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Development of *Diomus austrinus* Gordon (Coleoptera: Coccinellidae) on two mealybug prey species at five constant temperatures

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Abstract

Diomus austrinus Gordon is a generalist mealybug predator native to southern Florida. The objective of this study was to provide information on the development and survival of *D. austrinus* when reared on two common mealybug species [the Madeira mealybug, *Phenacoccus madeirensis* Green, and the citrus mealybug, *Planococcus citri* (Risso)] at five constant temperatures (15, 20, 25, 30, and 35 °C). Results of this study demonstrated the efficacy of *D. austrinus* as an egg predator of mealybugs within the temperature range found in greenhouses. *D. austrinus* successfully completed development on both mealybug species, with larval development requiring 1–4 days longer on the citrus mealybug than on the Madeira mealybug. *D. austrinus* feeding on the Madeira mealybug completed development in 15 days at 20 °C, 22 days at 25 °C, and 39 days at 30 °C. The lower and upper larval developmental thresholds of *D. austrinus* appeared to be 15 and 35 °C, respectively. Estimates of lower developmental thresholds using a linear model agreed with the observed results. Survival of *D. austrinus* from egg to adult among temperature/prey species combinations ranged from 60 to 90%. In all temperature/prey species combination, the cohorts were slightly female-biased, with an average proportion females of 0.53. Females reared on Madeira mealybugs at higher temperatures were generally larger than those reared on citrus mealybugs.

Keywords: Biological control; Coccinellidae; *Diomus austrinus*; Life history; *Phenacoccus madeirensis*; *Planococcus citri*; Pseudococcidae

1. Introduction

Scale insects and mealybugs are the most damaging pests in ornamental production and maintenance in Georgia, with the costs of damage and control exceeding $71 million in 2000 (Oetting et al., 2001). In greenhouse ornamental production and interiorscape maintenance in the southeastern United States, the most important mealybug species include the citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), the Madeira mealybug, *Phenacoccus madeirensis* Green, and the long-tailed mealybug, *Pseudococcus longispinus* (Targioni Tozzetti). The conventional management tactics for mealybugs in greenhouse ornamental production include regular applications of insecticides. Some of the most effective chemicals against the Madeira mealybug are organophosphates, pyrethroids, and insect growth regulators (Townsend et al., 2000). With some of these chemicals facing phase-out, and the rising environmental and economic concerns surrounding chemical control tactics, biological control presents a promising alternative to chemical control for greenhouse ornamental growers.

Biological control of mealybug in greenhouse production relies on augmentative releases of parasitoids and predators. The parasitic wasps *Leptomastix*

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dactylopii Howard and *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) are commercially available for the control of citrus mealybug. Generalist predators, such as green lacewings *Chrysoperla* spp. (Neuroptera: Chrysopidae), and a mealybug predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) are also marketed as biological control agents of mealybugs. Since no natural enemies are currently recommended for the control of the Madeira mealybug, efforts are currently underway in both Florida and Georgia to identify and evaluate possible biological control agents for the Madeira mealybug in greenhouse ornamental production.

Several genera of coccinellids in the subfamily Scymninae prey on mealybugs: *Cryptolaemus*, *Diomus*, *Hyperaspis*, *Nephus*, *Parasidis*, and *Sidis* (Hodek and Honěk, 1996). The genus *Diomus* has a cosmopolitan distribution (Gordon, 1976), with 18 indigenous or introduced *Diomus* species in the United States (Gordon, 1985). Most *Diomus* species are small, generalist predators feeding on mealybugs, coccids, aphids, aleyrodids and psyllids. Some *Diomus* have been studied or introduced as biological control agents of several homopteran pests. *Diomus pumilio* Weise, an introduced species from Australia, has established and provided control of the acacia psyllid, *Acizzia uncatoides* (Ferris and Klyver) (Hemiptera: Psyllidae) in California (Dreistadt and Hagen, 1994). Hentz and Nuessly (2002) provided important information on the biology of *Diomus terminatus* (Say), a potential biological control agent for the yellow sugarcane aphid, *Sipha flava* (Forbes) (Hemiptera: Aphididae). A five-day-rotation rearing system using the corn-leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae), was designed to mass-produce *D. terminatus* for augmentative aphid biological control in greenhouses (Hallborg, 2003). The life history of the Australian species *D. flavifrons* (Blackburn) feeding on the citrus mealybug has also been studied (Meyerdirk, 1983).

Diomus austrinus Gordon is a mealybug-feeding coccinellid native to southern Florida (Gordon, 1976). This species has been identified as a potential biological control agent of several mealybug species. It is important to understand the life history of *D. austrinus* to determine its suitability as a biological control agent of mealybugs in greenhouse conditions. The objective of this study was to provide life history information on *D. austrinus* when fed with two mealybug species that are pests in greenhouse settings at five constant temperatures.

2. Materials and methods

2.1. Sources of insects and colony maintenance

Sprouted russet potatoes (*Solanum tuberosum* L.) were used as host plants for rearing the citrus and Madeira mealybugs in an insectary at the University of Georgia, Griffin Campus, Griffin, GA. Ovisacs of both species were collected from the mealybug colonies. The outer wall of the mealybug ovisacs constructed of waxy filaments was removed before use, exposing the numerous eggs within the ovisacs. The mealybug eggs were used as the beetle larvae's food in the study.

