The introduction of the fire ant parasitoid Pseudacteon nocens *in North America: challenges when establishing small populations*

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The introduction of the fire ant parasitoid *Pseudacteon nocens* in North America: challenges when establishing small populations

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Abstract Several species of parasitoid phorid flies (*Pseudacteon* spp., Diptera: Phoridae) have been released into the United States as potential biological control agents for the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Here we report the first successful introduction and spread of *Pseudacteon nocens* Borgmeier at a site in Texas, USA. *Pseudacteon nocens* is an important natural enemy since it is a widespread and often abundant parasitoid of *S. invicta* in Argentina, where it attacks larger fire ant workers eliciting a strong defensive response. Several years of effort to establish this species previously failed, and here we provide a model to better understand the likelihood of founding new populations when introducing sequential batches of flies in field or laboratory cultures. We also report on a novel method of establishing new populations of phorids in the field using pupae burial boxes to overcome constraints of releasing adult flies or infected worker ants.

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Introduction

Parasitoids of the genus *Pseudacteon* Coquillett (Diptera: Phoridae) have been proposed as potential biological control agents for the red imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae) given their host-specificity within fire ants and several possible pathways of impact on fire ant populations (Orr et al. 1995). Direct impacts through worker mortality are low with field rates of parasitism usually less than 1% (Calcaterra et al. 2008; Morrison and Porter 2005) however indirect effects on colony health through reduced foraging efficiency and defenses (Feener and Brown 1992; Folgarait and Gilbert 1999) or even as pathogen vectors (Valles and Porter 2007) may provide important population level impacts, especially on environmentally stressed colonies.

Over 20 species of *Pseudacteon* decapitating flies are hosted by *S. invicta* or closely related species within the *S. saevissima*-complex (Patrock et al. 2009). Several species have been evaluated for introduction to the US for biocontrol, with successful establishment achieved for *P. tricuspis*, *P. curvatus*, *P. obtusus* and *P. litoralis* and here we report the first field establishment of *P. nocens* in the United

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States. A multi-species assemblage of phorids has been proposed for biological control of fire ants (Gilbert and Patrock 2002; Porter 1998) given that up to 14 species may co-occur, with each species differing in host-location cues, phenology, climate tolerance, and preferred host body size (Folgarait et al. 2007a, b).

Pseudacteon nocens Borgmeier was described in 1926 from Córdoba, Argentina and is hosted by *S. interrupta*, *S. invicta*, *S. macdonaghi*, *S. quinquecuspis*, *S. richteri*, and *S. saevissima* (Patrock et al. 2009). A small morph or cryptic species has also been reported (Folgarait et al. 2006) but the subject of this study is the nominal form. It is a widely distributed species found across much of northern Argentina, through Paraguay to southern Brazil (Calcaterra et al. 2005; Patrock et al. 2009) with climatic conditions ranging from mesic to arid. This species was found to tolerate areas with "Chaco"-type continental climates that had extreme temperatures and were arid (Folgarait et al. 2005).

P. nocens was reported as the most abundant phorid species throughout the year in Santiago del Estero (Azzimonti et al. 2004; Folgarait et al. 2007b). In that province it was the most common species at any time of the day, being 44.1% more likely to be found on an hourly basis than the second ranked species, *P. litoralis*. In another regional survey, it was present at nine of 30 sites, but when present it was among the most abundant species (Calcaterra et al. 2005). Absence from field surveys may be an artifact of collecting protocols since *P. nocens* is most prevalent in the early morning and late evening (Calcaterra et al. 2005; Folgarait et al. 2007a). However during fall and winter it may be quite abundant at midday (Azzimonti et al. 2004). Seasonally, *P. nocens* is more abundant in Fall (May–July) and again in Spring (Nov–Dec) (Calcaterra et al. 2005; Folgarait et al. 2007b). The crepuscular behavior corresponds to periods of high ant activity and is therefore relevant to biological control considerations since, apart from *P. litoralis*, the other phorid species already released are more active during other periods of the day.

