Influence of Bacteria on Larval Survival and Development in Rhagoletis (Diptera: Tephritidae)

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ABSTRACT The effect of the bacterium Klebsiella oxytocca on the fitness of Rhagoletis pomonella (Walsh) and R. suavis (Loew) was tested by removal and reinfestation experiments in the laboratory. The absence of the bacterium did not positively or negatively affect most components of fitness studied. These results suggest that larvae of Rhagoletis do not depend on gut microflora to provide essential nutrients or to detoxify plant secondary compounds.

KEY WORDS Insecta, Rhagoletis, symbiosis, Klebsiella oxytocca

INSECTS ARE OFTEN so intimately associated with specific bacteria or other microorganisms that the relationship can be considered symbiotic (Buchner 1965). The degree of intimacy can vary from eco-symbiotic relationships where a microorganism lives outside the body of the insect (Weber 1972, 1979) to intracellular endosymbiotic relationships (Awahmukalah & Brooks 1985, Ishikawa & Yamaji 1985) where the microorganism is housed in certain cells (mycocytes) or aggregations of cells (mycotomes). Perhaps the most common, but least understood, symbioses are extracellular endosymbioses, in which the microorganisms occur within the lumen of the insect’s digestive tract (Mansour 1954, Brooks 1963, Hill et al. 1976, Eymann & Friend 1983, Fitt & O’Brien 1985). The tendency among insect–microorganism symbiosis researchers has been to view extracellular endosymbioses as mutualistic, where the microorganism plays a role in the nutrition or protection of the host insect (Blewett & Fraenkel 1944, Jurzita 1979, Martin 1979, Ratner & Stoffolano 1984), and the insect provides food, environmental buffering, and increased dispersal to the microorganism (Jones 1984). Unfortunately, except for a few exhaustively studied cases such as the physiological interaction between termites and their digestive tract microbiota (Breznak 1982), experimental evidence in support of a mutualistic interaction between insects and extracellular endosymbionts is scant. Even more disturbing is the frequency with which an apparently obligate or close association between an insect species and a particular microorganism breaks down upon closer examination.

Perhaps no group illustrates the unsettled nature of our understanding of insect extracellular endosymbiotes better than the Tephritidae. Buchner (1965) contended that, among Diptera, the Tephritidae display the closest association with bacterial symbionts. Early work indicated that all tephritids are associated with one or more bacterial symbionts (Petri 1910, Stammer 1929, Allen et al. 1934, Hellmuth 1956) and that the flies possess morphological accommodations for these bacteria, with the Dacinae being most highly specialized (Stammer 1929, Girolami 1973). The best-studied tephritid, with regard to bacterial symbiosis, is Dacus oleae (Gmelin), the olive fruit fly. Initial studies suggested that D. oleae enjoys an intimate relationship with the olive knot bacterium, Pseudomonas savastanoi, and that the presence of the bacterium is essential for normal development, survival, and reproduction (Petri 1910, Stammer 1929, Fytizas & Tzanakakis 1966, Hagen 1966). However, more recent investigations have failed to recover P. savastanoi from the esophageal bulb of field-collected D. oleae (Yamvias et al. 1970, Luthy et al. 1983), and it now seems that D. oleae is not involved in a symbiotic association with a specific bacterium.

A similar pattern of change exists in the evidence relating to bacterial symbioses in the tephritid genus Rhagoletis. In the earliest work on the genus, T. C. Allen and his colleagues (Allen 1931, Allen & Riker 1932, Allen et al. 1934) demonstrated that a bacterium identified as Pseudomonas (Phytomonas) melophthora was associated with various life stages of the apple maggot, R. pomonella (Walsh). Based on Allen’s work, Boush and his co-workers interpreted the relationship between R. pomonella and P. melophthora as an obligate symbiosis and reported obtaining pure cultures of P. melophthora by streaking directly from macerated digestive tracts of R. pomonella larvae (Boush & Matsumura 1967, Miyazaki et al. 1968). However, the picture of a close relationship between R. pomonella and P. melophthora generated by all of the above work has not been supported by subsequent investigations.

Huston (1972), Dean & Chapman (1973), Rositer et al. (1983), and Howard et al. (1985) all failed to find P. melophthora during the course of extensive isolations from the digestive tracts and esophageal bulbs of field-collected and laboratory-reared R. pomonella. Typically, more than one bacterial species was isolated from an individual fly, and the predominant species varied even among

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R. pomonella originating from a single population.

