

Influence of Temperature, Humidity, and Plant Terpenoid Profile on Life History Characteristics of *Boreioglycaspis melaleucae* (Hemiptera: Psyllidae), a Biological Control Agent of the Invasive Tree *Melaleuca quinquenervia*

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ABSTRACT We investigated how environmental variables influence development and survivorship of *Boreioglycaspis melaleucae* Moore (Hemiptera: Psyllidae) by quantifying life history characteristics of adults, eggs, and nymphs when held at eight constant temperatures, four relative humidities, and on plants that differed in foliar terpenoid profiles. There is insufficient evidence to conclude that terpenoid profiles or humidity influence *B. melaleucae* development. Although longevity of adult psyllids is greater on plants that contain *E*-nerolidol versus viridiflorol profiles, this does not translate to a longer ovipositional period or increased fecundity. Similarly, humidity treatments had a limited and inconsistent effect on *B. melaleucae* developmental rates and nymphal survivorship. In contrast, developmental rates increased linearly with increasing temperature to an optimum 25°C, whereas greater temperatures caused total developmental rates to decrease. Temperature also affected nymphal survivorship, with no individuals completing development below 10 or above 30°C. Mean maximum daily temperatures in southern Florida commonly exceeded 30°C, with maximum temperatures ranging from 30 to 35°C (in Broward Co.) during 138 d in 2006. Therefore, we conclude that lethal upper temperature thresholds will limit population growth rates during summer.

KEY WORDS developmental rate, degree-days, adult longevity, chemotype, paperbark tree

Melaleuca quinquenervia (Cav.) Blake is a long-lived, evergreen tree that was introduced to Florida from Australia in the late 19th century for ornamental and agroforestry purposes (Dray et al. 2006). The tree exhibits prolific reproductive capabilities and forms dense monocultural stands (Rayamajhi et al. 2002). Since its introduction, the tree has invaded >200,000 ha of Florida wetlands, including portions of Everglades National Park (Turner et al. 1998). A biological control program targeting *M. quinquenervia* resulted in the establishment of the weevil *Oxyops vitiosa* Pascoe in 1997 (Center et al. 2000). Feeding by the weevil markedly reduces the tree's reproductive potential, growth, and survivorship (Pratt et al. 2005, Rayamajhi et al. 2008, Tipping et al. 2008), but *O. vitiosa* pupates in the soil so it is unable to thrive in permanently flooded habitats where some *M. quinquenervia* stands persist.

To enhance landscape-level suppression of *M. quinquenervia*, a second biological control agent, the psyllid *Boreioglycaspis melaleucae* Moore (Hemiptera:

Psyllidae), was released in Florida during spring 2002 (Center et al. 2006). *B. melaleucae* does not diapause and completes its life cycle entirely on the plant, resulting in less vulnerability to variation in hydrology (Wineriter et al. 2003). Like all psyllids, *B. melaleucae* passes through five instars. First instars are active but later stages are more sessile and congregate on leaves or stems, secreting copious amounts of white, waxy filaments from dorsal glands (Purcell 1997). Adults and nymphs feed by inserting stylets through stomatal pores to gain access to the phloem (Purcell et al. 1997). Both adults and nymphs feed on expanding buds as well as mature, fully expanded leaves. Psyllid herbivory induces leaf senescence, eventually resulting in mortality of coppicing stumps and seedlings (Franks et al. 2006, Morath et al. 2006, Center et al. 2007). *B. melaleucae* rapidly dispersed from release points, spreading an average of 4.7 km/yr but ranging as high as 10 km/yr (Center et al. 2006, Balentine et al. 2009). After establishment, common garden experiments confirmed that feeding and development by *B. melaleucae* was restricted to *Melaleuca* species, as predicted in quarantine-based host range testing, and posed no threat to native or economically important species (Center et al. 2007).

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Although *B. melaleuca* is considered an effective biological control agent of *M. quinquenervia*, little is known concerning the life history parameters of this species in its adventive range. Therefore, an experiment was designed to quantify the influence of temperature and humidity on the developmental times and survival of *B. melaleuca*. In addition, the influence of variation in the host plant's defensive chemistry on *B. melaleuca* development and survival was investigated. Wheeler (2005) documented the existence of two "chemotypes" of *M. quinquenervia* in Florida, each characterized by a particular suite of sesquiterpene compounds contained in plant tissues. The "viridiflorol" and the "E-nerolidol" chemotype each were named after the predominant terpenoid produced in plant tissues. Individual *M. quinquenervia* stands may contain plants of only one, or more commonly, a mixture of both chemotypes (Wheeler et al. 2007). Wheeler and Ordnung (2005) determined that *B. melaleuca* prefers to oviposit on viridiflorol as opposed to E-nerolidol leaves yet nymphal development was slightly slower when feeding on viridiflorol versus E-nerolidol leaves. However, the potential interaction between chemotype and temperature or humidity on instar-specific development has not been investigated for *B. melaleuca*.

The current study investigates the influence of temperature, humidity, and host plant chemotype on longevity, fecundity, development, and nymphal survival of *B. melaleuca*. To further analyze the relationship between temperature and developmental rate, we used linear and nonlinear models to determine the base temperature threshold, the lethal (upper) temperature threshold and the optimum temperature for development of *B. melaleuca*. This study is one in a series of post release evaluations of the introduced psyllid and is intended both to provide insight into the seasonal dynamics of *B. melaleuca* populations and to describe the influence of temperature on population growth and potential distribution.

