

Ovarian dynamics in relation to host quality in the Walnut-infesting Fly, *Rhagoletis juglandis*

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Summary

1. Reproductive behaviour is routinely studied with a view towards characterizing how an animal responds to variation in resource abundance and quality. This characterization is less commonly made with respect to reproductive physiology.

2. In the Walnut-infesting Fly, *Rhagoletis juglandis*, ovarian development is cued by the presence of the host fruit resource. In this study, I examined how ovarian development was affected by two host characteristics that relate to competition in the juvenile stages: fruit size and presence of conspecific larvae.

3. Large fruit promoted egg maturation more than small fruit, and uninfested fruit promoted maturation more than larval-infested fruit. Both effects were reproduced with artificial models of fruit.

4. The functional significance of these effects and ramifications for individual-level dynamics in oviposition behaviour are discussed.

Key-words: competition, host preference, oogenesis, Tephritidae

Functional Ecology (2005) **19**, 396–404

doi: 10.1111/j.1365-2435.2005.00993.x

Introduction

Behavioural ecologists routinely assess how an animal's behaviour responds to spatial and temporal variation in the quality and abundance of essential resources. Such analyses are less commonly made for physiological traits, even when traits have a strong impact on behaviour. For instance, whereas the manner in which egg-laying behaviour responds to variation in the quality and abundance of resources has long been a subject of keen interest, the influence of such variation on ovarian development has been much less studied. Nevertheless, in both vertebrates and invertebrates, resource stimuli modulate ovarian development much as they modulate egg-laying behaviour (Ligon 1978; reviewed by Papaj 2000). There is limited evidence that variation in resource quality or availability is associated with variation in ovarian development (Hopkins & Ekblom 1999).

Particularly poorly studied is how ovarian development responds to features of the resource that pertain to competition experienced by offspring. In insects that use host plants or parts of plants, for example, oviposition behaviour is highly sensitive to host size and presence of conspecifics (Nufio & Papaj 2001); yet if and how ovarian processes respond to such factors is essentially not known. The main objective of this study

therefore was to manipulate factors pertinent to competition among juveniles, and known to influence oviposition behaviour, and to determine how they influence ovarian development.

Flies in the Family Tephritidae are ideal for this evaluation because functional aspects of egg-laying behaviour in relation to larval competition have been well studied (Roitberg *et al.* 1984; Averill & Prokopy 1987a,b, 1989). The walnut-infesting tephritid fly, *Rhagoletis juglandis*, is appropriate for this study in several ways. First, as in other tephritids, females behave in a number of ways so as to minimize competition suffered by their young (Henneman & Papaj 1999; Nufio *et al.* 2000; Nufio & Papaj 2004a). Females prefer to lay eggs in large and/or ripe fruit when given the opportunity (Nufio *et al.* 2000; D. Papaj, unpublished observation). Females deposit a host-marking pheromone (HMP) on a fruit after oviposition that tends to deter egg-laying by females later visiting the fruit (Nufio & Papaj 2004a). Additionally, *R. juglandis* females, like females in other tephritid species, strongly avoid fruit infested by larvae in advanced stages, being deterred at least in part by visual characteristics of infested fruit (Henneman & Papaj 1999).

Second, host fruit has a strongly stimulating influence on egg maturation by *R. juglandis* females in the first ovarian cycle (Alonso-Pimentel *et al.* 1998; Lachmann & Papaj 2001). Females exposed to fruit mature more eggs and mature them faster in the first ovarian cycle than females deprived of fruit. Enhancement

of oogenesis is not a simple consequence of feeding on the fruit itself because the effect can be reproduced with artificial fruit models lacking nutritional value (Alonso-Pimentel *et al.* 1998).

Host cuing of ovarian development in *R. juglandis* presumably reflects a trade-off between survival and/or dispersal, on one hand, and production and maintenance of mature oocytes, on the other (Papaj 2000). A female that emerges beneath a tree bearing no fruit must find one bearing fruit. If making and maintaining eggs reduces the probability that a female will find a fruit-bearing tree, either by hindering dispersal or otherwise increasing chances of dying before fruit are found, the female may benefit by delaying egg maturation.

In this study, I sought to determine if the decision to delay egg maturation applies not only to the presence vs absence of fruit in the natal tree, but to the quality of those fruit for juvenile fitness. I evaluated two factors pertinent to the level of competition that a female's young might experience: the size of a fruit, and occupation by conspecific larvae. I predicted that patterns in facilitation of ovarian development would correspond roughly to patterns in oviposition preference: specifically, ovarian development would be facilitated more by large fruit than small fruit, and more by uninfested fruit than fruit infested by larvae in advanced stages.

