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SUPERPARASITISM OF *EPILACHNA VARIVESTIS* [*COL.: COCCINELLIDAE*] BY *PEDIOBUS FOVEOLATUS* [*HYM.: EULOPHIDAE*]: INFLUENCE OF TEMPERATURE AND PARASITOID-HOST RATIO (1)

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Superparasitism of *Epilachna varivestis* MULSANT larvae by the hymenopterous parasitoid, *Pediobius foveolatus* (CRAWFORD), occurred under laboratory conditions. However, *P. foveolatus* avoided previously parasitized larvae in a manner which was directly related to the number of times host larvae were initially parasitized. Increasing the parasitoid-host ratio also increased percent host mortality and highest overall host mortality occurred at 15.6° C when the parasitoid-host ratio was 10:10. Higher temperatures (22° and 28° C) and higher parasitoid-host ratios yielded higher numbers of parasitized larvae although a significant number of parasitoids failed to emerge at the highest parasitoid-host ratio. Higher temperatures along with increasing parasitoid-host ratios favored production of more male parasitoids.

The eulophid parasitoid, *Pediobius foveolatus* (CRAWFORD), has been shown to suppress populations of Mexican bean beetles (*Epilachna varivestis* MULSANT) in soybean fields (STEVENS *et al.*, 1975a). The biology of this parasitoid has been reported by LALL (1961), with additionnal reports by ANGALET *et al.* (1968) and STEVENS *et al.* (1975b). Parasitoids oviposit in late-stage larvae which mummify and produce an average of from 10.5 to 18.5 parasitoids per parasitized larva, depending upon the parasitoid-host ratio (STEVENS *et al.*, 1975b). LALL (1961) recognized that variations in the efficiency of *P. foveolatus* may be associated with climate, host density, and hyperparasitism (= superparasitism).

FRISKE (1910) first introduced the term superparasitism to denote conditions that result when any individual host is attacked by 2 or more species of primary parasites or by 1 species more than once. This definition was later restricted by SMITH (1916) to mean multiple attacks on 1 host by different parasitoids of the same species.

The number of parasitoids which can find ample nourishment to complete their development within a single host is limited. The adaptive significance of being able to recognize hosts which are unsuitable (i. e. already parasitized) for survival of parasitoids

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in nature is obvious. On a practical basis, avoidance of superparasitism is necessary in order to maintain viable stock colonies for experimentation and for rearing for innoculative or inundative releases in the field.

Salt (1937) provided the first critical study of superparasitism and pointed out the relationship of this phenomenon to "host marking" and the ability of parasitoids to discriminate between parasitized and healthy larvae. Numerous reports of host marking by parasitoids have since been published (Ullyett, 1949a, b, 1950; Bakker *et al.*, 1967; Price, 1972; and others).

Optimum parasite-host ratios for laboratory rearing of *P. foveolatus* are reported by Stevens *et al.* (1975), but information on superparasitism of *E. varivestis* has not been published. Also, there is a general paucity of information about temperature-parasitoid-host ratio interactions and the influence of these variables on superparasitism. The mymarid parasitoid, *Anaphes flavipes* (Foerster) was found to superparasitize its host, *Oulema melanopus* (L.), to the extent to which no parasitoid progeny were produced. Reports of changes in the sex ratio of certain Hymenoptera due solely to temperature have been published by Schread & Carmen (1934), DeBach (1943), Flanders (1945), Wilson & Woolcok (1960), Bowen & Stern (1966), and others.

The objectives of this study were to: 1) determine if superparasitism exists in *E. varivestis* larvae by *P. foveolatus*, 2) find out if the parasitoid will avoid previously parasitized *E. varivestis* larvae and relate the degree of avoidance by the parasitoids to the number of times individual *E. varivestis* larvae are previously parasitized, 3) investigate the influence of different constant temperatures and parasitoid-host ratios on superparasitism.

MATERIALS AND METHODS

P. foveolatus used in our experiments were mated females less than 48 h-old, which had been reared at 27° ± 2°C, 50 ± 10% RH and at a photoperiod of L:D 14:10. Adult parasitoids were fed honey by placing a drop on the top of the screened caps of rearing vials (10 × 3.5 cm). Hosts (*E. varivestis*) were reared in the greenhouse on snapbean (*Phaseolus* sp.) plants. All host larvae were 4th instars. Once parasitized, larvae were fed snapbean leaves until they formed pupae or mummies (parasitized larvae which died due to developing parasitoids inside them).

