Seasonal Distributions of Eggs and Larvae of *Rhagoletis indifferens* Curran (Diptera: Tephritidae) in Cherries[1]

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Abstract The seasonal distributions of eggs and first-, second-, and third-instar larvae of the western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), in sweet cherries were determined at three sites in central Washington in 2002 and 2003. The egg was the major stage during early, mid and late season. The distributions of eggs (i.e., the percentages of total immature stages that were eggs) were similar all season, but those of first, second and third instars were greatest in late season. First, second and third instars occurred in similar numbers in 2002, but third instars were the most abundant in 2003. Tree quadrant had no effect on egg and larval densities and distributions. The majority of infested fruit had only one egg or larva, but there were significant increases in percentages of fruit with two or $\geq$ three eggs or larvae as percentages of fruit that were infested increased during the season. When there were two larvae in a fruit, one was larger than the other in 90.8% of cases. Results indicate time of season but not location within trees (1.5 to 2 m above ground) has differential effects on egg and larval distributions in fruit and on female oviposition behaviors that may result in multiple infestations and larval interactions. Seasonal effects on immature stages are probably related to developmental times and stage-specific mortality; whereas, effects on adults may be related to reduced availability of unoccupied fruit for oviposition.

Key Words *Rhagoletis indifferens*, egg distributions, larval distributions, cherries

The western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), is the major pest of sweet cherries, *Prunus avium* (L.) L., in the Pacific Northwest of the U.S. The fly lays eggs into cherry fruit mostly in June and July, resulting in larval infestations that can prevent export of fruit to other countries or transport of fruit between states within the U.S. Eggs are usually laid singly into fruit, as an oviposition-deterring pheromone deposited by the female deters subsequent egg laying into the same fruit (Mumtaz 1976, Prokopy et al. 1976). After eggs hatch below the fruit surface, larvae tunnel towards the seed, feed on the flesh around it, complete development in about 2 to 3 wks, and exit the fruit, dropping as third instars that burrow into the soil to pupate (Frick et al. 1954).

Although the general biology of *R. indifferens* is known, the ecology of the egg and larval stages is poorly known. The majority of fruit has only one larva (Frick et al. 1954, Messina 1989, AliNiazee 1974), but egg densities, changes in the ratios of eggs to larvae, and larval instar distributions over the season have never been determined.

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These parameters are important for understanding characteristics and development of egg and larval *R. indifferens* populations. They are also important in understanding adult behaviors, particularly oviposition habits, which are difficult to observe in nature over the season. Egg infestations provide more direct inferences of oviposition habits than larval infestations if egg mortality and cannibalism of eggs and larvae occur (Messina 1989). Establishing egg distributions will provide clues to fundamental questions such as when and under what circumstances females oviposit multiple times in fruit.

In this study, the objectives were to determine the seasonal distributions of eggs and larvae of *R. indifferens* in cherries and the frequencies of fruit with multiple infestations over the season. Results are mostly discussed with respect to fly ecology, specifically the relative development of eggs and larvae, adult oviposition behaviors over the season and possible larval interactions. Implications for management and detection of larval infestations in fruit are also included.

Materials and Methods

Study sites and sample trees. Sweet cherry trees (mostly cv 'Bing') were sampled in 2002 and 2003 at three sites in central Washington: Kennewick (46° 13′ N, 119° 8′ W), Yakima (46° 34′ N, 120° 32′ W), and Ellensburg (47° 2′ N, 120° 31′ W). Because commercial orchards were essentially free of the fly, isolated unsprayed trees in residential yards were sampled. Different trees were sampled each year. Trees at all sites were 4.6 to 10.4 m tall and 3.6 to 10.0 m in diameter. In 2002, trees were chosen randomly, but not all these trees had infestations, so in 2003 only trees that had adults, based on sticky yellow panel catches, were used in the study.

