Parasitoid Density and Arena Size Effects on Progeny Production of *Anaphes iole* Girault (Hymenoptera: Mymaridae)[1]

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Abstract *Anaphes iole* Girault is a native, solitary egg parasitoid of *Lygus* bugs in North America. Ongoing research is considering factors that may lead to efficient mass rearing of *A. iole* for augmentative biological control. This study examined the effects of *A. iole* female density and arena size on progeny production. Production increased by a factor of 2.1 as parasitoid density increased from 5 to 10 and from 10 to 20 females per 8 L arena (rearing cage) with a host patch containing from 1,500 to 2,000 eggs of *Lygus hesperus* Knight (Heteroptera: Miridae). Sex ratios of mature progeny did not differ significantly between parasitoid densities of 10 versus 20 females. Arena size (≈1, 2, 4, or 8 L cages) had no effect on progeny production when 20 females were confined to the same cage. This research suggests that little or no measurable interference will occur between ovipositing *A. iole* females on shared host patches and cage size can be varied to increase rearing capacity.

Key Words *Anaphes iole, Lygus* bugs, biological control, interference, mass rearing

The solitary fairyfly, *Anaphes iole* Girault (Hymenoptera: Mymaridae), is the primary egg parasitoid of *Lygus hesperus* Knight (Heteroptera: Miridae) in the United States (Debolt 1987). Ongoing research is considering factors that may lead to efficient mass rearing of *A. iole* for augmentative biological control of *Lygus* bugs in *Bt*-cotton (Ruberson and Williams, III 2000), strawberries (Norton and Welter 1996), and perhaps in vegetables grown in greenhouses (Mc Gregor et al. 2000).

Despite the promise of *A. iole* as an effective biological control agent of *L. hesperus* (Udayagiri et al. 2000), only limited research has focused on mass rearing techniques. Preliminary research has shown that it may be feasible to mass produce *A. iole* (Jones and Jackson 1990). Knowledge of the searching and oviposition behaviors of conspecific females within confinement may contribute to the design of efficient rearing systems.

Searching parasitoids make decisions concerning which hosts to parasitize and which ones to reject. These decisions may become more complex when parasitoids forage in the company of conspecifics on the same host patch. The proportion of hosts that will generate parasitoid progeny may depend on the number of parasitoid—host encounters as well as parasitoid—parasitoid interactions in relation to host density (Knipling 1992).

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Any type of interaction between parasitoids (or predators) that reduces searching efficiency has been termed mutual interference (Hassell 1978). The loss of search time due to 'direct' mutual interference (i.e., disturbing, chasing, fighting, etc.) between parasitoids results in an immediate loss of searching efficiency. Mutual interference can also occur 'indirectly'—as a consequence of superparasitism of hosts (Visser and Driessen 1991). Superparasitism often occurs when host density is low or when parasitoid density is high (Waage 1986). This behavior may lead to reduced progeny production (Wylie 1965) or shifts in progeny sex ratios (Werren 1980, van Baaren et al. 1999) in an *in vivo* rearing system. However, variability in sex ratio can be unrelated to superparasitism, but rather a direct consequence of parasitoid density (Strand 1988).

The size of rearing cages may also influence the efficient rearing of parasitoids. Parasitoids may reduce searching time and increase oviposition rates in cages that restrict movement to only within close proximity of hosts (Thorpe and Dively 1985). Alternatively, cages that restrict parasitoid movement may cause reduced oviposition rates due to a reluctance of parasitoids to oviposit under crowded conditions. In contrast, spacious cages (i.e., those with excess horizontal or vertical space between host patches and cage walls or lid) can cause parasitoids to be less productive, if considerable time is spent searching in areas devoid of hosts.

The purpose of this research was to more clearly define the oviposition and searching behavior of lab-cultured *A. iole* females. I tested the hypothesis that parasitoid density per patch and cage size had no effects on progeny production. This research is important to the development of an efficient system for rearing this fairyfly.