The initial colony of *D. austrinus* was received from the Mid-Florida Research and Education Center, University of Florida, Apopka, FL, in May 2003. The adult beetles were released and subsequently established a viable population in a greenhouse at the Griffin Campus where they were supplied with a mixed culture of citrus and Madeira mealybugs maintained on coleus [*Solenostemon scutellarioides* (L.) Codd, var. 'Wizard Rose' (Labiatae)]. One hundred mating pairs were collected from the colony for this study. Each pair was isolated in a 60×15 mm polystyrene tissue culture dish (Becton–Dickinson Labware, Franklin Lake, NJ), and provided with an opened citrus mealybug ovisac on top of a piece of moistened paper towel. Twenty pairs were held in each of five environmental chambers (model I25L, Conviron, Winnipeg, Manitoba, Canada) maintained at one of the five constant temperatures (15, 20, 25, 30, and 35 ± 1 °C), $91 \pm 5\%$ RH, and 14:10 (L:D) h photoperiod. The mating pairs were examined every 24 h under dissecting microscopes and eggs laid on the paper towels were collected. Paper towels were replaced after each examination, and fresh mealybug ovisacs were added when the old ones were consumed.

2.2. Development and survivorship study

Female *D. austrinus* lay eggs by gluing them onto paper towels. Preliminary observations suggested that female beetles preferred to lay eggs in the indentations of paper towels than on the smoother surface of wax papers and filter papers. The only way to collect the eggs without damaging them was by cutting out the small section of paper towel around the eggs. Pieces of paper towel, each containing a single beetle egg, were placed on moistened paper towels in 60×15 mm tissue culture dishes, adjacent to excess eggs of either the citrus mealybug or the Madeira mealybug. More mealybug eggs were added to the paper towel when needed during the growth of the beetle larvae. Our observations suggested that *D. austrinus* larvae prefer to feed within a mealybug ovisac. To simulate the enclosed condition of a mealybug ovisac, a larger piece of paper towel was used to cover the beetle and the mealybug eggs. Fifty dishes were prepared in this manner for the 20, 25, and 30 °C treatments and 75 dishes were prepared for the 15 and 35 °C treatments. To maintain high humidity in the 30 and 35 °C treatments, the Petri dishes were kept in closed plastic boxes lined with additional moistened paper towels. All Petri dishes assigned to a specific

temperature treatment were placed in an environmental chamber maintained at the prescribed constant temperature, and examined every 24 h for development and survival of the beetle larvae. The duration to each successive molt, as evidenced by the presence of exuviae, was recorded for individual larvae. Sex ratio was determined upon emergence of adults for each temperature/prey species combination. The head width and body length of the emerged adults were measured with an eyepiece micrometer at 40× magnification.

2.3. Data analysis

Analysis of variance (ANOVA) carries the assumptions of normal distribution of error terms and equal variance of the data. The validity of these assumptions was tested with Quantile–Quantile plots for externally studentized residuals (PROC PLOT; SAS Insititute, 1985) and Brown and Forsythe's Test for Homogeneity (PROC GLM; SAS Insititute, 1985) before the ANOVA was performed. Results of the Brown and Forsythe's Test suggested that the data of the stage-specific duration of development violated the assumption of equal variance, and log-transformations of the data were performed to normalize the data distribution and equalize the variance. Head width was regressed against the body length to test for correlation between the two parameters (PROC REG; SAS Insititute, 1985). The main effects (temperature and prey species) were tested against the duration of each developmental stage or stadium, and the head width and body length of the adult beetles with a two way ANOVA (PROC GLM). When a significant difference between the means was detected, a Fisher's protected least significant difference (LSD) analysis with $\alpha = 0.05$ was performed to separate the means.

Development rate of insects is dependent upon the ambient temperature. One of the most common equations used to describe the relationship between ambient temperature and rate of development is the linear approximation or thermal summation model (Uvarov, 1931; Wagner et al., 1984). The thermal summation model generally takes the form

$$1/D = bT + a, \tag{1}$$

where D is the duration of development (in days) of a particular stage, T is the ambient temperature (in °C), and a and b are the regression parameters. The thermal summation equation is suitable for extrapolating the relationship between developmental rate and ambient temperature within a moderate range of temperatures, such as 15–30 °C (Gilbert et al., 1976). A study by Kontodimas et al. (2004) showed that the thermal summation equation accurately estimated ($r^2 > 0.99$) the development of two mealybug predators. To obtain the parameters a and b, Eq. (1) was fitted to the observed data using a linear regression procedure (PROC REG). Based on the regression parameters a and b, the lower developmental threshold ($t_{\min}$) and the thermal constant [K, or the sum of effective temperatures by Hodek and Honěk (1996)] can be calculated with the following equations:

$$t_{\min} = -\frac{a}{b} \tag{2}$$

and

$$K = \frac{1}{b}. \tag{3}$$

The lower developmental threshold ($t_{\min}$) is the temperature at which the insects cease further development, and the thermal constant (K) is the number of degree–days above $t_{\min}$ for the completion of a developmental stage. Campbell et al. (1974) provided equations to calculate standard errors (SE) of the lower developmental threshold and the thermal constant from linear models:

$$\mathrm{SE}_{t_{\min}} = \frac{\overline{1/D}}{b}\sqrt{\frac{s^2}{N\cdot(\overline{1/D})^2} + \left[\frac{\mathrm{SE}_b}{b}\right]^2} \tag{4}$$

