Duration of *Pseudacteon curvatus* Borgmeier (Diptera: Phoridae) Pupal Stages at Reduced Temperatures[1]

Larry G. Thead[2] and Douglas A. Streett

USDA, ARS, Biological Control of Pests Research Unit, P. O. Box 67, Stoneville, Mississippi 38776 USA

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Abstract Duration times were determined for *Pseudacteon curvatus* Borgmeier pupae subjected to various temperature regimes. Pupae that had initially developed 12-15 d in parasitized fire ant, *Solenopsis invicta* Buren, workers held at ~27°C were collected and subsequently held at different temperature regimes. Pupae exposed to a temperature of 26.7°C had the earliest peak fly emergence 15 d later. Pupae exposed to a lower temperature of 18.3°C for 12 or 19 d and then exposed to a temperature of 26.7°C required an additional 7 and 11 d to peak fly emergence, respectively. At 18.3°C peak fly emergence was delayed an additional 20 d. At 10°C for 12 or 19 d followed by 26.7°C, peak fly emergence was delayed an additional 12 and 18 d, respectively. Holding pupae at 10°C for 54 d followed by 26.7°C delayed peak fly emergence by an additional 51 d with a correspondingly high mortality. Fly survival rates tended to decrease as peak emergence was delayed. Models that predict fly emergence when pupae are held at reduced temperatures will prove useful in rearing programs where extended storage of phorid fly pupae is necessary for optimizing releases.

Key Words Phorid, rearing, imported fire ant, cold storage, *Solenopsis invicta*

During the last decade, an abundance of research has focused on phorid decapitating flies in the genus *Pseudacteon* that attack *Solenopsis* (Porter et al. 2003). The potential benefits of using phorid flies as biological control agents was recognized by Feener and Brown (1992) and Porter (1998). Since those studies, several species were released and have dispersed throughout areas of the U. S. infested with the red imported fire ant, *Solenopsis invicta* Buren, the black imported fire ant, *S. richteri* Forel and their hybrid, *S. invicta* X *richteri* (Porter et al. 2003, Vogt and Streett 2003, Graham et al. 2003, Thead et al. 2005).

Mass rearing of phorids has been conducted primarily at USDA, ARS facilities in Florida and Mississippi, the Florida Department of Agriculture and Consumer Services, Division of Plant Industry and the University of Texas in Austin (S. D. Porter, pers. comm.). Field releases have been made from progeny that were reared at one of these facilities. Rearing phorids in the laboratory is a labor-intensive process requiring elaborate attack box systems that demand precise environmental controls and require the collection of host ants from the field for parasitization (Vogt et al. 2003).

The ability to store phorid pupae over the short term would prove invaluable for researchers manipulating or targeting adult emergence to a particular date. Whereas

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[2]Address inquires (e-mail: LarryT@mdac.state.ms.us).

optimal conditions for laboratory rearing of some phorid species are known (Morrison et al. 1997, Folgarait et al. 2002, Vogt et al. 2003), studies that are targeted to delay development have either not been conducted or have not been published. Results of this study will provide useful information for short term storage of the phorid, *Pseudacteon curvatus* Borgmeier. The potential benefits of storing pupae over the short-term include increasing the number of flies available for releases and for research.

