Oviposition in *Yponomeuta cagnagellus*: the importance of contact cues for host plant acceptance

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Abstract. Small ermine moths (Yponomeutidae: Lepidoptera) are specialist herbivores. Species within the genus *Yponomeuta* are each specialized on a limited number of plant species, mainly within genera belonging to the Celastraceae. European *Yponomeuta* species have developed new specialized host affiliations, mainly on rosaceous hosts. Since these host shifts are reputed to be of consequence for speciation, the role of the ovipositing female is of particular interest. Study of the pre-oviposition behaviour of gravid *Y. cagnagellus* (Hb.) moths on host (*Euonymus europaeus*), non-host (*Crateagus monogyna*) and artificial oviposition substrates, provided information on the nature of the cues used for host plant acceptance and the insect’s perception of these cues. Host selection by adult females occurs with contact chemoreceptors probably located on the antennae or tarsi. MeOH-soluble, non-volatile phytochemical compounds washed from the host plant’s surface and applied on an artificial twig are sufficient to stimulate a complete sequence of behavioural elements leading to oviposition. Volatiles do not have a large effect on the pre-oviposition behaviour.

Key words. Host acceptance, Lepidoptera, moths, oviposition behaviour, specialization, *Yponomeuta cagnagellus*.

Introduction

Many phytophagous insect species are associated with a single or a few host plant species (Chapman, 1988; Mitter & Farrel, 1991). This relationship has been explored in theoretical studies, discussing the evolution of host specificity (e.g. Futuyma & Peterson, 1985), and practical studies of various specialist host plant systems elucidating the mechanisms that have contributed to the evolution of these systems (e.g. Bernays & Wcislo, 1994). Specialization involves adaptation of the insect to the single food source. Optimal digestion of the plant food requires physiological adaptation, and host specificity is mainly dependent on the recognition of the host plant as such and the ability to discriminate it from other, unsuitable, plant species (Thorsteinson, 1960; Dethier, 1982; Jermy, 1984; Miller & Strickler, 1984; Van Loon, 1996). Adaptation is dependent on a change in the, poorly studied, genes underlying these features (Coyne, 1992).

Among the phytophagous Lepidoptera, mono-and oligophagous species predominate (Ehrlich & Raven, 1964). In specialist Lepidoptera species the correct choice of the host plant by the adult female is critical. The adult female has to choose the food source of her offspring, but is not able to assess the nutritional quality directly because of the differing feeding habits of larvae and adults. The relatively immobile newly hatched larvae are not likely to locate an acceptable food source in the event of oviposition mistakes by the mother (Renwick & Chew, 1994).

The genus *Yponomeuta* (Yponomeutidae: Lepidoptera), or small ermine moths, contains phytophagous species with a wide, mainly palaeartic distribution. Host-associations are known for thirty-eight of seventy *Yponomeuta* species belonging to this genus (exclusive of American species). Of these, thirty-one are mono- or oligophagous within one genus of trees or shrubs mainly belonging to the Celastraceae (twenty-eight species occur on genera within this family: twenty-three feed exclusively on species within the genus *Euonymus*). Nine species occur in Western Europe; of these, three species are restricted to *Euonymus europaeus* (European spindle tree, Celastraceae). The remaining six European species are mono- or oligophagous on plants in the family of Rosaceae, Salicaceae or Crassulaceae (Gershenson & Ulenberg, 1998).

The results of a number of studies on the genus suggest that the European *Yponomeuta* species have evolved through host shifts from the ancestral host plant *E. europaeus* to the other,
mostly rosaceous, plant species (Menken et al., 1992; Menken, 1996). Although larvae will sometimes accept plant species other than their host, first-instar larvae are generally unable to develop to the adult stage on these non-hosts (Kooi, 1988; Kooi & Van de Water, 1988). Therefore, the selection ability of adult female Yponomeuta is a key factor in the maintenance of their host plant associations and evolution of new associations.

Little is known about oviposition in small ermine moths. Generally, the univoltine adult females deposit their eggs in masses of 50–100 eggs on the twigs of the woody host plants in late summer. The larvae hatch after approximately 2 weeks, and the first instars diapause under a protective shield until the first bud flush in spring provides them with food (Menken et al., 1992). Currently, the potential of a change in the selection behaviour of the adult moths, and its contribution to the occurrence of host shifts and subsequent specialization and host race formation are being studied (Menken & Roessingh, 1998). This involves an investigation of the genetic element associated with the ability of the adult females to deposit their egg masses on the correct host plant species. Information on the mechanisms of host recognition of adult moths is a prerequisite for this research.

