

## Host Utilization by the Walnut Fly, *Rhagoletis juglandis* (Diptera: Tephritidae)

CÉSAR R. NUFIO,<sup>1</sup> DANIEL R. PAPAJ,<sup>2, 3</sup> AND HENAR ALONSO-PIMENTEL<sup>2, 3</sup>

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**ABSTRACT** *Rhagoletis juglandis* Cresson is a specialist that deposits its eggs into the husks of developing walnut fruit. Like other walnut infesting flies in the *R. suavis* group, *R. juglandis* actively superparasitizes its larval hosts. However, little is known regarding the degree to which hosts are reused and the ecological context under which host reuse occurs. This field study examined the pattern of host utilization by *R. juglandis* and how fruit variables such as volume and penetrability affect the degree that hosts are reused. Fruit on four of five study trees were synchronously infested and within 2–2.5 wk all fruit on these trees were infested. Fruit on a fifth tree were significantly less penetrable than those found among the other trees in the study and this may explain why fruit on this tree were rarely used throughout the season. Walnut hosts were commonly multiply infested and reuse of hosts occurred in as few as 1–2 d after first infestation. Infestation levels within fruit appeared to stabilize 4–5 d after fruit were first used. Fruit volume was positively correlated with both the number of punctures on hosts and the infestation levels within hosts that had been infested for either 1–2 or 4–9 d. Large fruit were infested more quickly than small fruit, although this trend was stronger on some trees than others. Finally, despite a size-penetrability correlation among two of the five trees, penetrability itself did not explain either which fruit were preferentially used throughout the season or the infestation levels within fruit.

**KEY WORDS** *Rhagoletis juglandis*, walnut flies, superparasitism, marking pheromone, Tephritidae

CHOOSING WHERE OFFSPRING will develop is a simple form of maternal investment among insects. Ovipositional choices are especially important for insects whose larval stages are restricted to a particular environment or host. In such insects, offspring are themselves limited in their ability to acquire new resources if their natal resources become depleted (Messina and Renwick 1985, Smith and Lessells 1985). Maternal investment may involve avoiding laying eggs at sites previously used by conspecifics, a tendency often mediated by use of a marking pheromone (Prokopy 1981a, Roitberg and Prokopy 1987).

Frugivorous fruit flies in the family Tephritidae deposit egg clutches within the husks of developing fruit where their larvae are constrained to feed and develop. Females may possess visual and chemical mechanisms for assessing the quality of available hosts and for discriminating between previously infested and uninfested hosts (Prokopy et al. 1976, Prokopy and Roitberg 1984, Henneman and Papaj 1999). Females in the genus *Rhagoletis* assess and reject infested fruit on the basis of a marking pheromone that is deposited on the fruit surface after oviposition by previous females. Thus, marking pheromone in this system is believed to minimize larval competition by causing females to

distribute their clutches more uniformly within host patches than is expected by chance alone (Prokopy 1981a, 1981b; Bauer 1986; Averill and Prokopy 1989).

*Rhagoletis juglandis* (Cresson) is a member of the walnut-infesting *Rhagoletis suavis* group (Bush 1966). In southern Arizona this species is found on the Arizona walnut, *Juglans major* (Torr.), which can be found in montane canyons (1,200–2,700 m). These flies are univoltine and females deposit clutches of up to 30 eggs after puncturing the fruit surface with their ovipositor. The larval stages feed on the husk of developing fruit, pupate in the soil beneath the natal tree, diapause as pupae through the winter and spring, and emerge as adults during mid- to late summer. After deposition of a clutch, female *R. juglandis* drag their ovipositors on the fruit surface in a manner suggesting deposition of a marking pheromone. Despite displaying the genus-typical marking behavior, female walnut flies reinfest and often reuse the actual oviposition sites established by conspecifics (Papaj 1994). Although superparasitism, the use of hosts that already bear conspecific brood, is commonly associated with the lack of available hosts (Roitberg and Mangel 1988, Papaj et al. 1989), walnut flies prefer infested hosts early in the season when uninfested hosts are still available (Lalonde and Mangel 1994).

To explain superparasitism by walnut flies, researchers have proposed that the reuse of the oviposition sites provides females with direct benefits such as reduced ovipositor wear (Papaj 1993), reduced time to deposit clutches (Papaj and Alonso-Pimentel

<sup>1</sup> Interdisciplinary Program in Insect Sciences, University of Arizona, Tucson, AZ 85721.

