INFLUENCE OF TEMPERATURE AND DIET ON LONGEVITY AND FECUNDITY OF EDOVUM PUTTLERI GRISSELL (HYMENOPTERA: EULOPHIDAE)

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ABSTRACT—Longevity and fecundity were determined for *Edovum puttleri* Grissell, an egg parasitoid of *Leptinotarsa decemlineata* (Say), reared on diets of honey, honey-water mixture, and water alone at 20 and 24°C, respectively. Temperature and diet influenced both longevity and fecundity. For example, mated females lived about two days longer than unmated females or males over the duration of the tests. Parasitoids maintained at 24°C on a honey-water diet lived longer than those provided other diet sources at both temperatures. Females laid ca. 42 eggs during a 21-day ovipositional period with the highest number of eggs produced 7–10 days after mating. The parasitoid's inability to overwinter in Tennessee and its erratic efficiency in parasitizing the Colorado potato beetle during periods of temperature fluctuations, may severely limit its use as a potential biocontrol agent.

Leptinotarsa decemlineata (Say), the Colorado potato beetle (Coleoptera: Chrysomelidae), is a major pest of solanaceous vegetable crops including potato, tomato, and eggplant (Pedigo 1996). L. decemlineata is found from the Mississippi Valley and its tributaries, eastward to the Atlantic coast, northward into southern Canada, and southward to the Gulf of Mexico. Because of the massive use of insecticides, including arsenicals, chlorinated hydrocarbons, organophosphates, and carbamates during the last century against this species, L. decemlineata has developed resistance to many of these compounds (Gauthier et al. 1981, Hofmaster et al. 1965). As a result, incorporation of alternative control methods to suppress L. decemlineata populations has become more acceptable. The two primary natural enemies of L. decemlineata in North America, Perillus bioculatus (Fabricus) and Doryphorophaga doryphorae (Riley), are not considered effective biocontrol agents against this pest (Harcourt and LeRoux, 1967; Harcourt, 1971; Tamaki and Butt, 1978; Tamaki, 1981; and Lipa, 1985).

In 1980, Edovum puttleri Grissell (Hymenoptera: Eulophidae), an egg parasitoid of L. decemlineata, was collected from Colombia, South America, by Puttler (Grissell, 1981). Host specificity for E. puttleri was evaluated by Puttler and Long (1983), who concluded that E. puttleri had a host range restricted to species of Leptinotarsa. Lashomb et al. (1987) reported that E. puttleri preferred to parasitize L. decemlineata eggs of a specific age, while Janson et al. (1987) found this parasitoid to be successful in suppressing the 2nd generation of L. decemlineata on two cultivars of eggplant.

Because temperature and photoperiod are two key climatic factors that may affect permanent establishment of exotic natural enemies (Messenger, 1970), it was suggested by Obrycki et al. (1985) that developmental responses of *E. puttleri* in the United States may be better suited to a climate much warmer than that in the northeastern United States. Thus, Obrycki et al. (1985) proposed that the effectiveness of *E. puttleri*, especially at the beginning of the potato-growing season, may be limited. Obrycki

et al. (1985) also found that E. puttleri did not overwinter in the Northeast, and its survival was adversely affected by the life history of L. decemlineata. When the beetles entered diapause, neither immature nor adult parasitoids entered dormancy in either short day lengths or low temperatures in laboratory or field tests. Acosta and O'Neil (1999) reported survivability and fecundity of populations of this parasitoid from Honduras, Colombia, and Mexico differ depending upon temperature. Ruberson et al. (1987) found both the Columbian and Mexican biotypes oviposited less frequently in older host eggs; thereby making timing of releases into pest infested fields an important control consideration. Groden et al. (1989) developed a computer simulation to provide estimates of percent parasitism over time. Because information on survival rates and reproductive capabilities are major factors in determining the effectiveness of selected parasitoids (Knipling, 1979) as well as the ability to maintain populations for mass releases, the objective of our study was to assess the longevity and fecundity of E. puttleri fed three diets at two temperature regimes under laboratory conditions.

MATERIALS AND METHODS

A population of *L. decemlineata*, obtained from the University of Tennessee Plant Science Farm, Knox County, Tennessee, was established in the greenhouse on Irish potato (cv. Kenebec). Potato plants, grown in clay pots (15.4 cm diameter), and infested with 20-25 *L. decemlineata* adults, were placed into three wooden rearing cages with aluminum screened sides (50 cm \times 38 cm \times 20 cm).

