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OVIPOSITION IN *YPONOMEUTA CAGNAGELLUS*: WHEN, WHERE AND WHY THERE

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Summary

The choice of the hostplant by the adult female moths is a key-factor in host-shifts and subsequent speciation. Oviposition of *Y. cagnagellus* takes place during the scotophase. Preference for type and species of twig was tested in a bioassay. Eggbatches were mainly laid near buds and branches. Twigs of the host-species were preferred. Observed oviposition mistakes, caused by the presence of host twigs and behavioural observations suggest that *Y. cagnagellus* uses surface chemicals for host recognition.

INTRODUCTION

Yponomeuta cagnagellus (Lepidoptera: Yponomeutidae) belongs to the genus of phytophagous moths known as the small ermine moths. These moths are generally monophagous on trees and shrubs, with the majority of the species feeding on plants from the family Celastraceae; especially on the genus *Euonymus* (Menken, 1996). Of the species not feeding on Celastraceae, 4 feed on plants from the family Rosaceae; 2 feed on Salicaceae and 1 feeds on Crassulaceae. Apart from *Yponomeuta*, few other species feed on Celastraceae. One of the main hypotheses under investigation states that "the present affiliations in the genus *Yponomeuta* evolved from an ancestral association with Celastraceae, through allopatric speciation, mostly on *Euonymus*, and possibly through sympatric host shifts to other food plant genera" (Menken *et al.*, 1992).

The genus has been a topic of multidisciplinary study for more than 20 years. The behavioural and ecological studies have focused mainly on the host plant recognition of the larvae (Van Drongelen, 1979) and acceptance of host plants and non host plants by the larvae (Gerrits-Heybroek *et al.*, 1978; Herrebout *et al.*, 1987). It has been shown that the sugar alcohol dulcitol, which is the primary transport sugar in Celastraceae, has a phagostimulatory effect on larvae feeding on Celastraceous host plants. In Rosaceae, the primary sugar alcohol and phagostimulant is sorbitol, a stereo isomer of dulcitol (Herrebout *et al.*, 1987). However, some *Prunus* species (Rosaceae) contain low levels of dulcitol. The traces of dulcitol present in *Prunus* could have allowed the shift from Celastraceae to Rosaceae (Peterson *et al.*, 1990). The observation that *Y. cagnagellus* will accept *Prunus padus* twigs if they are impregnated with dulcitol (Kooi & Van de Water, 1988), further supports this hypothesis. However, it should be noted that the female's host plant recognition is the key factor of host selection. *Yponomeuta* lay their eggs in clusters on the twigs of the host plants; after emergence in spring, the larvae are small and unable to move very far.

So far, there has been very little work on the oviposition behaviour of *Yponomeuta*. Therefore, the host plant recognition and -acceptance in the adult moths is currently being investigated. This paper focuses on the oviposition behaviour of *Y. cagnagellus* and attempts to find answers to the questions When are the eggs laid? Where are they laid? and Why are they laid there?

The specific time period in which oviposition takes place, is important for pinpointing the best time for direct observation of pre-oviposition behaviour. Evaluation of the preferred oviposition sites on the host is needed to optimize a bioassay in which to test oviposition preference. We therefore studied the effects of twig-age and the presence of leaves on oviposition. To answer the question why the moths prefer a specific host, behavioural observations and oviposition choice tests were conducted. Observation of the pre-oviposition behaviour can give information about which signals the moth uses to recognise the host plant, and how they are detected.

EXPERIMENTS

Unless otherwise stated, the following methods apply to all experiments.
Fourth and fifth instar larvae of *Y. cagnagellus* were collected from Wageningen and Almelo and were allowed to pupate. After emergence the sexes were kept separately at 18 °C, LD 17:7. The moths were provided with food in the form of 1% honey water, and a twig from the host plant, *Euonymus europaeus*. The cut off twigs were kept fresh in small jars of water or 1% water-agar. In each experiment eggbatches were counted daily, and fresh twigs were provided every 10-14 days. Since *Y. cagnagellus* is nocturnal, all experiments were carried out under a reversed photoperiod, where the dark period was maintained during the daytime (LD 18:6 hours) at 22°C.

