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To the Graduate Council:

I am submitting herewith a thesis written by Nader M. Elkassabany entitled "Longevity and Fecundity of Edovum puttleri Grissell, with Notes on Its Effectiveness as a Biocontrol Agent in Tennessee." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology.

Paris L. Lambdin, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council

Vice Provost
and Dean of The Graduate School

LONGEVITY AND FECUNDITY OF *Edovum puttleri* GRISSELL, WITH NOTES ON ITS EFFECTIVENESS AS A BIOCONTROL AGENT IN TENNESSEE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Nader M. Elkassabany

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DEDICATION

This thesis is dedicated to my wife, Mrs. Amani Elkassabany, who is very understanding, supportive, and patient.

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ABSTRACT

In the laboratory, fecundity and longevity of Edovum puttleri Grissell (Hymenoptera: Eulophidae) were determined. Longevity was tested at two temperatures and with three different diets. The tested groups were mated females, virgin females, and males. Mean longevity was 17.9± 2.2 days, and parasite survival rate was highest under a combination of 24±1 °C and 50% water:50% honey diet. Mean fecundity for each female was 1.9±0.2 eggs/24 hr and 40.8±4.8 eggs in 21 days. In the summer of 1986, field releases were made in Knoxville and Crossville to determine the effectiveness of the parasite as a biocontrol agent. Counts of Leptinotarsa decemlineata (Say), Colorado potato beetle (CPB) (Coleoptera: Chrysomelidae), percent parasitized eggs and potato yields were the factors used to determine the effectiveness of the field treatments. At the rate of 12 female parasites/1 female CPB, there was no significant difference between the mass release treatment and the control. The parasite did not suppress the population of the CPB, while applying Ambush 112.5 g ai/ ha as a chemical treatment was effective.

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I. INTRODUCTION

Leptinotarsa decemlineata (Say), the Colorado potato beetle (CPB) (Coleoptera: Chrysomelidae), is a major common pest of solanaceous vegetable crops such as potato, tomato, and eggplant. It is found throughout the eastern part of the United States, in the Mississippi Valley and its tributaries, and eastward to the Atlantic coast, northward into southern Canada, and southward to the Gulf of Mexico.

Both larvae and adults of CPB feed on foliage (McIndoo 1935, Metcalf et al. 1962, Westcott 1964, Linduska 1978, Schalk and Stoner 1979) which results in an economic loss to the grower. The CPB is considered the major pest of potatoes in Tennessee. In 1985, Irish potatoes were planted on 2500 acres with a farm value of $2.85 million (TDA bull. 1986). Insecticides, including arsenicals, chlorinated hydrocarbons, organophosphates, and carbamates have been used since 1865 against this pest. As a result, CPB developed resistance to many insecticides (Gauthier et al. 1981) and finding an alternative control method became an urgent need. CPB has two known natural enemies in North America, the pentatomid predator, Perillus bioculatus (F.), and the tachinid parasite, Doryphorophaga doryphorae (Riley), but they are not effective as biocontrol agents of the CPB.

Edovum puttleri Grissell (Hymenoptera: Eulophidae), an

egg parasite of CPB, (Puttler and Long 1983), was collected in February and March, 1980, from Colombia, South America, by Puttler (Grissell 1981).

Because survival rates and reproductive capability of a parasite are major factors in determining the effectiveness of selective parasites (Knipling 1979), the objectives of this study were to (1) determine the longevity and fecundity of E. puttleri under laboratory conditions and (2) determine its effectiveness as a biocontrol agent of CPB in Tennessee.

II. LITERATURE REVIEW

The Colorado potato beetle (CPB) was found on potatoes in 1845 when settlers were making their advance westward and brought the cultivated potato, Solanum tuberosum (L.), into the edge of the habitat of CPB. The beetle's original host was buffalo-bur, Solanum rostratum (Dunal), which was introduced into North America through Spanish travel and commerce. The pest soon adapted to S. tuberosum. The original distribution of CPB was on the eastern slope of the Rocky Mountains, northward to the Canadian border, eastward into western Kansas and Nebraska, and southward into Texas and New Mexico (Tower 1906).

From 1865 to the present, hundreds of compounds, singly or in combination, were utilized to control CPB (Gauthier et al. 1981). Resistance of CPB to synthetic organic insecticides was first noted for DDT in New York state in 1952 (Quinton 1955). Resistance to other chlorinated hydrocarbons, including dieldrin, was recognized by (Hofmaster et al. 1965) in Virginia. In recent years, severe control problems due to the resistance to organophosphates and carbamate compounds such as carbaryl and carbofuran have been encountered in several northeastern states (Forgash 1981). This resulted in a search for biocontrol agents as an alternative method to suppress populations of CPB.

