LEARNING AND ITS EFFECT ON HOST-FINDING BY *EXERISTES ROBORATOR* (F.) (HYMENOPTERA : ICHNEUMONIDAE)

by

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ABSTRACT

In laboratory experiments females of the polyphagous, ichneumonid parasitoid *Exeristes roborator* (F.) were able to learn the colour, form, and odour of artificial host microhabitats in which they attacked hosts. The amount and timing of experience that females gained attacking hosts influenced the strength of their learned responses. Individuals learned from one host attack, but their learning was strengthened considerably by a second such attack, and increased somewhat more after further experience. Attacks on hosts carried out within the first 2 days after this activity began appeared to be most effective at causing learning. Females denied experience until more than 2 days had passed after first host attack still learned, but did not exhibit as strong learned responses as females gaining experience when younger. Parasitoids that attacked hosts first in one artificial microhabitat and then in a second learned to shift their responses to the second microhabitat, but did not transfer them completely. Apparently oviposition is not necessary reinforcement for learning, as attacks involving only host probing and feeding caused learning to occur. In laboratory and field cage experiments, parasitoids given experience with hosts in an artificial microhabitat subsequently exhibited significantly lower responses to a natural host/microhabitat system than inexperienced females. When females were given prior experience with the natural system, their responses to it were not increased over those of inexperienced insects, but they appeared to respond almost exclusively to this familiar system, while inexperienced females responded readily to other insect-infested vegetation present in the test arena. It is possible that the experimental conditions did not allow increased, learned responses to the natural system to be detected. Alternatively, learning may enhance the parasitoid's responses only to some natural host microhabitats, or may not significantly increase its responses to natural microhabitats. However, these results suggest that experience with

either a natural or an artificial host/microhabitat system prior to release may interfere with a biological control agent's post-release response to a different target system.

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GENERAL INTRODUCTION

Many factors are involved in the process by which parasitoids find and parasitise their hosts (Doutt 1959; Lewis *et al.* 1976; Vinson 1976, 1985; Arthur 1981; van Lenteren 1981; Weseloh 1981). Interest in the role of learning in this process is expanding as prior experience is found to influence responses to hosts, or host-related factors, by an increasing number of parasitoids (e.g. Arthur 1966, 1967, 1971; Vinson *et al.* 1977; Vet and van Opzeeland 1984, 1985; Lewis and Tumlinson 1988) and other insects (e.g. Prokopy *et al.* 1982, 1986; Traynier 1984, 1986; Bernays and Wrubel 1985; Papaj 1986a-d).

Learning by parasitoids presents applied entomologists with possibilities for either adversely affecting, or advantageously manipulating, parasitoid behaviour. Biological control often depends upon efficient host attack by released parasitoids. If reared or held in an insectary prior to release, these insects could become less responsive to target hosts, as a result of conditioning to factitious hosts or artificial environments. Alternatively, attack on target hosts by such parasitoids might be enhanced by experience with the host in its natural microhabitat prior to release. These ideas have been discussed in the literature for some time (Arthur 1967, 1981; Vinson *et al.* 1977; Wardle and Borden 1985), but have not been tested directly.

Different disciplines are not in complete agreement about the definition of the word learning. I have adopted a fairly broad definition of learning, which describes it as a process manifested by changes in behaviour that occur as a result of individual experience (Thorpe 1963).

Exeristes roborator (F.) is a polyphagous ichneumonid ectoparasitoid native to Europe. The majority of its natural hosts are lepidopterous larvae concealed in plant tissue (Thompson 1957). Females begin to attack hosts a few days after eclosion, initially just feeding on them, and subsequently both

feeding and ovipositing. In the laboratory oviposition rate varies considerably between individuals, with averages of 1-13 eggs/day (Baker and Jones 1934) and 8-10 eggs/day (Fox 1927) reported. The oviposition rate of females is probably much lower in the field (Baker and Jones 1934). The parasitoid does not appear to discriminate between unparasitised and parasitised hosts, and will readily accept the latter (Baker and Jones 1934; personal observation).

In North America, *E. roborator* has been released as a biological control agent against several pests (e.g. Baker *et al.* 1949; McLeod 1962; McGugan and Coppel 1962; Clausen 1978a,b; Clausen and Oatman 1978; Oatman 1978). Although surviving for some time (Baker *et al.* 1949; Arthur and Juillet 1961), the parasitoid did not become permanently established (Baker *et al.* 1949; McLeod 1962; Clausen 1978b; Clausen and Oatman 1978; Oatman 1978; Krombein *et al.* 1979). Reasons for these failures are not known, although in at least one case a lack of synchronisation between the life cycle of the host and of the parasitoid was believed to be the cause (Baker and Jones 1934).

In the laboratory, *E. roborator* can learn to respond to an artificial microhabitat from which hosts are absent with host-seeking behaviour when it has previously attacked hosts concealed in this microhabitat (Wardle and Borden 1985). Responding to a host's microhabitat is probably important in host-finding for many parasitoids (Doutt 1959, Vinson 1976, 1981, 1985). A host's immediate environment may provide stronger or more distinctive cues than the host itself, particularly when the latter is concealed within the microhabitat, as is the case for the hosts of *E. roborator*.

In this thesis host 'microhabitat' is used to refer to objects within which the hosts of *E. roborator* are concealed. The term 'habitat' has been used by some authors to refer to plants, plant parts, or other objects harbouring parasitoids' hosts (Doutt 1959, Vinson 1976, 1981), but this usage

can be confusing because the word also implies a much larger scale (Vinson 1984a).

The general objectives of my research were to investigate learning of host microhabitats by *E. roborator* in the laboratory, and to determine if such learning could influence the parasitoid's responses to hosts and their microhabitats under more natural conditions. An understanding of various aspects of learning could be useful to pest managers releasing this parasitoid, or others like it, as biological control agents. With accurate knowledge of the physical characteristics learned, of the experience required for learning, and of when a parasitoid learns, gained under controlled laboratory conditions, the importance of different features of rearing systems in causing learning by parasitoids exposed to them could be assessed. Evaluation of the effects of prior experience on a parasitoid's responses to a target host under more natural conditions than occur in the laboratory could facilitate prediction of the significance of pre-release learning for the parasitoid's performance in the field.

Wardle and Borden (1985) showed that young *E. roborator* exhibit stronger learned responses than old females, but did not identify the host microhabitat characteristics they learned, or elucidate the precise relationships between strength of learning and the nature and timing of experience. Therefore, learning of microhabitat features such as colour, form, and odour, all potential sources of distinctive host microhabitat cues for parasitoids (Vinson 1981), was examined. The amount and type of experience needed by *E. roborator* for learning was also investigated. In addition, the importance of the timing of this experience in relation to both the insect's readiness to attack hosts, and its other experiences with hosts, was assessed. Finally, field cage studies were undertaken to determine if prior experience attacking hosts in their natural microhabitat or in an artificial microhabitat,

respectively, could increase or decrease responses to, and attack upon, hosts in the natural microhabitat.

CHAPTER 1

LEARNING OF HOST MICROHABITAT COLOUR

INTRODUCTION

The ability to respond to the colour (wavelength) or brightness (intensity) of light reflected or transmitted by food sources or oviposition sites, or their surroundings, has been documented for phytophagous insects (Prokopy and Owens 1983), biting flies (Allan *et al.* 1987), and pollinating insects (Kevan 1978, 1983), and has been implicated for some predatory insects (Hagen *et al.* 1976). Limited evidence suggests that such cues may be involved in the responses of some parasitoids to their hosts (Takahashi and Pimentel 1967; Moore 1969; Richerson and DeLoach 1972; Weseloh 1972, 1986; Schmidt *et al.* 1978).

Insects' responses to particular wavelength or intensity cues can be innate, but may also be learned from prior experience with food or oviposition sites. The dronefly, *Eristalis tenax* L., (Ilse 1949) and the butterfly, *Heliconius charitonius* L., (Swihart and Swihart 1970) can be conditioned by feeding with sugar water or honey to visit preferentially artificial flowers of the same colour as those from which they previously fed. The bumble bees, *Bombus ternarius* Say and *B. terricola* Kirby, can learn to select artificial flowers from which to feed on the basis of their reflectance characteristics (Heinrich *et al.* 1977). Houseflies, *Musca domestica* L., fed while illuminated with light of specific wavelengths, learn to perform a feeding reflex in response to these wavelengths alone (Fukushi 1976). When fed against backgrounds transmitting specific wavelengths, the blowfly, *Lucilia cuprina* (Weidemann) (Fukushi 1985), and the desert ant, *Cataglyphis bicolor* F. (Kretz 1979), become trained to prefer locations transmitting these same wavelengths. Bernays and Wrubel (1985) found that prior experience of migratory grasshoppers, *Melanoplus sanguinipes* (F.), with food contained in boxes of a particular reflectance could influence their ensuing preference for coloured boxes in which to search for food. Female cabbage butterflies, *Pieris rapae*

(L.), given the opportunity to oviposit on both natural and artificial sites reflecting light with specific characteristics, subsequently prefer to oviposit on sites having the same reflectance characteristics (Traynier 1984, 1986). Best studied of all insects in this respect is the worker honey bee, *Apis mellifera* L., which can learn colours associated with food sources (von Frisch 1971; Wells 1973; Menzel and Erber 1978; Menzel 1985), and also the colours of landmarks surrounding feeding sites (Cheng *et al.* 1986).

Evidence for learning of colour in parasitoids is limited to the work of Arthur (1966), who showed that the ichneumonid, *Itoplectis conquisitor* (Say), can learn to distinguish artificial host microhabitats by the characteristic light they reflect. He stated that this parasitoid was learning the colour of these structures, and, since colour vision has been demonstrated for some insects, including hymenopterans (Menzel 1979), it is probable that *I. conquisitor* could see and learn the colour of its hosts' microhabitats. However, it could also have learned to discriminate between shelters solely on the basis of their brightness, i.e. the number of photons it perceives them reflecting or transmitting. If different host microhabitats reflect different total numbers of photons they will probably appear to a parasitoid to differ in brightness. Microhabitats reflecting equal numbers of photons, but at different wavelengths, could also appear to differ in brightness if the parasitoid's sensitivity to light of each different wavelength is not the same (Hawryshyn 1982).

My objectives were to test the hypothesis that *Exeristes roborator* could learn host microhabitat colour, and, if so, to determine if this learning could influence females' responses to hosts in microhabitats of both the learned colour and another colour.

MATERIALS AND METHODS

Insect rearing and maintenance

All female *E. roborator* came from a stock colony. Immature stages were reared on coddled larvae of a factitious host, the greater wax moth, *Galleria melonella* L., according to Syed's (1985) method. The numbers of females used in each experiment are given in Table 1.

All experiments were conducted in a small room illuminated with 'cool white' flourescent lights on a 8 h L:16 h D cycle. The temperature normally ranged between 22-26°C. Experimental females were held for pre-test treatment in 30 x 30 x 45 cm cages with water, honey-coated sugar cubes, and males so that they could drink, feed on a carbohydrate source, and mate. Test cages were identical to pre-test treatment cages, except that males were not present.

Learning of host microhabitat colour

Experiments 1 and 2

Pre-test treatments

Female *E. roborator* eclosing over a 2-day period were assigned randomly to 4 groups of 10-12 insects each and placed in pre-test treatment cages. Each of the 4 groups was subjected to a different treatment for 1 week (Table 1, Exp. 1 and 2). Parasitoids in all groups were exposed to 2 differently-coloured microhabitats. Hosts were presented to group I females in one of the microhabitats only, to allow subsequent determination of whether or not these females learned the colour of this microhabitat from attacking hosts in it. Parasitoids in group II were treated in an identical manner, except that they

Table 1. Pre-test treatments and testing regimes for *E. roborator* in Exp. 1-5.

Exp.	Group	N	Pre-test treatment[a,b]	Testing regime[b]
1	I	27	Given 1 blue egg cup containing hosts and 1 orange egg cup not containing hosts (hosts in blue cup)	Given 1 blue and 1 orange egg cup, neither containing hosts, simultaneously for 1 h
	II	26	Given 1 blue egg cup not containing hosts and 1 orange egg cup containing hosts (hosts in orange cup)	"
	III	27	Given 1 blue and 1 orange egg cup, both containing hosts (hosts in both cups)	"
	IV	24	Given 1 blue and 1 orange egg cup, neither containing hosts (cups alone)	"
2	I	27	As for group I, Exp. 1	Given 1 blue and 1 orange egg cup, both containing hosts, simultaneously for 1 h
	II	26	As for group II, Exp. 1	"
	III	26	As for group III, Exp. 1	"
	IV	25	As for group IV, Exp. 1	"
3	IV′	10	As for group IV, Exp. 1	As for Exp. 1
	V	10	Held without exposure to egg cups or hosts	"
4	VI	20	Given 1 black+blue egg cup containing hosts and 1 black+orange egg cup not containing hosts	Given 1 black+blue and 1 black+orange egg cup, neither containing hosts, simultaneously for 1 h
	VII	22	Given 1 black+blue egg cup not containing hosts and 1 black+orange egg cup containing hosts	"

Table 1. continued

Exp.	Group	N	Pre-test treatment[a,b]	Testing regime[b]
5	VIII	23	Given 1 light grey egg cup containing hosts and 1 dark grey egg cup not containing hosts (hosts in lt.grey cup)	Given 1 light and 1 dark grey egg cup, neither containing hosts, simultaneously for 1 h
	IX	24	Given 1 light grey egg cup not containing hosts and 1 dark grey egg cup containing hosts (hosts in dk.grey cup)	"
	X	22	Given 1 light and 1 dark grey egg cup, both containing hosts (hosts in both cups)	"
	XI	22	Given 1 light and 1 dark grey egg cup, neither containing hosts (cups alone)	"

[a]Fresh egg cups and hosts were placed in cages each day for 7 days.

[b]Hosts were 5 coddled larvae of *G. mellonella*.

were offered hosts only in the microhabitat of the other colour, to determine if this second colour could also be learned. As controls, insects in groups III and IV were offered hosts in both microhabitats, and microhabitats without hosts, respectively, to determine if general access to hosts in the microhabitats, or exposure to the microhabitats themselves, could produce behaviour resembling learning of either colour in females.

Two artificial host microhabitats differing in colour (Fig. 1) were created using 10 cm discs of broadcloth (65% polyester, 35% cotton) that had been painted blue or orange with interior latex paint (Cilux Superior Latex, C.I.L. Paints Inc., Montreal, Quebec) of royal blue (colour no. 4849-9) or geranium (colour no. 4622-9). Two m of fabric were submerged in a bath of paint diluted to 1/4 strength with water, and allowed to dry. Discs were then cut from this piece of fabric, so that all discs of a particular colour came from the same 'dye lot'. Total photons reflected from the 2 painted fabrics over the range of insect-visible wavelengths emitted by the lights in the experimental area were equalised at approximately 34% of incident photons so that both fabrics reflected light of equal overall intensity. This was done by adding small amounts of black interior latex paint (St. Clair Premium, colour no. 8061, St. Clair, Toronto, Ontario) to both paints (Appendix 1). The blue and orange paints were used because the dominant wavelengths they reflected were well separated while still falling within the insect-visible range (Menzel 1979).

A coloured fabric disc was placed over the inverted lid of a 150 ml styrofoam cup (Stax Plastics Ltd., Mississauga, Ontario) and held in place by the rim of the cup, which had been cut from its body. When hosts were required in this 'egg cup' apparatus, 5 coddled, late-instar larvae of *G. mellonella* were concealed beneath the fabric, through which female *E. roborator* probed with their ovipositors, fed, and oviposited on them

Figure 1. *Exeristes roborator* probing host larvae in blue and orange egg cups.

(Fig. 1). New cups and fresh larvae were placed in the cages each day, and the positions of the blue and orange cups were reversed each day to prevent possible learning of host microhabitat position.

When females were given hosts in 2 egg cups, 1 of each colour, they freely fed and oviposited on hosts in both cups.

At the end of their seventh day of pre-test treatment, females from each group were distributed randomly, as equally as possible, between 4 test cages. Due to variable mortality in the preceeding 7 days, test cages usually held 8-10 females. For individual and group identification each female in a test cage was marked on the thorax with a dot of paint (Testor Corp., Weston, Ontario) of a different colour.

Testing regimes

On the following day testing was carried out on all test cages in random order.

In Exp. 1, females in 2 randomly-chosen test cages were monitored for evidence that they had learned to distinguish host microhabitats on the basis of colour. These females were given 1 fresh egg cup of each colour simultaneously, neither of which contained hosts, (Table 1, Exp. 1) and their responses to each of the cups were observed for 1 h. A record was kept of whether or not each female contacted each egg cup, and, if she did, of how long she was in contact and how many times she probed with her ovipositor, a behaviour typical of *E. roborator* searching for hosts. A probe was counted if a female inserted her unsheathed ovipositor into any part of the cup. The positions of the blue and orange cups were reversed in the 2 test cages.

Parasitoids in groups I and II (Table 1, Exp. 1) were monitored to determine whether or not females exposed to hosts in a blue cup only or an

orange cup only, respectively, would subsequently concentrate their responses on cups of these colours to a significantly greater degree than females in the other groups, thus providing evidence that they learned host microhabitat colour. Control females in groups III and IV were tested to determine if a similar concentration of response on cups of one colour or the other could occur with general access to hosts in cups, or exposure to the cups alone.

In Exp. 2, females in the remaining 2 test cages were treated identically to females in Exp. 1, except that the 2 egg cups they were given simultaneously each contained 5 fresh coddled larvae of *G. mellonella* (Table 1, Exp. 2). These parasitoids were monitored to determine whether or not any learning revealed when females were tested with cups not containing hosts (Exp. 1) influenced females' activity when they had the opportunity to attack hosts in both cups.

Experiment 3

Pre-test treatments

To determine if repeated exposure of females in control group IV (Exp. 1 and 2) to blue and orange egg cups without reward could have decreased their responses to either cup, thus preventing detection of an innate attraction to one or the other, females eclosing over a 3-day period were divided randomly into 2 groups (Table 1, Exp. 3). Group IV' females were treated in an identical manner to females in group IV, Exp. 1. Group V females were not given hosts or egg cups so that responses by females with no previous exposure to the experimental system could be measured. After 7 days of pre-test treatment 10 females from each group were marked on the thorax with a dot of paint for identification and divided equally between 2 test cages.

Testing regime

On the following day, testing was carried out on the 2 cages in random order, as in Exp. 1 (Table 1, Exp. 3). A significantly greater and more concentrated response on one egg cup by females in group V than by females in group IV′ would indicate that exposure to cups without reward had reduced the responsiveness of the latter females to that cup.

Experiment 4

Pre-test treatments

To determine if *E. roborator* could be learning the chemical characteristics rather than the colour of the blue and orange pigments, females eclosing over a 2-day period were divided randomly into 2 groups. The pre-test treatments to which they were subjected (Table 1, Exp. 4) were identical to those for females in groups I and II (Exp. 1 and 2) in all but 1 detail. The 2 egg cups to which they were exposed were constructed with layers of nylon gauze fabric painted with black interior latex (St. Clair, Premium, colour no. 8061) to which had been added either the blue or the orange pigments from the paints used in Exp. 1-3. These pigments were present at concentrations equal to those found in the pure blue and orange paints. The black paint reduced, but did not entirely eliminate, colour differences between the pigments.

After 7 days of pre-test treatment the females in each group were marked on the thorax with a dot of paint and divided between 2 test cages.

Testing regime

On the following day, testing was carried out on the 2 cages in random order. Females in each cage were given 1 fresh black+blue and 1 fresh

black+orange egg cup, neither of which contained hosts, simultaneously for 1 h (Table 1, Exp. 4). Their behaviour was monitored as in Exp. 1.

Females in both groups were tested to determine whether or not they concentrated their responses on the egg cup in which they had previously attacked hosts. Such concentration, when visible differences between the cups were minimal, would suggest that *E. roborator* learned the chemical properties of the blue and orange pigments, rather than their colours.

Effect of experience with hosts in microhabitats of different reflectance intensities

Experiment 5

Pre-test treatments

To determine if *E. roborator* could learn the intensity of light reflected by a host microhabitat, females were assigned to 4 groups and subjected to pre-test treatments (Table 1, Exp. 5) as in Exp. 1 and 2. However, discs of 2 grey fabrics were used to make the artificial host microhabitats. Black and white interior latex paints (St. Clair Premium, colour nos. 8061 and 8057, St. Clair, Toronto, Ontario) were mixed in different proportions, diluted to 1/4 strength with water, and applied to cotton gauze fabric as in Exp. 1 to produce dark (22% reflectance) and light (44% reflectance) grey fabrics (Appendix 1). These reflectances encompassed the reflectances measured for the coloured fabrics used in Exp. 1 and 2. Moreover, if *E. roborator* possesses a spectral sensitivity similar to other hymenoptera (Menzel 1971; von Helversen 1972; Kretz 1979), the 2 reflectances provided an overall relative difference in intensity somewhat greater than any difference the parasitoid would be likely to be able to perceive between the blue and orange fabrics.

Testing regime

Females were prepared for testing and assessed for evidence of learning as in Exp. 1. They were given 1 fresh microhabitat of each reflectance intensity simultaneously, neither of which contained hosts (Table 1, Exp. 5). Learning of microhabitat intensity would be indicated if *E. roborator* experienced with hosts only in cups of one intensity concentrated their responses on cups of this same intensity significantly more than control females.

Statistical analysis

Except where indicated, nonparametric statistical procedures were employed for their robustness.

Experiments 1, 2, and 5

Females were classified according to whether or not they contacted and probed the cup of either colour (Exp. 1 and 2) or intensity (Exp. 5) only, both cups, or neither cup. For each experiment contingency tables of the numbers of females in these response categories were analysed with a 4 x 4 X^2 test. If the X^2 was significant (α=0.05), simultaneous 95% confidence intervals for differences between proportions were calculated (Miller 1981). These intervals were used to compare the proportions of females in groups exposed to hosts in a cup of one colour or intensity responding only to the cup of the same colour or intensity, with the proportions of females in the other 3 groups in the same experiment also responding only to the cup of that colour or intensity. Thus for each experiment 6 simultaneous intervals were calculated for each response type (contacting or probing). When intervals did not include 0 (when the lower confidence limit was >0) the proportions being compared were significantly different.

The mean proportions of total responses to egg cups that were directed at the blue (Exp. 1 and 2) or the light grey (Exp. 5) cup were determined for the responding females in each group. All females that contacted an egg cup at any time during the test period were counted as responders for calculation of mean proportion of total contact time spent on the blue or light grey cup. Only data from females that probed an egg cup during testing were included in the calculation of mean proportion of total probes executed on the blue or light grey cup. Within each experiment these means, and also mean total time spent in contact with, and mean total probes executed on, egg cups by all females in each group, were compared using the Kruskal-Wallis Test and multiple comparisons procedure of Conover (1980) (α=0.05). In Exp. 1 and 5 groups in which probing occurred were considered to differ from groups in which it did not occur in their probing responses to a cup of a particular colour or intensity whenever simultaneous 95% confidence intervals for the mean number of probes executed on each cup (Johnson and Wichern 1982) by probing females in a group did not include 0 for that cup. These groups were considered to differ from groups in which probing did not occur in their total probing responses to egg cups whenever the 95% confidence interval for mean total probes executed on cups (Zar 1984) by all females in a group did not include 0. Data were transformed by log(probes+1) for interval calculation.

Within each experiment the proportions of females in each group contacting and probing egg cups in total were compared using a test for comparing >2 proportions and a modified Newman-Keuls multiple comparisons procedure (Zar 1984) (α=0.05).

Experiments 3 and 4

Females were divided into 4 response categories as in Exp. 1, 2, and 5, and 2 x 4 X^2 analysis (α=0.05) was used to test for differences between groups within an experiment in the numbers of females in these categories.

The mean proportions of total response devoted to the blue (Exp. 3) or the black+blue (Exp. 4) cup by responding females in each group were determined as for Exp. 1, 2, and 5. These means, and mean total responses to cups by all females in each group, were compared within each experiment with the Mann-Whitney Test (Conover 1980) (α=0.05).

In each experiment, the proportions of all females in each group responding in total to cups were compared with the Fisher Exact Test (Zar 1984) (α=0.05).

RESULTS

Female *E. roborator* learned host microhabitat colour (Figs. 2, 3; Table 2), but not reflectance intensity (Fig. 4; Table 3).

In Exp. 1, females exposed to hosts only in an egg cup of one colour showed a subsequent preference for the cup of that colour, even when it did not contain hosts (Fig. 2; Table 2, Exp. 1). Highly significant differences (X^2 test, $p<0.001$) occurred among groups in the numbers of females contacting and probing either the blue or orange cup alone, both cups, or neither cup. With one exception, females in groups I and II responded exclusively to the cup they had previously experienced as a host microhabitat significantly more than females in the other groups (Fig. 2). The 58% of females in group II that probed only the orange cup was not significantly different from the 30% of control females in group III that probed only the cup of this colour.

Responding group I females spent >90% of their total contact time, and executed >90% of their ovipositor probes, on the blue cup (Table 2, Exp. 1). Therefore, they were directing <10% of their responses to the orange cup. In contrast, responding females in group II devoted >80% of their responses to the orange cup, as <20% of their contact time and ovipositor probes were directed at the blue cup (Table 2, Exp. 1). Responding control females in groups III and IV did not show a preference for cups of either colour (Table 2, Exp. 1).

Group IV females did not probe egg cups at all. Group I and II females differed from females in group IV only in probing responses to the coloured egg cup in which they had been given hosts during pre-test treatment, while group III females differed from group IV females in probing responses to cups of both colours (simultaneous 95% confidence intervals for mean probes on blue and orange egg cups by responding females in groups I-III).

Figure 2. Percent of *E. roborator* in groups I-IV in Exp. 1 responding to the blue or orange egg cup alone, both egg cups, or neither egg cup when hosts were not present in the cups. Bars marked with an asterisk are significantly different from all other bars in the same subgraph. Bar marked with a diamond is not significantly different from the next greatest bar, but is significantly different from the 2 lowest bars in the same subgraph [simultaneous 95% confidence intervals for differences between proportions (Miller 1981)]. Pre-test treatment for group I=hosts in blue cup, for group II=hosts in orange cup, for group III=hosts in both cups, and for group IV=cups alone.

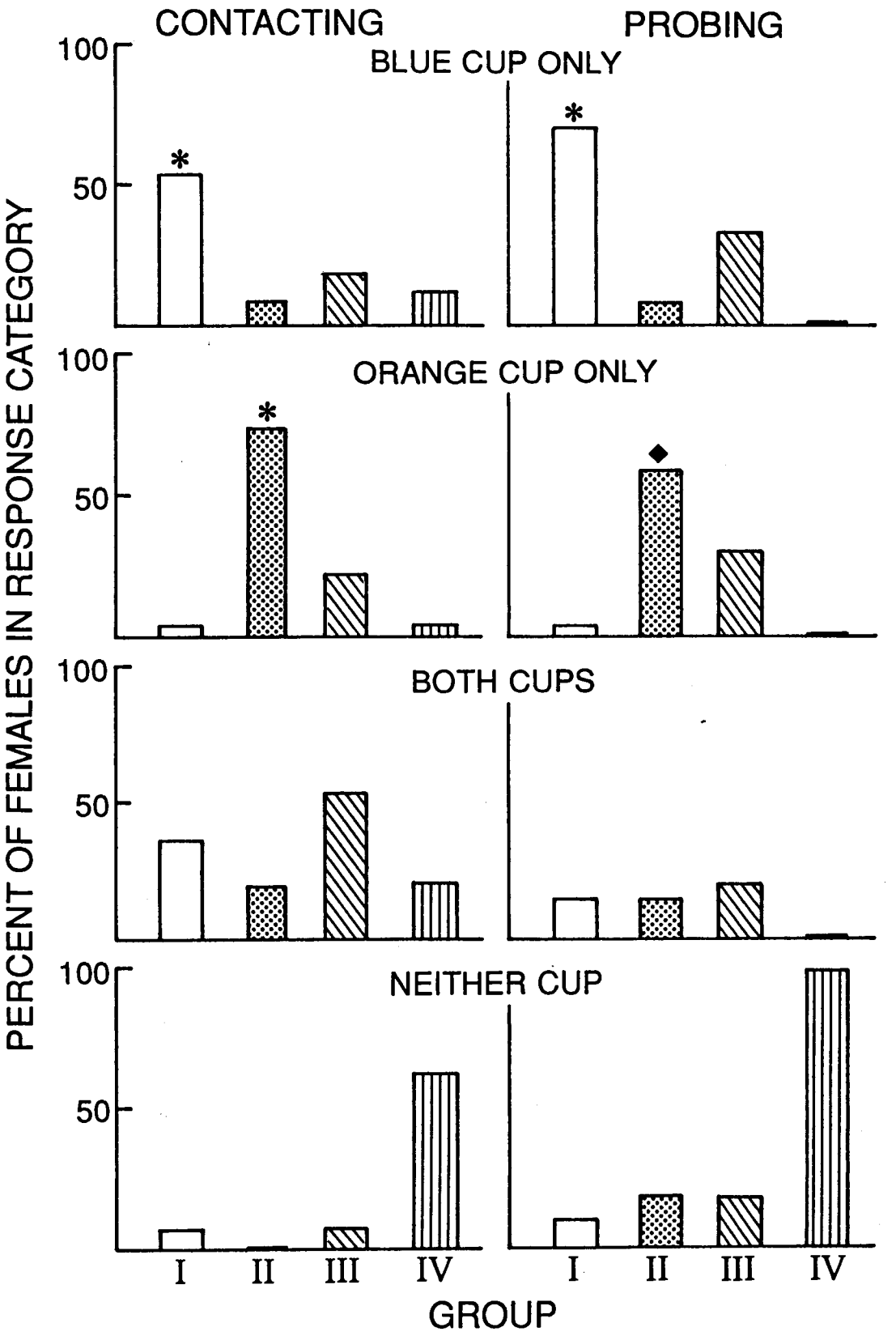

CONTACTING
PROBING
BLUE CUP ONLY
ORANGE CUP ONLY
BOTH CUPS
NEITHER CUP
100
50
PERCENT OF FEMALES IN RESPONSE CATEGORY
I
II
III
IV
GROUP

Figure 3. Percent of *E. roborator* in groups I-IV in Exp. 2 responding to the blue or orange egg cup alone, both egg cups, or neither egg cup when hosts were present in the cups. Bars marked with an asterisk are significantly different from all other bars in the same subgraph. Bar marked with a diamond is not significantly different from the next 2 greatest bars, but is significantly different from the lowest bar in the same subgraph [simultaneous 95% confidence intervals for differences between proportions (Miller 1981)]. Pre-test treatment for group I=hosts in blue cup, for group II=hosts in orange cup, for group III=hosts in both cups, and for group IV=cups alone.

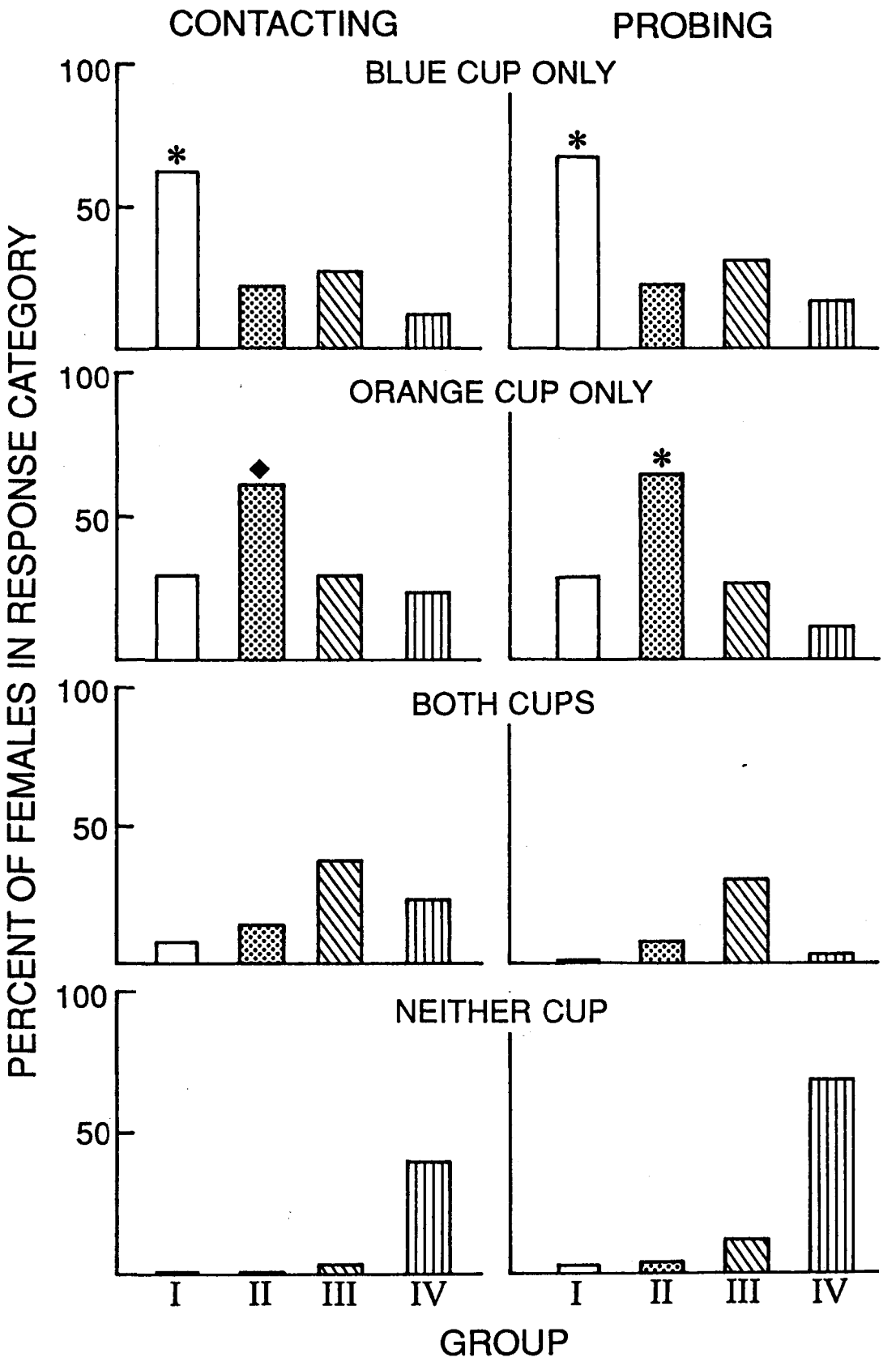

CONTACTING
PROBING
BLUE CUP ONLY
ORANGE CUP ONLY
BOTH CUPS
NEITHER CUP
100
50
PERCENT OF FEMALES IN RESPONSE CATEGORY
I
II
III
IV
GROUP

Table 2. Mean percent of total contacting and probing responses to egg cups directed at the blue cup by responding *E. roborator* in groups I-IV during testing in Exp. 1 (hosts not present in cups) and Exp. 2 (hosts present in cups).

Exp.	Group (pre-test treatment)	No. females contacting egg cups	Mean % (±S.E.) of total contact time spent on blue cup[a]	No. females probing egg cups	Mean % (±S.E.) of total probes executed on blue cup[a]
1	I (hosts in blue cup)	25	90.8 ± 4.4 a	24	91.4 ± 4.5 a
	II (hosts in orange cup)	26	13.6 ± 5.9 b	21	16.4 ± 7.3 b
	III (hosts in both cups)	25	50.1 ± 8.6 c	22	52.8 ± 9.7 c
	IV (cups alone)	9	55.5 ± 13.0 c	0	
2	I (hosts in blue cup)	27	68.3 ± 8.9 a	26	69.2 ± 9.2 a
	II (hosts in orange cup)	26	26.9 ± 8.3 b	25	26.6 ± 8.6 b
	III (hosts in both cups)	25	46.4 ± 8.5 ab	23	50.9 ± 9.1 ab
	IV (cups alone)	15	50.9 ± 12.0 ab	8	58.9 ± 18.0 ab

[a]Within each experiment means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05. Mean % of total response on orange egg cup = 100 - mean % of total response on blue egg cup.

Figure 4. Percent of *E. roborator* in groups VIII-XI in Exp. 5 probing the light or dark grey egg cup alone, both egg cups, or neither egg cup when hosts were not present in the cups. Bars marked with an asterisk are not significantly different from the 2 closest bars, but are significantly different from the lowest bar in the same subgraph [simultaneous 95% confidence intervals for differences between proportions (Miller 1981)]. Pre-test treatment for group VIII=hosts in lt.grey cup, for group IX=hosts in dk.grey cup, for group X=hosts in both cups, and for group XI=cups alone.

