HOST-HABITAT LOCATION AND HOST LOCATION BY *DIACHASMA ALLOEUM* MUESEBECK (HYM.; BRACONIDAE), A PARASITOID OF *RHAGOLETIS POMONELLA* WALSH (DIPT.; TEPHritidae)

by

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SUMMARY

Three laboratory experiments were designed to elucidate some of the stimuli that are involved in host micro-habitat searching, the stimulation of host-searching behaviour and in host location by *Diachasma alloleum*. Visual orientation was found to play an important role in the location of fruits (*Crataegus mollis*) by *D. alloleum*. Equal numbers of landings were obtained on host-infested and uninfested haws. A description is presented of the host-searching behaviour by *D. alloleum* on haws. It was found that probing activity and duration of stay on the fruit are strongly influenced by the presence of a *Rhagoletis pomonella* larva in the fruit. A quantification was made of the accuracy with which *D. alloleum* probes the fruit in search of hosts. It is concluded that host movement is the prime stimulus for the location of hosts. Females on haws containing a moving larva exhibited non-random probing. It was found that parasitization of the host by *D. alloleum* is preceded by paralysis of the host. When paralyzed larvae were offered in the same set-up, *D. alloleum* exhibited random probing.

INTRODUCTION

Many members of the braconid subfamily of Opiinae parasitize pre-pupal developmental stages of Tephritidae (Wharton & Marsh, 1978). The tephritid genus *Rhagoletis* is frugivorous on a variety of host plants in the holarctic and neotropical region of North America (Bush, 1969). *Rhagoletis pomonella* is restricted to Rosaceae. Female flies lay their eggs in host fruits wherein the subsequent larval stages feed before leaving the fruit and pupating in the soil. According to Wharton & Marsh (1978), six opiiine parasitoids have been reared from *R. pomonella*, one of which is *Diachasma alloleum*. In general, research on this parasitoid has been focussed on host records and parasitization percentages.

*Diachasma alloleum* is found on apple and hawthorn in the northeastern part of the U.S.A., and the bordering part of Canada. It is a solitary endoparasitoid which attacks 3rd (= last) instar larvae of *R. pomonella*. No records have been found of *D. alloleum* attacking any
other host than *R. pomonella*. Parasitization percentages on apple range from 0.1% to 20.1% (Boush & Baerwald, 1967; Rivard, 1967; Cameron & Morrison, 1977; Maier, 1982). A few authors report on behavioural observations. Courtship behaviour, suggesting the presence of a female sex-pheromone, is described by Boush & Baerwald (1967). Some aspects of the oviposition behaviour of *D. alloeum* are analyzed by Prokopy & Webster (1978).

Since Tephritidae cause commercial damage to fruit crop, interest has been focussed on the relation between the flies and their opine parasitoids. Some of the work on other Opiniae deals with the stimuli that are involved in the host-searching process. Generally, in this process, three stages are recognized: a) host-habitat location, b) host location and c) host acceptance. Vinson (1976) refers to these stages as ‘host selection’. Our research aimed at the elucidation of some of the stimuli that are involved in host-habitat location and host location by *D. alloeum*.

**MATERIALS**

Every fall, large quantities of fruits (hawthorn and apple), which are infested by *R. pomonella*, are collected from different localities around Amherst (Massachusetts). In the laboratory, *R. pomonella* larvae emerge from these fruits and pupate in moist vermiculite. The puparia are sifted out, transferred to plastic cups containing vermiculite, and stored at 4° C. In storage the pupae overwinter in diapause. Pupal development is initiated again by transferring cups to a climate room at 20 ± 2° C, 50 ± 5% RH, under cool fluorescent lamps with a 16 hour photoperiod, whereby they start to emerge after approximately one month.

Individual parasitoids were transferred to disposable petri dishes where they were kept separated from conspecifics. Droplets of a honey solution served as food, and water was provided by taping a moist wick onto the lid of the petri dish. The parasitoids were allowed to mate by confining them, in pairs, in a petri dish for one or two days. This was done according to the method of Boush & Baerwald (1967). They introduced 4 day old males with 24 hour old females. Only mature (2-3 week old) females were used in the various experiments.

