DEGREE-DAY ACCUMULATIONS AND SEASONAL DURATION OF THE PRE-IMAGINAL STAGES OF THE MEXICAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

DONALD B. THOMAS
U.S. Department of Agriculture, Agricultural Research Service
Subtropical Agriculture Research Laboratory, 2301 So. International Blvd.
Weslaco, TX 78596

ABSTRACT

Degree-day accumulations and puparial duration of the Mexican fruit fly, *Anastrepha ludens* (Loew), in the field was found to fit closely with a degree-day accumulation model developed by Leyva-Vazquez (1988) with laboratory data. Larval development time was more variable, however, and did not agree well with the laboratory based degree-day model. This may have been caused by a tendency of the larvae to remain in the fruit beyond the necessary development time and for subsequent egression to be spread over a period of weeks. Duration of the pre-imaginal stages is strongly a function of season. The puparial stage may be prolonged up to three months in the winter or be as brief as three weeks in the summer. There was no evidence of a winter diapause.

Key Words: Degree-days, population model, citrus pest, diapause, *Anastrepha ludens*

RESUMEN

Se encontró que las acumulaciones de grados-días y la duración del estado pupal en el campo de la mosca mexicana de las frutas, *Anastrepha ludens* (Loew), se ajustaban a un modelo de acumulación de grados-dias desarrollado por Leyva-Vázquez (1988) a partir de datos de laboratorio. El tiempo de desarrollo larval fue más variable y no se ajustó bien al modelo. Esto pareció deberse a la tendencia de las larvas a permanecer en el fruto más allá del tiempo de desarrollo necesario, y a que su salida del fruto ocurre durante un período de semanas. La duración de los estados preimaginales está fuertemente en función de la estación. El estado pupal podría prolongarse hasta tres meses en el invierno, o ser tan breve como de tres semanas en el verano. No hubo evidencia de diapausa de invierno.

Pre-imaginal development in the Mexican fruit fly, *Anastrepha ludens* (Loew), has been studied extensively under laboratory conditions (Darby & Kapp 1933, Baker 1944, Baker et al. 1944, Flitters & Messenger 1965, Celedonio-Hurtado et al. 1988). Flitter & Messenger (1965) state that development time for this species, egg to adult, ranges from 40-90 days under "normal" conditions.

Specific knowledge of pest phenology is an essential ingredient of effective pest management. Models based on population dynamics and the environmental parameters which drive them, almost invariably include the effect of temperature on development time. For example, the appearance of temperate tephritid pests, notably the apple maggot, *Rhagoletis pomonella* (Walsh), and the cherry fruit fly, *Rhagoletis indifferens* Curran, vary greatly from year to year but can be predicted by the standard degree-day method. Suppression operations are planned accordingly (Aliniazee 1976, Laing & Heraty 1984). The potential of a degree-day model for predicting outbreaks of the Mexican fruit fly, an intermittent but serious pest of citrus along the southern border of the United States, has long been recognized. The efforts to develop data for such a model are detailed in the present article and the efficiency of a degree-day based model of development time is assessed.

Leyva-Vazquez (1988) was the first to determine the degree-day accumulations for development time for each pre-imaginal stage under laboratory conditions. He reported 316 ± 10 degree-days for the puparial stage and 291 ± 57 combined degree-days for the egg and three larval instars. Using linear regression applied to the same data, the lower threshold of development was estimated to be at 9.4°C.

The purpose of the present investigation was to validate the degree-day calculations of Leyva-Vazquez (1988) obtained from laboratory experiments by determination of the duration and degree-day accumulations of the pre-imaginal stages under field conditions.

Materials and Methods

All insects used in these experiments were from laboratory cultures maintained at the USDA facility in Weslaco, Texas, using the rearing methods described by Spishakoff & Hernandez-Davila (1968).

Duration of the larval stage was determined by placing infested grapefruit in an outdoor screened enclosure located in the center of a grove of citrus at the Weslaco site. For the purposes of this experiment, the duration of the larval stage was measured from the day of oviposition until the day the larvae egressed the fruit. At 2-week intervals, three fresh grapefruits were placed in a laboratory cage containing 15-d-old adult Mexican fruit flies and exposed to oviposition for a period of 4 hours. At the end of this exposure period each grapefruit was placed separately in 5-liter plastic tubs containing 10 cm of clean sand. The sides and bottoms of the tubs were punctured to allow drainage. Two of the tubs were then placed in the outdoor enclosure, partly buried in the soil so that the level of the sand was at ground level. The third grapefruit was held in the laboratory at 25°C as a control. Beginning after 2 weeks, the minimum time for egg hatch and larval development, the sand in the tubs was sifted daily, except on weekends, to detect the presence of egressed larvae (larvae found on Monday were pooled and scored as if found on Saturday). The fruit was left in the tub until 2 weeks after the last larva had egressed. A recording hygrothermograph was maintained in the enclosure to provide ambient temperature data. This experiment began in July 1994 and continued through December 1995.

