

Temperature-Dependent Development, Survival, and Potential Distribution of *Ischnodemus variegatus* (Hemiptera: Blissidae), a Herbivore of West Indian Marsh Grass

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ABSTRACT The bug *Ischnodemus variegatus* (Signoret) (Hemiptera: Blissidae) is an adventive herbivore, native to South America that feeds in the invasive grass *Hymenachne amplexicaulis* (Rudge) Nees (Poaceae). This grass is a problematic weed in Florida and Australia, but it is a highly valued forage in Mexico, Cuba, and Venezuela. We studied the influence of nine constant temperatures (8–38°C) on the developmental time and survival of *I. variegatus*. Complete egg and nymphal mortality occurred at temperatures $\leq 20.5^\circ\text{C}$ and at 38°C . Developmental time decreased linearly with temperature until $28\text{--}30^\circ\text{C}$ and then increased at 33°C . Mortality of first, second, and third instars was high across all temperatures. Developmental time across all temperatures was greatest for eggs, first and fifth instars compared with other stages. Linear and Brière-1 nonlinear models were used to determine the lower temperature threshold at which the developmental rate ($1/D$) approached zero. The lower thresholds to complete development (egg to adult) estimated with the linear and nonlinear model were 14.6 and 17.4°C , respectively. The total degree-days required to complete development estimated by the linear model was 588. Using temperature data from Florida, a map was generated to project a prediction grid of *I. variegatus* generations per yr. Based on these predictions, the insect can complete three to five generations per year in areas currently invaded in Florida. Results of this study will be used to understand the potential distribution and population growth of *I. variegatus* in *H. amplexicaulis* infested regions.

KEY WORDS Blissidae, Poaceae, developmental rate, degree-days, biological control

Hymenachne amplexicaulis (Rudge) Nees (Poaceae) (West Indian marsh grass) is a robust, stoloniferous, semiaquatic, perennial grass, native to the Neotropics. The timing and pathway of introduction of this plant into Florida are unknown, but its quality as forage suggests that the introduction may not have been accidental. The grass also is established in Indonesia (Holm et al. 1979) and in Australia (Csurches et al. 1999) where it is considered a weed of national significance. The Florida Exotic Pest Plant Council listed the grass as a category I species, which are invasive exotics that are altering native plant communities by displacing native species, changing community structures or ecological functions, or hybridizing with natives (FLEPPC 2005). The aggressive growth of *H. amplexicaulis* is due in part to rapid adaptation to changes in water levels (Kibbler and Bahnisch 1999),

high production of stolons and perhaps the absence of effective natural enemies. Once the grass invades a wetland, it forms monotypic stands 2–3 m in height, with complete canopy cover. At the end of the growing season, this results in a massive accumulation of biomass. The grass disperses by seeds, which are produced in large quantities, and broken stolons, both of which can travel great distances during flooding events. Negative impacts of the grass in Australia affect the sugarcane industry, water resources, fisheries, and ecotourism (Csurches et al. 1999). Plant managers in Florida and Australia find it challenging to control this grass with herbicides due to regrowth from below ground stolons (Csurches et al. 1999).

H. amplexicaulis is considered a valuable forage grass in the Neotropics, particularly in Mexico, Cuba, and Venezuela. Important forage characteristics of this grass include high digestibility, high nitrogen content, and adaptation to changes in water levels (Antel et al. 1998, Kibbler and Bahnisch 1999). In the Brazilian pantanal, *H. amplexicaulis* occurs within four habitats: marsh ponds, waterlogged basins, tall grasslands, and forest edges (Pinder and Ross 1998). Observations in marshes at Myakka River State Park, Sarasota Co., FL (27.2°N , 82.2°W) suggest that when subject to inundation, *H. amplexicaulis* is capable of

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rapid stem elongation, increase in foliage volume and rapid nodal adventitious root production (R.D., personal observation). Kibbler and Bahnisch (1999) demonstrated that rapid elongation of the stem maintains the leaves above the water allowing emergent leaves to function at full photosynthetic capacity. In Venezuela, Tejos (1978) found a positive relationship between *H. amplexicaulis* growth and depth of flooding and that biomass production ranged from 5,911 to 18,162 t/ha/yr during the flood period and from 5,553 to 7,836 t/ha/yr during the dry season.