Seasonal egg and larval densities and distributions. Three to five trees (≥1.6 km apart) were sampled at each of the sites in both years, for 12 trees per year. To obtain seasonal data, a mean of 91 total cherries (range, 62-179) per tree was collected during each of early, mid, and late periods in the season (except in Ellensburg in 2003, when no sample was made during mid season). In Kennewick these respective periods were 3, 10, and 17 June 2002 and 17 May, 12 June, and 17 June 2003. In Yakima, these were 11, 18, and 25 June 2002 and 3, 11, and 24 June 2003. In Ellensburg, these were 19 and 26 June and 3 July 2002 and 18 June (no middle collection) and 2 July 2003. Fruit development was earliest in Kennewick, intermediate in Yakima, and latest in Ellensburg. To obtain tree spatial data, a mean of 23 cherries (range, 7-47, variation due to cherry availability) was sampled from each of the four tree quadrants (east, south, west, and north) per tree each period. Fruit were collected 1.5 to 2 m above ground (within heights that have highest infestations [Frick et al. 1954]) and their colors recorded visually. Fruit were immediately placed in 70% ethanol and transported to the laboratory where the diameters of five fruit from each quadrant were measured. The skin and pulp of each fruit were teased apart with forceps and thoroughly inspected under a dissecting microscope at 10 to 30×. Numbers of unhatched eggs (white and turgid), found ≤1 mm below the skin surface, and larvae, found around the seed and in the pulp, were recorded. A few larvae were soft, transparent and slender or dark and appeared to have been dead when fruit were collected (normal larvae were white or whitish), but were included in counts because this was not known for certain. All larvae were measured. First instars were 1 to 1.5 mm long, second instars were 2 to 4 mm, and third instars were 5 to 8 mm (Frick et al. 1954).

Infested fruit and distributions of fruit with multiple infestations. Percentages of fruit not infested during the season in 2002 and 2003 were calculated. In 2003,

larval respiration or exit holes in the outside of the fruit were also recorded at all sites for all periods except for Kennewick, where they were only recorded for the late period. The frequency distributions of fruit with different numbers of eggs and larvae (0, 1, 2, ≥3) were determined.

Statistics. Repeated-measures analysis of variance (SAS Institute 2001) was used to test for effects of (1) period and tree quadrant on egg and larval densities, (2) period, tree quadrant, site, and year on distributions of eggs and larvae (i.e., percentages of total immature stages that were eggs and larvae), (3) period on percent of fruit not infested, and (4) period on percent of fruit with multiple infestations. Percentages were subjected to a square-root-arcsine transformation. Each site was a replicate in which the same trees were repeatedly sampled over time (thus trees within sites were not independent units). Means ± SE are reported.

Results

Seasonal egg and larval densities and distributions. Mean fruit diameters from the three sites in early, mid, and late season in 2002 were 15.4 ± 1.4, 20.4 ± 0.5 and 21.7 ± 0.3 mm and in 2003 were 17.2 ± 1.0, 19.9 ± 1.0 and 20.9 ± 0.4 mm, respectively. Colors of the majority of fruit during these respective periods were yellow-orange, red and dark red. In 2002, egg and larval densities increased as the season progressed and fruit matured (eggs: $F = 51.7$, df = 1, 18; $P < 0.0001$; larvae: $F = 40.8$, df = 1, 18; $P < 0.0001$), but in 2003, egg densities were greater in early than late season ($F = 29.6$; df = 1, 14; $P < 0.0001$) while larval densities were greater in late season ($F = 129.7$; df = 1, 14; $P < 0.0001$) (Fig. 1). In both years, egg densities were greater than combined larval densities within each period, although they were nearly equal in late season in 2003 (Fig. 1). There was no effect of tree quadrant on egg or larval densities and no period x quadrant interaction ($P > 0.05$).

Eggs comprised the highest percentage of stages in 2002 and 2003, and were especially high compared with those of first instars in 2003 (Fig. 1, Table 1). Egg distributions were similar all season (50 to 80% of total stages), but distributions of each larval instar differed during the season, with larger differences among seasonal periods seen in third than first and second instars (Fig. 1, Table 1). In 2002, first and second instars were more abundant than third instars in mid season, but all occurred in similar numbers in late season (Fig. 1A). In 2003, third instars were more abundant than the other instars during both mid and late season (Fig. 1B). Tree quadrant had no effect on any stage, and there were no interactions between quadrant and period or site. A site difference was seen with eggs and with first instars and a year difference was seen with second and third instars (Table 1).

Infested fruit and distributions of fruit with multiple infestations. In 2002, 82.8 ± 9.6, 74.9 ± 14.4 and 46.7 ± 17.0% of fruit were not infested in early, mid, and late season, respectively ($F = 15.6$; df = 1, 5; $P = 0.0108$). In 2003, the trend was similar, with respective values of 83.1 ± 11.8, 53.6 ± 19.8 and 23.4 ± 8.5% ($F = 20.2$; df = 1, 4; $P = 0.0120$). Further inspections in 2003 indicated no fruit in early season had holes and few had eggs; whereas, by late season many fruit had one to four larval exit or respiration holes and most fruit had eggs or larvae (Table 2). Similarly, in early and mid season (two sites and one site, data not statistically analyzed), more eggs were found in (1) fruit that had no larvae and no holes than in (2) fruit with no holes but with larvae and in (3) fruit with holes (with or without larvae), but in late season eggs were found in equal frequency ($P > 0.05$) among these three fruit categories (Table 2).

