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Source: Florida Entomologist, 92(4): 578-581

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.092.0407

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## TEMPERATURE-DEPENDENT DEVELOPMENT OF THE CYCAD AULACASPIS SCALE, AULACASPIS YASUMATSUI (HEMIPTERA: DIASPIDIDAE)

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## ABSTRACT

Egg duration period, immature development time, and pre-oviposition period of the cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi, were measured at 9 constant temperatures in the laboratory. Egg duration period ranged from 15 d at 20°C to 7 d at 30°C. First instar development time was 30 d at 18°C but only 4 d at 35°C. No first instars completed development below 18°C or above 35°C. Development time of second instar females ranged from 19 d at 18 and 20°C to 9 d at 30°C. Development time of male second instar + pupa ranged from 19 d at 25 at 20°C to 9-10 d at 25-32°C. Pre-oviposition period averaged 14 d at 20°C to 8 d at 25-32°C; no females laid eggs at 18 and 35°C. The lowest temperature threshold for all stages ranged from 8 to 12°C and 538 degree-days were required for female immature development in a linear model. Development rates of the scale are compared to those of 3 of its natural enemies, *Cybocephalus nipponicus* Endrödy-Younga, *Rhyzobius lophanthae* (Blaisdell), and *Coccobius fulvus* (Compere and Annecke).

Key Words: armored scale, Cycas revoluta, development time, degree-days, natural enemies

## RESUMEN

Se midieron el período de duración del huevo, el tiempo de desarrollo de los inmaduros, y el período de pre-oviposición de la escama aulacaspis de las cícadas, *Aulacaspis yasumatsui* Takagi, a nueve temperaturas constantes en el laboratorio. El período de duración del huevo varió de 15 d a 20°C hasta 7 d a 30°C. El tiempo de desarrollo del primer estadío fue 30 d a 18°C pero solamente 4 d a 35°C. Ninguna ninfa en el primer estadío cumplió su desarrollo abajo de 18°C ni arriba de 35°C. El tiempo de desarrollo de hembras en el segundo estadío varió de 19 d a 18 y 20°C hasta 9 d a 30°C. El tiempo de desarrollo de machos en el segundo estadío + pupa varió de 15 d a 20°C hasta 9 d a 30°C. El tiempo de desarrollo de machos en el segundo estadío + pupa varió de 15 d a 20°C hasta 8 d a 25-32°C; El período de pre-oviposición tuvo un promedio de 14 d a 20°C hasta 8 d a 25-32°C; miguna hembra depositó huevos a 18 ni 35°C. Se estimó que el umbral térmico más bajo para todas las etapas varió de 8 a 12°C y se necesitaron 538 grados-días para el desarrollo inmaduro de las hembras en un modelo lineal. Se compara la tasa de desarrollo de la escama con las de tres de sus enemigos naturales, *Cybocephalus nipponicus* Endrödy-Younga, *Rhyzobius lophanthae* (Blaisdell), y *Coccobius fulvus* (Compere y Annecke).

Translation provided by the authors.

The cycad aulacaspis scale, Aulacaspis yasumatsui Takagi, is a native of Southeast Asia, and an invasive pest in Florida, Texas, Hawaii, West Indies, Costa Rica, New Zealand, and Ivory Coast (Germain & Hodges 2007) and in Guam where it is killing large numbers of the native Cycas micronesica K. D. Hill (Terry & Marler 2005). It was first detected in south Florida in 1998 and quickly spread throughout the state. The scale infests several species of cycads (Howard et al. 1999), but the king sago, Cycas revoluta Thunberg, a popular landscape plant due to its attractive form and minimal maintenance requirements, appears to be especially susceptible. Since 1998, large numbers of king sagos in south Florida have been destroyed by the cycad aulacaspis scale. A number of natural enemies are being studied in a biological control program to manage the pest. In order to better understand host-natural enemy relationships, the development time of the cycad aulacaspis scale was examined at 9 constant temperatures and compared to the development time of 3 natural enemies occurring in Florida, the parasitic wasp *Coccobius fulvus* (Compere and Annecke) (Hymenoptera: Aphelinidae) and the predatory beetles *Rhyzobius lophanthae* (Blaisdell) (Coleoptera: Coccinellidae) and *Cybocephalus nipponicus* Endrödy-Younga (Coleoptera: Cybocephalidae).