In 2000, the Neotropical bug *Ischnodemus variegatus* (Signoret) (Hemiptera: Blissidae) was discovered feeding and causing severe damage to *H. amplexicaulis* at Myakka River State Park. Scientists from the Florida Department of Agriculture and Consumer Services (FDACS) identified *I. variegatus* as a new record for the continental United States (Halbert 2000). The native distribution of *I. variegatus* includes Central and South America and collection records indicate *H. amplexicaulis* as the only host (Baranowski 1979, Slater 1987). Like other species in the Blissidae family (Hemiptera classification follows Henry 1997), *Ischnodemus* feeds on the sap of monocotyledonous plants (Slater 1976). Population outbreaks of this insect in central and south Florida occur from August to November. Feeding effects of *I. variegatus* diminish carbon dioxide assimilation, growth rate and biomass of *H. amplexicaulis* (Overholt et al. 2004). Despite its potential as a fortuitous biological control agent of *H. amplexicaulis*, there are no studies that address the basic biology of *I. variegatus* or its host range. The purpose of this study was to determine temperature-dependent developmental times and survival, and with this information generate a map depicting the predicted number of generations per yr of *I. variegatus* across Florida. This study is an initial step toward understanding the thermal requirements for *I. variegatus* establishment and population growth.

Materials and Methods

Source of *I. variegatus* and *H. amplexicaulis*. *I. variegatus* and *H. amplexicaulis* were collected in Myakka River State Park, and Fisheating Creek, Glades Co., FL (26.5° N, 81.7° W) and maintained at the Biological Control Research and Containment Laboratory (BCRCL), Fort Pierce, FL. Stolons of *H. amplexicaulis* were planted in two liter pots and placed in large trays filled with water to maintain permanent flooding conditions. Pots received 14g of Osmocote (Scotts, Marysville, OH) after transplanting and weekly applications of Miracle-Grow water soluble fertilizer (Scotts). Potted plants were placed in small, mesh screened cages (0.90 by 0.90 by 0.90 m) located within a walk-in rearing room maintained at 25–30°C, 50–80% RH, and a photoperiod of 14:10 (L:D) h. Field-collected *I. variegatus* were released in these cages and monitored every other day for nymphal survival and colonization. The maintenance of *I. variegatus* genetic variability was ensured by addition of field-collected individuals to the colony three times a year. Voucher specimens

of the plant and the insect were deposited in the Florida herbarium (accession no. 208823) and the Florida State Collection of Arthropods (accession no. E2002-6139), respectively.

Laboratory Studies. The lengths of eggs, nymphs, and adults were measured from randomly collected individuals from the insect colony. Pictures of individuals placed in a sealed petri dish were taken through a microscope using a digital camera fitted with Automontage software (Synchroscopy, Frederick, MD) and measured with National Institutes of Health ImageJ software (<http://rsb.info.nih.gov/ij/>). Length of eggs was measured along the longest axis. Nymphs and adults were measured from the tip of the rostrum to the most distal point of the abdomen. Behavioral observations were described from insect colonies and in field settings. Observations were performed only during the day at least once every 2 wk for a 2-yr period.

Developmental Time and Survival. Temperature development studies of *I. variegatus* were conducted in environmental chambers at 10 constant temperatures (8 ± 0.5, 13 ± 0.5, 18 ± 0.5, 20.5 ± 0.5, 23 ± 0.5, 25.5 ± 0.5, 28 ± 0.5, 30.5 ± 0.5, 33 ± 0.5, and 38 ± 0.5°C). Relative humidity and photoperiod were kept constant at 80% and 14:10 (L:D) h, respectively. Environmental variables were confirmed with HOBO data loggers (Onset Computer, Bourne, MA) placed in each chamber. Ten adult couples were placed in a small cage (0.3 by 0.3 by 0.3 m) and given stems of *H. amplexicaulis* for feeding and oviposition. Fresh eggs (≈1 d old) were collected from the stems and transferred individually to small (5-cm) petri dishes containing moist filter paper. Fifty eggs were placed at each temperature treatment. Egg development was monitored daily and hatching dates recorded.

Eggs collected from the adult colony were monitored daily and newly hatched nymphs were used for the nymphal development study. First instars were placed singly in 250-cm³ vials containing a small section of *H. amplexicaulis* whorl that was placed upright in wet sand. The center of the vial lid was removed in a circular shape and replaced by fine mesh to allow gas exchange. Nymphs were transferred to fresh plant material every 2 d by using a fine brush. Fifty individuals were exposed to each temperature treatment. Nymphal molts, confirmed by the presence of exuviae, and survival were recorded between 8 and 11 a.m. every other day until the last individual molted to the adult stage.

Developmental Rate and Degree-Day Requirement. Developmental time at different temperatures was analyzed using the general linear model procedure (PROC GLM, SAS Institute 1999) for each instar separately as well as the total immature stages combined. Whenever significant ($P < 0.05$) *F* values were obtained, means were separated using the Student-Newman-Keuls (SNK) test (SAS Institute 1999).