Materials and Methods

Host and parasitoid cultures. *Lygus hesperus* Knight was obtained from Biotactics, Inc. (Riverside, CA) and has been reared continuously for more than 4 yrs at the USDA-ARS, Biological Control and Mass Rearing Research Unit (BCMRRU), Mississippi State, MS. Adults and nymphs were reared on an artificial diet (Cohen 2000). Insects were held within an environmentally controlled room (25°C, 50 to 70% RH, 14:10 (L:D) h photoperiod) inside 8.3 L rectangular cages (12.7 × 26.0 × 37.5 cm; Rubbermaid®), topped with organdy cloth. Approximately 1,000 nymphs or adults were housed in each communal cage. This culture was propagated using oviposition packets (≈9 × 15 cm). Oviposition packets were made from two strips of Parafilm® (American Can Co., Greenwich, CT) that had been heat-sealed together. An aqueous solution of 2% Gelcarin® (FMC Corp., Food Ingredients Division, Rockland, ME) was enclosed within the Parafilm prior to sealing. This solution maintained a gelatinous consistency and provided a suitable substrate for deposition of *L. hesperus* eggs.

Anaphes iole was obtained from Biotactics, Inc. (Riverside, CA) and has been reared continuously from *L. hesperus* eggs on oviposition packets inside rearing cages (with the base and lid fitted with organdy cloth) for approximately 3 yrs at the USDA-ARS, BCMRRU, Mississippi State, MS. To facilitate successful emergence of *A. iole* adults from hosts, parasitized eggs (i.e., containing *A. iole* larvae) were rinsed from oviposition packets using lukewarm water. Rinsed eggs were then kept at ambient conditions (24 to 27°C, 40 to 50% RH, natural photoperiod) for 2 to 3 d to allow for evaporation of excess water from around the eggs. Next, single batches of rinsed eggs were placed inside clean 8.3 L rectangular 'emergence' cages (with plastic lids, but the sides of the cages were fitted with organdy cloth to allow for air circulation).

Cages were maintained in an environmentally-controlled room (27°C, 70 to 80% RH, 16:8 (L:D) h photoperiod, using 20-watt fluorescent lights) and were never provisioned with food or water. It might be more efficient to eliminate food from rearing cages under mass production, since food has been found to divert females away from host eggs (Jones and Jackson 1990). Without feeding, adults live no longer than 2 d at rearing conditions (EWR, unpubl. data). However, searching *A. iole* females deposit most of their eggs into available hosts within several days of adult life (Jones and Jackson 1990, Riddick 2003). Adult *A. iole* females may possess a lifetime complement of mature eggs in their ovaries upon emergence from hosts (i.e., are proovigenic). This has been demonstrated for several mymarid species (Jackson 1969, King and Copland 1969, Cronin and Strong 1993). Stoner and Surber (1969) found an average of 49 mature eggs in the ovaries of *A. iole* females, less than 3 h old.

Very little or no pre-oviposition period is required in this species and mating can occur within hours of emergence (Conti et al. 1996). Newly emerged, 0-2 d old, unfed, presumably mated, adult parasitoids were harvested three times per week as they flew into one of two plastic 'exit' tubes (39 mm diam × 85 mm length; BioQuip Products, Rancho Dominguez, CA). Exit tubes were affixed to one side of each emergence cage. Newly-emerged adults are phototactic and often fly into exit tubes facing in the direction of an artificial light source. Harvested adults were used for propagation of the colony or for experimentation.

