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Comparative Biochemistry and Physiology Part A 135 (2003) 625–634

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Comparative water relations of adult and juvenile tortoise beetles: differences among sympatric species

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Received 5 February 2003; received in revised form 20 May 2003; accepted 20 May 2003

Abstract

Relative abundance of two sympatric tortoise beetles varies between drought and 'wet' years. Differing abilities to conserve water may influence beetle survival in changing environments. Cuticular permeability (CP), percentage of total body water (%TBW), rate of water loss and percentage of body lipid content were determined for five juvenile stages and female and male adults of two sympatric species of chrysomelid beetles, the golden tortoise beetle, *Charidotella bicolor* (F.) and the mottled tortoise beetle, *Deloyala guttata* (Olivier). There were significant differences in %TBW and lipid content among juvenile stages. Second instars had the greatest difference in CP (37.98 and 11.13 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ for golden and mottled tortoise beetles, respectively). Mottled tortoise beetles had lower CP and greater %TBW compared with golden tortoise beetles, suggesting that they can conserve a greater amount of water and may tolerate drier environmental conditions. This study suggests that juvenile response to environmental water stress may differentially affect the survival of early instars and thus affect the relative abundance of adult beetles in the field. This is supported by the low relative abundance of golden tortoise beetle larvae in a drought year and the higher abundance in two 'wet' years.

Keywords: *Charidotella bicolor*; *Deloyala guttata*; Cuticular permeability; Water relations; Desiccation; Body water composition

1. Introduction

Water relations are critical to insects and other arthropods because of their large surface area to body volume ratios. The cuticle is generally the most important water efflux pathway (Edney, 1977) and is coated with a thin layer of epicuticular lipids that create a water-resistant barrier that reduces cutaneous water loss (Neville, 1975; Gibbs, 1998). In addition, the chemical composition of these epicuticular lipids, the structure of the cuticle itself, and active regulation by epidermal cells influence the rate at which water is lost (Edney, 1977; Hadley, 1994). Many mechanisms influence water loss prevention, attesting to its importance to insect survival.

Water loss or permeability of the cuticle can also vary with environmental conditions (temperature and humidity), age and developmental stage. Typically, arthropods living in wet or humid habitats often have greater cuticular permeabilities (CP) than arthropods living in more xeric or dry habitats (Edney, 1977; Hadley, 1994). One reason for this conservation differential is that in humid habitats, more water is available to diffuse passively into the body through the cuticle (Appel et

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1095-6433/03/$ - see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S1095-6433(03)00142-9

al., 1986) to replace what is lost due to diffusion and respiration (Haverty and Nutting, 1976).

However, the environment in which the animals are found does not dictate CP values (sensu Cohen and Pinot, 1977). Many adult tortoise beetles in the family Chrysomelidae, subfamily Cassidinae, have bright elytral colors that are lost following desiccation related changes in the thickness of the cuticle (Jolivet, 1994). Some adult tortoise beetles are able to actively change cuticular colors (Mason, 1929; Neville, 1977) in response to host plant chemistry (Kirk, 1971), disturbance (Vasconcellos-Neto, 1988) and sexual stimulation (Barrows, 1979). These color changes are not related to ambient relative humidity or photophase; therefore, it is possible that the CP values in adult beetles may be related to regulation of their metallic coloration and as well as adaptation to their environment.

In this study, we examined the water relations of two sympatric tortoise beetles, the golden tortoise beetle, *Charidotella bicolor* (F.) and the mottled tortoise beetle, *Deloyala guttata* (Olivier), (Chrysomelidae: Cassidinae), across life stages. Both golden and mottled tortoise beetles can be significant pests of sweet potatoes and tortoise beetles may be used as biological control agents for pest plant species in the family Convolvulaceae (Rosenthal and Carter, 1977; Baloch, 1977). As adults, golden and mottled tortoise beetles inhabit very similar habitats and feed on the same species of plants (Rausher et al., 1993; Capinera, 2001). Larvae feed in a similar manner as adults (Capinera, 2001), but the larvae of these two species are not found on the same host plant at the same time (pers. obs.). During an unusually dry year (2000) in Lee and Macon Counties, Alabama, USA average rainfall for the months May through August was 4.06 cm of rain per month (AWIS, 2002). Mottled tortoise beetle larvae abundance ranged from 15 to 20 individuals per square meter and were collected from the underside of leaves at field sites in Lee and Macon Counties, Alabama, USA. However, during the next 2 years rainfall averages for the same time period were 8.41 cm per month in 2001 and 7.60 cm per month in 2002. Golden tortoise beetle larvae abundance ranged from 18 to 30 individuals per m^2 at the same field sites and mottled tortoise beetle larvae were absent or patchily distributed (3–7 per m^2) (Hull-Sanders, unpublished data). If mottled and golden tortoise beetle larvae have different CPs, increased cuticular water loss may prevent golden tortoise beetles from developing or surviving during abnormally dry years and may partially account for differences in relative abundance.

