LIFE HISTORY STUDIES OF THE TOBACCO FLEA BEETLE, *EPITRIX HIRTIPENNIS* (MELSHEIMER) (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

The life history of the tobacco flea beetle, *Epitrix hirtipennis* (Melsheimer) (= *Epitrix parvula* Fab.) was studied under the controlled conditions of 27 ± 2.8°C, 80 ± 6% and a 14L:10D photophase. Eggs matured in ca. 4 days, the larval stage, including 3 instars, developed in 13 days, prepupal development took 3 days and the pupal stage lasted approximately 5 days. There was a 24 day interval between oviposition and adult emergence. Females laid 3.1 eggs/day with a 13 day period between adult emergence and first oviposition. The mean number of total eggs/female was 138.6 ± 14.7. Female oviposition continued until a few days before death and adult longevity was approximately 70 days. A visual means of distinguishing between male and female beetles was also developed.

Key Words: Tobacco flea beetle, *Epitrix hirtipennis*, tobacco, Chrysomelidae.

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INTRODUCTION

The tobacco flea beetle, *Epitrix hirtipennis* (Melsheimer) has long been recognized as a serious pest of flue-cured tobacco (*Nicotiana tabacum*) in the southeastern United States. In addition, the tobacco flea beetle has been recorded as a pest of tobacco in Canada, Mexico, Panama, Cuba, Bahamas, Puerto Rico, Ceylon, and the Philippine Islands. Tobacco appears to be its favorite cultivated host and in the United States the insect is likely to occur wherever tobacco is grown (Metcalf and Underhill 1919). *Epitrix hirtipennis* also feeds upon other species of Solanaceae. The tobacco flea beetle feeds on tomato (*Lycopersicon*), potato (*Solanum*), horse nettle (*Solanum carolinense*), ground cherry (*Physalis* spp.), eggplant (*Solanum melongena*), and jimson weed (*Datura stramonium*), and is frequently quite numerous on tomato and potato plants (Jewett 1926).

The most obvious damage to the tobacco plant occurs from feeding by the adult beetles. This damage is characterized by small pits, holes, or skeletonized leaves. Metcalf and Underhill (1919) observed that the flea beetle is capable of consuming 10.3 times its own weight of tobacco per day.

Overwintering adults emerge in early spring and adult females, deposit eggs on the soil surface around tobacco plants. After hatching larvae tunnel into the soil and feed on the tobacco roots. The mature larva constructs a small cell in the soil and pupates in it. Detailed descriptions of life stages of the tobacco flea beetle have been reported by Jewett (1926), Peterson (1967), Chamberlin et al. (1924) and Blatchley (1910).

Adults are found in tobacco throughout the growing season. The number of generations each year varies with respect to climate and geographical location.

After overwintering adults emerge and the first generation of beetles appear, it is difficult to distinguish one brood from another due to the relatively long lived adults. The objective of this research was to study the life history of the tobacco flea beetle under controlled conditions. Previous work focused primarily on field studies or rearing in unregulated insectory facilities. In the course of the investigation it became necessary to distinguish morphological characteristics of male and female beetles.

MATERIALS AND METHODS

The following procedure for rearing of all stages of the insect in the laboratory was developed to facilitate the study of the life history of the tobacco flea beetle.

Adult Rearing

Adults were collected from tobacco grown at the Coastal Plain Experiment Station, The University of Georgia, Tift County, GA. Containers (15.2 × 10.1 cm) were constructed to house the beetles. Holes were cut in the sides and tops of these containers and securely covered with batiste cloth to allow ventilation but prevented beetle escape. A thin layer of autoclaved soil was placed in the bottom of the containers and 2 ml of water were added as needed to prevent the soil from drying out. Beetles were collected with an aspirator and placed inside the containers. Tobacco leaves (NC2326) were added to the containers as needed. Each day the beetles were removed from the containers and the soil was collected for egg separation. Fresh soil, water and tobacco were then added to the containers, and the beetles were replaced. These containers were maintained in an incubator at approximately 27°C, 80% RH, and on a 14L:10D photophase cycle. Each container maintained a colony of about 500 adult flea beetles.

Egg Collection

Eggs were collected by floating them from the soil using a 20% aqueous magnesium sulfate solution (Horsfall 1956; Waldbauer and Kogan 1973). Samples were then examined under a dissecting microscope to locate the eggs which were removed using a camel hair brush. Eggs were placed in a petri dish which contained several layers of filter paper saturated with water. The petri dishes were then transferred to the above described incubator for ca. four days or until the eggs hatched. The filter paper in the petri dishes was checked daily for larvae and water was added as needed.