Multiple *A. iole* females and production in a 8.3 L rearing cage. The effect of parasitoid density (5, 10, 15, or 20 females) on progeny production by *A. iole* in rearing cages was determined in this experiment. *Lygus hesperus* eggs were embedded in host patches (*Lygus* oviposition packets, ≈9 × 15 cm) at densities of 1,500 to 2,000 eggs within 3 to 4 h of exposure to adults. Patches were randomly assigned to treatments and placed individually inside clean cages (i.e., arenas). The experimental unit was a single host patch per arena and a completely randomized block design was used with host patches replicated three times per treatment (parasitoid density) in four trials. Thus, each trial considered a total of 12 host patches. Date was used as a blocking factor; 31 October 2001, 7 November 2001, 14 November 2001, and 28 November 2001. For the entire experiment, there were 4 blocks and a total of 48 observations. A sample of approximately 400 *A. iole* adults were harvested from the BCMRRU colony and sexed, using a stereo zoom microscope (10 to 12 X). Parasitoids were from 0-2 d old, since adults live no longer than two days under rearing conditions at BCMRRU. Presumably, most females had mated before removal from cages.

Females, at densities of 5, 10, 15, or 20 individuals, were randomly assigned to treatments. Arenas were not provisioned with food or water. Arenas were placed inside a growth chamber (at 26.5°C, 60 to 70% RH, 16:8 (L:D) h photoperiod, using 20-watt fluorescent lights) for 24 h, then taken out to remove adult parasitoids.

Every other day, dishes were checked for *L. hesperus* nymphs that had hatched from unparasitized eggs. Nymphs were removed and discarded. Approximately 10 d after all host patches had been exposed to female parasitoids, parasitized eggs began to darken, eventually turning dark brown to black. This color change indicated the presence of a late stage *A. iole* pupa inside the host. The number of *A. iole* late stage pupae per treatment per patch was determined after 12 to 14 d.

Multiple *A. iole* females and progeny sex ratio. The effect of parasitoid density (10 versus 20 females) on sex ratio of progeny was determined in this experiment. The experimental unit was a host patch (≈19 × 15 cm) and a completely randomized

block design was used with host patches replicated three times per treatment in six trials. Date was used as a blocking factor; 5 September 2001, 11 September 2001, 19 September 2001, 25 September 2001, 2 October 2001, and 10 October 2001. For the entire experiment, there were 6 blocks and a total of 36 observations. Procedures of manipulating host patches and treatment females are the same as those mentioned in the previous experiment. Arenas were not provisioned with food or water. Parasitoids were exposed to hosts for 24 h, as before. After 7 to 10 d, parasitized eggs were rinsed from the patches, using the technique mentioned previously (see Host and parasitoid cultures). A sample of 150 to 170 parasitized eggs was taken from each density treatment. Eggs were placed individually inside a gelatin capsule (7 × 20 mm; BioQuip Products). Progeny sex ratio per treatment was determined as adults emerged from host eggs.

Arena size and progeny production. The effect of arena size on progeny production was determined in this experiment. Arenas were Rubbermaid® rectangular cages of the following sizes (and dimensions); 0.95 L (5.1 × 14.8 × 25.75 cm), 1.7 L (6.6 × 17.8 × 29.2 cm), 3.8 L (8.8 × 23.1 × 33.3 cm), and 7.95 L (12.8 × 26.6 × 38.1 cm). The base and lid of each cage was fitted with organdy cloth. *Lygus hesperus* eggs were embedded in the host patches at densities of 1,500 to 2,000 eggs within 3 to 4 h of exposure to adults. Patches were randomly assigned to treatments and placed individually inside clean arenas. The experimental unit was a host patch (≈9 × 15 cm) and a completely randomized block design was used with host patches replicated twice per treatment in three trials. Date was used as a blocking factor; 29 January 2002, 5 February 2002, and 19 February 2002. For the entire experiment, there were 3 blocks and a total of 24 observations. Parental *A. iole* adults were harvested from the BCMRRU colony and sexed, as described previously. Twenty females were randomly assigned to each arena. Arenas were not provisioned with food or water. Arenas were placed inside a growth chamber (at conditions mentioned previously) for 24 h, then taken out to remove adult parasitoids. The number of *A. iole* late-stage pupae per treatment per patch was determined after 12 to 14 d.