Reservoir Colony of E. puttleri—Parasitoids, obtained from the Beneficial Insect Introduction Laboratory (Agriculture Research Center, Beltsville, Maryland), were reared from eggs of L. decemlineata that had been exposed to adult parasitoids for 24 h. Egg masses were collected daily by cutting off egg-infested sections (ca. 0.5 cm²) of the foliage maintained in cages. Egg masses (10–12) were maintained on water-moistened filter paper

TABLE 1. Longevity of Edovum puttleri Grissell reared on various diets at two temperatures.

Temp. (°C)	Diet	Longevity ¹		
		Unmated females	Males	Mated females
24 ± 1	Water (100%)	$3.4 \pm 0.2 d$	4.2 ± 0.9 d	* 3.9 ± 0.2 d
	Honey - Water (50:50%)	$24.2 \pm 2.1 \text{ bc}$	$33.4 \pm 3.4 a$	$29.0 \pm 2.3 \text{ ab}$
	Honey (100%)	$22.9 \pm 3.5 \text{ bc}$	$20.9 \pm 3.7 \text{ bc}$	$27.8 \pm 3.9 \text{ abc}$
20 ± 1	Water (100%)	$5.7 \pm 0.6 d$	$3.7 \pm 0.2 d$	$6.8 \pm 0.5 \mathrm{d}$
	Honey - Water (50:50%)	$22.8 \pm 4.0 \text{ bc}$	$20.4 \pm 2.3 \text{ c}$	$21.7 \pm 1.6 \text{ bc}$
	Honey (100%)	$25.3 \pm 0.9 \text{ bc}$	$21.4 \pm 1.1 \text{ bc}$	$25.2 \pm 2.1 \text{ bc}$

¹ Means in columns followed by a common letter are not significantly different (P < 0.05).

placed on the bottom of a petri dish. The petri dish, containing one parasitoid, was placed in a plexiglass cage ($20~\rm cm \times 20~cm$) with five sides enclosed and the sixth side covered with thin mesh nylon. *L. decemlineata* eggs were exposed to the parasitoid for $24~\rm h.$

The parasitoid population was maintained in an environmentally controlled room at $24 \pm 2^{\circ}\text{C}$, 50--75% relative humidity, and 16 h light - 8 h dark photoperiod. Four vials (12 mm \times 35 mm), filled with distilled water and plugged with cotton, were placed on the bottom of each plexiglass cage. Paper towel strips (0.5 cm \times 1.0 cm), soaked in 80:20 dilution of honey and water by volume, also were placed on top of the cage. Parasitoids were added and removed from the cage by means of an aspirator.

L. decemlineata eggs were removed from the cage and observed daily for L. decemlineata larvae that hatched from unparasitized eggs. The number of L. decemlineata larvae that hatched was recorded and the L. decemlineata larvae were immediately removed to prevent their feeding on parasitized eggs.

Longevity of E. puttleri—Longevity of three groups of adult parasitoids (unmated males, mated females, and unmated females) fed three different diets (0:100, 50:50, and 100:0 honeywater by volume) was determined at two temperatures (20 and 24 \pm 1°C) under growth chamber conditions. Treatments consisted of soaking a paper towel strip (ca. 0.5 cm \times 3.0 cm) in the appropriate solution and placing the strip on the inside-top lid of the petri dish.

Sexes were distinguished with the use of a stereomicroscope. Upon emergence, approximately 50% of the adult females were removed daily from their cage to prevent mating. The remaining females were left in the cage for 24 h after emerging to mate. Each specimen was maintained in a petri dish and monitored daily until all parasitoids died. Treatments were arranged in a 3 \times 2 \times 2 factorial experimental design with means separation assessed using analysis of variance (SAS Institute Inc., 1985). This test was replicated ten times.

Fecundity of E. puttleri—Egg masses from each L. decemlineata rearing unit were collected daily to determine fecundity. Egg masses (< 24 h old and consisting of 30–35 eggs) were exposed to one female parasitoid in a petri dish for 24 h and then transferred to another petri dish. This procedure was repeated daily until the female died. Egg masses also were monitored until all parasitoids emerged. This test was replicated 15 times. Counts of emerged parasitoids from L. decemlineata eggs were recorded to determine the number of eggs laid every 24 h

and the cumulative number of eggs the female parasitoid laid during her lifetime.

RESULTS AND DISCUSSION

Longevity of E. puttleri—Longevity of parasitoids supplied only water differed significantly (P < 0.05) from all other treatments (Table 1). Males fed a honey-water solution at 24°C lived significantly longer (P < 0.05) than males fed other diets at 20 or 24°C. Also, males fed honey-water at 24°C lived significantly longer than females, except for mated females fed honey or honey-water at 24°C. Females (both mated and unmated) maintained at 20°C and fed 100% honey lived 2–3 days longer than those provided a honey-water combination and 18–19 days longer than those fed only water. However, this tendency was reversed for those females maintained at 24°C, where females fed a honey-water mixture lived on average five days longer than females fed only honey.