<sup>2</sup> Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721.

<sup>3</sup> Center for Insect Science, University of Arizona, Tucson, AZ 85721.

1997), and increased access to less penetrable fruit (Lalonde and Mangel 1994). Each of these benefits may indirectly increase female reproductive success by increasing the number of clutches that can be deposited in a female's lifetime. Still, work on other tephritid flies suggests that reuse would exact costs for larvae forced to compete with older or more numerous conspecifics. Such competition has always been posed as the primary reason that many tephritid species avoid laying eggs in previously infested fruit (Prokopy et al 1978, Prokopy 1981b).

To adequately address both the implications of host reuse on female and offspring fitness and to understand the potential function of a marking pheromone in this system, it is essential that researchers first examine how walnut flies use their walnut hosts in the field. This field study was designed to examine the time course of attack by female flies on host fruit as well as the level of attack suffered by those fruit. We were particularly interested in understanding how two fruit characteristics, size and ripeness, affect both which fruit within a given tree are used and the degree to which those fruit are reused. We chose to examine how fruit size affects levels of host reuse because this factor may determine the amount of available larval resources and thus may influence the levels of competition faced by later-laid clutches. We chose to examine fruit penetrability as this has been shown to influence host reuse in other walnut flies (Lalonde and Mangel 1994) and might influence the degree to which females reuse oviposition sites.

### Materials and Methods

**Fruit Characteristics and the Rate of Host Utilization.** In mid-June 1995, five *Juglans major* trees in Garden Canyon in Cochise County southern Arizona were selected for study. Trees were chosen for their relatively large fruit yields, with most fruit being easily accessible from the ground or with the aid of an 8 foot tall step ladder. Four of the trees were located in lower Garden Canyon (1,500 m in altitude) and one in upper Garden Canyon (1,700 m in altitude). Fruit on each tree were haphazardly chosen for census. Accessible branches and fruit were tagged with flagging tape placed at the base of the main branches as well as the base of the smaller branches that held the fruit. The fruit from a given tree used in this study constituted roughly 25–30% of the total fruit yield of that tree.

Walnut flies were first observed on a study tree on 10 July. Every few days thereafter, fruit were censused for the occurrence of oviposition punctures, which are created by females when depositing clutches within their hosts. After 19 July, when the first punctures were observed, study trees were censused every 2 d in the following manner. From 0900 to 1600 hours, tagged fruit within each tree were examined for signs of walnut fly oviposition punctures. With the use of calipers, minimum and maximum length was measured for each punctured fruit and for a haphazard sample of whatever unpunctured fruit remained on the tree. The minimum and maximum length measurements

were used to calculate the volume of a given walnut. This was done by assuming a walnut was spherical in shape, taking the average of the axes measurements as an estimate of sphere diameter and then computing fruit volume as  $4/3 \pi r^3$ , where  $r$  is the radius of the sphere.

Early in the season, fruit penetrability (a measure of ripeness) was assessed with the use of a hand-held spring penetrometer, calibrated in grams, for a haphazard sample of fruit from each study tree. Fruit penetrability was estimated as the mean of three penetrometer readings taken on a given fruit. Within the first two census dates of each tree, the penetrability of recently punctured fruit was compared with that of a sample of fruit that remained unpunctured. After the first two censuses on each tree we stopped recording penetrometer readings from unpunctured fruit in order to minimize the possible effects of the penetrometer punctures on female behavior. Finally, the proportion of tagged fruit used by females over the course of the study was also calculated for each tree.

**Volume and Penetrability.** The degree to which fruit volume and penetrability are correlated will influence our inferences regarding which of the two fruit characteristics most influences host reuse patterns. We therefore checked for a relationship between fruit penetrability and volume within each of the four trees. Within each tree we pooled volume and penetrability measurements for recently punctured fruit and unpunctured fruit that were both sampled during the first two census dates. Punctured fruit that contained hatched eggs were not included in this analysis.

**Infestation Levels.** Fruit were collected at three distinct periods: 1–2, 4–5, or 8–9 d after they were initially punctured by females. Fruit were immediately brought into the laboratory where volume and penetrability were measured with the use of calipers and a penetrometer as described above. Each fruit was then dissected and infestation levels in terms of total eggs and larvae present were recorded. The number of oviposition punctures on each fruit was also recorded. To examine the general relationship between fruit characteristics (penetrability and volume) and infestation levels, we pooled data across our study trees for analysis. This pooling strategy was also used when we examined how infestation levels changed as fruit remained on the trees either 1–2, 4–5, or 8–9 d on a tree.