Larval Rearing

PJA® plastic plant trays measuring 45.7 × 45.7 cm with 196 cavities each measuring 2.5 × 2.5 × 7.6 cm were used to germinate and grow tobacco seedlings. A Tifton loamy sand, characterized as fine loamy, siliceous, thermic *Plinthic Poleudults* with a pH of 6.6 was used as the plant growing medium. This soil had not been treated with herbicides or insecticides for at least a year and was autoclaved at 1476.5 g/m^2 (ATM) pressure and 121°C to prevent transmission of extraneous organisms into the growing medium.

The soil was dried and passed through a 40 mesh sieve to remove trash and to obtain uniform soil particle size. Each cavity in the plant tray was then filled with

moistened soil. A few tobacco seeds were placed on top of the soil, and a thin layer of soil was added to cover the seeds. The trays were again watered, placed in a greenhouse and watered later as needed. In ca. one week the seeds germinated, the plants were thinned to one plant per cavity, and 10 and 20 days later the plants were infested with larvae.

Five newly hatched larvae were placed near the base of each young tobacco seedling in the plant trays. Trays were then placed in an incubator programmed as described above and equipped with 40 watt flourescent plant growth lights. The plants were watered as needed.

Head capsule measurements were utilized to determine the number and the durations of larval instars (Dyar 1890). Each day a cohort of plants (with roots and soil) were removed from the trays and examined for larvae which were floated out of the soil in an aqueous magnesium sulfate solution. Enough plants were utilized to obtain at least 20 larvae/day. Larvae were stored in a vial of 90% ETOH until head capsule and body length measurements were taken using a calibrated ocular micrometer. This procedure was repeated daily until only adult beetles were found in the soil.

We also compared three soil moisture levels to determine the influence of moisture on oviposition. Three soil moisture levels (dry, moist and wet) were replicated five times in 15.2 by 10.1 cm containers. A colony of 50 male and 50 female tobacco flea beetles was maintained in each container at ca. 27°C, and 80% RH. "Dry soil" was autoclaved and placed in containers, and no water was added. "Moist soil," after autoclaving, was added to containers with approximately 0.5 ml of water/gram of soil. "Wet soil" was placed in containers with water added until the soil approached field capacity (Brady 1974). Containers were checked daily for eggs over a 5 day period. Data were analyzed using SAS (1982), Analysis of Variance and means separated using Duncan's multiple range tests (1955).

To determine adult longevity and fecundity, newly emerged larvae were collected, and placed near the base of tobacco plants (15.2 cm to 25.4 cm in height) grown in 15.2 cm pots filled with autoclaved soil. A cage was placed over each plant to capture adult beetles as they emerged from the soil. After emergence the beetles were sexed and 12 pairs (one male and one female) were placed in 29.6 ml rearing cups with a thin layer of autoclaved moistened soil and a tobacco leaf disc. Soil was removed daily and eggs were floated from the soil in an aqueous magnesium sulfate solution. The number of eggs oviposited was recorded every 24 h. The date that each beetle died was also recorded.

From 19 May through 4 August 1983, 500 flea beetle adults were collected weekly from a tobacco field and sexed to determine the sex ratio. All 500 beetles were collected the same day, in the same general area, and from all parts of the tobacco plant. Data were analyzed utilizing the Chi-square method.

RESULTS AND DISCUSSION

The male and female beetle were distinguished by examination under magnification of the ventral posterior portion of the abdomen. The following is a description of the abdominal sternites provided by Dr. Cecil Smith, Associate Curator for the Department of Entomology, University of Georgia, Athens, GA (pers. comm.). "Apparently the second and third sternites are fused in the Chrysomelidae (male and female) and the first segment is lost. The genital capsule

of the male is the 'pygidium' which is analogous to tergite VIII. In the female the sternites are similar to those of the male, and thus numbered in the same way, while the terminal segment is tergite VIII." The differences in the male and female abdomen are shown in Fig. 1.

