Host Preference of the Parasitoid *Trichopoda pennipes* (Diptera: Tachinidae) with *Euschistus servus* and *Nezara viridula* (Hemiptera: Pentatomidae)[1]

Grant L. Pilkay[2], Francis P. F. Reay-Jones[3], and Jeremy K. Greene[4]

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Abstract Laboratory studies were conducted to determine the host preference of the tachinid parasitoid fly *Trichopoda pennipes* (F.) for the brown stink bug, *Euschistus servus* (Say), and the southern green stink bug, *Nezara viridula* (L.). In choice and no-choice tests, 8-fold fewer eggs were laid on *E. servus* compared with *N. viridula.* Twenty-four *T. pennipes* emerged from 100 *N. viridula,* whereas only 2 larvae emerged from 100 laboratory-parasitized *E. servus.* Postmortem dissections of egg-bearing stink bugs without larval emergence revealed 20 *T. pennipes* larvae inside *N. viridula* but only 1 inside *E. servus.* These results confirm that *T. pennipes* prefers *N. viridula* as a host and is likely only an infrequent parasitoid of *E. servus.*

Key Words biological control, host selection, oviposition, superparasitism

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Trichopoda pennipes (F.) (Diptera: Tachinidae), an endoparasitoid of stink bug nymphs and adults, is one of the most-studied natural enemies of the southern green stink bug, *Nezara viridula* (L.), in the United States (Worthley 1924, Todd and Lewis 1976, Buschman and Witcomb 1980, Panizzi and Slansky 1985, Jones 1988). *Trichopoda pennipes* is native to North and South America and has been introduced to Hawaii, Australia, several Pacific islands, South Africa, Europe, and Israel for the control of *N. viridula* (Jones 1988, Colazza et al. 1996, Freidberg et al. 2011). *Trichopoda pennipes* produces up to 3 generations per year depending on location, with the last generation of the season remaining as second instars, overwintering inside the body of the host stink bug until late spring or early summer shortly after the stink bug breaks diapause (Dietrick and van den Bosch 1957). Each female fly lays 100 eggs on average, which are typically placed singly on late instars or adults (Pickett et al. 1996). The larva bores from the egg through the cuticle and into the stink bug, feeding on the host for about 2 weeks. After reaching the third instar, the larva exits the host between the posterior abdominal segments or the genitalia, killing the stink bug (Todd and Lewis 1976). Pupation takes place in the top 3 cm of soil. Upon emergence, adults feed on nectar (Tillman 2011).

Several mechanisms of host selection exist in insect parasitoids, with documented chemical and physical cues (Vinson 1976). Males of *N. viridula* produce an aggregation

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[1] Received 26 March 2013; accepted for publication 28 May 2013.

[2] Corresponding author (email: pilkayg@yahoo.com).

[3] Clemson University, School of Agricultural, Forest, and Environmental Sciences, Pee Dee Research and Education Center, 2200 Pocket Road, Florence, SC 29506

[4] Clemson University, School of Agricultural, Forest, and Environmental Sciences, Edisto Research and Education Center, 64 Research Road, Blackville, SC.

pheromone that attracts conspecific adults (both sexes) and late instars (Mitchell and Mau 1971, Harris and Todd 1980). This pheromone is also cross-attractive to the green stink bug, *Chinavia hilaris* (Say), and several egg and adult parasitoids including *T. pennipes* (Buschman and Whitcomb 1980, Colazza et al. 1999, Ehler 2000). The parasitoid uses the stink bug aggregation pheromone primarily to find its host, and secondarily as a mating kairomone, with both sexes of the fly being attracted to the chemical (Harris and Todd 1980, Aldrich et al. 1987, Tillman et al. 2010). Harris and Todd (1980) noted that, although the attraction of *T. pennipes* to *N. viridula* was continuous throughout the day, the attraction was strongest shortly before dark.

