

Effect of Low Temperatures on Mortality and Oviposition in Conjunction With Climate Mapping to Predict Spread of the Root Weevil *Diaprepes abbreviatus* and Introduced Natural Enemies

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ABSTRACT The tropical root weevil, *Diaprepes abbreviatus* (L.), has been a pest of citrus and ornamental plants since its introduction into Lake County, FL, in 1964. Since then, it has colonized the Florida peninsula to the south of its point of introduction but has not expanded its range to the north. A lower threshold for oviposition by *D. abbreviatus* was estimated as 14.9°C. Eggs were highly susceptible to cold, with 95% mortality ($LTime_{95}$) occurring in 4.2 d at 12°C. Relative susceptibility of life stages to cold was eggs > pupae > larvae > adults. Archived weather data from Florida were examined to guide a mapping exercise using the lower developmental threshold for larvae (12°C) and the lower threshold for oviposition (15°C) as critical temperatures for mapping the distribution of *D. abbreviatus* and the potential for establishment of egg parasitoids. Probability maps using the last 10 yr of weather data examined the frequency of at least 10, 15, 20, 25, or 30 d per winter when soil temperature was $\leq 12^\circ\text{C}$. The geographic area that experienced between 15 and 20 d per winter with mean daily soil temperature $\leq 12^\circ\text{C}$ closely approximated the northern limit of *D. abbreviatus* in Florida. Homologous maps of Arizona, California, and Texas predict the areas where soil temperatures favor establishment of *D. abbreviatus*. Successful establishment of egg parasitoids in Florida seems to be limited to southern Florida, where mean daily air temperatures fall below $15^\circ\text{C} < 25 \text{ d/yr}$. By this measure, we predict that egg parasitoids will not establish in Arizona, California, or Texas.

KEY WORDS *Diaprepes* root weevil, climate, oviposition threshold, lethal temperature, cold

Diaprepes abbreviatus (L.) was unintentionally introduced from the Caribbean to the mainland United States, first reported at Apopka (28.68° N, 81.51° W) in Orange County, FL, by Woodruff (1964). Since then, this highly polyphagous weevil has expanded its range in Florida southward >480 km (300 miles) to include most of peninsular Florida south of Apopka and northward to locations ≈ 48 km (30 miles) north of Apopka at approximate latitude 29° N (M. Thomas, personal communication). Twenty-three Florida counties are now considered infested (Nguyen et al. 2003, Weissling et al. 2004). In this region, *D. abbreviatus* is considered a major pest of citrus and ornamental plants. In 2000, *D. abbreviatus* was reported to be established in a citrus grove in the Rio Grande valley of Texas (Skaria and French 2001). During the period 1974–2003, inspectors of the California Department of Food and Agriculture reported >20 intercepts of *D.*

abbreviatus in plant material from Florida and Puerto Rico (Grafton-Cardwell 2005). In September 2005, it was discovered infesting ornamental palms in an urban area of Orange County, CA, and soon thereafter in an urban area of Long Beach in Los Angeles County (Grafton-Cardwell et al. 2004, Godfrey 2005). An official find has now been reported from San Diego County (CDFA 2006). The limited northern expansion of *D. abbreviatus* in Florida from its initial point of introduction suggests that this tropical species is limited by cool winter temperatures and may only be a threat to limited areas of Texas and California.

Adult *D. abbreviatus* oviposit on foliage where the eggs develop, but the neonate larvae fall to the ground and subsequent larval stages develop in the soil in association with plant roots. The lower threshold for neonate larval development was estimated to be 15°C (Lapointe 2000), and the lower developmental threshold for eggs was estimated to be 12°C (Lapointe 2001). In this paper, we report additional information about the effect of low temperatures on oviposition and survival of *D. abbreviatus* and predict the geographical areas of California and Texas that will be susceptible to infestation based on climate mapping of the current distribution of *D. abbreviatus* in Florida using the North Carolina State University-Animal Plant Health Inspection Service-Plant Pest Forecasting System

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(NAPFAST). NAPFAST is an internet-based system that uses simple biological models and climate databases to generate georeferenced output maps at a 10-km² resolution (Borchert and Magarey 2005).

Florida state, federal, and private industry entomologists have collaborated over the past decade to introduce species of hymenopteran egg parasitoids from the Caribbean to control *D. abbreviatus* (Hall et al. 2001). A survey of Florida locations during 1997 and 1998 found no evidence of egg parasitism of *D. abbreviatus* by native parasitoids, whereas parasitism of 36% was found at a site in Puerto Rico (Hall et al. 2001). In 2000, the endoparasitoid *Quadrastichus haitiensis* Gahan was introduced from Puerto Rico to Florida, and the ectoparasitoid *Aprostocetus vaquita-rum* Wolcott (Hymenoptera: Eulophidae) was introduced from the Dominican Republic; both are now considered established in parts of southern Florida (Jacas et al. 2005, Castillo et al. 2006). Recently, efforts have focused on gaining release permits for two other imported egg parasitoid species currently in quarantine and continued exploration of the Caribbean for new parasitoid species (Ulmer et al. 2006).

Successful establishment of egg parasitoids is likely to depend on year-round availability of prey egg masses. While egg masses and adults of *D. abbreviatus* can be found throughout the year in southern Florida and on Caribbean islands such as Puerto Rico (S.L., unpublished data), colder weather in central Florida presumably results in periods of weeks to months when no or few adult weevils and egg masses are observed in citrus groves. Here we report the effect of lethal temperatures on all life stages and the lower temperature threshold for oviposition behavior by adult *D. abbreviatus*. We predict areas in California, Arizona, and Texas where *D. abbreviatus* and its eggs parasitoids could establish based on comparative climate mapping.

