HOST FINDING MECHANISMS OF *COELOIDES BRUNNERI* VIERECK (HYMENOPTERA: BRACONIDAE)

by

Jim Vernon Richerson

B.A., University of Missouri, 1965

M.Sc., University of Missouri, 1968

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

in the Department

of

Biological Sciences

APPROVAL

Name: Jim Vernon Richerson

Degree: Doctor of Philosophy

Title of Thesis: Host finding mechanisms of Coeloides brunneri Viereck (Hymenoptera: Braconidae).

Examining Committee:

Chairman: Dr. G. H. Geen

J. H. Borden
Supervisor

T. Finlayson
Supervisor

B. P. Beirne

R. C. Brooke

P. Belton

H. F. Madsen
External Examiner
Research Scientist
Canada Dept. of Agriculture,
Summerland, British Columbia

Date Approved: Feb. 3, 1972

ABSTRACT

The host finding search patterns of Coeloides brunneri Viereck, a braconid parasite of Douglas-fir beetle larvae, were studied by visual observations and from tracings of the parasite's path on a plastic cylinder wrapped around a beetle-infested log on which the parasite was searching. The female parasite locates its host, lying under approximately .6 cm of bark, through four distinct phases of host finding: random search, nonrandom search, oviposition and nonsearch (resting and cleaning). Random search is characterized by large areas of bark being examined in a relatively straight path. Nonrandom search is a highly intensified examination of a small bark area and numerous turns of greater than 70°. Nonrandom search is initiated only at the end of a bark beetle gallery. Ovipositional probes are the culmination of successful host finding, in which the parasite paralyzes the host and deposits an egg. A stimulus associated with the end of a larval gallery was concluded to be the key stimulus by which the parasite recognizes its host.

Previous researchers had speculated or concluded that C. brunneri females detect their bark beetle hosts by perception of the vibrations or sound made by boring larvae. However, when placed on logs containing various actively mining stages of Dendroctonus pseudotsugae Hopkins, the parasite actively searched for the host only on logs infested with young or maturing brood larvae. Moreover, when offered larvae in logs that had been frozen at approximately -50°C and then allowed to thaw at room temperature for two days, they found the motionless, dead larvae, and oviposited through the bark onto them. Therefore, it was concluded that

C. brunneri is able to find its host by perception of some stimulus other than sound or vibration. Further experiments and observations produced no evidence that sound produced by the parasite (sonar), or odor of the host and/or its host tree are used in host finding. However, thermistor probe readings at the site of oviposition revealed that C. brunneri invariably oviposits on "hotspots" on the bark surface which are associated with the presence of bark beetle larvae underneath. Finally, in the absence of any bark beetle infestation, the parasite was induced to oviposit into bark that was heated by resistance wire probes placed beneath the outer bark. Therefore, it was concluded that C. brunneri locates its host by detecting host-associated temperature or infrared radiation (IR) differences in the bark.

A previously undescribed and unique placoid sensillum was identified as the sensillum most likely used in receiving host finding stimuli, based on amputation experiments of the parasite's antennae and inventory of the antennal sensilla. This elongate, dome-shaped plate organ was examined by light microscopy, and transmission and stereoscan electron microscopy. It differs from other plate organs in that it has two cuticular lamellae suspended internally from the dome of the sensillum. These lamellae separate the internal structure of the plate organ into three channels running the full length of the sensillum. Dendritic branches which enter the plate organ through an aperture in the center of the sensillum's cuticular floor fill the outer 2/3 of the median channel. A tormogen cell fills the two lateral channels and the basal third of the median channel. The linear structure and placement of these plate organs suggest that they are highly directional wave guides capable of perceiving IR in a manner that would enable C. brunneri to locate its host with a very high degree of accuracy.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to express my appreciation to Mr. G. Hollingdale, Dr. V. Bourne, Mr. M. Veto, and Dr. K. K. Nair for their assistance in the histological section of this study. Deep appreciation is extended to Dr. B. P. Beirne, Mrs. T. Finlayson, Dr. R. C. Brooke, Dr. P. Belton, Dr. H. Madsen, and especially Dr. John H. Borden for their advice, and help in the preparation of this manuscript.

INTRODUCTION

Biology of *Coeloides* spp.

Coeloides brunneri Viereck (Hymenoptera: Braconidae) is a parasite of larvae of the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins. While this parasite is considered to be a major mortality agent for the Douglas-fir beetle (Bedard 1933, Ryan 1961, Ryan and Rudinsky 1962), many forest entomologists consider its potential for control less effective than timber management practices that remove infested trees from otherwise healthy forests. However, *Coeloides* spp. are a major enemy of the Douglas-fir beetle and other subcortical insects (Table I), and as such warrant investigation. Many researchers (Bedard 1933, DeLeon 1935, Ryan and Rudinsky 1961) have studied the biology of *Coeloides* spp. but the parasitic behavior has been largely overlooked or examined only superficially.

The biology of *C. brunneri* as described by Ryan (1961) is as follows. This parasite has three complete generations a year with a portion of each generation overwintering in the fifth instar. The proportion which overwinters is different for each generation (approximately 5% in the first, 50% in the second, and 95% in the third generation).

The insect overwinters as mature larvae in cocoons constructed in the larval galleries of its host. During late spring, the larvae pupate and by late June the first adults may be found in the cocoons. Within one day after becoming adults, the parasites chew through the cocoons and bark to the outside, filling the cocoons with bark fragments as they emerge. More males than females emerge during the first half of the

Table I List of hosts parasitized by Coeloides spp. Majority of records from Bushing (1965). Other records from Thompson (1953).

Host Insect	Coeloides sp.	Coeloides brunneri Viereck	Coeloides dendroctoni Cushman	Coeloides pissodes (Ashmead)	Coeloides scolytivorus (Cresson)	Coeloides secundus Dalla Torre
Cerambycidae						
Unknown sp.		X				
Curculionidae						
Pissodes spp.				X		
P. approximatus Hpk.	X					
P. nemorensis Germar				X		
P. strobi (Peck)				X		
Scolytidae						
Unknown sp.	X					
Conophthorus monophyllae Hpk.		X				
Dendroctonus engelmanni Hpk.			X			
D. frontalis Zimmerman				X		
D. piceaperda Hpk.			X			X
D. ponderosae (=monticolae) Hpk.		X	X			
D. pseudotsugae Hpk.		X	X			
Ips sp.		X				
I. calligraphus (Germar)		X		X		
I. emarginatus (LeC.)			X			
I. grandicollis (Eichhoff)				X		
I. montanus (Eich.)			X			
I. paraconfusus Lanier		X				
I. perturbatus (Eich.)			X			
I. pilifrons Swaine			X			
I. pini (Say)	X					
I. radiatae Hpk.	X					
Leperisinus aculeatus (Say)					X	
Magdalis armicollis Say	X					
Melanophila drummondi (Kirby)		X				
Neoclytus acuminatus (F.)					X	
Orthotomicus sp.			X			
O. caelatus (Eich.)			X			
Pityogenes sp.			X			
Pseudohylesinus granulatus (LeC.)		X				
P. menziesii (Hpk.)		X				
Scolytus spp.		X				
S. muticus (Say)					X	
S. quadrispinosus Say					X	
S. tsugae (Swaine)		X				
S. ventralis LeC.		X	X			

emergence period and this is reversed during the latter half. Most of the parasites have emerged by the second week of July, although emergence continues for about three weeks. Females lay eggs on susceptible larvae through the bark of trees attacked by beetles in April through June. They live for only three weeks, usually dying before August. Approximately 95% of the adults developing from eggs laid during July emerge during August to parasitize susceptible larvae from later beetle invasions. The remaining 5% enter diapause in the fifth instar after cocoon formation and overwinter in this stage. Those parasites emerging in August lay eggs on larvae from May and June bark beetle invasions. Approximately 50% of the progeny of this second generation develop to the adult stage and emerge in September. The other 50% enter diapause and overwinter. A third generation develops in September and October from eggs laid in September by the adults of the second generation which emerged that month. Less than 5% of the third generation develop to the adult stage in September or October. The remainder enter diapause and overwinter. The percentage of overwintering insects of the third generation is greater than the second, but the absolute number is about the same since there are less third generation individuals than second. Thus, the overwintering individuals are from three different generations. Overwintering individuals from first, second and third generations emerge at approximately the same time in late June or early July of the following year.

Host Finding Mechanisms

Host selection is an essential and complex behavioral component of any host-parasite relationship. The organisms involved must be seasonally,

geographically, and ecologically coincident and physical, behavioral, or physiological barriers must not be present (Doutt 1965). It is widely accepted that the parasite is initially attracted to the host habitat and then to the host, which it then accepts or rejects. The classical phases of host selection are (1) host habitat finding, (2) host finding, (3) host acceptance, and (4) host suitability (Doutt 1965). Olfaction, hearing, vision, and touch are the most commonly reported perception mechanisms used in host finding.

Olfaction

Picard (1922) observed that Coccygominus instagator F. [=Pimpla instagator F.] females were stimulated to perform a piercing action with the ovipositor when exposed to paper cylinders soaked in the blood of their host (although no eggs were deposited). However, Thompson and Parker (1927) using Melittobia acasta Wilk. were unable to confirm Picard's results. Apanteles sesamiae Cam. could not find its host, Busseola fusca Fuller, in artificial burrows unless the host had fed, the odor from the frass being the critical factor (Ullyett 1936). Thorpe and Jones (1937) concluded that Venturia [=Nemeritis] canescens (Grav.) found its host, Ephestia kuhniella (Zeller), almost entirely by a sense of smell residing in the antennae. This parasite followed the odor trail of its true host in preference to an alternate host Meliphora spp. It was also found that the host habitat ordor (oatmeal) was attractive to V. canescens. Salt (1938) found that Trichogramma spp. could distinguish parasitized from unparasitized hosts by smell but not by sight, hearing, or touch. Female Nasonia vitripennis (Walker) were attracted to the odor of unwashed host puparia (Jacobi 1939) but not washed puparia (Edwards 1954,

Wylie 1958). Pimpla bicolor Bouché was reported to swarm around broken cocoons of its host Euproctis terminalis Wlkr. (Ullyett 1953). Vinson (1968) and Vinson and Lewis (1965) discovered that a secretion from the mandibular glands of Heliothis virescens (F.) stimulated the parasite Cardiochiles nigriceps Viereck to begin directed searching movements. Microplitis croceipes (Cresson) has been shown to use 13-methylhentriacontane, a chemical isolated from the feces and larvae of Heliothis zea (Boddie), to locate its host (Jones et al. 1971).

Not all host finding mechanisms utilizing odor are as direct as those mentioned above. Brachymeria mincita (L.) is apparently attracted by the specific odor of fermented host feces which do not occur until the host is in the third instar. The parasite will not visit fresh feces (Sycheskaya 1966). The fungus Amlostereum sp. is involved in host selection behavior of three parasitic species: Ibalia leucospoides (Hoch.), Rhyssa persuasoria (L.), and Megarhyssa nortoni nortoni (Cresson) (Madden 1968). This fungus is a symbiont of the siricid host of these parasites. Spradbery (1970a, b) demonstrated that drilling by the siricid parasites Ibalia drewseni Borries and Rhyssa persuasoria (L.) is elicited by the odor of siricid frass and the symbiotic host fungus and that washed larvae alone do not stimulate host searching behavior. Cultured fungal symbionts were the most attractive after three to four months, a period coinciding with the maturation of host larvae. Both the aqueous and ethanol extracts from the frass and fungus stimulated drilling by the parasites. Doutt (1957) reported that Macrocentrus ancylivorus Roh. probes into piles of frass that indicates the burrow of its host, the potato tuber worm; Apanteles

aristoteliae Vier. follows a similar frass trail of its host, the orange tortrix. Fischer (1959) concluded that Diadegmus chrysostictum (Gmelin) [=Horogenes chrisostictos Gmelin] is strongly attracted to the odor of flour and oatmeal but has little precise chemical guidance to the position of the host. Habitat odor stimulated search, and odor of both habitat and host stimulated further investigation and probing by the parasite's ovipositor. Stabbing with the ovipositor was thought to be random and continued for long periods of time if no host was encountered. Itoplectis conquisitor (Say) reportedly locates its host Rhyacionia buoliana (Schiff) by the odor of host food plant rather than by host odor (Arthur 1962). In the laboratory, I have observed that I. conquisitor will oviposit on host pupae in absence of host food plant material.

Hearing

DeLeon (1935) postulated that Coeloides dendroctoni Cushman, a parasite of Dendroctonus larvae, detected its host by vibrations caused by the host's chewing on the phloem tissue of the tree. Ryan and Rudinsky (1962) reported that C. brunneri finds its host in a similar way and that this parasite would "listen" for noises under the bark while searching infested logs. They reported that the parasite could be induced to oviposit through a piece of bark by scratching the undersurface of the bark with a pin. Spradbery (1970a) killed siricid larvae in situ by freezing and then thawing them and demonstrated that siricid parasites were able to detect and parasitize dead hosts in logs, thus eliminating host-produced sound as an essential stimulus for host finding. Allen et al. (1960) reported that it was possible to record sound production from adult Dendroctonus spp. but no mention was made of sound produced by the larvae.

Vision

Ullyett (1936) found that Dahlbominus fuscipennis (Zetterstedt) was first attracted to hosts by visual contact at a distance of five to six mm after which olfaction aided in recognition of the host. N. vitripennis (Edwards 1954) and Spalangia drosophilae (Ashm.) (Simmonds 1954) also find their hosts visually, although they are initially attracted to the odor of the host habitat. The great variation in physical characteristics of the host environments located by female N. vitripennis rules out any possibility that the hosts are recognized other than by sight (Wylie 1958). Perilitus coccinellae (Schrank) finds its adult beetle hosts by visual contact (Cushman 1913, Balduf 1926, Bryden and Bishop 1945, Walker 1961, Richerson and DeLoach 1972). Furniss (1968) reported that Karpinskiella paratomicobia Hagen and Caltagirone which oviposits in adult Douglas-fir beetles, finds its host through visual contact. Tomicobia tibialis Ashm. also a parasite of adult bark beetles, can detect movement of a walking beetle from as far away as four to five cm (Rice 1968). Once the host was attacked it was left alone by other parasites, indicating an odor response to already parasitized hosts.

Touch

Doutt (1957) observed that Solentus begini (Ashm.) walked across leaves and responded to the presence of serpentine leaf mines of Phytomyza atricornis Meig. The parasite traced the margins of the mine using its body and palpating its antennae over the leaf. This reaction was stimulated whether or not a susceptible host was present. Apanteles dignus Muesebeck, a parasite of the tomato pinworm, Keiferia lycopersicella

(Walsingham), searches across the surface of a leaf by tapping with its antennae. Upon contact with a leaf mine it stops walking and begins a circular movement, tapping the surface of the mine. It apparently responds to changes in the texture of the leaf over a mined area (Cardone and Oatman 1971).

Structure and Function of Sensilla Placodea

Sensilla placodea was first described by Hicks (1857, 1859), Leydig (1860) and Kraepelin (1883). Since then many different forms of plate organs have been described (Melin 1941). According to Snodgrass (1935) sensilla placodea present externally a thin cuticular plate, either elliptical, oval, or elongate in form, set over a large cavity in the cuticle. In the Hymenoptera, these plates are usually larger than eight microns and vary in form from an ellipse (Apis) to a narrow elongate oval (Cynips vespa). They are generally flush with the antennal surface but are sometimes elevated and may be surrounded by a deep groove. Bullock and Horridge (1965) and Chapman (1969), in addition to describing the generalized structure of the plate organs, briefly describe the cellular components of the sensillum. Electron microscope studies (Slifer and Sekhon 1960, 1961, Slifer 1969) on Apis and Nasonia have shown considerable structural diversity of placoid sensilla. However, the ultrastructural details remain undetermined and according to Slifer (1970) much of the earlier work should be redone using the improved techniques of fixation and staining now available.

Sensilla placodea have been hypothesized to serve as olfactory, and mechanoreceptors (Slifer 1960), but the hypotheses have generally been

unverified. However, the pore plates of Apis have been proven by electrophysiological investigation to be olfactory receptors (Lacher 1964, Lacher and Schneider 1963).

Objectives

The contradictory and tenuous evidence for many host-finding mechanisms indicate that much work is needed in the analysis of host-parasite relationships. It is evident that host-finding in many cases may be a single stimulus-response mechanism but that in other cases a complex of stimuli may be involved before a parasite is able to locate a host in any given habitat.

The objectives of this study were to describe the host finding behavior of C. brunneri and to analyze the mechanisms by which the parasite detects its host. A morphological description of a previously undescribed type of placoid sensilla, apparently used in host finding, is included.

REARING AND MAINTENANCE OF EXPERIMENTAL INSECTS

Host beetles and the parasites were reared in large screened cages from infested bark collected in the field. The adults of D. pseudotsugae and D. ponderosae were stored at 0 to 2 C until they were cultured in the laboratory on bolts of Douglas-fir or ponderosa pine, respectively.

Although Ryan (1961) stated that C. brunneri females cannot parasitize larvae that are under bark thicker than the length of their ovipositors, I observed C. brunneri emerging from very thick bark. To investigate this apparent anomaly further, samples of field collected Douglas-fir bark were separated according to bark thickness and held in rearing cages. Although the average length of the ovipositor of C. brunneri was determined to be 4.27 mm; (n=100; range, 4.03 to 6.56 mm), the greatest ratio of parasite to bark beetle emergence occurred from much thicker bark (Table II). This can be explained by the presence of fissures in the bark surface that provide thinner regions which are within reach of the parasite. In addition, late instar bark beetle larvae mine out toward the bark surface and, therefore, expose themselves to greater possibility of attack by the parasite.

The effectiveness of parasites of subcortical larvae is often limited by bark thickness (Ryan 1961, Ryan and Rudinsky 1962, Massey and Wygant 1954). However, Beaver (1967) observed that bark thickness does not have a significant effect on the level of parasitism by Coeloides scolyticida on Scolytus scolytus but the level is slightly reduced as bark thickness increases. In general, attempts to correlate bark thickness with the rate of parasitism have failed, probably due to a large variation in rates of

Table II Emergence of adult C. brunneri and D. pseudotsugae in relation to bark thickness.

Bark thickness (cm)	Location	Number of insects emerging from bark D. pseudotsugae	Number of insects emerging from bark C. brunneri	Number of C. brunneri per emerged beetle By location	Number of C. brunneri per emerged beetle $\bar{X}$
≤ .65	Pt. Roberts, Washington	238	33	.139	
	Pt. Roberts, Washington	1035	3	.003	.0283
≤ 1.27 > .65	Pt. Roberts, Washington	404	26	.064	
	UBC Forest, Haney, B.C.	36	5	.139	.070
≤ 1.95 >1.27	Fraser Canyon, B.C.	6	164	27.333	
	UBC Forest, Haney, B.C.	7	220	31.428	29.538
> 1.96	UBC Forest, Haney, B.C.	11	35	3.182	
	Pt. Roberts, Washington	1100	0	0.000	
	UBC Forest, Haney, B.C.	36	5	.139	.035

parasitism (Berisford et al. 1971). Invariably the highest level of parasitism is reported in the upper bole or thin barked regions of the tree (Ryan 1961, Ryan and Rudinsky 1962, DeLeon 1935, Beaver 1967). The data in Table II indicate that for C. brunneri, one should not automatically assume low levels of parasitism in bark thicker than the length of the ovipositor.

Adult parasites were held in a 20 X 20 X 40 cm mesh screen cage at 20-22 C. They were provided with water, an aqueous solution of honey, and raisins. Females were mated in a separate cage. Unmated females were not observed to search on infested bolts of Douglas-fir. To extend longevity, parasites were held at 4 C for three to four weeks without any discernable ill effects.

