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A modified rearing system for production of *Pseudacteon curvatus* (Diptera: Phoridae), a parasitoid of imported fire ants☆

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Abstract

A self-contained, climate-controlled box for exposing fire ants to attack by *Pseudacteon* parasitoids was developed. The initial system, which is being used to rear *Pseudacteon tricuspis* Borgmeier, consists of large (about 244 cm L × 97 cm W × 50 cm H), well-ventilated boxes (''attack boxes'') housed in a climate-controlled room (approx. 28 °C, 80% RH, 12:12 (L:D) h). Adult flies emerge into the boxes, which contain live host ants (*Solenopsis invicta* Buren). Host ants are confined in a series of trays within the box, each containing a pair of inverted cups under which the ants can hide. The cups alternate up and down, inducing the ants to trail back and forth within each tray. A modified system used for *Pseudacteon curvatus* Borgmeier consists of closed boxes, through which preconditioned air is pumped to maintain high relative humidity. Steam is used to generate humidity in a small, temperature-controlled room from which conditioned air is drawn, and infrared heating elements mounted above the attack box prevent condensation. This new system maintains high relative humidity (range 80–90%) crucial for activity and survival of *P. curvatus*. System performance was monitored in 4 ways: (1) environmental conditions within the system (RH, temperature, dew point, and light intensity); (2) production of *P. curvatus*; (3) successful development and emergence of *P. curvatus*; and (4) attack rates throughout the box. The modified system is capable of producing >1400 flies/day/attack box. This system will be useful for researchers with limited space in which rearing and research activities are conducted simultaneously.
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Keywords: Phorid; Parasitoid; Rearing; Environmental control

1. Introduction

The red and black imported fire ants (*Solenopsis invicta* Buren and *Solenopsis richteri* Forel, respectively) and their hybrid are considered serious pests of people, livestock, and wildlife in the US. Management of imported fire ants is primarily accomplished by use of contact poisons and various insecticidal baits. The relative expense of these control measures and rapid re-infestation capabilities of the ants have made wide-scale control impractical. Fire ant control in low-value, high-acreage systems (e.g., pasture) often cannot be justified economically (Drees et al., 1998).

Previous attempts to eradicate imported fire ants from the US were controversial and ineffective (reviewed by Klassen, 1989). When the black and red imported fire ants were accidentally introduced into the US sometime before 1918 and 1940, respectively, almost all of their natural enemies remained in South America (Porter et al., 1997). Natural enemies of fire ants in South America include about 20 species of dipteran parasitoids in the genus *Pseudacteon* (Porter and Pesquero, 2001).

☆ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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Williams and Whitcomb (1974) provided initial observations of phorid flies attacking South American *Solenopsis* spp., but early interest in using phorids as classical biological control agents was limited due to the apparently small percentage of ants attacked (Jouvenaz et al., 1981).

Recent work has suggested that parasitic phorid flies may exert more significant pressure on fire ant colonies than previously thought. Porter et al. (1995a) demonstrated the parasitic nature of the *Pseudacteon*/*Solenopsis* relationship. Fire ants exhibit specific defensive behaviors and postures when under phorid attack, presumably to make it more difficult for the female flies to oviposit (Cônsoli et al., 2001; Feener, 1987; Feener and Brown, 1992; Porter et al., 1995b; Williams and Banks, 1987; Williams et al., 1973); these behaviors suggest that phorids have exerted considerable evolutionary pressures on their hosts (Orr et al., 1995; Porter et al., 1995b). Feener and Brown (1992) hypothesized that parasitic phorids might be useful in suppressing fire ant populations in North America.

Maintaining cultures of phorid flies in the US for research, testing, and release against imported fire ants required development of a rearing system designed to address typical considerations (e.g., humidity and temperature control, lighting, etc.) as well as problems specific to use of a live host that happens to be an extremely active, aggressive, stinging insect. Rearing phorids on artificial media would present significant difficulties, especially related to collection of fertilized eggs, and the peculiar manner in which the developing flies pupate within the head capsule of the host ants (Cônsoli et al., 2001; Porter, 1997; Porter et al., 1995b). The majority of the pupal surface is unsclerotized, making water loss a concern. The pupa is situated within the host head capsule, under the tentorial arms, and the first three segments compress and harden to form a sclerotized plate that fills the oral cavity of the host (Porter et al., 1995a). The specialized handling such a pupa would require for successful development may present an insurmountable obstacle for practical in vitro mass-rearing.

