Pourable Artificial Diet for Rearing *Anoplophora glabripennis* (Coleoptera: Cerambycidae) and Methods to Optimize Larval Survival and Synchronize Development

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Ann. Entomol. Soc. Am. 98(4): 536–547 (2005)

ABSTRACT *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), is a recently introduced non-native invasive species in the United States that has the potential to destroy several tree species in urban and forest habitats. The ability to rear *A. glabripennis* in quarantine is critical to rapid progress on techniques for the exclusion, detection, and eradication of this pest. Survival and development were compared for larvae from two populations (Chicago, IL, and Queens, NY) on six diets containing varying levels of Fe (69–237 mg/liter) and for three populations (Illinois and New York plus Hohhot, Inner Mongolia, China) under four larval chill treatments (6, 9, 12, or 16 wk of development before chill). Larval survival and percentage pupation significantly decreased and developmental time slightly increased with increasing Fe levels in the diet. Larval survival and percentage of pupation were highest, adults weighed the most, and developmental time was shortest when larvae were reared on a pourable modification of the *Enaphalodes rufulus* (Haldeman) (ER) diet. Individuals from the China and Illinois populations were heavier than those from New York. On average, larvae from the Illinois population were ready to pupate sooner than those from New York or China. Some larvae that had not reached their critical weight for pupation before the chill period required a second chill period before initiating pupation. Overall survival increased as the developmental time period before chill increased. Further evaluation of the effects of temperature on development is needed to better understand the triggers for pupation and to predict the timing of various stages.

KEY WORDS *Anoplophora glabripennis*, artificial diets, iron, rearing

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae) is one of the more recently introduced non-native invasive species with potential to become a major pest in the United States. It was first discovered in the New York City area in August 1996, and additional infestations were discovered in the Chicago area (July 1998), Jersey City, NJ (October 2002), and Toronto and Vaughan, Ontario, Canada (September 2003) (Haack et al. 1997, Poland et al. 1998, Dodd 2004). In the United States, the USDA–Animal and Plant Health Inspection Service (APHIS) has implemented an eradication program whereby all trees with signs of beetle infestation (oviposition pits or exit holes) are removed and destroyed. The eradication program for *A. glabripennis* has greatly impacted the environments of the cities where this beetle has been found because of the removal of thousands of trees and millions of dollars in cost (Nowak et al. 2001). The United States has placed restrictions on trade to prevent further introductions (USDA–APHIS 1998). If the established populations of *A. glabripennis* are not eradicated, the beetle could threaten the maple sugar industry, fall-foliage tourism, natural ecosystems, recreational areas, and many beloved backyard and street trees (U.S. Dep. Agric 1999, Nowak et al. 2001).

A. glabripennis is widely distributed in China and is present in South Korea. In China, it is considered a major pest of several deciduous broadleaf tree species (Xiao 1992) and causes severe damage from 21° to 43° N latitude and from 100° to 127° E longitude (Yan 1985). Primary host trees of *A. glabripennis* in China include species of *Acer* (maple), *Populus* (poplar), *Salix* (willow), and *Ulmus* (elm), and it is reported to feed on >24 species of hardwood trees (Yang et al. 1995). Eggs are laid beneath the bark, and early instars feed under the bark, whereas later instars enter the wood. In China, *A. glabripennis* takes 1 or 2 yr to complete development, depending on the timing of adult emergence; later emerging adults lay eggs that may not hatch until spring the next year (Fan et al.

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1997), and some larvae require a chill period after they reach full size (Zhao et al. 1998).

Because not all research on *A. glabripennis* can be conducted in China or at North American sites where this beetle is being eradicated, the ability to mass rear *A. glabripennis* is critical to rapid progress on its exclusion, detection, and eradication techniques. Large numbers of beetles in all life stages are needed yearround. Mass rearing of *A. glabripennis* requires not only an artificial diet that supports larval survival and development but also one that can be dispensed quickly and in high volume. Although *A. glabripennis* develops and survives well on the diet of Zhao et al. (1998) as modified by Dubois et al. (2002), this diet is not easy to dispense into containers because of its low moisture content and rapid solidification. Therefore, a diet that is "pourable" before fully solidifying and that meets the nutritional requirements of *A. glabripennis* for development and survival is desirable.

In addition, ongoing research requires bioassaying even-aged and even-sized larvae, so methods to synchronize development are being sought. This diet and rearing information may be applicable for rearing closely related cerambycid species, such as *Anoplophora chinensis* (Förster) (=*A. malasiaca*) a serious orchard pest in Asia (Wang et al. 1996) that has recently been intercepted in bonsai plants in a nursery in Tukwila, WA (August 2001, Grob 2003) and useful as a model for developing diets and rearing methods for more distantly related cerambycids.

The objectives of the experiments described here were to develop a pourable version of the *Enaphalodes rufulus* (Haldeman) (ER) diet (Galford 1985) as modified for *A. glabripennis;* to determine the optimal dietary iron level for this pourable diet to promote maximal survival and rapid development of *A. glabripennis;* and to investigate methods for stockpiling eggs, synchronizing egg hatch, promoting pupation, synchronizing adult eclosion, and improving the resistance of the diet to microbial contamination.

Materials and Methods

Populations. The individuals used in these studies were from laboratory populations maintained on artificial diet originally established with either adults that emerged from infested branch sections obtained at eradication tree cuts in the United States or from larvae extracted from trees in China and shipped on artificial diet; both were 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, 041.58° N and 087.42° W) and in April 1999 from the Bayside, Queens, NY, infestation (384 adults, 040.45° N and 073.45° W). Larvae were obtained from Hohhot City, Inner Mongolia, China, in November 2001 (286 large larvae, 040.82° N and 111.60° E).

Larvae from 23 second generation mating pairs from New York and 17 second generation pairs from Illinois were used in the first experiment to compare larval survival and pupation on six diets. Larvae from 12 fourth generation mating pairs from the New York and 11 from the Illinois populations, and 13 first generation pairs from China were used in the second experiment to assess the effects of timing of larval chill on survival and pupation. Voucher specimens of the three *A. glabripennis* populations were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT.

Mating Adults and Obtaining Eggs. Virgin adults were held individually in 950-ml glass jars, until mated. *Acer saccharum* Marshall (sugar maple) twigs (3–7 mm in diameter), from which leaves had been removed, were added once a week as a food source; twigs were cut fresh weekly and stored in plastic bags at 5°C. Adults were fed for an average of 7 d before mating to ensure prompt egg laying afterward (Keena 2002). Each mating pair was held in a 3.8-liter glass jar. Freshly cut (i.e., held no >14 d at 10°C) *A. saccharum* bolts (3–7 cm in diameter and 20 cm in length) with both ends waxed were provided to the mating pairs for oviposition and fresh twigs as described above were added as a food source. Folded paper towels were placed in the bottom of the jars to collect frass and excess moisture. Two holes (1–2 mm in diameter) were drilled in the plastic lids used on the jars to allow airflow.