## MATERIALS AND METHODS

Eggs were collected from females on the day they were deposited. From 12-20 eggs were gently

transferred to a glass vial  $(9 \text{ mm} \times 50 \text{ mm})$  by using a fine camel hair brush and the vial was plugged with sterile cotton that was moistened daily with a drop of distilled water. Vials were placed in a Percival environmental chamber set with 50-60% RH, 14:10 h light:dark photoperiod, and one of the following constant temperatures: 18, 20, 25, 30, 32, or 35°C. Vials were examined daily for presence of crawlers, which were counted and killed, until all eggs hatched or became collapsed.

Cycas revoluta plants in 4.4-L pots were infested with crawlers by placing them next to plants with female scales that began ovipositing 6 d previous. The leaves were intermixed to allow movement of crawlers from infested to uninfested plants. One day after crawlers moved onto uninfested plants, the pots were placed individually in a Percival environmental chamber set with a constant temperature, 50-60% RH, and 14:10 h light:dark photoperiod. Experimental temperatures were 11, 18, 20, 25, 30, 32, 35, 38, and 42°C for females and 18, 20, 25, 30, and 32°C for males. Each settled crawler was assigned a number, the settling date was recorded, and its position on the leaflet was mapped. Each nymph was examined daily under a dissecting microscope. Nymphs were sexed when they molted to second instar. Females are recognized by the production of a round armor covering the nymph. Males are recognized by an initially 3-pronged scale cover that eventually becomes elongate and tricarinate. For males, dates of molting to second instar and emergence of adult from the scale cover were recorded. For females, dates of molting to second instar and to adult, or date of death prior to adulthood, were recorded. Starting 5 d after molting to adult, the armor of the female was gently lifted daily and the date of first egg produced was noted.

Since active crawlers require a day to settle, 1 d was added to the time period spent as a settled crawler to quantify the development time of the first instar. Means were statistically compared by analysis of variance and Student-Newman-Keuls test with  $\alpha = 0.05$ .

For the female nymphal stages, the linear portion of the developmental rate curve [R(T) = a + bT]was modeled by least squares linear regression (PROC GLM, SAS Institute 1999), where T was temperature, and a and b were estimates of the intercept and slope, respectively. Development rates that were not part of the linear portion of the curve were not included in the regression analysis. The base temperature threshold was estimated by the intersection of the regression line at R(T) = 0,  $T_0 =$ -a/b. Degree-day requirements for each stage were calculated from the inverse slope of the fitted linear regression line (Campbell et al. 1974).

#### RESULTS

The effect of temperature on egg hatching is shown in Table 1. No eggs hatched at  $18^{\circ}$ C (n = 25). Significant differences were detected among

TABLE 1. MEAN DEVELOPMENT TIME IN DAYS  $\pm$  SEM of immature stages, total nymphal development time, and pre-oviposition period of *A. yasumatsui* at 6 constant temperatures for females and 5 constant temperatures for males. Sample size for each mean is indicated in parentheses.