Statistical analysis. The general linear model (GLM) and analysis of variance (ANOVA) were used to test for significance of parasitoid density (5, 10, 15, or 20 females) and date on progeny production per patch. Simple linear regression was used to test for a significant relationship between parasitoid density and progeny production per patch. A Student's *t*-test was used to test for significance of parasitoid density (10 vs. 20 females) on progeny sex ratio. GLM and ANOVA were used to test for significance of arena size (≈1, 2, 4, or 8 L) and date on progeny production per patch. A Tukey HSD method was used for pairwise mean comparisons. Absolute data were square-root transformed and percentage data were arcsine-transformed prior to analysis (Zar 1999). Means were significantly different at $P \leq 0.05$. Statistical analyses were performed with SigmaStat (1994) and Systat (1998) computer software. Untransformed data are presented.

Results

Effect of multiple *A. iole* females on production and sex ratio. Female density had a significant effect on progeny production (Fig. 1; $F = 131.4$; df = 3,41; $P < 0.0001$). Production increased by a factor of 2.1 as density increased from 5 to 10 and from 10 to 20 parental females per arena. A regression analysis revealed a significant linear relationship between female density and progeny production (Fig. 1; $F = 278.7$;

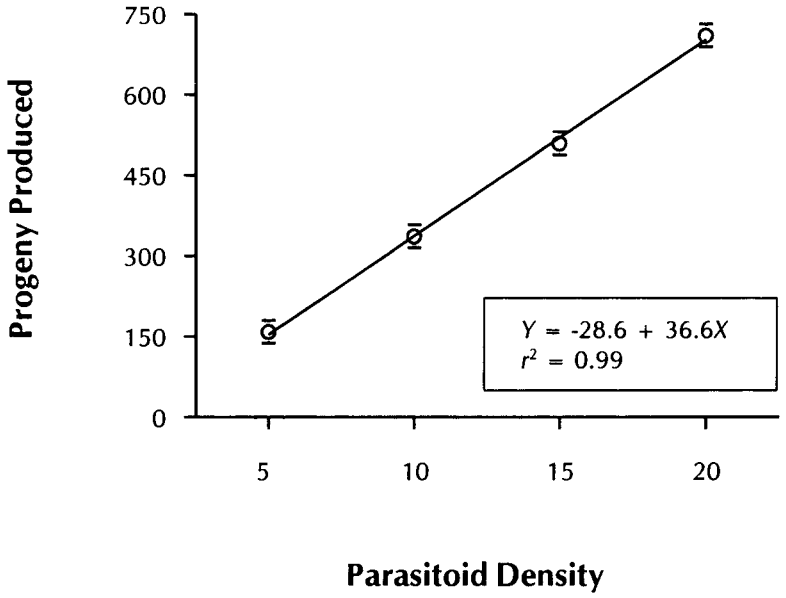

Fig. 1. Mean ± SEM number of *A. iole* progeny produced per patch per parasitoid density (5, 10, 15, 20 females) in 8 L cage arenas (n, 48 observations). Each patch contained from 1,500 to 2,000 *L. hesperus* eggs. The regression line defines the relationship between parasitoid density (*X*) and progeny production (*Y*). Simple linear regression; $Y = a + bX$; a, –28.6; b, 36.6; $r^2 = 0.99$ (n, 4 observations).

df = 1,2; $P = 0.004$). An estimate of the average number of progeny produced per female was 31.7, 33.6, 34.0, and 35.5 for the 5, 10, 15, and 20 female density treatments, respectively. Date had no significant effect on production ($F = 0.3$; df = 1,37; $P = 0.6$).

Sex ratio (i.e., production of female progeny) did not differ significantly between density treatments ($t = 1.0$; df = 34; $P = 0.3$). The mean ± SEM percent of female progeny per 10 and 20 density treatments was 49.6 ± 2.0 and 46.7 ± 2.1 (n = 18 observations per density), respectively.

