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Host-marking behaviour as a quantitative signal of competition in the walnut fly *Rhagoletis juglandis*

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Abstract. 1. Walnut-infesting flies in the *Rhagoletis suavis* species group actively re-use hosts for oviposition despite engaging in a genus-typical host-marking behaviour which, in other *Rhagoletis* groups, deters oviposition. In a study of the walnut fly, *R. juglandis* (Cresson), alternative hypotheses for the putative marking behaviour were evaluated.

2. The oviposition site attraction hypothesis proposes that the host mark guides females to oviposition sites on occupied fruit. The competition intensity signal hypothesis proposes that the host mark is an indicator of the level of competition to be incurred if fruit are re-used.

3. In a field cage, females were presented simultaneously with fruit previously exposed to 25 females that were also allowed to oviposit and engage in the putative marking behaviour, and control fruit on which females were allowed only to oviposit. The occurrence of host marking reduced a female's propensity to oviposit from 46% to just over 10%, consistent with the competition intensity signal hypothesis only.

4. In a laboratory assay, the duration of host marking was correlated positively with the size of a female's clutch. This result, also consistent with the competition intensity signal hypothesis, suggests that the amount of marking pheromone on a fruit is a reliable indicator of the number of eggs already deposited within.

5. In a second field-cage experiment, females were allowed to mark on fruit for 0, 10, 20, or 30 min and fruit were presented to test females. Whether or not females alighted on a particular host was not affected by the duration of marking; however, the frequency of both ovipositor probing and egg deposition decreased with increasing duration of marking. Consistent with the competition intensity signal hypothesis, this result suggests that the host mark permits females to assess the level of competition that a clutch will incur within re-used fruit.

Key words. Marking pheromone, oviposition deterrent, *Rhagoletis juglandis*, superparasitism, Tephritidae, walnut flies.

Introduction

The decisions that insects make about where their offspring will develop are especially important for species with larval stages that are restricted to a particular environment or host (Thompson, 1983; Smith & Lessells, 1985; Roitberg & Prokopy, 1987; Bernays & Chapman, 1994). Because insect larvae that develop within discrete hosts (e.g. flower buds, seeds, small fruit, stems, or other insects) may not be able to acquire more resources if natal hosts become depleted, larval development can be affected very strongly by the level of competition for food resources. Given that brood competition can reduce offspring survival, size, and reproductive potential and increase the time required to reach maturity (reviewed by Peters & Barbosa, 1977; Fox *et al.*, 1996; Blanckenhorn, 1998; Sweeney & Quiring, 1998;

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Allen & Hunt, 2001), it is not surprising that females in many systems have evolved mechanisms for avoiding use of previously exploited hosts (reviewed by Nufio & Papaj, 2001).

In order to reduce the competition for larval resources that their offspring may face, females of a variety of species reject previously exploited hosts on the basis of visual or tactile stimuli associated with eggs (Rausher, 1979; Shapiro, 1981; Williams & Gilbert, 1981; Takasu & Hirose, 1988) or larvae (Mappes & Mäkelä, 1993; reviewed by Nufio & Papaj, 2001). In addition to cues produced incidentally by the presence of conspecifics, a variety of entomophagous and phytophagous insects deploy signals of conspecific presence that are produced actively by the sender female. In almost all cases, the signal consists of a chemical termed a marking pheromone that has been deposited on or in the host; this marking pheromone almost always has a deterrent effect on oviposition (reviewed by Roitberg & Prokopy, 1987; Hofsvang, 1990; Landolt & Averill, 1999; Nufio & Papaj, 2001). In the apple maggot fly *Rhagoletis pomonella* (Walsh), for example, larval competition is intense as hawthorn berries typically yield no more than one or a few pupae, even if considerably more eggs are deposited into the fruit (Averill & Prokopy, 1987a; Feder *et al*., 1995). In *R. pomonella* the deposition of a marking pheromone reduces larval competition by causing females to distribute their clutches uniformly over host fruit (Prokopy, 1972; Averill & Prokopy, 1989). To date, 13 *Rhagoletis* species have been found to deposit a deterrent marking pheromone following egg deposition (reviewed by Landolt & Averill, 1999).

