

***Podisus distinctus* (Heteroptera: Pentatomidae) females are lighter feeding on *Tenebrio molitor* (Coleoptera: Tenebrionidae) Pupae subjected to ventral nerve cord transection**

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PODISUS DISTINCTUS (HETEROPTERA: PENTATOMIDAE) FEMALES ARE LIGHTER FEEDING ON TENEBRIO MOLITOR (COLEOPTERA: TENEBRIONIDAE) PUPAE SUBJECTED TO VENTRAL NERVE CORD TRANSECTION

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Abstract—*Podisus distinctus* (Stål, 1860) (Heteroptera: Pentatomidae) is a predator of defoliating caterpillars of *Eucalyptus* spp. (Myrtaceae). This South-American asopine can be reared in the laboratory using *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) pupae as alternative prey and released in the field. The movement observed in *T. molitor* pupae can be a defensive strategy. This makes it significant to study the development and reproduction of asopine predatory stinkbugs using transected pupae of this prey. The aim was to evaluate the performance of *P. distinctus* with transected or non-transected *T. molitor* pupae in the laboratory. The ventral nerve cord of *T. molitor* pupae was transected utilizing surgical forceps (T1, transected pupae), while the control included non-transected pupae (T2, non-transected pupae). These pupae were offered to *P. distinctus* nymphs and adults. We evaluated the duration and body mass of instars III, IV and V; duration from instar V to the adult stage; body mass of the newly emerged adults; adult sex ratio; total number of egg masses, eggs per female and egg mass; total nymphs per female and egg mass; percentage of nymphs hatched; female longevity, and the pre-oviposition, oviposition and post-oviposition periods of this predator. The body mass of *P. distinctus* adults was 7.29% greater with the non-transected *T. molitor* pupae; however, the other parameters showed similar values between treatments. Non-transected *T. molitor* pupae are preferred for rearing the predator *P. distinctus*.

Key words: Asopinae, development, transected prey, mass rearing, reproduction.

Larvae and pupae of holometabolous insects have limited defensive movements that can attract or deter predators. Adults of *Supputius cincticeps* (Stål, 1860) (Heteroptera: Pentatomidae) showed higher predation rates and easier handling with immobilized *T. molitor* larvae by inserting an entomological pin (15 mm) dorso-ventrally into the mesothorax (Azevedo and Ramalho, 1999). Furthermore, *Zelus longipes* L., 1767 (Heteroptera: Reduviidae) adults preyed on a greater number of smaller larvae of *Anagasta kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae), *Anticarsia gemmatilis* Hübner, 1818 and *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) than the medium and large ones. This was due to greater facility to catch smaller preys. The

predation preference on smaller caterpillars is important to control in advance of these insects (Cogni et al., 2002).

Predatory stinkbugs (Heteroptera: Pentatomidae: Asopinae) are biological control agents of defoliating caterpillars which feed on *Eucalyptus* spp. (Myrtaceae) (Herrick et al., 2008; Zanuncio et al., 2008a; Oliveira et al., 2011). The occurrence of *Podisus distinctus* (Stål, 1860) (Heteroptera: Pentatomidae) was recorded from Paraguay to French Guyana and in the Galapagos Islands (Ecuador) (Henry and Wilson, 2004; de Sá et al., 2013). This generalist predator attacks diverse pest species including *Thyrintina arnobia arnobia* Stoll, 1782 (Lepidoptera: Geometridae) larvae and pupae (Soares et al., 2009) and can be

released in the field through mass rearing in the laboratory using *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) and *Musca domestica* L., 1758 (Diptera: Muscidae) larvae and pupae as prey items (Neto et al., 2004; de Sá et al., 2013; Zanuncio et al., 2014). In comparing these two prey species, weight gain by *P. distinctus* was greater during nymphal stages when fed *T. molitor* pupae and larvae and when fed *M. domestica* larvae in instars I and II (Neto et al., 2004).

Tenebrio molitor larvae and pupae are the main alternative prey used for rearing asopine predators (Zanuncio et al., 2005, 2008b; Neves et al., 2010). The *T. molitor* larvae are reared on *Triticum aestivum* L. (Poaceae) bran in plastic trays (Morales-Ramos et al., 2011, 2013; Pereira et al., 2013) and pieces of vegetables are placed on the bran as energy and a water source (Grundy et al., 2000; Tavares et al., 2013b, 2013c).