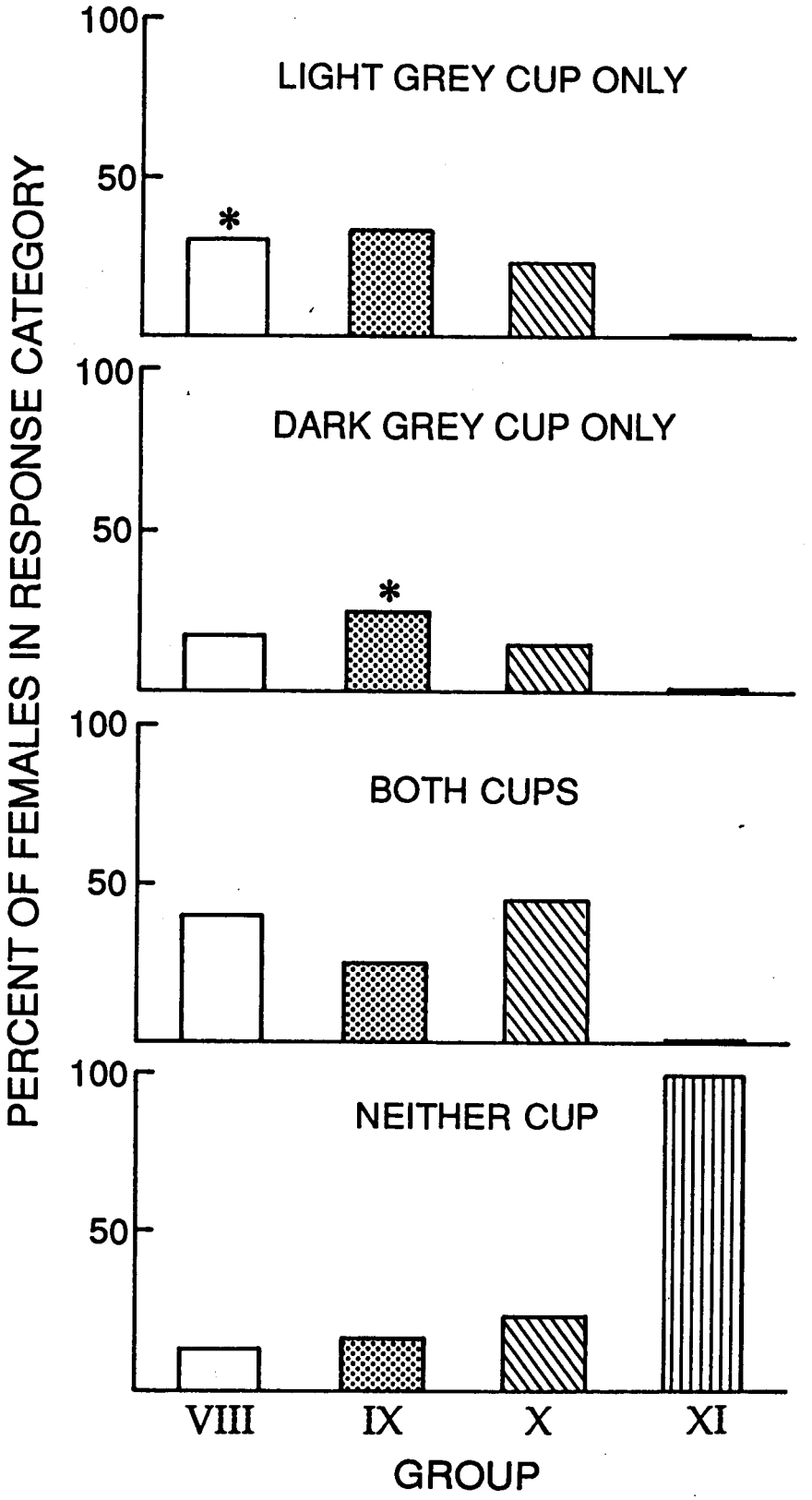

PERCENT OF FEMALES IN RESPONSE CATEGORY
100
50
LIGHT GREY CUP ONLY
*
100
50
DARK GREY CUP ONLY
*
100
50
BOTH CUPS
100
50
NEITHER CUP
VIII
IX
X
XI
GROUP

Table 3. Mean percent of total contacting and probing responses to egg cups directed at the light grey cup by responding *E. roborator* in groups VIII-XI during testing in Exp. 5 (hosts not present in cups).

Group (pre-test treatment)	No. females contacting egg cups	Mean % (±S.E.) of total contact time spent on light grey cup[a]	No. females probing egg cups	Mean % (±S.E.) of total probes executed on light grey cup[a]
VIII (hosts in lt.grey cup)	21	60.2 ± 7.5 a	20	60.6 ± 9.0 a
IX (hosts in dk.grey cup)	21	57.2 ± 9.2 a	20	57.5 ± 10.0 a
X (hosts in both cups)	20	56.6 ± 8.1 a	18	62.6 ± 9.0 a
XI (cups alone)	14	52.3 ± 11.0 a	0	

[a]Means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05. Mean % of total response on dark grey egg cup = 100 - mean % of total response on light grey egg cup.

In Exp. 2, the learned responses of females in groups I and II were weakened slightly when they were given the opportunity to attack hosts in egg cups of both colours (Fig. 3; Table 2, Exp. 2). Again, X^2 analysis of the numbers of females from each group in the 4 response categories revealed highly significant differences ($p<0.001$) between groups for both contacting and probing response patterns. Generally, even when hosts were present in both egg cups, females previously exposed to hosts only in a cup of 1 colour responded exclusively to the cup of that colour significantly more than females in other groups (Fig. 3). However, the 62% of group II females that contacted only the orange cup was significantly different only from the 24% of group IV females contacting only the cup of this colour (Fig. 3). Responding group I and II females still directed most of their responses to the cup of the colour they had previously experienced as a host microhabitat, with group I females devoting almost 70% of their activity to the blue cup and group II females directing a similar proportion of their responses to the orange cup (Table 2, Exp. 2). However, neither group I nor group II females differed significantly from control females in groups III and IV in the proportion of responses that they directed at the blue egg cup.

For groups I-III in Exp. 1 and 2, both total numbers of females responding and total response strength were similar, and significantly higher than for group IV (Table 4, Exp. 1 and 2). Thus, all females that were given hosts in egg cups during the pre-test treatment period appeared to learn equally to respond to egg cups as microhabitats. This general learning to respond to cups allowed differences to be revealed in specific responses to blue and orange cups.

Exposure to blue and orange egg cups without hosts for 7 days in Exp. 3 did not depress the responses of *E. roborator* to cups of either colour.

Table 4. Total contacting and probing responses to egg cups by *E. roborator* in groups I-IV in during testing in Exp. 1 (hosts not present in cups) and Exp. 2 (hosts present in cups), and by *E. roborator* in groups VIII-XI during testing in Exp. 5 (hosts not present in cups).

Exp.	Group (pre-test treatment)	Percent of females (±S.E.)[a] contacting cups	Percent of females (±S.E.)[a] probing cups	Mean total (±S.E.)[b] min in contact with cups	Mean total (±S.E.)[b] probes executed on cups
1	I (hosts in blue cup)	92.6 ± 5.0 a	88.9 ± 5.8 a	29.3 ± 3.4 a	17.3 ± 2.2 a
	II (hosts in orange cup)	100 a	80.8 ± 7.7 a	28.7 ± 3.7 a	15.3 ± 2.9 a
	III (hosts in both cups)	92.6 ± 5.0 a	81.5 ± 7.5 a	25.8 ± 3.7 a	14.3 ± 2.1 a
	IV (cups alone)	37.5 ± 10.0 b	0 b	0.7 ± 0.3 b	0 b
2	I (hosts in blue cup)	100 a	96.3 ± 3.7 a	44.7 ± 3.5 a	19.6 ± 2.2 a
	II (hosts in orange cup)	100 a	96.2 ± 3.8 a	43.6 ± 3.1 a	17.9 ± 1.8 a
	III (hosts in both cups)	96.2 ± 3.8 a	88.5 ± 6.7 a	41.5 ± 3.7 a	17.8 ± 1.8 a
	IV (cups alone)	60.0 ± 9.8 b	32.0 ± 9.3 b	14.0 ± 4.2 b	2.9 ± 1.1 b

Table 4. continued

Exp.	Group (pre-test treatment)	Percent of females (±S.E.)[a] contacting cups	Percent of females (±S.E.)[a] probing cups	Mean total (±S.E.)[b] min in contact with cups	Mean total (±S.E.)[b] probes executed on cups
5	VIII (hosts in lt.grey cup)	91.3 ± 6.2 a	87.0 ± 7.5 a	20.7 ± 2.4 a	14.3 ± 1.7 a
	IX (hosts in dk.grey cup)	87.5 ± 7.2 a	83.3 ± 8.3 a	21.7 ± 3.1 a	14.4 ± 2.0 a
	X (hosts in both cups)	90.9 ± 6.4 a	81.8 ± 9.1 a	18.8 ± 2.1 a	12.4 ± 1.7 a
	XI (cups alone)	63.6 ± 10.3 b	0 b	1.5 ± 0.4 b	0 b

[a]Within each experiment percentages in a column followed by the same letter are not significantly different, test for comparing >2 proportions and modified Newman-Keuls multiple comparisons procedure (Zar 1984), α=0.05.

[b]Within each experiment means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05. For data on probes executed 95% confidence intervals for mean total probes by females in groups I-III, Exp. 1, and groups VIII-X, Exp. 5, did not include 0; therefore, the data for these groups differed from those for group IV, Exp. 1, and group XI, Exp. 5, respectively.

Group IV' females did not differ significantly from group V females in numbers contacting the blue or orange egg cup exclusively, both cups, or neither cup (X^2 test, $p>0.05$), or in the proportion of contact time spent on the blue cup (Mann-Whitney Test, $p>0.05$). The total percentage of females contacting cups and the total time spent in contact were also similar for the 2 groups (Fisher Exact Test and Mann-Whitney Test, respectively, $p>0.05$), and very low. No females in either group probed the egg cups.

In Exp. 4, *E. roborator* did not discriminate between cups when visible differences between them were almost eliminated by mixing black with the blue and orange pigments. There were no significant differences between groups VI and VII in numbers of females contacting and probing (X^2 test, $p>0.05$) the black+blue or black+orange cup only, both cups, or neither cup. The mean proportions of their total responses that females in the 2 groups directed to each cup also did not differ significantly (Mann-Whitney Test, $p>0.05$), and their total contacting and probing responses to egg cups were similar (Fisher Exact Test and Mann-Whitney Test, respectively, $p>0.05$).

The total responses of females to light and dark grey egg cups without hosts in Exp. 5 again showed, as in Exp. 1 and 2, that all females given experience attacking hosts in cups (groups VIII-X) learned to respond to them as host microhabitats (Table 4, Exp. 5). However, females that had been given hosts only in the light grey (group VIII) or the dark grey (group IX) cup did not subsequently concentrate their responses on the cups of these intensities more than other females given access to hosts in egg cups (Fig. 4; Table 3).

There were no significant differences between groups in the numbers of females contacting either the light or the dark grey egg cup alone, both cups, or neither cup (X^2 test, $p>0.05$), or in the proportion of contact time females spent on the light grey cup (Table 3). There was a significant difference among groups in the numbers of females in each probing response category (X^2

test, $p<0.001$), but females in groups VIII and IX, respectively, did not probe the light or dark grey cup exclusively more than females in any other group except group XI (Fig. 4). Groups VIII-X also did not differ in their distribution of probes between the 2 egg cups (Table 3). Females in all 3 groups differed from females in group XI, that did not probe cups at all, in the strength of their probing responses to cups of both intensities (simultaneous 95% confidence intervals for mean probes on light and dark grey cups by responding females in groups VIII-X). Similarities between females in groups VIII-X in probing responses can be attributed to learning on their part to respond to egg cups in general as host microhabitats (Table 4).

DISCUSSION

The greater responsiveness of *E. roborator* in groups I and II to the blue and the orange egg cup, respectively, in Exp. 1 must have been the consequence of exposure to hosts only in a cup of one colour. Females in groups III and IV exposed to the same stimuli as females in groups I and II, but without specific pairing of hosts with a cup of one colour, did not subsequently display any colour preference. Nor did females inexperienced with hosts possess an innate attraction to cups of one colour or the other that could have been suppressed by prolonged exposure to the cups without reward (Exp. 3). Therefore, the colour preferences shown by both group I and II females resulted from learning of host microhabitat colour.

The results of Exp. 2 suggest that while this learning is strong, it is not so strong that learned preferences cannot be modified in response to the presence of hosts in microhabitats of other colours. Thus learning of one host microhabitat colour would not prevent females from finding hosts in microhabitats of other colours.

Both paints contained the same latex base, but chemical differences between the blue and orange pigments could have been detectable by *E. roborator*. However, when colour differences between the egg cups were greatly reduced in Exp. 4, females were unable to learn to discriminate between cups treated with blue or orange pigments. Therefore, it is very unlikely that *E. roborator* learned to use chemical cues to discriminate between blue and orange cups in Exp. 1 and 2.

If *E. roborator* possesses the characteristic hymenopteran spectral sensitivity (Menzel 1971; von Helversen 1972; Kretz 1979), it may not have perceived the orange and blue egg cups as being equally bright (Appendix 1). However, as there was no evidence in Exp. 5 for learned discrimination by the

parasitoid between egg cups of different reflective intensities, under the same conditions used in Exp. 1 and 2, it is very unlikely that the responses of females to blue and orange were caused by learning to distinguish between the 2 colours on the basis of perceived differences in intensity. With a spectral sensitivity similar to that of other hymenoptera, *E. roborator* probably would not have perceived intensity differences between the blue and orange fabrics greater than those tested between the light and dark grey fabrics (Appendix 1).

If *E. roborator* is similar to *A. mellifera*, which can detect intensity differences of 14-18% over a large range of illumination levels (Labhart 1974), it could have perceived the 2-fold difference in reflectance intensity between the light and dark grey egg cups. Thus its apparent lack of learning of this feature was probably not due to an inability to see this difference between the grey cups. The intensity of light reflected from a plant surface can vary considerably with angle and degree of illumination, and the perceived brightness of a plant can be affected by angle of view (Gates 1980; Prokopy and Owens 1983). Thus intensity of light may not be a reliable host micromicrohabitat cue for the parasitoid. However, intensity is more important than hue in host recognition by some insects (Owens and Prokopy 1986). Possibly *E. roborator* can learn host microhabitat brightness, but experimental conditions did not permit demonstration of such learning.

Foraging honey bees can readily distinguish many flowers from one another and from background vegetation or soil by their colours, and can use colour for long-range orientation to a food source (von Frisch 1971; Kevan 1978, 1983; Barth 1985). Although colour is not a unique characteristic of most flower types, learning of other characteristics such as odour or shape (Menzel 1985; Gould and Marler 1987) can be coupled with learning of colour,

so that bees can identify quite precisely flowers that are profitable sources of food (von Frisch 1971).

The many reported hosts of *E. roborator* (Thompson 1957) inhabit a diverse range of host plants and plant tissues that can differ considerably in colour from one another as well as from background vegetation. For example, late instar larvae of the codling moth, *Cydia pomonella* (L.), are found in ripe apples, which differ in colour from immature apples, those of other varieties, and apple foliage (Prokopy and Owens 1978; Owens and Prokopy 1986). Dying pine shoots, which mark the location of late-instar larvae of the European pine shoot moth, *Rhyacionia buoliana* Schiffermüller, differ in colour from live pine foliage. Possibly the parasitoid can distinguish these from other potential host microhabitats, and from their surroundings, in part by their colour. Thus learning of host microhabitat colour could function for *E. roborator* in a similar fashion to the way in which learning of flower colour is believed to function for foraging bees.

CHAPTER 2

LEARNING OF HOST MICROHABITAT FORM

INTRODUCTION

For some insects, cues involved in responses to food sources or oviposition sites can be provided by the form of these resources. Responses to aspects of host form such as size, shape, orientation, or pattern of arrangement of component parts occur in pollinators (Faegri and van der Pilj 1979), phytophagous insects (Prokopy and Owens 1983), and biting flies (Allan *et al.* 1987), and may also occur in predatory insects (Hagen *et al.* 1976). Such cues appear to be involved in the responses of a considerable number of parasitoids to their hosts, either through visual or tactile perception (Vinson 1976, 1985; Arthur 1981).

In a few cases, insects' responses to the form of feeding or oviposition sites or their surroundings are known to be influenced by learning. After contact with host plants or host-plant extracts applied to non-host plants the pipevine swallowtail butterfly, *Battus philenor* L., selectively searches for plants with similar leaf shape or leaf buds on which to oviposit (Papaj 1986a-d). Worker honey bees, *Apis mellifera*, can learn the size, shape, pattern, and orientation of elements of food source markers (von Frisch 1971; Wehner 1981; Gould 1984, 1985; Gould and Marler 1987). Ovipositional experience with a specific host fruit can affect the propensity of female apple maggot flies, *Rhagoletis pomonella* (Walsh), and Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), to respond with ovipositional behaviour to fruits and artificial fruit models of different sizes. Experienced flies may reject fruits and models not resembling the familiar host fruit in size (Papaj and Prokopy 1986; Prokopy and Fletcher 1987). Arthur (1967) showed that the ichneumonid parasitoid, *Itoplectis conquisitor*, could learn to discriminate between different host shelters on the basis of size and overall configuration, but not on the basis of orientation.

My objective was to document whether or not *E. roborator* could learn host microhabitat form, and, if so, to determine whether or not the parasitoid could be influenced by this learning when given the opportunity to attack hosts in microhabitats of different forms.

MATERIALS AND METHODS

Experiments 1 and 2

Pre-test treatments

To determine if *E. roborator* could learn host microhabitat form, females were reared, assigned to treatment groups, subjected to pre-test treatments (Table 5, Exp. 1 and 2), and prepared for testing as in the first 2 experiments in Chapter 1, except that the 2 artificial microhabitats placed in their cages each day during pre-test treatment differed in their form. These microhabitats (Fig. 5) were created using 2 styrofoam objects with approximately equal surface areas, a sphere 6.35 cm in diameter and a cylinder 2.54 cm in diameter and 14.6 cm in height. These forms were chosen because they resembled plant structures (fruits, shoots, stalks) in which some of the hosts of *E. roborator* are found. Each microhabitat was mounted on the tip of a disposable Pasteur pipette (Fisher Scientific, Toronto, Ontario), shortened to a length of 13 cm for spheres and 9 cm for cylinders. The blunt end of the pipette was attached to the centre of the overturned bottom half of a 60 x 15 mm disposable petri dish (Labtek, Miles Laboratories Inc., Naperville, Illinois). With this mount the microhabitats could be placed in cages with their centres at the same height, approximately 15 cm off the cage floor. Compartments in which hosts could be concealed were created in the surface of each microhabitat. A heated cork borer was used to melt 5 circular pits, 1.3 cm in diameter and 0.5 cm in depth. These depressions were arranged 1 cm apart in a straight line along the long axis of the cylinder, with the highest and lowest depressions 2 cm from the top and bottom of the cylinder, respectively. On the sphere, the depressions followed the surface curve around one side in a straight, vertical, line from a point 1 cm above the insertion of the Pasteur pipette to the top of the sphere. Each pit was covered by a taught, 1.5 x 1.5 cm piece of Kimwipe (Kimberly-Clark Ltd.,

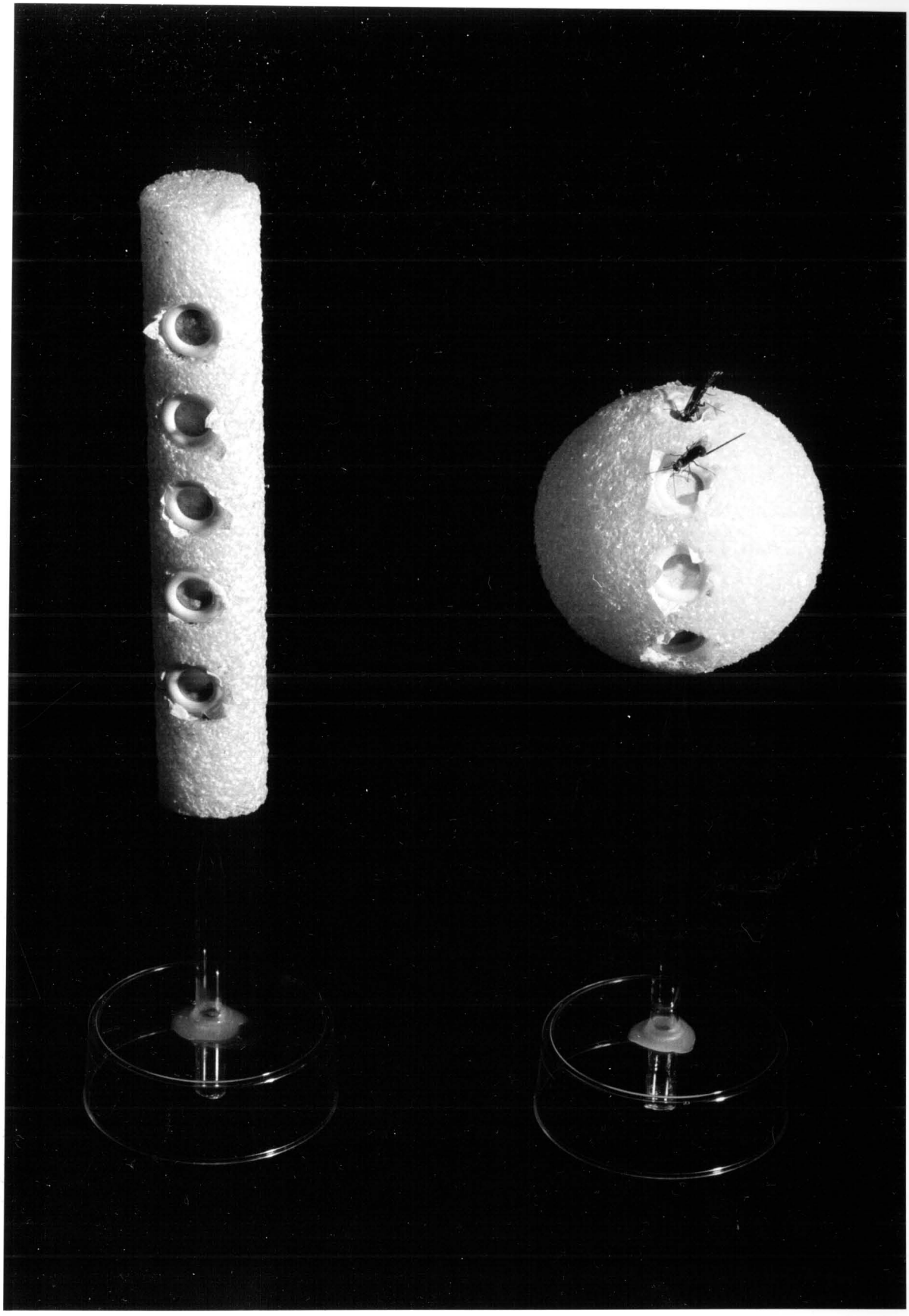
Table 5. Pre-test treatments and testing regimes for *E. roborator* in Exp. 1-3.

Exp.	Group	N	Pre-test treatment[a,b]	Testing regime[b]
1	I	24	Given 1 cylinder containing hosts and 1 sphere not containing hosts (hosts in cylinder)	Given 1 cylinder and 1 sphere, neither containing hosts, simultaneously for 1 h
	II	26	Given 1 cylinder not containing hosts and 1 sphere containing hosts (hosts in sphere)	"
	III	23	Given 1 cylinder and 1 sphere, both containing hosts (hosts in both microhabitats)	"
	IV	24	Given 1 cylinder and 1 sphere, neither containing hosts (microhabitats alone)	"
2	I	24	As for group I, Exp. 1	Given 1 cylinder and 1 sphere, both containing hosts, simultaneously for 1 h
	II	25	As for group II, Exp. 1	"
	III	24	As for group III, Exp. 1	"
	IV	23	As for group IV, Exp. 1	"
3	IV′	10	As for group IV, Exp. 1	As for Exp. 1
	V	10	Held without exposure to microhabitats or hosts	"

[a]Clean microhabitats and fresh hosts were placed in cages each day for 7 days.

[b]Hosts were 5 coddled larvae of *G. mellonella*.

Figure 5. Styrofoam microhabitats, with *E. roborator* probing host larvae in the sphere.



Toronto, Ontario) held in place with a 1.3 cm (outer diameter) white plastic ring (Tailorform, Symark Sales Co. Inc., Montreal, Quebec) pushed a few mm into the mouth of the pit. When hosts were required in the microhabitat, a single coddled larva of *Galleria mellonella* was concealed in each depression beneath the Kimwipe 'membrane'. Female *E. roborator* freely probed, fed, and oviposited on hosts in both the cylinder and the sphere (Fig. 5).

Each day clean microhabitats and fresh host larvae were placed in the cages. All microhabitats were cleaned between uses with 95% ethanol, and those used to present host larvae to females were never subsequently used without hosts.

Testing regimes

Females were assigned to Exp. 1 or 2 and assessed for evidence of learning as in the first 2 experiments in Chapter 1. In Exp. 1 females were simultaneously given 1 spherical and 1 cylindrical microhabitat, neither containing hosts, (Table 5, Exp. 1) and their responses to these microhabitats were monitored to determine if learning of microhabitat form, as indexed by contacting and probing responses, had occurred. Concentration of response by females in groups I and II on the cylinder and the sphere, respectively, when similar concentration by control group III and IV females did not occur, would indicate that the parasitoid had learned host microhabitat form. Females in Exp. 2 were tested in an identical manner, except that the 2 styrofoam microhabitats simultaneously placed in each test cage contained hosts (Table 5, Exp. 2). These females were monitored to determine whether or not any learning of microhabitat form revealed in Exp. 1 influenced females' choice of microhabitats in which to attack hosts.

The experimental procedures were repeated until >20 insects from each group had been tested in each experiment.

Experiment 3

Pre-test treatments

Exposure of control females in group IV (Exp. 1 and 2) to the cylinder and the sphere repeatedly without reward might have decreased their responsiveness and prevented detection of an innate attraction to one form or the other. To test this possibility females were reared, divided into 2 groups, subjected to pre-test treatments (Table 5, Exp. 3), and prepared for testing as in the third experiment in Chapter 1, except that group IV′ females were exposed to the same pre-test treatment as group IV females in Exp. 1 and 2 of this chapter (Table 5, Exp. 1 and 2).

Testing regime

Testing was carried out in random order on the 2 cages as in Exp. 1 (Table 5, Exp. 1 and 3). A reduction in responsiveness to a form due to exposure to the microhabitats alone would be suggested by significantly greater and more concentrated responses to either the cylinder or the sphere by group V females than by group IV′ females.

Statistical analysis

Response category data from Exp. 1-3 were analysed as in Exp. 1-3, Chapter 1, respectively, with females in each group within an experiment classified according to whether or not they responded to either microhabitat exclusively, both microhabitats, or neither microhabitat. When X^2 values were significant (α=0.05) for data from Exp. 1 and 2, simultaneous 95% confidence intervals were calculated for the differences between group I and groups II-IV in the proportion of females responding only to the cylinder, and between group II and groups I, III, and IV in the proportion of females responding only to the sphere. In addition, females in each group in Exp. 1 were

classified according to whether or not they contacted the cylinder or the sphere first, or did not contact either microhabitat, and 4 x 3 x^2 analysis (α=0.05) was used to detect differences between groups in the numbers of females in these first choice categories.

For all 3 experiments the mean proportions of total responses that were directed to the cylinder were calculated for the responding females in each group as in Chapter 1. These means, and also mean total responses to microhabitats by all females in each group, were compared (α=0.05) using the Kruskal-Wallis Test and multiple comparisons procedure of Conover (1980) for Exp. 1 and 2, and the Mann-Whitney Test (Conover 1980) for Exp. 3.

The percentages of all females in each group responding in total to microhabitats were compared (α=0.05) using a test for comparing >2 proportions and a modified Newman-Keuls multiple comparisons procedure (Zar 1984) for Exp. 1 and 2, and the Fisher Exact Test for Exp. 3.

RESULTS

Exeristes roborator learned host microhabitat form, although detection of form appeared to occur after contact with the microhabitats (Fig. 6; Table 6).

In Exp. 1, females exposed to hosts only in the cylinder (group I) or the sphere (group II) during pre-test treatment subsequently concentrated much of their host-seeking activities on these forms when they did not contain hosts (Fig. 6; Table 6). There were no significant differences between groups in the first contact choices of females (X^2 test, $p>0.05$), in the numbers of females contacting either microhabitat alone, both microhabitats, or neither microhabitat over the course of the test hour (X^2 test, $p>0.05$), or in the total numbers of females contacting microhabitats (Table 7, Exp. 1), suggesting that orientation to these microhabitats was not affected by prior experience with hosts in them. However, highly significant differences did occur in the numbers of females in the 4 probing response categories (X^2 test, $p<0.001$), with both group I and group II differing from one another and from control group IV in the proportions of females responding exclusively to the cylinder and the sphere, respectively (Fig. 6). However, group I and II females did not differ from control females in group III in this respect (Fig. 6). There were also significant differences between groups I and II in the distribution of responses by females between the cylinder and the sphere, with responding females in each group spending more than 70% of contact time and executing more than 80% of their ovipositor probes on the only microhabitat in which they previously had experience attacking hosts (Table 6). Neither group I nor group II differed from groups III and IV in distribution of contact time between forms, but responding females in group I favoured the cylinder with their probing responses more than responding females in both control groups (Table 6). Responding females in group II

Figure 6. Percent of *E. roborator* in groups I-IV in Exp. 1 probing the cylinder or sphere alone, both microhabitats, or neither microhabitat when hosts were not present in the microhabitats. Bars marked with an asterisk are not significantly different from the next lowest bar, but are significantly different from the 2 lowest bars in the same subgraph [simultaneous 95% confidence intervals for differences between proportions (Miller 1981)]. Pre-test treatment for group I=hosts in cylinder, for group II=hosts in sphere, for group III=hosts in both microhabitats, and for group IV=microhabitats alone.

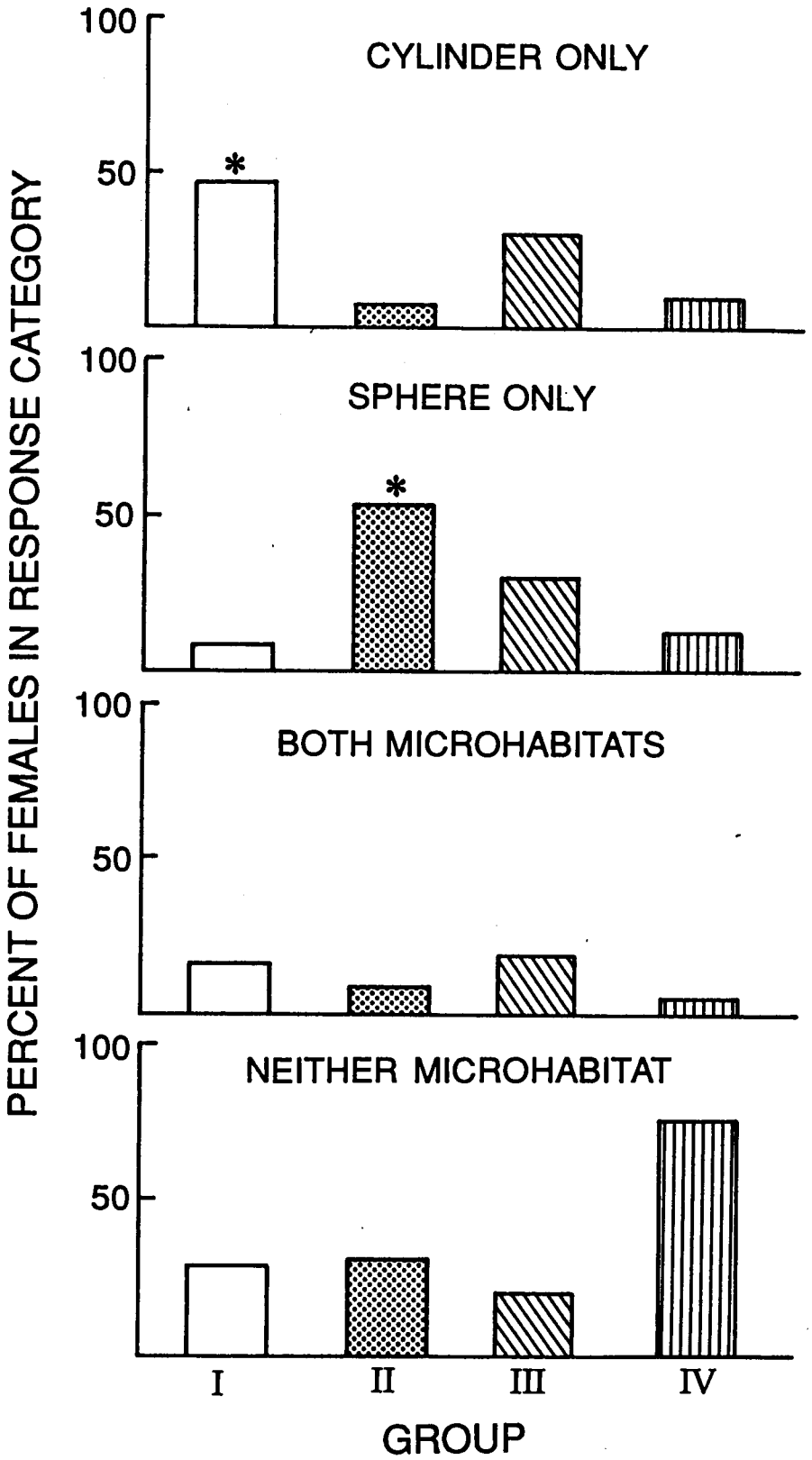

PERCENT OF FEMALES IN RESPONSE CATEGORY
100
50
CYLINDER ONLY
*
100
50
SPHERE ONLY
*
100
50
BOTH MICROHABITATS
100
50
NEITHER MICROHABITAT
I
II
III
IV
GROUP

Table 6. Mean percent of total contacting and probing responses to microhabitats directed at the cylinder by responding *E. roborator* in groups I-IV during testing in Exp. 1 (hosts not present in the microhabitats).

Group (pre-test treatment)	No. females contacting microhabitats	Mean % (±S.E.) of total contact time spent on cylinder[a]	No. females probing microhabitats	Mean % (±S.E.) of total probes executed on cylinder[a]
I (hosts in cylinder)	18	72.4 ± 8.8 a	17	81.1 ± 8.2 a
II (hosts in sphere)	20	24.4 ± 8.9 b	18	13.9 ± 7.7 b
III (hosts in both microhabitats)	18	54.3 ± 10.7 ab	18	52.0 ± 10.9 c
IV (microhabitats alone)	16	53.0 ± 11.5 ab	6	41.2 ± 20.0 bc

[a]Means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05. Mean % of total response on sphere = 100 - mean % of total response on cylinder.

Table 7. Total contacting and probing responses to microhabitats by *E. roborator* in groups I-IV during testing in Exp. 1 (hosts not present in microhabitats) and Exp. 2 (hosts present in microhabitats).

Exp.	Group (pre-test treatment)	Percent of females (±S.E.)[a] contacting microhabitats	Percent of females (±S.E.)[a] probing microhabitats	Mean total (±S.E.)[b] min in contact with microhabitats	Mean total (±S.E.)[b] probes executed on microhabitats
1	I (hosts in cylinder)	75.0 ± 8.8 a	70.8 ± 9.3 a	15.0 ± 2.9 a	9.3 ± 2.1 a
	II (hosts in sphere)	76.9 ± 8.3 a	69.2 ± 9.1 a	14.7 ± 3.4 a	8.5 ± 1.7 a
	III (hosts in both microhabitats)	78.3 ± 8.6 a	78.3 ± 8.6 a	18.7 ± 2.9 a	11.2 ± 1.8 a
	IV (microhabitats alone)	66.7 ± 9.6 a	25.0 ± 8.8 b	6.0 ± 1.7 b	1.7 ± 0.8 b
2	I (hosts in cylinder)	83.3 ± 7.6 a	83.3 ± 7.6 a	27.3 ± 3.6 a.	9.8 ± 1.7 a
	II (hosts in sphere)	76.0 ± 8.5 a	76.0 ± 8.5 a	25.2 ± 3.7 a	8.2 ± 1.5 a
	III (hosts in both microhabitats)	79.1 ± 8.3 a	79.1 ± 9.6 a	24.7 ± 3.4 a	10.3 ± 1.9 a
	IV (microhabitats alone)	69.6 ± 9.6 a	69.6 ± 9.6 a	18.7 ± 4.1 a	5.3 ± 2.1 b

[a]Within each experiment percentages in a column followed by the same letter are not significantly different, test for comparing >2 proportions and modified Newman-Keuls multiple comparisons procedure (Zar 1984), α=0.05.

[b]Within each experiment means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05.

differed significantly from those in group III in probe distribution, but the very large difference in distribution of probes between groups II and IV lacked significance statistically, probably due to the small number of probing females in group IV, and the large variation in their distribution of probes between the cylinder and sphere (Table 6). However, the significant difference between the 2 groups in propensity to probe only the sphere (Fig. 6) indicates that responding females in group II, like those in group I, showed a greater preference for the only microhabitat form in which they had previously attacked hosts.

In Exp. 1 females in groups I-III differed from group IV females in total numbers probing microhabitats, and in the strength of their total responses to microhabitats, but did not differ from one another in these responses (Table 7, Exp. 1). Thus exposure to hosts in microhabitats of different forms appeared to cause females in all 3 groups to learn equally to respond to these microhabitats. This general ability to learn to respond allowed disclosure of the differences caused by learning of specific microhabitat forms by females in groups I and II (Fig. 6; Table 6).

In Exp. 2, no influence of form learning could be detected in the responses of females in groups I and II to a cylinder and a sphere containing host larvae. There were no significant differences between groups in the numbers of females contacting or probing (X^2 test, $p>0.05$) either microhabitat alone, both microhabitats, or neither microhabitat, or in the distribution of contact time and ovipositor probes between the cylinder and the sphere by responding females (Kruskal-Wallis Test, $p>0.05$). Females with prior experience attacking hosts in styrofoam microhabitats (groups I-III) were superior to group IV females only in their total probing responses (Table 7, Exp. 2), indicating that the role of learning in responses to forms was much reduced when the forms contained hosts.