Linear Model. For the egg, nymphal and total immature stages (egg and nymphal stages combined), the linear portion (20–33°C) of the developmental rate curve [$R(T) = a + bT$] was modeled using the

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. The temperatures 18 and 38°C were not included in the regression analysis, because their values were not part of the linear portion of the curve. 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 using the inverse slope of the fitted linear regression line (Campbell et al. 1974).

Nonlinear Model. The nonlinear relationship between developmental rate $r(T)$ and temperature T was fitted to the Brière model, which allows the estimation of the upper and lower developmental thresholds (Brière et al. 1999). The Brière-1 model is defined as $R(T) = a T (T - T_0) (T_L - T)^{1/2}$, where R is the rate of development and is a positive function of the rearing temperature T , T_0 is the base temperature threshold, T_L is the lethal (upper) temperature threshold, and a is an empirical constant (Brière et al. 1999). The developmental rate of *I. variegatus* was modeled using the Marquardt algorithm of PROC NLIN (SAS Institute 1999), which determines parameter estimates through partial derivations. Temperature data used in the nonlinear model were from 18 to 33°C. Initial model parameters were calculated by the grid search method (SAS Institute 1999), with T_0 and T_L set between 13 and 18°C and 32 and 38°C, respectively.

Weather Data from Florida. Daily minimum and maximum temperatures from Florida were obtained from 98 weather stations recorded by the Applied Climate Information System (Climate Information for Management and Operational Decisions [CLIMOD], Southeast Regional Climate Center; <http://acis.dnr.sc.gov/Climod/>). Daily minimum and maximum temperatures were averaged for the last 5–11 yr depending on the availability of data, which provided 365 values for each temperature and station. The maximum period of weather data were from 1 January 1996 to 31 December 2006.

Calculation of Degree-Days and Number of Generations for Geographic Information System (GIS) Analysis. Accumulated degree-days for *I. variegatus* were obtained from DegDay version 1.01, which is an Excel (Microsoft, Redmond, WA) application developed by University of California-Davis (<http://biomet.ucdavis.edu/>). This application uses the upper and lower temperature threshold for an organism, and daily average of minimum and maximum temperatures to calculate the accumulated degree-days by using the single sine method (Baskerville and Emin 1969). The upper and lower temperature thresholds for *I. variegatus* immature stages (egg and nymphal stages) were estimated from the Brière-1 nonlinear model as 17.38 and 35.08°C, respectively. Degree-day requirements for *I. variegatus* were calculated from the fitted linear regression of the developmental rate function [$R(T) = a + bT$] as $K = 1/b$ (Campbell et al. 1974). The prediction of the number of generations per year was calculated by dividing the cumulative degree-days

per station by K , 588.24, required by *I. variegatus* immature stages to complete development.

Generation of GIS Map for Prediction of *I. variegatus* Generations in Florida. Weather station name, latitude, longitude and number of *I. variegatus* generations were tabulated in a Microsoft Excel spreadsheet, saved as IV dBase file and then imported into ArcGIS 9.0 (ESRI Inc., Redlands, CA). The imported file was converted to shapefile using the ADD X-Y DATA function followed by the selection of the State Plane Projection. A shapefile of Florida was obtained from AWhere Continental database (AWHERE, Inc., Denver, CO) to delineate the range of predictions.

The ArcGIS Geostatistical Analyst function (ESRI Inc.) was used to generate prediction grids of *I. variegatus* generations across Florida. Prediction values in unsampled locations were obtained by surface interpolation of sampled locations. The inverse distance weighted (IDW) deterministic method was used, whereby predictions are made from mathematical formulas that generate weighted averages of nearby known values. The IDW method gives closer points more influence on the predicted value than points that are farther away (hence the name inverse distance weighted). This method was used by Pilkington and Hoddle (2006) to predict the number of generations of an egg parasitoid in California. The parameters used in the IDW analysis were as follows:

1. The number of stations used for interpolation was set to 15 and with a minimum of 10. Due to the large number of weather stations, there were always 15 stations available for interpolation.
2. The Power Optimization option was selected generating a Power value $p = 1.7021$. This means that the weights of each weather station are proportional to the inverse distance raised to the power value p . Therefore, as the distance from the station increases, the weights decrease rapidly.
3. The search neighborhood shape was circular because there were no directional influences on the weighting of number of generations per station; thus, equal weight was given to each sample point regardless of the direction from the prediction location (ESRI Inc.). Ellipse parameters were set to the default values: angle, 0; major and minor semi-axis, 2.3878.

Results

Size and Behavior of Stages. Description of *I. variegatus* eggs and nymphs was reported by Baranowski (1979) and Slater (1987). The size, color, location, and behavior of the different stages found in our studies are given below.