The oviposition bolts were replaced weekly until a female died. Only one to seven of the weekly bolts from each female were available for use in these experiments because the other bolts were used for maintaining colonies and for other studies. After removal from the mating jars, the bolts were held at 25 ± 2°C and 60 ± 5% RH for an average of 3 d and then chilled at 10 ± 1 or 5 ± 1°C (half of the bolts in experiment 2) and 85 ± 10% RH for 3–80 d. In total, 113 bolts (68 from the New York population and 45 from the Illinois population) were chilled in experiment 1 and 86 bolts (31 New York, 17 Illinois, and 38 China) in experiment 2, an average of three bolts per female. Bolts were incubated for 12 d (experiment 1) and 6 d (experiment 2) at 25 ± 2°C and 60 ± 5% RH after removal from chill before the bark was stripped, and all eggs and larvae were counted. Eggs removed from under the bark were either placed together by bolt in a 60 by 15-mm petri dish (experiment 1) or individually in wells of a 24-well tissue culture plate (experiment 2) that was held in a water box (a 36 by 24 by 16-cm clear plastic box with grating in the bottom to support containers above the water) at 25 ± 2°C for 2 wk and checked daily for hatch.

Diet Preparation. All diets were made in a 12-liter steam-jacketed kettle (in 6–10-liter batches). Cool deionized water and agar were measured into the kettle and brought to a rolling boil. The premix ingredients (Table 1) were added while the agar/water mixture was boiling, and then the mixture was allowed to come back to a rolling boil for 30 s before turning off the heat. The diet continued to boil for several minutes. This procedure was necessary to kill bacteria and denature enzymes in the wheat germ that could degrade the diet. The diet was then mixed without

Table 1. Ingredients (amount per liter) for three diets tested for rearing *A. glabripennis*

	Ingredient	Diet		
		A. glabripennis		Modified *E. rufulus* (ER)
		Modification #1 (AG1)	Modification #2 (AG2)	
Initial ingredients	Distilled water (ml)	686.8	686.0	663.1
	Agar (*Gracileria* spp.) (g)	21.9	21.8	21.2
Premix added at boil	Sucrose (g)	15.6	15.6	6.6
	Raw wheat germ (g)	53.1	53.0	79.6
	Casein (80 mesh) (g)	9.4	9.4	6.6
	Cholesterol (g)	0.9	0.9	0.1
	Wesson's salt mixture w/o $FePO_4$ (g)[a]	2.8	2.8	2.0
	94% Amorphous $FePO_4$ (g)[b]	Variable[c]	0.0	0.1
	Torula yeast (g)	28.1	28.1	13.3
	Sodium propionate (g)	0.0	1.2	0.0
	Methyl paraben (g)[d]	0.8	1.6	1.3
	Sorbic acid (g)	1.6	1.6	1.3
Added in order	Wheat germ oil (g)	53.1	53.0	2.6
After 5-min cooling	Vitamin mixture (g)[e]	4.4	4.4	3.3
	Cellulose (g)[f]	121.7	121.6	199.0

The *E. rufulus* diet was developed by Galford (1985) and was modified for use with *A. glabripennis* by using raw (instead of toasted) wheat germ, Wesson's salt without ferric phosphate (adding desired quantity of ferric phosphate separately), a different vitamin mixture, and dropping the chloramphenicol in 95% ethanol. The *E. rufulus* diet was further modified to be a pourable diet for *A. glabripennis* here referred to as the "*A. glabripennis* modification."

[a] The Wesson's salt mixture was obtained from Purina Test Diets (Richmond, IN) without $FePO_4$ and varying quantities of ferric phosphate were added as indicated.

[b] Product made by Riedel-deHaen (Seelze, Germany) as iron(III) phosphate extrapure powder (04241).

[c] Four different amounts of $FePO_4$ were added to this diet: 0.0, 0.06, 0.12, and 0.18 g/liter.

[d] Full name *p*-hydroxybenzoic acid methyl ester.

[e] Product of Hoffman-La Roche (Fresno, CA) as premix #26862.

[f] Product from BioServ (Frenchtown, NJ) as 3425 (cellulose fiber).

heat for 5 min, allowing it to cool. After 5 min, the remaining ingredients were added one at a time in the order listed in Table 1. The ER diet was moved to an industrial strength pizza-dough mixer just before the cellulose was added because it became too thick for the mixing capabilities of the kettle. When the last ingredient had been thoroughly mixed in, the diet was poured into jars or spooned into trays (in the ER diet). Diet in the jars was allowed to cool to room temperature before storage in plastic bags at 15°C or cooler. Diet was typically used within 1 mo of preparation. Diet to be used for newly hatched larvae was covered after 15–30 min to prevent excessive moisture loss.

Holes appropriate for the instar size were cut in the diet, and it was warmed to room temperature before placing larvae in the diet. The ER diet was cut into jar-size chunks and placed in jars before holes were cut for the larvae. The holes cut in the diet were no wider than the larvae so that they could get enough traction to burrow into the diet. A wedge ≈2 cm in length, 1 cm in width, and no >2 cm in depth was cut in the diet by using a flamed metal spatula for first instars. After the first instars was placed in the hole, it was carefully covered with the diet wedge minus a small bit of the narrow tip. A larger wedge was cut for larvae that had been on the diet for 2 wk. Holes were cut with a flamed vegetable peeler for medium-sized larvae and with an apple corer that had been adjusted to cut a smaller diameter hole (16 mm) for large larvae. Medium-sized and large larvae were placed head first into the holes and not covered with diet. Lids without holes were used for jars containing first instars because small larvae desiccate easily, and two small holes were drilled into jar lids used for all other larvae.

Experiment 1: Diet Comparisons. There were six diet treatments: *A. glabripennis* modification #1 (AG1, Table 1) containing 68, 122, 179 and 239 available Fe mg/liter of diet, the same diet containing 180 Fe mg/liter with 3.6 ml of a 10% chloramphenicol in 95% ethanol solution added, and the ER diet (175 Fe mg/liter). The amount of bioavailable Fe in each diet was determined using methods developed by Willis and Montgomery (1994). No $FePO_4$ was added to obtain the 68 Fe mg/liter level and the other amounts are listed in Table 1.

It was not possible to completely randomize the larvae because they chew on each other when held together, even by bolt. All larvae and eggs found when the bolts were stripped of bark were held by bolt in a 60 by 15-mm petri dish. Larvae from each family with at least six larvae (15 New York families and eight Illinois families) were weighed, and equal numbers from each family were assigned to each diet to exclude any genotype bias that may exist (some genotypes may survive better and develop faster in one diet than others). This resulted in one to six larvae from each family on each diet, because of differences in the total number of larvae available from each family. In total, 60 larvae were assigned to each diet treatment, with 25 from the Illinois population and 35 from the New York population. Larvae were initially held individually at 25 ± 2°C and 60 ± 5% RH in the dark in 59-ml clear plastic jars with 1.0 cm of the assigned diet. The diet was changed biweekly (except during the chill when

it was changed only once at 4 wk), and the container size and diet height were increased as larvae grew. At the first diet change, larvae were placed in 59-ml jars with 2.0 cm of diet; at the second change, they were moved to a 118-ml jar with 2.0 cm of diet; at the third, they were again placed in a 118-ml jar but with 3.5 cm of diet; and at the fourth and fifth, they were placed in a 237-ml jar with 3.5 cm of diet. For all subsequent changes, they were placed in a 237-ml jar with 5.0 cm of the assigned diet. Larvae were weighed at weeks 2, 4, 6, and 12. All jars were checked weekly for larval mortality, and rarely the diet was replaced early if excessive fungal or bacterial growth was evident on the surface. Larvae were moved to 10 ± 1°C and 85 ± 10% RH for 70 d when they reached full size (i.e., 3.5–4.5 cm in length). This move to lower temperature took place 14 wk after being placed on diet for four of the AG1 diets. Larvae on the diet containing 239 Fe mg/liter were moved at 16 wk, and those on the ER diet were moved at 20 wk. After 70 d, all larvae were returned to 25 ± 2°C and 60 ± 5% RH to complete development.