and

$$\mathrm{SE}_K = \frac{\mathrm{SE}_b}{b^2}, \tag{5}$$

respectively. The term $\overline{1/D}$ is the mean developmental rate of a particular life stage, s^2 is the residual mean square of developmental rate, and SE_b is the standard error of the regression parameter b. The 95% confidence intervals of the lower developmental threshold and thermal constant of each life stage can be calculated by the equations:

$$t_{\min} \pm t_{\alpha/2,\mathrm{df}}\mathrm{SE}_{t_{\min}} \tag{6}$$

and

$$K \pm t_{\alpha/2,\mathrm{df}}\mathrm{SE}_K, \tag{7}$$

respectively, where $t_{\alpha/2,\mathrm{df}}$ is the t-statistic calculated from the data.

Stage-specific survival was calculated for each temperature/prey species combination by dividing the numbers of insects that successfully molted to the next developmental stage by the total number of insects presented at the beginning of a developmental stage. Sex ratio was determined as the proportion of adults that were females for each temperature/prey species combination. Proportion of survival and sex ratio were arcsine-transformed to normalize data distribution. Since all Petri dishes from a specific temperature/prey species combination were held within an environmental chamber, only one true replicate was recorded for each prey species factor in a temperature factor. For a two-factorial experiment with only one replication in each factor combination, no degree of freedom is available for estimating error. As a result, normal ANOVA with analysis

of interactions is not appropriate for analyzing the treatment effects in design with single replicate in each factorial combination. Tukey one-degree-of-freedom model for interaction (or transformable nonadditivity) is appropriate for the analyses of the main effects in factorial experiments with single replicate by transforming the interactions and replacing the true estimate of error with a higher-order surrogate error, called the Tukey's error in this case (Oehlert, 2000; Tukey, 1949). Tukey suggested that correcting or reducing nonadditivity with a simple transformation on a correct scale is equivalent to adding one degree of freedom term to the model (Tukey, 1949). The Tukey's one-degree-of-freedom test for nonadditivity was performed within PROC GLM to test for the main effects of temperature and prey species on the arcsine-transformed survival and sex ratio.

3. Results

3.1. Development

Significant prey species effects on *D. austrinus* development were detected in larval and total developmental durations but not in egg, pre-pupal and pupal durations (Table 1). At every temperature, the larval developmental period of *D. austrinus* reared on the citrus mealybugs was 1–3 days longer than those reared on the Madeira mealybug. The difference between prey species in egg to adult developmental time was greatest at the lower temperature: 6 days at 20 °C compared to 2 days at 30 °C.

Between 20 and 30 °C, the duration of each developmental stage was significantly decreased with each incremental increase in temperature (Table 1). Data were not available for every developmental stage reared at 15 and 35 °C due to low survival. The egg incubation period was six times longer at 15 °C than at 30 °C. Larval development was fastest at 30 °C, and ranged from 11 to 36 days between 30 and 20 °C. No larvae completed development at 15 and 35 °C. Pupal duration at 20 °C was nearly three times as long as that at 30 °C. Adult beetles reared on the Madeira mealybugs completed development in 15 days at 30 °C, which was 2.5 and 1.5 times shorter than at 20 and 25 °C, respectively.

The thermal summation equation fitted very well to the data of the current study, as indicated by the high r^2 values ($r^2 > 0.90$ for all developmental stages, Table 2 and Figs. 1 and 2). The lower developmental threshold estimated from the regression parameters (a and b) for the egg stage of *D. austrinus* was estimated as 12 °C, and 70 degree-days were required for hatching (Table 2). The mean $t_{\min}$ for each of the larval, pupal, and egg to adult development were all approximately 14 °C. The cumulative degree-days required to complete development from egg to adult on Madeira mealybug was 240, with an additional 21 degree-days for beetles developing on citrus mealybugs.

Male body length ranged from 1.4 to 1.6 mm and the head width ranged from 0.43 to 0.55 mm (Table 3), and was best described by the equation ($r^2 = 0.4931$, $P < 0.0001$; Fig. 3A)

$$\text{Male body length} = 0.78 + 1.43\ (\text{male head width}). \quad (8)$$

Females generally had greater body length (1.4–1.8 mm) and head width (0.45–0.65 mm) than the males (Table 3). The female body length and head width was positively correlated ($r^2 = 0.5540$, $P < 0.0001$; Fig. 3B), and the regression was

$$\text{Female body length} = 0.70 + 1.69\ (\text{female head width}). \quad (9)$$

The effects of temperature and prey species treatments were significant only for body length and head width of adult females. Female *D. austrinus* reared on the Madeira mealybug at 30 °C had the largest body length (>1.6 mm) and head width (>0.55 mm). Only the body length of males reared on the two mealybug species was significantly different.

