Superparasitism Occasionally Predisposes *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) to Develop Gregariously in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)[1]

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J. Entomol. Sci. 37(1): 1-9 (January 2002)

Abstract Experiments were designed to estimate the incidence of superparasitism of *Spodoptera exigua* (Hübner) and gregarious development of *Cotesia marginiventris* (Cresson). Dissections of host larvae revealed that superparasitism was common at all host densities (5, 10, or 20 larvae per 0.05 liter arena). At least two parasitoid eggs were present per host on day 1 after parasitoid oviposition; eggs were scarcely found on day 2 and never found on days 3, 4 or 5. More than one parasitoid first instar was present per host on day 2 at all densities, although more were found in hosts of the 5 density rather than the 20 density treatment. Second instars were present in hosts on days 3, 4 and 5, and at least one was present per host on day 5. Third instars (the final instar stage) were not present inside hosts by day 5. Rearing of host larvae demonstrated that gregarious development was rare, since less than 6% of parasitized hosts yielded two third instars that spun cocoons. Neither host density nor exposure time (24 h, 48 h) had an effect on the occurrence of gregarious development. The adult emergence rate was 64% and the sex ratio was male-biased for parasitoids that developed gregariously. The adult emergence rate was 73% and the sex ratio was unbiased for those that developed solitarily. This research suggests that superparasitism of hosts by *C. marginiventris* can occasionally predispose parasitoid larvae to develop gregariously. Frequent superparasitism of hosts may be detrimental to the long-term propagation of *C. marginiventris*.

Key Words Solitary, gregarious, superparasitism, mass rearing, biological control

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Cotesia marginiventris (Cresson), formerly in the genus *Apanteles* Foerster (Mason 1981), is an important natural enemy of the beet armyworm, *Spodoptera exigua* (Hübner), and other lepidopteran pests in agroecosystems (McCutcheon et al. 1990, Ruberson et al. 1994, Cecilia and Luna 1996). It has been used as a model organism for studies on tri-trophic interactions between crop plants, pests and their natural enemies (Loke and Ashley 1984, Dmoch et al. 1985, Turlings et al. 1990, 1991, Farmer 1997). This species has been known previously as a solitary endoparasitoid (Boling and Pitre 1970, Tillman 2001), in that only 1 offspring can complete its development from the resources provided by a single host (Salt 1934). Most species of

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[1] Received 22 December 2000; accepted for publication 17 June 2001. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by USDA for its use.

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Cotesia Cameron are gregarious, but approximately 25% of them are solitary (Mason 1981).

In gregarious endoparasitoids, more than 1 offspring can complete its development in a single host (Godfray 1994). Superparasitism has been shown to occasionally predispose larvae of another braconid, *Aphaereta genevensis* Fischer, which normally develops solitarily, to develop gregariously inside dipteran hosts (Mayhew and van Alphen 1999). Superparasitism occurs when a parasitoid deposits two or more eggs in the same host during two or more distinct attacks. If only a single female of a species is involved, the behavior is termed self-superparasitism; when two or more females are involved, it is called conspecific superparasitism (van Dijken and Waage 1987). Self-superparasitism in solitary parasitoids may often result in a waste of time and eggs; however, it can be an adaptive behavior when the presence of two or more eggs per host increases the overall chance that one parasitoid will survive (van Alphen and Visser 1990). Conspecific superparasitism can be adaptive if the offspring of the second female to attack the host has a probability of greater than zero of surviving to the adult stage (van Alphen and Visser 1990). Superparasitism behavior may result from the inability of searching parasitoids to discriminate between previously parasitized and unparasitized hosts (van Lenteren and Bakker 1975, Hubbard et al. 1999).