The two spotted stink bug, Perillus bioculatus (F.), was found to be an important predator of CPB (Knight 1923). Perillus bioculatus can apparently decrease the rate of defoliation when the population of CPB is small, but as the pest population increases, the effectiveness of the predator is diminished (Harcourt and LeRoux 1967, Harcourt 1971, and Tamaki and Butt 1978). Perillus bioculatus feeding behavior also limits its effectiveness as a biocontrol agent because the predator has the tendency to feed to fulfillment and individuals engage in aggeragate feeding (Tamaki 1981). In eastern Europe, hibernating P. bioculatus adults become active in the spring much earlier than CPB and die due to lack of food. However, taking into consideration that mass rearing of P. bioculatus is time consuming and costly, its use for seasonal colonization is quite impractical (Lipa 1985).

The tachinid parasite, Doryphorophaga doryphorae (Riley), a parasite of CPB larvae, has little effect on the population dynamics of CPB in the field (Harcourt 1971). All efforts to introduce and acclimatize this parasitic fly in Poland and USSR failed and work in this direction has stopped (Lipa 1985).

In February and March 1980, Puttler made a search in Colombia, South America, for potential natural enemies to use against the CPB. He discovered an egg parasite of L. undecimlineata (Stal.) (Grissell 1981), a beetle that does

not occur in the Nearctic Region, but is widely distributed in the Neotropical Region (Tower 1906). In 1981, Grissell examined the parasite and concluded that it represented a new genus and species which he described as Edovum puttleri, Grissell. Initially, E. puttleri had been misplaced in the genus Emersonopsis (Sanchez 1973, Pasada and Garcia 1976). Puttler's primary purpose for collecting E. puttleri was to determine whether the parasite might accept eggs of CPB as a host for oviposition and development.

Host specificity for E. puttleri was evaluated by Puttler and Long (1983), who concluded that E. puttleri has a restricted host range with probable specificity to species of Leptinotarsa. Temperature and photoperiod are two key climatic factors, in addition to seasonal occurrence affecting the permanent establishment of exotic natural enemies (Messenger 1970). Developmental responses of E. puttleri in the United States appear to be suited to a climate much warmer than the temperature in the northeastern United States (Obrycki et al. 1985). Thus, the effectiveness of E. puttleri, especially at the beginning of the potato growing season, would be limited. They found that E. puttleri did not overwinter in that area and its survival is directly affected by CPB's life history. When the beetles entered diapause, neither

immature nor adult parasites entered dormancy in either short day lengths or low temperatures under laboratory and field test conditions. Pediobius foveolatus (Crawford), an imported eulophid parasite of the Mexican been beetle, Epilachna varivestis Mulsant, is very effective as a larva parasite, but the parasite is unable to overwinter in the U.S. It can be effectively maintained in the laboratory during the winter and increased sufficiently for releases in the spring and early summer (Stevens et al. 1975).

In addition, (Obrycki et al. 1986) found that six pesticides which he evaluated had adverse effects on adults or young immature parasites.

III. MATERIALS AND METHODS

A population of Colorado potato beetle (CPB) was established in the greenhouse on Irish potato (cv. Kenebec). The colony was started from 50 adult CPB, collected in 1985 from the U.T. Plant Science Farm, Knoxville, TN. Potted potato plants were placed in wooden rearing cages with aluminum screen sides (50.8 cm × 38.1 cm × 20.8 cm) as needed to feed the 20-25 CPB adults maintained in each cage (De Wild 1957).

To maintain a parasite reservoir population, newly laid eggs of CPB were exposed to adult parasites for 24 h. Egg masses from the cages were collected daily by cutting off sections of the foliage with eggs attached. Approximately 10-12 egg masses were placed in a petri dish with moist filter paper in the bottom to regulate humidity. The petri dish was placed in a plexiglass cage (20.3 cm × 20.3 cm) with five sides enclosed and the sixth side covered with thin mesh nylon to prevent escape of a thousand newly emerged adult parasites.

After CPB eggs were exposed to the parasites for 24 hr, they were removed from the cage and observed for CPB larvae that hatched from the unparasitized eggs. These were removed in order to prevent them from feeding and destroying the parasitized eggs.

The parasite reservoir population was maintained in a

room at 24±1 °C and 50-75% RH with a 16L-8D photoperiod. Inside the cage, 3-4 vials (12 × 35 mm) containing water and plugged with cotton were placed on the bottom and paper towel pieces soaked in 80:20 dilution of honey and water by volume were placed on the top. Parasites were added and removed from the cage by means of an aspirator.

Longevity of adult parasites was measured in the laboratory at two different temperatures (24±1 and 20±1 °C), fed three different diets (0:100, 50:50, and 100:0 of honey and water by volume), and three different groups (males, mated females and virgin females). Water was supplied in a (12 × 35 mm) vial plugged with cotton for all treatments. In treatments in which undiluted honey was the diet source, a piece of paper towel was soaked in 100% honey and placed on the inner surface of the lid in the dish. Those treatments using 50-50% honey and water as the diet source, the piece of paper towel was soaked in 50:50 dilution of honey and water by volume, placed inside on the top of the dish.

Sexes were distinguished with the use of a stereoscopic microscope. Females were removed from their cage within the first 6 hr of emergence to prevent mating. Other females were left in the cage for 24 hr after emerging to mate. Each of the 18 treatments was replicated 10 times in a completely randomized design, with each individual specimen maintained in a petri dish. A total of 60 males,

60 virgin females, and 60 mated females was the tested population. Longevity data were recorded daily based on survival rate of the parasite population until all parasites died.