DESCRIPTION OF HOST FINDING BEHAVIOR

Description and Use of a Lazy Susan Observation Chamber

An apparatus was designed to provide a simultaneous visual observation and a permanent record of the search behavior of C. brunneri. Infested bolts (approximately 25 X 30 X 50 cm) were placed on a Rubbermaid 38 cm diameter lazy Susan. A cylinder of clear acetate (.1 X 53 X 90 cm) was placed around the log with a space of approximately three cm between the log and the plastic (Figs. 1-2). Cardboard discs were used as a lid and bottom to the plastic cylinder. A female parasite was released into the chamber and its movement was traced with a Staedtler No. 317 pen. Time markers were made on the tracings every 10 minutes. The direction of movement as well as all stops and ovipositions by a parasite were marked on the tracing. As the parasite moved over the log, the lazy Susan was turned so the parasite was facing the observer. This movement had no observable effect on the parasite's behavior.

Immediately following each series of five replications (i.e. the search behavior of five female C. brunneri), the test log was removed, debarked and replaced on the lazy Susan. The bark beetle gallery pattern visible on the exposed sapwood was traced on a clear plastic cylinder which was overlaid on the tracings of the parasite search patterns. Search patterns and oviposition sites were then correlated with the presence or absence of bark beetle galleries and larvae (Figs. 3-4).

This apparatus was used to qualitatively and quantitatively describe C. brunneri search patterns and in the following comparison experiments: behavior over 24 hours versus behavior in a 90-minute test period, and

Fig. 1, 2 Lazy Susan observation chamber. Disassembled (Fig. 1) to show Rubbermaid lazy Susan, cylinder of clear polyethylene, cardboard discs, and infested log, and (Fig. 2) fully assembled and in operation.

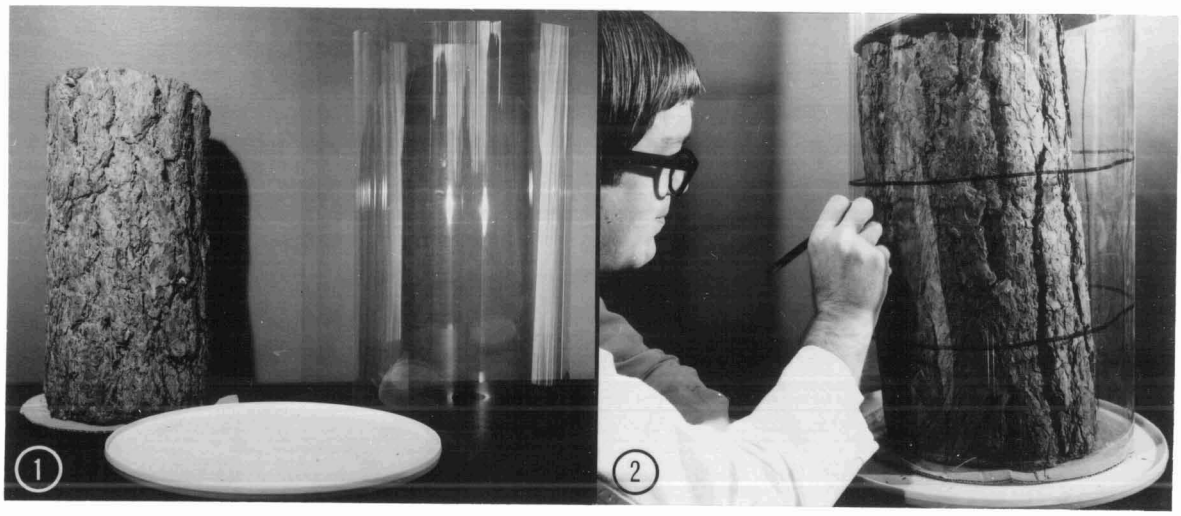

1
2

Fig. 3 Search patterns of five C. brunneri females on Douglas-fir infested with D. pseudotsugae during a 90-minute test period. Numeral indicates start of search. Oviposition site designated by OV.

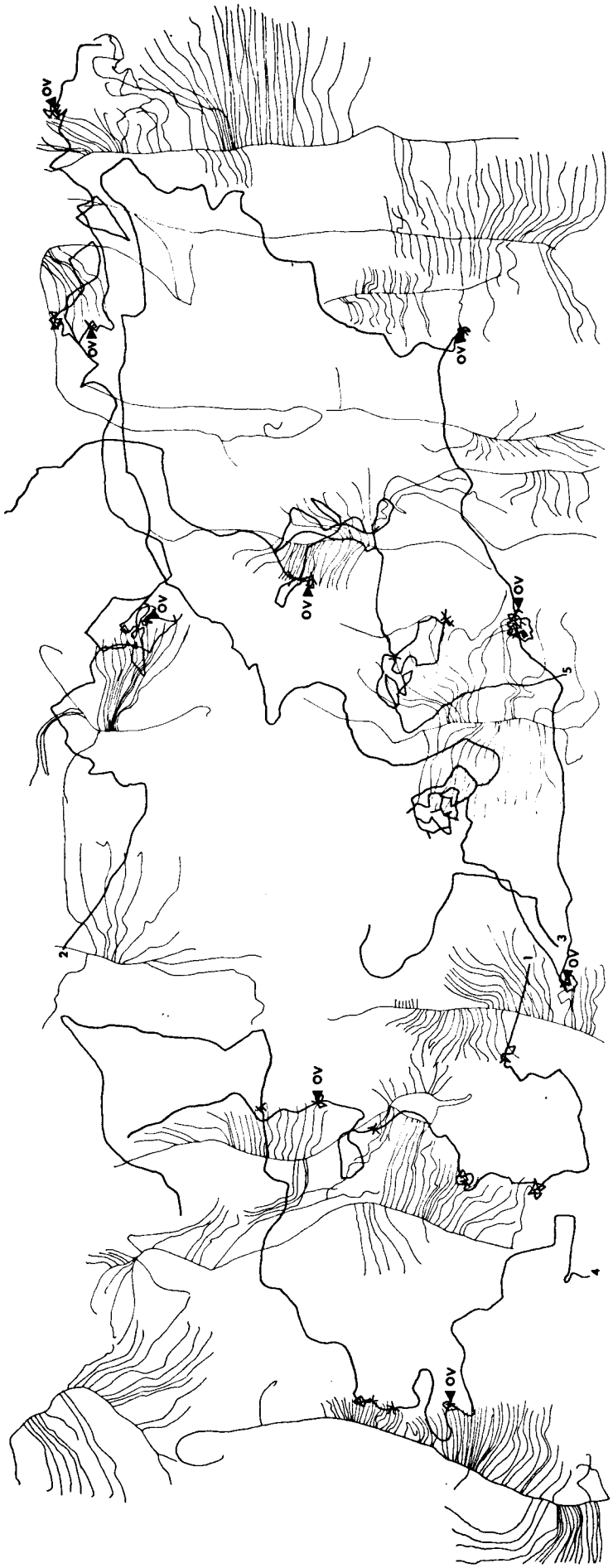
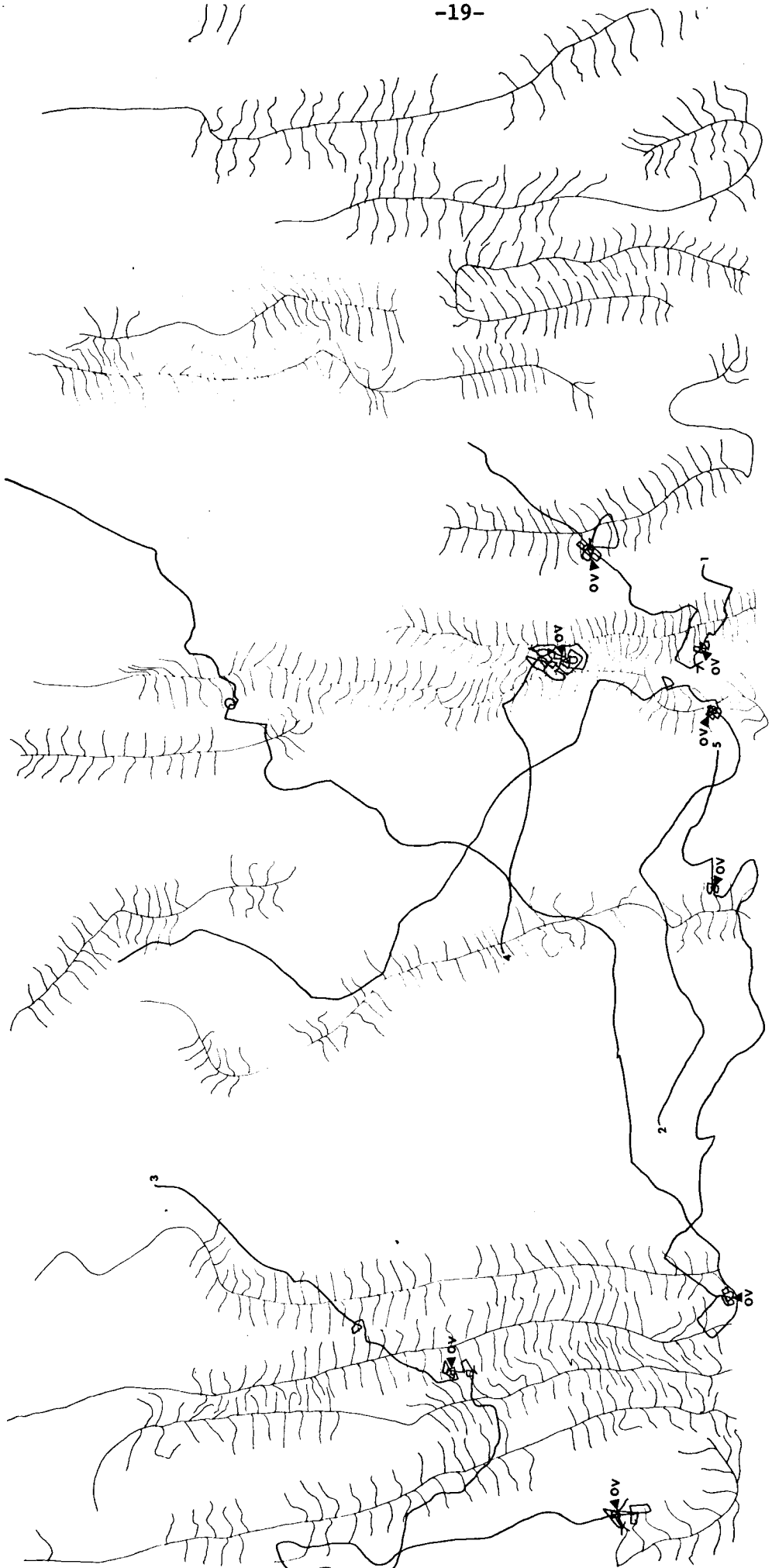


Fig. 4 Search patterns of five C. brunneri females on ponderosa pine infested with D. ponderosae during a 90-minute test period. Numeral indicates start of search. Oviposition site designated by OV.


OV
1
2
3
4
5

response to D. pseudotsugae in Douglas-fir versus D. ponderosae in ponderosa pine. Selected parameters were analyzed to identify key stimuli which maintain or induce change in the search pattern.

Qualitative Description of Search Behavior

Previous descriptions of search behavior of parasites which attack subcortical insects have stressed the randomness or nonrandomness of the search pattern. DeLeon (1935) reported that a C. dendroctoni female moved over the bark at random while "sounding" with her antennae, but did not use bark beetle entrance, ventilation, or exit holes as a means of approaching the host or finding an unobstructed path for the ovipositor. Moreover, C. dendroctoni did not respond to exposed larvae. Spradbery (1970b) observed that the ichneumonid parasites of siricid larvae randomly surveyed the bark of infested timber with the antennae and exhibited sustained antennal activity (palpating) in areas of special interest. Probes with the ovipositor were not random and the majority were made in response to siricid tunnels. Megarhyssa sp. began oviposition only after considerable walking and exploratory behavior followed by a more intensive stationary exploration of a single spot (Heatwole, Davis and Wenner 1964). Doutt (1965) and Laing (1937) reported that Nasonia vitripennis and Trichogramma spp., respectively, explored in straight lines and turned sharply only when a host was found. In an olfactometer, N. vitripennis began a twisting path when the odor of contaminated liver was introduced (Edwards 1954). Ryan and Rudinsky (1962) reported that female C. brunneri were slow but thorough searchers that paused to "listen" for noises or vibrations under the bark surface. The parasite followed natural crevices in the bark and stopped searching in strong light.

C. brunneri search patterns as recorded using the lazy Susan technique are similar to those of Rhyssa persuasoria (Spradbery 1970a). Both species exhibit random and nonrandom search patterns with intermittent periods of rest and preening. There are four distinct phases of host finding for C. brunneri: nonsearch, random search, nonrandom search, and oviposition. The four phases are sequential and were never observed out of sequence except for nonsearch behavior which occurs before and during random search. Nonsearch is characterized by a cleaning activity of the body by a stationary parasite. Random search is an extended traverse in which turns, when they occur, are gradual and sweeping. This phase is typically in a vertical or vertical-oblique direction and is influenced by the parasite's tendency to follow crevices in the bark. Nonrandom search is characterized by many sharp turns and a small area of bark examined (Figs. 3-4).

Once nonrandom search is initiated there are apparent periods of nonsearch but these are not spent cleaning or resting. Rather, they appear to be for receiving and interpreting stimuli. During nonsearch stops in nonrandom search the antennae are held at approximately a 45° angle from the body. While the parasite is in motion in either random or nonrandom search the antennae move side to side in parallel so that an area of bark approximately one cm wide is examined. The apical three segments of each antenna will be curved down and pass very close to or occasionally touch the bark surface. The remainder of the antenna rarely touches the bark. Like C. dendroctoni (DeLeon 1935) C. brunneri does not respond to entrance, emergence, or ventilation holes in the bark. The parasite does not follow larval galleries to the end of the gallery where the host is located.

Oviposition is characterized by the drilling of the ovipositor into the bark. When a host larva is located, it is paralyzed by the injection of a venom, and then an egg is deposited. If a larva is not located another probe is made until a host is located and paralyzed. However, an oviposition was recorded if a single ovipositional probe occurred. The oviposition phase is a culumination of previous response behavior that ultimately commits the parasite to select one site as a potential host location.

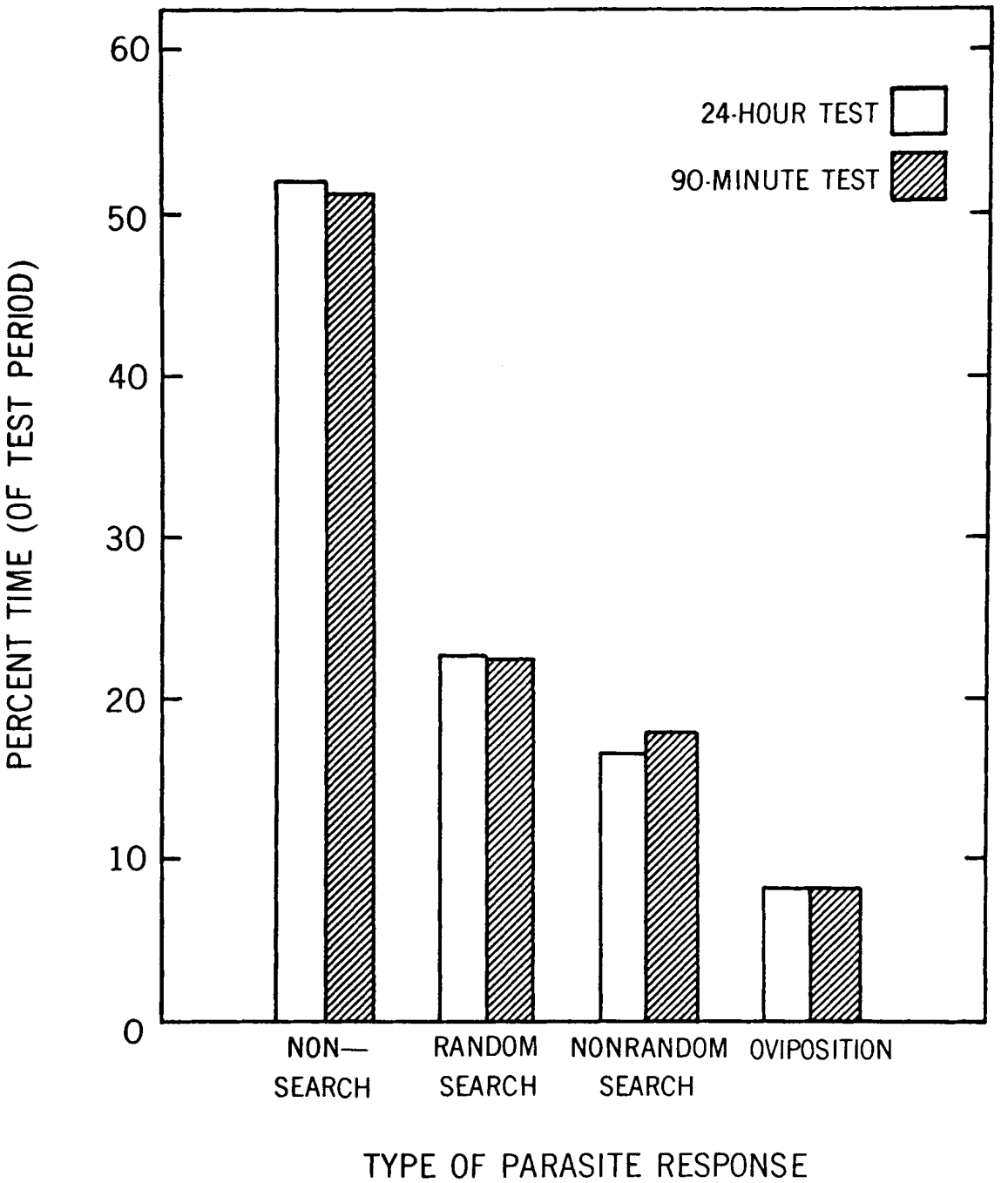
After oviposition the parasite usually moves to one side of the area just searched and preens for 15-40 minutes before resuming a random search pattern. The female rarely returns to the area where she last deposited an egg, unless she returns during a random search. This is contrary to the observation that C. scolyticida continued to search the area where it had previously searched and oviposited (Beaver 1967).

Comparison Between 24-hour and 90-minute Test Periods

Test time length can be critically important to any behavioral study. To determine if a period of 90 minutes would allow for typical parasite behavior, search patterns of parasites during 90-minute and 24-hour test periods were compared. Five replicates were run using a single parasite in each 90-minute or 24-hour observational period. Two bolts of Douglas-fir infested by approximately the same number of D. pseudotsugae were used, one for each test duration.

There was no difference between the percent of time spent in each of the four phases of host finding in either test duration (Fig. 5). Therefore, all further experiments were conducted in 90-minute periods.

Fig. 5 Percent of time spent by five C. brunneri females in the four phases of host finding during 24-hour and 90-minute test periods on Douglas-fir infested with D. pseudotsugae.


60
50
40
30
20
10
0
24-HOUR TEST
90-MINUTE TEST
PERCENT TIME (OF TEST PERIOD)
NON—
SEARCH
RANDOM
SEARCH
NONRANDOM
SEARCH
OVIPOSITION
TYPE OF PARASITE RESPONSE

Quantitative Description of Search Behavior

Five female C. brunneri on Douglas-fir during a 90-minute period spent approximately 36 to 46 minutes in nonsearch, 20 to 30 minutes in random search, 15 to 16 minutes in nonrandom search and eight to nine minutes ovipositing (Figs. 5-6). On ponderosa pine the average times were 51, 23, 12 and 5 minutes in nonsearch, random search, nonrandom search, and oviposition, respectively (Fig. 6).

The progressively less time spent in each of the four sequential phases of search behavior suggests that when the parasite nears its objective, stimuli are sufficiently strong that less time is required to respond and to move to the next phase. The least time is spent in oviposition, where no locomotory search is required since the location of the host is known. The short oviposition period is probably of adaptive significance to the parasite since there would be a greater chance of predation by clerid and ostomid beetles or birds the longer it remained immobile on the bark.