Members of the *Rhagoletis suavis* group, the so-called walnut flies, differ from members of other *Rhagoletis* species groups in that they show an oviposition preference for egg-infested fruit. Not only do females deposit a significant number of eggs into previously egg-infested fruit, they also commonly deposit eggs directly into previously established oviposition cavities formed within the walnut husks (Papaj, 1993, 1994; Lalonde & Mangel, 1994; Nufio *et al*., 2000). In *Rhagoletis juglandis*, the focus of the work reported here, re-use of egg-infested fruit is common despite the fact that females engage in what appears to be the genus-typical host-marking behaviour (Papaj, 1994; Nufio *et al*., 2000). This behaviour involves dragging the ovipositor on the surface of the fruit following the deposition of a clutch.

In walnut flies, the reuse of oviposition cavities, in particular, apparently provides females with direct benefits such as reduced ovipositor wear (Papaj, 1993), reduced time to deposit clutches (Papaj & Alonso-Pimentel, 1997), and increased access to less penetrable fruit (Lalonde & Mangel, 1994); however, the existence of such benefits begs the question of why *R. juglandis* females appear to mark fruit in a manner almost identical to that observed in *Rhagoletis* species that strongly avoid re-use of occupied fruit. The potential effectiveness and function of the marking behaviour is further complicated by the fact that, although they are all specialists on walnuts, in the lab, other members of the *R. suavis* group either do not engage in ovipositor dragging following clutch deposition (*R. suavis*), ovipositor drag infrequently following clutch deposition (*R. boycei*), or ovipositor drag for short bouts directly around previously made oviposition sites (*R. completa*) (C. R. Nufio, unpubl. data). The latter marking behaviour is different than that of *R. juglandis* in that *R. juglandis* circles the whole host fruit as it ovipositor drags.

The following two hypotheses for the occurrence of this behaviour in *R. juglandis* are put forward. First, a host mark might serve to guide females to existing oviposition cavities. If re-use of oviposition cavities confers direct benefits on females, perhaps a female might benefit from marking such sites, if the mark facilitated later use of that site by that female or its kin or if progeny benefit from being placed in the company of conspecific larvae by being better able to overcome host associated toxins. In either case, the site attraction hypothesis predicts that females would be attracted to or arrested on fruit that bear the putative mark.

Second, while walnut flies may benefit from reusing larval hosts by being able to deposit more eggs in their life time and by being able to maximise the utilisation of available hosts (Nufio *et al*., 2000), it is conceivable that the mark signals a cost associated with re-use, specifically a cost in terms of losses in larval fitness associated with competition. Based on what might be termed the competition intensity signal hypothesis, a female's propensity to re-use a previously exploited host is expected to decline with increasing levels of a marking pheromone, which should be correlated with numbers of eggs in the fruit. This expectation leads in turn to three predictions that are tested here. (1) The effect of the marking behaviour should generally be to deter oviposition. (2) Females should deposit an amount of mark that is proportional to the size of their clutch. (3) Females should be deterred by the mark in proportion to the amount of mark deposited.

Materials and methods

Natural history

Rhagoletis juglandis is a member of the walnut-infesting *Rhagoletis suavis* group (Bush, 1966). In southern Arizona, U.S.A., this species is found on the Arizona walnut *Juglans major* (Torrey), which occurs in montane canyons between 1200 and 2700 m. These flies are univoltine and females deposit clutches of 16 eggs (Nufio *et al*., 2000) after piercing the fruit surface with their ovipositor and hollowing out a small cavity in the walnut husk. The larval stages feed on the husk of a single fruit, after which they emerge and pupate in the soil beneath the natal tree. Pupae diapause through the winter and spring, and adults emerge during mid to late summer of the subsequent year.

General protocol

Adult female flies used in the laboratory and field cage experiments originated as larvae from fruit collected

1–2 years prior to their emergence in the laboratory from Garden Canyon in the Huachuca Mountains in southern Arizona (31°21.7′N, 110°20.4′W). Upon emergence, flies were reared in 3.79-l plastic containers and provided with *ad libitum* water, sugar, and slips of a yeast hydrolysate and sugar mixture. Flies were stored in a room with a LD 14:10 h cycle and a day/night temperature of 32–28 °C. Fruit used in all tests were ripe Arizona walnut *Juglans major* fruit and were collected several days prior to their use from several localities in Pima and Cochise counties, Arizona.