Also every fall, uninfested haws (*Crataegus mollis*) are collected from localities where trees are regularly sprayed with insecticide. The fruits are stored at 4° C before preparing them for experiments. For each experiment haws were taken from cold storage, and a number of fruits was infested by allowing *R. pomonella* females to oviposit in them. Both infested and uninfested fruits were then transferred to a climate room (see above), where the *R. pomonella* eggs developed into 3rd instar larvae.
METHODS AND RESULTS OF THE DIFFERENT EXPERIMENTS

Fruit Choice Experiment

The objective of this experiment was to determine which stimuli are involved in the location of fruits by *D. alloeum* females.

The experimental set up is illustrated in Fig. 1. The screen cage contained 3 fruits: 1) a haw (*C. mollis*: Ø = 20 mm.) containing one *R. pomonella* larva, 2) an uninfested haw, and 3) an artificial haw, constructed of modelling clay and dipped in red-dyed ceresin wax. Each test run consisted of releasing 9 or 10 *D. alloeum* females in the cage and recording landings for a period of 90 minutes. Six such runs were conducted. To avoid the possibility that the parasitoids would orientate to stimuli outside the cage, the cage was rotated 90° after each 5th landing.

![Diagram](image)

Fig. 1. Experimental set up of the fruit-choice experiment. A screen cage containing: 1) a haw containing a 3rd instar larva, 2) an uninfested haw, 3) an artificial haw.

The data from the fruit choice experiment in Table 1 show that there is no significant difference between the number of landings on the infested fruit and the uninfested fruit. Although the number of landings on the artificial fruit is significantly smaller in comparison with the landings on real fruits, it must be noted that the number of approach flights towards the artificial fruit was much higher than the number of actual landings suggests. In observing the approach flights it became clear that frequently females did fly towards the artificial
fruit only to a distance of 1-2 cm. But decided not to land on it. This was not observed with any of the real fruits. If approaches without landing would be added to the landings, the P-values would rise above the 5% significance level (e.g. 2 additional landings on artificial fruits; infested vs. artificial: P = 0.08; uninfested vs. artificial: P = 0.06). Therefore we conclude that, in this experiment, odours emanating from the real fruits did not play a significant role in the initial orientation of the females. Once the females had landed, they stayed much longer on the infested fruit than on the uninfested fruit and artificial fruit, although probes in the later two were also observed.

**Table 1**

Number of landings by *D. alloorum* on different fruits in the fruit-choice experiment.

<table>
<thead>
<tr>
<th>Number of females</th>
<th>Landings on infected haw</th>
<th>Landings on uninfested haw</th>
<th>Landings on artificial haw</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>35</td>
<td>19</td>
</tr>
</tbody>
</table>

Infested vs. uninfested P = 0.91*; infested vs. artificial P = 0.04*; uninfested vs. artificial P = 0.05*; *Chi-square test.

Since the initial orientation towards all three types of fruit was identical and often the parasitoids approached the fruits in a straight line, we conclude that visual orientation plays an important role in fruit location by *D. alloorum*, and the parasitoids do not selectively land on fruits containing hosts.

**Oviposition Experiment**

This experiment was designed in order to describe and quantify searching behaviour of *D. alloorum* on infested and uninfested haws (*C. mollis*)

In a little cage, which was constructed from a disposable glass, two haws were hung (Fig. 2); a test haw which was either infested or uninfested, and a control haw which was uninfested. The fruits hung approximately 2 cm. apart. A test consisted of allowing a female to walk from a vial onto the test haw, after which her behaviour on the
fruit was continuously recorded. A test was terminated when a female had either flown to the uninfested control haw, or when a female had left the test haw and was sitting somewhere in the cage and did not return to the test haw within 5 minutes. None of the 8 females that were tested had had any oviposition experience prior to the first time they were tested. The order in which the females (A to H) were tested on infested (1) and uninfested (0) test haws was as follows: A-0, B-0, B-1, C-1, C-1, E-1, C-0, F-1, D-1, C-1, F-0, F-1, E-1, G-1, H-1. The number of larvae inside the haws was verified by allowing the *R. pomonella* larvae to emerge from the fruits and pupate. After this the fruits were dissected to check on remaining larvae.

![Diagram](image)

**Fig. 2.** Experimental set up of the oviposition experiment. A plastic cage containing: 1) the test haw which can either be infested or uninfested, 2) the control haw which is uninfested.

