PROC. ENTOMOL. SOC. WASH.
85(4), 1983, pp. 806–810

OBSERVATIONS ON THE BIOLOGY OF *LIRIOMYZA TRIFOLIEARUM* (DIPTERA: AGROMYZIDAE)

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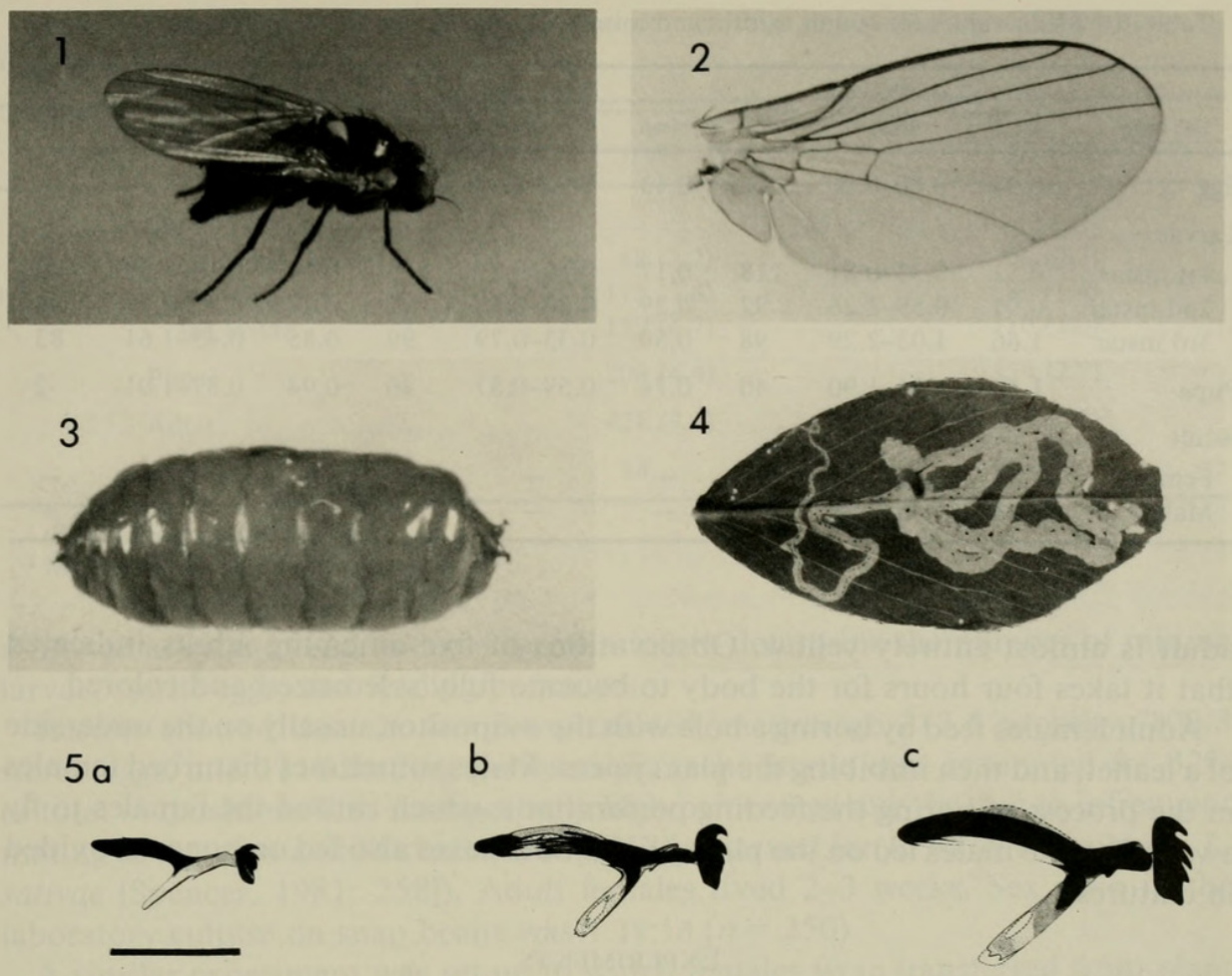
Abstract.—*Liriomyza trifoliearum* Spencer is a native serpentine leafminer attacking alfalfa and other legumes in the USA and Canada. In laboratory tests, fecundity was 213.5 progeny/♀, and number of feeding perforations was 1116/♀. Adult longevity was 2–3 weeks, with a preoviposition period of 4–6 days. The times for development of stages and instars at 21.1°C and 25.6°C were determined. The size of eggs, larvae, adults, and mine widths for each instar was measured. The cephalopharyngeal skeleton of each instar was measured and illustrated. The leafminer was found useful as a host for laboratory rearing of several native and introduced parasite species.

