BIOLOGY OF *ARIDELUS RUFOTESTACEUS* (HYMENOPTERA: BRACONIDAE), PARASITOID OF SOUTHERN GREEN STINK BUG NYMPHS

John R. Ruberson
Russell J. Ottens
Melissa D. Thompson
Dept. of Entomology
Univ. of Georgia
Tifton, GA
Scott R. Shaw
Dept. of Renewable Resources, Univ. of Wyoming
Laramie, WY

Abstract

Development and survival of the adventive braconid parasitoid *Aridelus rufotestaceus* were studied in relation to temperature (20, 25, and 30° C), and to life stages (second, third, fourth, fifth, and adult stages) of the host stink bug *Nezara viridula*. In addition, parasitoid adult longevity and fecundity, and offspring sex ratio were also assessed at 25°C. Parasitoid developmental time was inversely related to temperature (approximately 59 to 65 days from egg to adult at 20°C, 40 to 41 days at 25°C, and 35 to 36 days at 30°C), and parasitoid emergence was comparable across all temperatures, although emergence tended to decline at 30° C. Parasitoid development times were unaffected by host stage within temperatures, but in all cases parasitoid emergence from the fifth instar nymph and adult stages was reduced, demonstrating that this parasitoid is a true nymphal parasitoid. Adult female A. rufotestaceus lived on average 25.8 ± 13.38 days, with the longest-lived female surviving 48 days. Females produced an average of 144.4± 52.60 offspring, with most reproduction constant and concentrated in the first 10 days following emergence. Females are pro-ovigenic, and begin parasitizing hosts almost immediately following emergence from the cocoon. Parasitoid offspring are almost entirely female, with only 9 (or 1.4%) of the 669 offspring reared in the study being male. Production of sons appears to be limited to the later portion of the reproductive period. The parasitoid *A. rufotestaceus* has promise as a biological control agent of stinkbugs in the United States, but several of its life history attributes may limit its efficacy, most notably its slow development and possible temperature limitations.

Introduction

A complex of stinkbug species have become serious pests of a variety of crops in the southeastern United States. They present a series of challenges to managers. First, finding the bugs in the field can be a real challenge, particularly when one is trying to quantify the number of bugs in relation to the damage caused. Second, it is important to find the damage in time to be able to make corrections. Because the injury caused by stinkbug is often quite cryptic, it may be easier to detect injury sometime after it has already occurred, which may lead to revenge treatments. Third, stinkbugs range across the landscape in a variety of domesticated and wild plants. This creates significant management difficulties, as they are highly mobile, especially in the adult stage. Finally, the variety of tools available to manage the bugs is quite limited by both the variety of tools available and the variability in the susceptibility of stinkbug species to the insecticides.

Biological control of stinkbugs has been studied for many years throughout the world (e.g., Clausen 1978). Although predation of stinkbugs is known, it has been poorly investigated primarily due to difficulties in such studies. Nevertheless, several predator species have been identified as possibly being important. Ehler (2000, 2002) indicated that ants, spiders, lady beetles, lacewings, and pillbugs (among others) could consume low to moderate numbers of stink bug eggs or young nymphs in the lab and/or field. Ruberson and Olson (2010) reported that fire ants and long-horned grasshoppers were the dominant predators of Southern green stink bug eggs in cotton, peanuts, and soybeans in Georgia. The role of predators in managing stinkbug populations remains poorly understood.

Considerably more effort has been focused on understanding the role of parasitoids in shaping stinkbug population dynamics. The parasitoid complex of stinkbugs is dominated by egg parasitoids and adult parasitoids, with limited parasitoid activity reported against nymphs (Jones 1988). For example, Buschman and Whitcomb (1980) reported that 10% of *Nezara viridula* nymphs were parasitized by the predominantly adult parasitoid *Trichopoda pennipes*

(Diptera: Tachinidae). The basis for this gap is not understood, but may involve challenges of overwintering for the parasitoid in the Temperate Zone. Most temperate stinkbugs overwinter as adults, but the egg stage can also be quite resilient as an overwintering host.