Exposure to the styrofoam cylinder and sphere without hosts for 7 days did not reduce the responses of *E. roborator* to microhabitats of either form. In Exp. 3, group IV′ and V females did not differ in numbers contacting or probing (X^2 test, $p>0.05$) either microhabitat alone, both microhabitats, or neither microhabitat, in the proportions of total contact time and ovipositor probes they directed to the cylinder, or in the strength of their total responses to microhabitats (Mann-Whitney Test, $p>0.05$). Similar total numbers of females also contacted and probed microhabitats in both groups (Fisher Exact Test, $p>0.05$).

DISCUSSION

The respective concentration of ovipositor probes by female *E. roborator* in both groups I and II in Exp. 1 on cylindrical and spherical microhabitats without hosts must have been caused by learning of microhabitat form. Females in groups III and IV, that together were exposed to the same stimuli as females in groups I and II, but without specific pairing of hosts with one form, did not concentrate their probing activities on either microhabitat. The lack of a strong response to either the cylinder or the sphere by group IV females was not the result of prolonged exposure to the microhabitats without reward, because females given this experience did not differ in their responses from females never exposed to the microhabitats (Exp. 3).

Considerable examination of the microhabitats during initial contact, and possibly initial probing, probably was necessary for clear discrimination between them by group I and II females, since insects in these 2 groups differed from those in both control groups only in their probing responses to the cylinder and the sphere. Thus, females probably devoted most of their probing activity to a microhabitat after distinguishing its form.

After contact, females could not have learned to use some distinguishing feature other than form, as the cylinder and sphere did not differ in their surface texture or appearance, consistency, or chemical composition.

The microhabitats of the natural hosts of *E. roborator* (Thompson 1957) can differ considerably in form from both surrounding vegetation and each other. Examples include apples (the codling moth, *Cydia pomonella*), cotton bolls (the pink bollworm, *Pectinophora gossypiella* (Saunders)), corn stalks (the European corn borer, *Ostrinia nubilalis* Hubner), and 'shepherd's crook' pine shoots (the European pine shoot moth, *Rhyacionia buoliana*). If such differences in form are perceptable to *E. roborator*, they could be useful to

the parasitoid in the identification of host microhabitats. While these natural host microhabitats are not unique examples of plant architecture, learning of their form, when combined with learning of other distinctive features, could contribute to recognition by the parasitoid of plant structures that are likely to contain suitable hosts.

Since learning did not affect females' responses to a host microhabitat until after contact in Exp. 1, it probably did not influence females in Exp. 2 because they detected the presence of larvae of *G. mellonella* on or before contact, and probed for them without assessing the form of the objects that housed them. Thus, learning of host microhabitat form expressed after contact would probably only influence responses to plant structures in natural situations in which hosts are not immediately detected. A female might search for a longer time on a structure of familiar form, even if she did not initially detect a host in that microhabitat.

The apple maggot, *Rhagoletis pomonella*, can learn to discriminate after contact between fruit models of different sizes (Papaj and Prokopy 1986). Städler (1977) has suggested that proprioreceptors in the leg joints and mechanoreceptors on the ovipositor of this and other insects could be involved in the perception of the size and surface curvature of objects. Similar mechanisms may have allowed female *E. roborator* to determine the form of the cylinder and sphere when in contact with them. Alternatively, the parasitoid could also have been using features that it detected visually during contact. Visual or tactile assessment of the form of an object after contact with it could have several advantages. The insect would not need a clear line of sight from a distance to the object, or need to have it contrast in some way with its background. The form perceived by the insect would not depend on its angle of view, and tactile senses might allow detection of details too subtle to be distinguished visually.

In some cases, learning of form by insects affects their response to an object before contact. For example, the shapes and sizes of flowers provide distinctive visual cues that worker honey bees, *Apis mellifera*, learn to use in conjunction with other flower features to identify profitable resources from a distance (von Frisch 1971; Gould 1984; Gould and Marler 1987). The lack of effect of form learning on orientation to host microhabitats from a distance by *E. roborator* could either reflect the experimental conditions or the fact that the parasitoid does not learn to use form in this way.

CHAPTER 3

LEARNING OF HOST MICROHABITAT ODOUR

INTRODUCTION

Olfactory cues are involved in the responses of many insects, including parasitoids, to food sources or oviposition sites (Vinson 1976, 1981, 1984b, 1985; Kogan 1977; Städler 1977; Galun 1977; Greany and Hagen 1981; Weseloh 1981; Williams 1983; Miller and Strickler 1984; Visser 1986). These volatile chemical cues can be constituents of food or oviposition sites or their surroundings, or can result from the interaction of these resources with other components of their environment.

Learning acquired through prior experience with food or oviposition sites may govern the responses of some insect species to olfactory cues. When offered a choice of foods, larvae of the tobacco hornworm, *Manduca sexta* (Johanssen), orient preferentially to the odours of foods on which they have previously fed (Saxena and Schoonhoven 1978, 1982). Induced preferences for familiar foods are reduced when the olfactory organs of *M. sexta* larvae are removed (Hanson and Dethier 1973). Prior feeding experience enhances the responses of adults of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, to potato volatiles (Visser and Thiery 1986). Adult houseflies, *Musca domestica*, (Fukushi 1979, 1983) and fruit flies, *Drosophila melanogaster* Meigen, (Tempel *et al.* 1983), worker honey bees, *Apis mellifera*, (von Frisch 1971; Wells 1973; Menzel 1985; Gould and Marler 1987), and females of the American cockroach, *Periplaneta americana* (L.), (Balderrama 1980) can learn and become attracted to odours associated with sugar sources upon which they feed. Some odours loose their repellency for *D. melanogaster* and other insects when associated with larval foods, or environments in which adults feed (Thorpe 1939; Crombie 1941, 1944; Hershberger and Smith 1967; Manning 1967). These results may not depend on feeding by the insect, however, as exposure to the odour alone can have similar effects (Crombie 1944; Manning 1967).

Lewis and Tumlinson (1988) have demonstrated that the braconid parasitoid, *Microplitis croceipes* (Cresson), learns odours associated with host faeces during contact with the faeces. Vet and van Opzeeland (1984) have shown that *Asobara tabida* (Nees) and *A. rufescens* (Foerster), 2 braconids that attack drosophilids, learn to distinguish the odour of infested host microhabitats through experience attacking hosts in these microhabitats. As well, the microhabitat odour preferences of females of these 2 species (Vet and van Opzeeland 1984) and of 2 eucoilid parasitoids of drosophilids, *Leptopilina heterotoma* (Thomson) (Vet and van Opzeeland 1985) and *L. clavipes* (Hartig) (Vet 1983), can be modified by experience with hosts in different microhabitats. For *L. clavipes*, rearing on hosts in a particular microhabitat, or exposure of adults to traces of it upon eclosion, can increase females' subsequent attraction to the odour of that host microhabitat (Vet 1983). In the braconid, *Aphaereta minuta* (Nees), the same experience influences the ability of females to distinguish between the odours of infested and uninfested microhabitats (Vet 1985b). The odour of larvae of the wax moth, *Achroia* (=*Melliphora*) *grisella* (F.), becomes attractive to females of the ichneumonid *Venturia* (=*Nemeritis*) *canescens* (Gravenhorst) when they have been reared on this factitious host, or exposed to it or its odour as adults (Thorpe and Jones 1937; Thorpe 1938). These females also become almost indifferent to a strongly repellent odour, that of cedar wood oil, when it is present in environments in which they feed (Thorpe 1938), and become attracted to a non-host odour, geraniol, when they are given the opportunity to attack hosts in association with it (Arthur 1971).

This chapter describes research undertaken to investigate the hypothesis that *E. roborator* can learn the odour of host microhabitats, and, if it does, to try to determine if this learning plays a role in responses to host microhabitats by the parasitoid.

MATERIALS AND METHODS

Learning of host microhabitat odour

Experiment 1

Pre-test treatments

To determine if *E. roborator* could learn host microhabitat odour, females were reared, divided into groups, and subjected to pre-test treatments (Table 8, Exp. 1) as in the first 2 experiments in Chapter 1, except that the 2 fresh artificial host microhabitats placed in their cages each day differed in their odour. These microhabitats were constructed by securing a folded Kimwipe between the inverted lid and rim of a 150 ml styrofoam cup, as was done with fabric discs (Fig. 1). Excess tissue was trimmed from outside the cup rim. To give one of these egg cups the scent of ripe apples, a 10 x 10 cm piece of Parafilm (American Can Co., Greenwich, Ct.) that had been stretched around a ripe Red Delicious apple (Papaj and Prokopy 1986) and left for 24 h at room temperature was compressed and placed beneath the folds of the Kimwipe. This scent was chosen as representing the odour of the microhabitat of one of the natural hosts of *E. roborator*, the codling moth, *Cydia pomonella* (Thompson 1957). A stretched 10 x 10 cm piece of Parafilm that had not been treated was compressed and placed beneath the Kimwipe of the second egg cup. Five coddled larvae of *G. mellonella* were concealed between the folds of the Kimwipe when the presence of hosts in an egg cup was required. Female parasitoids probed, fed, and oviposited on hosts through the Kimwipe.

After 7 days of pre-test treatment all females were marked on the thorax with a dot of paint to identify their treatment group and were placed together in a 30 x 30 x 45 cm holding cage in a room used only for olfactory

Table 8. Pre-test treatments and testing regimes for *E. roborator* in Exp. 1-3.

Exp.	Group	N	Pre-test treatment[a]	Testing regime
1	I	42	Given 1 egg cup with apple-scented Parafilm containing hosts and 1 egg cup with untreated Parafilm not containing hosts (hosts in apple-scented Parafilm cup)	Given 10 min in 2-choice static-air olfactometer with apple-scented and untreated Parafilm odour sources
	II	42	Given 1 egg cup with apple-scented Parafilm not containing hosts and 1 egg cup with untreated Parafilm containing hosts (hosts in untreated Parafilm cup)	"
	III	42	Given 1 egg cup with apple-scented Parafilm and 1 egg cup with untreated Parafilm, both containing hosts (hosts in both cups)	"
	IV	42	Given 1 egg cup with apple-scented Parafilm and 1 egg cup with untreated Parafilm, neither containing hosts (cups alone)	"
2	IV′	10	As for group IV, Exp. 1	As for Exp. 1
	V	10	Held without exposure to egg cups or hosts	"
3	I′	23	As for group I, Exp. 1	Given 1 egg cup with apple-scented Parafilm and 1 egg cup with untreated Parafilm, neither containing hosts, simultaneously for 1 h
	II′	22	As for group II, Exp. 1	"

[a]Fresh egg cups and hosts were placed in cages each day for 7 days. Hosts were 5 coddled larvae of *G. mellonella*.

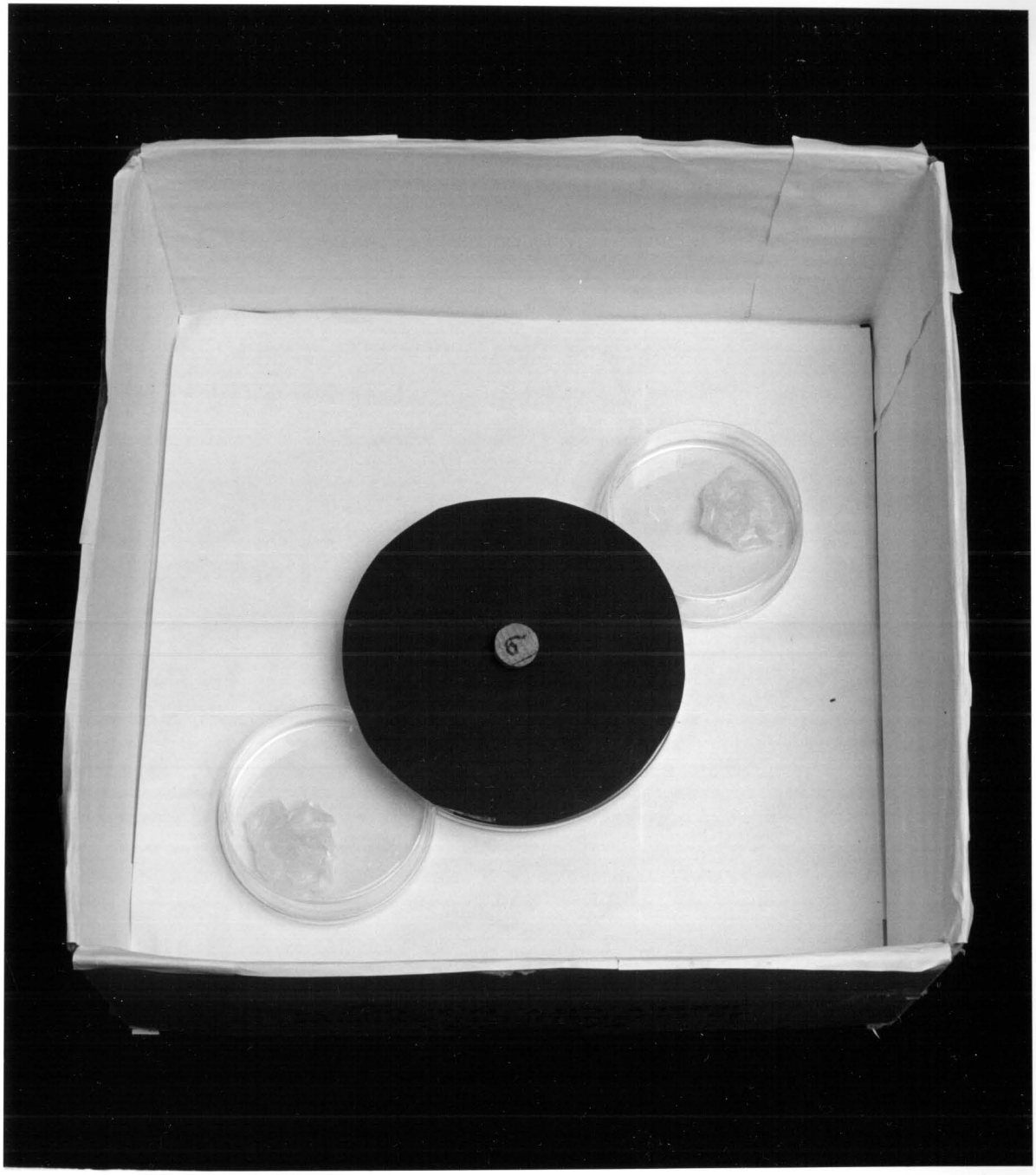
experiments. Otherwise, conditions were similar to those in the pre-test treatment room.

Testing regime

On the following day females were tested individually in one of three 2-choice, static-air olfactometers (Table 8, Exp. 1). These devices (Fig. 7) consisted of a central release chamber made from a 150 x 25 mm disposable Labtek petri dish and 2 smaller odour chambers made from 100 x 25 mm disposable Labtek petri dishes (Miles Laboratories Inc., Naperville, Illinois). The walls of the dishes were cut so that the odour chambers could be attached to opposite sides of the central release chamber, leaving 2, 40 x 25 mm rectangular openings 180° apart from one another through which females could leave the release chamber and enter the odour chambers. The 3 joined bodies of the petri dishes separated as a group from their joined lids, so that the olfactometer could be opened to remove insects or replace odour sources. The walls and ceiling of the central release chamber were covered with black construction paper. A 17 mm hole in the centre of the ceiling of the release chamber provided the entry point for test insects.

During testing, the olfactometer was placed in a 33 x 33 x 15 cm box lined with white paper to prevent external visual stimuli from influencing test results. One chamber contained a piece of apple-scented Parafilm identical to that used in pre-test treatments, while the other contained a piece of untreated Parafilm. These odour sources were visually identical. Six females from each group were tested in the olfactometer in a day. Each was removed from the holding cage in a capped 5 ml black-painted shell vial at the beginning of the preceeding test, and usually spent 10-20 min in the vial before she was released by inverting the opened vial over the hole in the ceiling of the central chamber of the olfactometer. She was given 10 min to leave the vial and 10 min in the olfactometer from time of entry. A record

Figure 7. Two-choice, static-air olfactometer in which *E. roborator* in Exp. 1 and 2 were tested.



was kept of whether or not she entered either odour chamber during this latter 10 min period, and of how long she spent in each chamber. If a female did not leave the vial within 10 min she was replaced with another, identically-handled member of the same group.

Parasitoids in groups I and II (Table 8, Exp. 1) were tested to determine if prior experience with hosts only in cups with apple-scented or untreated Parafilm, respectively, would cause them to prefer to visit and spend time near these odour sources to a significantly greater degree than females in the other groups, thus providing evidence that they had learned the odour of their host's microhabitat. Control females in groups III and IV were tested to determine if females would exhibit a similar preference for one odour source or the other after access to hosts in cups or exposure to cups alone, respectively, without specific pairing between hosts and cup odour.

Each day females were tested in random order within 6 quartets made up of one female from each group. One olfactometer was used to test 2 quartets, after which it was replaced with a clean apparatus. For each quartet, fresh pieces of Parafilm were placed in the olfactometer, and the position of the odour chambers was reversed by rotating the apparatus 180°.

At the end of each day, olfactometers were washed with Sparkleen detergent (Fisher Scientific Co., Nepean, Ontario) and rinsed with 95% ethanol. Odour chambers that contained apple-scented Parafilm on one test day contained untreated Parafilm on the next day and *vice versa*, so that the position of odour sources within one apparatus was reversed each time it was used for testing.

Forty-two females from each group were tested in the olfactometer.

Experiment 2

Pre-test treatments

Repeated exposure of females in control group IV (Exp. 1) to the unrewarded odour sources could have lowered their response to either one, and prevented detection of an innate preference for one of the odours. To determine if this had occurred, females were reared, divided between 2 groups, and subjected to pre-test treatments (Table 8, Exp. 2) as in the third experiment in Chapter 1, except that group IV′ females were exposed to the same pre-test treatment as group IV females in Exp. 1 of this chapter (Table 8, Exp. 1). At the end of pre-test treatment, 10 females from each group were prepared for olfactometer testing, as described for Exp. 1.

Testing regime

The testing procedure was the same as that used in Exp. 1 (Table 8) except that females were tested in random order within pairs made up of 1 individual from each group. One olfactometer was used to test 5 pairs. For each pair fresh pieces of Parafilm were placed in the odour chambers, and the apparatus was rotated 180°. If group V females showed a significantly greater preference than group IV′ females for either odour source, it would suggest that exposure to the unrewarded odours had suppressed an innate tendency of the latter females to respond to that odour.

Effect of odour learning on response to egg cups in a cage bioassay

Experiment 3

Pre-test treatments

To determine if odour learning could influence the choice by *E. roborator* of microhabitat in which to search for hosts, females reared as in Exp. 1 were divided randomly into 2 groups (I′ and II′) that were subjected to pre-test treatments identical to those experienced in Exp. 1 by groups I and II, respectively (Table 8.). After 7 days, the females in each group were marked with paint for identification and divided randomly between 2 test cages.

Testing regime

On the following day, females were monitored for their responses to egg cups as in Chapter 1, except that they were given simultaneously one fresh egg cup with apple-scented Parafilm and one fresh egg cup with untreated Parafilm, neither of which contained hosts (Table 8, Exp. 3). If females concentrated their responses on the egg cup in which they had attacked hosts during pre-test treatment, this would suggest that they were responding to cups on the basis of learned odours.

Experimental procedures were repeated until >20 insects were tested in each group.

Statistical analysis

Experiment 1

Females in each group were classified according to whether or not they entered either chamber alone, both chambers, or neither chamber, and this

response category data was analysed as in Exp. 1, 2, and 5 in Chapter 1. Where X^2 values were significant (α=0.05), simultaneous 95% confidence intervals (Miller 1981) were calculated for the differences between group I and groups II-IV in the proportion of females entering only the apple-scented Parafilm chamber, and between group II and groups I, III, and IV in the proportion of females entering only the untreated Parafilm chamber.

The mean proportion of total time spent in odour chambers that was spent in the apple-scented chamber was determined for all responding parasitoids (females entering at least one odour chamber) in each group. These means were compared with the Kruskal-Wallis Test and multiple comparisons procedure of Conover (1980) (α=0.05).

Experiment 2

Females were classified according to whether or not they entered either odour chamber alone or both odour chambers (all females entered at least 1 chamber), and 2 x 3 X^2 analysis (α=0.05) was used to detect differences between groups in the numbers of females in these categories. Mean proportions of total time spent in chambers that were spent in the apple-scented chamber by the females in the 2 groups were compared with the Mann-Whitney Test (Conover 1980) (α=0.05).

Experiment 3

Females in each group were classified according to whether or not they contacted or probed either egg cup alone, both cups, or neither cup, and the numbers of females in these response categories were compared as in Exp. 3 and 4 in Chapter 1. Mean proportions of total contacting and probing responses to cups that were directed to the apple-scented egg cup, and mean total responses to egg cups were also calculated and compared as in Exp. 3 and 4 in Chapter 1.

RESULTS

Female *E. roborator* were able to learn the odour of a host microhabitat (Fig. 8; Table 9), but this learning did not influence their response to host microhabitats.

In Exp. 1, only females exposed to hosts exclusively in the egg cup with apple-scented Parafilm (group I) preferred this odour (Fig. 8; Table 9). The pattern of entry into odour chambers differed significantly between groups (X^2 test, $p<0.001$), with females in group I entering only the apple-scented Parafilm chamber significantly more than females in the other 3 groups (Fig. 8). Females in group II, however, did not enter the untreated Parafilm chamber exclusively significantly more than females in any other group except group I (Fig. 8). There were also statistically significant differences between groups in the distribution of time spent in odour chambers, with responding group I females spending almost 75% of their total chamber time in the apple-scented chamber (Table 9). Females in the other 3 groups spent <50% of their total chamber time in this chamber, and did not differ from each other in the distribution of their time between odour chambers (Table 9).

In Exp. 2, exposure to the 2 egg cups without hosts for 7 days did not alter the responses of *E. roborator* to apple-scented or untreated Parafilm. Group IV′ and V females did not differ significantly in the numbers of females entering either odour chamber alone or both chambers (X^2 test, $p>0.05$), or in their distribution of time spent in odour chambers between the apple-scented and untreated Parafilm chambers (Mann-Whitney Test, $p>0.05$).

Figure 8. Percent of *E. roborator* in groups I-IV in Exp. 1 entering the apple-scented Parafilm or untreated Parafilm odour chamber alone, both odour chambers, or neither chamber in a 2-choice static-air olfactometer. Bar marked with an asterisk is significantly different from all other bars in the same subgraph. Bar marked with a diamond is not significantly different from the next 2 greatest bars, but is significantly different from the lowest bar in the same subgraph [simultaneous 95% confidence intervals for differences between proportions (Miller 1981)]. Pre-test treatment for group I=hosts in apple-scented Parafilm cup, for group II=hosts in untreated Parafilm cup, for group III=hosts in both cups, and for group IV=cups alone.

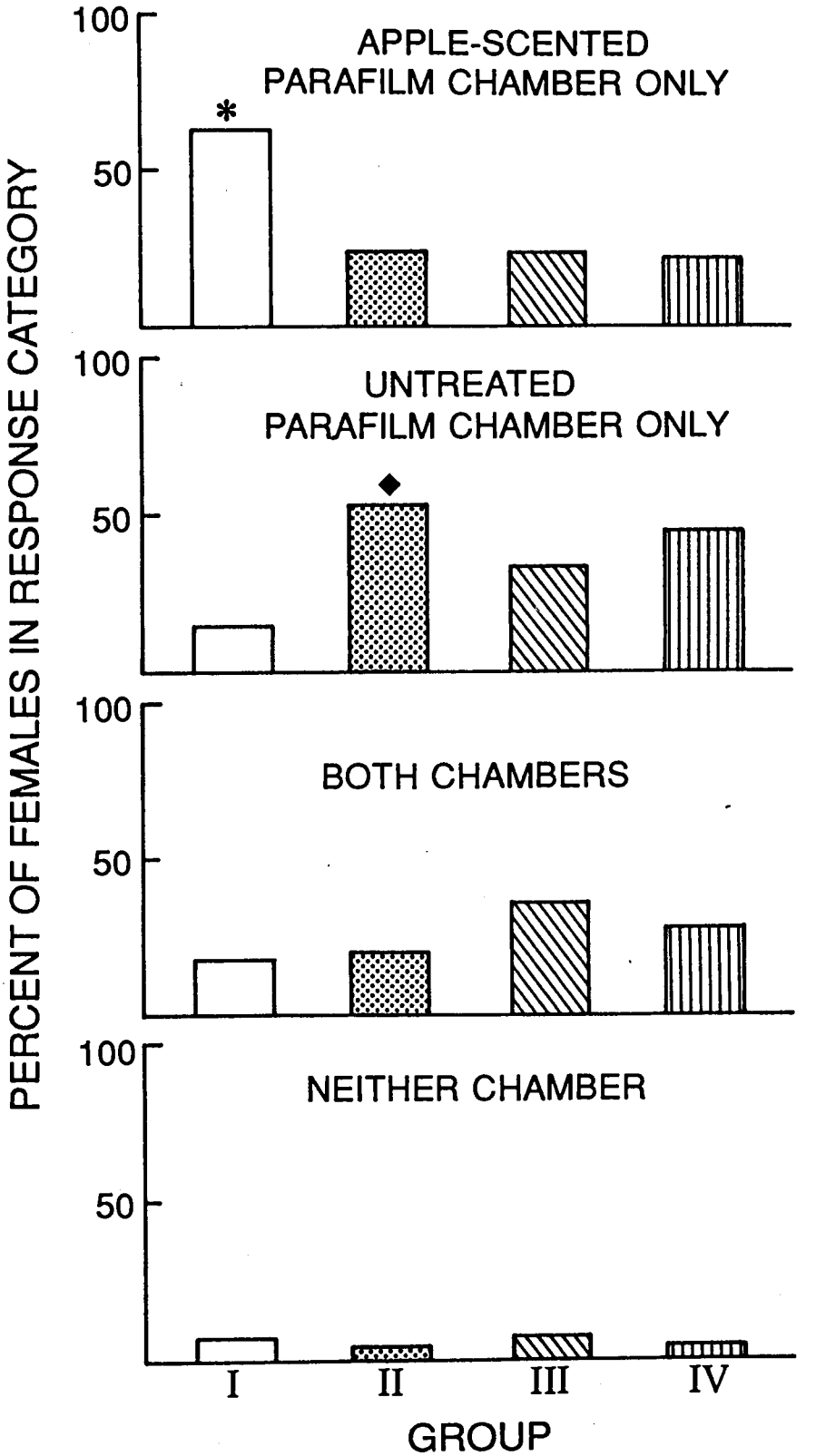

APPLE-SCENTED
PARAFILM CHAMBER ONLY
UNTREATED
PARAFILM CHAMBER ONLY
BOTH CHAMBERS
NEITHER CHAMBER
100
50
PERCENT OF FEMALES IN RESPONSE CATEGORY
I
II
III
IV
GROUP

Table 9. Mean percent of total time in olfactometer odour chambers spent in apple-scented Parafilm chamber by responding *E. roborator* in groups I-IV during testing in Exp. 1.

Group (pre-test treatment)	No. females entering odour chambers	Mean % (±S.E.) of total time in odour chambers spent in apple-scented Parafilm chamber[a]
I (hosts in apple-scented Parafilm cup)	39	74.3 ± 6.4 a
II (hosts in untreated Parafilm cup)	40	33.9 ± 7.1 b
III (hosts in both cups)	39	46.4 ± 6.6 b
IV (cups alone)	40	40.8 ± 6.9 b

[a]Means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05. Mean % of total time in odour chambers spent in untreated Parafilm chamber = 100 - mean % of total odour chamber time spent in apple-scented Parafilm chamber.

In Exp. 3, females exposed to hosts only in an egg cup of one odour did not show a preference for the cup with that odour in the cage bioassay. There were no significant differences between groups I' and II' in the numbers of females contacting or probing (X^2 test, $p>0.05$) the apple-scented Parafilm or untreated Parafilm egg cup alone, both egg cups, or neither cup, or in the total responses of females to egg cups (Mann-Whitney Test and Fisher Exact Test, respectively, $p>0.05$).

DISCUSSION

In Exp. 1 the elevated response of group I females to the apple-scented Parafilm odour source must have resulted from learning of host microhabitat odour, because control females in groups III and IV, that between them were exposed to the same stimuli as group I females, but without specific pairing between hosts and the apple-scented egg cup, did not display the same preference. The extended period of exposure to this odour source without reward experienced by group IV females did not cause their lack of preference for this odour, as was shown by the similarity of response between group IV' and V females in Exp. 2. Group II females apparently did not learn to respond to untreated Parafilm, probably because it had no detectable odour. However, a situation in which females were able to detect but unable to learn the odour of untreated Parafilm could arise if odour learning in *E. roborator* reflects a bias similar to that in worker honey bees, that learn to associate flower odours with food sources more readily than odours that have no natural significance for them in this context (Barth 1985; Menzel 1985; Gould and Marler 1987).

The lack of a significant difference between groups I' and II' in response to egg cups (Exp. 3) demonstrated that females were not responding to learned microhabitat odour under the bioassay conditions. The results of Exp. 1 show that females could determine whether or not an egg cup was the source of an odour, as females in group I must have been able to pinpoint the scented egg cup in which they attacked hosts in order to learn that apple scent marked host microhabitats. In additional cage bioassays with 8 other volatile compounds *E. roborator* exposed to hosts only in egg cups having one specific odour also showed no subsequent tendency to favour cups with the same odour (unpublished results).

Two hypotheses could explain the lack of evidence for learning of odour in the egg cup bioassay. Possibly, it did not provide a situation in which odour learning would be used by the parasitoid. Alternatively, visual cues from the two visually-identical egg cups may simply have provided such strong stimuli that the odour cues were overridden.

The time spent by group I females in the apple-scented chamber of the olfactometer could have reflected a response to the 'taste' of apple-treated Parafilm. Females could have learned this 'taste' while attacking hosts in egg cups, since their antennae, tarsi, and ovipositors, all of which may possess contact chemoreceptors (Vinson 1985), could have penetrated the porous Kimwipe covering the Parafilm. However, possible learning of Parafilm 'taste' through such contact did not appear to influence females' behaviour towards these odour sources, because group I' and II' females in Exp. 3 did not differ in their responses after contacting egg cups without hosts. Moreover, when in the apple-scented odour chamber in the olfactometer, group I females did not spend appreciable amounts of time in contact with the Parafilm, but instead spent their time walking around or standing in the chamber.

Little is known about the physiological ability of parasitoids to detect the odours of their hosts' microhabitats, and to distinguish them clearly from other olfactory stimuli. Phytophagous insects are believed to be able to detect plant odours and distinguish even subtle differences between them, both through the detection of unique volatiles released by some plants, and through the ability to assess the composition of volatile blends, which are released in characteristic ratios by different plants (Städler 1977; Finch 1980; Miller and Strickler 1984; Visser 1986). Behavioural evidence suggests that parasitoids may possess similar abilities to detect and distinguish different plant odours (Thorpe and Caudle 1938; Monteith 1955, 1960, 1966; Arthur 1962; Herrebout and van der Veer 1969; Read *et al.* 1970; Camors and Payne 1972;

Schuster and Starks 1974; Shajahan 1974; Kennedy 1979; Nettles 1979, 1980; Elzen *et al.* 1983, 1984a,b, 1986, 1987; Powell and Zhang Zhi-Li 1983), as well as the odours of other host microhabitats (Laing 1937; Thorpe and Jones 1937; Vet 1983, 1985b; Vet *et al.* 1983, 1984; Vet and van Opzeeland 1984, 1985). While nothing is known about the general ability of *E. roborator* to detect the odours of plants and plant parts, it is obvious from my results that the parasitoid can detect the odour of ripe apples, the microhabitat of one of its hosts, the codling moth. As well, the food plants of 2 of its other hosts, the cotton bollworm, *Pectinophora gossypiella*, and the European pine shoot moth, *Rhyacionia buoliana*, possess odours detectable to other ichneumonids that attack hosts on these plants (Arthur 1962; Elzen *et al.* 1983, 1984a,b, 1986, 1987). The ichneumonid *Itoplectis conquisitor*, which also attacks *R. buoliana*, can distinguish between the odours of different pine species (Arthur 1962).

As in *A. mellifera* (von Frisch 1971), learning of host microhabitat odour could act in conjunction with learning of visual cues to increase the precision with which *E. roborator* distinguishes profitable host microhabitats. It could also act alone to allow the parasitoid to detect microhabitats when visual cues are obstructed, or when a host microhabitat does not have distinctive visible characteristics. Due to their susceptibility to disruption by air currents, odour cues may be much less multidirectional than visual ones (Miller and Strickler 1984). However, since odour can be carried away from its source by air currents it might provide cues to the parasitoid over a greater distance than visual cues.

CHAPTER 4

HOW AND WHEN *EXERISTES ROBORATOR* LEARNS

INTRODUCTION

For different insect species there can be considerable variation in the nature of experiences with feeding or oviposition sites that result in learning which influences subsequent responses to these resources. The importance of the timing of these experiences also varies between species.

Foraging honey bees, *Apis mellifera*, require few rewarded visits to learn how to handle even complex flowers properly (Weaver 1956, 1965). They learn the odours of most food sources accurately after only one rewarded visit, and learn most colours after 1-5 such visits (Menzel and Erber 1978; Gould 1984; Gould and Marler 1987). However, up to 15 visits are needed to learn the shapes of food sources with similar accuracy (Gould 1984, 1985; Gould and Marler 1987). The bee's taste receptors must be stimulated for colour learning to occur, but, provided the sugar reward is above threshold, its concentration does not influence learning (Menzel and Erber 1972). After either very few or many rewarded visits to a coloured food source, honey bees will readily change to another sugar source of a different colour after only one reward, although they are more reluctant to switch if they have made an intermediate number of rewarded visits to the first sugar source (Menzel 1985).

In contrast, bumble bees appear to require many visits to learn how to handle complex flowers efficiently (Heinrich 1979a; Laverty 1980), and to learn the visual characteristics of food sources (Heinrich *et al.* 1977). They also do not appear to learn to shift between food sources with different reflectance characteristics as readily as honey bees (Heinrich *et al.* 1977).

Like honey bees, houseflies, *Musca domestica*, learn food odours accurately after a single feeding experience (Fukushi 1983), and blowflies, *Lucilia cuprina*, learn colours with considerable accuracy after 2 feeding

trials (Fukushi 1985). However, the learning performance of flies can be affected by the concentration of the reward (Fukushi 1983).

American cockroaches, *Periplaneta americana*, also learn the odour of food quickly, and learn to switch to new food sources readily as well. Two short rewarded visits to a scented sugar source, coupled with a visit to a negatively reinforced, different odour source cause marked learning. This experience is also sufficient to cause reversal of earlier learning (Balderrama 1980).

For phytophagous insects, learned responses to food sources are acquired after variable periods of feeding. The grasshoppers, *Melanoplus sanguinipes* and *Schistocerca americana* (Drury), respectively, can learn characteristics of the environment in which they feed, and can learn to avoid foods associated with an aversive stimulus, after one meal (Bernays and Wrubel 1985; Bernays and Lee 1988). After 24 h of feeding *ad lib.* larvae of the corn earworm, *Heliothis zea* (Boddie), show an induced preference for familiar food plants. On the other hand, 48 h of feeding does not induce a food plant preference in larval tobacco hornworms, *Manduca sexta*, although feeding for an entire stadium brings about this induction (Jermy *et al.* 1968; Hanson and Dethier 1973).

In female lepidoptera searching for oviposition sites, contact with a substrate exhibiting suitable chemical characteristics can be sufficient to induce learning; successful oviposition is not necessarily required as reinforcement. The pipevine swallowtail, *Battus philenor*, can learn to associate aspects of plant form with host plant chemicals detected during a single landing (Papaj 1986a), and the cabbage butterfly, *Pieris rapae*, learns the reflectance characteristics of host plants and substrates treated with sinigrin after a single contact with its tarsi (Traynier 1984). For *P. rapae*, reversal of learning can occur after contact with some, but not all, host

plants (Traynier 1984), and the strength of learning can be influenced by the concentration of sinigrin on the substrate (Traynier 1986). Learned preferences of *B. philenor* for one host can be readily reversed by contact with another (Papaj 1986 a,d; Papaj and Rausher 1987a), but females appear to become more conservative about changing hosts as they gain experience with one host (Papaj 1986c).

True fruit flies learn quickly from the experience of ovipositing in one fruit type. Females of the apple maggot fly, *Rhagoletis pomonella*, and the Mediterranean fruit fly, *Ceratitis capitata*, distinguish between familiar and unfamiliar fruits after 4 and 5 ovipositions into 1 fruit type, respectively. For both flies, the same number of ovipositions into a different host subsequent to initial conditioning causes a complete reversal in learned responses (Prokopy *et al.* 1982; Cooley *et al.* 1986).

Female parasitoids also learn from varying experiences with hosts. *Bracon mellitor* Say can learn to associate a chemical with host larvae after 35 min of exposure to contaminated hosts. Modification of this learning does occur with further, different experience, although initial acquisition of learning appears to occur more readily (Vinson *et al.* 1977). With as little as 1-3 h of opportunity to oviposit on their drosophilid hosts in various microhabitats, braconid and eucoilid parasitoids can usually learn chemical characteristics of these hosts and their microhabitats (Vet 1983, 1985a; Vet and van Opzeeland 1984, 1985). A similar amount of further experience with a different host/microhabitat system can reverse learning in braconid parasitoids (Vet and van Opzeeland 1984).

The ichneumonid, *Venturia canescens*, develops an attraction to the odour of a factitious host if exposed to its odour alone for 1.5-2 days (Thorpe 1938); the attraction is just as strong as that developed when the parasitoid is allowed to contact the host (Thorpe and Jones 1937). Direct attack upon a

host is not necessary for learning of host-associated cues by the braconid, *Microplitis croceipes* (Cresson). Drost *et al.* (1986) showed that a short period of contact with host faeces increases orientation by this parasitoid to a feeding host in a wind tunnel. Lewis and Tumlinson (1988) subsequently demonstrated that one bout of antennal contact with host faeces causes *M. croceipes* to learn to associate odours with host-specific chemicals present in the faeces.