Experiment 1: female responses to marked fruit

In this field cage experiment, attempts were made to determine whether the walnut fly's ovipositor dragging behaviour, which accompanies clutch deposition, might be consistent with the placement of a chemical marker on the host surface. More specifically, whether a gravid female's propensity to oviposit into a host was enhanced, reduced, or unaffected by whether it encountered a previously *marked* host relative to when it encountered an *unmarked* host.

To generate marked and unmarked hosts, fruit (30–35 mm diameter) were paired for size then assigned randomly to a *dragged on* or *not dragged on* treatment respectively. Fruit in the not dragged on treatment were wrapped in Parafilm©, whereas those in the dragged on treatment were not. Females could oviposit readily into the Parafilm©-wrapped fruit; however, during ovipositor dragging after oviposition, the ovipositor could not make contact with the fruit surface. Experiments on related fruit fly species have indicated that such treatment prevents deposition of marking pheromone on a fruit surface (Papaj *et al.*, 1989). Both members of a replicate pair contained five punctures made with a #00 insect pin and spaced haphazardly over the fruit surface. The punctures were intended to stimulate and facilitate egg deposition especially for the fruit wrapped in Parafilm©. Fruit in both treatments was hung within separate 3.79-l plastic containers that contained *ad libitum* water, sugar, and slips of a yeast hydrolysate and sugar mixture. Twenty-five medium to large flies were placed into each cage for 48 h and allowed to deposit clutches. After 48 h, fruit were removed from the cages, not dragged on treatment fruit were unwrapped and both fruit types were marked below with a black permanent marker for easy identification. Fruit were gently placed into separate egg cartons and transported to the field cages were they would immediately be used. Six fruit (three of each treatment) were distributed uniformly among the branches of the test trees in the field cage. Probing attempts and initiation of oviposition by individually marked females on fruit of each treatment were recorded (see *Behavioural observations* below). This experiment was terminated after 54 females had alighted either on the control or experimental fruit and this was accomplished after 8 test days.

Experiment 2: relationship between clutch size and time spent engaged in a putative host-marking behaviour

In this experiment, attempts were made to determine whether a female's putative host-marking behaviour carries information about the size of the female's clutch and two factors, egg load and female size, that may be confounded with clutch size. Previous studies on congeners indicated that ovipositor dragging is a reasonable predictor of the amount of marking pheromone deposited on a fruit (Averill & Prokopy, 1980). Fruit used in this experiment was 26–34 mm in diameter. Flies used in laboratory experiments were 12–30 days, post-eclosion. Groups of 10–15 mature females were placed within single clear 473-ml plastic cups, fitted with Petri dish lids, in which they were provided with *ad libitum* water, sugar, and a yeast hydrolysate and sugar mixture. A walnut fruit was hung from the top of each of the cages and females were permitted to oviposit. After a female initiated oviposition, the fruit on which the fly oviposited was removed from the cup. Any other females present on that fruit were removed by aspiration. The fruit was then hung from the top of an empty cup cage and the female was allowed to complete oviposition. After oviposition was completed, the length of time(s) that a female dragged its ovipositor over the walnut surface was recorded. Female walnuts flies typically ovipositor-drag for several seconds to minutes, then stop and often groom or walk about, then resume ovipositor-dragging behaviour. In order to minimise the chances that an ovipositor-dragging session was ended prematurely, in the experiments where marking duration were measured, the session was ended only if a female flew from the host on which it was ovipositor-dragging and spent 15 consecutive minutes off the fruit. Total duration of marking did not include pauses that occurred between dragging bouts, whether those pauses occurred on or off the fruit.

After an observation session for a particular female was terminated, the female was placed into a labelled 1.5-ml plastic snap-cap vial and frozen at −4 °C. After the experiment was completed, all females were dissected in saline under a stereoscope and the number of mature eggs remaining in their ovaries was counted. With the use of an ocular micrometer, female size was estimated by measuring the length of the medial vein bordering the anterior portion of the discal medial cell. This wing measure was used as an estimate of female size because previous laboratory experiments showed that it was strongly correlated with other indicators of female size such as thorax size, head width, and femur length (A. Lachman, unpubl. data) and because of the relative ease of using the wing measure as opposed to other indicators. A female's egg load at time of testing was estimated as the sum of the number of eggs deposited into the fruit and the number of mature eggs remaining in its ovaries.