The majority of infested fruit had only one egg or larva regardless of the period of

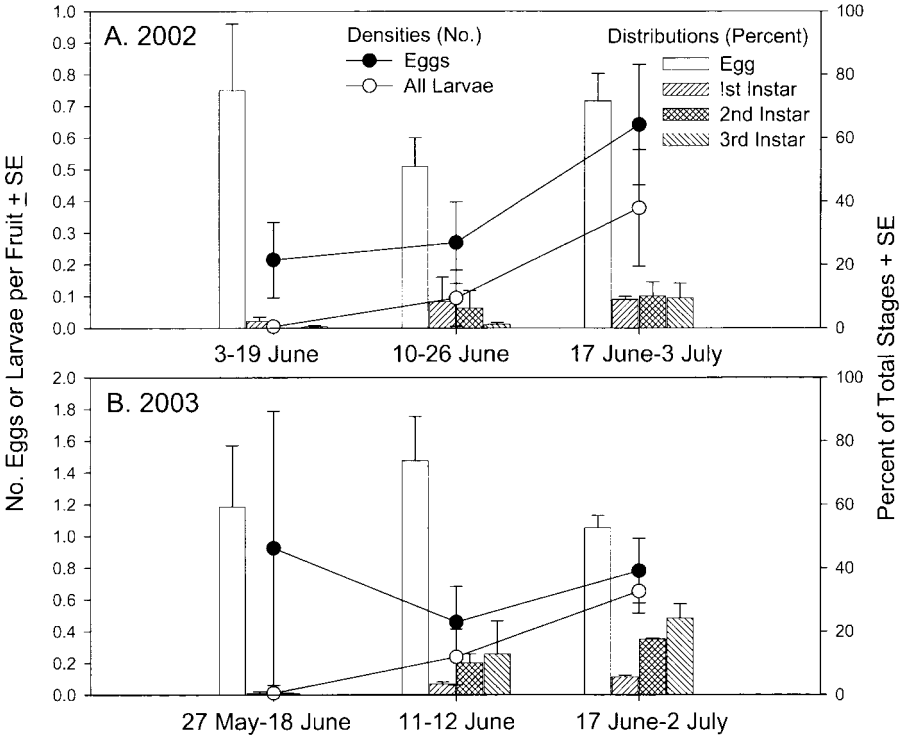

Fig. 1. Mean egg and larval densities and distributions of *Rhagoletis indifferens* in sweet cherry fruit during three periods in A) 2002 and B) 2003 in Kennewick, Yakima, and Ellensburg, WA. Overlapping dates were a result of different fruit development times among sites.

the season (Table 3), but there were significant increases in percentages of fruit with two or ≥3 eggs or larvae as the season progressed in 2002 (eggs: $F = 16.2$; df = 1, 5; $P = 0.0101$; larvae: $F = 7.0$; df = 1, 5; $P = 0.0460$) and 2003 (eggs: $F = 6.2$; df = 1, 4; $P = 0.0731$; larvae: $F = 21.0$; d = 1, 4; $P = 0.0105$). These increases in egg and larval density per fruit coincided with increases in percentages of fruit that were infested from early to late season (Fig. 1, Tables 2 and 3). The maximum numbers of eggs and larvae in a fruit in late season were 9 and 5, respectively. However, in early and mid season 2003, 2 to 8 eggs were seen in single fruit from 5 of 8 trees even though 29.5 to 70.4% of non-infested fruit had no holes. When there were two larvae, one was larger than the other in 90.8% of 184 fruit. The larger and smaller larva were 5.2 ± 0.1 and 3.0 ± 0.1 mm long, respectively ($n = 167$ pairs). The most extreme larval size difference, 6 to 8 mm and 1 to 2 mm, was seen in 14.4% of the fruit.

