

# Rearing Protocol and Life History Traits for *Poecilus chalcites* (Coleoptera: Carabidae) in the Laboratory<sup>1</sup>

Jonathan G. Lundgren,<sup>2,3</sup> Jian J. Duan,<sup>4</sup> Mark S. Paradise<sup>4</sup> and Robert N. Wiedenmann<sup>2</sup>

Northern Grain Insects Research Laboratory, USDA-ARS, Brookings, SD, 57006, USA

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**Abstract** A rearing protocol for the predaceous ground beetle, *Poecilus chalcites* (Say), is described. The effects of dietary constituents, substrate moisture content, and substrate type on larval developmental rates and size were examined in the laboratory. The protocol was successful in obtaining nearly 80% pupation rates, although adult size was smaller than field-collected beetles, and laboratory-produced adults did not lay eggs. We determined experimentally that some of the components of the meridic diet used for colony production could be removed without compromising larval size or developmental rates, but that nutrition beyond cat food was necessary to increase larval size. We found a positive correlation of larval size with increased moisture content using vermiculite substrate at three moisture levels (33.3, 50.0 and 66.7% by weight). Untreated Fer-Til® (GreenGro Products, Jackson, WI) soil resulted in the highest pupation rate (70 to 80%) and had one of the shortest developmental periods of the five soils tested. Steaming or sifting Fer-Til soil compromised its ability to support larval development until pupation.

**Key Words** Biological control, ground beetles, nutrition, predator, semi-artificial diet

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Ground beetles are abundant entomophagous predators and granivores in agricultural systems (Lövei and Sunderland 1996, Kromp 1999, Menalled et al. 2005). In addition to their role in pest management, ground beetle communities are excellent bioindicators of habitat qualities (Lövei and Sunderland 1996, Perner 2003). Although ground beetles are generally recognized as being important fauna of agroecosystems, little is known concerning their life history strategies relative to other predators. A number of factors are responsible for discrepancies in the knowledge of ground beetles, including behavioral and physiological heterogeneity within the family (Thiele 1977), difficulties associated with the taxonomy of adults of some groups and larvae of most species, and the inability to maintain most species of ground beetles in culture in the laboratory (Tomlin 1975, Goulet 1976). A rearing protocol for carabids that is based on an artificial diet would be useful for understanding life history attributes of ground beetles, and for developing laboratory assays to assess the dietary toxicity of insecticidal substances to adults and larvae.

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<sup>2</sup>Center for Ecological Entomology, Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL, 61820, USA.

<sup>3</sup>Address inquiries (e-mail: jlundgren@ngirl.ars.usda.gov).

<sup>4</sup>Monsanto Company, Ecological Technology Center, 800 N. Lindbergh Blvd, St. Louis, MO, 63167, USA.

*Poecilus chalcites* (Say) is a carnivorous ground beetle that is commonly encountered in crop habitats in North America. This species is recorded from 35 states east of the Rocky Mountains in the U.S., and in Ontario and Quebec in Canada (Bousquet and Larochelle 1993). *Poecilus chalcites* prefers moist soils characteristic of cropland and has been reported in cotton (Whitcomb and Bell 1964), corn (Kirk 1971a, Hsin et al. 1979), soybeans (Wiedenmann et al. 1992), and alfalfa (Los and Allen 1983). Adults and larvae are believed to be exclusively predatory, and *P. chalcites* adults have been observed to feed on several crop pests, including western corn rootworm (*Diabrotica virgifera virgifera* LeConte, Kirk 1975), corn earworm (*Helicoverpa zea* [Boddie], Lesiewicz et al. 1982), black cutworm (*Agrotis ipsilon* Hufnagel, Best and Beegle 1977, Lund and Turpin 1977), and armyworm (*Pseudaletia unipuncta* Haworth, Laub and Luna 1992, Clark et al. 1994). In Illinois farmland (field corn, soybeans and organic vegetable fields), we found *P. chalcites* to be the most abundant ground beetle species during most of the growing season (J. G. L., unpubl. data), an observation noted in other states as well (Kirk 1975, Hsin et al. 1979, Best et al. 1981).