The most common inhabitant of the digestive tract and esophageal bulb according to all four studies was Klebsiella oxytoca (assuming that the taxon XII of Huston [1972] represents K. oxytoca).

Howard et al. (1985) also characterized the esophageal bulb inhabitants of six other Rhagoletis species—R. mendax Curran, R. cornitana Bush, R. electromorpha Berlocher, R. tabellaria (Fitch), R. suavis (Loew), and R. completa Cresson. They discovered a diverse microbial flora, although K. oxytoca predominated in every species of fly except R. tabellaria. Enterobacter agglomerans, another species of the family Enterobacteriaceae, was the bacterial species most frequently isolated from R. tabellaria.

Thus, the bulk of recent evidence supports the view that species of Rhagoletis do not enter into symbiotic relationships with microorganisms. We regard a symbiotic relationship as the permanent or semipermanent association between two forms of life (Steinhaus 1967). In general, the microbial flora of an individual fly consists of enteric bacteria that are widely distributed in nature, and even K. oxytoca, the bacterium most frequently associated with Rhagoletis, is not found in every fly. The cosmopolitan nature of the bacteria found in the digestive tract of Rhagoletis suggests that microorganisms are acquired from the environment. This suggestion is bolstered by the failure of Huston (1972) to isolate bacteria from 8 of 20 eggs and 48 of 70 larvae of R. pomonella collected from apples in the field, and by the finding that K. oxytoca occurs on the surface of apples in the field (unpublished data). However, an eventual association between Rhagoletis and bacteria appears to be virtually inevitable, in that bacteria have been isolated from all adult Rhagoletis studied.

Given the certainty that Rhagoletis will encounter and ingest bacteria, what is the nature of the interaction between the two groups? This question is of vital importance in the light of suggestions that bacteria represent an important food for tephritids (Drew et al. 1983, Drew & Lloyd 1987) and that bacteria may help to mediate host shifts in Rhagoletis (Howard et al. 1985). As noted earlier (Howard et al. 1985), an understanding of the effect of bacteria on the fitness of Rhagoletis calls for studies comparing the fitness of flies separated from bacteria and flies associated with bacteria. Here we report the effect of bacterial removal and reinfection on the development and survival of a laboratory strain of R. pomonella and on the development and survival of R. suavis, the walnut husk fly.

Materials and Methods

Rhagoletis pomonella. The first experiment was carried out on a laboratory population of R. pomonella, designated as Strain 1. This strain origi-
containing two larvae that had been exposed to the same treatment as the experimental larvae. At least three larvae from each set of five apples were removed 10 d after insertion and checked for the presence or absence of bacteria, allowing us to monitor the success of our reinfecion and sterilization techniques. Dissected digestive tracts were macerated in 0.2 ml sterile phosphate buffer as were samples of surrounding apple tissue. Undiluted and serial 10-fold dilution platings of digestive tract homogenate and apple tissue homogenate spread on brain heart infusion agar revealed that 100% of the reinfected larvae harbored bacteria in their digestive tracts (1 × 10^4 to 1 × 10^6 cells), whereas no bacteria were detected in the digestive tracts of axenic and untreated larvae. Similarly, the apple tissue surrounding reinfected larvae invariably contained a large number of bacterial cells, whereas no bacterial cells were found in the apple tissue surrounding axenic and untreated larvae. We characterized 42 colonies isolated from reinfected larvae or from apple tissue surrounding reinfected larvae by using the API 20E (Analytab Products, Plainview, N.Y.) microbial identification system, which includes 23 standard biochemical tests. At least one colony came from each reinfected larva and from each sample of rot. All colonies keyed out to *K. oxytoca* and matched the biochemical profile of isolate #172.

We monitored larval emergence and pupation by sifting the vermiculite lining the trays containing the apples with a #14 sifter (U.S. Standard Sieve Series) every 48 h. Immediately before sifting, the apples were transferred to new vermiculite-lined trays. Pupae were removed individually from the sifter and weighed on a Mettler Type H6T Digital Balance (Hightstown, N.J.), before being placed into sterile Petri dishes (100 by 15 mm) (one Petri dish for each replicate of each treatment) filled with sterilized vermiculite.