3.2. Survival and sex ratio

Survival of each developmental stage was significantly influenced by temperature, except for the pupal stage where 100% of the pupae reared on Madeira mealybug yielded adults (Table 4). No difference in survival between the two prey species treatments was detected. On average, 5% of the eggs held at each temperature and prey species combination were infertile. Egg survival was the lowest at the 15 °C treatment, ranging from 1 to 3%. At 35 °C, 17–21% of beetle eggs hatched. At extreme temperatures, some embryos (45% at 15 °C and 65% at 35 °C) achieved a certain level of embryonic development as evidenced by the appearance of body segments and simple eyes, but failed to complete development. At 15 and 35 °C, hatching neonate larvae were inactive and often failed to locate and consume food, eventually dying from starvation and/or desiccation. Eggs incubated at 25 °C had the highest average egg hatch (94%) and adult eclosion (97%) rates, while 100% of larvae reared at 30 °C survived to pupation. The lowest survival rate from egg to adulthood (54%) was observed at 20 °C with citrus mealybug as prey. Overall survival rates were more than 75% in other temperature/prey species combinations.

Sex ratio, presented as proportion females in each temperature/prey species combination, was not significantly influenced by either temperature or prey species (Table 4). The cohorts were slightly female-biased, with a proportion females of 0.53.

Table 1
Means (±SE) of stage-specific duration of development of *D. austrinus* feeding on two mealybug species at five constant temperatures

Prey	Temp (°C)	Developmental stage								
		Egg	Larval instar						Pupal	Egg to adult
			First	Second	Third	Active fourth	Pre-pupal[a]	Total fourth[b]		
P. citri	15	21.0[c] aA	—	—	—	—	—	—	—	—
	20	10.1 ± 0.1 bA	6.6 ± 0.3 aB	4.0 ± 0.3 aA	4.2 ± 0.2 aA	8.1 ± 0.4 aA	2.5 ± 0.2 aA	10.6 ± 0.5 aA	9.5 ± 0.1 aA	45.0 ± 0.7 aA
	25	5.8 ± 0.1 cA	3.4 ± 0.2 bB	2.4 ± 0.1 bA	2.8 ± 0.1 bA	3.9 ± 0.1 bA	1.7 ± 0.1 bB	5.7 ± 0.2 bA	5.4 ± 0.1 bA	25.6 ± 0.3 bA
	30	3.6 ± 0.1 eA	2.4 ± 0.1 cB	1.9 ± 0.1 cA	1.8 ± 0.1 cA	2.4 ± 0.2 cA	1.2 ± 0.1 cA	3.6 ± 0.2 cA	3.5 ± 0.1 cA	16.7 ± 0.2 cA
	35	3.9 ± 0.1 dA	2.7 ± 0.3 bcA	1.5 ± 0.5 cA	2.0[c] bc	3.0[c] bc	—	—	—	—
P. madeirensis	15	22.7 ± 0.3 aA	—	—	—	—	—	—	—	—
	20	10.0 ± 0.1 bA	5.0 ± 0.2 aB	3.0 ± 0.1 aB	3.2 ± 0.1 aB	5.9 ± 0.2 aB	2.4 ± 0.2 aA	8.4 ± 0.3 aB	9.2 ± 0.1 aA	38.5 ± 0.5 aB
	25	5.9 ± 0.1 cA	2.7 ± 0.1 bB	1.7 ± 0.1 bB	2.1 ± 0.1 bB	2.6 ± 0.1 bB	2.1 ± 0.1 aA	4.7 ± 0.1 bB	5.4 ± 0.1 bA	22.4 ± 0.2 bB
	30	3.6 ± 0.1 dA	1.9 ± 0.1 cB	1.3 ± 0.1 cB	1.6 ± 0.1 cA	2.1 ± 0.1 cA	1.1 ± 0.1 bA	3.2 ± 0.1 cA	3.3 ± 0.1 cA	14.8 ± 0.4 cB
	35	3.8 ± 0.1 dA	2.0[c] bcA	—	—	—	—	—	—	—
P (Temp)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P (Prey)		0.8208	<0.0001	<0.0001	<0.0001	<0.0001	0.4587	<0.0001	0.2877	<0.0001
P (Temp × Prey)		0.5732	0.9776	0.5247	0.1468	0.0008	0.0610	0.0890	0.7034	0.2322

Note. All data were log-transformed before 2-way ANOVA was performed. Means duration of a developmental stage followed by the same small letter were not significantly different among the temperature treatments within a prey species treatment. Means duration of a developmental stage followed by the same capital letter were not significantly different between the prey species within a temperature treatment. All mean separation tests were performed on Fisher's protected LSD with $\alpha = 0.05$ (SAS Insititute, 1985).

[a] The pre-pupal stadium is a part of the fourth larval instar, when the larvae become inactive in preparation for pupation.

[b] Total duration of fourth larval instar = duration of active fourth larval instar + duration of prepupal.

[c] Only one larva survived.

Table 2
Regression parameters and statistics of Eq. (1) lower developmental threshold (t_{min}) and thermal constant (K) of *D. austrinus* reared on two mealybug species