Specifically, this study determined CP, total body water composition (%TBW), rates of body mass and percentage of total body water content loss in golden and mottled tortoise beetles. Each measure was compared among juvenile life stages and between male and female adults to determine how water relations may change throughout the lifecycle of these beetles. In addition, whole body lipid content (%lipid) was compared between species and among stages. Whole body lipid content affects cutaneous water loss and is inversely related to CP (Loveridge, 1973; Tucker, 1977; Appel et al., 1986).

2. Materials and methods

2.1. Insects

Tortoise beetle adults and larvae were collected from Lee and Macon counties in Alabama, USA during the summers of 2000 and 2001 to establish continually breeding colonies. Colonies were maintained at 25.0 ± 2 °C, 80 ± 10% RH, and a photoperiod of 16:8 (L:D) h, fed 3–10 leaves of morning glories, *Ipomoea* spp., 3 d per wk, and provided water on a cotton ball ad libitum. Beetles were separated by species and kept in 0.95-l plastic containers with lids that had been modified by cutting out the central portion and hot gluing Tight Weave No-See-Um mosquito netting over the opening. Ten to 15 adults were reared together. All animals used in this study were reared in the colony from eggs. First instar larvae were collected from the colony within 2 h after handing and before their first feeding or production of their first fecal mass. Larvae of tortoise beetles retain their feces on small anal projections called urogomphi. These fecal masses are used as defense shields against predators. Second instars, third instars, pre-pupae and pupae were selected from the colony and fecal masses were removed with fine forceps from the urogomphi thereby ensuring all water loss recorded was from body tissue. All specimens of each life stage were within 2 d of age of each other. Adult females and males were identified and removed from the colony during copulation. Sexes can only be accurately identified during copulation. Females and males were placed

in separate containers for at least 24 h prior to experiments.

2.2. Water relations experiments

One measure of water loss through the cuticle, or CP, can be quantified under a constant set of conditions as the mass of water lost per unit body surface area (cm^2) per unit time (e.g. h) per unit saturation deficit (Torr or mmHg) (Edney, 1977), but see Noble-Nesbitt (1991) for additional units. A total of 20 adults (ten of each gender) and 50 juveniles (ten of each stage of each species) were selected randomly. Water content, CP ($\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$), rate of percentage of total body water loss (%TBW), and percentage of body lipid content of freshly killed tortoise beetles were determined gravimetrically with a digital balance (0.01 mg sensitivity) (Appel et al., 1983; Mack and Appel, 1986). Beetles were killed by a 15 min exposure to HCN gas. Dead beetles were used to determine cuticular water loss without confounding measures of respiratory and fecal water loss. Initial mass was determined and individual beetles were placed in uncovered 30 ml glass vials and held at 30.0 ± 0.1 °C in an 11-l desiccator over $\approx$2.3 kg anhydrous $CaSO_4$ ($\approx$0–2% RH, saturation deficit of 31.824 mmHg). Mass losses were measured at 2, 4, 6, 8, 10, 12 and 24 h. Dry mass was determined after the specimens were dried in an oven at $\approx$50 °C for $\geqslant$72 h. Lipids were extracted from beetle bodies by first breaking individual beetles into several pieces and extracting lipids with 5 ml chloroform for $\geqslant$72 h (Appel et al., 1986). The chloroform supernatant was removed by suction filtration and beetle pieces were placed into $\approx$50 °C oven. Lipid-free masses were measured 24 h after drying.