On one occasion, Kunnalaca and Mueller (1979) found two *C. marginiventris* larvae developing inside the same green cloverworm, *Plathypena scabra* (F.). Although both larvae emerged from their shared host, neither one developed to the adult stage. During preliminary experiments to develop efficient techniques for rearing *C. marginiventris,* I noticed that two third instars occasionally emerged from the same beet armyworm and then proceeded to spin separate cocoons. More rigorous experiments were designed to determine the frequency of superparasitism of *S. exigua* and gregarious development of *C. marginiventris.*

Materials and Methods

Insect cultures. *Cotesia marginiventris* adults were from a colony at the USDA, REE, ARS, MSA, Biological Control and Mass Rearing Research Unit (BCMRRU), Mississippi State, MS. This parasitoid has been in production for more than 60 continuous generations. An automated system for rearing this parasitoid is currently under investigation. The host, *S. exigua,* has been reared at BCMRRU for more than 160 continuous generations, using well-established procedures (see Tillman et al. 1997). Host larvae were reared on an agar - soybean flour - wheat germ meridic diet (after King and Hartley 1985).

Detecting superparasitism. The effects of host density and day-after-oviposition on the number of parasitoid eggs, first and second instars detected in each *S. exigua* larva were determined in two trials. For both trials, host density was 5, 10 or 20 *S. exigua* late first to second instars per parasitoid female per arena, and the exposure time was 24 h. Arenas were 0.05 L plastic centrifuge tubes. A 2-cm diameter hole was bored into the cap of each tube and covered with nylon mesh to provide air circulation. Meridic diet (≈20 ml) was poured into the base of each tube. It hardened in 30 min. *Spodoptera exigua* larvae were added at random to each tube per treatment. Next, a 1 to 2-d-old *C. marginiventris* female was added at random to each tube. A total of 30 tubes were used, 10 per host density treatment.

Tubes containing hosts and parasitoid were held in a growth chamber at 26.5°C, 60-70% R.H., 16:8 h (L:D) cycle. After 24 h, ovipositing parasitoids were removed and tubes were returned to the growth chamber. For the next 5 consecutive days, one tube per density treatment was removed from the chamber and stored at −13 to −22°C for dissection at a later date.

Spodoptera exigua were dissected while submerged in sterile water (in a watch glass) using forceps and a stereo zoom microscope (≥20X magnification). Immature stages were found in the hemocoel, and were rendered visible by applying 2 to 3 drops of Double Stain™ (a solution of acid fuchsin and lignin pink, BioQuip Products, Gardena, CA). A total of 350 *S. exigua* larvae were dissected. The number and stage of parasitoid immatures found inside each host were recorded. Immature stages of *C. marginiventris* have been described and illustrated previously (Boling and Pitre 1970).

Detecting gregarious development. The effects of host density and exposure time on the percentage of hosts yielding any *C. marginiventris* progeny were determined. This rearing experiment consisted of 3 identical trials. Host density of 5, 10 or 20 *S. exigua* late first to second instars per parasitoid female were used per arena (0.05 L centrifuge tube). Host larvae were exposed to a parasitoid female for 24 h or 48 h. The procedures of adding meridic diet to each tube, then placing host larvae and a single female parasitoid in each tube were identical to those used for the detection of superparasitism experiment. Sixty tubes were used for each host density, with half at each exposure time in each trial, for a total of 180 tubes for the entire experiment.

Tubes were held in a growth chamber at 26.5°C, 60-70% R.H., 16:8 h (L:D) cycle. After 24 h or 48 h, parasitoid females were removed and tubes were returned to the growth chamber. At 4 to 5 d after the date that host larvae were exposed to the parasitoid, all host larvae were removed from tubes and placed, individually, into a clean Petri dish (10 mm depth × 35 mm diam), with lid, supplied with ≈4 g of fresh, meridic diet, and then allowed to develop undisturbed in these dishes for 5 to 6 additional days inside the rearing room. After 10 d from the initial ‘sting date’, healthy parasitoid larvae had emerged from hosts and constructed a silken cocoon at the base, side, or on the lid of the dish arena. The number of cocoons present in each dish was recorded.