Experiment 2: Timing of Larval Chill. The *A. glabripennis* modification #2 (AG2, Table 1), AG1 with more antimicrobials added, was the only diet used in this experiment. There were four chill timing treatments: larvae were moved to 10 ± 1°C and 85 ± 10% RH after 6, 9, 12, and 16 wk of development on diet. These times corresponded to the mean developmental times plus 7 d for larvae to reach the fifth, sixth, seventh, or eighth instar, respectively, on this diet at 25°C (unpublished data). Newly hatched larvae found when bark was stripped or those hatching from eggs found during stripping were segregated by population and family. They were then equally divided among all treatments with one to nine larvae per treatment per family (because of differences among families in the number of available larvae) and held at 25 ± 2°C with 60 + 5% RH, and a photoperiod of 16:8 (L:D) h. Forty-one larvae from the China population and 36 each from the Illinois and New York populations were used in each treatment. Larvae to be chilled at 6 wk were placed in 118-ml jars with 3.5 cm of diet, and larvae in all other treatments were placed in 59-ml jars with 2.0 cm of diet. At 25°C, larvae were moved every 6 wk until pupation, except while in chill, to 5 cm of fresh diet in a 237-ml jar. Larvae were checked for mortality every 1 to 2 wk. They were weighed and placed in 118-ml jars with 3.5 cm of fresh diet just before being chilled. All larvae were held 84 d at 10 ± 1°C and 85 ± 10% RH. After the 84 d, larvae were moved to fresh diet at 25°C to complete development. If the larvae had not pupated by week 48 on diet, they were chilled as described previously for an additional 84 d and then returned to 25°C to complete development.

Handling Pupae and Emerging Adults. Larvae were checked for prepupation weekly for the last few weeks before chilling and 2 to 3 times per week after being returned to 25°C. When a larva neared pupation, it shrank so that the distance between intersegmental areas decreased, it became soft, and the underlying tissues near the head became clear. Accompanying the physical changes were behavioral changes; the larva stopped eating and created a larger than normal cell in the diet, often with a trench that was open to the top, or the larvae remained motionless on the diet surface. When a larva became a prepupa, it was moved to a 50-ml centrifuge tube with one or two pin holes in the lid and a piece of damp paper towel to act as a wick. The tube was then placed in a tightly closed water box (i.e., 50 by 40 by 25-cm opaque plastic box with grating in the bottom to support containers above the water) until adult emergence. Neither prepupae nor pupae developed properly if the water in the boxes was allowed to completely evaporate. Prepupae and pupae were checked daily for eclosion. Once an adult emerged, it was held in a dry dark box for 4 d to allow the cuticle to fully sclerotize before it was sexed, weighed, and given twigs to eat. Prepupae, pupae, and teneral adults were all very fragile and were handled as little and as gently as possible.

Statistical Analyses. In both experiments, the percentage hatch of eggs under the bark on the chilled bolts was analyzed by REML (PROC MIXED, SAS Institute 1999). The model for the bolts from experiment 1 used chill duration group, population, and the interaction between the two as fixed effects and family as a random effect. For experiment 2, the fixed effects were chill duration group, population, and chill temperature, and family was a random effect.

In a few cases where the larvae pupated in the diet, 17 d (median pupation duration) was subtracted from the adult emergence date to estimate the pupation date and days from chill to pupation (no pupation duration was recorded). In experiment 1, the following variables were analyzed by REML (PROC MIXED, SAS Institute 1999): initial larval weight; larval weight gain at 2, 4, 6, and 12 wk; time to pupation; time from chill to pupation; pupation duration; and adult weight. The model used treated diet treatment, population and diet treatment by population as fixed effects, whereas family was treated as a random effect. For adult weights, gender also was added as a random effect. The ER diet treatment was omitted from all pupal and adult variable analyses because only one individual completed development. In experiment 2, the following variables were analyzed by REML (PROC MIXED, SAS Institute 1999): larval weight at chill, time to pupation, time from chill to pupation, pupation duration, adult weight, and longevity, and family was a random effect. The same model was used as in experiment 1, substituting chill treatment for diet treatment. The pupation chill groups (no chill, one chill, and two chills) were analyzed separately for time to pupation, time from chill to pupation, and adult weights because of obvious differences among these groups. Adult weights for the 16-wk treatment were analyzed separately with chill group and population as fixed effects, and family and gender as random effects. In both experiments, means were separated with least squares tests with $\alpha = 0.05$ and a Bonferroni correction (SAS Institute 1999) was used.

Table 2. Comparison of mean ± SE initial weight and weight gain of *A. glabripennis* larvae from the New York and Illinois populations on artificial diets containing varying quantities of available iron

Fe (mg/liter) in diet	Initial weight ± SE			Mean weight gain ± SE (mg)[a]			
	Diet[b]	*n*	mg	2 wk	4 wk	6 wk	12 wk
Bayside, Queens, NY							
69	AG1	35	4.3 ± 0.1a	69 ± 6a	354 ± 18a	640 ± 28a	1,554 ± 64a
122	AG1	35	4.4 ± 0.1a	70 ± 5a	278 ± 16ab	538 ± 27ab	1,262 ± 61b
179	AG1	35	4.4 ± 0.1a	69 ± 6a	323 ± 17ab	570 ± 27ab	1,221 ± 63b
239	AG1	35	4.4 ± 0.1a	67 ± 6a	249 ± 17b	388 ± 28c	688 ± 66c
180	AG1 + C	35	4.5 ± 0.1a	62 ± 5a	265 ± 16b	541 ± 26ab	1,256 ± 64b
175	ER	35	4.4 ± 0.1a	68 ± 6a	293 ± 17ab	477 ± 27bc	714 ± 70c
Ravenswood, Chicago, IL							
69	AG1	25	4.8 ± 0.2a	93 ± 7a	415 ± 20ab	760 ± 33a	1,608 ± 77ab
122	AG1	25	4.8 ± 0.2a	88 ± 7a	381 ± 20ab	790 ± 32a	1,703 ± 74a
179	AG1	25	4.7 ± 0.2a	105 ± 7a	446 ± 21a	775 ± 35a	1,742 ± 82a
239	AG1	25	4.8 ± 0.2a	83 ± 7a	410 ± 20ab	734 ± 32a	1,398 ± 73b
180	AG1 + C	25	4.8 ± 0.2a	80 ± 7a	341 ± 21b	726 ± 35a	1,581 ± 81ab
175	ER	25	4.8 ± 0.2a	86 ± 7a	375 ± 21ab	570 ± 36b	948 ± 96c

[a] Mean larval weights within populations for each weighing time followed by the same letter are not significantly different based on the least squares mean separation test with $\alpha = 0.05$ and with a Bonferroni correction (SAS Institute 1999).