In addition to *N. viridula*, *C. hilaris*, and the brown stink bug, *Euschistus servus* (Say), the parasitoid has been documented using the squash bug, *Anasa tristis* (DeGeer), (Worthley 1924), and the red-banded stink bug, *Piezodorus guildinii* (Westwood), (Panizzi and Slansky 1985) as hosts. Parasitism rates, determined by identifying *T. pennipes* eggs on the cuticle of the stink bug, were documented for *N. viridula* as high as 44% by Todd and Lewis (1976), whereas *E. servus* collected simultaneously had parasitization rates of only 0.29%. Limited data have been collected as to the rates of successful emergence of *T. pennipes* in *E. servus*. Buschman and Whitcomb (1980) did not collect any *T. pennipes* emerging from 130 *E. servus* in Florida. McPherson et al. (1982) documented 5 tachinid species (including *T. pennipes*) emerging from *E. servus* collected in Louisiana, though rates were not reported. Whereas published survey data show that *N. viridula* is more commonly parasitized by *T. pennipes* than *E. servus*, no study has yet attempted to identify *T. pennipes* preference behavior among host species. This study aims to clarify the host preference and parasitoid emergence rates of *T. pennipes* with *N. viridula* and *E. servus* in the laboratory.

Materials and Methods

Stink bugs used in the preference trials originated from laboratory colonies at the Clemson University Edisto Research and Education Center (REC) in Blackville, SC. Tachinids were reared from field-collected *N. viridula* and *E. servus*, known hosts of several tachinid species (Eger and Ables 1981, Jones et al. 1996). Collections of stink bugs occurred in fallow areas, wheat, *Triticum aestivum* (L.), and corn, *Zea mays* (L.), from mid-May to mid-July 2012 at the Clemson University Edisto REC. All pupae were reared to adulthood in a plastic rearing cage (30 × 30 × 30 cm) lined with paper towels in an environmental chamber (25°C, 14:10 L:D). A dish of cotton balls saturated in a 30% sugar water solution was provided to feed any adults that emerged (Modi and Tesh 1983). Upon emergence, *T. pennipes* flies were isolated in a separate cage to observe mating activity, and were provided with sugar water and droplets of honey.

Laboratory-reared stink bugs used in trials were held for approx. 1 wk after the end of nymphal development for cuticular scleritization before being offered to *T. pennipes*. Two cohorts of mated (based on observations) females of *T. pennipes* were used in choice and no-choice trials in 5 arenas formed from 5 plastic cups (9 cm in diam × 7 cm high) with screen mesh lids. A small dish with cotton soaked in sugar water was provided as a source of moisture and carbohydrates for *T. pennipes* and a single green bean, *Phaseolus vulgaris* (L.), was placed in the arena as food for the stink bugs. For no-choice trials, *E. servus* and *N. viridula* were offered separately in male-only and female-only pairs (n = 20 pairs per cohort). Choice trials consisted of

N. viridula and *E. servus* being offered in pairs of different species or a male and a female of the same species ($n = 30$ pairs per cohort). A single mated female of *T. pennipes* was placed in each arena. Trials were repeated 5 times per cohort for a total of 10 replications. Arenas were washed with soap and water and dried between trials. Female flies of each cohort (11 and 9 flies per cohort) were returned to the rearing cage between trials and randomly selected for each arena and trial to randomize exposure to arenas and stink bugs.

After 24 h, stink bugs were removed from an arena and the number of *T. pennipes* eggs deposited per stink bug was counted. Stink bugs were isolated in standard plastic specimen cups fitted with a mesh lid and provided green beans for diet until the emergence of a parasitoid larva or until death. In cases where the stink bug died but a larva did not emerge, the stink bug was held for an additional 24 h for any larval parasitoids to complete their development. If none were observed, the stink bug was dissected to determine if a larva was present inside. Dates of egg deposition were recorded, as were the dates of stink bug death or parasitoid emergence. Additionally, external signs indicative of parasitism were noted. Voucher specimens were deposited with the arthropod collection at Clemson University.

Numbers of *T. pennipes* eggs per stink bug were analyzed for significant departure from random by performing chi-square tests (Zar 1999). The expected frequency of eggs per stink bug was one-half for choice trials (df = 1) and one-fourth for no-choice trials (df = 3).

Results

Field-collected *T. pennipes* emerged from *N. viridula* exclusively. All pupae obtained from field-collected *E. servus* were identified upon adult emergence as *Cylindromyia euchenor* (Walker) (Diptera: Tachinidae). One specimen of an unknown species of *Trichopoda* also was obtained from *N. viridula*, but was not included in laboratory trials.

