EFFECT OF *TRICHOPODA PENNIPES* PARASITIZATION ON *NEZARA VIRIDULA*[1]

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ABSTRACT - *Trichopoda pennipes* parasitization had a strong negative effect on *Nezara viridula* adult longevity, fecundity and feeding. A parasitized female lived half the time of an unparasitized one and for a parasitized male this reduction in longevity was even greater. Unparasitized females laid four times more eggs than parasitized one. Parasitization had no significant effect on egg fertility. Feeding intensity by *N. viridula* is reflected by the number of punctured soybean seeds. Caged plants exposed to unparasitized adults produced only one half as many unpunctured seeds compared to cages with parasitized adults. The percentage of normal seeds (with commercial value) was 31.1% higher in cages with parasitized adults.

Index terms: feeding behaviour, longevity, fecundity, parasitism.

EFEITO DA PARASITAÇÃO DE *TRICHOPODA PENNIPES* EM *NEZARA VIRIDULA*

RESUMO - A parasitação de *Trichopoda pennipes* teve um forte efeito negativo sobre a longevidade, fecundidade e alimentação de *Nezara viridula*. Fêmeas parasitadas viveram a metade do tempo que viveram as não-parasitadas; e em machos, a redução da longevidade foi ainda maior. Fêmeas não-parasitadas ovipositaram quatro vezes mais do que as parasitadas. A parasitação não teve efeito significativo na fertilidade dos ovos. A intensidade de alimentação de *N. viridula* é refletida pelo número de puncturas na semente de soja. Plantas em gaiolas, expostas a adultos não-parasitados, produziram somente a metade das sementes sem puncturas em relação às plantas expostas a adultos parasitados. A percentagem de sementes normais (com valor comercial) foi de 31,1% maior em gaiolas com adultos parasitados.

Termos para indexação: comportamento alimentar, longevidade, fecundidade.

INTRODUCTION

The effectiveness of *Trichopoda pennipes* as a biocontrol agent against *Nezara viridula* is dubious. Some consider this parasite ineffective (Capeluto 1949) because it deposits more than one egg per individual host and the host continues to mate and oviposit despite being parasitized. Other believe it is effective (Harris & Todd 1982) because longevity of parasitized *N. viridula* is reduced causing significant reductions in population levels of this pest. These opinions were based on observations in particular situations such as a particular crop within a season. Any control tactic may succeed or fail or have a reduction in its potential for controlling an insect depending on several factors which vary from year to year.

In the literature, the range in seasonal average level of parasitization of *N. viridula* by *T. pennipes* is reported to be between 40-90%, but during a season parasitization, levels can vary between 0-100%. *Trichopoda pennipes* is an active parasite present during almost all year in the southeastern United States, and is the most widely distributed species in both North and South America and among adjacent islands. The key conditions for successful parasitism as stated by Bouletreau (1986) are based on the persistence of parasite-host interaction on the parasites ensuring the dispersion of propagules to new hosts, and the acquisition of sufficient energy from the host to assure growth and

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survival of the host until reproductive maturity of the parasite. The presence of the parasite and host in the same habitat is the result of active behavior of the parasites (Bouletreau 1986).

Shahjahan (1968) studying the effect of *T. pennipes* parasitization on *N. viridula* reported reduced fecundity and longevity of parasitized compared with unparasitized bugs. Harris & Tood (1982) showed that parasitization by *T. pennipes* caused a 49% reduction in the longevity of male and female *N. viridula*. Egg fertility and egg mass size were not reduced by parasitization, and fecundity of parasitized females was not reduced in relation to that of unparasitized females, during a time period equal to the lifetime of parasitized females. Lifetime fecundity of unparasitized females was 3.8 times the lifetime fecundity of parasitized females. They concluded that since *N. viridula* mate and oviposit throughout their lives, parasitization by *T. pennipes* can cause significant reductions in population levels of this pest.