Brontocoris tabidus (Signoret, 1852), *Podisus maculiventris* (Say, 1832), *Podisus nigrispinus* (Dallas, 1851), and *S. cincticeps* reveal adequate development and reproduction when fed *T. molitor* pupae and/or larvae (Vivan et al., 2003; Shapiro and Legaspi, 2006; Zanuncio et al., 2011). The movements of the abdominal segments of intact *T. molitor* pupae can be blocked by transecting the ventral nerve cord (VNC) (Ichikawa, 2008; Kurauchi and Ichikawa, 2009). The pupae and larvae of transected prey could be preyed upon more easily and with less energy costs to the predator to immobilize because mobile insects can camouflage, make body movements and escape, bite and spit unpalatable substances, as well as cause injuries and even death to asopine predators (Gentry and Dyer, 2002; Lemos et al., 2005; Silva et al., 2012).

The aim of this work, therefore, was to study the performance of *P. distinctus* using *T. molitor* pupae that have been subjected to VNC transection to improve the rearing of this predator for biological control programs.

METHODS

The experiment was conducted in the Laboratory of Biological Control of Insects (LCBI) of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil at $25 \pm 2^\circ\text{C}$, $70 \pm 12\%$ relative humidity and 12 (light): 12 (dark) hours photoperiod.

Healthy *T. molitor* pupae (93.70 ± 1.13 mg, $n = 10$) up to one-day-old were used and were mass

reared by the LCBI where the prey is fed with *T. aestivum* bran (75% carbohydrates, 12% proteins, 11% minerals/sugars, and 2% lipids). Wheat bran was placed in 60 cm long \times 40 cm wide \times 12 cm high white plastic trays (Morales-Ramos et al., 2011, 2013; Pereira et al., 2013). Three *Saccharum officinarum* L. (Poaceae) pieces (about 100 g each) and two of *Sechium edule* (Jacq.) Swartz (Cucurbitaceae) (around 150 g each) were placed in each tray on the wheat bran as an energy and water source for the insects (Grundy et al., 2000; Tavares et al., 2013b, 2013c). Two newspaper sheets were placed on the substrate per tray to reduce the light and insect movements (Barbosa et al., 2012; Tavares et al., 2011, 2013a).

A small portion of the VNC was exposed outside the body through a tiny incision in the ventral area of the second abdominal segment of the *T. molitor* pupae and transected between the first and second abdominal ganglia after being held and twisted with the tip of surgical forceps (T1). The control used non-transected pupae (T2). Abdominal pulsation of the pupae ceased immediately after the VNC was severed and the oozing hemolymph caused insect death (Ichikawa, 2008). The transected or non-transected pupae were offered to *P. distinctus* nymphs from instar II, because those of instar I are not predatory, feeding only on egg waste and sucking up water (Neto et al., 2004; Soares et al., 2009).

Podisus distinctus egg masses were obtained from the mass rearing of LCBI where this predator received intact *T. molitor* pupae and water in a moistened cotton wool wad. These egg masses were placed on thin cotton wool strips in plastic Petri dishes (14.5 cm diameter \times 1.2 cm high) with a wad of moistened cotton wool to facilitate nymph hatching, which were maintained here until instar II.

One second instar *P. distinctus* nymph was placed per plastic Petri dish (9.0 cm diameter \times 1.2 cm high) with a transected or non-transected *T. molitor* pupa and changed every three days with a moistened cotton wool wad until the adult stage, representing the T1 and T2 treatments, respectively. Pupae began to oxidize and darken two days after the transection. The adults obtained were fed, similar to the nymphs. Two-days-old males and females were paired and placed in a 500 mL plastic pot with a transected or non-transected *T. molitor* pupa and changed every two days with a water moistened cotton wool wad. Dead males

Table 1. Duration (days) of instars III, IV and V, and from instar V to the adult stage (V inst.-adult); body mass (mg) of instars III, IV and V and of the adult females; sex ratio [number of females ÷ (number of adults)]; total number of egg mass per female, eggs per female and per egg mass, nymphs per female and per egg mass; nymph hatching (%); female longevity (Long. females) and pre-oviposition, oviposition and post-oviposition periods (mean ± standard error of the mean) of *Podisus distinctus* (Heteroptera: Pentatomidae) with transected or non-transected *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae.