Materials and Methods

Sources of Insect and Plant Material. All *B. melaleuca* individuals used in this study were collected as needed from a field population that was originally established in 2002 at the Invasive Plant Research Laboratory in Fort Lauderdale, Broward Co., FL (Wineriter et al. 2003, Center et al. 2006). *M. quinquenervia* trees used in this study were vegetatively propagated in 3.8-liter pots during summer 2002 by using cuttings from stock plants and housed in 6.1-m³ screen cages under ambient conditions until the conclusion of experiments in spring 2007. The chemotype of each sapling was determined (E-nerolidol or viridiflorol) by gas chromatography-mass spectroscopy as described by Wheeler (2005), and trees were periodically pruned to a height of ≈ 1 m. Plants were sprayed with insecticidal soap (Garden Safe Brand, Schultz, Bridgeton, MO) weekly and with a fungicide (Spec-tracide, Immunox, St. Louis, MO) twice annually until initiation of the experiments. Approximately 30 g of

controlled release fertilizer (Nutricote Total Type 270, 13N-13P-13K, Chisso-Asahi Fertilizer Co., Ltd., Tokyo, Japan) were added to each pot semiannually.

Adult Longevity, Fecundity, and Egg Development. *Longevity.* Fifth-instar nymphs were incubated in environmental cabinets at 25°C, 75% RH, and a photoperiod of 12:12 (L:D) h (model I-36LLVL, Percival Scientific, Perry, IA; reported error, $\pm 0.5^\circ\text{C}$, $\pm 10\%$ RH). Individuals were sexed within 6 h of molting to the adult stage. One male-female pair was then transferred to each of 10 replicate *M. quinquenervia* branches per chemotype by using a cotton swab and held in environmental cabinets at 10, 15, 20, 25, or 30°C, 75% RH, and a photoperiod of 12:12 (L:D) h. To prevent escape by adult psyllids, branches were enclosed in plastic cylindrical cages that contained five screen-covered holes to allow air flow. Adults were checked daily for survivorship by their response to gentle prodding with a camel's-hair brush.

Fecundity. Fifth-instar nymphs were incubated as described above. Within 6 h of emergence, one female was paired with three field-collected males inside plastic cages on each of 10 replicate *M. quinquenervia* branches per chemotype. These plants were held in environmental cabinets at 10, 15, 20, 25, or 30°C, 75% RH, and a photoperiod of 12:12 (L:D) h. Each day, eggs were counted, males were removed, and females were transferred to fresh branches along with three newly collected males. This process was continued until the females died.

Egg Development. Seven field-collected females were placed within sleeve cages on branches of each chemotype for 24 h at 25°C, 75% RH, and a photoperiod of 12:12 (L:D) h. Females and cages were removed from branches and the resulting eggs were held in environmental cabinets at 10, 15, 20, 25, 30, or 35°C; 75% RH; and a photoperiod of 12:12 (L:D) h. At each temperature, five eggs on each of 16-29 replicated branches were monitored daily for development of eye spots and eclosion.

Nymphal Development and Survivorship. Five groups of 100 adults (90 females, 10 males) each were caged on *M. quinquenervia* trees of both chemotypes in environmental cabinets maintained at 25°C, 75% RH, and a photoperiod of 12:12 (L:D) h. Adults were removed after 24 h, and the resulting eggs were held until eye spots developed (≈ 2 wk). Egg-bearing leaves were then excised at random, and leaf petioles were inserted into moist floral foam to prolong leaf turgor. These excised leaves were held within environmental cabinets under the conditions described above, and eggs were checked daily for eclosion by using a dissecting microscope.

For the subsequent nymphal development study, all excised leaves provided to nymphs were mature, fully expanded leaves of <1 yr of age. Within 24 h of egg eclosion, 720 neonates each were transferred randomly to one of 720 excised leaves, 360 of each chemotype, by using a camel's-hair brush. The petiole of each excised leaf was then inserted into moist floral foam, and each leaf was placed in a 10-dram plastic vial. Five screen-covered holes in each vial allowed for

equilibration with external conditions. A pair of vials containing a leaf of each chemotype was then randomly assigned to an airtight container (155 by 65 mm). Approximately 200 g of supersaturated salt solution of either K_2SO_4 , NaCl, $Mg(NO_3)_2$, or $MgCl_2$ was placed into each container to maintain relative humidities of 97, 75, 54, and 33%, respectively, within the enclosed environment. Solutions were prepared as described by Winston and Bates (1960). Humidities were verified using hygrometers (OaktonLog Data Logger, model 35710-10 [OAKTON Instruments, Vernon Hills, IL], reported error, $\pm 3\%$ RH), which were calibrated using a wet- and dry-bulb psychrometer (model 3312-40, Cole-Parmer Instrument Co., Vernon Hills, IL; reported error, $\pm 3\%$ RH). Vials were placed on a plastic surface so as to provide a barrier to the salt solution below. Ten replicated humidity containers for each of the four humidity conditions were held at 5, 10, 15, 20, 25, 27.5, 30, 35, or 45°C and a photoperiod of 12:12 (L:D) h in environmental chambers.

A fresh leaf was placed into each vial daily to allow nymphs to self-transfer, and if vacated, the old leaf was removed. Nymphs were monitored daily for ecdysis, which was determined by the presence of exuviae, the developmental changes of wing buds and nymph length. Dead nymphs were replaced until the supply of neonates was exhausted.