Discussion

The seasonal increases and differences in egg and larval densities of *R. indifferens* followed clear patterns and have several explanations. The seasonal increases

Table 1. Results of repeated-measures analysis of variance testing effects of various factors on percentages of immature stages of *Rhagoletis indifferens* in sweet cherry fruit

Factor	Numerator df	Egg		1st Instar		2nd Instar		3rd Instar	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Period*	1	0.56	0.457	22.47	<0.001	83.52	<0.001	93.98	<0.001
Tree Quadrant**	3	1.23	0.308	0.43	0.734	0.30	0.827	0.45	0.721
Site	2	39.47	<0.001	5.17	0.009	0.02	0.976	0.44	0.644
Period × Quadrant	3	0.90	0.448	0.41	0.748	0.20	0.895	0.11	0.954
Period × Site	2	30.05	<0.001	1.27	0.290	1.09	0.345	4.77	0.013
Site × Quadrant	6	0.16	0.986	0.50	0.806	0.78	0.593	0.78	0.587
Year	1	1.43	0.237	1.59	0.213	10.29	0.002	25.48	<0.001

Denominator df = 49.

* Early, mid and late season.

** East, south, west and north sides of trees.

Table 2. Percentages of cherries with eggs or larvae absent or present ±SE related to prior infestations as indicated by larval exit or respiration holes and larval presence in 2003

	Eggs or larvae absent		Eggs present	Eggs present	Eggs present
Period**	No holes	With holes	No holes no larvae	No holes with larvae	With holes*
Early	78.6 ± 20.0	0.0 ± 0.0	18.4 ± 17.0	1.2 ± 1.2	0.0 ± 0.0
Mid	76.4	2.2	16.9	0.5	0.3
Late	8.9 ± 2.3	14.3 ± 8.5	10.0 ± 5.3	15.2 ± 7.3	17.3 ± 1.9

* Either with or without larvae.

** Early: two sites, eight trees; Mid: one site, five trees; Late: three sites, 12 trees. Each site considered a replicate.

Table 3. Percentages of uninfested cherries and of cherries with 0, 1, 2, or ≥3 eggs or larvae (all instars combined) ±SE in 2002 and 2003 in early, mid and late season

		2002		2003	
Period	Number	Eggs	Larvae*	Eggs	Larvae*
Early	0	83.2 ± 9.2	99.5 ± 0.4	83.2 ± 11.4	98.8 ± 0.9
	1	12.9 ± 7.1	0.5 ± 0.4	11.0 ± 6.3	1.2 ± 0.9
	2	2.9 ± 1.5	0.0 ± 0.0	3.8 ± 3.3	0.0 ± 0.0
	≥3	1.0 ± 0.6	0.0 ± 0.0	1.9 ± 1.9	0.0 ± 0.0
Mid	0	80.1 ± 9.7	90.9 ± 8.4	67.6 ± 14.0	77.3 ± 16.3
	1	15.3 ± 7.4	8.5 ± 7.8	21.5 ± 7.3	18.6 ± 12.7
	2	3.7 ± 2.2	0.6 ± 0.6	8.4 ± 5.0	3.4 ± 3.2
	≥3	0.8 ± 0.5	0.0 ± 0.0	2.4 ± 1.8	0.6 ± 0.4
Late	0	59.0 ± 12.1	71.1 ± 13.4	51.5 ± 8.5	44.1 ± 10.1
	1	26.3 ± 8.1	21.3 ± 10.8	25.6 ± 1.5	44.5 ± 6.1
	2	10.2 ± 2.8	5.7 ± 3.4	16.7 ± 5.9	10.8 ± 4.7
	>3	4.4 ± 1.5	1.9 ± 1.7	6.1 ± 2.6	0.5 ± 0.1

Means from three sites each year, three to five trees per site.

* All instars combined.

clearly reflect adult emergence patterns (Frick et al. 1954) that coincided with increased fruit maturity (Messina et al. 1991). Densities of eggs were greater than of larvae perhaps because of high oviposition levels and the relatively long developmental time of the egg, which at 24 to 26°C averaged 6 days, compared with 2 to

5 d, 4 d and 8 d for first, second, and third instars, respectively (Frick et al. 1954). The greater egg than larval densities suggest that larval numbers alone (Messina 1989, Messina et al. 1991) underestimate true oviposition levels and that studies using this variable to infer oviposition habits need to account for this.

The seasonal differences between egg and larval distributions and densities also suggest stage-specific mortality. In particular, large discrepancies between numbers of eggs and first instars suggest first instar mortality was high. Few dead first instars (at collection time) were seen, but these could have deteriorated and remained undetected. Low egg, high early instar, and moderate late instar mortality (0.4, 65 and 45%, respectively) have been noted for *Rhagoletis pomonella* (Walsh) in apples (Leroux and Mukerji 1963). Mortality agents of *R. indifferens* larvae in cultivated fruit have not been confirmed, but high temperatures in late season were associated with larval deaths (Frick et al. 1954). Perhaps high heat has more adverse effects on first than third instars, as first instars cannot create large mines to the outside that are used by third instars for breathing or possibly for cooling. The greater numbers of third than first instars in late season (in 2003) may be related to lower mortality in addition to the longer developmental time of the third instar.