For butterflies and moths in general, finding the host plant can involve several sensory modalities. The final discrimination is the product of complex and often interrelated factors, involving various senses, plant cues and the insect’s physiological state. Vision and olfaction may be used for long-range cues, often plant surface compounds (e.g. Ramaswamy, 1988; Honda, 1995).

Recent work on the discrimination behaviour of adult Y. cagnagellus points to the importance of non-volatile chemicals on the twig surface for host recognition (Hora & Roessingh, unpublished data). An extract of twig surface chemicals of E. europaeus, obtained by washing the twigs in CH₂Cl₂ and MeOH and sprayed on artificial glass twigs, has a stimulatory effect on the oviposition of Y. cagnagellus, which is specialized on this host.

In insects, the sensory equipment for short-range host selection consists of contact chemoreceptors on appendages that contact the substrate, e.g. antennae, tarsi and ovipositor. This study investigates the pre-oviposition behaviour of Y. cagnagellus to identify the receptor groups and plant cues involved in host discrimination. The sequential use of the appendages containing sensilla, and more important, the stages of this sequence where it is interrupted when the oviposition substrate is not acceptable for oviposition, indicates which receptor groups are probably used for host discrimination. The behavioural sequence prior to oviposition of gravid female Y. cagnagellus on the host plant, E. europaeus, and a non-host Crataegus monogyna (Rosaceae), were studied. The role of volatile compounds was investigated by comparing the behaviour on host twigs and artificial twigs treated with host surface extracts, either with or without the addition of host volatiles.

Materials and Methods

Insects

Yponomeuta cagnagellus (Hb.) (Yponomeutidae: Lepidoptera) moths were reared in the laboratory from egg masses collected in the field from its host E. europaeus. First-instar larvae were provided with young E. europaeus leaves in plastic Petri dishes and reared at 23 ± 1°C, LD 17:7 h until pupation. Alternatively, fifth-instar larvae close to pupation were collected in the field. All larvae pupated and ecedosed at 18 ± 1°C, LD 17:7 h. Shortly after emergence of the moths, males and females were separated and kept in the climate room where the observations took place (at 22 ± 1°C, LD 18:6 h).

Yponomeuta cagnagellus is not sexually mature upon eclosion (Hendrikse, 1979), and in Yponomeutidae fertilization is required before oviposition is possible (Taylor, 1967). Since the physiological condition of insects (in particular egg load) can have considerable influence on their behaviour (Minkenberg et al., 1992), two different methods were used to standardize the physiological state of the Y. cagnagellus females. For the identification of the behavioural elements, female moths were kept in glass vials (8 cm high, 2 cm diameter) closed with cotton wool, accompanied by a male of the same age, for three weeks after eclosion and fed with a solution of 1% honey in water on a piece of filter paper which was renewed every 2–3 days. Mating should take place within this period (Hendrikse, 1979). On the last day of these 3 weeks, moths were used in the experiment or kept at 5°C and the same light regime as the observational chamber for at most 1 week before observation.

This method yielded relatively low numbers of mated and ovipositing females and was changed in the later experiments, for which males and females were kept separate in glass jars (15 cm high, 10 cm diameter) in groups of ten to thirty, provided with fresh water and a 10% honey solution in 1% water agar in 0.5-ml Eppendorf tubes. The moths were checked regularly for signs of sexual maturity. Female moths were considered sexually mature when they were observed calling at the end of the scotophase, males when they showed ‘wing fanning’ behaviour (Hendrikse, 1986). To mate these moths, ten adults of each sex were placed in a large PVC cage (70 cm high, 30 cm diameter), 1 h prior to the end of the scotophase. During the ensuing 2-h period, mating couples were taken out of the cage and put individually in glass vials (8 cm high, 2 cm diameter). After copulation the males were removed and the females were kept in the separate glass vials for 5–6 days. On the last day, they were used for observation or transferred to 5°C and the same light regime as the observational chamber for at most one week before observation. Females tested before the fifth day after copulation rarely showed any activity. Following this method, a higher percentage of moths exhibited pre-oviposition activity than did those in the first observations.

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Table 1. Behavioural elements of *Y. cagnagellus* after contact with the host up to and including oviposition

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Rest'</td>
<td>Resting posture. Antennae folded alongside the body.</td>
</tr>
<tr>
<td>'Stand'</td>
<td>Standing still, moving the antennae.</td>
</tr>
<tr>
<td>'Preen'</td>
<td>Preening the antennae and palpi while standing.</td>
</tr>
<tr>
<td>'Antennae sweep'</td>
<td>Walking, touching the surface with the antennae.</td>
</tr>
<tr>
<td>'Ovipositor sweep'</td>
<td>Walking, touching the surface with both antennae and ovipositor.</td>
</tr>
<tr>
<td>'Ovipositing'</td>
<td>The actual egg deposition.</td>
</tr>
</tbody>
</table>
Table 2. Twig Contact Duration (percentage of time spent on the twig), search activity (percentage of time walking and ovipositing) of Y. cagnagellus on different twig types (Activity on twig) and search activity of Y. cagnagellus on the arena (Activity on arena) in the different experimental situations.