Methods

NATURAL HISTORY

In southern Arizona, *R. juglandis* uses Arizona Walnut, *Juglans major*, exclusively as its host. There is a single generation per year. Flies eclose between July and September, depending on elevation, from puparia in the soil beneath their natal tree. First matings take place in the foliage, but males move within a week of emergence to fruit where they establish territories and defend them from other males. Soon after, gravid females begin arriving and ovipositing in fruit, and multiple mating on fruit is common (Alonso-Pimentel & Papaj 1996). Like males in other walnut-infesting members of the genus, *R. juglandis* males guard oviposition punctures and females show a strong propensity to lay eggs within previously established oviposition cavities (Papaj 1993, 1994; Lalonde & Mangel 1994). After oviposition, females deposit an HMP that deters oviposition (Nufio & Papaj 2004a). Eggs hatch within 4 days, and larvae develop over the course of 2 weeks. Given timing of female emergence, egg deposition and egg hatch, some females could be expected to emerge within trees bearing fruit containing eggs or larvae. Larvae are constrained to develop within the fruit in which they were deposited as eggs. Development is usually completed after the rotting fruit fall to the ground, with larvae leaving the fruit to form puparia in the soil beneath the natal tree. Puparia enter an obligate diapause until the following year.

GENERAL METHODS

Flies used in this study originated from larval-infested fruit from a variety of sites in southern Arizona in the previous year. Pupae were harvested from these fruit, stored at 4 °C for at least 4 months to break diapause, and warmed to room temperature (*c.* 29 °C) approximately 5 weeks before eclosion.

Unmated females within 2 days of eclosion were placed into clear plastic treatment cups (12 cm high, 10 cm in diameter at top, tapering to 6 cm at bottom; Solo brand) containing *ad libitum* water, yeast extract and sugar cubes. Between 10 and 20 females were placed in a given cup, the exact number being uniform among treatments and among replicate cups within a given experiment, but varying across experiments according to fly availability. All cups were surrounded by white cardboard barriers to control for effects of extraneous visual stimuli. Flies were held in cups for 8 days, chosen because previous experiments (Alonso-Pimentel *et al.* 1998) indicated that this period is long enough to observe an effect of host fruit on egg maturation, if present, but short enough to ensure that females lay few, if any, eggs. Several times over the 8-day period, the positions of the cups were switched haphazardly to control for spatial differences in light or temperature.

Mortality was consistently light (<10%) and not obviously biased towards particular treatments. After 8 days, all surviving flies were frozen at -10 °C. Under stereoscopy, I dissected females and counted the number of oocytes contained in the ovaries. In counts, I used a follicle staging system of vitellogenesis developed for this species by Lachmann & Papaj (2001). This system recognizes five stages distinguished by degree of yolk deposition. In this study, I counted oocytes in the last two stages, Stages D and E. Stage E oocytes are chorionated and fully yolked, and ready to be laid; Stage D oocytes are unchorionated and more than 50% filled with yolk granules, and will ordinarily progress to Stage E within hours. I did not count oocytes in Stages A–C. Stage A oocytes lack yolk altogether and are too small to census reliably. Oocytes in Stages B and C contain yolk, but the stages are so ephemeral that such oocytes are rarely observed.

I additionally measured body size in all females because numbers of oocytes in many insects, including this species, are positively correlated with body size (Alonso-Pimentel *et al.* 1998). Wing vein measurement was recorded as a proxy for size, correlating well with total wet weight, thorax width and other body size measures (H. Alonso-Pimentel & D. Papaj, unpublished observation).

Data were analysed using general linear models with JMP statistical exploration software (SAS Inc., Cary, NC), separately analysing patterns in number of mature oocytes (Stage E) and total number of yolked oocytes (Stages D and E). I nested individual female data within replicate treatment cups. I entered female wing vein length as a variable into the model, to control for

chance variation in female size between treatments; as in many insects (Papaj 2000), number of mature oocytes (often termed 'egg load') and body size are generally positively related in *R. juglandis*.

FRUIT SIZE ASSAYS

Fruit were collected from trees at a variety of sites in southern Arizona, and separated into small, medium and large categories. Small fruit were intermediate between the smallest fruit on a tree and fruit of average size (mean small fruit (SE): 23.45 (± 0.25) mm diameter). Large fruit were intermediate between the largest fruit on a tree and fruit of average size (mean large fruit (SE): 30.93 (± 0.66) mm diameter). Medium fruit were discarded. Large or small fruit were hung one to a cup from the tops of the cups, suspended by wire tied around the stems. Fruit were replaced with fruit of like condition on the fourth day of the experiment. Four replicate cups were set up for each fruit size treatment in each of two trials; as a control, four replicate cups with no fruit were set up in each trial.

