Season, Hour of Release and Holding Time as Determinants of Persistence of Mass Reared, Sterilized Mexican Fruit Flies (Diptera: Tephritidae) at a Release Site[1]

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Abstract Persistence of mass-reared, radiosterilized Mexican fruit flies, *Anastrepha ludens* (Loew), released at a target site was measured by trap-back. Released flies persisted in greater numbers during the winter months, with lowest numbers recovered in the summertime. Nocturnal releases were equally effective as morning releases in terms of persistence. Afternoon releases yielded better results in the winter but should be avoided in summer. Lowest persistence occurred in months with temperature extremes.

Key Words *Anastrepha,* fruit flies, sterile insect technique, biocontrol, trapping

The Mexican fruit fly, *Anastrepha ludens* (Loew), is a pest of citrus throughout Central America, Mexico, and the southern border of the United States. In Mexico and the U.S. the sterile insect technique is used to suppress fruit fly populations in commercial growing regions and as an eradication tool for isolated outbreaks (Holler et al. 1984, Gutierrez-Samperio et al. 1990). In Texas the sterile release program is monitored with an extensive grid of McPhail traps to measure coverage of the target area by the released flies, and for detection and surveillance of the pest population (Nilakhe et al. 1991). The sterile flies are marked prior to release with a fluorescent dye so they can be distinguished from wild flies. The sterile flies have an expected mean life span of 5 to 10 d following release (Thomas and Loera 1998), necessitating weekly dispersals for maintaining a high sterile to fertile fly ratio. The reduction in sterile fly captures with time following release is due mainly to mortality, but also, dispersal out of the release area. Therefore, the parameter measured by trap-back is persistence at the release site. Persistence is assumed to be a valid measure of the number of sterile flies in the target area and a low trap-back rate indicates potential program failure (Krafsur 1999). In Texas, with a McPhail trap density of 1 per 50 ha, the summertime releases typically have a much lower trap-back rate (~0.01%) than those in winter (~0.03%) (Thomas et al. 1999). One of the factors thought to reduce persistence of the flies is unfavorable ambient conditions at the time of release. Consequently, program managers schedule dispersals for the less stressful, cooler, hours of the day, usually early morning, in order to enhance persistence.

A series of experiments was conducted for the purpose of determining the effectiveness of this strategy by comparing morning releases to afternoon releases. An

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alternative to the early morning release option is to disperse the flies at night. Although it is known that flies will not fly in darkness, nocturnal releases could position the flies to disperse themselves at sunrise. Therefore, nighttime releases were included in these experiments using trap-back numbers to measure persistence of the sterile population at the target site.

Materials and Methods

Study site. All releases were made in a 55-ha commercial citrus grove with mature, fruit-bearing, valencia orange trees (*Citrus aurantium* L.) at Linares, Nuevo Leon, Mexico. The release site was located in the center of the grove at a point traversed by an irrigation canal bordered by mature pecan trees, *Carya pecan* (Marsh.) Engl. & Graebon. The release site was selected because of the availability of shade, water, and vehicular access. A hygrothermograph and rain gauge were operated at this grove throughout the 2-yr test period.

Sterile flies. Pupae were obtained from the USDA-APHIS Mexican Fruit Fly production facility in Mission, TX. The insects were radio-sterilized in the puparial stage, then held through eclosion to final transport in 16-L plastic boxes with screened windows at the USDA-ARS laboratory in Weslaco, TX. Each box contained 10,000 pupae and was provisioned with a gelled slab containing fructose, water, and yeast hydrolysate (Martinez et al. 1987). The flies were 3 to 4 d old at the time of transport and release. The puparia were powdered with a fluorescent dust which marked the eclosing adults for identification. Based on the experience in Texas, the failure rate of this marking method is less than one in 10,000 flies. The flies for each monthly series, consisting of 30,000 puparia from the same production batch, were separated into three boxes and marked with a different color dust for each release: yellow for the morning, green for the afternoon and blue for night.

