	1	1
Total	243		111	

The cultured symbiotic fungus was bioassayed 30 to 120 days after inoculation.
9 replicates using 3 females per replicate.

Table V. Comparison of Different Materials on Host-searching Behaviour

Test	Experimental details: Contents of cavity	Cavities per sheet	No. of replicates	♀♀ per replicate	Results: No. of palpations	No. of drills
1	a *Serropalpus* frass	5	10	3	8	2
	b Siricid frass	5			49	24
2	a Siricid frass with 1 siricid larva present	4	8	3	148	18
	b Siricid frass without larvae	4			93	21
3	a Washed siricid larvae	6	4	4	(not	0
	b Siricid frass only	6			recorded)	300

Table VI. The Response of Females to Frass from Different Parts of Siricid Tunnels

	Distance of frass samples from siricid larva (cm)				
	0–1	1–2	2–3	3–4	4–5
No. of drills	317	213	124	118	88
% of total drills	36·8	24·7	14·4	13·7	10·2
Mean	63·4	42·6	24·8	23·6	17·6
S.E. of mean	(±13·5)	(±10·8)	(±7·7)	(±5·4)	(±12·6)

5 replicates, using 4 females per replicate.
Frass from a different tunnel used in each replicate.

ion between 1st, 2nd, and 3rd cm of frass were significant ($P<0·001$), but there were no significant differences between 3rd, 4th and 5th cm. There was some difference in attraction between 3rd and 5th cm of frass ($P<0·02$).

To determine the effect of frass moisture content on host-searching behaviour, frass was mixed and dried, and 0·1, 0·2, and 0·4 ml of water added to it in the cavities, to make the frass damp, moderately wet, and soaking,

respectively. Samples of dry frass were also tested. In nine replicates, soaked frass accounted for 37 per cent of the 1236 drills, wet and damp frass for 26 per cent each, and dry frass for 9 per cent. The differences in attraction between soaked and moderately wet frass, and between damp and dry treatments were significant ($P<0{\cdot}001$). When untreated 1st cm frass was compared with soaked 5th cm frass, 71 per cent of the 370 drills were made into 5th cm of frass (eleven replicates).

The effects of fungi on host-searching behaviour. Several experiments were made to clarify the role of the symbiotic fungus and other fungi in the detection of hosts.

To determine whether the age of fungal symbionts influences their attractiveness to the parasite, fungal cultures of *S. juvencus* and *Urocerus gigas* (L.) symbionts*, aged 0 to 6 months, were compared using five cavities per monthly sample in ten replicates. As can be seen from Fig. 3, there was an increase in the attractiveness of both symbionts with age, a maximum

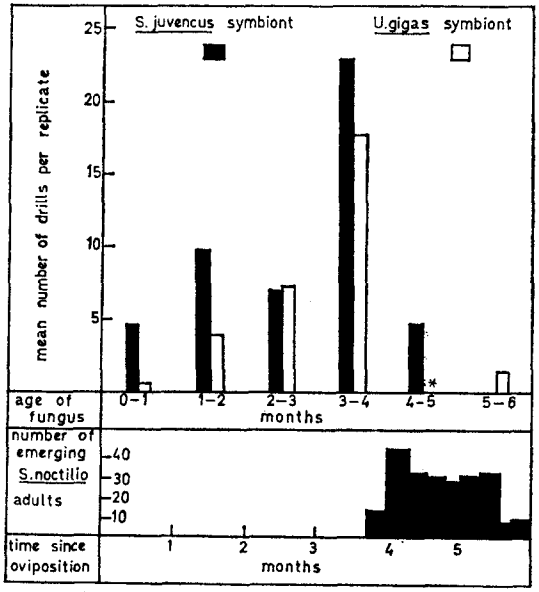

Fig. 3. Attractiveness of symbionts as a function of age.

being reached at 3 to 4 months. This was followed by a sharp decrease by 4 to 5 months (in *S. juvencus*) and attractiveness being negligbile after 5 months. The between-month differences were significant ($P<0{\cdot}001$) except between 1 to 3 and 2 to 3 month *S. juvencus* symbionts. The emergence of *S. noctilio* adults from culture logs maintained at 25°C are given in Fig. 3 and illustrate that maximum attractiveness of the symbiont coincides with the maturation of siricid larvae prior to emergence.