In a survey comparing prevalences of *Pseudacteon* species at disturbed mounds or at foraging trails, *P. nocens* was twice as common at disturbed mounds (Folgarait et al. 2007a). It was also associated with shaded microsites compared to sunny or partially shaded sites (Folgarait et al. 2007a). *P. nocens* is an aggressive parasitoid that elicits a particularly strong response from workers resulting in foraging ants abandoning food resources in the presence of ovipositing females (Folgarait et al. 2006).

The development of *P. nocens* was found to vary with temperature, host species, source of flies, and host size (Folgarait et al. 2006). Development times were 17–32% longer at 22°C compared to 28°C and pupal mortality was also lower at 22°C. Fly sizes differed according to source (Corrientes > Santiago del Estero). Mean female development times were 56 days at 22°C, and 44 days at 28°C in *S. invicta.* Development times are over 30% slower in *S. richteri* than in *S. invicta* (Folgarait et al. 2002). Female development time is about two–eight days longer than males (Folgarait et al. 2002, 2006). The emergence rate for over 6,000 pupae was 61.2% (Folgarait et al. 2002).

Field sex ratios are generally male-biased typically 2:1–3:1 (Folgarait et al. 2006, 2007a), potentially an outcome of differing gender-host size associations (Morrison et al. 1999). Preferred host size is ~0.6 mm head width, however *P. nocens* will utilize workers with a range from 0.5–1.1 mm head width. Accordingly, *P. nocens* adult body sizes range considerably (0.2–0.66 mm male, 0.27–0.63 mm female thoracic width), with slight regional differences in mean body size (Folgarait et al. 2006).

In sequential, no choice tests, 30% of *P. nocens* females attacked *S. geminata* following exposure to *S. invicta*, but at only 1/6th of the rate with *S. invicta* (Estrada et al. 2006). Even at these levels, *P. nocens* had lower non-host attraction than *P. curvatus* which has already been released (Porter and Gilbert 2004) and a decision was made to proceed with introductions of *P. nocens*.

A major challenge posed when initiating new populations of a novel species in the laboratory or in the field is the potential for disruptive impacts from a range of demographic, developmental and environmental factors, as encountered when rearing parasitoids in other systems (van Lenteren 2003). Allee effects in very small populations may result in reduced fitness when conspecific densities are low through positive correlations between abundance and per capita growth rates (Taylor and Hastings 2005). Several early attempts to begin a laboratory culture of *P. nocens* by our team and also by USDA-ARS (S. Porter pers. comm.) failed to generate positive population growth. Typical conditions for raising

other species of phorid flies in laboratory cultures have been described (Pesquero et al. 1995; Porter et al. 1997; Vogt et al. 2003), but these conditions and modifications thereof (described below) failed to yield viable populations of *P. nocens*.

Considering that Allee effects would decrease per capita growth rates of very small populations, an option was to increase the number, frequency and size of incoming pupae shipments. As reported below, a significant shipment and importation effort was made to establish a laboratory culture but this population failed to grow or survive beyond 15 months.

These experiences required us to re-evaluate the prospects for successfully introducing *P. nocens* to North America and we developed a novel field release method to overcome the failure of laboratory cultures of this species. In this paper we report on the first successful field establishment of *P. nocens*, along with two novel contributions to methodology by using 1) a new field release method and 2) a simulation model to evaluate challenges associated with rearing this species in a laboratory culture.

Methods

Field sites of sources and introduced populations

Pseudacteon nocens pupae were originally generated by field attacks on *Solenopsis* fire ants at several sites in Argentina. *Solenopsis invicta* and *S. richteri* were used as host species since prior studies had shown both to be competent hosts (Folgarait et al. 2006). Initial collections made in Corrientes province (27.78°S, 58.08°W) (2003–2004) were used for host-specificity testing and for initial efforts to establish laboratory cultures. From 2004 to 2010, flies were sourced in Santiago del Estero province (28.27°S, 63.95°W). The alternate site was chosen because *P. nocens* was locally abundant there, and also to seek an alternate biotype from a drier habitat after the initial lack of success in developing a laboratory culture using Corrientes flies (Porter and Briano 2000). Santiago del Estero has a similarly arid climate to that of south-central Texas, and it has been proposed (Folgarait et al. 2005) that matching biotypes from climatically similar sites of origin to sites of release may be an important factor for successful introductions of *Pseudacteon*. Further shipments of pupae obtained in Folgarait's laboratory using flies from Santiago del Estero were used both for laboratory trials and for release into the field in Texas (Table 1).

