Effects of Temperature on *Anoplophora glabripennis* (Coleoptera: Cerambycidae) Larvae and Pupae

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ABSTRACT Developmental thresholds, degree-days for development, larval weights, and head capsule widths for each larval instar and the pupal stage of *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) were studied at eight constant temperatures (5, 10, 15, 20, 25, 30, 35, and 40°C) for two source populations (Ravenswood, Chicago, IL [IL], and Bayside, Queens, NY [NY]). The estimated lower threshold temperature for development of instars 1–5 and the pupal stage was near 10°C and was near 12°C for the higher instars. Developmental rate was less temperature sensitive for instars 5–9 compared with instars 1–4. Development for all but the first instar was inhibited at constant temperatures >30°C, and all instars failed to develop at 40°C. Although the two source populations had similar responses to temperature, IL larvae were heavier than those from NY. Temperature and its influence on larval weight had profound impacts on whether a larva proceeded to pupation. Based on the temperature effects detailed here, larval development and pupation should be possible in most of the continental United States where suitable hosts are available. These data can be used to develop a degree-day model to estimate beetle phenology; however, at least 2°C should be added to air temperatures to adjust for the mediation of temperature by the wood. These data provide a basis for predicting the potential geographical range of this species and for developing phenological models to predict the timing of immature stages, both of which are important for management programs.

KEY WORDS temperature, survival, pupation, development, degree-days, Asian longhorned beetle

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae), referred to as the Asian longhorned beetle, is widely distributed in China (Lingafelter and Hoebeke 2002) and is present in South Korea (Williams et al. 2004). In China, it is considered a major pest of several deciduous broadleaf tree species (Yan and Qin 1992) and causes severe damage from 21 to 43° N latitude and from 100 to 127° E longitude (Yan 1985). The corresponding cold hardiness zones in North America extend from southern Canada to southern Mexico. The *A. glabripennis* infestations that have been discovered in North America to date are in the New York City area (August 1996), the Chicago area (July 1998), northern New Jersey (October 2002), the Toronto area, Ontario, Canada (September 2003), and most recently in Worcester, MA (August 2008) (Haack et al. 1997, Poland et al. 1998, Massachusetts Introduced Pests Outreach Project 2008, APHIS 2009). In the United States, the USDA Animal and Plant Health Inspection Service (APHIS) has implemented an eradication program using the only currently effective method for limiting its spread: the removal and destruction of all trees with signs of beetle infestation (e.g., oviposition pits or exit holes). The eradication program for *A. glabripennis* has resulted in the removal of thousands of trees and has cost millions of dollars (Nowak et al. 2001). However, if the established populations of *A. glabripennis* are not eradicated, the beetle could potentially move into urban, suburban, and forested areas throughout the range of its known hosts, causing damages in excess of $600 billion (Nowak et al. 2001) in urban tree costs alone. The potential for economic damage to the hardwood (e.g., lumber and maple sugar) and recreational industries (e.g., fall tourism) that depend on *A. glabripennis* host trees may be even greater.

Temperature has profound effects on insect life history processes such as development, survival, and reproduction. The response of insects to temperature can be important in predicting the potential geographical range of a species and in developing phenological models to predict population dynamics and the timing of various stages for planning control or survey programs. Little information exists on the effects of temperature on the life history of *A. glabripennis*. Previous research on the effects of temperature on *A. glabripennis*, both in China and in the laboratory, have focused on documenting the upper and lower temperature limits for activity (such as flight) and fecundity (Keena 2006, Zhou et al. 1984) among adult beetles. In China, *A. glabripennis* is either univoltine or bivoltine,

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primarily determined by the climate and the timing of oviposition; eggs that are laid in the fall may not hatch until the next year (Hua et al. 1992, Yan and Qin 1992). Although field studies in China have produced estimates of accumulated degree-days and lower developmental thresholds to complete a generation, more detailed data have been lacking (Yang et al. 2000). Keena (2006) and Zhang et al. (1995) have evaluated these parameters for eggs and first and second instars in the laboratory, but a complete assessment of the impacts of temperature on the complete life cycle of *A. glabripennis* has not been done. Effects of temperature on egg hatch and individual instars of *Anoplophora malasiaca* (Thomson) [now considered synonymous with *Anoplophora chinensis* (Forester) see Lingafelter and Hoebeke 2002], a closely related species, at 20, 25, and 30°C in citrus bolts were evaluated by Adachi (1994), but similar work on *A. glabripennis* larvae has not been reported.

In the United States, *A. glabripennis* females lay eggs from July to November (Lingafelter and Hoebeke 2002). First-instar larvae feed in the rotting cambium, second and early third instars feed on healthy phloem and outer layers of xylem, and late third and later instars feed on the xylem (Yan and Qin 1992). The ultimate instar creates a chamber near the outer bark where it pupates (usually leaving ≈1 cm of xylem for the adult to chew through). Most *A. glabripennis* pass the winter in the larval stage, and pupation usually occurs in the spring or summer (Haack et al. 2006). Thus, most of the larval period is spent well protected and insulated by the xylem from the outside environment. This life history makes it difficult to directly observe development in the field and makes it necessary to take the insulating effects of the wood into account when using laboratory-generated data to predict phenology in the field.

The purpose of this study was to determine the relationships between larval and pupal life history parameters and temperature for *A. glabripennis.* Time in instar/stage, weight, and head capsule widths were recorded over a range of constant temperatures. The mathematical relationships between temperature and developmental rates were developed to facilitate use of this information in developing phenology models and to compare the thermal responses of *A. glabripennis* with those of closely related species previously reported.

Materials and Methods

Populations and Temperatures. Individuals were from the fourth or fifth laboratory generations of populations established from adults that emerged from infested branch sections obtained at eradication tree cut sites in the United States and transported under permit to the USDA Forest Service quarantine facility in Ansonia, CT. The infested branch sections were obtained in February 1999 from the Ravenswood, Chicago, IL, infestation (1,450 adults, 41.58° N and 87.42° W) and April 1999 from the Bayside, Queens, NY, infestation (384 adults, 40.45° N and 73.45° W). Voucher specimens of both populations were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT.

Both populations were subjected to eight constant temperatures for these studies: 5, 10, 15, 20, 25, 30, 35, and 40°C, with temperature fluctuations within the environmental chambers typically remaining within 1°C of the set value. Beetles were subjected to a photoperiod of 16:8 (L:D) h. Humidity was passively maintained by placing open buckets (with 896 cm^2 of surface area) of water in the bottom of each chamber. One chamber (25°C), which had full humidity controls, did not require the open humidity source. The humidity in the chambers averaged 80 ± 10, 85 ± 15, 70 ± 10, 80 ± 5, 60 ± 5, 45 ± 5, 68 ± 4, and 10 ± 5% RH at 5, 10, 15, 20, 25, 30, 35, and 40°C, respectively. Actual humidity in the diet-filled petri dishes was likely much higher than in the chamber, because the diet was moist, and the petri dishes were held in opaque boxes. Because there were not enough chambers to run all of the temperatures simultaneously, the studies were set up over a period of 2 yr. In the first year, larvae from each population were held at 10, 15, 20, 25, and 30°C. In the second year, larvae were held at 5, 25 (for comparison with the data from year 1), 35, and 40°C. Additionally during the second year, some larvae were reared at 25°C to the third or fifth instar and then moved to 10, 15, 35, or 40°C to get an estimate of developmental time for these instars at temperatures near the lower or upper developmental thresholds. This method of moving larvae to assess development was previously used in studies on *Plutella xylostella* L. (Liu et al. 2002).

Obtaining Newly Hatched Larvae. Virgin adults were held individually in 950-ml glass jars with *Acer saccharum* Marshall (sugar maple) twigs (3–7 mm diameter with leaves removed) as a food source until they were single pair mated in 3.8-liter glass jars and provided an *A. saccharum* bolt (3–7 cm diameter and 20 cm long) with both ends waxed as an oviposition substrate. Twigs were cut fresh biweekly and held in plastic bags, bolts were cut monthly, and both were stored at 10°C and ≥80% RH until used for food or oviposition. Both twigs and bolts were changed weekly. Folded paper towels were placed in the bottoms of the jars to collect frass and excess moisture. Two holes (1–2 mm diameter) were drilled in the plastic jar lids to allow airflow. In the first year, some of the pairs were part of the study described in Keena (2006) and were held at 15, 20, or 25°C, whereas all of those for the second year were held at 25°C.