In 1998, a braconid parasitoid was reported in Italy attacking nymphs of *Nezara viridula* (Shaw et al. 2001). This was the first report of a nymphal parasitoid of this stinkbug species, and the wasp was a Braconid that had originally been described in 1986 as *Aridelus rufotestaceus*, from the coastal region of Georgia, south of Russia (Tobias 1986). The parasitoid was first found in the United States in Georgia in 2007, where it was reared from one nymph of *N. viridula* (Ruberson and Wickings 2008) and identified by Dr. Scott Shaw (Univ. of Wyoming). It has subsequently been reared from nymphs in four counties in Georgia (Coffee, Mitchell, Sumter, and Tift) in 2008 and 2009, indicating that it is established and relatively widespread. Parasitism rates by the parasitoid have been low, however, accounting for less than 1% of all stink bugs collected.

The purpose of the present study was to elucidate several facets of the biology of this parasitoid. First, the role of temperature and host stage on parasitoid survival and development was examined. Second, longevity, fecundity, and offspring sex ratio were studied at a single temperature. The studies provide additional insights into the biology of the parasitoid and possible function as a biological control agent.

Materials and Methods

Insect sources and maintenance.
A parasitoid colony was established from 8 adults reared from hosts collected in 2009. Parasitoids were reared on Southern green stink bugs (SGSB) that were maintained in the laboratory on green bean pods and shelled sunflower seeds (25± 1°C, L:D 14:10). Third instar nymphs were exposed to parasitoids to obtain parasitoid offspring.

Experimental approach – Temperature effects on parasitoid development and survival.
SGSB nymphs of instars 2-5 and adult were individually exposed to parasitoids and stinging was observed before stink bugs were removed from parasitoids. Stung bugs were placed in one of three temperatures (20, 25, and 30°C; each L:D 14:10) and monitored for development and survival host and parasitoid. At least 30 bugs were stung for each instar/temperature combination, with the exception of adults, which the parasitoids were extremely hesitant to sting (548 bugs stung in total). Bugs were provided fresh bean pod pieces and shelled sunflower seeds every two days until parasitoids emerged or until 10 days after last parasitoid emerged from the respective stung cohort (at which point stung bugs were discarded). Developmental times of parasitoids to cocoon formation and subsequent adult formation were calculated. Bug development following parasitism was also calculated for bugs from which parasitoids successfully emerged.

Experimental approach – Parasitoid longevity and fecundity.
Newly emerged parasitoid females (n=7) were provided 30 3rd-instar SGSB nymphs daily (along with a 5% honey-water solution) and held at 25±1°C, L:D 14:10 until the female died. Females were monitored daily for survival. Exposed nymphs (held at 25±1°C, L:D 14:10) were provided fresh bean pod pieces and shelled sunflower seeds every two days until parasitoids emerged or until 10 days after last parasitoid emerged from the respective stung cohort (at which point stung bugs were discarded). Female fecundity was calculated based on the number of parasitoid offspring that successfully emerged and on the number of parasitoid larvae recovered in dissections of stink bugs that died prematurely. Sex ratio of parasitoid offspring was calculated from successfully emerged parasitoids.

Results

Temperature Effects.
As expected, parasitoid development was significantly affected by temperature, with development accelerating with increasing temperature. The relationship is nonlinear, with development slowing proportionately greater as the temperature changed from 25 to 20C relative to the change from 30 to 25C (Tables 1 and 2). Within temperatures, parasitoid development was unaffected by host instar (Tables 1 and 2). Similarly, parasitoid developmental success was reduced somewhat at 30C relative to 20 and 25C, and appeared to decline in the 5th instar relative to other host instars (Table 3).

Table 1. Developmental times (egg to larval emergence, in days) of *Aridelus rufotestaceus* after oviposition into various host instars, and at three temperatures (photoperiod L:D 14:10). Host species used was the Southern green stink bug, *Nezara viridula*. No significant differences were noted between instars within temperatures. NE = no emergence.

Host instar	Temperature (ºC)		
	20	25	30
2	30.3 ± 0.50 30	19.3 ± 0.49 30	18.0 ± 1.05 30
3	29.4 ± 1.42 47	19.3 ± 0.45 38	17.0 ± 0.63 44
4	30.9 ± 0.65 40	19.2 ± 0.39 40	17.2 ± 0.56 65
5	32.8 ± 4.06 44	19.2 ± 1.30 42	18.4 ± 1.52 47
Adult	NE	NE	17 1

Table 2. Developmental times (egg to adult, in days) of *Aridelus rufotestaceus* after oviposition into various host instars, and at three temperatures (photoperiod L:D 14:10). Host species used was the Southern green stink bug, *Nezara viridula*. No significant differences were noted between instars within temperatures. NE = no emergence.

