Moisture requirements of the ladybird beetle *Stethorus nigripes* in relation to habitat preference and biological control

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Introduction

Various species of the ladybird beetle genus *Stethorus* Weise (Coleoptera: Coccinellidae) have been used throughout the world as biological control agents of mites [particularly *Tetranychus urticae* Koch and *Panonychus ulmi* (Koch)] on a variety of crops (Gordon & Chapin, 1983). The Australian species *S. nigripes* Kapur is of renewed interest for biological control of spider mites because of its new-found establishment in mite-infested corn fields in the southern plains (Texas, Oklahoma, and Kansas) of the United States (Gordon, 1993; Pollock & Michels, 2002). This beetle is a fairly recent introduction from Australia, intended for application in Californian almond orchards (Richardson, 1977; Hoy & Smith, 1982). Interestingly, *S. nigripes* has established itself in at least one corn field in Texas (Gordon, 1993) and has expanded its range northward through areas of corn production (Pollock & Michels, 2002), which follows the distribution of its main prey, the grass mite, *Oligonychus pratensis* (Banks), a pest of corn, sorghum, and wheat (Gilstrap et al., 1980). Adult beetles are especially small, about 1 mm in length, and move about quite actively, crawling in large numbers along the central rib on the underside of the corn leaf, mainly within the mites' webbing where they feed on mite eggs (preferred), nymphs, and adults. Larvae probe directly through the webbing in search of mites, feed, and pupate nearby, and thus all life history stages of *S. nigripes* are concentrated along the central rib on the underside of the leaf (Pollock & Michels, 2002). Few experiments have

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focused on the biology and physiology of *Stethorus* species in general, and *S. nigripes* in particular. The current study seeks to provide a contribution in that area.

Thriving in a particular environment depends upon the ability to maintain constant levels of body water, so-called 'water balance' (Wharton, 1985), and is important for arthropod natural enemies that are being considered for classical biological control programs pertaining to their suitability for release and establishment in a new geographic region (Yoder & Hoy, 1998). Indeed, climatic adaptation is recognised as a problem in classical biological control programs, and water balance strategies of arthropod natural enemies have been explored in mites (Gaede, 1992; Yoder, 1998; Yoder & Houck, 2001), and have only recently begun for beetles (Yoder et al., 1999) and wasps (Yoder & Hoy, 1998). The main problem is water loss due to small body size (large surface area to volume; Hadley, 1994), and this would be a particular problem for the minute *S. nigripes*. Water gain by ingestion of moist food, blood and tissue fluids, drinking free water, or absorbing water vapor from the air counters water loss so that water balance can be achieved (Wharton, 1985). Typically, water balance relationships match moisture requirements in a given environment, and most information is garnered by analyzing water loss rates (Hadley, 1994).

The central purpose of this study was to better understand: (1) habitat preference and suitability for *S. nigripes*, (2) the potential for spread into new regions, (3) features crucial for survival, and (4) to gain new information about the biology of this beetle, with the goal of enhancing its application to biological control programs. Water balance characteristics of free-living female adults were determined, and include water content, water loss rate, and ability to tolerate dehydration. Studies were also aimed at determining the beetle's primary source of water, by examining

whether female adults drink free water and also by examining whether they could take up water vapor from the air. Larvae (final instar) and pupae were also investigated to provide a more comprehensive analysis of the water relations of *S. nigripes*.

Materials and methods

Insects

Stethorus nigripes were field-collected in June–July from corn fields in northern Texas (Pollock & Michels, 2002). Adults and larvae were free ranging on corn leaves; pupae were attached to the undersurface of the corn leaf predominately at the mid-line central rib. Age of the insects was not known, but all stages were taken at the same time of collection. It is also assumed that only non-diapausing stages were used in the experiment, because the collection was during mid-summer (June–July) and because of the occurrence of adults (suspected diapause stage) on corn leaves rather than on the surface of the ground. Prior to experiments, beetles were deprived of water and food (spider mites) for 12 h (85% r.h., 22–24 °C) to eliminate effects of ingestion, excretion, defecation, and reproduction on mass changes (Wharton, 1985).