When Scots pine sawdust was added to cultures of *S. juvencus* symbiont, its attractiveness was considerably increased (Table VII).

Table VII. Relative Attractiveness of Scots Pine Sawdust and Symbiotic Fungus

Materials tested	No. drills	Per cent of total
S. juvencus symbiont plus sawdust	378	73
S. juvencus symbiont only	134	26
Sterile sawdust only	4	1

10 replicates, using 3 females per replicate.

To determine differences in the responses of the parasite from three siricid host species to the symbiotic fungi associated with these siricids, two experiments were made. The results of the first (Table VIII, test 1) showed a preference by all parasites for the *S. noctilio* symbiont and a low level of attractiveness to the *U. gigas* symbiont, irrespective of the host source of the insect. Similarly in a comparison of *S. juvencus* and *U. gigas* symbiont, (Table VIII, test 2) the latter proved less attractive, particularly to parasites reared from *U. gigas* hosts.

Several fungi of the genus *Stereum*, which are associated with timber, were compared with *S. juvencus* symbiont, using three cavities per species in each of ten replicates. The results (Table IX, test 1) demonstrated the dominant attractiveness of the symbiont, although *S. chailletii* accounted for 47 per cent of the drills made in response to the named *Stereum* species. When *S. chailetii* and two European species of *Amylostereum* were compared with the symbionts (Table IX, test 2), the *S. juvencus* symbiont proved the most attractive and *U. gigas* symbiont the least attractive. The differences in attractiveness between the named fungi were not significant but significant differences were found between them and the two symbiont species ($P<0{\cdot}001$).

To determine whether other species of fungi associated with woodlands are attractive to

*The symbiotic fungi associated with different *Sirex* species and *U. gigas* are characteristically different in appearance and growth rates, and are probably different strains or species.

Table VIII. Attractiveness of Symbiotic Fungi from Different Siricid Species to Females from Different Siricid Host Species

Test	Host origin of parasite	No. replicates	Age of cultures (days)	No. ♀♀ per replicate	Per cent of total drills into symbionts from			Total drills
					S. noctilio	*S. juvencus*	*U. gigas*	
1	*S. noctilio*	9	20	3	54	41	5	78
	S. juvencus				54	32	14	50
	U. gigas and *S. juvencus*				48	35	17	82
	Total drills				108	77	25	210
2	*S. juvencus*	8	20–60	5		55	45	365
	U. gigas					70	30	107
	Total drills					277	195	472

Table IX. Attraction of Females to Different Species and Strains of Symbiotic Fungi, *Stereum* and *Amylostereum*

Fungus	Test	No. of replicates (age of fungus (in days)	No. ♀♀ per replicate	No. drills	Per cent of total drills
S. juvencus symbiont	1	16 (10–46)	4	117	44
S. chailletii (Pers.) Fr.				68	26
S. purpureum Fr.				48	18
S. pini Fr.				23	9
S. sanguinolentum (Alb. and Schw.) Fr.				7	3
Total				263	100
S. juvencus symbiont	2	10 (49–56)	5	88	41
A. laevigatum (Fr.) Boid.				50	24
A. areolatum (Fr.) Boid.				31	15
S. chailletii				28	13
U. gigas symbiont				16	7
Total				213	100

female parasites, several fungi were bioassayed after 10, 24 and 50 days incubation. The results (Table X) demonstrated that *S. juvencus* symbiont exerted the dominant attractiveness, and that *Lenzites abietana* was the most attractive of the remainder. When *L. abietana* was compared directly with *U. gigas* symbiont, sixty-five of the ninety-two drills (four replicates) were made in response to the former.