Duration of the puparial stage was determined by sprinkling 100 10-d-old larvae into each of five 5-liter capacity plastic tubs containing clean sand to a depth of 10 cm. Before placing the larvae, a small amount of water was sprinkled on the sand and holes poked in the surface with a narrow rod. The larvae were allowed to inter themselves in the sand, a process which normally required less than 10 minutes. Four of the tubs were then transported to the field and placed outdoors. The experiment was conducted from May 1992 to April 1993 at two sites in the state of Nuevo Leon, Mexico, an area in which the Mexican fruit fly is indigenous. One of the sites was a citrus orchard located near the town of General Teran. The other was a grove of wild citrus, *Sargentia greggi* Wats., in a mountain canyon 15 km west of the town of Linares. The experiment was also replicated at the Weslaco site between June 1993 and June 1994, again in the screened enclosure. Details of the environment at these locations and data on seasonal survival rates of the immature stages of the Mexican fruit fly have been described in a separate study (Thomas 1995). Briefly, freezing temperatures are rare in this region and during this study occurred on only one winter night when the temperatures reached -1°C. The winters and springs are typically dry with most rainfall in the summer months.

After two weeks of exposure a pyramid-shaped emergence cone, 60 cm^2 at the base, was placed over each of the individual tubs. Emerged adults accumulated in an inverted glass jar on the top of the cone. These cones were checked daily throughout the study, except on weekends. A recording hygrothermograph was maintained at each site to provide ambient temperature data.

Degree-days (°D) were calculated using the standard weather bureau formula, also known as the Means Method (Pruess 1983, Fry 1983),

$$[\text{Max} + \text{Min}/2] - \text{base}.$$

This formula was used by Leyva-Vazquez (1988) for the Mexican fruit fly and has been used successfully for other tephritid pests as well (Aliniazee 1976, Reissig et al, 1979). All temperature values were rounded to the nearest °C including the base, for which a value of 9°C was used. For statistical analysis, the correlations between degree-days and development time and day length and development time, were calculated using least squares regression (Sokal & Rohlf 1973). In the regression equation, day length was represented as the difference, in days, between the oviposition date and the summer solstice.

Results and Discussion

Laboratory studies have shown that temperature is a dominant factor determining larval development time. Flitters & Messenger (1965) reported 11-12 days larval development time at constant 27°C but were able to extend the larval stage to 125 days using a 12° ± 10°C temperature regime. In the present field study over the course of the year, the duration of the larval stage in grapefruit was found to range from as few as 19 days in May to as many as 69 days for an oviposition in mid-November (Table 1). Although this result would seem to be in accord with the expected effect of seasonally prevailing temperatures, temperature alone may not have been the most dominant factor. There was typically a 1-2 week lag between the first and last larval egress in each test although presumably these larva were exposed to at least similar temperatures. In one test, with an oviposition date in mid-February, there was a 19 day spread between the first and last larval egress. Even in the controls, infested fruit maintained at constant temperature (24°C) in the laboratory, mean larval duration was 23.1 to 37.3 days, with an overall range from 19 to 54 days. It is noteworthy that some larvae in May, a warm month, did not egress until 29 days post-oviposition, while in November, a cool month, the first larva egressed also in 29 days. Accordingly, the time spent inside the fruit by any particular larva is not necessarily reflective of development time per se. Under optimal conditions the egg and larva can complete development in 16 days. It would appear that conditions inside the fruit were not uniform, or at least, did not induce uniformity in the behavior of the larvae with respect to egression. Some reports suggest that larvae egress the fruit in response to environmental cues, rather than as a conclusion to the completion of development or depletion of the food source. McPhail & Bliss (1933) likewise found a range of 18 to 35 days for the larval stage in mangoes held in the laboratory. In field-collected mangoes (Cuernavaca, Mexico) left outdoors (exact date of oviposition unknown) the maximum crawl off date was 44 days. They noted that egression from mangoes was stimulated by rainfall, and that this effect could be induced by drumming or vibrating the fruit.

TABLE 1. DURATION OF THE LARVAL STAGE IN GRAPEFRUIT IN AN OUTDOOR ENCLOSURE OVER THE COURSE OF THE YEAR AT WESLACO, TEXAS: FIRST, LAST AND MODAL EGRESS IN DAYS AND DEGREE-DAYS.