Liriomyza trifoliearum Spencer is a native serpentine leafminer originally described in Spencer and Stegmaier (1973: 107–108). Its range is probably Nearctic on alfalfa, *Medicago sativa* L. (Hendrickson, 1979). Since alfalfa was introduced into North America from Eurasia, it appears this somewhat polyphagous leafminer has accepted alfalfa as an additional host plant. Other reported host plants are *Pisum sativum* L., *Trifolium incarnatum* L., and *T. repens* L. (Spencer, 1973: 99). The leafminer is not an economic pest, with the possible exception of a report by Jensen and Koehler (1970). They observed moderate to heavy infestations of mixed *L. pictella* (Thomson) and *L. munda* Frick (probably *L. sativae* Blanchard and *L. trifoliearum* respectively (Spencer, 1973: 100; Spencer, 1981: 258)) on alfalfa in California. They also reported six parasite species from the two host species. Hendrickson (1979) recovered 19 species of parasites from *L. trifoliearum* in northeastern USA; he also described the low population densities usually encountered in the field. The purpose of this paper is to present notes on the behavior and biology of the leafminer, and on the usefulness of the leafminer as a host for rearing native and introduced parasites in a biological control program against alfalfa blotch leafminer (ABL), *Agromyza frontella* (Rondani). Mass rearing of parasites using ABL as a host is difficult because large numbers of pots of relatively slow growing alfalfa are required, but *Liriomyza trifoliearum* can be abundantly reared on snap beans, *Phaseolus vulgaris* L.

BEHAVIORAL OBSERVATIONS

Oviposition may take place on either the upper or lower epidermis of an alfalfa leaflet, but the lower is preferred. Eggs are positioned between the leaflet epidermis



Figs. 1–5. *Liriomyza trifoliearum.* 1, Adult female. 2, Wing. 3, Puparium. 4, Typical serpentine mine in alfalfa leaflet; a third-instar larva is visible in center. 5, Cepahlopharyngeal larval skeletons: a, first instar; b, second instar; c, third instar. Bar = 0.1 mm.

and the mesophyll. Larvae mine in these same layers until shortly before pupation when they frequently cross over to the opposite epidermis. Newly eclosed larvae usually mine toward the proximate end of the leaflet, then turn, following the central vein for part or all of its length. Some larvae mine in an apparently random manner, even crossing over their own mine and pupate in the distal part of the leaflet. As many as 4 larvae in a single alfalfa leaflet have been observed in the field. Mining larvae sometimes cross over mines of other larvae but remain solitary. Frass is deposited in lumpy trails.

The larva is white at eclosion but soon takes on a yellow cream color. Like other agromyzids, there are three instars. In the late first or early second instar, green gut contents become visible. Large amounts of what appears to be adipose tissue accumulate in the late third (mature) instar and may obscure the cephalopharyngeal skeleton, but otherwise the skeleton is clearly visible throughout larval maturation. During a brief prepupal period, the larva contracts and assumes an ellipsoid shape. Puparia are cream colored initially, darkening to various shades of brown. Pupation occurs within the leaf.