Host instar	Temperature (ºC)		
	20	25	30
2	63.6 ± 4.70 15	40.2 ± 1.84 17	36.0 1
3	59.2 ± 2.37 18	40.7 ± 0.91 21	35.2 ± 1.11 12
4	63.5 ± 2.25 24	40.0 ± 0.73 20	35.7 ± 1.27 11
5	65.3 ± 3.20 16	41.1 ± 1.33 20	36.3 ± 0.58 3
Adult	NE	NE	NE

Table 3. Emergence success (egg to larval emergence, in days) of *Aridelus rufotestaceus* after oviposition into various host instars, and at three temperatures (photoperiod L:D 14:10). Host species used was the Southern green stink bug, *Nezara viridula*.

Host instar	Temperature (ºC)		
	20	25	30
2	50	66.7	30
n	30	30	30
3	38.3	68.4	36.4
n	47	38	44
4	60	77.5	26.2
n	40	40	65
5	52.3	54.8	10.6
n	44	42	47
	0	0	0.59
Adult	17	18	17

Parasitoid longevity and fecundity.

Adult parasitoids lived an average of 25.8 ± 13.38 days, with the longest-lived female surviving 48 days. Females produced an average of 144.4± 52.60 offspring, with most reproduction constant and concentrated in the first 10 days following emergence (Fig. 1). Females are pro-ovigenic, and begin parasitizing hosts almost immediately following emergence from the cocoon. Of the 669 offspring that became adults, 9 (or 1.4%) were male. Two of the females failed to produce any sons. Of the five females producing sons, one female produced one son each when she was 3 and 4 days old, respectively, and none of the other females produced sons before being 8-days old.

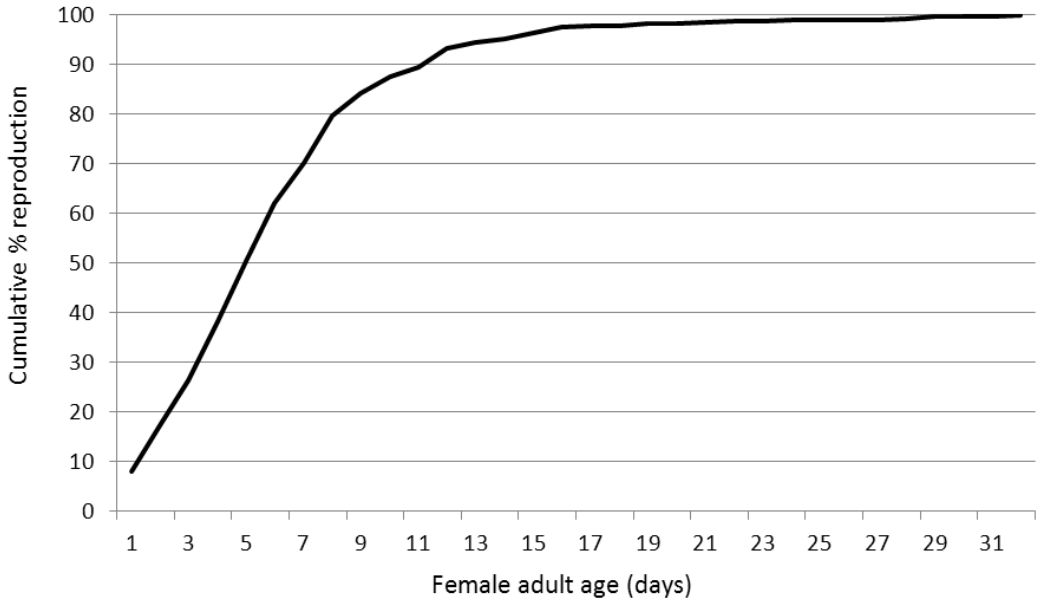

Fig. 1. Rate of reproduction by female *A. rufotestaceus* in response to parasitoid adult age (25°C).

Discussion

Developmental times of *A. rufotestaceus* were relatively long when compared to a number of other parasitoid wasps, but this long development synchronizes the parasitoid with appropriate stages of its host, which may require 35 to 50 days to go from egg to egg at 25°C (Harris and Todd 1980). Parasitoid emergence was generally good across all temperatures tested, although it appeared to decline somewhat at 30°C. It is possible that parasitoid success may be constrained somewhat at temperatures above 30° C, which could limit their effectiveness in the southeastern United States where average summer temperatures can exceed 30°C.