Eggs. Length is 2.97 ± 0.13 mm (mean \pm SD) ($n = 25$) (Fig. 1A). Eggs are laid in masses (12 eggs per mass, range 1–38) between the leaf sheath and culm preferentially near the node. Newly deposited eggs (0–5 d) are white and older eggs (6–10 d) turn bright red. An egg parasitoid, *Eumicrosoma* sp. (Hymenoptera: Scelionidae) was found in Myakka River State Park and later identified as a possible introduced spe-

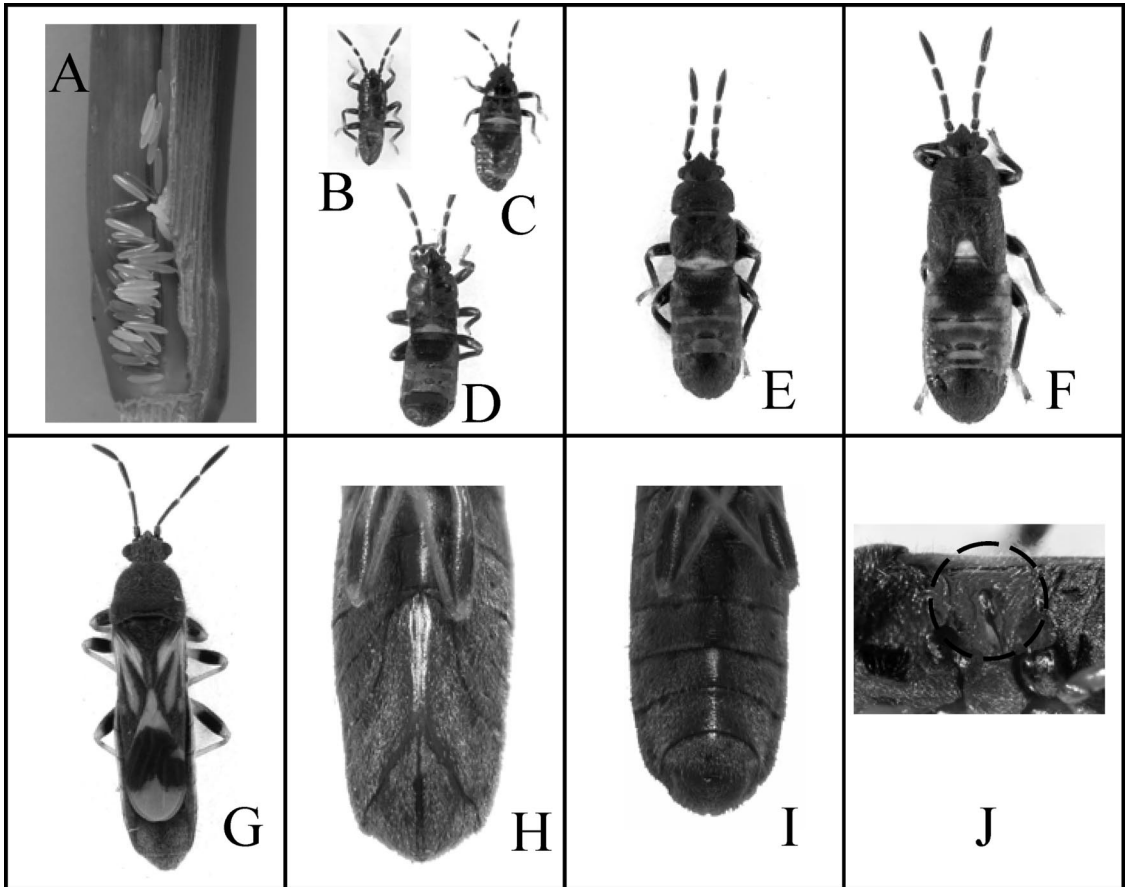


Fig. 1. Life stages of *I. variegatus* and length in mm (mean \pm SD). (A) Egg mass on *H. amplexicaulis* culm, 2.97 ± 0.13 ($n = 25$). (B) First instar, 1.45 ± 0.28 ($n = 23$). (C) Second instar, 2.70 ± 0.39 ($n = 47$). (D) Third instar, 3.06 ± 0.31 ($n = 42$). (E) Fourth instar, 3.95 ± 0.32 ($n = 53$). (F) Fifth instar, 5.45 ± 0.43 ($n = 46$). (G) Female, 7.23 ± 0.56 ($n = 28$); male, 6.05 ± 0.22 ($n = 49$). (H) Female sclerites at ventral tip of abdomen. (I) Male sclerites at ventral tip of abdomen. (J) Scent glands in thorax of adult.

cies for North America (T. Nuhn, personal communication). This parasitoid attacks young and old eggs (R.D., unpublished data), and its presence can be detected by the black coloration of the eggs. Because eggs are immobile and take longer to develop than other stages, it seems to be the most vulnerable stage for parasitization or predation. The impact of *Eumicrosoma* sp. on *I. variegatus* population is unknown but previous studies have demonstrated that egg parasitism on hemipterans play an important role on regulation of populations (Buschman and Whitcomb 1980, Irvin and Hoddle 2007).