An identical assay using artificial models mimicking fruit in shape and colour was conducted. Models consisted of wooden spheres painted with a green that was spectrophotometrically matched to the colour of ripe Arizona walnut fruit. Gravid females will attempt to oviposit in such models, but the wood is impenetrable and eggs cannot be laid. Large models were 37 mm in diameter; small models were 26 mm in diameter. These sizes are well within the minimum and maximum dimensions of fruit found on wild *Juglans major* trees in southern Arizona. Four replicate cups were established for each treatment; as a control, four replicate cups with no fruit were set up in each trial.

LARVAL INFESTATION ASSAYS

Larval-infested fruit were collected from trees at several sites in southern Arizona, and separated into heavily infested and lightly infested classes. Infested fruit are readily recognized by a characteristic brown and black surface rot that is distinct from other sources of fruit damage. Lightly infested fruit showed minimal rot no greater than 1 cm in diameter and localized to the site of oviposition. Such fruit typically contain first-instar larvae and some eggs. In contrast, the rot on heavily infested fruit covered *c.* 30–50% of the fruit surface. Such fruit typically contain mainly last-instar (= third-instar) and some second-instar larvae. Infested fruit in a given trial, whether lightly or heavily infested, came from the same tree, so as to prevent confounding of fruit properties such as size, ripeness and trichome density which are known to vary among trees. Fruit were hung from the top of experimental cups, suspended by a wire harness consisting of two loops of 20 gauge wire wrapped around the fruit. Fruit were replaced with fruit of like condition on the fourth day of the experiment. Eight replicate cups were established for

each treatment; as a control, eight replicate cups with no fruit were set up in each trial.

I conducted a similar assay using artificial models that mimicked uninfested and larval-infested fruit in shape and colour. Based on results of the live-fruit assay, I chose to mimic heavily infested fruit, but not lightly infested fruit. Uninfested fruit consisted of 37 mm diameter black plastic spheres painted with a green that had been matched spectrophotometrically to the colour of ripe Arizona walnut fruit. Gravid females will attempt to oviposit in such models, but the plastic is impenetrable and eggs cannot be laid. Models of heavily infested fruit consisted of the same type of plastic spheres on which a mosaic of green, brown and black paint had been applied with an artist's brush. The green consisted of the same paint used for uninfested fruit. Paint mimicking brown colours of the rot had been spectrophotometrically matched to the brown of actual rot, and was applied along with black paint to the fruit surface in patches resembling the patches on larval-infested fruit in area and shape. Matching was conducted in the human-visible range, since, conveniently, neither uninfested walnut fruit nor rotting patches on walnut fruit reflect appreciably in the ultraviolet range. The models used here had been used previously in a field assay, with infested-fruit mimics being shown to be significantly less attractive to *R. juglandis* females than uninfested-fruit mimics (Henneman & Papaj 1999). Six replicate cups were established for each treatment; as a control, six replicate cups with no fruit were set up in each trial.

Results

EFFECT OF FRUIT SIZE

In each of two trials, the nesting effect on number of mature oocytes or total number of yolked oocytes was not significant (Tables 1 and 2). In both trials, the number of mature or yolked oocytes was significantly positively related to mid-vein length, a proxy for body size. In both trials, the overall treatment effect was highly significant, with respect both to mature and yolked oocytes (Tables 1 and 2). In Trial 1, the number of mature oocytes in the large fruit treatment was strongly significantly greater than in either the small-fruit or the no-fruit treatment (Fig. 1; Tukey's HSD, large vs small, $Q = 6.91$, $P < 0.0001$; large vs none, $Q = 7.34$, $P < 0.0001$). The number of mature oocytes in the small fruit treatment was not significantly different from that in the no-fruit treatment (Fig. 1; Tukey's HSD, $Q = 0.43$, $P = 0.95$). Similarly, the number of yolked oocytes in the large fruit treatment was significantly greater than in either the small-fruit or the no-fruit treatment (Fig. 1; Tukey's HSD, large vs small, $Q = 7.07$, $P < 0.0001$; large vs none, $Q = 7.48$, $P < 0.0001$). The number of yolked oocytes in the small fruit treatment was not significantly different from that in the no-fruit treatment (Fig. 1; Tukey's HSD, $Q = 0.41$, $P = 0.95$).