[b] Diet: AG1, *A. glabripennis* modification #1 diet; AG1 + C, AG1 diet with chloramphenicol in ethanol added; and ER, *E. rufulus* diet with no chloramphenicol (Galford 1985).

Survival curves for the larvae were analyzed using the Cox proportional hazard model (PHREG, SAS Institute 1999). Only treatment and population were included in the model because the interaction term did not meet the P value < 0.25 decision level chosen. Larvae that were accidentally killed or that remained alive when the study was terminated were coded as censored data with their last observed live time. Larvae that pupated were coded as censored data with pupation date as the last observation time. In experiment 1, the four AG1 diet treatments without antibiotics were compared, and then the diet treatments with similar amounts of bioavailable iron (179 and 180) but differing in whether antibiotics were added or not were compared separately. Larval survival on the ER diet was obviously different from and not parallel to that on the AG1 diets and was evaluated separately. Percentage of pupation was compared using pair wise Pearson's χ^2 analysis (Statistix 2003) between selected treatments within a population.

Results

Experiment 1: Diet Comparisons. Average percentage of hatch of eggs laid under bark on the chilled bolts was 76.2 ± 2.3% of which 43.9 ± 3.0% had hatched by the time the bark was stripped off. There were no significant differences in percentage of hatch among populations ($F = 0.78$, df = 1, $P = 0.38$) or groups experiencing different chill durations (11–30, 31–60, and 61–80 d) ($F = 0.66$, df = 2, $P = 0.52$). There were no significant differences in initial larval weight either between diet treatments ($F = 0.71$, df = 5, $P = 0.62$) or population ($F = 3.42$, df = 1, $P = 0.07$), or the interaction of the two ($F = 0.80$, df = 5, $P = 0.55$).

Larvae from Illinois had gained significantly more weight than those from New York at 2 wk ($F = 14.70$, df = 1, $P < 0.01$). At week 4, Illinois larvae had gained significantly more weight than those from New York ($F = 57.32$, df = 1, $P < 0.01$), and diet treatment also had a significant effect on larval weight gain ($F = 6.97$, df = 5, $P < 0.01$). At weeks 6 and 12, the interaction between diet treatment and population had a significant effect on larval weight gain (week 6: $F = 6.10$, df = 5, $P < 0.01$; week 12: $F = 6.73$, df = 5, $P < 0.01$).

New York larvae on the AG1 diets without antibiotics showed a significant Fe dose response in 12-wk weight gain; larvae reared on diet containing 69 mg/liter weighed more than those on 122 or 179 mg/liter (Table 2). The same dose response was not evident for weight gain in Illinois larvae at 12 wk (Table 2). Weight gain of larvae on the ER diet was less than on all the AG1 diets for the Illinois larvae and less than all but the AG1 diet containing 239 mg/liter for the New York larvae (Table 2). There was no significant difference in larval weight gain between the AG1 diet with antibiotic and the comparable diet without the antibiotic (179 mg/liter, Table 2).

There were differences between the AG1 diets and the ER diet in larval survival rates. Before chill, percentage live larvae on the ER diet decreased by >4% per week, whereas percentage live larvae only decreased by ≈1% per week on all the AG1 diets (Fig. 1). The sharper decline in percentage live larvae on the AG1 diets after chill was due to many individuals pupating (Figs. 1 and 2). The amount of Fe in the AG1 diets without antibiotic had a significant effect on the larval survival rate (hazard ratio = 1.53, $\chi^2 = 2.04$, df = 1, $P < 0.01$), whereas population did not ($\chi^2 = 2.04$, df = 1, $P = 0.15$). Compared with larvae on the AG1 diet with 69 Fe mg/liter, those on the diet with 122 Fe mg/liter were 53% less likely to survive, those on the 179 Fe mg/liter diet were 133% less likely to survive, and those on the 239 Fe mg/liter diet were 255% less likely to survive. When larval survival on the AG1 diets with 179 Fe mg/liter and with 180 Fe mg/liter plus antibiotic were compared there was no significant difference due to diet ($\chi^2 = 0.39$, df = 1, $P = 0.53$) or population ($\chi^2 = 0.01$, df = 1, $P = 0.90$).

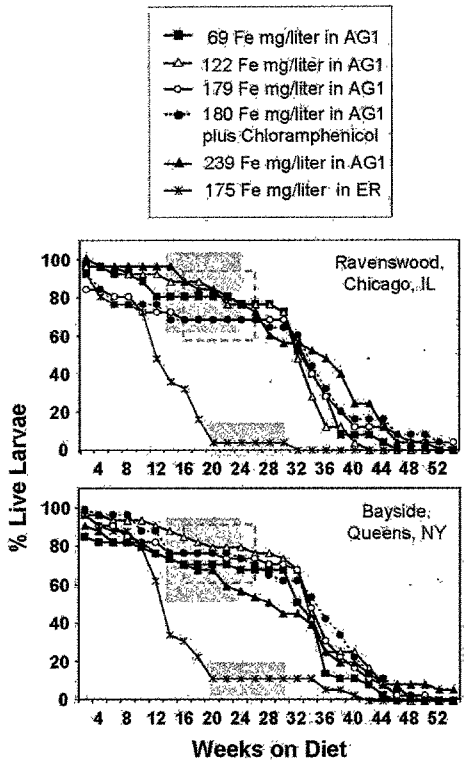

Fig. 1. Percentage of live larvae of *A. glabripennis* from New York and Illinois over 52 wk on artificial diets containing varying quantities of available iron. The diets were AG1 and the modified ER. The shaded area indicates the chill period at 10 ± 1°C and 85 ± 10% RH, and the dashed box indicates the chill period for the diet containing 239 Fe mg/liter. Declines in percentage live larvae before chill are primarily due to mortality, whereas declines after chill are primarily due to pupation.