In the fecundity test, egg masses from each CPB rearing unit were collected daily. Egg masses (1-2/female) were exposed to one female parasite in a petri dish and transferred to another petri dish at the end of 24 hr exposure to the female. This procedure was repeated daily until the same female died. Egg masses were kept under observation until parasites emerged; the process was replicated 15 times. Counts of emerged parasites from parasitized eggs were recorded to determine how many eggs were laid every 24 hr and later to determine the cumulative number of eggs the female parasite lays during her lifetime.

In field tests, potato seed pieces (cv. Kenebec) were planted on 24th and 29th April, 1986, at Plant Science Experiment station, Knoxville and the Plateau Experiment Station in Crossville, respectively. Three treatments were arranged in a randomized complete block design replicated four times. Plots consisted of 2 rows, each row was 3 m long, plants were spaced 10-12 cm apart. Rows were 91.5 cm wide and 101.5 cm apart. Blocks were separated by 3 m alleyways.

Permethrin (Ambush EC), with 112.5 g ai/ha was applied by means of a compressed air pressure sprayer on June 2nd and 25th, 1986 in Knoxville and on 4th and 29th June in Crossville. Chemically treated plots were 64 m away from the control plot and the parasite release plots to eliminate the effect of chemical drift on the other two treatments.

A release rate of 12 female parasites/1 CPB female was made based on CPB fecundity which is 350-500 eggs/female. The number of parasites needed for release was based on the precount of CPB adult population. In Knoxville, 650 parasites were released on June 2, 12, and 22, 1986, respectively. In Crossville, 560 parasites were released on June 5, 15, and 25, 1986, respectively.

Absolute counts for all stages of CPB on the foliage of each potato plant were made every 4-5 days in both locations. Counts were made by examining both sides of the potato leavies closely in the treatment plots. It took about 10-15 min to count and to record all numbers in a row. Means for CPB population numbers were calculated to determine differences between effectiveness of treatments. Three egg mass samples collected randomly by collected an egg mass observed every 3-4 feet within the plot, this procedure was repeated each week from the mass release plots and were returned to the insectary at U.T. Farm in Knoxville, TN. Egg masses were maintained separately in

marked petri dishes to determine the percentage of parasitized eggs at different times and locations. Egg masses were observed for CPB eclosion. CPB larvae were removed daily so as they would not damage the other eggs. After the parasite emerged, counts were made and means were calculated for percent of parasitized eggs from the Knoxville and Crossville populations.

Potato yields were used to determine the effectiveness of treatments. Potatoes were harvested and weighed on July 31, 1986 in Knoxville and on August 4, 1986 in Crossville.

ANOVA and general linear model procedures with the new Duncan's multiple range for mean separation were used to analyze the longevity and the yield data (SAS Institute Inc. 1985).

IV. RESULTS AND DISCUSSION

LONGEVITY OF *E. puttleri*

Mean longevity for all females was 18.3± 2.8 days and the mean for all males was 17.3±1.8 days; however, mated females lived 19.0±1.8 and virgin females lived 17.4±2.4 days. All individuals tested at 24± 1 °C lived 17.5±1.8 days and the other individuals tested at 20±1 °C lived 16.2±2.2 days.

When the three food resources were compared, the mean longevity number for all individuals was highest when provided 50% water-50% honey and lowest when provided 100% water (Table 1). Water was not a sufficient diet for *E. puttleri* survival for more than 4.6±0.5 days. Longevity data indicated that the best laboratory condition to maintain the parasite was at 24±1 °C and a diet of 50% water-50% honey. Survival rate under laboratory conditions was affected by both diet and temperature.

Means of the 18 treatment combinations were compared in (Table 2). All treatments which were supplied water as a food resource were significantly different from all other treatments. All other treatment groups exhibited no significant difference on longevity of *Edovum puttleri* in the test.

Table 1. Longevity of adult Edovum puttleri supplied three different diets.

Diet	Rate	Days lived±SE
Water	100%	4.6±0.45 c
Water - Honey	50% - 50%	25.2±3.2 b
Honey	100%	21.9±2.0 a

Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. Longevity of adults Edovum puttleri at 2 temperatures and 3 diets.

		Longevity [mean±SE]		
Temp(C)	Diet	Virgin females	Males	Mated females
24±1	Water 100%	3.4±0.2 d	4.2±0.9 d	3.9±0.2 d
	Water+Honey 50-50%	24.2±2.1 bc	33.4±3.4 a	29.0±2.3 ab
	Honey 100%	22.9±3.5 bc	20.9±3.7 bc	27.8±3.9 abc
20±1	Water 100%	5.7±0.6 d	3.7±0.2 d	6.8±0.55 d
	Water+Honey 50-50%	22.8±4.0 bc	20.4±2.3 c	21.7±1.6 bc
	Honey 100%	25.3±3.9 bc	21.4±1.1 bc	25.2±2.1 bc

Means in columns and rows followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

FECUNDITY OF *E. puttleri*

The mean number of eggs laid/female/24 hr was 1.95±0.5 eggs while the mean number of eggs laid by a female over her 21 days ovipositional period was 42.2±3.4 eggs. The ovipositional period was determined when the last live female from the 15 mated females tested stopped laying eggs. Oviposition began on the 3rd day with a majority of eggs deposited over the next 9 days (Figure 1). When egg production terminated after 21 days, 40% of the females remained alive for an additional 6 days.