Experiment 3: oviposition responses to fruit marked for variable periods of time

Generating fruit in dragging duration treatments. In this field cage experiment, the responses of gravid females that encountered walnut hosts on which ovipositor-dragging behaviour had occurred for 0, 10, 20, or 30 min was measured. Fruit used in this field cage study was 30–35 mm in diameter. Fruit in the four dragging duration treatments were generated as follows. Four walnuts, similar in size and ripeness, were assigned randomly to be marked for a particular duration (0, 10, 20, or 30 min). Fruit were then hung from the tops of 473 ml clear, plastic cup cages containing 10–15 gravid females (12–30 days post-eclosion). Females were permitted to deposit clutches into the fruit and then to engage in ovipositor-dragging behaviour. Variation in clutches and number of cavities was controlled for by having all fruit (even the fruit assigned to be in the unmarked fruit treatment) receive three clutches deposited into the same oviposition cavity. To ensure that clutches were placed into the same cavity, gravid females were brushed gently towards previously created cavities. After one or several attempts at new sites, females usually encountered the previously made oviposition site and re-used it.

For fruit assigned to the 0 min dragging treatment, females were removed immediately after deposition of a clutch before any ovipositor dragging behaviour commenced. For fruit assigned to the remaining treatments, following an oviposition event females were permitted to drag their ovipositors over the fruit surface until they stopped or until 10, 20, or 30 min of aggregate marking duration on the fruit had been achieved. If the total duration of ovipositor-dragging behaviour by the first female did not reach the criterion for the treatment to which the fruit was assigned, the fruit was returned to a cup cage of females and the above cycle was repeated. This procedure continued either until the dragging duration criterion was reached or until a fruit had received three clutches within the same cavity. Sometimes, the aggregate dragging duration on a fruit reached the criterion before three clutches were deposited. In that case, females were permitted to add clutches to the existing cavity but were not permitted to ovipositor-drag after completion of the clutch.

Usually, fruit that required 10, 20, and especially 30 min of marking required more than three females (and hence more than three clutches) to mark the fruit. In order to reach the ovipositor-dragging duration criterion, females were allowed to deposit clutches into non-treatment fruit, than they were placed onto treatment fruit on which they were allowed to engage in ovipositor-dragging behaviour. This was done as follows. After these females finished ovipositing into non-treatment fruit, they were gently coaxed onto a thin filter paper tip placed at the end of a 15.2 cm long wooden dowel that had been dipped into a sucrose solution. These females were then placed onto fruit that had reached the three clutch maxima but still required females to drag ovipositors on the fruit to reach the time criterion. Females transferred in this way readily engaged in ovipositor-dragging behaviour on the new host (e.g. Prokopy *et al.*, 1982). Once the criterion dragging duration was reached on a given fruit, females were aspirated from the fruit.

After two replicates of the four treatments had been generated on a single day (for a total of 18 replicates in the study), fruit were marked below with a black marker for easy identification and hung overnight in a refrigerator to preserve the efficacy of a putative mark. The fruit treatments were used in experiments the following morning (see *Behavioural observations* below). In order to minimise cross-contamination of the treatments during transportation, fruit from the two replicates were hung within two separate clear 3.79-l plastic containers. These fruit were spaced apart in such a way as to prevent them from physically contacting each other during transport. To prevent researchers from contaminating the treatments throughout the experiment, fruit were only handled by the metal wires that were tied to their stems. It was also assumed that as in other tephritid fruit flies, any putative marking pheromone would act primarily as a contact pheromone that would be perceived by tarsal chemoreception (Crnjar & Prokopy, 1982).

