Effect of Fruit Screening Method on Estimating Number of Oriental Fruit Flies, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), in Host Fruit

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ABSTRACT. The effect of fruit screening method on estimations of the numbers of oriental fruit fly, *Bactrocera dorsalis* (Hendel), in host fruit was studied, using papaya (*Carica papaya* L., variety Kapoho Solo) as the test fruit. In this paper, "fruit screening method" is defined as a collective method of sifting fruits and rearing medium for larvae and pupae of fruit flies, rearing the recovered immature stages to adults, and recovering the emerged fruit fly and parasitoid adults. Six calculation methods were used to estimate fruit fly numbers. Each method simulated a different fruit screening method. Data showed that estimates of numbers of fruit flies in host fruit varied significantly with the fruit screening method (or with the method of calculating the number of flies in infested fruit). This report recommends that fruit screening methods be carefully evaluated against the objectives of the study being conducted, and the proposed application of the data being gathered.

Accurate estimate of the numbers of oriental fruit fly, *Bactrocera dorsalis* (Hendel), in host fruits is of prime importance in surveys to evaluate the efficacy of suppression and eradication control treatments. Methods to estimate the numbers of oriental fruit fly, or of any tephritid fruit fly, in host fruits vary, and consequently, so does the accuracy of the estimate. In surveys where sample size and level of accuracy are not the main concerns, fruits are dissected in the field at the time of sampling, and the presence of developed larvae is recorded (e.g., DeWoskin 1981). A more common method is to hold the fruit in rearing containers for several days to a few weeks, allowing mature larvae to emerge from the fruit. Then the resulting pupae are reared to adults, and either the number of emerged adults or the sum of adults and dead pupae is used as an estimate of population (Harris et al. 1986, Harris & Lee 1989, Liquido et al. 1990, Nishida et al. 1985, Vargas et al. 1983a,b, Wong et al. 1983, 1989). Another method of fruit screening is to sift the fruit and rearing medium, for both living and dead larvae and pupae. The total of emerged adults and recovered dead immatures is then used as the estimate of fruit fly population (Liquido et al. 1989, Liquido & Cunningham 1990). None of these methods, however, consider the number of emerged parasitoids. Logically, in estimating the number of fruit flies in host fruit, a single parasitoid should count as one fruit fly. I outline several other methods which include parasitoid counts in population estimates.

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I evaluated the effect of fruit screening methods on the estimate of oriental fruit fly population in host fruit using papaya (*Carica papaya* L.) as the test fruit. In fruit fly field ecology, "fruit screening method" is defined as a collective method of sifting fruits and rearing medium for larvae and pupae, rearing the recovered immature stages to adults, and recovering adult fruit flies and parasitoids. This paper follows that definition.

MATERIALS AND METHODS

The data presented in this report were extracted from a data base consisting of papayas, variety Kapoho Solo, randomly collected from orchards in Nanawale, District of Puna, Hawaii, between September 1985 and December 1989. Each record in the data base represents one papaya fruit. Each fruit was characterized by degree of visual ripeness based on extent of yellow coloration of the skin (Liquido et al. 1989), and by data on fruit fly infestation. Using DATA STEP and PROC STEP (SAS Institute 1985a), each fruit was assigned a random number. Afterwards, 50 three-quarters to fully ripe fruit with oriental fruit fly numbers ≥ 1 were randomly selected. This process was done 10 times to generate 10 sets of data, each with 50 papaya samples.

Fruit Holding and Screening for Numbers of Oriental Fruit Fly. All papaya samples in the data base were uniformly held and screened as described below. Immediately after field collection, fruit were brought to the laboratory and each was placed in an individual plastic bucket (3.78 liter) that contained a 5-cm layer of wheat bran at the bottom. A 0.64-cm wire mesh, measuring 13 by 13 cm and bent at each corner, served as the platform to hold the fruit 5 cm above the bran. The wheat bran absorbed fruit exudate and served as the pupation medium for mature larvae (Armstrong et al. 1984, Liquido et al. 1989).

Fruit samples were held in buckets for 2 wk. Living and dead larvae and pupae were separated from the bran and rotting fruit with sieves of increasingly smaller mesh. Dead larvae and pupae were preserved in 75% ethyl alcohol. Live pupating larvae and pupae were placed in plastic cups (0.25 liter) containing a small amount of sand for pupation. After 2 wk, emerged fruit flies and parasitoid adults were collected from the rearing container, killed and preserved in alcohol. Dead larvae and pupae were sifted from the sand and also preserved in alcohol. Dead larvae and pupae were individually examined under the microscope and identified using Hardy (1949). Adult parasitoids were identified using Beardsley (1961).

Data Analyses. Data were summarized in the following catagories: number of oriental fruit fly adults, adult parasitoids, and dead fruit fly larvae and pupae. The total number of fruit flies in each fruit was estimated using six methods of calculation (Table 1). These methods of calculation simulated different fruit screening methods (or methods of determining and recording fruit fly infestation data). Methods I, III, and V simulated fruit screening procedures in which adult parasitoids are not recovered and counted. In methods II, IV, and VI, each parasitoid was counted as one

TABLE 1. Methods of estimating number of oriental fruit flies in host fruit.