Materials and Methods

Solenopsis invicta workers and brood were collected from Neshoba Co., MS, during February 2004 using the collecting and preparation procedures described by Vogt et al. (2003). Ants and brood were floated from the soil (Banks et al. 1981), and workers were passed through a 20-mesh sieve to separate smaller workers that are preferred by *P. curvatus* (Morrison and Gilbert 1998). Colonies were held in the laboratory at ~27°C on a 12:12 (L:D) h photoperiod and maintained with water, sugar water, and crickets (*Acheta domestica* L.) *ad lib* for 2 to 3 wks prior to use. Ants and brood were placed inside the attack box described by Vogt et al. (2003) for rearing *P. curvatus* at the USDA, ARS, Biological Control and Mass Rearing Research Unit, MS State, MS, and exposed to laboratory-reared *P. curvatus*, Formosa, Argentina biotype, (S. D. Porter, pers. comm.) for parasitization. After 3 d, the ants were removed from the attack box and placed inside a sealed and ventilated Rubbermaid® Servin' Saver® (Newell Rubbermaid Inc. Freeport, IL) container (30.5 cm L × 21.5 cm W × 8.5 cm H) that had been lined with Fluon® (Asahi Glass Ltd., Chadds Ford, PA) to prevent ant escape. The container received one saturated humidity block [cylindrical Castone block (Castone dental plaster, Dentsply International, York, PA) about 6 cm diam × 3 cm H], one nest cell (150 × 25 mm Petri dish lined with about 1 cm saturated Castone), and a sugar source [2 M sugar solution soaked into a crumpled Techwipe® (Horizon Industries, Tyler, TX) and dried]. The container was held in an environmentally-controlled room at ~27°C, 60% RH, and on a 12:12 (L:D) h photoperiod. Three times per week, dead ants were collected and evaluated for parasitism. Thirteen days after the ants were first exposed to *P. curvatus* attacks, 400 individual ant heads containing puparia with hardened sclerotized plates (Porter 1998), as evidenced by their darkened color, were carefully removed for use in the experiment and held for 2 d on wet castone trays inside a control room maintained at ~27°C, 80% RH and on a 12:12 (L:D) h photoperiod (Vogt et al. 2003). Phorid pupae were separated into treatments, each with 50 pupae, that were subjected to 8 temperature regimes. The 50 parasitized ant heads in each treatment were placed individually onto 5-mm depressions drilled into castone-lined Petri dishes. A 3.5-cm diam × 1.0 cm deep depression was molded into the castone for adding water as needed (usually daily) to avoid movement of parasitized ant heads which could occur if water was applied directly to the castone surface. Each dish was placed into a ventilated plastic tray (56 × 44 × 12 cm), covered tightly with a clear Plexiglas top, and placed inside Percival® Scientific Series 36 Controlled Environment Chambers (Model: E-36HO, Perry, IA) maintained at 80% RH, 12:12 (L:D) h photoperiod, and either 26.7°C, 18.3°C or 10.0°C or in the environmentally-controlled room. A Hobo® data logger (Onset Computer Corporation, Pocasset, MA) was placed inside each tray to monitor temperature and humidity. Pupae from three of the treatments were held until emergence either at 26.7°C, 18.3°C or 10°C. Pupae from two of the treatments were held until emergence at 18.3°C for either 12 d or 19 d and then returned to 26.7°C. In two treatments at a

lower temperature of 10.0°C, the pupae were monitored for either 12 d or 19 d and then returned to 26.7°C until emergence. The control treatment was held inside the control room for 12 d and then returned to 26.7°C until emergence.

Evaluations of individual parasitized ant heads were made (<4 min/treatment) each successive day to determine emergence of flies. An empty head capsule with an open sclerotized cap indicated a fly had emerged and was recorded as emerged. Flies that did not develop, i.e., remained creamy colored in appearance, were recorded as aborted. A fly that began development, as determined by adult features visible through puparium, but died or died during emergence was recorded as preemerged. Total numbers of emerged, aborted and preemerged flies were recorded for all treatments. A single experiment was conducted because phorid production was limited and prioritized for use in field releases. Emergence data as a function of different temperature regimes were subjected to ANOVA, correlation and regression analysis using PROC INSIGHT (Schlotzhauer and Littell 1997).

Results and Discussion

The data loggers recorded temperatures in the trays inside the 10, 18.3 and 26.7°C environmental chambers ranging from 9.0-10.2°C, 17.1-18.3°C and 24.8-26.7°C. The temperature in the tray inside the control room ranged from 25.6-27.5°C. Humidity in the environmental chambers ranged from 80-85%. Humidity in the sealed trays ranged from 80-100%.

