Ovipositional preferences of the walnut husk fly, *Rhagoletis completa* (Diptera: Tephritidae) on various fruits, vegetables and varieties of walnuts

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ABSTRACT

The ovipositional preferences of the walnut husk fly, *Rhagoletis completa,* were studied using different fruits, vegetables, and walnut cultivars. Among the fruits and vegetables tested, pear was the substrate most often chosen for egg deposition, followed by nectarine, apple, green pepper, potato, and tomato. The eggs hatched equally well in all the substrates, but the larvae completed their growth and development only in nectarine. Among the varieties of walnuts tested, the most egg punctures per nut were in CVs 'Spurgeon' and 'Manregian' and the most eggs per nut were deposited in 'Manregian'. The females made the fewest egg punctures deposited the fewest eggs per nut in 'Hartley'.

Key words: Oviposition, development, walnut, walnut husk fly, *Rhagoletis completa.*

INTRODUCTION

Tephritid fruit flies have been cultured throughout the world for different research purposes, and have been reared from different hosts. Multivoltine tropical fruit flies are relatively easy to rear (Steiner and Mitchell 1966), whereas the univoltine temperate flies including the walnut husk fly (WHF), *Rhagoletis completa* Cresson (Diptera: Tephritidae) are difficult to culture in the laboratory. Different techniques such as wax domes, gelatin balls, paraffin foam balls, and agar balls have been tried for oviposition, with varying degrees of success (Hagen *et al.* 1963, Prokopy 1966, Haisch and Boller 1971). Foam rubber spheres of about 5 cm diam, wrapped in parafilm, were developed by Hagen as oviposition devices for the WHF (Cirio 1972). We have tested different artificial diets for rearing WHF larvae without much success; consequently, this insect has never been satisfactorily reared in the laboratory on artificial diets. Larval diet seems to be the major difficulty to continuous rearing.

The WHF is a major pest of walnut, but has also been recorded from peaches and nectarines. We have found breeding populations of WHF from a peach orchard in Washington state, although such occurrences are rare. Differential susceptibility of walnut varieties to WHF was shown by Boyce (1934) in California. Reported here are the results of an ovipositional preference study dealing with different fruits, vegetables and selected varieties of walnuts. The larval development and survival in the different substrates is also reported.

MATERIALS AND METHODS

Different fruits and vegetables were tested including the 'D'Anjou' pear *(Pyrus communis),* 'Golden Delicious' apple (*Malus domestica*), 'Supreme' nectarine (*Prunus* *persica*), 'Pontiac' red potato (*Solanum tuberosum*), 'Jupiter Premarilla' green bell pepper (*Capsicum annum*), and ripe 'Roma' tomato (*Lycopersicon esculentum*) using a randomized complete block design to test for ovipositional preference. Adult WHF were maintained at 24±1° C, 80±10% RH and 16:8 L:D photoperiod. A diet consisting of a mixture of yeast hydrolysate, fructose (Nutritional Biochemicals Co., Cleveland, Ohio) and distilled water at a ratio 4:7:10 by weight, respectively, as described by Tsiropoulos (1978), was provided for adults. This diet and distilled water were offered to the flies continuously on sterile absorbent cotton. Twelve mated pairs of mature flies (18-20 days old) were confined in each of four wooden-frame cages (35x25x40 cm) containing one each of the six substrates described above. The flies were given 48 hr to oviposit. The experiments were repeated 4 times, and the substrates were examined for numbers of punctures, and eggs in each puncture. The data were analyzed using ANOVA and means were separated by Duncan's multiple range test (significant at $p \leq 0.05$).

Substrates provided to the flies for egg laying were dissected to obtain eggs. One hundred eggs (<24 hr old) were removed randomly from each substrate and placed separately on moist filter paper in petri dishes, for each substrate. The petri dishes were placed in cardboard cartons and then transferred to a growth chamber at 24±1°C, and 80±10% RH. The eggs were examined daily until the 8th day after first hatch, then discarded.