We checked the Petri dishes for adult emergence once every 24 h. Upon emergence, adults were transferred into sterilized 4-liter glass jars containing autoclaved wet absorbent cotton, autoclaved sugar, and an autoclaved yeast strip. Because of very serious fungal and bacterial contamination problems in these bottles, we did not attempt to compare systematically the adult survivorship differences among the three treatment groups.

*Rhagoletis suavis*. Infested walnuts were collected from Ingham County, Michigan in the fall of 1983 and held in trays until the larvae emerged and pupated in moist vermiculite. Diapausing pupae were stored at 4°C for 7 mo in vermiculite-filled Petri dishes. Diapause was broken by transferring pupae to a 25°C chamber. The protocol for the experiment with *R. suavis* closely resembled the protocol we used for *R. pomonella*, except for the following differences.

Because of difficulty obtaining eggs from *R. suavis* in the laboratory, only one replicate of each treatment group could be set up. Each treatment group of 20 larvae were inserted into a single black walnut. In the field, one black walnut husk often will harbor 20 or more larvae. Reinfecions were carried out with isolate #317, a representative of *K. oxytoca* obtained from *R. suavis*. To facilitate emergence, the three walnut husks were broken open 17 d after the insertion of the larvae. More than 50% of all larvae in each treatment group pupated within the next 24 h. Six weeks after the walnut husks were broken open, the pupae were placed into a 4°C refrigerator. After 4 mo of storage, diapause was broken by transferring pupae to a 25°C chamber. Adult emergence was monitored daily.

To monitor the success of our reinfecion and sterilization techniques, we set aside three walnuts, each containing five larvae that were either axenic, reinfected, or untreated. Three larvae from each walnut were removed 10 d after insertion and checked for the presence or absence of bacteria by using the protocol previously described for *R. pomonella*. The three reinfected larvae contained a large number of bacteria in their digestive tracts (3.0 × 10^6 to 4.4 × 10^4 cells), whereas no bacteria were found in the digestive tracts of axenic and untreated larvae. All colonies isolated from the reinfected larvae were morphologically identical. Six colonies (two from each larva) were tested with the API 20E microbial identification system and all matched the biochemical profile of isolate #317. Thus, our reinfecion and sterilization techniques appear to have been successful.

**Statistical Analysis.** To test for the effects of the three treatments on survival to pupation and survival to adulthood of *R. pomonella* larvae, we used a loglinear model (BMDP4F [Dixon 1983]). Loglinear models are designed to study the interrelations between cross-classified variables in multiway tables (Dillon & Goldstein 1984). In the two data sets analyzed here, the model is analogous to a three-dimensional contingency table analysis. The advantages of loglinear models over χ² analyses of contingency tables are that they can handle very low expected values and multidimensional designs.

For development time to pupation, we used a two-way analysis of variance (ANOVA) (BMDP2V [Dixon 1983]) to test for effects of treatment differences, replicate differences, and interactions between these factors. We did not use a nested design with replicate apples nested within treatment and block combinations because individual apples were not followed in the experiment. It is important to note that this analysis is more likely to find differences among treatments than is a nested design. Data were square root transformed before analysis to improve their fit to a normal distribution. We used a three-way ANOVA (BMDP4V [Dixon 1983]) to test for the effects of treatment differences, replicate differences, sex, and interactions between these factors on development time to adulthood. Again data were square root transformed before analysis.
There was a bimodal distribution of the data on the mass of *R. pomonella* pupae (likely caused by a difference in size between males and females) that could not be eliminated by transformation, so we calculated cell means and performed a one-way ANOVA (BMDPIV [Dixon 1983]) on the nine measurements to test for differences among the three treatments.

We did not perform multivariate ANOVA on the data from *R. pomonella* because we did not follow individuals through the course of the experiment.

For *R. sucuris*, we used a one-way ANOVA to test for the effect of treatment differences on development time to pupation and on development time to adulthood. In both cases, the data were square root-transformed before analysis. We also used a one-way ANOVA to test for the effect of treatment differences on the mass of pupae.