Behavioural observations

Females used in experiments 1 and 3 were treated as follows. Once a cohort of females was 12–25 days old, the females were chilled at 4 °C for 7–10 min and marked individually by placing two dots of non-toxic tempura paint on their thorax. By using four colours in any combination, test females were assigned unique marks. After females were marked, they were placed into a separate 3.79-l plastic container containing *ad libitum* water, sugar, and slips of a yeast hydrolysate and sugar mixture, as well as two ripe walnuts. Females were allowed to deposit clutches over a 24-h period, in order to reduce egg load and provide them with egg-laying experience. This procedure was designed to heighten their sensitivity to host-marking pheromone because, in other species, egg load (van Randen & Roitberg, 1996) and previous experience (Roitberg *et al.*, 1993; Potting *et al.*, 1997) has been found to affect a female's responses to marked hosts.

After being exposed to a walnut host for 24 h, 30–40 females were released into a 3 m wide × 3 m tall cylindrical, nylon-screen field cage erected within an evaporator-cooled greenhouse at the University of Arizona Agriculture Experimental Station in Tucson, and allowed to acclimate for 24–48 h before the experiment commenced. During this time, females had access to slips of a yeast hydrolysate and sugar mixture, and small water bottles with extended cotton wicks distributed among the branches of two 2–3 m high potted walnut trees placed close to one another inside the cage.

On the mornings of the test, treatment fruit were removed from the refrigerator and allowed to warm for 90 min before use in field cage tests. Fruit were then distributed uniformly among several branches. To control for position effects, fruit were rotated over positions within

the trees every 45 min. Behavioural observations typically began at 09.00 hours and ended anywhere between 12.00 and 15.30 hours, depending on female activity levels.

After distributing treatment fruit, a single observer that moved about the field cage and could see all treatments from several vantage points recorded the data. This observer recorded the first fruit on which a particular female alighted and whether the female probed the fruit with her ovipositor or initiated oviposition into the fruit, or rejected the host fruit. Probing consisted of a female curling its abdomen and poking the fruit firmly with the end of its ovipositor. The initiation of an egg-laying or oviposition event was noted when a female placed its ovipositor into an oviposition cavity constructed previously by females or less often when a female initiated the construction of her own oviposition cavity into the fruit. Creation of an oviposition cavity occurred when a female inserted its ovipositor into the walnut husk and then pivoted repeatedly around the ovipositor. At the point at which a test female stopped pivoting and became quiescent, it was removed immediately from the fruit in order to prevent it from depositing eggs into the fruit. During the experiment, greenhouse temperature and humidity ranged from 22 to 27 °C and 40% to 50% respectively. Because of the amount of time required to set up each of the dragging duration treatments, replicate fruit were typically used for two consecutive observation periods. All statistical analyses were conducted in JMP version 4 (SAS, 2000).

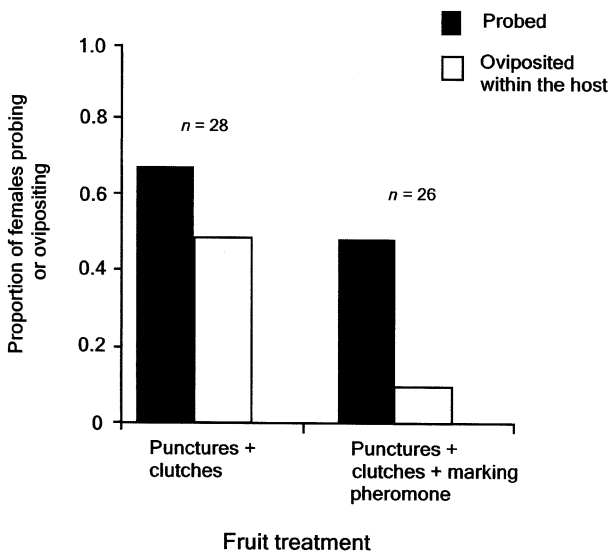

Fig. 1. The proportion of females that probed the fruit surface with their ovipositors and the proportion of these females that initiated egg laying into a fruit in relation to the number of females that alighted on each fruit. *n* – the number of females alighting on a given treatment fruit.

Results

Experiment 1: female responses to marked fruit

Females alighted on all models with more or less equal frequency (Fig. 1), which is not consistent with the site attraction hypothesis. Females that alighted on a fruit typically walked along the fruit surface and commonly made contact with the fruit surface with their mouthparts. Such behaviour commonly preceded any probing and oviposition attempts. The tendency of a female that landed on a fruit to probe the fruit was, however, not greater on marked fruit verses unmarked fruit as predicted by the site attraction hypothesis ($\chi^2_1 = 0.09$, $P =$ NS).