To determine the development of larvae in different substrates, oviposited substrates (four of each kind) with 1-2 oviposition punctures were placed in plastic containers (18.5x13x11 cm) (Tri-State Plastics, Dixon, NY) with sifted vermiculite in the bottom so that mature larvae could pupate. These containers were placed in a rearing room maintained at 24±1°C, 80±10% RH, and 16:8 L:D photoperiod, for larval development. Pupae collected from nectarines, were stored at 3°C for four months and then moved to a rearing room for adults to emerge.

Eggs laid in apples in the laboratory were removed and surface sterilized in 0.01% sodium hypochlorite solution for 3 min, rinsed in 70% ethyl alcohol and then washed in distilled water (Neilson 1969, AliNiazee and Brown 1977). These eggs were then placed on wet filter paper in petri dishes and transferred to a growth chamber maintained at 24±1°C, and 80±10% RH for hatching. Newly hatched larvae were transferred with a fine camel hair brush to two different artificial diets developed for the closely-related larvae of the western cherry fruit fly, *Rhagoletis indifferens* (AliNiazee and Brown 1977) and the apple maggot, *Rhagoletis pomonella* (Neilson 1969) in 5 cm diam petri dishes and larval development was checked.

To determine the oviposition of WHF on five walnut varieties, a randomized complete block design was used for the treatments, replicated four times. The experiment was set up in a rearing room maintained at 24±1°C, 80±10% RH, with a photoperiod 16:8 L:D. Nuts of varieties 'Franquette', 'Spurgeon', 'Mayette', 'Manregian', and 'Hartley' were obtained from unsprayed orchards near Junction City, Oregon. They were thoroughly checked for oviposition punctures, and clean nuts with 5 cm twigs were placed in 35 mL plastic cups with water. One nut of each variety was hung in its cup in each of four wooden cages (35x25x40 cm). Five mated pairs of mature flies (18-20 days old) were released in each cage. Food and water were provided to flies as described. After 48 hr the nuts were examined for oviposition punctures, and eggs in the punctures. The data were analyzed using ANOVA and means were separated by Duncan's multiple range test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The data (Table 1) show a significant ($p \leq 0.001$) difference in ovipositional response of WHF to different fruits and vegetables provided for oviposition. The highest numbers of egg punctures per substrate were in pear (8.7±0.5, mean±SD), followed by nectarine (5.5±0.9), apple (3.0±0.4), green bell pepper (1.7±0.5), potato (1.2±0.2), and tomato (0.5± 0.3). The number of eggs laid was also significantly different. The highest total number of eggs per substrate was laid in pear (227.5±10.4, mean±SD) followed by nectarine (69.7±16.6), apple (37.7±8.6), pepper (17.0±3.7), potato (10.0±2.2), and tomato (5.5±3.28). The substrate with the highest number of punctures also had the most eggs and the substrate with the lowest number of punctures had the fewest eggs.

Table 1

Ovipositional preferences of female *Rhagoletis completa* for various substrates in the laboratory.

OVIPOSITIONAL SUBSTRATE	PUNCTURES/ SUBSTRATE MEAN ± SD	EGGS/ SUBSTRATE MEAN ± SD	EGGS HATCHED (%)
Pear	8.7 ± 0.5 a	227.5 ±10.4 a	92
Nectarine	5.5 ± 0.9 b	69.7 ±16.6 b	92
Apple	3.0 ± 0.4 c	37.7 ± 8.9 c	90
Pepper	1.7 ± 0.5 cd	17.0 ± 3.7 cd	84
Potato	1.2 ± 0.2 de	10.0 ± 2.2 cd	86
Tomato	0.5 ± 0.3 e	5.5 ± 3.3 e	88

Means in a column followed by different letters are significantly different (Duncan's multiple range test), $p<0.05$.

