Effect of Parasitoid and Host Age on Oviposition and Emergence of *Microplitis croceipes* (Hymenoptera: Braconidae) an endoparasitoid of *Helvicoverpa zea* (Lepidoptera: Noctuidae)[1]

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ABSTRACT The effect of parasitoid age and two instars of the host *Helicoverpa zea* (Boddie) were investigated for the endoparasitoid, *Microplitis croceipes* (Cresson). Third and fourth instars of *H. zea* were exposed to three different age ranges (3 to 5, 6 to 8, and 12 to 15-d-old) of mated adult female *M. croceipes*. No significant differences were shown in rate of parasitism regardless of parasitoid age or host instar. Significant differences ($P < 0.05$) were found between parental age and adult wasp emergence, sex ratio, and number in pupal stage entering diapause. Six to 8-day-old parasitoids parasitizing third instar hosts yielded the highest percentage of adult emergence (47.5 ± 14%; $\overline{X}$ ± SD), the highest percentage of females (79%), and the lowest percentage (4.5 ± 6%; $\overline{X}$ ± SD) entering diapause during the test.

KEY WORDS Insecta, *Microplitis croceipes, Helicoverpa, zea, Heliothis* spp., biological control, corn earworm.

Corn earworm, *Helicoverpa zea* (Boddie), is a major pest of soybean, corn, cotton, and other row crops in the southeastern United States (Metcalf et al. 1962). Concern over the ability of *H. zea* and the tobacco budworm, *Heliothis virescens,* to develop resistance to insecticides, increasing cost of chemical control, and insecticide residue problems have stimulated interest in seeking alternative methods of control (Stadelbacher et al. 1984). As a result, it is important to identify viable endemic natural control agents of *H. zea* and integrate their use into pest management practices to strategically avoid destruction of natural enemy populations (Zehnder et al. 1990).

Microplitis croceipes (Cresson) is an important native parasitoid of *Heliothis/Helicoverpa* spp. (Bryan et al. 1969) and has potential for use in biological control programs (Lewis and Brazzel 1968). *M. croceipes* has been reported to parasitize *Heliothis/Helicoverpa* spp. in many cultivated crops in the U. S., including alfalfa, soybeans, cotton, corn, grain sorghum (Smith et al. 1976, Pair et al. 1982), and tomato (Lewis and Brazzel 1968). *M. croceipes* will attack and can develop in all instars of *Heliothis/Helicoverpa* spp. (Tillman and Powell 1989), however, third instars are preferred (Lewis 1970b). The first and second

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instars of *Heliothis/Helicoverpa* spp. are more difficult for *M. croceipes* to find than the third, and the females are more reluctant to attack the larger fourth and fifth instars (Lewis 1970b). The same results were shown by Hopper and King (1984) on cotton in field cages and in the laboratory (Hopper 1986).

M. croceipes life cycle requires approximately 14.5 d at 30°C (Bryan et al. 1969, Lewis and Snow 1971). Bryan et al. (1969) reported that *M. croceipes* has a short preoviposition period (about 24 h between emergence and egg laying). The parasitoid oviposits a single egg per host and the ontogeny includes three instars which feed on the host's hemolymph. After reaching the third instar the larva bores out of its host and spins a cocoon (Lewis 1970a, Vinson and Dahlman 1989). The host larva does not feed or develop further and soon dies (Bryan et al. 1969, Lewis 1970a).

M. croceipes seems to be able to regulate its host's development (Lewis 1970b). Early host instars are allowed to develop, thus providing sufficient food supply and allowing pupal cell formation. However, host development stops before the pupal stage is reached (Lewis 1970b). The calyx fluid that coats the parasitoid egg appears to play a role in this regulation of host physiology (Lewis 1970b, Jones and Lewis 1971). *M. croceipes* diapauses (at temperatures below 25°C) in the prepupal stage inside the parasitoid cocoon once it has exited the body of the host (Bryan et al. 1969).

In addition to its endemic status, *M. croceipes* adults are also especially attractive as control agents because of their propensity for a relatively high level of tolerance to certain insecticides which are highly effective against *Heliothis/Helicoverpa* spp. (Bull et al. 1989). Studies show that some insecticides, such as pyrethroids, and certain carbamates and organophosphates have potential for use in a management program that emphasizes conservation of natural enemies (Powell and Scott 1985, 1991, Powell et al. 1986, Katayama et al. 1987, Elzen et al. 1987, 1989, Zehnder et al. 1990, Herbert 1993).