Oviposition Date	No. Pupae	Range (Days)	Mode (Days)	First (°D)	Modal (°D)
Jan 03	65	42-59	58	392	565
Jan 26	244	39-56	41	339	360
Feb 16	153	33-52	44	338	470
Mar 09	189	26-36	28	312	338
Mar 23	70	25-32	26	316	334
Apr 13	200	21-39	25	327	402
May 04	41	19-29	27	332	473
Jun 14	7	33-33	33	659	659
Jul 31	32	23-33	33	381	552
Aug 15	117	22-28	22	420	420
Sep 02	177	20-28	26	346	445
Sep 07	192	22-33	27	388	477
Oct 01	231	20-32	28	323	446
Oct 19	9	39-56	41	447	463
Nov 10	66	29-41	32	395	410
Nov 14	25	63-69	63	479	479

Mean °D = 387 ± 87; 456 ± 84.

They reported that in the absence of rainfall the larvae typically egressed in the early morning hours, which would be the time of highest humidity. Thus, one might conclude that in the absence of a specific entrainment, the larvae trickle out of the fruit over a period of weeks rather than making a synchronized mass exodus. In accordance with the findings of Baker et al. (1944), there was no evidence of a gender related difference in development time as has been reported for *Anastrepha suspensa* (Loew) by Sivinski & Calkins (1990). Of the 616 flies which eclosed on the earliest emergence date of each replicate in the Mexican field studies, 317 were females and 299 males.

The actual temporal spread in egression was mainly a function of the number of larvae produced by the fruit. Among the June replicates only one fruit produced larvae and in this fruit only seven larvae completed development. In this case, all larvae egressed on the same day. Evidently the high summer temperatures inhibit survival, possibly because of dessication of the fruit. Of the twelve replicates between May 24 and August 18, only three produced larvae that egressed and pupariated, while the control fruit in the laboratory each produced in excess of 100 larvae per fruit. By contrast, during the preceding springtime replicates, eleven out of twelve fruit produced larvae. The numbers ranged from 1 to 201 larvae per fruit with a mean of 75 larvae egressing to pupariate per fruit. Not surprisingly, larger numbers of surviving larvae produce a wider egression pattern. This was especially obvious in the control fruit where optimal temperature conditions and a lack of environmental cues triggering egression resulted in as many as 353 larvae developing in one fruit and a spread of as much as 32 days between the first and last larval egress.

Under these circumstances, degree-days was not a good predictor of development time as defined here, time between oviposition and larval egression. Leyva-Vazquez (1988) determined the mean accumulation of degree-days for egg + larval development to be 291 ± 57°d. In this study the mean accumulation for the first egressing larvae in each replicate was 387 ± 87°d and for the modal egression date, 456 ± 84°d. Thus, the larval stage was prolonged relative to that which would be determined from ambient temperature alone and accumulated degree-days was not a good predictor of larval egress. The coefficient of determination (r^2) between degree-days and modal egress was only 0.565, and for first larval egress only 0.490. Those values were not much higher than the predictive value of calendar date relative to day length. The coefficient of determination (r^2) for day length vs modal egress was 0.448. Reissing et al. (1979) also found the degree-day method to be a better predictor of emergence than mean historical calendar date for the apple maggot.

One well known cause of disparity between degree-day predictions and actual development time is the Kaufmann or Rate Summation effect (Worner 1992). Often in the field, development at low temperatures is faster, and development at high temperatures slower than predicted from laboratory studies. This is especially true of motile, herbivorous insects. Various explanations have been offered to account for this effect (Wagner et al. 1984; Hagstrum & Milliken 1991), but it is unlikely that the Kaufmann effect can be evoked as the cause of the disparity. Firstly, the prolongation of the larval stage occurred even in those replicates wherein the ambient temperatures were far from the extremes at which development was retarded in the laboratory (less than 9°C or in excess of 31°C). Secondly, it is doubtful that the temperatures inside a grapefruit resting on the ground would reach the extremes that were experienced in ambient temperatures. Moreover, since the duration of the larval stage in the controls was also extended beyond the minimum development time with egression spread out over a period of weeks, it is doubtful that any temperature based effect was the dominant factor determining the delay in the date of egress.

In contrast with the results from larval duration, the puparial development time closely paralleled the degree-day predictions from the laboratory studies. Leyva-Vazquez (1988) reported 316 ± 10°d mean accumulation between pupariation and adult eclosion. In the field studies conducted at Weslaco the mean puparial stage duration was 304 ± 25°d for the first adult eclosion and 310 ± 24°d for the modal adult eclosion (Table 2). These values were very close to predicted and thus temperature was the dominant factor determining intra-puparial development time. Furthermore, the correlation between eclosion and degree-day accumulation was very high. For earliest eclosion the coefficient (r^2) was 0.966 and for modal eclosion 0.979.