The effects of frass and symbiotic fungus extracts. Aqueous and alcoholic extracts of frass and fungus were bioassayed, using 1·5 cm diameter filter paper discs impregnated with concentrated extracts, on the gauze roofs of the cages. Two standard extracts were prepared using 0·5 g and 3·0 g of frass, and 1 cm^2 and entire plates of 4-month old *S. juvencus* symbiont in 10 ml and 50 ml of water and 100

Table X. The Relative Attractiveness of Different Species of Woodland Fungi

Species of fungus	Number of drills	Per cent of total drills
S. juvencus symbiont	68	53
Lenzites abietana (Bull.) Fr.	20	16
Trametes odorata Wulf	13	10
Fusarium oxysporum Fr.	12	10
Coniophora puteana (Fr.) Karst.	7	5
Trichoderma sp.	7	5
Fomes annosus (Fr.) Cooke	1	1
Polyporus abietinus Fr.	0	0

15 replicates, using 3 females per replicate.
3 cavities with each fungus used per replicate.

per cent ethanol respectively. Extraction was made at 25°C for 5 days and the solutions centrifuged and evaporated under vacuum until almost dry. The extracts elicited intense palpating activity, and also the ovipositor probing response (Table XI).

Table XI. The Attractiveness of Aqueous and Alcoholic Extracts of Frass and Symbiotic Fungi

Material extracted	Solvent	Total number of palpations	Total number of drills
Frass	water	29	22
Frass	ethanol	34	24
Fungus	water	27	3
Fungus	ethanol	49	12
Total		139	61

12 replicates, using 3 females per replicate.

The influence of the host on searching behaviour. Experiments were made to assess the role of host larvae and their by-products on the subsequent attractiveness of frass, cellulose or fungus. The experimental details and results are summarized in Table XII. They demonstrated that the presence of siricid larvae in frass or on fungal cultures increased the attractiveness of the substrate. Similarly, powdered cellulose contaminated with excretory and/or secretory products of siricid larvae, elicited much ovipositor probing. Although siricid gut extracts increased the attractiveness of fungal cultures, pure uric acid on plates had an inhibitory effect, siricid head extracts had no effect, and chitinase increased their attractiveness.

Discussion

The specific factors that attract a parasite to its host's environment and enable it to locate the host have aroused much speculation and study. Parasites are often attracted by their host's food rather than by the host itself (Laing 1937). Many parasites find their hosts by first detecting host indicators; thus the frass beside the burrows of the potato tuber worm attracts *Macrocentrus ancylivorus* Roh. (DeBach 1964), and *Solenotus begini* Ashmead finds its host, *Phytomyza atricornis* Meigan, by progressing along the length of the leaf-mine (Doutt 1957). Sound emanating from the chewing larvae of woodborers could play a role in their detection by parasites, but Heatwole *et al.* (1963, 1964), from studies on *Megarhyssa* species parasitizing *Tremex columba* L. concluded that sound plays no part in host detection, though they state that males aggregate in response to the chewing sounds of the emerging females.

Madden (1968) has shown that *Megarhyssa nortoni nortoni* Cresson and *R. persausoria* respond to paper that had been in contact with siricid-infested wood, and also to extracts of the paper, of frass and of fungal cultures, and he concluded that the symbiotic fungus is involved in host location behaviour. The present study confirmed that fungus-infested wood stimulates the female to oviposition behaviour, and demonstrated that the attractiveness of fungal symbionts is considerably increased when sawdust is present. All this evidence points to a fungus-produced odour in the timber being responsible for attracting the parasite. If the symbiotic fungus plays a major role in the production of attractants, the comparative lack of response to other common woodland fungi illustrates the rather specific nature of the attractants involved. It is possible that sight plays a part in host habitat detection although infested trees are often visually indistinguishable from dead but uninfested trees.