From the oviposition experiment a generalized sequence of behavioural stages on haws which contain a host can be described: 1) The female walks over the fruit while drumming the surface with the subterminal part of the antennae which are slightly curved upward (Fig. 3A). Bouts of high walking and drumming activity are sometimes alternated with periods of low activity when the parasitoid sits still most of the time. Occasionally the female can be seen to groom or touch the fruit surface with her mouthparts. 2) Ovipositor probing (Fig. 3B) was frequently observed. Three types of probes could be recognized: a; a probe which lasts only a few seconds after which the ovipositor is retracted, b; a probe which lasts much longer, during which the ovipositor is put in and out of the puncture hole without retracting it completely, and c; a probe during which the female is almost motionless for 1-4 minutes. Based on dissection of the hosts it
can be concluded that the type-c probe is an actual oviposition. 3) The oviposition posture is illustrated in Fig. 3C. 4) After an oviposition, without exception, a behaviour was observed the function of which remains obscure. We refer to this stage as 'excreting' since the female can be seen to walk over the fruit, regularly pressing the tip of her abdomen onto the fruit surface, whereby, on every one of the 7-15 times she does this, a minute drop of a clear fluid is excreted (Fig. 3D).

Fig. 3. Oviposition behaviour on hawthorn. The different stages are described in the text.
### Table II

Measures of *D. alleum* activity on haws in the oviposition experiment: the time spent on the haw, the number of type-a, type-b and type-c probes (see text), and the probing frequency.

<table>
<thead>
<tr>
<th></th>
<th>Number of hosts in haw</th>
<th>Female (nr. exp.)</th>
<th>Time spent on fruit (seconds)</th>
<th>Number of type-a, b probes</th>
<th>Number of type-c probes (ovipositions)</th>
<th>Probing frequency (P/min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I no host</td>
<td></td>
<td>0 A(1)</td>
<td>233</td>
<td>3</td>
<td>0</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 B(2)</td>
<td>232</td>
<td>1</td>
<td>0</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 C(7)</td>
<td>1014</td>
<td>18</td>
<td>0</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 F(11)</td>
<td>678</td>
<td>11</td>
<td>0</td>
<td>0.97</td>
</tr>
<tr>
<td>II no oviposition</td>
<td></td>
<td>1 C(5)</td>
<td>5930</td>
<td>476</td>
<td>0</td>
<td>4.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 F(8)</td>
<td>3666</td>
<td>113</td>
<td>0</td>
<td>1.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 F(12)</td>
<td>1127</td>
<td>22</td>
<td>0</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 E(13)</td>
<td>2998</td>
<td>104</td>
<td>0</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 G(14)</td>
<td>2937</td>
<td>115</td>
<td>0</td>
<td>2.35</td>
</tr>
<tr>
<td>III ovipositions</td>
<td></td>
<td>1 B(3)</td>
<td>3061</td>
<td>108</td>
<td>4</td>
<td>2.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 C(4)</td>
<td>3431</td>
<td>141</td>
<td>2</td>
<td>2.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 E(6)</td>
<td>7899</td>
<td>332</td>
<td>5</td>
<td>2.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 D(9)</td>
<td>1562</td>
<td>28</td>
<td>2</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 C(10)</td>
<td>8484</td>
<td>385</td>
<td>1</td>
<td>2.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 H(15)</td>
<td>5915</td>
<td>136</td>
<td>5</td>
<td>1.38</td>
</tr>
</tbody>
</table>

I (3, 4, 6) vs. II (3, 4, 6) P<0.01 Mann-Whitney U-test; I (3, 4, 6) vs. III (3, 4, 6) P<0.01 Mann-Whitney U-test; II (3, 4, 6) vs. III (3, 4, 6) n.s. (α = 0.05) Mann-Whitney U-test.

As can be seen in Table II (columns 1 and 5), only 6 out of 11 oviposition tests on haws containing a host resulted in one or more successful ovipositions. Whether this is due to rejection of the hosts by the parasitoids, or to their inability to locate the hosts, remains uncertain. The data in column 3 substantiate the conclusion from the fruit-choice experiment that *D. alleum* stays longer on fruits with hosts than they do on uninfested fruits (Mann-Whitney U-test; P<0.01). The oviposition experiment also shows that oviposition activity is strongly elicited by the presence of a host. Both the number of probes and the probing frequencies (Table II; columns 4 and 6) were significantly higher on haws containing hosts than on uninfested haws (Mann-Whitney U-test; P<0.01). Furthermore it was found that, although *D. alleum* is a solitary parasitoid, some hosts were parasitized more than once. It is not known whether *D. alleum* can discriminate between parasitized and unparasitized hosts and avoids to oviposit in the former. The females that were used in the oviposition experiment...
were not provided with hosts other than the single host offered in each experiment. In this set up the ability to discriminate may have been obscured by the parasitoids inability to find other hosts.