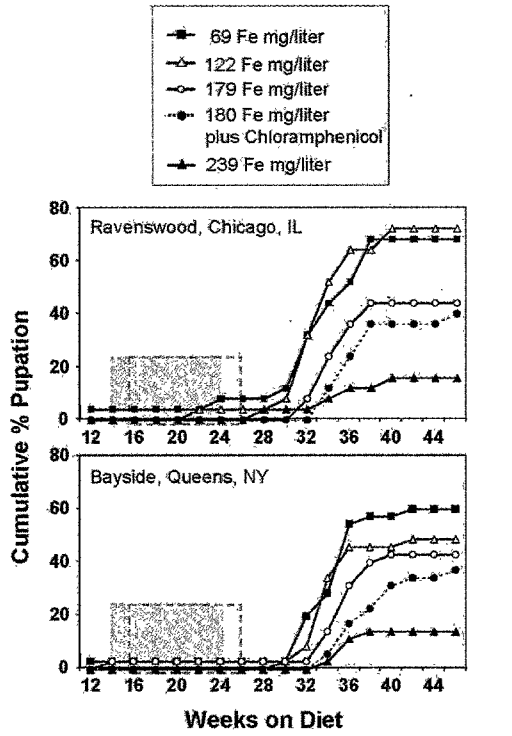

Fig. 2. Cumulative percentage pupation of *A. glabripennis* from New York and Illinois on *A. glabripennis* diet modification #1 containing varying quantities of available iron. The shaded area indicates the chill period at 10 ± 1°C and 85 ± 10% RH, and the dashed box indicates the chill period for the diet containing 239 Fe mg/liter.

Percentage of pupation was higher on the AG1 diet than on the ER diet for both populations (Fig. 2). In general, percentage pupation declined with increasing Fe in the AG1 diet (Fig. 2). The exception to this trend was that there was no significant difference in pupation rates for larvae on diets with 122 and 179 Fe mg/liter for New York individuals ($\chi^2 = 0.72, P = 0.39$) and 69 and 122 Fe mg/liter for Illinois individuals ($\chi^2 = 0.38$, $P = 0.54$) (Fig. 3). There was no significant difference in percentage pupation between the AG1 diets that contained 179 Fe and 180 Fe mg/liter with antibiotic for either population (New York: $\chi^2 = 0.75$, $P = 0.39$; Illinois: $\chi^2 = 1.30$, $P = 0.25$; Fig. 2).

The time from the end of the chill period to pupation differed significantly among AG1 diet treatments ($F = 5.88$, df = 4, $P < 0.01$) but not between populations ($F = 2.12$, df = 1, $P = 0.15$) or the interaction between the two ($F = 0.12$, df = 4, $P = 0.97$) (Table 3). The time from the end of the chill period to pupation was significantly longer for the AG1 diet containing antibiotic than for all other diets, except the one containing 179 Fe mg/liter (Table 3). Diet treatment also had a significant effect on total days to pupation ($F = 3.93$, df = 4, $P = 0.01$), whereas population ($F = 0.94$, df = 1, $P = 0.34$) and the interaction between the two ($F = 0.12$, df = 4, $P = 0.97$) did not have significant effect. In general, days to pupation increased with increasing Fe mg/liter in the diets. Larvae on the AG1 diet containing antibiotic took significantly longer to pupate than those on the diets containing 69 or 122 Fe mg/liter (Table 3). These between treatment differences were due in part to larvae that pupated before chill. One individual from each population on the 69 Fe mg/liter AG1 diet and one from the New York population on the 179 Fe mg/liter AG1 diet pupated early. In addition, two Illinois larvae pupated during the chill period, one each on the AG1 diets with 69 and 122 Fe mg/liter (Fig. 2).

There were significant differences in pupal duration between diet treatments ($F = 3.46$, df = 4, $P = 0.01$) and populations ($F = 4.48$, df = 1, $P = 0.04$) (Table 3). Pupal duration tended to decrease with increasing Fe mg/liter in the diet for New York pupae but not for Illinois pupae.

Adult weights differed significantly due to an interaction between diet treatments and populations ($F = 5.00$, df = 4, $P < 0.01$). Adult weights tended to decrease with increasing Fe mg/liter in the AG1 diet for New York individuals (Table 3). The mean adult

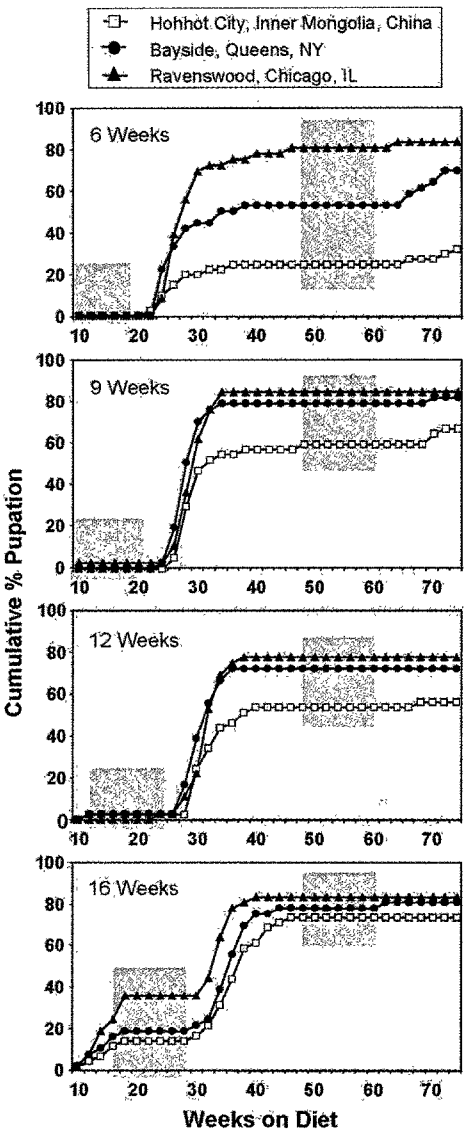

Fig. 3. Cumulative percentage pupation of *Anoplophora glabripennis* from New York, Illinois, and China on *A. glabripennis* diet modification #2 with varying start times for the first larval chill period. Larvae were held at 25 ± 2°C and 60 ± 5% RH except during the shaded time periods when they were chilled at 10 ± 1°C and 85 ± 10% RH.

weights for the Illinois individuals may be affected by the percentage females because females on average weighed 380 mg more than males.

Experiment 2: Timing of Larval Chill. Percentage of hatch of eggs laid on chilled bolts differed significantly between populations ($F = 3.57$, df = 2, $P = 0.04$) but not between chill temperatures ($F = 3.43$, df = 1, $P = 0.07$) or groups experiencing different chill durations (1–50 and 51–73 d) ($F = 3.28$, df = 1, $P = 0.08$). Mean percentage of hatch was 67.3 ± 4, 56.4 ± 4, and 51.2 ± 6 for China, New York, and Illinois eggs, respectively. The percentage of hatch at the time that the bark was stripped was 3.1% for China eggs, 4.8% for New York eggs, and 5.8% for Illinois eggs. Percentage of hatch averaged 63.9 ± 3 for bolts held at 10°C and 52.7 ± 4 for bolts held at 5°C.

Population had a significant effect on larval survival rate (hazard ratio = 0.76, $\chi^2 = 5.69$, df = 1, $P = 0.02$), whereas chill treatment did not ($\chi^2 = 2.46$, df = 1, $P = 0.12$). Compared with the China larvae, the New York larvae were 24% more likely to survive and the Illinois larvae were 58% more likely to survive. Survival to the beginning of the chill period was consistently high across treatments and populations (Table 4); most of the prechill larval mortality was due to first instars that failed to establish on the diet. China larval survival rate dropped after chill in the 6-, 9-, and 12-wk treatments and pupal survival was lower in the 16-wk treatment (Table 4). Across all populations and treatments, larval survival during chill was >90%. The survival rate of New York larvae was lower after chill only for the 6-wk treatment, and survival of Illinois larvae was consistently high as evidenced by >75% pupation in all treatments (Table 4).