<table>
<thead>
<tr>
<th>Oviposition substrate</th>
<th>n</th>
<th>Twig Contact Duration (%)</th>
<th>Activity on twig (%)</th>
<th>Activity on arena (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavioural components</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. europaeus</td>
<td>15</td>
<td>49.4 a</td>
<td>53.6 a</td>
<td>24.6 a</td>
</tr>
<tr>
<td>Comparing host and non-host twigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. europaeus</td>
<td>20</td>
<td>81.6 b</td>
<td>52.0 a</td>
<td>22.3 b</td>
</tr>
<tr>
<td>C. monogyna</td>
<td>20</td>
<td>70.9 c</td>
<td>11.1 b</td>
<td>12.7 b</td>
</tr>
<tr>
<td>Effect of volatile compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. europaeus twig odour</td>
<td>20</td>
<td>71.1 a</td>
<td>7.6 a</td>
<td>28.9 a</td>
</tr>
<tr>
<td>Artificial twig odour</td>
<td>20</td>
<td>65.7 a</td>
<td>17.0 a</td>
<td>15.9 a</td>
</tr>
<tr>
<td>E. europaeus twig no odour</td>
<td>15</td>
<td>72.4 a</td>
<td>37.8 a</td>
<td>22.8 a</td>
</tr>
<tr>
<td>Artificial twig no odour</td>
<td>15</td>
<td>75.9 a</td>
<td>24.1 a</td>
<td>14.4 a</td>
</tr>
</tbody>
</table>

n = number of females observed. Differences between TCD and activity of individual females in the two observations were tested for significance using Wilcoxon’s matched pair rank test (2-tailed P < 0.05). When differences were determined between different groups of individuals, a Mann-Whitney U-test was applied (2-tailed P < 0.05). The observation on volatile compounds is not compared to the first two observations because of the shorter observation period. Data followed by the same letter are not significantly different.

Observations

Yponomeuta cagnagellus females oviposit throughout the scotophase (Bremner et al., 1997). To facilitate behavioural observations, the scotophase started at 10.00 hours and ended at 16.00 hours. During the observation periods the climate room was illuminated with red light (red Philips light bulb, 60 W, 11 Lux). The arena in which the behaviour was observed, consisted of an open ended Perspex tube (15 cm long, 4 cm diameter) fixed at an angle of approximately 45°. The twigs of the host, non-host and the artificial twigs were fixed in the central axis of the tube using a cotton wool plug at the lower part of the arena. A constant airflow was generated in the arena by entering pressurized air at the bottom at a rate of 320 ln/h. Host odour could be added to this airflow by placing a Perspex tube containing an intact twig with all leaves between the filter and the airflow meter. To be able to observe the moth on the twig at the side opposite of the observer, a mirror was placed under the arena.

In all experiments, six behavioural elements (Table 1) and the location of the moth on either the arena or different parts of the twig, were recorded using ‘The Observer 3.0’ (Noldus Information Technology, Wageningen, The Netherlands), registering duration and frequencies of each element.

Identification of behavioural elements

A freshly cut, 12-cm long twig of E. europaeus was fixed in the centre of the arena after all leaves but one were removed to provide a clear view of the insect. The twig was kept fresh by placing the cut end in a 1.5-ml centrifuge tube with 1% water agar. As mating was rarely observed, females were tested prior to the observation to assess their inclination to oviposit. A vial containing a female showing locomotor activity was placed into the airflow at the upper opening of the arena and left there for a maximum of 15 min. Females that were inclined to walk into the arena were used for the observations. Observation started when the moths walked into the arena and was discontinued when the female began ovipositing or when the moth did not attempt oviposition within the next 2 h. Observations during which the moth did not move for 30 min were not used for analysis. Out of forty-two moths tried, fifteen matched these criteria. To standardize the data the first hour of each observation was analysed.

Comparing host and nonhost twigs

The behaviour on host and non-host twigs was compared using twigs of intact plants as oviposition substrate. The arena tubes were fixed with cotton wool plugs on the lignified parts of flexible twigs of potted plants of two species, E. europaeus and Crataegus monogyna. Crataegus monogyna (Hawthorn) is the host of Yponomeuta padellus, which is closely related to Y. cagnagellus. In bioassays, Y. cagnagellus does not accept C. monogyna for oviposition (Bremner et al., 1997). All leaves but one were removed at least 24 h prior to the experiment. The object of the experiment was to study the effect of contact cues of host and non-host. To compensate for the odour possibly produced by the leaf on the host twig, E. europaeus odour was passed through the arena in all cases to provide a constant background of host odour.