On infested Douglas-fir logs there are many significant differences between random and nonrandom host-finding behavior (Table III). In random search there is a significantly greater distance travelled, more time spent, larger bark surface area searched, and significantly fewer galleries crossed than in nonrandom search. Nonrandom search clearly has characteristics of a more concentrated search pattern, such as a much tighter turning pattern, and the linear search area (path 1 cm wide along the tracing) is much greater than the bark surface area searched (linear search area minus area recrossed). Therefore, the parasite must retraverse considerable areas in nonrandom search. The similarity in the number of galleries encountered for the first time indicates that there is a nearly equal chance of crossing a new gallery in either search pattern. Infested

Fig. 6 Comparison of the percent of time spent by five *C. brunneri* females in the four phases of host finding on two species during a 90-minute test period.

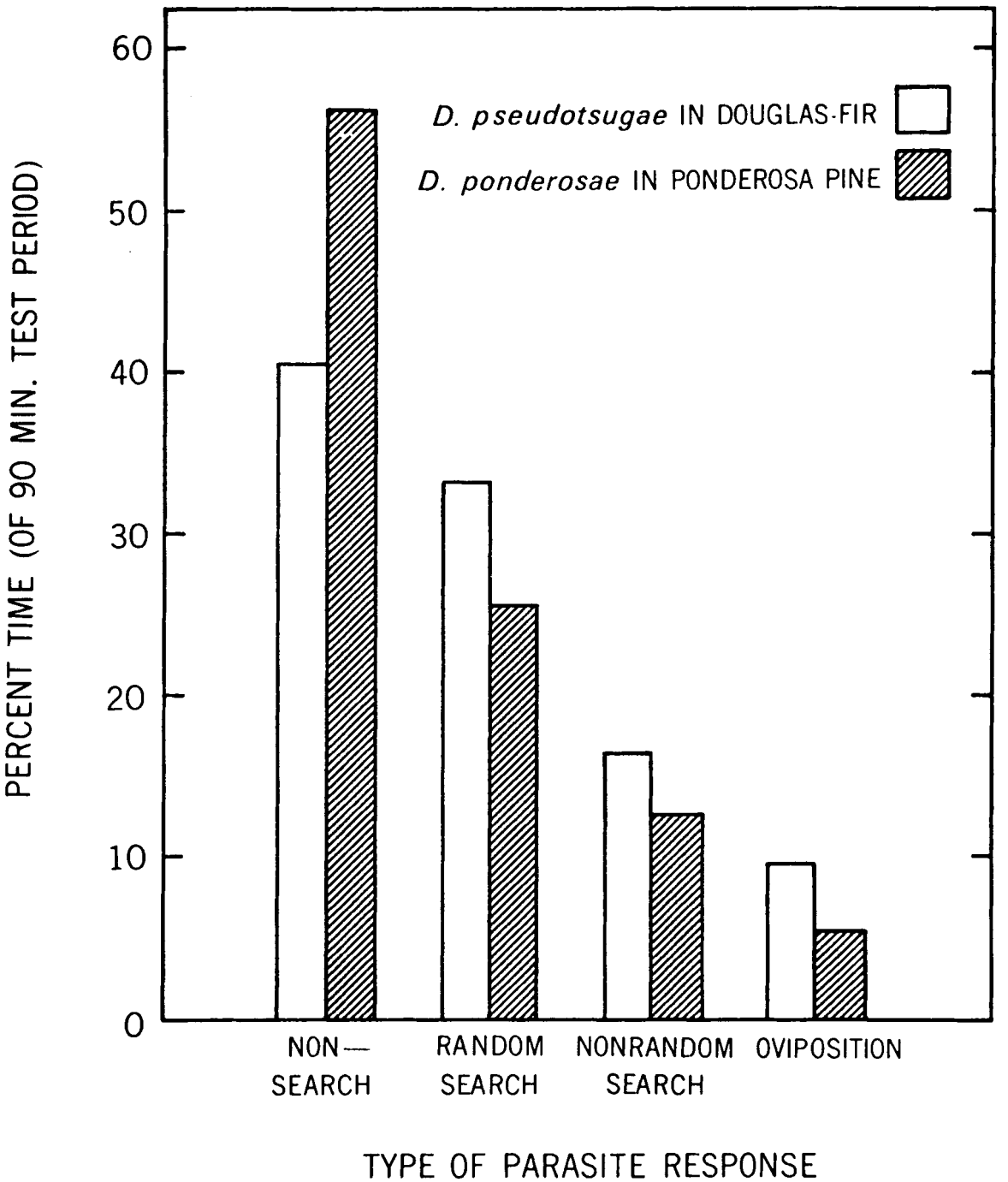

60
50
40
30
20
10
0
PERCENT TIME (OF 90 MIN. TEST PERIOD)
D. pseudotsugae IN DOUGLAS-FIR
D. ponderosae IN PONDEROSA PINE
NON— SEARCH
RANDOM SEARCH
NONRANDOM SEARCH
OVIPOSITION
TYPE OF PARASITE RESPONSE

Table III Comparison between random and nonrandom search by *C. brunneri* females on Douglas-fir infested with *D. pseudotsugae*.

Search character	Random Search $\bar{X}$ [a]	Nonrandom Search $\bar{X}$ [a]	t Probability
Distance (cm)	74.46	25.84	<.10
Time (min)	29.80	14.90	<.01
Rate (cm/min)	2.49	1.72	<.50
Number of turns >70°	16.80	46.80	<.02
Area searched (cm^2)	856.15	26.82	<.001
Linear area searched (cm^2)	938.20	325.58	<.01
Galleries encountered first time	15.60	17.00	>.50
Galleries crossed	17.00	44.80	<.01
Number of ovipositions	---	8.40	

[a] Mean of five observations.

ponderosa pine bolts elicit the same significant differences as Douglas-fir (Table IV). This indicates the parasite has a comparatively similar search ability on both species, thus substantiating its recorded occurrence from both D. pseudotsugae and D. ponderosae (Table I).

Comparison Between Response to D. pseudotsugae and D. ponderosae

A comparison of search patterns between two host trees indicate that a more efficient search is made on D. pseudotsugae. Approximately 28 percent more time is spent in inactive nonsearch on pine than on Douglas-fir (Fig. 6). The parasite, however, spends more time in meaningful active search patterns on Douglas-fir than on pine.

The only significant difference in random search on Douglas-fir and ponderosa pine (Table V) is in the number of turns. This difference may be caused by the rougher Douglas-fir bark which forces the parasite to turn more often. Otherwise, search is no different on pine than on Douglas-fir.

The greater time and more efficient search patterns in the nonrandom search (Table VI), also indicates that D. pseudotsugae is a superior host for C. brunneri. There is a greater number of gallery crossings and ovipositions on Douglas-fir than on pine. Because D. pseudotsugae larval galleries are longer and much closer together than those of D. ponderosae, there is a greater chance for initial encounter and recrossing of the former in a concentrated search. Thus, the gallery pattern of the host beetle may enhance host finding ability.

Table IV Comparison between random and nonrandom search by *C. brunneri* females on ponderosa pine infested with *D. ponderosae*.

Search character	Random search $\bar{X}$ [a]	Nonrandom search $\bar{X}$ [a]	t probability
Distance (cm)	67.66	18.34	<.05
Time (min)	23.10	11.70	<.05
Rate (cm/min)	2.75	1.48	<.50
Number of turns >70°	7.40	27.80	<.01
Log area searched (cm^2)	743.54	46.31	<.001
Linear area searched (cm^2)	841.18	231.08	<.02
Galleries encountered first time	12.80	6.00	>.50
Galleries crossed	14.60	20.40	<.02
Number of ovipositions	---	7.00	

[a] Mean of five observations.

Table V Comparison of random search patterns of C. brunneri between two species of tree and host beetle (five female parasites tested on each host).

Search character	D. pseudotsugae in Douglas-fir $\bar{X}$ [a]	D. ponderosae in Ponderosa Pine $\bar{X}$ [a]	t probability
Distance (cm)	74.46	67.66	> .50
Time (min)	29.80	23.10	< .50
Rate (cm/min)	2.49	2.75	> .50
Number of turns >70°	16.80	7.40	< .01
Area searched (cm^2)	856.15	743.54	> .50
Linear area searched (cm^2)	938.20	841.18	< .50
Galleries encountered first time	15.60	12.80	> .50
Galleries crossed	17.00	14.60	> .50

[a] Mean of five observations.

Table VI Comparison of nonrandom search patterns of C. brunneri between two species of tree and host beetle (five female parasites tested on each host).

Search character	D. pseudotsugae in Douglas-fir $\bar{X}^a$	D. ponderosae in Ponderosa pine $\bar{X}^a$	t Probability
Distance (cm)	25.84	18.34	<.50
Time (min)	14.90	11.70	<.50
Rate (cm/min)	1.72	1.48	>.50
Number of turns >70°	46.80	27.80	<.50
Log area searched (cm^2)	26.82	46.31	<.50
Linear area searched (cm^2)	25.84	18.34	<.50
Galleries encountered first time	17.00	6.00	<.50
Galleries crossed	44.80	20.40	<.01
Number of ovipositions	8.40	7.00	<.05

[a] Mean of five observations.

Correlation of Search Characteristics with Maintenance of or Change in Search Pattern

Since nonrandom search is vitally important as an obligate prerequisite to oviposition, selected characteristics of this search pattern were analyzed. When the number of galleries encountered the first time and the time spent in nonrandom search (Fig. 7) are correlated, an increase in the number of galleries encountered on Douglas-fir does not induce more time spent in nonrandom search. However, the parasite, on pine, increases the amount of time spent in nonrandom search if more galleries are encountered, probably indicating a response to increased numbers of the host at low host density. Such a response probably does not occur on Douglas-fir because of the greater number and proximity of larval mines. Nonrandom search on Douglas-fir appears time dependent given a minimum threshold stimulus indicative of high host density. This mechanism is beneficial since the parasite will stop searching in a given time and move on, thus avoiding persistent responses to artifacts or to unsuitable hosts.

Increasing a possible oviposition stimulus (galleries encountered) has only a slight effect in increasing the number of ovipositions (Fig. 8). Thus, it appears that oviposition rate is more probably limited by the capacity of the parasite to lay eggs.

In random search there is no increase in the total number of galleries crossed when the number of galleries encountered for the first time are increased, which indicates that there are few or no galleries recrossed (Fig. 9). However, in nonrandom search, increasing gallery encounters

Fig. 7 Correlation of the minutes of nonrandom search with the number of larval galleries encountered by five female C. brunneri per host tree in a 90-minute test period.

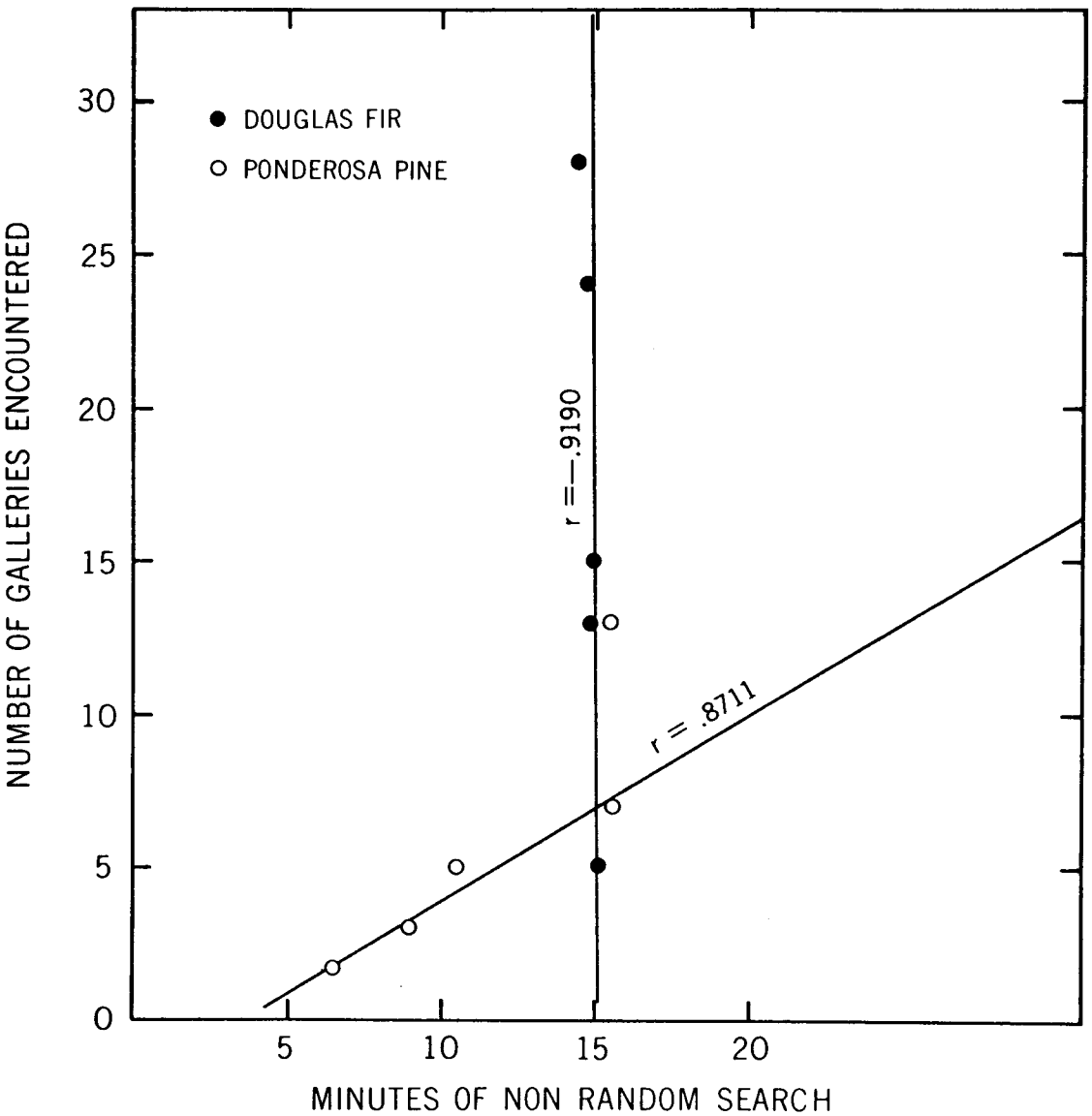

DOUGLAS FIR
PONDEROSA PINE
r = -.9190
r = .8711
NUMBER OF GALLERIES ENCOUNTERED
MINUTES OF NON RANDOM SEARCH
0
5
10
15
20
25
30
5
10
15
20

Fig. 8 Correlation of the number of ovipositions with the number of larval galleries encountered by five female C. brunneri per host tree in a 90-minute test period.

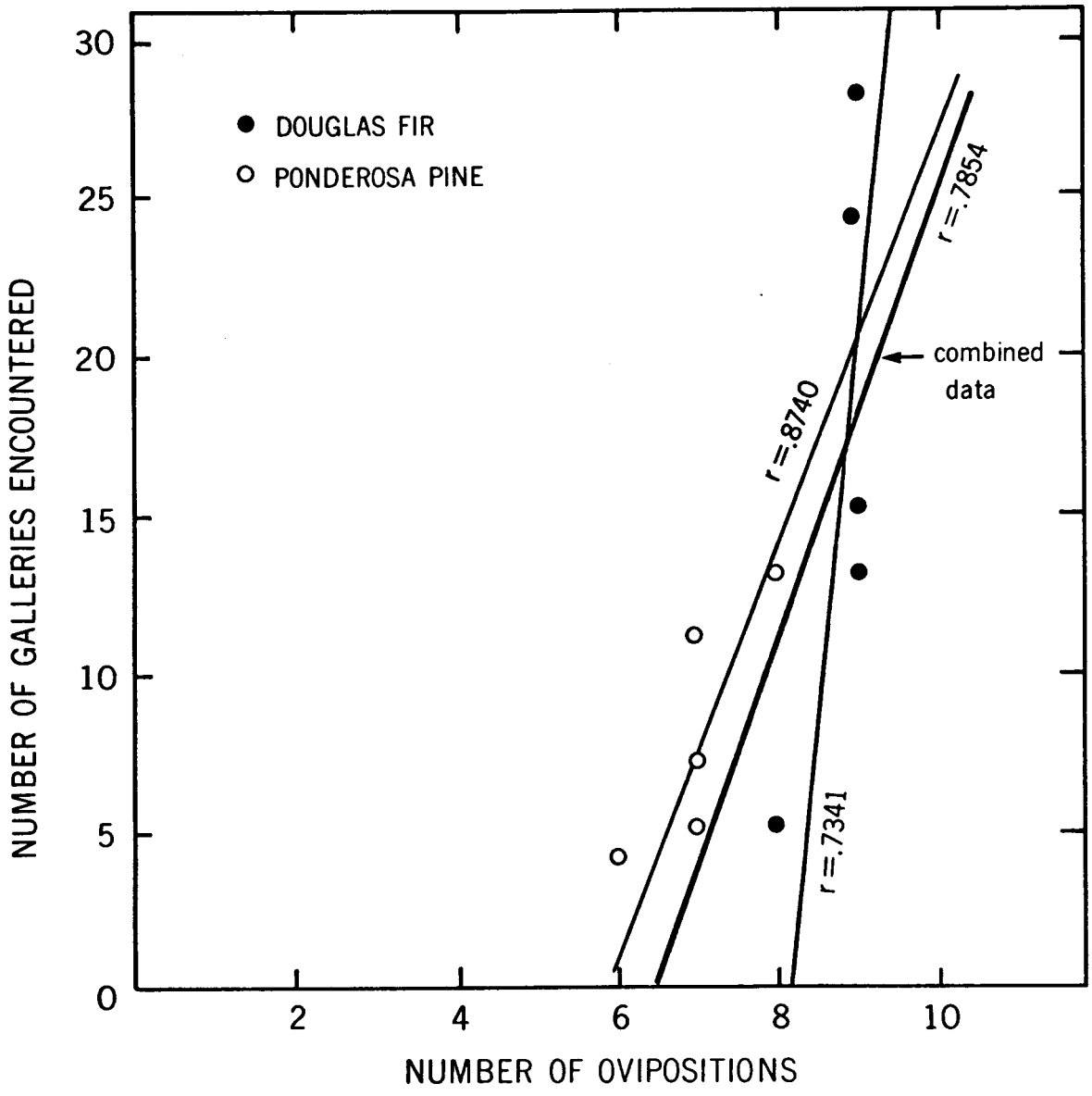
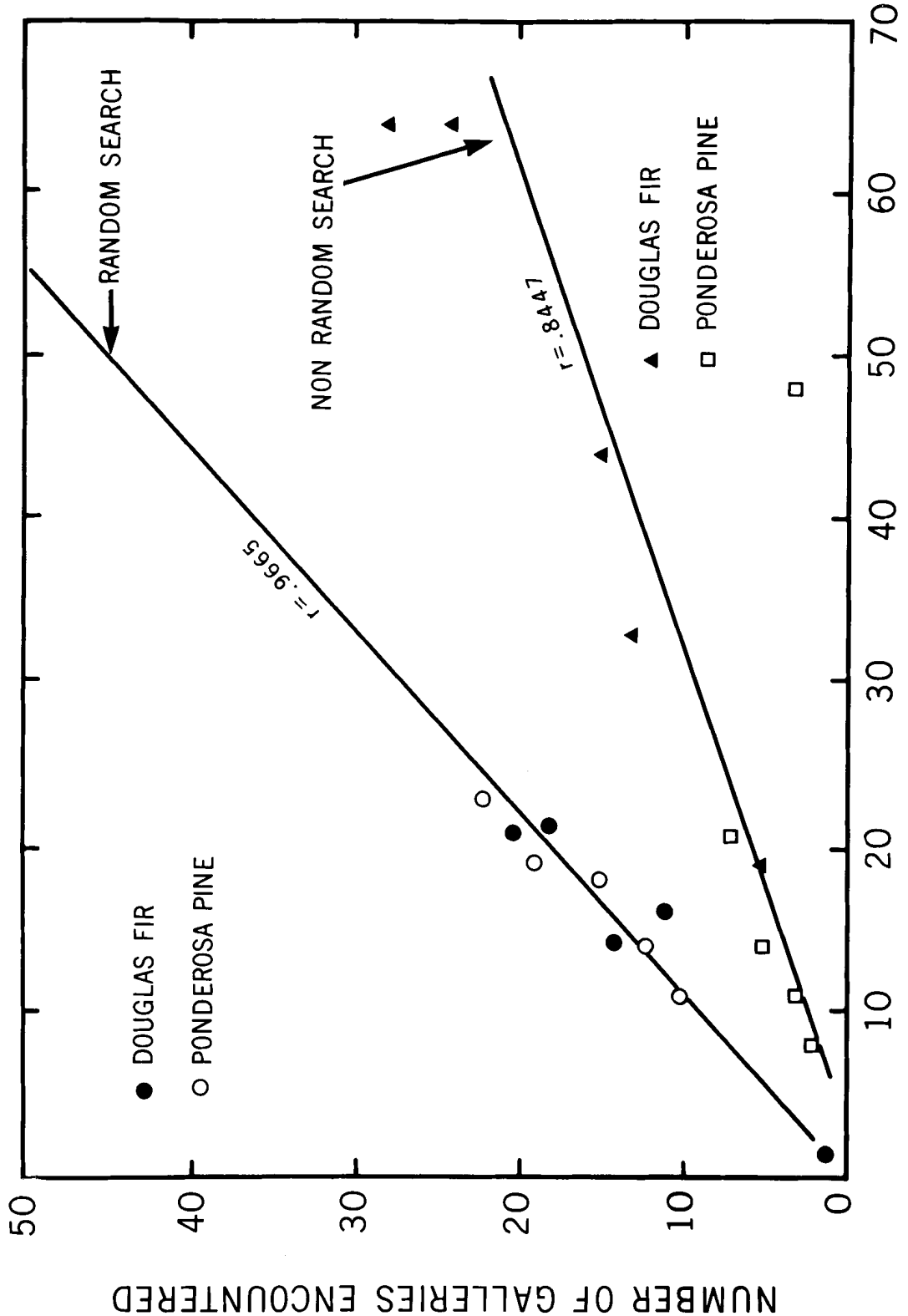

DOUGLAS FIR
PONDEROSA PINE
r=.8740
r=.7854
r=.7341
combined data
NUMBER OF GALLERIES ENCOUNTERED
NUMBER OF OVIPOSITIONS
0
5
10
15
20
25
30
2
4
6
8
10

Fig. 9 Correlation of the number of larval gallery crossings with the number of larval galleries encountered by five female C. brunneri per host tree in a 90-minute test period.