Effect of arena size on production. Arena size had no significant effect on progeny production per arena size when 20-females were confined to the same arena (Fig. 2; $F = 1.04$; df = 3,18; $P = 0.40$). An estimate of the average number of progeny produced per female was 39.4, 38.2, 36.6, and 35.3 for the 1, 2, 4, and 8 L arenas, respectively. Date had no significant effect on production per arena size ($F = 2.65$; df = 2, 18; $P = 0.10$).

Discussion

The observation that production increased by a factor of 2.1 as parasitoid density increased from 5 to 10 and 10 to 20 females per host patch per arena suggests that parasitoids did not interfere, significantly, with one another at this scale. Since host

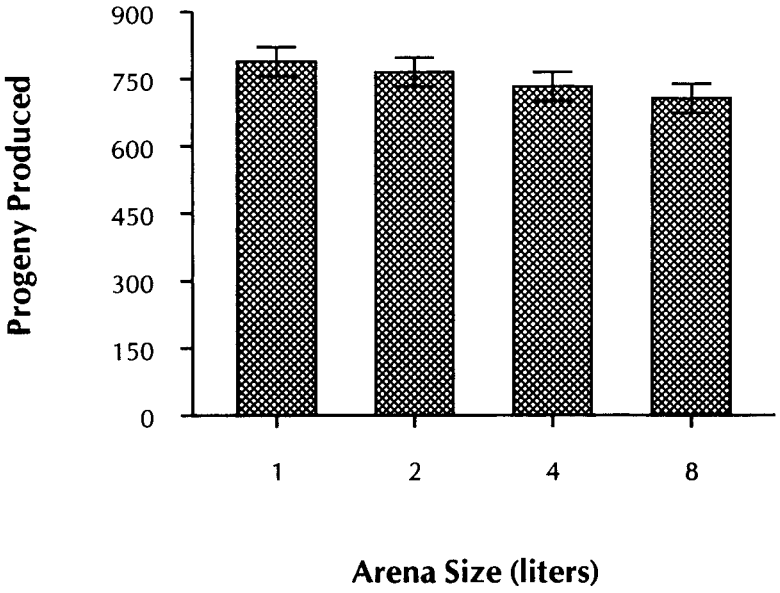

Fig. 2. Mean ± SEM number of *A. iole* progeny produced per patch by 20 parasitoid females in 1, 2, 4, or 8 L arenas (n, 24 observations). Each patch contained from 1,500 to 2000 *L. hesperus* eggs.

density greatly exceeded the combined production potential of parasitoids at all treatment densities, the occurrence of superparasitism of hosts by conspecifics was likely very low in this experiment. Jackson (1986) mentioned that *A. ovijentatus* (= *A. iole*) usually deposited only one egg per host when an abundance of host eggs was available. Females may recognize a chemical substance (i.e., marker), deposited on the exterior egg surface by the first female to attack and oviposit in the host, and avoid ovipositing into the same egg (Roitberg and Mangel 1988, Conti et al. 1997, Wu and Nordlund 2002).

Cronin and Strong (1993) discovered that an increase in density of *Anagrus delicatus* Dozier (Mymaridae) females resulted in a decrease in parasitism rate and per-capita number of hosts parasitized per patch. Superparasitism of hosts at higher wasp densities was responsible for the decline. Sagarra et al. (2000) observed mutual interference between females of *Anagyrus kamali* Moursi (Encyrtidae), when attacking third-instar nymphs and adult females of the hibiscus mealybug. Oviposition rates decreased with increasing parasitoid density, perhaps as a mechanism of avoiding excessive superparasitism. However, superparasitism did not affect the sex ratio of progeny upon emergence from hosts (Sagarra et al. 2000).