Finally, to estimate the number of eggs deposited by females during an oviposition event we collected fruit on which we observed females establishing a new oviposition puncture and subsequently engaging in what appeared to be host-marking behavior. Host marking behavior consisted of females dragging their ovipositors along the fruit surface. Such behavior has been demonstrated in numerous *Rhagoletis* species to be indicative of the deposition of host marking pheromone (Prokopy 1981b). The recently punctured fruit were collected, dissected, and the number of eggs within the puncture counted. We compared the number of eggs from a single oviposition event with in-

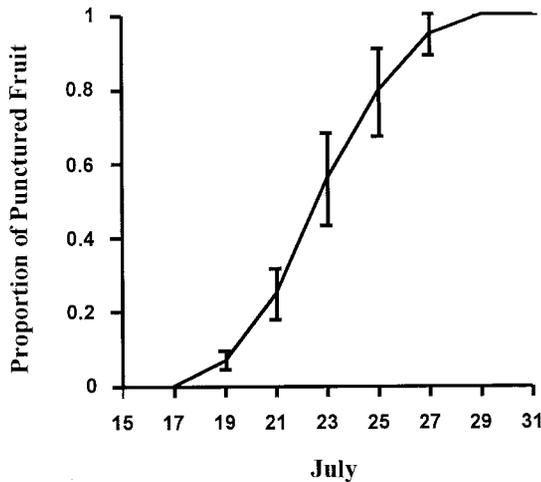


Fig. 1. Proportion of study fruit punctured over the season ( $n = 232$ ). By 31 July, all fruit on each of the study trees were punctured.

festation levels found in fruit that had been infested for either 1–2, 4–5 or 8–9 d.

### Results

Although five trees were in the original design, one tree (labeled A4 and located in lower Garden Canyon) was eventually eliminated because we observed few *R. juglandis* individuals in mid-July and almost no flies in this tree during any other census date. By the end of the season, only 12 fruit, which included tagged and untagged fruit, were found to be punctured. Even at the end of the season the fruit on this tree were smaller ( $F = 46.6$ ;  $df = 4, 180$ ;  $P < 0.0001$ ; Tukey HSD,  $P < 0.05$  for each of four comparisons) and less penetrable ( $F = 38.2$ ;  $df = 4, 70$ ;  $P < 0.0001$ ; Tukey HSD,  $P < 0.05$  for each of four comparisons) than that of any other study tree in the beginning of the season.

**Fruit Volume and Host Utilization.** To examine the pattern of host fruit utilization, we analyzed how the proportion of tagged fruit with oviposition punctures changed over time. Within each tree, 100% of tagged fruit were punctured over an  $\approx 9$ -d period (Fig. 1). By the 14th day after the beginning of the census, all fruit on each study tree were infested. It was not possible to estimate precisely the exact time over which all fruit became infested after the first fruit on a tree was punctured because fruit that exceeded the range of our 8-foot step ladder may have been infested before those lower on the tree. Still, a conservative estimate of the time required to puncture all fruit on a given tree is no more than 2–2.5 wk.

To establish whether larger fruit were used during each census period, we compared the volumes of fruit that remained unpunctured on the tree with those of recently punctured fruit. We found that, during most of the censuses on trees A3 and A5, volumes of newly infested fruit were significantly greater than volumes of haphazardly sampled unpunctured fruit. In con-

trast, in trees A1 and A2, infested fruit volumes were significantly greater than those from the haphazard sample only for the first census date in which fruit were punctured (Fig. 2).

**Fruit Penetrability and Host Utilization.** To establish whether fruit penetrability was related to whether or not fruit were used by the flies during the first two census dates, we compared the penetrability of fruit that remained unpunctured on a tree with that of recently punctured fruit. Although we did not control for any possible effects of punctures on fruit ripeness, we found that on a tree-by-tree basis the penetrability of fruit used during the first two census periods was not significantly different from that of the haphazard unpunctured sample collected during the same period (Wilcoxon-signed rank,  $P > 0.05$  for each of four within tree comparisons).