Nymphs. The length of each immature stage is shown in Fig. 1. Upon hatching first instars are bright red (Fig. 1B), and they remain aggregated near the eggs and then migrate to tightly oppressed spaces between leaves and stems. Feeding and resting occurs in tight spaces between the leaf sheath and culm, and in the inner whorl. Fourth and fifth instars are darker than early instars (Fig. 1E and F). Laboratory and field observations showed the first to fourth instars are more often found in aggregations, whereas fifth instars

and adults can be observed exploring as individuals. If nymphs or adults are disturbed, they secrete a strong odor from the scent glands located in the thorax (Fig. 1J) and abdomen. After molting, the cuticles of nymphs and adults are bright red and delicate, but after a couple of hours they darken and harden.

Adults. Females are larger than males (Fig. 1) and both genders have a distinctive "M" pattern at the base of the hemelytra. The sclerites at the ventral tip of the abdomen of females are triangular in shape whereas in males the last sclerites are rounded (Fig. 1H and I). Adults become highly active during the hottest part of the day and mating individuals can be found in aggregations. Despite having fully developed wings, adult flying was restricted to short hops of a few meters or less. Gravid females mostly walked, possibly due the larger size of their abdomens. Oviposition behavior can be described as follows: females locate the fold of the leaf sheath by walking around the stem while performing antennation of the surface. Once a fold is located, the proboscis is extended and briefly inserted at the site for probing; if the site is accepted then the

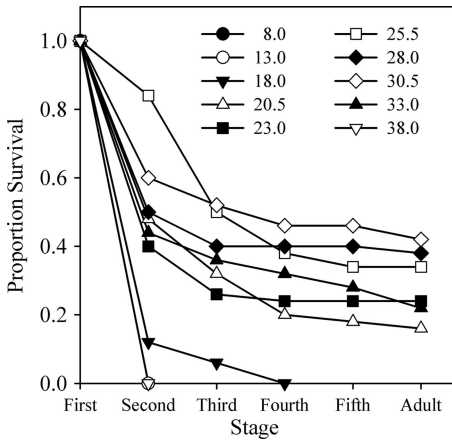


Fig. 2. Proportion survival of *I. variegatus* stages at constant temperatures (°C).

female extends and inserts the ovipositor at the site and starts laying eggs.

Immature Survival and Developmental Time. The survival of *I. variegatus* nymphs varied with temperature (Fig. 2). Nymphs could not complete development at extreme low and high temperatures (8, 13, and 38°C) and died before molting to the second instar (Fig. 2). At 8, 13, and 18°C, first instars typically survived several weeks before dying, whereas at 38°C they usually died a few days after eclosion (Table 1). At 18°C, a few nymphs molted to the second instar, but none survived to the third instar. First instar survival increased between 20.5 and 33°C and it was the highest, 84%, at 25.5°C (Fig. 2). Nymphal survival decreased up to the third instar, after which survival stabilized. The percentage of nymphs that molted to the adult stage was highest, 42%, at 30.5°C and lowest, 16%, at 20.5°C.

Temperature affected the developmental time for eggs, nymphs, and total immature stages (egg and

Table 2. Linear regression parameter estimates describing the relationship between temperature and developmental rates (1/D) of *I. variegatus* stages

Stage	Intercept	Slope	R ²	n	Threshold (°C)	Degree-days ^a
Egg	-0.144	0.0087	0.9507	4	16.55	114.94
Nymph	-0.028	0.002	0.9498	5	14.00	500.00
Egg to adult	-0.025	0.0017	0.9478	5	14.70	588.24

^a Total degree-day to complete development.

nymphal stages combined) (Table 1). Mean developmental time from egg to adult was longest, 122 d, at 20.5°C and shortest, 40 d, at 30.5°C. Egg, first, and fifth instars had the longest developmental times at each temperature indicating critical stages for *I. variegatus* survival. Developmental time of each stage significantly decreased between 20.5 and 30.5°C (Table 1). At 33°C, there was a slight increase in developmental time, which could indicate the initiation of stressful conditions.

Both linear and nonlinear models were used to determine the relationship between developmental rate (1/D) and temperature (T). Developmental rates of each stage and total immature stages (egg and nymphs) were estimated between 20.5 and 30.5°C where the relationship with temperature was approximately linear. 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 14 to 16.55°C and total degree-days required for immature development was 588.