Table 1. Statistics for effects of host resource on number of mature oocytes (= egg load)

Host fruit character	Host type	Treatment effect		Replicate effect		Fly size effect	
		F_{df}	P	F_{df}	P	F_{df}	P
Fruit size	Live fruit, Trial 1	17.56 _{2,81}	0.0001	1.67 _{9,81}	0.11	13.21 _{1,81}	0.0005
	Live fruit, Trial 2	4.33 _{2,75}	0.016	1.33 _{9,75}	0.24	11.41 _{1,75}	0.0012
	Model	13.58 _{2,77}	0.0001	3.02 _{9,77}	0.004	3.79 _{1,77}	0.05
Presence of larvae	Live fruit	9.83 _{3,344}	0.0001	0.98 _{20,344}	0.49	31.45 _{1,344}	0.0001
	Model	4.22 _{2,102}	0.017	1.98 _{15,102}	0.02	11.74 _{1,102}	0.0009

Treatment, nesting and fly size effects in a general linear model for five assays are presented. **Bold** indicates P -values of 0.05 or less.

Table 2. Statistics for effects of host resource on total number of yolked oocytes

Host fruit character	Host type	Treatment effect		Nesting effect		Fly size effect	
		F_{df}	P	F_{df}	P	F_{df}	P
Fruit size	Live fruit, Trial 1	18.30 _{2,81}	0.0001	1.56 _{9,81}	0.14	12.68 _{1,81}	0.0006
	Live fruit, Trial 2	6.42 _{2,75}	0.003	1.13 _{9,75}	0.36	12.33 _{1,75}	0.0008
	Model	13.47 _{2,77}	0.0001	2.70 _{9,77}	0.009	4.56 _{1,77}	0.05
Presence of larvae	Live fruit	9.98 _{3,344}	0.0001	0.94 _{20,344}	0.54	30.06 _{1,344}	0.0001
	Model	4.99 _{2,102}	0.009	2.11 _{15,102}	0.015	14.19 _{1,102}	0.0003

Treatment, nesting and fly size effects in a general linear model for five assays are presented. **Bold** indicates P -values of 0.05 or less.

Identical patterns were observed in Trial 2. The number of mature oocytes in the large fruit treatment was significantly greater than in either the small-fruit or the no-fruit treatment (Fig. 1; Tukey's HSD, large vs small, $Q = 4.06$, $P = 0.01$; large vs none, $Q = 3.63$, $P < 0.03$). The number of mature oocytes in the small fruit treatment was not significantly different from that in the no-fruit treatment (Fig. 1; Tukey's HSD, $Q = 0.43$, $P = 0.95$). Similarly, the number of yolked oocytes in the large fruit treatment was significantly greater than in either the small-fruit or the no-fruit treatment (Fig. 1; Tukey's HSD, large vs small, $Q = 4.53$, $P = 0.0056$; large vs none, $Q = 4.73$, $P < 0.0036$). The number of yolked oocytes in the small fruit treatment was not significantly different from that in the no-fruit treatment (Fig. 1; Tukey's HSD, large vs small, $Q = 0.20$, $P = 0.99$). I was unable to find evidence of eggs laid in any of the fruit, though it is possible that a small number of clutches were missed.

Results obtained with fruit-mimicking green wooden spheres differing in size, in which eggs could not be laid, paralleled results with live fruit (Fig. 2). Number of mature or yolked oocytes was significantly positively related to mid-vein length (Tables 1 and 2). The overall treatment effect was again significant, both for mature and yolked oocytes. In contrast, the nesting effects on number of mature and yolked oocytes were significant (Tables 1 and 2). Number of mature oocytes in the large sphere treatment was significantly greater than in either the small-sphere or the no-sphere treatment (Fig. 1; Tukey's HSD, large vs small, $Q = 6.47$, $P <$

0.0001; large vs none, $Q = 6.95$, $P < 0.0001$). The number of mature oocytes in the small fruit treatment was not significantly different from that in the no-fruit treatment (Fig. 1; Tukey's HSD, $Q = 0.48$, $P = 0.98$). Similarly, the number of yolked oocytes in the large sphere treatment was significantly greater than in either the small-sphere or the no-sphere treatment (Fig. 1; Tukey's HSD, large vs small, $Q = 6.66$, $P < 0.0001$; large vs none, $Q = 6.73$, $P < 0.0001$). The number of yolked oocytes in the small sphere treatment was not significantly different from that in the no-sphere treatment (Fig. 1; Tukey's HSD, $Q = 0.08$, $P > 0.99$).