Besides the direct damage caused by the southern green stink bug to soybean seeds, it also causes indirect (secondary) damage to plants and consequently to seed quality. Secondary effects include delayed leaf maturation, retention of leaves and development of abnormal leaflets and pods close to the main stem (Todd & Herzog 1980, Panizzi & Slanky Junior 1985). These secondary effects are normally present when extensive stink bug injury occurs during seed development.

The objectives of this study were to evaluate the direct effects of parasitization by *T. pennipes* on aspects of *N. viridula* longevity, fecundity and feeding in soybean.

MATERIALS AND METHODS

The experiment was conducted in 1987 on soybean variety Bradford, maturity group VII, in a very uniform area in a soybean field in Newberry, Florida, USA. It was a caged experiment. Cages were similar, made of black plastic 0.5 mm mesh screen placed over a "tomato wire-frame" and supported by wooden post. The cone-shaped frame was placed up side down over the plants, i.e. the larger portion (opening) toward the soil surface. The cone was formed by 3 lateral wires, one of which was inserted in a hole in the stick and used as support for the cage. The two remaining wires were maximally extended to expand the overal diameter of the frame.

A rectangular screen bag (60 x 1.20 m) with Velcro the length of one side was placed over the frame and firmly adjusted around the frame. The screen cage was gathered and fastened with clothespins at the base of the plant stems in such a way that there was no opening. A heavy layer of tanglefoot TM(sticky material) was applied to the bottom of the cage and plant stems down to the soil surface. This material was replaced or reapplied every 2-3 days or immediately after a rain to prevent ants and other predators from entering the cages. Each cage enclosed 3-5 uniform plants. Cages were set up at the beginning of the R4 soybean growth stage to avoid possible injury to pods or seeds by stink bugs from the field population. The cages were placed in pairs 2 m apart along rows. One row was skipped between rows with cages. Several plants adjacent to the cage were removed to prevent leaves from touching the screen and being used as a bridge by ants for entering the cages. Before the cages were set up, all spiders, insects and eggs were removed from the plants by shaking and hand-picking. Thereafter, regular checks were made to insure that no insects were in the cage before commencement of the experiment.

Parasitized adults were obtained by exposing individuals of the same age (7-10 day old) to fly parasites for a 24 h period, after which each bug was checked for presence of tachinid eggs on the body. Those with two or more eggs were removed and paired. No effort was made to pair the sexes by size or color. To replace dead or injured bugs, the same procedure was conducted with a large number of adults maintained on pole bean pods at 27°C, 70-80% RH, 14 h photophase. Unparasitized pairs were obtained in the same manner with insects being taken from laboratory rearing cages. *Nezara viridula* nymphs were reared on pole bean pods and peanuts and adults maintained on pole bean pods. Each pair, parasitized and unparasitized, was placed in a small container with a piece of soft tissue paper and immediately taken to the field and introduced into cages. This procedure avoided or minimized stress caused by higher temperatures, excessive handling or other physical injuries. At the beginning of R5 growth stage on September 9, one female and one male adult *N. viridula* were introduced into each cage. Nine cages received a parasitized pair and nine received an

unparasitized pair. The paired cages (parasitized and unparasitized) were set up at the same time, and the sequence of cage pair infestation was done at random (choice from 1-9).

The experiment was conducted over a period of 53 days, and 52 daily observations in cages were made.

If mortality occurred for any adult during the first four days after introduction into the cage the pair was removed (both dead and alive) and replaced by another pair from the laboratory stock of the same age and time of parasitization.

Each cage was observed daily during the morning hours (9-11). Three days a week (Monday, Wednesday, Friday) the cages were opened on the Velcro side and inspected for egg masses, and conditions of adults were observed. On other days, external observation through the screen was made to note conditions of adults. Dead stink bugs were removed from the cage on the day of discovery and brought to the laboratory to record parasitization. Egg masses were also removed when observed and the number of eggs per egg mass and egg fertility were determined in the laboratory. Egg masses were maintained in plastic petri dishes (15 x 90 mm) with a water saturated dental cotton roll inside each petri dish to maintain high humidity and stored in a rearing chamber at 27°C, 70-80% RH and 14 h photophase. Each cage was checked until both insects died, and thereafter only maintenance was done (Tanglefoot TM maintained and dead leaves removed). The cages were removed and plants were cut off at the bottom of the stem on the day of harvest. All pods were picked by hand. In the laboratory the number of filled and empty pods were counted. Seeds were removed from pods and number of seeds, weight and number of punctures per seed were recorded. These observations were made separately for each cage.