CP was calculated as the microgram of water lost (between 0 and 2 h) per unit body surface area (cm^2) per unit time (h) per unit saturation deficit (mmHg) in the desiccator chamber. Surface area was estimated for each specimen using Meeh's formula (Meeh, 1897):

$$S = 12M^{2/3},$$

where S, body surface area (cm^2) and M, initial mass (g) (Edney and McFarlane, 1974). CP values of 0–30 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$ indicates a low amount of water is lost through the cuticle and the arthropod may be adapted to xeric habitats. Arthropods found in mesic environments usually have permeabilities between 31 and 60 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$ and those found in hygric (water saturated) habitats have permeabilities >60 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$ (Edney, 1977).

Percentage of TBW was calculated as the difference between initial mass and the mass after drying, dividing by the initial mass and then multiplying by 100. Percentage of lipid content was calculated as the difference between dry mass and the mass after lipid extraction divided by the initial mass and then multiplied by 100. Mean hourly mass loss and %TBW were used to calculate rates of mass and %TBW loss.

2.3. Data analysis

Experiments were conducted in a randomized complete block design to evaluate differences in initial masses, %TBW, percentage of lipid content, and CP between species, among non-adult life stages (first, second, third instars, pre-pupae and pupae), and adult genders. Data for each stage were tested for normalcy and heterogeneity of variance and were not transformed (Sokal and Rohlf, 1995). Data were analyzed by analysis of variance (ANOVA) with species, block and stage as main effects using SAS software (SAS Institute, 1999–2001). ANOVA tests were followed by the Bonferroni (Dunn) mean separation test to detect differences within juvenile stages within species and differences within and between species for adults. Contrasts were performed to separate differences between juvenile stages between species. To test if Meeh's formula provided an unbiased estimation of surface area, CP values were regressed against initial mass. Percentage of total body water lost and percentage of initial mass loss by juveniles were regressed against time using power function of the form $y = a(1 - e^{(-bx)})$, where y is percentage of total body water lost (or percentage of initial mass lost), x is desiccation time (h), a is the y-axis intercept and b is the rate constant (%TBW loss/time(h)) (SigmaPlot, SPSS, 2001). This function was chosen because it represents an exponential rise to a maximum (1 or 100%); the maximum percentage of mass or %TBW any organism can lose is 100%. Percentage of total body water lost and percentage of initial mass lost by adults were regressed against time using a linear function of the form $y = a + bx$, where y is percentage of total body water lost (or

Table 1
Mean (±S.E.) initial mass, percent total body water (%TBW), CP and percentage of total lipid content (%lipid) of three larval, pre-pupal and pupal stages of cassadinid beetles

Species	Stage	*n*	Initial mass (mg)	%TBW	CP ($\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$)	%Lipid
Golden tortoise beetle	First instar	10	0.34±0.93a	74.18±0.64a*	17.32±5.97acd	4.80±0.86a
	Second instar	10	3.20±0.93b	77.41±0.64b**	37.98±5.97b*	0.91±0.86b
	Third instar	10	11.84±0.76c*	76.12±0.52b***	23.16±4.88ac**	1.85±0.70b*
	Pre-pupae	10	17.40±0.82d†	73.30±0.56a†	19.93±5.24ac†	2.98±0.76ab
	Pupae	10	14.85±0.74e‡	74.62±0.50a‡	4.30±4.72ad	2.75±0.68ab
Mottled tortoise beetle	First instar	10	0.40±0.52v	78.37±1.44x*	11.71±1.91x	3.86±0.78x
	Second instar	10	1.96±0.52w	81.03±1.36x**	11.13±1.91x*	3.02±0.70x
	Third instar	10	9.41±0.52x*	79.82±1.36x***	9.09±1.91x**	4.23±0.70x*
	Pre-pupae	10	11.58±0.52y†	79.55±1.36x†	6.77±1.91xy†	3.86±0.70x
	Pupae	10	10.00±0.52xz‡	79.52±1.36x‡	2.93±1.91y	4.63±0.70x

Means followed by the same letter within a group (abc or xyz) are not significantly different (Bonferroni mean separation (SAS Institute, 1999–2001)). Means followed by the same symbol (*, **, ***, †, or ‡) are significantly different within the same instar (contrast difference (SAS Institute, 1999–2001)).

percentage of initial mass lost), x is desiccation time and a is the y-axis intercept and b is the rate constant (%TBW loss/time (h)) (SigmaPlot, SPSS, 2001). Data are expressed as means±S.E. A significance level of $P \leqslant 0.05$ was used for all tests.