FIELD TEST

In pretreatment counts in Knoxville, an average of 13.5 adults, 0 larvae, and 2.4 egg masses/treatment plots were recorded. In Crossville, an average of 11.5 adults, 0 larvae, and 1.7 egg masses/treatment plot were recorded.

Counts of the Colorado potato beetle (CPB) population throughout the experiment in Knoxville indicated no significant difference between the parasite release treatment and the control (Table 3). There was a significant difference between the chemical treatment and the parasite release and the control treatments. In Crossville, population numbers for all stages of the CPB were similar to the numbers counted in Knoxville (Table 4). There was no significant difference between release treatment and the control. There was a significant difference between the chemical treatment and the other two

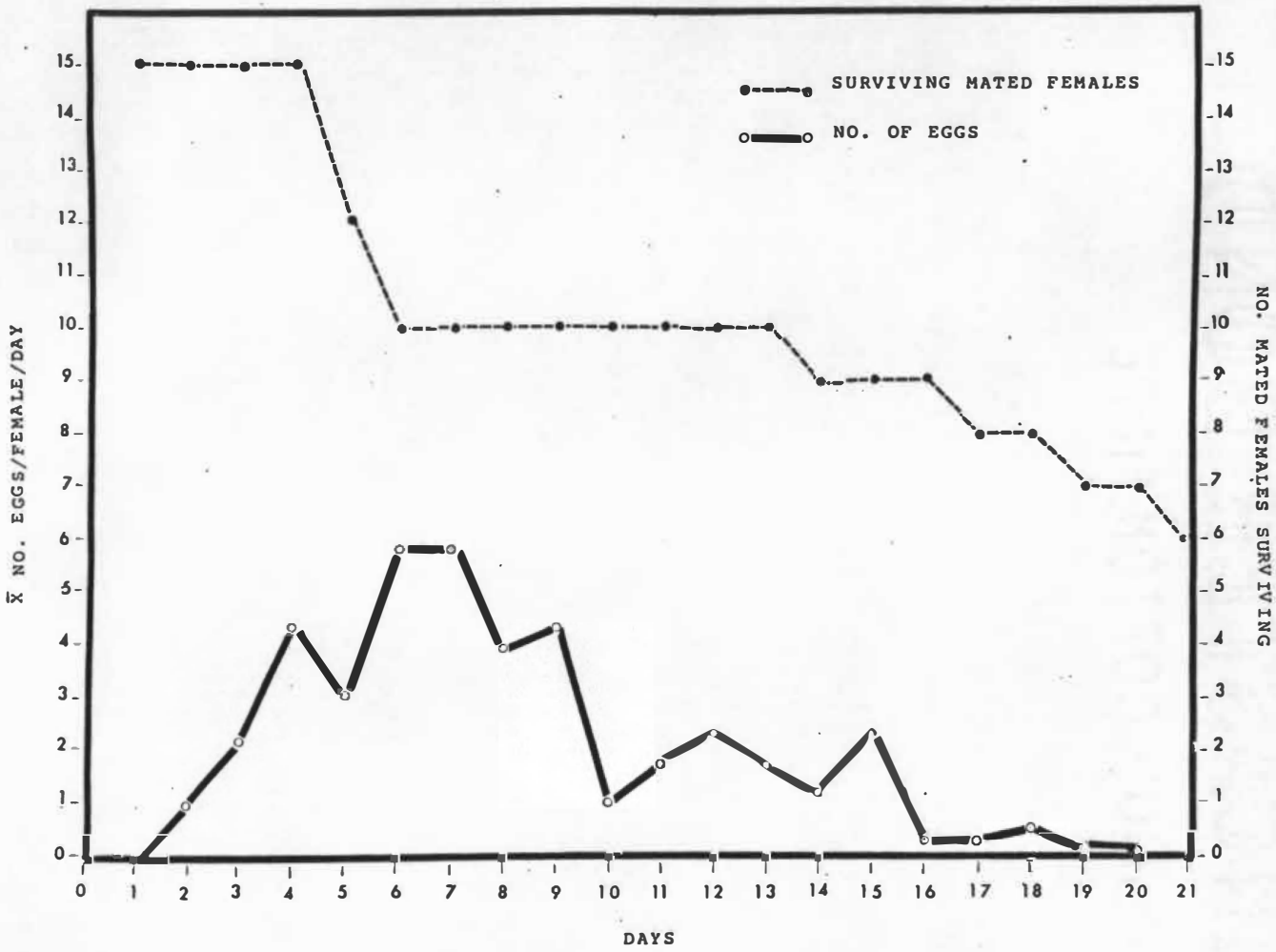

Figure 1. Oviposition pattern and longevity of Edovum puttleri mated females.