A weak but significant negative correlation between fruit volume and fruit penetrability was found for both the unpunctured haphazard samples and fruit punctured on the first two census dates within trees A1 and A2 (regression coefficient =  $-0.0173$ ,  $t = -4.81$ ,  $df = 52$ ,  $r^2 = 0.31$ ,  $P = 0.0004$ ; regression coefficient =  $-0.01$ ,  $t = -2.5$ ,  $df = 55$ ,  $r^2 = 0.10$ ,  $P = 0.015$ ; trees A1 and A2, respectively). There were no significant differences between the slopes of the fruit volumes versus penetrability data within these trees for the unpunctured sample versus the recently punctured fruit ( $F = 2.00$ ;  $df = 1, 50$ ;  $P = 0.79$ ;  $F = 2.85$ ;  $df = 1, 53$ ;  $P = 0.10$ , trees A1 and A2, respectively). There was also no significant differences in the slopes of the fruit volumes versus penetrability data between trees A1 and A2 for the unpunctured sample and the recently punctured fruit ( $F = 0.96$ ;  $df = 1, 92$ ;  $P = 0.33$ ). To simplify the graphic representation between penetrability and fruit volume within trees A1 and A2, we pooled the unpunctured haphazard sample and recently punctured fruit data from both trees (regression coefficient =  $-0.02$ ;  $t = -5.82$ ,  $df = 94$ ,  $r^2 = 0.26$ ,  $P < 0.0001$ ) (Fig. 3). No correlation between penetrability and fruit volume was found for trees A3 and A5 (regression coefficient =  $0.004$ ,  $t = -1.47$ ,  $df = 47$ ,  $r^2 = 0.04$ ,  $P = 0.15$ ; regression coefficient =  $-0.01$ ,  $t = -0.83$ ,  $df = 41$ ,  $r^2 = 0.02$ ,  $P = 0.41$ ; trees A3 and A5, respectively).

Within each of the pooled age cohorts, penetrability was neither correlated with infestation levels (Spearman's correlation,  $P > 0.05$ ) nor with the number of punctures found on a particular fruit (Spearman's correlation,  $P > 0.05$ ).

**Infestation Level and Fruit Volume.** Our estimate of the average ( $\pm$ SE) clutch size deposited by females during a single oviposition event was  $15.7 \pm 1.5$ . The average infestation level of hosts increased with age of fruit. The number of eggs deposited in a single oviposition event was significantly less than the infestation levels found in fruit collected 1–2, 4–5, or 8–9 d after they were first infested ( $F = 33$ ;  $df = 3, 24$ ;  $P < .0001$ ) (Fig. 4).

To establish whether a relationship between infestation levels and fruit volumes existed at our study site, we pooled the 1–2 d cohorts across trees and pooled 4–5 with the 8–9 d cohorts across trees. By pooling among cohorts, we attempted to both facilitate our