Determination of water balance characteristics

Mass changes were monitored gravimetrically using an electrobalance (CAHN 25, Ventron Co., Cerritos, CA; precision of 0.2 μg SD and accuracy of ± 6 μg at 1 mg). Each specimen was weighed singly without enclosure and without anesthesia. Transfer to the weighing pan was done with an aspirator and was accomplished in < 1 min. Test percentage relative humidities (% r.h.) were generated with glycerol–water mixtures (Johnson, 1940) and saturated salt solutions (Winston & Bates, 1960); calcium sulfate generated 0% r.h. (Toolson, 1978). All test atmospheres were measured with a hygrometer (± 3% r.h.; Taylor Scientific, St. Louis, MO). Basic observations were conducted at 22–24 °C, L14:D10, in an environmental room. Selection of these conditions was based on previous water balance literature so that comparisons could be made.

The amount of water available for exchange (water mass, m) was determined according to standard methods (Wharton, 1985; Hadley, 1994). Briefly, beetles were weighed and placed at 0% r.h., 22–24 °C, until 6–8% of body mass was lost (initial mass); thus, changes in mass reflect changes in water pool (Wharton, 1985). Consecutive mass determinations (0% r.h., 22–24 °C) were made at hourly intervals for five readings of mass, then specimens were transferred to 80 °C, 0% r.h., for drying and were monitored periodically until they reached constant mass (dry mass; 3–4 days plus 2 extra days of drying). The water

mass (m) was calculated by subtracting initial and dry masses. Intermediate mass values were fitted to Wharton's (1985) exponential model (eqn 1) to determine water loss rates.

$$m_t = m_0 e^{-kt} \qquad (eqn\ 1),$$

where m_t is the water mass at any time t, m_0 is the initial water mass, and k is the amount of water loss in unit time, or $\ln(m_t/m_0) = -k_t$ (Wharton, 1985).

The point during dehydration at 0% r.h., 22–24 °C, where adult beetles failed to right themselves and crawl one body length when prodded was defined as the critical activity point. This approximates the dehydration tolerance limit, an irreversible lethal amount of water loss, and was expressed as a percentage of water mass lost. The critical activity point for larvae was defined as the amount of water lost that prevented pupation, and the critical activity point for pupae was defined as the amount of water lost that prevented adult eclosion (Yoder & Hoy, 1998).

Water was stained with 1% Evans blue and offered to beetles in a 9-cm diameter Petri dish (15 beetles per plate) for 24 h to determine whether beetles drank free water. Beetles were dissected in 10% NaCl using a light microscope and examined for the presence of blue coloration (Yoder & Smith, 1997). As an additional possible water resource, the ability to absorb water vapor from the air was determined by monitoring the water mass of the beetles at various relative humidities (85%, 93%, and 98% r.h.) at 12-h weighing intervals. The ability to maintain a relatively stable water mass (m) in subsaturated air was taken as evidence of being able to counter water loss with gains from the atmosphere and is a good estimate of the critical equilibrium humidity (CEH), the lowest relative humidity from which water vapor absorption takes place (Wharton, 1985).

Statistics

Data (15 per replicate; n = 3) were compared by an analysis of variance (ANOVA) following an arcsin transformation in the case of percentages. Parameters derived from regression lines were compared using Sokal & Rohlf's (1981) test for the equality of slopes of several regressions.

Results and discussion

Water balance profiles of larvae, pupae, and female adults of *S. nigripes* are presented in Table 1. The most distinguishable feature is the extremely high water loss rate of female adults, which was roughly three-times faster than larvae and six-times faster than pupae (ANOVA; P < 0.05; Figure 1). The importance of the high water loss rate for adult females is that it is the best predictor of habitat preference and suitability for *S. nigripes* because this stage

Table 1 Water balance profiles of beetle larvae (L), pupae (P), and female adults (A) of *Stethorus nigripes*. Free water drinking was assessed by exposing insects to droplets of Evans blue stained water and dissection; CEH, critical equilibrium humidity is the relative humidity above which water gain can occur. Data represent mean ± SE (15 per replicate; n = 3)

Characteristic	L	P	A
Water Pool			
Initial mass (mg)	0.221 ± 0.07	0.187 ± 0.05	0.146 ± 0.08
Water mass (mg)	0.142 ± 0.03	0.122 ± 0.09	0.093 ± 0.04
Water content (%)	64.3 ± 2.4	65.2 ± 3.1	63.7 ± 2.7
Water loss			
Water loss rate (%/h) (Figure 1)	0.95 ± 0.017	0.44 ± 0.021	2.82 ± 0.013
Amount lost at death (%)	36.11 ± 1.9	26.82 ± 2.6	25.02 ± 2.2
Water gain			
Free water drinking	no evidence	no evidence	blue dye in gut
CEH (% r.h.) (Figure 2)	> 98% r.h.	> 98% r.h.	> 98% r.h.

is free-living; the occurrence of larvae and pupae are non-dispersal stages and restricted to the underside of the corn leaf. Thus, the ability to retain water (water loss rate) contributes little to the ability by *S. nigripes* to function in their environment, which places the emphasis on water gain (modified from Wharton, 1985). From a water balance perspective (Hadley, 1994), *S. nigripes* is hydrophilic with regard to water balance and requires a moisture-rich, humid environment.