When the adult emerges from the puparium, it passes through either the upper or lower leaflet epidermis, the choice apparently random. The newly emerged

Table 1. Mean values for length, width, and mine width for *Liriomyza trifoliearum*.

Life stage	Length (mm)			Width (mm)			Mine width (mm)		
	Mean	Range	*n*	Mean	Range	*n*	Mean	Range	*n*
Egg	0.24	0.20–0.26	20	0.13	0.10–0.14	20	—	—	—
Larvae									
1st instar	0.51	0.33–0.81	118	0.17	0.10–0.27	119	0.19	0.10–0.39	119
2nd instar	0.91	0.59–1.26	92	0.29	0.20–0.40	92	0.44	0.23–0.79	86
3rd instar	1.66	1.03–2.29	98	0.50	0.33–0.79	99	0.85	0.43–1.61	83
Pupa	1.59	1.31–1.90	40	0.74	0.59–0.87	40	0.94	0.87–1.01	2
Adult									
Female	1.55	1.41–1.61	7	—	—	—	—	—	—
Male	1.41	1.33–1.56	8	—	—	—	—	—	—

adult is almost entirely yellow. Observations of five emerging adults indicated that it takes four hours for the body to become fully sclerotized and colored.

Adult females feed by boring a hole with the ovipositor, usually on the underside of a leaflet, and then imbibing the plant juices. Males sometimes disturbed females in the process of boring the feeding perforations, which caused the females to fly away, then the males fed on the plant juices. Both sexes also fed on honey provided in cultures.

Experiments

The laboratory culture of *L. trifoliearum* was maintained on snap beans. Other host plants acceptable in the laboratory but less productive were fava bean, *Vicia faba* L., lima bean, *Phaseolus limensis* Macf., and red clover, *Trifolium pratense* L. (When the latter three species were grown for a summer next to an alfalfa field lightly infested with the leafminer, no mining took place on them, suggesting *L. trifoliearum* has a strong preference for alfalfa as host plant under field conditions.) The leafminer culture was maintained at 25.6°C, 60 ± 5% RH, and 16L:8D.

A single experiment was conducted to determine fecundity, number of sterile eggs, number of feeding perforations, and adult female longevity. Virgin females were isolated by holding bean-leaf pieces containing puparia in individual 5 × 1.2 cm sealed petri dishes with moistened filter paper to maintain humidity. Petri dishes were examined each morning to collect six newly emerged virgin females. A newly emerged female, with two males selected at random from the host culture, were confined on potted alfalfa (25–30 cm height in a plexiglass cylinder [12.7 cm diam × 30 cm]). Ventilation was provided through the top of the cylinder and 16 ventilation holes (2.5 cm diam) which were covered with organdy fabric. Honey was provided as food.

Each female was confined to potted alfalfa for one week, then transferred to a fresh alfalfa plant with two newly collected male flies until the female died. It was determined from other tests that eggs hatched in less than one week, so we allowed the plant to remain unexamined for an additional week before looking at the leaflets. After the female was removed from the pot, and a week for egg hatch was allowed, all leaflets (including dehisced leaflets at the bottom of the pot) were

Table 2. Mean times from oviposition to onset of each stage or larval instar of *Liriomyza trifoliearum* at 21.1°C and 25.6°C.

Stage or instar	Time of onset (h)	
	21.1°C	25.6°C
Larva		
1st instar	88 (2.2)[1]	56 (0.2)
2nd instar	118 (3.8)	89 (1.4)
3rd instar	159 (4.7)	110 (2.0)
Pupa	208 (4.4)	134 (2.7)
Adult	428 (7.7)	386 (0.5)[2]
n	14	26

[1] SE of mean in parentheses.
[2] Mean and SE of 15 observations.

removed and examined under magnification to determine the number of mining larvae, sterile eggs, and feeding punctures.