Use of live hosts, however, also presents problems. In particular, fire ants frequently exhibit a typical "freezing" behavior when exposed to phorid attack, becoming immobilized and assuming defensive postures (Cônsoli et al., 2001; Porter et al., 1995a). Containment of fire ants is an important consideration in the laboratory. In this paper we describe a rearing system developed for use with *Pseudacteon* flies. The initial system is in use at USDA-ARS, CMAVE in Gainesville, FL, and the APHIS rearing effort at the Florida Department of Agriculture's Division of Plant Industry, Biocontrol Rearing Facility, Gainesville, FL. It uses ambient conditions in a humidity- and temperature-controlled room to create favorable conditions in large "attack boxes" in which adult flies parasitize fire ant hosts. These large, automated attack boxes were designed by Nordlund and Smith to scale up mass rearing, and were based on smaller automated units constructed for rearing *Pseudacteon tricuspis* (Porter, 2000). The modified system is in place at the USDA-ARS Biological Control and Mass Rearing Research Unit, Mississippi State, MS. Temperature and humidity within the attack box are controlled by a combination of pumping conditioned air through the box, and use of infrared heating elements. The modified system was developed for use in an interior room of the facility where it was desirable to maintain ambient conditions favorable for conducting other tasks. The automated attack box used for rearing *Pseudacteon curvatus* is presented first, followed by the basic rearing procedure, and performance of the system.

2. Materials and methods

2.1. Humidity control

Pseudacteon curvatus flies require high relative humidity for development and survival (Porter, unpublished data). Humidity is generated using steam (approx. 25 psi) released into a small (1.8 m × 2.7 m × 2.4 m) temperature-controlled room via a solenoid actuated steam valve. The steam line is fully insulated, and a bucket trap minimizes water output from the steam outlet. A small oscillating fan (about 40 cm diam.) prevents condensation on the floor and circulates air within the room. More complex (and expensive) configurations are available (e.g., steam-jacketed outlets), but our system has proven sufficient for the conditions required. Relative humidity is controlled using an Omega RHCN-1C RH/Temperature controller (Omega Engineering, Stamford, CT). The room is maintained at 29 °C and approx. 85–95% RH.

2.2. Attack box

A list of basic materials used for construction of the attack box is provided in Table 1. Box dimensions are illustrated in Fig. 1A and B. Essentially, ants are confined in a series of 15 trays within the box (only 13 shown in Fig. 1B to save space). Each tray has a pair of "lifter cups" (Fig. 2) that alternately raise and lower, inducing the ants to trail back and forth within the trays while seeking shelter under the cup in the "down" position, and in the "up" position exposing them to attack by ovipositing phorids (see Porter and Briano, 2000). Each cup is suspended on a string with an S-hook for easy installation and removal. A pair of inverted, Fluon-coated cups provides insurance against ants climbing up the strings. The top of the attack box is constructed from a single piece of 6.35 mm thick Plexiglas. A