Discrimination between parasitised and unparasitised hosts by *Ephedrus californicus* Baker improves after experience with several unparasitised aphids (Chow and Mackauer 1986). Learning to discriminate between parasitised and unparasitised hosts after once ovipositing in an unparasitised host has been postulated for *Leptopilina heterotoma* and *Trichogramma embryophagum* Hartig (van Lenteren and Bakker 1975; Klomp *et al.* 1980), but van Alphen *et al.* (1987) have shown that such discrimination by these parasitoids does not depend upon this experience.

Prior experience with hosts can affect insects' responses in both larval (e.g. Jermy *et al.* 1968; Wiklund 1973; Cassidy 1978) and adult (e.g. Arthur 1966; Prokopy *et al.* 1982; Vet and van Opzeeland 1984; Papaj 1986a; Lewis and Tumlinson 1988) stages. Learning from larval feeding does not appear to influence adult responses to food or oviposition sites for most insects (Salt 1935; Crombie 1941; Monteith 1962; Arthur 1965; Wiklund 1974; Phillips 1977; Copp and Davenport 1978; Stanton 1979; Weseloh 1980; Tabashnik *et al.* 1981; Wasserman 1981; Jaenike 1982, 1983; Rausher 1983; Vet *et al.* 1984; Vet and van Opzeeland 1985; Prokopy *et al.* 1986). Pre-imaginal learning may affect some adult insects' responses (Thorpe and Jones 1937; Thorpe 1939; Cushing 1941; Crombie 1944; Hershberger and Smith 1967; Manning 1967; Yamamoto *et.al* 1969; Phillips and Barnes 1975; Vinson *et al.* 1977; Smith and Cornell 1979; Kudon and Berisford 1980; Vet 1983, 1985b; Luck and Uygun 1986), but this influence

could also have a genetic basis in some cases, or be brought about by exposure of adults to the larval food upon eclosion (Jaenike 1982, 1983; Papaj and Rausher 1983).

Experience with hosts at certain times of larval or adult life may be more effective at causing learning than the same experience at other times. If age is important, younger insects often learn more readily than older ones. The food plant preferences of the Indian stick insect, *Carausius morosus* (Brunn.), are induced in the latter half of the first nymphal stadium. As the insect ages its preferences become progressively less susceptible to modification by further experience with different foods (Cassidy 1978). Wardle and Borden (1985) found that female *Exeristes roborator* exposed to hosts in artificial microhabitats for a period immediately after eclosion show stronger learned responses to these microhabitats than older females similarly treated. Vet (1983) also detected a possible effect of age on host microhabitat odour learning in *L. clavipes;* females first exposed to hosts when 2 days old demonstrate a slightly stronger learned attraction to host microhabitat odours than females first exposed to hosts when 1 month old.

The general objective of the work reported in this chapter was to examine the effect of the nature and timing of experience with hosts on learning by female *Exeristes roborator.* Specifically, experiments were conducted to determine how much experience of a given type is needed before this parasitoid exhibits learned behaviour, and to pinpoint more accurately the period in its early adult life during which experience produces the greatest effect on its behaviour. As well, an experiment was conducted to test the hypothesis that this parasitoid learns to transfer its host-seeking activities from one host microhabitat to another.

MATERIALS AND METHODS

Exeristes roborator were reared and held as described in Chapter 1, except that in Exp. **B**1-**E**1 each female was held individually with 2 males in a 16 x 23 x 31 cm cage for both pre-test treatment and testing. Egg cups used as host microhabitats in Exp. **B**1-**E**1 were constructed as described in Chapter 3, but without the inclusion of parafilm. Coddled larvae of *Galleria mellonella* (Syed 1985) were used as hosts in all experiments. Females not attacking hosts in egg cups by the end of their seventh day post-ecosion were discarded from all groups in Exp. **B**2, **C**1-**C**3, and **D**1-**D**3.

A. Behaviour of *E. roborator* towards egg cups containing hosts

To determine how *E. roborator* gained experience with hosts in egg cups, newly-eclosed females kept as in Exp. **B**1-**E**1 were offered an egg cup containing 2 host larvae for 8 h each day for 6 days, and their behaviour towards the cup was observed continuously. The duration of all visits to the cup, and the activities performed during each visit, were recorded for 27 females, with 6-8 individually-caged females observed at one time.

B. Tests for learning from exposure to hosts in egg cups prior to host attack

Pre-test treatments

*Experiment **B**1*

To test the hypothesis that female *E. roborator* learn from exposure to hosts before they begin attacking them, newly-eclosed females were assigned randomly to 3 groups and subjected to pre-test treatments for 7 days (Table 10). Parasitoids in group **B**I were given hosts in an egg cup and observed closely. As soon as each female first began to probe the larvae with her

Table 10. Pre-test treatments for *E. roborator* in Exp. **B**1 and **B**2.

Exp.	Group	N	Pre-test treatment[a]
B1	**B**I	26	Given an egg cup containing hosts until the start of host attack, then given an egg cup not containing hosts until the end of the 7th day post-eclosion (hosts in cup/cup alone)
	BII	23	Given an egg cup containing hosts for 7 days (hosts in cup)
	BIII	24	Given an egg cup not containing hosts for 7 days (cup alone)
B2	**B**I′	21	As for group **B**I, Exp.**B**1
	BIV	20	Given an egg cup containing hosts until the start of host attack, then held without exposure to egg cups or hosts for approximately 1/2 h
	BV	23	Given an egg cup containing hosts until the start of host attack, then held without exposure to egg cups or hosts until the following day

[a]Fresh egg cups and hosts were placed in cages each day. Hosts were 2 coddled larvae of *G. mellonella*.

ovipositor, the cup was replaced with an empty cup (one not containing hosts) for the rest of the treatment period. This prevented females from gaining experience attacking hosts, so that the effect of their early exposure could be assessed. Group BII females had access to hosts in an egg cup for the entire treatment period, as a control to ensure that learning could be measured under the experimental conditions. Females in group BIII were exposed to an empty egg cup for 7 days to determine the response level during testing of females given no opportunity to learn. At the end of the seventh day cups were removed from all cages, and females were left for testing on the following day. If group BI and BIII females did not differ in their test responses to the egg cup, while group BII females exhibited significantly higher responses, it would indicate that *E. roborator* does not learn from exposure to hosts prior to attack.

Experiment B2

To determine whether or not learned responses to the egg cup could have been acquired by females in group BI, Exp. B1, but subsequently lost when these females were exposed to a cup without hosts for a period of time before testing, newly-eclosed females were assigned randomly to 3 groups for pre-test treatment (Table 10). Females in group BI′ were treated as females in group BI (Exp. B1) had been. Females in groups BIV and BV were also offered an egg cup containing host larvae until they first probed these hosts, but at this time the cup was removed from their cages, and they were left without exposure to an egg cup or hosts until they were tested for learning. Group BIV females were tested approximately 1/2 h after removal of the egg cup, to determine if females exhibited learning soon after exposure to hosts. Group BV females were tested on the following day, in case the disturbance caused by cup removal at the time of first probing interfered with the performance of learned behaviour by group BIV females. No difference between the test

responses of females in the 3 groups would indicate that exposure to the empty egg cup after initiation of host attack had not caused loss of learned responses by females in group BI (Exp. B1).

Testing regime

In both experiments testing was carried out by placing a fresh egg cup without hosts in each cage for 20 min. A record was kept of whether or not the female contacted and probed the cup, and, if so, of how long she spent in contact and how many times she probed. In Exp. B1 all females were tested in random order. In Exp. B2, group BIV females were tested as they became available, while all group BI' and/or group BV females ready for testing on a given day were processed in random order. The procedure was repeated until at least 20 females had been tested in each group in the 2 experiments.

C. Importance of experience with hosts after commencement of host attack

Pre-test treatments

Experiment C1

To determine if females acquired learning from one day's experience with hosts after beginning to attack them (the equivalent of 1 host attack), newly-eclosed females were divided randomly into 2 groups for pre-test treatment (Table 11). Parasitoids in both groups were initially offered an egg cup containing host larvae each day. These cups were left in the cages of group CI females until the end of the day on which they began to probe for hosts, and then removed. This was considered to be 1 day's experience with hosts. As soon as females in group CII began to probe the larvae, their cups were replaced with empty cups for the rest of the day. This was considered to be no experience. Testing was done on the following day. A significanty greater

Table 11. Pre-test treatments for *E. roborator* in Exp. C1-C5.

Exp.	Group	N	Day post-eclosion of first host attack	Pre-test treatment[a]
C1	CI	27	3-7	Given an egg cup containing hosts until the end of the first day of host attack (1 day's experience)
	CII	23	3-7	Given an egg cup containing hosts until the start of host attack, then given an egg cup not containing hosts for the rest of the day (no experience)
C2	CI'	25	3-7	As for group CI, Exp. C1
	CIII	26	3-7	Given an egg cup containing hosts until the end of the 2nd day of host attack (2 days' experience)
	CIV	25	3-7	Given an egg cup containing hosts until the end of the 3rd day of host attack (3 days' experience)
	CV	22	3-7	Given an egg cup containing hosts until the end of the 4th day of host attack (4 days' experience)
	CVI	20	3-7	Given an egg cup containing hosts until the end of the 5th day of host attack (5 days' experience)
C3	CIII'	21	3-7	As for group CIII, Exp. C2
	CVII	22	3-7	Given an egg cup containing hosts until the end of the first day of host attack, then given an egg cup not containing hosts for 1 day (1 day's experience/cup alone 1 day)
	CVIII	25	3-7	Given an egg cup containing hosts until the end of the first day of host attack, then held without exposure to egg cups or hosts for 1 day (1 day's experience/no hosts or cup 1 day)

Table 11. continued

Exp.	Group	N	Day post-eclosion of first host attack	Pre-test treatment[a]
C4	CIX	18	3	Given an egg cup containing hosts until the end of the 5th day post-eclosion
	CX	26	4	Given an egg cup containing hosts until the end of the 6th day post-eclosion
	CXI	21	5	Given an egg cup containing hosts until the end of the 7th day post-eclosion
	CXII	19	6	Given an egg cup containing hosts until the end of the 8th day post-eclosion
	CXIII	9	7	Given an egg cup containing hosts until the end of the 9th day post-eclosion
C5	CXIV	20	3	As for group CI, Exp. C1 (3 days old; 1 day's experience)
	CXV	20	3	As for group CIII, Exp. C2 (3 days old; 2 days' experience)
	CXVI	20	3	As for group CIV, Exp. C2 (3 days old; 3 days' experience)
	CXVII	20	5	As for group CI, Exp. C1 (5 days old; 1 day's experience)
	CXVIII	20	5	As for group CIII, Exp. C2 (5 days old; 2 days' experience)
	CXIX	20	5	As for group CIV, Exp. C2 (5 days old; 3 days' experience)

[a]Fresh egg cups and hosts were placed in cages each day. Hosts were 2 coddled larvae of *G. mellonella*.

test response to the egg cup by group CI than by group CII females would indicate that *E. roborator* learned from 1 day's experience attacking hosts.

Experiment C2

To test the hypothesis that the strength of learned responses would increase as experience with hosts increased, newly-eclosed females were assigned randomly to 5 groups and subjected to pre-test experience attacking hosts for 1-5 days (Table 11). Each day every female was offered a fresh egg cup containing hosts. For females in group CI', the cup was removed at the end of the day on which host attack began, as had been done for group CI (Exp. C1), and testing was carried out on the following day. Females in groups CIII-CVI were given egg cups containing hosts for 1-4 additional days, respectively. At the end of the appropriate period of experience, females were prepared for testing on the following day by removing the egg cups from their cages. Significant increases in test responses with increasing experience would suggest that host attacks subsequent to the first one also contributed to learning.

Experiment C3

Experiment C3 evaluated whether or not the increased responses of females given 2 days' or more experience after initial host attack in Exp. C2 could have been due to differences in factors other than amount of pre-test experience, such as average test age, average amount of exposure to the egg cup itself, and amount of time elapsed between initial host probing and testing. Newly-eclosed females were divided randomly into 3 groups (Table 11). As a representative example, females in group CIII' were given 2 days' experience as described for group CIII (Exp. C2). Females in groups CVII and CVIII were given 1 day's experience as described for group CI, Exp. C1. On the next day females in group CVII were exposed to an egg cup without

hosts, while females in group CVIII were not exposed to hosts or a cup. At the end of the pre-test treatment period cups were removed from the cages of females in groups CIII′ and CVII. Parasitoids in all groups experienced the same passage of time between the start of host attack and testing, and were of similar average test ages. Females in group CVII were also exposed to the egg cup for as long, on average, as females in group CIII′. Group CVIII was included as a control in case exposure to the empty egg cup prior to testing decreased the test responses of group CVII females. If the test responses of group CIII′ females remained significantly higher than those of group CVII and CVIII females, the pre-test treatment differences in factors other than experience (outlined above) probably did not affect test responses in Exp. C2.

Experiment C4

Females in Exp. C1 and C2 were tested over a range of ages. Variation in this factor could have reduced the precision of the results if age affects the strength with which the insect responds. To determine if the age at which females were tested influenced their responses to the egg cup, newly-eclosed females were offered a cup containing host larvae each day, and were divided into 5 groups according to the day post-eclosion that they began to probe hosts (Table 11). Each female was then given an egg cup with hosts on the following 2 days so that she gained a total of 3 days' experience, as described for Group CIV (Exp. C2). At the end of the third day of experience cups were removed from cages in preparation for testing. Parasitoids beginning to attack hosts on their third-seventh days were tested. A lack of significant differences between the groups of females would indicate that age did not influence their test responses.

Experiment C5

Females in Exp. C1 and C2 also varied considerably in the age at which they first attacked hosts. If this factor influences acquisition of learning, it too might have reduced the precision of the results in these experiments. To determine if the amount of experience required for initial acquisition of learning was the same for females beginning host attack at different ages, parasitoids were offered an egg cup containing hosts each day, and females first attacking hosts on their third or fifth days post-eclosion were selected. Within each age category, females were randomly divided into 3 pre-test treatment groups (Table 11), whereby females in groups CXIV and CXVII were given 1 day's experience with hosts, those in groups CXV and CXVIII were given 2 days' experience, and those in groups CXVI and CXIX were given 3 days' experience. Egg cups were removed from all cages at the end of the appropriate period of experience, in preparation for testing. If all females beginning host attack at different ages, but given the same experience, did not differ significantly in their test responses, it would indicate that age at first host attack did not influence the acquisition of learning.

Testing regime

In Exp. C1-C5 females were tested as in Exp. B1 and B2. Within an experiment, testing was carried out in random order on all females ready for testing on a given day; at least 20 females were tested in each group in all but Exp. C4.

D. Time at which experience most influences behaviour

Pre-test treatments

Experiment D1

Experiment **D1** assessed the effect of delaying extensive experience with hosts on the strength of the parasitoid's learned responses, so that the period in early adult life during which it learns most readily could be identified. Newly-eclosed females were divided randomly into 5 groups and subjected to pre-test treatments involving delays of 0-4 days between initial host probing and a further 3 days' experience with hosts (Table 12). All females were offered an egg cup containing hosts until they began to probe these larvae. As a control, parasitoids in group **DI** were allowed to complete these initial attacks, and were offered a cup with hosts on the following 2 days, so that they gained 3 days' experience with hosts immediately after commencement of host probing. As soon as each female in groups **DII-DV** began to probe hosts, the cup with larvae was removed from her cage and replaced by a cup without hosts for 1-4 days, depending on her group. After this, a cup with hosts was again placed in her cage for 3 consecutive days. Cups were removed from all cages at the end of the third day of experience in preparation for testing. Significantly lower test responses by females in groups experiencing delays than by females in group **DI** would indicate that the period during which the former females were given hosts was not as important for learning as all or part of the period during which they were deprived of hosts. When the responses of females experiencing sequentially longer and longer delays ceased to differ significantly form one another, it would indicate that the limit of the sensitive period for learning had been exceeded by all their deprivation periods.

Table 12. Pre-test treatments for *E. roborator* in Exp. D1-D4.

Exp.	Group	N	Day post-eclosion of first host attack	Pre-test treatment[a]
D1	DI	21	3-7	As for group CIV, Exp. C2 (Table 11) (no delay/3 days' experience)
	DII	24	3-7	Given an egg cup containing hosts until the start of host attack, then given an egg cup not containing hosts for the rest of the day. Again given an egg cup containing hosts on the following 3 days (1-day delay/3 days' experience)
	DIII	24	3-7	Given an egg cup containing hosts until the start of host attack, then given an egg cup not containing hosts for the rest of the day and the next day. Again given an egg cup containing hosts on the following 3 days (2-day delay/3 days' experience)
	DIV	26	3-7	Given an egg cup containing hosts until the start of host attack, then given an egg cup not containing hosts for the rest of the day and the next 2 days. Again given an egg cup containing hosts on the following 3 days (3-day delay/3 days' experience)
	DV	23	3-7	Given an egg cup containing hosts until the start of host attack, then given an egg cup not containing hosts for the rest of the day and the next 3 days. Again given an egg cup containing hosts on the following 3 days (4-day delay/3 days' experience)
D2	DIII'	21	3-7	As for group DIII, Exp. D1
	DVI	24	3-7	Given an egg cup containing hosts until the start of host attack, then held without exposure to egg cups or hosts for the rest of the day and the next day. Again given an egg cup containing hosts on the following 3 days (2-day delay (no egg cup)/3 days' experience)

Table 12. continued

Exp.	Group	N	Day post-eclosion of first host attack	Pre-test treatment[a]
	DVII	22	3-7	Given an egg cup containing hosts until the end of the day after the start of host attack, then given an egg cup not containing hosts for next 2 days. Again given an egg cup containing hosts on the following day (2 days' experience/2-day delay/1 day's experience)
	DVIII	21	3-7	Given an egg cup containing hosts until the end of the day after the start of host attack, then held without exposure to egg cups or hosts for the next 2 days. Again given an egg cup containing hosts on the following day (2 days' experience/2-day delay (no egg cup)/ 1 day's experience)
D3	DV'	20	3-7	As for group DV, Exp. D1
	DIX	22	3-7	Given an egg cup containing hosts until the start of host attack, then given an egg cup not containing hosts for the rest of the day and the next 6 days (no experience)
D4	DXI	20	4	As for group DII, Exp. D1 (4 days old; 1-day delay/3 days' experience)
	DXII	20	4	As for group DIII, Exp. D1 (4 days old; 2-day delay/3 days' experience)
	DXIII	20	4	As for group DIV, Exp. D1 (4 days old; 3-day delay/3 days' experience)
	DXIV	20	6	As for group DII, Exp. D1 (6 days old; 1-day delay/3 days' experience)
	DXV	20	6	As for group DIII, Exp. D1 (6 days old; 2-day delay/3 days' experience)
	DXVI	20	6	As for group DIV, Exp. D1 (6 days old; 3-day delay/3 days' experience)

[a]Fresh egg cups and hosts were placed in cages each day. Hosts were 2 coddled larvae of *G. mellonella*.

Experiment D2

Experiment D2 evaluated whether or not the decreased responses of females given delayed experience with hosts in Exp. D1 could have been due to differences in factors other than continuity of experience, such as average test age, average amount of total exposure to the egg cup, amount of elapsed time between initial host probing and testing, and time of exposure to an empty cup during delays in experience. Newly-eclosed females were divided randomly into 4 groups (Table 12). Parasitoids in all groups experienced the same passage of time between first host attack and testing, and were of similar average test ages. Females in group DIII' were given 3 days' experience after a 2-day delay exactly as for females in group DIII (Exp. D1), as a representative example of females in Exp. D1. Females in group DVI were treated similarly, except that they were not exposed to an empty egg cup during the 2-day delay in experience after initial host attack, to determine if reduced responses occurred in the cup's absence. Parasitoids in groups DVII and DVIII were given 2 days' uninterrupted experience attacking hosts as described for females in group CIII (Table 11, Exp. C2). Group DVII females were then exposed to an egg cup without hosts for a further 2 days, while group DVIII females were left for 2 days without exposure to an egg cup or hosts. On their final day of pre-test treatment females in both groups were again exposed to an egg cup containing hosts. Insects in group DVII experienced similar average overall times of exposure to the egg cup, and also the same amount of exposure to an empty egg cup, as group DIII' females. These 2 groups differed only in the timing of the 2-day delay in experience. Group DVIII females controlled for the possibility that exposure to an empty egg cup after 2 days' experience would reduce the learned responses of group DVII females, making their learning appear similar to that of group DIII' females.

At the end of the last day of the pre-test treatment period cups were removed from the cages of females in all 4 groups, in preparation for testing. If the test responses of females in group DIII' and DVI remained significantly below those of females in groups DVII or DVIII, pre-test treatment differences in factors other than delay length probably would not have affected test responses in Exp. D1.

Experiment D3

To determine if a long (4-day) delay in experience immediately after commencement of host attack led to reduced, but still detectable, learning, newly-eclosed females were divided randomly into 2 groups (Table 12). Parasitoids in group DV' were treated as were those in group DV (Exp. D1). Females in group DIX were given an egg cup with hosts until they first probed these larvae, and then were given a cup without hosts for the remainder of that day and for the following 6 days. At the end of the final day of treatment cups were removed from all cages. Significantly greater test responses by females in group DV' than by females in group DIX would indicate that females still learned to some degree from experience gained after long delays.

Experiment D4

As in experiments in section C, *E. roborator* in experiments in section D varied in the age at which they first attacked hosts. To determine if this variation could have reduced the precision of the results in these experiments, Exp. D4 tested the hypothesis that females beginning host attack at different ages had different periods during which they were most sensitive to the effects of experience. Newly-eclosed females were offered an egg cup containing hosts each day. Those females first probing hosts on their fourth and sixth days post-eclosion were selected and, within each age category,

divided randomly into 3 pre-test treatment groups subjected to 1-, 2-, and 3-day delays between initial host probing and a further 3 days' experience with hosts (Table 12). Delays experienced by females were: Groups DXI and DXIV, 1 day; groups DXII and DXV, 2 days; and groups DXIII and DXVI, 3 days. At the end of the third day of experience, cups were removed from all cages, in preparation for testing. If all females beginning host attack at different ages, but subjected to the same delay, did not differ significantly in their test responses, it would indicate that age at initial host attack did not influence the length of the sensitive period for learning.

Testing regime

In Exp. D1-D4 females were tested as described for Exp. B1 and B2. Within an experiment, testing was carried out in random order on all females available on a given day. At least 20 females were tested in each group in all experiments.

E. Learning a second host microhabitat

Experiment E1

First and second pre-test treatments

To determine if *E. roborator* could learn to shift its responses from one host microhabitat to another, parasitoids eclosing over a 2-day period were randomly divided into 4 pre-test treatment groups (Table 13). Every day for 7 days females in each group were exposed to both a blue egg cup identical to those used in Chapter 1, and a white cylinder identical to those used in Chapter 2. For groups EI and EII, the blue egg cup contained 5 host larvae, while for groups EIII and EIV the white cylinder contained an equal number of hosts. The positions of the cup and cylinder were reversed each day. After 7

Table 13. First and second pre-test treatments for *E. roborator* in Exp. **E**1.

Group	N	First pre-test treatment[a]	Second pre-test treatment[b]
EI (hosts in cup/ hosts in cup)	29	Given 1 blue egg cup containing hosts and 1 white cylinder not containing hosts	Repeat of the first pre-test treatment
EII (hosts in cup/ hosts in cylinder)	33	As for group **E**I	Given 1 blue egg cup not containing hosts and 1 white cylinder containing hosts
EIII (hosts in cylinder/ hosts in cylinder)	35	Given 1 blue egg cup not containing hosts and 1 white cylinder containing hosts	Repeat of the first pre-test treatment
EIV (hosts in cylinder/ hosts in cup)	32	As for group **E**III	Given 1 blue egg cup containing hosts and 1 whitw cylinder not containing hosts

[a]Fresh egg cups, cylinders, and hosts were placed in cages each day for 7 days. Hosts were 5 coddled larvae of *G. mellonella*.

[b]As in [a], except that treatment was carried out for 4 days.

days females from each group were marked with paint for identification and divided randomly among 4 test cages, as described in Chapter 1. On the following day all females were subjected to their first test, to determine if they had learned these microhabitats. Such learning would be indicated by a significant concentration of test responses by females on the microhabitat in which they had been given hosts during pre-test treatment.

Beginning on the day after the first test, females were subjected to a second, 4-day, pre-test treatment period (Table 13). These treatments were identical to the first treatments, except that females in group EII were offered hosts in the white cylinder, and those in group EIV were offered hosts in the blue egg cup, so that learning of each object as a second host microhabitat could be evaluated. Groups EI and EIII acted as controls, allowing the measurement of the responses of females that had been exposed to the hosts and microhabitats for the same length of time, without undergoing a shift in host location. At the end of the second pre-test treatment period females from each cage were re-marked with paint if necessary and again randomly distributed among 4 test cages in preparation for the second test on the following day. A significant shift in concentration of test responses from the blue cup to the white cylinder by group EII females would indicate that they had learned the cylinder as a host microhabitat. A similar shift away from the cylinder in favour of the cup by females in group EIV would indicate that they too had learned the second host microhabitat they experienced.

First and second tests

First and second tests were identical. Females were offered a blue egg cup and a white cylinder simultaneously for 1 h, and their responses to both were assessed. Neither artificial microhabitat contained hosts, and the positions of the cup and cylinder were reversed in successive test cages,

which were chosen in random order. Responses were recorded as in experiments in Chapters 1-3.

Statistical analysis

For Exp. **B**1-**B**2, **C**2-**C**4, and **D**1-**D**2 the proportions of females in each group within an experiment contacting and probing the egg cup were compared with a test for comparing >2 proportions and a modified Newman-Keuls multiple comparisons procedure (Zar 1984) (α=0.05). For these experiments mean time spent in contact with the cup and mean number of ovipositor probes executed on it by all the females in each group within an experiment were compared with the Kruskal-Wallis Test and multiple comparisons procedure of Conover (1980) (α=0.05).

In each of Exp. **C**1 and **D**3, proportions of responding females were compared using the Fisher Exact Test (Zar 1984) and mean responses were compared with the Mann-Whitney Test (Conover 1980) (α=0.05).

For Exp. **C**5 and **D**4 mean responses to the egg cup were compared using a nonparametric, 2-factor analysis of variance (Zar 1984) (α=0.05). To obtain equal sample sizes for analysis, data were randomly deleted from groups in which >20 individuals had been tested (Zar 1984). If the analysis indicated that a factor had a significant effect on parasitoid response, a modified Newman-Keuls procedure was used to compare the levels of that factor (Zar 1984).

In Exp. **E**1, data from the first and second tests were analysed in the same way. Females were categorised according to whether or not they contacted and probed either microhabitat alone, both microhabitats, or neither microhabitat. Where 4 x 4 X^2 analysis of the numbers of females in each response category indicated that significant differences occurred between

groups (α=0.05), the proportions of females in all groups responding only to the egg cup, and also only to the cylinder, were compared using simultaneous 95% confidence intervals for differences between proportions (Miller 1981). The mean proportion of total responses directed at the blue egg cup by contacting and probing females in each group, and the mean total responses to microhabitats by all females in each group were compared with the Kruskal-Wallis Test and multiple comparisons procedure of Conover (1980) (α=0.05). The proportions of females in each group responding in total to microhabitats were compared using a test for comparing >2 proportions and a modified Newman-Keuls multiple comparisons procedure (Zar 1984) (α=0.05).

RESULTS

Experience with hosts during the first 2 days after initial host attack markedly affected the strength of learned responses to the egg cup as a host microhabitat by *E. roborator*. Most females made 1 attack on hosts in the cup on each of these 2 days, probing the larvae with their ovipositor and feeding upon them (Table 14). The first such day of experience resulted in learning on the part of females, but learned responses were significantly strengthened after a second day of experience (Table 15, Exp. **C1-C3**; Table 16). A lack of exposure to hosts during this period reduced the learned responses of females significantly (Table 17, Exp. **D1-D2**; Table 18).

When exposed to an egg cup containing hosts, females gradually increased the number of visits they made to the cup, as well as the time they spent in contact with it and in searching and, occasionally, probing it, in the days up to the time they began to attack hosts (Table 14). On the first 2 days that females attacked hosts they continued to make several visits to the egg cup. They usually only attacked hosts during one of these visits unless disturbed by males, in which case they would leave the cup, returning later to complete the attack. All attacks involved ovipositor probing and feeding on host tissues. The amount of time spent in contact with the cup and in host-seeking activities on the cup not involving direct contact with a host increased considerably after the first host attack. After the second day of host attack females increased the number of visits involving host contact, and began to oviposit as well as feed upon hosts; most females performed both activities by the fourth day after host attack began (Table 14).

Exeristes roborator did not learn to respond to the egg cup from exposure to hosts in it before the commencement of host attack (Table 15, Exp. **B1**). Group **BI** females exposed to hosts in a cup only until the time they first probed these larvae did not respond to the egg cup without hosts in

Table 14. Behaviour of *E. roborator* towards an egg cup containing host larvae before and after initial host attack.

Day[a]	No. females observed[b]	Mean (±S.E.) total visits to cup	visits involving attack upon hosts in cup	total min spent in contact with cup	min spent in host-seeking behaviour[c] on cup not involved in host attack	min spent attacking hosts in cup	percent of observed females feeding only	ovipositing only	feeding and ovipositing
-5	1	1	/	1	0	/	/	/	/
-4	3	4.0 ± 0.6	/	4.7 ± 0.9	0.3 ± 0.3	/	/	/	/
-3	10	3.0 ± 0.7	/	3.1 ± 0.6	0.8 ± 0.3	/	/	/	/
-2	27	4.7 ± 0.7	/	12.0 ± 2.2	1.1 ± 0.3	/	/	/	/
-1	27	9.6 ± 1.1	/	22.4 ± 3.2	2.3 ± 0.4	/	/	/	/
1	27	4.3 ± 0.6	1.2 ± 0.1	132.2 ± 13.8	11.7 ± 3.2	71.3 ± 7.1	100	0	0
2	26	4.2 ± 0.6	1.3 ± 0.1	129.9 ± 15.6	11.7 ± 2.3	64.7 ± 6.5	100	0	0
3	24	8.4 ± 0.8	5.0 ± 0.5	150.0 ± 12.7	22.0 ± 3.8	75.8 ± 8.4	50	8	42
4	17	10.7 ± 1.5	6.8 ± 1.0	201.4 ± 16.0	32.7 ± 3.3	115.2 ± 15.2	0	24	76

[a]Relative to initial host attack, which occurred on day 1.

[b]The same 27 females were observed repeatedly for 6 days each; initial host attack occurred on the 3rd day of observation for 17 females, on the 4th day of observation for 7 females, on the 5th day of observation for 2 females, and on the 6th day of observation for 1 female.

[c]Intensive antennation and ovipositor probing.

Table 15. Contacting and probing responses to the egg cup by *E. roborator* in groups BI-BIII, groups CI-CII, groups CI'-CVI, and groups CIII'-CVIII, during testing in Exp. B1, C1, C2, and C3, respectively.

Exp.	Group (pre-test treatment)	Percent of females (±S.E.)[a] contacting cup	Percent of females (±S.E.)[a] probing cup	Mean (±S.E.)[b] min in contact with cup	Mean (±S.E.)[b] probes executed on cup
B1	BI (hosts in cup/ cup alone)	26.9 ± 8.7 a	11.5 ± 6.3 a	1.0 ± 0.5 a	0.5 ± 0.3 a
	BII (hosts in cup)	95.7 ± 4.2 b	87.0 ± 7.0 b	11.7 ± 1.4 b	7.0 ± 1.1 b
	BIII (cup alone)	37.5 ± 9.9 a	20.8 ± 8.3 a	1.7 ± 0.6 a	0.8 ± 0.4 a
C1	CI (1 day's experience)	55.6 ± 9.6 a	37.0 ± 9.3 a	5.0 ± 1.2 a	3.0 ± 1.0 a
	CII (no experience)	39.1 ± 10.1 a	13.0 ± 7.0 a	1.3 ± 0.5 b	0.6 ± 0.4 b
C2	CI' (1 day's experience)	68.0 ± 9.3 a	44.0 ± 9.9 a	5.2 ± 1.0 a	2.9 ± 0.9 a
	CIII (2 days' experience)	88.5 ± 6.3 a	69.2 ± 9.1 b	9.0 ± 1.3 b	5.3 ± 1.1 b
	CIV (3 days' experience)	84.0 ± 7.3 a	80.0 ± 8.0 bc	10.0 ± 1.6 b	7.2 ± 1.5 bc
	CV (4 days' experience)	81.8 ± 8.2 a	72.7 ± 9.5 bc	8.1 ± 1.6 ab	6.0 ± 1.3 bc
	CVI (5 days' experience)	90.0 ± 6.7 a	85.0 ± 8.0 c	11.1 ± 1.6 b	8.6 ± 1.6 c

Table 15. continued

Exp.	Group (pre-test treatment)	Percent of females (±S.E.)[a] contacting cup	probing cup	Mean (±S.E.)[b] min in contact with cup	probes executed on cup
C3	CIII' (2 days' experience)	76.2 ± 9.3 a	61.9 ± 10.6 a	8.1 ± 1.5 a	5.0 ± 1.0 a
	CVII (1 day's experience/cup alone 1 day)	50.0 ± 10.7 a	31.8 ± 9.9 a	2.6 ± 0.9 b	1.5 ± 0.6 b
	CVIII (1 day's experience/no hosts or cup 1 day	60.0 ± 9.8 a	40.0 ± 9.8 a	4.4 ± 1.2 b	2.5 ± 0.7 b

[a]Within each experiment percentages in a column followed by the same letter are not significantly different. Exp. B1, C2, C3: Test for comparing >2 proportions and modified Newman-Keuls multiple comparisons procedure, Exp. C1: Fisher Exact Test (Zar 1984), α=0.05.

[b]Within each experiment means in a column followed by the same letter are not significantly different. Exp. B1, C2, C3: Kruskal-Wallis Test and multiple comparisons procedure, Exp. C1: Mann-Whitney Test (Conover 1980), α=0.05.

Table 16. Mean contacting and probing responses to the egg cup by *E. roborator* in groups CXIV-CXIX during testing in Exp. C5.

Group (pre-test treatment)	Mean (+S.E.)[a] min in contact with cup	probes executed on cup
CXIV (3 days old; 1 day's experience)	6.1 ± 1.7	3.3 ± 1.4
CXV (3 days old; 2 days' experience)	8.5 ± 1.7	5.6 ± 1.7
CXVI (3 days old; 3 days' experience)	10.4 ± 1.9	6.7 ± 1.6
CXVII (5 days old; 1 day's experience)	4.6 ± 1.5	2.3 ± 0.9
CXVIII (5 days old; 2 days' experience)	9.1 ± 1.7	6.0 ± 1.5
CXIX (5 days old; 3 days' experience)	9.5 ± 1.7	5.7 ± 1.2

[a]Responses were significantly affected by amount of experience with hosts after initial host attack, but not by age at initial host attack; there was no significant interaction between the 2 factors [nonparametric 2-factor analysis of variance (Zar 1984), α=0.05]. Females with 1 day's experience (groups CXIV and CXVII) differed significantly from females with 3 days' experience (groups CXVI and CXIX) in min spent in contact with cups, and from females with 2 (groups CXVI and CXVIII) or 3 days' experience in probes executed on the cup; responses by females with 2 or 3 days' experience did not differ significantly [modified Newman-Keuls nonparametric multiple comparisons procedure (Zar 1984), α=0.05].

Table 17. Contacting and probing responses to the egg cup by *E. roborator* in groups DI-DV, groups DIII'-DVIII, and groups DV'-DIX during testing in Exp. D1, D2, and D3, respectively.

Exp.	Group (pre-test treatment)	Percent of females (±S.E.)[a] contacting cup	Percent of females (±S.E.)[a] probing cup	Mean (±S.E.)[b] min in contact with cup	Mean (±S.E.)[b] probes executed on cup
D1	DI (no delay/3 days' experience)	85.7 ± 7.6 a	76.1 ± 9.3 a	10.5 ± 1.6 a	8.1 ± 1.7 a
	DII (1-day delay/3 days' experience)	79.2 ± 8.3 a	62.5 ± 9.9 b	8.6 ± 1.5 ab	5.4 ± 1.4 ab
	DIII (2-day delay/3 days' experience)	66.7 ± 9.6 a	45.8 ± 10.2 c	5.6 ± 1.4 bc	3.4 ± 1.1 bc
	DIV (3-day delay/3 days' experience)	57.7 ± 9.7 a	38.5 ± 9.5 c	3.5 ± 0.9 c	2.1 ± 0.7 c
	DV (4-day delay/3 days' experience)	52.0 ± 10.4 a	39.1 ± 10.2 c	3.1 ± 0.9 c	2.4 ± 0.9 c
D2	DIII' (2-day delay/3 days' experience)	52.4 ± 10.9 a	52.4 ± 10.9 a	4.5 ± 1.3 a	3.7 ± 1.4 a
	DVI (2-day delay [no egg cup]/3 days' experience)	70.8 ± 9.3 a	41.7 ± 10.1 a	5.9 ± 1.3 ab	3.0 ± 0.9 a
	DVII 2 days' experience/2-day delay/1 day's experience	81.8 ± 8.2 a	77.3 ± 8.9 b	8.8 ± 1.4 bc	6.4 ± 1.4 b
	DVIII 2 days' experience/2-day delay [no egg cup]/ 1 day's experience)	85.7 ± 7.6 a	85.7 ± 7.6 b	10.9 ± 1.5 c	7.2 ± 1.2 b

Table 17. continued

Exp.	Group (pre-test treatment)	Percent of females (±S.E.)[a] contacting cup	probing cup	Mean (±S.E.)[b] min in contact with cup	probes executed on cup
D3	DV′ (4-day delay/3 days′ experience)	60.0 ± 11.0 a	30.0 ± 10.2 a	3.9 ± 1.2 a	2.2 ± 1.0 a
	DIX (no experience)	36.4 ± 10.3 a	18.2 ± 8.2 a	1.5 ± 0.6 b	0.5 ± 0.3 a

[a]Within each experiment percentages in a column followed by the same letter are not significantly different. Exp. D1-D2: Test for comparing >2 proportions and modified Newman-Keuls multiple comparisons procedure, Exp. D3: Fisher Exact Test (Zar 1984), α=0.05.