Materials and Methods

Oviposition Threshold. Male and female adult *D. abbreviatus* were obtained from a laboratory colony maintained by the U.S. Horticultural Research Laboratory, Fort Pierce, FL (Lapointe and Shapiro 1999). These were caged (one pair per cage) on citrus foliage (*Citrus macrophylla* Wester) and provided with strips of wax paper for oviposition (Wolcott 1933). Clear plastic cages (14 by 20 by 10 cm) with screened lids were held in an environmental chamber at constant temperatures (12, 15, 18, 21, 27, and 30°C), 90% RH, and 12:12 L:D. Each cage was considered a replicate. Newly emerged (≤ 24 -h-old) adult weevils ($n = 10$ pairs per temperature but $n = 20$ pairs at 15°C) were taken from diet cups, placed in the cages (one male = female pair per cage), and held at 30°C until oviposition was observed in all cages or until 2 wk had passed. Pairs that failed to produce eggs during that period were discarded. When pairs of *D. abbreviatus* were observed to be producing eggs in all cages, the temperature of the chamber was lowered to the test temperature and held constant for 2 wk. The temperature

was returned to 30°C at the end of the test period. Wax paper strips were collected every second or third day from the cages and the number of eggs counted. The number of eggs per female per day was calculated and analyzed by regression (PROC GLM; SAS 2002) to estimate the lower threshold for oviposition behavior.

Lethal Temperatures. Eggs, larvae, pupae, and adults were obtained from the USDA colony as described above and placed in environmental chambers at constant temperatures (0, 3, 6, 9, and 12°C). Chambers containing eggs and adults were kept at 12:12 L:D, whereas larvae and pupae were kept in continuous dark.

Eggs were exposed to 0, 3, 6, 9, or 12°C for 1, 2, or 3 d or to a control temperature of 26°C. Eggs of uniform age were obtained by placing wax paper oviposition strips in cages containing adult *D. abbreviatus* and removing them after 24 h. Five egg masses (113 ± 15 eggs per egg mass or 562 ± 16 eggs [SE] per temperature regimen for a total 12,374 eggs) were placed in plastic vials capped to maintain humidity and assigned to one of the temperature regimens. Five egg masses were removed from each temperature regimen each day and placed in a chamber at 26°C. The number of viable eggs, neonate larvae, and dead eggs was recorded daily for egg masses subjected to each temperature regimen until no further hatch was observed. Percent egg mortality at each temperature was regressed on the length of exposure to that temperature using Proc GLM (Legg et al. 2000, SAS 2002).

Ninety diet cups (PC100, 1-oz. cups and lids; Jet Plastica, Harrisburg, PA) containing a total of 547 11-d-old larvae (≈ 6 larvae/cup) and 90 cups containing a single immature (unscerotized) pupa each were placed in a dark environmental chamber at 0, 3, 6, 9, or 12°C for periods of 1–30 d. Three cups of larvae and three pupae were removed from each temperature regimen daily and placed in a chamber at 26°C chamber until survival could be assessed. For larvae, this was usually 3–5 d after transfer to 26°C. For each temperature regimen, the number of live and dead larvae was recorded, and percent mortality was calculated as the number of dead larvae divided by the initial number of larvae. Larvae were removed from the temperature/exposure treatments daily for ≤ 30 d or until complete mortality was observed. After each temperature/exposure regimen, pupae were maintained at 26°C until death or emergence as adults. Percent larval mortality for each temperature was regressed against the period of exposure.

We observed that low temperatures can cause death of pupae and moderate to severe deformities in the emerging teneral adults. Deformities result in adults of reduced viability and inability to mate. Therefore, pupal morbidity (defined here as the sum of dead pupae and deformed teneral adults) was calculated for each temperature/exposure treatment. The data are presented as the time to 100% morbidity ($LTime_{100}$) because the limited availability of pupae at a similar developmental stage resulted in an insufficient number of replications (three) at each treatment combination of temperature and exposure to calculate a

regression. Control groups for each temperature treatment consisted of 30 pupae maintained at 27°C.

Adult weevils (one male-female pair per cage) were held at the test temperatures (75–95% RH) for up to 30 d and checked daily for mortality. Cages were provided with fresh citrus leaves as needed. At the end of the temperature-exposure treatment, adults were transferred to a chamber at 26°C for 1 wk to assess survival.

Field Oviposition. During the course of field studies reported elsewhere (Lapointe et al. 2006), data were collected on the seasonal fluctuation in the number of egg masses laid by *D. abbreviatus* on citrus trees over 3 yr at two sites in St. Lucie County, FL, from 2001 to 2003. Visual inspections of trees for egg masses were conducted approximately weekly on budded citrus trees (*Citrus sinensis* ‘Midsweet’ on *C. volkameriana* ‘Volkamer lemon’) that were planted in February 2001, at the University of Florida’s Indian River Research and Education Center at Fort Pierce, FL. The grove was located on a poorly drained hydric (Winder) soil, known to be infested by *D. abbreviatus* and the citrus root weevil *Pachnaeus litus* (Germ.). We made no attempt to differentiate between egg masses of the two species because their egg masses are indistinguishable in the field. The total number of adult root weevils counted over 3 yr was 6,125, of which 5,537 (90.4%) were *D. abbreviatus* and 588 (9.6%) were *P. litus* (Lapointe et al. 2006).

Weather Data and Climate Homology. Archived air (60 cm) and soil (10 cm) temperature data were downloaded from the University of Florida’s FAWN website (Florida Automated Weather Network; <http://fawn.ifas.ufl.edu/>) for representative weather station sites in peninsular Florida: Homestead (25.51° N), Fort Pierce (27.43° N), Okahumpka (near Leesburg and approximately the northern limit of *D. abbreviatus*’ range; 28.68° N), and Alachua (Gainesville; 29.80° N). Mean (\pm SEM) daily air (60 cm) and soil (10 cm) temperatures were calculated for each Julian date based on archived data available from 1998 (1999 in the case of Alachua) through 2006. For 11 selected sites in California (Figs. 4 and 5), mean daily air temperatures were downloaded from the University of California integrated pest management (IPM) Online website (<http://www.ipm.ucdavis.edu/WEATHER/wxretrieve.html>). Mean daily air temperatures for Weslaco, TX, were downloaded from the Texas A&M University weather site (<http://etweather.tamu.edu/>). Mean weekly air temperatures were calculated to smooth data and facilitate comparison of Florida and California sites (Fig. 3).