	Non-transected		Transected		F	VC
Duration (days)						
III instar	3.48 ± 0.13	22 ^a	3.87 ± 0.12	24 ^a	0.10 ^{ns}	23.04%
IV instar	4.07 ± 0.86	21 ^a	4.40 ± 0.85	21 ^a	3.72 ^{ns}	51.55%
V instar	6.35 ± 1.38	20 ^a	7.02 ± 1.44	19 ^a	0.03 ^{ns}	56.55%
V inst.-adult ^d	43.24 ± 20.15	17 ^a	43.76 ± 23.15	13 ^a	1.46 ^{ns}	41.33%
Body mass (mg)						
III instar	6.41 ± 3.53	22 ^b	6.32 ± 3.41	24 ^b	0.16 ^{ns}	51.48%
IV instar	18.95 ± 8.48	21 ^b	15.42 ± 8.20	21 ^b	1.18 ^{ns}	21.27%
V instar	41.54 ± 15.53	20 ^b	34.23 ± 15.74	19 ^b	2.42 ^{ns}	30.84%
Adult females	77.00 ± 16.84 a	17 ^b	71.39 ± 18.54 b	13 ^b	4.12*	68.05%
Sex ratio	0.54 ± 0.02	33 ^c	0.54 ± 0.02	25 ^c	-	-
Total						
Egg mass/female	13.35 ± 3.57	8 ^b	12.20 ± 4.64	6 ^b	0.21 ^{ns}	41.45%
Eggs/female	274.11 ± 96.29	8 ^b	204.06 ± 83.25	6 ^b	0.87 ^{ns}	51.11%
Eggs/egg mass	20.04 ± 4.35	8 ^b	16.30 ± 2.18	6 ^b	0.01 ^{ns}	19.73%
Nymphs/female	212.70 ± 78.77	8 ^a	172.26 ± 78.04	6 ^a	0.60 ^{ns}	41.67%
Nymphs/egg mass	15.24 ± 4.03	8 ^a	12.23 ± 3.38	6 ^a	0.83 ^{ns}	27.65%
Hatching (%)	94.35 ± 3.06	8 ^a	86.75 ± 10.79	6 ^a	1.73 ^{ns}	6.78%
Long. females	50.05 ± 8.87	8 ^b	46.60 ± 10.22	6 ^b	0.45 ^{ns}	19.38%
Periods						
Pre-oviposition	10.76 ± 2.02	8 ^b	14.00 ± 4.60	6 ^b	0.01 ^{ns}	27.02%
Oviposition	35.00 ± 9.79	8 ^b	28.66 ± 11.93	6 ^b	0.35 ^{ns}	17.12%
Post-oviposition	4.29 ± 2.31	8 ^b	3.93 ± 2.15	6 ^b	0.33 ^{ns}	54.01%

Means followed by the same small letter per line do not differ by the F test (one-way ANOVA). ^{ns} = not significant; * = significant; ^a = mean of two insects; ^b = mean of two couples; ^c = total number of insects; ^d = males and females; VC = variation coefficient.

were replaced with living and healthy ones from the same treatment until the female's death. Petri dishes and pots for rearing the insects were cleaned daily with a water-moistened cotton wool swab when the preyed upon pupae were removed from the containers.

We recorded the duration of instars III, IV and V and from instar V to the adult stage; body mass of instars III, IV and V instar and newly-emerged adults; sex ratio [number of females ÷ (number of adults)]; total number of egg masses, eggs per female and per egg mass; percentage of nymphs hatched; total of nymphs per female and per egg mass; female longevity and pre-oviposition, oviposition and post-oviposition periods of *P. distinctus*.

The design was completely randomized (CRD) with two treatments [non-transected (T1) or transected (T2) *T. molitor* pupae]. A total of 25 replications were used, each with two nymphs or two *P. distinctus* pairs of a male and female adult.