Cirio (1972) reported that the female WHF probably selects an ovipositional substrate based on visual stimuli, such as color, shape, size, and other surface characteristics and to a certain extent on the internal humidity. It appears that pear must closely resemble walnuts in physical qualities to elicit such a high ovipositional response from WHF (Table 1). Although no walnuts were included in this trial for direct comparison, indirect comparison of data shown in Table 2, taken under the same conditions, suggests that more eggs were laid in pears than in walnuts.

Eggs obtained from various substrates hatched on moist filter paper (Table1), and there were few apparent differences. For example, about 92% of the eggs obtained from pears and nectarines hatched followed by 90% from apples, and 84% from green bell peppers. However, larval survival was not uniform. In potatoes, tomatoes and green peppers the larvae died soon after hatching, whereas in pear and apple, some larvae reached the second and even third instars but died before pupation. In nectarine the larvae pupated successfully. In an earlier study, Boyce (1934) had found peach to be a suitable host for this insect, while other fruits tested including naval orange, tangarine, grapefruit, apple, pear, grape, potato, egg plant, bell pepper and tomato were unsuitable.

The WHF larvae did not develop on the artificial diets suitable for cherry fruit fly and apple maggot larvae, although some larvae moulted to the second and even third instars but all died before pupation. These media might support larval development with some additional ingredients and changes. Unusual growth of mold on artificial diets may also have interfered with larval growth.

Rhagoletis flies are well known for developing races specific to particular hosts (Bush 1969, Boller and Bush 1974). Bush (1969) speculated that the apple maggot developed different sympatric races as a result of shifts to unexploited hosts. The shift of apple maggot from its native host hawthorn (*Crataegus* spp.) to domestic apple (*Malus domestica*) in eastern North America over one hundred years ago, has now resulted in partial reproductive isolation for these two populations (Feder *et al.* 1988). McPherson *et al.* (1988a,b) found significant differences in allele frequencies between apple maggot fly populations raised on apple and hawthorn, and these siblings have been cited as example of sympatric speciation through divergence in host plant association (Bush 1969). Seasonal asynchrony caused by different fruit developing at different times is perhaps sufficient to initiate and maintain a restricted gene flow among different fly populations from different hosts thus leading to new host races (Smith 1988). Although no host race formation has been reported in *R. completa,* some genetic changes in different WHF populations collected from walnuts have already been reported by Berlocher (1984) as a result of the introduction of this species to different areas of the western United States.

The data on varietal preference presented in Table 2 show that female flies made significantly ($p \leq 0.05$) more punctures per nut in cultivars 'Spurgeon' and 'Manregian' and deposited the highest numbers of eggs in these two varieties. Significantly fewer eggs were deposited per nut in 'Mayette' and 'Franquette'. The fewest punctures and eggs were found in 'Hartley', which appeared to be highly resistant to oviposition. In a field study, Shelton and Anderson (1990) showed that 'Hartley' was much less susceptible to WHF attack than other varieties tested, including CVs 'Ashley', 'Payne', or 'Serr', over a 6-year study period in California.

Table 2

Ovipositional preferences of female *Rhagoletis completa* for walnut varieties.

VARIETY	PUNCTURES/NUT	EGGS/NUT	EGGS/PUNCTURE
	MEAN ± SD	MEAN ± SD	MEAN ± SD
Hartley	0.5 ± 0.3 a	4.7 ± 2.7 a	9.5 ± 0.5 a
Mayette	1.0 ± 0.0 a	14.2 ± 1.1 bc	14.2 ± 1.1 bc
Franquette	1.0 ± 0.0 a	26.7 ± 5.3 cd	28.0 ± 4.8 d
Spurgeon	3.2 ± 0.2 c	43.7 ± 7.0 de	13.2 ± 1.1 bc
Manregian	2.2 ± 0.2 b	51.0 ± 8.7 e	20.2 ± 2.0 cd

Means in a column followed by different letters are significantly different (Duncan's multiple range test), $p<0.05$.

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