The oviposition bolts were replaced weekly until a female died or all the larvae needed for the study had hatched. After removal from the mating jars, the bolts were held at the same temperature as the mating pair to which they were exposed or moved to 10°C and held for 1–5 wk to stockpile eggs for use in this and other studies. Chilled bolts were incubated as needed at 25°C to obtain hatch. When not chilled, bolts were held 14, 8, and 6 d at 15, 20, and 25°C, respectively, before the bark was stripped and all eggs were counted and removed. This allowed all eggs to at least partially

embryonate, thereby reducing egg mortality caused by handling. Eggs removed from under bark were placed individually in the wells of a 24-well tissue culture plate or in 35 by 10-mm petri dishes with vented lids and held in a water box at the temperature at which the egg was laid. Eggs were checked daily for hatch and larvae were inserted into diet within 24 h of hatch.

Larval Set up and Monitoring. The artificial diet used in these studies was the *A. glabripennis* modification AG1 in the first year for the first 150+ d (depending on when the larva was set up) and AG2 for the rest of the first year diet changes and all of the second year work, both with $\leq$0.06 g $FePO_4$ added per liter of diet (Keena 2005). The change to the AG2 diet was made because of the increased antimicrobial properties that increased survival and reduced the frequency of diet changes. In addition, there were no known affects on larval developmental rate. For the 10–30°C temperature treatments during the first year, 1–7 larvae from each of 22 NY (66–73 total larvae per temperature) and 28 IL female parents (103–106 total larvae per temperature) were used. For the 25°C temperature treatment in the second year, 1–8 larvae from 17 NY (53 total larvae) and 25 IL (105 total larvae) were used. In the second year, progeny from 13–14 of the same females from each population (NY and IL) as used for the 25°C treatment were put into the 35 (1–5 larvae each, 104 total), 40 (1–3 larvae each, 66–67 total), and 5°C (1–3 larvae each, 66–67 total) treatments. An additional five sets of 1–4 larvae from each of 13 females from each population (total of 32 larvae per population) were set up at 25°C and reared to either the third or fifth instar and then moved to other temperatures. Larvae were set up over 207 and 103 d in the first and second year, respectively. The numbers of larvae used from each female and the number of females per population for all parts of the study were based on availability.

The newly hatched larvae were individually weighed and assigned a temperature treatment on a rotating basis for each mating pair independently (to keep equal numbers per treatment). Larvae were placed in a hollow (next to the dish bottom) created in a 1-cm^2 chunk of diet cut from the center of the 35 by 10-mm petri dish (one per dish) and then put back in place. The petri dishes were kept in an opaque box (45.7 by 27.9 by 15.2-cm heavy plastic box with overlapping lid) and held at the assigned temperature. These boxes kept the larvae in the dark except when opened to check for molts. The diet was changed after each larval molt or when the diet was unusable because of excessive tunneling, discoloration from fungal or bacterial growth, or excessive dehydration. The diet was changed at least every 7, 10, 14, 21, 30, and 30 d at 40, 35, 25, 20, 15, and 10°C, respectively. The diet was changed once after 200 d for larvae held at 5°C. The fresh diet used for set up or replacement was brought to the temperature of the larva for temperatures $<$20°C and to room temperature for temperatures $\geq$20°C before use. Extra dishes of diet were stored at 10 or 15°C until they were needed. All petri dishes used were of the vented type to prevent condensation and allow air exchange. They were not completely filled with diet to allow for frass build up. First- and second-instar larvae were set up in 35 by 10-mm, third instar in 60 by 15-mm, fourth instar in 100 by 15-mm, and fifth instar and up in 150 by 15-mm petri dishes. Second- through fourth-instar larvae were inserted in the diet the same way as the first instars, using larger chunks of diet with larger hollows created in the center; later instars were placed in canoe-shaped openings cut in the middle of the diet.

All larvae were observed daily, except at 5 and 10°C, where they were checked weekly, to determine whether they had molted, died, or required a diet change. The time that the larvae were at room temperature was kept to a maximum of 15 min to prevent the diet temperature from changing significantly and affecting the developmental rate. Larvae were weighed after each molt, and the shed head capsule from each larva was saved in a separate 1.5-ml microcentrifuge tube. If the shed head capsule could not be found, but the larva had obviously molted (as evidenced by an incompletely sclerotized head capsule), it was recorded as a molt that was not confirmed. Unconfirmed molts were carefully checked to determine whether the normal amount of time between molts had occurred. If the assumption of a molt was not supported by the data, the larva was dropped from the study. If a larva appeared to be dead, the date was recorded, and the larva was held for 2 d to ensure death had occurred (occasionally when larvae are near a molt they appear dead). If the larva died because of human error (i.e., smashed or damaged), it was dropped from the study, and if possible, was replaced with a newly hatched larva from the same population and female.

First instars held at 5°C were weighted after 200 d, and larvae that survived to 300 d were moved to 25°C and monitored daily until they molted. All larvae held at a constant 10°C were moved to 25°C at 390 d after the start of the study (all surviving larvae were at least 300 d old at the time). One half of the larvae that had not pupated at 15°C were moved to 25°C at 737 d after the start of the study (all surviving larvae were at least 500 d old at the time). After another 107 d, any larvae held at 15°C that had not pupated were moved 25°C. All larvae that were moved to 25°C were allowed to complete development to determine sex, some requiring a chill at 10°C before they would pupate. At the other temperatures, year 1 larvae were chilled 48 h after molting to the ninth instar or if they spent 120+ d in an instar without a molt. One half of the year 2 larvae held at 25°C were chilled at the seventh instar, and the other half were only chilled if they spent $>$120+ d in an instar without a molt. These protocols were put in place because some larvae apparently require a chill period before they will progress to pupation, and larvae held at low temperatures may never molt or pupate without a period at a higher temperature.

When the larva became a prepupa (larva shortened, became limp, and began to clear just behind the head),

it was moved to a 50-ml container with a piece of damp paper towel and placed in an opaque water box (same box as use for the larvae but with a piece of plastic egg crating in the bottom to hold the containers above the water) to help maintain the moisture level. Pupae were weighed 24 h after they were first observed then returned to the 50-ml container. Once the adult emerged, it was kept in a dry opaque box until it was fully sclerotized and began to chew the paper towel. The adult was sexed, weighed, and given twigs to feed on. Adults were used for other studies as needed.

Statistical Analyses. The following dependent variables were analyzed in PROC MIXED (SAS Institute 1999) using restricted maximum likelihood (REML) estimation methods, which is a statistical approach to provide unbiased estimates of variance in unbalanced designs: developmental time (d), larval/pupal weights (mg), and head capsule widths (mm) for each temperature. Females and males were analyzed separately in all cases. The model used temperature, population (New York or Illinois) and the interaction between the two as fixed effects, whereas maternal family was treated as a random effect. Year was included for the 25°C temperature treatment by distinguishing the 2 yr in the data and accounted for by controlling for the maternal families that were different between years. Differences among means were determined using the Tukey-Kramer test with $\alpha = 0.05$ (SAS Institute 1999).

The relationship between larval weight at the beginning of the instar and the width of the head capsule was described using a least squares linear regression (Statistix 2008) after the data had been log transformed. The relationships between temperature (t) and larval developmental rate or pupal development (r) were described using linear regression where $r = a + bt$. Temperatures near the lower developmental threshold (T_L) or above the optimal temperature were not included; only temperatures from the linear portion of the curve were used. The lower developmental threshold (T_L) was estimated as $-a/b$, where a and b were estimated by least squares regression (Statistix 2008). A nonlinear relationship (Briere et al. 1999) was evaluated but not ultimately used because of its poor fit to the data, especially near the optimal temperature. The relationship between instar and the slope estimated from temperature versus development regression was also described using two separate linear regressions (Statistix 2008): one for instars 1–4 and a second for instars 4–9.

The cumulative distributions of time in instar at 15, 20, 25, 30, and 35°C and degree-days needed for completion of each instar/stage were calculated. The degree-days for each instar/stage were calculated for each larva separately by subtracting the estimated T_L from the constant holding temperature and then multiplying by the number of days in that instar/stage. Temperatures close to the upper or lower thresholds of development were not used because plots of temperature and development produced polytonic curves, which may indicate genetic variation in response. The data for the NY and IL populations were pooled to provide the most robust estimations of degree-day requirements. The cumulative proportion of individuals (P) over accumulated degree-days (DD) was described using a Gompertz function,

$$P = \exp[-\exp(-b\mathrm{DD} + a)]$$

in which a and b are the lag and the rate of increase, respectively (Brown and Mayer 1988, PROC NLIN and Marquardt convergence method, SAS Institute 1999). Predicted accumulated degree-days for 10, 50, 90, and 99% of the population to complete each instar/stage were calculated.