In contrast to the effects of season, there was no effect of tree quadrant on egg and larval densities or distributions, which agrees with previous work (Frick et al. 1954, Messina 1989). Oviposition seemed to occur uniformly in all locations within a tree (at least 1.5 to 2 m above ground), despite the varying adult densities in these locations at different times of the day (Yee 2002). Flies may, however, oviposit less on upper than lower parts of trees (Frick et al. 1954). The equal densities and distributions among quadrants are consistent with the hypothesis that the oviposition-deterring pheromone, which is effective for at least 4 and possibly 15 to 20 days (Mumtaz and AliNiazee 1983), causes dispersal and uniform spacing of eggs (Messina 1989).

The amounts of infested fruit and the frequency distributions of fruit with multiple infestations over the season suggest that as more fruit become occupied over the season, females become less discriminating in their choice of oviposition sites. The presence of eggs in fruit with larvae suggests this was the case. It is possible females cannot discriminate between fruit with and without larvae in late season, but *R. pomonella* were deterred from laying into sour cherry and hawthorn fruit containing a single second or third-instar larva (Averill and Prokopy 1987a). Multiple infestations were also seen earlier in the season when many fruit were unoccupied, suggesting females sometimes oviposit multiple times in a single visit without exploring other fruit. Perhaps such behavior represents a vestige of *Rhagoletis* evolution in which ancestors similar to walnut-infesting *R. completa* Cresson (Boyce 1934) oviposited multiple times in fruit. Another explanation is that fewer fruit were susceptible to oviposition in early season.

In fruit with two larvae, one was almost always larger than the other, suggesting egg hatch times were staggered or that negative larval interactions occurred in which one larva reduced the growth of the other through physical or chemical mechanisms. Frick et al. (1954) found that 8 to 16 larvae successfully emerged from single cherries and pupated, but larval deaths were also noted and pupal sizes were not mentioned. In *R. pomonella*, survivorship and pupal sizes were reduced when more than one larva developed per hawthorn fruit (12 to 20 mm diam), possibly through interference competition (Averill and Prokopy 1987b). In *R. indifferens*, the habit of laying single eggs in individual cherries probably evolved in response to the small fruit (8 to 10 mm diam) of native bitter cherry (Mumtaz and AliNiazee 1983), *Prunus emarginata*

(Dougl. ex. Hook) D. Dietr., but maintenance of such a habit may not be as vital in fly populations attacking large cultivated cherries.

Results have implications for management and detection of *R. indifferens* infestations. There is a zero tolerance for larvae in fruit in Washington State, forcing growers to treat with insecticides weekly and requiring that fruit be inspected at packinghouses, even though most commercial orchards have few or no flies. Systemic insecticides such as dimethoate that kill small larvae (Ministry of Agriculture, Food, and Fisheries 2004) may affect the egg and larval instars differently. If so, application rates need to differ depending on the time of season. With respect to larval detection, the current method used is the brown sugar flotation technique (Hass 2001), which depends on inspectors identifying larvae that float to the surface of a sugar solution after cherries are crushed. Its accuracy may be compromised if the majority of larvae during harvest were small and difficult to see, especially if larval densities are low. However, the results show that large larvae occur as frequently as or more frequently than small larvae during late season. This suggests the probability of detecting larvae is relatively high, which should reduce human error associated with the use of the technique.

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The first line of the article is printed in error and should read, "The western cherry fruit fly, *Rhagoletis indifferens* Curran, is the major pest of sweet cherries, *Prunus avium* (L.) L., in the Pacific Northwest of the United States."

Hesler, L. S., Z. Li, T. M. Cheesbrough and W. E. Riedell. 2005. Nymphiposition and population growth of *Rhopalosiphum padi* L. (Homoptera: Aphididae) on conventional wheat cultivars and transgenic wheat isolines. J. Entomol. Sci. 40: 186-196.

The generic name for the bird cherry-oat aphid, *Rhopalosiphum padi* L. (Homoptera: Aphididae), was inadvertently misspelled throughout the article.

Asaro, C., C. W. Berisford, M. J. Dalusky, J. L. McLaughlin, and C. Czokajlo. 2005. Preliminary tests of an attracticide formulation for control of the Nantucket pine tip moth (Lepidotera: Tortricidae). J. Entomol. Sci. 40: 240-245.

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