Calculation Methods	References
I. No. adults	Harris & Lee 1989, Harris et al. 1986 Liquido et al. 1990 Nishida et al. 1985
II. No. adults + parasitoids	
III. No. dead pupae + adults	Vargas et al. 1983a,b Wong et al. 1983, 1989
IV. No. dead pupae + adults + parasitoids	
V. No. dead larvae + dead pupae + adults	Liquido et al. 1989 Liquido & Cunningham 1990
VI. No. dead larvae + dead pupae + adults + parasitoids	

oriental fruit fly. Methods I and II simulated fruit screening procedures which do not recover and count dead larvae and pupae. Methods I, II, III, and IV simulated fruit screening procedures which disregard dead larvae. Method VI simulated the detailed fruit screening technique outlined in this paper.

The variation in number of oriental fruit flies due to different methods of calculation was determined by a completely randomized design analysis of variance, with the ten randomized data sets serving as replicates. Prior to the analysis, the homogeneity of variances was tested by the F_{max} method (Sokal & Rohlf 1981). Means were separated by Duncan's (1955) multiple range test. PROC GLM and MEANS were used for statistical analyses (SAS Institute 1985a,b).

RESULTS AND DISCUSSION

The mean number of oriental fruit flies per fruit varied significantly with the methods of calculation ([Homogeniety test: $F_{max} = 2.11$; df = 6,9; $P > 0.05$) (ANOVA: $F = 25.43$; df = 5,54; $P < 0.05$)]). Mean numbers of oriental fruit fly per fruit based on calculation methods V and VI were significantly > methods III and IV > method II > method I (Table 2). The estimate based on emerged adults alone (Method I) was the lowest. In contrast, the highest estimate included counts of fruit fly larvae which failed to pupate, pupae which failed to emerge, and emerged oriental fruit fly adults and parasitoids (method VI). Analysis of data, therefore, showed that exclusion of dead immatures and parasitoids (each counts as one fruit fly) results in underestimation of fruit fly density in host fruit.

The braconid parasitoids reared from oriental fruit fly included *Biosteres arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead). The combined parasitization rates by these parasitoids ranged from 6-14% when fly density was calculated by method VI. However, when density was calculated by methods II and IV, parasitization rates were 15-26% and 10-17%, respec-

TABLE 2. Estimates of the number of oriental fruit flies in three-quarters to fully ripe papaya (Kapoho Solo) fruits using different calculation methods.

Calculation methods	Mean no. oriental fruit fly per fruit ± SEM[1]
I. No. adults	25.57a ± 1.28
II. No. adults + parasitoids	30.40b ± 1.33
III. No. adults + dead pupae	42.47c ± 1.91
IV. No. adults + dead pupae + parasitoids	44.15c ± 1.94
V. No. adults + dead pupae + dead larvae	47.30d ± 1.94
VI. No. adults + dead pupae dead larvae + parasitoids	48.99d ± 1.98

[1]Means followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).

tively. These differences in parasitization rates indicate that the method of screening fruit flies and parasitoids from host fruit (or the method of calculating the fruit fly density) should be carefully evaluated in assessing the performance of parasitoids in area-wide augmentation or inundation programs.

The accuracy of estimates of numbers of oriental fruit fly, or any other species of frugivorous tephritid fruit fly, in fruit samples depends not only on procedures used in fruit screening, but also on the method of holding fruit in rearing containers. For instance, fruit in rearing containers can be held singly or in group (either a set number of fruit per container or as many fruit as can fit in a container). Furthermore, either sand, vermiculite, or wheat bran (placed at the bottom of the fruit holding container) is generally used as an absorbent of fruit exudate and as a larval pupation medium. The method of fruit holding is known to influence survival of larvae and pupae (N.J.L. unpublished date). So, if the fruit holding method causes high larval and pupal mortality, estimates based on calculation methods I-IV can be very misleading. Whenever possible, available labor permitting, I strongly recommend holding fruit individually, and the use of bran as the fruit moisture absorbent and pupation medium (Liquido et al. 1989, Liquido & Cunningham 1990).

In summary, I have found that the fruit screening procedures and, consequently, the method of calculating the number of fruit flies in host fruit, significantly affect population estimates. I recommend that procedures for recovering fruit flies and their parasitoids be carefully evaluated against the objectives of the research being conducted, and the intended use of the data being gathered. In Table 3, recommendations for proper fruit screening or calculation methods for some specific field studies are listed. Although the data presented here are for oriental fruit fly, these recommendations may be applied to melon fly *Bactrocera cucurbitae* (Coquillett); Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and

TABLE 3. Recommendations for choosing the appropriate method for estimating the number of fruit flies in host fruit, based on the objective of the study.

Objectives	Fruit Screening or Calculation Methods
1. Monitor area-wide efficacy of male annihilation treatments and sterile insect releases	1, 3[a]
2. Evaluate seasonal and temporal trends in population density	1, 3[a]
3. Evaluate efficacy of parasitoid inundative and augmentative releases	2, 4, 6[a]
4. Evaluate competition between parasitoids for hosts in different habitats	2, 4, 6[a]
5. Compare infestation rates among different varieties of hosts	5, 6[a]
6. Estimate number of fruit flies in different commodities for developing quarantine treatments	5, 6[a]

[a]Method highly recommended.

Malaysian fruit fly, *Bactrocera latifrons* (Hendel), because of their similar life histories and niche overlap.

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