EFFECT OF LARVAL PRESENCE

The number of oocytes matured per females was lower overall in this experiment than in other experiments, owing to use of flies of comparatively small body size. The number of mature or yolked oocytes was again significantly positively related to mid-vein length, a proxy for body size (Tables 1 and 2). The nesting effect on number of mature or yolked oocytes was not significant. The overall treatment effect was significant (Fig. 3; Tables 1 and 2). The number of mature or yolked oocytes diminished progressively, from uninfested to lightly infested to highly infested to no-fruit treatments (Fig. 3). The number of mature oocytes in the uninfested fruit treatment was significantly greater than in the heavily infested treatment and no-fruit treatment (Fig. 3; Tukey's HSD, uninfested vs heavily infested, $Q = 3.98$, $P < 0.03$; uninfested vs none, $Q = 7.23$, $P <$

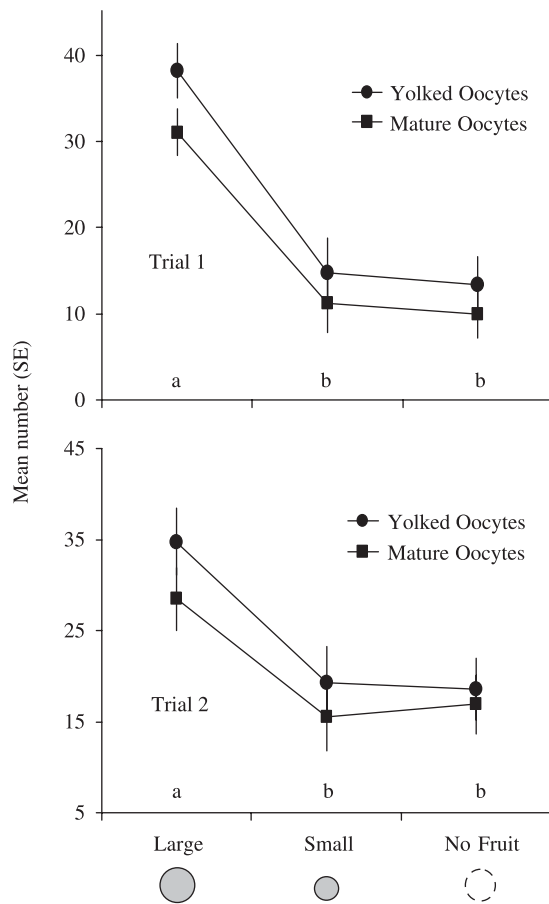


Fig. 1. Effect of fruit size on mean egg load, defined in terms either of mature (fully yolked, chorionated) oocytes or yolked oocytes, for each of two trials. Letters indicate significant differences for either mature or yolked oocytes, using Tukey's HSD. See text for *P*-values.

0.0001). The number of mature oocytes in the uninfested fruit treatment was not significantly different from that in the lightly infested fruit treatment (Tukey's HSD, $Q = 0.72$, $P = 0.96$). The number of mature oocytes for females held with lightly infested fruit was significantly greater than the number for females held without fruit (Tukey's HSD, $Q = 6.57$, $P < 0.0001$), but not significantly different from that for females held with heavily infested fruit (Tukey's HSD, $Q = 3.01$, $P = 0.15$). The number of mature oocytes in the heavily infested fruit treatment was greater than in the no-fruit treatment, but the difference was not significant (Tukey's HSD, $Q = 3.24$, $P = 0.10$).

An identical pattern was observed with respect to number of yolked oocytes. Number of yolked oocytes in the uninfested fruit treatment was significantly greater than in the heavily infested treatment and no-fruit treatment (Fig. 3; Tukey's HSD, uninfested vs heavily infested, $Q = 3.82$, $P < 0.04$; uninfested vs none, $Q = 7.39$, $P < 0.0001$). The number of yolked oocytes in the uninfested fruit treatment was not significantly different from that in the lightly infested fruit treatment (Tukey's HSD, $Q = 0.81$, $P = 0.94$). The number of yolked oocytes for females held with lightly infested

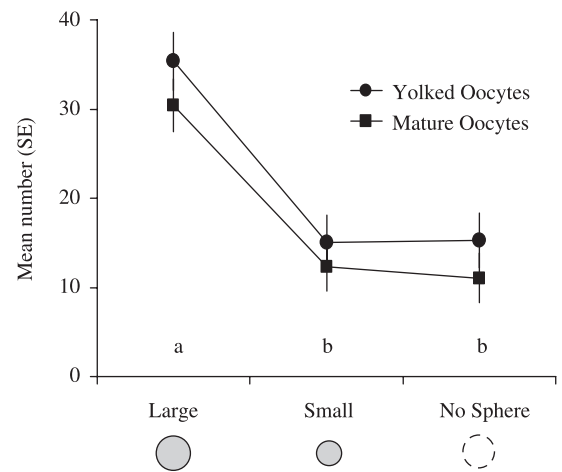


Fig. 2. Effect of fruit model size on mean egg load, defined in terms either of mature (fully yolked, chorionated) oocytes or yolked oocytes. Letters indicate significant differences for either mature or yolked oocytes, using Tukey's HSD. See text for *P*-values.