The larval weights at the time of chill varied significantly by treatment ($F = 113.53$, df = 3, $P < 0.01$) and population ($F = 14.98$, df = 2, $P < 0.01$) but not by an interaction of the two ($F = 2.053$, df = 6, $P = 0.06$). Larvae chilled at 6 wk weighed significantly less than those chilled at 9 wk, which in turn weighed less than those chilled at 12 or 16 wk (Table 5). Larvae from New York weighed significantly less than those from Illinois or China (Table 5).

The time from the end of the first chill period to pupation was significantly affected by treatment ($F = 3.80$, df = 3, $P = 0.01$) but not by population ($F = 0.59$, df = 2, $P = 0.55$) or the interaction of the two ($F = 1.07$, df = 6, $P = 0.38$). The time from the end of the first chill period to pupation for larvae in the 6-wk treatment (61.9 ± 3 d) was significantly longer than for those in the 12- (49.7 ± 3 d) or 16-wk treatments (47.8 ± 3 d) and intermediate for those in the 9-wk treatment (52.1 ± 3 d).

Time to pupation for larvae that received one chill was significantly affected by chill treatment ($F = 63.7$, df = 3, $P < 0.01$) but not population ($F = 0.35$, df = 2, $P = 0.71$) or the interaction between the two ($F = 1.08$, df = 6, $P = 0.38$). Population also was not significant for the larvae in the 16-wk treatment that pupated without chill ($F = 1.12$, df = 2, $P = 0.37$). Average time to pupation significantly increased with increasing time before larval chill above 9 wk: 189 ± 3, 199 ± 3, 218 ± 3, and 244 ± 3 d for treatments of 6, 9, 12, and 16 wk, respectively. The average time to pupation for larvae that pupated with no chill was 92 ± 3 d and for larvae experiencing two chills was 477 ± 7 d.

Total percentage pupation varied by chill treatment, between populations, and in the timing of the pupation relative to the larval chills (Fig. 3; Table 5). Some of the China, New York, and Illinois larvae from the 12- and 16-wk chill treatments pupated before chilling, with significantly more pupating in the 16- than the 12-wk group (Table 4). Some Illinois larvae

Table 3. Comparison of mean ± SE time to pupation, pupal duration, percentage of females, and adult weight of *A. glabripennis* from the New York and Illinois populations on artificial diets containing varying quantities of available iron

Fe (mg/liter)	Diet	*n*	Time to pupation (d)	Chill to pupation (d)	Pupal duration (d)	*n*	% ♀	Adult wt (mg)
Bayside, Queens, NY								
69	AG1	21	231 ± 6a	72 ± 5a	18.1 ± 0.3a	21	52	1,126 ± 189a
122	AG1	17	236 ± 7a	69 ± 5a	17.9 ± 0.4a	16	47	1,089 ± 191a
179	AG1	15	237 ± 8ab	79 ± 5ab	17.5 ± 0.4ab	15	57	921 ± 192a
239	AG1	5	244 ± 13ab	61 ± 8a	15.8 ± 0.6b	5	40	472 ± 212b
180	AG1 + C	14	259 ± 8b	90 ± 6b	17.4 ± 0.4ab	13	44	1,147 ± 195a
Ravenswood, Chicago, IL								
69	AG1	17	223 ± 7a	68 ± 6a	18.1 ± 0.4a	17	31	1,190 ± 191a
122	AG1	18	227 ± 7a	62 ± 5a	18.6 ± 0.4a	18	53	1,278 ± 190a
179	AG1	11	238 ± 9ab	69 ± 6ab	18.7 ± 0.5a	10	60	1,334 ± 195a
239	AG1	4	236 ± 15ab	52 ± 10a	17.8 ± 0.7a	4	100	1,184 ± 213a
180	AG1 + C	10	253 ± 9b	81 ± 7b	16.9 ± 0.5a	10	44	1,147 ± 195a

Diet: AG1, *A. glabripennis* modification #1 diet as described in this study: AG1 + C, AG1 diet with chloramphenicol added; and ER, *E. rufulus* diet with no chloramphenicol (Galford 1985).

Means within populations for each parameter followed by the same letter are not significantly different based on the least squares mean separation test with $\alpha = 0.05$ and with a Bonferroni correction (SAS Institute 1999).

from the 9-wk chill treatment also pupated before chill (Table 4). A few China larvae from the 6-, 9- and 12-wk treatments, a few New York larvae from the 6- and 9-wk treatments, and one Illinois larva from the 6-wk treatment did not pupate until after they had received a second chill (Fig. 3). The highest total percentage pupation of the China and New York larvae occurred in the 9- and 16-wk treatments, and there were no apparent treatment differences for the Illinois larvae. However, pupal mortality due to fungal infection, failure to shed the last head capsule, or incomplete pupation varied between treatments and populations, reducing the percentage of adults emerging, especially for the China population (Table 5).

Pupal duration for larvae receiving one chill differed significantly by chill treatment ($F = 4.91$, df = 3, $P = 0.00$) but not population ($F = 1.88$, df = 2, $P = 0.16$) or the interaction of the two ($F = 0.17$, df = 6, $P = 0.99$). The mean pupal duration for larvae in the 6-wk chill treatment was significantly less (16.6 ± 0.2 d) than that for larvae in the 9- (17.5 ± 0.1 d), 12- (17.4 ± 0.1 d), or 16-wk (17.5 ± 0.2 d) chill treatments. There was no population effect on pupal duration for larvae in the 16-wk chill treatment that pupated before chill ($F = 0.44$, df = 2, $P = 0.57$). The mean pupal duration for these larvae was 16.5 ± 0.9 d. There was a weak positive correlation between pupal duration and adult weight, with pupal duration tending to increase with increasing individual weight.

Adult weights for larvae that were chilled once differed significantly by chill treatment ($F = 12.38$, df = 3, $P < 0.01$) and population ($F = 11.83$, df = 2, $P < 0.01$) but not the interaction between the two ($F = 1.46$, df = 6, $P = 0.19$). Adults from New York weighed less (1,183 ± 228 mg) than those from Illinois (1,347 ± 228 mg) or China (1,355 ± 228 mg) (Table 5). Adults in the 6-wk treatment group were lighter on average (1,152 ± 229 mg) than all other treatment groups (9 wk: 1,262 ± 228 mg; 12 wk: 1,388 ± 228 mg; and 16 week 1,376 ± 228 mg) (Table 5). For adults from the 16 wk

Table 4. Comparison of percentage larval and pupal survival and percentages of individuals reaching the pupal and adult stages of *A. glabripennis* reared on artificial diet and with different larval chill times for the China, New York, and Illinois populations

Wk 1st chill	*n*	% live larvae		% pupation			Total % pupal survival	% adult emergence
		Beginning 1st chill	Beginning 2nd chill	Prechill	Post 1st chill	Post 2nd chill		
Hohhot City, Inner Mongolia, China								
6	41	81	22	0	24	10	71	24
9	41	90	12	0	59	7	78	51
12	41	81	15	2	51	5	80	46
16	41	71	2	15	59	0	67	49
Bayside, Queens, NY								
6	36	97	17	0	53	17	84	58
9	36	89	3	0	78	3	100	81
12	36	83	3	3	69	0	100	72
16	36	69	0	19	61		83	67
Ravenswood, Chicago, IL								
6	36	92	3	0	81	3	97	81
9	36	81	0	3	81		97	81
12	36	83	3	3	75	0	93	72
16	36	61	0	36	47		97	81

The diet used was the *A. glabripennis* modification #2 as described in this study. The second chill occurred at week 48 for all treatments. No value indicates there were no larvae from that population that received a second chill.