The chi-square test indicated a departure from random in no-choice tests (Fig. 1), with 8.3-fold more eggs deposited on *N. viridula* than on *E. servus*. Significant differences between the 2 species also held for choice trials, but differences were not

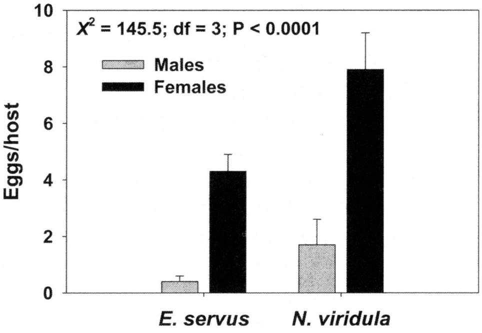

Fig. 1. Average numbers of eggs (± SEM) laid per host by *Trichopoda pennipes* in no-choice (same species, same gender) trials.

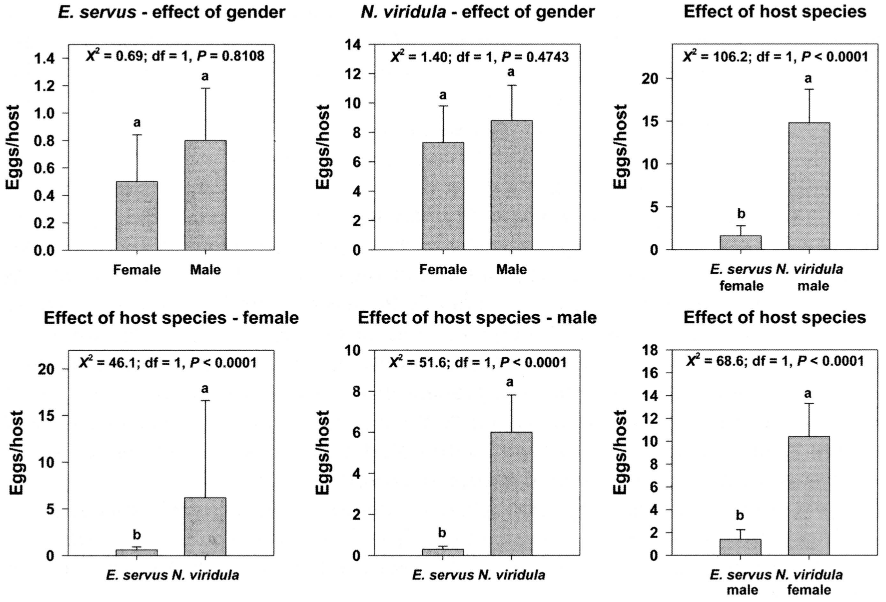

Fig. 2. Effect of gender and species (*Nezara viridula* and *Euschistus servus*) on average numbers of eggs (± SEM) laid per host by *Trichopoda pennipes* in choice trials. Means followed by the same letter are not significantly (*P* > 0.05) different based on chi-square tests.

significant between genders of the same species (Fig. 2). A total of 779 eggs was laid on *N. viridula* across choice and no-choice trials, compared with 94 on *E. servus*.

Out of 200 (100 *N. viridula*, 100 *E. servus*) stink bugs tested, one or more tachinid eggs were deposited on 92 *N. viridula* and 26 *E. servus*. Twenty-six larvae emerged from egg-bearing stink bugs (24 from *N. viridula* and two from *E. servus*). Of the 24 larvae from *N. viridula*, 20 of the egg-bearing stink bugs had 7 eggs or fewer, whereas 4 larvae emerged from stink bugs with more than 7 eggs. Larvae emerged from *E. servus* with 1 egg each. The other 92 egg-bearing stink bugs (68 *N. viridula*, 24 *E. servus*), dissected after death, yielded 21 living larvae (1 from *E. servus* in a no-choice trial, and 20 from *N. viridula*, including 1 with 2 larvae). Of the 82 stink bugs with no visible eggs after the 24-h trial, none had evidence of parasitism after death, based on dissections.

Numbers of tachinid eggs deposited on stink bugs varied considerably among individual stink bugs, ranging from 0 - 43 per host. Flies laid eggs on nonhost surfaces as well as on hosts, with eggs found on rearing cage walls and on seven *T. pennipes* pupae that had not yet emerged as adults. One emerging pupa had 24 eggs on it as the fly attempted to leave the pupal case.