Parasitoid density had no apparent effect on the sex ratio of mature progeny that had emerged from hosts in this study. The fact that female progeny did not exceed 50% of emerging adults suggests that some parental females were unmated. Females were harvested at random from the colony (at BCMRRU) and used in experiments. Sex ratios of progeny from mated *A. iole* females have been slightly female-biased (Jackson 1986). Also, 0 d old mated females generated slightly female-biased

(59.6% females) and 1 d old mated females generated unbiased (51.0% females) progeny sex ratios (Riddick 2003).

Unfavorable shifts in progeny sex ratio have been a major impediment to mass rearing of 'quality' natural enemies (Waage 1986). Shifts in sex ratios may occur in response to encounter rates and the sequence of oviposition into hosts rather than in response to conspecifics foraging on the same host patch and ovipositing into parasitized hosts (Strand 1988). There is also evidence that females reared under crowded conditions produce unbiased sex ratios, whereas females reared in isolation produce female-biased sex ratios (Strand 1988), as predicted by the Local Mate Competition model of Hamilton (1967).

The observation that progeny production was not affected by arena size, as it varied from approximately 1 to 8 liters, was not expected. Apparently, ovipositing females do not interfere with one other, to any measurable extent, even at the lowest arena size, and parasitoids do not waste searching time in areas devoid of hosts in the larger arenas. Since cage (arena) size did not significantly effect progeny production, it might be possible to increase rearing capacity by redesigning the cages. Cages that have the capacity to hold 200 host patches might help conserve shelf space and also reduce labor costs associated with maintenance of a large number of small cages. Rearing capacity as well as the production of quality insects are thought to provide keys to success in mass rearing of biological control agents (Nordlund 1998).

In conclusion, this study suggests that little or no measurable interference will occur between ovipositing *A. iole* females on shared host patches and cage size can be varied to increase rearing capacity.

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References Cited

Cohen, A. C. 2000. New oligidic production diet for *Lygus hesperus* Knight and *L. lineolaris* (Palisot de Beauvois). J. Entomol. Sci. 35: 301-310.

Conti, E., W. A. Jones, F. Bin and S. B. Vinson. 1996. Physical and chemical factors involved in host recognition behavior of *Anaphes iole* Girault, an egg parasitoid of *Lygus hesperus* Knight (Hymenoptera: Mymaridae; Heteroptera: Miridae). Biol. Control 7: 10-16.

Conti, E., W. A. Jones, F. Bin and S. B. Vinson. 1997. Oviposition behavior of *Anaphes iole,* an egg parasitoid of *Lygus hesperus* (Hymenoptera: Mymaridae; Heteroptera: Miridae). Ann. Entomol. Soc. Am. 90: 91-101.

Cronin, J. T. and D. R. Strong. 1993. Superparasitism and mutual interference in the egg parasitoid *Anagrus delicatus* (Hymenoptera: Mymaridae). Ecol. Entomol. 18: 293-302.

Debolt, J. W. 1987. Augmentation: rearing, release, and evaluation of plant bug parasites, Pp. 82-87. *In* R. C. Hedlund and H. M. Graham [eds.], Economic importance and biological control of *Lygus* and *Adelphocoris* in North America. USDA, Agric. Res. Serv. ARS-64.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science 156: 477-488.

Hassell, M. P. 1978. The dynamics of arthropod predator-prey systems. Princeton Univ. Press, Princeton, NJ.

Jackson, D. J. 1969. Observations on the female reproductive organs and the poison apparatus of *Caraphractus cinctus* Walker (Hymenoptera: Mymaridae). Zool. J. Linn. Soc. 48: 59-81.

Jackson, C. G. 1986. Effects of cold storage of adult *Anaphes ovijentatus* on survival, longevity, and oviposition. Southwest. Entomol. 11: 149-153.

Jones, W. A. and C. G. Jackson. 1990. Mass production of *Anaphes iole* for augmentation against *Lygus hesperus:* effects of food on fecundity and longevity. Southwest. Entomol. 15: 463-468.