The percentage of *S. exigua* larvae successfully yielding any *C. marginiventris* progeny (cocoons) was calculated from the initial host density per tube, for both exposure times. The percentage of parasitized *S. exigua* yielding 2 cocoons was estimated from this data set. The emergence rate and sex ratio of adults produced solitarily and gregariously were calculated.

Statistical analysis. A 3 × 5 factorial analysis of variance (ANOVA; general linear model) was used to test the significance of host density and day-after-oviposition on the presence of parasitoid progeny (eggs, first and second instars) inside *S. exigua* larvae. A 3 × 2 factorial ANOVA (general linear model) was used to test the significance of host density and exposure time on the percentage of *S. exigua* yielding any *C. marginiventris* cocoons as well as the percentage of *S. exigua* yielding two cocoons. The Tukey’s HSD method was used for pairwise comparisons of means after the ANOVA (Zar 1999). Percentage data were arcsine-transformed and absolute data were square-root transformed prior to analysis (Sokal and Rohlf 1981). Statistical analyses were performed with SigmaStat (1994) or Systat (1998) software. For all experimental analyses, means were considered significantly different if $P \leq 0.05$. Untransformed data are illustrated.

Results

Superparasitism. Two or more *C. marginiventris* eggs were often found inside *S. exigua* larvae on day 1 at all host densities (Fig. 1A). Host density had no significant effect on the abundance of eggs per host (F = 0.25; df = 2, 335; P = 0.78). But, day-after-oviposition had a significant effect (F = 95.5; df = 4, 335; $P < 0.0001$); more eggs were found on day 1 than on any other day. The host density and day-after-oviposition interaction was not significant (F = 0.13; df = 8, 335; P = 0.99).

Cotesia marginiventris first instars were most often found inside hosts on days 2 and 3. Host density (F = 3.1; df = 2, 335; P = 0.047) and day-after-oviposition (F = 40.4; df = 4, 335; $P < 0.0001$) significantly affected the number found inside hosts (Fig. 1B). On average, more than one parasitoid first instar was present per host on day 2 at all host densities. Also, more first instars were present in hosts of the 5 density treatment rather than the 10 density or 20 density treatments on day 3. However, the interaction of host density and day-after-oviposition was not significant (F = 1.9; df = 8, 335; P = 0.06). All first instars had enlarged heads and sickle-shaped mandibles. One *C. marginiventris* first instar was found with its mandibles embedded in the body of another first instar in the same host on three separate occasions (once