Here, we describe a method to rear *P. chalcites* from eggs to adulthood and examine the effects of varying the dietary constituents, substrate moisture content, and substrate type on larval fitness. This rearing method can be used for assays to evaluate the toxicity of insecticidal substances via dietary exposure and to explore the behavior and physiology of this species.

### Materials and Methods

**Colony origin and maintenance.** All the insects (eggs and larvae) used in this study originated from adults of *P. chalcites* collected with dry pitfall traps between April and September in agricultural habitats (corn, soybean, and organic vegetable) in Champaign, IL, 2001–2003. Upon collection, *P. chalcites* was identified using morphological keys (Lindroth 1966), and voucher specimens were placed in the arthropod collection of the Illinois Natural History Survey, Champaign, IL (accession # 44366–44370). Adult carabids were placed in 0.45-L glass jars (Ball® jars, 8 cm diam × 12 cm high, Alltrista Corp., Muncie, IN) that contained approximately 0.18-L unsteamed Fer-Til® potting soil (GreenGro Products, Jackson, WI) which served as a rearing and oviposition substrate. This soil mix consisted of peat, sand, humus, and soil, with initial water content of approximately 35% (w/w). Fer-Til soil, labeled as sterile, contained a number of organisms, including algae, moss, nematodes, and fungus gnats. Soil in the jars was replaced once per week. For the adult colony, photoperiod was 14:10 (L: D) h, temperature was 28°C, and relative humidity (RH) was 20 to 30%. Adults were reared on artificial diet (Table 1, diet A) or water-saturated cat food (Iams Original Adult Formula™; The Iams Company, Dayton, OH). Two pieces of cat food or 0.5 g of artificial diet A were provided on an inverted plastic lid (BioServ product #9053, BioServ, Frenchtown, NJ), and food was changed every other day. Water was present in the diet and soil, and as condensation on the sides of the jars, so additional water was not supplied. In the laboratory, adults survived for a mean of 30 d without food, and more than a year in the laboratory when food was provided (J. G. L., unpubl. data). Five adult *P. chalcites* of mixed sex ratio were reared per jar. The sexes of this species can be distinguished by examining the foretarsi under 50X magnification (J. G. L., pers. obs.). The most proximate foretarsus in males is heart-shaped; in females this foretarsus is comparatively thin. Male foretarsi appear generally more stout and setose than in females.

Females readily oviposit white, 1.5-mm long eggs directly into soil, often partially burying the eggs into clods of soil with high levels of clay. After the weekly soil change for adults, soil containing carabid eggs was transferred to a 0.45-L glass jar with a tight-fitting lid. Eggs were allowed to hatch in the sealed jars, and newly-hatched larvae were collected from the soil twice per week. Females must mate multiply to achieve maximum fecundity; one preliminary study showed the greatest fecundity to be approximately 53 eggs per female (J. G. L., unpubl. data). Most females (approximately 90%) oviposited in the laboratory, and fertilized eggs were produced for up to 25 d after collection, regardless of how many times females were mated (J. G. L., unpubl. data).

Larvae were reared individually in 32-ml plastic cups with tight-fitting plastic lids (BioServ product #9051); cups were filled with 16 ml of unsteamed Fer-Til potting soil. Attempts to rear multiple larvae in a single container were unsuccessful. *Poecilus chalcites* larvae are cannibalistic; despite varying the larval densities in different volumes of soil, only a single larva remained in each cup after 1 wk (J. G. L., unpubl. data). Also, larvae were strongly dependent on the diet for nutrition, and died within 7 d if left unfed (J. G. L., unpubl. data). A moist environment was critical for larval survival; exposing larvae to 30% RH outside the soil for as little as 1 h was fatal (J. G. L., unpubl. data). Larvae were provided with 0.1 g of artificial diet A, and diet was changed every 24 to 48 h. Water was present in the diet and soil, and as condensation inside the cups, so additional water was not supplied. Soil was changed approximately every 7 d. Larvae were allowed to pupate in the soil; ordinarily they pupated in a cell they created in the soil at the bottom of the cup, or at the soil surface.