The second, third, and fourth instars of the stinkbug appear to be most suitable for parasitoid development and survival. Parasitoid emergence declined in the fifth instar, and only a single parasitoid successfully emerged from the adult stage. In addition, the parasitoids were recalcitrant in parasitizing adult stinkbugs. Therefore, it is possible that when the wasps stung stinkbug adults, most failed to deposit eggs. Alternately, the host may successfully suppress the developing parasitoid egg or larva.

The adult parasitoids live for approximately 3-4 weeks, but concentrate the bulk of the reproduction in the first 10 days following emergence. This concentration is advantageous for biological control in that survivorship probability of the parasitoid in the field is likely inversely related to parasitoid longevity. Therefore, the greater the span of time over which the parasitoid is reproductive, the less likely it is to achieve its full reproductive potential prior to death.

The capacity of *A. rufotestaceus* to produce almost exclusively daughters is beneficial for biological control in that the death of virtually all hosts by parasitism will result in a parasitoid that is capable of killing additional hosts, and which will not need to mate. The mechanisms for this thelytokous sex determination are unknown at present, but may be due to sex-modifying bacteria. If this is the case, then there is the possibility that overall effectiveness of the parasitoid as a biological control agent may be reduced. A growing body of literature indicates that such bacteria can adversely affect the biology of parasitoids, reducing overall fecundity and with it the number of hosts killed (e.g., Hohmann et al. 2001). Additional work needs to be done in this area.

The parasitoid *A. rufotestaceus* has promise as a biological control agent of stinkbugs in the United States, but several of its life history attributes may limit its efficacy, most notably its slow development and possible temperature limitations.

Acknowledgments

We appreciate funding support for this project from Cotton Incorporated and the Georgia Cotton Commission.

References

Buschman, L. L., and W. H. Whitcomb. 1980. Parasities of *Nezara viridula* (Hemiptera: Pentatomidae) and other Hemiptera in Florida. Fla. Entomol. 63: 154-162.

Clausen, C. P. (Ed.) 1978. Introduced parasites and predators of arthropod pests and weeds: a world review. Agriculture handbook number 480. Agricultural Research Service, United States Department of Agriculture, Washington DC.

Ehler, L.E. 2000. Farmscape ecology of stinkbugs in Northern California. Thomas Say Publications in Entomology, Memoirs. Entomological Society of America, Lanham, Maryland.

Ehler, L.E. 2002. An evaluation of some natural enemies of *Nezara viridula* in northern California. BioControl 47: 309-325.

Harris, V. E., and J. W. Todd. 1980. Duration of immature stages of the Southern green stinkbug, *Nezara viridula* (L.), with a comparative review of previous studies. J. Ga. Entomol. Soc. E5: 114-124.

Hohmann, C. L., R. F. Luck, and R. Stouthamer. 2001. Effect of *Wolbachia* on the survival and reproduction of *Trichogramma kaykai* Pinto and Stouthamer (Hymenoptera: Trichogrammatidae). Neotropical Entomol. 30: 607-612.

Jones, W.A. 1988. World review of the parasitoids of the Southern green stink bug, *Nezara viridula* (L.)(Heteroptera: Pentatomidae). Ann. Entomol. Soc. Am. 81: 262-273.

Ruberson, J. R., and K.G. Wickings. 2008. Importance of natural enemies for stink bug control in Georgia: 2007. In T. Grey, M. Toews, and C. Perry (eds.), Cotton Research-Extension Report 2007, pp. 111-121. Univ. of Georgia Coop. Ext. Publ. 6.

Shaw, S. R.; Salerno, G.; Colazza, S.; Peri, E. 2001. First record of *Aridelus rufotestaceus* Tobias (Hymenoptera: Braconidae: Euphorinae) parasitizing *Nezara viridula* nymphs (Heteroptera: Pentatomidae) with observations on its immature stages and development. Journal of Hymenoptera Research, 10: 131-137

Tobias, V. I. 1986. Subfamily Euphorinae. In: G.S. Medvedev (ed.), Identification key for insects of the European part of the U.S.S.R., Volume 3. Hymenoptera 4:181-250. Nauka Publishing House, Leningrad (in Russian).