Results

Developmental Time and Mortality. At temperatures ≤15°C, development was slow or did not occur, and mortality was variable. At 5°C, no first-instar larvae molted after being held for 300 d. The 39.4 and 63.6% (NY and IL populations, respectively) of the larva that survived to 300 d took 8.31 ± 0.56 and 7.43 ± 0.28 d (NY and IL populations, respectively) to molt to the second instar after being moved to 25°C. This is longer than it would normally take at 25°C, despite the fact that the larvae did gain weight at 5°C, as evidenced by the 200-d weights (7.1 ± 2.0 mg for NY and 6.6 ± 1.0 mg for IL; see Table 5 for average hatch weights). Some first-instar larvae held continuously at 10°C were able to molt one to four times (Table 1), but 86.1 and 68.0% of the NY and IL larvae, respectively, had died by the end of the treatment. Many of the third-instar larvae that were moved to 10°C molted (69% of the NY and 45.2% of the IL; Table 2). Of the remaining larvae, 13.8 and 45.2% of the NY and IL larvae, respectively, died, and the others were alive but had not molted by the end of the treatment (Table 2). Mortality of the fifth-instar larvae that were moved to 10°C was low (0.0% for the NY and 12.5% for the IL), and only about one quarter (27.6% for the NY and 25.0% for the IL) molted before the treatment was ended (Table 2). One of the fifth-instar NY larvae that did not molt became a prepupa at 10°C and remained in that stage for the 136d it was held at 10°C; it only progressed to pupation once it was moved to 25°C. Most of the fifth-instar larvae moved to 15°C molted (80.0% NY and 60.9% IL; Table 2) or pupated (16.0% NY and 21.7% IL), and only 4% from each population died.

The number of days spent in each instar/stage generally decreased as temperature increased from 10 to 30°C (Table 1; Figs. 1 and 2). Developmental time for the immature stages differed significantly between temperatures or the interaction between population and temperature was significant for all but the 13th and 14th instars (Table 1). Mortality was lowest at 25°C and increased at temperatures both above and below that. For example, after 52 wk, the mortality was 52.9 and 39.8 at 15°C, 41.4 and 49.0 at 20°C, 31.7 and 27.2 at 25°C, and 76.8 and 68.0% at 30°C for NY and IL, respectively.

The relationships between temperature and developmental rate and the estimated lower (T_L) thresholds for each immature stage are given in Table 3. The T_L for the first five instars and the pupa was close to

Table 1. Developmental time (d) of immature stages of *A. glabripennis* at seven constant temperatures [mean ± SE (*n*)]

F-statistics	Population	Instar/ stage	Temperature (°C)						
			10	15	20	25	25 y2	30	35
Pop. × Temp: $F = 13.68$, df = 6, 726, $P < 0.0001$	IL	1	158.2 ± 1.1a (39)	15.7 ± 0.8c (84)	8.5 ± 0.8df (73)	5.3 ± 0.8defg (90)	4.5 ± 0.7efg (98)	4.1 ± 0.8g (83)	3.4 ± 1.1g (45)
	NY	1	139.4 ± 1.7b (16)	15.2 ± 1.0c (50)	8.7 ± 1.0de (54)	5.3 ± 0.9defg (61)	4.3 ± 1.0defg (49)	4.0 ± 1.0fg (54)	3.3 ± 1.1g (49)
Pop. × Temp: $F = 2.16$, df = 6, 659, $P = 0.0448$	IL	2	67.5 ± 2.8a (21)	33.6 ± 1.6b (76)	12.8 ± 1.6c (70)	7.6 ± 1.5cd (89)	7.4 ± 1.4cd (97)	5.5 ± 1.5d (80)	5.9 ± 2.2cd (33)
	NY	2	65.9 ± 4.2a (9)	41.9 ± 2.0b (44)	11.0 ± 1.9cd (52)	6.9 ± 1.7cd (60)	7.1 ± 2.0cd (46)	4.7 ± 1.8cd (53)	6.1 ± 2.1cd (43)
Pop. × Temp: $F = 6.09$, df = 5, 553, $P < 0.0001$	IL	3	70.3 ± 4.9b (9)	97.5 ± 1.9a (65)	17.0 ± 1.9c (66)	9.9 ± 1.6cd (88)	10.6 ± 1.6cd (94)	7.8 ± 1.7d (77)	NA
	NY	3	64.9 ± 6.59b (5)	79.5 ± 2.5b (36)	16.7 ± 2.2cd (48)	10.1 ± 2.0cd (57)	10.4 ± 2.2cd (46)	7.6 ± 2.1cd (50)	10.0cd (1)
Temp: $F = 97.35$, df = 5, 519, $P < 0.0001$	IL	4	NA	40.4 ± 1.6a (62)	24.8 ± 1.6b (63)	15.6 ± 1.4c (86)	15.3 ± 1.4c (92)	12.0 ± 1.5c (73)	NA
	NY	4	66.0a (1)	48.3 ± 2.1a (34)	26.4 ± 1.9b (46)	15.6 ± 1.7c (54)	15.3 ± 1.9c (45)	13.9 ± 1.9c (48)	NA
Pop. × Temp: $F = 10.37$, df = 4, 491, $P < 0.0001$	IL	5	NA	56.4 ± 3.5b (57)	29.2 ± 3.4cd (59)	19.0 ± 2.9cd (83)	19.4 ± 2.8cd (91)	14.9 ± 3.1d (69)	NA
	NY	5	NA	97.1 ± 4.7a (30)	32.4 ± 3.9c (45)	19.0 ± 3.6cd (52)	21.7 ± 4.0cd (43)	14.9 ± 3.9d (44)	NA
Pop. × Temp: $F = 3.8$, df = 4, 464, $P = 0.0045$	IL	6	NA	111.1 ± 5.3b (56)	44.6 ± 5.2d (58)	23.0 ± 4.5d (79)	24.3 ± 4.2de (91)	20.7 ± 5.0e (63)	NA
	NY	6	NA	143.1 ± 8.0a (25)	72.6 ± 6.1c (43)	20.8 ± 5.6de (51)	33.1 ± 6.1de (42)	18.7 ± 6.5de (37)	NA
Pop. × Temp: $F = 5.2$, df = 4, 415, $P = 0.0004$	IL	7	NA	127.4 ± 7.1a (47)	74.3 ± 6.6b (53)	28.0 ± 5.6c (76)	37.3 ± 5.1c (90)	22.5 ± 6.5c (56)	NA
	NY	7	NA	95.8 ± 11.7ab (17)	116.1 ± 7.8ab (38)	38.5 ± 6.9c (49)	37.0 ± 8.2c (35)	20.2 ± 8.4c (33)	NA
Temp: $F = 22.4$, df = 4, 308, $P < 0.0001$	IL	8	NA	98.6 ± 8.8a (35)	107.1 ± 8.1a (42)	33.3 ± 6.2cd (74)	46.2 ± 6.8bcd (61)	23.8 ± 7.5d (49)	NA
	NY	8	NA	92.1 ± 18.0abc (8)	76.7 ± 11.2ab (21)	35.6 ± 8.0bcd (43)	47.6 ± 10.7bcd (24)	24.4 ± 10.4d (25)	NA
Pop. × Temp: $F = 3.9$, df = 4, 184, $P = 0.0044$	IL	9	NA	104.6 ± 10.0a (13)	57.5 ± 8.4bc (22)	31.1 ± 6.2d (69)	40.1 ± 8.4c (29)	24.7 ± 7.3d (34)	NA
	NY	9	NA	137.0abcd (1)	86.2 ± 11.0ab (11)	38.8 ± 7.8cd (37)	115.9 ± 14.1ab (9)	20.1 ± 9.2cd (20)	NA
Pop. × Temp: $F = 8.8$, df = 34, 127, $P < 0.0001$	IL	10	NA	31.0 ± 17.6ab (2)	47.2 ± 10.4b (11)	38.0 ± 7.7b (59)	54.4 ± 11.0ab (21)	29.7 ± 8.3b (31)	NA
	NY	10	NA	**NA**	105.2 ± 12.0a (7)	33.0 ± 9.4b (26)	20.0 ± 34.2ab (2)	35.3 ± 10.3b (17)	NA
Temp: $F = 7.2$, df = 4, 38, $P = 0.0002$	IL	11	NA	56.0b (1)	NA	32.2 ± 7.7b (22)	59.4 ± 8.7ab (17)	26.2 ± 8.3b (19)	NA
	NY	11	NA	**NA**	175.0a (1)	36.9 ± 13.6b (7)	106.0 ± 25.5ab (2)	22.5 ± 12.7b (8)	NA
Temp: $F = 48.9$, df = 3, 18, $P = 0.0002$	IL	12	NA	165.0a (1)	NA	32.3 ± 4.2b (10)	26.1 ± 5.3b (8)	26.9 ± 3.5b (14)	NA
	NY	12	NA	**NA**	NA	28.6 ± 8.5b (2)	17.0b (1)	19.6 ± 6.6b (5)	NA
NS	IL	13	NA	53.0a (1)	NA	44.3 ± 6.1a (9)	23.3 ± 6.9a (7)	26.8 ± 5.3a (12)	NA
	NY	13	NA	**NA**	NA	30.5 ± 13.0a (2)	16.0a (1)	18.8 ± 8.2a (5)	NA
NS	IL	14	NA	55.0a (1)	NA	43.3 ± 10.5a (7)	22.3 ± 11.3a (6)	30.0 ± 8.8a (10)	NA
	NY	14	NA	**NA**	NA	15.0a (1)	16.0a (1)	43.4 ± 12.4a (5)	NA
Temp: $F = 237.0$, df = 4, 226, $P < 0.0001$	IL	Pupa	NA	47.4 ± 0.9a (11)	26.4 ± 0.4b (47)	17.5 ± 0.4c (59)	17.8 ± 0.4c (53)	12.4 ± 1.0d (9)	NA
	NY	Pupa	NA	54.0a (1)	26.4 ± 0.6b (38)	17.1 ± 0.6c (37)	17.2 ± 0.6c (32)	13.2 ± 0.9d (11)	NA
Temp: $F = 49.0$, df = 4, 227, $P < 0.0001$	IL	Adult	NA	703.9 ± 24.3a (11)	409.0 ± 12.6b (48)	358.3 ± 11.6c (59)	245.4 ± 12.2e (53)	299.9 ± 27.1ce (9)	NA
	NY	Adult	NA	521.0abcd (1)	427.4 ± 14.1b (38)	339.8 ± 14.2c (37)	258.3 ± 15.5de (32)	294.7 ± 25.1ce (11)	NA