When does Y. cagnagellus *oviposit ? Actometer experiments and behavioral observations.*
An actometer (Syntech, Hilversum, the Netherlands) was used to determine when oviposition activity took place. This device, consisting of an enclosed chamber with a high frequency transmitter and a receiver, uses Doppler interference to measure movement. The transmitter sends out a stable frequency which is picked up by the receiver. Movement within the chamber alters the received frequency. Mated females were put in the actometer for periods of 20 - 60 hours, with food and a host twig. The actometer was linked to a paper recorder, which showed the frequency changes (activity) as a series of peaks per unit time. In the analysis the activity was quantified as: No peaks = 0; Less than 5 peaks per hour = 1; 6-20 peaks per hour = 2; More than 20 peaks per hour = 3. Data from 21 females was pooled to give the average activity per hour. In our setup the actometer only recorded the fact that the moths are active; so direct observation was required to correlate details of the signal with oviposition behaviour. In a preliminary study of the pre-oviposition behaviour, four female moths were observed. A red light was used as the observations were carried out during the scotophase. The moths had been mated and were deprived of host plants for two to three days. They were observed for periods of 20-60 minutes in large glass tubes 25 cm by 3 cm. These had 30 ml of 1% agar in the bottom to hold a host plant twig, and were covered with gauze.

Figure 1 shows the average activity of the moths over a 24 hour period. Most activity was registered during the scotophase and at the end of the photophase. In addition, 26 moths were seen ovipositing. Of these only 3 were observed ovipositing in the photophase. During oviposition, the moths sit almost motionless, with only the ovipositor moving. This makes it difficult to detect oviposition behaviour from the actometer recordings. However, in one case oviposition was observed during the actometer recording. The oviposition took place in the scotophase, and lasted 40 minutes. In the three hours prior to this there was a period of very high activity consisting of bouts of activity lasting between 8 and 28 minutes, interspersed with 8-24 minute periods of little or no activity. Possibly the high levels of activity found prior to oviposition correspond to the assessment of the host twig by the moth, as described in Hora & Roessingh (1996). This behaviour consist of running up and down the twig, touching the twig surface with antennae and/or ovipositor.

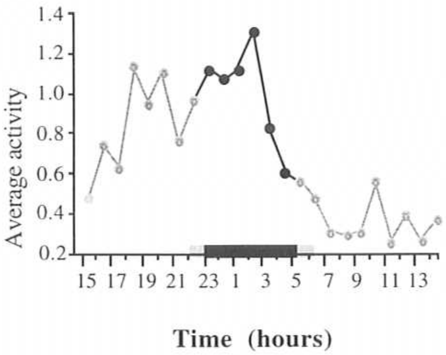

Figure 1. The average activity of 21 female Y. cagnagellus *during 24 hours. The black bar represents the scotophase, the gray bars represents dusk or dawn .*

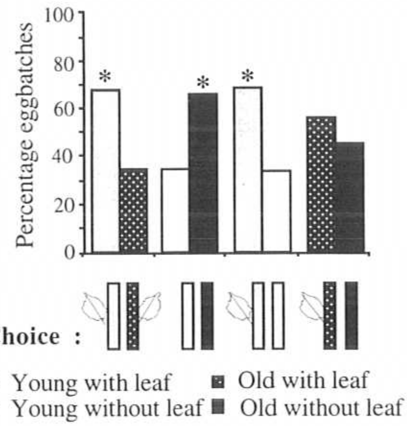

Figure 2*. The percentage of eggbatches in each of the four twig choice situations (see text) . For each choice 7, 7 , 9 and 9 females were tested respectively . (Chi* ^{2} $p< 0.05$*).*

Where does Y. cagnagellus *oviposit ? Choice experiments with host plant twigs.*

A twig choice experiment was set up, giving the moths a choice between two types of 10-15 cm lengths of *Euonymus* twig in glass jars 20 cm high, 10 cm diameter, with gauze lids. There were one or three pairs of moths per jar. Four combinations of twig types were used: 1. Young (green, flexible shoots) or old twigs (lignified twig parts), with leaves. 2. Young or old twigs, without leaves. 3. Young twigs with or without leaves. 4. Old twigs with or without leaves. Each combination was replicated three times, resulting in a total of 32 females. In all experiments the position of the eggbatches on the twig was noted.