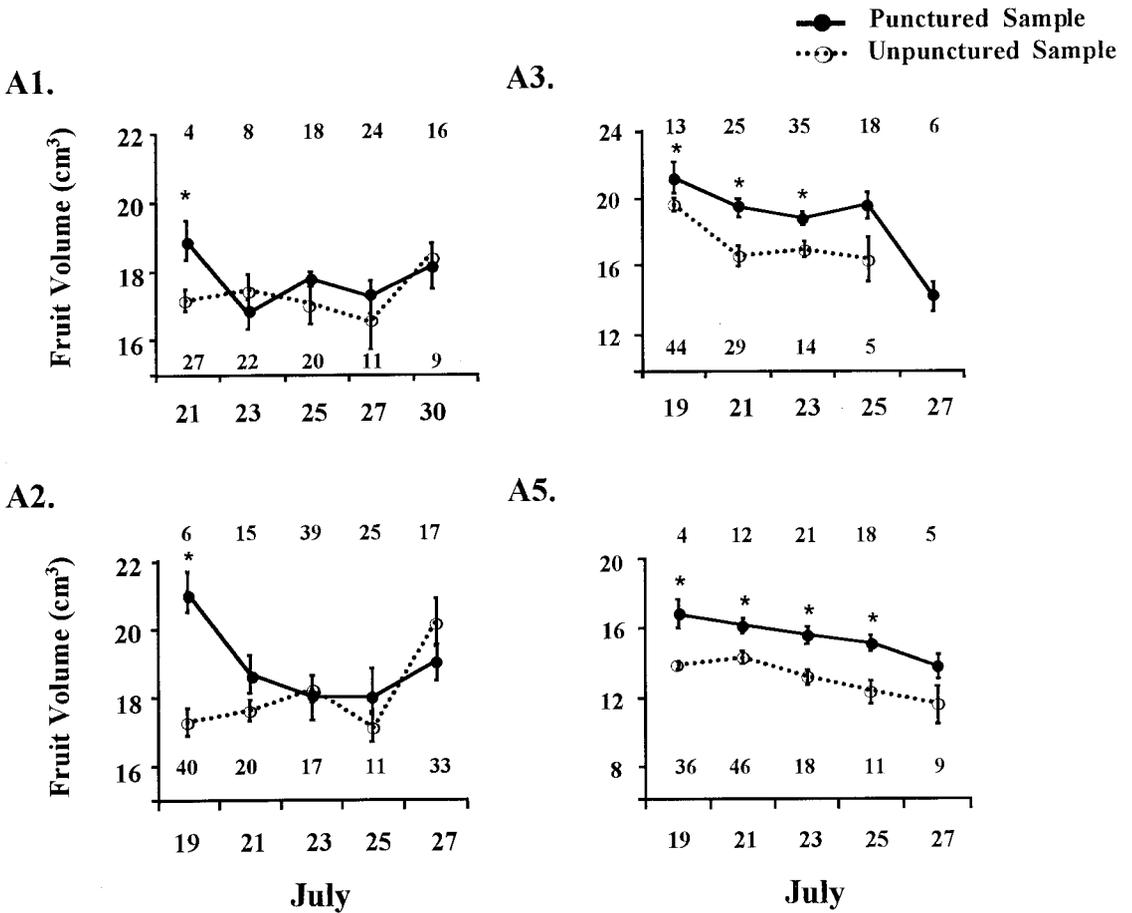


Fig. 2. Volume of both fruit from each of the study trees that were punctured and those of a haphazard sample that remained unpunctured throughout the season. Sample sizes are given for each point. (\*, significant difference between the punctured and unpunctured fruit volume during a particular census date; Tukey honestly significant difference (HSD) for within tree comparisons,  $P < 0.05$ ).

analyses and increase the sample size per treatment. The appropriateness of this *a posteriori* pooling strategy we used was supported by our findings that there

was no difference between the infestation levels and volumes of the day 4–5 and 8–9 d cohorts and these latter cohorts had significantly different infestation levels than their respective 1–2 d cohort (Fig. 4).

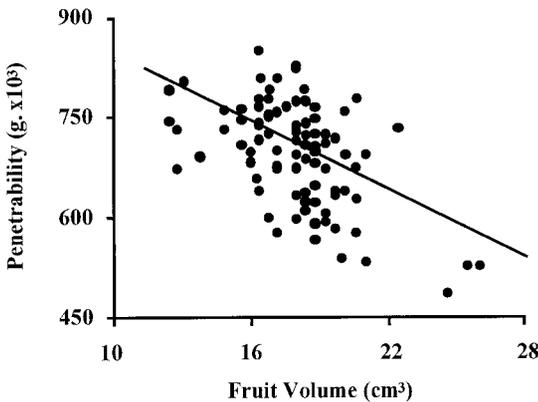


Fig. 3. Relationship between fruit volume and penetrability using pooled data from trees A1 and A2. Regression lines drawn for diagrammatic purposes.

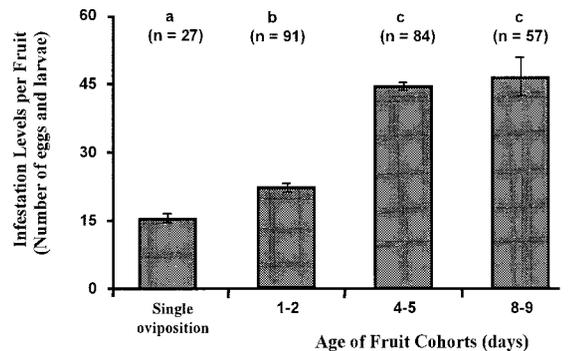


Fig. 4. Median number of eggs deposited during a single oviposition and the median infestation levels ( $\pm$ SE) as a function of fruit cohort age. Bars sharing the same letter are not significantly different (Tukey HSD,  $P < 0.05$ ).