3. Results

3.1. Juvenile stages

Mean initial body mass of juveniles ranged from 0.34 to 17.40 mg for golden tortoise beetles and from 0.40 to 11.58 mg for mottled tortoise beetles (Table 1). For both species, pre-pupae had the greatest mean initial body mass, although in golden tortoise beetles pre-pupal mass was significantly different from first, second and third instars, but not from pupae (Table 1). Between species, third instar, pre-pupal and pupal golden tortoise beetles had significantly greater body mass than mottled tortoise beetles ($P<0.01$). Mean %TBW of first instars ranged from 74.18% for golden tortoise beetles to 78.37% for mottled tortoise beetles. Percentage of total body water of second instars ranged from 77.41 to 81.03%, third instars ranged from 76.12 to 79.82%, pre-pupae ranged from 73.30 to 79.55% and pupae ranged from 74.62 to 79.52% in golden and mottled tortoise beetles, respectively (Table 1). There were significant differences in %TBW between species ($F=54.45$, d.f.=1, $P<0.0001$); however, there was not a significant species by instar interaction ($F=0.64$, d.f.=4, $P=0.63$) indicating that the instars vary in a similar manner between species. Contrasts indicated that %TBW was significantly different ($P<0.01$) between species within each instar. Mottled tortoise beetles had a significantly greater mean %TBW for each instar compared with golden tortoise beetles.

CP values using Meeh (1897) surface area model estimates were not significantly ($P>0.05$) related to initial mass for all stages (Table 2), indicating that the surface area estimation model did not bias CP values. Juvenile mean CP ranged from 4.30 to 37.98 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ for golden tortoise beetles and from 2.93 to 11.71 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ for mottled tortoise beetles (Table 1). There were significant differences within golden tortoise beetle instars ($F=4.77$, d.f.=4, $P=0.0024$) and only pupal CP was significantly lower in mottled tortoise beetles. Golden tortoise

Table 2
Results of the relationship of CP to mass as a determinant of bias in Meeh's formula ($S=12M^{2/3}$) estimation for surface area

Species	Stage	*n*	*F*	d.f.	*P*
Golden tortoise beetle	First instar	10	0.68	1	0.4334
	Second instar	10	4.13	1	0.0766
	Third instar	10	3.78	1	0.0737
	Pre-pupae	10	0.95	1	0.3514
	Pupae	10	3.69	1	0.0753
	Adults	20	0.00	1	0.9904
Mottled tortoise beetle	First instar	10	1.97	1	0.2552
	Second instar	10	5.52	1	0.1004
	Third instar	10	0.41	1	0.5681
	Pre-pupae	10	1.36	1	0.3282
	Pupae	10	3.42	1	0.1015
	Adults	20	0.21	1	0.6551

Table 3
Mean (±S.E.) initial mass, percent total body water (%TBW), CP and percent total lipid content (%lipid) for adult female and male cassadinid beetles

Species	Sex	*n*	Initial mass (mg)	%TBW	CP ($\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$)	%Lipid
Golden tortoise beetle	Female	10	22.47±0.55a	48.14±1.65a	9.92±0.92a	13.53±1.87a
	Male	10	15.07±0.55b	48.61±1.56a	10.06±0.92a	10.57±1.77a
Mottled tortoise beetle	Female	10	15.71±0.55b	54.39±1.56b	8.06±0.92a	8.30±1.77a
	Male	10	11.29±0.55c	54.11±1.56ab	8.76±0.92a	7.53±1.77a

Means followed by the same letter are not significantly different (Bonferroni mean separation (SAS Institute, 1999–2001)).

beetle pupal CP was significantly less than either second, third or fourth instars ($P<0.05$). Between species, second, third and pre-pupal golden tortoise beetle CP was greater than for mottled tortoise beetles ($P<0.05$). Mean percentage of lipid content for juveniles ranged from 0.91 to 4.80% for golden tortoise beetles and 3.02–4.63% for mottled tortoise beetles (Table 1). There were significant differences in percentage of lipid between instars of golden tortoise beetles ($F=3.37$, d.f.=4, $P=0.016$), but there were no significant differences in percentage of lipid between instars of mottled tortoise beetles. Within golden tortoise beetles, the largest difference occurred between first and second instars and was significant. Between species, only third instars were significantly different ($P=0.02$).