The exploratory route over the surface of infested logs exhibited some features of the kinokinetic response; little turning in areas which elicited few if any palpations and drills, and considerable turning in areas where drilling was stimulated. This behaviour is suggestive of orientation to a diffuse stimulus where a

Table XII. The Effect of Various Treatments of Frass, Cellulose and Fungus on the Drilling Response

Test	Substrate (controls)	Age (days)	Treatment		Duration (days)	No. replicates	Drilling activity		
			Quantity	Additive			No. drills	Per cent of drills into exp. group	Significance *P*
1	*U. gigas* symbiont	63	1	Siricid larva	8	6	273	60	0·01
2	*S. juvencus* symbiont	38	1	„ „	8	6	127	92	0·001
3	Siricid frass	—	1	„ „	14	5	169	57	0·05
4	Powdered cellulose	—	5	Siricid larvae	14	8	180	82	0·001
5	*S. juvencus* symbiont	25	2 ml	Siricid head extract	30	9	347	50	ns
6	*S. juvencus* symbiont	25	2 ml	Siricid gut extract	30	9	265	70	0·001
7	*S. juvencus* symbiont	54	0·7 mg	Uric acid	30	8	280	40	0·01
8	*U. gigas* symbiont	23	0·7 mg	Chitinase	50	6	78	70	0·001

Siricid larvae surface-sterilized in 100 per cent ethanol before use.
Siricid extracts using 13 larval heads and guts each in 15 ml water, and extracted for 5 days at 0°C.

steep gradient is lacking, or simply that the active material acts as an arrestant during exploratory behaviour. When the parasite probes the wood with the ovipositor, it possibly avoids extraneous odours associated with the bark and obtains further information on the distribution and concentration of stimuli in the wood.

Complete insertion of the ovipositor into the wood is not a prerequisite to parasitization, in apparent contrast to the three species of *Megarhyssa* studied by Heatwole *et al.* (1964), which only parasitized larvae 'which are at a depth in the wood equal to the length of the female's ovipositor'. Despite the non-randomness of drilling, the proportion of drills resulting in successful detection of hosts was small (11 per cent).

Although antagonistic behaviour between females on infested logs resulted in the acquisition of loosely-defined territories, previous drilling by one female did not apparently inhibit other females from drilling in the same area.

The female parasitized dead larvae in timber, demonstrating that active hosts are not necessary to stimulate drilling, and confirming that sounds made by larvae are not essential for host detection.

Drilling is elicited by a substance which is most potent in siricid frass and not by the host larva itself, and successful detection depends on locating the end of the tunnel near the host. Bioassay of frass demonstrated an increasing attractiveness, from oviposition shaft to host chamber, even when the same quantities were compared, although in timber the volume of frass per unit length of tunnel increases towards the host end. Madden (1968) found a high moisture content associated with recently deposited frass, and the current study shows that the attractiveness of frass was considerably enhanced when its moisture content was increased. Nevertheless, the sequence of drills made by females in logs gave no indication that they followed concentration gradients of attractants in individual tunnels.

The symbiotic fungus elicited the drilling response, attractiveness increasing with age up to 3 to 4 months. The changes in attractiveness of fungal cultures could be due to the accumulation of attractants caused by autolysis of hyphae and bacterial action, or a sequential production of attractive substances. The maximum attractiveness of fungal cultures coincided with the maturation of siricid larvae cultured at

similar temperatures, and if this correlation applies to conditions in the forest, trees containing mature larvae would exert the greatest attraction to the searching parasite. Within the timber, Coutts (1965) has observed the fungus growing most densely in the wood bordering larval tunnels. It is of interest that fungus-decayed wood attracts termite foragers, probably assisting in food detection and collection (Esenther & Coppel 1964).