Three field sites in Texas were selected to optimize prospects of success based on the following criteria. All sites were in southern or coastal Texas, along temperate riverine woodlands embedded in arid mesquite or oak savanna. The goal was to provide sites with year round warmth and moisture, and with a high abundance of host fire ants. River corridors are considered likely to act as refuge source populations in times of drought, and also to serve as corridors to aid the spread of introduced flies (Plowes et al. 2011). The three sites used were in Kenedy (27.16°N, 97.96°W), Gonzales (29.31°N, 97.38°W) and Dimmit (28.50°N, 99.63°W) counties. The Kenedy county site was abandoned after twice being flooded during the initial release efforts. Weather records for the three sites were extracted from the nearest weather stations on http://wunderground.com.

Pupae releases in buried boxes

Given the difficulties of developing laboratory cultures of *P. nocens* (see next section), we decided to test an alternate method of release and introduction. With previously introduced species of lab cultured adult flies of *P. tricuspis*, *P. curvatus* and *P. obtusus*, we either released adult flies or we parasitized colonies of *S. invicta* in the lab and returned the infected workers to the field (Gilbert et al. 2008; Plowes et al. 2011). Faced with small lab cultures of *P. nocens* we tried a new method whereby imported pupae were placed in a buried, insulated box from which enclosing flies could emerge through an escape tube. It was assumed that flies would emerge using natural cues such as temperature, humidity or barometric pressure.

The boxes contained only pupariating flies in the host head capsule that had been sterilized with solutions of methyl paraben and bleach solution to minimize potential development of fungi. The pupae were set out on 9 × 6 cm trays with 1 cm Denstone© plaster bases that were moistened. The pupae trays were placed into an insulated Coleman© cooler box (60 × 40 × 40 cm, L × W × H) with an internal lining of shop towel moistened with 0.5% bleach solution to minimize fungal growth and maintain

Table 1 Shipment and release summary for *P. nocens*

(a) Date of shipment	(b) Pupae shipped: total all spp.	(c) Estimated *nocens* females shipped	(d) Emergence rate	Estimated number of adult *P. nocens* females					(j) Capture dates (& numbers) at Dimmit site	(k) Notes
				(e) Retained in lab	(f) Emerged in field	(g) Kenedy Co.	(h) Dimmit Co.	(i) Gonzales Co.		
Dec 2006	2,792	106	0.38	4	36	36				1
Feb 2007	415	24	0.25	6						
April 2007	2,226	327	0.23	25	50	50				
May 2007	2,378	99	0.35	15	20	20				
June 2007	2202	582	0.52	55	248	248				
Oct 2007	1,990	380			133	133				
Dec 2007	2,339	468			210	210				
Apr 2008	1,043	209			135	135				1
May 2008	2,371	474			308	308				1
Jul 2008	3,397	679			441	441				1
Nov 2008	6,485	991	0.35	169	178		178			
May 2009	3,503	718	0.36	259						
Jun 2009	3,106	621	0.48	298						
Oct 2009	3,016	1,175	0.27	111	206			206	20-Oct-09 (2)	
Oct 2009	3,452	1,003	0.44	263	178			178		
Nov 2009	3,487	951	0.46	264	173			173	19-Nov-09 (1)	
Nov 2009	2,610	844	0.34	172	115		115			
Dec 2009	1,784	450	0.51	230				4 cols		2
Jan 2010	2,093	632	0.44	278						
Feb 2010								2 cols	22-Feb-10 (2)	2
Apr 2010								6 cols	23-Apr-10 (1)	2
May 2010								100	6-Jun-10 (2)	2
Nov 2010	1,611	448			214		214			
Mar 2011									7-Mar-11 (1)	
Jun 2011									9-Jun-11 (27)	