Females							
Egg	$1^{ m st}$ instar	2 <sup>nd</sup> instar	Total nymph	Pre-oviposition period	Egg to adult		
	30.8 ± 1.0 a (18)	19.0 ± 2.0 a (2)	46.5 ± 1.5 a	no eggs	_		
$15.5 \pm 0.5 a (2)$	$17.8 \pm 0.8 \text{ b} (17)$	19.3 ± 0.3 a (15)	$36.8 \pm 0.9 \text{ b}$	$13.6 \pm 0.5 a (14)$	52.6		
$9.6 \pm 0.2 b (27)$	$11.0 \pm 0.2 c (72)$	$11.9 \pm 0.2 \text{ bc} (72)$	22.9 ± 0.3 c	$8.1 \pm 0.3 b (52)$	32.5		
$6.9 \pm 0.1 c (63)$	$10.4 \pm 0.2 c (140)$	$9.3 \pm 0.2 c (110)$	19.7 ± 0.2 c	$7.9 \pm 0.2 \ b \ (54)$	26.6		
$7.4 \pm 0.2 \text{ c} (18)$	$8.9 \pm 0.1 d (30)$	$12.7 \pm 0.3 \text{ bc} (30)$	$21.6 \pm 0.4 c$	$7.7 \pm 0.5 \ b \ (15)$	29.0		
$7.9 \pm 0.1 c (51)$	—	—	—	no eggs	—		
		Males					
Egg	$1^{ m st}$ instar	2 <sup>nd</sup> instar + pupa	Total nymph		Egg to adult		
	29.6 ± 0.5 a (45)	_		_			
_	16.4 ± 0.5 b (20)	14.6 ± 1.2 a (5)	29.6 ± 1.8 a	_	46.5		
_	$10.8 \pm 0.2 c (31)$	$9.7 \pm 0.3 b (27)$	$20.4 \pm 0.4$ b	_	30.1		
_	$10.3 \pm 0.3 c (50)$	$9.7 \pm 0.7 b (12)$	$19.9 \pm 0.6$ b	_	26.9		
_	$8.6 \pm 0.2 d (59)$	$9.4 \pm 0.2 b (59)$	$18.0 \pm 0.2$ c	_	25.4		
	$ \begin{array}{c} - \\ 15.5 \pm 0.5 a (2) \\ 9.6 \pm 0.2 b (27) \\ 6.9 \pm 0.1 c (63) \\ 7.4 \pm 0.2 c (18) \\ 7.9 \pm 0.1 c (51) \end{array} $	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		

Means within a column for each sex followed by the same letter are not significantly different (P > 0.05; Student-Newman-Keuls test).

the egg duration periods at the other 5 temperatures tested (F = 78.93, df = 4, P < 0.001). As expected, increasing temperature decreased hatching time up to 30°C, but 32° and 35°C did not decrease hatching time. Percentage egg hatch at 30°C (84.0%, n = 75) was greater than percentage egg hatch at 35°C (62.2%, n = 82), more than double that at 25°C (40.9%, n = 66), and nearly 3 times the percentage at 32°C (29.5%, n = 61).

No development of the first instar occurred at 11, 38, and 42°C. Significant differences were detected among the development times of female first and second instars and total development times of female nymphs at the other 6 temperatures (F = 402.3, 83.8, and 148.2, respectively, df =5, P < 0.001) (Table 1). First instars developed slowest at 18°C and fastest at 35°C. There was no significant difference between first instar development times at 25 and 30°C, both of which were significantly different from development times at 20 and 32°C. Female second instars required significantly more days to reach adulthood at 18 and 20°C than at temperatures  $\geq 25$ °C. Many female nymphs produced deformed armors during the molt to second instar at 18 and 35°C, and only 3 individuals at these temperatures reached adulthood. Total female nymphal development times at 25°C and higher were not significantly different but were significantly different from the development time at 20°C. Female nymphs required more than twice as many days to complete development at 18°C than at  $\geq 25$ °C.

Females that reached a dulthood at 18 and 35°C died without producing eggs. Pre-oviposition period was significantly longer at 20°C (F = 35.1, df = 3, P < 0.001), but there were no significant differences among the pre-oviposition periods at the other 3 temperatures (Table 1).

Significant differences were detected among the development times of male first instar (F = 645.7, df = 4, P < 0.001), second instar + pupa (F = 14.4, df = 3, P < 0.001), and total development times of male nymphs (F = 59.3, df = 5, P < 0.001) (Table 1). First instar males developed slowest at 18°C and fastest at 32°C. There was no significant difference between first instar development times at 25 and 30°C, both of which were significantly different from development time at 20°C. Second instar + pupal development time was significantly longer at 20°C than at the 3 higher temperatures, which were not significantly different among themselves; no males completed development after attaining the second instar at 18°C. Total male immature development time was slowest at 20°C and fastest at 32°C, with no difference in times between 25 and 30°C.