Nymphal development and survivorship also were studied on leaves that remained attached to the plant to validate the assumption that measured parameters were not influenced by the detached leaf experimental design. For both chemotypes, 20 neonate replicates were each randomly assigned to an attached individual leaf and plants were placed into an environmental cabinet at 20, 25, or 30°C; 75% RH; and a photoperiod of 12:12 (L:D) h. The petiole of each experimental leaf was encircled with a sticky coating (Tangle-Trap, The Tanglefoot Company, Grand Rapids, MI) to prevent the escape of nymphs from leaves. Nymphs were monitored daily for survivorship, which was characterized by movement after a light prod with a camel's-hair brush. Dead nymphs were replaced as described above.

Statistical Analysis. Analysis of variance (ANOVA) (PROC GLM, SAS Institute 1999) was used to examine the influence of temperature on adult longevity and reproductive performance. Analysis of covariance (ANCOVA) was used to compare the influence of chemotype and gender on adult longevity as well as chemotype on reproductive parameters of females after accounting for the variation of the dependent variable by including the temperature range (10–30°C) as the covariate (Littell et al. 2002). The assumption of homogeneity of slopes was tested by including the interaction of the covariables in the model. ANOVA (PROC GLM) was used to examine the influence of excised versus attached leaves. To account for repeatedly measuring the same individual over time, the PROC MIXED statement was used to quantify the influence of temperature, humidity, chemotype, and gender on nymphal developmental rates (Littell et al. 2002). Nymphal stage was used as the

repeated variable, and individual nymphs were nested under the temperature variable in the repeated measures statement. The covariance structure was the autoregressive of order 1. For all dependent variables, least square means were calculated and differences among treatments were analyzed using Tukey's test (PDIFF ADJUST = TUKEY, Littell et al. 2002). Logistic ANOVA (binomial distribution and logit link function) was used to investigate the influence of temperature, humidity, and chemotype on stadium-specific survivorship (PROC GENMOD, Littell et al. 2002). Survivorship among temperatures and humidities was conducted as described above. A three-way ANOVA was used to quantify the effects of temperature, chemotype, and gender on adult longevity, female fecundity, and egg development. To meet assumptions of normality and homogeneity of variances, longevity and fecundity data were transformed by $\log(x) + 1$. Post hoc analyses were conducted as described above, and data are presented as means \pm SE.

Modeling Developmental Rate and Number of Generations. Linear Models. For the egg stage, each nymphal stadium, and for total juvenile development the linear portions (15–25°C) of the developmental rate curves were modeled as

$$R(T) = a + bT \quad [1]$$

using the least square linear regression (PROC GLM; SAS Institute 1999), where R is the rate of development and is a positive function of the rearing temperature T , and a and b were estimates of the intercept and slope, respectively. The temperatures 5, 10, 27.5, 30, and 35°C were not included in the regression analyses because their values were consistently not part of the linear portion of the curve. Setting $R(T)$ to zero in equation 1, the base temperature threshold was estimated as

$$T_0 = -\frac{a}{b} \quad [2]$$

Nonlinear Models. The nonlinear relationship between developmental rate $R(T)$ and temperature T was fitted to the Brière model that allows the estimation of the upper and lower developmental thresholds (Brière et al. 1999). The Brière-2 model defines developmental rate as

$$R(T) = aT(T - T_0)(T_L - T)^{\frac{1}{m}} \quad [3]$$

where T_0 is the base temperature threshold, T_L is the lethal (upper) temperature threshold, and a and m are empirical constants (Brière et al. 1999). The parameters a , m , T_0 , and T_L were estimated by iterative nonlinear regression using the Marquardt algorithm of PROC NLIN (SAS Institute 1999). This method uses partial derivatives of developmental rate with respect to each parameter to guide the curve-fitting process (Lactin et al. 1995). Temperature data used in the nonlinear model were from 5 to 35°C. Initial model parameters were calculated by the grid search method (SAS Institute 1999) with T_0 and T_L set between 5–10° and 30–35°C, respectively. The optimum temperature

Table 1. Mean \pm SE adult longevity, preovipositional period, and total eggs per female of *B. melaleuca* as a function of five constant temperatures and two different chemotypes

Source	Level ($^{\circ}$ C)	Adult longevity (d)	Preovipositional period (d)	Total ovipositional period (d)	Eggs per ♀ per d	Total eggs per ♀
Temp	10	63.1 (7.70)a	10.7 (2.63)a	23.7 (5.38)ab	1.1 (0.45)a	32.3 (10.57)a
	15	65.7 (4.17)a	5.3 (0.76)b	28.5 (3.75)a	5.6 (0.47)b	164.7 (28.38)b
	20	40.8 (2.98)a	3.6 (0.58)b	28.1 (3.54)a	9.9 (0.87)c	275.7 (49.20)c
	25	21.6 (1.98)b	2.3 (0.17)b	14.7 (2.6)b	11.5 (1.60)c	175.9 (32.36)b
	30	16.5 (0.75)b	2.5 (0.24)b	14.7 (1.77)b	13.7 (0.93)c	191.7 (21.88)bc
Chemotype	<i>E</i> -nerolidol	44.6 (3.56)a	5.7 (1.21)a	21.5 (2.29)a	8.9 (1.01)a	169.9 (22.92)a
	Viridiflorol	38.3 (2.93)b	4.4 (0.64)b	23.2 (2.82)a	7.4 (0.78)a	154.9 (22.54)a

Means followed by different letters are significant at $P = 0.05$.