Cumulative emergence rates from *P. curvatus* pupae that had developed for 12-15 d after first exposure to parasitism by phorid flies varied depending on the rearing temperature regimen (Fig. 1). First emergence of flies occurred at 14 d from pupae exposed to 26.7°C or from pupae held in the control room. Peak emergence occurred at 15 d from pupae held in the control room or the 26.7°C treatment with an average

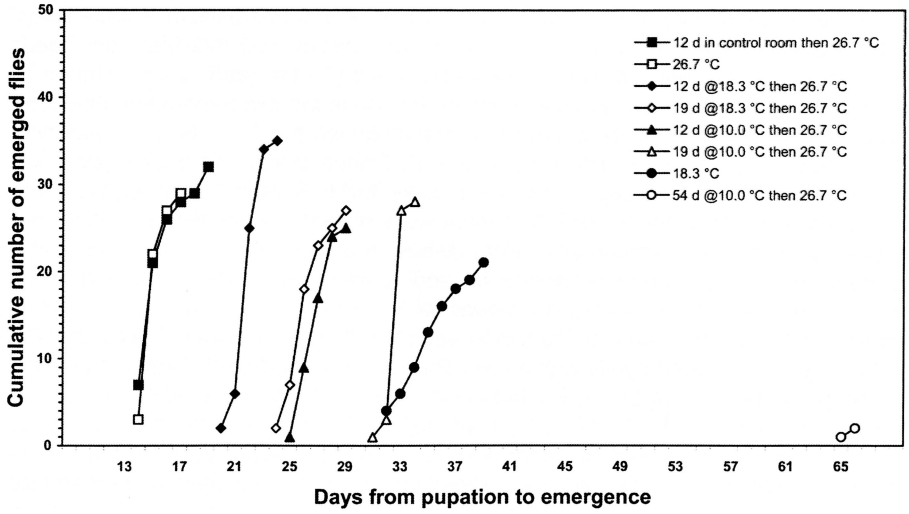

Fig. 1. Cumulative phorid emergence over time.

emergence of 15.5 ± 1.5 d (± SD) from the control room and 15.2 ± 0.7 d from the 26.7°C treatment. Emergence rates from pupae held in the control room were not statistically different from pupae held in the 26.7°C chamber ($F = 1.14$, df = 59, $P > 0.29$, $R^2 = 0.02$). This was expected as the temperatures and relative humidity within the control room and the chamber were calibrated to be comparable. The 26.7°C treatment represents the typical rearing temperature regimen for *P. curvatus* (Vogt et al. 2003) and was used as the control for this study. The flies which emerged appeared normal and attacked ants when made available.

Pupae reared for 12 d at 18.3°C and then at 26.7°C, had the first and peak fly emergence delayed to 20 and 22 d with an average emergence of 22.1 ± 0.9 d. Approximately a 7 d delay in emergence was observed with pupae held in this treatment regimen. First and peak emergence were delayed to 24 and 26 d when pupae were held for 19 d at 18.3°C and then at 26.7°C with an average emergence of 26.2 ± 1.3 d. Fly development was nearing completion at 12 d because the additional 7 d within the 18.3°C chamber only added 4 d to the time of emergence. First and peak emergence were delayed to 32 and 35 d with an average emergence of 35.0 ± 2.2 d when pupae were held at 18.3°C. Pupal mortality increased with time with a 1.4 fold increase in aborted and preemerged pupae as compared with the 26.7°C control. Flies from each of the 18.3°C treatments appeared normal and attacked ants when available.

Pupae held in a temperature regimen of 10°C for 12 d followed by 26.7°C had first and peak emergence delayed to 25 and 27 d with an average emergence of 27.0 ± 1.0 d. Lengthening the time that pupae remained inside the 10°C chamber to 19 d followed by 26.7°C increased first and peak emergence to 31 and 33 d with an average emergence of 32.9 ± 0.5 d. This treatment increased time to peak emergence by 18 d with no effect on total emergence. Flies from both of these treatments appeared normal and attacked ants. It was intended at the beginning of the experiment to evaluate the 10°C treatment until emergence; however, daily observations through the 54 d evaluation revealed that fly development did not seem to be occurring even though they appeared to be viable. After 54 d the pupae were moved to the 26.7°C chamber to continue development. First emergence did not occur until 65 d with an average emergence of 65.5 ± 0.7 d. This treatment had 2 normal looking flies. Ant attack by the 2 flies was not observed. Ten partially emerged flies that died before emergence appeared normal, and death was not attributed to lack of moisture or equipment malfunction. The delay in development at the low temperature may have reduced the energy reserves required for emergence. Temperatures of 10°C for 54 d had a detrimental effect on pupal viability, reducing viable emerged flies 14.5 fold.