**Rearing evaluation for colony.** As an initial evaluation of this rearing procedure, we recorded the duration of the larval and pupal stages, and compared the sex ratio and adult weights with field-collected populations. Prepupal and preadult survivorships of larvae ( $n = 28$ ) were recorded. Adults that were produced in the laboratory were reared under the same conditions as described for field-collected adults. Means of sex ratio and post-mortem dry weight were compared between field-collected adults ( $n = 60$ ) and laboratory-reared adults ( $n = 73$ ) using standard least squares models run with JMP software (SAS 1998). The ages of field-collected adults were unknown, and ages of laboratory-produced adults at the time of weighing ranged from 1 to 2 months.

**Diet component study.** An artificial diet was used to maintain a *P. chalcites* colony. The diet contained a mixture of several ingredients (Table 1) that have been used previously to rear carabids in the laboratory (Kirk 1975, Tomlin 1975, Goulet 1976, Weseloh 1998). The importance of some of the major nutritional components of this diet for *P. chalcites* larval survival and development were investigated experimentally.

Larvae (1 to 3 d old;  $n = 23$  for each treatment) were placed individually into 32-ml plastic cups, and then fed one of four diets. Diets A–D are presented in Table 1; diets B–D are derivations of diet A, with major ingredients being sequentially replaced by an appropriate ratio of cat food and water in order to maintain the texture of diet A. Diet was replaced every 48 to 72 h, and each feeding consisted of 0.25 g of diet. The substrate was unsteamed Fer-Til potting soil, which was sifted using standard U.S. number 10 sieves (2 mm openings). The initial substrate moisture content was approximately 35%, and substrate was changed weekly. This experiment was conducted in an environmentally-controlled chamber at 30°C, with  $65 \pm 5\%$  RH, 16:8 (L: D) h.

Every 7 d for 42 d, the cups were checked for the presence of dead larvae, pupae, and adults. At 35 d, surviving larvae were temporarily removed from the substrate and weighed to the nearest 0.1 mg, and then were returned to the experiment. Similarly, the weights of living, newly-emerged adults and the eclosion rates were recorded. For each diet, the proportion of survivors at 45 d was recorded. A proportional hazards model (SAS Institute 1998) was used to compare survivorship among the samples reared on different diets. Standard least squares models (SAS Institute 1998) were used to compare larval and adult weights among the diet treatments. A nominal regression model (SAS Institute 1998) was used to compare pupation rates among different diet treatments.

**Substrate evaluation.** We hypothesized that the substrate used in rearing could affect the fitness of carabid larvae and pupae, and thus we evaluated the suitability of several substrates for rearing *P. chalcites* in the laboratory. Substrates evaluated were (1) untreated Fer-Til soil, (2) Fer-Til soil that had been steamed for 30 to 60 min at 82°C, (3) OECD soil, and (4) SARPY 91 soil. The OECD soil is produced by Massachusetts Research Center, Springborn Laboratories, (Wareham, MA) and contained 35% coarse sand, 35% fine sand, 20% kaolin clay, and 10% peat moss by weight. The SARPY 91 soil originated from New Bloom Field, MO, and stored at Monsanto Company (Chesterfield campus) since 2001; this sandy loam soil contained 71% sand, 22% silt and 7% of clay; pH was 8.1; and it contained 8.7% w/w. In addition to the aforementioned substrates, we determined in preliminary studies that a commercial variety of top soil, silica sand (Mallinckrodt Baker, Inc. Phillipsburg, NJ), Hartke compost (Hartke Family Nursery, St. Louis, MO), moistened filter paper (Whatman International Limited, Maidstone, United Kingdom) and TeraSorb Hydrogel (Plant Health Care, Inc., Pittsburgh, PA) were unsuitable for larval development (J. G. L., J. J. D. and M. S. P., unpubl. data). Similarly, Kirk (1971b, 1975) found that using moistened paper led to less than 50% pupation; eclosion rates were not provided.