[b]Within each experiment means in a column followed by the same letter are not significantly different. Exp. D1-D2: Kruskal-Wallis Test and multiple comparisons procedure, Exp. D3: Mann-Whitney Test (Conover 1980), α=0.05.

Table 18. Mean contacting and probing responses to the egg cup by *E. roborator* in groups DXI-DXVI during testing in Exp. D4.

Group (pre-test treatment)	Mean (±S.E.)[a] min in contact with cup	probes executed on cup
DXI (4 days old; 1-day delay/3 days' experience)	7.5 ± 1.6	5.1 ± 1.3
DXII (4 days old; 2-day delay/3 days' experience)	6.0 ± 1.7	4.0 ± 1.3
DXIII (4 days old; 3-day delay/3 days' experience)	5.0 ± 1.6	2.0 ± 0.9
DXIV (6 days old; 1-day delay/3 days' experience)	9.0 ± 1.6	5.9 ± 1.4
DXV (6 days old; 2-day delay/3 days' experience)	6.3 ± 1.6	4.3 ± 1.3
DXVI (6 days old; 3-day delay/3 days' experience)	3.2 ± 1.2	2.8 ± 1.1

[a]Responses were significantly affected by length of delay in experience with hosts after initial host attack, but not by age at which delay was experienced; there was no significant interaction between the 2 factors [nonparametric 2-factor analysis of variance (Zar 1984), α=0.05]. Responses by females experiencing a 1-day delay in experience (groups DXI and DXIV) differed significantly from responses by females experiencing a 3-day delay (groups DXIII and DXVI); responses by females experiencing a 2-day delay (groups DXII and DXV) did not differ significantly from responses by females experiencing 1- or 3-day delays [modified Newman-Keuls nonparametric multiple comparisons procedure (Zar 1984), α=0.05].

significantly higher numbers, or with significantly greater intensity, than group BIII females offered only an empty egg cup during pre-test treatment. In all respects the responses of parasitoids in both groups were significantly inferior to those of group BII females, that had learned to respond to the cup from exposure to hosts in it throughout pre-test treatment. Wardle and Borden (1985) showed previously that exposure to hosts does not in itself cause *E. roborator* to respond to similar egg cups at high levels.

In Exp. B2, females exposed to hosts in an egg cup only until initial host probing and then tested for learned responses almost immediately (group BIV), or on the next day (group BV), did not differ significantly in any responses to an empty egg cup from group BI females given an empty egg cup for several days following their first probe [test for comparing >2 proportions (Zar 1984), and Kruskal-Wallis Test (Conover 1980), α=0.05]. Therefore, the low response to the egg cup of females in group BI (Exp. B1) was not the result of exposure to a cup without reward after initial host attack.

One day's experience with hosts in an egg cup immediately upon commencement of host attack was sufficient to cause significant learning (Table 15, Exp. C1). Group CI females, given hosts in a cup for the day on which they began host attack, responded with significantly greater intensity, although not in significantly greater numbers, to a cup without hosts than control females (group CII), that did not have access to hosts after initial host probing.

A second, successive day's experience for females in group CIII (Exp. C2) (Table 15) resulted in further significant increases in intensity of response and also in numbers of females probing the egg cup. Responses to the egg cup generally tended to increase somewhat with additional experience, but only after 5 days' exposure to hosts in a cup was there a further significant

increase in any response, with group CVI females probing the egg cup in greater numbers and with greater intensity than group CIII females .

Females in the different treatment groups in Exp. C2 differed in average test age, time of overall exposure to the egg cup, and time between initiation of host attack and testing, as well as in amount of experience with hosts. However, the former 3 factors were probably not involved in the increased responses to the cup by females with greater experience with hosts. In Exp. C3 (Table 15), females in group CIII' given 2 days' experience responded with significantly greater intensity to the egg cup than females in groups CVII and CVIII, that were given 1 day's experience but were tested at the same ages and same time after initiation of host attack as group CIII' females, and, for group CVII females, given the same amount of exposure to an egg cup. The rather low responses of group CVII females may have been due to the day of unrewarded exposure to an egg cup they experienced before testing.

Experiment C4 provided evidence that the age at which females were tested did not affect their responses to the egg cup during testing. When females began host attack on their third-seventh day post-eclosion, and were 6-10 days old when tested, they did not differ significantly in numbers responding [test for comparing >2 proportions (Zar 1984), α=0.05] or in intensity of response [Kruskal-Wallis Test (Conover 1980), α=0.05].

The day post-eclosion on which females began to attack hosts also did not appear to influence their initial acquisition of learning. In Exp. C5, there was no significant effect of age at commencement of attack (3 or 5 days post-eclosion) on the intensity with which females given 1, 2, or 3 days' experience responded to the cup during testing; nor was there a significant interaction between age of initial attack and amount of experience (Table 16). However, as in Exp. C2 (Table 15), the degree of experience with hosts possessed by females influenced the strength of their responses (Table 16).

A delay of at least 2 days from the time females began to probe hosts until they were again given hosts led to a significant reduction in the learned responses of *E. roborator* to the egg cup as a host microhabitat (Table 17, Exp. D1). Group DIII females experiencing a 2-day delay probed the cup in significantly fewer numbers, spent significantly less time in contact with it, and executed significantly fewer ovipositor probes on it than group DI females, given no interruption. Longer delays of 3 or 4 days (group DIV and DV females, respectively) did not lead to further significant reduction in responses. The responses of group DII females, subjected to a 1-day delay, were generally intermediate between those of group DI and DIII females.

Although females in the different groups in Exp. D1 differed from one another in average test age, duration of overall exposure to the egg cup, time between initial host attack and testing, and time of exposure to an egg cup without hosts during delays in experience, Exp. D2 indicated that none of these factors appeared to be responsible for the differences that occurred between groups in responses to the cup. Females in all groups in Exp. D2 were tested at the same average age and same time after initiation of host attack. In addition, group DIII′ and DVII females, and group DVI and DVIII females, had the same average total exposure to the egg cup. The probing responses of females in groups DIII′ and DVI remained significantly lower than those of females in groups DVII and DVIII, however (Table 17, Exp. D2). The similarities in response between females in groups DIII′ and DVI, and between females in groups DVII and DVIII, showed that the presence of an empty egg cup in cages during delays in exposure to hosts in Exp. D1 was not a determining factor in responses. Therefore, only timing of the delay in experience with hosts could have produced the observed differences in response.

Learned responses were reduced considerably, but not entirely eliminated, if females were not given hosts when first ready to attack them.

In Exp. D3, group DV′ females subjected to a 4-day delay in exposure to hosts, but subsequently given 3 days' experience with hosts, spent significantly more time in contact with the egg cup during testing than group DIX females given an empty cup after initial host probing (Table 17, Exp. D3). Although the responses of group DV′ females were larger in general than those of group DIX females, the 2 groups did not differ significantly in any other respect.

The duration of the period after initial host probing during which females acquired strong learned responses to the egg cup did not appear to be influenced by their age when they began this activity (Table 18). In Exp. 11, there was no significant effect of age at first host attack (4 or 6 days post-eclosion) on intensity of response to the cup for females subjected to delays in exposure to hosts of 1-3 days. There was also no significant interaction between the age of initial host attack and the length of the interruption. However, as in Exp. D1 (Table 17), responses of females of both ages were significantly lower when they were deprived of hosts for 3 days immediately after initial attack, than when they were deprived for 1 day (Table 18).

In Exp. E1, female *E. roborator* learned first one host microhabitat, and then a second, but did not transfer their responses completely from one to the other. After initial exposure to hosts in either a blue egg cup or a white cylinder, females showed a marked preference for the microhabitat without hosts in which they had previously attacked larvae. In all groups females responded to this microhabitat exclusively in significantly higher numbers (Fig. 9, first test), and with a significantly greater proportion of their total activity (Table 19, first test), than females in groups exposed to hosts in the other microhabitat. Experiments conducted in Chapters 1 and 2 showed that under similar experimental conditions *E. roborator* has no innate attraction to either of the microhabitats. Thus the responses to both the

blue egg cup and the white cylinder in Exp. E1 must have been the result of learning.

There was no difference between groups in total numbers of females responding [test for comparing >2 proportions (Zar 1984), α=0.05] or in mean total responses to microhabitats [Kruskal-Wallis Test (Conover 1980), α=0.05], indicating that females were able to perform the learned responses equally on both the cup and the cylinder.

After a second period of exposure to hosts and microhabitats, females in groups EI and EIII, which had been given hosts in the same microhabitat in both parts of the experiment, maintained their strong preference for the microhabitat in which they had continued to attack hosts (Fig. 9, Table 19, second test). In contrast, females in groups EII and EIV, which had experienced a reversal in host location, for the most part did not respond exclusively to one microhabitat or the other in large numbers (Fig. 9, Table 19, second test).

Evidently, females in groups EII and EIV had learned the second microhabitat to some extent, as their responses to this object were significantly higher than those of females that had not been exposed to hosts in it. During the second test group EII females probed the white cylinder only in significantly higher numbers, and directed a significantly higher proportion of their total responses to this microhabitat than group EI females, and the same differences occurred between group EIV and EIII females in their responses to the blue egg cup (Fig. 9, Table 19, second test).

Females experiencing a change in host microhabitat generally did not develop as strong a response to the second microhabitat as did insects that had attacked hosts only in this same microhabitat for the whole course of the experiment. Group EII females contacted the white cylinder only less than

Figure 9. Percent of *E. roborator* in groups **E**I-**E**IV in Exp. **E**1 responding to the blue egg cup or the white cylinder alone, both artificial microhabitats, or neither microhabitat in the first and second tests. Bars in the same subgraph marked with the same letter are not significantly different [simultaneous 95% confidence intervals for differences between proportions (Miller 1981)]. First/second pre-test treatments for group **E**I=hosts in cup/hosts in cup, for group **E**II=hosts in cup/hosts in cylinder, for group **E**III=hosts in cylinder/hosts in cylinder, and for group **E**IV=hosts in cylinder/hosts in cup.

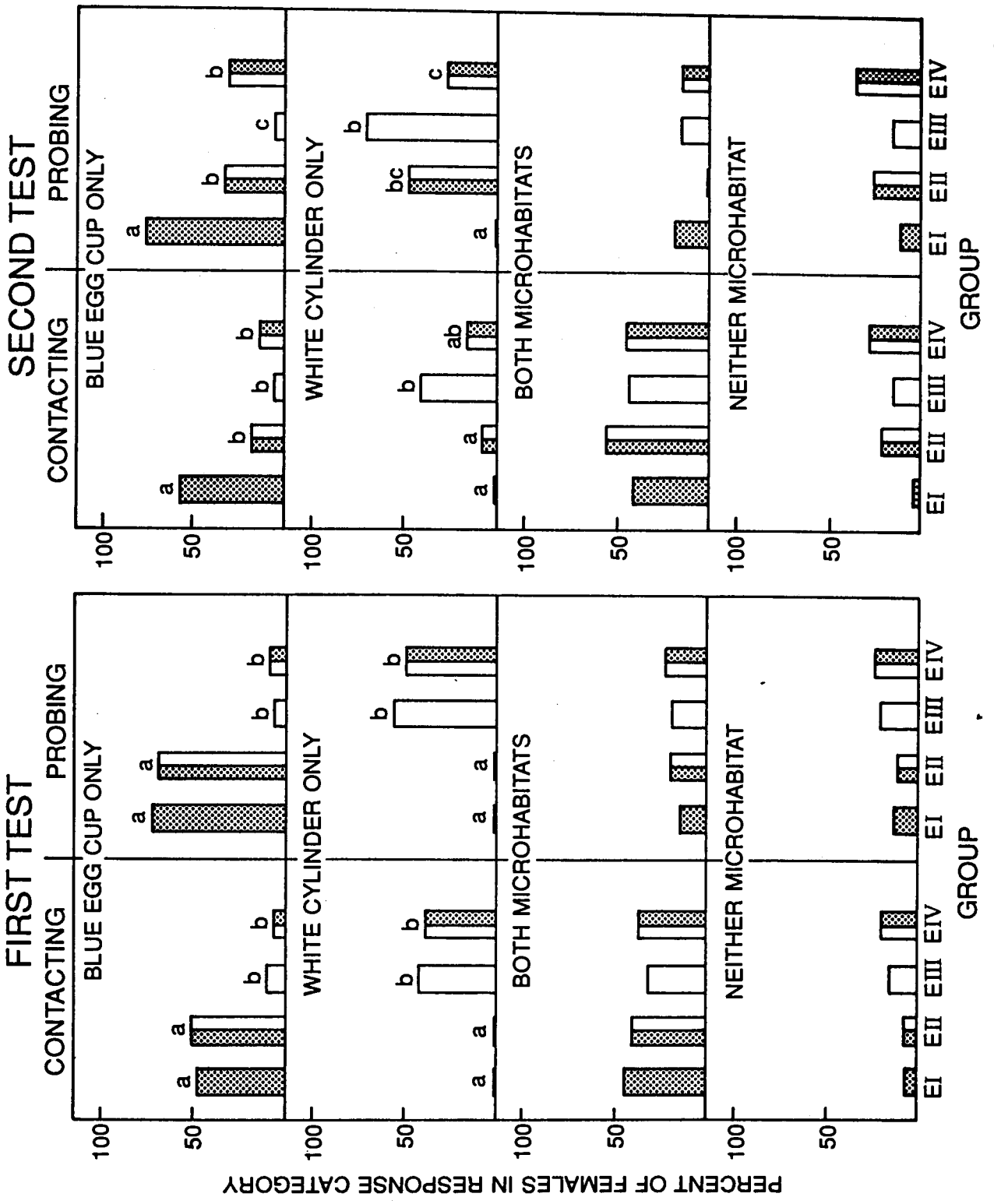

FIRST TEST
SECOND TEST
CONTACTING
PROBING
BLUE EGG CUP ONLY
WHITE CYLINDER ONLY
BOTH MICROHABITATS
NEITHER MICROHABITAT
PERCENT OF FEMALES IN RESPONSE CATEGORY
100
50
EI EII EIII EIV
GROUP

Table 19. Mean percent of total contacting and probing responses to artificial host microhabitats directed at the blue egg cup by responding *E. roborator* in groups EI-EIV in the first and second tests of Exp. E1.

Test	Group (first/second pre-test treatments)	No. females contacting habitats	Mean % (±S.E.) of total contact time spent on blue cup[a]	No. females probing habitats	Mean % (±S.E.) of total probes executed on blue cup[a]
First	EI (hosts in cup/ hosts in cup)	27	84.2 ± 5.2 a	25	92.0 ± 4.5 a
	EII (hosts in cup/ hosts in cylinder)	30	90.6 ± 2.6 a	29	94.3 ± 2.4 a
	EIII (hosts in cylinder/ hosts in cylinder)	29	19.4 ± 6.1 b	28	15.0 ± 5.4 b
	EIV (hosts in cylinder/ hosts in cup)	26	18.9 ± 6.0 b	24	17.7 ± 6.6 b
Second	EI (hosts in cup/ hosts in cup)	28	92.2 ± 3.3 a	26	95.0 ± 2.6 a
	EII (hosts in cup/ hosts in cylinder)	26	49.3 ± 8.0 b	25	40.0 ± 10.0 b
	EIII (hosts in cylinder/ hosts in cylinder)	30	8.8 ± 3.6 c	30	7.3 ± 3.8 c
	EIV (hosts in cylinder/ hosts in cup)	23	46.9 ± 9.0 b	21	48.6 ± 10.3 b

[a]Within each test means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05. Mean % of total response on white cylinder = 100 - mean % of total response on blue egg cup.

group EIII females, and directed a significantly smaller part of their responses to this microhabitat, although they did not probe the cylinder exclusively in significantly lower numbers than females in the latter group (Fig. 9, Table 19, second test). The responses of group EIV females to the blue cup were significantly lower than those of group EI females in all cases (Fig. 9, Table 19, second test).

Females experiencing a reversal virtually always responded to the microhabitat in which they had first attacked hosts significantly less than females that had been given hosts in this same microhabitat for the whole experiment (Fig. 9, Table 19, second test). Nonetheless they retained some of their initial learning through the second pre-test treatment period, because their responses to this microhabitat remained higher than those of females never given hosts in it. In the second test females in group EII probed the blue egg cup in higher numbers, and directed a significantly higher proportion of their total contact time and ovipositor probes to this microhabitat, than females in group EIII. The responses of group EIV and EI females to the white cylinder differed significantly in the same way (Fig. 9, Table 19, second test).

In the second test, switched females did not decrease their overall performance of host-seeking behaviour, i.e. their total responses to microhabitats [test for comparing >2 proportions (Zar 1984) and Kruskal-Wallis Test (Conover 1980), α=0.05]. Therefore shifts in the proportion of total responses directed to each microhabitat by group EII and EIV females in this test (Table 19) cannot have been due simply to a decrease in learned response to the first host microhabitat, creating the false impression that response to the second microhabitat had increased.

DISCUSSION

These experiments suggest that once female *E. roborator* begin to attack hosts, they rapidly learn host microhabitats from direct contact with hosts in them. In some parasitoids, such direct contact is not necessary for learning to occur (Thorpe 1938; Dmoch *et al.* 1985; Drost *et al.* 1986; Lewis and Tumlinson 1988), but it is unlikely that *E. roborator* would have learned in these experiments without the reinforcement of host contact. The coddled larvae of *Galleria mellonella* used as hosts were not metabolically active, or damaging a live host of their own, and there was no larval silk or frass present with them in the cups. Thus most of the host-associated factors thought to cause learning (Dmoch *et al.* 1985; Drost *et al.* 1986; Lewis and Tumlinson 1988) were absent.

Nonetheless, larval odour alone could have caused modification of females' behaviour once they were ready to attack hosts, much as it altered the behaviour of *Venturia canescens* (Thorpe 1938). However, females given 1 day's experience with hosts in Exp. C1, C2, and C5 showed similar learned responses to the egg cup, regardless of whether or not they carried out their initial host attack early or late in the day. Females that had begun host attack within the first 2 h of the photoperiod (N=24) spent 5.3 $\pm$ 1.3 min in contact with the cup and probed it 3.0 $\pm$ 0.9 times, while females that had begun host attack in the last 2 h of the photoperiod (N=15) spent 6.2 $\pm$ 1.7 min in contact and probed 2.7 $\pm$ 1.1 times (test responses from all 3 experiments pooled). Approximately 60% of females contacted the cup, and approximately 67% of these probed it, in both groups. Thus, the continued presence of a cup containing hosts in the cage after direct host contact had ended did not appear to influence the behaviour of females that completed host attack early. As well, the fact that the parasitoid's behaviour towards the cup was not affected by exposure to hosts in it, along with any attendent host

odours, before initial host contact supports the conclusion that host odour alone did not affect the parasitoid.

Virtually all female *E. roborator* carried out 1 host attack on each of the first 2 days that they performed this activity, and all these initial attacks involved ovipositor probing and feeding only. Therefore, I conclude that females learned to respond to host microhabitats from 1 such attack, and that this learning was strengthened by subsequent attacks. Oviposition did not appear to be necessary for learning to occur, since it was only on the third or subsequent host attack that females oviposited. Oviposition may have contributed to a strengthening of learning, since responses to the egg cup continued to increase with experience gained after the first 2 host attacks, but as females often both fed and oviposited during these later attacks it is not possible to separate the contributions of feeding and oviposition to the strength of learned responses.

Under more natural conditions *E. roborator* carries out host attacks on a similar schedule to that observed here, although it does not both feed and oviposit on the same host larva in later host attacks (personal observation). Thus learning under natural conditions could also follow a similar schedule. It is possible, however, that additional experiences, such as contact with host-related factors, could cause learning in *E. roborator* exposed to a less artificial host/microhabitat system than the one used in these experiments.

Learning from a limited amount of experience with hosts, such as seen in *E. roborator*, is typical of parasitoids and other insects (e.g. Menzel and Erber 1978; Balderrama 1980; Prokopy *et al.* 1982; Fukushi 1983, 1985; Vet 1983; Vet and van Opzeeland 1984, 1985; Traynier 1984; Bernays and Wrubel 1985; Papaj 1986a; Cooley *et al.* 1986; Bernays and Lee 1988; Lewis and Tumlinson 1988). Investing a large amount of time in acquiring learning would not be advantageous to these relatively short-lived animals. Learning from

initial experiences with hosts may quickly focus the host-seeking activities of *E. roborator* on one of many hosts it has the potential to attack, perhaps increasing the efficiency with which it uses the host available to it.

The significant decrease in learning by *E. roborator* deprived of hosts during the time they would normally complete their first 2 host attacks shows that experience with hosts during this period was most important for host microhabitat learning. These results support those obtained by Wardle and Borden (1985), which showed that adult females held for 5 or 10 days before being given hosts did not learn as well as females exposed to hosts from the time they eclosed. If many females held without hosts for 5 days in the latter experiments were ready to attack hosts on their third or fourth day post-eclosion, then for them the 2-day sensitive period for learning would have passed by the time they were allowed to carry out attacks. The time of initial host attack was not monitored by Wardle and Borden (1985), but assessment of activity in a representative group of females conservatively showed that at least 40% were actively attacking hosts by their fourth day post-eclosion.

In Exp. B1-D4, careful observation of many females showed that times of initial host attack varied considerably within the range of 3-7 days post-eclosion, both within and between different batches of females. This variation could reflect slight differences in the developmental rates of females, either for genetic reasons, or because of slight fluctuations in rearing conditions. Whatever its basis, variation in the age of first host attack did not lead to variation in the basic learning profile of females.

In both Exp. D3 and earlier work (Wardle and Borden 1985), *E. roborator* held without access to hosts in early adulthood still learned to a limited degree when later given hosts in egg cups, and thus the sensitive period for learning did not have an absolute endpoint. The decrease in learning could

have occurred because total host deprivation during a critical period interfered with the development of normal learning ability, or because of a programmed decrease in learning capacity with age, no matter what the insect's prior access to hosts (Wardle and Borden 1985). Heinrich (1984) has speculated that the nervous system of honey bees might retain the plasticity required for learning because it does not reach the stage of maturation that produces 'hard-wired' responses to stimuli, permitting environmental input to interact with genetic direction in the shaping of circuits. Perhaps in *E. roborator* and other insects with sensitive learning periods in early adult life (Jaisson 1980), maturation of the nervous system after eclosion reduces the potential influence of environmental factors as the insect ages. In *E. roborator* this process might somehow be initiated by the onset of readiness to attack hosts.

If the sensitivity of *E. roborator* to the effects of experience with other host/microhabitat systems follows a similar profile to that seen here, the parasitoid's attacks on hosts in early adult life (or lack of them) could have an important influence on its later patterns of host use in nature or in applied biological control. However, caution is necessary in concluding from these results that sensitivity would always follow the same course. Birds appearing to have a very discrete sensitive period for song learning when trained with tape-recorded songs (Marler 1970) do not necessarily exhibit such a restricted learning period when exposed to live tutors (Baptista and Petrinovich 1984). *Exeristes roborator* might learn in ways not revealed here when exposed to live hosts in natural microhabitats.

Exeristes roborator showed some degree of flexibility in its learning of host microhabitats, although it appeared to acquire initial learning more readily than it learned a second host microhabitat. At the end of Exp. 1, females with 5-9 days' experience attacking hosts in one microhabitat

throughout the experiment showed strong learned preferences for that object. In contrast, females with 1-5 days' experience attacking hosts in one microhabitat and then 4 days' experience attacking hosts in another microhabitat, neither retained an absolute preference for the first object, nor developed a full preference for the second object. These females did not have as much opportunity to acquire a learned preference for either microhabitat as females that did not experience such a change, but the similarity of responses between the first and second tests for the latter females showed that more, similar experience did not make a great deal of difference to the strength of learned preferences.

Papaj (1986c) has pointed out that strong initial learning may not readily be displaced by new learning, if the initial learning prevents or interferes with the gaining of experience necessary to reinforce new learning. However, during the second pre-test treatment period in Exp. E1, casual observation of *E. roborator*, and examination of feeding damage to and oviposition on hosts in microhabitats removed from cages each day, indicated that switched females readily attacked hosts in the second microhabitat. Thus strong initial learning did not appear to prevent the parasitoid from gaining the experience necessary for learning of the new microhabitat. The lack of development of a strong learned preference for the second host microhabitat by these females could have occurred because by the time most of these females encountered hosts in this microhabitat they were past their sensitive period for learning. This interpretation would support the hypothesis that the mechanism behind the parasitoid's sensitive period is a decrease in learning capability with age, rather than the alternative hypothesis that development of learning in *E. roborator* is hampered by host deprivation during the critical period. However, females experiencing a host microhabitat change did not completely forget the first microhabitat learned, and this may have prevented them from displaying the full extent of their learning of the second

microhabitat during the second test in Exp. 1. Heinrich *et al.* (1977) suggested that the readiness with which bumble bees forget what they have learned may determine whether or not they readily shift their foraging activities from one sugar source to another.

Heinrich *et al.* (1977) stated that conservative switching behaviour in short-lived worker bumble bees is an advantage if learning is costly in time and energy. For similar reasons, fidelity to an initially-learned host microhabitat might be advantageous to *E. roborator*, but not enough is known about the costs of learning for this parasitoid, or about its interactions with its natural hosts, to conclude this definitely (Wardle and Borden 1985). Retention of some ability to learn to shift to new resources, such as seen in *E. roborator*, would still allow an insect to take advantage of situations in which the costs of learning a new resource were outweighed by the overall benefits of exploiting it.

It is possible that *E. roborator* might not be conservative about transfering its host-seeking activities to new microhabitats under natural conditions. Although females were still quite responsive to the first host microhabitat learned after 4 days' unrewarded exposure to it, they readily shifted their attack to hosts available in the new microhabitat during the second pre-test treatment period, in spite of their lack of preference for this microhabitat when it did not contain hosts.

CHAPTER 5

EFFECT OF PRIOR EXPERIENCE ON THE RESPONSE OF *EXERISTES ROBORATOR* TO A NATURAL HOST AND MICROHABITAT

INTRODUCTION

It is often proposed that learning from prior experience with hosts gives insects the flexibility to track unpredictable or variable resources, either as individuals or as successive generations (e.g. Vinson 1976; Rausher 1978; Heinrich 1979a; Arthur 1981; Jaenike 1982, 1985; Prokopy *et al.* 1982; Papaj and Rausher 1983; Vet 1983; Vet and van Opzeeland 1984, 1985; Papaj 1986a,d). Learning initiated and reinforced by encounters with an abundant or suitable food source or oviposition site may concentrate an insect's foraging activities on this resource.

For a parasitoid intended for release as a biological control agent, learning could potentially be manipulated to enhance its attack upon a target pest, and potentially its success in controlling the pest (Arthur 1967, 1981; Vinson *et al.* 1977), but could also interfere with the parasitoid's response to the pest (Wardle and Borden 1985).

Numerous laboratory studies suggest that host-associated learning could influence attack upon target hosts by parasitoids (e.g. Thorpe and Jones 1937; Monteith 1963; Arthur 1966, 1967, 1971; Samson-Boshuizen *et al.* 1974; Taylor 1974; Vinson *et al.* 1977; Cornell and Pimentel 1978; Sandlan 1980; Strand and Vinson 1982; Vet 1983, 1985a,b; Vet and van Opzeeland 1984, 1985; Dmoch *et al.* 1985; Wardle and Borden 1985; Chow and Mackauer 1986; Drost *et al.* 1986; Luck and Uygen 1986; Lewis and Tumlinson 1988), although parasitoids' responses to hosts are not always affected by prior experience (Weseloh 1984). In order to assess the significance of parasitoid learning for biological control, its effects must be measured under conditions similar or identical to those encountered in environments in which parasitoids would be released. Such studies have not been undertaken to assess directly the effects of learning on the responses of parasitoids to target pests. Nonetheless, learning from contact with host frass (Lewis and Tumlinson 1988) may have been involved in

enhancing searching of host microhabitats and parasitisation by released *Microplitis croceipes* in field and greenhouse studies (Gross *et al.* 1975). Similarly, learning from exposure to hosts or host-related factors could conceivably also have played a role in increasing parasitisation rates in greenhouse and field releases of other parasitoids (Gross *et al.* 1975, 1981; Loke and Ashley 1984).

Detailed field studies of the effects of prior experience on insects' subsequent responses to hosts are plagued by numerous problems. It is difficult to observe most insects for sufficiently long to obtain reliable information about the effects of experience on their host-finding behaviour. Also, many insects forage in environments that can be observed only with considerable disruption. Thus the most extensive field studies have involved butterflies searching for oviposition sites on low vegetation in relatively open terrain (Rausher 1978; Papaj and Rausher 1983, 1987a,b; Stanton 1984; Papaj 1986c,d). More limited field observations on the effect of learning on the foraging behaviour of bumble bees, *Bombus* spp., (Heinrich 1979a; Laverty 1980) and the honey bee, *Apis mellifera*, (Weaver 1956, 1965) have been made, and the possible or real influence of experience on aspects of host utilisation by various diptera has also been examined in field settings (Prokopy *et al.* 1982; Jaenike 1985, 1986, 1988; Hoffmann and Turelli 1985; Prokopy and Fletcher 1987; Hoffmann 1988; Prokopy and Papaj 1988). As an alternative to the true field setting, productive experiments have been carried out in large field cages, which allow some control over conditions of observation, but retain many features that foraging insects encounter in the field (Heinrich 1979a; Laverty 1980; Papaj and Rausher 1983, 1987 a,b; Papaj 1986 a,b,d).

Results of these studies suggest that prior experience with a food source or oviposition site can affect the responses by some insects to hosts

in nature. The accuracy with which 3 sulphur butterflies, ***Colias philodice*** *eriphyle* W.H. Edwards, *C. meadii* W.H. Edwards, and ***C. alexandra*** **W.H. Edwards,** distinguish host from non-host plants is increased over the short term by prior foraging experience, although the butterflies appear to 'forget' quickly what they have learned (Stanton 1984). Learning also causes the pipevine swallowtail, *Battus philenor*, to improve its rate of host discovery and to concentrate its search for oviposition sites on the most suitable host plants (Rausher 1978; Papaj and Rausher 1983, 1987a; Papaj 1986a-d). Foraging bees learn to concentrate their activities on flowers perceived to be most rewarding, and also learn how to improve their handling of different flowers (Weaver 1956, 1965; Heinrich 1979a; Laverty 1980), thus maximising their profits (Heinrich 1979b). Learning from exposure to a particular food might increase the numbers of females of the fruit flies, *Drosophila tripunctata* Loew and *D. melanogaster*, subsequently settling at that food (Jaenike 1985, 1986, 1988; Hoffmann 1988), although such experience does not appear to result in learning by the flies in all instances (Hoffmann and Turelli 1985; Jaenike 1985; Hoffmann 1988). Increased settling at some foods may also be caused by starvation rather than learning (Hoffmann and Turelli 1985). Prokopy *et al.* (1982), Prokopy and Fletcher (1987), and Prokopy and Papaj (1988) have shown that prior experience with 1 host fruit type probably affects host acceptance in the field by the apple maggot fly, *Rhagoletis pomonella*, and the Queensland fruit fly, *Dacus tryoni* (Froggatt). Laboratory studies indicate that this experience often leads to increased rejection of unfamiliar fruit types, although increased acceptance of familiar hosts can also occur (Prokopy *et al.* 1986; Papaj and Prokopy 1986; Prokopy and Fletcher 1987; Prokopy and Papaj 1988).

The research described in this chapter was undertaken primarily to determine whether or not prior experience attacking hosts could influence the host-seeking behaviour and success in attacking hosts of *Exeristes roborator*

under field-cage conditions. This parasitoid can learn to respond to and concentrate its host-finding behaviour on artificial host microhabitats in the laboratory (Wardle and Borden 1985; Chapters 1-4). Therefore, I examined the effects of prior experience with a natural host, the European pine shoot moth, *Rhyacionia buoliana*, in both natural and artificial microhabitats, on the parasitoid's subsequent host-seeking and -attacking responses in a simulated natural environment.

MATERIALS AND METHODS

Parasitoids, hosts, and host microhabitats

For all experiments *E. roborator* were reared on coddled larvae of *G. mellonella* according to Syed's (1985) method, and in all pre-test treatment cages females had access to honey-coated sugar cubes, water, and males.

Except where noted, larvae of *R. buoliana* and pine shoots used in experiments were collected from 7 ornamental plantings of Scots pine, *Pinus sylvestris* (L.), in or near Vancouver, British Columbia. Expanding pine shoots infested with late-instar larvae were clipped from trees along with a length of adjoining branch. Shoots and larvae not used within a few days of collection were stored in plastic bags at 4°C for up to 8 weeks, until 2-3 days before use. Uninfested pine shoots were collected and handled in the same way.

Uninfested potted 3- and 4-year-old Scots pines were purchased from commercial nurseries (Clay's Nurseries and E.J. Murray and Son, Langley, B.C.). Shoot moth infestations were established on these trees as noted for each experiment.

Galleria mellonella larvae used as hosts in Exp. 1 and 2A were coddled (Syed 1985). Egg cups used as artificial host microhabitats were constructed as described in Chapter 3, but without the inclusion of Parafilm.

Preliminary test of the effect of prior experience with hosts in natural and artificial microhabitats on the response of *E. roborator* to Scots pines infested with *R. buoliana*

Experiment 1

To gain an indication of whether or not prior experience affected the responses of *E. roborator* to the natural host/habitat system, a preliminary experiment was conducted from mid-April to mid-May, 1985, in a greenhouse illuminated on a 16 h L:8 h D cycle. Natural light was available for most of the photophase. The temperature normally ranged between 18 and 24°C.

Pre-test treatments

Newly-eclosed females were marked on the thorax with a dot of paint, as in Chapter 1, to identify their eclosion date, divided randomly into 3 groups, and placed in 30 x 30 x 45 cm cages where they were exposed to 3 pre-test treatments (Table 20) for 12-14 days. To give parasitoids in group I experience with the natural system, freshly-cut pine shoots infested with larvae of *R. buoliana* were presented to them in a flask of water. Infested shoots were obtained from 4-year-old potted Scots pines on which female *R. buoliana* had oviposited the previous summer. Six weeks before the experiment, the development of shoots and host larvae was accelerated by moving some of the pines from outside into a greenhouse kept at approximately 25°C. A constant supply of expanded shoots infested with host larvae was maintained by moving new pines into the greenhouse at weekly intervals. As controls, females in group II were not exposed to any hosts or microhabitats, so that the responses of naive females could be measured. Group III females were presented with factitious hosts in an artificial microhabitat, to allow assessment of the effects of this experience on females' responses to the natural system.

Table 20. Pre-test treatments and testing regime for *E. roborator* in Exp. 1.

Group	N	Pre-test treatment[a]	Testing regime
I	25	Given 5 cut shoots of Scots pine, each infested with 1 larva of *R. buoliana*, each day for 12-14 days (*R. buoliana* in pine)	Individuals released for 1 h in an indoor cage containing 1 Scots pine heavily infested with larvae of *R. buoliana*
II	25	Held without exposure to hosts or microhabitats for 12-14 days (no hosts or microhabitats)	"
III	25	Given 1 egg cup containing 5 coddled larvae of *G. mellonella* each day for 12-14 days (*G. mellonella* in egg cup)	"

[a]Fresh, infested pine shoots and egg cups containing hosts were placed in cages each day.

At the end of their final day of pre-test treatment, 2 females were removed from each cage and placed individually in 60 ml glass jars, where they remained overnight.

Testing regime

On the following day these females were released individually into a 1 x 1 x 1 m observation cage containing 1 potted Scots pine heavily infested with late-instar shoot moth larvae (Table 20), to test the hypothesis that prior experience with hosts in different microhabitats had affected their responses to the natural host/microhabitat system. The infested pine came from the same pool of trees from which material was obtained for the pre-test treatment of group I females. The order of testing was randomised within each trio of the 3 or 6 females tested each day.

An opened jar containing a female was placed in the northwest corner of the observation cage. Most females left the jar soon after it was opened. Each female's behaviour was observed and recorded on a microcassette recorder for 1 h after release. A record was kept of whether or not the female contacted the infested pine and responded to it by performing typical host-seeking and -attacking behaviour, such as intensive antennation, ovipositor probing, and feeding, on it. If she did, records were also made of the amount of time spent in each of these activities, the number of ovipositor probes executed, and the number of pine shoots showing external signs of infestation (host webbing and frass) that she searched and probed (attacked), as measures of the intensity of her response. Experimental procedures were repeated until 25 insects from each group had been tested. The test pine was replaced after every fifth trio of females was tested.

Significant elevation of the responses of group I females, and/or depression of the responses of group III females, when compared to the

responses of parasitoids in group II, would suggest that prior exposure to one or both systems influenced females' behaviour towards the natural system.