Figure 2 shows the percentages of the eggbatches in each choice situation. When leaves are present, a significantly higher proportion of eggbatches is laid on the younger twig. In contrast, the older twig seems to be preferred when leaves are removed. The presence of leaves induced in a higher frequency of oviposition only when the twigs were young. Of the 575 eggbatches laid on *Euonymus*, 57% were laid close to leaf buds, leaves or side twigs. A large proportion of eggbatches (30%) were laid on the stem, away from buds or side branches. The remaining thirteen percent of the eggbatches were laid close to, or on other eggbatches.

Why does Y. cagnagellus *oviposit there? Choice experiments with hosts and non hosts.*

A host choice experiment was performed, giving the moths a choice between *Euonymus* (the host), and an alternative twig from the host plant of a closely related moth; either *Malus domestica* (host of *Y. malinellus*) or *Crataegus monogyna* (host of *Y. padellus*). The oviposition on twigs both kept in the same jar of water was compared to the oviposition on twigs in separate jars.

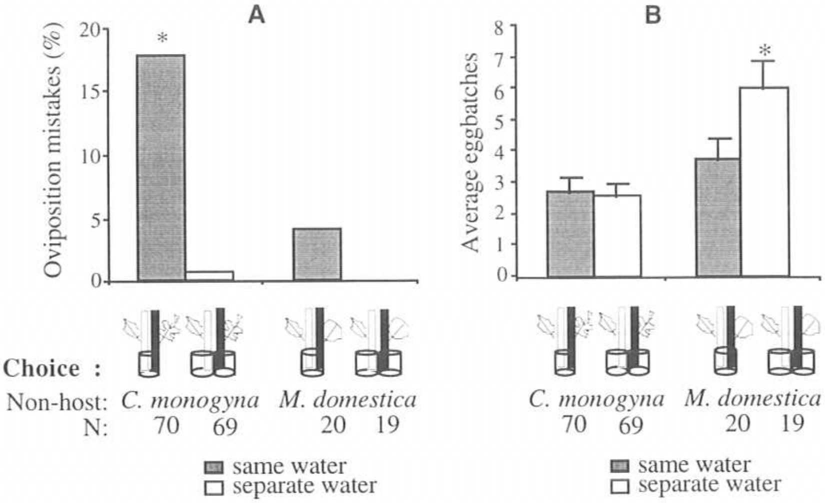

***Figure 3. a)** The percentage of oviposition mistakes on non-hosts when twigs of host and non hosts were offered in the same jar of water or in seperate jars. Wilcoxon/ Mann-Whitney $p<0.05$. **b)** The average number of eggbatches per female laid in the same experiment. (eggbatches on host and non host combined) Wilcoxon/ Mann-Whitney $p<0.05$.*

In a preliminary experiment it was found that eggbatches were laid on the non-host *Crataegus* when both twigs were placed in the same jar of water. No oviposition mistakes occurred when the twigs were in separate jars. This experiment was repeated three times - once in 1995 and twice in 1996 with *Crataegus* only. Again, oviposition mistakes were only made when the *Crataegus* twig was in the same water as *Euonymus* (figure 3a). A very small percentage of eggbatches was laid on *Malus* when it was in the same water as *Euonymus*. However, there is a significant reduction in the total number of eggbatches laid on *Euonymus* when *Malus* was in the same water, compared to when the twigs were seperate. This effect was not found for *Crataegus* (figure 3b).

There appeared to be no pattern to the timing or place of the eggbatches laid on *Crataegus*; the *Euonymus* twigs were healthy in all but two cases, and the eggbatches were not noticeably smaller than those laid on *Euonymus*. Not all of the moths laid eggbatches on *Crataegus*, but of those that did, some laid several eggbatches, while others only laid one. There was no specific time period either; eggbatches were laid on *Crataegus* as early as 3 days after mating, or as late as 17. The eggs were viable; if the egg shield was removed, the larvae were found to be alive.

DISCUSSION

This study of the oviposition behaviour of *Y. cagnagellus* aimed to find answers to the questions; When does oviposition take place? Where are the eggs laid? and Why are they laid there?

Oviposition of *Y cagnagellus* seems to occur at the beginning of the scotophase, although there was no direct evidence of a set time in the scotophase for egglaying. However, similar timing of oviposition is found in a number of moths (Hattori, 1988; Jallow & Zalucki, 1996; Peterson *et al.*, 1994).