RANDOM SEARCH
NON RANDOM SEARCH
r=.9665
r=.8447
DOUGLAS FIR
PONDEROSA PINE
DOUGLAS FIR
PONDEROSA PINE
NUMBER OF GALLERIES ENCOUNTERED
NUMBER OF GALLERY CROSSINGS
0
10
20
30
40
50
10
20
30
40
50
60
70

induces the parasite to recross galleries. Therefore, the search pattern is intensified where the chance of finding the host is maximal.

Encountering the end of a larval gallery for the first time induces the parasite to enter nonrandom search (Fig. 10). The poor correlation indicates that there is not always a cause and effect relationship. For example, a parasite walking over the end of a gallery may not detect the presence of a host in the bark. Alternatively, larvae at the end of many galleries may not be suitable hosts.

The most important significant correlation, between the number of times nonrandom search is initiated at the end of a gallery and the number of times nonrandom search is initiated (Fig. 11), indicates that the stimulus inducing nonrandom search is definitely associated with the end of a larval gallery. Thus, a parasite never begins nonrandom search unless it is over the end of a larval gallery (Fig. 11). It was noted previously (Figs. 3-4) that the parasite does not encounter a larval mine and follow it to the end where a potential host larva is located. This observation is consistent with the extremely high correlation in Fig. 11. The parasite must respond only to the stimulus emanating from the end of a larval gallery. The existence of this stimulus appears to be the key to successful host finding in C. brunneri.

Fig. 10 Correlation of the number of times nonrandom search is initiated with the number of times the end of a gallery is encountered the first time by five female C. brunneri per host tree in a 90-minute test period.

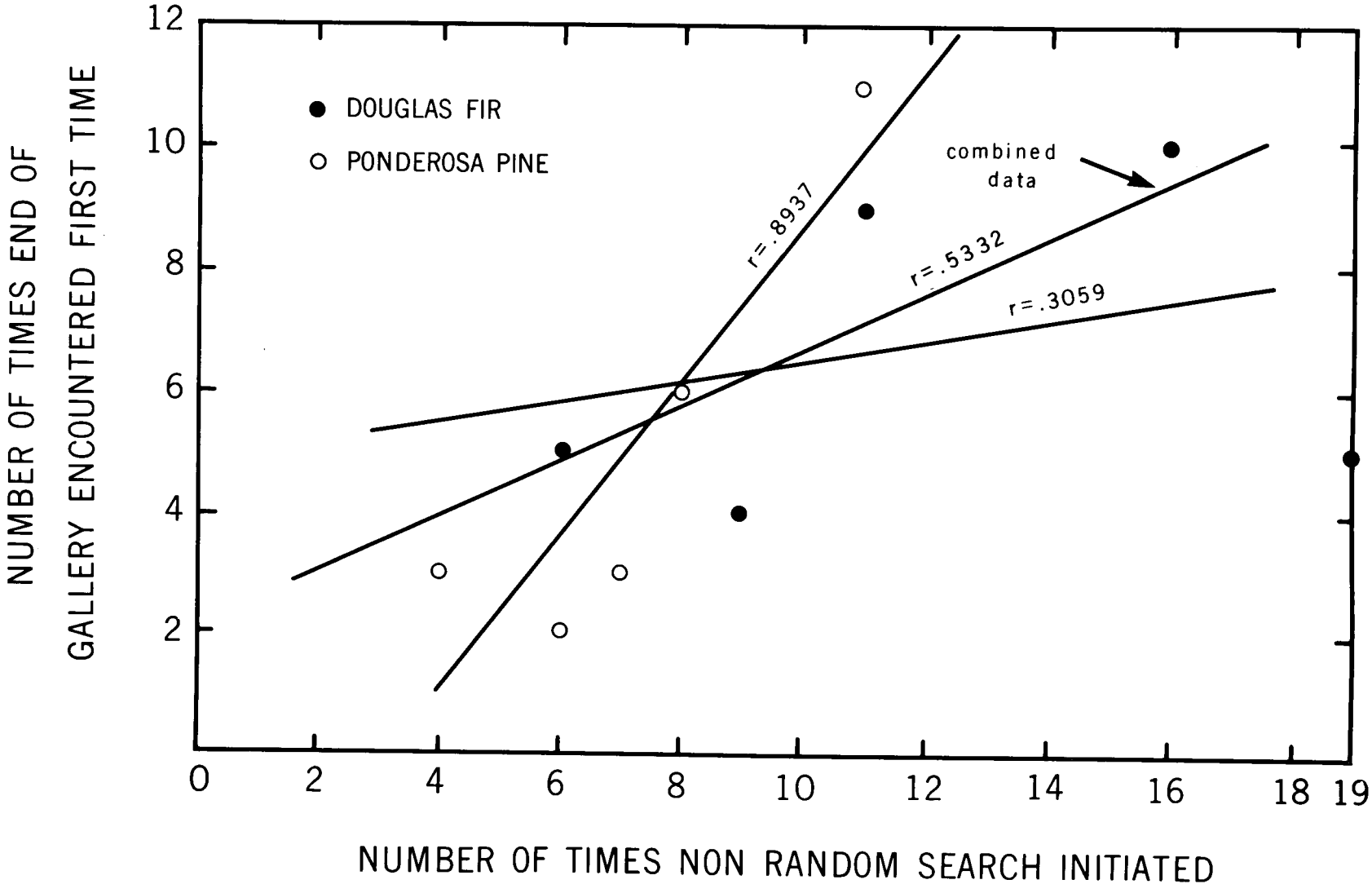
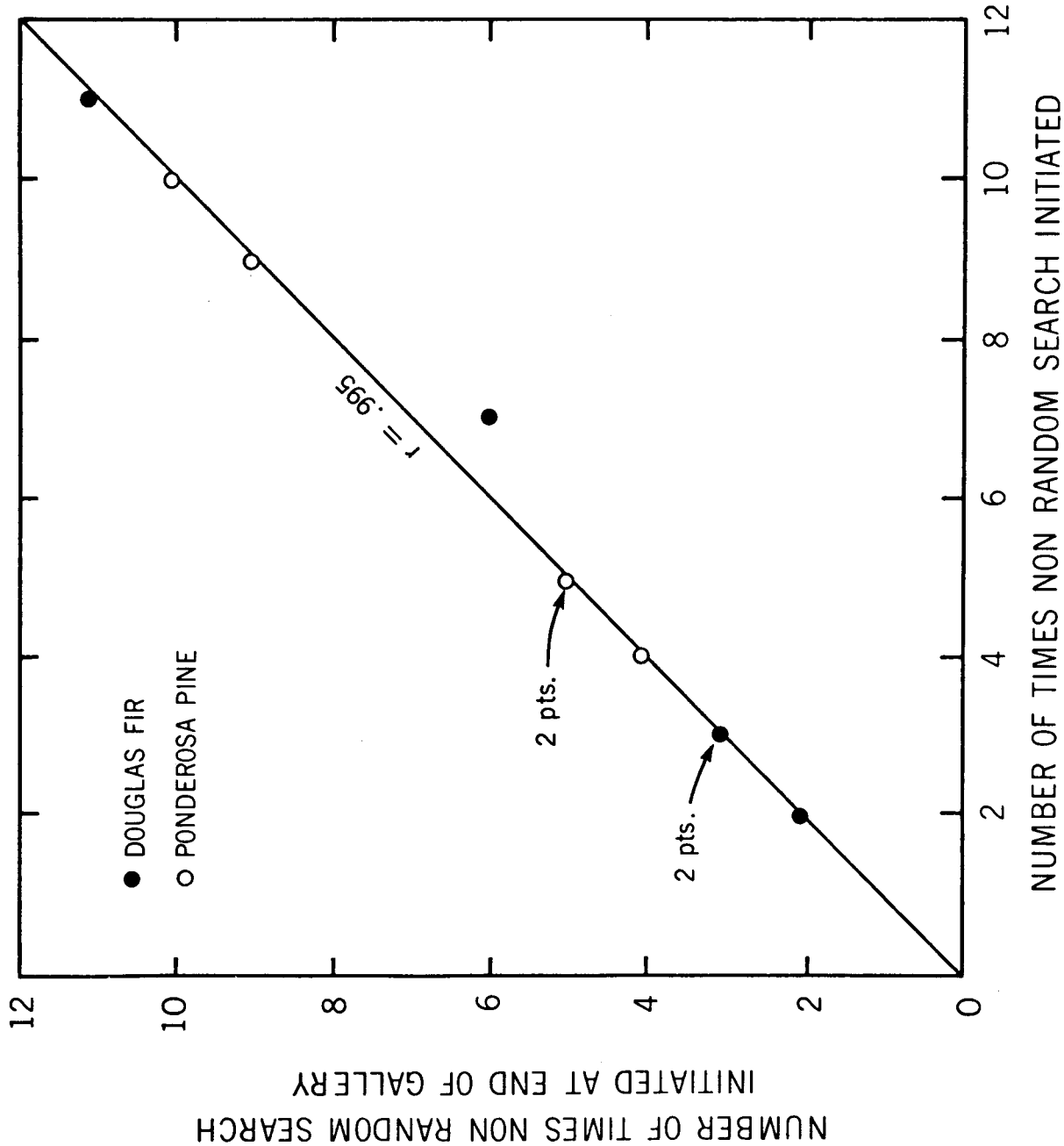

NUMBER OF TIMES END OF
GALLERY ENCOUNTERED FIRST TIME
12
10
8
6
4
2
0
2
4
6
8
10
12
14
16
18
19
DOUGLAS FIR
PONDEROSA PINE
combined
data
r=.8937
r=.5332
r=.3059
NUMBER OF TIMES NON RANDOM SEARCH INITIATED

Fig. 11 Correlation of the number of times nonrandom search is initiated with the number of times nonrandom search is initiated over the end of a larval gallery by five female C. brunneri per host tree in a 90-minute test period.


NUMBER OF TIMES NON RANDOM SEARCH INITIATED AT END OF GALLERY
NUMBER OF TIMES NON RANDOM SEARCH INITIATED
0
2
4
6
8
10
12
● DOUGLAS FIR
○ PONDEROSA PINE
r = .995
2 pts.
2 pts.

MECHANISMS OF HOST FINDING

The mechanisms by which a parasite locates its host may be elucidated by two approaches. One is to locate the site on the parasite of stimulus reception. The nature of a receptor organ may then reveal the type of stimulus perceived. The other approach is to identify as many types of stimuli as possible and, by various experiments or observations, to determine to which stimuli the parasite responds. Both approaches were followed in this study.

Fifty unmated female C. brunneri were treated as follows: 20 were antennectomized, 20 had approximately half of the 45 antennal segments removed from both antennae, and 10 were left with antennae intact. They were observed for two days to detect possible postoperative behavioral changes. On the second day males were introduced and all females copulated. Even the completely antennectomized females fed, mated, and flew, indicating that there was no apparent physiological or behavioral postoperative shock. On the third day, 10 females from each treatment were selected and exposed, one at a time, on an infested Douglas-fir log.

Antennectomized females failed to search while those with half or whole antennae were able to detect hosts and oviposit on them (Table VII). There was no significant difference between the time spent in each search pattern between normal and partially antennectomized females. Two conclusions can be made: (1) the antennae are necessary in host finding, (2) the sensilla used in host finding are present on both the distal and proximal regions of the antennae.

Table VII Effect of antennal amputations on search behavior of female C. brunneri on D. pseudotsugae in Douglas-fir. N=10 parasites, each tested for 90-minutes for each treatment.

Search Characteristic	Percent Time[a]		
	Fully Amputated	1/2 Antennae	Normal Antennae
Nonsearch	100	43.1	51.3
Randon Search	---	27.4	22.3
Nonrandom Search	---	20.7	18.0
Oviposition	---	8.8	8.4

[a] No significant difference (t-test, P>.50) between search patterns of insects with normal antennae and those with 1/2 antennae.

Possible Host Finding Stimuli

Many types of host detection systems could be used by a parasite of subcortical insects. Therefore, a systematic examination of possible host finding stimuli for C. brunneri was undertaken to investigate: sound and vibration produced by the host, parasite-produced sound, odor, temperature, and infrared radiation (IR). The role of magnetic fields was briefly investigated.

In exploratory experiments with artificial substrates such as paper and plastic, C. brunneri exhibited normal search patterns only on bark of infested trees. Therefore, all experiments were designed to provide the parasite with infested bark.

Sound and Vibration Produced by the Host

The host finding mechanism most frequently hypothesized for C. brunneri is to detect and orient to the sound produced by the host larva chewing on plant tissues (Ryan 1961).

Infested logs in the field and the laboratory were examined to determine the developmental stage of the parasitized host. These examinations disclosed a small number (estimated <1%) of parasitized D. pseudotsugae pupae which would not have been able to produce sound or vibrations to guide a searching parasite. As C. brunneri paralyses its host, thereby arresting development (Ryan and Rudinsky 1962), these hosts could not have transformed to the pupal stage following oviposition by the parasite.

Bolts of Douglas-fir (approximate dimensions: 0.5 m long, 30 cm in diameter, 1 cm bark thickness) were inoculated with 20 mating pairs of D. pseudotsugae. The resulting broods were allowed to develop to various

stages before the bolts were exposed to parasites. The response of two to three day-old female parasites was observed for 90 minutes on bolts with parent beetles only, first and second instar larvae, third to fourth instar larvae, emerging brood (predominantly callow adults as well as pupae), and uninfested bolts. Five replications were made for each of the five log treatments (i.e. five parasites for 90 minutes each per treatment for a total of 2,250 parasite-minutes of observation).

Logs containing D. pseudotsugae larvae stimulated C. brunneri females to pursue sustained, random and nonrandom search (Fig. 12). However, only bolts containing potential hosts (i.e. third to fourth instar larvae) stimulated the parasites to follow nonrandom search with oviposition (Fig. 12). The parasites were clearly able to discriminate between suitable host larvae and all other life stages of the host despite the fact that beetles in all other stages except pupae actively mine in the phloem tissue and could possibly produce detectable sounds and/or vibrations.

A 1 m-long bolt of Douglas-fir was inoculated with 20 pairs of D. pseudotsugae. After four weeks, when the larvae were predominantly third to fourth instar, the bolt was sawn into two equal lengths. One half was exposed to five, two to three-day-old female parasites for 90 minutes, and the number of ovipositions was recorded. The other half was frozen at approximately -50 C for two days, killing all larvae in the bolt. The frozen log was allowed to thaw at room temperature for two days and then exposed to parasites in an identical manner as the unfrozen log. After the test the two halves were debarked and the condition of the host larvae observed. Five replications were made using five different parasites per replication.

Dead (freeze killed) as well as live larvae were attacked by C. brunneri (Table VIII). There were 42 ovipositions in the unfrozen logs and 36 in the thawed logs. There was no significant difference between the number of ovipositions in either treatment. Examination of debarked logs after the treatment showed that all larvae in the thawed logs were dead. Some parasite eggs were recovered from decomposing larvae. Most of the larvae in the unfrozen control logs were alive.

The thickness of the bark, its sound carrying capacity, the presence of more than one sound-producing stage of Dendroctonus, and the presence of sound-producing subcortical insects other than those parasitized by Coeloides indicate that sound or vibration are not reliable and effective host finding stimuli. The presence of parasitized pupae, discrimination between sound-producing host stages, and oviposition on dead larvae provide sufficient evidence to reject the hypothesis that sound or vibrations are obligatory stimuli for host finding by C. brunneri.

Although sound or vibrations are not obligatory-host finding stimuli (Fig. 12, Table VIII), C. brunneri could use host-produced sound as a facultative stimulus. Therefore, efforts were made to detect host sound using a sensitive sound detection system.

A BSR stereophonic ceramic cartridge type C1 ST3, sensitive from five to 30 kilahertz (kHz), was connected to an amplifier and attached to the bark of a bolt of Douglas-fir infested with third to fourth instar D. pseudotsugae. A total of 150 locations were tested. No sound of larvae chewing was detected. The log was then debarked and the larvae were observed to be alive, vigorous and feeding. A bolt of Douglas-fir infested

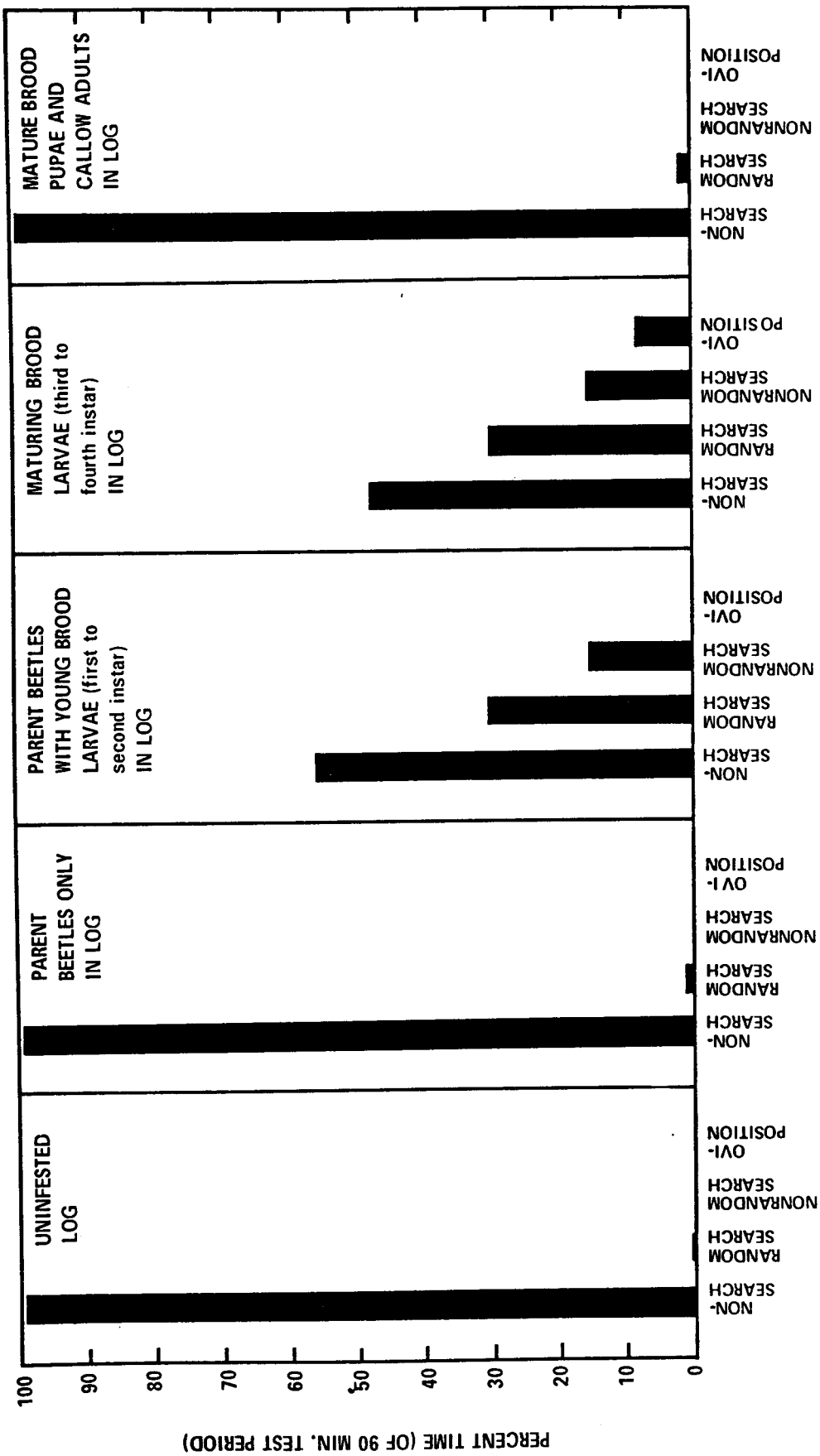
Table VIII Comparative ovipositional response by C. brunneri on live and freeze-killed D. pseudotsugae larvae in either half of 5 test logs.