Table 3. Effect of the treatments on the population numbers of L. decemlineata, Knoxville, TN.

Treatment	Mean±SE of CPB		
	Adults	Larvae	Egg masses
Parasite	12.10±0.9 a	33.05±11.3 a	3.9±0.4 a
Ambush	1.40±1.0 b	00±0 b	0.48±0.28 b
Control	10.48±0.7 a	33.5±10.8 a	2.67±0.23 a

Means in a column followed by a common letter are not significantly different at the level 5% level by Duncan's multiple range test.

Table 4. Effect of chemical and biological treatments on L. decemlineata populations, Crossville, TN.

Treatment	Mean±SE of CPB		
	Adults	Larvae	Egg masses
Parasite	9.96±0.2 a	27.70±10.1 a	1.92±0.3 a
Ambush	1.15±0.9 b	0.0±0 b	0.30±0.1 b
Control	8.44±0.4 a	28.38±10.3 a	1.80±0.3 a

Means in a column followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

treatments. This indicated that treating with Ambush 112.5 g ai/h was highly effective against CPB in Tennessee.

After treatment of plants with Ambush, there was a significant reduction in the adult population, followed by an increase about 20 days later due to the emergence of the second generation. Then the second treatment was applied (Figure 2). The number of CPB adults in the parasite release plots parallel the adult numbers in the control. The same trend occurred with the egg mass numbers (Figure 3).

High populations of CPB larvae occurred during early June due to the number of larvae which hatched from the first generation's eggs in the field. However, no larvae were found on plants treated with Ambush (Figure 4).

Mean percentage of parasitized eggs from the total number CPB eggs collected in Knoxville was 22.61% (Figure 5). The highest percentage of parasitized eggs occurred on June 26, four days after the last parasite release. In Crossville a slightly higher rate of 25.87% was obtained (Figure 6) and again the highest point on the curve was on June 30, five days after the last parasite release.

Obrycki et al. (1985) indicated that the percentage of eggs parasitized in the field is affected by host plant. E. puttleri parasitized 41% of the CPB eggs on Solanum tuberosum compared to 13% of the CPB eggs on Solanum berthaultii a wild potato species.

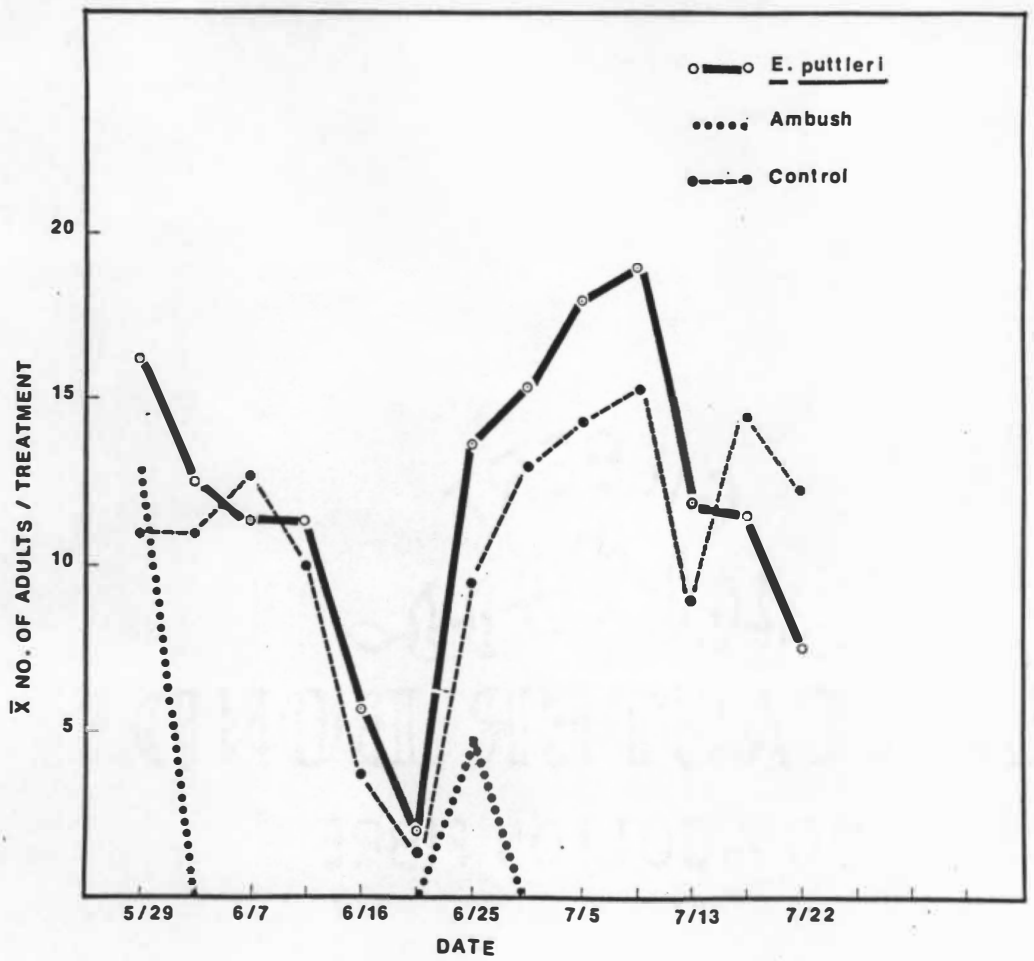

Figure 2. Average level of Colorado potato beetle adults (data were pooled over a treatment), Knoxville, Tennessee.
Chemical treatment (Ambush EC) 112.5 g ai/h was applied on June 2nd and 25th 1986.