Regression analysis of the data from the various treatment regimes provided models (Fig. 2) that accurately predict days to phorid fly emergence, within the range of temperatures evaluated, as a function of pupae held at constant temperature, pupae held at constant temperature for 12 d and then at 26.7°C and pupae held at constant temperature for 19 d and then at 26.7°C. Other models (Fig. 3) accurately predict days to fly emergence, within the range of temperatures evaluated, as a function of the number of days pupae were maintained at either 18.3°C or 10°C before exposure to a temperature of 26.7°C. The highly significant models can be used for short-term phorid pupal storage for research and release programs.

Pseudacteon release programs benefit when emergence can be delayed and targeted to a specific date. Increasing the numbers of flies emerging on a chosen date

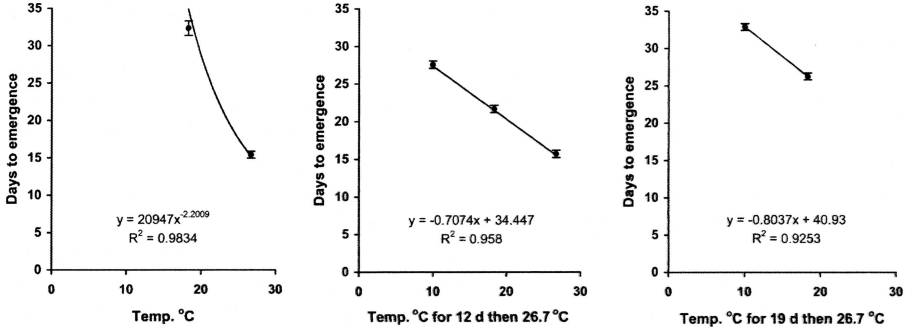

Fig. 2. Days from phorid pupation to adult emergence as a function of constant temperature ($F = 2004.57$, df = 48, $P < 0.0001$); constant temperature for 12 d followed by a temperature of 26.7°C ($F = 1986.64$, df = 87, $P < 0.0001$); constant temperature for 19 d followed by a temperature of 26.7°C ($F = 656.94$, df = 53, $P < 0.0001$).

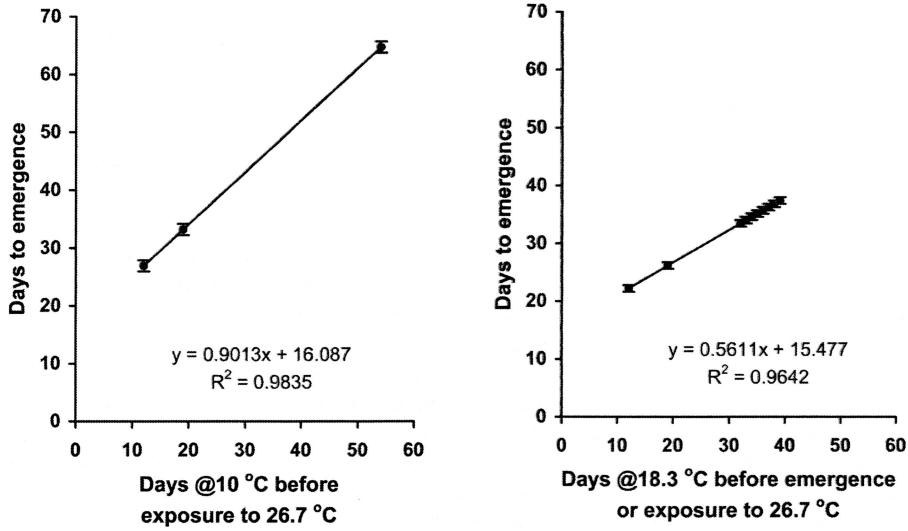

Fig. 3. Days from phorid pupation to adult emergence as a function of days pupae were maintained at 10°C before exposure to 26.7°C ($F = 3159.87$, df = 53, $P < 0.0001$); days pupae maintained at 18.3°C before emergence or exposure to 26.7°C ($F = 2178.97$, df = 81, $P < 0.0001$).

can expose ants to increased parasitization so that more ants containing *Pseudacteon* parasitoids will be returned to their home mounds. Adult emergence can also be delayed so that more flies are available for release on a desired date. Utilizing the described storage methods and models will benefit future release efforts.

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