Table 1
Materials needed for construction of attack box

Item	Qty	Source	Approx. cost ($)
10 ft 2 in. × 6 in., spruce	6	N/A	35.70
8 ft 2 in. × 4 in., spruce	4	N/A	13.60
Casters, 3 in.	2	N/A	14.40
Casters, 3 in. locking	2	N/A	16.60
5/8 in. plywood, 4 ft × 8 ft	3	N/A	81.80
1/2 in. plywood, 4 ft × 8 ft	1	N/A	22.50
Foil back insulation (for bottom), 4 ft × 8 ft, 3/4 in.	1	N/A	8.50
Plexiglas, 4 ft × 8 ft, 3/4 in.	1	N/A	119.50
Clamp lights	4	N/A	37.20
Carlon box 12 in. × 12in. × 6 in.	1	N/A	47.70
5X852F 8 pin socket	1	Dayton	3.40
8 ft fluorescent lamp fixtures	3	N/A	87.50
Lamp bulbs, daylight, 8 ft	6	N/A	29.20
4C440 Blower	1	Dayton	44.80
TORK SPST Timer	1	N/A	51.90
Foil duct to pipe in preconditioned air	Varies	N/A	25.00
Temp. controller Orion E5C2	1	Grainger	89.80
Thermocouple 6A843	1	Grainger	16.70
1/2 in. pillow blocks	2	N/A	9.90
Time delay relay 2A179	1	Grainger	58.40
Pneumatic motor MS-153-DA	1	Whitey	202.10
Mounting bracket MS-MB-133	1	Whitey	25.90
1/4 in. solenoid valve 2F985-2	1	Grainger	49.30
Coil connector 2G-503-2	1	Grainger	8.80
Mini flow filter 6B306	1	Grainger	69.20
Time delay relay 1A 367-0	1	Grainger	54.70
Strap hinges	8	N/A	6.00
Pull handles	4	N/A	8.60
Gray paint	1 gal	N/A	23.00
Sash locks	8	N/A	13.60
8 pin relay R4105 DPDT	1	Nesco	11.60
Stainless steel rod, 1/4 in.	9 ft	N/A	25.00
Infrared heating elements, 60W	4	IR Salamander	100.00
Screws, ext. cords, wiring supplies	N/A	N/A	100.00
		Total	1511.90

stainless steel rod runs the entire inside length of the box, supported by bushings on either end and a pillow block on each of 2 supports across the top, just under the Plexiglas. Fourteen individual cams (7.6 cm dia and 2.54 cm wide) attached to the steel rod raise and lower the lifter cups in each of the trays, by means of attached strings that run through screw eyes installed on the inner surface of the Plexiglas (Fig. 1B). The rod is turned by a pneumatic motor, which is timed to operate from 09:00 to 18:00 h at 10 min intervals. Humidified air is delivered from the room described above (*Humidity Control*) via a small duct fan connected to a rheostat to control speed, and is returned to the room through a length of 10.2 cm diam. insulated, flexible duct.

A smaller box constructed to accommodate 8 pupae trays (see *Collecting Parasitized Ants* below) is attached to one end of the attack box, and adult flies enter the attack box through a hole joining the two boxes.

The attack box has a false bottom to accommodate the 15 trays. We use white, ribless nesting totes in our attack box (model 300-5N, Del-Tec/Panel Controls, Greenville, SC), lined with Fluon on the inner walls to prevent ant escape. Tray dimensions (at the top) are 27.5 cm wide (23.5 cm inner) × 41.75 cm long (38.25 cm inner) × 12 cm deep. The walls are slightly tapered, so the dimensions of the bottom are approx. 22 cm × 36 cm. A shallower tray is available from the manufacturer; tray depth does not appear to be critical to rearing success, but a shallower tray would allow for narrower access doors in the sides of the attack box. Additionally, the doors could be offset to allow for easier removal and installation of trays. In the initial design, the holes cut in the false bottom of the attack box were large enough that the trays rested with the lip flush to the inside bottom of the box. However, this made it possible for newly emerged, unsclerotized flies to walk into the trays, where they became easy prey for the ants (Porter, unpubl. data). The height of the false bottom should be adjusted so that the tray lip is approx. 0.5–1 cm above the false bottom. The space directly in front of the area where adult flies emerge is empty to allow newly emerged flies an area to rest while they harden.