Only female adults were capable of drinking free water from droplets. There was no evidence of blue coloration in gut diverticula in dissections of larvae and pupae after they had been exposed to dots of Evans blue stained water. None of the beetles examined were capable of absorbing water vapor, as indicated by their failure to keep water content steady at 85%, 93%, and 98% r.h. (Table 1; Figure 2). Water loss could not be balanced by gains from ambient air. When expressed in activities, the activity of the beetle's body water (a_w) = 0.99a_w (Wharton, 1985), thus *S. nigripes* can achieve an equilibrium water content (water gain = water loss; i.e., CEH) only when the water vapor activity (a_v, a_v = % r.h./100) of the surrounding air is > 1.00 a_v or 100% r.h. For *S. nigripes*, the fact that the CEH is > 98% r.h. indicates that water must be imbibed as a liquid (Wharton, 1985). By contrast, arthropods that use water vapor as a primary source of water, such as mites, ticks, and certain larval beetles, maintain an equilibrium water content by pumping water against the activity gradient that exists between the a_w of the body water and ambient a_v using solute-driven uptake mechanisms (Hadley, 1994). Only the larval stage of *S. nigripes* is shown in Figure 2 (CEH determination) because among beetle life cycle stages it is the one most likely to absorb water vapor, but *S. nigripes* larvae apparently lack this ability. Free water drinking is anticipated for an adult beetle, pupae do not drink because they lack a functional mouth, and the source of water for larvae is probably their food, i.e., the blood and tissue fluids obtained by preying on mites (modified from Hadley, 1994). Mites (food) probably represent an additional water resource for the adult.
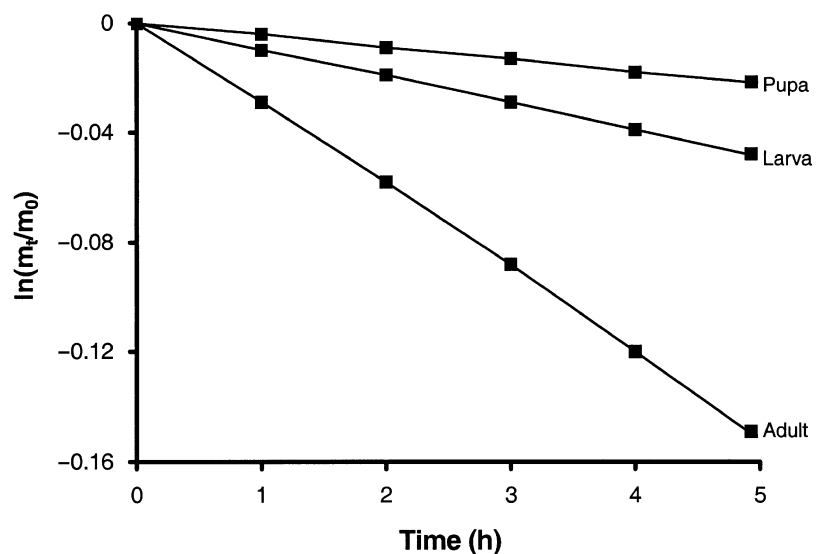