Replicate	Ovipositions in half of test log not frozen [a]	Ovipositions in half of test log frozen, then thawed [a]
1	9	8
2	9	9
3	8	6
4	10	9
5	6	4
Mean ovipositions per log	8.4	7.2[b]

[a] Total number by five parasites.

[b] No significant difference between means (t Test, t = .068, P <.50).

Fig. 12 Percent of test time spent by C. brunneri females in various responses on uninfested logs and on bolts of Douglas-fir containing D. pseudotsugae in four life stages. Five replications (one replicate = one parasite for 90-minutes) for each treatment.


PERCENT TIME (OF 90 MIN. TEST PERIOD)
100
90
80
70
60
50
40
30
20
10
0
UNINFESTED LOG
PARENT BEETLES ONLY IN LOG
PARENT BEETLES WITH YOUNG BROOD LARVAE (first to second instar) IN LOG
MATURING BROOD LARVAE (third to fourth instar) IN LOG
MATURE BROOD PUPAE AND CALLOW ADULTS IN LOG
NON-SEARCH
RANDOM SEARCH
NONRANDOM SEARCH
OVI-POSITION
TYPE OF PARASITE RESPONSE

with adult bark beetles was also tested and their chewing and stridulation were detected with the cartridge.

The failure to detect the sound of chewing larvae, even though they were of the appropriate age and size for parasitism by C. brunneri, further indicates that sound could not be used as a host finding stimulus. Therefore, the hypothesis that C. brunneri uses host-produced sound or vibrations to detect its host (DeLeon 1935, Ryan and Rudinsky 1962) can be rejected.

Parasite-Produced Sound

Another possible host finding mechanism which might be employed by parasites of subcortical insects is to locate the hidden host by a type of sonar. Sound waves reaching a larva or its feeding chamber would presumably be reflected differently from those impinging upon phloem or xylem tissue.

A Bruel and Kjar (type 2204) impulse precision sound level meter with a type 4135 quarter inch condenser microphone, sensitive from 0 to 100 kHz was used to determine if any sound was produced by C. brunneri while searching an infested Douglas-fir bolt. Tests were run between 1:00 and 3:00 A.M. to minimize the amount of background noise. In 10 test-hours, there was no evidence of sound produced by searching C. brunneri females. Thus, it is unlikely that any type of sound detection is used in host finding by C. brunneri.

Magnetism

R. H. Wright (personal communication)[1] suggested that perception of or orientation within magnetic fields by a parasite such as C. brunneri

[1] British Columbia Research Council, Vancouver, B.C.

may be important in host finding. To test this suggestion five female C. brunneri were exposed individually for 90 minutes to an infested bolt of Douglas-fir. A 2.54 cm magnet used as a magnetic stirrer was held directly over each searching parasite at a height of one cm. All parasites continued their normal search patterns and oviposited on host larvae. There was no observable effect of the magnet, and it is concluded that magnetic fields have no association with host finding mechanisms in C. brunneri.

Odor

Creation of an experimental system in which candidate odors would be released under bark was considered unlikely to produce meaningful results. Odor perception by the parasite could potentially be confused by the natural odors from the bark itself. In addition, if odor were to diffuse through approximately 4 to 6 mm of bark the resulting odor field might be too large, and, therefore, would not provide a sufficiently precise mechanism of host location for C. brunneri. It has been hypothesized that the siricid parasite R. persuasoria locates its host in part by detection of frass and fungus odor diffusing through the bark and wood from the host galleries (Spradbery 1970a). Unlike C. brunneri, R. persuasoria locates a host which is more spacially removed from its neighbors than bark beetle larvae. Moreover, it successfully parasitizes less than 15% of the hosts it detects (Spradbery 1970b), and thus does not utilize a precise method of host finding.

Nevertheless, it was decided to test the hypothesis that C. brunneri utilizes odor to find its host. Since saturation of an atmosphere with moth pheromone odor will prevent successful orientation to natural pheromone

sources (Gaston, Shorey and Saario 1967) a similar disruption of searching success could occur if C. brunneri uses a specific odor in locating its host.

A cage (.5 X .5 X 1 m) was sealed with plastic wrap and made air tight. An infested bolt of Douglas-fir which was attractive to female parasites was debarked in a room held at 0 C. All bark, bark beetle larvae, bark beetle frass, and uninfested phloem tissue were removed. The bolt was washed with distilled water and the resulting mixture of host tree and host insect material and wash was placed in the airtight cage. The odor in the cage was extremely strong to the human nose. Another infested log was placed on top of the debris. Five female C. brunneri were released in the sealed cage and were observed for 90 minutes. Three replications of the test were made using fresh host material and searching parasites each time.

In spite of the very strong odor of host tree and insect material from another attractive log the parasites searched normally and oviposited successfully on host larvae (Table IX). Under saturated odor conditions, the parasite could respond in one of two ways. Sensory neurons could adapt and there would be no ovipositional response to a given stimulus. Alternatively, if there were a response, it would be confused and non-directional, and oviposition would occur at sites without hosts. However, ovipositions did occur and dissection of the bark showed that all ovipositions made on the log were made over host larvae (Table IX).

The results of the saturated air experiment strongly suggest that C. brunneri does not utilize odor as a host-finding stimulus. However, the odor of an infested host tree could be a major stimulus in host habitat

Table IX Host finding by female C. brunneri in 90-minute tests on infested Douglas-fir logs in an air-tight cage saturated with the odor of an infested log.

Observations	Experimental: Logs in air saturated with odor of infested log				Control log in clear air
	Log 1	Log 2	Log 3	$\bar{X}$	
No. females tested	5	5	5	5	5
No. females observed ovipositing	5	5	5	5	5
No. ovipositions per female	1.8	1.6	1.8	1.73	1.8
No. oviposition sites (marked on outer bark)	9	8	9	8.67	9
No. successful ovipositions (verified by dissections after one week)	9	8	9	8.67	9

finding (Doutt 1965). It is possible that the odor of an infested host tree is the stimulus which induces the commencement of random search, but only if logs infested with bark beetle larvae have a different odor from logs infested with other stages of beetles.

Temperature and Infrared Radiation

Two factors which remain as possible stimuli to host finding in C. brunneri are temperature and IR. Preliminary experiments were designed to determine if there is a range of temperature at which successful searching can occur and whether there is a detectable temperature difference associated with host larvae.

Five bolts of infested Douglas-fir, previously shown to be attractive to female C. brunneri, were exposed for 45 - 60 minutes in a horizontal postion to solar radiation (ambient temperature 32 C), until a constant bark temperature was reached. Temperature readings were made on the bark surface of four quadrants: upper, lower, shaded side, and exposed side. Prior to testing parasites on the logs, a number from one to four was assigned to the four positions on which any one quadrant of the horizontal log could be placed. On each of the five test logs, the hottest surface (exposed upper quadrant) was placed according to the number taken from a single random draw of the four numbers (e.g. a draw of 2 would mean turning the host surface until it was facing downward). The bolts were placed horizontally on a stand to allow a 3 cm space beneath the log. One female C. brunneri was released on each test log and and its behavior was observed for 90 minutes. The cage in which the logs were placed for this test had relatively diffuse light.

The parasites searched over all portions of the logs except the very hot areas previously exposed to direct solar radiation. Only when these hot bark areas had cooled down to about 35 C would the female parasites search and oviposit on them, whereas search and oviposition occurred on the cooler areas from the beginning of the test (Table X).

Ryan (1962) observed that C. brunneri is photonegative during search, and avoids the upper surface of horizontal logs. In this and preceding tests the C. brunneri did not respond to light once a search pattern was started. In addition, search and oviposition occurred on all surfaces of the log (Table X). These data indicate that C. brunneri is thermosensitive, not photonegative. Between 15 and 35 C there is normal search behavior and oviposition, but above and presumably below this range, nonsearch (basking or cold stupor) predominates.

Possibly, at high or low temperature extremes, C. brunneri females are unable to search for and find their host, not because they are immobilized by these temperatures, but because they are unable to detect their host. If a parasite was detecting a difference in temperature or IR associated with a host, the difference might be obliterated at fairly high or low bark temperatures.

Three bolts of Douglas-fir infested with maturing brood of D. pseudotsugae were exposed to 15 female parasites, five to each log. The parasites were interrupted in the act of oviposition and the oviposition site marked. A telethermometer (Yellow Springs Instrument Co. model no. 43 TD, with a range of 0 to 50 C) and a thermistor (model no. 421, 1/4 inch diameter) were used to measure the temperature at each oviposition site and at one antennal length in each of four directions surrounding it. The bolts were

Table X Oviposition by C. brunneri and temperature decline on four surfaces of logs after exposure to solar radiation. Mean of five logs with one parasite female per log.

Region of Log	Time in Minutes	Bark Surface Temperature	Number of ovipositions in preceding 15-minutes Total	Mean per Female
Exposed Upper Quadrant	0	39.5	-	
	15	39	-	
	30	37.5	-	
	45	36.5	-	
	60	35.5	-	
	75	35	5	1
	90	34	10	2
Shaded Lower Quadrant	0	31	-	
	15	31	5	1
	30	31	10	2
	45	31	-	
	60	31	-	
	75	31	5	1
	90	31	5	1
Exposed Side Quadrant	0	39	-	
	15	38.5	-	
	30	37.5	-	
	45	36.5	-	
	60	36	-	
	75	35.5	-	
	90	34	10	2
Shaded Side Quadrant	0	35	-	-
	15	35	5	1
	30	34	5	1
	45	34	5	1
	60	33.5	-	-
	75	33	10	2
	90	32	5	1

debarked and the marked oviposition sites were correlated with the presence of host larvae.

The temperature at each of the 15 oviposition sites was the highest of the five temperatures measured. Moreover, the mean difference in temperature between the oviposition site and the other four sites was significantly higher (t-test, P= .01) than the mean difference in temperature between one of the other four sites and any of the other three (Fig. 13). A host larva was found under each oviposition site. Therefore, a detectable temperature difference does exist and the C. brunneri apparently selects these 'hotspots'.

A 30 cm bolt of uninfested Douglas-fir was split in half lengthwise. Twelve holes, 1 cm diameter, arranged every 4 cm in two parallel rows of six holes each, were bored from the flat side of the split log to the cambial layer (approximately 6 mm from the surface of the bark). The rows were 4 cm apart and the resulting pattern resembled the distribution of D. pseudotsugae larvae at a stage susceptible to C. brunneri. The holes were evenly spaced to ensure that artificially created 'hotspots' would be discrete and not fuse into a larger warm area. Into each hole was placed a resistance wire probe constructed of #30 Nichrome wire rolled into a 1 X .6 cm coil, simulating the size of a susceptible D. pseudotsugae larva (Fig. 14). All the probes were connected to a variable transformer. Sufficient voltage (1.7 volts) was passed through the probe to generate a 'hotspot' on the outer surface of the bark over each probe (Table XI). The bark surface temperatures over the probes and at points midway between them were recorded with the thermistor used in the preceding experiments.

Fig. 13 Comparison of the surface temperature differences between the oviposition site of C. brunneri and four sites one antennal length away on Douglas-fir bolts infested with D. pseudotsugae. Mean of fifteen measurements taken at fifteen different oviposition sites.

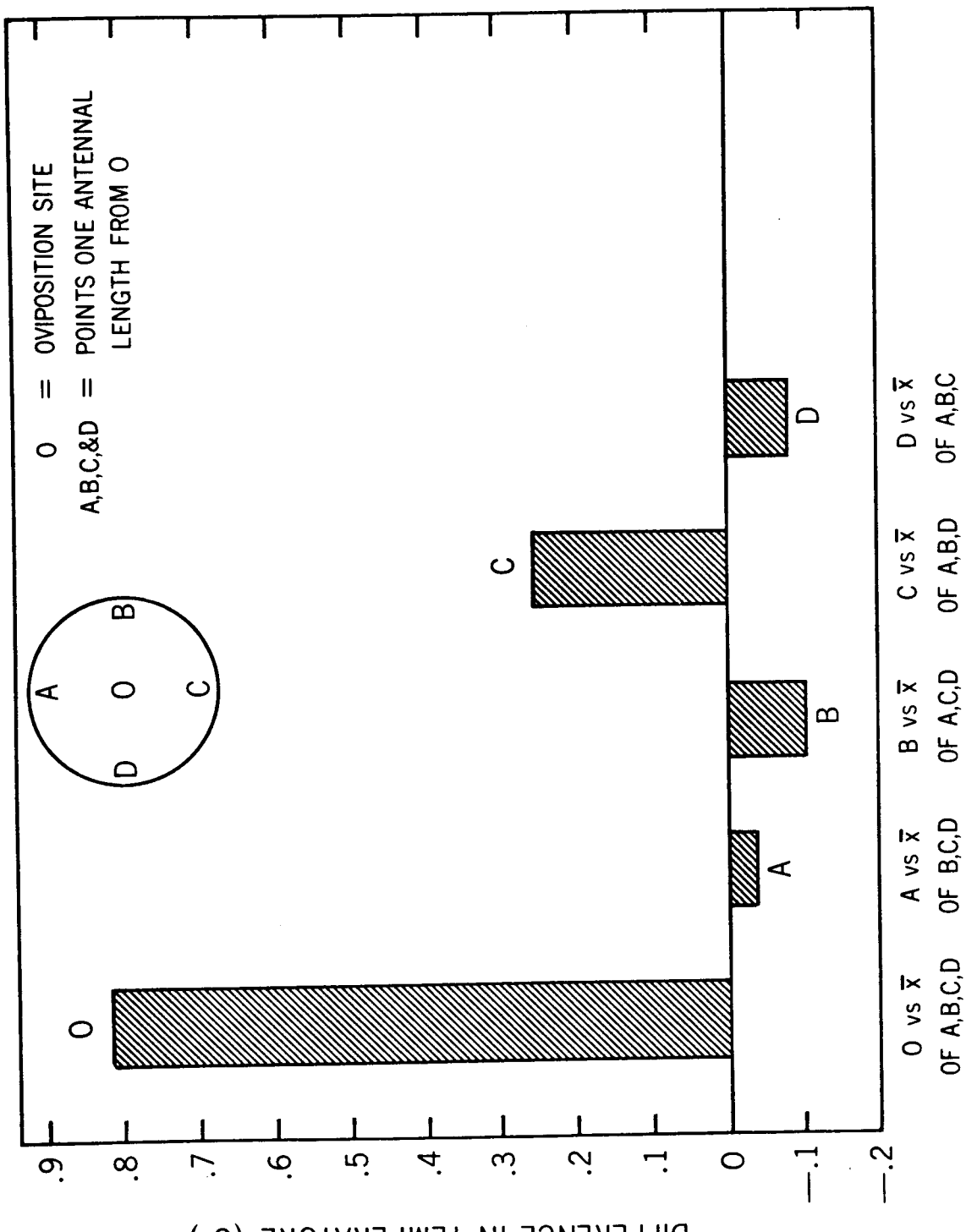

DIFFERENCE IN TEMPERATURE (C°)
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
—.1
—.2
O = OVIPOSITION SITE
A,B,C,&D = POINTS ONE ANTENNAL LENGTH FROM O
A
B
C
D
O
O
A
B
C
D
O vs x̄ OF A,B,C,D
A vs x̄ OF B,C,D
B vs x̄ OF A,C,D
C vs x̄ OF A,B,D
D vs x̄ OF A,B,C

Fig. 14 Apparatus used in the heated resistance wire experiment. Portion of the test log is cut away to show arrangement of the holes and the placement of resistance wire coil.

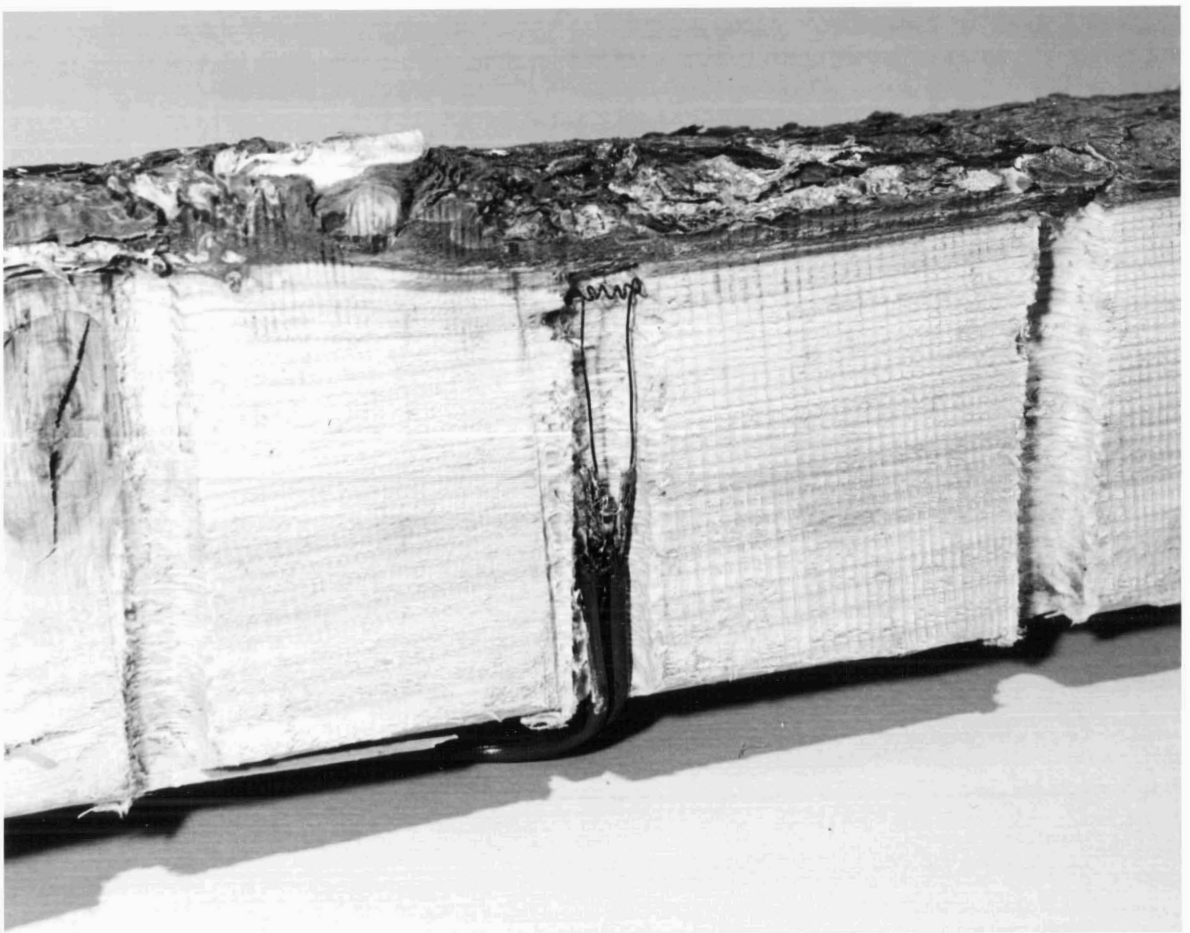


Table XI Response of five female C. brunneri to artificial 'hotspots' created by resistance wire probes in the bark of uninfested Douglas-fir. Test duration is 90-minutes per parasite.