(Brière et al. 1999) for *B. melaleuca* development was then calculated as

$$T_{opt} = \frac{(2mT_L + (m + 1)T_0) + \sqrt{4m^2T_L^2 + (m + 1)^2T_0^2 - 4m^2T_0T_L}}{4m + 2} \quad [4]$$

Generation Predictions. Daily minimum and maximum temperatures from Florida were obtained from 98 weather stations recorded by the Southeastern Regional Climate Center (NOAA 2007). Daily temperature values for minimum and maximum were averaged for 2001 through 2006 for each station. Accumulated degree-days for *B. melaleuca* were calculated using the single sine method (DegDay version 1.01, University of California Davis, Davis, CA; <http://biomet.ucdavis.edu/>). From the upper and lower temperature thresholds for *B. melaleuca* estimated here, degree-day requirements 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 number of generations per year was predicted by dividing the cumulative degree-days per station by K , the days required by *B. melaleuca* to complete development.

Predictions for *B. melaleuca* generations across the sampled landscape were developed with ArcGis 9.1 (Geostatistical Analyst function, ESRI Inc., Redlands, CA). Prediction values in unsampled locations were surface interpolated by the Inverse Distance Weighted deterministic method using the criteria: 1) the number of stations used for interpolation was set to 15; 2) power optimization was used to ensure the weights of each weather station were proportional to the inverse distance; and 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 (Pilkington and Hoddle 2006, Diaz et al. 2008).

Results and Discussion

Adult Longevity, Fecundity, and Egg Development. Temperature affected adult longevity ($F = 25.33$; $df = 4, 171$; $P < 0.0001$), with significantly shorter life spans

occurring at 25 and 30 $^{\circ}$ C versus the remaining temperatures (Table 1). When analyzed across all temperature treatments, female longevity was greater than that of males (ANCOVA: $F = 3.97$; $df = 1, 177$; $P = 0.0478$). Females held at 25 $^{\circ}$ C survived 25.8 ± 2.93 d, on average, whereas male longevity was 19.2 ± 2.55 d at the same temperature. These data are consistent with previous developmental studies of *B. melaleuca*, which also determined that females survived ≈ 3 wk when held at 22–24 $^{\circ}$ C and generally outlived their male counterparts (Purcell et al. 1997, Wineriter et al. 2003).

The preovipositional period for *B. melaleuca* was greatest when held at 10 $^{\circ}$ C, but no differences were observed among the remaining temperatures ($F = 8.13$; $df = 1, 171$; $P < 0.0001$) (Table 1). Temperature also influenced the psyllid's total ovipositional period ($F = 3.21$; $df = 4, 171$; $P = 0.0181$), with the moderate temperatures 15 and 20 $^{\circ}$ C providing the longest period, whereas the higher temperature treatments were markedly shorter (Table 1). The daily ovipositional rate increased concomitantly with temperature ($F = 26.67$; $df = 4, 171$; $P < 0.0001$), but the total oviposition per lifetime ranged more widely among temperatures ($F = 7.61$; $df = 4, 171$; $P < 0.0001$) (Table 1). Oviposition, for example, was observed at 10 $^{\circ}$ C, the coolest temperatures tested here, but the number of eggs deposited was markedly lower than the remaining temperatures (Table 1). There was no clear influence of temperature, however, on total egg production when comparing 15–30 $^{\circ}$ C (Table 1), indicating that *B. melaleuca* oviposits within a broad temperature interval.

Adult longevity across all temperatures was greater when held on plants of the *E*-nerolidol chemotype compared with viridiflorol ($F = 6.38$; $df = 1, 177$; $P = 0.0124$). However, chemotype did not influence the preovipositional period ($F = 2.01$; $df = 1, 72$; $P = 0.1610$), total ovipositional period ($F = 0.11$; $df = 1, 72$; $P = 0.7455$), the number of eggs deposited per female per day ($F = 2.66$; $df = 1, 72$; $P < 0.1072$), or total eggs oviposited per female per lifetime ($F = 0.08$; $df = 1, 72$; $P < 0.7784$). Although longevity of adult psyllids was greater on *E*-nerolidol, this did not translate to a greater ovipositional period and, more importantly, greater fecundity (Table 1).

Table 2. Mean \pm SE developmental time (days) of immature stadia of *B. melaleuca* at six constant temperatures, four constant humidities, and two different chemotypes

Source	Level	Egg	Nymphal stage					Total nymphal development	Egg to adult
			1	2	3	4	5		
Temp (°C)	10		35.3 (2.48)a	16.6 (4.45)a	24.00a				
	15	37.3 (0.36)a	11.1 (0.32)b	8.2 (0.18)b	8.4 (0.22)b	8.9 (0.24)a	13.1 (0.37)a	48.8 (0.52)a	86.1
	20	18.0 (0.19)b	4.8 (0.16)c	4.8 (0.16)c	3.4 (0.07)c	4.0 (0.08)c	5.8 (0.10)c	22.8 (0.24)b	40.9
	25	12.3 (0.19)c	3.9 (0.07)dc	2.3 (0.06)d	2.3 (0.11)c	2.6 (0.07)d	4.2 (0.07)d	15.2 (0.16)d	27.4
	27.5		4.7 (0.17)dc	3.3 (0.14)dc	3.8 (0.16)c	4.92 (0.21)b	6.5 (0.40)bc	21.7 (0.66)bc	
RH (%)	30	10.2 (0.10)d	3.8 (0.06)d	2.9 (0.08)d	3.8 (0.13)c	5.2 (0.15)b	7.2 (0.24)bc	20.7 (0.40)c	30.9
	97		6.4 (0.64)a	4.6 (0.38)a	4.7 (0.23)a	5.6 (0.25)a	7.4 (0.41)a	26.8 (1.51)a	
	75		4.9 (0.25)c	3.8 (0.22)bc	3.9 (0.21)b	4.8 (0.23)b	6.5 (0.37)a	23.6 (1.28)a	
	53		6.7 (0.68)a	4.5 (0.46)ab	3.9 (0.30)b	4.6 (0.23)b	6.7 (0.43)a	23.1 (1.24)a	
	33		5.5 (0.44)b	3.5 (0.24)c	3.9 (0.24)b	4.6 (0.27)b	7.2 (0.45)a	23.9 (1.63)a	
Chemotype	<i>E</i> -nerolidol	20.6 (0.81)a	5.9 (0.40)a	4.0 (0.24)a	3.9 (0.14)a	4.9 (0.17)a	6.8 (0.29)a	24.3 (1.03)a	44.9
	viridiflorol	18.1 (0.89)a	6.0 (0.39)a	4.3 (0.26)a	4.3 (0.20)a	4.9 (0.18)a	7.1 (0.30)a	24.5 (0.98)a	42.6