Mean longevity of all males and females was 17.3 ± 1.8 and 18.2 ± 2.8 days, respectively. However, mated females lived 19.1 ± 1.8 days and unmated females lived only 17.3 ± 2.4 days. Although not significantly different, those individuals (both males and females) maintained at $24 \pm 1^{\circ}$ C lived a mean of 18.9 ± 1.8 days compared to those held at $20 \pm 1^{\circ}$ C that lived a mean of 17.0 ± 2.2 days.

Fecundity of E. puttleri—The mean number of eggs laid/female/24 h was 2.0 ± 0.5 . Females laid an average of 42.0 ± 3.4 eggs during a 21 day ovipositional period. Oviposition began on the second day with a majority of the eggs laid during the next 7–8 days (Fig. 1). When egg production terminated after 21 days, 40% of the females remained alive for an additional six days.

The survival rate of *E. puttleri* on *L. decemlineata* under laboratory conditions was affected by both diet and temperature. In general, females outlived males, except for those fed a honeywater solution at 24°C, and as expected, water alone was not sufficient to keep the parasitoids alive more than 5 (3–7) days. These data indicate that the combination of 24 ± 1 °C and a honey-water mixture was the best laboratory condition and diet to economically and effectively maintain *E. puttleri*.

Females laid an average of 42 eggs during their lifespan, which indicates that 8-12 female parasitoids are needed to parasitize the 350-500 eggs produced by one *L. decemlineata* female. The parasitoid's inability to overwinter in Tennessee and

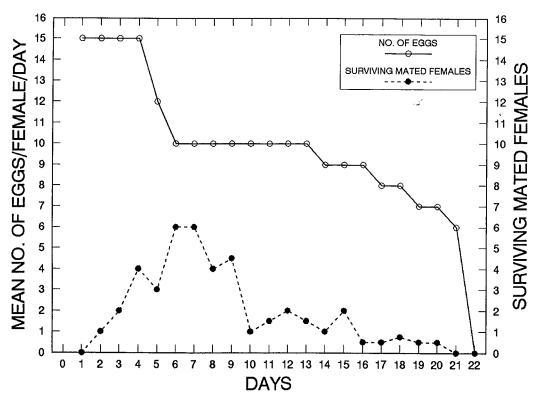


FIG. 1. Fecundity and longevity of Edovum puttleri Grissell adult females.

its erratic efficiency in parasitizing the Colorado potato beetle during periods of temperature fluctuations, may limit its use as a potential biocontrol agent. Although this condition may potentially be overcome by mass rearing and storing parasitoid eggs for making annual releases (Schroder, 1997), the expense involved compared to the available conventional control methodologies may be prohibitive. Schroder (1985) estimated the cost for mass-rearing the parasitoid at ca. \$10/1000 wasps. Although E. puttleri is relatively easy to rear and maintain under laboratory conditions, accessibility, lack of practical procedures to incorporate and establish the parasitoid in the field, labor involved to monitor the populations, and expense to purchase the parasitoids may inhibit its widespread use as a primary control agent for L. decemlineata (Tipping et al., 1999) in both home gardens and commercial fields in Tennessee.

Additional field studies are needed to evaluate the impact of mass releases of *E. puttleri* to suppress *L. decemlineata* populations in Tennessee. Because of the difficulty in controlling *L. decemlineata*, production conditions, and market factors, a steady decline has occurred in the acreage of potatoes grown in Tennessee from a high of 10,760 ha in 1981 to ca. 2,000 ha in 1990 (Brantner, 1990) to less than 1,700 ha projected for the 1996 growing season. Should availability of this parasitoid be made cost effective as an alternate control tactic for *L. decemlineata*, perhaps it may encourage growers to again increase potato acreage within the state.

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LITERATURE CITED

ACOSTA, N. M., AND R. J. O'NEIL. 1999. Life history characteristics of three populations of *Edovum puttleri* Grissell (Hymenoptera: Eulophidae) at three temperatures. Biol. Cont., 16:81–87.

BRANTNER, R. 1990. Tennessee Agriculture Statistics 1990. Tennessee Agric. Stat. Serv. Nashville, Tennessee.

GAUTHIER, N. L., R. N. HOFMASTER, AND M. SEMEL. 1981. History of Colorado potato beetle control. Pp. 13–31 *in* Advances in potato pest management (J. H. Lashomb and R. Cassagrande, eds.). Hutchinson Ross, Stroudsburg, Pennsylvania.