Parasite	Time of oviposition Min.	Sec.	Temperature (C)[a] oviposition site (O)	A	B	C	D	$\bar{X}$ difference O VS $\bar{X}$ of A, B, C, D.
1	8	14	31.5	28.5	28.5	29.0	28.0	3.000
	31	25	33.5	30.0	31.0	29.5	30.5	3.250
2	11	31	32.5	30.5	31.0	31.0	31.0	1.625
	53	57	34.0	32.5	32.0	32.0	32.5	1.750
3	20	05	34.0	32.5	33.0	33.0	33.5	1.000
	72	30	34.5	33.5	33.5	33.5	33.5	1.000
4	13	58	27.5	25.5	26.0	26.0	25.0	1.875
	67	11	33.5	31.0	31.0	32.0	31.0	2.250
5	31	16	31.0	28.5	29.0	28.5	28.5	2.375
	84	42	34.5	31.5	31.0	30.5	31.5	2.375

[a] Oviposition site (O) and sites A, B, C, and D identical to description in Fig. 14.

The log with artificial 'hotspots' was placed in a 35 X 15 X 15 cm cage and a single mated female parasite was released into the cage through a cloth sleeve in the cage door. A different parasite was used in each of the five 90-minute experimental and four control replicates. In the control a parasite was released on the log without heating up the resistance wires. Three of the control parasites were later tested for ability to respond to artificial 'hotspots'.

Each experimental insect oviposited twice in the 90-minute period (Table XI). Each oviposition was made over a resistance wire probe. Two ovipositions were made over the same probe by different parasites. In a preliminary experiment with larger resistance wires, oviposition was recorded by four additional parasites within a 20-minute period before the 'hotspots' were obliterated by an uncontrollable, general warming of the bark.

All parasites showed the four phases of host-finding behavior. The duration of the initial random search phase was shorter than on naturally infested bark and the nonrandom search more intense with numerous turns over the 'hotspots'. No eggs were laid since oviposition ended abruptly as the penetrating ovipositor neared the hot probe. In each case there was a significantly warmer bark temperature at the oviposition site than at one antennal length's distance (Table X). No oviposition occurred in the control runs. All three control parasites tested for response ability after the 90-minute control period were observed to search and begin oviposition over the probes.

In the absence of all other stimuli, the presence of a 'hotspot' on the bark apparently stimulated C. brunneri to oviposit, the final phase of host finding. Therefore, it can be concluded from these data that C. brunneri finds its host by contact or radiant (IR) temperature detection.

DESCRIPTION OF C. BRUNNERI ANTENNAE AND A UNIQUE SENSILLUM PLACODEUM

The external surface and cuticular structure of the antennae of female C. brunneri was examined by light and scanning electron microscopy. For light microscopy, antennae were removed from living insects, cleared in 10% KOH and mounted on microscope slides. Antennae were prepared for scanning microscopy by vacuum coating with gold. Elucidation of the internal cuticular structure of the antennae was made possible by slicing an antennal section in half longitudinally and digesting out the cellular material with 10% KOH, before the gold coating was applied. The antenna was examined in a Cambridge Stereoscan II Microscope.

In preparation for light and transmission electron microscopy, antennae were removed from the live insect, cut into three- or four-segment lengths in cold 3% phosphate buffered glutaraldehyde at pH 7.2, fixed in same for 24 hours and post fixed in 2% phosphate buffered osmium tetroxide at pH 7.2 for two hours. The tissue was then dehydrated through ethanol and propylene oxide before embedding in Araldite.

Sections were cut on a Reichert Om U2 microtome. Thin sections were mounted on 200 mesh copper grids, stained with uranyl acetate and lead citrate and examined on an RCA EMU-3H microscope. Light microscope sections were stained with methylene blue and mounted in Permount. Unstained sections were examined with a phase contrast microscope.

A light microscope survey of the antennal surface of 100 female and 100 male C. brunneri revealed that there were only three dominant types

of sensilla present: (1) sensilla chaetica, (2) thin-walled "chemoreceptor" hairs as described by Slifer (1970), and (3) an extremely elongated type of sensillum placodeum. The chemoreceptor sensilla were present on the apical and first 10-20 segments of the antennae. There were only one to three per segment, the apical segment possessing three. Many sensilla chaetica were present on the apical and all other segments. They appear as guard setae around the placoid sensilla (15-24 per sensillum). The placoid sensilla are found on all but the basal four segments of the antennae. The apical segment has four to 10 plate organs with a mean of six. The second and third segments have from eight to 12 per segment with a mean of 10. The fourth to the 44th segments have 13-15 plate organs with a mean of 15. No other sensilla were observed either in light or electron microscope sections or in whole mounts of the antennae.

The cuticular structure of the sensilla placodea was reconstructed from various preparations and sections and is shown in a semidiagrammatic representation in Fig. 15. The morphology is unique in that the placoid sensillum of C. brunneri has two prominent lamellae suspended internally from the dome of the sensillum. Consequently, this sensillum does not resemble the classic placoid sensilla of Slifer (1960, 1969), Melin (1941), Snodgrass (1935), or Bullock and Horridge (1965). Lamellae in a placoid sensillum have been noted in only one other insect, also a hymenopteran, by Ruland (1888).

The plate organs are evenly distributed around the entire periphery of each antennal segment (Fig. 16). They extend the full length of the segment but are not connected between segments (Figs. 17-19). The dome is surrounded on the outside by a furrow-like groove and guard setae (Fig. 18).

Fig. 15 Semidiagrammatic representation of the placoid sensillum from the antennae of Coeloides brunneri showing the cuticular structure in various cross sections.

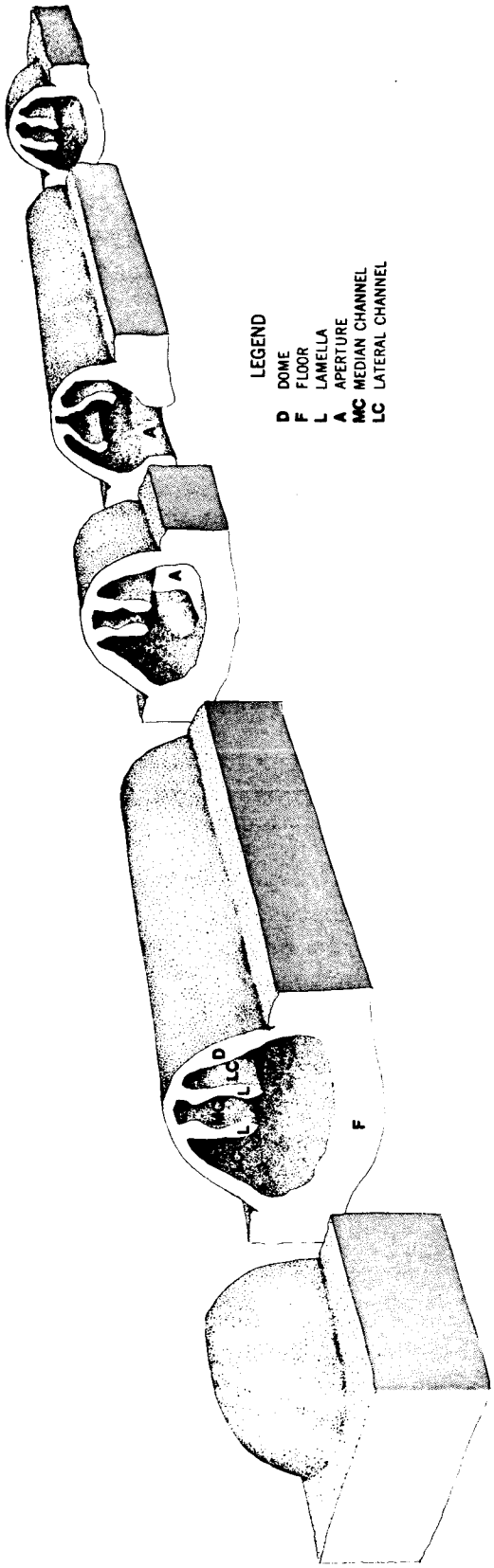
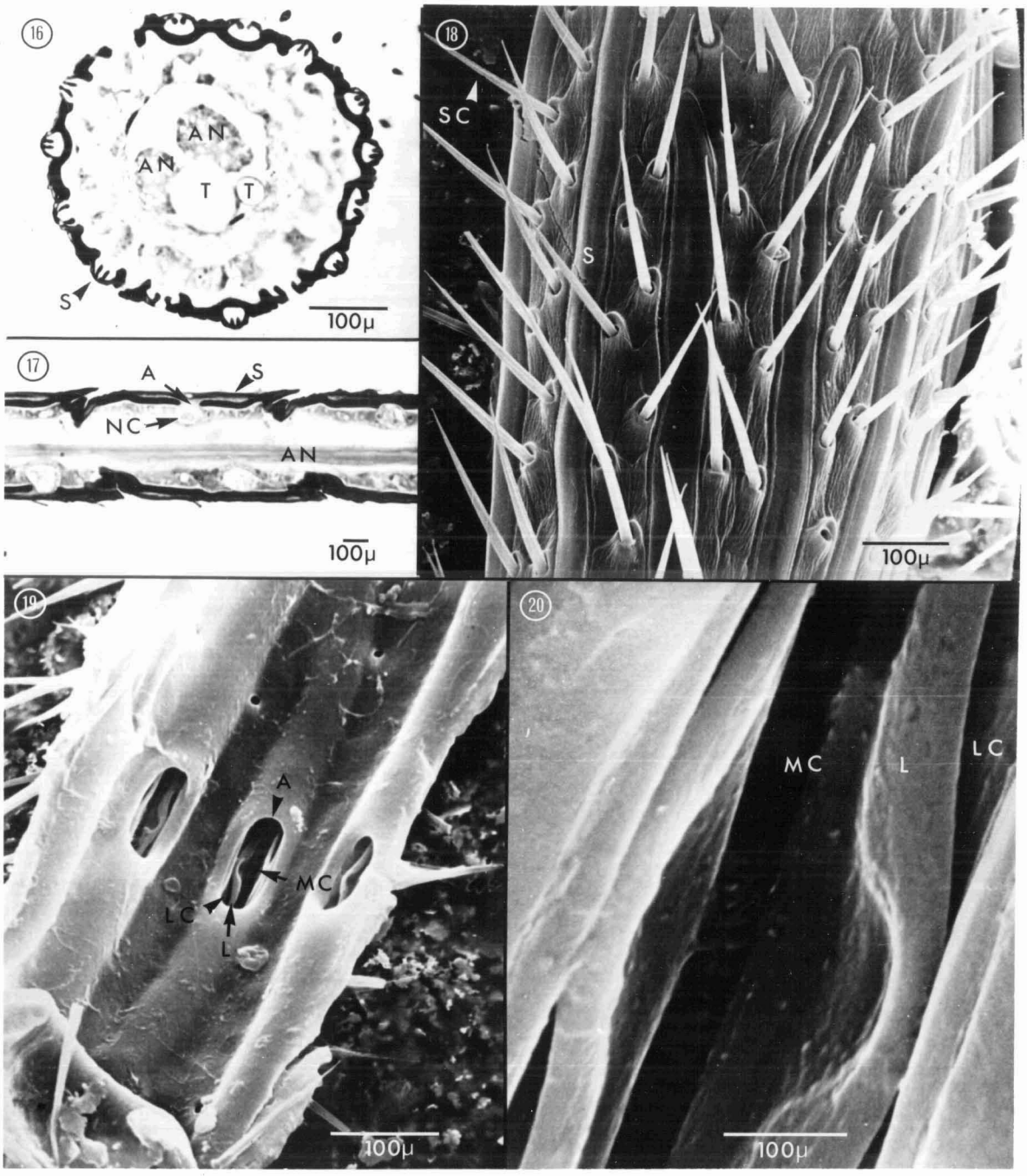

LEGEND
D DOME
F FLOOR
L LAMELLA
A APERTURE
MC MEDIAN CHANNEL
LC LATERAL CHANNEL
D
L
LC
L
F
A
A
A

Fig. 16 Cross section of antennal segment through the midregion showing arrangement of the placoid sensilla (S) around the entire periphery, and in the lumen, the double Antennal Nerve (AN) and Tracheae (T).

Fig. 17 Longitudinal section of antennal segment showing placement of the placoid sensilla (S) within the segment, the Aperture (A) in the cuticular floor, the location of the sensory Nerve Cell Bodies (NC) below the cuticular floor, and the Antennal Nerve (AN).

Fig. 18 Integument of the third antennal segment from the distal end showing the dome-shaped placoid sensilla (S) surrounded by a furrow-like indentation and by Sensilla Chaetica (SC). The crack in the segment and the collapse of the median channel in one plate organ are artifacts of preparation.

Fig. 19-20 Scanning electron micrographs of the cuticular structure on the inside of an antennal segment showing the Aperture (A) into the sensillum, the pouch of the median channel and the Lamellae (L). Note small apertures leading to the guard setae (Fig. 19) and interior ridges on the dome above the Median Channel (MC) (Fig. 20). Lateral Channel (LC).


16
AN
AN
T T
S
100µ
17
A
S
NC
AN
100µ
18
SC
S
100µ
19
A
MC
LC
L
100µ
20
MC
L
LC
100µ

The cuticle on the inside of an antennal segment is perforated by the apertures into the plate organ through which the lamellae are clearly visible (Figs. 19-20). The lamellae diverge to form a "pouch" just above the aperture. The plate organ is tubular in shape as evidenced by swelling on the inside of the antennal segment (Fig. 19). On the interior surface of the dome at the apex of the median channel and extending throughout the length of the sensillum are small ridges running perpendicular to the long axis of the sensillum (Fig. 20). The function of these ridges is unclear.

Measurements of the placoid sensilla taken from whole mounts of the antennae from 15 female C. brunneri shows a relatively consistent width but some variation in length of the sensillum (Table XII). On all but the apical segment each of the placoid sensilla extend the full length of the antennal segment. The average sensillum is 106 μ long and 7.1 μ wide. Measurements taken from cross sections of 25 sensilla in the midregion of the antennae show variations in the size of the sensillum (Table XIII).

In whole mounts of the antennae there are three areas of transluscence within the plate organ. The largest is produced by the transmission of light through the aperture. The second largest is light passing through the lamellar pouch in the median channel just above the aperture, and the smallest region is caused by light passing through the median channel throughout the entire length of the sensillum. The measurements of these regions are given in Table XIV. These regions are important because through them pass the sensory dendrites and their branches. While the gross dimensions of the sensilla are somewhat variable particularly in the apical three segments, these areas through which the neural material passes up through and into the sensillum are of relatively constant dimensions segment to segment (Table XIV). Therefore, any sensory function which would be depend-

Table XII Measurements of sensilla placodea on 15 female C. brunneri taken from whole mount of the antennae.[a]

Segment	Number sensilla measured	Length in microns range	Length in microns mean	Width in microns range	Width in microns mean
Apical	114	74-112	102	6-13	7.7
2	178	79-101	93.5	6-9	7.3
3	180	84-105	95.7	5-8	7.0
20	225	94-123	105.1	5-8	6.9
40	225	126-143	136.3	5-9	7.0

[a] Measurements are from all sensilla placodea on the segment examined.

Table XIII Measurements of the inner cuticular structures of 375 sensilla placodea taken from cross sections of the antennae of female C. brunneri.[a]

Structure measured	Measurement in microns Range	Mean
Floor width	17.7-22.8	18.2
Sensillum height	11.4-17.4	14.4
Lamella height	4.5- 7.2	5.4
Width of Apex of median channel	2.4- 6.6	3.5
Width of Middle of median channel	1.8- 7.8	3.3
Width of Bottom of median channel	2.4- 8.1	3.3
Lamella thickness	2.7- 6.7	3.5

[a] Figure 15 illustrates the inner cuticular structures measured.

Table XIV Measurements of the cuticular structures of 1246 placoid sensilla taken from whole mounts of the antennae of 25 female C. brunneri.

	Aperture				Lamellar Pouch				Median Channel	
	Length in microns		Width in microns		Length in microns		Width in microns		With in microns	
Segment	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
1	17.3-29.9	22.1	6.1-8.9	7.7	4.7-10.3	7.2	2.8-3.2	2.9	2.3-2.5	2.3
2	22.0-23.4	22.9	6.5-8.4	7.6	7.9- 8.1	7.9	2.8-3.3	2.9	2.3-2.8	2.4
3	18.7-20.6	19.3	7.0-7.9	7.6	5.6- 7.0	6.1	2.8-3.3	2.9	1.8-2.3	2.0
20	18.2-20.6	20.8	6.5-8.9	7.5	7.5- 8.4	7.7	2.8-3.3	3.1	1.9-2.3	2.2
40	16.4-22.5	19.9	7.0-7.5	7.4	7.5- 8.4	7.8	2.8-3.8	3.4	1.9-2.3	2.2

ent on constant dimensions of cuticular structures would not be influenced by the location on the antenna or the gross dimensions of a particular sensillum.

The dendrites of the sensory neurons enter the sensillum only through the aperture (Figs. 15-17, 19-20). A tormogen cell lines all of the inner surfaces of the sensillum except the apical 2/3 of the median channel. This cell completely fills the lateral channels (Fig. 21). The trichogen cell lies immediately below the lamellae and possesses microvilli which apparently secrete an acellular matrix into the median channel (Fig. 21). An acellular matrix, probably also produced by the trichogen cell, fills the remainder of the sensillum.

In the region of the aperture the dendrites pass up into the median channel (Figs. 22, 24) and once there progressively branch into fibers approximately .09 μ in diameter, each containing a neurotubule. These dendritic branches fill the apical 2/3 of the channel (Figs. 21, 23).

The structure of the plate organ neurons is similar to previously described insect sensory neurons (Slifer 1960, Melin 1941). The nerve cell body, lying below the cuticle adjacent to one of the two antennal nerves (Fig. 17), produces a dendritic process (Fig. 25) which passes towards the plate organ (Figs. 22, 24, 26). The dendritic process forms the ciliary rootlets, proximal and distal basal bodies and the ciliary collar (Fig. 26). The ciliary region of the dendrites is enclosed in a cuticular sheath. Within each dendrite are numerous neurotubules. The entire ciliary region is surrounded by the microvilli of the trichogen cell (Fig. 26). A vacuole is present in the ascending bundle of dendritic

Fig. 21 Transmission electron micrograph of cross section of placoid sensillum at a point some distance from aperture. The Trichogen Cell (Tr), Tormogen Cell (To), Dendritic Branches (DB), Microvilli (Mv), Acellular Matrix (Ma) and cuticular structures, Dome (D), Lamellae (L), and Floor (F), are shown.

Fig. 22-23 Cross sections of sensillum through the center of the Ciliary Region (C). Basal bodies (B^1 and B^2) of the Dendritic Processes (DP) lie below the aperture, before the dendrites enter the median channel (Fig. 22). Fig. 23, dendrites within median channel, showing Cuticular Sheath (CS) and dendritic branching with various numbers of Neurotubules (Nt) in the Dendritic Branches (DB). Microvilli (Mv), Tormogen Cell (To), Dome (D), Lamellae (L).