Means followed by different letters are significant at $\alpha = 0.05$.

Total eggs per female ranged from 32 to 276, markedly greater than those reported for *B. melaleuca* previously (Table 1). Purcell et al. (1997), for example, reported an average of 78 ± 14 eggs per lifetime, and Wheeler and Ordnung (2005) recorded 105 ± 16.5 . One explanation for the disparity between previous reports and those presented in this study may be related to ovipositional behavior. Here, we supplied females with new plant material daily in contrast to previous studies, which maintained the same plant material throughout the study (≈ 20 d). This latter method may have modified the ovipositional behavior of *B. melaleuca* that may have been influenced by egg presence or density (Nufio and Papaj 2001). The cabbage seedpod weevil, *Ceutorhynchus assimilis* (Paykull), for example, marks oilseed rape, *Brassica napus* L., pods after oviposition with a pheromone, which inhibits egg deposition by conspecifics (Ferguson et al. 1999). Although it remains uncertain whether *B. melaleuca* produces an ovipositional deterrent or uses some other mechanism to avoid competition, it is clear that previous reports underestimated the psyllid's reproductive potential.

Temperature influenced egg development ($F = 2474.84$; $df = 3, 292$; $P < 0.0001$), with the duration required to hatch decreasing with increasing temperature (Table 2). Eggs held above 30°C or below 15°C failed to eclose. Eggs did not develop and collapsed within a few days at 35°C but collapsed after several weeks at 10°C , suggesting that *B. melaleuca* has a greater tolerance to extreme temperatures that approach the lower versus upper thresholds (Table 2). Internal structures of *B. melaleuca* eggs also were influenced by temperature. Eye spot development was shortest at 30°C (5.83 ± 0.10 d) $< 25^\circ\text{C}$ (7.72 ± 0.17 d) $< 20^\circ\text{C}$ (11.35 ± 0.07 d) $< 15^\circ\text{C}$ (24.18 ± 0.48 d) ($F = 949.23$; $df = 3, 292$; $P < 0.0001$). Similarly, the egg tooth was apparent earliest at 30 (5.46 ± 0.14 d) and 25°C (6.37 ± 0.29 d), latest at 15°C (22.49 ± 0.38 d), and intermediate for 20°C (10.85 ± 0.14) ($F = 757.24$; $df = 3, 292$; $P < 0.0001$). There was no effect of plant chemotype on egg developmental rate ($F = 1.77$; $df = 1, 292$; $P = 0.1845$).

Nymphal Development and Survivorship. No differences were observed in nymphal developmental rates between the attached and detached leaf treatments when held at 25°C ($F = 1.62$; $df = 1, 71$; $P = 0.21$) or 30°C ($F = 2.15$; $df = 1, 22$; $P = 0.16$). Nymphs completed development somewhat faster on attached versus detached leaves at 20°C ($F = 25.84$; $df = 1, 48$; $P < 0.0001$), with a significant treatment \times gender interaction ($F = 14.68$; $df = 5, 48$; $P = 0.0004$). When gender was analyzed separately, developmental rate differed between leaf treatments for males ($F = 53.48$; $df = 1, 48$; $P < 0.0001$), but females were not affected ($F = 0.54$; $df = 1, 48$; $P = 0.47$). Survivorship was not influenced by excised leaves at either of the two temperatures. In summary, the development of male nymphs was influenced when feeding on excised versus intact leaves at one of the two temperatures tested, and survivorship was not affected by leaf treatment for either gender or at either temperature. We therefore concluded that the excised leaf experimental design provided a reasonable approximation of nymphal performance on intact leaves, and we proceeded with the excised leaves for the remainder of our study.

Temperature influenced development of *B. melaleuca* ($F = 567.99$; $df = 1, 1,780$; $P < 0.0001$) (Fig. 1), with total nymphal development ranging from 15.2 d at 25°C to 48.8 d at 15°C (Table 2). These data are similar to those reported by Wineriter et al. (2003), who determined that nymphal developmental time ranged from 3 to 4 wk when held at 22 – 24°C . Instar-specific developmental rates here increased with increasing temperature up to 25°C , whereas temperatures of 27.5 and 30°C caused total developmental rates to decline (Table 2). Nymphs failed to develop at temperatures below 10 and above 30°C . The fifth nymphal stadium was always longest in duration, regardless of test temperature, humidity, or chemotype. As a general trend, the first stadium followed the fifth stadium in terms of duration, whereas the second stadium required the least amount of time to molt to the subsequent stage (Table 2).