Table 5. Comparison of mean (± SE and [n]) weight of chilled larvae, time from the end of chill to pupation, time to pupation, adult weight, and percentage of females of *A. glabripennis* reared on artificial diet and with different larval chill times for the China, New York, and Illinois populations

Wk 1st chill	Chill wt (mg)	Chill to pupation (d)	Time to pupation (d)			% ♀	Adult wt (mg)		
			No chill	After 1st chill	After 2nd chill		No chill	After 1st chill	After 2nd chill
Hohhot City, Inner Mongolia, China									
6	1,097 ± 72 [33]d	57 ± 8 [8]a		183 ± 8 [8]c	493 ± 34 [2]	70		1,197 ± 24 [7]ab	916 ± 44 [2]
9	1,467 ± 68 [37]c	51 ± 5 [19]a		198 ± 5 [19]bc	487 ± 4 [2]	67		1,239 ± 23 [19]b	1,422 ± 43 [2]
12	1,861 ± 72 [33]b	51 ± 5 [17]a	81 [1]	219 ± 5 [17]b	467 [1]	63	1,033 [1]	1,464 ± 23 [17]ab	1,544 [1]
16	2,318 ± 78 [28]a	51 ± 6 [15]a	99 ± 8 [5]	247 ± 6 [15]a		25	1,085 ± 11 [4]	1,518 ± 23 [15]a	
Bayside, Queens, NY									
6	834 ± 71 [35]c	58 ± 5 [17]a		187 ± 5 [18]c	468 ± 9 [3]	50		1,044 ± 23 [18]a	1,479 ± 32 [2]
9	1,321 ± 74 [32]b	49 ± 4 [28]a		196 ± 4 [28]c	492 [1]	57		1,203 ± 23 [28]a	1,567 [1]
12	1,558 ± 76 [30]ab	48 ± 5 [24]a	74 [1]	217 ± 5 [25]b		38	746 [1]	1,262 ± 23 [25]a	
16	1,768 ± 87 [22]a	50 ± 5 [16]a	88 ± 7 [6]	247 ± 5 [18]a		50	929 ± 21 [6]	1,221 ± 23 [18]a	
Ravenswood, Chicago, IL									
6	1,067 ± 74 [33]c	71 ± 4 [28]a		197 ± 4 [28]a	445 [1]	55		1,216 ± 23 [26]b	1,009 [1]
9	1,580 ± 77 [30]b	57 ± 4 [27]ab	63 [1]	204 ± 4 [28]ab		48		1,344 ± 23 [28]ab	
12	1,909 ± 76 [30]ab	50 ± 4 [26]b		216 ± 4 [26]b		36		1,437 ± 23 [25]a	
16	2,003 ± 91 [20]a	43 ± 5 [17]b	100 ± 5 [12]	239 ± 5 [17]a		59	1,187 ± 20 [12]	1,389 ± 23 [17]ab	

The diet used was the *A. glabripennis* modification #2 as described in this article. The second chill occurred at week 48 for all treatments. In some cases, the exact date of pupation was not known or the adult was not weighed. Values given for chill to pupation time are only for those larvae that received only one chill. Values for each parameter within each population followed by the same letter are not significantly different based on the least squares mean separation test with $\alpha = 0.05$ and with a Bonferroni correction (SAS Institute 1999). No value indicates that no individuals were present in that group.

chill treatment, whether the larvae pupated before or after chill had a significant effect on adult weight ($F = 7.52$, df = 1, $P = 0.00$) as did the population from which they came ($F = 7.52$, df = 2, $P < 0.01$). Adult weights of larvae that pupated before chill were significantly lower (1,152 ± 235 mg) than those of larvae that pupated after chill (1,374 ± 231 mg).

Adult longevity differed significantly among populations ($F = 5.19$, df = 2, $P = 0.01$) but not by treatment ($F = 0.25$, df = 3, $P = 0.86$) or the interaction of treatment and population ($F = 0.36$, df = 6, $P = 0.90$). New York adults lived for a significantly shorter period (81 ± 6 d) than did China (112 ± 8 d) or Illinois (93 ± 6 d) adults.

Discussion

Chilling oviposition bolts containing eggs for up to 80 d at 10°C provides a useful means of stockpiling eggs and synchronizing hatch. The percentage of hatch from the bolts used in experiment 1 was comparable with the hatch from unchilled bolts from the same generation and populations (Keena 2002). The egg hatch from the bolts chilled at 5°C in experiment 2 was not significantly lower than from bolts chilled at 10°C, but in other experiments higher egg morality has resulted when 5°C was used (unpublished data). Thus, chilling at 5°C is less desirable. The bolts used in experiment 2 also were drier (as evidenced by splitting during chill), and this resulted in lower hatch from even the bolts chilled at 10°C. Thus, if bolts are to be chilled, they should have relatively high initial moisture content.

The AG2 diet was the best diet evaluated because a higher percentage of larvae survived and pupated, usually in a shorter time. The pourable AG diets overall helped in reducing the amount of preparation necessary and production time and overall effort. The adjustments in the amounts of each ingredient (i.e., effectively increasing the lipid, protein, and sugar content while reducing bulk) and increase in relative water content of the AG diets over the ER diet resulted in faster development, higher weights, and lower larval mortality. The lower Fe levels in the AG2 diet (same as in the AG1 69 mg/liter diet) improved larval survival, increased pupation rate, and shortened the developmental time as evidenced by pupation occurring before chill. The higher levels of preservatives in the AG2 diet, compared with the AG1 diet, were so effective at reducing contamination by saprophytes that the time between diet changes could be lengthened from 2 to 6 wk, resulting in higher survival of larvae. The adults produced seemed normal in behavior, size, and morphology compared with field-collected individuals. Together, these changes have reduced the cost (i.e., supplies, salaries, and equipment maintenance) of rearing a single individual from egg (including holding a mated pair to produce the egg) to adult by 50%. A single beetle now costs $21 if startup (reusable holding containers) and overhead costs are not included (totaling $10). The AG2 diet has

now been used for two additional generations with similar results to those reported here.