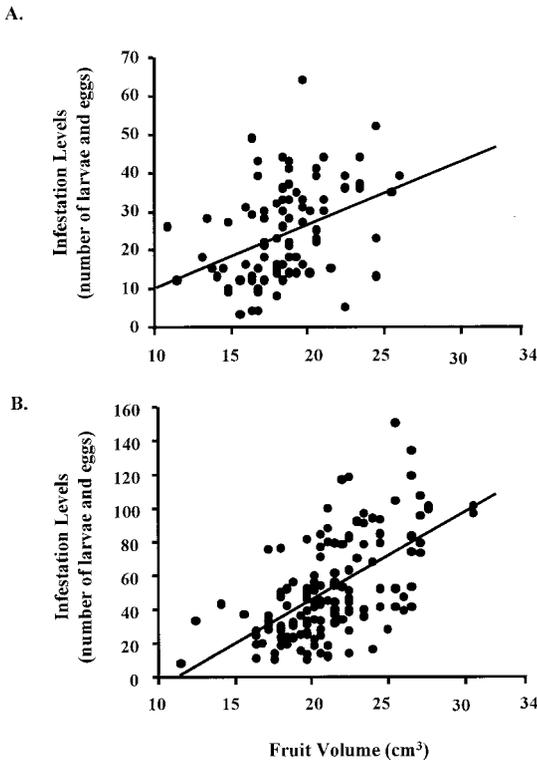


Fig. 5. Relationship between fruit volume and infestation levels. (A) Pooled 1-2 d cohorts. (B) Pooled 4-9 d cohorts. Note the difference in scale between the infestation levels of the two cohorts. Regression lines drawn for diagrammatic purposes.

Hereafter, the pooled 1-2 d cohorts and the pooled 4-5 and 8-9 d cohorts will be referred to as the 1-2 d cohorts and 4-9 d cohorts respectively.

We found a positive correlation between fruit volume and infestation levels for the 1-2 d and for the 4-9 d cohorts ( $r_s = 0.384$ ,  $n = 91$ ,  $P = 0.0001$ ;  $r_s = 0.60$ ,  $n = 141$ ,  $P = 0.0001$ ; 1-2 and 4-9 d cohorts, respectively) (Fig. 5A and B).

The number of oviposition punctures was positively correlated with infestation levels within the 1-2 d cohorts ( $r_s = 0.54$ ,  $n = 91$ ,  $P < 0.0001$ ) and 4-9 d cohorts ( $r_s = 0.70$ ,  $n = 141$ ,  $P < 0.0001$ ). Fruit from the 1-2 d cohorts had  $1.0 \pm 0.04$  punctures compared with  $2.0 \pm .11$  punctures on the 4-9 d cohorts (Mann-Whitney  $U(91,141) = 2613$ ;  $P < 0.0001$ ). The number of punctures on a fruit was positively correlated with fruit volume in the 1-2 ( $r_s = 0.24$ ,  $n = 91$ ,  $P < 0.05$ ) and 4-9 d cohorts ( $r_s = 0.56$ ,  $n = 141$ ,  $P < 0.0001$ ).

When the number of oviposition punctures and their corresponding infestation levels were pooled across all fruit cohorts, a significant positive correlation between these two variables was found ( $r_s = 0.78$ ,  $n = 232$ ,  $P < 0.0001$ ) (Fig. 6). The average infestation level of hosts containing a single puncture ( $24.7 \pm 1.1$ ) was significantly greater than the estimated 16 eggs laid in a single oviposition event ( $F = 11.86$ ;  $df = 1, 155$ ;  $P < .0001$ ). On average, each additional puncture

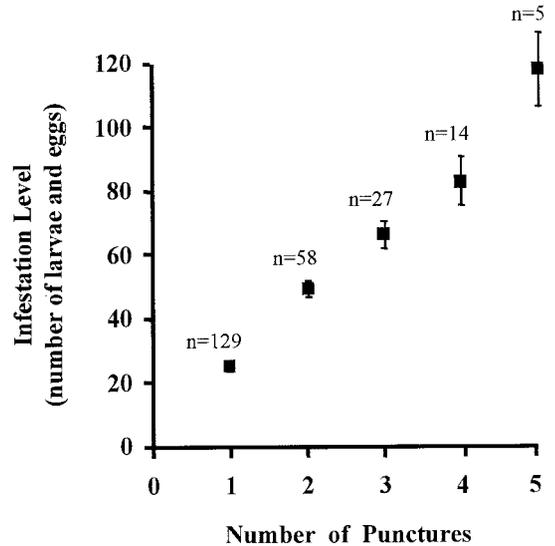


Fig. 6. Relationship between the number of oviposition punctures on a fruit and their corresponding infestation levels.

corresponded to a net infestation level increase of 23 ( $\pm 4.5$ ) individuals. If a single clutch is composed of 16 eggs then on average a single oviposition site contains 1.7 clutches and each additional puncture adds 1.5 clutches to a host. If this is the case, a fruit with four punctures contains roughly six clutches and a fruit with five punctures contains roughly eight clutches.