Females attempted oviposition significantly less often into fruit potentially bearing a mark than on unmarked fruit ($\chi^2_1 = 11.47$, $P < 0.001$). The presence of a mark reduced a test female's propensity to oviposit from nearly 46% to 10%. This result is thus consistent with the competition intensity hypothesis as that hypothesis predicts that females will be deterred by the putative mark.

Experiment 2: effects of clutch size on time spent engaged in a putative marking behaviour

In this experiment, whether ovipositor dragging duration was related to a female's clutch size, as predicted by the competition intensity hypothesis, was measured. Body size (estimated from wing vein length) and egg load (calculated as the sum of the eggs deposited into a fruit and the number of mature eggs remaining in a female's ovaries), two factors which might be confounded with clutch size were also measured. Linear regression analyses indicated that deposited clutch size was related significantly to egg load ($r^2 = 0.26$, $F_{1,37} = 12.34$, $P = 0.001$) but not to body size ($r^2 = 0.004$, $F_{1,37} = 0.15$, $P =$ NS). In this experiment, larger females had marginally higher but not statistically significantly higher egg loads than smaller females ($r^2 = 0.10$, $F_{1,37} = 4.19$, $P = 0.052$). On average, female egg loads consisted of 31 mature eggs ($\pm$SE 2.2, $n = 38$) and females deposited 33% of the eggs they carried.

All females in this experiment dragged their ovipositors along the fruit surface following clutch deposition. A multiple regression analysis including clutch size, egg load, and female size as independent variables, and marking duration as the dependent variable, showed that these variables as a group significantly explained female marking duration [$F_{3,34} = 4.59$, $P = 0.008$; marking time (s) $= 201.7 + 31.6 \times$ (clutch size) $- 5.0 \times$ (egg load) $- 57.6 \times$ (female size)]. However, when controlling for the other two independent variables, the estimate of host-marking duration was significantly improved by adding clutch size ($F_{1,34} = 13.42$, $P = 0.001$) but neither egg load ($F_{1,34} = 3.89$, $P =$ NS) nor female size ($F_{1,35} = 0.07$, $P =$ NS) significantly improved the overall model. By itself, deposited clutch size significantly explained marking duration ($r^2 = 0.17$, $F_{1,53} = 10.33$, $P = 0.002$; Fig. 2) and marking duration commonly exceeded 5 min [mean $\pm$ SE $= 285.5\,\text{s} \pm 25.3\,\text{s}$ ($n = 54$), range 22–734 s].

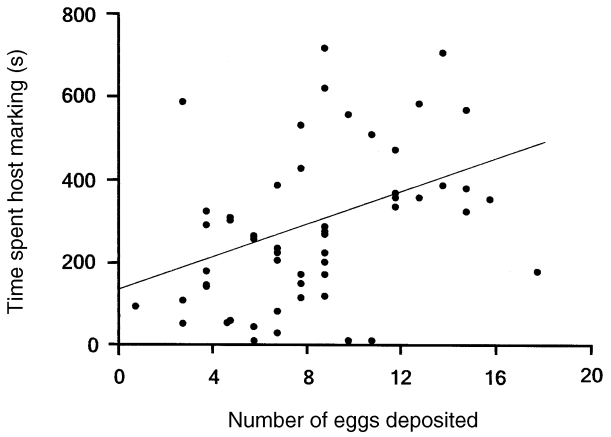

Fig. 2. The relationship between clutch size and duration of ovipositor-dragging behaviour.

Experiment 3: oviposition responses to fruit on which females ovipositor-dragged for 0, 10, 20, or 30 min

In this field cage experiment, attempts were made to determine whether oviposition responses to marked fruit declined with the aggregate time previous females spent engaged in the putative host-marking behaviour. Females were found to alight on all models with more or less equal frequency (Fig. 3). Once having landed, however, the tendency of a female to probe the fruit with its ovipositor decreased as the aggregate duration of ovipositor-dragging behaviour increased (Fig. 3). A logistic regression relating propensity to probe a fruit (yes or no) to the amount of time that females ovipositor-dragged on a host (0, 10, 20, 30 min), suggested a potential effect of dragging duration on probing propensity ($\chi^2 = 5.35$, d.f. $= 1$, $P < 0.03$; critical alpha set at 0.025 due to the number of non-independent comparisons).