### Results

**Rhagoletis pomonella**—**Fate**. The number of larvae surviving to pupation in each replicate of each treatment is given by the symbol "n" in Fig. 1. It is clear that an association with *K. oxytoca* is not essential for larvae of *R. pomonella* to survive to pupation. A large number of larvae survived to pupation in the two treatment groups (untreated and axenic) that were consistently free of micro-

![Fig. 1](image)

**Fig. 1.** Number of pupae of *R. pomonella* found in vermiculite-lined trays; trays were sifted every 2 d. n, total number of pupae obtained from each replicate.

![Fig. 2](image)

**Fig. 2.** Number of adults of *R. pomonella* found in Petri dishes; dishes were checked every day. n, total number of adults obtained from each replicate.

organisms (see Materials and Methods). Tests of marginal and partial association indicate the order of magnitude of the change in goodness of fit produced by entering a given effect into a loglinear model. Two effects were highly significant (*P < 0.005*): fate (more larvae pupated than died); and more important, the interaction between treatment and fate (more untreated larvae than axenic larvae and reinfeclted larvae survived to pupation).

The data for survival from pupation to adulthood are displayed in Table 1. Here, differences among the three treatments that were evident in survival-to-pupation data have largely disappeared. There is no longer a significant interaction between treatment and fate according to loglinear models. These findings indicate that the presence or absence of bacteria during the larval stage of development has no effect on the survival of pupae, and that the adverse effects of the benzalkonium chloride wash do not extend into the pupal stage of development.

**Rhagoletis pomonella**—**Development Rates**. An association with bacteria does appear to accelerate larval development in *R. pomonella*. There were significant differences among the three treatment groups in time to pupation (*F* = 3.10, *P < 0.05*) with the reinfeclted group reaching pupation more quickly than the untreated group, which in turn developed more quickly than the sterilized group (Fig. 1). However, the effects of the treatment differences were small and were overshadowed by the differences among the rep-

### Table 1. Fate of pupae from larvae of *R. pomonella*

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Fate</th>
<th>Untreated</th>
<th>Axenic</th>
<th>Reinfeclted</th>
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<tbody>
<tr>
<td>1</td>
<td>Adulthood</td>
<td>15</td>
<td>14</td>
<td>6</td>
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<tr>
<td></td>
<td>Death</td>
<td>2</td>
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<td></td>
<td>Total</td>
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<td>16</td>
<td>8</td>
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<tr>
<td>2</td>
<td>Adulthood</td>
<td>16</td>
<td>10</td>
<td>13</td>
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<td></td>
<td>Death</td>
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<td>3</td>
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<td>Total</td>
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<td></td>
<td>Death</td>
<td>1</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>19</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

Untreated, reared without treatment; axenic, reared without gut microflora; reinfeclted, reared with gut microflora.
licates ($F_{1,120} = 14.73, P < 0.005$). There was no significant interaction between replicates and treatments.

The effects of the difference in larval development rates are hardly apparent in the time-to-adulthood data (Fig. 2). Adults emerged slightly sooner in the reinfected treatment group than in the other two groups, but the difference was not statistically significant ($F_{1,160} = 0.31, P > 0.50$). Sex was the only factor with an effect on time to adulthood approaching significance ($F_{1,160} = 3.047, P = 0.10$). Females tended to develop faster than males. Females also emerge before males in the field (Boller & Prokopy 1976).

Because we measured the same individuals to obtain the time-to-pupation data and the time-to-adulthood data, we do not view our ANOVA tests as independent. Nevertheless, they do provide insight into the two data sets and are therefore worthy of reporting. We could not use a repeated-measures ANOVA because we did not follow individuals through the course of the experiment.

*Rhagoletis pomonella*—*Mass of Pupae*. There were no significant differences in the mass of pupae among treatment groups (Table 2) ($F_{1,29} = 1.337, P > 0.32$).

*Rhagoletis suavis*. The number of *R. suavis* larvae surviving to pupation in each treatment group is shown in Fig. 3. It is clear that the presence of bacteria in the gut is not essential for *R. suavis* larvae to metabolize the walnut husk and develop to the pupal stage. There was no significant deviation from random in the distribution of numbers of pupae among the three treatment groups ($\chi^2, P > 0.1$). Moreover, there were no significant differences in the mass of pupae among the three treatments (mean mass of untreated, 0.0146 g; axenic, 0.0142 g; reinfected, 0.0156 g; $F_{1,2,40} = 0.85, P > 0.25$).