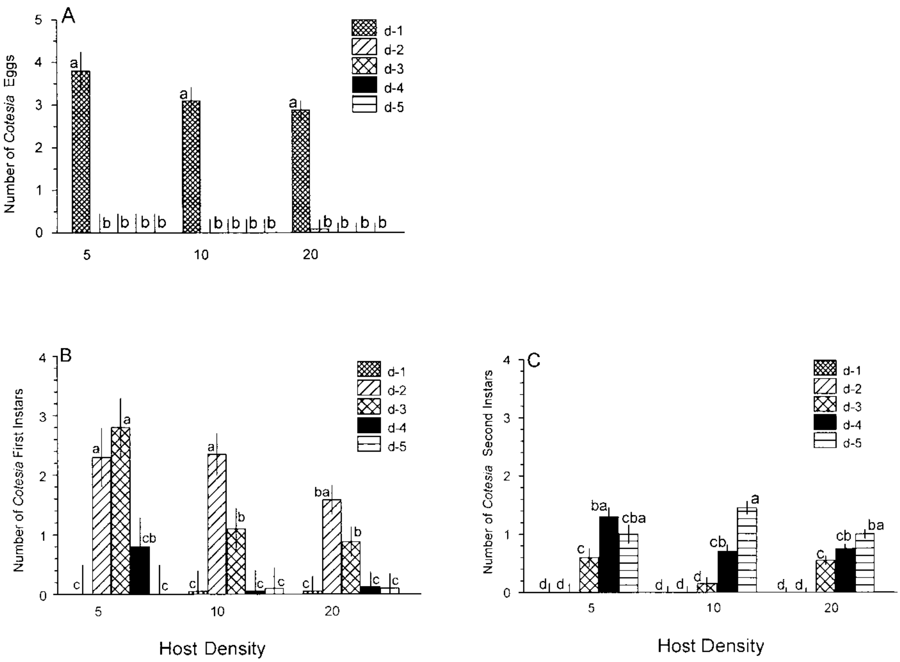

Fig. 1. Least square mean ± SEM number of *C. marginiventris* eggs (A), first instars (B), and second instars (C) found inside *S. exigua* larvae on days (1-5) after parasitoid oviposition. n, 350 total observations. Bars with different letters are significantly different (Tukey's HSD method, $P \leq 0.05$).

at the 5, 10, and 20 density treatments) on day 3. Evidence of cannibalistic behavior between first instars was not found on any other day.

Cotesia marginiventris second instars were present inside hosts on days 3, 4, and 5 (Fig. 1C). There was a significant interaction between host density and day-after-oviposition ($F = 3.1$; $df = 8, 335$; $P = 0.002$). Significantly more second instars were present per host of the 10 density treatment on day 5 rather than on day 4 or day 3. Similarly, more second instars were found per host of the 20 density treatment on day 5 rather than day 3. Host density had no effect on the number found inside hosts ($F = 2.1$; df = 2, 335; $P = 0.12$), but day-after-oviposition did ($F = 75.4$; df = 4, 335; $P < 0.0001$). Third instars were not present in hosts on day 5.

Gregarious development. The interaction of host density and exposure time was significant ($F = 7.6$; df = 2, 173; $P = 0.001$). A greater percentage of hosts were parasitized at the 5 density/24 h treatment level than at any other level (Fig. 2A). Nearly 70% of hosts of the 5 density/24 h treatment level yielded one or two *C. marginiventris* third instar(s) that spun cocoons. Host density had no effect on the percentage of *S. exigua* larvae yielding one or two *C. marginiventris* cocoons ($F = 2.0$; df = 2, 173; $P = 0.13$); nor did exposure time ($F = 0.24$; df = 1, 173; $P = 0.62$).

Host density had no effect on the percentage of parasitized *S. exigua* yielding two *C. marginiventris* cocoons ($F = 0.38$; df = 2, 161; $P = 0.69$); nor did exposure time ($F = 1.6$; df = 1, 161; $P = 0.21$). The host density x exposure time interaction was not significant ($F = 2.8$; df = 2, 161; $P = 0.06$). The incidence of gregarious development was less than 6% and it was not influenced by any of the treatment levels (Fig. 2B). For the entire experiment, 32 *S. exigua* larvae yielded two *C. marginiventris* cocoons for a total of 64 cocoons arising from gregarious development. The adult emergence rate from these cocoons was 64.1% (n = 41 adults). This included 25 males and 16 females; the sex ratio was 1: 0.64 (males: females). In comparison, 939 *S. exigua* larvae yielded 1 *C. marginiventris* cocoon for a total of 939 cocoons arising from solitary development. The adult emergence rate from these cocoons was 72.9% (n = 685 adults). This included 339 males and 346 females; the sex ratio was 1: 1.02 (males: females).

Discussion

Superparasitism. Dissections of *S. exigua* larvae that were exposed to *C. marginiventris* females for 24 h provided evidence of superparasitism. The presence of at least two eggs per host at all host densities could indicate that the host densities were too low or the exposure time was too long in this study. In either case, parasitoid females did not discriminate between parasitized and unparasitized hosts. Failure to discriminate could have been due to naivety (van Lenteren and Bakker 1975, Dmoch et al. 1985), since parasitoid females had no prior experience with hosts before placement into experimental arenas.

At least two first instars were present per host of the 5 density and 10 density treatments on day 2, although the abundance of first instars declined significantly by days 4 and 5 at all densities. The decline could have been a result of physical combat between first instars, since cannibalism was apparent between first instars in the same host in this study. Another reason for the decline was that first instars (those that escaped from being eaten or injured) had molted into second instars.