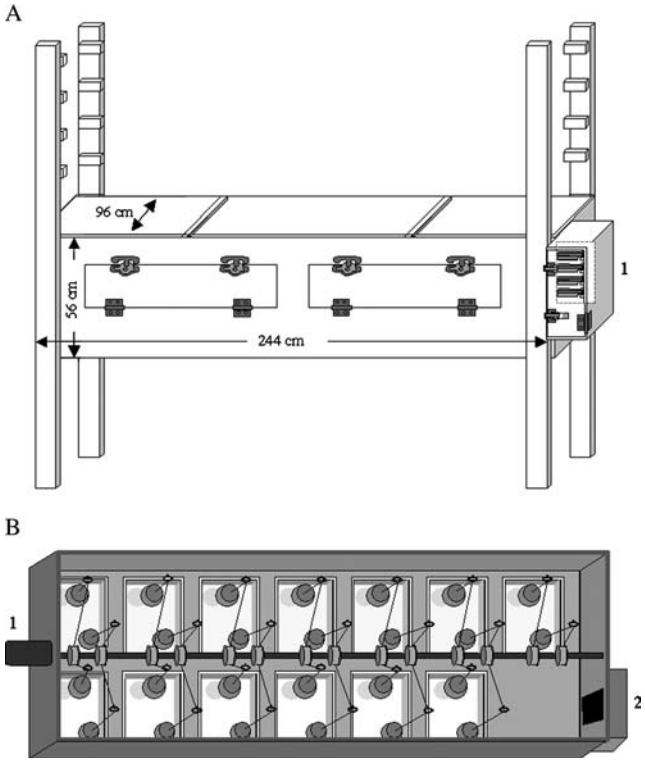

Fig. 1. General appearance and dimensions of attack box from side (A) and top (B). Cutaway view in (A) shows pupal trays in emergence box on the right side of main attack box (1). In (B), the lifter cups are pictured with the upper cup in each tray in the up position. A pneumatic motor (1) operates the cams that raise and lower the lifter cups. Flies enter the attack box from the emergence box (2).

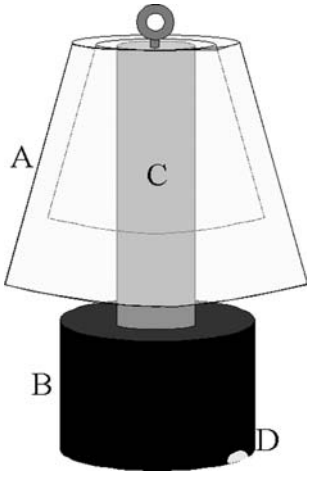

Fig. 2. Lifter cup. The bottom of the cup (paint can lid) is filled to within approx. 0.75 cm with paraffin. Inner and outer baffles (5 and 9 oz plastic cups) (A) and inverted paint can cap (B) are coated with Fluon. A wooden dowel (C) separates the baffles from the inverted cap. Two small openings (D) allow ants access to the space under the cup. A pair of strings is attached to the bottom of each lifter to allow ants to climb down when the lifter switches to the up position (not pictured).

Light was initially provided by three 2-tube, 244 cm fluorescent fixtures (“daylight” tubes) mounted above the box. Two additional 91.4 cm single tube fixtures were added after about five months of production, one at each end of the attack box (perpendicular to the main bank of lights), to even out light distribution. The lights could be adjusted by moving them up or down on supports built into the box frame (Fig. 1A; lights not shown). Lights were controlled with a 24 h timer and set for a 12:12 (L:D) h photoperiod. Four infrared heating elements (60 W, Infrared Internationale, UK) were installed in standard clamp-lights with reflective shades and clamped to the frame above the box. The configuration of lights and infrared elements could be altered to suit different indoor conditions. The infrared elements were wired to a thermocouple within the box and set to 28 °C. The elements operated primarily at night, to combat condensation.

2.3. General rearing procedure

Host ants (*S. richteri* and *S. invicta* × *richteri*) were maintained in the laboratory at about 28 °C with a 12:12 (L:D) h photoperiod. Colonies were fed a standard diet of crickets, and 2 M sugar water offered in test tubes plugged with cotton. Supplements of boiled egg yolk and a standard entomophage diet (Cohen Entomophage Diet, US Patent #5,834,177) were offered occasionally. Colonies were periodically collected from the wild to replenish laboratory stock. Host ants were collected in an area dominated by hybrid fire ants (Oktibbeha County, MS), and were used within 1 month of collection.

2.4. Changing out the attack box

Every 3–4 d, ants in the attack box were removed and replaced with fresh, unparasitized workers. To minimize fly escape, when opening the attack box, the air supply was turned off and lights in the room (with the exception of the attack box lights) were turned off. Parasitized ants were removed from each tray in the box and put aside in a larger, Fluon-lined tray for about 30 min to 1 h. During this time, they tended to create discrete piles of debris (dead brood, dead workers, cotton from sugar, and water tubes), making it easy to remove this material. Refuse can also be removed from the individual trays with an aspirating device prior to changing out the box. Brood were separated and set aside, workers were placed in a Fluon-lined container with a tight-fitting lid (about 19 cm × 29 cm × 6 cm) with a 1 cm × 4 cm screened vent in the top. Brood were separated using the method described by Banks et al. (1981), except ants were not anesthetized. Each container received one saturated humidity block [cylindrical Castone block (Castone dental plaster, Dentsply International, York, PA), about 6 cm diam. × 3 cm high], one nest cell (125 mm × 25 mm petri dish lined with about 1 cm saturated Castone), and a sugar wad (2 M sugar solution soaked into crumpled lab wipes and dried). Humidity blocks served to

maintain near saturation humidity within the containers. Containers were marked with dates exposed, and placed in a holding room (60% RH, 27 °C).