For a monophagous insect like *Y. cagnagellus*, the female's choice of oviposition site is very important, given the limited mobility of the larvae. When ovipositing on *Euonymus*, the moths preferred young twigs with leaves or old twigs without leaves. This may represent a preference for the most suitable type of twig, as in a natural situation young twigs always have leaves, whereas older twigs do occur without leaves. This implies that the moth selects the optimal parts of the host plant for oviposition. Although much of the within-hostplant species discrimination by ovipositing butterflies is obscure, behavioural patterns observed in some Lepidoptera are highly suggestive of such discrimination. For example, the costarican pierid *Perrhybris pyrra* has been observed comparing individual leaves of an accepted shrub, before choosing the one to oviposit on (Singer, 1984).

Y. cagnagellus lays 1-10 eggbatches during her lifespan. This suggests that particular care should be taken to ensure the success of the larvae in one batch, as it constitutes at least 10 % of her offspring. This care is reflected in the preference of the moths to lay eggbatches near buds, leaves and side branches, which results in placing the emerging offspring close to a source of food, after bud flush in the following spring. The positioning of eggbatches away from the buds in our experiments, could be due to shortage of more optimal places. Alternatively, it could indicate that the larvae are able to migrate on the host, even if they are unable to move to a new host plant.

Y. cagnagellus lays eggbatches almost exclusively on her host plant *E. europaeus*. However, when the moths are in a host choice situation it appears that the oviposition behaviour can be influenced by the presence of non-host twigs in the same jar of water. *Crataegus* can be made more acceptable by the presence of *Euonymus*, whereas *Euonymus* will be less accepted in the presence of *Malus*. The most simple hypothesis to explain these results is that the behavioural change could be due to the transfer of one or more chemicals which have an affect on oviposition from one twig to the other. This hypothesis assumes that chemicals are indeed passing from twig to twig, and that the moths are able to detect them when they are assessing the twig. Twigs are known to take up chemicals, as Kooi & Van de Water (1988) impregnated twigs with sugar alcohols by standing them in a solution for 24 hours. Substances also pass out from the cut end of the twigs, as *Malus* twigs cause water and agar to turn orange.

What is the nature of the hypothetical compound which is causing *Y. cagnagellus* to accept *Crataegus* instead of *Euonymus*? It is known that moths use chemicals for host plant identification. These can be volatile compounds (e.g. Binder *et al.*, 1995) or surface phytochemicals (e.g. Derridj *et al,* 1989, Thibout & Auger, 1996). The larval phagostimulant dulcitol is a possible candidate oviposition stimulant for *Yponomeuta*. Assuming that the moths can detect it as well, dulcitol passing from *Euonymus* to *Crataegus* it could make *Crataegus* more acceptable. The reduction in eggbatches laid when *Malus* is present could be due to a deterrent, for instance phloridzin. This compound, found in *Malus*, is a feeding deterrent for many species (but not for *Y. malinellus*). An experiment was conducted to test these assumptions: In two repetitions, a total of 58 moths were offered a choice between *Crataegus* twigs in a 1% dulcitol solution and *Crataegus* twigs in a 1% mannitol (a neutral sugar alcohol) solution. Only 8 eggbatches were laid, divided over both the dulcitol- and the mannitol impregnated twigs. In contrast, 62 eggbatches were laid on *Euonymus* by the same amount of females in a control trial during the same period. Therefore, it seems that dulcitol on its own does not stimulate oviposition.

Behavioural patterns identified during observation involved the moth running up and down the twig, dragging its antennae and ovipositor over the surface. Similar behaviours have been recorded in other moth species (e.g. Hattori, 1988; Thibout & Auger, 1996). The direct observation of the moths suggest that in *Y. cagnagellus* both the antennae and ovipositor might be used in assessing the twig, but further observation of pre-oviposition behaviour is needed.

Further study should involve more detailed phytochemical work, as well as analysis of the chemoreceptors on antennae and ovipositor. Study of interspecific differences in the host discrimination of adults of different *Yponomeuta* species, will add to the knowledge of the evolution of these species. Oviposition behaviour is clearly one of the key fields to be investigated to gain a true understanding of the mechanisms involved in host shifts and the wider questions relating to speciation.

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