The parameter estimates for the Brière-1 nonlinear model are shown in Table 3. The lower temperature threshold predicted for the immature stages ranged from 16.8 to 17.9°C (Table 3) which may be slightly low, because laboratory studies showed that nymphs did not complete development at 18°C. The upper

Table 1. Mean developmental time in days (mean ± SE) of immature *I. variegatus* stages at 10 constant temperatures

Stage ^a	Temp (°C)									
	8	13	18	20.5	23	25.5	28	30.5	33	38
Egg				35.7 ± 0.21a	15.51 ± 0.32b	12.94 ± 0.10c	10.41 ± 0.20d	11.43 ± 0.07e	14.15 ± 0.23f	
N1	80	80	80	80	80	80	80	80	80	80
	2-19	3-55	38.33 ± 5.75a	21.79 ± 1.80b	13.40 ± 1.57c	11.81 ± 0.56c	7.52 ± 0.48d	5.83 ± 0.46d	8.32 ± 0.37e	5-7
N2	50	50	6	24	20	42	25	30	22	50
			29.67 ± 8.17a	19.13 ± 2.57b	11.31 ± 1.25c	10.76 ± 1.24c	5.80 ± 0.54c	4.85 ± 0.40c	5.78 ± 0.48c	
N3			3	16	13	25	20	26	18	
				12.50 ± 2.00a	7.83 ± 0.80b	7.32 ± 0.95b	5.25 ± 0.46b	4.96 ± 0.43b	3.56 ± 0.30c	
N4				10	12	19	20	23	16	
				10.89 ± 0.73a	7.08 ± 1.11b	5.53 ± 0.48b	5.15 ± 0.55b	4.87 ± 0.37b	5.00 ± 0.35b	
N5				9	12	17	20	23	14	
				21.75 ± 1.33a	10.75 ± 1.11b	8.59 ± 0.53c	7.47 ± 0.38c	8.29 ± 0.40c	7.82 ± 0.50c	
Only nymphal stage				8	12	17	19	21	11	
				71.38 ± 6.20a	47.83 ± 2.40b	46.12 ± 2.97b	31.58 ± 1.07c	28.81 ± 1.01c	30.09 ± 0.76c	
Egg to adult				121.76	65.88	56.95	41.6	40.23	44.63	

Means within a row followed by different letters are significantly different ($P < 0.05$; SNK). Analysis of variance of eggs ($F = 2204.04$; $df = 4, 395$; $P = 0.0001$), N1 ($F = 46.88$; $df = 6, 162$; $P < 0.0001$), N2 ($F = 21.37$; $df = 6, 114$; $P < 0.0001$), N3 ($F = 12.17$; $df = 5, 94$; $P < 0.0001$), N4 ($F = 10.91$; $df = 5, 89$; $P < 0.0001$), N5 ($F = 46.79$; $df = 5, 82$; $P < 0.0001$), and nymphal stage ($F = 41.95$; $df = 5, 82$; $P < 0.0001$).

N1, nymphal first instar; N2, second instar; N3, third instar; N4, fourth instar; and N5, fifth instar.

Table 3. Parameter estimates (a, T₀, T_L)^a for the Brière-1 nonlinear model describing the relationship between temperature and developmental rate (1/D) of *I. variegatus* stages

Stage	Parameters estimates (°C) ^b					R ²
	a	T ₀	95% confidence	T _L	95% confidence	
Egg	0.00016	17.9000	16.74–19.06	32.5171	31.12–33.87	0.9900
Nymph	0.00004	16.7993	13.71–19.89	36.1051	32.41–39.80	0.9527
Egg to adult	0.00003	17.3837	15.84–18.93	35.0794	33.70–36.46	0.9820

^a a, empirical constant; T₀, lower temperature threshold; T_L, upper temperature threshold.

^b Degrees centigrade except for a.

temperature threshold for immatures was predicted to be between 32.5 and 36.1°C (Fig. 3). The rate of development increased with temperature until the curve reached an optimum and then decreased rapidly as temperatures reached the upper temperature threshold (Fig. 3).

GIS Mapping of *I. variegatus* Generations in Florida. A grid map indicating the predicted number of *I. variegatus* generations was generated for Florida (Fig. 4). Overall, the predicted grids followed a thermal gradient across the state. Predicted number of generations ranged from 2.36 to 4.84 in Florida counties. Florida counties located below Lake Okeechobee had the highest number of generations per year ranging from 3.52 to 4.84. Florida counties located between Orlando and Lake Okeechobee had fewer generations per year (3.21–3.52). Counties where the average maximum temperature in January was below 17°C were excluded, because laboratory studies and nonlinear models predicted high *I. variegatus* mortality at constant low temperatures.