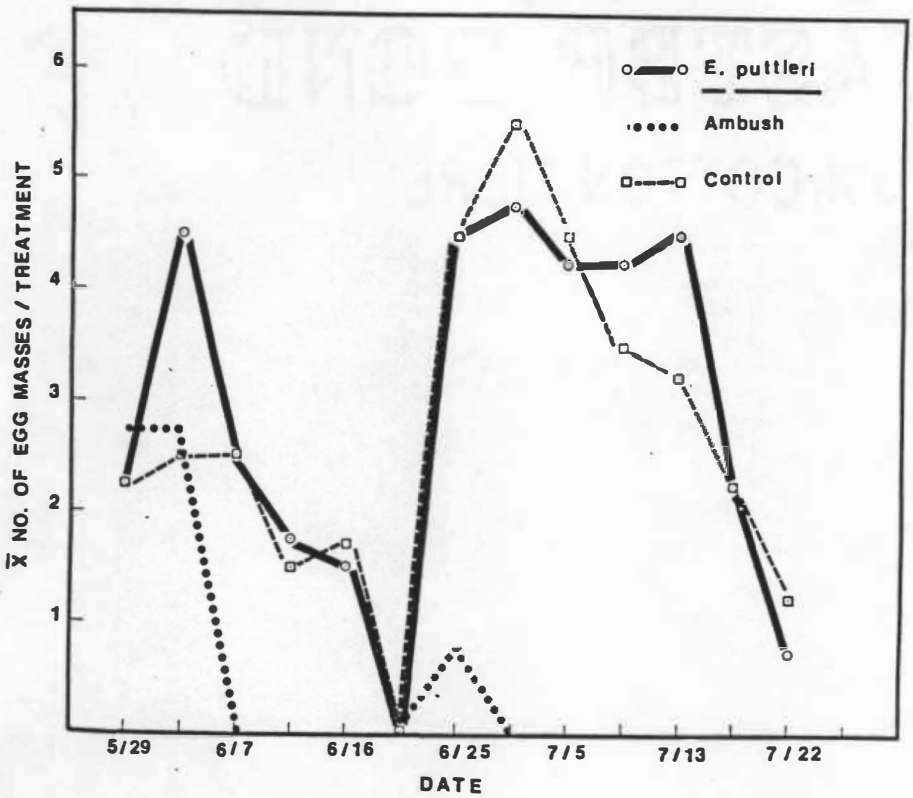

Figure 3. Average level of Colorado potato beetle egg masses (data were pooled over a treatment), Knoxville, Tennessee.
Chemical treatment (Ambush EC) 112.5 g ai/h was applied on June 2nd and 25th 1986.