Effect of prior experience with hosts in natural and artificial microhabitats on the short-term response of *E. roborator* to Scots pines infested with *R. buoliana* in a field cage

These experiments were conducted out-of-doors from late spring to early summer over a 3-year period. *Exeristes roborator* were exposed to pre-test treatments under ambient outdoor conditions, and then were tested for their responses to Scots pines infested with late-instar larvae of *R. buoliana* in a 2.5 x 3.5 m screened field cage with a translucent, fibreglass roof, 1.7 m high at the sides and 2.6 m high at the peak.

Experiment 2A - 1985

Pre-test treatments

Newly-eclosed females were marked with paint as in Exp. 1, divided randomly into 6 groups, placed in 30 x 30 x 45 cm cages in a screened enclosure, and exposed to 6 different pre-test treatments (Table 21, Exp. 2A) for 12-14 days. Parasitoids in groups IV and VIII were allowed to attack *R. buoliana* in its natural microhabitat and in the artificial microhabitat, respectively, to determine if these experiences would increase or decrease their respective responses to infested pines under simulated natural conditions. Females in groups V and VII were given the pine and egg cup microhabitats alone, respectively, to separate any effects of exposure to the microhabitats themselves from the effects of experience attacking hosts in them. Group VI permitted the responses of females with no exposure to the host or microhabitats to be assessed. Group VIII was included to determine if changes in response seen in group III (Exp. 1) were due to exposure to a factitious host, rather than experience attacking hosts in the egg cup.

Table 21. Pre-test treatments and testing regimes for *E. roborator* in Exp. 2A-C and Exp. 3.

Exp.	Group	N	Pre-test treatment[a]	Testing regime[b]
2A	IV	20	Given 5 cut shoots of Scots pine, each infested with 1 host, for 12-14 days (*R. buoliana* in pine)	Individuals released for 1 h in a field cage containing 4 Scots pines heavily infested with hosts
	V	20	Given 5 uninfested cut shoots of Scots pine for 12-14 days (pine alone)	"
	VI	20	Held without exposure to hosts or microhabitats for 12-14 days (no hosts or microhabitats)	"
	VII	20	Given 1 egg cup containing no hosts for 12-14 days (egg cup alone)	"
	VIII	20	Given 1 egg cup containing 5 hosts for 12-14 days (*R. buoliana* in egg cup)	"
	IX	20	Given 1 egg cup containing 5 coddled larvae of *G. mellonella* for 12-14 days (*G. mellonella* in egg cup)	"
2B	IV′	27	Given the terminal growth of a Scots pine infested with 10 hosts for 12-14 days (*R. buoliana* in pine)	Individuals released in a field cage containing alders and 4 Scots pines lightly infested with hosts until they flew to the walls or roof
	V′	27	Given the uninfested terminal growth of a Scots pine for 12-14 days (pine alone)	"
	VI′	27	As for group VI, Exp. 2A (no hosts or microhabitats)	"
	VII′	27	As for group VII, Exp. 2A (egg cup alone)	"
	VIII′	27	Given 1 egg cup containing 10 hosts for 12-14 days (*R. buoliana* in egg cup)	"

Table 21. continued

Exp.	Group	N	Pre-test treatment[a]	Testing regime[b]
2C	IV''	25	Given haemolymph and the terminal growth of a Scots pine infested with 10 hosts for 10-12 days (*R. buoliana* in pine)	Individuals released in a field cage containing alders and 4 Scots pines lightly infested with hosts for 1 h or until they ceased host-related activities on pine for 20 min
	V''	25	Given haemolymph and the uninfested terminal growth of a Scots pine for 10-12 days (pine alone)	"
	VI''	25	Given haemolymph and held without exposure to hosts or microhabitats for 10-12 days (no hosts or microhabitats)	"
	VII''	25	Given haemolymph and 1 egg cup containing no hosts for 10-12 days (egg cup alone)	"
	VIII''	25	Given haemolymph and 1 egg cup containing 10 hosts for 10-12 days (*R. buoliana* in egg cup)	"
3	IV''	6	As for group IV'', Exp. 2C	Groups of 5 females released for 23 h in a field cage arranged as in Exp. 2C
	V''	6	As for group V'', Exp. 2C	"
	VI''	6	As for group VI'', Exp. 2C	"
	VII''	6	As for group VII'', Exp. 2C	"
	VIII''	6	As for group VIII'', Exp. 2C	"

[a]Fresh pine shoots, egg cups, and hosts were placed in cages each day.

[b]Hosts were larvae of *R. buoliana* except as noted for group IX, Exp. 2A.

All pine shoots were presented to females in groups IV and V in flasks of water. Collected shoots showing external signs of infestation were examined to ensure that each contained a suitable host larva before they were offered to females in group IV. Uninfested shoots offered to females in group V were also examined to ensure that they were undamaged by insects. For females in group VIII, late-instar larvae of *R. buoliana* were removed from collected pine shoots and placed between the folds of the Kimwipe in egg cups just before presentation to females.

At the end of their final day of pre-test treatment, 2 females were removed from each cage and placed individually in 60 ml glass jars. These jars were stored overnight in the field cage in which testing was carried out on the following day.

Testing regime

The field cage contained 4 potted Scots pines placed equidistant from the 4 corners, approximately 1 m apart. All trees were heavily infested with late-instar larvae of *R. buoliana*. The shoot moth infestation had been established on these pines as in Exp. 1, except that the pine shoots and larvae had been allowed to develop to the appropriate stage out of doors. Pines for use in the later weeks of the experiment were moved into storage at approximately 4°C for 1-3 weeks to retard further development. Each week a fresh set of infested pines was removed from storage, held 2-3 days, and set out in the field cage in place of the spent pines.

Testing (Table 21, Exp. 2A) was carried out throughout June on days when the temperature in the field cage remained $\geq$18°C, a temperature at which *E. roborator* is reported to be normally active (Fox 1927; Baker and Jones 1934). The females from each group were tested in random order between 0900 h and 1700 h, Pacific Daylight Time (PDT). Each female was released

individually from an opened jar, which was placed on a low wooden platform in the centre of the field cage. Her behaviour was observed and recorded for 1 h, as in Exp. 1. In addition, any pine shoots attacked (searched and probed) by a female were examined at the end of the hour to determine whether or not they contained hosts, and the success of females in attacking larvae (paralysing them and, usually, feeding or ovipositing on them) was noted. Experimental procedures were repeated until 20 females from each group had been tested.

Significant enhancement of the responses of group IV females over those of group V and VI females, and/or reduction of the responses of group VIII and IX females below those of group VI and VII females, would suggest that prior experience attacking hosts in one or both of the microhabitats affected females' responses to the natural system in the field cage.

Experiment 2B - 1986

Pre-test treatments

Newly-eclosed females were marked as in Exp. 1, divided randomly into 5 groups, and placed in 45 x 45 x 90 cm cages kept in the screened outdoor enclosure as in 1985. For 12-14 days these females (groups IV'-VIII') were exposed to 5 different pre-test treatments corresponding to the treatments employed in Exp. 2A for groups IV-VIII (Table 21, Exp. 2A and 2B).

Females in group IV' were exposed to the terminal growth (leader, upper whorl of side branches, and internode between this and the next whorl) of a 3-year-old Scots pine infested with 10 late-instar larvae of *R. buoliana*. Compared to the 1985 treatment this exposure more closely resembled the host microhabitats encountered in the field cage and increased the rewards available to females. When the buds began to expand, infestations on the

terminal growth of pines were created by placing on them small shoot moth larvae from infested pines used the previous year. One day before the terminal growth was to be used for the first time in a pre-test treatment it was clipped from the tree and placed in a flask of water. Infested shoots were checked to ensure that there were 10 healthy late-instar larvae of *R. buoliana*. Pupae or small larvae were removed and replaced with suitable larvae removed from collected infested shoots. They were allowed 24 h to settle into infested sites. After this the terminal growth was placed for 24 h in the cage of group IV females. It was then removed and host corpses and parasitoid eggs were excised from infested sites, and fresh shoot moth larvae were again allowed 24 h to infest vacant sites. Two infested terminal growths treated in this manner were presented on alternate days to females in group IV′ over the period of a week, after which they were replaced with similar, fresh material. A continuous supply of terminal foliage with expanding, infested shoots was ensured by holding some pines at 4°C for up to 4 weeks and, in the case of trees used at the very end of the experiment, in a cool, high-altitude forest for a further few weeks to delay bud expansion, before establishing shoot moth larvae on them. This method of presenting hosts to females in group IV′ allowed considerable control over host quality.

Females in group V′ were exposed on alternate days to 2 terminal growths clipped from uninfested 3-year-old Scots pines. The trees from which these clippings were taken were handled in the same manner as infested pines.

Females in groups VI′-VIII′ were exposed to pre-test treatments identical to those experienced by females in groups VI-VIII in Exp. 2A, except that the egg cup given females in group VIII′ contained 10, rather than 5, host larvae.

At the end of their last day of pre-test treatment, 2 females from each group were placed individually in 100 x 15 mm disposable petri dishes and held overnight in the field cage.

Testing regime

The field test cage contained 4 potted, infested Scots pines placed as in 1985, but, to diversify the vegetation, the pines were interspersed with 5 red alders, *Alnus rubra* Bongard. Each pine was deliberately infested with only 5 late-instar larvae of *R. buoliana*, a much lower host density than in 1985. These infestations were developed and extended over the term of the experiment as for the pre-test terminal growth clippings presented to group IV' females, so that the quality of infestations available for females to attack was more consistent than it had been in 1985. Each week a fresh set of 4 infested pines was placed in the field cage.

Testing (Table 21, Exp. 2B) was carried out from early June to late July, on days when the field cage temperature remained ≥ 18°C. Individual females were released between 0900 h and 1600 h PDT from opened petri dishes placed on the release platform in the centre of the cage. Petri dishes were used instead of glass jars because the insects in them warmed faster after cool nights, and left them more readily than the deeper, narrow-necked jars. The behaviour of females was observed and recorded as in Exp. 2A from the time they left the opened dish until they flew to the cage walls or roof and remained there for 20 continuous min, rather than for the arbitrary 1 h period used in 1985. This procedure allowed better assessment of the persistence and success of females searching for and attacking hosts. In addition to the data recorded in Exp 2A, a record was kept of females' host-seeking behaviour (searching and probing) on the alders, which unexpectedly harboured larval populations of a leaf roller (tentatively identified as *Archips* sp.) and a sawfly, the red-backed sawfly, *Eriocampa ovata* L..

Before release of a female, any shoot moth larvae successfully attacked by the previous female were replaced with late-instar larvae removed from collected infested shoots.

Usually, 1 female/group was tested in a day, and the order in which these females were released each day was randomised. On several occasions a complete replicate of 5 females could not be tested in 1 day because released females spent several hours on the vegetation. On these occasions observations ceased when the last female released before 1600 h spent 20 min on the cage walls or roof, and resumed with a female from the next randomly-selected group on the next suitable day. On such days testing was carried out only on females from the remaining groups in the series. A few females did not fly to the cage walls or roof by 1900 h. None of these females was actively searching for or attacking hosts, and observations were terminated at this time.

Twenty-seven females in each group were tested.

Experiment 2C - 1987

Pre-test treatments

Newly-eclosed females were treated as in Exp. 2B except that they were exposed to pre-test treatments for 10-12 days (Table 21, Exp. 2B and 2C). The pre-test treatment period was shortened to reduce mortality. In addition, females in all groups were allowed to feed on the haemolymph of coddled late-instar *G. mellonella*, in an attempt to eliminate differences between host-experienced and inexperienced females in capacity to develop mature eggs, and thus to oviposit on hosts, without giving females in groups V''-VII'' prior experience attacking host larvae to feed (Appendix 2). Four tubes, each containing the haemolymph of 3 larvae of *G. mellonella* coddled at 65°C

(Appendix 2), were suspended from a clear acetate frame and placed in the centre of the floor of each cage every second day for approximately 8 h.

Infested and uninfested pine clippings and egg cups containing hosts were prepared as in Exp. 2B. At the end of their final day of pre-test treatment, 2 females with swollen abdomens, indicating that they had fed on hosts or haemolymph, were removed from each cage, marked on the thorax with a dot of paint to identify their group, and placed in a 30 x 30 x 45 cm holding cage inside the field cage. There they had access to water and honey-coated sugar cubes, and had the opportunity to adjust to the field cage microclimate. After 24 h they were placed individually in 100 x 15 mm disposable petri dishes in preparation for testing on the following day.

Testing regime

The preparation of the infested and uninfested pines, and the field cage organisation were virtually the same as in 1986, except that each pine in the field cage harboured 8 late-instar larvae of *R. buoliana*. The alders in the field cage were not infested with leaf rollers, but were heavily attacked by *E. ovata*.

Testing (Table 21, Exp. 2C) was carried out from late May to early July, on days when the field cage temperature remained $\geq$20°C and the weather was clear and sunny. In Exp. 2B, *E. roborator* had appeared to be most active under such weather conditions, and less active at the 18° threshold used previously. Between 1100 h and 1900 h each day, females from groups IV''-VIII'' were released in random order as in Exp. 2B, and observed for at least 60 min. Observation of females that did not perform any host-seeking activities on the pines was ended after 60 min. Females that responded to the pines with host-seeking behaviour were observed until they ceased these activities or attacks on hosts for 20 consecutive minutes, or until the end of

the 60 min observation period, whichever came later. I chose this cut-off point because very few females in Exp. 2A and 2B that searched for and attacked hosts on the pines and then remained inactive on the pine foliage for 20 min or longer subsequently resumed these activities. Responses to pines and alders, and success in attacking hosts were recorded as in Exp. 2B, and host larvae successfully attacked by 1 female were replaced as in Exp. 2B before the next female was released.

Twenty-five females in each group were tested.

Effect of prior experience with hosts in natural and artificial microhabitats on the long-term (23 h) success of *E. roborator* in attacking *R. buoliana* infesting Scots pines in a field cage.

Experiment 3

Pre-test treatments

Females from groups IV''-VIII'' (Table 21, Exp. 2C and 3) were used. Just before 1100 h on test days, 5 females were removed from each pre-test treatment cage and placed in a 60 ml jar.

Testing regime

A field cage similar to that used in Exp. 2C was used, except that the 4 Scots pines it contained were infested with a total of 15 late-instar larvae of *R. buoliana*, with 3 or 4 larvae/tree in marked locations. Preliminary tests had shown that 5 females did not exhaust this number of hosts in a 23 h period.

Testing (Table 21, Exp. 3) was conducted from late May to mid July, 1987, on days when the field cage temperature was $\geq$20°C by 1000 h and when the 24 h forecast was for sunny, clear weather. At 1100 h the 5 test females were

allowed to leave the opened jar, which was placed on a ledge on the rear wall of the cage. They were left for 23 h, and retrieved at 1000 h on the following day.

Each marked infested pine shoot was then examined to determine if its occupant was still alive and, if not, to determine if it had been attacked and paralysed by *E. roborator*. When a larva was missing, the trees were thoroughly searched to determine if it had moved to a new location. The remains of all dead larvae were removed from infestations along with any eggs and haemolymph-stained webbing, and late-instar larvae of *R. buoliana* removed from collected infested shoots were placed in all infestations from which hosts had been removed. They were allowed to settle for approximately 1 h. Any pupae were also replaced.

One quintet of females from each group was tested in random order in each of 6 series of releases. In the random release order of each series a 23 h period during which no parasitoids were present in the screenhouse was included as a 'blank' control, to determine whether or not shoot moth larvae went missing or exhibited symptoms similar to those resulting from attack by *E. roborator* in the absence of the parasitoid. If the weather deteriorated during a release, no data were collected, and if bad weather forced a break in a release series, the series was resumed when the weather permitted. In such cases infested sites were checked for the presence of suitable hosts before the next release was started.

Influence of learning on choice of microhabitat in which to attack hosts by *E. roborator*

Experiment 4

Pre-test treatments

Newly-eclosed females were marked on the thorax with paint as in Exp. 1, divided randomly into 5 groups (X-XIV), placed in 30 x 30 x 45 cm cages under insectary conditions as in Chapter 1, and subjected for 10 days to 5 different pre-test treatments (Table 22). Females in groups X-XIII were exposed to the pine and egg cup microhabitats simultaneously. Parasitoids in groups X and XI were given hosts only in one of these microhabitats, to determine if learning would influence their subsequent choice of microhabitat in which to attack hosts. As controls, insects in group XII were offered hosts in both microhabitats, and females in group XIII were given the microhabitats without hosts, to determine if the responses of group X or XI females could be due simply to access to hosts, or exposure to the microhabitats alone, rather than experience attacking hosts in a specific microhabitat. As an additional control, parasitoids in group XIV were not exposed to any hosts or microhabitats.

Collected pine shoots were examined to ensure that they provided the correct infested or uninfested stimulus before being placed in a flask of water and presented to females as in Exp. 2A. Larvae of *R. buoliana* were removed from collected shoots and presented to females in egg cups as in Exp. 2A-C. The positions of the pine shoots and egg cups given simultaneously to females in groups X-XIII were reversed each day. Females freely attacked hosts in both microhabitats.

At the end of their final day of pre-test treatment, 5 females were removed from each cage and re-marked on the thorax with paint for group

Table 22. Pre-test treatments and testing regime for *E. roborator* in Exp. 4.

Group	N	Pre-test treatment[a,b]	Testing regime[b]
X	20	Given 5 cut shoots of Scots pine, each infested with 1 host, and 1 egg cup containing no hosts (hosts in pine)	Given 5 cut shoots of Scots pine, each infested with 1 host, and 1 egg cup containing 5 hosts simultaneously for 1 h
XI	20	Given 5 uninfested cut shoots of Scots pine, and 1 egg cup containing 5 hosts (hosts in egg cup)	"
XII	20	Given 5 cut shoots of Scots pine, each infested with 1 host, and 1 egg cup containing 5 hosts (hosts in both microhabitats)	"
XIII	20	Given 5 uninfested cut shoots of Scots pine and 1 egg cup containing no hosts (microhabitats alone)	"
XIV	20	Held without exposure to hosts or microhabitats (no hosts or microhabitats)	"

[a]Fresh pine shoots, egg cups, and hosts were placed in cages each day for 10 days.

[b]Hosts were larvae of *R. buoliana*.

identification. One female from each group was placed in each of 5 test cages identical to pre-test treatment cages except for the absence of males.

Testing regime

On the following day, testing (Table 22) was carried out on the groups of females in the test cages in random order. Females in each cage were presented simultaneously for 1 h with 5 fresh infested Scots pine shoots in a flask of water and 1 fresh egg cup containing 5 larvae of *R. buoliana*. These larvae had been removed from collected shoots and placed in egg cups immediately before the cups were placed in test cages. A record was kept of whether or not females contacted each host microhabitat and probed it with their ovipositors, of how long females spent in contact with each microhabitat, and of how many times they probed it. The position of the pine shoots and egg cup were reversed in successive cages. Twenty females from each group were tested.

Significantly greater concentration of response on the infested pine or the egg cup containing hosts by females in groups X and XI, respectively, than by all control females would indicate that learning had influenced the parasitoid's choice of microhabitat in which to attack hosts.

Statistical analysis

Experiments 1 and 2A-C

Within each experiment the proportions of females in each group contacting infested pines and searching and probing on them were compared using a test for comparing >2 proportions and a modified Newman-Keuls multiple comparisons procedure (Zar 1984). In Exp. 2A-C this test was also used to compare the proportion of females in each group successfully killing hosts.

The Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980) was used to compare mean times spent in contact with pines and spent in host-seeking and attacking activities, mean numbers of ovipositor probes executed, mean numbers of infested shoots (showing external signs of infestation for Exp. 1, and containing host larvae for Exp. 2A-C) searched and probed, and, for Exp. 2A-C, mean numbers of host larvae killed. These comparisons were made both for all females in each group and for those females in each group responding to pines (searching and probing on them). In Exp. 2B-C, this test was also used to compare the mean proportions of total time spent host-seeking and -attacking, of total probes executed, and of total attacks, that were directed at infested pine shoots, as well as to compare the mean proportions of attacks on infested pine shoots that were successful, and the mean times spent in first feeding attacks. In Exp. 2C mean times spent in first ovipositional attacks, and mean numbers of hosts oviposited on by successful females were also compared. If one or more samples were <5, critical values for the Kruskal-Wallis test statistic were calculated using Wallace's (1959) Beta approximation.

For all statistical tests α=0.05.

Experiment 3

The mean numbers of host larvae attacked by females in each group were compared with the Kruskal-Wallace Test and multiple comparisons procedure (Conover 1980) (α=0.05).

Experiment 4

Data were analysed as in Chapters 1-3. Females were classified according to whether or not they contacted or probed either host microhabitat alone, both microhabitats, or neither microhabitat, and 5 x 4 X^2 analysis was

used to determine if groups differed in the numbers of females in these response categories. When this X^2 was significant (α=0.05), simultaneous confidence intervals were calculated for the differences between group X and groups XI-XIV in the proportion of females contacting or probing only infested pine shoots, and between group XI and groups X and XII-XIV in the proportion of females contacting or probing only egg cups containing hosts.

The mean proportions of total host microhabitat contact time spent in contact with infested pine shoots by all females that contacted at least 1 host microhabitat, and the mean proportions of total ovipositor probes executed on infested pine shoots by all females that probed at least 1 microhabitat, were calculated, and compared by groups with the Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980) (α=0.05). Mean responses to both host microhabitats in total were also compared using this test.

The proportions of females in each group responding in total to host microhabitats were compared with a test for comparing >2 proportions and a modified Newman-Keuls multiple comparisons procedure (Zar 1984) (α=0.05).

RESULTS

Experiment 1

Prior experience with *G. mellonella* in egg cups had a strong adverse effect on the innate tendency of female *E. roborator* to respond to infested Scots pine. In terms of both the percentage of females responding (Fig. 10) and the overall intensity of response (Table 23), females as a whole in group III were inferior to both inexperienced group II females and group I females experienced with *R. buoliana* in cut pine shoots. Parasitoids in group III that responded to the pine with searching and probing behaviour were inferior to both responding group I and group II females in the time they devoted to host-seeking and -attacking behaviour, and to responding group II females in the number of ovipositor probes they executed; however, they did not attack significantly fewer pine shoots showing external signs of infestation than responding females in the other 2 groups (Table 23).

Group I females experienced with *R. buoliana* infesting Scots pine shoots responded to the infested pine in higher numbers than inexperienced females in group II (Fig. 10), suggesting a positive effect of prior experience with this host and microhabitat. However, the responses of females in the 2 groups did not differ in intensity (Table 23), with the exception that responding parasitoids in group I were actually inferior to responders in group II in the number of ovipositor probes they executed (Table 23). Therefore, prior experience attacking *R. buoliana* in cut Scots pine shoots apparently only slightly enhanced the innate tendency of female *E. roborator* to respond to this host and microhabitat.

Figure 10. Percent of *E. roborator* in groups I-III in Exp. 1 contacting a Scots pine infested with larvae of *R. buoliana* and searching and probing on it in an indoor cage. Bars in the same subgraph marked with the same letter are not significantly different [test for comparing >2 proportions and modified Newman-Keuls multiple comparisons procedure (Zar 1984), α=0.05]. Pre-test treatment for group I=*R. buoliana* in pine, for group II=no hosts or microhabitats, and for group III=*G. mellonella* in egg cup.

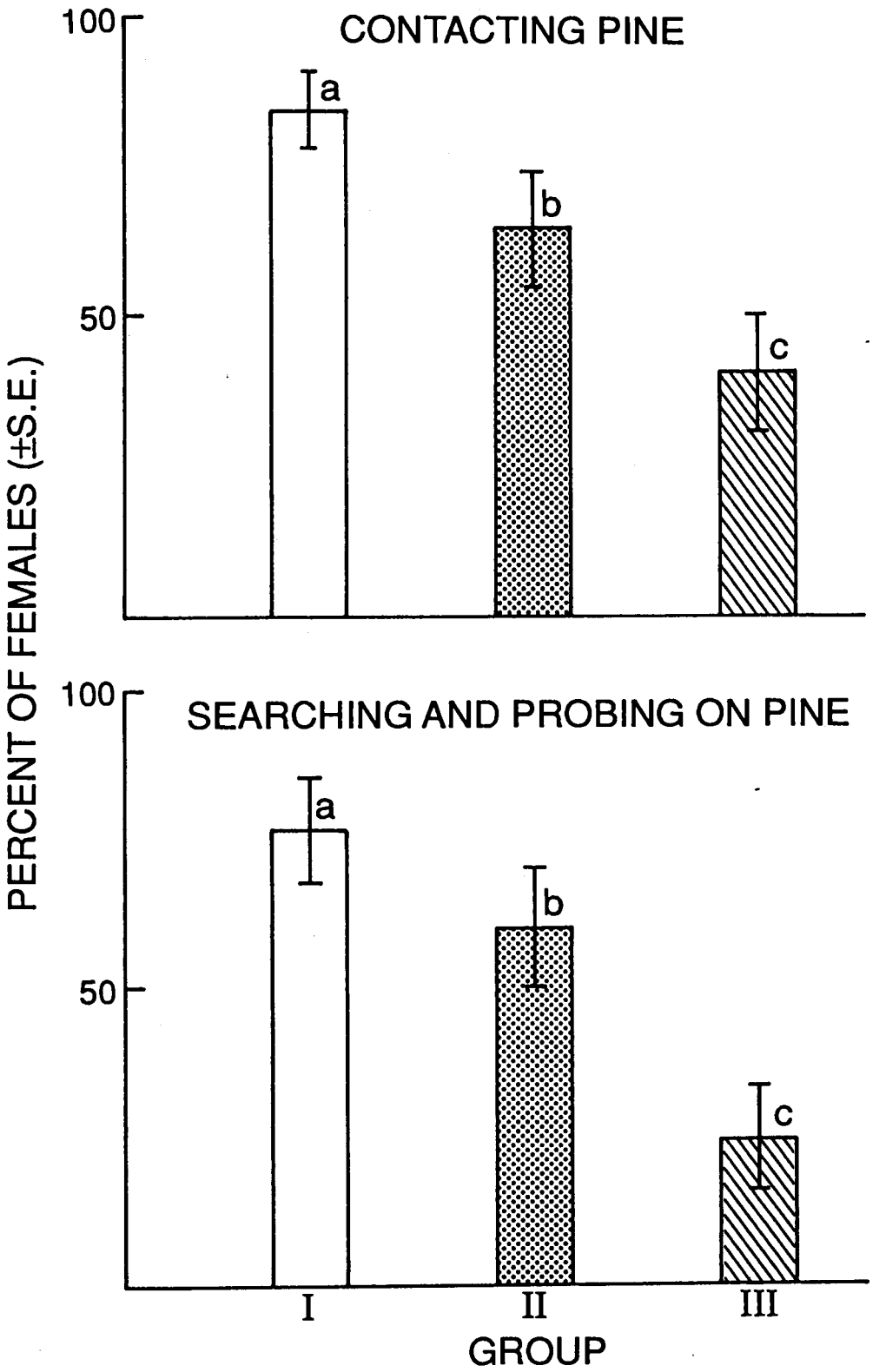

CONTACTING PINE
100
50
a
b
c
SEARCHING AND PROBING ON PINE
100
50
a
b
c
PERCENT OF FEMALES (±S.E.)
I
II
III
GROUP

Table 23. Mean responses by *E. roborator* in groups I-III in Exp. 1 to a Scots pine infested with larvae of *R. buoliana* in an indoor cage.

Category	Group (pre-test treatment)	Mean time (min ±S.E.)[a] in contact	Mean time (min ±S.E.)[a] in host-seeking and -attacking behaviour[b]	Mean (±S.E.)[a] probes executed	Mean (±S.E.)[a] infested[c] shoots searched and probed
All females	I (*R. buoliana* in pine)	32.6 ± 3.8 a	24.7 ± 4.1 a	12.9 ± 3.1 a	1.3 ± 0.3 a
	II (no hosts or microhabitats)	21.6 ± 4.2 a	18.2 ± 3.6 a	17.9 ± 4.2 a	1.4 ± 0.4 a
	III (*G. mellonella* in egg cup)	7.8 ± 2.7 b	3.4 ± 1.4 b	2.1 ± 1.1 b	0.4 ± 0.2 b
Responding females[d]	I (*R. buoliana* in pine)	40.3 ± 3.0 a	32.4 ± 4.0 a	17.0 ± 3.6 a	1.7 ± 0.3 a
	II (no hosts or microhabitats)	35.9 ± 3.7 a	30.3 ± 3.3 a	29.8 ± 4.9 b	2.3 ± 0.5 a
	III (*G. mellonella* in egg cup)	26.8 ± 5.8 a	14.1 ± 3.6 b	8.8 ± 3.7 a	1.5 ± 0.3 a

[a]Within each category means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05.

[b]Intensive antennation, ovipositor probing, and feeding.

[c]Showing external signs of infestation (shoot moth webbing and frass).

[d]Females that searched and probed on the pine. N = 19 for group I, 15 for group II, and 6 for group III.

Experiment 2A-C

Female *E. roborator* in Exp. 2A-C responded to shoot moth-infested Scots pines in the field cage (Fig. 11; Tables 24, 25) essentially as in the greenhouse trial (Fig. 10; Table 23). Females experienced with hosts in the artificial egg cup microhabitat responded less to the infested pines than females in all other groups, but females experienced with *R. buoliana* in pine shoots generally did not respond to infested pines more than inexperienced females.

Experiment 2A

Females in groups VIII and IX, that had been exposed to *R. buoliana* and *G. mellonella*, respectively, in egg cups, were inferior to females in all other groups in virtually every behaviour when the collective responses of all females were compared. The participation rates (Fig. 11, Exp. 2A), and overall intensities of response (Table 24, Exp. 2A) of females in these 2 groups were significantly lower than those of females in groups IV-VII. Moreover, group VIII and IX females killed significantly fewer larvae than all other females save those in group V (Table 24, Exp. 2A). Since females in groups VIII and IX were similar to one another in all aspects of behaviour towards infested pines (Fig. 11, Exp. 2A; Tables 24 and 25, Exp. 2A), the species of host they were offered in egg cups did not play a role in producing the adverse effect of pre-test treatment on their response to the natural host and microhabitat.

Responding females in groups VIII and IX were significantly inferior to responders in most other groups in the time spent in host-seeking and -attacking behaviour and in the number of ovipositor probes executed, but, while they attacked fewer infested pine shoots and killed fewer host larvae than females in groups IV-VII, they were not significantly inferior in these

Figure 11. Percent of *E. roborator* in groups IV-IX in Exp. 2A, groups IV′-VIII′ in Exp. 2B, and groups IV′′-VIII′′ in Exp. 2C contacting Scots pines infested with larvae of *R. buoliana*, searching and probing on them, and successfully attacking at least 1 larva in a field cage. Bars in the same subgraph marked with the same letter are not significantly different [test for comparing >2 proportions and modified Newman-Keuls multiple comparisons procedure (Zar 1984), α=0.05]. Pre-test treatments for groups IV, IV′, and IV′′=*R. buoliana* in pine, for groups V, V′, and V′′=pine alone, for groups VI, VI′, and VI′′=no hosts or microhabitats, for groups VII, VII′, and VII′′=egg cup alone, for groups VIII, VIII′, and VIII′′=*R. buoliana* in egg cup, and for group IX=*G. mellonella* in egg cup.

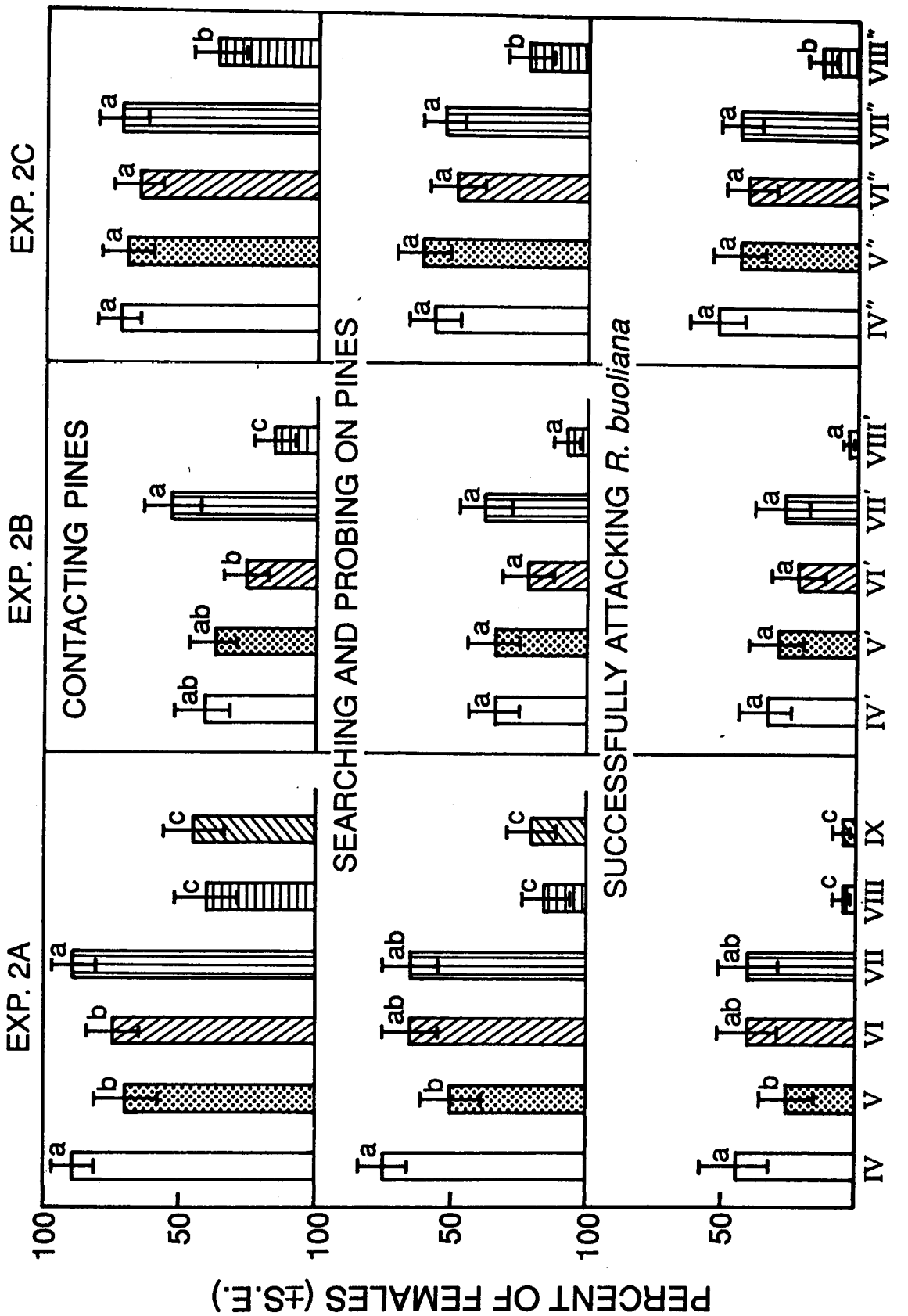

EXP. 2A
EXP. 2B
EXP. 2C
CONTACTING PINES
SEARCHING AND PROBING ON PINES
SUCCESSFULLY ATTACKING R. buoliana
PERCENT OF FEMALES (±S.E.)
100
50
GROUP
IV V VI VII VIII IX
IV' V' VI' VII' VIII'
IV" V" VI" VII" VIII"

Table 24. Mean responses by all *E. roborator* in groups IV-IX in Exp. 2A, groups IV'-VIII' in Exp. 2B, and groups IV''-VIII'' in Exp. 2C to Scots pine infested with larvae of *R. buoliana* in a field cage.