Much work has been done on the parasitoid-host relationship between *M. croceipes* and *H. zea,* however, many questions remain on the biology of the parasitoid. Objectives of this study were to determine if the age of the parasitoid, when initially allowed to oviposit, influences parasitization of the host, adult progeny emergence or progeny sex ratio. A second objective was to determine if parasitoid age affects preference for host (*H. zea*) instar.

Materials and Methods

General Rearing Procedures. *H. zea* eggs and *M. croceipes* pupae were obtained from the Southern Insect Management Laboratory, USDA, ARS, Stoneville, MS. *H. zea* eggs held for hatch and first instars placed on a Nutrisoy/wheatgerm diet (King and Hartley 1985) were maintained in an environmental chamber controlled at 27°C, 50% RH, and a 14:10 (L:D) photoperiod. *M. croceipes* pupae held for emergence were maintained at these same conditions. Adult wasps were held in 0.5-liter clear plastic rearing/mating cages with lids removed and replaced with wire mesh and 1.5-cm stoppered holes in the sides for the extraction of the parasitoids. Wasps were provided with water and a 50:50 honey-water solution as a food source.

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Experimental Procedures. Third and fourth instar *H. zea* (determined by presence of molted head capsules) were exposed to three different age ranges of presumed mated female *M. croceipes*. Third and fourth instars were used because *M. croceipes* prefers these sizes for oviposition (Hopper and King 1984). The ages (days post-eclosion) of the parasitoids were: 3 to 5 d (group 1), 6 to 8 d (group 2), and 12 to 15 d (group 3). Mating occurs as soon as both sexes are present (Bryan et al. 1969, Lewis 1970a, Lewis and Burton 1970), thus male and female wasps held together in mating cages for 48 h were presumed mated. Fifty presumed mated females (hereafter referred to as mated females) were identified and held together with accompanying males throughout the test period.

Oviposition was evaluated by exposing 10 third instar *H. zea* to one mated female wasp in a 15 × 100 mm Petri dish. Females were aspirated out of the mating cages and into the petri dishes containing the 10 larvae. Chips of the Nutrisoy/wheatgerm medium had been placed in the dish to serve as food and discourage cannibalism by *H. zea*. Once female wasps were introduced into the oviposition arena they began searching behavior. The dish could then be partially opened and closed with relative ease without losing the parasitoid. Larvae were removed as soon as stinging was observed and oviposition was suspected. Once larvae were stung, they were then placed individually in 30-ml plastic cups containing the Nutrisoy/wheat germ diet. Individual diet cups were placed in styrofoam cell trays which were inverted after five days in the case of group 1, and after six days for groups 2 and 3, and held under the continuous development condition described above. If stinging was not observed within 30 min, the female was replaced. Average duration of exposure time for a female to sting 10 host larvae was 12 min. No female was used twice. Fourth instars were exposed under the same conditions, except only five larvae were placed in a petri dish at one time. The test procedure was modified due to the large size of these larvae and the tendency of larger larvae to fight with the wasp more than smaller larvae. The tests were replicated five times for each age group and instar combination.

Recording and Analyzing Data. The cups containing stung larvae were monitored daily and observations were recorded regarding parasitoid larval emergence, successful cocoon formation, emergence of adult parasitoids, determination of sex, mortality, incidence of diapause, or host pupation. Most adults emerged within 4 to 5 days after pupation. *M. croceipes* cocoons were considered to be in diapause if adult parasitoid emergence did not occur after two weeks and the parasitoid prepupae inside the cocoon were not dead (based on subsample dissections). *H. zea* larvae that showed arrested development and did not pupate after 21 d were dissected to determine the presence of partially developed parasitoids. If the host development was not arrested, the host larva was allowed to complete development to the pupal stage and then was discarded. Percent successful parasitism was determined by dividing total larvae exposed by the number parasitized, as evidenced by parasitoid emergence and dissections.

A three-factor analysis of variance was used to determine average percent parasitization, adult emergence, sex ratio, and percentage diapause of *M. croceipes* progeny as influenced by parental age and host instar. When significant differences occurred, means were separated by Tukey's studentized range test ($P = 0.05$) (MEANS/TUKEY portion of the General Linear Model Procedure [SAS Institute 1985]).