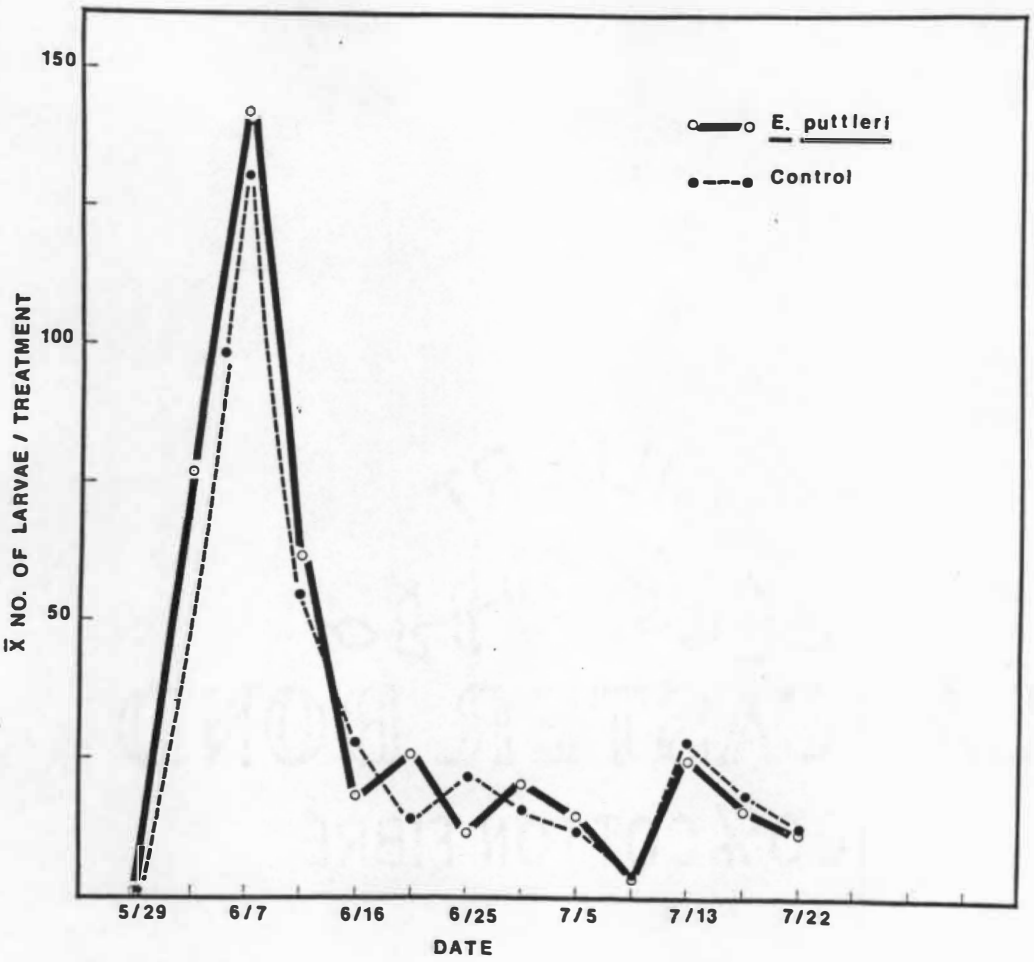

Figure 4. Average number of Colorado potato beetle larvae of the parasite mass releases treatments, Knoxville, Tennessee.

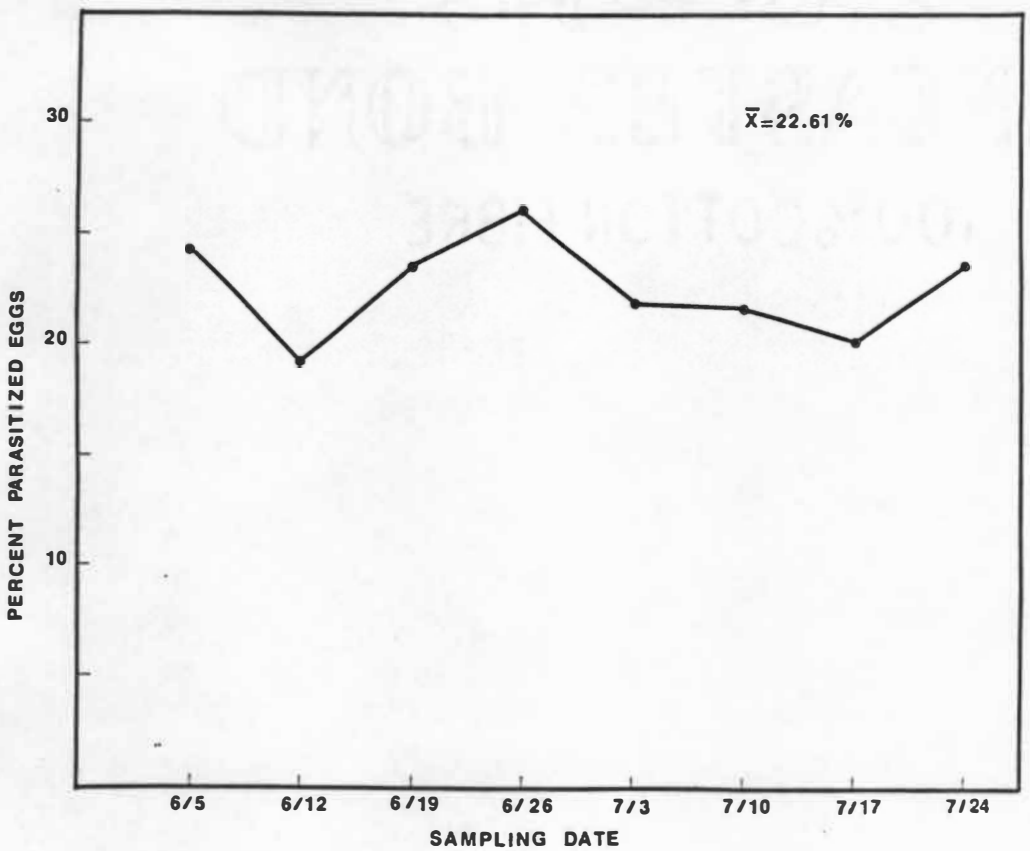

Figure 5. Percent parasitized Colorado potato beetle eggs in mass releases plots in Knoxville, Tennessee. Adult parasites were released on 2nd, 12th, and 22nd June 1986.