Fig. 24 Longitudinal section of sensillum slightly off center in the region of the aperture. Lateral portion of one of the Lamellae (L) in the pouch region has been removed in sectioning. The Basal Bodies (B) of the Dendritic Process (DP) lie just below the aperture in the floor of the sensillum. Ciliary Region (C), Ciliary Collar (CC), Dome (D).

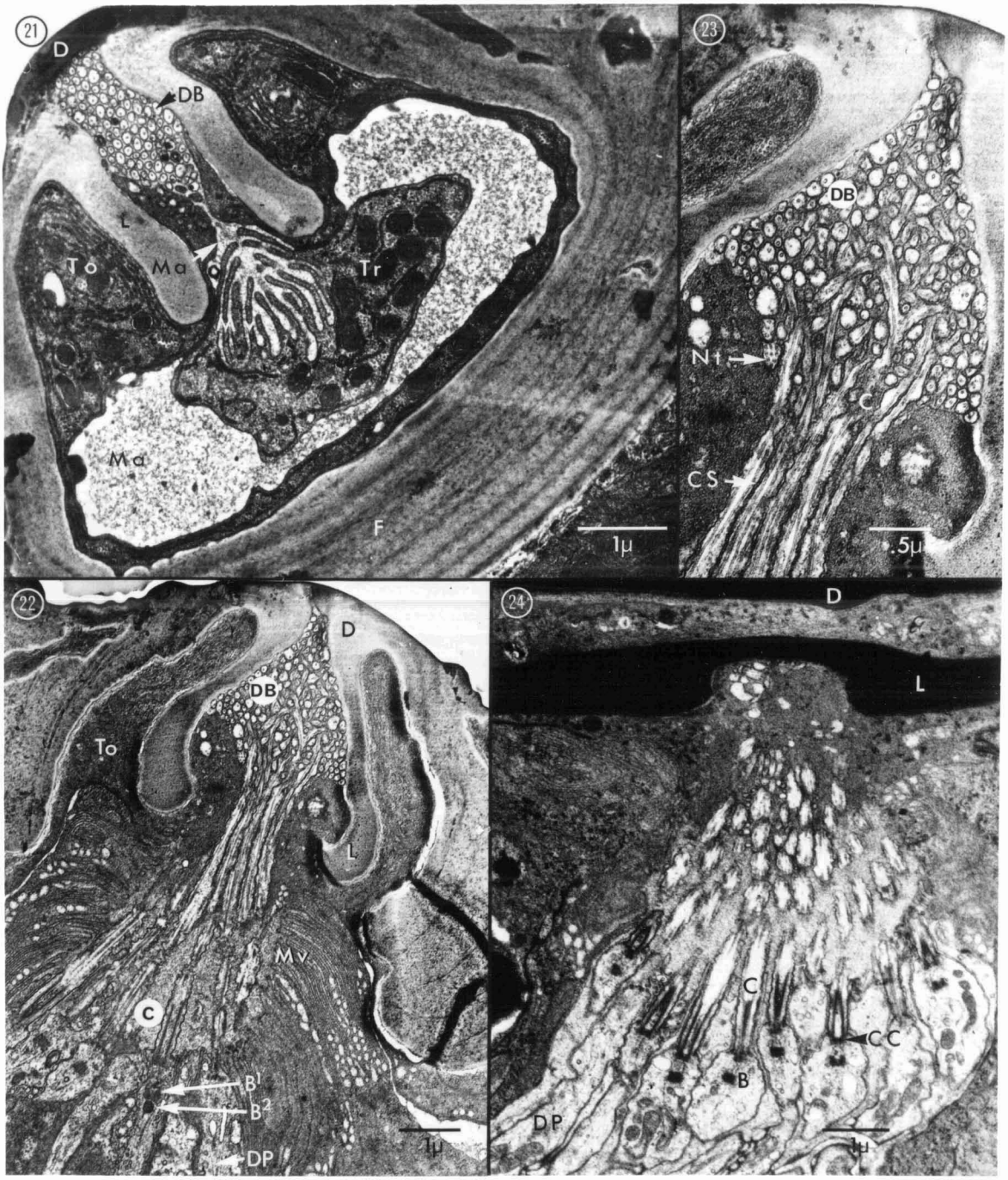

21
D
DB
L
To
Ma
Tr
Ma
F
1μ
23
DB
Nt
C
CS
.5μ
22
D
DB
To
L
Mv
C
B1
B2
DP
1μ
24
D
L
C
CC
B
DP
1μ

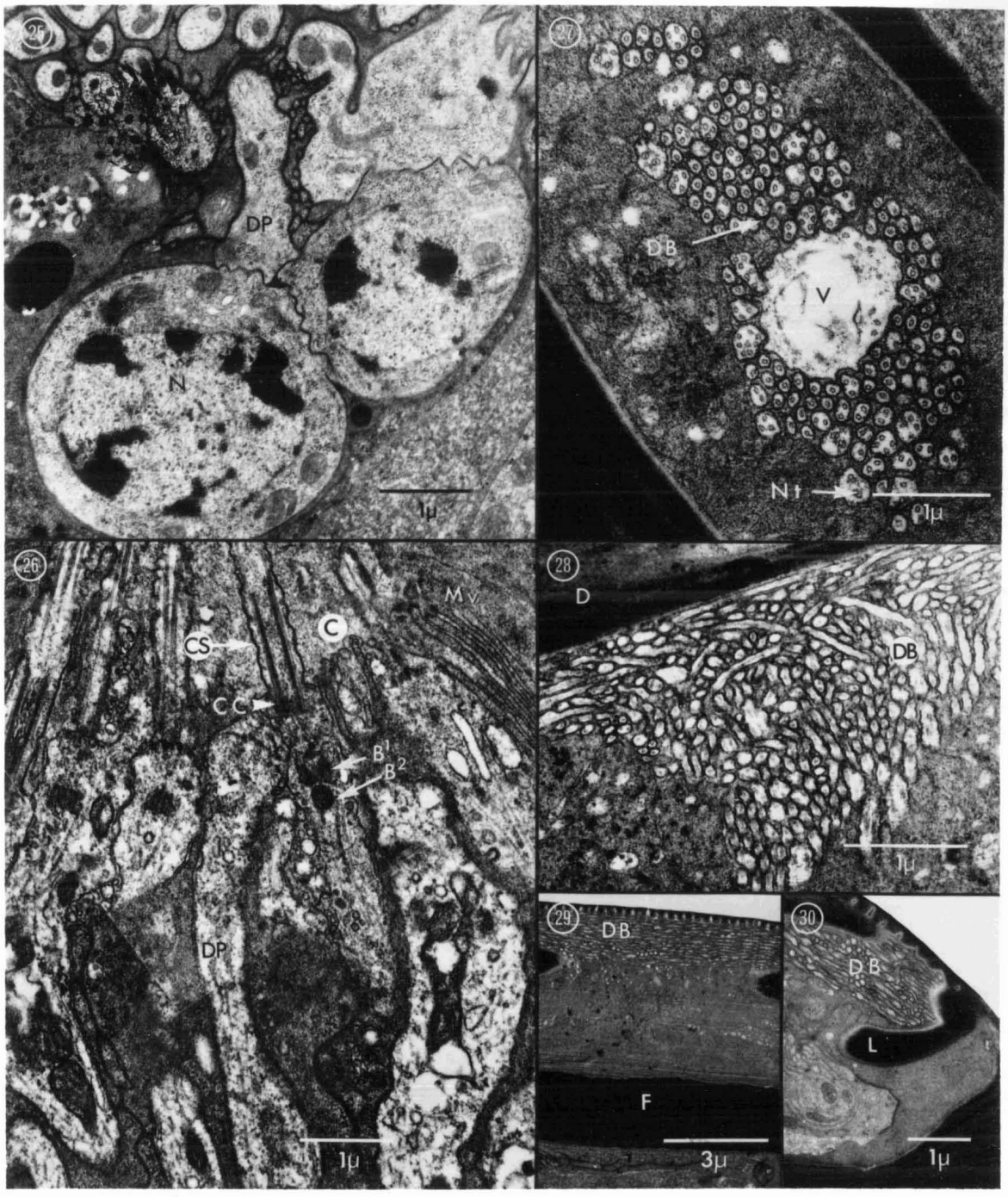
Fig. 25 Longitudinal section showing nerve cell body forming the Dendritic Process (DP). Nucleus (N).

Fig. 26 Cross sections showing the structure of the dendrite in the basal body and Ciliary Regions (C). Dendritic Process (DP), Distal (B^1) and Proximal (B^2) Basal Bodies, Ciliary Collar (CC), Cuticular Sheath (CS), Microvilli (Mv).

Fig. 27 Saggital section of sensillum demonstrating the presence of a Vacuole (V) in the ascending bundle of Dendritic Branches (DB) in the median channel. Varying number of Neurotubules (Nt) is evidence of dendritic branching.

Fig. 28 Longitudinal section in the middle of the median channel, directly above the aperture showing Dendritic Branches (DB) reaching the inner surface of the cuticle at the top of the Dome (D) and orientating longitudinally in the median channel.

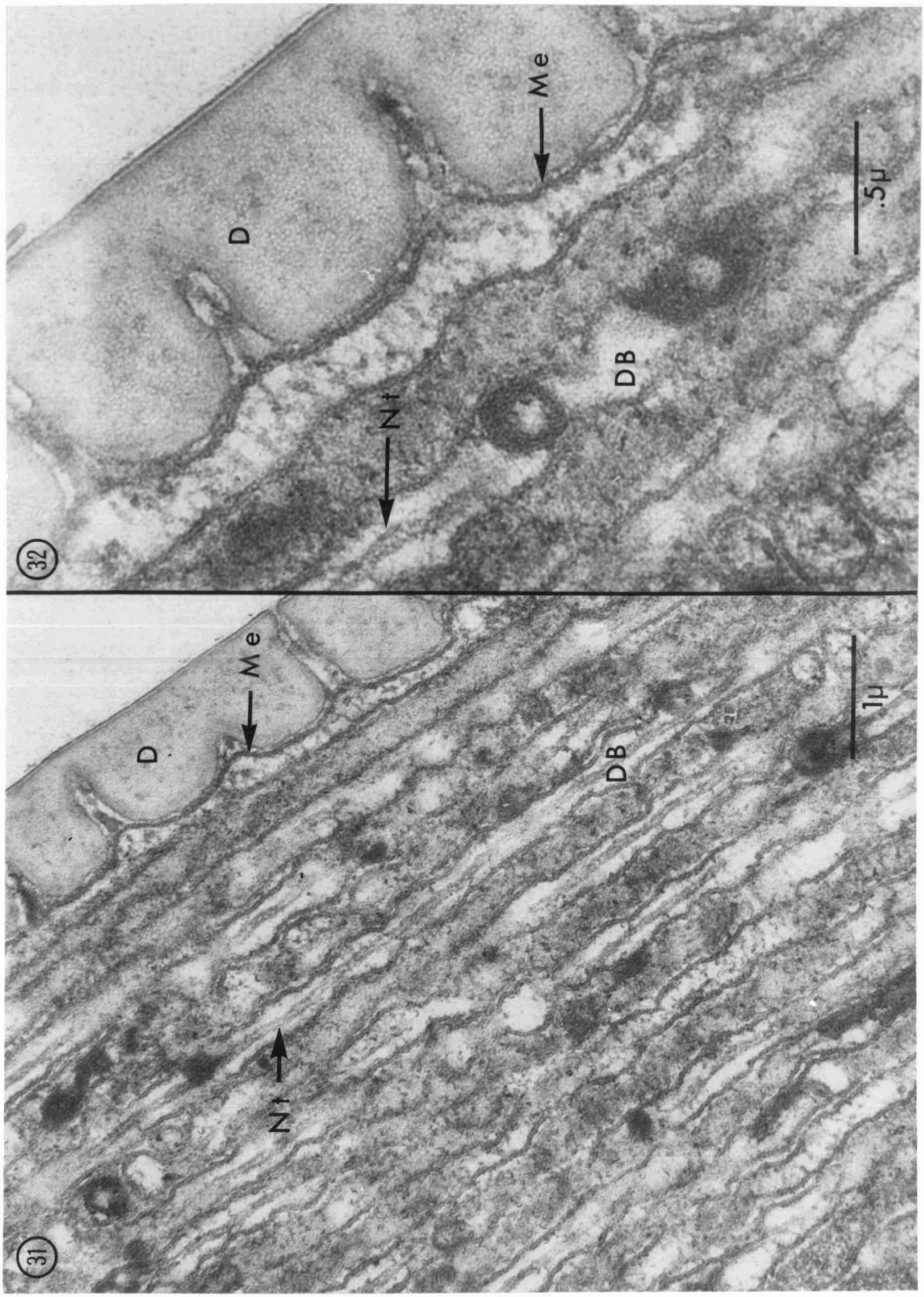
Fig. 29-30 Longitudinal sections showing Dendritic Branches (DB) reaching the inner surface of the cuticle at the top in the median channel extending further down the length of the sensillum (Fig. 29), eventually reaching the extreme end (Fig. 30). Note ridges (see Fig. 20) in the cuticle of the median channel. Floor (F), Lamellae (L).


25
C
DP
N
1μ
27
DB
V
Nt
1μ
26
MV
CS
C
CC
B1
B2
DP
1μ
28
D
DB
1μ
29
DB
F
3μ
30
DB
L
1μ

branches within the median channel directly above the aperture (Fig. 27).

Once the dendritic branches enter the upper 2/3 of the median channel they begin to turn and orient along the length of the median channel (Fig. 28), filling the entire median channel to both ends of the sensillum (Figs. 29-30). The dendritic branches reach the inner surface of the one-micron-thick cuticular dome (Figs. 31-32). Unlike many verified olfactory receptors (Slifer 1970, Steinbrecht and Müller 1971) there is no expansion of the pores in the cuticle to form a chamber, nor are there the characteristic dendritic filaments extending into the .16μ wide pore lumen. Moreover, the sensillum did not take up crystal violet dye (Slifer 1960), a diagnostic criterion of many olfactory receptors (Slifer 1970). The entire inner surface of the cuticle is lined with a subcuticular membrane (Fig. 32). Each of the dendritic branches is enclosed is a membrane sheath and contains a single neurotubule (Figs. 31-32).

Fig. 31-32 Longitudinal sections showing Neurotubules (Nt) in the Dendritic Branches (DB) and the location of these dendritic branches just below the inner surface of the Dome (D). Note the absence of neurofilament connections with the cuticular pores. Subcuticular Membrane (Me).


(31)
D
Me
Nt
DB
1μ
(32)
D
Me
Nt
DB
.5μ

CONCLUDING DISCUSSION

This study has elucidated the behavioral and morphological factors involved in host finding by C. brunneri and has provided the first evidence that host finding by a parasitic hymenopteran is accomplished by detecting temperature differences associated with its host. The behavioral and morphological data obtained in this study provide a basis on which to hypothesize a sequence of events that occur in C. brunneri host finding, i.e. from the time a female C. brunneri alights on an infested tree until she oviposits on a host larva. The mated parasite, once attracted to a bark beetle-infested tree, will initiate random search immediately, or after a period of cleaning and resting (nonsearch). During random search the parasite follows a relatively straight path, except for diversions around or along bark crevices. Passing over the end of a gallery containing an actively mining larva the parasite begins nonrandom search, characterized by a series of short sharp turns which orient the parasite so that the host larva will lie parallel to the long axis of the parasite. This concentrated search phase is initiated only when the end of a larval gallery is crossed (Figs. 3, 4, 11), although there are instances where nonrandom search is not initiated while making this crossing (Fig. 10).

Once the parasite has oriented itself directly over a possible host it begins to drill the ovipositor through the bark until contact is made with the larva or until the full length of the ovipositor has been extended and no contact has been made. These ovipositional probes continue until the larval cuticle of the host has been located and a venom has been

injected into the host. The parasite then withdraws its ovipositor part of the way out of the chamber, once again makes contact with the larva and deposits an egg on the host cuticle. Then, the parasite will resume random search in an adjacent area either immediately, or after a period of rest at the site of oviposition. The parasite was never observed to concentrate search in the area of the original oviposition suggesting that field observations of nearly 100% parasitism of individual bark beetle broods is not the activity of an individual parasite. This supposition is supported by the fact that a C. brunneri female produces about 21 eggs in her lifetime (maximum of nine per day) (Ryan 1961), considerably less than the average fecundity of a D. pseudotsugae female.

The systematic and precise search pattern of C. brunneri coupled with its low fecundity indicate that the parasite is well adapted to its host and relies on accurate host finding rather than a more random encounter with its host for successful reproduction.

Several observations lead to the conclusion that C. brunneri locates its host by detecting warm areas (hotspots) in the bark associated with host larvae. In particular, C. brunneri females invariably oviposited at sites warmer than the surrounding bark (Fig. 14). These hotspots were later verified to be directly over larval chambers. More conclusively, in the absence of all other possible stimuli, female parasites were stimulated to oviposit on bark with hotspots artifically created by resistance wire probes (Table 10).

The most probable cause of these hotspots in naturally infested logs is metabolic heat. From the time D. pseudotsugae larvae hatch they are

actively metabolizing and mining in the inner bark. These larvae pack their frass densely in the gallery behind them, presumably by turning around in the larval gallery and "bulldozing" the frass into place with the head capsule as in Alniphagus aspericollis (LeConte) (Borden 1969). This activity provides a frass-free chamber within which the larvae continue to eat the tissue in front of them. These air-filled chambers containing the actively metabolizing larvae would be expected to retain the metabolic heat generated by the larvae. Apparently, the chambers of the maturing larvae, for which C. brunneri shows a preference (Fig. 12), are sufficiently warmer than the surrounding bark and are of a sufficient size and depth to induce oviposition.

One might question why C. brunneri is not induced to oviposit on younger bark beetle larvae (Fig. 12), which would also be expected to generate considerable metabolic heat. There are several possible explanations. Firstly, the very young larvae are deep in the phloem tissue mining at the bark-wood interface. Any temperature difference associated with such larvae would be much more difficult to detect than that from a larger larva, which by its very bulk would provide a gallery ceiling much closer to the outer surface of the bark even if it continued to mine at the bark-wood interface. Moreover, older larvae often turn their galleries outward, thus bringing them closer to the surface presumably rendering them more detectable by the parasite, and placing them within the range of parasite's ovipositor. Secondly, the early instar host larvae may not generate a stimulus which would allow the parasite to locate a host accurately. Thus, early instar larvae which mine very close together in a

broad, expanding front may warm up a general area of bark, sufficient to induce nonrandom search, but unlike the more discrete galleries of older larvae, would not provide a small enough hotspot to induce oviposition (Fig. 12). A third possible explanation is that C. brunneri oviposition stimuli must be of a certain area as well as temperature difference. Thus, a parasite would initiate nonrandom search when it detected a warm area of bark, but would oviposit only on a hotspot of dimensions which are characteristic of a later instar host.

At the bark surface and a few millimeters above where the parasite is detecting temperature differences the effect of solar radiation and shading will affect the actual temperature difference (Baake 1968). Solar heated logs would be warmed up to the point where there is no detectable temperature difference between the area above the larval chambers and the uninfested tissues. Alternatively, in shaded conditions there will be a greater difference between the hotspots and the shaded uninfested bark and a predictable increase in parasite activity would result (Table 10).

Oviposition on freeze-killed larvae (Table 8) would appear to be a contradiction to the use of temperature differences in host finding by C. brunneri. However, the microbial activity in the larval chamber, as indicated by the decay of the dead larvae, would provide a source of metabolic heat within the larval chamber.

Supporting evidence for the hotspot method of host detection also came from incidental observations. In the laboratory cages, parasites were observed in ovipositional stances around such nonhost objects as nails in wooden parts of the cage, grooves where a plastic door fit in, and metal

support staples in the corners of the cage. This behavior was exhibited only on those areas of the cage warmed by the sun (near windows). These objects were presumably heated up by solar radiation and the difference between the temperature of the wood and the metal objects or the grooves were sufficient to stimulate the ovipositional attempts at nonhost targets.