The effect of ovipositor-dragging duration on oviposition was due at least in part to an effect on the propensity of a female to initiate probing events before leaving the fruit (Fig. 3). Actual oviposition events declined as host-marking duration increased. A logistic regression relating propensity to probe the fruit (yes or no) to the amount of that time females were allowed to mark a particular host (0, 10, 20, 30 min) showed the effect of dragging duration on oviposition to be significant ($\chi^2 = 7.82$, d.f. $= 1$, $P = 0.005$). The resulting logistic regression model estimated that every minute of ovipositor dragging on a given fruit reduced a female's propensity to initiate probing into that fruit by 4.1% ($\pm$ 1.5%).

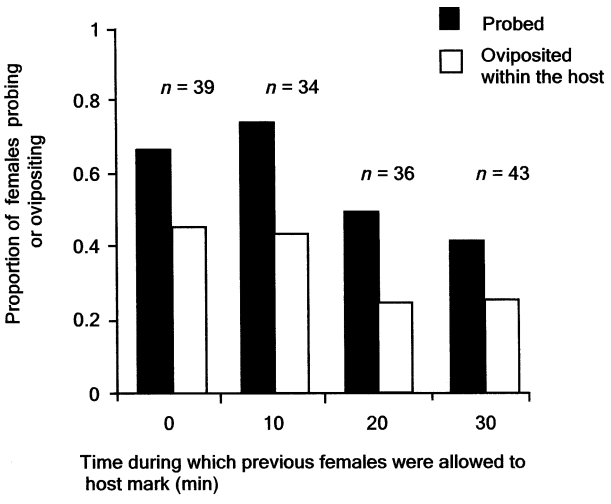

Fig. 3. The proportion of females that probed the fruit surface with their ovipositors and the proportion of these females that initiated egg-laying into a fruit in relation to the number of females that alighted on each fruit. n – the number of females alighting on a given treatment fruit.

Discussion

Evidence for host marking and oviposition deterrence

Of the two hypotheses presented above, the results provide support only for the competition intensity signal hypothesis. More specifically, ovipositor-dragging behaviour in *R. juglandis* was found to be a bona fide host-marking behaviour, with the mark deterring oviposition in relation to the number of eggs deposited into a fruit (Figs 2 and 3). In this respect, both the form of the host-marking behaviour and the deterrent consequences of that behaviour are similar to those described for numerous other *Rhagoletis* species (reviewed by Landolt & Averill, 1999; Prokopy & Papaj, 1999). Noteworthy about the current finding is the fact that this pattern of host-marking behaviour and response to the mark occurs despite the fact that walnut flies in general actively re-use fruit and even reuse existing oviposition cavities (Papaj, 1993, 1994; Lalonde & Mangel, 1994).

Nufio and Papaj (2001) identified a number of lines of evidence commonly used to document the existence of a marking pheromone. This study gathered some but not all of these lines of evidence. This study, for example, identified a behaviour pattern that is logically consistent with the deposition of a chemical mark. While attempts were made to rule out the effects of other potential stimuli, such as the eggs themselves or recent fruit damage associated with oviposition (by having each treatment contain an equal number of clutches and punctures), it still remains to be shown that a chemical is deposited on the fruit during ovipositor-dragging behaviour. For example, this study did not use extracts of the marking pheromone to show that, when applied to uninfested fruit, such extracts deter oviposition. Such information is important to obtain; it would rule out, for example, the possibility that ovipositor-dragging behaviour does not involve deposition of a chemical but rather generation of some kind of damage to the fruit surface.

In the case of a species such as *R. juglandis*, such an alternative seems highly unlikely. Abundant evidence gathered for other, relatively closely related *Rhagoletis*

species (reviewed by Landolt & Averill, 1999) indicates that deposition of a mark is involved in a behaviour very similar to that observed in *R. juglandis*. While the marking pheromone of *R. pomonella* appears to last for at least a week in the field (Averill & Prokopy, 1987b), such information is lacking in most tephritid systems and should be addressed in future studies.