Next, the box was stocked with fresh ants. Because *P. curvatus* flies attack small fire ant workers (Morrison and Gilbert, 1998), workers were allowed to pass through a #20 sieve prior to use. Worker ants were separated from brood and weighed out into individual Fluon-lined containers (about 10 cm × 18 cm, ×5 cm, 0.8 g ants/container). Brood from one or more colonies (including brood retrieved from the previous batch of host ants if it still appeared healthy) were added to the containers (1 g brood/container). Brood is necessary to induce the ants to trail back and forth between the lifter cups while under attack by the flies (Porter, unpubl. data). If using brood from a different source colony than the workers, the brood and workers were allowed a minimum of 30 min to bond before proceeding further. The ants and brood were then placed in the trays within the attack box and remained there for exposure to attacking phorids.

2.5. Collecting parasitized ants

Every 2–3 d (Mon., Wed., and Fri.) dead and moribund ants were collected from colony fragments previously exposed to attacking flies. Each colony fragment was inspected for "boneyards" (piles of dead ants), which were removed with an aspirator. Humidity blocks were moistened as necessary, and sugar wads moistened or replaced. Dead ants and debris collected from colony fragments <8 d from first date of attack were discarded, and colony fragments >35 d from the last date of attack were discarded. The remaining material collected from the colony fragments (primarily consisting of dead ant bodies, and detached heads containing phorid pupae or last instar larvae) was scattered on a tray (41 cm × 17 cm × 2 cm) lined with about 1 cm of Castone. Each tray had three equally spaced holes (2.5 cm diam.) cut in the bottom so that excess water could be removed from the plaster by placing the tray on a towel. The Castone was saturated with water beforehand, taking care not to leave water standing on the surface. The surface was divided into 24 equally-sized cells using a pencil, allowing for production and emergence estimates (see Section 3).

Trays with dead ants and phorid pupae were placed in larger (about 56 cm × 43 cm × 13 cm) trays, with a 5 cm × 11 cm screened hole on each end for ventilation. The lip of the larger tray was lined with foam weather stripping, and a Plexiglas top was held in place with large binder clips. Trays were initially watered daily, but after examination of emergence rates the watering regime was changed to once per week (see Section 3). Trays of pupae collected >4 d prior to watering received a 1% household bleach solution to reduce fungal growth, newer trays received Millipore water. Water was gently applied to the trays with a wash bottle, taking care not to leave a film of water standing on the surface. Trays were held for 13 d at 27 °C; on day 14 they were placed in the emergence box on the side of the attack box (see Fig. 1A). They remained there for 10 d, and were watered lightly each afternoon (15:00 h) with 1% household bleach solution.

2.6. Box performance

Performance of the modified system was evaluated in several ways. Hobo dataloggers (Onset Computer Corporation, Bourne, MA) were used to measure RH, temperature, dew point, and light intensity (1 m) at three points within the attack box (one in the center, and one at each end). Measurements proceeded for a period of 14 d. Additionally, light intensity (lux) within the bottoms of the trays was measured using an Extech 407026 light meter (Extech Instruments, Waltham, MA). Periodic measurement of temperature and RH were also taken using a portable temperature/humidity meter (see below).

2.7. Attack rates and production estimates

Attack rates of *P. curvatus* were estimated using timed observations of the number of flies actively attacking ants in each tray. During each sampling period, an observer noted time of day, temperature and RH in the attack box, and the number of attacking flies/15 s observation in each tray. Data were used to determine how the attacking flies were distributed within the box, and to estimate fly activity throughout the day. Observations were made on days when several (>100) adult flies were present in the box, because peaks in emergence with low activity in between were occurring in the laboratory colony.