The absence of bacteria also does not affect the rate of development to pupation (Fig. 3). Larvae in the reinfected group pupated slightly sooner than did larvae in the sterilized and untreated groups, but the differences were not statistically significant. In all three treatment groups, the largest number of pupae were found on day 18, the day after the walnut husks had been broken open.

Survival of *R. suavis* larvae to adulthood was relatively poor in all treatment groups (Fig. 4). Regardless of the poor performance, there was no significant deviation from random in the distribution of numbers of adults among the treatment groups ($\chi^2, P > 0.1$), indicating that the presence or absence of bacteria during the larval stage of development does not significantly affect survival to maturity. In addition, there were no significant differences among the three treatment groups in time to adulthood (Fig. 4) ($F_{1,2,19} = 1.42, P > 0.25$). Thus, development rate to adulthood is also not affected by the presence or absence of bacteria during larval development.

**Discussion**

**Bacteria as a Nutrient Source.** The larvae of *Rhagoletis* are highly host-specific frugivores (Boller & Prokopy 1976), whereas the adults are believed to feed primarily on homopterous honeydews (Hagen & Tassan 1972). Because both types of food are low in nitrogen (Burroughs 1970, Hagen & Tassan 1972) and have been reported to lack one or more essential amino acids (Miyazaki et al. 1968, Boush et al. 1969, Salamo & Risik 1969, Burroughs 1970), much attention has focused on the possibility of...
that bacteria provide amino acids and other nutrients to *Rhagoletis*, either by secreting the nutrients into the environment or by being digested.

In one study assessing the nutritional significance of bacteria to *Rhagoletis*, Miyazaki et al. (1968) demonstrated that a bacterium isolated from *R. pomonella* and identified as *P. melophthora* could synthesize methionine and cystine, two essential amino acids. The tentative conclusion of the investigation was that *P. melophthora* might provide amino acids to *R. pomonella*. Adopting a more direct approach, Tsipouridou (1981) reported that the incorporation of antibiotics into a number of chemically defined diets adversely affected adult survival, the preoviposition period, and the fecundity of *R. completa*. He went on to infer that microbial associates provide vitamins, amino acids, and possibly minerals to the fly. Unfortunately, Tsipouridou included no controls in his experiments for toxic reactions to antibiotics. There also is a more general problem with the use of chemically defined media in removal experiments. Such media rarely, if ever, mimic the texture, aroma, or nutrient composition of an insect's natural food substrate. Thus, they are poor substitutes for a natural diet when one is attempting to make an initial assessment of the nutritional significance of microbial associates to an insect.

The results reported here show that when *R. pomonella* larvae are reared on apples (a natural food) in the laboratory, they do not appear to be nutritionally dependent on bacteria. In the majority of the components of fitness studied (survival from pupation to adulthood, development time to adulthood, mass of pupae), we found no significant differences among untreated larvae, larvae reared axenically, or larvae reinfected with a clone of *K. oxytoca* (see Tables 1 and 2, Fig. 2). There were significant differences among the three groups in the numbers of larvae surviving to puation; the untreated group displayed greater survivorship than the axenic group, which in turn did better than the reinfected group (Fig. 1). Because the testing program carried out during the course of the experiment indicated that untreated larvae were not carrying bacteria (see Materials and Methods), differences between the untreated and axenic groups should be attributed to the effects of a benzalkonium chloride wash rather than to the presence or absence of bacteria. The influence of bacteria would manifest itself in differences between the reinfected group and the axenic group. Thus, the presence of bacteria appears to have no effect or a slightly detrimental effect on larval survival.

The only component of fitness that was potentially positively affected by the presence of gut microflora was development time from hatching to puation (Fig. 1). Individuals reinfected with *K. oxytoca* reached puation faster than axenic individuals. A faster development rate to puation would be selectively advantageous if larvae of *R. pomonella* are more susceptible to predation and parasitism than pupae, or if flies lacking bacteria develop so slowly that they are unable to complete development in various midseason and late-season fruits before the onset of freezing temperatures. At present, the validity of these suppositions is unknown. It also must be noted that the differences among the three treatment groups were less dramatic than the differences among the three replicates.

Perhaps the most impressive aspect of the *R. pomonella* data is the similarity among the treatment groups in the various parameters measured. One implication of this similarity is that *R. pomonella* does not depend on bacteria to provide larvae with nutrients necessary for development and survival to adulthood. A second implication is that *R. pomonella* is not harmed by the presence of *K. oxytoca* in its gut.