Releases. Releases were made in the first week of each month for 23 consecutive months from February 1998 to December 1999. Transport time from the lab in Weslaco to Linares was approximately 5 h. The boxes were transported to the release site in an air-conditioned van with the first release made soon after arrival. All three releases were made within a 24-h period at programmed times. The morning release was between 0800 and 0900 h, the afternoon release at 1500 ± 1 h, and the night release between 2100 and 2200 h. In 1998, the afternoon release was first with the other boxes held in a covered, open-air, tractor shed on site until release that night and the following morning. In 1999, the regimen was changed by scheduling arrival time to the evening so that the nocturnal release was first, followed by the morning and afternoon releases, respectively. This change in regimen was made to test for the influence of holding time in the boxes.

Trap-back. The release point was at the center of a 200-m diameter ring of 12 McPhail traps. A ring configuration was used because wind direction or some other factor might influence the directionality of dispersal. All traps were situated within the grove rather than at the margins to avoid edge effects. Because the grove was trapezoidal in shape some traps were closer to the edge of the grove than others. However, the study grove abutted with neighboring groves at its narrow end, also minimizing the edge effect. Each trap was hung in an orange tree, baited with a 300 ml aqueous slurry of torula yeast with borax. The traps were in continuous operation throughout the 2-yr study, serviced on Friday of each week when the flies were removed and the bait renewed. The dead flies were transported to the laboratory

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where they could be checked under ultraviolet light to separate marked flies from wild flies prior to counting.

Statistical analysis. Student's *t*-test was used for pairwise comparisons of means. Model I ANOVA was used to compare group means. Probabilities of the *t* and *F* values were computed with the software programs TPROB and FPROB (Speakeasy Computing 1987, Chicago, IL). Least squares linear regression was used to test correlations between weather variables and capture success.

Results and Discussion

The persistence of the flies at the release site was consistent with expectations. In this study 99.6% of the captures were made within the 3 wks immediately following release. Of the total 5,595 marked flies captured, 80.7% were caught in the first week, 14.0% in the second week, and 4.9% in the third week after release. This pattern reinforces the conclusion from previous trap-back studies that it is necessary to release flies at weekly intervals to maintain effective pressure on the target population.

There were large differences in trap-back among monthly replicates, from a high of 9.0% of the released, sterile flies in August 1998, to a low of 0.04% in August 1999 (Table 1). On a seasonal basis, the summer months (May-July) had the lowest capture rate with only 1.9% of the released flies trapped back. The winter months (December-January) had the highest trap-back, 3.9% of the flies released, with the intervening spring and fall months at 2.2% and 2.4%, respectively. This result followed the pattern regularly observed in Texas. However, there was so much variance among the monthly rates overall, that when group comparisons were made using ANOVA, the seemingly large differences among seasons were not statistically significant ($F = 0.496$; df = 3, 19; $P = 0.689$).

Weather and trap success. It was postulated that an underlying cause of monthly variation in captures could be the ambient conditions at the release site. Table 2 shows the range of temperatures and precipitation attained during the critical first week following each monthly release. Analysis of these data failed to show a statistically definable effect attributable to weather conditions. There was no correlation between average weekly temperature and fly captures ($r^2 = 0.022$), nor between the high temperature on the day of release and fly captures ($r^2 = 0.17$). There were more flies captured in the hottest weeks (average high > 35°C), (mean = 285.7 ± 349.1, n = 6) than in the coldest weeks (average temp <20°C), (mean = 203.1 ± 229.4, n = 7), but the difference was not statistically significant ($t = 0.512$, df = 11, $P = 0.309$). Likewise, differences in rainfall did not explain the variation. Weeks with precipitation averaged fewer fly captures than dry weeks (220.9 ± 145.3, n = 12; vs 267.5 ± 293.4, n = 11), but again, the difference was not statistically significant ($t = 0.489$, df = 21, $P = 0.315$).