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Results

No significant differences in mean percent parasitization were found due to parasitoid age or host instar exposed. Age of the parasitoid did not affect percentage parasitization of either host instar (Table 1). The highest percentage of successful parasitization occurred in the third instar hosts by parasitoids aged for 12 to 15 d (group 3). Percentage of parasitization included host larvae in which the parasitoid did not complete development, this was confirmed by dissection. The percentage of host larvae with parasitoids that died before exiting the host body cavity accounted for 1.0% of the total number parasitized.

Table 1. Mean percentage parasitization of two instars of *H. zea* by *M. croceipes* at three parasitoid ages and resulting mean percentage emergence of *M. croceipes* adults*.

Parasitoid age (days)	n		% Parasitization ± SD		% Adult parasitoid emergence ± SD	
	3rd	4th	3rd instar	4th instar	3rd instar	4th instar
1) 3-5	50	50	74.0 ± 14 a	76.0 ± 14 a	20.4 ± 14 a	11.7 ± 11 b
2) 6-8	50	41	80.0 ± 14 a	81.4 ± 19 a	47.6 ± 14 a	44.9 ± 15 a
3) 12-15	50	50	82.0 ± 4 a	74.0 ± 9 a	43.3 ± 18 a	42.8 ± 12 a

* Means followed by differing letters in each column are significantly different ($P < 0.05$; Turkey [SAS 1985]).

Percentage emergence of *M. croceipes* adults was much lower than the percentage parasitized. Significant differences ($P < 0.05$) in adult emergence occurred between parasitoid age groups (1, 2, and 3) in the fourth instar (Table 1). Group 1 (3 to 5 d) was significantly different ($P < 0.05$) from the other two groups within the fourth instar. The highest adult emergence was in parasitoids from group 2 (6 to 8 d) for both instars, the lowest in group 1 (3 to 5 d) (Table 1). Cocoons were not spun by 43% of the parasitoids that emerged from hosts; the parasitoid died if it did not spin a cocoon within 2 h after exiting the host. The highest proportion that did not spin cocoons was in group 1 (3 to 5 d) with 57.4 and 68.3% for the third and fourth host instars, respectively (Table 1). Group 2 (6 to 8 d) had 44.2% of third instar and 39.2% of fourth instar that failed to spin cocoons. Group 3 (12 to 15 d) had 19.7 and 32.0% non-spinning parasitoid larvae for the third and fourth host instars, respectively. Group 3 was significantly different ($P < 0.05$) from the other two age groups within the third instar.

More males than females were produced in the majority of the parasitoid age group and host instar combinations (Table 2). The percentage of *M. croceipes* progeny entering diapause appeared to be affected by parental age (Table 2). Group 2 had the lowest percentage entering diapause and group 3 had the highest; differences between group 2 (6 to 8 d) and group 3 (12 to 15 d) within the third instar were significant ($P < 0.05$) (Table 2).

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Table 2. Adult sex ratio, and percentage entering diapause of *M. croceipes* progeny at three age ranges parasitizing two host instars.*

Parasitoid age (days)	n		Sex ratio (F:M)		% Diapause ± SD	
	Instar					
	3rd	4th	3rd	4th	3rd	4th
1) 3-5	50	50	1: 3.0	1:0.7	18.8 ± 14 ab	20.0 ± 19 a
2) 6-8	50	41	1: 0.7	1:1.5	4.5 ± 6 b	9.0 ± 8 a
3) 12-15	50	50	1: 17.0	1:1.7	32.2 ± 17a	19.7 ± 12 a

* Values followed by differing letters in each column are significantly different ($P < 0.05$; Tukey [SAS 1985]).

Discussion

Parasitism of *H. zea* by *M. croceipes* was successful regardless of the age of the parasitoid or of the two host instars used in the test. When the female was allowed to search the area containing larvae, the hosts were stung as they were encountered. No differences were observed in readiness to sting compared with parasitoid age, even though the fourth instar host larvae were more aggressive in attacking the wasps. When the fourth instars fought the wasp, that activity seemed to stimulate the parasitoid to oviposit. Once the female wasps encountered host frass, an earnest search began, which generally led to finding a larva and tapping with the antenna in a curved position. Upon contact with the host, she immediately stung the larva. This ovipositional activity concurs with previous descriptions (Lewis 1970a).