*Host Location Experiment*

The purpose of this experiment was to see if, in analogy with other Opiinae that attack larval stages of Tephritidae (Lathrop & Newton, 1933; Nishida, 1956; Lawrence, 1981), *D. alboeum* use larval movement as a host-location stimulus. The following technique was used: A haw (*C. mollis*; Ø ± 18 mm.), containing a *R. pomonella* larva, was cut in two halves. Stones and larva were removed. By equally spreading

![Diagram A](image1)

![Diagram B](image2)

Fig. 4. A) Set up of the host-location experiment. A half haw in a petri dish. B) View of the haw from above. Indicated are: the larva; the numbered subsequent probes; an example of how the α’s are measured.
out frass and faeces through both fruit halves, using a spatula, the
halves were modelled into little 'fruit cups'. A larva was put in one of
the halves. This half was transferred to a disposable petri dish (Fig.
4A). One *D. alloeum* female was introduced into the petri dish. This set
up allowed us to observe, under a binocular microscope, both the
behaviour of the parasitoid and that of its host.

A test run started when a female had climbed onto the fruit. After
this a continuous recording was made of all ovipositor probes whereby
their position on the fruit surface, relative to the position of the host,
was recorded by making sketches indicating every subsequent probe
(Fig. 4B). A test run was terminated either when the host had been
successfully paralyzed and parasitized, or when the host had been
paralyzed but managed to escape from the ovipositor before the
paralytic became effective. A control run was conducted using the
paralyzed host and the same female. The motionless host was trans-
ferred to the other fruit half, and a clean petri dish was used. Again every
probe was recorded. A control run was terminated either when the
host had been parasitized (again) or after 60 minutes (after approx-
imately 45 minutes, the host slowly recovers from paralysis).

The probing accuracy was determined by measuring the angle *α*
of every probe as is illustrated in Fig. 4B. We used the host's mandibulae
as a reference point since in the test runs the hosts were constantly
scraping the fruit flesh at the edge of the 'fruit cup'. In the control runs
the paralyzed hosts were placed in the fruit in a similar position.

A series of 10 test runs and 10 control runs was conducted. None of
the 3 females that were available at the time had had any oviposition
experience prior to the first time they were tested. The females (A, B,
C) were tested in the following order: A, B, A, B, C, C, B, C, C, B.

Also a series of 10 test runs was conducted using McIntosh apples as
a host medium. The set up and method of probe recording was similar
to the test runs on hawthorn. A hawthorn-reared larva was inserted
2 mm. under the fruit skin of a circular slice of apple (diameters:
between 53 and 66 mm.), devoid of larvae or frass and faeces. The
tests were terminated either after paralyzing/parasitizing the host, or
after 60 minutes. Four inexperienced females (D, E, F, G,) were
tested in the following order: D, D, E, E, E, G, G, G, G.

The results of the host-location experiment are illustrated in Figs. 5,
6 and 7. Comparing Fig. 5 and 6 clearly shows that *D. alloeum*
exhibited non-random probing on haws containing a moving larva,
whereas random probing was exhibited on haws containing a para-
lyzed larva. This proves that host vibration indeed is an important stimulus in host location by *D. alloecum*, a fact which is corroborated by the probe distribution of *D. alloecum* on apples containing a moving larva and no frass or faeces other than that which had been produced by the inserted host (Fig. 7).

The probe distribution on apple (Fig. 7) seems to reflect a higher degree of location accuracy by the parasitoids on apple than on hawthorn (Fig. 5). However, because of the difference in diameter between the fruits, the same tangential distance on the surface of a small haw will result in a much larger $\alpha$ than it will on the surface of a big apple. Therefore, in this respect, the Figs. 5 and 7 are not comparable.

![Fig. 5. Distribution of probes in haws with a moving larva. Probing scores in 10° sections on either side of $\alpha = 0$ are represented by the bars as percentages of the total number of probes (N).](image)

![Fig. 6. Distribution of probes in haws containing a paralyzed larva. Probing scores in 10° sections on either side of $\alpha = 0$ are represented by the bars as percentages of the total number of probes (N).](image)
TABLE III
Number of probes before locating a host in hawthorn (host-location experiment).