Gravid moths were placed on the twig in the arena by carefully shaking them out of the glass vials. They were allowed to recover from this manipulation for 5–10 min. The observation started when the moth began to walk and lasted 60 min. To minimize behavioural differences due to natural variation in pre-oviposition behaviour, each moth was observed on both E. europaeus and C. monogyna twigs.
enabling comparison of the behaviour of the same individual on both host and non-host. During a 3-h interval between these two observations, the moth was kept in a glass vial. Two moths were observed during one scotophase. The order of presentation of the twigs was alternated. To be able to observe selection behaviour of moths that began ovipositing on *E. europaeus* before being observed on *C. monogyna*, their egg deposition was interrupted by removing the moth from the twig after it had been observed laying the first five eggs. None of these moths continued oviposition after being removed from the twig. When the moth had not oviposited within either observation period, it was kept in the arena on an *E. europaeus* twig until it had. All moths did so within the current scotophase or the one after the observation.

A total of twenty-three observations were made of moths on *E. europaeus* and twenty-two on *C. monogyna*. Of these, twenty individuals were observed on both plants: three moths were allowed to continue ovipositing on *E. europaeus* and to complete the deposition of the egg mass. These could not be used for subsequent observation on *C. monogyna*. Two moths were observed solely on *C. monogyna.

**Effect of volatile compounds**

In this experiment the same protocol was followed as in the experiment comparing host and non-host twigs. Instead of *C. monogyna* twigs, artificial twigs were used. These were constructed from Pasteur capillary pipettes covered with paper tape (TESSA, Beiersdorf BDF, Germany). The artificial twigs were treated with methanolic extract of *E. europaeus* twig surface chemicals. This extract was obtained by washing twigs briefly first in CH$_2$Cl$_2$ and then in MeOH, following the protocol of Städtler & Roessingh (1991) (Hora & Roessingh, 1996). To obtain an accurate comparison with the bare artificial twigs, all leaves were removed from the *E. europaeus* twigs. The artificial twigs were treated with host-extract over a length of 7 cm, therefore the *E. europaeus* twigs were made of similar length by shortening the arena with cotton wool plugs.

The experiment was divided into two blocks: in the first, air containing *E. europaeus* odour was passed over both real and artificial twigs. In the second block only purified and humidified air was used. Within each block one individual was observed on both the artificial and the real twig. In between both observations the moths were kept in a glass vial for 3 h. The sequence of the observation was systematically alternated. To enable three observations within one scotophase, the duration of each observation was shortened to 40 min.

Oviposition capability of the females and attractiveness of the artificial twig were verified by keeping the females in the arena with the artificial twig until they had deposited an egg mass. Only observations of the moths that oviposited within 48 h following the observation were used for analysis. Out of forty-two moths observed in this experiment, thirty-five oviposited within 48 h after the observation. Twenty of these were observed in with host odour added to the arena and fifteen in arenas with a purified airflow.

**Data analysis**

As *Y. cagnagellus* females oviposit only once every two to three scotophases, the actual oviposition is rarely observed and cannot serve as a measure for preference in this experiment. To quantify the pre-oviposition behaviour, three different variables were therefore calculated from the raw observation data.

1. **Twig Contact Duration (TCD).** Time in contact with twig divided by the total observation time. This variable is indicative of the moth’s oviposition drive. Differences between TCD of individual females in the two observations were tested for significance using Wilcoxon’s matched pair rank test (2-tailed $P<0.05$). When differences were determined between different groups of individuals, a Mann–Whitney U-test was applied (2-tailed $P<0.05$).

2. **Activity.** Time spent showing active pre-oviposition behaviour and ovipositing on the twig (or arena) divided by the total time spent on the twig (or arena). This variable was used as a measure for the attractiveness of the twig. Differences in activity were analysed in the same manner as differences in TCD.

3. **First order transitional frequencies between the behavioural elements.** The differences in the behavioural sequence in each experimental situation were analysed using contingency table analysis (Colgan & Smith, 1978). The analysis was conducted on the total number of transitions between pairs of behavioural elements. From these, an $i \times j \times k$ contingency table was constructed, in which the first variable represented $i$ preceding behavioural elements, the second variable represents $j$ following behavioural elements and the third the $k$ experimental situations (in our case either different locations or different kinds of twigs). For such a 3-way table, eight log–linear models can be formulated that predict transitional frequencies under all possible levels of interaction of these three variables. The models range from complete independence (i.e. total absence of behavioural sequences and no effect of the experimental situation), to a total 2-way interaction model, in which behavioural elements interact significantly and different behavioural sequences occur in each experimental situation. Using G-tests it can be determined which of the possible predictions of the interaction amongst the variables explain the observed data best. A complete description of these models is given by Colgan & Smith (1978).