There is no apparent pre-imaginal conditioning of the parasite to the strain of fungal symbiont associated with the host; indeed females reared exclusively from *U. gigas* hosts preferred samples of *S. juvencus* symbiont. Cartwright (1938) considered that the siricid symbiont closely resembles *Stereum sanguinolentum* but in a comparison of named *Stereum* species *S. sanguinolentum* elicited the least response. Recent studies by Talbot (1964) strongly suggest that the symbiont associated with *S. noctillo* in Australia is a species of *Amylostereum*, and *S. chailletii* (recently transferred to the genus *Amylostereum*) proved the most attractive of the *Stereum* species. When *S. chailletii* was compared with *A. areolatum*, *A. laevigatum*, and the symbionts, *S. juvencus* symbiont elicited more drilling than the named fungi which were not distinguished by the parasite. This suggests that the symbiotic fungus is closely related to but not identical with the named *Amylostereum* spp. Marked differences between the symbiotic fungi of *S. noctilio*, *S. juvencus* and *U. gigas* were also demonstrated.

Siricid and beetle frass were readily distinguished by the parasite, possibly due to specific host factors. Siricid larval gut extracts and also chitinase increased the attractiveness of fungal cultures, but head extracts did not. This suggests that the increase was not simply due to the addition of nitrogeneous products, although Madden (1968) found that the frass immediately behind larvae was characterized by high nitrogen content. It is possible that extra-corporal digestion of fungus preceding ingestion, and also larval excretory products, contribute directly or indirectly to an increase in the production of attractants. Certainly, powdered cellulose contaminated by active siricid larvae elicited intense drilling. Logs containing dead larvae are less attractive, possibly because of a cessation of larval secretory and excretory activities, although a masking of attractive odours by decomposition may be responsible.

Attractants, which are most potent in the tunnel behind the host larva, presumably diffuse through the wood, their movements influenced by factors such as wood density, aeration, and moisture content. Madden (1968) has pointed out that moisture gradients in the timber may modify the concentration of attractants which are released. Odour concentration or emission would probably be greater at cracks or fissures in the wood. If the parasite locates tunnels by comparison of odour concentrations with the tips of the antennae and ovipositor, the odour diffusion gradient should be sufficiently steep and steady to permit comparisons to be made at different points on the log surface. Although the parasite does not follow individual tunnels, it does distinguish those parts of the log with tunnels from uninhabited areas, demonstrating an initial non-random search for host material. When the host area has been located, drilling becomes more random within the vicinity of hosts, and relatively few drills result in successful host detection.

Summary

1. Exploratory behaviour over the bark of infested timber comprises a survey of the surface with the antennae, sustained antennal activity (palpating) in areas of special interest, and ovipositor probing to a depth of 1 to 3 mm into the wood.

2. Drilling to a depth of more than 5 mm is elicited by stimuli emanating from within the timber, with surface features such as cracks in bark and wood, influencing the point of ovipositor insertion. Siricid oviposition punctures do not attract the parasite or stimulate drilling.

3. Antagonistic behaviour between females results in the acquisition of loosely defined territories.

4. The majority of drills are made in response to siricid tunnels and are not random, but the parasite does not apparently follow concentration gradients along individual tunnels.

5. Females are able to detect and parasitize dead hosts in logs, demonstrating that sound is not a necessary stimulus for drilling, and is not essential for host detection.

6. The majority of drills are elicited by siricid frass; washed siricid larvae alone stimulate no host-searching response. The frass nearest the host is most attractive, and the attraction of the frass decreases with increase in distance from the host. Wet frass elicits more drilling than dry frass.

7. Cultured fungal symbionts 3 to 4 months

old are the most attractive, coinciding with maturation of host larvae in timber under similar conditions. There is no evidence that the species of tree influences attraction, or of pre-imaginal conditioning of the parasite to its host's symbiont. Comparison of the symbiont with several common woodland fungi demonstrated the specific attractiveness of the symbiont.

8. Comparison of named *Stereum* and *Amylostereum* species with the symbionts demonstrated a marked preference for the *S. juvencus* symbiont which is probably closely related to *Amylostereum* species.

9. Aqueous and ethanolic extracts of frass and fungus stimulate drilling.

10. When siricid larvae are maintained on frass or fungus medium, or when siricid larval-gut extract, and chitinase are incorporated into the medium, the attractiveness of the substrate is increased.

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