Exp.	Group (pre-test treatment)	Mean time (min ±S.E.)[a] in contact	Mean time (min ±S.E.)[a] in host-seeking and -attacking behaviour[b]	Mean (±S.E.)[a] probes executed	Mean (±S.E.)[a] infested[c] shoots searched and probed	Mean (±S.E.)[a] hosts successfully attacked[d]
2A	IV (*R. buoliana* in pine)	36.6 ± 4.7 a	22.2 ± 4.8 a	15.4 ± 3.2 a	1.3 ± 0.3 a	0.7 ± 0.2 a
	V (pine alone)	25.1 ± 5.5 a	18.3 ± 4.9 a	14.5 ± 4.7 a	0.6 ± 0.2 b	0.3 ± 0.1 ab
	VI (no hosts or microhabitats)	29.3 ± 5.6 a	22.1 ± 4.6 a	17.1 ± 4.4 a	0.8 ± 0.2 ab	0.4 ± 0.1 a
	VII (egg cup alone)	32.9 ± 4.6 a	17.7 ± 3.9 a	12.0 ± 3.2 a	0.9 ± 0.2 ab	0.4 ± 0.1 a
	VIII (*R. buoliana* in egg cup)	7.6 ± 3.4 b	1.6 ± 1.2 b	0.9 ± 0.6 b	0.1 ± 0.07 c	0.05 ± 0.05 b
	IX (*G. mellonella* in egg cup)	6.1 ± 2.8 b	2.0 ± 1.6 b	1.0 ± 0.6 b	0.1 ± 0.07 c	0.05 ± 0.05 b

Table 24. continued

Exp.	Group (pre-test treatment)	Mean time (min ±S.E.)[a] in contact	Mean time (min ±S.E.)[a] in host-seeking and -attacking behaviour[b]	Mean (±S.E.)[a] probes executed	Mean (±S.E.)[a] infested[c] shoots searched and probed	Mean (±S.E.)[a] hosts successfully attacked[d]
2B	IV′ (*R. buoliana* in pine)	84.3 ± 28.1 a	24.1 ± 8.0 a	10.4 ± 3.9 a	0.9 ± 0.3 a	0.8 ± 0.3 a
	V′ (pine alone)	56.1 ± 18.1 ab	21.1 ± 6.4 a	6.0 ± 2.5 a	0.4 ± 0.1 b	0.4 ± 0.1 a
	VI′ (no hosts or microhabitats)	47.3 ± 20.4 ab	13.7 ± 5.7 a	3.3 ± 1.9 a	0.2 ± 0.1 ab	0.2 ± 0.1 a
	VII′ (egg cup alone)	67.0 ± 24.6 a	21.2 ± 7.2 a	5.6 ± 2.1 a	0.4 ± 0.1 a	0.3 ± 0.1 a
	VIII′ (*R. buoliana* in egg cup)	9.1 ± 8.1 b	2.0 ± 1.5 a	0.3 ± 0.26 a	0.04 ± 0.04 b	0.04 ± 0.04 a

Table 24. continued

Exp.	Group (pre-test treatment)	Mean time (min ±S.E.)[a] in contact	Mean time (min ±S.E.)[a] in host-seeking and -attacking behaviour[b]	Mean (±S.E.)[a] probes executed	Mean (±S.E.)[a] infested[c] shoots searched and probed	Mean (±S.E.)[a] hosts successfully attacked[d]
2C	IV'' (*R. buoliana* in pine)	78.1 ± 14.9 a	47.0 ± 10.5 a	28.8 ± 7.7 a	1.6 ± 0.4 a	1.3 ± 0.4 a
	V'' (pine alone)	46.2 ± 8.9 a	31.0 ± 6.6 a	21.3 ± 4.9 a	1.1 ± 0.3 a	0.5 ± 0.1 a
	VI'' (no hosts or microhabitats)	43.5 ± 9.0 a	25.3 ± 6.0 a	17.4 ± 4.7 a	0.9 ± 0.3 a	0.6 ± 0.2 a
	VII'' (egg cup alone)	55.0 ± 10.2 a	30.4 ± 8.0 a	20.3 ± 6.2 a	0.7 ± 0.2 a	0.5 ± 0.1 a
	VIII'' (*R. buoliana* in egg cup)	11.2 ± 3.9 b	4.1 ± 2.0 b	2.5 ± 1.2 b	0.1 ± 0.07 b	0.1 ± 0.07 b

[a]Within each experiment means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05.

[b]Intensive antennation, ovipositor probing, and feeding.

[c]Containing larvae of *R. buoliana*.

[d]Paralysed and (usually) fed or oviposited upon.

Table 25. Mean responses by responding (searching and probing) *E. roborator* in groups IV-IX in Exp. 2A, groups IV'-VIII' in Exp. 2B, and groups IV''-VIII'' in Exp. 2C to Scots pines infested with larvae of *R. buoliana* in a field cage.

Exp.	Group	N	Mean time (min ±S.E.)[a] in contact	Mean time (min ±S.E.)[a] in host-seeking and -attacking behaviour[b]	Mean (±S.E.)[a] probes executed	Mean (±S.E.)[a] infested[c] shoots searched and probed	Mean (±S.E.)[a] hosts successfully attacked[d]
2A	IV (*R. buoliana* in pine)	15	41.1 ± 4.8 a	29.6 ± 5.0 ab	20.5 ± 3.3 a	1.7 ± 0.3 a	0.9 ± 0.2 a
	V (pine alone)	10	46.4 ± 4.0 a	36.6 ± 5.2 a	29.0 ± 6.7 a	1.1 ± 0.2 a	0.5 ± 0.2 a
	VI (no hosts or microhabitats)	13	44.9 ± 4.2 a	34.0 ± 4.2 a	26.4 ± 5.1 a	1.2 ± 0.2 a	0.6 ± 0.1 a
	VII (egg cup alone)	13	40.0 ± 3.7 a	27.1 ± 3.9 abc	18.5 ± 3.9 ab	1.3 ± 0.2 a	0.6 ± 0.1 a
	VIII (*R. buoliana* in egg cup)	3	22.2 ± 11.4 a	10.7 ± 6.6 bc	5.7 ± 2.6 bc	0.7 ± 0.3 a	0.3 ± 0.3 a
	IX (*G. mellonella* in egg cup)	4	22.6 ± 10.7 a	10.0 ± 7.6 c	4.8 ± 2.6 c	0.5 ± 0.3 a	0.3 ± 0.3 a

Table 25. continued

Exp. Group	N	Mean time (min ±S.E.)[a] in contact	Mean time (min ±S.E.)[a] in host-seeking and -attacking behaviour[b]	Mean (±S.E.)[a] probes executed	Mean (±S.E.)[a] infested[c] shoots searched and probed	Mean (±S.E.)[a] hosts successfully attacked[d]
2B IV′ (*R. buoliana* in pine)	9	205.2 ± 52.9 a	72.3 ± 13.8 a	31.2 ± 8.4 a	2.8 ± 0.5 a	2.4 ± 0.5 a
V′ (pine alone)	9	168.1 ± 29.1 a	63.3 ± 7.9 a	18.0 ± 6.0 a	1.2 ± 0.2 b	1.1 ± 0.2 b
VI′ (no hosts or microhabitats)	6	208.3 ± 55.6 a	61.8 ± 13.4 a	15.0 ± 6.8 a	1.0 ± 0 b	1.0 ± 0 b
VII′ (egg cup) alone)	10	178.3 ± 50.2 a	57.3 ± 13.0 a	15.2 ± 4.2 a	1.0 ± 0.1 b	0.7 ± 0.2 b
VIII′ (*R. buoliana* in egg cup)	2	121.5 ± 97.5 a	27.5 ± 7.5 a	4.0 ± 3.0 a	0.5 ± 0.5 b	0.5 ± 0.5 b

Table 25. continued

Exp. Group	N	Mean time (min ±S.E.)[a] in contact	Mean time (min ±S.E.)[a] in host-seeking and -attacking behaviour[b]	Mean (±S.E.)[a] probes executed	Mean (±S.E.)[a] infested[c] shoots searched and probed	Mean (±S.E.)[a] hosts successfully attacked[d]
2C IV'' (*R. buoliana* in pine)	14	126.4 ± 16.8 a	84.0 ± 11.1 a	51.4 ± 10.2 a	2.9 ± 0.5 a	2.3 ± 0.5 a
V'' (pine alone)	15	75.1 ± 8.5 bc	51.6 ± 6.9 b	35.5 ± 5.6 a	1.8 ± 0.3 ab	0.9 ± 0.2 b
VI'' (no hosts or microhabitats)	12	81.4 ± 9.4 ab	52.7 ± 5.6 ab	36.3 ± 6.2 a	1.9 ± 0.3 ab	1.3 ± 0.3 ab
VII'' (egg cup alone)	13	89.9 ± 12.1 ab	58.5 ± 10.5 ab	39.1 ± 9.4 a	1.4 ± 0.2 bc	0.9 ± 0.1 b
VIII'' (*R. buoliana* in egg cup)	5	42.4 ± 5.6 c	20.4 ± 6.5 c	12.4 ± 3.9 a	0.6 ± 0.2 c	0.6 ± 0.2 b

[a]Within each experiment means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05.

[b]Intensive antennation, ovipositor probing, and feeding.

[c]Containing larvae of *R. buoliana*.

[d]Paralysed and (usually) fed or oviposited upon.

respects (Table 25, Exp. 2A). Thus, prior experience with hosts in an egg cup had no significant effect on the success of these responders in attacking hosts over the 1 h observation period.

Inexperienced females in group VI did not differ from females in groups IV, V, and VII in virtually all behaviours recorded (Fig. 11, Exp. 2A; Tables 24 and 25, Exp. 2A). However, the percent of females contacting the infested pines was significantly higher for groups IV and VII than for group VI (Fig.11, Exp. 2A). Therefore, prior exposure to *R. buoliana* in Scots pine shoots (group IV), to the pine shoots alone (group V), or to the egg cup alone (group VII) had little effect on females' responses to infested pine. Significant differences occurred between groups IV and V in some responses (Fig. 11, Exp. 2A; Table 24, Exp. 2A), indicating that prior exposure to infested and uninfested pine shoots may have affected females' behaviour towards infested pines. However, since only in numbers contacting the pines was there a significant difference between females in either of these 2 groups and inexperienced group VI females (Fig. 11, Exp. 2A), such effects must have been very slight.

Experiment 2B

In 1986 there were almost no significant differences between groups IV'-VIII' in responses to the infested pines in the field cage (Fig. 11, Exp. 2B; Tables 24 and 25, Exp. 2B), but trends in the data generally parallelled results obtained in 1985. However, when responding females in Exp. 2B were allowed time to complete sessions of host-seeking behaviour and attacks on hosts, *E. roborator* experienced with *R. buoliana* on pine (group IV') attacked significantly more infested shoots, and killed significantly more hosts, than females in all other groups (Table 25, Exp. 2B). This result occurred even though these group IV' females did not devote significantly more host-seeking or -attacking time or effort to the infested pines than females in the other

groups (Table 25, Exp. 2B). Neither did they devote greater mean proportions of the time spent in these activities, the probes they executed, or the attacks they carried out, to infested pine shoots, as opposed to dried and cracked sites on the pines damaged by other causes (Kruskal-Wallis Test, α=0.05). Group IV′ females also did not perform specific attacking behaviours more quickly than other females. For females that fed on hosts, the mean duration of first feeding attacks from the time searching began until feeding was complete did not differ between groups (Kruskal-Wallis Test, α=0.05). As well, group IV′ females that attacked infested pine shoots were no more successful in killing the occupants than females in other groups. For females that initiated attacks on infested shoots, the mean proportions of successful attacks did not differ between groups (Kruskal-Wallis Test, α=0.05). Thus, responding females in group IV′ did not appear to be superior at distinguishing suitable sites for attack, nor were they quicker or more adept at accepting or handling the microhabitat or host because of their prior experience.

Females in groups V′-VII′ were incapable of oviposition because they were denied hosts on which to feed during pre-test treatment (Appendix 2). Thus they made only feeding attacks on hosts during testing. However, 14 of the 22 successful attacks by group IV′ females, that had fed upon hosts during pre-test treatment, resulted in oviposition. The mean duration of these ovipositional attacks by group IV′ females was 18.0 $\pm$ 2.5 min, while the mean duration of feeding attacks by these females was 40.8 $\pm$ 7.9 min (N=7). Mean duration of feeding attacks by group V′-VII′ females were somewhat higher, ranging from 52.0 $\pm$ 6.7 min (N=10) for females in group V′ to 63.6 $\pm$ 14.9 min (N=7) for group VII′ females. The 1 successful female in group VIII′ spent 35 min in a feeding attack on a single host. Thus, ovipositional attacks appeared to occur much faster than feeding attacks. In addition to making the fastest attacks, ovipositing females appeared to have the capacity to execute

more attacks in succession than feeding females. Eight of the 9 successful group IV′ females oviposited on hosts; of these 8 females, 6 killed 2-5 larvae before they ceased searching (the other female fed on a single host). In contrast, of the 22 successful females from other groups (that only fed on attacked hosts), just 2 killed more than a single larva. Each of these 2 females fed on 2 hosts before stopping searching and attacking behaviour.

Group IV′ responders probably investigated significantly more infested shoots and attacked significantly more hosts than group V′-VII′ responders, without expending significantly more time and effort (Table 25, Exp. 2B), because females that were ovipositing on hosts were capable of carrying out a higher number of more rapid attacks than females that were only feeding. Responding group VIII′ females, that also had the capacity to oviposit, having previously fed on hosts in egg cups, probably did not have an elevated attack rate (Table 25, Exp. 2B) because their experience with hosts in egg cups interfered with normal responses to infested pines.

Females in groups IV′-VIII′ also differed in their responses to the leaf-roller- and sawfly-infested alders in the field cage. None of the females experienced with *R. buoliana* in either pine shoots or egg cups (groups IV′ and VIII′) responded to the alders with host-seeking behaviour, while significantly higher numbers of females from groups V′-VII′ searched and probed on these trees (Fig. 12, Exp. 2B).

Experiment 2C

In 1987 *E. roborator* in groups IV′′-VIII′′ again responded to infested pines in the field cage as females in groups IV-VIII in Exp. 2A had done. For females in group VIII′′, exposure to *R. buoliana* in egg cups decreased overall response to the pines and success in attacking hosts significantly below that of females in all other groups (Fig. 11, Exp. 2C;

Figure 12. Percent of *E. roborator* in groups IV′-VII′ in Exp. 2B and groups IV′′-VIII′′ in Exp. 2C searching and probing on alders. Bars in the same subgraph marked with the same letter are not significantly different [test for comparing >2 proportions and modified Newman-Keuls multiple comparisons procedure (Zar 1984), α=0.05]. Pre-test treatments for groups IV′ and IV′′=*R. buoliana* in pine, for groups V′ and V′′=pine alone, for groups VI′ and VI′′=no hosts or microhabitats, for groups VII′ and VII′′=egg cup alone, and for groups VIII′ and VIII′′=*R. buoliana* in egg cup.

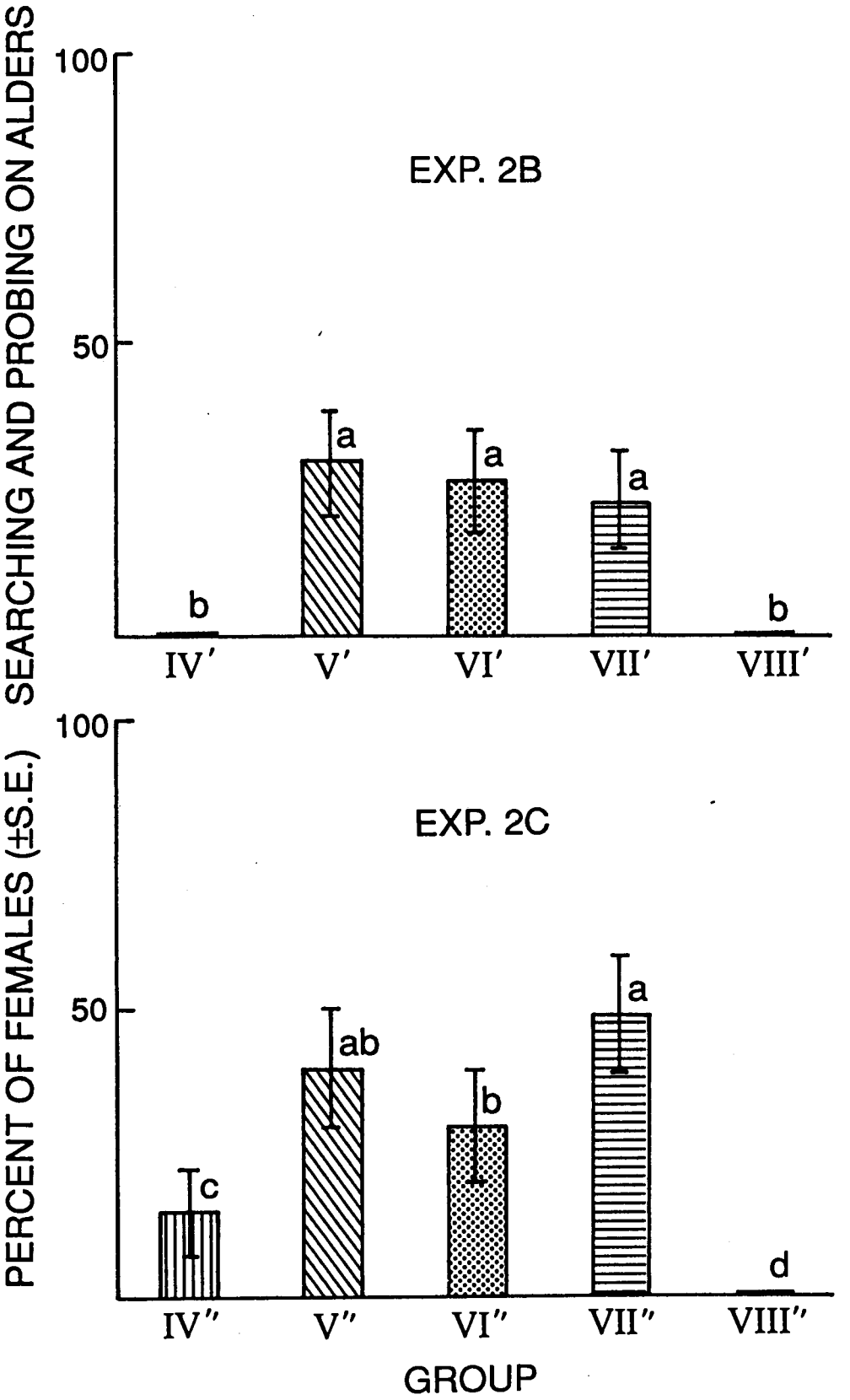

PERCENT OF FEMALES (±S.E.) SEARCHING AND PROBING ON ALDERS
100
50
EXP. 2B
a
a
a
b
b
IV′
V′
VI′
VII′
VIII′
100
50
EXP. 2C
a
ab
b
c
d
IV″
V″
VI″
VII″
VIII″
GROUP

Table 24, Exp. 2C). On the other hand, experience with this host infesting Scots pine, and exposure to pine or egg cups alone, had no significant effect on overall responses to infested pines or success in attacking hosts for females in groups IV''-VII'' (Fig. 11, Exp. 2C; Table 24, Exp. 2C).

Females from group VIII'' that responded to the infested pines performed some host-seeking and -attacking behaviours at lower intensities than females in other groups (Table 25, Exp. 2C). They also directed significantly lower proportions of their host-seeking activities towards infested pine shoots (Table 26). However, their ultimate success in attacking hosts was not significantly different from that of responding inexperienced females in groups V''-VII''(Table 25, Exp. 2C).

Although responding females in groups IV''-VII'' differed little in the intensity of their responses to the infested pines (Table 25, Exp. 2C), some differences occurred between group IV'' and V'' females, again suggesting that prior exposure to infested pine shoots and to pine alone might have affected females' behaviour towards infested pine in a way too subtle to be detected through comparison with females exposed to nothing.

When all females were fed haemolymph of *G. mellonella*, the superiority of responding females experienced with infested pine in successful host attacks (seen in Exp. 2B) was reduced but not entirely eliminated. Responding group IV'' females still killed significantly more hosts than responding females in groups V'', VII'', and VIII'' (Table 25, Exp. 2C).

As in 1986, responding females experienced with *R. buoliana* in pine shoots did not differ significantly from responding group V''-VII'' females in the proportions of host-seeking and -attacking activities devoted to infested shoots (Table 26). There were also no significant differences between groups in the mean times females that oviposited or fed on hosts spent in their first

Table 26. Mean percent of total host-seeking and -attacking responses directed at infested pine shoots by responding (searching and probing) *E. roborator* in groups IV''-VIII'' during testing in Exp. 2C.

Group (pre-test treatment)	N	Mean % of total (±S.E.)[a]		
		time in host-seeking and -attacking activities[b] spent on infested[c] shoots	probes executed on infested[c] shoots	attacks carried out on infested[c] shoots
IV'' (*R. buoliana* in pine)	14	90.9 ± 4.9 a	87.6 ± 5.9 a	88.6 ± 5.4 a
V'' (pine alone)	15	94.3 ± 3.2 a	93.7 ± 2.5 a	85.3 ± 5.6 a
VI'' (no hosts or microhabitats)	12	98.8 ± 8.6 a	95.3 ± 3.2 a	95.8 ± 3.0 a
VII'' (egg cup alone)	13	95.8 ± 2.6 a	94.3 ± 4.1 a	90.4 ± 5.3 a
VIII'' (*R. buoliana* in egg cup)	5	42.5 ± 19.6 b	36.9 ± 18.4 b	40.0 ± 18.7 b

[a]Means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05.

[b]Intensive antennation, ovipositor probing, and feeding.

[c]Shoots containing larvae of *R. buoliana*.

attacks of each type, or in the mean proportions of attacks on infested shoots that were successful (Kruskal-Wallis Test, α=0.05. Group VIII'' was not included in the comparison of mean times spent in first feeding attacks, as no females in it fed on hosts.). Therefore, while responding group IV'' females were still somewhat superior at causing host mortality (Table 25, Exp. 2C), an enhanced ability to distinguish suitable sites for attack or to handle or accept the microhabitat or host, resulting from prior experience, again did not appear to be at the root of this superiority.

Although ovipositional attacks occurred in all groups when females were fed the haemolymph of *G. mellonella*, parasitoids in each group still did not oviposit equally on attacked hosts. Successful group IV'' females oviposited on their victims significantly more than successful females in groups V'' and VII''.[1] As in Exp. 2B, these ovipositional attacks tended to be shorter in duration than feeding attacks. For females in groups IV''-VII'', which both fed and oviposited on hosts, the mean duration of feeding attacks ranged from 44.9 $\pm$ 6.4 min (N=9) for group VI'' to 65.9 $\pm$ 12.9 min (N=8) for group VII'', while the average length of all ovipositional attacks ranged from 21.3 $\pm$ 4.9 min (N=6) for group VI'' to 34.0 $\pm$ 2.0 min (N=2) for group VII''. For group VIII'' females, ovipositional attacks (the only kind executed) lasted 22.7 $\pm$ 9.4 min (N=3) on average. Again, in this experiment females that oviposited tended to carry out more host attacks than females that did not. Of the 48 females from all groups that attacked hosts, 22 oviposited, and 11 of these females carried out successful attacks on 2-7 larvae. Group IV'' females carried out 7 of these multiple host attacks, and the remaining 4 were carried out by group VI'' females. Of the 26 successful females that did not oviposit, only 3 killed more than a single larva (each of these 3 females killed 2 larvae). These non-ovipositional single and multiple attacks were distributed among groups IV''-VII'', although most of them occurred in groups V''-VII''.

Therefore, the superiority of responding females experienced with infested pine in successfully attacking hosts could again be explained by differences between these females and females without prior exposure to hosts in tendency to oviposit. Group IV'' females oviposited more often than group V''-VII'' females[1], even though the latter females were fed, and the shorter duration of ovipositional attacks would again mean that more hosts could be killed without a significant elevation in the time spent in completing attacks (Table 25, Exp. 2C). As in 1986, responding group VIII'' females, that were also experienced with hosts, probably did not show this enhanced success in attacking hosts (Table 25, Exp. 2C) because exposure to hosts in egg cups had interfered with the parasitoid's normal responses to infested pine (Fig. 11, Exp. 2C; Tables 24 and 25, Exp. 2C, and 26).

As in Exp. 2B, females in Exp. 2C that were experienced in attacking *R. buoliana* in pine shoots or egg cups (groups IV'' and VIII'') showed little or no tendency to perform host-seeking behaviour on alders, while significantly higher numbers of females without prior experience with hosts (groups V''-VIII'') searched and probed on these trees (Fig. 12, Exp. 2C).

Experiment 3

When given 23 h in which to attack *R. buoliana* infesting Scots pines, *E. roborator* experienced with this host in egg cups (group VIII'') continued to show reduced success (Table 27). The success of females that had prior experience attacking *R.buoliana* infesting pine (group IV'') was no greater than that of females with no previous exposure to hosts (groups V''-VII'') over this duration, and these latter females also did not differ from one another in this respect (Table 27).

Shoot moth larvae did not die or leave infestations without re-establishing themselves elsewhere on the pines when *E. roborator* were not

Table 27. Mean number of larvae of *R. buoliana* attacked by quintets of *E. roborator* from groups IV''-VIII'' in Exp. 3. over a 23 h period in a field cage.

Group (pre-test treatment)	N	Mean no. larvae dead + missing (±S.E.)[a]
IV'' (*R. buoliana* in pine)	6	9.0 ± 1.3 a
V'' (pine alone)	6	8.0 ± 1.3 a
VI'' (no hosts or microhabitats)	6	6.5 ± 1.0 a
VII'' (egg cup alone)	6	7.2 ± 0.5 a
VIII'' (*R. buoliana* in egg cup)	6	2.3 ± 0.4 b

[a]Means followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05.

present in the field cage. Therefore, all missing or dead larvae were attributed to the activities of the parasitoid, and were counted as successful attacks.

Experiment 4

Exeristes roborator presented with larvae of *R. buoliana* only in egg cups responded to the host in this microhabitat almost exclusively, virtually ignoring hosts in their natural microhabitat when offered a choice of infested pine shoots or egg cups containing host larvae (Fig. 13; Table 28). Females given larvae of *R. buoliana* only in pine shoots subsequently concentrated their responses on hosts in this microhabitat, but only to a significantly greater degree than females entrained to cups. Females given no prior exposure to hosts showed a strong innate response to infested pine shoots (Fig. 13; Table 28).

Highly significant differences occurred among groups in the numbers of females contacting and probing (X^2 test, $p<0.001$) either host microhabitat alone, both microhabitats, or neither microhabitat, with a greater proportion of females from group XI than from any other group restricting their responses to egg cups containing host larvae (Fig. 13). The highest proportions of females contacting and probing only the infested pine shoots were found in group X, but these proportions were significantly different only from the proportions of females in group XI that responded to this host microhabitat exclusively (Fig. 13).

Responding females in group XI devoted only approximately 6% of their contact time and ovipositor probes to infested pine shoots, significantly less than any other group (Table 28). Thus they devoted almost all their responses to egg cups containing *R. buoliana*. Although responding group X females directed more than 80% of their contact time and ovipositor probes to infested

Figure 13. Percent of *E. roborator* in groups X-XIV in Exp. 4 responding to infested Scots pine shoots only, an egg cup containing larvae of *R. buoliana* only, both host microhabitats, or neither microhabitat. Bars marked with an asterisk are significantly different from all other bars in the same subgraph. Bars marked with a diamond are significantly different only from the lowest bar in the same subgraph [simultaneous 95% confidence intervals for differences between proportions (Miller 1981)]. Pre-test treatment for group X=hosts in pine, for group XI=hosts in egg cup, for group XII=hosts in both microhabitats, for group XIII=microhabitats alone, and for group XIV=no hosts or microhabitats.

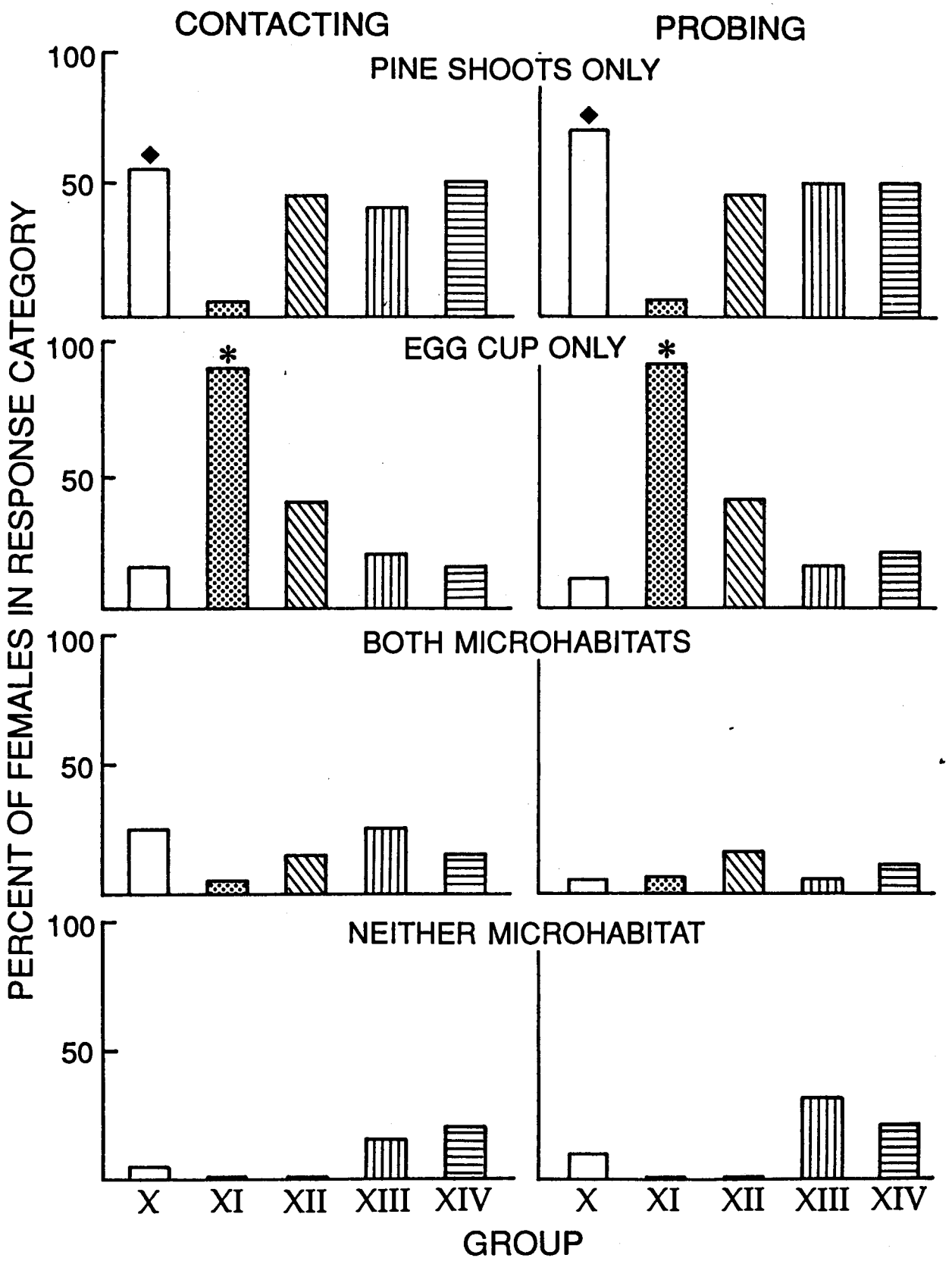

CONTACTING
PROBING
PINE SHOOTS ONLY
EGG CUP ONLY
BOTH MICROHABITATS
NEITHER MICROHABITAT
100
50
PERCENT OF FEMALES IN RESPONSE CATEGORY
X
XI
XII
XIII
XIV
GROUP

Table 28. Mean percent of total contacting and probing responses to microhabitats of larvae of *R. buoliana* directed at infested pine shoots by responding *E. roborator* in groups X-XIV during testing in Exp. 4.

Group (pre-test treatment)	No. females contacting microhabitats	Mean % (±S.E.) of total contact time spent on infested pine shoots[a]	No. females probing microhabitats	Mean % (±S.E.) of total probes executed on infested pine shoots[a]
X (hosts in pine)	19	82.4 ± 8.5 a	18	88.7 ± 7.6 a
XI (hosts in egg cup)	20	6.4 ± 5.1 b	20	6.0 ± 5.0 c
XII (hosts in both microhabitats)	17	53.7 ± 10.9 a	20	53.8 ± 10.9 b
XIII (microhabitats alone)	20	72.5 ± 10.6 a	14	74.3 ± 11.6 ab
XIV (no hosts or microhabitats)	16	69.9 ± 10.9 a	16	67.6 ± 11.2 ab

[a]Means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05. Mean % of total response on egg cup = 100 - mean % of total response on pine shoots.

pine shoots, group XIII and XIV females also directed similar proportions of their responses to the infested shoots (Table 28).

The total responses of females in groups XI and XII to host microhabitats were similar and, after contact, significantly higher than the responses of females exposed to the microhabitats but not hosts (group XIII), or not exposed to hosts or microhabitats (group XIV) (Table 29). The intensity of total response to host microhabitats by group X females was also similar to that of group XI and XII females (Table 29), suggesting that learning might be playing a role in the responses to microhabitats of females in all 3 groups. However, while the total responses of group X females to host microhabitats were consistently higher than those of group XIII and XIV females, differences between these groups generally were not significant (Table 29).

Table 29. Total contacting and probing responses to microhabitats of larvae of *R. buoliana* by *E. roborator* in groups X-XIV during testing in Exp. 4.

Group (Pre-test treatment)	Percent of females (±S.E.)[a] contacting microhabitats	Percent of females (±S.E.)[a] probing microhabitats	Mean total (±S.E.)[b] min in contact contact with microhabitats	Mean total (±S.E.)[b] probes executed on microhabitats
X (hosts in pine)	95.0 ± 4.9 a	90.0 ± 6.7 b	31.6 ± 3.9 ab	17.3 ± 2.5 ab
XI (hosts in egg cup)	100 a	100 a	39.9 ± 3.8 a	20.5 ± 3.0 a
XII (hosts in both microhabitats)	100 a	100 a	38.2 ± 3.4 a	21.0 ± 2.2 a
XIII (microhabitats alone)	85.0 ± 8.0 a	70.0 ± 10.2 c	26.7 ± 4.6 b	11.9 ± 3.1 b
XIV (no hosts or microhabitats)	80.0 ± 8.9 a	80.0 ± 8.9 bc	24.9 ± 4.5 b	13.8 ± 2.3 b

[a]Percentages in a column followed by the same letter are not significantly different, test for comparing >2 proportions and modified Newman-Keuls multiple comparisons procedure (Zar 1984), α=0.05.

[b]Means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05.

DISCUSSION

The reduction in overall response to infested pines and attack upon *R. buoliana* by *E. roborator* exposed to *G. mellonella* (Exp. 1 and 2A) or *R. buoliana* (Exp. 2-3) in egg cups must have been due to experience attacking hosts in this artificial microhabitat, because the responses and success of females exposed to *R. buoliana* in pine, or exposed to the egg cup alone, were not similarly depressed.

This negative influence on response to infested pines was probably a result of learning of the egg cup host microhabitat by females experienced in attacking hosts in it. From this experience *E. roborator* learns to respond to egg cups without hosts with normal host-seeking behaviour (Wardle and Borden 1985). In Exp. 4, such learning caused parasitoid females to ignore infested pine and concentrate their attacks on *R. buoliana* in egg cups. This preference must have been due to microhabitat learning on the part of females, because no other females in the experiment, no matter what their experience, showed the same preference for egg cups containing shoot moth larvae. Therefore, the preference cannot have resulted from general exposure to hosts or microhabitats. Nor can it have been an innate response of inexperienced females that was suppressed by prolonged exposure to microhabitats without reward during pre-test treatment. Finally, it could not have been due to learning of host cues, since both egg cups and pine shoots contained the same hosts during pre-test treatment and testing.

Experience attacking hosts in egg cups did not impair females' ability to perform host-seeking behaviour, since in Exp. 4 the total responses of all females that were experienced with hosts to host microhabitats were similar, regardless of where they had previously attacked hosts. Neither is it likely that the responses to infested pines by females experienced with hosts in egg cups only appeared to be significantly depressed because deprivation caused

inexperienced females not given hosts during pre-test treatment to respond to the pines at elevated levels. In insectary tests even 3-4 day old females that had never been exposed to hosts showed substantially greater responses to cut, infested pine shoots than females that had prior experience with hosts in egg cups.[2] These young, inexperienced females were just beginning to search for hosts (Wardle and Borden 1985), and so would not have been suffering from the effects of deprivation. Females held for 12 days without access to hosts did not show a greater response to cut infested pine shoots than the younger inexperienced females.[2]

Experience attacking hosts in egg cups appeared both to prevent females from responding to infested pines, and to reduce their execution of appropriate responses on the pines. When females exposed to hosts in egg cups did respond, it was often at a lower intensity, and with less accuracy, than females in other groups, although they were not so greatly affected that their attack on larvae of *R. buoliana* was significantly curtailed.

The lack of significant reduction in response to infested pines by females experienced with *R. buoliana* in egg cups in Exp. 2B (1986) was probably due to cool, unsettled weather over the course of the experiment, which caused activity levels to be low in all groups. A warm, sunny June in 1985 caused the temperatures in Exp. 2A to be well above 18°C on most test days. In contrast, temperatures were rarely much above the 18°C minimum required for testing on test days in Exp. 2B. During Exp. 2C, for which experimental procedures were virtually identical to those for Exp. 2B, testing was once again done in warm, stable weather, and the results were similar to those in Exp. 2A. Thus changes in experimental procedure between Exp. 2A and 2B were not responsible for the low activity levels of females in the latter experiment.

The effect of depression of response to infested pines due to egg cup learning on females' overall success in attacking hosts was not transitory, as the total number of hosts killed by females possessing this learning was still low after 23 h in Exp. 3.

Prior experience with *R. buoliana* infesting pine shoots caused no apparent short- or long-term increase in the numbers of *E. roborator* responding to infested pines, or in the intensity of their responses, since in almost all behaviours assessed in Exp. 1-4 parasitoids with this experience did not differ significantly from inexperienced females. However, when females were allowed to complete host finding and attacking in the field cage in Exp. 2B and 2C, responding females with prior experience with this host and microhabitat tended to be superior in both discovery of infested shoots (Exp. 2B) and attack on hosts (Exp. 2B and 2C). These superior responses could have indicated a learning effect, in which experienced females had become 'expert' at recognizing infested shoots, or were particularly efficient and adept at handling or accepting the microhabitat or hosts, and thus in a given duration could investigate more infested shoots and carry out more host attacks than other females. However, in neither experiment were females experienced with R. buoliana in pine shoots superior to inexperienced females at any of these functions.

A more probable reason for the higher success rate of females experienced with hosts in pine could be that experienced and inexperienced females differ in their ability, and possibly their tendency, to oviposit on hosts. Ovipositing females appeared to be able to carry out more host attacks in less time than females that only fed on hosts. In Exp. 2B, ability to oviposit was limited to those females that had been given hosts during pre-test treatment (Appendix 2). Thus differences in reproductive maturity could

have caused the observed differences in success between females experienced with hosts in pine shoots and inexperienced females.