The maps for predicting areas favorable for *D. abbreviatus* establishment were created using NAPPFAST at a 10-km² resolution (Borchert and Magarey 2005). The climate database contains daily weather observations from 1,879 weather stations typically located at airports in North America, starting from 1 January 1978 to the present. There are 98 stations in California, 72 in Texas, 20 in Arizona, 98 in Mexico, 24 in New Mexico, and 51 in Florida. The maps were created using data from all stations available then

Table 1. Mean \pm SEM no. eggs laid per female *D. abbreviatus* per day at six constant temperatures (n = no. females)

Temperature (°C)	n	No. eggs/d
12	10	0.0 \pm 0.0
15	20	7.4 \pm 2.3
18	10	15.3 \pm 4.1
21	10	42.8 \pm 11.3
27	9	91.9 \pm 17.1
30	59	93.3 \pm 7.0

interpolated using the Barnes method (Barnes 1964). The Barnes method places a distance weight to each station and a radius of influence that each station has.

We hypothesized that *D. abbreviatus* has not been able to expand its northern range in Florida because of the frequency of soil temperatures <12°C in northern Florida based on published lower developmental thresholds for larvae and eggs (Lapointe 2000, 2001). Using NAPPFAST, we created a model that recorded the number of days each year when average daily soil temperature (10 cm) was \leq 12°C. Probability maps were generated using the latest 10 yr of weather data examining the frequency of at least 10, 15, 20, 25 or 30 of 365 d when soil temperature was 12°C or lower. The maps, which encompass all of North America, were imported into Arc GIS 9.0. The 10-, 15-, 20-, 25- or 30-d maps were overlaid and incorporated with information on the northernmost report of *D. abbreviatus* in Florida.

We created a second model that recorded the number of days per year when average daily air temperature was below the 15°C functional lower limit for oviposition by *D. abbreviatus* determined and reported here. Probability maps were generated using the last 10 yr of weather data examining the frequency of \geq 25 or \geq 35 of 365 d where the average daily air temperature was \leq 15°C. The maps were imported into Arc GIS 9.0, where data were excluded when the frequency of occurrence was <5 of 10 yr.

Statistical Analysis. Response variables (oviposition and mortality/morbidity) were analyzed by regression (Proc GLM; SAS 2002) on temperature. For oviposition data, the lower temperature threshold for oviposition was estimated from the regression. The regression equations were used to estimate the time required to cause 50 and 95% mortality (LTime₅₀ and LTime₉₅, respectively) at each temperature tested for eggs, larvae, and adults.

Results

Oviposition Threshold. No oviposition by adult *D. abbreviatus* held at 12°C was observed (Table 1). Adult weevils exposed to 12°C for 2 wk survived and resumed oviposition when the temperature was returned to 30°C. However, the eggs produced during the post-12°C treatment period at 30°C were nonviable. Oviposition (mean number of eggs per female) increased linearly with increasing temperatures between 15 and 27°C ($y = 7.4x - 109.5$; $r^2 = 0.97$; $df =$

Table 2. Mean no. days required to produce 50 (LTime₅₀) and 95% (LTime₉₅) mortality in eggs, larvae, and adults or 100% mortality (LTime₁₀₀) in pupal *D. abbreviatus* at constant temperatures

Temperature (°C)	Eggs		Larvae ^a		Pupae ^b	Adults ^c	
	LTime ₅₀	LTime ₉₅	LTime ₅₀	LTime ₉₅	LTime ₁₀₀	LTime ₅₀	LTime ₉₅
0	<1.0	<1.0	5.4	8.7	<1.0	7.2	10.7
3	0.7	2.0	6.4	10.1	<1.0	22.1	26.5
6	2.0	3.6	9.4	14.6	6.5	>30.0	>30.0
9	2.3	4.1	>30.0	>30.0	12.5		
12	3.7	4.2			>30.0		

^a Line fits for eggs—3°C: $y = 2.56x^3 - 26.48x^2 + 89.70x + 0.73$; $R^2 = 0.99$; $F_{1,3} = 57.4$; $P_r > F = 0.01$; 6°C: $y = 27.06x - 3.26$; $R^2 = 0.95$; $F_{1,3} = 54.4$; $P_r > F = 0.005$; 9°C: $y = 26.34x - 11.87$; $R^2 = 0.94$; $F_{1,3} = 48.5$; $P_r > F = 0.006$; 12°C: $y = 0.41e^{1.31x}$; $R^2 = 0.98$; $F_{1,3} = 188.6$; $P_r > F = 0.0008$. Line fits for larvae—0°C: $y = 13.3x - 21.5$; $R^2 = 0.67$; $F_{1,5} = 8.3$; $P_r > F = 0.04$; 3°C: $y = 12.25x - 28.1$; $R^2 = 0.65$; $F_{1,6} = 9.3$; $P_r > F = 0.03$; 6°C: $y = 7.7x - 21.4$; $R^2 = 0.91$; $F_{1,9} = 81.5$; $P_r > F < 0.0001$.

^b Mortality included dead and deformed pupae and teneral adults.

^c Line fits for adults—0°C: $y = 12.8x - 42.3$; $R^2 = 0.95$; $F_{1,5} = 145.4$; $P_r > F < 0.0001$; 3°C: $\log(y) = 0.14x + 3.9$; $R^2 = 0.90$; $F_{1,21} = 198.7$; $P_r > F < 0.0001$.

1,4; $P_r > F_{\text{slope}} = 0.01$). Extrapolation of the regression yielded a mean functional lower threshold for oviposition of 14.9°C ($P_r > t_{\text{intercept}} = 0.03$).