Physical evidence for emergence of the larvae from the egg was noticed in the dissections. Eggs laid on *N. viridula* often showed a dark spot where the parasitoid larvae had burrowed through the cuticle of the host after hatching. As this was noticed late in the experiment, no data were kept of the numbers of stink bugs demonstrating these spots, or how many occurred per number of eggs on the stink bug, but it was generally noted that no signs of cuticular penetration were observed on *E. servus*, even after several days of carrying eggs.

Discussion

Trichopoda pennipes exhibited a clear preference for *N. viridula* over *E. servus*, with 8.3-fold more eggs laid on *N. viridula.* The presence of *T. pennipes* eggs on the cuticle has been used as a reliable method of determining parasitized status in past studies with a high level of accuracy in *N. viridula* (Harris and Todd 1981). All stink bugs without eggs had no external or internal evidence of parasitism, indicating that the eggs were likely not present at any point, as opposed to being dislodged in handling or movement. The low number of tachinid larvae in or emerging from *E. servus* provided further evidence of the unsuitability of *E. servus* as a frequent host, as did the lack of entry wounds on the cuticle underneath the eggs. In our study, the presence of *T. pennipes* eggs on *E. servus* was not a reliable indicator of host suitability.

Prior studies have noted *T. pennipes* as using the male *N. viridula* aggregation pheromone to find its host (Mitchell and Mau 1971). Therefore, we expected oviposition to be higher around male *N. viridula*, as documented by Todd and Lewis (1976), which did not occur. The limited selection of hosts in the arena, as well as the fact that *T. pennipes* mating had already occurred away from *N. viridula*, might have influenced these results.

Superparasitism has been documented in *T. pennipes* when high populations of parasitoids occur relative to host bugs (Shahjahan 1968). Superparasitism occurs when multiple larvae enter and attempt to develop in the same host. Only 1 larva typically can survive in a single bug, and superparasitization decreases parasitoid survival rate (Shahjahan 1968). In that study, the percentage of emerged larvae dropped from 64 - 78% at 2 - 5 larvae per bug to 38% when 7 larvae attempted to develop in a host. The host's fecundity was about 59% that of unparasitized hosts, but egg fertility remained unaffected (Shahjahan 1968). Adult parasitism rates of *N. viridula* by *T. pennipes* have been as high as 69% in South Carolina (Shahjahan 1968). Of 112 overwintering adults of *N. viridula* in the same study, 22% had at least 1 *T. pennipes* egg attached to the cuticle (Jones et al. 1996). In Blackville, SC, where our experiments took place, parasitism rates of *N. viridula* had previously been documented at 37 - 65% (Jones et al. 1996). In our experiments, multiple *T. pennipes* eggs laid on a single stink bug occurred in 106 out of 200 stink bugs tested (17 for *E. servus* and 88 for *N. viridula*). This level of superparasitism could have been attributed to the limitations imposed by the arena. In the wild, the parasitoid would have had the freedom to leave after depositing an egg on a host. Of 36 *N. viridula* hosting more than 7 eggs, only 4 larvae (11%) emerged from the host, suggesting that superparasitism might have been responsible for the low emergence rate. Larval emergence from 56 stink bugs hosting 1 - 7 eggs increased to 36%. The 2 *T. pennipes* larvae that emerged from *E. servus* were from stink bugs hosting 1 egg each.

The deposition of eggs on nonhost surfaces has been documented previously in tachinids (Stireman et al. 2006). The eggs are placed on surfaces that the host will frequent, and parasitism takes place when the host crawls over the egg-bearing surface, triggering the egg to hatch. *Trichopoda pennipes* is not one of the species for which this behavior had been observed. Whereas it is unknown what prompted this ovipositional behavior, there is no evidence that this behavior was anything other than an artifact of confinement and it is likely incidental.

Many tachinid flies have localized variations in host preference, with some populations using different hosts in different areas of the parasitoid range (Jones 1988, Ehler 2000). *Trichopoda pennipes* is likely a complex of cryptic species, as different hosts have been documented in different regions (Jones 1988, Panizzi and Slansky 1985, Pickett et al. 1996). Our results with parasitoids in South Carolina might not be valid elsewhere. More research is required to determine if our observed host preferences are consistent over a wide geographic area. If the species complex theory is valid, and *E. servus* is found to be a desirable host for some regional variations of *T. pennipes*, procedures to differentiate these cryptic species and their host preferences could be a fruitful avenue of investigation.

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