Differences in developmental rates across relative humidity levels also were observed ($F = 30.30$; $df = 3,$

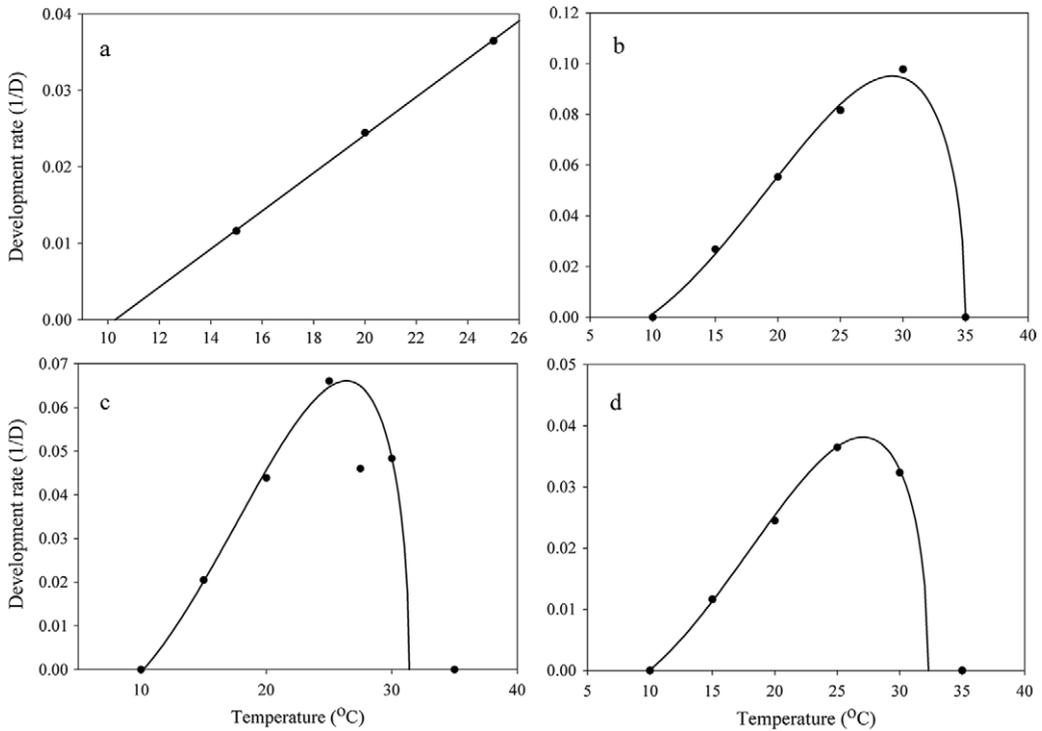


Fig. 1. Developmental rates of *B. melaleuca* at different temperatures. (a) Linear regression of total immature development (egg to adult, 1/D). (b-d) Developmental rates for egg, nymph, and total development, respectively, and predicted values (line) based on Briere-2 nonlinear model.

1,780; $P < 0.0001$), with a significant temperature \times relative humidity interaction ($F = 7.91$; $df = 13, 1,780$; $P < 0.0001$). To investigate these interactions, relative humidity was analyzed (PROC GLM) separately for each temperature level ($n = 6$) within a nymphal stage ($n = 5$). Of the resulting analyses, relative humidity significantly influenced developmental rates in seven of 30 temperature \times humidity levels. Differences in development of first-instar nymphs held at 25°C were observed ($F = 11.85$; $df = 3, 74$; $P = <0.0001$), with individuals developing faster at 97% RH, but no differences were observed at the other temperature and humidity levels for this stage ($P > 0.05$). In contrast, second instars held at 25°C ($F = 11.84$; $df = 3, 74$; $P = <0.0001$) developed slowest at 97% RH compared with the other humidities, whereas developmental rate for individuals held at 30°C ($F = 3.45$; $df = 3, 74$; $P = 0.0188$) was faster at 53 versus 97% RH ($P < 0.05$), with the remaining humidities intermediate ($P > 0.05$). Development of third- and fourth-instar nymphs held at both 27.5°C (third: $F = 6.83$; $df = 3, 77$; $P = 0.0004$; fourth: $F = 11.84$; $df = 3, 70$; $P = <0.0001$) and 30°C (third: $F = 8.42$; $df = 3, 114$; $P = <0.0001$; fourth: $F = 6.55$; $df = 3, 96$; $P = 0.0005$) was slowest for 97% RH compared with the remaining humidities ($P < 0.05$), which were not different from one another ($P > 0.05$). Relative humidity only influenced total nymphal developmental time when individuals were held at 27.5°C ($F = 4.21$; $df = 3, 48$; $P = 0.0104$), where

again, development was slowest at 97% RH compared with the other humidity levels ($P < 0.05$).