Lethal Temperatures. Eggs were highly susceptible to temperatures of 0–12°C with 95% mortality occurring in <1–4.2 d (Table 2). Egg mortality was complete after 24 h at 0°C, and no regression was attempted. At 3°C, egg mortality increased asymptotically with increasing exposure (Fig. 1). The line was fit with a third-order polynomial and predicted LTime₉₅ at 2.9d (Table 2). At 6 and 9°C, linear regression was used to estimate LTime₉₅ at 3.6 and 4.1 d, respectively. Data for egg mortality at 12°C were fitted with an exponential function (Fig. 1). LTime₉₅ at 12°C was estimated at 4.2 d (Table 2). Mean control mortality of eggs ($n = 4172$) maintained at 26°C was 0.9%.

Larvae suffered low levels of mortality when held at temperatures >6°C (Table 2). Mortality of larvae never exceeded 5.4% when exposed to 9°C for up to 30 d. Similarly, no mortality was observed among larvae exposed for up to 30 d at 12 or 26°C. Larvae held at 0, 3, and 6°C suffered mortality that increased linearly with increasing time at the test temperature.

Regression analysis yielded LTime₉₅ values ranging from 9 to 15 d at 0 and 6°C, respectively (Table 2).

Pupae were more susceptible to low temperatures than adults or larvae (Table 2). Control groups for pupae held at 26°C suffered no mortality and produced only one deformed adult from a total of 150 pupae. Pupal morbidity was 100% (three of three) after 24 h and all longer durations at 0 and 3°C and after 6.5 and 12.5 d at 6 and 9°C, respectively (Table 2).

Adult survival at 26°C (control) was 100% ($n = 29$). Adult LTime₉₅ at 0 and 3°C was 10.7 and 26.5 d, respectively. Adults held at constant temperatures of 6, 9, and 12°C survived until the end of the experimental period of 30 d (Table 2).

Field Oviposition. The winters of 2001–2002 and 2002–2003 were the warmest and coldest, respectively, during the period of 1998 through 2006 as measured by the number of days with mean daily temperatures below 15°C at the four Florida weather stations (Gainesville, Leesburg, Fort Pierce, and Homestead). At Fort Pierce, the accumulated number of days with mean daily air temperature <15°C was 23 in 2001–2002 and 45 in 2002–2003 (Fig. 2). The first and last days with mean daily temperature <15°C occurred on 20

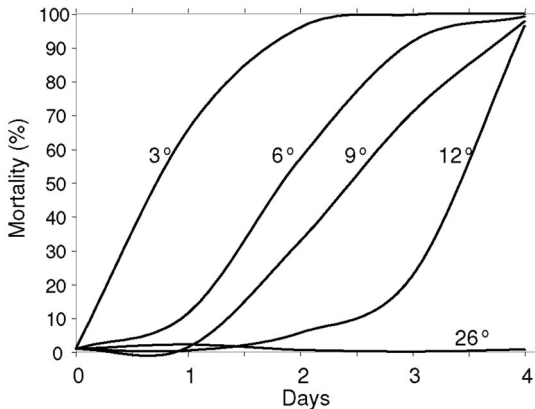


Fig. 1. Percent mortality of eggs of *D. abbreviatus* held at five constant temperatures for up to 4 d. Equations describing the lines used to predict LTime₅₀ and LTime₉₅ are given in Table 2.

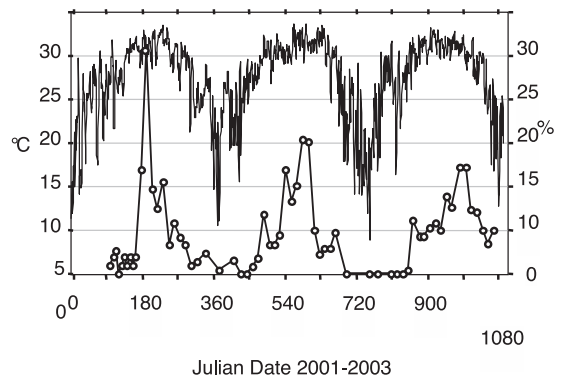


Fig. 2. Mean daily air temperature (top line) at Fort Pierce, FL, over 3 yr and seasonal distribution of oviposition by root weevils in a citrus grove. Circles represent counts of egg masses expressed as a percentage of the total number of egg masses per calendar year. Julian date 1 = 1 January 2001.

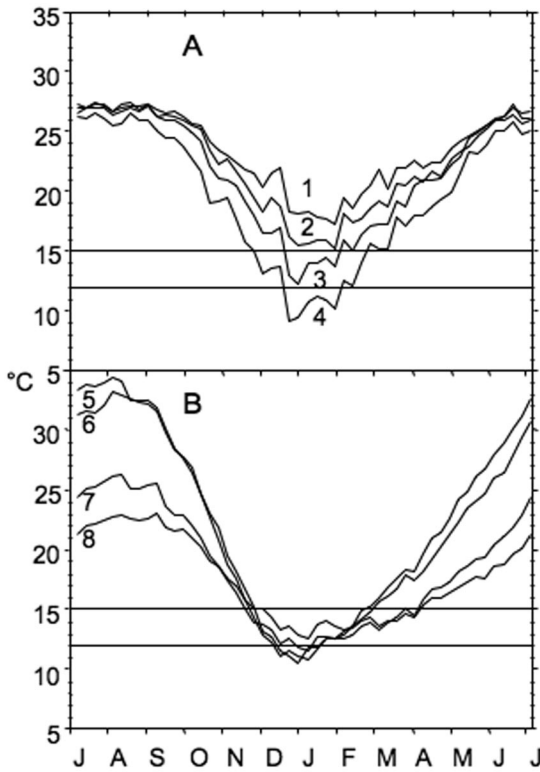


Fig. 3. Mean weekly air temperatures at four representative sites in Florida (A) and four sites in California (B). Florida sites 1-4: Homestead, Ft. Pierce, Leesburg, and Gainesville, respectively. California sites 5-8: Blythe, Brawley, Riverside, and Chula Vista, respectively. Horizontal reference lines at 15 and 12°C represent the lower threshold temperature for oviposition and lower developmental threshold (LDT) for eggs of *D. abbreviatus*, respectively.