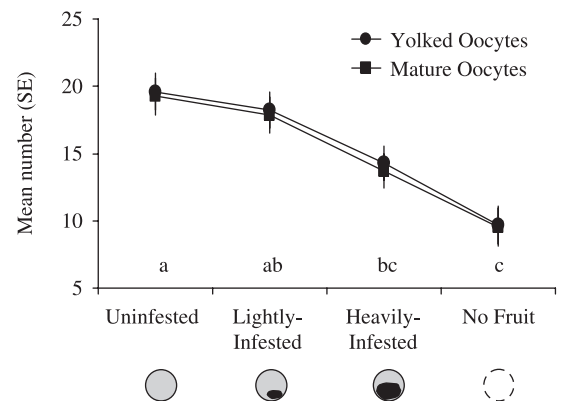


Fig. 3. Effect of larval infestation of fruit on mean egg load, defined in terms either of mature (fully yolked, chorionated) oocytes or yolked oocytes. Letters indicate significant differences, using Tukey's HSD. See text for *P*-values.

fruit was significantly greater than the number for females held without fruit (Tukey's HSD, $Q = 8.33$, $P < 0.0001$), but not significantly different than for females held with heavily infested fruit (Tukey's HSD, $Q = 3.01$, $P = 0.15$). The number of yolked oocytes in the heavily infested fruit treatment was greater than in the no-fruit treatment, but the difference was marginally insignificant (Tukey's HSD, $Q = 3.56$, $P = 0.06$). I was unable to find evidence of eggs laid in any of our fruit, though it is possible that a small number of clutches were missed.

An effect of larval infestation on egg maturation was also obtained in assays of visual mimics of infested and uninfested fruit, in which eggs could not be laid (Fig. 4). The number of mature or yolked oocytes was again significantly positively related to mid-vein length, a proxy for body size (Tables 1 and 2). The nesting effects on number of mature and yolked oocytes were

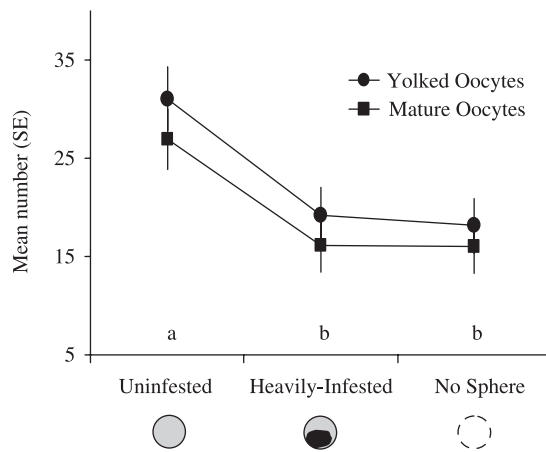


Fig. 4. Effect of visual mimicking of larval infestation on mean egg load, defined in terms either of mature (fully yolked, chorionated) oocytes or yolked oocytes. Letters indicate significant differences for either mature or yolked oocytes, using Tukey's HSD. See text for *P*-values.

significant, owing apparently to different replicate groups being established using flies of slightly different sizes (Tables 1 and 2). The overall treatment effect was significant (Tables 1 and 2). The number of mature oocytes in the uninfested mimic treatment was significantly greater than in the infested mimic treatment and the no-model treatment (Fig. 4; Tukey's HSD, uninfested *vs* infested, $Q = 4.11$, $P = 0.013$; uninfested *vs* none, $Q = 4.16$, $P < 0.011$). The number of mature oocytes in the infested mimic treatment was not significantly different from that in the no-fruit treatment (Tukey's HSD, $Q = 0.05$, $P > 0.99$). An identical pattern was observed for yolked oocytes. The number of yolked oocytes in the uninfested mimic treatment was significantly greater than those in the infested mimic treatment and the no-model treatment (Fig. 4; Tukey's HSD, uninfested *vs* infested, $Q = 4.24$, $P = 0.009$; uninfested *vs* none, $Q = 4.63$, $P = 0.004$). The number of yolked oocytes in the infested mimic treatment was not significantly different from that in the no-fruit treatment (Tukey's HSD, $Q = 0.39$, $P = 0.96$).

Discussion

UNDERLYING MECHANISMS

Although the fruit exposure regimen was timed such that few eggs would be laid in fruit, and although I did not find eggs, it is possible that females held with high-quality fruit (i.e. large fruit or uninfested fruit) matured more eggs simply because females laid more eggs in such fruit. In Diptera, oviposition stimulates egg maturation (Adams *et al.* 1968). However, the possibility of oviposition was absolutely precluded in the fruit model experiments, because the spheres used are impenetrable to female ovipositors. Females deprived of a penetrable sphere will not lay eggs elsewhere in a cage, and hence absolutely no oviposition occurred

in model experiments. Yet results using models, with respect both to fruit size and to level of infestation, closely paralleled results with live fruit. I therefore conclude that effects of fruit quality on egg maturation do not require oviposition for their expression. Still to be resolved is the extent to which a female's lifetime production of eggs is regulated by host sensory cuing *vs* effects of oviposition.