Additionally, an index of dissimilarity (ID) can be calculated between the expected values under the assumptions of each model, and the observed values. The ID provides an alternative method to determine the fit of a model. It represents the percentage of observed transitions that would have to be relocated to make the observed data fit the $i \times j \times k$ table predicted by the model exactly (Messina & Dickinson, 1993); therefore its values can range between 0 and 100. A program designed by B. Tran in EXCEL 7.0 (‘Contable’, Parr et al., 1996), performing the complete analysis was kindly provided by Martin Parr.

When a sequence of elements can be shown to exist with this analysis, the next question is what elements participate in this sequence and in which order. To identify those transitions that are significantly part of a sequence of behavioural

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elements, standardized residuals ((\(\text{Observed}_{ijk} - \text{Expected}_{ijk}\))/\(\sqrt{\text{Expected}_{ijk}}\)) of the observed transitions were calculated when compared to a model that does not include the assumption of dependence of following and preceding behavioural elements (a sequence). Thus, high positive residuals for certain transitions indicate that these are particularly discrepant with this model, and therefore a significant part of the behavioural sequence. The significantly positive transitions [those for which positive residuals were obtained which are higher than the calculated threshold (\(\sqrt{\chi^2_{ijkl}/(\text{total number of cells})}\)], were used to construct kinetograms of the pre-oviposition behaviour.

Where interaction of the sequence with the experimental situation was found, the differences in sequences of behaviour elements between those experimental situations were identified by calculating the standardized residuals under the model that lacks this assumption of the experimental situation influencing the behavioural sequence. The positive residuals then identify those transitions in a particular experimental situation which occur more frequently than would be expected if the behaviour remained unchanged in different experimental situations.

Results

Identification of behavioural elements

The behaviour shown by gravid \(Y. \text{cagnagellus}\) females on their host plant \(E. \text{europaeus}\) can be divided into several behavioural elements (Table 1). Often the moth stands still with the antennae held besides the body at 45–90° to the body axis. During these pauses the antennae are moved in a circular movement (‘Stand’, 36.7% of the observation duration). Also the antennae and palpi can be preened (‘Preen’, 2.4% of the observation duration). While walking, the antennae are stretched forward and held at a slight angle from the body axis, gently stroking the surface with the anterior segments (‘Antennae sweep’, 15.7% of the observation duration).

When contact is made with the host plant twig, the moth usually extends her abdomen, bending it ventrally, while the terminal abdominal segments are protruded from their normal retracted position. The ovipositor touches the twig surface, making small lateral movements while the moth is walking up and down the stem, often within the limits of two or more nodes. During these ‘runs’, the antennae continue to touch the surface ahead of the moth (‘Ovipositor sweep’, 29.7% of the observation duration). Occasionally the moth slows down or halts, while stretching her abdomen and intensifying the contact of the ovipositor with the surface of the stem. This is mostly done on the stem near nodes or other irregularities of the stem surface. This phase is broken by periods of rest, in which the moth preens her antennae and palpi or stands still with her antennae moving in the air.

During oviposition, the moth stands still with her abdomen opposite a node or bud. The antennae are eased alongside the body, and the ovipositor tip is kept in close contact with the

<table>
<thead>
<tr>
<th>Oviposition substrate</th>
<th>First experience</th>
<th>(n)</th>
<th>Twig Contact Duration (%)</th>
<th>Activity on twig (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E. \text{europaeus})</td>
<td>(E. \text{europaeus})</td>
<td>12</td>
<td>91.9 a</td>
<td>36.4 a</td>
</tr>
<tr>
<td>(C. \text{monogyna})</td>
<td>(E. \text{europaeus})</td>
<td>11</td>
<td>72.8 a</td>
<td>50.3 a</td>
</tr>
<tr>
<td>(C. \text{monogyna})</td>
<td>(E. \text{europaeus})</td>
<td>9</td>
<td>79.6 a</td>
<td>11.9 a</td>
</tr>
<tr>
<td>(C. \text{monogyna})</td>
<td>(C. \text{monogyna})</td>
<td>13</td>
<td>62.3 a</td>
<td>4.4 a</td>
</tr>
</tbody>
</table>

\(n\) = number of females observed. Significance is determined by Mann–Whitney \(U\)-test between different groups of moths on the same twig species (2-tailed \(P<0.05\)). Data followed by the same letter within a column are not significantly different.