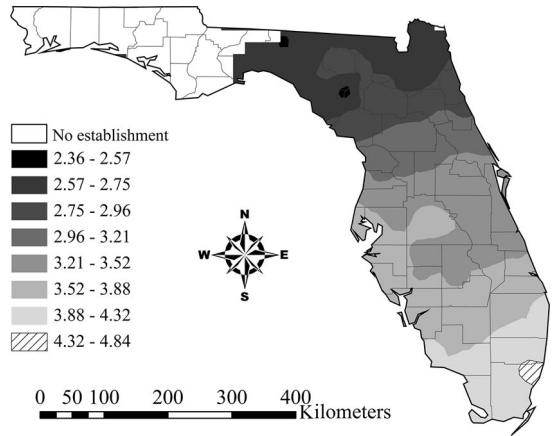


Fig. 4. Geographical information system map showing the predicted number of generations of *I. variegatus* in Florida.

Discussion

Developmental time and survival of eggs as well as immature stages were affected by temperature. No survivorship was observed at extreme low and high temperatures (Table 1). Nymphs died within a few days at 38°C and after weeks at lower extreme temperatures, suggesting that *I. variegatus* has a broader lower temperature threshold compared with the upper threshold. A wider range of lower lethal temperature threshold is common for insects (Heinrich 1981, Bayoh and Lindsay 2004). The overall high mortality observed in the first three instars may have been due to the rearing of *I. variegatus* as individuals in our experiments, as opposed to typical aggregations observed in the field. Harrington (1972) observed that *Ischnodemus* species were strongly gregarious and

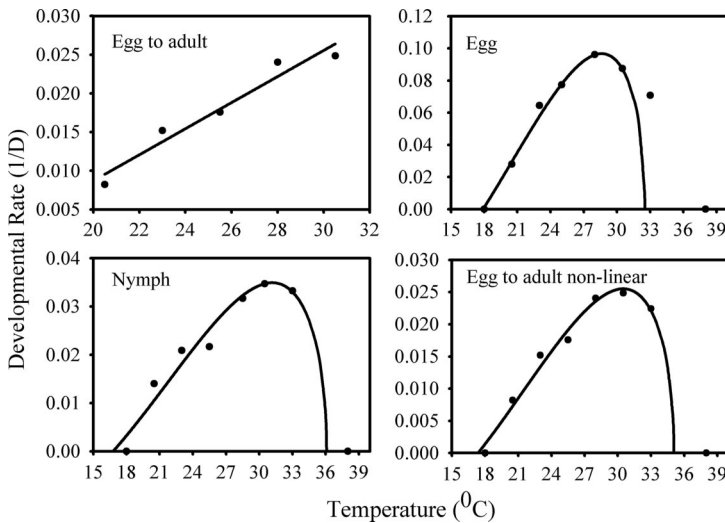


Fig. 3. Developmental rates (1/D) of *I. variegatus* at different temperatures (°C). Linear regression of eggs to adult stages and observed and predicted values by Brière-1 nonlinear model.

nymphs reared in isolation died sooner than nymphs reared in groups. The benefits of aggregations on survival in early instars is unclear but may be related to an increase in humidity as shown for German cockroach, *Blattella germanica* (L.) (Dambach and Goehlen 1999) and the southern green stink bug, *Nezara viridula* (L.) (Lockwood and Story 1986). Another explanation for the high mortality at extreme temperatures could be constant conditions at which the insects were exposed. Extreme temperatures such as 8, 13, and 38°C are typically present for only a few hours a day in the subtropics. During certain hours in winter and summer, temperatures in *I. variegatus* infested regions in Florida could reach 0 and 40°C, respectively (CLIMOD 2007). Despite these conditions, field sampling confirms that *I. variegatus* is present throughout the year (R.D., unpublished data) demonstrating that this insect can survive extremes under existing environmental variability. Eggs, first, and fifth instars took longer to develop than other stages, indicating their importance for *I. variegatus* development. The length of time spent during the fifth instar could be explained by the larger amount of food that insects require during the last immature stage before reproduction (Scriber and Slansky 1981, He et al. 2003, Bommireddy et al. 2004). Longer developmental time in stages before reproduction also was found in *Ischnodemus falicus* (Say) and *Ischnodemus slossoni* Van Duzee (Harrington 1972), which have temperate distributions. Developmental time from egg to adult was 3 times less at 30.5°C (40 d) compared with 20.5°C (122 d), demonstrating clearly the influence of temperature on development. The range of temperatures where development was fastest occurred between 28 and 33°C (Table 1; Fig. 3), which is in agreement with immature survival (Fig. 1). These ideal conditions for *I. variegatus* development are typical in central Florida from April to October. Developmental rates of *I. variegatus* increased almost linearly with temperature until reaching an optimum at 28–30°C and then decreasing rapidly (Fig. 3). This pattern has been observed in other hemipteran (Scott and Yeoh 1999), and non-hemipteran insects (Ponsonby and Copland 1996, Mazzei et al. 1999, Herrera et al. 2005). Our results of developmental rates were obtained at constant temperatures; however, there is a possibility that our values are underestimated, because some insects develop faster at variable temperatures (Worner 1992).