The columns report *a* month of shipment, *b* total number of pupae shipped, all species and sexes, *c* estimated number of female *P. nocens* in the shipment; *d* emergence rate per capita; *e–i* estimated numbers of adult *P. nocens* females retained in the lab (for checks, experiments or cultures), or emerged in the field at three sites; *j* dates and captures of female *P. nocens* at the Dimmit Co. site

The emergence rate and female content of field released colonies were estimated from parameters of subsamples retained and tracked in the lab. Estimates are provided for six cases with no subsample, using the mean emergence rate (0.38, range 0.25–0.52) and mean female content (21%, range 15–38%)

1 Site extensively flooded post-release, 2 Releases of colonies or pupae from Brackenridge Field Laboratory lab culture

humidity. A 50 cm long, vertical, PVC-made escape pipe 4 cm diameter was drilled and glued to the lid of the box. The escape pipe terminated in a downward facing pipe section with a metal mesh screen (2 mm hole size) to prevent predator and rain ingress. After placing the pupae trays into the box and sealing it with aluminum foil tape, the box was placed into a plastic garbage bag and sealed with only the escape tube emerging to prevent ingress of water, dirt and other arthropods. This apparatus was then buried into the ground such that the lid was about 10 cm below ground level and the excavated soil and other plant material was mounded over the box to provide 30 cm of insulating material. Boxes with pupae were left buried for a minimum of three months before excavation and inspection. Following field releases using the burial boxes, we periodically monitored for the presence of flies at four disturbed fire ant nests and at four trays of fire ants brought from the laboratory (Table 1). During each monitoring event, all flies attracted to the disturbed mound or tray were recovered by aspiration, identified and stored in vials with 90% ethanol. Monitoring started between 3–4 pm, and continued until 30 min after dusk or 30 min after recovery of the first *P. nocens* female fly. In June 2011 we used sticky fly traps (LeBrun et al. 2009) to determine expansion from the release site. Pairs of traps were placed at $\sim$500 m intervals up to 2 km east and west of the release site, set out at 3–4 pm and recovered by 9 am the following day.

During initial trials, one box was buried at Brackenridge Field Station in Austin, Texas, for 30 days in September 2007 to evaluate how well the box buffered against ambient temperature and humidity fluctuations and to determine the pupal emergence rates of a sister species, *P. tricuspis*, compared to samples maintained in the laboratory. An ethanol filled tube was attached to the escape tube opening to capture emerging flies. A Hobo© data logger was included to record temperature and humidity.

Key demographic parameters (species content, emergence rate, sex ratio) were estimated from subsamples of shipments retained in the laboratory. We generally retained around 10% of each shipment for tracking, although six shipments were sent directly to the release sites with no tracking samples retained and in these six cases we estimated demographic parameters using mean emergence rates and sex ratios from other shipments.

Laboratory cultures

A series of efforts were made to start laboratory cultures of *P. nocens* both by the University of Texas, Austin laboratory, and by USDA-ARS. We followed the same protocols used for successfully rearing three other phorid species, *P. tricuspis*, *P. obtusus* and *P. curvatus* (Pesquero et al. 1995; Plowes et al. 2011; Porter et al. 1997; Vogt et al. 2003). These efforts failed and a series of modifications to the protocols was attempted to overcome potential issues such as high larval mortality, mating failures, and low oviposition rates. We did record several instances of highly successful oviposition rates, but these were often accompanied by high levels of aborted fly larvae emerging from the ant head capsule just prior to pupariation (Fig. 3). We also suspected that mating conditions may not have been optimal since low numbers of ovipositing females were observed compared to the total numbers that emerged. To simulate the crepuscular activity period of *P. nocens* in an effort to improve mating conditions and oviposition rates, we tested a lighting schedule and lamp combination with 2-h periods of low light using warm-white fluorescent tubes and infra-red lamps to simulate sunset lighting, but with no overall improvement in parasitism rate. In other species, the newly enclosed adult flies take several hours to mature prior to mating and so we tested several options including keeping flies one or two days before allowing them to mate, and by using "mating tubes" where a female and a male were placed in 10 cm of plastic tubing to ensure close contact. On the suggestion of S. Porter (USDA-ARS) we included strips of black fabric in the fly chambers since the flies were observed to aggregate on these strips. We also included a variety of plants with different leaf types since other species of phorids have been reported to swarm or perform courtship routines on leaves (Disney 1994). None of these modifications resulted in long term improvements to the population growth rate.