December and 5 March and 18 November and 1 April for the winters of 2001-2002 and 2002-2003, respectively (Fig. 2). During the winter of 2001-2002, egg masses were found at low but consistent numbers during the winter, with the exception of two dates encompassing a period of 14 d (5 and 19 March 2002). During the winter of 2002-2003, there was a period of 141 d from 26 November through 16 April when no egg masses were found in the field (Fig. 2).

Weather Data and Climate Homology. Florida weather stations presented a clear gradation of decreasing mean daily winter air temperatures with increasing latitude, with only one of the four sites, Alachua (Gainesville) having mean daily temperatures <12°C (Fig. 3). Alachua is the only one of the four stations located north of the known distribution of *D. abbreviatus* (Fig. 6). The weather data showed that, in the humid subtropical environment of Florida, winter temperatures varied to a greater extent compared with summer temperatures, whereas in California, summer temperatures varied to a greater extent compared with winter temperatures (Fig. 3). Eight of 11 locations studied in California and Weslaco, TX, recorded <30 d/yr with mean daily soil temperatures

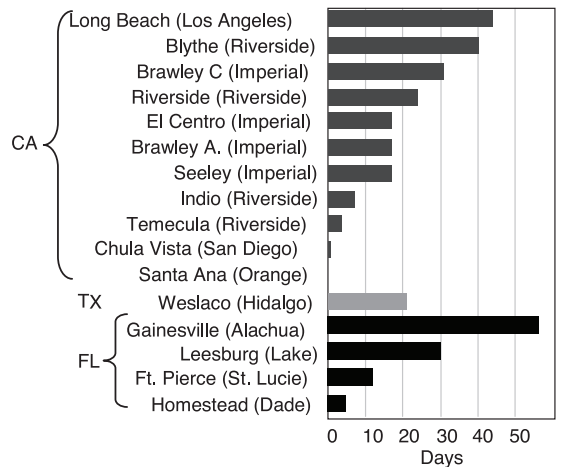


Fig. 4. Mean number of days per year with mean daily soil (10 cm) temperatures $\leq 12^\circ\text{C}$ at 4 sites in Florida, 11 sites in southern California, and 1 site at Weslaco, TX.

$\leq 12^\circ\text{C}$ (Fig. 4). Leesburg, the approximate northern limit of *D. abbreviatus* in Florida, recorded a mean of 30 d/yr with mean daily soil temperature $\leq 12^\circ\text{C}$ (Fig. 4). All sites we studied in southern California recorded >60 d/yr with mean daily air temperature <15°C, the oviposition threshold for *D. abbreviatus* determined here. Weslaco, TX, recorded a mean of 41 d/yr with mean daily air temperature $\leq 15^\circ\text{C}$ (Fig. 5).

Based on these observations and data presented here on lethal and threshold temperatures, two critical temperatures, 12°C soil temperature and 15°C air temperature, were selected for analysis and generation of the NAPPFAST maps. The first critical temperature, 12°C, was selected as the lower threshold for development of eggs and larvae (Lapointe 2000, 2001). The Florida map of >15 cumulative d with mean soil tem-

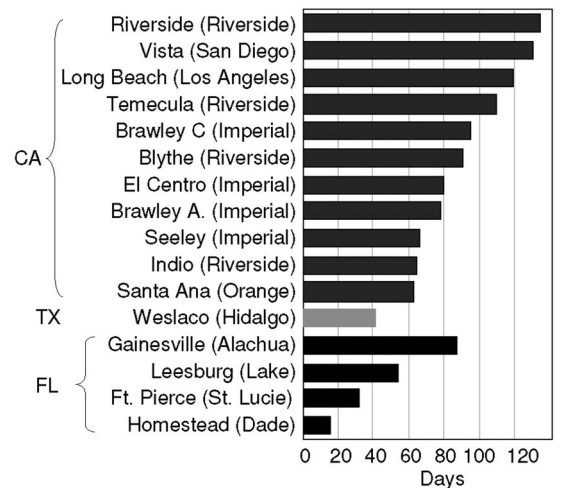


Fig. 5. Mean number of days per year with mean daily air (60 cm) temperatures $\leq 15^\circ\text{C}$ at 4 sites in Florida, 11 sites in southern California, and 1 site at Weslaco, TX.

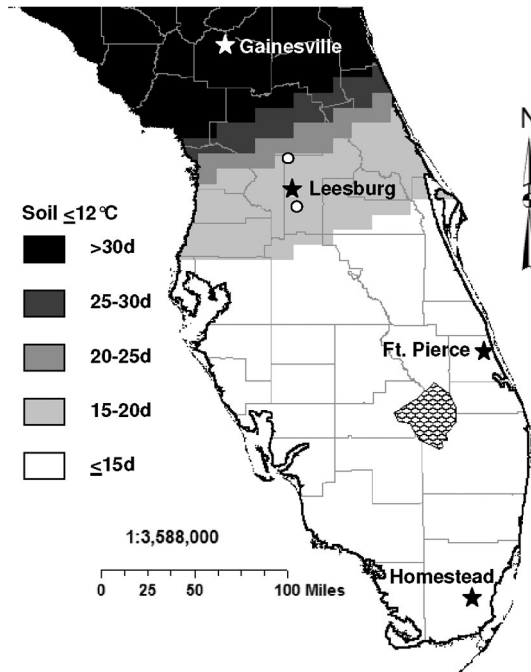


Fig. 6. Distribution map of the occurrence ≥ 5 of the last 10 yr of >30 , $25\text{--}30$, $20\text{--}25$, $15\text{--}20$, and ≤ 15 d/yr with average daily soil temperatures $<12^{\circ}\text{C}$ in Florida. Locations (stars) are University of Florida weather stations. Circles represent the northernmost record for *D. abbreviatus* in the state (Lake County, north of Leesburg at southern border of Marion County) and the site of the original discovery of the species at Apopka, south of Leesburg (Woodruff 1964).

peratures of $\leq 12^{\circ}\text{C}$ (Fig. 6) agreed closely with the observed distribution of *D. abbreviatus* in Florida, whereby Lake County is the northernmost county with documented establishment of the weevil (Nguyen et al. 2003). The second critical temperature, 15°C , was selected based on the experimentally determined lower threshold for oviposition reported here.