Soybean seeds were separated and classified into categories described by Jensen & Newson (1971). The four categories were based on the amount of visual damage from the southern green stink bug, such as: N = seeds with no visual damage, L = seeds showing punctures but without shriveling of the seedcoat, M = seeds with some shriveling of the seedcoat, H = seeds with extensive or total shriveling of the seedcoat. These seed categories were adopted and used. Seeds were also categorized according to the number of punctures per seed: 0, 1-3, 4-7, and more than seven punctures per seed.

After all seeds of each cage were classified, using the two classification systems, they were vigorously mixed and passed through a seed laboratory sieve nº 9. They were separated into normal (not passed through the sieve) and abnormal (passed) seed. All seeds of each category were counted and weighed.

A microscope and an illuminated magnifier were used to search for punctures and determine the damage category. One person made all of the observations. For the purposes of this study, fecundity refers to the number of eggs laid, fertility is indicated by change in egg color from yellowish-white to orange-red or hatched, and infertility is indicated by eggs not changing color hue (except yellow) or unhateched after two weeks.

RESULTS AND DISCUSSION

Results reported were obtained from nine cages with unparasitized pairs, and, from eight cages with parasitized pairs.

Longevity (number of days lived) was significantly different for females, males and total days lived for the pair. Unparasitized insects lived much longer than parasitized ones (Table 1). Parasitized females lived a mean of 15.0 ± 2.7 days, with a range of 7-33 days, while unparasitized females lived a mean of 29.0 ± 4.4 days with a range of 13-48 days. Parasitized males lived a mean of 12.0 ± 2.4 days, with a range of 5-16 days, compared to unparasitized ones that lived 29.2 ± 4.9 days with a broader range of 5-52 days. Longevity for total adults, the total number of days that both sexes in a pair lived, was much higher for unparasitized insects, with a mean of

TABLE 1. Effect of *Trichopoda pennipes* parasitization on *Nezara viridula* adult longevity.

Condition		Longevity (days)		
		Mean	Range	SE
Unparasitized	Female	29.0**	13-48	4.4
	Male	29.2**	5-52	4.9
	Total	58.2**	18-100	6.4
Parasitized	Female	15.0	7-33	2.7
	Male	12.0	5-26	2.4
	Total	27.0	13-59	4.9

T - test (P=0.05) ** Significant

59.0 ± 6.4 days (range 34-89 days). Parasitized adults lived a mean of 27.0 ± 4.9 days (range of 13-59).

Parasitization of *N. viridula* adults by *T. pennipes* affects female and male longevity, reducing their life span by 44.9% and 36.4%, respectively. Number of cumulative days lived for each sex and for each combined pair was calculated. It was assumed that this combined value represented the amount of time that plants in each cage were potentially exposed for feeding. It also was assumed that the higher the number of cumulative days lived, the greather the feeding potential.

Parasitized females, males and total adults had a mean number of cumulative days lived (feeding days) of 15.0, 120 and 27.0 days, respectively. These values for unparasitized ones were 29.7, 29.3 and 58.2 days, respectively. The number of feeding days for each pair was calculated by adding the number of days lived by the female and male in the same cage (cumulative number of days lived for the pair). Parasitized cages had a mean number of feeding days of 27.0 (range 13-59 days) and unparasitized had more than double the number of days, 59 with a range of 18-100 days.