It is unlikely that ambient conditions do not influence survival or activity of the released flies. A greater proportion of months with temperature extremes (high or low) during the week of release had fewer (<70) fly captures (6 of 13 releases), compared to months with moderate temperatures, wherein only one of ten such months had fewer than 100 flies captured. That is, low capture rates most often occurred in months with unfavorable temperatures, but other factors seemed to confound the end result, such that not all weeks with temperature extremes had low trap-back.

The lack of statistically significant trends linking weather variables to fly captures

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Table 1. Monthly trap-back of flies released at three different times as percent of captures (numbers captured in parentheses)

Month-Year	Morning releases	Afternoon releases	Nocturnal releases	Total flies
FEB 98	16.4 (10)	44.3 (27)	39.3 (24)	61
MAR 98	19.8 (59)	57.0 (170)	23.2 (69)	298
APR 98	27.1 (70)	55.0 (142)	17.8 (46)	258
MAY 98	8.0 (4)	54.0 (27)	38.0 (19)	50
JUN 98	0.0 (0)	97.3 (36)	2.7 (1)	37
JUL 98	25.9 (127)	37.9 (186)	36.2 (178)	491
AUG 98	33.7 (302)	29.9 (268)	36.4 (326)	896
SEP 98	33.1 (150)	40.2 (182)	26.7 (121)	453
OCT 98	33.1 (89)	42.4 (114)	24.5 (66)	269
NOV 98	27.5 (19)	46.4 (32)	26.1 (18)	69
DEC 98	33.2 (82)	41.7 (103)	25.1 (62)	247
JAN 99	27.7 (69)	33.3 (83)	39.0 (97)	249
FEB 99	20.0 (6)	64.4 (25)	15.6 (5)	36
MAR 99	40.7 (188)	30.6 (142)	28.8 (134)	464
APR 99	20.0 (46)	33.5 (77)	46.5 (107)	230
MAY 99	46.6 (76)	26.4 (43)	27.0 (44)	163
JUN 99	44.3 (104)	28.9 (68)	26.8 (63)	235
JUL 99	45.0 (77)	25.7 (44)	29.2 (50)	171
AUG 99	27.3 (3)	27.3 (3)	45.4 (5)	11
SEP 99	12.5 (3)	70.8 (17)	16.7 (4)	24
OCT 99	43.7 (76)	24.1 (42)	32.2 (56)	174
NOV 99	34.1 (15)	40.9 (18)	25.0 (11)	44
DEC 99	32.6 (217)	32.2 (214)	35.2 (234)	665
Total flies	1792	2063	1740	5595
Monthly mean	77.9	89.7	75.6	
Percent of total	32.0	36.9	31.1	
Mean monthly percent	28.4	42.8	28.8	

in this study is not unprecedented. Eskafi (1988), Celedonio et al. (1995), and Aluja et al. (1996) reported a lack of correlation of weather with trap success with this species. This is attributable to confounding influences. Hot, dry weather may have a negative influence on survival and activity because food and moisture are less available. But the same influences tend to make the aqueous protein bait used in McPhail traps more attractive, thus increasing trapability (McPhail 1937, Cunningham et al. 1978). A mitigating factor in the present study was that the release site, deliberately chosen because it afforded shade and surface water, allowed the flies the opportunity to select favorable microhabitats and, to some extent, escape the vagaries of adverse weather.