The IL population was from Ravenswood and the NY population was from Bayside. Within instars (across all temperatures and for both stains), means followed by the same letter are not significantly different based on Bonferroni test with $\alpha = 0.05$ (SAS Institute 1999). The data for 10–30°C were obtained in the first year and the 25 y2, and 35°C data were obtained in the second year. The days spent in chill at 10°C were subtracted from the total for the instar in which they occurred.

NA, parameter not applicable because there were no larvae or no molts at these temperatures for these instars; NS, no significant findings.

Table 2. Developmental time (d) of immature stages of *A. glabripennis* moved from 25°C to four constant temperatures at either the beginning of the third or fifth instar [mean ± SE (*n*)]

Population	Instar	Temperature (°C)			
		10	15	35	40
IL	3	261.9 ± 5.0 (14)	NA	NA	NA
NY	3	241.9 ± 4.2 (20)	NA	NA	NA
IL	5	282.2 ± 16.0 (6)	203.1 ± 9.8 (14)	17.6 ± 8.7 (23)	All 23 died
NY	5	304.8 ± 10.7 (8)	190.8 ± 8.9 (20)	20.7 ± 9.0 (21)	All 22 died

The IL population was from Ravenswood and the NY population was from Bayside. These data were obtained in the second year, and larvae are from the same families as used in the constant temp work.

NA, parameter not applicable because no larvae at these temperatures for these instars.

10°C, whereas T_L for the higher instars was closer to 12°C. Larvae in the fifth instar and higher responded less to temperature changes than did those in the lower instars, as evidenced by the estimated slopes of the developmental rate curves (Fig. 3). The linear relationship for the first four instars was described by the following formula: slope of developmental rate = (0.0149 ± 0.0003) + (−0.00278 ± 0.0009) × instar (adjusted R^2 = 0.997). The linear relationship for instars 4–9 was described by the following formula: slope of developmental rate = (0.0050 ± 0.0003) + (−0.00031 ± 0.00005) × instar (adjusted R^2 = 0.895).

The relationship between the instar at which larvae received a chill and the number of instars they went through before pupation for the 15–30°C range is given in Table 4. In addition to the variation in ultimate instars, several larvae from the first year of the experiment spent >100 d in either their ultimate instar or in one of the two preceding instars; 0 larvae at 30°C, 3–4 at 25°C, 30 at 20°C, and 15–16 at 15°C per population went through an extended instar. The number of larvae with extended instars was higher in the 25°C group from the second year; 11 NY and 13 IL individuals spent >100 d in their ultimate or penultimate instar. Although Table 1 only provides data up to the 14th instar, at 25 and 30°C, 23 larvae went through 15–21 instars before they died, and another 2 pupated in the higher instars (Table 4).

At temperatures >30°C, larvae survived for a short period of time and development either did not occur or larvae that molted were small and more translucent. All the larvae held at 40°C died without molting, first instars survived 9.35 ± 0.88 and 9.33 ± 0.61 d, and moved fifth instars survived 14.59 ± 1.11 and 13.17 ± 0.91 d for the NY and IL populations, respectively. At 35°C, most first instars were able to molt at least twice (Table 1), but larvae survived only a total of 38.70 ± 1.87 and 38.45 ± 2.47 d for the NY and IL populations, respectively. Most fifth-instar larvae moved to 35°C molted (Table 2) and a few died without molting (NY, 16.0%; IL, 8.0%). The upper developmental thresholds for all the instars seems to be between 35 and 40°C based on these development and mortality data, al-

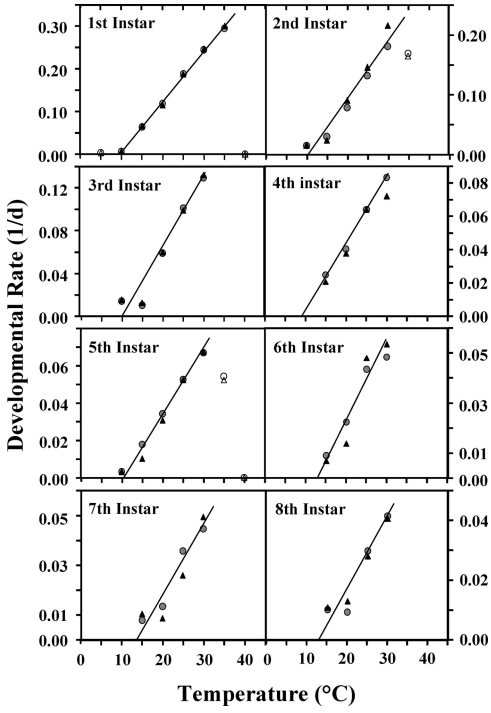

Fig. 1. Relationships between temperature and mean developmental rate (1/d) for each larval instar of *A. glabripennis*. The line represents the estimated linear relationship using both populations (Ravenswood, IL, circles; Bayside, NY, triangles) and the observed points (only the solid markers were used for line estimation) are shown.

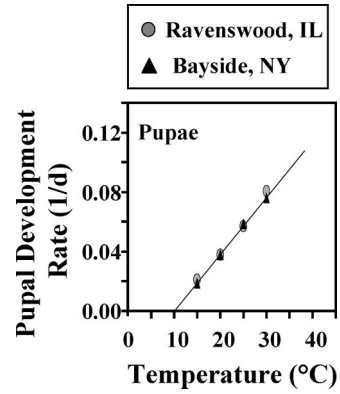

Fig. 2. Relationship between temperature and mean pupal development rate (1/d) for *A. glabripennis*. The line represents the estimated linear relationship using both populations and observed points are shown.

Table 3. Parameter values for linear models used to describe the relationships between temp and time spent in each immature stage of *A. glabripennis*

Instar/stage	n^a	Slope ± SE	Intercept ± SE	F	df	P	Adjusted R^2	Temperature range (°C)	T_L (°C)
1	10	0.0120 ± 0.0002	−0.1160 ± 0.0043	3514.5	8, 9	<0.0001	0.997	10–30	9.66
2	10	0.0095 ± 0.0008	−0.0988 ± 0.0166	149.8	8, 9	<0.0001	0.943	10–30	10.32
3	10	0.0064 ± 0.0006	−0.0648 ± 0.0125	119.0	8, 9	<0.0001	0.929	10–30	10.11
4	8	0.0038 ± 0.0003	−0.0346 ± 0.0064	187.4	6, 7	<0.0001	0.964	15–30	9.10
5	10	0.0033 ± 0.0001	−0.0327 ± 0.0030	560.2	8, 9	<0.0001	0.984	10–30	9.81
6	8	0.0031 ± 0.0004	−0.0397 ± 0.0092	62.3	6, 7	0.0002	0.897	15–30	12.67
7	8	0.0027 ± 0.0004	−0.0355 ± 0.0089	47.8	6, 7	0.0005	0.870	15–30	13.30
8	8	0.0022 ± 0.0003	−0.0264 ± 0.0067	57.0	6, 7	0.0003	0.889	15–30	12.06
9	8	0.0024 ± 0.0003	−0.0293 ± 0.0061	83.1	6, 7	0.0001	0.921	15–30	12.26
Pupa	8	0.0039 ± 0.0001	−0.0394 ± 0.0026	1240.0	6, 7	<0.0001	0.994	15–30	10.10

Parameter values are means followed by SEs. In the table, T_L = lower temp threshold. The temperature range used did not always include all the data points because some were not part of the linear potion of the response line based on preliminary plots.