Prey	Developmental stages	Regression parameters		Regression statistics		$t_{min} \pm SE_{t_{min}}$ (95% CI)	$K \pm SE_K$ (95% CI)
		b	a	P	r^2		
P. citri	Egg	0.0141	−0.1717	0.0119	0.9095	12.5 ± 0.7 (11.1–13.9)	69.8 ± 3.5 (62.8–76.8)
	Larval	0.0066	−0.0941	0.0150	0.9996	14.2 ± 0.2 (13.7–14.7)	150.8 ± 2.7 (145.5–156.1)
	Pupal	0.0199	−0.3020	0.0419	0.9957	14.9 ± 0.7 (13.6–16.2)	50.3 ± 3.0 (44.3–56.3)
	Egg to adult	0.0038	−0.0552	0.0388	0.9963	14.5 ± 0.3 (13.9–15.1)	261.8 ± 6.3 (249.4–274.2)
P. madeirensis	Egg	0.0143	−0.1795	0.0091	0.9243	12.2 ± 0.6 (11.0–13.4)	71.1 ± 2.9 (65.3–76.9)
	Larval	0.0075	−0.0963	0.0127	0.9996	12.9 ± 0.3 (12.4–13.4)	134.0 ± 3.7 (126.7–141.3)
	Pupal	0.0199	−0.2069	0.0778	0.9851	15.2 ± 0.4 (14.4–16.0)	50.2 ± 1.8 (46.7–53.7)
	Egg to adult	0.0042	−0.0578	0.0376	0.9965	13.9 ± 0.2 (13.5–14.3)	240.4 ± 4.0 (232.4–248.4)

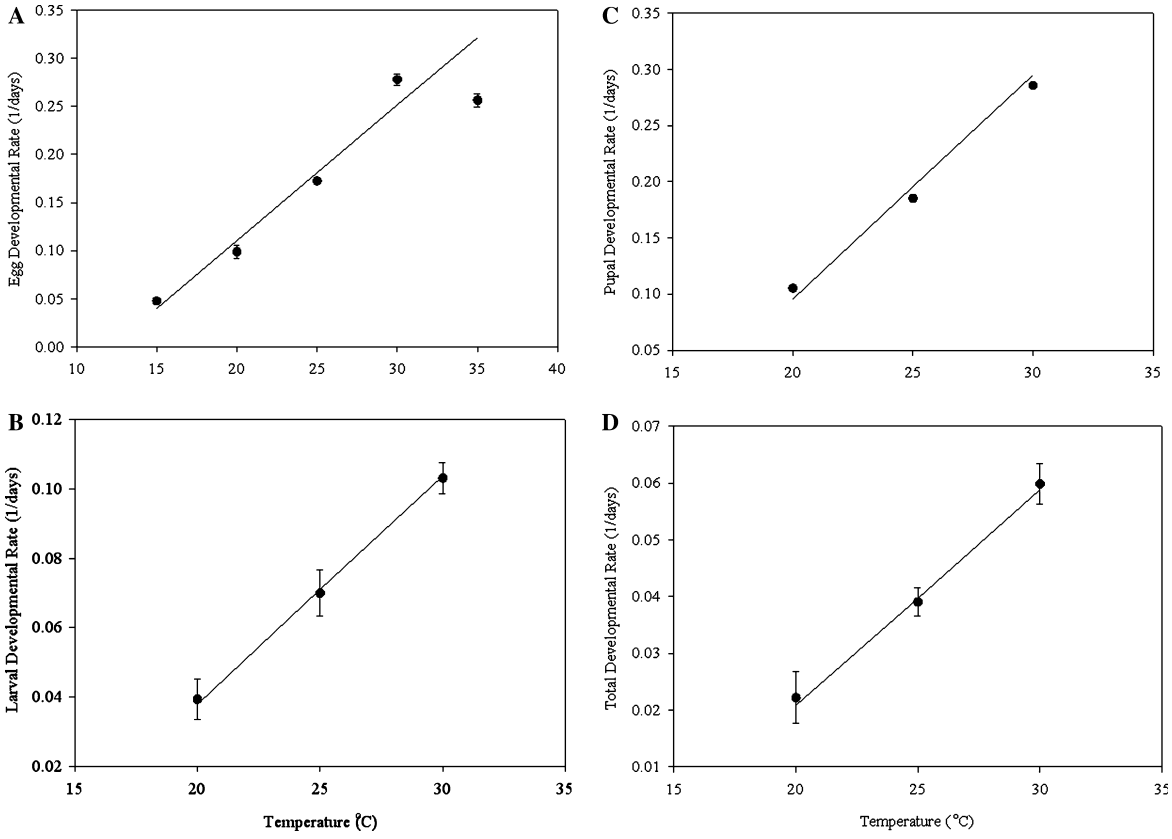

Fig. 1. Mean (±SEM) developmental rates of eggs (A), larvae (B), pupae (C), and egg to adult (D) of *D. austrinus* reared on *P. citri* at various constant temperatures. The solid line represents the regression line fitted to Eq. (1).

4. Discussion

Diomus austrinus was shown in this study to be an efficient egg predator of the Madeira mealybug and the citrus mealybug, two of the most commonly encountered mealybug pest species in greenhouse ornamental production of the southeastern United States. The beetle successfully completed development on eggs of the two mealybug species between 20 and 30 °C. The Madeira mealybug and the citrus mealybug represent prey of different quality to *D. austrinus*. Individuals reared on the Madeira mealybug achieved high developmental rate and were larger than those feeding on the citrus mealybug.