King, P. E. and M. J. W. Copland. 1969. The structure of the female reproductive system in the Mymaridae (Chalcidoidea: Hymenoptera). J. Nat. Hist. 3: 349-365.

Knipling, E. F. 1992. Principles of insect parasitism analyzed from new perspectives: practical implications for regulating insect populations by biological means. U.S. Dept. Agric., Agric. Handbook No. 693.

McGregor, R. R., D. R. Gillespie, D. M. J. Quiring and D. Higginson. 2000. Parasitism of the eggs of *Lygus shulli* and *Lygus elisus* (Heteroptera: Miridae) by *Anaphes iole* (Hymenoptera: Mymaridae). J. Entomol. Soc. Br. Col. 97: 89-93.

Nordlund, D. A. 1998. Capacity and quality: keys to success in the mass rearing of biological control agents. Nat. Enemies Ins. 20: 169-179.

Norton, A. P. and S. C. Welter. 1996. Augmentation of the egg parasitoid *Anaphes iole* (Hymenoptera: Mymaridae) for *Lygus hesperus* (Heteroptera: Miridae) management in strawberries. Environ. Entomol. 25: 1406-1414.

Riddick, E. W. 2003. Factors affecting progeny production of *Anaphes iole* Girualt (Hymenoptera: Mymaridae). Biocontrol 48: 177-189.

Roitberg, B. D. and M. Mangel. 1988. On the evolutionary ecology of marking pheromones. Evol. Ecol. 2: 289-315.

Ruberson, J. R. and L. H. Williams, III. 2000. Biological control of *Lygus* spp.: a component of areawide management. Southwest. Entomol. Suppl. 23: 96-110.

Sagarra, L. A., C. Vincent and R. K. Stewart. 2000. Mutual interference among female Anagyrus kamali Moursi (Hymenoptera: Encyrtidae) and its impact on fecundity, progeny production and sex ratio. Biocontrol Sci. Technol. 10: 239-244.

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

Stoner, A. and D. E. Surber. 1969. Notes on the biology and rearing of *Anaphes ovijentatus,* a new parasite of *Lygus hesperus* in Arizona. J. Econ. Entomol. 62: 501-502.

Strand, M. R. 1988. Variable sex ratio strategy of *Telenomus heliothidis* (Hymenoptera: Scelionidae): adaptation to host and conspecific density. Oecologia 77: 219-224.

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

Thorpe, K. W. and G. P. Dively. 1985. Effects of arena size on laboratory evaluations of the egg parasitoids *Trichogramma minutum, T. pretiosum,* and *T. exiguum* (Hymenoptera: Trichogrammatidae). 14: 762-767.

Udayagiri, S., S. C. Welter and A. P. Norton. 2000. Biological control of *Lygus hesperus* with inundative releases of *Anaphes iole* in a high cash value crop. Southwest. Entomol. Suppl. 23: 27-38.

van Baaren, J., B. L. Landry and G. Boivin. 1999. Sex allocation and larval competition in a superparasitizing solitary egg parasitoid: competing strategies for an optimal sex ratio. Funct. Ecol. 13: 66-71.

Visser, M. E. and G. Driessen. 1991. Indirect mutual interference in parasitoids. Neth. J. Zool. 41: 214-227.

Waage, J. K. 1986. Family planning in parasitoids: adaptive patterns of progeny and sex allocation, Pp. 63-95. *In* J. K. Waage and D. Greathead [eds.], Insect parasitoids. Academic, London.

Werren, J. 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. Science 208: 1157-1159.

Wu, Z. X. and D. A. Nordlund. 2002. Superparasitism of *Lygus hesperus* Knight eggs by *Anaphes iole* Girault in the laboratory. Biol. Control 23: 121-126.

Wylie, H. G. 1965. Some factors that reduce the reproductive rate of *Nasonia vitripennis* (Walk.) at high adult population densities. Can. Entomol. 97: 970-977.

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