[a] This n is the no. of observations used in the linear model, equivalent to the no. of temperatures used multiplied by the no. of populations included (always 2).

though larvae can survive for a time at these temperatures.

Larval Weights and Head Capsule Sizes. First-instar larvae from the IL population were generally heavier than those from the NY population (Table 5), but within each of the first year temperature treatments, there were no significant difference in weights between populations. Larval weights within an instar differed significantly by temperature (except for instar 14), and pupal weights differed significantly by sex within population within each temperature treatment (Table 5). By the third instar, the weights of the larvae held at constant 20–30°C were significantly heavier than those held at temperatures above or below that range. Head capsule widths differed significantly by temperature (except for instar 14; Table 6). The linear relationship between the log of the larval weight at the beginning of an instar (WT) and the log of the head capsule width (HC) was described by the following formula: log (HC) = (0.67 ± 0.0008) + (0.28 ± 0.0006) × WT (adjusted $R^2 = 0.980$).

Degree-Days Required for Each Instar and Stage. The temperature ranges used for each instar/stage had to be carefully chosen. Some of the cumulative proportion versus DD curves for temperatures near the T_L had what appeared to be a second plateau in the middle of the curve (see Fig. 4 for the 15°C curve for the third instar). The Gompertz function provided an excellent fit to the data for the temperature ranges chosen, with an adjusted $R^2 \approx 0.99$ for all but the ultimate instar (Table 7). The predicted degree-day accumulations at which 50 and 90% of the population will complete each instar are only separated by ≈30 DD in the lower instars and reach a maximum separation in the ultimate instar (>500 DD), which tends to vary substantially in length (Table 7).

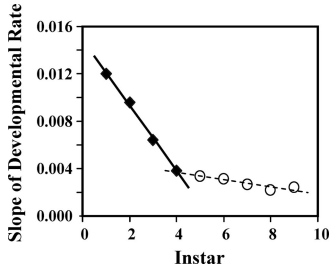

Fig. 3. Relationships between the slopes of the linear developmental rate versus temperature (Fig. 1) and instar for *A. glabripennis*. The solid line was fitted for instars 1–4 ($y = -0.0028x + 0.0149$, $R^2 = 0.99$) and the dashed line for instars 4–9 ($y = -0.0003x + 0.0051$, $R^2 = 0.95$).

Discussion

Temperature had a significant impact on all the life history parameters assessed in this study. The relationship between temperature and development duration in days was linear between 10 and 30°C, and the lower threshold for development was estimated to be between 9 and 13°C for the immature stages. As temperatures increased above 30°C, the rate of larval development decreased, and the lethal upper threshold was between 35 and 40°C. Larvae in instars 5–9 had a lower developmental response to increasing temperatures than those in the first four instars. The two populations had very similar responses to temperature, but the NY larvae survived and developed better at 30°C than the IL larvae, and the reverse was true at 5°C. The two populations also differed in weight; the IL population consistently weighed more at the beginning of each instar, although it was only significantly different for the 25°C treatment in the second year for instars 4–9. These results represent the first complete description of these relationships for all the immature stages over this broad a temperature range and will greatly facilitate development of phenology models and predictions of potential geographic range that are essential for effective control and eradication efforts.

The lower developmental thresholds estimated in this study are lower than those estimated for *A. glabripennis* larvae in the field in Shangdong, China (15.2 ± 1.9°C, Yang et al. 2000) and for first (11.7 ± 0.9°C) and second instars (11.4 ± 0.8°C) in the laboratory in Inner Mongolia, China (Zhang et al. 1995).

Table 4. Number of *A. glabripennis* larvae that pupated at each instar, with (112 d at 10°C) and without (NC) a chill period at the instar indicated, for four constant temperatures

Ultimate Instar	Population	Rearing temperature (°C)/larval instar at chill																	
		15°C	20°C					25°C first year					25°C second year						30°C
		NC	6	7	8	9	NC	7	8	9	10	NC	6	7	8	9	10	11	NC	9
6	IL						3												1	
7	IL	3					6												7	
8	IL	5			1		16		1			1		22	3				7	
9	IL	3				9	1			8				21		1			5	
10	IL					11				34							1			6
11	IL									9	1					1		3	2	1
12	IL									1								1		2
13	IL									1[a]										
15	IL									1[a]										
16	IL									2										
6	NY	1	2				2						3						3	
7	NY		1	2			10	2						8					2	
8	NY						11	1	2					10	1				3	
9	NY					3	1			10				1		3				1
10	NY					6				17										9
11	NY					1				4	1								1	1

Year 1 larvae held at 20–30°C were chilled at the ninth instar or after 120+ d without a molt at a lower instar. One half of the year 2 larvae held at 25°C were chilled at the seventh instar and the other half were only chilled if they spent >120+ d in an instar without a molt. Only individuals that made it successfully to adult were included (an additional 21 larvae pupated, all temperatures combined, but died before becoming adults).

[a] Both these larvae were chilled a second time during the instar in which they finally pupated.

The lower developmental thresholds reported for *A. malasiaca* for instars 1–7 are similar to those we obtained for *A. glabripennis*, although the trend for the lower threshold to be higher for larvae in instars ≥6 than for the earlier instars was not seen in *A. malasiaca* (Adachi 1994). These differences could be because the other laboratory studies used only the 20–30°C temperature range in the estimations. In addition, differences in the larval nutritional substrate could have influenced developmental rate in the other studies, and *A. glabripennis* larvae seem to require longer to complete instars greater than four than larvae of *A. malasiaca.*

The estimated degree-days for first and second instars done in the laboratory in Inner Mongolia, China, were greater than those estimated here, whereas our estimated degree-days for the pupal stage was higher (Zhang et al. 1995). This is probably partly because of the differences in our estimates of the lower developmental thresholds. Smith et al. (2004) estimated that 50% of the adults would emerge under natural conditions in China after 950 DD (when the accumulation started on 1 January and an arbitrary lower threshold of 10°C was used). Their field estimates correspond fairly well with estimates of the degree-days necessary for 50% of our larvae chilled after they reached the seventh instar (924.6 DD) to reach adult and are less than that for the larvae chilled after they reached the ninth instar (1,195.1 DD). This would suggest that *A. glabripennis* under natural conditions in China went though 7 or 8 instars, which were also the ultimate instars of the major of the larvae that proceeded to pupation without a chill.

Temperature and its influence on larval weight had strong impacts on whether larvae proceeded to pupation. Larvae had to reach at least 500 mg to pupate, and most pupated between 1,000 and 2,000 mg when reared on the artificial diet used in this study. This could indicate that there is a threshold weight for pupation, as has been documented for another Lamiinae, *Psacothea hilaris* (Pascoe) (Coleoptera: Cerambycidae, Munyiri et al. 2004). At 15°C, it took much longer for larvae to reach this size. Moreover, even when they did reach the size larvae normally pupate at (1,000–2,000 mg), many did not proceed to pupation even in 2 yr. This suggests there may be a lower temperature (that varies between individuals) that must be exceeded for larvae to progress to pupation. At 20°C, most of the larvae were able to proceed to pupation without either a chill period or exposure to higher temperatures. Only a few larvae at 25°C and no larvae at 30°C were able to proceed to pupation without a chill period, and the timing of the chill period determined the instar at pupation. Most larvae that were chilled pupated either without molting or after a single molt. Therefore, the threshold weight would have to be reached before the chill or with little additional growth after chill. *A. malasiaca* larvae also did not proceed to pupation at constant 25 or 30°C and died after going through many supernumerary molts (Adachi 1994). The threshold weight combined with the temperature gates would prevent adult emergence at the wrong time of year and aid in further synchronizing the adults. Thus, larvae that are too small to pupate in the spring would grow through the summer and overwinter before progressing to pupation, creating the biennial portion of the population documented in cooler regions of China (Hua et al. 1992).