The mean number of days per winter with mean daily air temperatures below 12°C within the latitudes infested with *D. abbreviatus* in Florida range from 4.7 d at Homestead to 29.6 d at Leesburg (Okahumpka), the approximate northern limit of the range of *D. abbreviatus* (Table 3). The Alachua weather station (near Gainesville), located ≈ 100 km north of Leesburg, experienced an average of 56.1 d per winter $<12^{\circ}\text{C}$ from

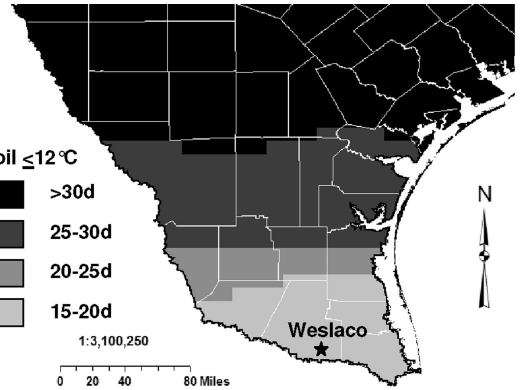


Fig. 7. Distribution map of the occurrence ≥ 5 of the last 10 yr of >30 , $25\text{--}30$, $20\text{--}25$, and $15\text{--}20$ d/yr with average daily soil temperatures $<12^{\circ}\text{C}$ in south Texas, including the location of the current infestation by *D. abbreviatus* at Weslaco.

1998 through 2006. Soil temperatures $<12^{\circ}\text{C}$ occurred between 15 and 20 d/yr at the northern limit of *D. abbreviatus* (Fig. 6). The corresponding area in Texas encompasses three counties (Cameron, Hidalgo, and Willacy) and southern portions of three counties (Brooks, Kenedy, and Starr; Fig. 7) including the current location of infestation, Weslaco (Skaria and French 2001). In California, the model based on 12°C soil temperature predicted that Imperial County and portions of Riverside and San Diego Counties are susceptible to infestation by *D. abbreviatus* (Fig. 8). Of particular interest, the model predicted a small area on the coast of Orange and Los Angeles Counties that includes a location currently infested [California Department of Food and Agriculture (CDFA) 2006] that should be only marginally susceptible to infestation based on corresponding data from the Florida map.

Compared with Florida, temperature data from California do not follow a simple north-south gradient (Fig. 8). Locations within Riverside County, for example, range from an average of 0 d/yr with mean daily air temperature $<12^{\circ}\text{C}$ (Santa Ana, Orange County) to 44 d/yr at Long Beach, Los Angeles County (Fig. 4). Variation within Riverside County can be seen by comparing Temecula (4 d/yr $<12^{\circ}\text{C}$) with Blythe (40 d/yr $<12^{\circ}\text{C}$).

In Texas, temperature conditions at Weslaco seem to be favorable for establishment of *D. abbreviatus*. Air temperatures at Weslaco are intermediate to those at Fort Pierce and Leesburg, FL (Fig. 4). Similarly, soil temperatures in southern Texas (Fig. 5) correspond

Table 3. Accumulated mean \pm SEM (range) no. days per winter with daily mean temperature below two threshold temperatures related to the biology of *D. abbreviatus* at four representative locations in Florida

Location (County)	Database	Latitude	d $< 15^{\circ}\text{C}$	d $< 12^{\circ}\text{C}$
Homestead (Dade)	1998–2006	25°28'	16 \pm 2 (9–26)	5 \pm 1 (3–10)
Fort Pierce (St. Lucie)	1998–2006	27°27'	32 \pm 3 (23–45)	12 \pm 1 (7–19)
Leesburg (Lake)	1998–2006	28°48'	54 \pm 4 (36–72)	30 \pm 3 (17–42)
Gainesville (Alachua)	1999–2006	29°39'	87 \pm 4 (72–98)	56 \pm 3 (45–68)

Data retrieved from Florida Automated Weather Network (FAWN).

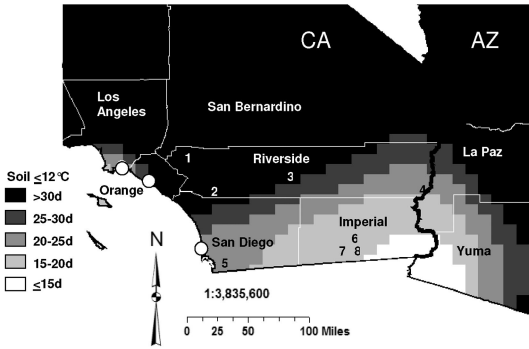


Fig. 8. Distribution map of the occurrence ≥ 5 of the last 10 yr of >30 , 25–30, 20–25, 15–20, and ≤ 15 d/yr with average daily soil temperatures $< 12^\circ\text{C}$ in southern California and Arizona (Yuma and La Paz Counties). Circles are the location of three known infestations by *D. abbreviatus*. Numbers represent weather stations: Riverside (1), Temecula (2), Indio (3), Blythe (4), Chula Vista (5), Brawley (6), Seeley (7), El Centro (8)

to those at Lake County, within the current range of *D. abbreviatus*.

The maps of air temperatures show a gradation in southern Florida of the number of days with mean daily air temperature $< 15^\circ\text{C}$, the lower threshold for oviposition by *D. abbreviatus*, from 10 to 15 d/yr in south Florida (Homestead) to 15 to 25 d/yr at Fort Pierce (Fig. 9). The results of release and recovery of egg parasitoids of *D. abbreviatus* in Florida have shown successful establishment only in extreme southern Florida (Hall et al. 2001, Peña et al. 2005). Our model suggests that egg parasitoid species cannot establish in areas subject to $15\text{--}25$ d/yr $\leq 15^\circ\text{C}$. The model predicts no establishment of egg parasitoids in California or Texas (Fig. 10). Mean daily air temperature data (Fig. 4) support this conclusion; mean number of days per winter with mean daily air temperature $< 15^\circ\text{C}$ at all sites sampled in California (range, 63–134 d) greatly exceed that at Fort Pierce, FL (32 d).