The developmental rates of *D. austrinus* increased with increases in temperature to an upper threshold where the developmental rate slowed down. This phenomenon is commonly observed among other coccinellids that prey on aphids (e.g., Uygun and Atlihan, 2000), whiteflies (Ren et al., 2002), scale insects

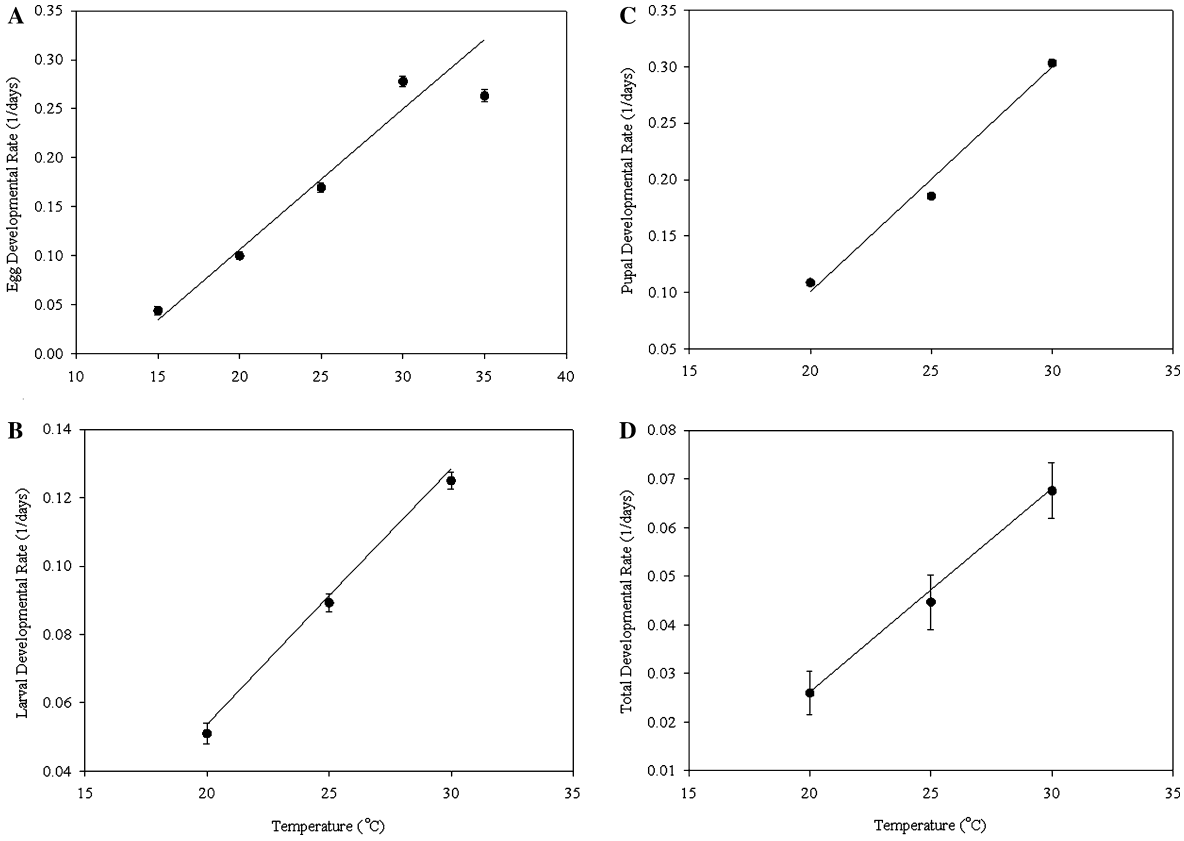

Fig. 2. Mean (±SEM) developmental rates of eggs (A), larvae (B), pupae (C), and egg to adult (D) of *D. austrinus* reared on *P. madeirensis* at various constant temperatures. The solid line represents the regression line fitted to Eq. (1).

Table 3
Means (±SE) of head width (mm) and body length (mm) of adult *D. austrinus* reared on two mealybug species at three constant temperatures

Prey	Temperature (°C)	Female		Male	
		Body length	Head width	Body length	Head width
P. citri	20	1.49 ± 0.03 bA	0.50 ± 0.02 bA	1.48 ± 0.02 aA	0.50 ± 0.01 aA
	25	1.63 ± 0.03 aA	0.52 ± 0.01 bA	1.47 ± 0.03 aB	0.49 ± 0.01 aA
	30	1.53 ± 0.02 bB	0.53 ± 0.01 bB	1.49 ± 0.02 aA	0.51 ± 0.01 aA
P. madeirensis	20	1.55 ± 0.02 bA	0.52 ± 0.01 bA	1.50 ± 0.02 aA	0.50 ± 0.01 aA
	25	1.66 ± 0.01 aA	0.53 ± 0.01 abA	1.52 ± 0.02 aA	0.50 ± 0.01 aA
	30	1.63 ± 0.02 aA	0.56 ± 0.01 aA	1.53 ± 0.02 aA	0.50 ± 0.01 aA
P (Temp)		<0.0001	0.0103	0.8325	0.3049
P (Prey)		<0.0001	0.0052	0.0285	0.6337
P (Temp × Prey)		0.2188	0.7481	0.6512	0.2142

Note. Means of each parameter measured followed by the same small letter were not significantly different among the temperature treatments within a prey species treatment. Means of each parameter measured followed by the same capital letter were not significantly different between the prey species within a temperature treatment. All mean separation tests were performed on Fisher's protected LSD with $\alpha = 0.05$ (SAS Insititute, 1985).