Various authors disagree about the presence of specific thermoreceptors in insects. Bullock and Horridge (1965), Chapman (1969), and Melin (1941) agree that there is no receptors. However, Schneider (1964), Schafer (1970) and Rose (1967) state that many if not all insects possess thermoreceptors. Most of the thermoreceptors described to date have been sensilla trichodea or coeloconica (Rose 1967, Schafer 1970), while sensilla placodea have been assumed and in one case, demonstrated to be olfactory in function (Schneider 1969).

Two of the few organisms known to possess the ability to detect IR radiation are insects. Evans (1965) reported a prothoracic IR receptor on the buprestid, Melanophila acuminata DeGeer. Callahan (1965) and Hsaio and Susskind (1970) contend that Heliothis zea (Boddie) uses IR to locate its mate and host plants. Griffith and Susskind (1970) demonstrated that the sensilla trichodea of the antennae of H. zea are capable of perceiving radiation with a wavelength of about four microns. The principal vertebrate in which the ability to perceive IR is known is the oriental pit viper in which the pit organs of the head are proven IR receptors (Terashima, Goris and Katsuki 1970; Block 1950; Bullock and Diecke 1956). It is clear that much work must be done to clarify the question of infrared reception by insects and other animals.

A strong argument can be made that C. brunneri locates its host through IR perception rather than thermoreception. An equally strong argument can be made that the elongate sensilla placodea on the antennae are IR receptors.

At rest C. brunneri holds its antennae out at a 45° angle, but in random or nonrandom search it moves the antennae side to side in parallel, sweeping them through a 180° arc. This action effectively scans the area of bark immediately in front and to the side of the parasite's head. Rarely do the antennae touch the bark surface as would be expected were the parasite "feeling" for hotspots on the bark surface. Rather, it usually scans one millimeter above the surface on the bark which would require perception of differential warming of the air above the bark if the parasite were employing a thermoreceptor.

Callahan (personal communication)[2], Hsaio and Susskind (1970) and Gerritsma and Haanstra (1970) report infrared windows (wavelengths of radiation that are not absorbed by the atmosphere, particularly carbon dioxide and water vapor) in the environment. All of these researchers report IR windows in the six to 14 micron range and smaller windows down into the one micron range. Thus it would be possible for C. brunneri to employ IR in one to three micron range in host finding.

---

[2] Agricultural Research Service, U.S.D.A., Insect Attractants, Behavior, and Basic Biology Research Lab., Gainesville, Florida 32601.

Antennal amputations demonstrated that C. brunneri antennae are essential in host finding (Table 7). Inventory of the antennal sensilla revealed only two types of sensilla in addition to the abundant elongate placoid sensilla. These were the numerous socketed sensilla chaetica or guard setae which are obvious tactile receptors, and a very few "chemoreceptor" pegs. Thus the only sensilla likely to be used in host finding are the elongate placoid sensilla.

The ultrastructure of these sensilla does not support a chemoreceptor or other function, but does provide considerable evidence to support an IR receptor function. The median channel is filled with dendritic branches packed into a channel sufficiently wide to receive up to two micron radiation, within the range of known environmental IR windows. The cuticular lamellae apparently act as shields against any stimulus contacting the dendritic branches everywhere but in the narrow median channel. The linear structure and placement of the placoid sensilla running the full length of the antennal segment and located all around the periphery, suggest that they are highly directional wave guides capable of precisely perceiving IR stimuli that lie within the insect's path. This structure and arrangement would enable a parasite moving its two antennae in parallel, not only to perceive IR emanating from a hotspot in the bark, but to delineate its boundaries and dimensions. Such a capability would explain the extremely accurate host finding ability of C. brunneri compared with other parasites of subcortical insects such as R. persuasoria (Spradbery 1970a). In addition, it would explain the ability of the parasite to orient parallel to the long axis of the larval gallery.

Conclusive evidence that the plate organs on C. brunneri are infrared receptors must be resolved through electrophysiological means. It should also be proven (possibly by IR photography) that naturally infested logs emit infrared radiation and that this radiation will stimulate ovipositional behavior in C. brunneri.

LITERATURE CITED

Allan, D.G., R.R. Michael, and S.A. Stone. 1960. Sounds of Douglas-fir beetle activity. Ore. Forest Lands Research Center. Research Note 36, 19 pp.

Arthur, A.P. 1962. Influence of host tree on abundance of Itoplectis conquisitor (Say) (Hymenoptera: Ichneumonidae), a polyphagous parasite of the European pine shoot moth, Rhyaciona buoliana (Schiff) Lepidoptera: Olethrentidae). Can. Ent. 94(4): 337-47.

Bakke, Alf. 1968. Ecological studies on bark beetles (Coleoptera: Scolytidae) associated with scots pine (Pinaes sylvestris L.) in Norway with particular reference to the influence of temperature. Meddelelser fra Det Norske Skogfur soksuesen. No. 83, 21: 444-602.

Balduf, W.V. 1926. The bionomics of Dinocampus coccinellae Schrank. Annals of the Entomological Society of America. 19: 465-498.

Beaver, R.A. 1967. The regulation of population density in the bark beetle Scolytus scolytus (F.). J. Animal Ecology 36: 435-451.

Bedard, W.D. 1933. The Douglas-fir beetle - its seasonal history, biology, habits, and control. U.S. Division of Forest Insect Field Station, Coeur d'Alene, Idaho. Office Report. 67 pp.

Berisford, C.W., H.M. Kulman, R.L. Pienkowski, and H.J. Heikkenen. 1971. Factors affecting distribution and abundance of hymenopterous parasites of Ips spp. bark beetles in Virginia (Coleoptera: Scolytidae). Can. Ent. 103(2): 235-239.

Block, M.J. 1950. Function and operation of the facial pit of the pit vipers. Nature, 165(4190): 284-285.

Borden, J.H. 1969. Observations on the life history and habits of Alniphagus aspercollis (Coleoptera: Scolytidae) in southwestern British Columbia. Can. Ent. 101: 870-878.

Bryden, J.W. and M.H.W. Bishop. 1945. Perilitus coccinellae (Schrank) (Hym., Braconidae) in Cambrdigeshire. Entomologist's Monthly Magazine 81: 51-52.

Bullock, T.H. and F.P.J. Diecke. 1956. Properties of an infrared receptor. J. Physiol. 134: 47-87.

Bullock, T.H. and G.A. Horridge. 1965. Structure And Function In The Nervous System Of Invertebrates. Vol. II. San Francisco. W. H. Freeman & Co. pp 1610.

Bushing, R.W. 1965. A synoptic list of the parasites of Scolytidae (Coleoptera) in North America North of Mexico. Can. Ent. 97(5): 449-492.

Callahan, P.S. 1965. Intermediate and far infrared sensing of nocturnal insects. Part II. The compound eye of the corn earworm, Heliothis zea, and other moths as a mosaic optic-electromagnetic thermal radiometer. Ann. Ent. Soc. Amer. 58(5): 746-756.

Cardona, Cesar and E.R. Oatman. 1971. Biology of Apanteles dignus (Hymenoptera: Braconidae) a primary parasite of the tomato pinworm. Ann. Entomol. Soc. Amer. 64(5): 996-1007.

Chapman, R.F. 1969. The Insects Structure And Function. The English Universities Press Ltd., London. 819 pp.

Cushman, R.A. 1913. Biological notes on a few rare or little known parasitic Hymenoptera. Proceedings of the Entomological Society of Washington. 15: 153-161.

DeLeon, Donald. 1935. The biology of Coeloides dendroctoni Cushman (Hymenoptera: Braconidae) an important parasite of the mountain pine beetle (Dendroctonus monticolae Hopk.). Annals of the Ent. Soc. Amer. 28(4): 411-24.

Doutt, R.L. 1957. Biology of Solenotus begini (Ashmead). Jour. Econ. Ent. 50: 373-74.

Doutt, R.L. 1959. The biology of parasitic Hymenoptera. Ann. Rev. Ent. 4: 161-82.

Doutt, R.L. 1965. Biological characteristics of entomophagous adults. pp 145-167 in P. DeBach (ed.) Biological Control Of Insect Pests and Weeds. Reinhold Pub. Co., New York. pp 844.

Edwards, R.L. 1954. The host-finding and oviposition behavior of Mormoniella vitripennis (Walker) (Hym.: Pteromalidae), a parasite of muscoid flies. Behavior 7: 88-113.

Evans, W.G. 1965. The orientation of Melanophila acuminata (Coleoptera, Buprestidae) to fires. Proc. XII Int. Congr. Entomol.: 286.

Fisher, R.C. 1959. Life history and ecology of Horogenes chrysostictos Gmelin (Hymenoptera, Ichneumonidae), a parasite of Ephestia sericarium Scott (Lepidoptera, Phycitidae). Can. J. Zool. 37: 429-46.

Furniss, M.M. 1968. Notes on the biology and effectiveness of Karpinskiella paratomicobia parasitizing adults of Dendroctonus pseudotsugae. Ann. Entomol. Soc. Amer. 61(6): 1384-1389.

Gaston, L.K., H.H. Shorey, and C.A. Saario. 1967. Insect population control by the use of sex pheromones to inhibit orientation between the sexes. Nature, 213(5081): 1155.

Gerritsma, C.J. and J.H. Haanstra. 1970. Infrared transmission of air under laboratory conditions. Infrared Physics, 10: 79-90.

Griffith, P.H. and C. Susskind. 1970. Electromagnetic communication versus olfaction in the corn earworm moth, Heliothis zea. Ann. Ent. Soc. Amer. 63(3): 903-905.

Heatwole, H., D.M. Davis, and A.M. Wenner. 1964. Detection of mates and hosts by parasitic insects of the genus Megarhyssa (Hymenoptera: Ichneumonidae). Am. Midl. Nat. 71:374-81.

Hicks, J.B. 1857. On a new structure in the antennae of insects. Linnean Society Transactions. XXII: 147-154.

Hicks, J.B. 1859. On certain sensory organs in insects, hitherto undescribed. Royal Society Proceedings X: 25-26.

Hsiao, H.S. and C. Susskind. 1970. Infrared and microwave communication by moths. IEEE Spectrum. March: 69-76.

Jacobi, E.F. 1939. Uber Lebensweise, Auffinden des Wirtes und Regulierung der Individuenzahl von Mormoniella vitripennis Walker. Archives Neerland Zoologica. 3: 197-282.

Jones, R.L. and W.J. Lewis. 1971. Substance that stimulates host-seeking by Microplitis croceipes (Hymenoptera: Braconidae), a parasite of Heliothis spp. Annals of the Entomological Society of America 64(2): 471-473.

Kraepelin, K. 1883. Ueber die Geruchsorgnne der Gliedertiere. Entl. Abit. Realsch. Johan. Hamburg.

Lacher, V. 1964. Elektrophysiologische untersuchungen an einzelnen Rezeptoren fin Geruch, Kohlendioyd, Luftfeuchtigkeit und Temperatur auf der Antennen der Arbeitsbiene und der Drohne (Apis mellifera L.). Z. Vergleich. Physiol. 48: 587-623.

Lacher, V. and D. Schneider. 1963. Elektrophysiologischer Nachweis der Reichfunktion von Porenplatten (Sensilla placodea) auf den Antennen der Drohne und Arbeitsbiene (Apis mellifera L.). Z. Vergleich. Physiol. 47: 247-78.

Laing, J. 1937. Host-finding by insect parasites. 1. Observations on the finding of hosts by Alysia manducator, Mormoniella vitripennis, and Trichogramma evanescens. J. Animal Ecol. 6: 298-357.

Leydig, F. 1860. Ueber Geruchs-und Gehörorgane der Krebse und Insecten. Reichert, Archiv.: 265-314.

Madden, J.L. 1968. Behavioral responses of parasites to the symbiotic fungus associated with Sirex noctilis F. Nature 218: 189-90.

Massey, C.L. and N.D. Wygant. 1954. Biology and control of the engelmann spruce beetle in Colorado. U.S. Dept. Agriculture, Forest Service, Circular No. 944.

Melin, D. 1941. The function of the pore-plates in Hymenoptera. Zoologiska Bidrag fran Upsala, 20: 303-344.

Picard, F. 1922. Contribution à l'Etude des parasites de Pieris brassicae L. Bull. Biol. de la France et de la Belgigue. 56(1): 54-130.

Rice, R.E. 1968. Observations on host selection by Tomicobia tibialis Ashmead (Hymenoptera: Pteromalidae). Contrib. Boyce Thompson Inst. 24: 53-56.

Richerson, J.V. and C.J. DeLoach. 1972. Some aspects of host selection by Perilitus coccinellae (Hym.: Braconidae). In press.

Rose, A.N. 1967. Thermobiology. Academic Press. London. 653 pp.

Ruland, F. 1888. Beiträge zur kenntnis der antennalen Sinnesorgane der Insekten. Zeitschr. wiss. Zoologie, 46: 602-628.

Ryan, R.B. 1961. A biological and developmental study of Coeloides brunneri Vier., a parasite of the Douglas-fir beetle, Dendroctonus pseudotsugae Hopk. Ph.D. thesis. Ore. State Univ., Corvallis. 172 pp (No. 61-3939, Univ. Microfilms Inc. No. First St. Ann Arbor, Michigan.

Ryan, R.B. and J.A. Rudinsky. 1962. Biology and habits of the Douglas-fir beetle parasite, Coeloides brunneri Viereck (Hymenoptera: Braconidae), in western Oregon. Can. Ent. 94: 748-763.

Salt, G. 1938. The sense used by Trichogramma to distinguish between parasitized and unparasitized hosts. Proc. Roy. Soc. London 122: 57-75.

Schafer, R. 1970. An electrophysiological survey of antennal sense organs in the cockroach, Leucophaea moderae. Am. Zool. 10: 310.

Schneider, Dietrich. 1964. Insect antennae. Annual Review of Entomology 9: 103-122.

Simmonds, F. 1954. Host finding and selection by Spalangia drosphilae (Ashm.). Bull. ent. Res. 45: 527-37.

Slifer, E.H. 1960. A rapid and sensitive method for identifying permeable areas in the body wall of insects. Ent. News. 71: 179-82.

Slifer, E.H. 1969. Sense organs on the antennae of a parasitic wasp, Nasonia vitripennis (Hymenoptera, Pteromalidae). Biological Bulletin 136: 253-63.

Slifer, E.H. 1970. The structure of arthropod chemoreceptors. Annual Review of Entomology 15: 121-142.

Slifer, E.H. and S.S. Sekhon. 1960. The fine structure of the plate organs on the antenna of the honey bee, Apis mellifera Linneaus. Experimental Cell Research 19: 410-14.

Snodgrass, R.E. 1935. Principles of Insect Morphology. McGraw-Hill Book Co., New York. 667 pp.

Spradbery, J.P. 1970a. Host finding by Rhyssa persuasoria (L.), an ichneumonid parasite of siricid wood wasps. Animal Behavior 18, 1: 103-114.

Spradbery, J.P. 1970b. The biology of Ibalia drewseni Borries (Hymenoptera: Ibaliidae), a parasite of siricid wood wasps. Proc. Royal Entomol. Soc. London 45(A): (7-9) 104-113.

Steinbrecht, R.A. and B. Müller. 1971. On the stimulus structures in insect olfactory receptors. Z. Zellforsch 117: 570-575.

Sychevskaya, V.I. 1966. Biology of Brachymeria minuta (L.) (Hymenoptera: Chalcidoidea), a parasitoid of synanthropic flies of the family Sarcophogidae (Diptera). Ent. Review 45: 424-29.

Terashima, Shin-ichi, R.C. Goris, and Y. Katsuki. 1970. Structure of warm fiber terminals in the pit membrane of vipers. J. Ultrastructure Research 31: 494-506.

Thompson, W.R. 1953. A catalogue of the parasites and predators of insect pests. Commonwealth Inst. Biological Control, Ottawa, Canada.

Thompson, W.R. and H.L. Parker. 1927. Etudes sur la biologie des insectes parasites: la vie parasitaire et la notion morphologique de l'adaption. Ent. Sr. Fr., Ann. 96(30): 113-46.

Thorpe, W.H. and F.G.W. Jones. 1937. Olfactory conditioning in a parasitic insect and its relation to the problem of host selection. Proc. R. Soc. London 124(B): 56-81.

Ullyett, G.C. 1936. Host selection by Microplectron fuscipennis, Zett. (Chalcididae: Hymenoptera). Proc. R. Soc. London (B): 256-291.

Ullyett, G.C. 1953. Biomathematics and insect population problems. A critical review. Memoirs of the Entomological Society of South Africa 2: 1-89.

Vinson, S.B. 1968. Source of a substance in Heliothis virescens (Leopidoptera: Noctuidae) that elicits a searching response on its habitual parasite Cardiochiles nigriceps (Hymenoptera: Braconidae). Ann. ent. Soc. Amer. 61(1): 8-10.

Vinson, S.B. and W.J. Lewis. 1965. A method of host selection by Cardiochiles nigriceps. Jour. Econ. Ent. 58(5): 869-71.

Walker, M.F. 1961. Some observations on the biology of the ladybird parasite, Perilitus coccinellae (Schrank), with specific reference to host selection and recognition. Entomologist's Monthly Magazine 97: 240-244.

Wylie, H.G. 1958. Factors that affect host finding by Nasonia vitripennis (Walk.) (Hymenoptera: Pteromalidae). Can. Ent. 90: 597-608.

CURRICULUM VITAE

Jim V. Richerson

PERSONAL INFORMATION

Birthdate: September 22, 1943
Married, one child
U. S. Citizen

EDUCATION

1961 Graduated from William Chrisman High School, Independence, Missouri
1965 B.A. (Botany), University of Missouri, Columbia, Missouri
1968 M.Sc. (Entomology), University of Missouri, Columbia, Missouri

SOCIETIES

Entomological Society of Canada
Entomological Society of America

WORK EXPERIENCE

1965-68 Research technician, USDA Biological Control Laboratory, Columbia, Mo. Responsibilities included three summers of field work in agricultural entomology, studies on the populations of insects of cole crops and alfalfa, and biology of aphid parasites. As federal employee (1967-68), assumed supervisory capacity over other technicians. M.Sc. research on coccinellid parasite Perilitus coccinnellae done in conjunction with research lab and while performing continued studies on aphid biology and effects of pesticides on aphid parasites and predators.

1968-71 Teaching Assistant, Simon Fraser University, mainly as Laboratory Assistant and Tutorial Leader. Courses taught include: Introductory Biology, Introductory Ecology, Insect Biology, Entomology.

PUBLICATIONS

M.Sc. Thesis:

Parasitism of Coccinellids by Perilitus coccinellae.

Publication in refereed journals:

Richerson, J.V. 1970. A world list of parasites of Coccinellidae. J. Ent. Soc. B.C. 67: 33-48.

Richerson, J.V. and C.J. DeLoach. 1972. Some aspects of host selection by Perilitus coccinellae. Ann. Ent. Soc. Amer. (in press).

Richerson, J.V. and J.H. Borden. 1971. Sound and vibration are not obligatory host finding stimuli for the bark beetle parasite, Coeloides brunneri (Hymenoptera: Braconidae). Entomophaga, 16(1): 95-99.

Manuscript in preparation:

Richerson, J.V. and C.J. DeLoach. Seasonal parasitism of coccinellids in Missouri. (In review proceedings, U.S. Dept. of Agriculture)

PAPERS PRESENTED AT SCIENTIFIC MEETINGS

Richerson, J.V. 1968. Factors in host selection of coccinellids by Perilitus coccinellae. Central Branch Meetings, Entomological Society of America, Denver, Colo.

Richerson, J.V. 1971. Host finding behavior of Coeloides brunneri. Annual Meeting, Entomological Society of Canada, Victoria, B.C. August, 1971.

Richerson, J.V., J.H. Borden and G. Hollingdale. 1971. Ultrastructure of antennal "placoid" sensilla on the bark beetle parasite Coeloides brunneri (Hymenoptera: Braconidae). Annual Meeting, Entomological Society of America, Los Angeles, Calif., December, 1971.

AWARDS

Graduate Student Award, Pacific Branch, Entomological Society of America. December, 1971.