A confirmation of our results can be found in the earlier unpublished work of Huston (1972). Huston disinfected eggs of *R. pomonella* to eliminate bacteria and then monitored the development and survival of larvae reared in apple, either axenically or with a variety of bacteria isolated from the fly. He discovered that the presence or absence of bacteria did not affect larval survival, larval development rate, or the mass of pupae. However, there is a major difference between Huston's methods and those reported here. The bacteria he used for reinfection are only infrequently associated with *R. pomonella*, which left open the possibility that he missed the beneficial effect of a common microbial associate. Our results render this a less likely possibility.

**Bacteria as Detoxification Agents.** Boush & Matsumura (1967) mentioned the possibility that the bacteria associated with *Rhagoletis* help to detoxify harmful host plant compounds, thereby protecting the fly; and they demonstrated that a bacterial culture (identified as *P. melophthora*) could break down a variety of pesticides, apparently through the hydrolytic action of strong esterases. Because we used picked, mature apples (which are low in phenolics [Hulme & Rhodes 1971]) in the *R. pomonella* removal experiment, the results are not particularly pertinent to the issue of host plant detoxification. However, our work with *R. suavis* is germane to the issue.

The larvae of *R. suavis* eat the husks of *Juglans* (walnut) species, a food that is extremely rich in phenolics and quinones (Leistner 1981), such as juglone. The consumption of such a potentially toxic diet would suggest that if any species of *Rhagoletis* needs help with food detoxification, it is *R. suavis*. Yet, larvae of *R. suavis* reared axenically did not exhibit diminished fitness relative to larvae reared with bacteria (see Results, Fig. 3 and 4). Apparently, *R. suavis* larvae do not require gut microflora to metabolize or detoxify the walnut husk. Given that *R. suavis* possesses the biochemical machinery necessary to deal with the toxic compounds present in walnut husks, it seems unlikely
that other species of *Rhagoletis* require help from bacteria for food detoxification.

**Adult Fitness.** We did not rigorously compare components of adult fitness among our treatment groups because of contamination problems in the axenic groups. However, Huston (1972) reported that the presence of a strain of *Serratia marcescens* in the digestive tract of apple maggot adults resulted in an increase in fecundity compared with axenic adults. Unfortunately, *Serratia marcescens* has only rarely been isolated from apple maggots (Huston 1972, Dean & Chapman 1973, Howard et al. 1985), and contamination problems prevented Huston from assessing the effects of bacteria more commonly associated with *Rhagoletis*. Obviously, to reach a full understanding of the relationship between *Rhagoletis* and their microbial associates, the effect of bacteria removal on adult fitness must be more carefully examined. The importance of such work is underscored by results with *Dacus tryoni* Froggatt, which suggest that in subtropical and tropical Dacinae, bacteria from the alimentary canal are introduced onto the host tree where they may play a significant role in fruit fly biology, as an adult food and as enhancers of host attractiveness (Drew & Lloyd 1987).

Many aspects of the relationship between *Rhagoletis* and microorganisms remain to be studied. For example, in addition to the question of adult reliance on bacteria, there is the question of whether gut bacteria protect the digestive tract of *Rhagoletis* against invasion by pathogenic microorganisms. We feel comfortable in concluding from our results only that *Rhagoletis* larvae do not depend on bacteria to break down refractile compounds in host fruits, to provide essential nutrients, or to detoxify secondary plant compounds.

But these conclusions have important implications for biologists studying *Rhagoletis*. Research on *Rhagoletis*–microorganism symbioses has grown out of efforts to understand host specificity and host shifts among species of *Rhagoletis*, in particular the factors mediating survival on a host fruit. The reasoning has been that if bacterial symbionts play a vital role in larval nutrition or in host fruit detoxification, then it may be the ability of the bacteria to grow in a host fruit that limits the host range of the fly. However, with the premise of a bacterial symbiosis becoming more unlikely (Huston 1972, Dean & Chapman 1973, Rossiter et al. 1983, Howard et al. 1985) and the possibility of larval dependence on bacteria increasingly less tenable, the potential influence of associated bacteria on host shifts by *Rhagoletis* appears to be diminished.

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