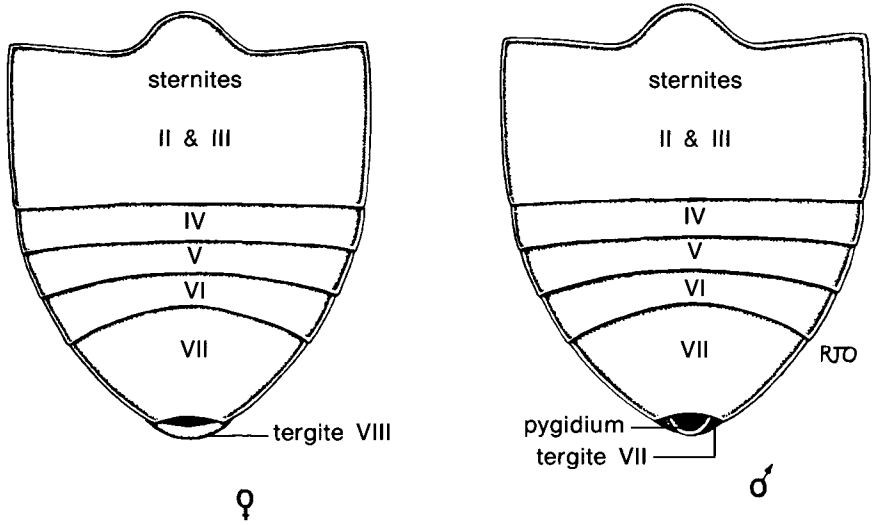

Fig. 1. Ventral view of abdomen of male (Right) and female (Left) tobacco flea beetle, *Epitrix hirtipennis* (Melsheimer).

The mean number (± S.D.) of eggs per 50 females was significantly ($P \leq 0.05$) greater in the wet soil (419.8 ± 44.7) than in the moist (26.6 ± 6.9) or dry soil (13.6 ± 4.6). The range of means were 369 - 480, 18 - 36, and 8 - 20, respectively. In this test soil moisture was not standardized before autoclaving or before additional water was added. So these moisture regimes represent only relative soil moisture conditions. Eggs positioned on wet filter paper, hatched in four days ca. 27°C and 80% RH. Chamberlin et al. (1924) stated that egg incubation periods range from 3 - 11 days in the field, with longest periods being in late winter or early spring when overwintered flea beetles begin oviposition and the shortest periods (3 to 5 days) during the summer months. Egg hatch in less than three days has not been reported.

The mature egg has a yellowish appearance and the head capsule and the posterior edge of the anal plate of the larva can be observed. The larvae ate through or burst a hole in the end of the egg. By constant movement the larvae pull free from the egg and immediately begin searching for food or burrowing into the soil. As larvae are placed on young tobacco seedlings, most begin to crawl down the stalk and disappear into the soil. However, some larvae were observed feeding on young tobacco leaves or on the main stem before moving into the soil.

Larval feeding habits were observed by placing young tobacco plants with washed roots attached in a petri dish containing moist filter paper with a number of larvae. The majority of the young larvae fed on the outside of the young tender

roots of the seedling. A few larvae were observed tunneling into the root and became completely hidden inside. In other instances, larvae were observed severing a root, and then moving on to another. Potential larval damaged resulting in feeding lesions may not only damage the root, but may allow one or more secondary pathogens to enter the root system (Semtner 1984).

Under controlled conditions, the tobacco flea beetle had three instars, a prepupal, and a pupal stage before emerging as adults. The mean width (± S.D.) of 85 first instar, 142 second instar and 87 third instar head capsule measurements was 0.126 ± 0.005, 0.168 ± 0.007 and 0.210 ± 0.005, respectively. The range of means were 0.106 - 0.128 for first instar, 0.148 - 0.170 for second instar and 0.191 - 0.212 for third instar head capsule measurements. Larval development was completed in 13 days with prepupal and pupal development totaling 8 days (Table 1). The length of the larval stage in the field varies with soil temperature and moisture levels.

Table 1. Mean number days (± S.D.) required for the tobacco flea beetle, *Epitrix hirtipennis*, to complete each larval, prepupal, and pupal stage, and adult longevity at 27 ± 2.8°C and 80 ± ± 6% RH.

	Instar					Adult
	1st	2nd	3rd	Prepupa	Pupa	longevity
Mean ±S.D.	3.1 ± 2.6	5.1 ± 2.5	4.5 ± 1.7	3.1 ± 1.4	5.5 ± 2.2	69.6 ± 17.9
Range	3.0 - 5.2	4.3 - 9.0	3.0 - 5.8	1.0 - 4.0	4.0 - 6.0	6 - 89

Newly emerged first instar larvae had a head capsule width of 0.106 mm. As the head capsule completed expansion and sclerotization the measurement increased to 0.126 mm. The length of the first instar varied between 0.55 and 1.28 mm, and averaged 1.10 ± 0.28 mm. As the larvae emerged from the first molt the head capsule measured 0.148 mm, but was 0.168 mm after expansion. The body length of the second larval instar ranged from 1.19 to 3.10 mm and averaged 2.10 ± 0.48 mm. The third instar head capsule, immediately following the second molt measured 0.191 mm and was 0.210 after expansion. The length of the third instar varied between 3.06 and 4.87 mm and averaged 3.63 ± 0.67 mm. These results show that larval length varied too much to be useful in identifying and determining the number of larval instars, but head capsule measurements are adequate to do so.