Larval food quality or condition apparently can reduce the need for chill at the higher temperatures because more larvae proceeded to pupation at 25°C in the second year on the diet with improved antimicro-

Table 5. Weights (mg) at the beginning of the immature stages of *A. glabripennis* at seven constant temperatures [mean ± SE (*n*)]

F-statistics	Population	Instar/ stage	Temperature (°C)						
			10	15	20	25	25 y2	30	35
Pop.: $F = 19.8$, df = 1, 1149,	IL	1	5.3 ± 0.1abc (105)	5.5 ± 0.1ab (103)	5.4 ± 0.1abc (106)	5.4 ± 0.1abc (105)	5.9 ± 0.2a (105)	5.5 ± 0.1abc (105)	5.9 ± 0.2a (51)
$P < 0.0001$	NY	1	4.9 ± 0.2c (73)	4.9 ± 0.2c (72)	4.9 ± 0.2c (73)	4.9 ± 0.2c (66)	5.0 ± 0.2bc (54)	4.8 ± 0.2c (73)	5.3 ± 0.2abc (53)
Temp.: $F = 4.4$, df = 6, 725,	IL	2	13.3 ± 0.7ab (39)	13.0 ± 0.5b (84)	13.5 ± 0.5ab (73)	13.7 ± 0.5ab (90)	15.8 ± 0.5a (98)	14.0 ± 0.5ab (83)	14.5 ± 0.7ab (45)
$P = 0.0002$	NY	2	10.9 ± 1.0b (16)	11.9 ± 0.6b (50)	12.5 ± 0.6b (54)	12.8 ± 0.6b (61)	14.4 ± 0.7ab (49)	12.9 ± 0.6ab (53)	14.3 ± 0.8ab (49)
Temp.: $F = 43.6$, df = 6, 658,	IL	3	34.1 ± 3.3gh (21)	41.1 ± 1.9efgh (76)	51.5 ± 2.0cd (70)	60.1 ± 1.8ab (89)	65.8 ± 1.8a (97)	56.5 ± 1.9bc (80)	38.7 ± 2.8fgh (33)
$P < 0.0001$	NY	3	25.3 ± 4.9h (9)	37.2 ± 2.4gh (44)	43.0 ± 2.3defg (52)	50.0 ± 2.2cdef (59)	60.1 ± 2.5abc (46)	50.54 ± 2.3bcde (53)	35.1 ± 2.7gh (43)
Temp.: $F = 61.0$, df = 6, 550,	IL	4	61.4 ± 15.0f (9)	97.8 ± 6.4f (65)	163.2 ± 6.3cd (66)	183.0 ± 5.7bc (88)	225.1 ± 5.6a (94)	170.3 ± 6.0cd (76)	NA
$P < 0.0001$	NY	4	70.4 ± 19.8ef (5)	94.3 ± 8.2f (34)	143.0 ± 7.3cd (48)	153.0 ± 6.8cd (57)	206.7 ± 7.6ab (46)	139.9 ± 7.2de (50)	56.0bcdef (1)
Temp.: $F = 84.8$, df = 5, 515,	IL	5	63.0def (1)	214.9 ± 15.5f (61)	402.7 ± 15.2ab (63)	436.6 ± 13.4bc (86)	573.7 ± 13.3a (92)	351.3 ± 14.4de (71)	NA
$P < 0.0001$	NY	5	NA	217.0 ± 19.7f (34)	366.0 ± 17.7cd (45)	374.8 ± 16.4cd (54)	506.1 ± 18.2ab (45)	286.1 ± 17.3ef (48)	NA
Temp.: $F = 82.8$, df = 4, 485,	IL	6	NA	456.5 ± 32.6e (56)	756.5 ± 32.1bc (58)	767.8 ± 28.1b (82)	1,062.5 ± 27.7a (91)	568.2 ± 30.0de (69)	NA
$P < 0.0001$	NY	6	NA	432.4 ± 43.8e (26)	615.9 ± 35.7cd (45)	638.1 ± 33.9bcd (52)	983.3 ± 38.5a (42)	442.8 ± 36.1e (44)	NA
Temp.: $F = 72.9$, df = 4, 436,	IL	7	NA	755.0 ± 49.6cd (50)	1,062.9 ± 47.9b (55)	1,076.3 ± 41.9b (78)	1,562.4 ± 40.5a (90)	736.9 ± 45.4cd (63)	NA
$P < 0.0001$	NY	7	NA	715.0 ± 74.7cd (18)	815.3 ± 55.2c (39)	915.1 ± 50.4bc (50)	1,415.1 ± 59.9a (42)	578.7 ± 57.1d (36)	NA
Temp.: $F = 51.4$, df = 4, 336,	IL	8	NA	966.8 ± 77.1cd (37)	1,280.7 ± 72.3b (46)	1,259.2 ± 63.4b (74)	1,883.7 ± 68.3a (90)	809.3 ± 68.6cd (56)	NA
$P < 0.0001$	NY	8	NA	940.0 ± 126.6bcd (10)	964.2 ± 91.0bcd (23)	1,089.7 ± 74.1bc (47)	1,811.3 ± 100.6a (35)	651.3 ± 83.2d (32)	NA
Temp.: $F = 33.8$, df = 4, 210,	IL	9	NA	1,013.7 ± 115.1cde (15)	1,429.3 ± 98.0bc (24)	1,381.8 ± 69.0bc (72)	2,006.2 ± 95.5a (61)	843.1 ± 78.5de (47)	NA
$P < 0.0001$	NY	9	NA	1,204.0abcde (1)	896.8 ± 132.3cde (11)	1,177.8 ± 86.6cd (39)	1,801.8 ± 154.3ab (24)	654.7 ± 101.8e (24)	NA
Temp.: $F = 24.8$, df = 4, 133,	IL	10	NA	860.5 ± 2286.1bcd (2)	1,533.0 ± 124.9b (12)	1,507.5 ± 60.5b (60)	2,115.6 ± 97.5a (29)	928.7 ± 76.1cd (34)	NA
$P < 0.0001$	NY	10	NA	1280.0abc (1)	1,394.0 ± 155.1bc (7)	1,449.8 ± 85.3b (27)	1,804.7 ± 24.6ab (9)	808.9 ± 100.1d (19)	NA
Temp.: $F = 10.9$, df = 4, 47,	IL	11	NA	761.0abc (1)	NA	1,608.0 ± 103.6b (25)	2,166.9 ± 119.1a (21)	1,065.7 ± 104.1c (24)	NA
$P < 0.0001$	NY	11	NA	**NA**	1,246.0abc (1)	1,540.7 ± 188.8abc (7)	2,048.4 ± 361.8ab (2)	702.3 ± 180.7c (8)	NA
Temp.: $F = 6.9$, df = 3, 22,	IL	12	NA	961.0abc (1)	NA	1,451.3 ± 109.8b (12)	2,205.6 ± 120.3a (17)	1179.9 ± 92.3bc (17)	NA
$P = 0.0019$	NY	12	NA	**NA**	NA	1,109.0 ± 269.0bc (2)	1,485.9 ± 380.5abc (2)	730.7 ± 143.8c (7)	NA
Temp.: $F = 3.4$, df = 3, 15,	IL	13	NA	913.0ab (1)	NA	1,518.0 ± 147.1ab (10)	2,119.1 ± 191.7a (8)	1299.2 ± 128.3ab (12)	NA
$P = 0.0443$	NY	13	NA	**NA**	NA	1,245.3 ± 297.2ab (2)	1,438.0ab (1)	880.1 ± 231.1b (5)	NA
NS	IL	14	NA	991.0a (1)	NA	1,609.9 ± 174.5a (8)	2,096.1 ± 201.9a (8)	1,438.2 ± 141.5a (12)	NA
	NY	14	NA	**NA**	NA	1,358.6 ± 336.1a (2)	1,512.0a (1)	1,029.3 ± 240.3a (5)	NA
Sex × Pop. × Temp.:	IL	Pupa ♂	NA	777.8 ± 140.0e (4)	1,081.1 ± 64.2e (26)	1,245.7 ± 58.2de (33)	1,554.1 ± 52.3de (43)	1,178.4 ± 1115.3e (6)	NA
$F = 2.8$, df = 11, 237,									
$P = 0.0016$	IL	Pupa ♀	NA	1,163.9 ± 115.9de (3)	1,502.7 ± 68.9de (21)	1,897.1 ± 65.7bc (24)	2,251.0 ± 59.6a (32)	1,422.0 ± 160.9cde (3)	NA
	NY	Pupa ♂	NA	711.0de (1)	773.6 ± 79.1e (53)	1,186.4 ± 69.9e (20)	1,402.8 ± 70.8de (23)	912.6 ± 141.5e (4)	NA
	NY	Pupa ♀	NA	NA	1,183.1 ± 70.5e (21)	1,547.8 ± 76.0bcd (17)	1,967.4 ± 87.8abc (13)	1,289.3 ± 116.7de (7)	NA

The IL population was from Ravenswood and the NY population was from Bayside. Within instars or stage (across all temperatures and for both stains and sexes), means followed by the same letter are not significantly different based on Bonferroni test with $\alpha = 0.05$ (SAS Institute 1999). The data for 10–30°C were obtained in the first year and the 5, 25 y2, and 35 °C data were obtained in the second year. The first instar weights of the 5°C larvae were 6.0 ± 0.2ab (33) and 5.2 ± 0.2abc (33) for the IL and NY larvae, respectively.