Also unresolved is the precise mechanism by which host fruit quality affects oogenesis. Although females need to feed on exogenous protein, the effect of fruit quality seems unlikely to be due to differential feeding on the fruit itself. Host feeding has never been observed in *R. juglandis*; rather, females feed away from the host, probably on bird faeces, aphid honeydew and surface bacteria, as has been reported in *R. pomonella* (Hendrichs *et al.* 1993). Results with fruit models further suggest that effects do not require any feeding that might occur on fruit, as the models had no conceivable nutritional value. It remains possible that a high-quality host stimulates protein feeding more than a low-quality host, albeit away from the fruit. Consistent with this idea, data in hand indicate that the general effect of host fruit presence on oogenesis is mediated by increased protein consumption (D. Mayntz and D. Papaj, unpublished observation).

FUNCTIONAL SIGNIFICANCE OF PATTERNS

The pattern of effects of the quality of the host fruit resource, in terms of fruit size and level of infestation, on egg maturation corresponded roughly with the pattern of effects of host quality on oviposition behaviour. There are reasons to expect that oviposition and ovarian development will often, but not always, respond in similar ways to variation in the host resource (Papaj 2000). Both oviposition and ovarian development should be sensitive to factors relating to juvenile success. However, oviposition and ovarian development should respond differently in terms of the scale of variation in the resource to which each process is sensitive, owing to a difference in the time scale over which each process takes place. Compared with the act of oviposition, ovarian development generally occurs over a longer time scale, particularly in a species such as *R. juglandis* whose females must forage for an exogenous protein source in order to make new eggs. As such, ovarian development would be expected to respond to environmental variation at a coarser scale. For example, whereas a female may withhold egg-laying on a given fruit, based on the expectation of soon finding a more suitable fruit within the same tree, she will probably not delay egg maturation on that basis. Egg maturation takes too long for such a delay to be of benefit. However, delays in maturation can be of benefit in decisions at a larger spatiotemporal scale, such as the decision to stay within the tree or disperse to a new one.

This difference in scale can potentially drive a difference in the trade-offs involved for oviposition *vs* ovarian

development. A different trade-off, with different costs and benefits, is involved in withholding oviposition in a given fruit and searching for a more suitable fruit than is involved in delaying egg maturation and dispersing to a more suitable tree. The former may relate to a trade-off, say, in allocation of handling time (i.e. the time required to lay a clutch in a fruit) *vs* search time, whereas the latter may relate to a trade-off, say, in allocation of energy and nutrients to dispersal ability or longevity, *vs* reproductive capacity. In the following sections, we apply this reasoning to an accounting of patterns observed in our experiments.

Fruit size

Females matured eggs faster when held with larger fruit. This pattern parallels the strong oviposition preference for large fruit observed in laboratory and field assays of these flies (D. Papaj, unpublished observation). In the field, larger fruit consistently receive more eggs than smaller fruit, but despite bearing more eggs, yield higher per-capita survival and larger pupae (the latter a strong predictor of lifetime female fecundity) (Nufio & Papaj 2001, 2004b; Nufio 2002).

The fitness advantage of large fruit is complemented by a possible advantage in delaying egg maturation and dispersing to trees bearing such fruit. In southern Arizona, host *Juglans major* trees vary markedly in the mean size of the fruit they bear, over even relatively small spatial scales (Nufio & Papaj 2001; Nufio 2002). Fruit bearing large fruit could therefore be within dispersal distance of a tree bearing small fruit. As such, a female emerging beneath a tree bearing small fruit may benefit by dispersing in search of a tree bearing large fruit. If dispersal or survival trades off against egg maturation, females making a decision to disperse should mature eggs more slowly in the presence of small fruit *vs* large fruit. This was the effect obtained here.

The fact that females held with small fruit or fruit models matured no more eggs than females held with no fruit at all reflects a discrepancy between patterns in oviposition and ovarian development, given that small fruit, though less preferred, are nevertheless utilized in nature. This discrepancy may be indicative of how trade-offs associated with ovarian development differ from those associated with oviposition. From an oviposition standpoint, as unutilized large fruit become increasingly rare, the fitness return associated with laying at least some eggs in small fruit must eventually exceed the fitness return associated with waiting to find the rare large fruit. Females then lay eggs in small fruit, as observed. From an ovarian standpoint, in contrast, our present results imply that the fitness return associated with maturing and laying eggs in a tree bearing small fruit may routinely be less than the fitness return associated with delaying maturation and dispersing to a tree bearing larger fruit. Hence, exposure to small fruit does not significantly enhance oogenesis.