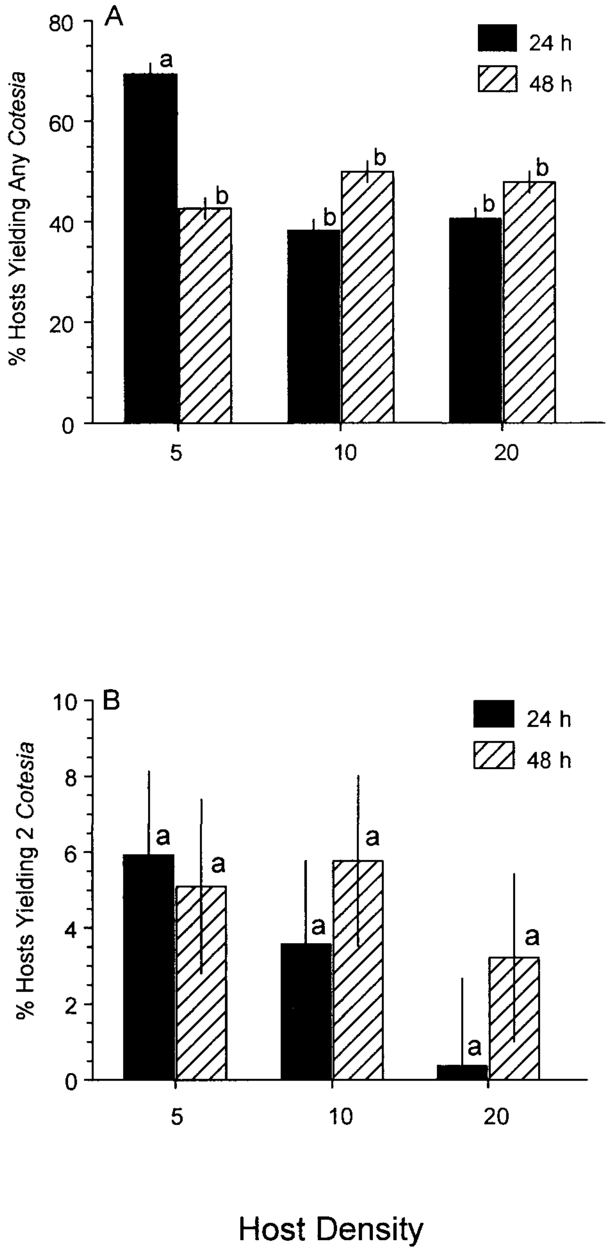

Fig. 2. (A) Least square mean ± SEM percentage of *S. exigua* yielding any *C. marginiventris* progeny (cocoons) per host density and exposure time in each arena. *n*, 180 total observations. (B) Least square mean ± SEM percentage of *S. exigua* yielding two parasitoid progeny. *n*, 167 total observations. Bars with different letters are significantly different (Tukey's HSD method, $P \leq 0.05$).

Host density had no effect on the abundance of second instars, but more were found in hosts on day 5 than on day 3 at both the 10 density and 20 density treatments, because some had not yet molted to the second instar by day 3. Although second instars are not cannibalistic (Boling and Pitre 1970), they probably vie for limited amounts of nutrients and oxygen, both of which are provided by the host. Physiological suppression by starvation or asphyxiation (see Mackauer 1990) could prevent supernumerary second instars from developing into third instars within a shared host.

If host size correlates positively with the amount of food or oxygen accessible to parasitoids, it is conceivable that two *C. marginiventris* third instars have a greater prospect of emerging successfully from a larger-sized host than from a smaller-sized one. Some solitary braconids (species of *Apanteles* Foerster and *Cotesia*) routinely develop inside large hosts and do not exhaust all available resources (Le Masurier 1987). Some solitary species develop in hosts that are large enough to support broods of 20 or more gregarious larvae (Le Masurier 1987).