Experiments designed to show superparasitism and avoidance of previously parasitized larvae by *P. foveolatus* were conducted by varying the length of time between initial and subsequent parasitization and by varying the number of ovipositions by the parasitoid. Time intervals between initial and subsequent ovipositions were 1, 5, and 24 h for experiments 1, 2, and 3, respectively. Each test container (replicate) held 1 female parasitoid and 3 host larvae. One larva had been parasitized once, 1 parasitized 6 times and a 3rd larva, which served as a control, had not been parasitized. There were 24 combined replicates for the 3 tests. Individual larvae were recognized by marking them with a small amount of fluorescent pigment. Visual observations were made of the number of times individual *P. foveolatus* oviposited in each larva. When oviposition occurred, the parasitized larva was immediately replaced.

Observations were made using rectangular clear plastic containers which were 19 × 13 × 8 cm for experiments 1 and 3, and for experiment 2, the cylindrical containers were 3.5 × 8.75 cm. Observation times (exposure of *P. foveolatus* to each group of 3 larvae) were 70, 120 and 65 minutes for experiments 1, 2, and 3, respectively. An attack or oviposition was recorded when the parasitoid mounted the larva, inserted its ovipositor, and remained in this position for 2 or more minutes. All encounters of less than 2 minutes duration were not recorded.

Tests involving parasitoid-host ratio and influence of temperature on superparasitism were conducted using 6 replicates of parasitoid-host ratios of 1:10, 4:10 and 10:10 at constant temperatures of 15.6, 22.0 and 28.2°C. The appropriate number of parasitoids and hosts (4th stage larvae) were confined in 13.5 × 14.5 cylindrical plastic containers with screened lids for 24 h. Food for host larvae was provided by inserting the petioles of a bouquet of snapbean leaves into a 50 ml Erlenmeyer flask of water. Individual flasks were taped to the bottom of each test container. Host larvae were allowed to feed for 6-8 days after initiation of the test. Three to 4 days before expected emergence of the parasitoids, each host larva was placed inside a No. OO transparent gelatin capsule. Emergence of the parasitoids was monitored for 8-10 days following first emergence to allow sufficient time for parasitoids to emerge.

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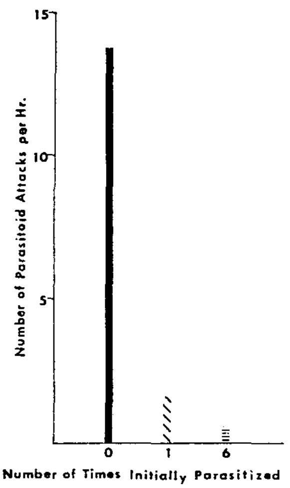

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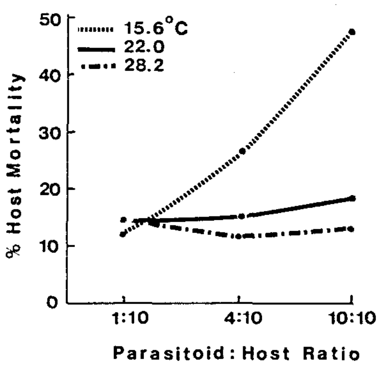

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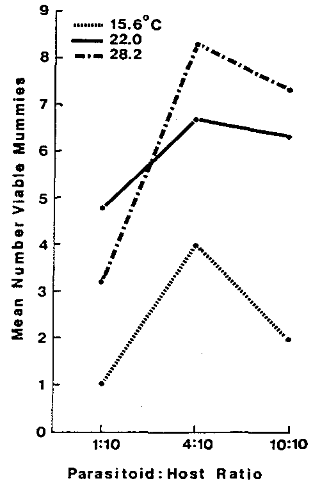

FIG. 1. Attacks by *P. foveolatus* on parasitized or unparasitized 4th-stage larvae of *Epilachna varivestis*

FIG. 2. Mortality of *E. varivestis* larvae at different temperatures and parasitoid (*P. foveolatus*)-host ratios.

FIG. 3. Mean numbers of viable mummies (*E. varivestis* larvae which produce live adult parasitoids) at different temperatures and parasitoid-host ratios.

Records were kept on the numbers of: 1) dead and viable mummies (those producing live parasitoids), 2) male and female parasitoids from each parasitized larva, and 3) male and female parasitoids which did not emerge. This last determination was made using a dissecting microscope.