Percentage of TBW lost by juveniles increased curvilinearly with desiccation time (Table 4, Fig. 1), with first instars generally losing a greater percentage of mass than other instars. In golden tortoise beetles, the rate of %TBW loss was not different between first and second instars, third and pre-pupa, nor pre-pupae and pupa (Fig. 1a). After 24 h, mean %TBW lost was 96.84±0.58% for first instars, 84.48±1.70% for second instars, 49.94±2.15% for third instars, 35.37±1.82% for pre-pupae and 16.57±0.98% for pupae of golden tortoise beetles (Fig. 1a). In mottled tortoise beetles, the rate of %TBW loss was not different between first and second instars, second and third instars, nor third instars, pre-pupae and pupae (Fig. 1b). After 24 h, mean %TBW lost was 83.74±1.28% for first instars, 62.39±2.38% for second instars, 33.17±1.74% for third instars, 28.43±0.53% for pre-pupae and 12.94±0.89% for pupae of mottled tortoise beetles (Fig. 1b).

3.2. Adults

Initial adult masses ranged from 22.47 mg for female golden tortoise beetles to 11.29 mg for male mottled tortoise beetles (Table 3). Body mass of adult female beetles was significantly greater ($F=116.29$, d.f.=1, $P<0.001$) than adult males and was also significantly different between species ($F=92.4$, d.f.=1, $P<0.0001$). However, there was no significant difference between the mean mass of male golden tortoise beetles and female mottled tortoise beetles. Adult body water content ranged from 48.14% in female golden tortoise beetles to 54.39% in female mottled tor-

Table 4
Power function regression coefficients (mean±S.E.) for percentage of total body water lost over time for three larval, pre-pupal and pupal stage of cassadinid beetles

Species	Stage	*a*	*b*	*F*	*P*	R^2
Golden tortoise beetle	First instar	104.17±6.30	0.10±0.01	448.81	<0.0001	0.987
	Second instar	86.43±7.63	0.11±0.02	159.92	<0.0001	0.964
	Third instar	63.05±6.70	0.06±0.01	368.75	<0.0001	0.984
	Pre-pupae	42.30±4.14	0.07±0.01	333.03	<0.0001	0.982
	Pupae	134 386.80±543.75	0.00±0.01	740.47	<0.0001	0.992
Mottled tortoise beetle	First instar	136.20±23.49	0.04±0.01	400.79	<0.0001	0.985
	Second instar	151 792.00±370.65	0.00±0.01	342.71	<0.0001	0.983
	Third instar	188 360.01±824.07	0.00±0.01	1006.64	<0.0001	0.994
	Pre-pupae	152 389.24±632.81	0.00±0.00	2381.32	<0.0001	0.998
	Pupae	21.19±2.71	0.04±0.01	809.49	<0.0001	0.993