Gregarious development. A greater percentage of hosts yielded at least one *C. marginiventris* cocoon at the 5 density/24 h treatment level, rather than any other treatment level (see Fig. 2A). This observation suggests that females attacked most, if not all, of the hosts at this low density and that the offspring had a good chance of surviving and emerging from these hosts. But, the time of exposure appeared important. The yield of cocoons was considerably less for those hosts exposed to parasitoid females for 48 h at the low density. An increase in exposure time can lead to a decrease in emergence of progeny from hosts and increase the mortality rate of progeny still inside hosts (Ueno 1997).

The observation that host density, exposure time, and the interaction of these two factors had no effect on the percentage of parasitized hosts yielding two *C. marginiventris* cocoons was not expected (see Fig. 2B). It suggests that behavioral and physiological interactions between host and developing brood were as important as adult oviposition behavior in the successful rearing of two rather than the typical one parasitoid from a single host.

Adult emergence from cocoons produced gregariously was lower than expected in this study. One plausible explanation is that *C. marginiventris* immatures died inside cocoons prior to pupation or maturation. Reasons for developmental failure may or may not be due to some nutritional defect - as an outcome of sharing the same host.

For those adults that successfully emerged from cocoons produced via gregarious development, the sex ratio was male-biased. The preponderance of male progeny could indicate that ovipositing females laid more unfertilized eggs than fertilized eggs in hosts that were already parasitized. Nevertheless, gregarious development can be detrimental to the continuous propagation of *C. marginiventris* if emergence rates are consistently lower than expected and sex ratios are frequently male-biased.

Conclusion. The results of this investigation are most pertinent to the design of efficient systems for rearing *C. marginiventris*. The results should not be used to predict or estimate the oviposition behavior and larval development of this parasitoid under natural conditions in the field. From the standpoint of rearing, efforts should be made to reduce superparasitism. Perhaps, exposing only experienced females rather than naive ones to hosts at densities in excess of 20 larvae per parasitoid may significantly reduce superparasitism and the incidence of gregarious larval development.

Acknowledgments

I thank Mary Catherine Tate (USDA-ARS, Mississippi State, MS) for her technical assistance and Nick J. Mills (Division of Insect Biology, Univ. California, Berkeley, CA) and Pedro Barbosa (Department of Entomology, University of Maryland, College Park, MD) for their comments on an earlier version of this manuscript. This manuscript was approved for publication as Journal Article No. J-9789 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University.

References Cited

Boling, J. C. and H. N. Pitre. 1970. Life history of *Apanteles marginiventris* with descriptions of immature stages. J. Kansas Entomol. Soc. 43: 465-470.

Cecilia, N. M. and M. G. Luna. 1996. Parasitism, survivorship, sex ratio, and developmental time of *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), parasitizing *Rachiplusia nu* (Guennée) (Lepidoptera: Noctuidae) larvae in soybean crops in Argentina. Acta Entomol. Chilena 20: 23-28.

Dmoch, J., W. J. Lewis, P. B. Martin and D. A. Nordlund. 1985. Role of host-produced stimuli and learning in host selection behavior of *Cotesia* (= *Apanteles*) *marginiventris* (Cresson). J. Chem. Ecol. 11: 453-463.

Farmer, E. E. 1997. New fatty acid-based signals: a lesson from the plant world. Science 276: 912-913.

Godfray, H. C. J. 1994. Parasitoids: behavioral and evolutionary ecology. Princeton Univ. Press, Princeton, NJ.

Hubbard, S. F., I. F. Harvey and J. P. Fletcher. 1999. Avoidance of superparasitism: a matter of learning? Anim. Behav. 57: 1193-1197.

King, E. G. and G. G. Hartley. 1985. *Heliothis virescens,* Pp. 323-328. *In* P. Singh and R. F. Moore [eds.], The handbook of insect rearing, Vol. II. Elsevier, NY.