Data were subjected to analysis of variance and means separated by least significant difference test.

RESULTS AND DISCUSSION

Results from our experiments demonstrate that *P. foveolatus* avoided *E. varivestis* larvae which has been previously parasitized (fig. 1). Further, there was a direct relationship between the degree of avoidance and number of times hosts had been previously parasitized. Parasitization of control (non-parasitized) larvae accounted for 87.4% of the attacks and *E. varivestis* larvae which had been parasitized 6 times were subsequently parasitized less than once per hour for the entire test period. Variation in oviposition among individual parasitoids ranged from none to 3 within a 65 min. observation period.

These findings indicate the possible presence of a host marking pheromone which is deposited on the host by the parasitoid at the time of oviposition. The presence of this material tends to deter further oviposition by other parasitoids. The suppression of superparasitism via this mechanism prevents, 1) production of mummies which contain inadequate food resources for the large number of developing parasitoid larvae, 2) egg wastage, and 3) may dictate dispersal and/or distribution of progeny in the field. Inadequate food resources results in death of developing *P. foveolatus* before they reach maturity or causes a reduction in size and viability (our unpublished data). Under laboratory conditions, with high parasitoid-host ratios, we were able to produce mummies which never produced adult parasitoids, i.e. although *P. foveolatus* preferred non-parasitized larvae, the propensity to oviposit was greater than the ability to refrain from it in the absence of healthy (non-parasitized) hosts. WESELOH (1976) demonstrated this with *Apanteles melanoscelus* RATZEBURG which parazitized the gypsy moth, *Porthetria dispar* (L.).

We found no significant differences in the number of attacks by the parasitoid regardless of the time (1-24 h) which had lapsed between initial and subsequent parasitization. Thus if a marker substance was placed on hosts during oviposition this material was relatively non-votatile.

Overall % host mortality, which includes all causes of mortality, was essentially the same at all temperatures when the parasitoid-host ratio was 1:10. Increasing the ratio to 4:10 and 10:10 also increased host mortality, especially at a 15.6°C where % host mortality was significantly ($P < 0.05$) higher than at either 22 or 28°C (fig. 2). Almost 50% of the host larvae expired at the parasitoid-host ratio of 10:10 when the temperature was 15.6°C. These findings suggest an interaction between cool temperatures and high parasitoid-host ratios. Because of the reduction in development at cooler temperatures (and higher humidities), some mortality may be attributed to invasion of host larvae by bacteria, fungi, etc. Infection and eventual mortality by these agents may be expedited by the increased number of ovipositions at the higher parasitoid-host ratios by providing sites for invasion of these microorganisms.

TABLE 1

Average number of mummies (parasitized larvae) per container at different temperatures and parasitoid-host ratio (a) (b)

Parasitoid-host ratio	Temperature (° C) 15.6	22.0	28.2
1:10	2.7a	6.0a	4.3a
4:10	8.2b	8.8b	9.7bc
10:10	8.4b	9.5b	10.0c

(a) Parasitoid = *P. foveolatus*, host = *E. varivestis*.

(b) Averages followed by the same letter in each column are not significantly different according to the test for least significant difference (LSD) ($P < 0.05$).

Higher temperatures and parasitoid-host ratios yielded higher number of parasitized larvae (table 1). The significant reduction ($P < 0.05$) in numbers of mummies produced at 15.6°C may have been due, in part, to the reduction of oviposition activety or mutual interference by the parasitoids at this temperature. Differences between numbers of parasitized larvae in our study were not significant at the parasitoid-host ratios of 4:10 and 10:10 or between temperatures of 22° and 28.2°C. However, many of the parasitized larvae did not produce parasitoids, especially at the highest parasitoid-host ratio. Dissection of mummies from which all live parasitoids had emerged revealed that a significant number of the parasitoids had failed to emerge when the parasitoid-host ratio was 10:10 (table 2). Superparasitism was probably the reason for this reduction in numbers of parasitoids. Competition among the developing parasitoid larvae, whether by physical combat or for food, often results when the host cannot supply resources for the supernumerary parasitoids. Reduction in numbers of viable mummies (those which yield parasitoids) at the ratio of 10:10 is illustrated in figure 3. Under conditions of our tests 4 parasitoids and 10 hosts yielded higher numbers of viable mummies at all temperatures.