Both linear and nonlinear models overestimated the lower temperature threshold, because laboratory results indicated that eggs and nymphs did not complete development below 20.5°C. The partial nymphal development at 18°C could be an indication that *I. variegatus* can develop at this temperature for short periods making the prediction of an absolute lower threshold not possible (Herrera et al. 2005). However, because there is a narrow temperature range between 18 (no development) to 20.5°C (complete development), we can safely predict that the lower threshold of *I. variegatus* occurs within this range. The predictions of both models for the lower threshold for eggs and nymphs were different (Tables 2 and 3). Egg and

nymphal lower thresholds ranged from 16.6 to 19.1°C and from 13.7 to 19.9°C, respectively. This indicates a greater susceptibility of eggs to lower temperatures than nymphs. Although nymphs can move and locate microclimates suitable for development (e.g., plant structures, conspecific aggregations), eggs are immobile and successful development depends on local conditions. This greater resistance to lower temperatures of nymphs compared with eggs also has been observed in other heteropteran insects (He et al. 2003, Bommireddy et al. 2004).

The degree-days (588) required to complete development from egg to adult could be underestimated, because the lower threshold is probably higher than 14.7°C (Table 2). The lower threshold for development of *I. variegatus*, 18–20.5°C, explains its mostly tropical and partially subtropical distribution. The preoviposition period of *I. variegatus* is ≈ 7 d at 28°C (R.D., unpublished data), and it was not included in the calculations of degree-days. Therefore, the present model probably overestimates the number of *I. variegatus* generations. A future model could be improved by including the preoviposition period at different temperatures to accurately predict the degree-day requirement to complete one generation. Outside of Florida, *I. variegatus* has been reported from as far north as Honduras in Central America, as far east as the Dominican Republic and Trinidad in the West Indies and as far south as northern Argentina and Uruguay (Slater and Wilcox 1969, Baranowski 1979, Slater 1987, Baranowski and Slater 2005).

The current distribution of *H. amplexicaulis* and *I. variegatus* in the continental United States is limited to central and south Florida (Wunderlin and Hansen 2004, University of Florida Herbarium 2007). Further studies on cold tolerance of *H. amplexicaulis* and *I. variegatus* would provide a better understanding of the potential distribution on United States. Prediction of the potential range and population growth of herbivores could decrease some of the uncertainty about potential ranges of introduced biological control agents. This study estimated the number of *I. variegatus* generations based on long-term data of 98 weather stations across Florida and the degree-days required to complete egg-to-adult development. The GIS map shows spatially the areas across Florida where *I. variegatus* could establish and the potential number of generations.

The use of degree-days for mapping insect voltinism has been used recently for insect conservation (nymphalids butterflies, Bryant et al. 2002), pest management (western corn rootworm, Hemerik et al. 2004), and biological control (egg parasitoid, Pilkington and Hodde 2006). The current northern and southern invasion fronts of *H. amplexicaulis* are the St. Johns River (28.08° N, 80.75° W, Brevard Co.) and Big Cypress National Park (25.92° N, 81.3° W, Collier Co.), respectively. The lower winter temperatures in north Florida could be a climatic barrier for the invasion of *H. amplexicaulis* and *I. variegatus*, which are mostly restricted to the tropics. Degree-day accumulation in central and especially in south Florida, provides ideal

conditions to sustain nearly five *I. variegatus* generations per year (Fig. 4), which could facilitate its establishment in case *H. amplexicaulis* invades the Everglades National Park. If *I. variegatus* arrives in Australia, the tropical climate of the northern regions would likely provide ideal conditions for its development and population growth. Other countries where climatic conditions for *I. variegatus* may be ideal include Mexico, Puerto Rico, Venezuela and Cuba, where *H. amplexicaulis* is highly valued as forage. Evaluation of herbivores for weed biological control programs includes studies on climate matching between native and adventive range, host specificity of the agent, and effectiveness in reducing weed density. Temperature–development studies of weed biological control agents provide baseline knowledge that facilitates agent rearing, colonization, and prediction of population growth. Temperature experiments revealed that optimal conditions for *I. variegatus* ranged from 28 to 30°C, which explains the occurrence of outbreaks late in summer in Florida (R.D., unpublished data) and its subtropical to tropical distribution. Ongoing studies on population dynamics, host range testing and impacts to *H. amplexicaulis* will elucidate the importance of *I. variegatus* as a fortuitous biological control agent in Florida.

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