## Discussion

Our study shows that *R. juglandis* commonly superparasitizes walnut hosts in the field. From field collections, we estimated that this walnut fly lays a clutch size of  $\approx 16$  eggs. As presented above (see *Results*), many walnuts had significantly  $>16$  eggs within 1-2 d of the initial oviposition event. Therefore, many fruit are reused within 1-2 d of first being attacked.

The exact degree to which the 1-2 d fruit cohorts are being reused is difficult to calculate because clutch size varies among females and we have no way of distinguishing clutches that are laid at the same site. Our study also did not address whether females deposit larger clutches into larger fruit, a process that could contribute to an early positive relationship between fruit volume and infestation level. Even if females adjust clutch size to fruit size, our data demonstrates that reuse of hosts by multiple females must still be an important factor leading to increases in infestation levels. Because the mean infestation levels of fruit that remained on the tree 4-9 d were significantly greater than that estimated for a single clutch and that found in fruit which were infested for only 1-2 d, females had to be reusing many fruit to some extent.

Our tree censuses showed that all fruit on four of five trees were infested within 2-2.5 wk (Fig. 1). Fruit on a fifth tree were virtually untouched. This result suggests that fruit within a given tree are either nearly

all acceptable or all unacceptable during the flight season and further that although our sample size is admittedly limited to just five trees most trees fall into the 'all fruit acceptable' category. Finally, our study finds that 'all fruit acceptable' trees are synchronously infested and that all fruit on each of the trees are infested within 2–2.5 wk.

**Fruit Characteristics and Host Utilization.** Fruit volume appears to influence not only fruit that are used throughout the season, but also the degree to which fruit are superparasitized. Our field data show that in two of four trees, for all but the last census dates, the mean volume of fruit that were used exceeded the mean volume of fruit that remained unpunctured (Fig. 2). In the remaining two trees, we found that the mean fruit volume of recently punctured fruit was greater than that of unpunctured fruit only during the first census. Although not consistent among trees, it still appears that fruit volume may sometimes influence fruit use.

Fruit volume appears to not only influence which fruit are used by walnut flies but also the degree to which hosts are superparasitized. In our study we found a positive correlation between fruit volume and their respective infestation levels (Fig. 5). This positive correlation was not only found for fruit that remained on the tree 4–9 d after they were first infested but also fruit that had only been infested for 1–2 d. Although females appear to superparasitize larger fruit to a greater extent than smaller fruit, density-dependent factors leading to higher offspring mortality in smaller fruit could also explain the relationship between fruit size and infestation levels. Our measure of infestation level was the number of eggs and larvae present within a host. By not being able to count eggs as soon as they were laid, we may have inadvertently neglected to count individuals that had hatched but died as early instars. To address this latter issue, we conducted a field experiment the following year in which we specifically defined infestation levels as the number of eggs and egg husks (the latter produced by individuals that had hatched and moved away from their egg chorion) present at oviposition sites. In this second study we confirmed our previous finding that the number of eggs placed within a host is significantly correlated with its volume (C.R.N. and D.R.P., unpublished data).

During the first two censuses in each of our study trees we did not find that recently punctured fruit were significantly more penetrable than sampled fruit that remained unpunctured on the same trees. This finding suggests that fruit penetrability was not a factor that determined which fruit were preferentially used by the walnut flies early in the season. However, although fruit ripeness was not found to influence which fruit within a tree were selectively punctured, it does seem to influence which trees will be used by the walnut flies. In our study, for example, few flies were surveyed and few walnuts were used in one of the study trees, a tree that consistently contained fruit that uniformly were less penetrable than those found on other trees. These findings for the walnut fly appear

to be consistent with those found for the apple maggot fly. This is because although fruit ripeness influences which trees are preferentially used by the apple maggot flies (Averill and Prokopy 1989, Murphy et al. 1991), once in a tree these flies may not discriminate among hosts on the basis of ripeness (Prokopy and Papaj 1989).