NA, parameter not applicable because no larvae or no molts at these temperatures for these instars; NS, no significant findings.

Table 6. Larval head capsule widths (mm) for *A. glabripennis* at five constant temperatures [mean ± SE (*n*)]

F-statistics	Population	Instar/ stage	Temperature (°C)				
			10	15	20	25	30
Temp.: $F = 7.2$, df = 4, 520, $P < 0.0001$	IL	1	0.99 ± 0.01ad (38)	0.96 ± 0.01abc (83)	0.98 ± 0.01abc (69)	0.97 ± 0.01abc (85)	0.95 ± 0.01bc (79)
	NY	1	0.99 ± 0.02ab (16)	0.96 ± 0.01abc (48)	0.94 ± 0.01abc (51)	0.93 ± 0.01cd (60)	0.93 ± 0.01c (47)
Temp.: $F = 8.0$, df = 4, 472, $P < 0.0001$	IL	2	1.39 ± 0.02b (21)	1.39 ± 0.02b (71)	1.46 ± 0.02a (70)	1.47 ± 0.01a (84)	1.42 ± 0.02ab (74)
	NY	2	1.34 ± 0.03b (9)	1.39 ± 0.02b (42)	1.40 ± 0.02ab (52)	1.40 ± 0.02ab (56)	1.35 ± 0.02b (46)
Temp.: $F = 40.73$, df = 4, 419, $P < 0.0001$	IL	3	1.79 ± 0.06e (8)	1.93 ± 0.03de (62)	2.18 ± 0.02ab (66)	2.21 ± 0.02a (84)	2.12 ± 0.02abc (66)
	NY	3	1.83 ± 0.08e (5)	1.85 ± 0.03e (34)	2.05 ± 0.03cd (47)	2.07 ± 0.03bc (54)	2.00 ± 0.03cd (43)
Temp.: $F = 65.4$, df = 4, 394, $P < 0.0001$	IL	4	NA	2.43 ± 0.04e (61)	2.96 ± 0.04ab (61)	3.03 ± 0.03a (81)	2.84 ± 0.04bcd (67)
	NY	4	1.92e (1)	2.36 ± 0.05e (33)	2.78 ± 0.04cd (45)	2.86 ± 0.04abc (52)	2.67 ± 0.05d (41)
Temp.: $F = 68.6$, df = 3, 375, $P < 0.0001$	IL	5	NA	3.11 ± 0.05d (57)	3.75 ± 0.05a (56)	3.74 ± 0.04a (78)	3.44 ± 0.04b (64)
	NY	5	NA	3.01 ± 0.06d (30)	3.46 ± 0.05b (45)	3.52 ± 0.05b (52)	3.17 ± 0.06d (40)
Temp.: $F = 46.7$, df = 3, 352, $P < 0.0001$	IL	6	NA	3.75 ± 0.05cde (56)	4.27 ± 0.05a (58)	4.25 ± 0.05a (76)	3.87 ± 0.05bcd (59)
	NY	6	NA	3.63 ± 0.07de (25)	3.95 ± 0.06bc (43)	4.02 ± 0.06b (49)	3.58 ± 0.07e (32)
Temp.: $F = 46.8$, df = 3, 307, $P < 0.0001$	IL	7	NA	4.20 ± 0.06bc (46)	4.67 ± 0.06a (51)	4.62 ± 0.05a (76)	4.11 ± 0.06c (53)
	NY	7	NA	3.98 ± 0.09cd (17)	4.25 ± 0.07bc (35)	4.37 ± 0.06b (45)	3.82 ± 0.07d (29)
Temp.: $F = 41.2$, df = 3, 234, $P < 0.0001$	IL	8	NA	4.46 ± 0.07bc (31)	4.90 ± 0.06a (39)	4.87 ± 0.05a (72)	4.27 ± 0.06cd (46)
	NY	8	NA	4.43 ± 0.13bcd (8)	4.45 ± 0.09bc (18)	4.65 ± 0.06ab (41)	4.04 ± 0.08d (22)
Temp.: $F = 39.8$, df = 3, 154, $P < 0.0001$	IL	9	NA	4.62 ± 0.10bcd (14)	5.07 ± 0.09a (22)	5.07 ± 0.06a (65)	4.37 ± 0.07cd (33)
	NY	9	NA	4.80abcd (1)	4.74 ± 0.11abc (11)	4.90 ± 0.07ab (32)	4.19 ± 0.10d (18)
Temp.: $F = 29.6$, df = 3, 81, $P < 0.0001$	IL	10	NA	4.44abc (1)	5.23 ± 0.15a (6)	5.29 ± 0.07a (47)	4.55 ± 0.80bc (26)
	NY	10	NA	**NA**	4.96 ± 0.14ab (7)	4.96 ± 0.10a (17)	4.36 ± 0.11c (13)
Temp.: $F = 11.5$, df = 3, 25, $P < 0.0001$	IL	11	NA	4.65ab (1)	NA	5.38 ± 0.10a (21)	4.65 ± 0.011b (16)
	NY	11	NA	**NA**	4.90ab (1)	5.19 ± 0.21a (4)	4.17 ± 0.19b (6)
Temp.: $F = 6.5$, df = 2, 12, $P = 0.0121$	IL	12	NA	5.00ab (1)	NA	5.25 ± 0.14a (9)	4.94 ± 0.10ab (13)
	NY	12	NA	**NA**	NA	5.20 ± 0.23ab (2)	4.34 ± 0.15b (5)
Temp.: $F = 6.7$, df = 2, 9, $P = 0.0163$	IL	13	NA	5.00ab (1)	NA	5.25 ± 0.14a (6)	4.94 ± 0.10ab (12)
	NY	13	NA	**NA**	NA	5.20 ± 0.23ab (2)	4.34 ± 0.15b (5)
NS	IL	14	NA	5.15a (1)	NA	5.31 ± 0.14a (6)	5.04 ± 0.11a (10)
	NY	14	NA	**NA**	NA	5.00a (1)	4.47 ± 0.16a (5)
Temp.: $F = 22.2$, df = 3, 167, $P < 0.0001$	IL	Ultimate	NA	4.79 ± 0.07bc (29)	4.94 ± 0.07b (39)	5.32 ± 0.07a (37)	4.51 ± 0.19bcd (5)
	NY	Ultimate	NA	4.36 ± 0.09d (19)	4.51 ± 0.08cd (33)	5.01 ± 0.09ab (23)	4.55 ± 0.15bcd (9)

The IL population was from Ravenswood and the NY population was from Bayside. Within instars (across all temperatures and for both stains), means followed by the same letter are not significantly different based on Bonferroni test with $\alpha = 0.05$ (SAS Institute 1999). The data for 10–30°C were obtained in the first year and the 25°C data from the second year are not shown.

NA, parameter not applicable because no larvae or no molts at these temperatures for these instars; NS, no significant findings.

bial properties than in the first year. This could have been because of some improvement in the quality of the diet or because of lower moisture in the diet. A lower moisture content was a consequence of having to change the diet less frequently in the second year. Larvae exposed to lower moisture in wood, caused by tree decline or being in cut wood, could use moisture as an effective cue to quickly proceed to pupation to escape declining conditions that could lead to death. These results show the great plasticity of this insect to adapt to variable environmental conditions.

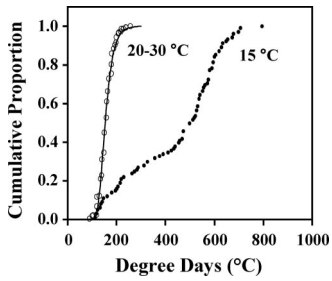

Fig. 4. Cumulative proportion of *A. glabripennis* larvae molting from the third to fourth instar for 20–30 (○) and 15°C (●) over accumulated degree-days. The solid line over the 20–30°C represents the fitted equation (Table 5) for this instar.