Discussion

We assumed that soil temperature, particularly mean daily soil temperatures below the lower developmental limit of larvae, has limited the northern expansion of the range of *D. abbreviatus* in the state of Florida. The concentration of commercial citrus groves in central and southern Florida may also have influenced the southerly movement of *D. abbreviatus* from its original place of introduction at Apopka in the 1960s. However, given the wide host range of this weevil and the broad distribution of noncommercial citrus, citrus relatives, and other hosts north of Apopka, the lack of northerly dispersal suggests that climate plays a dominant role in determining geographic range. For this reason, we studied the effect of temperatures below the known developmental thresholds of eggs and larvae for this species on survival and oviposition behavior to generate a first es-

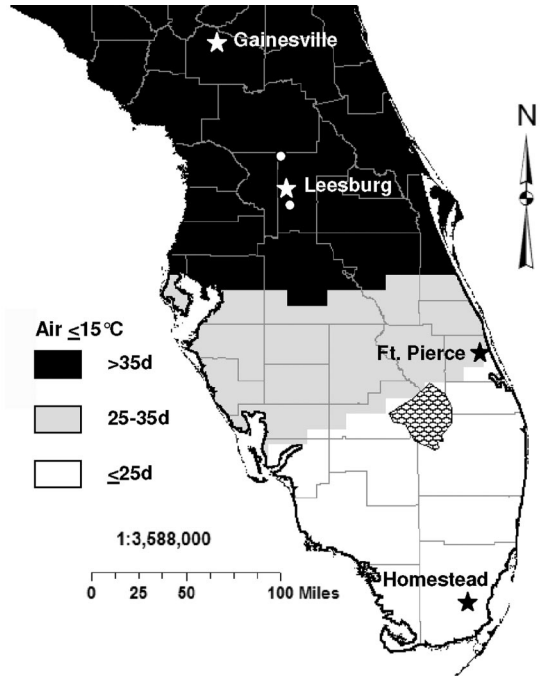


Fig. 9. Distribution map displaying the occurrence ≥ 5 of the last 10 yr of >35 , 25–35, and ≤ 25 d/yr with average daily air temperature $< 15^\circ\text{C}$ in Florida. Locations (stars) are University of Florida weather stations. Circles represent the northernmost record for *D. abbreviatus* in the state (north of Leesburg) and the original discovery of the species in the state at Apopka (south of Leesburg) by Woodruff (1964).

timete of the potential spread of *D. abbreviatus* in the recently infested states of California and Texas.

As is common among broad-nosed weevils (Otiorynchinae), two of the four life stages of *D. abbreviatus* are subterranean (larvae and pupae) and two are aerial (adults and eggs). Pupae were more susceptible to low temperatures compared with larvae.

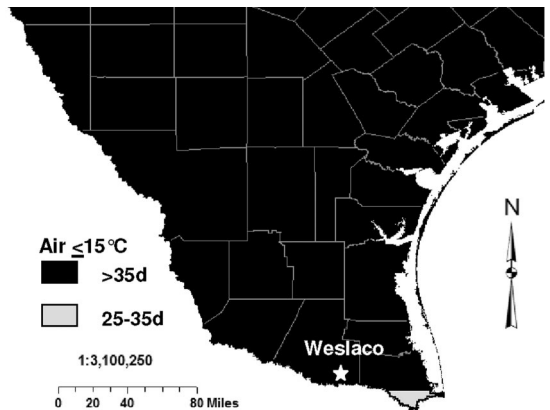


Fig. 10. Distribution map displaying the occurrence 5 or more years out of the last 10 of >35 , and 25–35 d per year with average daily temperature $< 15^\circ\text{C}$ in southern Texas.

Pupal morbidity was complete when held at 9°C for 14 d, whereas larvae suffered no measurable mortality at that temperature when exposed for up to 30 d (Table 2). The effect of temperatures $\leq 9^\circ\text{C}$ on pupae included mortality and deformities in the emerging adult, most typically expressed as deformed flight wings and elytra that render the adult nonviable, flightless, more susceptible to predation, or unable to mate. In addition to direct mortality and disruption of normal development, low temperatures under natural conditions may increase the susceptibility of larvae and pupae to mortality factors such as predators and pathogens as the insect becomes quiescent. We made no attempt to measure this.

The map of Florida based on 12°C soil temperature (Fig. 6) closely described the current distribution, particularly the northern extent, of *D. abbreviatus* that has been stable since its introduction into the state in the 1960s. Soil temperatures at Weslaco, TX, are similar to those in the northern range of *D. abbreviatus* in Florida around Leesburg. For this reason, Fig. 8 predicts favorable conditions of soil temperature for establishment at Weslaco and the counties immediately adjacent. Soil temperatures in the southern portions of Imperial County (CA) and Yuma County (AZ) resembled those of southern Florida. These areas may be highly susceptible to infestation by *D. abbreviatus*, whereas portions of Riverside and San Diego (CA) and La Paz (AZ) Counties may experience infestation to a lesser extent. Of particular interest was the limited area predicted by the climate homology model in Los Angeles, Orange, and San Diego Counties (CA) that corresponds to the currently known sites of establishment of *D. abbreviatus* in California (Fig. 7). This occurrence seems to validate the soil temperature model, at least for the time being. We expect that the map of California will be useful for prioritizing the deployment and use of resources for detection and current eradication efforts in that state. In south Texas, winter soil temperatures favorable for establishment occur in four counties (Cameron, Hidalgo, Starr, and Willacy) and the southern portions of four more (Brooks, Jim Hogg, Kenedy, and Zapata; Fig. 7).