These data indicate that *B. melaleuca* can develop under a broad range of humidity conditions and, unlike temperature, there is no optimal humidity level for nymphal development. Interestingly, there is limited evidence to suggest that nymphal stages responded differently to relative humidity levels. First instars held at 25°C, for example, developed faster at 97% RH, but later instars (2-4) developed slower at 97% RH level and select temperatures at or above 25°C. These differences may be related to the dispersive nature of first-instar nymphs, which are highly active after hatching and probably experience great susceptibility to desiccation than the older life stages. In contrast, all other nymphal instars are sedentary and exhibit evolutionary adaptations that permit the herbivore to buffer fluctuations in humidity levels (Danks 2002). Nymphs, for example, are dorsoventrally flattened, allowing the insects to reside within the leaf's humid boundary layer (Schowalter 2000). In addition, these later nymphal stages produce copious amounts of waxy flocculence that envelop the insect for much of its life cycle (Wineriter et al. 2003). Although the selection for wax production may be attributed to other sources (i.e., predation), flocculence probably serves to moderate the herbivore's microclimate (Gullan and Kosztarab 1997, Danks 2002). Under high humidity levels (97% RH) and

Table 3. Linear regression parameter estimates describing the relationship between temperatures and developmental rates (1/D) of *B. melaleuca*

Stage	Intercept	Slope	R ²	Threshold (°C)	Degree-days ^a
Egg	-0.042	0.0048	0.99	8.84	209.1
Nymphs	-0.048	0.0046	0.99	10.45	219.8
Egg to adult	-0.026	0.0025	0.99	10.27	402.1

^a Total degree-days to complete development.

warm temperatures ($\geq 25^{\circ}\text{C}$), however, these adaptations may be detrimental as the microclimate exceeds suitable developmental conditions. Considering that the majority (77%) of temperature \times humidity interactions were nonsignificant (>0.05), the influence of relative humidity across all temperatures also was analyzed. In this analysis, developmental time of first instars was shortest at 75% RH, but rates converged among the three lower relative humidity levels for nymphal stages 2–4 (Table 2). Developmental times at 97% RH, however, were significantly longer for nymphal stages 3 and 4 (Table 2) as described above.

The effect of plant chemotype on nymphal development was highly significant ($F = 10.74$; $df = 1, 1,780$; $P = 0.001$), with a significant chemotype \times temperature interaction ($F = 3.47$; $df = 5, 1,780$; $P = 0.004$). This interaction was attributed to single temperature (27.5°C ; GLM: $F = 6.52$; $df = 1, 90$; $P < 0.0124$) for which nymphs feeding on *E-nerolidol* (4.3 d) developed faster than those feeding on *viridiflorol* (5.2 d). However, it is unclear how this effect, found at only one temperature, would be biologically relevant. The magnitude of difference in developmental time (5.9 d on *E-nerolidol* versus 6.0 d on *viridiflorol*) when analyzed across all temperatures was not convincing, and this effect became insignificant when the interaction of temperature and chemotype was omitted from the model ($P = 0.0896$). Although robust differences were not found between plant chemotype and total developmental rate, nymphs of all five *B. melaleuca* stadia

consistently developed slightly faster on average when feeding on *E-nerolidol* leaves (Table 2). This trend, although nonsignificant, is similar to that presented by Wheeler and Ordung (2005), who reported marginally shorter nymphal developmental time when held on *E-nerolidol* (12.7 d) versus *viridiflorol* (13.4 d) leaves.

Two- and three-way interactions between main effects did not influence nymphal survivorship ($P > 0.05$). Similarly, total nymphal survivorship was not affected by relative humidity ($\chi^2_3 = 5.53$, $P < 0.1369$) or chemotype ($\chi^2_1 = 0.96$, $P < 0.3284$). Temperature, in contrast, influenced nymphal survivorship (Table 3), with no individuals completing development above 30 or below 15°C (Fig. 2). Total nymphal survivorship was greatest at 25°C (95%, $\chi^2_1 = 140.12$, $P < 0.0001$); lowest at 27.5°C (35%, $\chi^2_1 = 6.86$, $P = 0.0088$); and intermediate at 15, 20 and 30°C ($\chi^2_1 = 1.84$, $P = 0.1750$). At 10°C , nearly 14% of first-instar nymphs survived to the second instar, but none survived beyond the third instar (Fig. 2).

Modeling Developmental Rate and Number of Generations. Developmental rates for eggs, nymphs, and total development from egg-to-adult stage are presented in Table 4. The linear model estimated that the lower temperature threshold for all measured stages ranged from 8.8 to 10.5°C , and total degree-days required for development was 402. Consistent with linear estimates, the lower temperature threshold predicted by nonlinear models for egg and nymphal stages ranged from 9.6 to 10.2°C (Table 4). Developmental rates for eggs increased with increasing temperatures until the curve reached an optimum near 30°C , with an estimated T_{opt} of 28.8°C (Fig. 1; Table 4). With a lower optimum temperature, nymphs seemed more sensitive to temperatures above 25°C than eggs (Fig. 1; Table 4).

The predicted number of *B. melaleuca* generations across the Florida landscape indicates that the psyllid can complete multiple generations per year throughout the geographic range of the target weed *M. quinque-neria* (Fig. 3). Overall, spatial variation in gener-

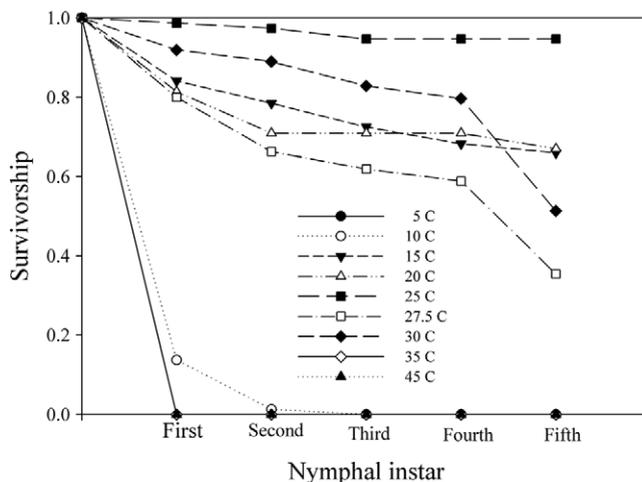


Fig. 2. Stage-specific survivorship for *B. melaleuca* nymphs when held at nine different constant temperatures.