Adult parasitoid emergence was low for all treatments and instars. This may be accounted for by two factors: failure of the parasitoid to spin a cocoon once it exited the host, and secondly, the number of parasitoids entering diapause. Percentage of parasitoids not spinning cocoons was highest for the youngest group tested (3 to 5 d). The method of inverting the cups after six days may have reduced cocoon spinning. The cups were inverted to avoid the host larva boring into the diet to form a death cell. The parasitoid emerges in this cell and the diet is usually too moist to allow spinning of a cocoon (Bryan et al. 1969). If the cups are inverted, the host larva is forced onto the lid, and is provided a dry place for the wasp to spin its cocoon. A six-day period was too long to wait for inversion. All replications of group 1 were inverted after six days, and all replications of the other two age groups were inverted after five days.

More males were produced as older parasitoids were used. Group 3 (12 to 15 d) was the only group where both instars produced more males, suggesting that older adults produced more male progeny. This fact is consistent with findings by Lewis and Snow (1971) that *M. croceipes* exhibits facultative parthenogenesis or arrhenotoky. Group 1 (3 to 5 d) for the fourth instar and group 2 (6 to 8 d) for the third instar, produced the highest female:male sex ratio 1:0.66 and

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1:0.72, respectively. These results suggest that adult *M. croceipes* aged 3 to 8 d, would be preferable for rearing parasitoid adult females.

The youngest (3 to 5 d) and oldest (12 to 15 d) *M. croceipes* age groups entered diapause in higher percentages than group 2 (6 to 8 d). Group 2 in third instar had the lowest percentage entering diapause (4.5%) which was significantly different ($P < 0.05$) from group 3 (12 to 15 d). Group 3 in third instar had the highest (32.2%). Progeny developing from eggs deposited by the youngest and oldest adult parasitoids seemed to result in greater incidence of diapause than those deposited by 6-8-d-old wasps.

The results of this study showed that of the age ranges tested, *M. croceipes* will parasitize third and fourth instars of *H. zea*, its preferred host instars, equally across those ranges. However, the youngest and oldest parasitoids tested produced more males in third instar hosts. The highest number of males was in the oldest group, third instar. This may indicate lowered effectiveness of mating in older females that had not previously oviposited. The oldest group and (third instar) hosts, also had the highest numbers entering diapause under these rearing conditions. Overall, group 2, with third instar hosts, gave the highest percentage adult emergence (47.5 ± 14%; $\overline{X} \pm SD$), a significant difference ($P < 0.05$) in the percentage of females produced (79%), and a significantly lower ($P < 0.05$) percentage (4.5 ± 6%) of the wasps entering diapause during the test period. This indicated that 6-8 d mated female *M. croceipes* may yield better results when used in rearing and field release programs where third and early fourth instar *H. zea* infestations occur. Further studies are needed to evaluate the fitness of *M. croceipes* progeny developing in the field after augmentative releases.

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References Cited

Bryan, D. E., C. G. Jackson and R. Patana. 1969. Laboratory studies of *Microplitis croceipes,* a braconid parasite of *Heliothis* spp. J. Econ. Entomol. 62: 1141-1144.

Bull, D. L., E. G. King and J. E. Powell. 1989. Effects and fate of selected insecticides after application to *Microplitis croceipes.* Suppl-Southwestern Entomol. 12: 59-70.

Elzen, G. W., P. J. O'Brien and J. E. Powell. 1989. Toxic and behavioral effects of selected insecticides on the *Heliothis* parasitoid *Microplitis croceipes.* Entomophaga 34: 87-94.

Elzen, G. W., P. J. O'Brien, G. L. Snodgrass and J. E. Powell. 1987. Susceptibility of the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae) to field rates of selected cotton insecticides. Entomophaga 32: 545-550.

Herbert, D. A., Jr., G. W. Zehnder, J. Speese and J. E. Powell. 1993. Parasitization and timing of diapause in Virginia *Microplitis croceipes* (Hymenoptera: Braconidae): Implications for biocontrol of *Helicoverpa zea* (Lepidoptera: Noctuidae) in soybean. Environ. Entomol. 22: 693-698.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-02 via free access

Hopper, K. R. 1986. Preference, acceptance, and fitness components of *Microplitis croceipes* (Hymenoptera: Braconidae) attacking various instars of *heliothis virescens* (Lepidoptera: Noctuidae). Environ. Entomol. 15: 274-280.

Hopper, K. R. and E. G. King. 1984. Preference of *Microplitis croceipes* (Hymenoptera: Braconidae) for instars and species of *Heliothis* (Lepidoptera: Noctuidae). Environ. Entomol. 13: 1145-1150.