Production estimates (pupae/day) were obtained by counting the number of healthy pupae on 25% of the surface area of each pupae tray, multiplying by 4, and dividing by the number of days since the last collection. Emergence estimates were obtained by counting pupae that emerged (as evidenced by an empty, but undamaged head capsule), pupae that died as preemerged adults, and pupae that aborted development sometime before maturation.

All means are reported as means ± SE.

3. Results and discussion

3.1. Environmental conditions

Environmental conditions within the box (temperature, RH, dew point) cycled daily. Conditions were in-

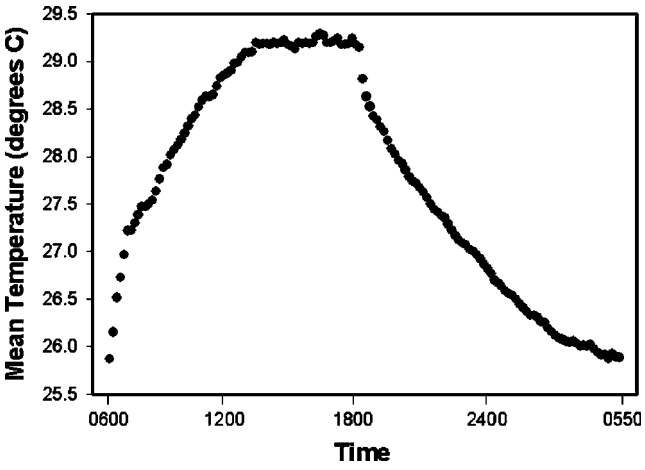

Fig. 3. Temperature in the attack box. During lights on (A), temperature cycled up, during lights off (B) temperature dropped.

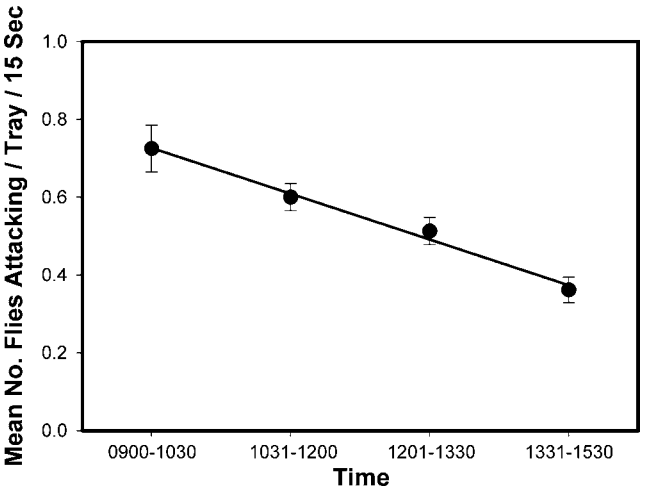

Fig. 4. Attack rates declined approx. 50% during the day ($y = 0.84 - 0.12x$, $r^2 = 0.9900$).

distinguishable between locations within the box, so data were pooled and mean conditions modeled by time of day. Temperature rose very quickly for the first 50 min after lights on, from 25.9 to 27.2 °C; temperature then increased at a slower rate until about 400 min after lights on (12:40 h), and remained relatively constant (about 29.2 °C) until lights off at 18:00 h. Temperature decayed exponentially following lights out to a minimum of about 25.9 °C (Fig. 3). Temperature never fell below dew point after installation of the infrared elements above the box; prior to that, condensation was noted on the inner surface of the Plexiglas top before lights on. Relative humidity within the attack box also cycled daily. Humidity levels initially remained at 76–78% RH during lights on, and approached 85% RH at night. Humidity has since been adjusted to remain at about 80–85% RH during lights on, and approach 90% RH at night. Humidity averaged about 83% RH during lights on as measured by a separate, traceable probe placed in the box (Model HI 91601F, Hanna Instruments, Woonsocket, RI).