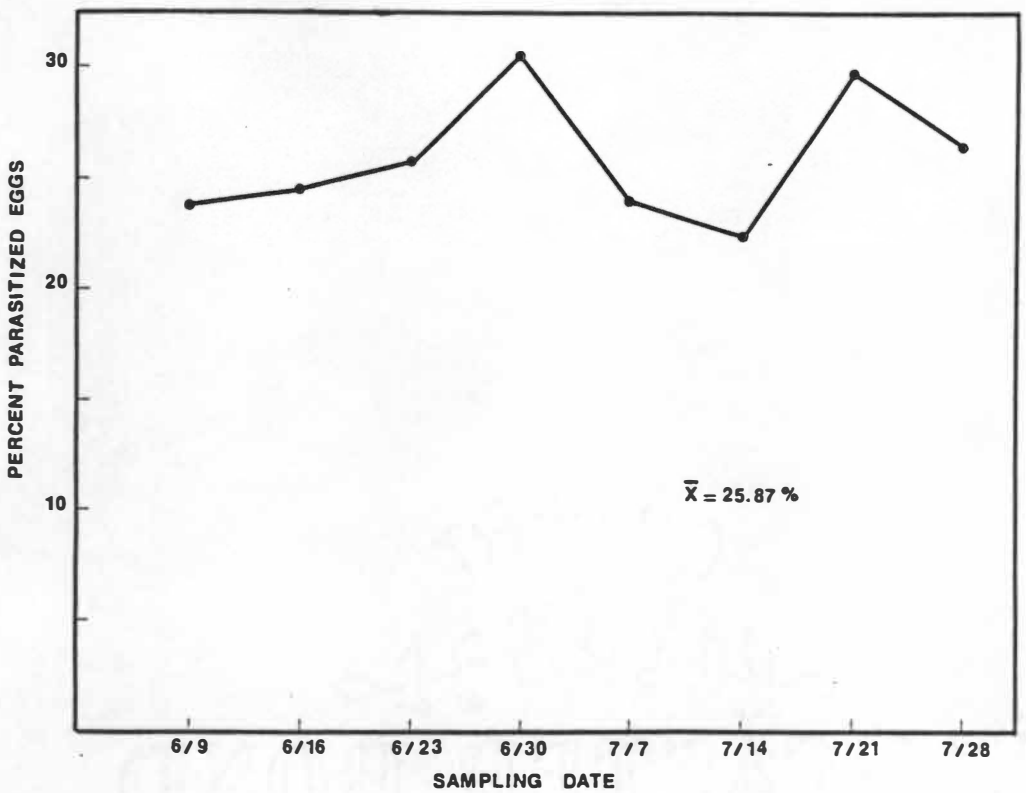

Figure 6. Percent parasitized Colorado potato beetle eggs in mass releases plots in Crossville, Tennessee. Adult parasites were released on 5th, 15th, and 25th June 1986.

Significantly higher potato yields were obtained from plots treated chemically at Knoxville and Crossville (Table 5). No significant differences were noted between the means of the parasite release treatment plots and the control.

Table 5. Influence of E. puttleri mass releases and chemical treatments on potato yields

Treatment	Knoxville		Crossville	
	kg/plot	kg/h	kg/plot	kg/h
Parasite	11.8 b	3292 b	10.8 b	3013 b
Ambush	23.2 a	6473 a	18.2 a	5078 a
Control	12.6 b	3515 b	9.8 b	2734 b

Means in a column followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

Population counts of the CPB and the potato yield from field test in Knoxville and Crossville indicated that the parasite at the rate of 12 female parasite/female of CPB did not effectively suppress the CPB population. This agrees with results by Cantwell et al. (1985) who pointed out that E. puttleri by itself was unable to reduce the CPB population.

V. CONCLUSION

Edovum puttleri Grissell is a newly discovered egg parasite of the Colorado potato beetle (CPB); in fact, this is the first time that the parasite was introduced to Tennessee, which is one of the eleven states currently researching the parasite. It is relatively easy and inexpensive to rear and maintain under laboratory conditions. However, the parasite's inability to overwinter in this region and the host specificity to the genus Leptinotarsa severely limits its use as a potential biocontrol agent. Host specificity is a major factor in determining the effectiveness of a biocontrol agent. When a parasite has more than one host, its chances to establish itself increase. The inability of the parasite to overwinter may be overcome by mass rearing and making annual releases. Pesticides have a great influence on limiting the parasite population and its ability to establish itself in the field.

The survival rate was affected by the diet and temperature. Water was not sufficient to keep the parasite alive more than an average of 5 days. A female parasite laid an average of 41$\pm$5 eggs during their lifespan, which means that theoretically 10-12 female parasites were needed to parasitize 350-500 eggs produced by one CPB female. Mass releases of the parasite in the field at a rate of 12

female parasites/female CPB were not effective in suppressing the CPB population. Perhaps a higher rate of the parasite release would be more successful in reducing the CPB; however, further tests to determine the required release rate are needed, taking into consideration the cost. In the mass release plots, although the parasite parasitized 22%-25% of the CPB eggs, there was no significant difference between yields from the control plots and yields from mass release plots. Cantwell et al. (1985) found that when Bacillus thuringiensis subsp. thuringiensis was used in combination with E. puttleri, it significantly reduced the CPB populations in tomato plots which indicated that further tests are needed to determine if the parasite can be a component in an IPM program rather than use the parasite by itself.

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