Results showed that six females produced an average 213.5 progeny, 100.3 sterile eggs, and 1116 feeding perforations each. Sterile eggs accounted for 32% of total oviposition. This unexpectedly high percentage may be due to infrequent mating as Oatman and Michelbacher (1958) observed for *L. pictella* (probably *L. sativae* [Spencer, 1981: 258]). Adult females lived 2–3 weeks. Sex ratio in the laboratory culture on snap beans was 1.1♀:1♂ (n = 250).

A similar experiment was set up in which females were transferred from plant to plant daily. Since no eggs were oviposited by any female during the first 4–6 days, we concluded that the species has a pre-oviposition period.

In another experiment, we examined immature development. Potted alfalfa was exposed to the leafminer culture for one-half or one hour. The plants were then removed, covered with a cylindrical cage (described in the first experiment) and placed at either 21.1 ± 1°C or 25.6 ± 1°C. Plants were observed several times daily until hatching occurred. Leaves with larvae were then placed on moistened filter paper to maintain humidity in a plastic petri dish with a tight fitting lid. Observations were made 1–3 times daily until adult emergence occurred. During observation, insects were removed from controlled temperature conditions for about 15 minutes. Measurements taken at each observation were: larval length and width, mine width, cephalopharyngeal skeletal length, and pupal length and width (Table 1). In some instances, measurements could not be taken due to larval curl, crossed mines, etc. Only individuals reaching the adult stage were included in the analyses.

The mean time from oviposition to onset of each stage or larval instar at 21.1°C and 25.6°C was determined by averaging for all insects observed (Table 2). If a molt occurred between observations, as was the usual case, the average of the pre- and post-molt times was taken as the time of molting.

Illustrated in Figs. 1–4 are the female adult, female wing, puparium, and typical serpentine mine. Larvae were cleared overnight in chlorophenol and mounted in Hoyer's medium for illustration of the cephalopharyngeal skeleton of each instar (Fig. 5). Each skeleton has two dorsal processes and a single ventral process. The

species has closely appressed left and right mandibles, each with two teeth in the third (mature) instar. The mandibles are asymmetric, so that when viewed laterally, all four teeth appear. The mandibles are fused at their most ventral point.

Use as a Host for Rearing Parasites

We reared seven native and two European parasite species on *L. trifoliearum* on snap beans for biological studies, mass releases, or shipments to cooperators. The native parasite species were Braconidae: *Opius dimidiatus* (Ashmead); Eucoilidae: *Cothonaspis* poss. n. sp.; Eulophidae: *Chrysocharis clarkae* Yoshimoto, *C. giraulti* Yoshimoto, *Diglyphus intermedius* (Girault), *D. pulchripes* Crawford, and *Pnigalio minio* (Walker); and the European species *Chrysocharis melaenis* (Walker) and *Diglyphus isaea* (Walker). A rearing technique for the last species was briefly described by Hendrickson (1975).

We also experimented with *L. sativae* Blanchard and *L. trifolii* (Burgess) as host species. Both these species pupated in the potting mix which had to be kept moist for high percentage emergence of either leafminers or pupal parasites. This moisture requirement allowed contaminant arthropods to reach annoying levels even with sterilized potting mix. The advantage of *L. trifoliearum* as a host is that larvae pupate in the leaves rather than in the potting mix. Thus, pots can be allowed to dry out which greatly reduces the abundance of contaminant species, or leaves can be cut and placed in cages for insect emergence and the potting mix thrown out.

Acknowledgments

We thank Glenn P. Zelluk, undergraduate in the Department of Entomology and Applied Ecology, University of Delaware, Newark, for his assistance in this research, and R. J. Dysart, R. W. Fuester, R. A. Byers, and S. L. Poe for reviews. We appreciate the assistance of G. C. Steyskal (Agromyzidae), P. M. Marsh (Braconidae), and G. Gordh (Eulophidae: *Pnigalio, Diglyphus*) of the Systematic Entomology Laboratory, USDA, Washington, D.C., and C. M. Yoshimoto (Eucoilidae and Eulophidae: *Chrysocharis*) at the Biosystematics Research Institute, Agriculture Canada, Ottawa, for identification of the hosts and parasites.

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