Oviposition, displaying intensive lateral movements until the first egg is deposited. While depositing an egg, pumping movements are made with the abdomen, pressing the ovipositor closely to the stem surface (‘Ovipositing’, 14.9% of the observation duration). When the egg is laid the abdomen is brought further under the body, still held closely to the stem surface, while the pumping movements accelerate. After 34 ± 1 s (\(n=98\) eggs, \(n=6\) females), the ovipositor is released from the surface and relocated to the oviposition site. The location for the next egg is then determined by moving the tip of the ovipositor over the newly laid egg mass, until the next egg is added to the mass (30 ± 3 s, \(n=98\) eggs, \(n=6\) females). In one mass up to 150 eggs can be laid in a compact pattern at a rate of approximately one egg per minute. Directly after deposition of the egg mass the moths do not show further host examination behaviour but will move a few steps from the newly laid egg mass and assume a resting posture, in which the antennae and the posterior pair of legs are folded backwards alongside the abdomen (‘Rest’, 0.3% of the observation duration). A next egg mass is generally laid two or three nights later (Karalius & Büda, 1995; K. H. Hora, personal observation).

The moths spent half of the observation period on the twig. When on the twig, the moths displayed more active behaviour than on the arena wall (Table 2). However, substantial individual variation in activity was observed, varying from minimally 0.6% to maximally 98% active behaviour during the entire observation period. The main difference in behaviour on the twig and the arena wall lies in the behaviour changes from ‘antennae sweep’ to ‘ovipositor sweep’ and the occurrence of oviposition. The latter two behavioural elements are shown exclusively on the host twig.

Comparing host and non-host twigs

A number of differences are apparent in the behaviour of moths on host and non-host twigs. Of the observations on
E. europaeus, twelve resulted in oviposition during the observation period. Oviposition on C. monogyna was observed only once, with a moth which had attempted oviposition during previous observation on E. europaeus. No moths in the group first observed on C. monogyna, oviposited on C. monogyna. No significant differences were found in TCD or activity on the same plant-species between the two groups of moths with different experience (Table 3). Therefore, all observations on the same plant species were pooled for analysis.

The Twig Contact Duration (TCD) was higher on the host twigs, E. europaeus, compared to that on the non-host C. monogyna (Table 2). The activity of the moths on the E. europaeus twig was considerably higher than on C. monogyna. The activity on the arena wall in the presence of E. europaeus or C. monogyna did not differ significantly, nor did the moths show more active behaviour on C. monogyna twigs than on the arena (Table 2).

For the contingency table analysis all first-order transitions were pooled for the two plant species (Fig. 1). Although the total two-way interaction model does not explain the observed transitions ($\chi^2_{10} = 28.32$, NS), it does results in a low ID of 3.71. This implies the existence of a behavioural sequence which is dependent on the plant species. The behavioural patterns on the twigs of both plant species differ mainly in the proportion of transitions from ‘antennae sweep’ to ‘ovipositor sweep’ (Fig. 1). On E. europaeus this transition is more likely to occur, and the moths display the latter behaviour more often. On the non-host the behavioural sequence is often cut off after ‘antennae sweep’, which is then followed by ‘stand’.

The lack of occurrence of the ‘ovipositor sweep’ behaviour on C. monogyna is also clearly demonstrated by the significant difference in the percentage of time spent on the twig in which this behaviour is displayed on both twigs (Fig. 1). Moreover, this behaviour on C. monogyna was only shown by four of the twenty-two moths, whereas it was displayed by twenty-one of the twenty-three moths on E. europaeus.

Effect of volatile compounds

The moths on real or artificial twigs, with or without the addition of host volatiles, did not differ significantly in TCD, activity on the twig or in the activity on the arena wall (Table 2). Analysis of the behavioural transitions yields
A. *E. euonymus* twig with odour

B. artificial twig with odour

C. *E. europaeus* twig without odour

D. artificial twig without odour
minor differences in behaviour in the four different experimental situations. Low ID’s (2.20–8.70) and low \( \chi^2_{24} \)-values (13.05–26.53, NS) for models lacking the assumption of an effect of the experimental situation on the behavioural sequence, show that the experimental situation generally does not affect the occurrence of the behavioural elements. However, the effect of the experimental situation is not insignificant in all comparisons: on the real twig addition of odour seems to affect the sequence slightly (\( \chi^2_{24} = 71.00, P<0.05; \) ID = 8.70). Similar results are obtained with other models including the assumption of an effect of the behavioural sequence, but little influence of the experimental situation.

To identify transitions that cause the slight variation of the behavioural sequence in the four experimental situations, all four were compared simultaneously in a 5 \( \times \) 6 \( \times \) 4 table. No oviposition had been observed with the twenty moths in the block with odour added to the arena during the observation period. Of the fifteen moths observed in the block lacking these volatiles, four moths oviposited during the observational period: one on both the real and the artificial twig and three on the real twig only. These showed a tendency to complete the transition from ‘antennae sweep’ to ‘ovipositor sweep’ more often than the group of moths introduced by host odour (asterisks in Fig. 2). In this group the ‘ovipositor sweep’ also displayed longer. These differences, however, could be an effect of variations in the physiological state between the two groups of moths. Within groups of moths, no significant differences could be found in the percentage of time of each element on real or artificial twig (Wilcoxon matched pair rank test, \( P<0.05 \)).