TABLE 2

Average numbers of unemerged P. foveolatus *per container at different temperatures and parasitoid-host ratios.*

Temperature	Parasitoid-host ratio		
(° C)	1:10	4:10	10:10
—	—	—	—
15.6	6.7	20.0	20.8
22.0	8.8	26.8	32.5
28.2	15.0	14.2	66.2 (a)

(a) Significantly different from all other means ($P < 0.05$).

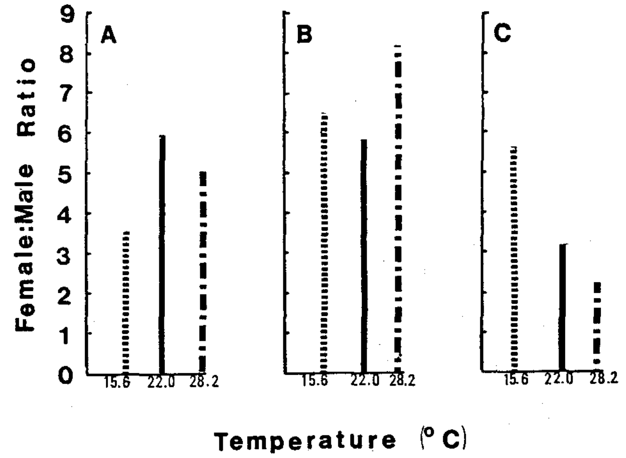

FIG. 4. Emergence of adult female and male *P. foveolatus* parasitoids at different temperatures and parasitoid-host ratios. A = 1:10, B = 4:10, C = 10:10

Increasing temperatures coupled with increasing parasitoid-host ratios favored production of more male parasitoids (fig. 4) The alteration of sex ratio in Hymenoptera due to either high or low temperature is not uncommon (BOWEN & STERN, 1966 and others). BOWEN & STERN (1966) attribute this shift in sex ratio to changes in the primordial germ cells during maturation of the oocytes. We question this as an explanation for the sex ratio change in *P. foveolatus* because the females were reared at 26.7°C and were reproductively active at the time our tests were initiated. Also, the female parasitoids were exposed for a relatively short time (24 h) to the respective temperatures. It may be more probable that the higher production of males was induced during development of the parasitoid embryo or larva within the host tissue.

In conclusion, consideration should be given to both temperature and parasitoid-host ratio when studying quantitative relationships between *E. varivestis* and *P. foveolatus*. Although superparasitism exists, the mechanism employed by *P. foveolatus* to avoid it may be a stronger deterrent in the field than in the laboratory. Where adequate numbers of host larvae are present, superparasitism probably does not limit the growth of parasitoid populations but may serve to disperse its members. Cool temperatures coupled with high parasitoid-host ratios induced a high degree of host mortality which could not be attributed entirely to superparasitism. The change in the male-female ratio due to temperature is important in the dynamics of these species in the field and laboratory. Warmer temperatures (especially 28.2°C), at high-parasitoid-host ratios, prevented emergence of a significant number of parasitoids.

These findings may provide inputs for developing computer simulation models of *E. varivestis* and *P. foveolatus*. Also, additional insights into the biology of these species have been gained which should be beneficial in rearing *P. foveolatus* for control of *E. varivestis*.

RÉSUMÉ

Superparasitisme d'*Epilachna varivestis* [*Col. : Coccinellidae*] par *Pediobius foveolatus* [*Hym. : Eulophidae*] : influence de la température et de la proportion de parasitoides et d'hôtes.

Le superparasitisme des larves d'*Epilachna varivestis* MULSANT par l'hyménoptère parasite, *Pediobius foveolatus* (CRAWFORD) se produit au laboratoire. Cependant *P. foveolatus* évite les larves déjà parasitées à un degré directement en rapport avec le nombre de fois que les hôtes ont été initialement parasités. L'augmentation du rapport parasites-hôte accroît le pourcentage de mortalité de l'hôte et la mortalité la plus élevée a lieu à 15° 6 C pour un rapport parasite/hôte de 10:10. Des températures plus fortes (22° et 28° C) et des rapports plus importants aboutissent à plus de larves parasitées mais beaucoup de parasites ne parviennent pas à quitter l'hôte lorsque le rapport parasite/hôte est le plus grand. L'élévation de la température accompagnée d'une augmentation de ce rapport favorise la production de mâles du parasite.

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