For testing, larvae from the colony (1 to 2 d old) were randomly assigned to one of the four substrates ( $n = 30$ ). Larvae were reared individually in 32-ml plastic cups that contained 16 ml of the respective substrate. Experimental conditions were 14: 10 (L: D) h, 28°C, and 20 to 30% RH. The developmental status of immature insects was checked every 48 h, and old diet was replaced with 0.1 g of artificial diet A, and 2 to 4 ml of distilled water was added. The substrate was replaced every 7 d. The proportions of immatures that pupated and eclosed were compared among treatments with likelihood-ratio tests, and the durations of the larval stages were compared among treatments with Van der Waerden test for normal quantiles (SAS Institute 1998).

**Substrate moisture assays.** Larvae of *P. chalcites* are sensitive to desiccation (as suggested by Kirk 1975 and observed by J. G. L.), and experiments were devised to evaluate the most suitable soil-moisture content for larval development and survivorship. Experimental conditions were 30°C, 65 ± 5% RH, 16: 8 (L: D) h. Larvae (1 to 3 d old) were isolated from the colony into individual 32-ml plastic cups that contained 16 ml of moistened vermiculite with a particle size <2 mm, (lot #3K22A2, Zonolite™, Grace Construction Products, Cambridge, MA) and 0.25 g of diet A (Table 1). Each larva was randomly assigned to one of three substrate treatments that contained 33.3, 50.0, or 66.7% (w/w) ( $n = 20$ ). Diet was replaced every 48 h, and vermiculite was replaced every 7 d. The moisture contents of the different treatments correspond to the initial substrate moisture content when new vermiculite was added; moisture content decreased between weekly substrate changes. Cups were checked

**Table 1. Meridic diets evaluated for *Poecilus chalcites*\***

Ingredients	Diet A	Diet B	Diet C	Diet D
Cat food (Iam's adult maintenance formula)	35 g	38.3 g	46.6 g	65 g
Distilled water (soak the cat food in water until soft)	70 ml	76.7 ml	93.3 ml	130 ml
Chicken liver	25 g	25 g	—	—
Raw chicken egg	1	1	1	—
Lepidopteran larvae (7th instar <i>G. mellonella</i> )	10 g	—	—	—
Vitamin solution**	1.5 ml	1.5 ml	1.5 ml	1.5 ml
Sorbic acid	1 g	1 g	1 g	1 g
Tetracycline	0.5 g	0.5 g	0.5 g	0.5 g
Blend the above ingredients for 3 min, add the following ingredients, and blend for an additional 1 min				
Agar		3 g		
Boiling water		70 ml		

\* Diet A was used to maintain the *P. chalcites* colony in the laboratory. All of these recipes yield approximately 300 ml of diet.

\*\* Vitamin solution was a mixture of the following ingredients: distilled water (100 ml), niacinamide hydrochloride (100 mg), calcium pantothenate (100 mg), riboflavin (50 mg), thiamine hydrochloride (25 mg), pyridoxine hydrochloride (25 mg), folic acid (25 mg), biotin (2 mg), vitamin B12 (0.2 ml) (from Attallah and Newsom 1966).

every 7 d for 42 d, and larvae were recorded as dead, pupated, or eclosed. At 35 d, surviving larvae were weighed to the nearest 0.01 mg, and then were returned to rearing cups. Survivorship was compared among treatments using a proportional-hazards model. The proportions of larvae that survived to pupation and eclosion were compared using likelihood-ratio tests, and larval weights at 35 d were compared among treatments using the Kruskal-Wallis rank-sums test.