### Discussion

This investigation presents a detailed description of the pre-oviposition behaviour of *Y. cagnagellus*, giving information on the nature of the host plant cues used for host plant recognition and the insect’s perception of these cues. The results indicate that host discrimination in adult females can occur after alighting on the host, with the aid of receptors probably located on the antennae and perhaps the tarsi. MeOH-soluble, non-volatile phytochemical compounds of the host plant on an artificial twig alone are sufficient to trigger a complete sequence of behavioural elements up to and including oviposition. In contrast, volatiles do not have a large effect on the insect’s pre-oviposition behaviour.

### Behavioural sequence

Behaviour after alighting on the plant prior to oviposition is still relatively unexplored in moths. As Renwick & Chew (1994) point out in their review: ‘The contact evaluation behaviour of moths on a plant has not been studied to the same extent as it has been for butterflies’. Recently, however, descriptions of detailed observations of the oviposition behaviour have become available for some moth species such as the leek moth *Acrolepiopsis assectella* (Plutellidae) (Thibout & Auger, 1996), the pod-borer *Etiella zinckenella* (Pyralidae) (Hattori, 1988) and *Plutella xylostella* (Plutellidae) (Justus & Mitchell, 1996). These bear resemblance to the ‘antennae sweep’ and ‘ovipositor sweep’ behaviour of *Y. cagnagellus*.

### Plant cues used for host discrimination

In *A. assectella* and *P. xylostella* as well as in *Y. cagnagellus*, close examination of the plant surface with the antennal tips in combination with the ovipositor appears to be typical. The importance of contact phytochemicals for host selection has been shown for both *A. assectella* and *P. xylostella* (Justus & Mitchell, 1996; Spencer, 1996; Thibout & Auger, 1996). This suggests that in *Y. cagnagellus* host recognition is also based on contact cues such as plant surface compounds.

However, *Y. cagnagellus* spends a considerable amount of time with the antennae rotating in the air. This is probably an olfactory-related behaviour: the antennae of *Y. cagnagellus* (and many other moths) contain multiporous sensory hairs that have an olfactory function and are able to detect a number of volatile phytochemical compounds (Van der Pers, 1982; Cuperus, 1983). Orientation based on volatiles might be involved in long-range detection of the host.

Host odour can also act as ‘backup information’, minimising the probability of oviposition mistakes. These could considerably affect the fitness of the female: under laboratory conditions, *Y. cagnagellus* lays on average 3.8 \( \pm \) 0.4–4.5 \( \pm \) 1.0 egg masses (Karalius & Büda, 1995), but females have been observed laying as many as ten egg masses (K. H. Hora, personal observation). Even assuming the maximum of ten egg masses is achieved in the field, this implies that one oviposition mistake would imperil minimally 10% of the total offspring. It is therefore conceivable that host selection will involve other, seemingly redundant, sensory inputs besides contact chemoreception. On the other hand, oviposition

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*Fig. 2.* Significantly positive first-order transitions (solid arrows) of the behavioural elements of *Y. cagnagellus* on *E. europaeus* twigs and artificial twigs with *E. europaeus* odour added (A and B, \( n = 20 \)) and on the same comparison without host volatiles (C and D, \( n = 15 \)) in the experiment investigating the effect of volatile compounds on the pre-oviposition behaviour. Decimal numbers associated with the arrows represent the probability of each transition given the preceding behaviour. \( * \) = significantly different transition between experimental situations (see text). Dotted lines represent first order transitions not significantly part of the sequence. The percentage occurrence of each behavioural element is given below its category definition: \( * \) = significantly higher percentage between groups with or without odour (Mann–Whitney U-test, 2-tailed \( P<0.05 \)). The thickness of the arrows indicates the frequency of occurrence of this transition.

mistakes might be caused by adjacent non-hosts presenting confusing odours, perhaps even masking those of the host, or host-odor enveloping adjacent non-hosts (Asatt & O’Dowd, 1976; Thierry & Visser, 1986). In the present study volatile compounds do not seem to have a large effect on host acceptance by Y. cagnagellus, since the behaviour shows only minor differences between absence and presence of volatiles and these may be attributed to differences in physiological state (egg load) of the two groups of moths.