Figure 1 Water loss (SE ≤ 0.002) of *Stethorus nigripes* larvae, pupae, and female adults at 0% r.h. and 22–24 °C. The slope of the line through the plot is the rate of water loss; m_t, water mass at any time t and m_0, initial water mass (15 per replicate; n = 3).
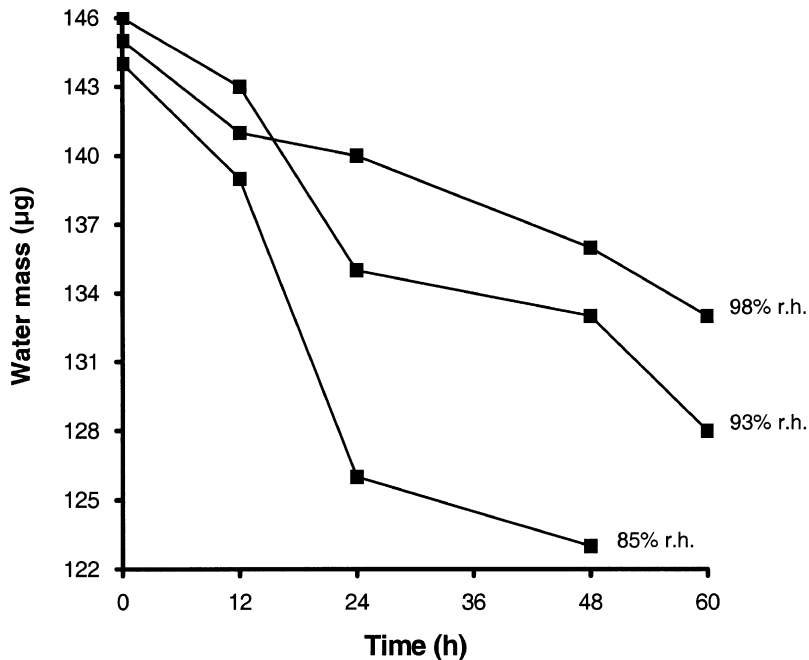
Figure 2 Water mass loss (SE ≤ 0.08) by larvae of *Stethorus nigripes* in subsaturated air at 22–24 °C. Inability to maintain a constant water mass indicates a failure to maintain equilibrium (gain ≠ loss) by absorbing water vapor from the air (15 per replicate; n = 3). Female adults and pupae similarly failed to maintain equilibrium water mass and showed similar declining trends as beetle larvae.

Pupae had the lowest water loss rates when compared to adults and larvae (ANOVA; P < 0.05), and this is consistent with the view that the pupa is a major water retaining stage (Hadley, 1994), because it cannot replenish water stores by drinking or feeding (Yoder & Hoy, 1998). In addition to enhanced water conservation, survival of *S. nigripes* pupae without being able to eat or drink is facilitated by having a relatively brief stadium, averaging 5 days (D. Pollock, unpublished). Water loss rates for *S. nigripes* also fit the trend that water loss rates are typically higher for larvae that are actively feeding, and the water loss rates for adults are high due to greater flying activity and smaller body size (modified from Hadley, 1994). In general, the water balance strategies throughout the life history of *S. nigripes* compare favorably to patterns observed in other insects. The only distinction is the fast water loss rate of female adults.

Approximately the same amount of water (~64%) was present in larvae, pupae, and female adults (ANOVA; P > 0.05), despite significant differences in body size and water mass (larvae > pupae > adults; ANOVA; P < 0.05). In all cases, water mass correlated positively with dry mass (R ≥ 0.93; ANOVA; P < 0.001). That pupae and adults have similar water content is a common occurrence and approaches the mean water content (70%) of most insects (Hadley, 1994). Female adults and pupae of *S. nigripes* can experience only about a quarter loss of their body water before they succumb to desiccation, which is within the 20–30% range of dehydration tolerance limits for most insects (Hadley, 1994). The 64% water content of

S. nigripes larvae is unusual for an insect. Typically, larvae have a 10–20% higher water content than mature stages, which reflects the higher water content of their food, such as decaying or fresh animal and plant tissue (Hadley, 1994). Larvae can withstand 10–15% greater loss of water than pupae or adults, attributed to a higher fat content (dry mass) (Hadley, 1994), and this is precisely what was observed with *S. nigripes* larvae (Table 1). Little, if any, variation was noted in size, shape, and water content within larva, pupa, and female adult groups, suggesting that the differences that we note are stage-specific. Female adults lose water the fastest, larvae can tolerate a greater loss, and pupae are maximized for water retention, although all stages require about the same amount of body water to function.

Stethorus nigripes possesses several characteristics that make it an effective natural enemy in classical biological control programs (Richardson, 1977). These include the ability to survive at low prey densities, adequate intrinsic rates of increase, behavioral and reproductive synchrony with prey and habitat, and strong functional and numerical response to density of mite prey. The high moisture requirement of *S. nigripes*, as shown by our study, may be one of the drawbacks when using this particular beetle species broadly for spider mite control. The bulk of the work on suitability of various *Stethorus* species for integrated pest management of mites has involved testing the beetles' sensitivity to pesticides (Walters, 1976; Field, 1979). This study on *S. nigripes* indicates that moisture requirements

and water balance represent an additional, important consideration for establishment of any *Stethorus* species.

Acknowledgements

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