Larvae on the AG2 diet and the *A. glabripennis* diet (Zhao et al. 1998) modification recommended by Dubois et al. (2002) (AGD) developed at about the same rate and had similar percentage of pupation, even though the rearing temperature on the AGD diet was 2.5°C lower. As reported by Dubois et al. (2002), Illinois larvae were placed on the AGD diet when they were equivalent in weight to larvae at 6 wk on the AG2. Adding 40 d to the number of days to pupation gives 87 and 104 d (male and female, respectively), which is similar to the time period for Illinois larvae that pupated without chill on the AG2 diet. Adult weight on the AG2 diet was slightly higher than that on the AGD diet, possibly due to a slightly higher water content. Increasing the water content in the AGD diet was reported to result in higher weights (Dubois et al. 2002). Adult weight has been shown to be correlated with fecundity (Keena 2002), so larger individuals are desirable for a rearing program. In addition, the AG2 diet is easier to prepare and can be dispensed into many containers more quickly than can the drier AGD diet.

The fact that increasing iron had deleterious effects on *A. glabripennis* is interesting because iron modulates critical processes in insects, such as synthesis of nucleic acids, electron transfer in cellular respiration, cuticle formation and tanning, and steroid production, and it is important in several rate-limiting metabolic processes (Locke and Nichol 1992). When dietary iron is deficient for two successive generations in gypsy moth, *Lymantria dispar* L., egg hatch is reduced, larval development slows, and larval mortality increases (Keena et al. 1998). Too much iron can be equally deleterious because it serves as a prooxidant that can reduce the nutritive value of the diet and as a toxin that can have direct affects on insect survival (Cohen 2003). Unlike gypsy moth, *A. glabripennis* apparently does not need more iron than is provided in the wheat germ and other diet components, so adding $FePO_4$ has a deleterious effect. This effect could be explained by the levels of iron in woody tissues being generally low (Haack and Slansky 1987) so that *A. glabripennis* is adapted to live on a diet that contains little iron. In addition, the high fiber and tannin content of woody tissues reduces the bioavailability of protein and minerals, such as iron (Mattson and Scriber 1987). This might partly explain why the increased protein and decreased fiber of the AG diet compared with that of the ER diet resulted in improved larval survivorship and development.

Chloramphenicol, a compound with antibacterial activity, had no apparent effect on *A. glabripennis*, and the three antifungal compounds in the AG2 diet seemed to improve survival and development (see Merck 1996 for antimicrobial activity of compounds). This suggests that *A. glabripennis* does not have any symbiotes that might be killed by these compounds. Some yeast-like organisms have been found in the mycetomes of Cerambycidae that seem to function as symbiotes, but their exact role is debatable (Linsley 1959). These organisms are held in small tissue masses or invaginations of the gut wall in larvae and in intersegmental pouches of the ovipositor, and then smeared on the eggs as they are oviposited to pass them to the next generation (Linsley 1959). However, they have not been found in cerambycid larvae that live in fresh wood of deciduous trees as *A. glabripennis* does (Schomann 1937). Whether these yeast-like organisms or other symbiotes that are not killed by the antimicrobials used in artificial diets exist in *A. glabripennis* remains uncertain.

Significant differences among the populations in larval and adult weights, time to pupation, and response to the timing of larval chill were documented. The larvae and adults from the Illinois and China populations were significantly heavier than those from New York. This is consistent with the adult weight differences reported for the Illinois and New York populations by Keena (2002). The lower larval survival and pupation in the China population may have been due, in part, to this population being only in its second laboratory generation and thus experiencing some difficulty in adapting to artificial diet or laboratory rearing conditions.

On average, larvae from the Illinois population were ready to pupate sooner than those from the other two populations. This is most clearly illustrated by the numbers of Illinois larvae that pupated without chill in the 16-wk treatment of experiment 2 and by the minimal effect of the various chill treatments in experiment 2 on total percentage of pupation. The China population was the most sensitive to the timing of chill, possibly indicating that larvae from this population tend to pupate at a later instar, more may require a chill to complete development, or some may require >1 yr to complete development. If larvae that pupated before or during chill in each treatment in experiment 2 were in the instar that should have been completed in the previous treatment (e.g., fifth instars and the 9-wk treatment; sixth instars and the 12-wk treatment), then 3% of the Illinois larvae pupated in the fifth instar, 3% (in chill) in the sixth instar, 30% in the seventh instar, and the remainder in the eighth or higher instars (Table 4). In comparison, 2% of the China larvae pupated in the sixth instar, 13% in the seventh instar and the rest in the eighth or higher instars. Chilling larvae at 12 wk minimizes prechill pupation and results in good overall pupation rates if synchrony of adult emergence is desirable. However, if a shorter time to pupation is desired, this can be accomplished by delaying chill until most of the larvae not requiring a chill to complete development have pupated (18–20 wk).

A larval chill period is not required by all the larvae, which is consistent with previous findings (Zhao et al. 1998, Dubois et al. 2002). However, the percentage of individuals in a population that requires one or more chills may vary as indicated by the results in experiment 2. Hua et al. (1992) indicated that the percentage of larvae that require 2 yr to complete development varies from province to province in China, with the highest percentages occurring in the more northern

areas. In addition to the need of a chill period for pupation, two other factors are likely involved: the critical body size required for pupation and the timing of the chill period in relation to body size attained. When the period of time before the onset of chill is shorter than needed for the larva to develop to its final instar or reach its critical body size for pupation, the larva will resume feeding and development after chill, before it is ready to pupate. In *Anoplophora chinensis* (form *malasiaca*) (Forster), a species closely related to *A. glabripennis*, a larger percentage of larvae from eggs laid later in the summer compared with early in the summer pupate only after the second winter and pupate at higher instars (Adachi 1994). If this development is not completed before summer temperatures are experienced, the larva may continue molting and wait to pupate until after the next chill period (winter or cool fall), depending on population/temperature interactions. At a constant 25°C, some *A. glabripennis* larvae molt and gain weight until a critical size is attained and then continue to feed a little and molt with little weight gain until conditions for pupation exist (i.e., a chill period followed by warmer temperatures) (unpublished data). Adachi (1994) also found that *A. chinensis* larvae held at a constant 25 or 30°C continued to molt and did not pupate. The critical weight for pupation seems to vary both within and between populations, which could indicate a high degree of plasticity or genetic variation for this trait (Slansky and Scriber 1985). Further evaluation of the effects of temperature on development is needed to better understand the triggers for pupation and to predict the timing of various stages for eradication and control treatments. In addition, the effects of different larval chill temperatures and intervals still need to be evaluated because shortening the chill period shortens the total developmental time and further reduces rearing costs.

Acknowledgments

I thank Ann Hajek, John Tanner, David Williams, and the anonymous reviewers for the critical reviews of this paper, and John Brown and John Stanovick for statistical review and assistance. Mark Hood, Jeff Horn, Walter Major III, Geoffrey Martino, Paul Moore, Steve Ulanecki, Alice Vandel, and Yvette Williams provided technical assistance. I also thank USDA–APHIS personnel for coordinating the efforts to obtain infested logs from both populations.

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Received 30 June 2004; accepted 15 April 2005.