Data were subjected to analysis of variance (one way ANOVA) and the means were compared using the F test with the SAS INSTITUTE software (1989) (Supplier: UFV).

RESULTS

The duration of instars III, IV and V, from instar V to the adult stage and of the adult stage of *P. distinctus* females was similar among transected and non-transected *T. molitor* pupae (Table 1).

The body mass of instars III, IV and V of *P. distinctus* was similar between treatments and that of the adult females was 7.29% lower with the transected *T. molitor* pupae (Table 1). Feeding on non-transected *T. molitor* pupae, therefore, increased the *P. distinctus* body mass (Table 1).

The sex ratio and total number of egg masses, eggs and egg masses per female of *P. distinctus* were similar among transected and non-transected *T. molitor* pupae (Table 1).

The number of nymphs per female and per egg mass, percentage of nymphs hatched, female longevity, and pre-oviposition, oviposition and post-oviposition periods of *P. distinctus* were similar among the transected and non-transected *T. molitor* pupae (Table 1).

DISCUSSION

The similar duration of instars III, IV and V, instar V to the adult stage, and the adult stage of *P. distinctus* females fed with transected or non-transected *T. molitor* pupae was not an expected result as the predator supposedly spends more energy to immobilize non-transected pupae (Neto et al., 2004; Soares et al., 2009; de Sá et al., 2013). However, the defense mechanisms of non-transected *T. molitor* pupae may be ineffective against predators (Ichikawa, 2008; Kurauchi and Ichikawa, 2009; Silva et al., 2012), despite the observation that larger larvae of this species and other alternative hosts may show greater resistance to predation (Santos et al., 1995; Provost et al., 2006; Pires et al., 2009). This was evident by the longer duration of the nymphal period of *Podisus connexivus* Bergroth, 1891 (= *P. nigrispinus*) with larger *Alabama argillacea* (Hübner, 1823) (Lepidoptera: Noctuidae) larvae, which was attributed to their greater defense (Santos et al., 1995). The feeding of the nymphs and adults of *P. nigrispinus* is facilitated because *T. molitor* pupae do not move around and only present a circular motion (Ichikawa, 2008; Kurauchi and Ichikawa, 2009). This is important because the degree of defense varies among the prey, as demonstrated by the lower predation rate of *P. maculiventris* on *A. gemmatalis* and *Plathypena scabra* (Fabricius, 1798) (= *Hypena scabra* Fabricius, 1798) larvae than on those of *Trichoplusia ni* (Hübner, 1800-1803), *Pseudoplusia includens* (Walker, 1858) (= *Chrysodeixis includens* Walker, 1858) and *Heliothis zea* Boddie, 1850 [= *Helicoverpa zea* (Boddie, 1850)] (Lepidoptera: Noctuidae) due to higher agility of the first two species (Marston et al., 1978). *Bombyx mori* (Linnaeus, 1758) (Lepidoptera: Bombycidae) and *T. arnobia arnobia* larvae showed strong and active head and body movements in response to *Podisus rostralis* (Stål, 1860) (Heteroptera: Pentatomidae) (Lemos et al., 2005) and *S. cincticeps* (Silva et al., 2012) attacks. Moreover, immobilized prey may occur less frequently, which reduces the biological parameters of the natural enemies (Eubanks and Denno,

2000). This was true for *Geocoris punctipes* (Say, 1832) (Heteroptera: Geocoridae) with 11 attacks on mobile nymphs and five on immobilized ones of *Acyrtosiphum pisum* Harris, 1776 (Hemiptera: Aphididae), suggesting visual orientation and response to prey movement (Eubanks and Denno, 2000). Moreover, the consumption and reproduction of *P. distinctus* were similar with free or immobilized *M. domestica* larvae (Pires et al., 2009).