## Results

**Rearing evaluation.** In the sample of neonate larvae from the laboratory colony ( $N = 28$ ), the proportions that pupated and eclosed were 0.857 and 0.679, respectively. Mean  $\pm$  SEM durations of the egg stage was  $3.59 \pm 0.05$  d, larval duration was  $34.65 \pm 7.00$  d, and pupal duration was  $7.62 \pm 2.23$  d. Male and female immatures developed at similar rates ( $t_{61} = 0.701$ ,  $P = 0.49$ ). The sex of beetles and whether they were laboratory-reared or collected from the field affected adult dry weight ( $F_{sex} =$

4.06,  $df = 1$ ,  $P = 0.02$ ;  $F_{\text{location}} = 271.43$ ,  $df = 1$ ,  $P < 0.0001$ ;  $F_{\text{sex} \times \text{location}} = 0.004$ ,  $df = 1$ ,  $P = 0.95$ ) (Table 2). Sex ratio (proportion males) of laboratory-produced adults was 0.34 ( $n = 73$ ); sex ratio of field-collected adults was 0.36 ( $n = 305$ ). Under these rearing conditions, laboratory-produced adults could not be induced to lay eggs.

**Diet component study.** The proportional-hazards model did not reveal any significant differences in survivorship of insects reared on any of the four diets ( $\chi^2_3 = 0.40$ ,  $P = 0.94$ ). There were significant effects of diet on larval weight at 35 d ( $F_3 = 3.78$ ,  $P = 0.013$ ). Larvae reared on diet B weighed significantly more than those fed diet D (Fig. 1). Diet type did not significantly affect the pupation rate of larvae ( $\chi^2_3 = 1.01$ ,  $P = 0.80$ ), or the size of eclosed adults ( $F_3 = 0.96$ ,  $P = 0.45$ ). Mean  $\pm$  SEM percentage of individuals that pupated in 45 d was  $26.05 \pm 3.84\%$ , and the mean  $\pm$  SEM adult weight was  $37.27 \pm 1.12$  mg (data pooled over treatments).

**Substrate evaluation.** There was significant variation in the proportion of larvae that pupated in the different substrates ( $\chi^2_{3,116} = 36.20$ ,  $P < 0.0001$ ). Larvae reared in unsteamed Fer-Til soil had the highest pupation rate; 70.0, 33.3, 20.0, and 3.3% of larvae pupated in unsteamed Fer-Til, SARPY 91, steamed Fer-Til, and OECD substrates, respectively (Fig. 2). Similarly, eclosion rates were significantly affected by the substrate type ( $\chi^2_{3,116} = 23.42$ ,  $P < 0.0001$ ), and the highest eclosion rate was for larvae and pupae reared on unsteamed Fer-Til soil. Eclosion rates for the different treatments were 30.0, 3.3, 0, and 0% in unsteamed Fer-Til, OECD, steamed Fer-Til, and SARPY 91 substrates, respectively.

Substrate type significantly influenced the duration of preimaginal development. Development was significantly shorter for larvae in unsteamed Fer-Til, steamed Fer-Til, and SARPY 91 substrates than for larvae in the OECD soil ( $\chi^2_3 = 8.72$ ,  $P = 0.03$ ) (Fig. 2). Mean  $\pm$  SEM (n) larval development periods were  $27.67 \pm 0.74$  (21),  $28.1 \pm 0.66$  (10),  $30.17 \pm 1.74$  (6), and 40 (1) d for larvae reared in unsteamed Fer-Til, SARPY 91, steamed Fer-Til, and OECD soils, respectively (Fig. 2). Except for one exception in the OECD soils, pupae only eclosed in unsteamed soil; mean  $\pm$  SEM duration of the pupal stage in this treatment was  $5.44 \pm 0.38$  d ( $n = 9$ ).