There are also indications that there may be genetic variation in response to temperature as evidenced by the within- and between-population differences in responses to temperature. This could lead to selection of traits appropriate to new climates, as might occur when the beetle is moved in cut wood. A possible example of this is the observation that larvae from a population collected from the Worcester, MA, infestation (a more northern location) can pupate at 10°C (M.K., unpublished data), whereas larvae from the two populations used in these studies were unable to

Table 7. Parameter values for predicted for the relationship between proportion of *A. glabripennis* progressing to the next instar or stage and no. of accumulated degree-days above the lower threshold for each immature stage

Instar/stage	n^a	a ± SE	b ± SE	F	df	P	Adjusted R^2	Temperature range (°C)	Predicted accumulated degree-days for larvae completing of the instar			
									10%	50%	90%	99%
1	53	0.0710 ± 0.0026	5.19 ± 0.20	13522	50, 52	<0.0001	0.988	15–35	61.4	78.3	104.8	137.9
2	65	0.0569 ± 0.0019	5.99 ± 0.21	14964	62, 64	<0.0001	0.989	15–35	90.6	111.7	144.8	186.1
3	32	0.0456 ± 0.0016	6.61 ± 0.24	8086	29, 31	<0.0001	0.994	20–30	126.7	153.0	194.3	245.9
4	51	0.0251 ± 0.0005	5.92 ± 0.12	27952	48, 50	<0.0001	0.997	20–30	202.8	250.6	352.7	419.3
5	58	0.0210 ± 0.0002	5.16 ± 0.07	65469	55, 57	<0.0001	0.998	20–30	227.6	284.8	374.5	486.4
6	95	0.0131 ± 0.0002	3.29 ± 0.04	65260	92, 94	<0.0001	0.997	20–30	187.6	279.2	423.0	602.4
7	103	0.0110 ± 0.0001	3.11 ± 0.03	93344	100, 102	<0.0001	0.998	20–30	206.7	315.9	487.1	700.8
8	85	0.0111 ± 0.0001	3.69 ± 0.04	72428	82, 84	<0.0001	0.998	20–30	257.7	365.8	535.5	747.2
Ultimate	174	0.0035 ± 0.0001	2.10 ± 0.06	12155	171, 173	<0.0001	0.971	15–30	365.8	711.8	1254.7	1931.8
Pupa	29	0.0645 ± 0.0029	16.13 ± 0.74	4654	26, 28	<0.0001	0.990	15–30	237.1	255.7	284.9	321.4

This relationship was described using a Gompertz function, $P = \exp[-\exp(-b\mathrm{DD} + a)]$ in which a and b are the lag and the rate of increase, respectively (Brown and Mayer 1988, PROC NLIN and Marquardt convergence method, SAS Institute 1999). The data for the New York and Illinois populations were pooled. The data for ultimate instars were removed from the specific instar and lumped into the "Ultimate" category because many larvae spend a longer time in the last instar than would be normal for that instar. Temperatures too close to the lower or upper limits for each instar were not included in the calculations.

[a] This n is the no. of unique degree-day values (with varying proportions of the population associated with each) that were used in the calculations.

pupate at this low a temperature, although some did become prepupae at 10°C. Accordingly, caution should be used in applying predictions based only on temperature relationships developed in the laboratory to field conditions, given the possible food quality effects and genetic variability in response to temperature.

The thermal environment that the larva is exposed to in the wood can be very different depending on location in the tree, depth within the wood, wood density, solar exposure, topographic position of the tree, seasonality, etc. During the day, direct solar radiation, reflected solar radiation, and thermal radiation in addition to air temperature and wind speed affect tree temperature. At night, stored thermal radiation continues to keep wood temperature higher than the ambient air temperature. Also, temperatures deeper in the trunk can take minutes to hours to equilibrate after external conditions have changed (Derby and Gates 1966). In *Pinus contorta* Douglas ex Loud., phloem temperatures on the south side of a tree were up to 33°C warmer than on the north side and up to 21°C higher than air temperature, with the largest differences occurring in the late summer and fall (Bolstad et al. 1997). Daily minimum phloem temperatures for all sides of the tree averaged 2°C higher than daily air temperatures (Bolstad et al. 1997). Similar temperature differences were measured in hardwood trees; cambial temperature in aspen in April averaged 2–10°C higher than air temperatures (Derby and Gates 1966). Logan and Powell (2001) measured actual phloem temperatures that *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae) was exposed to and found that it was almost always higher than ambient temperature. When actual phloem temperature was used rather than ambient, the predicted time for one generation of *D. ponderosae* went from 4 to 2 yr (Logan and Powell 2001). Winter studies in Ontario, Canada, suggest that the temperature inside a large diameter sugar maple may reach as much as 10°C warmer than external air temperature (Roden et al. 2008). When using the degree-day estimates from our studies to predict the phenology of this beetle, at least 2°C should be added to both day and night air temperatures or to the lower threshold when making these calculations.

The thermal environment in the tree may also explain the differences in response to temperature we documented between first- through fourth-instar larvae and larvae in instars greater than or equal to fifth. Late third- and fourth-instar larvae begin feeding almost exclusively on the xylem (Yan and Qin 1992), initially still near the phloem; as they grow larger, they tunnel deeper into the wood, and the final-instar larva creates a chamber near the outer bark where it pupates in the spring (Lingafelter and Hoebeke 2002). Larvae in or near the phloem as well as pupae would be potentially exposed to a broader range of temperatures and more temperature fluctuations. In addition, some small larvae and eggs, laid too late to hatch in the fall, overwinter (Hua et al. 1992, Haack et al. 2006). Therefore, the smaller larvae need to be able to tolerate a broader range of temperatures (development from 10 to 35°C was documented), and it would be beneficial to them to be able to resume development as early in the year as possible (lower T_L estimated). Large larvae, however, would not be exposed to as great a temperature fluctuation because they are deeper in the wood and thus buffered. They also should be less responsive to small temperature changes so that they progress to pupation at the right time, a process that may help synchronize emergence. Larger larvae also seem to be freeze tolerant and are able to survive temperatures below their supercooling point for short periods of time, thus giving them the ability to survive across the broad range of conditions within the range of their host species (Roden et al. 2008).

Given the temperature effects detailed here, *A. glabripennis* larval development and pupation should be possible in most of the continental United States where suitable hosts are available. In New York and Chicago, growing conditions are optimal, with long periods of temperatures ideal for development and a few months of chill that will allow larvae to proceed to pupation and synchronize adult emergence. In cooler climates, like Vermont, where the number of months that development can take place are reduced, it will likely take >1 yr for the beetle to complete development. Monthly mean temperatures at Burlington International Airport, VT, were 14, 18, 21, 20, and 15°C during May, June, July, August, and September, respectively, whereas the corresponding maximum temperatures were 20, 24, 27, 26, and 20°C, respectively (30-yr normals, 1961–1990, National Climatic Data Center). This should provide adequate heat over the course of >1 yr, and the larvae should be able to survive the average winter minimum temperature (−14°C in January), which is above the supercooling point (−25.8 + 1.1°C, Roden et al. 2008).

In warmer climates, like Florida, temperatures <20°C may be a limiting factor, because these temperatures would be required to induce larvae to progress toward pupation. Summer temperatures could reach lethal levels for long enough periods of time (2–4 wk at or above 35°C depending on the instar) to kill larvae. Monthly mean temperatures at Miami International Airport, FL, were 21, 20, 20, and 22°C during December, January, February, and March, respectively, (30-yr normals, 1961–1990, National Climatic Data Center), which should provide enough chill to allow larvae to pupate. Monthly maximum temperatures at Miami International Airport, FL, only reached 30, 31, 32, 32, 31, and 29°C during May, June, July, August, September, and October, respectively (30-yr normals, 1961–1990, National Climatic Data Center). These maximum average summer temperatures for Miami, FL, are not in the lethal range but could result in some larval mortality and lower larval weight gains. Larvae should be able to complete development in these warmer areas, and it is uncertain whether they would adapt so that they could develop and emerge continuously throughout the year. These data on temperature effects on life history of larvae and pupae of *A. glabripennis*, combined with similar published data for adults and eggs (Keena 2006), will aid in developing potential geographical range maps for this species and phenological models to predict the timing of all life stages. This information is critical for developing *A. glabripennis* management programs.

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