The similar body mass of instars III, IV and V of *P. distinctus* between treatments and the lower mass of adults fed transected *T. molitor* pupae suggests a higher selectivity of the adults of this predator for the prey, which had less body mass than pupae and larvae of alternative prey species (Neto et al., 2004; Soares et al., 2009; de Sá et al., 2013). The feeding on non-transected *T. molitor* pupae with circular movements increased the *P. distinctus* body mass. Body mass accumulated during nymphal stage allows the predator to develop into an adult. This shows a higher consumption of the *T. molitor* pupae during instar V, as reported for the predator *P. maculiventris* with *Manduca sexta* (Linnaeus, 1763) (Lepidoptera: Sphingidae) larvae (Stamp et al., 1991). Predators may prefer mobile prey because they enable easy location, as found for *G. punctipes* orientated by vibrations of *A. sativum* (Eubanks and Denno, 2000). Moreover, *P. nigrispinus* consumed a greater number of defenseless *S. frugiperda* larvae due to the higher aggressiveness of mobile ones (Zanuncio et al., 2008a). Hemolymph leakage from wounded *T. molitor* pupae which resulted from transecting the VNC, accelerated its degeneration with a blackening of its body (oxidation), which may reduce the food quality (Ichikawa, 2008; Kurauchi and Ichikawa, 2009). In addition, the hemolymph on the body surface of the prey may reduce its chances of selection by *P. distinctus* because Asopininae may avoid damaged larvae (Zanuncio et al., 2008a; Pires et al., 2009). These predators can recognize prey with their antennae directed forward, as observed for *P. rostralis* with *B. mori* larvae (Lemos et al., 2005). Asopininae touch their prey with their antennae and legs, suggesting the use of tactile cues in prey selection (Silva et al., 2012). Prey quality is important for egg production because Asopininae have a greater body mass and, consequently, higher fecundity, with adequate nutrition (Evans, 1982; Lemos et al., 2005; Oliveira et al., 2005).

The similarity in sex ratio and total number of egg masses, eggs and egg masses per female of *P. distinctus* with transected or non-transected *T. molitor* pupae, concur with that reported for this predator with immobilized or mobile *M. domestica* larvae (Pires et al., 2009). However, developmental time, and number of eggs per female and per egg mass, and egg masses per female of *Pristhesancus plagipennis* Walker, 1873 (Hemiptera: Reduviidae) were lower with *T. molitor* larvae than with those of *Helicoverpa armigera* (Hübner, [1809]) (Lepidoptera: Noctuidae) killed using hot water. This was explained by the fact that larvae of the latter have thinner cuticular membrane than those of *T. molitor*. Thus, upon being soaked with water, fluid leaks and adheres to the abdomen, making feeding difficult and causes loss of vigor and ultimately death of this predator (Grundy et al., 2000). Although the means were not significantly different, mean egg values were consistently higher in *P. distinctus* that fed on non-transected *T. molitor* pupae. This probably accounted for much of the difference in female weight.

The similar number of nymphs per female and per egg mass, percentage of nymphs hatched, female longevity, and pre-oviposition, oviposition and post-oviposition periods of *P. distinctus* with transected or non-transected *T. molitor* pupae shows that the mobility (rotary motion) of this prey did not influence these biological parameters. The pre-oviposition and post-oviposition periods of the predators were similar with larvae or pupae of this prey (Lacerda et al., 2004), which were either mobile or not, and the longevity of *P. nigrispinus* was similar with smaller or medium-sized *A. argillacea* larvae, suggesting a higher defense capability of the larger ones (Ramalho et al., 2008). However, mobility can be significant as the *T. molitor* larvae are less aggressive than those of *S. frugiperda* and *T. arnobia arnobia* (Silva et al., 2012). The latter two species move abruptly, using their head and body to thwart the attack and thus reducing the biological parameters of *S. cincticeps* (Silva et al., 2012). *Thyrinteina arnobia arnobia* larvae are more aggressive than those of *S. frugiperda*; the former presents a more flexible body and uses jaws as its defensive strategy and, therefore, suffers fewer attacks (Silva et al., 2012).

The VNC transection of each *T. molitor* pupae took approximately 30 seconds, which increased the working time of rearing *P. distinctus* in the

biofactories. The yield was 120 pupae transected per hour and 960 per day, assuming an eight-hour work day. Despite the extra preparation efforts, female adults of *P. distinctus* weigh less when fed *T. molitor* pupae subjected to VNC transection. Therefore, this predator should be reared with non-transected *T. molitor* pupae, which enables females to have a greater body mass. The low defense capacity of *T. molitor* pupae did not affect other biological parameters of *P. distinctus*.

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