(Ponsonby and Copland, 1996), mealybugs (Kontodimas et al., 2004), and spider mites (Roy et al., 2003). Larval *D. austrinus* failed to survive to pupation at 15 and 35 °C. According to our results, 15 and 35 °C appeared to be below and above the lower and upper larval developmental thresholds of *D. austrinus*, respectively. The lack of studies on the life history of other *Diomus* species makes comparisons within the genus *Diomus* difficult. The two existing studies on the biology of *Diomus* were conducted at only a single constant temperature. *D. flavifrons* (Blackburn) feeding on the citrus mealybug required 29 days to complete development at 24 °C (Meyerdirk, 1983). At 27.5 °C, the aphidophagous *D. terminatus* (Say) completed development in 15 days, with the egg (4 days) and pupal (5 days) stages having the longest developmental periods (Hentz and Nuessly,

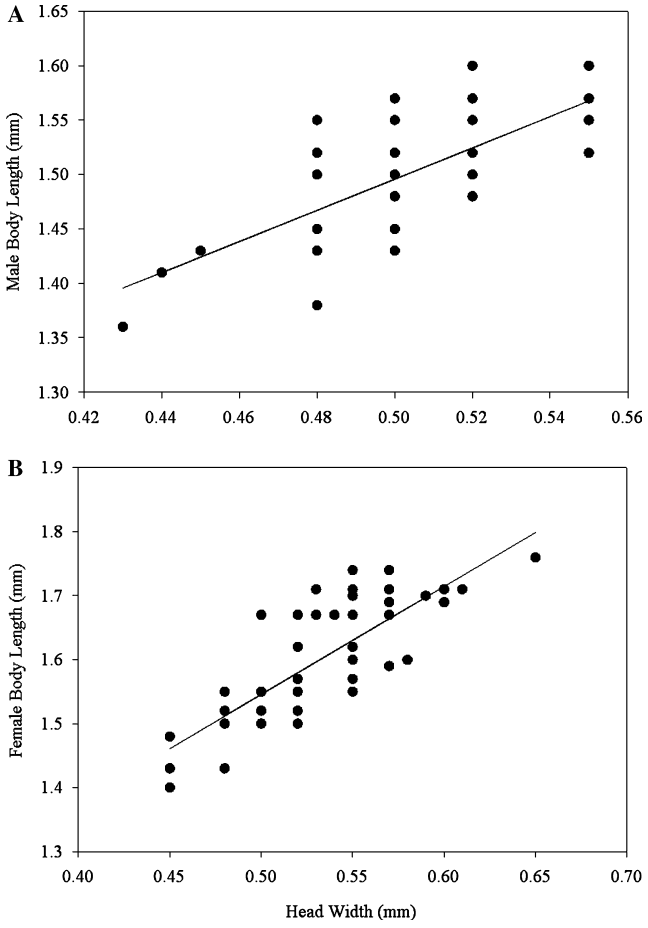

Fig. 3. Regressions between body length and head width of adult males (A) and females (B) of *D. austrinus*. The solid line represents the best-fitted linear regression line.

2002). Thus the developmental period of *D. austrinus* seems to fall within the range of *D. flavifrons* and *D. terminatus*.

The theoretical estimates of the lower developmental threshold for the larval and pupal stages of *D. austrinus* were above 14 °C, and agreed with the observations of this study. Comparing with other coccinellids, the theoretical lower developmental threshold for egg to adult development in *D. austrinus* (14 °C) was found to be lower than *Chilocorus nigritus* (F.) (16.6 °C; Ponsonby and Copland, 1996), but higher than *Cryptolaemus montrouzieri* Mulsant (13.7 °C; estimated from Babu and Azam, 1987), *Nephus reunioni* Fürsch (11.9 °C; Izhevsky and Orlinsky, 1988), *Nephus bisignatus* (Boheman) (9.4 °C; Kontodimas et al., 2004), *Nephus includens* (Kirsch) (10.9 °C; Kontodimas et al., 2004), and *Propylea dissecta* (Mulsant) (10.4 °C; Omkar and Prevez, 2004). Among these coccinellid species, *C. mountrouzieri*, *N. bisignatus*, *N. includens*, and *N. reunioni* are mealybug predators. *D. austrinus* appeared to require higher temperature than these mealybug-feeding coccinellids to complete egg to adult development. The lower developmental threshold may determine the climatic tolerance and thus the geographical distribution of the species. *N. bisignatus*, having a lower theoretical developmental threshold than *N. includens*, is distributed in Northern Europe while *N. includens* is restricted to Southern Europe (Kontodimas et al., 2004). In the United States, *C. montrouzieri* which has a lower developmental threshold similar to that of *D. austrinus*, is distributed in California and Florida (Gordon, 1976). The known distribution of *D. austrinus* is restricted to southern Florida (Gordon, 1976). With an egg to adult development threshold of 14 °C, we believe that the natural distribution range of *D. austrinus* may be restricted to southern United States. In greenhouses maintained above 15 °C, adults and larvae of *D. austrinus* remain active year-round (Chong, personal observation).