GRISSELL, E. E. 1981. *Edovum puttleri* n. g., n. sp. (Hymenoptera: Eulophidae), an egg parasite of the Colorado potato beetle (Chrysomelidae). Proc. Entomol. Soc. Wash., 83:790–796.

GRODEN, E. F., A. DRUMMOND, R. A. CASAGRANDE, AND J. H. LASHOMB. 1989. Estimating parasitism of Colorado potato beetle eggs, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), by *Edovum puttleri* (Hymenoptera: Eulophidae). Great Lakes Entomol., 22:47–54.

HARCOURT, D. G., AND E. J. LEROUX. 1967. Population regulation in insect and man. Amer. Sci., 55:400-415.

HARCOURT, D. G. 1971. Population dynamics of *Leptinotarsa* decemlineata (Say) in eastern Ontario. III. Major population process. Can. Entomol., 103:1049–1061.

HOFMASTER, R. N., R. L. WATERFIELD, AND J. C. BOYD. 1965. Insecticides applied to the soil for control of eight species

- of insects on Irish potatoes in Virginia. J. Econ. Entomol., 60:1311-1318.
- JANSSON, R. K., J. LASHOMB, E. GRODEN, AND R. BULLOCK. 1987. Parasitism of *Leptinotarsa decemlineata* [Coleoptera: Chrysomelidae] by *Edovum puttleri* [Hymenoptera: Eulophidae] in different cultivars of eggplant. Entomophaga., 32: 503-510.
- KNIPLING, E. F. 1979. The basic principles of insect population suppression and management. Agric. Handbook No. 512. USDA. Wash., DC
- LIPA, J. J. 1985. Progress in biological control of the Colorado potato beetle (*Leptinotarsa decemlineata*) in eastern Europe. OEPP/EPPO Bull., 15:207–211.
- LASHOMB, J., D. KRANAICKER, R. K. JANSSON, Y. S. NG, AND R. CHIANESE. 1987. Parasitism of *Leptinotarsa decemlineata* (Say) eggs by *Edovum puttleri* [Hymenoptera: Eulophidae]: effects of host age, parasitoid age, and temperature. Can. Entomol. 119:75–82.
- MESSENGER, P. S. 1970. Bioclimatic inputs to biological control and pest management programs. Pp. 84–102 *in* Concepts of pest management. (R. L. Rabb and F. E. Guthrie, eds.). North Carolina St. Press, Raleigh, North Carolina.
- OBRYCKI, J. J., M. J. TAUBER, C. A. TAUBER, AND B. GOL-LANDS. 1985. *Edovum puttleri* (Hymenoptera: Eulophidae), an exotic egg parasitoid of the Colorado potato beetle (Coleoptera: Chrysomelidae): Responses to temperature zone conditions and resistant potato plants. Environ. Entomol., 14: 48–54.
- PEDIGO, L. P. 1996. Entomology and Pest Management. Prentice Hall, 2nd ed. Upper Saddle River, New Jersey.

- PUTTLER, B., AND S. H. LONG. 1983. Host specificity tests of an egg parasite, *Edovum puttleri* (Hymenoptera: Eulophidae), of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Proc. Entomol. Soc. Wash., 85:384–387.
- RUBERSON, J. R., M. J. TAUBER, AND C. A. TAUBER. 1987. Biotypes of *Edovum puttleri* (Hymenoptera: Eulophidae): Responses to developing eggs of the Colorado potato beetle (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Amer., 80:451–455.
- SAS INSTITUTE INC. 1985. SAS/STAT user's guide, version 5. SAS Institute, Cary, North Carolina.
- SCHRODER, R. F. W. 1985. In this corner of the pinhead. Agricultural Research. USDA. Agric. Res. Ser., 33:4–5.
- ——. 1997. Feasibility of using cryostored Colorado potato beetle (Coleoptera: Chrysomelidae) eggs for rearing *Edovum puttleri* (Hymenoptera: Eulophidae). Great Lakes Entomologist., 30:177–180.
- TAMAKI, G. 1981. Biological control of potato pests, Pp. 190–191 *in* Advances in potato pest management (J. H. Lashomb and R. Casagrande, eds.). Hutchinson Ross, Stroudsburg, Pennsylvania.
- TAMAKI, G., AND B. BUTT. 1978. Impact of *Perillus bioculatus* on the Colorado potato beetle and plant damage. USDA, Wash., DC Tech. Bull. No. 1581.
- TIPPING, P. W., C. A. HOLKO, B. ABDUL, A. AREF, AND J. R. ALDRICH. 1999. Evaluating *Edovum puttleri* Grissell and *Podisus maculiventris* (Say) for augmentative biological control of Colorado potato beetle in tomatoes. Biol. Cont., 16:35–42.