3.2. Attack rates and production estimates

Data (mean no. flies attacking/15 s/tray) were assigned to time categories (09:00–10:30, 10:31–12:00, 12:01–13:30, and 1331 + h), averaged within each time category, and subjected to regression analysis to test for the effect of time of day on attack rates, yielding an estimate of fly activity during the day. With respect to time, attack rates decreased some 50% between 09:00 and 15:30 h (Fig. 4). This decline is less than published estimates of mortality (approx. 30% of flies surviving after 4 h of attack in the laboratory) (Porter, 1997), but is not directly comparable, as other factors (such as behavioral periodicity, temperature changes, or potential fecundity) might be partially responsible for the decline. *P. curvatus* flies have been observed attacking throughout the day in the field (Vogt, unpubl. data) and the rise of about 2 °C in temperature from 09:00 to 12:00 h would be expected to increase attack rates (Porter, unpubl. data). Host ants were active throughout the day in the rearing system, unlike ants in the field, which tend to slow or cease activity during the hottest times of the day (Porter and Tschinkel, 1987).

Fly emergence was initially relatively low (about 60%), but rose to about 85% following a change from daily watering of pupal trays to weekly watering. Placing pupal trays within the larger trays described above prevented them from drying out between watering times. The increase in emergence may have been due to decreased disturbance and/or suffocation from frequent watering. Current peak production with this system exceeds 2500 flies/day/box. Colony growth in an attack box is illustrated in Fig. 5. During this period of production, all pupae were allowed to develop and emerge

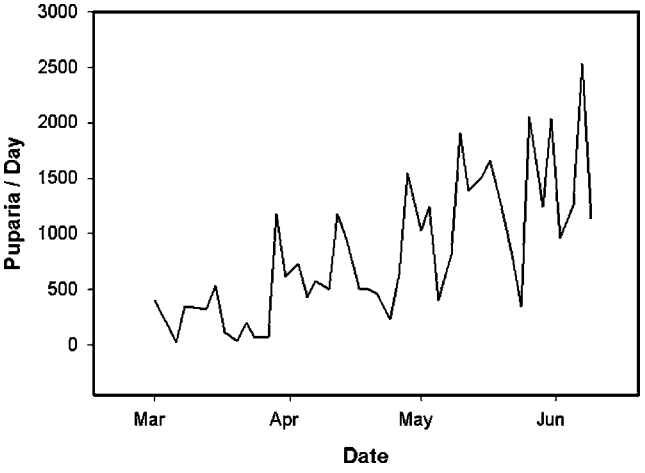

Fig. 5. Production of *P. curvatus* in a new attack box.

as new adults into the rearing system, so Fig. 5 represents a natural population growth dynamic within the system. Mean production during the last month of growth was 1441 ± 152 flies/box/day. Production is somewhat variable, but can be smoothed by holding a portion of the pupae at cool temperatures (≈20 °C) for a few days to delay emergence (Porter, unpubl. data). Natural variability in developmental time will also smooth peaks in pupariation and emergence.

Researchers interested in rearing phorids with this system should bear several things in mind. First, maintaining near-saturation humidity during pupation is critical to rearing success, as evidenced by a dramatic increase in production following a reduction in size of vents in containers used to house parasitized ants, and corresponding rise in RH (Vogt, unpubl. data). Secondly, while the system of lifter cups described herein does induce ants to trail (thus exposing them to attack by the flies), ants are sometimes reluctant to trail during periods of heavy attack. The attack box should be checked periodically and ants that exhibit "freezing" behavior should be agitated. Finally, balancing humidity requirements of pupating phorids with effective use of Fluon (which is not effective at high RH) is difficult at best. Fluon quickly regains effectiveness when exposed to dry air (as in opening containers that house parasitized ants); however, it contains a surfactant and should not be applied to the lower 1–2 cm of the inner walls of containers to avoid suffocation of ants and/or developing phorid pupae should it become wet.

This rearing system offers several advantages over previous methods. Progression from individual, small rearing trays (Porter, 2000) to the large, automated attack boxes resulted in time savings of approx. 26 person-h/week for similar production (from ≈34 h total to ≈8 h total, assuming production of 900 flies/day). In particular, the need to transfer adult flies to individual rearing trays (≈7 person-h/week) and collect individual pupae (≈10.5 person-h/week) was entirely eliminated. In the modified system, environmental conditions within the attack boxes can be changed relatively easily for experimental manipulation. Maintaining a comfortable environment outside the boxes allows for other research activities in close proximity to the fly colony, so that researchers with limited space could consider maintaining an attack box within their laboratory. Mass production of phorid flies on a very large scale will require further innovation; however, this system should prove useful for inoculative releases, maintenance of research colonies, and further experiments on phorid biology and rearing.

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