Yponomeuta cagnagellus is monophagous on E. europaeus (Gerrits-Heybroek et al., 1987) and this monophagy is apparent in the host discrimination behaviour of the adult female. Oviposition rarely takes place on C. monogyna or other non-hosts, even in no-choice situations (Bremner et al., 1997). The behavioural differences shown by the moths on host (E. europaeus) and non-host (C. monogyna) twigs, reflect this strict host discrimination. It is unlikely that these differences are caused by long-distance cues since odour of E. europaeus was provided with both host and non-host twigs. It is therefore likely that contact cues, present on the twig surface are responsible for the observed behavioural differences.

Observation of Y. cagnagellus females on the artificial twig has shown that these moths are able to recognize the host by contact chemostimuli alone. Currently, the phytochemical compounds responsible for the stimulatory effect on the methanolic twig surface extracts are being identified.

Receptor groups used by Y. cagnagellus

The use of the ovipositor during the runs up and down the stem by Y. cagnagellus, suggests some chemosensory function. Indeed chemosensory sensilla have been found on the ovipositor of a number of moths (Chadha & Roome, 1980; Valencia & Rice, 1982; Faucheux, 1988b; Marion-Poll et al., 1992; Qui et al., 1998). It has been suggested that these chemoreceptors may act to prevent egg laying on chemically unacceptable places (Chadha & Roome, 1980; Valencia & Rice, 1982).

In Y. cagnagellus, the ovipositor is frequently brought into contact with the twig surface on the host, less on a non-host and hardly ever on a neutral surface such as the arena wall. Similar observations were made by Spencer (1996), who saw P. xylostella probing the surface with the ovipositor only after encountering an oviposition stimulant with its tarsi or antennae. Under these conditions oviposition could occur without the ovipositor being in contact with the chemical compounds. In Y. cagnagellus, the ovipositor also seems to be of less importance for actual host acceptance, as this organ is not used in the evaluation of the non-host twig. This implies that the recognition of the unsuitable oviposition substrate has already taken place using organs other than the ovipositor, e.g. the tarsi or the antennae.

As the ovipositor of moths generally contains many mechanosensory sensilla besides chemoreceptors, its function seems more likely the determination of the suitable oviposition site based on structural cues (Chadha & Roome, 1980; Valencia & Rice, 1982; Faucheux, 1988b; Marion-Poll et al., 1992). Plant surface texture appears to be more critical for moths than for butterflies in the evaluation of potential oviposition sites (Renwick & Chew, 1994). Yponomeuta cagnagellus shows a strong preference for depositing the egg masses near buds, side shoots or any other irregularities on the twig surface (Bremner et al., 1997) and pays considerable attention to these structures during the pre-oviposition behaviour.

Many diurnal butterfly species exhibit a so-called ‘drumming’ behaviour when examining plants, which involves drumming the oviposition surface repeatedly with the front tarsi (Ilse, 1956; Fox, 1966). The behaviour is widely believed to provide chemical information about the plant through tarsal sensilla. These sensilla and their importance for host discrimination, have been identified in a number of butterfly species (Renwick & Chew, 1994; Van Loon, 1996 and references therein).

Moths do not generally exhibit this drumming behaviour (Renwick & Chew, 1994). However, there is some evidence that tarsal receptors in moths are involved in plant acceptance, e.g. in Trichoplusia ni (Noctuidae) (Renwick & Radke, 1982), Heliothis virescens (Noctuidae) (Ramawamy et al., 1987) and P. xylostella (Qui et al., 1998). Putative chemoreceptors have also been found on the tarsi of Manduca sexta (Sphingidae) and Ostrinia nubilalis (Pyralidae), but their function in host selection is not clear (Kent & Griffin, 1990; Marion-Poll et al., 1992).

The presence and importance of chemosensory sensilla on the antennae in host discrimination has been shown for some moths. For example, contact chemoreceptors have been described on antennae of Acrolepiopsis assectella (Faucheux, 1988a) and H. virescens (Ramawamy, 1988). Antennetected misfemale Homoeosoma electellum (Pyralidae) were no longer able to discriminate between oviposition sites, indicating that chemoreceptors involved in oviposition site selection are located on the antennae (Delisle et al., 1989). Ablation studies in P. xylostella also point out their importance in detection of deterrents (Qui et al., 1998).

The ‘antennae sweep’ behaviour as described here for Y. cagnagellus is likely to be the behaviour that allows for perception of contact cues through sensilla located on the antennae, although sensilla on the tarsi may also be involved. On both appendages contact chemoreceptors have been identified (Cupres, 1983), but no electrophysiological evidence on their importance for host plant recognition has been published so far. The prominent use of the antennae during examination of the twig, however, is an indication that the antennae perform an important role in host discrimination of Y. cagnagellus. The role of the antennal chemoreceptors is currently being studied with electrophysiological methods.

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