Jones, R. L. and W. J. Lewis. 1971. Physiology of the host-parasite relationship between *Heliothis zea* and *Microplitis croceipes*. J. Insect Physiol. 17: 921-927.

Katayama, R. W., C. H. Cobb, J. G. Burleigh and W. R. Robinson. 1987. Susceptibility of adult *Microplitis croceipes* (Hymenoptera: Braconidae) to insecticides used for *Heliothis spp*. (Lepidoptera: Noctuidae) control. Flor. Entomol. 70: 530-532.

King, E. G. and G. G. Hartley. 1985. *Heliothis virescens,* pp. 323-328. *In* P. S. Singh and R. F. Moore [ed.], Handbook of insect rearing, Vol 2. Elsvier Sci. Publ. Soc. Amsterdam.

Lewis, W. J. 1970a. Life history and anatomy of *Microplitis croceipes* (Hymenoptera: Braconidae), a parasite of *Heliothis* spp. (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 63: 67-70.

1970b. Study of species and instars of larval *Heliothis* parasitized by *Microplitis croceipes*. J. Econ. Entomol. 63: 363-365.

Lewis, W. J. and J. R. Brazzel. 1968. A three-year study of parasites of the bollworm and the tobacco budworm in Mississippi. J. Econ. Entomol. 61: 673-676.

Lewis, W. J. and R. L. Burton. 1970. Rearing *Microplitis croceipes* in the laboratory with *Heliothis zea* as hosts. J. Econ. Entomol. 63: 656-658.

Lewis, W. J. and J. W. Snow. 1971. Fecundity, sex ratios, and egg distribution by *Microplitis croceipes*, a parasite of *Heliothis*. J. Econ. Entomol. 64: 6-8.

Metcalf, C. L., W. P. Flint and R. L. Metcalf. 1962. Destructive and useful insects: their habits and control. McGraw-Hill Book Company, New York. pp. 1087.

Pair, S. D., M. L. Laster and D. F. Martin. 1982. Parasitoids of *Heliothis* spp. (Lepidoptera: Noctuidae) larvae in Mississippi associated with sesame interplantings in cotton, 1971-1974: Implications of host-habitat interaction. Environ. Entomol. 11: 509-512.

Powell, J. E., E. G. King and C. S. Jany. 1986. Toxicity of insecticides to adult *Microplitis croceipes* (Hymenoptera: Braconidae). J. Econ. Entomol. 79: 1343-1346.

Powell, J. E. and W. P. Scott. 1985. Effect of insecticide residues on survival of *Microplitis croceipes* adults (Hymenoptera: Braconidae). in cotton. Environ. Entomol. 68: 692-693.

1991. Survival of *Microplitis croceipes* (Hymenoptera: Braconidae) in contact with residues of insecticides on cotton. Environ. Entomol. 20: 346-348.

SAS Institute. 1985. SAS User's Guide: Statistics, 1985 edition. SAS Institute, Inc. Cary, N. C.

Smith, J. W., E. G. King and J. V. Bell. 1976. Parasites and pathogens among *Heliothis* species in the Central Mississippi Delta. Environ. Entomol. 5: 224-226.

Stadelbacher, E. A., J. E. Powell and E. G. King. 1984. Parasitism of *Heliothis zea* and *H. virescens* (Lepidoptera: Noctuidae) larvae in wild and cultivated host plants in the delta of Mississippi. Environ. Entomol. 13: 1167-1172.

Tillman, P. G. and J. E. Powell. 1989. Comparison of acceptance of larval instars of the tobacco budworm (Lepidoptera: Noctuidae) by *Microplitis croceipes, Microplitis demolitor, Cotesia kazak* (Hymenoptera: Braconidae), and *Hyposoter didymator* (Hymenoptera: Ichneumonidae). J. Agric. Entomol. 6: 201-209.

Vinson, S. B. and D. L. Dahlman. 1989. Physiological relationship between braconid endoparasites and their hosts: the *Microplitis croceipes-Heliothis* spp. system. Suppl-Southwestern Entomol. 12: 17-37.

Zehnder, G. W., D. A. Herbert, R. M. McPherson, J. Speese III and T. Moss. 1990. Incidence of *Heliothis zea* (Lepidoptera: Noctuidae) and associated parasitoids in Virginia soybeans. Environ. Entomol. 19: 1135-1140.

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