Our predictive model of areas susceptible to invasion by *D. abbreviatus* is based on several assumptions. Among these, the primacy of temperature as a determinant of range of *D. abbreviatus* must be questioned. Low soil moisture may also limit larval development (Lapointe and Shapiro 1999) in areas where soil temperature is favorable. Similarly, a lack of nutritionally adequate foliage for adults in arid areas may limit adult dispersal where air temperatures are favorable. While many of the microclimatic areas of California that are likely to be exposed to *D. abbreviatus* are drier than Florida, irrigation of suburban and agricultural lands are likely to create adequate conditions for establishment.

We also assumed that the current distribution of *D. abbreviatus* in Florida resulted primarily from sub-optimal or lethal soil temperatures in northern Florida. A contributing factor that may have acted to constrain the movement of *D. abbreviatus* is the dis-

tribution of commercial citrus groves in Florida. Commercial citrus largely, but not completely, disappeared from northcentral Florida after catastrophic freezes in the 1980s. However, Simpson et al. (1996) reported 157 genera and ≈ 270 species in 59 plant families capable of serving as partial or complete hosts of *D. abbreviatus* including crops such as peanut (*Arachis hypogaea* L.), maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), ornamental trees, and others species. These are widely distributed in northern Florida. Also, there is no evidence to suggest that citrus is a preferred host of *D. abbreviatus*. Larval growth and survival of *D. abbreviatus* were greater on the legume *Cajanus cajan* Millspaugh (pigeon pea) compared with citrus and equivalent to that on citrus when reared on *Crotalaria pallida* Ait. (rattlebox), a naturalized legume found in most Florida counties and five other southeastern states (Lapointe 2003). Rather, it is likely that the presence of large contiguous groves of irrigated and fertilized citrus creates soil moisture and host quality conditions favorable to *D. abbreviatus*, and therefore, this generalist weevil seems to be a pest of citrus.

The lower developmental threshold for eggs of *D. abbreviatus* was estimated to be 12°C (Lapointe 2001). In that study, mortality of eggs held continuously at 12°C for >28 d was 100%, but no attempt was made to estimate lethal time (LTime). In this study, LTime₉₅ for eggs at 12°C was 4.2 d and declined to <24 h at 0°C. Adults were more resistant to cold, similar to larvae, and survived >7 d at 0°C (Table 2). Female *D. abbreviatus* ceased oviposition immediately when placed at 12°C and resumed oviposition when returned to 30°C. In addition, eggs oviposited during the week after the 2-wk exposure of adults to 12°C were nonviable, showing that air temperature $\leq 12^\circ\text{C}$ is not only lethal to eggs postoviposition but also to eggs present in the ovaries before oviposition, thereby constraining both the reproduction of *D. abbreviatus* during winter months and survival of egg parasitoid populations.

Efforts to establish biological control agents of *D. abbreviatus* in Florida are ongoing and include releases of the Eulophid egg parasitoids *Q. haitiensis* from Puerto Rico and *A. vaquitarum* from the Dominican Republic (Hall et al. 2001). Although *Q. haitiensis* and *A. vaquitarum* seem to have established in extreme southeastern Florida after multiple releases since 2000 (Peña et al. 2005), these species have not expanded their range into central Florida (Castillo et al. 2006). Castillo et al. (2006) estimated the lower development threshold for *Q. haitiensis* to be 16°C, or 4°C higher than that reported for eggs of *D. abbreviatus* (Lapointe 2001). Based on the oviposition threshold temperature (15°C) of *D. abbreviatus* and the failure of egg parasitoid establishment in central Florida, we conclude that winter air temperatures in Arizona, California, and Texas (>25 d/yr with mean daily temperatures $<15^\circ\text{C}$) are unfavorable for establishment of egg parasitoids throughout those states.

Castillo et al. (2006) suggested that winter temperatures in central Florida limit establishment of

Q. haitiensis when they fluctuate below the lower developmental threshold of the parasitoid. Our data reinforce the supposition that egg parasitoid distribution will be limited to southern Florida because of the absence of prey eggs for prolonged periods of time during the winter months in central and northcentral Florida where *D. abbreviatus* occurs. Fort Pierce appears to be the approximate northern limit to egg parasitoid establishment, as shown by the 2 yr of data from that location (Fig. 2). The winters of 2001–2002 and 2002–2003 were the warmest and coldest, respectively, during the period of 1998 through 2006 as measured by the number of days with mean daily temperatures below the lower threshold for oviposition by *D. abbreviatus* (Table 3). Our field counts of egg masses during those two winters show the effect of low temperatures on egg availability in the field (Fig. 2). During the warmer winter (2001–2002), there was a constant but low number of egg masses recorded throughout the winter. During the colder winter (2002–2003), there was a period of 141 d when no egg masses were observed. We predict that egg parasitoids of *D. abbreviatus* will experience periodic extinctions in the Fort Pierce area if releases are continued. These field results seem to validate the model for egg parasitoid establishment based on 15°C air temperature (Fig. 9).

Shipment of ornamental and nursery plants from Florida and the Caribbean infested with *D. abbreviatus* is the most likely means of spread of this weevil to other U.S. states. Because eggs and pupae are susceptible to cold at relatively high temperatures, the possibility of using cold to eliminate these life stages from palm trees, for example, may be considered. Eggs of *D. abbreviatus* are unlikely to survive if plants are held at 12°C for 5 d, whereas both eggs and pupae could be 95% controlled by a treatment of 3°C for 3 d. Unfortunately, considerably longer periods would be required to control larvae (>10 d) or adults (>25 d) at 3°C (Table 2).

The immediate application of the predictions our model has generated will be to inform the survey and eradication programs for the states of Arizona, California, and Texas of the areas with a high probability of permanent establishment of *D. abbreviatus*. We emphasize that this is a first attempt to delineate areas of high risk for establishment, and we are aware of the much higher degree of climate variability in California where locally unique microclimates may favor establishment outside of the zones indicated on our map (Fig. 8). Also, *D. abbreviatus* may confound our estimates by adapting to new environmental conditions over time. Empirical data on future movement and establishment of *D. abbreviatus* could be used to refine these predictions and to determine if adaptation is occurring.

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