Kunnalaca, S. and A. J. Mueller. 1979. A laboratory study of *Apanteles marginiventris,* a parasite of green cloverworm. Environ. Entomol. 8: 365-368.

Le Masurier, A. D. 1987. A comparative study of the relationship between host size and brood size in *Apanteles* spp. (Hymenoptera: Braconidae). Ecol. Entomol. 12: 383-393.

Loke, W. H. and T. R. Ashley. 1984. Potential uses of kairomones for the behavioral manipulation of *Cotesia marginiventris* (Cresson). J. Chem. Ecol. 10: 1377-1384.

Mackauer, M. 1990. Host discrimination and larval competition in solitary endoparasitoids, Pp. 41-62. *In* M. Mackauer, L. E. Ehler and J. Roland [eds.], Critical issues in biological control. Intercept, Andover.

Mason, W. R. M. 1981. The polyphyletic nature of *Apanteles* Foerster (Hymenoptera: Braconidae): a phylogeny and reclassification of Microgastrinae. Mem. Entomol. Soc. Canada 115: 1-147.

Mayhew, P. J. and J. J. M. van Alphen. 1999. Gregarious development in alysiine parasitoids evolved through a reduction in larval aggression. Anim. Behav. 58: 131-141.

McCutcheon, G. S., S. G. Turnipseed and M. J. Sullivan. 1990. Parasitization of lepidopterans as affected by nematicide-insecticide use in soybean. J. Econ. Entomol. 83: 1002-1007.

Ruberson, J. R., G. A. Herzog, W. R. Lambert and W. J. Lewis. 1994. Management of the beet armyworm (Lepidoptera: Noctuidae) in cotton: role of natural enemies. Florida Entomol. 77: 440-453.

Salt, G. 1934. Experimental studies in insect parasitism. II. Superparasitism. Proc. R. Soc. (Lond.) Ser. B. 114: 455-476.

SigmaStat. 1994. SigmaStat user's guide, version 1.0. Jandel Scientific, San Rafael, CA.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry: the principles and practice of statistics in biological research. 2nd ed. Freeman, New York.

Systat. 1998. Systat 8.0 Statistics user's manual. SPSS Inc., Chicago, IL.

Tillman, P. G. 2001. Factors affecting parasitization of *Spodoptera exigua* (Lepidoptera: Noctuidae) and sex ratio of the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). J. Entomol. Sci. 36: 188-198.

Tillman, P. G., G. McKibben, S. Malone and D. Harsh. 1997. Form-fill-seal machine for mass rearing noctuid species. MS Agric. For. Expt. Sta. Tech. Bull. 213: 1-4.

Turlings, T. C. J., W. A. Scheepmaker, L. E. M. Vet, J. H. Tumlinson and W. Lewis. 1990. How contact foraging experiences affect preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). J. Chem. Ecol. 16: 1577-1589.

Turlings, T. C. J., J. H. Tumlinson, F. J. Eller and W. J. Lewis. 1991. Larval-damaged plants: source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. Entomol. Exp. Appl. 58: 75-82.

Ueno, T. 1997. Effects of superparasitism, larval competition, and host feeding on offspring fitness in the parasitoid *Pimpla nipponica* (Hymenoptera: Ichneumonidae). Ann. Entomol. Soc. Am. 90: 682-688.

Van Alphen, J. J. M. and M. E. Visser. 1990. Superparasitism as an adaptive strategy for insect parasitoids. Annu. Rev. Entomol. 35: 59-79.

Van Dijken, M. J. and J. K. Waage. 1987. Self and conspecific superparasitism by the egg parasitoid *Trichogramma evanescens*. Entomol. Exp. Appl. 43: 183-192.

Van Lenteren, J. C. and K. Bakker. 1975. Discrimination between parasitized and unparasitized hosts in the parasitoid wasp *Pseudocoila bochei*: a matter of learning. Nature 254: 417-419.

Zar, J. H. 1999. Biostatistical analysis. Fourth ed. Prentice Hall, NJ.