When all females were fed haemolymph of *G. mellonella* in Exp. 2C to make them capable of ovipositing on hosts (Appendix 2), *E.* roborator experienced with *R. buoliana* in pine were significantly superior to some, but not all, host-inexperienced females in successfully attacking hosts, indicating that differences in reproductive status had been at least partly responsible for differences in success between these groups in the previous year. Nonetheless, in Exp. 2C females experienced with *R. buoliana* infesting pine oviposited on attacked hosts significantly more often than most inexperienced females, and the success of the former females in attacking hosts still reflected this difference. There are 2 possible explanations for this result. Either the haemolymph diet fed upon by females during pre-test treatment had not actually equalised the reproductive capacity of experienced and inexperienced females, or *E. roborator* has a strong tendency to feed upon the first hosts it attacks, regardless of its reproductive capacity. The diet was nutritionally equivalent to intact hosts under insectary conditions, and enabled females to produce many eggs (Appendix 2). During pre-test treatment in Exp. 2C, females readily fed on the haemolymph they were given. Over the 10-12 days they had the opportunity to feed 5-6 times, and probably fed as much as they had in the insectary experiments described in Appendix 2. It is unlikely that the addition of shoot moth larvae to the diet of experienced females could have imparted a greater reproductive capacity than in inexperienced females, in light of the considerable egg production of females fed haemolymph (Appendix 2). However, warm temperatures in the screened enclosure could have caused the haemolymph to spoil, rendering it ineffective as a source of nutrients for egg maturation. Therefore the reproductive capacities of experienced and inexperienced females in Exp. 2C could have differed. Differences in oviposition rate between fed, inexperienced and

experienced females could also have reflected differences in tendency to oviposit on hosts, but if *E. roborator* does possess a strong tendency to feed rather than oviposit on the first hosts it attacks, it is unclear why females fed haemolymph oviposited so readily on *G. mellonella* larvae (Appendix 2). Possibly preventing females tested in Appendix 2 from feeding on the hosts they were offered in egg collection devices was enough to divert them to oviposition behaviour, or possibly the tendency was not elicited by dead, factitious host larvae.

It is difficult to conclude definitely that increased success was not an effect of learning, since most multiple host attacks were carried out by females that were also experienced with shoot moth larvae in pine. However, in Exp. 2C these attacks were not strictly limited to females in this group, but were also carried out by inexperienced group VI'' females. Therefore, they did not appear to be linked solely to experience with hosts.

In both Exp. 2B and 2C, responding *E. roborator* given *R. buoliana* in egg cups had the same opportunities to feed upon hosts during the pre-test treatment as females given larvae in pine shoots, yet they did not show the same enhanced host attack and oviposition rates during testing. This result does not invalidate the above argument that females experienced with infested pine shoots had a greater tendency to attack hosts during testing than inexperienced females because of an increased capacity for oviposition. The experience of attacking hosts in egg cups interfered with females' normal responses to infested pines, and probably prevented them from finding many hosts on which to oviposit.

Since consistent increases in response to infested pines by females experienced with this host/microhabitat system did not occur, learning did not enhance females' responses to this resource in the way it enhances their

responses to hosts in artificial microhabitats (Wardle and Borden 1985). There are several possible explanations for this result.

Firstly, it is possible that the experimental procedures did not facilitate demonstration of such enhancement. There is some suggestion in the data that prior experience with *R. buoliana* in pine shoots might increase the responses of *E. roborator* to infested pine, but the parasitoid may have responded for the most part directly and innately to host-associated cues during testing, obscuring any role played by the microhabitat in host-finding. An unsuccessful attempt to eliminate this possibility was made in Exp. 2B and 2C by decreasing the host density and increasing the diversity of the vegetation present. Alternatively, in Exp. 1-3, females exposed to cut infested pine in small cages may have acquired learning that was of little consequence in host-finding in larger cages containing intact pines. An attempt to increase the general resemblance between host microhabitats in pre-test treatment and testing by using a larger cut portion of pine in Exp. 2B and 2C did not enhance females' responses, but increasing the size of pre-test treatment cages to accomodate an intact infested pine was impractical. However, because the responses of females that had been exposed to infested pines during pre-test treatment were not reduced below those of inexperienced females, it is evident that these experienced females did not learn to regard cut pine as a host microhabitat different from the intact pines to which they were exposed in test cages. As well, in Exp. 4, pre-test treatment and testing cages and infested pine shoots were virtually identical. Although females experienced with infested pine shoots appeared to favour them more than inexperienced females, the differences were not significant, again suggesting no effect of learning. Thus, the evidence indicates that dissimilarities between pre-test treatment and test infested pine and cages in Exp. 1-3 did not cause the apparent lack of enhancement of response.

Another explanation for this lack of enhancement might be found in the history of the insects. Parasitoids used in these experiments came from a stock colony kept for many years, and eggs for colony maintenance have been collected on hosts in a device similar to egg cups. Years of culturing in this way have not produced a strain of *E. roborator* capable only of learning to respond to egg cups, as several experiments have demonstrated that the parasitoid can learn other artificial host microhabitats (Chapter 2, Appendix 2), and natural microhabitat characteristics (Chapter 3). However, after generations of exposure to artificial host microhabitats the parasitoid's ability to learn to respond to natural host microhabitats may have been reduced to such an extent that it was no longer an effective force in the insects' response to infested pine. Culturing has been shown to affect learning in the Mediterranean fruit fly, *Ceratitis capitata*, probably through selective pressures exerted on learning of host fruit size (Papaj *et al.* 1987). Possibly members of this population of *E. roborator* that learn the features of the egg cup (and thus similar artificial microhabitats) well, rather than the features of natural host microhabitats, have been favoured by rearing procedures. This would require genetically-based individual variation in learning of host microhabitat features by *E. roborator*, but it is not known if this occurs.

A third explanation could be that the results reflect the way in which learning occurs in *E. roborator*. Learning may not play a strong role in host microhabitat finding by the parasitoid under natural conditions. Alternatively, prior experience simply may alter the parasitoid's responses to some host microhabitats, but not to others. For several insects, experience with some resources has little or no discernable effect on subsequent responses to those resources, while responses to other resources are considerably altered by prior experience with them (Jaenike 1982, 1983, 1985; Prokopy *et al.* 1982, 1986; Vet and van Opzeeland 1984;

Bernays and Wrubel 1985; Cooley *et al.* 1986; Prokopy and Fletcher 1987; Papaj *et al.* 1987; Hoffmann 1988). Often, the resources to which responses are most affected by prior experience are less preferred than those to which responses are little or not at all changed by familiarity (Jaenike 1982, 1983, 1985; Prokopy *et al.* 1982, 1986; Vet and van Opzeeland 1984; Bernays and Wrubel 1985). This was true for *E. roborator*. In Exp. 4, inexperienced *E. roborator* showed a much stronger response to *R. buoliana* infesting Scots pine than to the same host in egg cups. After prior experience with hosts in egg cups this preference was strikingly reversed, but it was not significantly affected by experience attacking hosts in pine shoots.

Vet and van Opzeeland (1984) have suggested that one function of learning is to provide insects with an escape when their normal hosts are rare. Insects whose usual or preferred hosts are reasonably abundant and predictable may have strong innate responses to these hosts, which allow efficient utilization of them when they are available. However, if the supply of these hosts is in any way uncertain, the ability to learn to attack other less frequently utilized hosts to a greater degree may be an advantage to the insect. My results may indicate that host microhabitat learning allows *E. roborator* to escape onto unusual or less preferred hosts, rather than simply enhancing the responses of the parasitoid to all hosts. A study using several normal and unusual or less preferred natural host/microhabitat systems could determine if such a trend is apparent in the responses of experienced *E. roborator* to familiar natural systems.

Learning from prior experience can become evident when it reduces an insect's responses to unfamiliar resources, without necessarily also enhancing its responses to familiar resources (Cooley *et al.* 1986; Prokopy *et al.* 1986; Papaj and Prokopy 1986; Papaj *et al.* 1987; Prokopy and Papaj 1988). The advantage of such learning is not clearly understood, but it may prevent

insects from expending foraging time and effort on rare resources, and thus increase their sampling of abundant hosts. Improved discrimination among members of a host species may result, allowing the insect to exploit host individuals of the highest quality available (Cooley *et al.* 1986; Prokopy *et al.* 1986; Prokopy and Fletcher 1987). Learning of this type might also be occurring in *E. roborator*. The depressed responses to infested pines by parasitoids experienced with hosts in egg cups in these experiments could indicate that these females were actively searching only for cups, or that they had learned to avoid unfamiliar host microhabitats, or both. As well, the lack of response to alders in Exp. 2B and 2C by *E. roborator* experienced with hosts in either pine or egg cups could indicate that the parasitoid learns to avoid unfamiliar resources. However, it could also be interpreted as showing that females held without exposure to hosts were less discriminating in their responses than parasitoids given hosts prior to testing. Insects subjected to resource deprivation can become less discriminating in their host responses (Singer 1982; Hoffmann and Turelli 1985). To determine if the parasitoid learns to avoid unfamiliar natural host/microhabitat systems, the responses of females experienced with one natural system to other systems could be compared to the responses of naive females, and also to those of females not deprived of hosts but not familiar with a single system (i.e. offered hosts in all test systems during pre-test treatment).

If *E. roborator* does learn to avoid unfamiliar resources, this learning apparently did not have one of the consequences proposed by Prokopy *et al.* (1986) and Cooley *et al.* (1986). Females experienced with infested pine did not appear to 'sample' (search and probe) it more than inexperienced females, that were 'sampling' both infested pines and alders.

[1] Mean numbers of hosts (±S.E.) oviposited on by successful females:

Group	IV''	1.4 ± 0.3 a	(N=13)
Group	V''	0.1 ± 0.1 b	(N=11)
Group	VI''	0.6 ± 0.2 a	(N=10)
Group	VII''	0.2 ± 0.1 b	(N=11)
Group	VIII''	1 a	(N=3)

Means followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05.

[2] Responses of *E. roborator* to infested cut pine shoots over 20 min:

Group	N	Percent of females (±S.E.) contacting	Percent of females (±S.E.) probing	Mean (±S.E.) min in contact	Mean (±S.E.) probes
3-4 day old inexperienced females	6	66.7 ± 19.2	66.7 ± 19.2	8.3 ± 3.4	5.2 ± 2.6
12 day old inexperienced females	10	80.0 ± 12.6	70.0 ± 14.5	9.6 ± 2.7	6.9 ± 2.1
12 day old females experienced with hosts in egg cups	13	30.8 ± 12.8	7.7 ± 7.4	1.9 ± 1.5	0.9 ± 0.9

CONCLUSIONS

Exeristes roborator is able to learn the colour, form, and odour of artificial host microhabitats. If these features can be learned for natural host microhabitats, they could be used, either singly or in conjunction, in the identification of potentially profitable plants and plant parts to search for hosts. Very little experience attacking hosts in artificial microhabitats is required for the acquisition of host microhabitat learning. This experience has the greatest influence on the parasitoid's behaviour when it is gained in the first 2 days of adult life.

Learning of an artificial host microhabitat can interfere with the responses of *E. roborator* to a natural host/microhabitat system. The numbers of hosts attacked by the parasitoid can remain depressed for a considerable length of time because of such learning. If exposed to hosts in an artificial insectary system even for a short time prior to release, this parasitoid and others like it could become significantly less efficient at attacking a target host in its natural microhabitat after release. Once *E. roborator* learns one artificial host microhabitat, it does not appear to learn as readily to transfer its host-seeking activities to a second one. Thus, if the parasitoid learned an artificial insectary system, it probably would not readily learn to divert its attack to a target host after release.

Microhabitat learning did not appear to enhance the responses of *E. roborator* to Scots pine infested with larvae of *R. buoliana*. This result could be an artifact of the experimental procedure, or of the history of the culture from which experimental individuals were obtained, but it could also be a true reflection of the effect of prior experience with natural host/microhabitat systems on the parasitoid's subsequent responses to them. Thus, it would be useful to know how learning functions in nature for a parasitoid, when trying to predict whether or not prior experience with a target host might enhance its performance against that host.

An increase in immediate response to a target host might not necessarily be the only beneficial effect of prior experience with that host. If given prior exposure to a target host and microhabitat, a parasitoid's subsequent responses might be restricted to that host for the most part, and its effectiveness against a pest might be increased over the long term by this fidelity. If this effect were coupled with a lack of ready learning to switch from one host microhabitat to another, such as is seen in *E. roborator*, it would be unlikely that a parasitoid would learn to concentrate its attack on other possible hosts present in the release area.

For a parasitoid such as *E. roborator*, which must feed on hosts to reach reproductive maturity, exposure to a diet that promoted egg maturation without causing adverse learning could have practical benefit. Release of individuals capable of immediate oviposition might enhance the parasitoid's initial attack upon a target population. If exposure to the target host and microhabitat for this purpose was impractical, an artificial diet might serve in its place. Alternatively, the parasitoid could be exposed to a nontarget system in a way that would not cause adverse learning. For instance, it might be possible to give *E. roborator* access to a nontarget system for feeding after the sensitive period for learning had passed, without significantly depressing its responses to the target system.

Entomophagous insects may be reared for biological control using target host/microhabitat systems. However, nontarget natural, factitious, or artificial systems often must be employed for reasons of economy or ease of handling, or because target systems cannot be adapted for use in rearing facilities (Morrison and King 1977; Waage *et al.* 1985). In some production procedures, adult insects can be exposed to the rearing system prior to release, either through deliberate use of females for egg collection, or because adults eclose in the presence of the rearing host and are not

immediately removed (Morrison and King 1977; Singh and Moore 1985). Learning from this exposure might be influencing the insects' performance in the field. This possibility should be assessed, so that procedures can be adjusted, if feasible, to exploit beneficial learning or avoid adverse learning by insects destined for release. In future development of rearing systems, learning by entomophagous insects should also be taken into consideration. However, the possibly conflicting demands of economy and practicality, and of maximising beneficial or avoiding adverse learning, should be balanced to produce the most effective overall natural enemy for a given cost.

APPENDIX 1

REFLECTANCE OF LIGHT BY HOST MICROHABITATS

Measurement and calculation of reflectance of photons

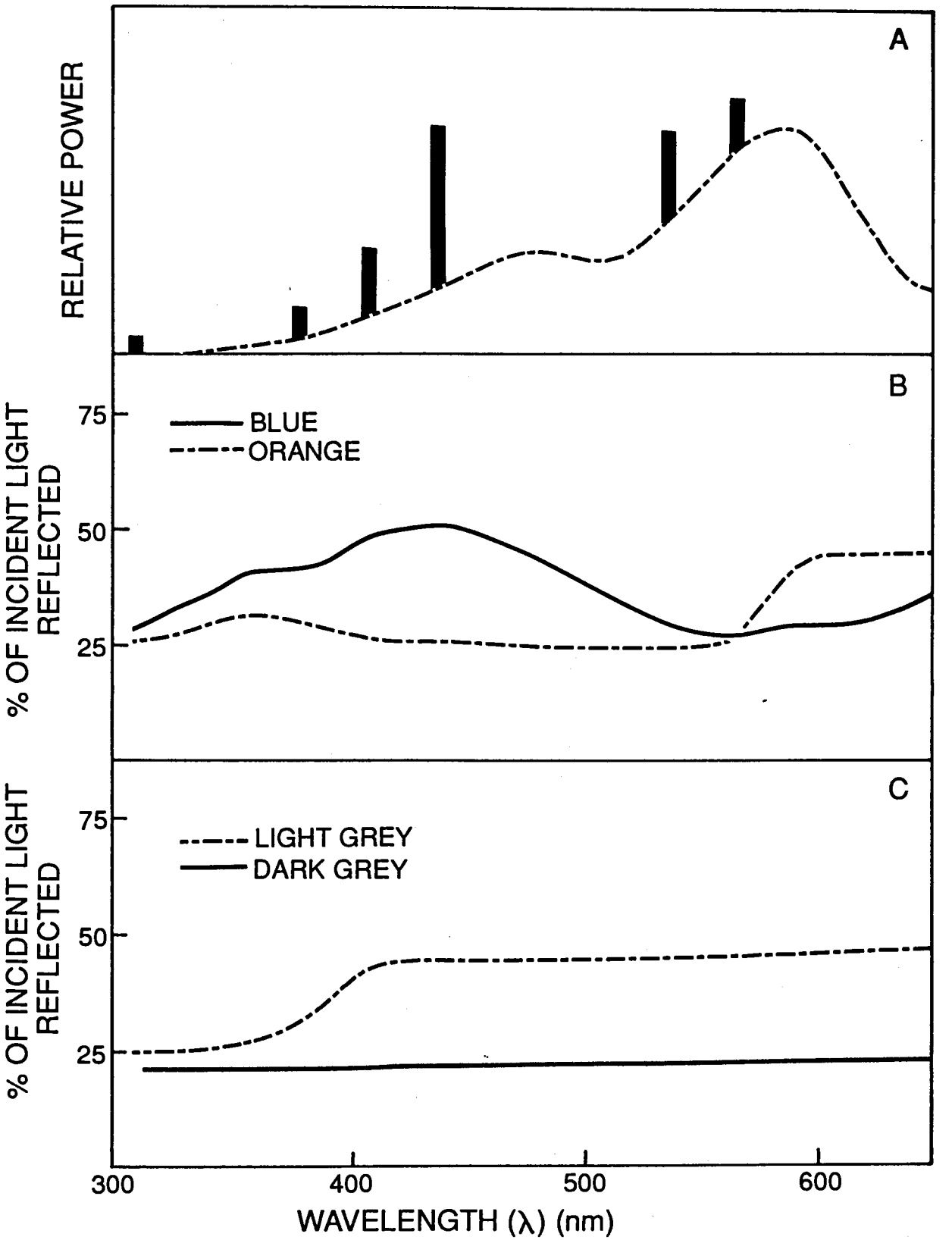
Reflectance spectra for painted fabrics used to construct egg cups were obtained with a Cary 17 spectrophotometer with integrating sphere. A white MgO standard was used.

The emission spectrum of the lights in the experimental area (General Electric 'cool white' flourescent lights, 40 W) (Fig. 14A) was obtained from Jagger (1977). These lights emit wavelengths from 310-750 nm; however only those wavelengths from 310-650 nm, the range to which insect eyes are sensitive (Menzel 1979), were considered when calculating overall reflectances.

Since the energy of a photon is inversely proportional to its wavelength, the relative number of photons incident upon egg cups could be calculated by multiplying the relative power of the light source by wavelength (λ) at 10 nm intervals to determine relative photons incident at each interval, and then summing these products for all intervals. Relative photons reflected by each painted fabric were calculated by multiplying relative photons incident at each 10 nm interval by the percent reflectance of the fabric at the interval wavelength, and summing these products for all intervals (Jagger 1977; Billmeyer and Saltzman 1981). Overall percent of incident photons reflected by each fabric was calculated by dividing relative photons reflected by relative photons incident, and multiplying by 100. The whole calculation is described by the equation:

$$\frac{\sum_{\lambda=310}^{650} \text{relative power} \times \lambda \times \text{reflectance}}{\sum_{\lambda=310}^{650} \text{relative power} \times \lambda} \times 100 = \% \text{ of incident photons reflected}$$

Figure 14A-C. Emission spectrum of General Electric 'cool white' flourescent lights (40W) (from Jagger 1977) (**A**), and reflectance spectra for blue and orange (**B**), and dark grey and light grey (**C**) fabrics.


A
RELATIVE POWER
B
BLUE
ORANGE
% OF INCIDENT LIGHT REFLECTED
75
50
25
C
LIGHT GREY
DARK GREY
% OF INCIDENT LIGHT REFLECTED
75
50
25
300
400
500
600
WAVELENGTH (λ) (nm)

Equalisation of photon reflectance (brightness) of blue and orange fabrics

The blue fabric used in egg cups reflected 34% of incident photons over the wavelength range 310-650 nm, and the orange fabric reflected 33% of incident photons in this range.

To achieve these reflectances a 1:99 v/v mixture of black:blue latex and a 1:9 v/v mixture of black:orange latex were made up and diluted for the paint baths. Reflectance spectra for both coloured fabrics are given in Fig. 14B.

Production of fabrics reflecting different intensities of light

A 9:1 v/v mixture and a 2:8 v/v mixture of black and white latex produced reflectances of 22% (dark grey) and 44% (light grey) of incident photons, respectively, when diluted to 1/4 strength and applied to the cotton fabric. Reflectance spectra for both grey fabrics are given in Fig. 14C.

Calculation of relative brightness of painted fabrics for 3 species of hymenoptera

The spectral sensitivity curves for *Apis mellifera* (von Helversen 1972), *Paravespula germanica* F. (Menzel 1971), and *Cataglyphis bicolor* F. (Kretz 1979) were used to determine what the probable relative brightness of the 4 painted cloths used in Exp. 1-3 and 5 would be for each of these 3 members of the hymenoptera.

To calculate the relative number of photons reflected by each fabric that a species would be capable of detecting, the relative number of photons reflected by the fabric was multiplied by the spectral sensitivity of the species at each 10 nm interval within the 310-650 nm range, and the products

for all intervals were summed (Billmeyer and Saltzman 1981). The number of photons reflected from each fabric that would be detected by the species under consideration was expressed as a multiple of the number of photons this species would detect being reflected from the dark grey fabric, which was the darkest fabric for all 3 insects, in order to determine the relative brightnesses of all 4 fabrics for this insect. The calculation is described by the equation:

$$\frac{\sum_{\lambda=310}^{650} \text{relative power} \times \lambda \times \text{reflectance (A)} \times \text{spectral sensitivity (B)}}{\sum_{\lambda=310}^{650} \text{relative power} \times \lambda \times \text{reflectance (DG)} \times \text{spectral sensitivity (B)}}$$

$$= \text{relative perceived brightness of (A) for (B)}$$

where A = blue, orange, or light grey fabric
B = *A. mellifera*, *P. germanica*, or *C. bicolor*
DG = dark grey fabric

These calculations showed that, for all 3 species, the probable difference in brightness was larger for the dark and light grey fabrics than for the blue and orange fabrics. Specifically, for *A. mellifera* and *C. bicolor* the orange fabric was 1.3x brighter, the blue fabric was 1.8x brighter, and the light grey fabric was 1.9x brighter than the dark grey fabric. For *P. germanica* the orange fabric was 1.3x brighter, the blue fabric was 1.6x brighter, and the grey fabric was 2x brighter than the dark grey fabric.

APPENDIX 2

A HAEMOLYMPH DIET AS A SUBSTITUTE FOR LARVAL HOSTS OF *EXERISTES ROBORATOR*

INTRODUCTION

Female *E. roborator* feed upon the first few hosts they attack. Approximately 2-3 days after their first such meal they begin to oviposit upon hosts. Thereafter they may either feed or oviposit on attacked hosts. Females probably obtain nutrients necessary for egg production from feeding upon hosts (Fox 1927; Baker and Jones 1934; personal observation), although this point has not been rigourously examined. Differences in general vigour do not appear to occur between host-experienced and inexperienced females as a result of these nutritional differences (Fox 1927), but the reproductive status of individuals that have had access to hosts almost certainly differs from that of individuals that have not.

Results of Exp. 2B in Chapter 5 indicated that females with prior experience attacking *R. buoliana* infesting Scots pine shoots were more successful than inexperienced females in attacking these hosts, in spite of the fact that in other aspects of host selection experienced and inexperienced females were equal (Fig. 11, Exp. 2B; Tables 24 and 25, Exp. 2B in Chapter 5). Experienced individuals may have attacked more hosts in the time they spent responding to the pines than inexperienced females because they were able to oviposit.

If inequality in reproductive status between experienced and inexperienced females was at the root of differences between them in success attacking hosts, it should be possible to eliminate these differences by feeding inexperienced females a diet making them reproductively equal to experienced females. This feeding would have to be done without causing females to learn to respond to the device in which they were given the diet as a host microhabitat, as such learning could interfere with their subsequent responses to host microhabitats.

Artificial diets have been developed on which female ichneumonids can reach reproductive maturity (Bracken 1965, 1966, 1969; House 1980). However, since female *E. roborator* feed largely on the haemolymph and fat body of hosts (Fox 1927; Baker and Jones 1934; personal observation), I hypothesised that a diet of haemolymph alone could be administered in such a way as to provide females with a source of nutrients equivalent to intact hosts, without causing them to learn to respond to the container of this diet as a host microhabitat. The experiments described here were undertaken to test this hypothesis.

MATERIALS AND METHODS

Pre-test treatments

Experiments 1 and 2

Newly-eclosed females were placed individually in 16 x 23 x 31 cm cages, each containing honey-coated sugar cubes, water, and 2 males, and held under the conditions described in Chapter 1. They were assigned randomly to 3 different pre-test treatments (Table 30) lasting 6 days.

The feeding device to which females in all groups were exposed was made from a disposable Pasteur pipette heated where it began to narrow into its tip, bent to produce an elbow slightly greater than 90°, cut to a length of approximately 5.5 cm, and suspended upright from a cork glued to a clear acetate stand so that the bent tip was approximately 1 cm off the cage floor (Fig. 15A).

One intact larva of *G. mellonella*, coddled at 65°C and inserted head-first into the tip of a feeding tube so that its posterior half remained exposed, was offered each day to each female in group I, as a complete host diet. To feed host haemolymph alone to each female in group II, 3 such coddled larvae were decapitated each day and the haemolymph thus released was sucked into the bent tip of the feeding tube by capillary action. Females in both groups fed freely from these devices. Parasitoids in group III were not fed any host material, but were exposed to an empty feeding apparatus each day to control for the effects of repeated exposure to the device. At the end of the final day of pre-test treatment the feeding apparatus was removed from each cage, and the females were left in the same cages for testing.

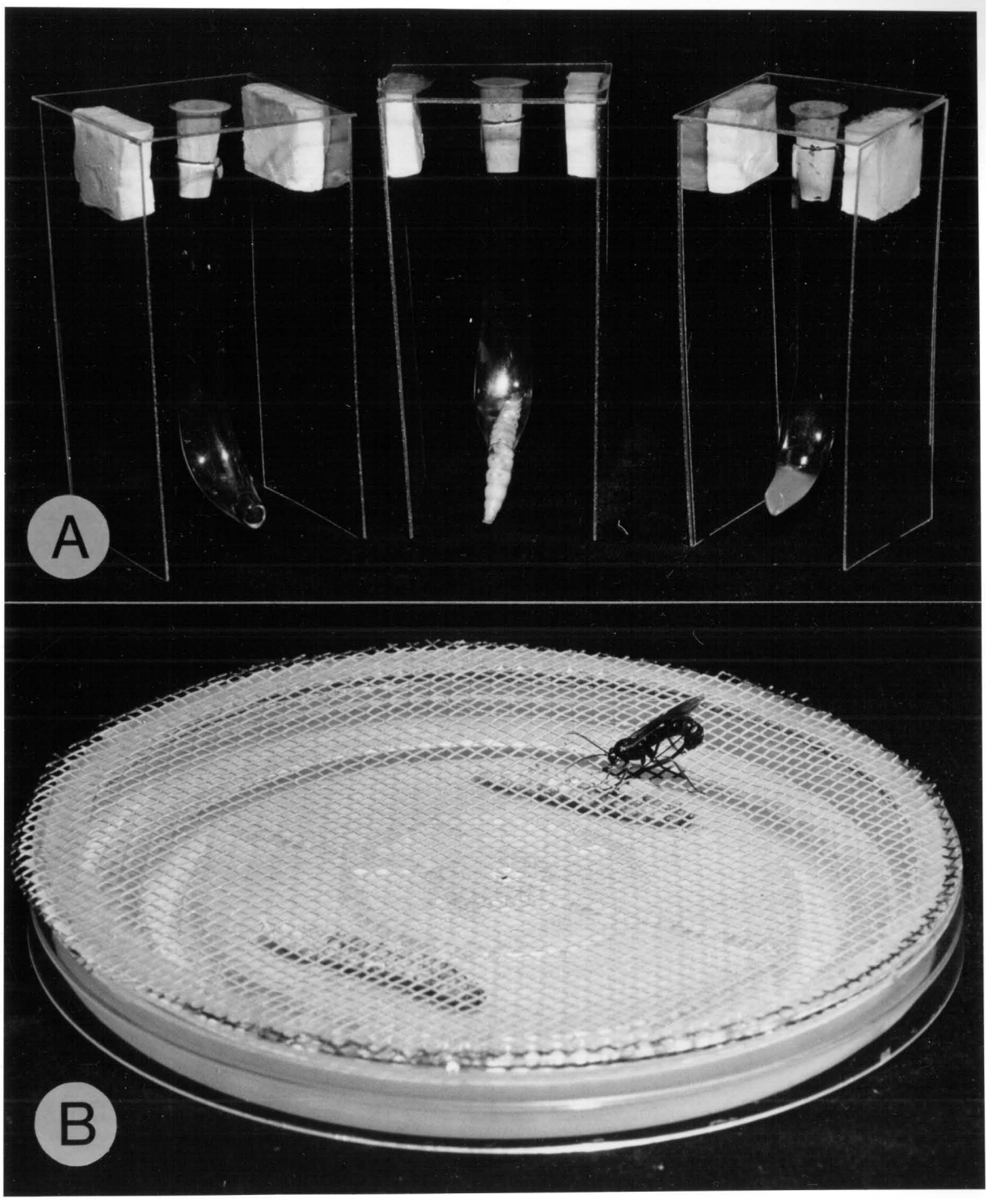
Feeding devices were washed with Liqui-Nox detergent (Alconox Inc., New York, New York) and rinsed with ethanol between uses. Tubes used to offer

Table 30. Pre-test treatments for *E. roborator* in Exp. 1 and 2.

Group	N	Pre-test treatment[a]
I	35	Given a feeding device containing 1 intact host (intact host)
II	37	Given a feeding device containing only the haemolymph of 3 hosts (haemolymph alone)
III	32	Exposed to an empty feeding device (no diet)

[a]Clean feeding devices and fresh food were placed in cages each day for 6 days. Hosts were coddled larvae of *G. mellonella*.

Figure 15A-B. **A.** Feeding devices to which *E. roborator* were exposed during pre-test treatment: left, empty (group III); centre, containing a coddled larva of *G. mellonella* (group I); and right, containing haemolymph of coddled *G. mellonella* (group II). **B.** *Exeristes roborator* ovipositing on a larva of *G. mellonella* in an egg collection device used in Exp. 2.


A
B

food to females in groups I and II were never placed in the cages of group III females.

Testing regimes

Experiment 1: Test for learning of feeding devices

On the day following removal of the feeding apparatus, females were tested individually in random order for evidence that they had learned to respond to this device as a host microhabitat. A clean, empty device was placed in each cage for 20 min, and a record was kept of whether or not females contacted the device and probed it with their ovipositors. I also recorded how long they spent in contact and how many times they probed. A lack of significant difference between the responses of females in groups II and III when such a difference occurred between the responses of females in group I and those of females in the other 2 groups would indicate that *E. roborator* was able to learn to respond to the device, but did not learn from feeding on haemolymph in it.

Over 30 females from each group were tested. Feeding devices were rinsed with ethanol between uses, and devices used for testing were not used in pre-test treatments.

Experiment 2: Test of fecundity

After testing in Exp. 1, all females remained in their cages without exposure to food or the feeding device until the following day. An egg collection device was then placed in each female's cage. The base of this device consisted of the inverted lid of a 150 ml styrofoam cup to which 2 coddled larvae of *G. mellonella* were firmly attached with Krazy Glue (F.P. Feature Products Inc., Mississauga, Ontario). A piece of fibreglass hardware cloth (1.7 mm mesh) was glued to the lid of a 100 x 15 mm disposable petri

dish, from which most of the centre had been removed, leaving only the rim. This rim fitted snuggly over the cup lid and held the hardware cloth screen several mm above the host larvae glued to the lid (Fig. 15B). Female *E. roborator* could detect the presence of the larvae through the screen and oviposit on them, but could not get close enough to feed on them.

After 4 h the egg collection apparatus was removed from each cage and the number of eggs laid by each female was recorded. Eggs were collected 3 times for each female, on alternate days. Fresh cup lids and host larvae were used each time, and hardware cloth covers were rinsed with ethanol beetween uses.

After fecundity testing females were preserved in 70% ethanol and dissected to determine how many mature eggs $\geq$2.3 mm in length (Fox 1927) their ovaries contained.

All eggs laid by females during fecundity testing were held individually at 23°C on coddled larvae of *G. mellonella* in gelatin capsules (no. 00; Parke, Davis, and Co. Ltd., Brockville, Ontario). As eggs of *E. roborator* normally hatch within 2 days at 23°C (Baker and Jones 1934; personal observation), eggs that did not hatch within 3 days of laying were considered to be inviable.

If *E. roborator* in groups I and II were similar in the parameters assessed while group III females produced not eggs, the hypothesis that haemolymph is equivalent to intact hosts as a source of nutrients for normal egg production would be confirmed.

Statistical analysis

In Exp. 1, the proportions of females in each group responding to the feeding device were compared with a test for comparing >2 proportions and a modified Newman-Keuls multiple comparisons procedure (Zar 1984). Mean

responses to the device by all females in each group were compared with the Kruskal-Wallis Test and multiple comparisons procedure of Conover (1980).

In Exp. 2, the total number of eggs produced by each female in groups I and II was determined by adding together the numbers of eggs she laid and the number of mature eggs her ovaries contained. The percentage of the eggs each female in the 2 groups laid that were viable was determined by dividing the total number of eggs hatched by the total number of eggs laid. Mean total eggs produced and mean percentage of eggs viable were compared with the Mann-Whitney Test (Conover 1980). Females in group III produced no eggs. If the confidence intervals for mean numbers of eggs produced by females in groups I and II did not include 0, these groups were considered to differ significantly from group III in this capacity.

RESULTS

Females fed haemolymph alone did not learn to respond to the feeding device (Table 31), but produced as many eggs as females provided with intact larvae (Table 32).

In Exp. 1, females in group II did not contact or probe the empty feeding device in greater numbers or with greater intensity than females in group III (Table 31), indicating that the experience of feeding on host haemolymph in this device had not increased its attractiveness for females, or caused females to behave towards it as though it were a host microhabitat. In contrast, group I females consistently responded to the device at significantly higher levels than females in the other 2 groups (Table 32), showing that experience attacking intact hosts in the apparatus caused them to learn that hosts could be found in it.

In Exp. 2, no significant differences occurred between females fed intact hosts and females fed haemolymph alone in the overall numbers of eggs produced, or in the quality of these eggs, as measured by the proportion of eggs laid that were viable (Table 32). Thus haemolymph appeared to be equal to intact hosts as a source of nutrients for reproductive development, at least over the term of the experiment. Unfed group III females did not lay any eggs, nor did their ovaries contain mature eggs. This result indicates that females were not able to produce mature eggs except when they had access to host fluids.

During the 6 days of the pre-test treatment period some females in group I began to lay eggs on larvae provided in the feeding device. The number of eggs laid under these conditions was small, and did not change the statistical relationships between groups.

Table 31. Contacting and probing responses to the feeding device by *E. roborator* in groups I-III during testing in Exp. 1.

Group (pre-test treatment)	Percent of females (+S.E.)[a] contacting device	probing device	Mean (+S.E.)[b] min in contact with device	probes executed on device
I (intact host)	71.4 ± 7.6 a	65.7 ± 8.0 a	4.8 ± 0.8 a	2.9 ± 0.5 a
II (haemolymph alone)	35.1 ± 7.8 b	2.7 ± 2.66 b	1.1 ± 0.3 b	0.08 ± 0.08 b
III (no diet)	43.8 ± 8.8 b	6.3 ± 4.3 b	1.1 ± 0.3 b	0.09 ± 0.07 b

[a]Percentages in a column followed by the same letter are not significantly different, test for comparing >2 proportions and modified Newman-Keuls multiple comparisons procedure (Zar 1984), α=0.05.

[b]Means in a column followed by the same letter are not significantly different, Kruskal-Wallis Test and multiple comparisons procedure (Conover 1980), α=0.05.

Table 32. Egg production by *E. roborator* in groups I-III during testing in Exp. 2.

Group (pre-test treatment)	Mean total (±S.E.) eggs produced[a]	Mean percent (±S.E.) of laid eggs viable[a]
I (intact host)	14.8 ± 1.1 a	77.2 ± 3.8 a
II (haemolymph alone)	13.1 ± 1.0 a	83.1 ± 2.4 a
III (no diet)	0 b	/

[a]Means in a column followed by the same letter are not significantly different, Mann-Whitney Test (Conover 1980), α=0.05. For data on eggs produced, 95% confidence intervals for mean total eggs produced by females in groups I and II did not include 0. Therefore the data for these groups differed significantly from those for group III.

DISCUSSION

The response of *E. roborator* in group I to the empty feeding device (Exp. 1) shows that females were capable of learning to respond to this apparatus. Therefore, the lack of significant response to the device on the part of haemolymph-fed females (group II) indicates a lack of learning on their part that the device was a source of host material. Host recognition by *E. roborator*, which is an ectoparasitoid, could well depend on external cues (Arthur 1981; Vinson 1985), which would be lacking in haemolymph.

Insect haemolymph is a rich source of nutrients for egg production often utilised by parasitoids (House 1977, 1980). While *E. roborator* offered intact hosts feed upon both haemolymph and fat body (Fox 1927; Baker and Jones 1934; personal observation), there are many similarities between the 2 substances, particularly in protein components (Keeley 1985). Thus females feeding on haemolymph alone probably had access to essentially the same nutrients necessary for egg production as females feeding on intact hosts.

The equal viability of the eggs produced by group I and II females shows that egg quality was not affected by the nature of the diet. The lack of production of eggs by unfed females (group III) confirms the observation that females of *E. roborator* that have not fed on hosts normally do not oviposit (Fox 1927; Baker and Jones 1934).

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