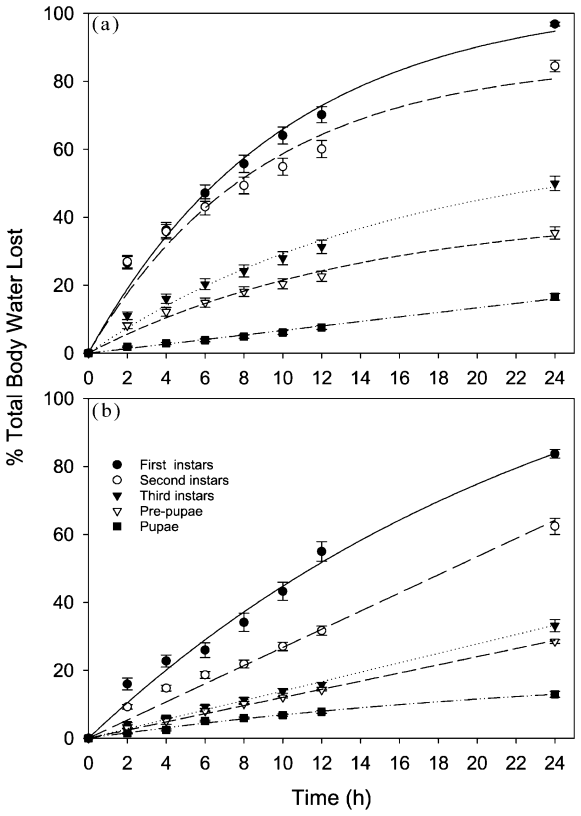

Fig. 1. Percentage of total body water lost over time in golden tortoise beetles (a) and mottled tortoise beetles (b).

toise beetles. Percentage of body water was significantly greater in mottled tortoise beetles than in golden tortoise beetles ($F=13.73$, d.f.$=1$, $P=0.0007$). Bonferroni mean separation indicated that female mottled tortoise beetles had significantly greater %TBW than either female or male golden tortoise beetles, but was not significantly different than male mottled tortoise beetles (Table 3).

Adult CP values using Meeh (1897) surface area models were not significantly related to initial mass (Table 2), indicating that the surface area estimation model did not bias CP values. Mean adult CP ranged from 8.06 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$ in female to 10.06 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$ in male golden tortoise beetles (Table 3). There were no significant differences in CP between either species or gender. Adult mean percentage of lipid ranged from 7.53% in male mottled tortoise beetles to 13.53% in female golden tortoise beetles (Table 3). There were no significant differences in percentage of body lipid between either species or gender.

Percentage of TBW lost by adults increased linearly with desiccation time (Fig. 2), with male mottled tortoise beetles generally losing a greater percentage of water than females. After 24 h, mean %TBW lost was $50.38\pm1.82\%$ in female and $50.34\pm1.93\%$ in male golden tortoise beetles (Fig. 2a). Mean %TBW lost after 24 h in mottled tortoise beetles was $59.47\pm2.53\%$ for females and $80.62\pm2.39\%$ for males (Fig. 2b). Rate of %TBW loss was not different between golden tortoise beetle males and females and mottled tortoise beetle females (Table 5). However, all were significantly different from mottled tortoise beetle males.

4. Discussion

Mottled and golden tortoise beetles inhabit the same environment and utilize the same food sources (Rausher et al., 1993; Capinera, 2001); however, their relative abundance in the field

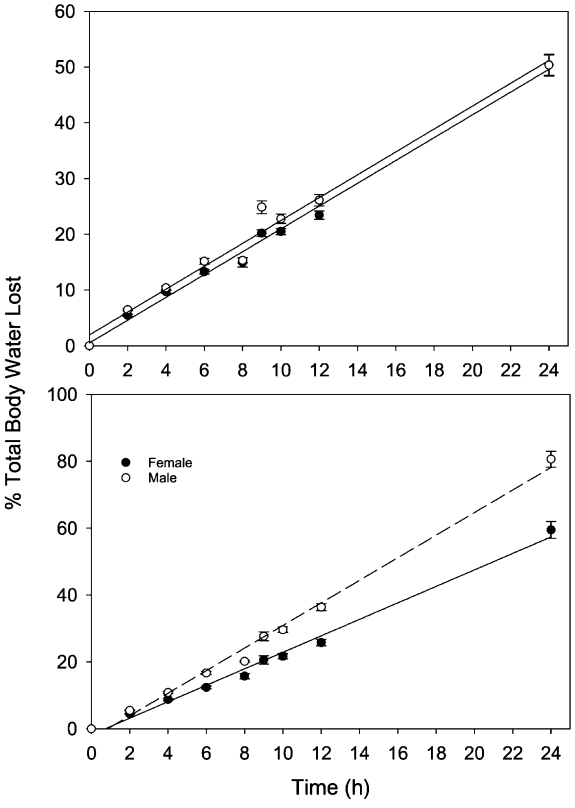

Fig. 2. Percentage of total body water lost over time of male and female golden tortoise beetles (a) and mottled tortoise beetles (b).