Fecundity, expressed by the number of egg masses or eggs produced, was statistically different ($P < 0.05$) between parasitized and unparasitized females. Unparasitized females laid more egg masses and eggs. Mean numbers of egg masses and eggs per unparasitized female were 2.5 ± 0.53 and 199.3 ± 15.0, respectively, and for parasitized females these values were 0.6 ± 0.19 egg mass and 51.2 ± 47.9 eggs, giving a significant mean difference ($P < 0.05$) of 1.9 egg masses and 148.1 eggs per female (Table 2). Egg fertility was not statistically different ($P < 0.05$), but was higher for unparasitized females (mean egg fertility 78.2%) than parasitized ones (mean egg fertility 48.7%).

Considering these parameters to indicate fecundity (number of egg masses and eggs) and fertility (percent viable eggs) it was possible to demonstrate a drastic effect of *T. pennipes* parasitization on *N. viridula* females. This effect could strongly influence population growth, parasitization effects fecundity, reducing by 82.1% the number of egg masses and 77.1% the total number eggs produced.

TABLE 2. Effect of *Trichopoda pennipes* parasitization on *Nezara viridula* fecundity and fertility.

Condition	Mean	Range	SE
		Number egg masses	
Unparasitized	2.5**	0-5	0.53
Parasitized	0.6	0-1	0.19
		Number eggs	
Unparasitized	199.3**	0-458	15.0
Parasitized	51.2	0-86	47.9
		Percent fertility	
Unparasitized	78.2NS	0-100	14.4
Parasitized	48.7	0-100	16.7

T - Test (P=0.05)
** Significant, NS not significant

Feeding activity of *N. viridula* adults had a direct effect on seed quantity and quality. Unparasitized adults lived much longer than parasitized, consequently they had more feeding days and higher feeding activity. This affected pod and seed quantity and quality. There was no statistically significant difference ($P < 0.05$) between mean number of pods filled in cages with parasitized and unparasitized pairs, but cages with parasitized bugs had larger numbers of filled pods (11.8%), suggesting that parasitized adults fed less during the early stages of seed formation. The number of seeds produced in cages with parasitized and unparasitized adults were not statistically different, but again cages with parasitized had a higher mean number of seeds.

The mean number of unpunctured seeds and, probably as a direct consequence, number of normal seeds were significantly different. The largest differences were observed between means for these two variables. Cages with parasitized adults had nearly twice the number of unpunctured seeds and 1.45 times more normal seeds. Cages with parasitized adults had

23.8% fewer seeds punctured (30.9% of seeds punctured by parasitized bugs and 52.5% by unparasitized). According to the usual seed analysis for seed laboratory evaluation (França Neto & Henning 1984), cages with parasitized *N. viridula* had 93.8% normal seeds, and 98.4% of seed weight was from normal seeds. In cages with unparasitized *N. viridula* these values were 78.1% and 94.0%, respectively. Cages with parasitized adults had 7.4% of the seeds unacceptable for sale as seeds (1.6% of the total seed weight) and unparasitized had 18.4% (6% of total seed weight). It was recorded the principal effects of feeding activity of parasitized and unparasitizied *N. viridula* adults (Table 3). Total seed weight was not statistically different ($P < 0.05$) between means of treatments with parasitized and unparasitized individuals, but cages with parasitized adults had a narrower range of variability in seed weight. This difference might be important when extrapolated to a larger amount of seeds, for example, to the amount produced in a hectare.

TABLE 3. Effect of *T. pennipes* parasitization on *N. viridula* adult feeding.

Variable	Condition			
	Unparasitized		Parasitized	
	Mean	Range	Mean	Range
Pods filled	86.7NS	54-104	102.1	74-137
Seeds unpunctured	68.3**	13-122	122.4	41-216
Seeds punctured	75.4NS	40-134	54.7	14.151
Total seeds	143.8NS	93-194	177.1	128-239
Normal seeds	112.3**	6-167	163.1	99-228
Seed weight-mg	127.4NS	62.3-166.1	141.8	130.2-166.2

T - Test ($P = 0.05$)
** Significant, NS not significant

CONCLUSION

Trichopoda pennipes parasitization has a strong negative affect on *Nezara viridula* adult longevity, fecundity and feeding.

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