The thermal constant estimate of *D. austrinus* (240–260 degree-days) was lower than *C. nigritus*

Table 4
Stage-specific survivorship (%) and sex ratio (proportion of females) of *Diomus austrinus* reared on two mealybug species at five constant temperatures

Prey	Temperatures (°C)	Developmental stages				Sex ratio (proportion of females)
		Egg	Larval	Pupal	Egg to adult	
P. citri	15	1	0	—	0	—
	20	72	80	94	54	0.47
	25	92	98	98	88	0.56
	30	82	100	94	76	0.50
	35	18	0	—	0	—
P. madeirensis	15	3	0	—	0	—
	20	88	86	100	76	0.51
	25	96	92	100	88	0.54
	30	90	100	100	90	0.53
	35	21	0	—	0	—
P (Temp)		0.0012	0.0007	<0.0001	0.0040	0.6665
P (Prey)		0.0760	0.8517	<0.0001	0.1920	0.2432
P (Tukey's error)		0.2793	0.7499	<0.0001	0.3728	0.3339

(324.8 degree-days), *P. dissecta* (465.11 degree-days), *N. includens* (490.5 degree-days), and *N. bisignatus* (614.3 degree-days). Comparisons of the thermal constant suggested that *D. austrinus* has a shorter biological cycle within the moderate temperature range, thus is capable of completing more generations per year than these coccinellid species.

The effects of temperature on survival rates of *Diomus* beetles were not investigated in Meyerdirk (1983) and Hentz and Nuessly (2002). Extreme temperatures appeared to be deleterious for the survival of eggs and young larvae of *D. austrinus*. At 15 and 35 °C, less than 25% of eggs hatched and no larvae completed development to adulthood. *D. austrinus* demonstrated high survival rates at 25 and 30 °C. Similar results were obtained from studies on other coccinellids feeding on aphids and scale insects. The survival rates were highest at a temperature range of 22–30 °C for all developmental stages of *Chilocorus nigritus* (F.) developing on the scale insect *Abgrallaspis cyanophyllii* (Signoret) (Hemiptera: Diaspididae) (Ponsonby and Copland, 1996). The stage-specific survival rates were also the highest at 25 and 30 °C for *P. mediterraneus* Fabre feeding on the black scale, *Saissetia oleae* Oliver (Hemiptera: Coccoidae), in Morocco (M'Hamed and Chemseddine, 2001). Egg to adult survival of *P. dissecta* peaked at 27 °C and decreased at 20 and 35 °C (Omkar and Prevez, 2004).

Different prey species have been known to influence the development, survival and reproduction of coccinellids. Hodek and Honěk (1996) made a distinction between essential prey and alternative prey for coccinellid predators. Consumption of essential prey allows the growth and development of larvae and reproduction by adults. Alternative prey provide needed energy and nutrients but do not contribute to the development and reproduction of the coccinellids. The eggs of both Madeira mealybug and citrus mealybug were suitable for the development of *D. austrinus*, and thus partially satisfied the requirements as an essential prey. It has been demonstrated that there are variations in the suitability of essential prey for coccinellids (Hodek and Honěk, 1996; Kalushkov and Hodek, 2004). Madeira mealybugs appeared to be a more suitable prey for *D. austrinus* than citrus mealybugs by allowing higher developmental rates, especially for larvae. The incubation periods of egg and pupal stages were not influenced by prey species. Studies of other scale insect feeding coccinellids similarly suggested that the larval duration of development was most significantly influenced by different prey species. The total duration of development of the coccinellid *Chilocorus bipustulatus* (L.) differed significantly among the three diaspidid scale species tested, and the greatest difference was detected in the duration of the pre-oviposition period (Uygun and Elekçioğlu, 1998).

Immature survival, as well as larval growth and adult reproduction, of coccinellids are linked to the quality of prey species (Hodek and Honěk, 1996). The enhanced performance of coccinellid larvae on the suitable prey may be due to higher protein levels or increased consumption (Omkar and Srivastava, 2003). Immature survival of two cohorts of *D. austrinus* feeding on eggs of the citrus mealybug and the Madeira mealybug were not significantly different. Other studies, however, had demonstrated the significant influence of prey species on the survival of coccinellids. The egg to adult survival of *C. bipustulatus* varied among the three dispidid scale species tested, and was the highest (47%) when feeding on *Pseudaulacaspis pentagona* (Targioni Tozzetti) (Uygun and Elekçioğlu, 1998). Among the 13 aphid species tested, all were suitable for the development of *Coccinella septempunctata* L. but seven species caused higher mortality (Kalushkov and Hodek, 2004). Using six aphid species, Omkar and Srivastava (2003) showed that the immature survival of *C. septempunctata* was highest when feeding on *Lipasphis erysimi* (Kaltenbach). The same six species of aphids tested in Omkar and Srivastava (2003) were also tested for the development of *Coccinella transversalis* F. (Omkar and James, 2004). In both studies, coccinellid larvae developing on *Aphis nerii* Boyer de Fonsclombe had the lowest survival rate (38% for *C. transversalis* and 44% for *C. septempunctata*).

Our study did not investigate the effects of prey species on the reproduction of *D. austrinus*, which is another criterion for an essential prey. Adults of *D. austrinus* reared on the Madeira mealybugs were larger than those reared on the citrus mealybug. The effects of prey species on the adult size or weight, and the relationship between the adult size or weight and per capita fecundity, were well-established (Hodek and Honěk, 1996). Generally, a large female is more fecund than a smaller female. One example is *Cycloneda sanguinea* (L.), which was heavier and more fecund when developing on the brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae), than on the green citrus aphid, *Aphis spiraecola* Patch (Michaud, 2000). The quality of offspring may also be influenced by the choice of prey species as suggested by the higher fertility of eggs produced by *C. sanguinea* when fed on *A. spiraecola* than on *T. citricida*. More studies on the reproduction of *D. austrinus* should be performed before we can make a conclusion on the suitability of the two mealybug species on reproduction of *D. austrinus*.

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