Table 5
Power function regression coefficients (mean ± S.E.) for percentage of total body water lost over time for adult cassadinid beetles

Species	Sex	*a*	*b*	*F*	*P*	R^2
Golden tortoise beetle	Female	0.49 ± 0.67	2.05 ± 0.07	1059.07	< 0.001	0.993
	Male	1.94 ± 1.20	2.05 ± 0.11	334.17	< 0.001	0.980
Mottled tortoise beetle	Female	−1.77 ± 0.92	2.46 ± 0.09	818.32	< 0.001	0.992
	Male	−2.87 ± 1.23	3.38 ± 0.12	856.64	< 0.001	0.992

differed between years (Hull-Sanders, unpublished data). Adult tortoise beetle CP was not significantly different between species or gender (Table 3) and while this is expected of similar insects with similar lifestyles, the low CP values of these mesic species are consistent with other beetles that inhabit desert environments (Ahearn and Hadley, 1969). Low abundance of adult golden tortoise beetles cannot, therefore, be attributed to the significantly drier abiotic conditions of 2000 (ANOVA mean rainfall May–August, 2000, 2001, 2002, $F = 5.6271$, d.f. = 2, $P = 0.0179$). Juvenile CP differed between species for second, third and pre-pupal instars; golden tortoise beetle juvenile CP was at least twice that of mottled tortoise beetle juvenile CP (Table 1). These results suggest golden tortoise beetle juveniles are more vulnerable to desiccation and cannot tolerate drier environmental conditions.

Percentage of TBW content of the three larval and one pre-pupal stage in this study (73.30–81.03%) was similar to the juvenile range of 78–81% found in the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), that inhabit similar environments as tortoise beetles (Edney, 1977; Hadley, 1994). Pupal TBW content (74.62–79.52%) was also similar to pupal *P. japonica* (74%), but was greater than the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (61%), a species that inhabits xeric environments (Marcuzzi, 1960). Adult %TBW content ($\approx$48% for golden tortoise beetles and $\approx$54% for mottled tortoise beetles) was similar to the range reported for other Coleoptera (40.8–81% for adults) (Hadley, 1994). For both species, second instar larvae had the greatest %TBW and were significantly different from first instars, pre-pupae and pupae in golden tortoise beetles (Table 1). Any sublethal dehydration can adversely affect the developmental process (Wigglesworth, 1972); therefore, greater water contents may allow for moderate amounts of desiccation and still allow normal development.

Body lipid and water contents are inversely proportional in many arthropods (Edney, 1977; Mack and Appel, 1986; Steinbauer, 1998). Body lipid and water contents were also inversely correlated in this study (Fig. 3). First instar larvae of golden tortoise beetles and pupae of mottled tortoise beetles had the greatest lipid content among juveniles. Golden tortoise beetle first instar larvae migrate away from the natal leaf before feeding; whereas, first instar mottled tortoise beetles feed immediately upon emergence or risk desiccation (pers. obs.). Golden tortoise beetle females may allocate more lipid resources to their eggs than mottled tortoise beetles, allowing first instar larvae to survive longer until finding a suitable host leaf to feed upon. High body lipid content is a prerequisite for long-distance migration (Downer and Matthews, 1976; Schneider et al., 1995; Mason et al., 1990). Finding a suitable host plant may constitute a migration for 0.34 mg larvae. High body lipid content may also occur in response to food stress (Legaspi et al., 1996). Food stress results from either a lack of food or poor quality food. While beetles were given abundant leaves to feed upon in culture, the lack of whole plants may be an artificial stress that is a result of raising beetles within a colony. Female golden tortoise beetles may allocate more lipids because of this artificial stress.