The linear model provided a good means to describe the relationship between developmental rate (1/D) and temperature (T). Table 2 shows the lower threshold temperature and total degree-days required to complete development of each immature stage. The linear model estimated that the lower temperature threshold for all stages ranged from 8 to  $12^{\circ}$ C and 538 degree-days were required for female immature development. The number of day-degree requirements for first and second instars were very similar.

DISCUSSION

Cycad aulacaspis scales had great difficulty developing beyond the first instar below 20°C. Average minimum temperatures are below this level in south Florida during the months Nov to Apr, so scales settled on the plant leaves would suffer increased mortality or develop very slowly, if at all. Scales under megasporophylls or on the roots, however, may be protected from the lower ambient temperatures. The optimal temperature for female development is 30°C. Average maximum temperatures approximate or slightly exceed this level in south Florida during the months May to Oct, thus supporting greater population growth and plant infestation in this period (unpublished data). Egg incubation period at this temperature is 7 d. Therefore, the time interval from egg to egg at 30°C is on average 34.5 d for this insect. The linear model underestimated the lower temperature threshold because laboratory results confirmed that eggs and nymphs did not complete development below 18°C.

In Florida and elsewhere, the 3 principal natural enemies of the cycad aulacaspis scale are *C. fulvus*, *R. lophanthae* and *C. nipponicus*. The development time of female *C. fulvus* parasitizing the arrowhead scale, *Unaspis yanonen*-

TABLE 2. PARAMETER ESTIMATES DESCRIBING THE RELATIONSHIP BETWEEN TEMPERATURES AND DEVELOPMENTAL RATES (1/D) of A. YASUMATSUI FEMALES.

Stage	Intercept	Slope	$\mathbb{R}^2$	Threshold °C	$\mathbf{Degree-days}^1$
Egg	-0.096	0.0080	0.99	12.0	124.3
$1^{st}$ instar	-0.050	0.0051	0.90	9.9	194.9
2 <sup>nd</sup> instar	-0.041	0.0050	0.96	8.2	202.4
Egg to adult	-0.017	0.00186	0.95	9.3	537.6

<sup>1</sup>Total degree-days to complete development.

sis Kuwana, is 52, 27, and 26 d at 19, 25, and 30°C, respectively (Ogata 1987). Rhyzobius lo*phanthae* development from egg to egg is 44, 32, and 24 d when preying on Aspidiotus nerii Bouché (Stathas 2000) and 48, 34, and 27 d when feeding on *Chrysomphalus aonidum* (L.) (Stathas et al. 2002) at 20, 25, and 30°C, respectively. In comparison, the life cycle times from egg to egg for A. yasumatsui at the same 3 temperatures are 50, 31, and 28 d, respectively. These data indicate that C. fulvus and R. lophanthae develop at similar rates or slightly more quickly than the cycad aulacaspis scale and may produce as many or a few more generations than their host within a defined period of time, which is a favorable characteristic of biological control agents. The time interval for development from egg to egg of C. nipponicus feeding on the euonymus scale, Unaspis euonymi (Comstock), is 48 d at 22°C (Alvarez & van Driesche 1998), whereas the time interval is 44 d for the predator feeding on A. yasumatsui at 25°C (Smith & Cave 2006). The development rate of A. yasumatsui at this temperature is more rapid (31 d). Therefore, A. yasumatsui can build up population numbers faster than C. nipponicus, which, coupled with the fact that C. nipponicus pupae are parasitized by Aphanogmus albicoxalis Evans and Dessart (Evans et al. 2005), may limit the effectiveness of this predator as a biological control agent of the scale. The fecundity of A. yasumatsui is yet unstudied, but the prolific reproduction of this pest may be one reason why its natural enemies are not providing adequate control on king sagos in Florida.

### ACKNOWLEDGMENTS

Celia Branch, Michael Burton<sup>†</sup>, Julieta Castillo, Veronica Manrique, and Greta Thorson are gratefully acknowledged for assistance in data collection. This research was supported by grants from the Florida Department of Agriculture and Consumer Services and the Department of Interior Cooperative Conservation Initiative.

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