As larvae approached maturity, they stopped feeding and a small earthen cell was constructed approximately 1.3 - 7.6 cm below the soil surface. The duration of this prepupal stage varied (range = 1.0 - 4.0 days) even under controlled conditions. Body length during this period was reduced to almost half that of the mature larvae and measured 1.9 - 2.7 mm ($\bar{x}$ = 2.4 mm). This stage resembled a shrunken larva.

The pupa remained in the small earthen cell. Age of the pupa may be approximated by its appearance. The pupal stage took 5 - 6 days to develop at 27 ± 2.8°C. On the day after the third molt, pupae were completely white; on the second day they appeared to have a slight pigmentation of the eyes; on the third day the eyes were reddish-brown and the tips of the mandibles were a brownish color; the 4-day-old pupae had almost black eyes, darkened legs and mandibles, and the ventral portion of the abdomen was either a dull grey or a light brown color. The pupae continued to sclerotize until adult eclosion. The only part of the

Table 2. Mean daily egg production for twelve pairs of tobacco flea beetles, *Epitrix hirtipennis,* at 27 ± 2.8°C, 80 ± 6% RH, and 14L:10D photophase.

	Pair number (1 male - 1 female)												Grand
	1	2	3	4	5	6	7	8	9	10	11	12	mean
Mean daily no. eggs produced	2.9	3.5	2.7	3.9	2.8	3.1	2.9	3.5	2.8	3.1	3.4	2.8	3.1
Range	0 - 8	0 - 9	0 - 9	0 - 7	0 - 9	0 - 6	0 - 8	0 - 8	0 - 7	0 - 11	0 - 10	0 - 11	0 - 8.58
Total no. eggs produced during oviposition period	123	160	133	163	134	116	124	138	139	147	153	133	138.58
Preoviposition period (days)	13	14	12	15	13	12	15	15	12	14	16	15	13.8
Oviposition period (days)	43	46	49	42	48	38	43	40	49	47	45	48	44.8

pupae not darkened by the fifth or sixth day was the dorsal portion of the abdomen concealed by the elytra and wings. This remained a greyish color for a day or more even after adult eclosion.

As adult beetles emerged from the soil in the plant trays, they actively began to search for food. Newly emerged adults fed on the underside of the tobacco leaves that were touching the ground or near it.

In order to estimate adult longevity and fecundity 12 female and 12 male beetles were captured as they were emerging from the soil around a tobacco plant on 20 July 1983. No eggs were obtained until 12 days post-ecolosion. All females began oviposition by 16 days post-ecolosion, and a fairly constant rate of egg production continued until 52 - 57 days post-ecolosion, during which a sharp decline was noted. Egg production had ceased entirely by 60 days post-ecolosion, and most beeltes were dead by day 70 post-ecolosion. These findings on longevity are slightly higher than estimates by Chamberlin et al. (1924) and somewhat lower than estimates by Jewett (1926). Tobacco flea beetles have survived as long as 161 days (Chamberlin 1924). The mean number of eggs deposited by the female beetles during the oviposition period also varies (Table 2).

The sex distribution of the tobacco flea beetle over a 12 week period is presented in Table 3. In all but one sampling period there were significantly ($P < 0.05$) more females than males collected, and even in that sample (28 July) counts favored females. This agrees with Elsey (1976) who observed a higher number of female than male beetles in field collections. These beetles were collected at random from all parts of the tobacco plant, through-out the day and in the same general location.

Table 3. Sex distribution in field populations of the tobacco flea beetle, *Epitrix hirtipennis* sampled over a 12 week period. Tift Co., GA, 1983.

Week	No. collected		Ratio: Female/Male	X^2
	Female	Male		
May 19	286	214	1.34	10.37†
May 26	303	197	1.54	22.47†
June 2	305	195	1.56	24.20†
June 9	334	166	2.01	56.45†
June 16	318	182	1.75	36.99†
June 23	310	190	1.63	28.80†
June 30	298	202	1.48	18.43†
July 7	274	226	1.21	4.61*
July 14	321	179	1.79	40.33†
July 21	289	211	1.37	12.17†
July 28	262	238	1.10	1.15
August 4	320	180	1.78	39.20†

* $P < 0.05$.
† $P < 0.01$ (Chi-square).

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