**Why Do Flies Superparasitize Their Larval Hosts?** Reuse of walnut hosts by *R. juglandis* may be influenced by three factors. First, reuse of walnut hosts may be related to their size. Most *Rhagoletis* species use relatively small hosts (e.g., hawthorn berries, cherries, blueberries, and dogwood berries [Bush 1966]) that appear to offer fewer resources for developing offspring than do walnut fruit. Studies of *R. pomonella* (Walsh), for example, have shown that rarely do more than three or four pupae emerge from a hawthorn berry, even when more are deposited within them (Averill and Prokopy 1987; Feder et al. 1995). In contrast, it is not unusual for a walnut host to yield several dozen *R. juglandis* or *R. boycei* (Cresson) pupae (C.R.N. and D.R.P., unpublished data). The ability of walnut hosts to support greater infestation levels may also explain why members of the *R. suavis* clade deposit clutches rather than single eggs at oviposition sites, the latter being the rule in most other species within the genus.

Within walnut hosts, variation in size may determine the degree to which these hosts can be reused with minimal or acceptable costs associated with larval competition. Measured as either infestation levels within a fruit or the number of punctures on a fruit (a conservative estimate of host reuse), we found that larger fruit were superparasitized more often than smaller fruit (Fig. 5). We also found that 4–5 d after fruit were initially infested, fruit were often no longer reused. Thus, we hypothesize that by 4–5 d, infestation levels reach a point at which the costs of larval competition, due to a reduction in available larval resources and age asymmetries between competing broods, may outweigh any potential benefits of reuse. Females may reject hosts that have been previously infested 4–5 d or longer by detecting changes in marking pheromone concentration, changes in host quality associated with the presence of conspecific broods (Fitt 1984), or a combination of the two. Although it is unlikely that a host's response to being infested requires 4–5 d to accumulate this process might also help to explain why females do not reuse hosts previously infested 4–5 d or more.

If the availability of larval resources within hosts is important to larval survival or fitness, we might expect that females would preferentially use larger fruit. In two of four study trees we did find that larger fruit were consistently more heavily attacked over most census dates (Fig. 2). The preference for large fruit does not seem to be explained by a tendency for large fruit to be more penetrable: in two of the four trees in which larger fruit were preferentially used, we did not find a correlation between fruit size and penetrability. Furthermore, penetra-

bility did not explain much of the variation in the degree to which fruit were reused.

The second factor that may influence the reuse of hosts by walnut flies is the ephemeral nature of these larval resources. We propose that because nearly all walnut hosts within an area will be synchronously used within 2–2.5 wk, there will be both a spatial and temporal limit on the total amount of larval resources available to a population of walnut flies. On an individual level, this may mean that females are time limited and must maximize the number of clutches deposited within the limited window of larval resource availability. One way to maximize the number of clutches deposited within the allotted time may be to superparasitize hosts as they ripen and become accessible to females. Superparasitizing hosts to maximize the number of clutches deposited may again be a viable strategy for walnut flies because walnut husks can support the development of more than a few clutches (C.R.N. and D.R.P., unpublished data).

The third factor that may influence superparasitism by walnut flies concerns the benefits that females may gain by not simply reusing a host fruit but by reusing the actual oviposition punctures created by previous females. By reusing oviposition punctures, females may save time (Papaj and Alonso-Pimentel 1997), decrease the wear to their ovipositors (Papaj 1993), or gain access to fruit that are relatively impenetrable (Lalonde and Mangel 1994). These benefits have been proposed to increase the number of clutches a female can lay over a lifetime. Although not designed to test the benefits associated with reusing oviposition punctures, our study suggests that reuse of oviposition sites is common, with each puncture containing 1.5–1.7 clutches on average. Benefits associated with reuse of oviposition sites, therefore, may be commonly experienced by females throughout a season. Still, although reuse of oviposition sites seemed to be occurring, it explained only a portion of reuse of a fruit and the establishment of new oviposition sites appears to contribute more to total infestation levels within a host.

Our field study was designed to examine the patterns of host utilization by *R. juglandis*. The results of our study suggest that fruit characteristics, namely fruit volume and penetrability are important factors that influence host utilization by walnut flies. To understand how host use patterns emerge it is important to directly examine female oviposition behavior (van Lenteren 1981) and how factors such as fruit characteristics influence the choices females make. Another important factor to examine is the use of a potential marking pheromone in this system. Preliminary field cage assays suggest that *R. juglandis* utilizes a marking pheromone which decreases reuse (C.R.N. and D.R.P., unpublished data). Future studies will directly examine female oviposition behavior and how marking pheromone may influence patterns of host utilization in the field.

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