Effect of release time. Interestingly, nocturnal releases were as efficacious as morning releases, in terms of trap-back. Contrary to expectations, flies from the afternoon releases were more persistent at the target site than the flies from the morning releases. A mean monthly rate of 43% of the recaptures were of flies re-

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Table 2. Weather data and monthly fly captures—1998-99. Temperatures (degrees C) and rainfall (mm) during first week following release

Month	Hi	Mean Hi	Lo	Mean Lo	Avg	Rain	Flies
FEB 98	34	26	1	3	14	—	61
MAR 98	35	21	2	11	16	20	298
APR 98	37	33	12	16	25	—	258
MAY 98	41	38	16	20	29	—	50
JUN 98	43	38	20	23	30	10	37
JUL 98	43	40	21	28	34	12	491
AUG 98	40	39	21	22	30	—	896
SEP 98	37	32	20	20	26	20	453
OCT 98	32	29	11	15	22	25	269
NOV 98	29	21	10	14	17	15	69
DEC 98	31	26	5	15	20	12	247
JAN 99	34	29	2	8	18	—	249
FEB 99	32	24	–3	7	15	—	36
MAR 99	39	34	8	17	25	—	464
APR 99	44	40	22	26	33	—	230
MAY 99	39	33	14	18	26	28	163
JUN 99	33	31	19	19	25	178	235
JUL 99	32	31	17	18	24	155	171
AUG 99	38	37	15	18	27	—	11
SEP 99	33	31	12	14	23	—	27
OCT 99	35	30	12	15	23	51	174
NOV 99	27	27	7	12	19	5	44
DEC 99	26	21	–1	3	12	—	665

leased in the afternoon compared to the morning and nocturnal releases at 28 and 29% each (Table 1). This difference was statistically significant using ANOVA (F = 8.31; df = 2, 67; $P < 0.001$). However, this difference only emerged when the capture rates were expressed as a mean monthly percent. Overall, the afternoon flies accounted for 36.9% of the total captures; only slightly higher than the 32.0 and 31.1% from the morning and nocturnal releases, respectively. The greater difference when expressed as mean monthly percent was due to months with lower persistence overall. In the eight months with the lowest total captures (May and June 1998, August and September 1999, and February and November of both years), the afternoon releases were disproportionately represented in the trap-back, accounting for 56% of all captures. These data suggest that whatever factor is depressing the released fly population, it affects the flies released in the afternoon less than those released at other times.

Holding time and persistence. The target of a sterile insect release program is the sexually active adult. The male Mexican fruit fly is not sexually active until approximately 4 d after eclosion (Dickens et al. 1982). For that reason the factory-reared insects are held until they are 3 to 4 d old prior to release. Because each replicate consisted of flies from the same rearing cohort, inevitably some flies had to be held longer in order to be released at different times. The longer holding time might have

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had a negative influence on trap-back success. During the first year of the test the afternoon release was made immediately upon arrival at the site, with the remaining flies held in their boxes awaiting release later that evening and the following morning. In ten of the 11 replicates that year the afternoon flies provided the highest capture rates. Therefore, we reversed the procedure in the second year so that the afternoon release was last. Nonetheless, in the second year of the study the afternoon releases still accounted for more, 36% of the captures, compared to a 33 and 31% share for the morning and night releases. However, a holding time effect was still detectable, because the afternoon released flies accounted for the most captures in only three of the 12 months in 1999, and provided the least captures in five of the 12 months. Thus, holding time, though not an overriding influence, probably accounts for some of the unexplained variation in trap-back success. In a study of releases of sterile screwworms, *Cochliomyia hominivorax* (Coquerel), it was similarly concluded that efficiency in transport and handling prior to release is one of the important factors contributing to persistence of insects at a target site (Thomas and Mangan 1992).

Season and release time. When the effect of release time is examined as a function of season, a trend does emerge. Afternoon releases in the summertime (June to August) were less successful than the morning and night releases: 29.9 vs 35.0 mean monthly percent of flies captured, although this difference was only marginally significant ($t = 1.34$, df = 13, $P = 0.10$). Moreover, compared to other months, the afternoon releases in the summertime resulted in lower mean captures (29.9% vs 43.4%), and this difference was clearly significant ($t = 2.21$, df = 20, $P = 0.02$). Therefore, afternoon releases would be preferred during most of the year, but should be avoided during the summer months when morning releases, or even the nocturnal release option, would be preferable.

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