Previous studies have focused on adult arthropods and used CP to predict the distribution (Appel et al., 1983, 1999) or the activity (Roberts et al., 1994; Webb and Telford, 1995) when encountering various combinations of temperature and humidity. Other studies that have been conducted on juvenile stages have focused on over-wintering (Ramloov and Lee, 2000) or inactive stages (Danks, 2000) when water uptake is restricted. This study included the inactive pupal stage in which CP was found to be the lowest and not significantly different between species (Table 1). However, %TBW was significantly higher and the rate at which water was lost lower (Fig. 1) in mottled tortoise beetles

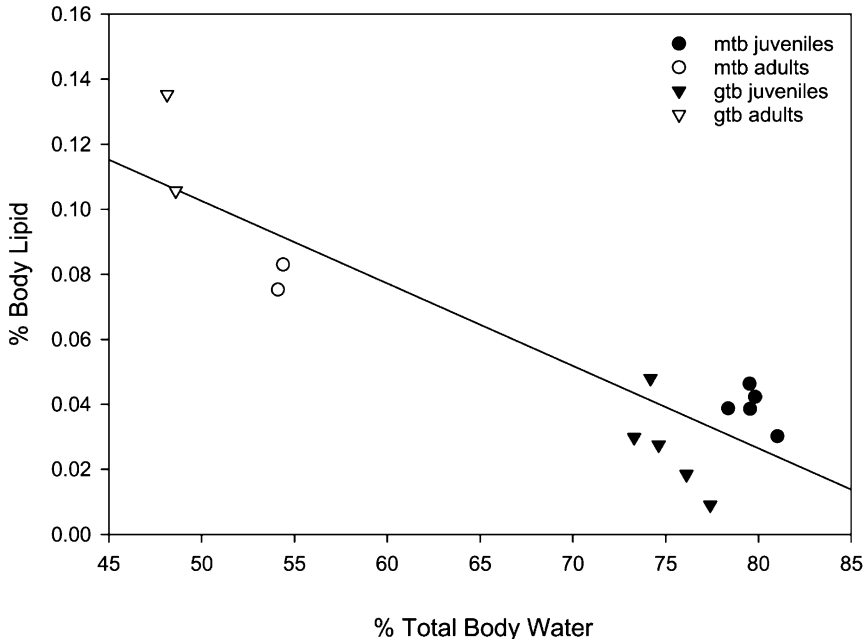

Fig. 3. Correlation between percent total body water and percent body lipid for two sympatric species of cassadinid beetles, the mottled tortoise beetles (mtb) and the golden tortoise beetles (gtb).

perhaps indicating the mottled tortoise beetle's ability to remain inactive longer.

Both mottled and golden tortoise beetle adults have metallic elytral coloration; mottled tortoise beetles achieve their coloration at sexual maturity (Barrows, 1979) and do not lose it until after death. Once the are sexually mature, golden tortoise beetles can actively regulate elytral water content to change from gold to orange and back again (Jolivet, 1994). This active regulation requires water to be moved between the lamellae layers (Hinton, 1960). Any loss of water through the cuticle would interrupt the color and decrease the benefit these colors provide to the golden tortoise beetles. The similarity of CP values of adult mottled and golden tortoise beetles may indicate habitat adaptation in mottled tortoise beetles, but may be an artifact of color regulation in golden tortoise beetles.

This is the first study to compare the water relations of beetles that utilize the same food resources, but differ in relative abundance between wet and dry years. We found that the juvenile instars have higher CP values than adults, indicating that juveniles may be more vulnerable to desiccation. In addition, mottled tortoise beetle larvae had significantly lower CP than golden tortoise beetle larvae suggesting that they may be able to withstand drier conditions than the golden tortoise beetle larvae. In addition, mottled tortoise beetle larvae had a greater %TBW than golden tortoise beetles suggesting a conservation of water as well.

Predicting the distribution and relative abundance of arthropods cannot be based solely on adult physiology. In this study, adult CP values indicated that mottled and golden adult tortoise beetles could withstand extremely dry conditions similar to desert environments. Based on the juvenile CP values, however, the two species of beetles would be expected to diverge in their habitat requirements. Mottled tortoise beetle juvenile CP values are consistent with beetles found in drier habitats, but the golden tortoise beetle juvenile CP values are consistent with beetles found in more moist habitats. CP values can be used in part to predict arthropod distribution in response to environment, but each lifestage must be used to determine the most vulnerable stage to abiotic conditions.

Acknowledgments

We thank Marla J. Tanley (Auburn University) for technical assistance. We also thank Auburn

University for their funding of this research and the support of the Department of Entomology and Plant Pathology as well as the support of Stephen Sanders, without whom this research would not be possible.

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