**Substrate moisture assays.** The proportional-hazards model did not reveal any significant differences in survivorship curves of larvae reared at different moisture concentrations ( $\chi^2_2 = 0.44$ ,  $P = 0.80$ ) (Fig. 3a). However, after 35 d, larvae reared in vermiculite with 66.7% water weighed significantly more than larvae in the other treatments ( $\chi^2_2 = 11.32$ ,  $P = 0.004$ ) (Fig. 3b). Furthermore, after 35 d the only larvae to pupate ( $n = 3$ ) and eclose ( $n = 2$ ) were reared in the vermiculite substrate containing 66.7% water.

**Table 2. Mean  $\pm$  SEM adult dry weights (mgs) for *Poecilus chalcites* reared in the laboratory or collected in the field**

	Field collected (n = 60)	Laboratory reared (n = 73)
Male	62.2 $\pm$ 1.8Aa	36.3 $\pm$ 1.3Ba
Female	67.0 $\pm$ 1.8Ab	40.1 $\pm$ 1.1Bb

Values within columns followed by different lowercase letters were significantly different, and values within rows followed by different capital letters were significantly different ( $\alpha = 0.05$ , Tukey-Kramer means comparison).

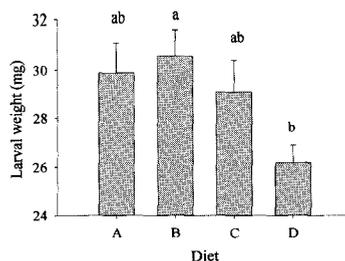


Fig. 1. The effect of diet on *Poecilus chalcites* larval weight. Diets A–D are described in Table 1 and text, and the experiment was conducted on sifted Fer-Til substrate. Larval weights were recorded from living individuals at approximately 35 d of age. Bars with different letters above them are significantly different ( $\alpha = 0.05$ , Tukey-Kramer means comparison).

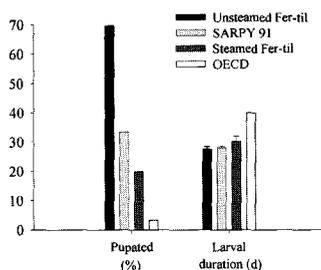


Fig. 2. Pupation rates and larval durations for *Poecilus chalcites* reared on different substrates. The pupation rate bars represent observations on single populations for each treatment, and so no error bars are included. The pupation rates and larval development rates were significantly different among the substrates ( $P < 0.05$ ).

## Discussion

The protocol for rearing *P. chalcites* in the laboratory presented here is adequate for attaining a high level of larval survival sufficient for rearing more than 80% of larvae to pupation. Diet constituents, substrate types, and moisture levels in the substrates all affected larval fitness. Exploring diapause in this species will be critical to maintaining a continuous culture in the laboratory.

Our diet evaluation shows that the original diet (A) (Table 1) can be simplified without affecting the growth of laboratory-reared individuals. Similar pupation rates and larval weights in larvae fed diets A, B and C suggest that fitness is not compromised by the removal of chicken liver and lepidopteran larvae from the carabid artificial diet. That said, we should note that a low proportion of larvae survived to pupation in this study (around 26%), and the impact of diet simplification on pupation rates may be clarified by using more suitable rearing conditions such as unsifted soil

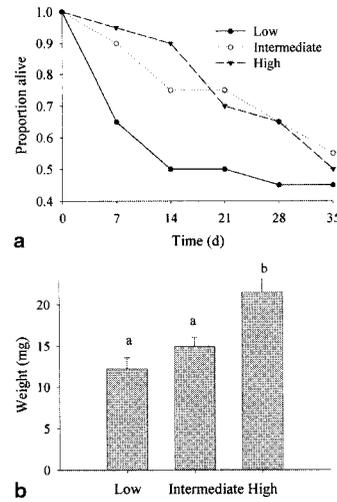


Fig. 3. Survivorship and weights for *Poecilus chalcites* larvae reared under different substrate moisture levels. Low, intermediate, and high substrate moisture contents correspond to 33.3, 50.0, and 66.7% w/w. **3a.** Survivorship was not significantly different among the three treatments ( $\alpha = 0.05$ , proportional-hazards model). **3b.** Larval weights were recorded from live individuals that were approximately 35 d old, and bars with different letters above them are significantly different ( $\alpha = 0.05$ , Tukey-Kramer means comparison).

or increased initial soil moisture, as shown by the substrate evaluation and substrate moisture assays of this study/article. We found that rearing larvae on a diet consisting primarily of cat food (diet D) resulted in comparatively lower larval weights. This indicates that the additional nutrition from the egg or chicken liver was important to larval development. More information on the nutritional requirements for this species will help to optimize the rearing diet, and help to understand the feeding behavior of *P. chalcites* in the field.