<table>
<thead>
<tr>
<th>Moving larva</th>
<th>Paralyzed larva</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
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<tr>
<td>17</td>
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<td>2</td>
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<tr>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>93</td>
<td>190</td>
</tr>
</tbody>
</table>

T-test (related samples): $P < 0.05$.

Fig. 7. Distribution of probes in apples containing a moving larva. Probing scores in $10^6$ sections on either side of $\alpha = 0$ are represented by the bars as percentages of the total number of probes (N).
Table III gives the number of probes which females required for locating (paralyzing/parasitizing) moving larvae and paralyzed larvae in haws. In 2 out of 10 control runs the host was not located within 60 minutes, therefore Table III includes only 8 pairs of probe numbers. A t-test for related samples proves that non-random host location took fewer probes than random probing (P<0.05).

DISCUSSION AND CONCLUSION

Here we will discuss our findings in the light of the work of other workers on Opiine host-selection (i.e. on Bisteris spp. and Opius spp.). The fact that D. alloeum in the fruit-choice experiment landed equally on infested and uninfested fruits indicates that the host medium is attractive to this parasitoid regardless of the presence of the host. This was also found for several other Opiniacae (Nishida, 1956; Nishida & Napompeth, 1974; Greany et al., 1977). Our findings indicate that visual orientation towards fruits, as was found for the host R. pommonela (Prokopy, 1976) is also present in D. alloeum. Although in general little is known about habitat location by parasitoids (Vinson, 1981), chemical orientation seems to play a major role. Visual orientation in host-habitat location has not often been found. Some examples are: Itolectis conquistor which can be conditioned to associate colour with the presence of hosts (Arthur, 1966), and Mesopolobus spp. that see host galls on oak (Askew, 1961). Greany et al. (1977) found that olfactory stimuli play an important role in host-habitat location by B. longicaudatus. On this point, no information is available for D. alloeum. Visual orientation to fruits within host plants may very well be preceeded by olfactory orientation, for example in locating host plants. More experimental work is needed to reveal the relative importance of visual and olfactory orientation in long-range and short-range host-habitat location by D. alloeum and other Opiniace.

Our findings from the oviposition experiment with D. alloeum qualitatively agree with those of Prokopy & Webster (1978), although both the recorded duration of stay and the number of probing attempts in our experiment were much higher. The fact that haws devoid of hosts did not retain the parasitoids and were hardly probed agrees with the findings for O. fletcheri (Nishida, 1956), O. canaliculatus (Prokopy & Webster, 1978) and B. longicaudatus (Lawrence, 1981).

The ‘excretion’ behaviour by D. alloeum, following oviposition, deserves special interest. One is inclined to draw a parallel between this behaviour and that of the tephritid host species which, after oviposition, deposit a oviposition-deterring pheromone on the fruit by dragging the extended ovipositor over the fruit surface (Prokopy,
1976). Marking a haw with an oviposition deterring pheromone could be functional for D. alloceum in hawthorn, since it is a solitary parasitoid and most haws only contain one host. Host discrimination has been observed in B. longicaudatus (Lawrence et al., 1978), but no information is presented on the mechanism by which this parasitoid avoids superparasitism.

The host-location experiment resulted in very direct evidence that vibrations caused by the gnawing of the host stimulate oviposition behaviour and also serves as a location stimulus eliciting non-random probing by D. alloceum. Vibration as a location stimulus has been found before in B. melicus (Lathrop & Newton, 1933), O. fletcheri (Nishida, 1956) and B. longicaudatus (Lawrence, 1981).

Interestingly enough, in our host-location experiment, D. alloceum females were actively probing in fruits containing a paralyzed larva. If vibration is the sole oviposition-behaviour stimulus, this would not be expected. Since the fruits did however contain frass and faeces, we suggest that the presence of each may also elicit oviposition behaviour. Further research should corroborate this.

In many habitats where the larval parasitoid D. alloceum is found, the opine egg parasitoid O. canaliculatus, which is phylogenetically closely related to D. alloceum, is exploiting the same population of R. pomonella. Prokopy & Webster (1978) found that in O. canaliculatus the oviposition-deterring pheromone of R. pomonella, as well as the fly's boring punctures in the fruit skin, act to retain the parasitoids to the fruit and stimulate probing activity. It was also found that D. alloceum did not react as such to these stimuli. Our finding that D. alloceum uses vibration caused by the host as a location stimulus illustrates that these two closely related parasitoids which can be found to parasitize the same host species in the same habitat, show 'fine tuned' behavioural adaptations which are clearly correlated with their preferred host stage.

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