Table 4. Development parameters for the empirical constants a , lower (T_o) and upper (T_L) temperature thresholds, and well as optimal temperature (T_{opt}) based on Briere-1 nonlinear model describing the relationship between temperature and developmental rate ($1/D$) of *B. melaleuca* stages

Stage	Parameter estimate (°C)				
	a	T_o	T_L	T_{opt}	R^2
Egg	0.000069	9.6	35.0	29.1	0.99
Nymphs	0.000069	10.2	31.4	26.4	0.96
Egg to adult	0.000036	9.95	32.3	27.1	0.99

ation number is minimal, and the predictions followed a thermal gradient across the state. The highest number of generations was predicted to occur in southern Florida, ranging from 12 to 13 per annum. In central Florida, *B. melaleuca* is predicted to complete approximately nine to 11 generations versus eight to nine generations in the northern most reaches of the tree's distribution in the state (Fig. 3).

These data also have relevance to seasonal fluctuations of *B. melaleuca* densities in the field, with higher populations observed during winter versus summer (Center et al. 2007). Causes for observed seasonal fluctuations in *B. melaleuca* densities are probably attributed to complex interactions between biotic (i.e., natural enemies, plant phenology) and abiotic (i.e., precipitation, temperature, humidity)

factors. There is little evidence that Florida's minimum daily temperatures limit *B. melaleuca* densities. Of the 20 weather stations south of Lake Okeechobee, where the highest concentrations of *M. quinquenervia* occur, fewer than half the stations reported an average minimum temperature below 10°C, and no locations remained below 18°C during any given 24-h period (based on data from 2001 to 2006). Mean maximum daily temperatures for the coolest regions of the state consistently exceeded the lower temperature thresholds for all stages of the herbivore, indicating continued psyllid development during Florida's mild winters.

Among the abiotic factors tested here, seasonal fluctuations in psyllid densities may be most strongly influenced by negative population growth rates associated with upper temperature thresholds. The mean maximum daily temperatures commonly exceeded 30°C south of Lake Okeechobee, corresponding to temperatures at which high levels of nymphal mortality were observed (Fig. 2). In Fort Lauderdale (Broward Co.), for example, maximum temperatures ranging from 30 to 35°C were recorded on 138 d during 2006 (NOAA 2007). It should be noted, however, that these extreme temperatures were brief, and it is unclear how short periods of exposure to temperatures >30°C affect development and survival of *B. melaleuca*.

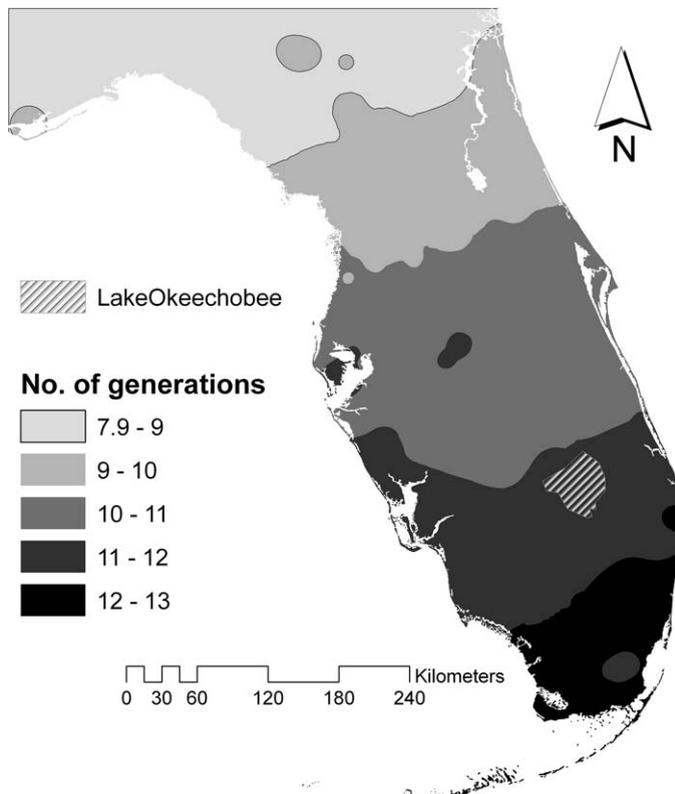


Fig. 3. Predicted number of generations for the introduced psyllid *B. melaleuca* across the geographic distribution of its host *M. quinquenervia*.

cae. An additional factor not addressed here but probably to contribute to seasonal fluctuations of *B. melaleuca* is precipitation. Precipitation levels and temperature are temporally correlated in south and central Florida; frequent and often heavy rainfall events are typical of the warmer summer months whereas rain falls less frequently during the cooler winter months. Casual observations in the field suggest summer rains may wash away flocculence, perhaps increasing the vulnerability of nymphs to environmental and predatory dangers, and may even dislodge younger instars from host plants. Although empirical evidence is lacking, it stands to reason that rain could directly cause mortality of psyllids. This may be particularly true in the juvenile stages that would have limited dispersal capabilities to find a new host if dislodged from an *M. quinquenervia* leaf by heavy rain.

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