Presence of larvae

Females matured significantly fewer eggs in fruit infested by larvae in advanced stages than in uninfested fruit and, in statistical terms at least, no more eggs than females held without any fruit at all. This result parallels patterns in fly behaviour in nature. Males do not defend heavily infested fruit and females avoid them absolutely (Henneman & Papaj 1999). A female's eggs are unlikely to survive in fruit infested by larvae in advanced stages, which will consume virtually the entire husk before leaving and pupating. In southern Arizona, virtually 100% of the fruit on a given tree are infested within *c.* 10 days (Nufio & Papaj 2001). However, trees vary markedly in the timing of fruit ripening and, thus, trees bearing uninfested, freshly ripened fruit could be within dispersal distance of trees bearing heavily infested fruit (Nufio & Papaj 2001). A newly emerged female that encounters larval-infested fruit might therefore disperse in search of neighbouring trees bearing newly ripened, uninfested fruit. If dispersal or survival trades off against egg maturation, females should delay maturation in response to larval-infested fruit, relative to uninfested fruit. This was the effect observed here.

That lightly infested fruit enhanced oogenesis to a degree comparable to uninfested fruit is not wholly inconsistent with patterns of oviposition in this particular *Rhagoletis* species. Although *R. juglandis* females deposit a host-marking pheromone on fruit after oviposition, and although HMP has a deterrent effect on egg-laying (Nufio & Papaj 2004a), females nevertheless oviposit multiply into walnut fruit. In a field study, we estimated that the average fruit contained *c.* five clutches after 5–6 days of the first appearance of damage, and it is not uncommon to find eggs in fruit bearing first- or even second-instar larvae. Multiple oviposition is a consequence in part of the ample volume of food available in a walnut husk relative to the size of individual clutches (Nufio & Papaj 2004b). Additionally, as in all walnut-infesting *Rhagoletis* species that have been examined, *R. juglandis* females not only oviposit multiply into fruit but frequently oviposit directly into pre-existing oviposition cavities (Papaj 1993, 1994). This peculiar behaviour appears to be favoured by direct female benefits such as time saved in oviposition or reduction of ovipositor wear (Lalonde & Mangel 1994; Papaj 1994; Papaj & Alonso-Pimentel 1997). In short, the balance of costs in terms of larval competition and benefits in terms of time savings or ovipositor wear may render a lightly infested only marginally less attractive than uninfested fruit.

CONSEQUENCES OF HOST CUIING FOR OVIPOSITION BEHAVIOUR

The effects of host quality on ovarian development can be expected to have direct consequences for oviposition behaviour, because insects with many eggs to lay

(i.e. with higher egg load) behave differently from conspecifics with few eggs to lay. They forage more persistently, lay larger clutches, and are less discriminating about where to lay their eggs, all of which are functional adjustments to current egg load (Mangel 1987, 1989; reviewed by Minkenberg *et al.* 1992; reviewed by Mayhew 1997). Such effects are common in tephritid flies (Prokopy *et al.* 1995; Duan *et al.* 1995; van Randen & Roitberg 1996). *R. juglandis* females with higher egg load are more likely to mate, are more likely to be found on fruit, and lay larger clutches (Alonso-Pimentel & Papaj 1996; Carsten & Papaj 2005; D. Papaj, unpublished observation).

In the context of the present results, some testable predictions can be made. For example, females in trees bearing large fruit should lay larger clutches in those fruit and 'fill' those fruit with eggs faster than females in trees with average-sized fruit, but should also be less discriminating over the range of variation in the size of those large fruit. Also, females in trees bearing lightly infested fruit should lay smaller clutches in those fruit and fill fruit more slowly than females in trees with mainly uninfested fruit, but should also be more discriminating over the range of variation in infestation levels. The effects on clutch size and discrimination are adaptive in both cases.

In closing, the connection between egg maturation and oviposition has been at the heart of a debate in the parasitoid behaviour literature regarding the occurrence of egg limitation *vs* time limitation in such insects (Rosenheim 1996; Heimpel *et al.* 1996; Heimpel & Rosenheim 1998; Sevenster *et al.* 1998; Rosenheim 1999; Rosenheim *et al.* 2000). One implication of the present study and others like it (Rivero-Lynch & Godfray 1997; Hopkins & Ekbom 1999) is that the spectrum of trade-offs that needs to be considered when accounting for patterns in oviposition behaviour will probably be broader when ovarian development is itself a dynamic process sensitive to resource abundance and quality.

Acknowledgements

Heather Mallory is gratefully acknowledged for technical assistance. M. L. Henneman designed the models in the infested fruit model assay. The project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2001-35302-09977.

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Received 21 July 2004; revised 28 January 2005; accepted 17 February 2005