The use of appropriate substrates is critical to a successful rearing program for carabid beetles. Inherent factors of the substrates undoubtedly played a role in the success or failure of different substrates to sustain larval development. For example, the abrasiveness of substrates may have affected the larval cuticle or interfered with molting, the consistency of some substrates restricted the movement of carabid larvae, and the relative inability of some substrates to retain water at appropriate levels also affected larval development (J. G. L., pers. obs.). Finally, there may be some important nutritional factors present in some soils that promote larval development.

Findings from this study support the use of unsteamed Fer-Til soil as a substrate for larval rearing (Fig. 2), although it is not clear what component of this soil is contributing to the relatively high level of pupation we observed. Steaming and sifting Fer-Til soil reduced its ability to support larval development; for instance, pupation rates dropped from 70 to 80% in untreated Fer-Til soil to <30% in soil that was steamed or sifted (Fig. 2, and see methods and results in the diet evaluation experi-

ments). This suggests that there may be some biotic factor in the unsteamed soil that is supplementing the nutrition of *P. chalcites* larvae and allowing them to reach pupation. It is possible that providing antibiotics to the beetles in the diet is eliminating microbial symbionts that facilitate digestion in ground beetles (J. G. L., unpubl. data), and these endosymbionts may be restored when the beetles that are reared in unsterilized soil in which microorganisms are allowed to persist. Whatever the soil is contributing to the nutrition or physiology of *P. chalcites*, larvae are dependent on the diet for the bulk of their nutrition.

We found that high water content of soils favored larval development (Fig. 3), and our research quantitatively supported previous published claims that larvae of *P. chalcites* are sensitive to humidity and substrate water content (Lindroth 1966, Kirk 1975). The susceptibility of *P. chalcites* larvae to desiccation may help to explain their habitat preferences and the spatial dynamics of *P. chalcites* within agricultural habitats (Kirk 1975), and why this species does not occur in some habitats (Wiedenmann et al. 2004). Adults of *P. chalcites* are much more mobile than the larval stage, and likely encounter many habitats that vary in their suitability for their progeny. Creating soil environments that are conducive to moisture retention may help to conserve this predator and encourage the suppression of certain agronomic pests through predation.

*Poecilus chalcites* adults require a diapause period in order to achieve potential fecundity, and understanding this diapause will be necessary to maintain *P. chalcites* in continuous culture. By dissection, Kirk (1975) determined that *P. chalcites* remain sexually immature until overwintering occurred; this may explain why the laboratory-produced adults from our rearing colony did not produce eggs. Kirk (1975) found that holding *P. chalcites* adults at 5°C for 30 d was sufficient to prompt each field-collected female to produce an additional 200 eggs. His exact methods concerning photoperiod, environmental conditions, and substrates used for diapause were not presented, and we have not been able to replicate those experiments. In unpublished research, we have discovered that a high proportion of field-collected and laboratory-reared adults can survive exposures of 30 to 60 d at temperatures as low as -5°C, but that when brought out of diapause, these adults did not lay eggs. Nevertheless, *P. chalcites* undoubtedly overwinter as adults (Kirk 1975, Lindroth 1966, J. G. L., pers. obs.), and oviposit in the spring and summer, so there must be some physiological, nutritional, or environmental cue that is used to stimulate egg production. More research into this area will allow us to keep *P. chalcites* in continuous culture in the laboratory.

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