Marking pheromone as an indicator of brood numbers

Walnut flies differ from other *Rhagoletis* species in which host marking has been studied in that eggs are laid in batches rather than singly. The tendency for females to lay eggs in batches suggests a possible function of a marking pheromone, namely to convey information about the number of eggs deposited into fruit. In this study, evidence that a female's mark may encode information about clutch size was obtained. Specifically, the amount of time that a *R. juglandis* female spends dragging its ovipositor on the fruit surface is proportional to the size of the clutch that it has just laid (Fig. 2). Studies on related *Rhagoletis* species suggest that the duration of ovipositor-dragging behaviour is a reliable indication of the amount of marking pheromone deposited on the fruit surface (Averill & Prokopy, 1980). If this also applies to host marking in *R. juglandis*, it follows that the amount of marking pheromone deposited by a *R. juglandis* female is correlated positively with the size of the clutch. Moreover, results of the field-cage experiment in which females were presented with fruit varying in the aggregate duration of host marking suggest that females are indeed sensitive to variation in the amount of marking pheromone found on a fruit. In short, *R. juglandis* appears potentially able to signal not only the presence of eggs in a fruit but also their number.

Duration of host marking may, however, be an imperfect indicator of the amount of marking pheromone on a fruit. Congruence between duration of marking and the amount of marking pheromone placed on a host may be affected by variation in the speed at which females dragged their ovipositors on the fruit surface, the width of the deposited trail, the consistency in the amount of marking pheromone deposited along a trail, and female diet (Hendry, 1976; Averill & Prokopy, 1988). These factors could account in part for the considerable scatter in the relationship between duration of host marking and clutch size (Fig. 2).

It is thought that there is only one other insect for which a possible relationship between clutch size and duration of marking has been suggested. A correlation between marking time and clutch size deposited within a host was inferred for the gregarious egg parasitoid *Telenomus fariai* (Lima) (Bosque & Rabinovich, 1979). In this study, however, clutch size was not measured directly but inferred from the number of progeny emerging from a host. These authors also did not examine how the amount of time a female spent dragging on the egg surface affected the behaviour of other females that were exposed to such hosts. In a few other cases in which females make a graded assessment of level of infestation within a host, the underlying mechanism has not been conclusively established (Bakker *et al*., 1972, 1990; van Lenteren & Debach, 1981; van Dijken & Waage, 1987).

Is the potential to encode and transmit information about clutch size realised during signalling by *R. juglandis* under field conditions? Re-use in the field occurs to such an extent that such information would be useful to females. Because females actively re-use hosts in the field, after 4–5 days a fruit may contain 45 eggs (1 clutch = 16 eggs), and it is not unusual to find fruit into which females deposited 80 or more eggs (Nufio *et al*., 2000; C.R. Nufio & D.R. Papaj, unpubl. data). Host re-use, however, appears to have negative consequences from the perspective of any given larva, decreasing larval survival and reducing the weight at which offspring will pupate (C.R. Nufio & D.R. Papaj, unpubl. data). In laboratory studies, this reduction in pupal weight translates to a reduction in lifetime female fecundity (C.R. Nufio & D.R. Papaj, unpubl. data).While there are apparent costs to larvae forced to compete with conspecifics, re-using hosts may also initially be associated with direct and indirect benefits that increase the number of clutches females may deposit over a lifetime (Papaj, 1993, 1994; Lalonde & Mangel, 1994; Papaj & Alonso-Pimentel, 1997). Ecological factors, such as the size of walnut hosts relative to those used by other flies in the genus, and the temporal and spatial ephemerality of hosts in the field may also influence the degree to which females to re-use hosts (Nufio *et al*., 2000). Given the shifting benefits and costs associated with re-using a host, females would seem to benefit from a sensitivity to the accumulating costs associated with re-using a host, a sensitivity that was detected in the field cage assays. In the field, there also appears to be a relationship between fruit size and infestation levels, a pattern that suggests that females are allocating clutches in a way that minimises competition (Nufio *et al*., 2000; C.R. Nufio & D.R. Papaj, unpubl. data). What remains to be demonstrated is whether a sensitivity to the presence of a marking pheromone is directly influencing clutch distribution patterns under field conditions.

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