The results obtained from the Mexican portion of the study were more ambivalent. The mean accumulation for the modal egression date at the citrus grove near General Teran was 329 ± 42°d, in reasonably close agreement with the results from the laboratory and the Texas field study. However, the mean accumulation for the modal egression date at the mountain canyon site was substantially higher, 410 ± 46°d. The

TABLE 2. DURATION OF THE PUPARIAL STAGE IN THE SOIL OF AN OUTDOOR ENCLOSURE OVER THE COURSE OF THE YEAR AT WESLACO, TEXAS: FIRST, LAST, AND MODAL TIME TO ECLOSION IN DAYS AND DEGREE-DAYS.

Pupariation Date	No. Flies	Range (Days)	Mode (Days)	First (°D)	Modal (°D)
Jan 10	112	43-50	43	345	345
Jan 24	121	36-42	38	277	297
Feb 07	165	29-36	32	284	299
Feb 22	157	29-34	30	289	305
Mar 07	173	28-31	28	313	313
Mar 21	136	22-24	22	281	281
Apr 04	106	22-24	22	302	302
Apr 18	4	21-21	21	313	313
May 02	65	17-18	17	288	288
May 16	1	16-16	16	277	277
May 23	21	14-15	15	283	305
Jun 28	7	15-15	15	279	279
Sep 09	4	18-18	18	329	329
Sep 20	3	16-17	16	271	271
Oct 04	3	16-21	18	276	303
Oct 18	4	31-35	35	316	350
Nov 01	1	35-35	35	336	336
Nov 15	8	36-37	36	326	326
Nov 29	3	43-43	43	323	323
Dec 13	115	45-56	50	322	328
Dec 27	133	49-52	49	349	349

Mean °D = 304 ± 25; 310 ± 24.

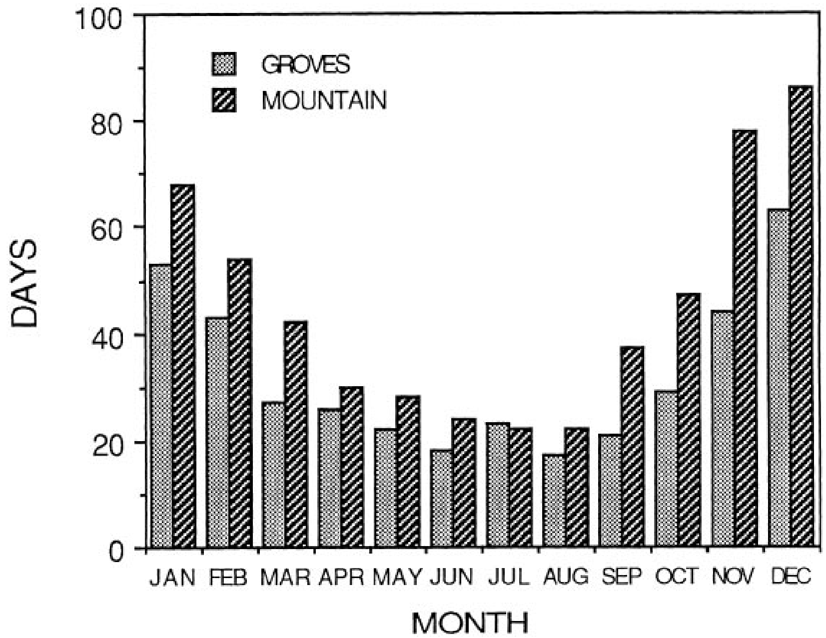

Fig. 1. Length of the puparial stage of the Mexican fruit fly at two localities in northern Mexico: a citrus grove and a mountain canyon.

effect of the slightly cooler mean temperatures at the mountain location is reflected in the graphic representation of these results (Fig. 1). The puparial stage is uniformly prolonged at the higher elevation site relative to the commercial citrus grove location in accordance with expectations. The duration of the puparial stage was shortest (about 3 weeks) during the warmest summer months, and longest during the winter season (prolonged as much as 3 months). Since degree-day accumulations predict this prolongation of the puparial stage, and it can be duplicated in the laboratory, the data suggest that the Mexican fruit fly naturally overwinters in the puparial stage, but not in a true diapause. The greater apparent accumulation of degree-days at the mountain site suggests that actual thermal unit accumulation was less than that calculated by the Means Method. Yellow chapote grows on the east facing slope of the Sierra Madre Oriental, and there may be a montane shadow effect that causes cooler temperatures in the late afternoon compared to the open lowland sites where commercial citrus is cultivated. If so, then an hourly rather than daily heat unit accumulation model may be necessary to predict adult eclosion date in this habitat.

In summary, the results of these studies indicate a significant seasonal effect on generation time. Ultimately the prediction of demographic events such as adult eclosion, seasonality of infestation and number of annual generations will have to incorporate data from the adult reproductive cycle. Temperature is more strongly influential of puparial stage duration than of larval stage duration. Puparial development is so prolonged by low temperatures that overwintering in this stage naturally results without induction of diapause.

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