Simulation model for growth of small populations

Given the difficulties encountered initiating laboratory cultures of *P. nocens* we developed a simulation model to evaluate the shipment strategies needed to successfully start a population of *P. nocens*.

The model uses a general approach that may be applied to populations in the field or laboratory since it allows flexible testing of the effects on population growth of different generation times, adult longevity, differing male and female development periods, development period distributions, and per capita reproductive rates. The algorithm is a Markov-chain formulation based on a projection of the number of offspring of the current cohort of adult flies for a particular day, using the aforementioned demographic parameters. A further projection is made for each successive day, based on the number of emerging males and females that form the daily cohort. The algorithm is initialized using a schedule for the emergence of the founding population (which may be one or more pulses of adults emerging from a shipment or release of flies). The model may be applied to either laboratory or field populations by adjustment of relevant demographic parameters, which may be affected by various environmental and ecological conditions.

When applied to the case of *P. nocens* in the laboratory, we simulated the following conditions that were expected to span the range of likely parameters. The response variable (Fig. 4 y-axis) is the resulting population size after 20 generations of simulation for a range of demographic parameters: a) the lag between female and male emergence (Fig. 4 x-axis) was varied from zero to four days; b) each simulation chart shows a set of outcomes when varying female per capita replacement rates between 1.2 and 2.0. The chosen parameter ranges were based on the following laboratory observations: a) the distribution of individual development times was modeled as a log series, per laboratory observations of an initial peak followed by a long tail; b) observed female–male development delays were about two days; c) female replacement rate for *P. curvatus* is about 1.57 (Vogt et al. 2003), and 1.1 for *P. obtusus* (RMP unpublished); d) the male:female sex ratio of laboratory cultures is approximately 2:1. For the simulation, generation time was assumed to be 30 days, with all shipments occurring within that 30 day window. We simulated several shipment strategies, to show the effect of varying the timing and number of initial shipments made within the first generation period (Fig. 4a, b, c). We considered three cases, of one, two and four shipments during the 30 day window of one generation.

Results

Establishment in the field

We report the first establishment and spread of a field population of *P. nocens* in North America. The releases of buried pupae in November 2008, 2009 and 2010 at the Nueces River in Dimmit County, Texas established successfully and *P. nocens* was recorded there seven times between October 2009 and June 2011 (Fig. 1a; Table 1), with spread over 3 km recorded in June 2011.

At the Kenedy County release site, we twice recorded *P. nocens* at that release point within the first generation period in 2008, but no further flies have been detected since then during multiple surveys and we assume that the species failed to establish. This site was subject to widespread flooding following several release events with water ingress into the burial boxes, and potential flooding of any pupae in the habitat (Fig. 1b). No flies have been recovered from the Gonzales County site during four subsequent surveys. The Gonzales site underwent severe winter conditions in the months following the primary release activities (Fig. 1c).

Evaluation of buried box release method

A test box containing pupae of *P. tricuspis* confirmed the efficacy of this novel method. The emergence rate of adult flies from pupae was 51.6% in the box, compared to 69.7% for a control set of pupae held in the laboratory. The sex ratio of emerging flies from the box was 1:1.54 (male:female) compared to 1:1.40 in the control sample (lab cultured populations are manipulated to be female biased). Environmental conditions inside the box were found to be well regulated compared to ambient, with a daily mean temperature 0.7°C cooler, maximum 4.6°C cooler, and minimum 2.6°C warmer than outside (Fig. 2). Importantly, the buffered conditions also resulted in a constant high humidity (92–96% RH) while ambient humidity ranged from 32 to 90% RH (mean 66.4%). High humidity (over 90%) is essential to fly pupal development.

Laboratory culture results

A culture of *P. nocens* was maintained at the Brackenridge Field Laboratory from November 2008

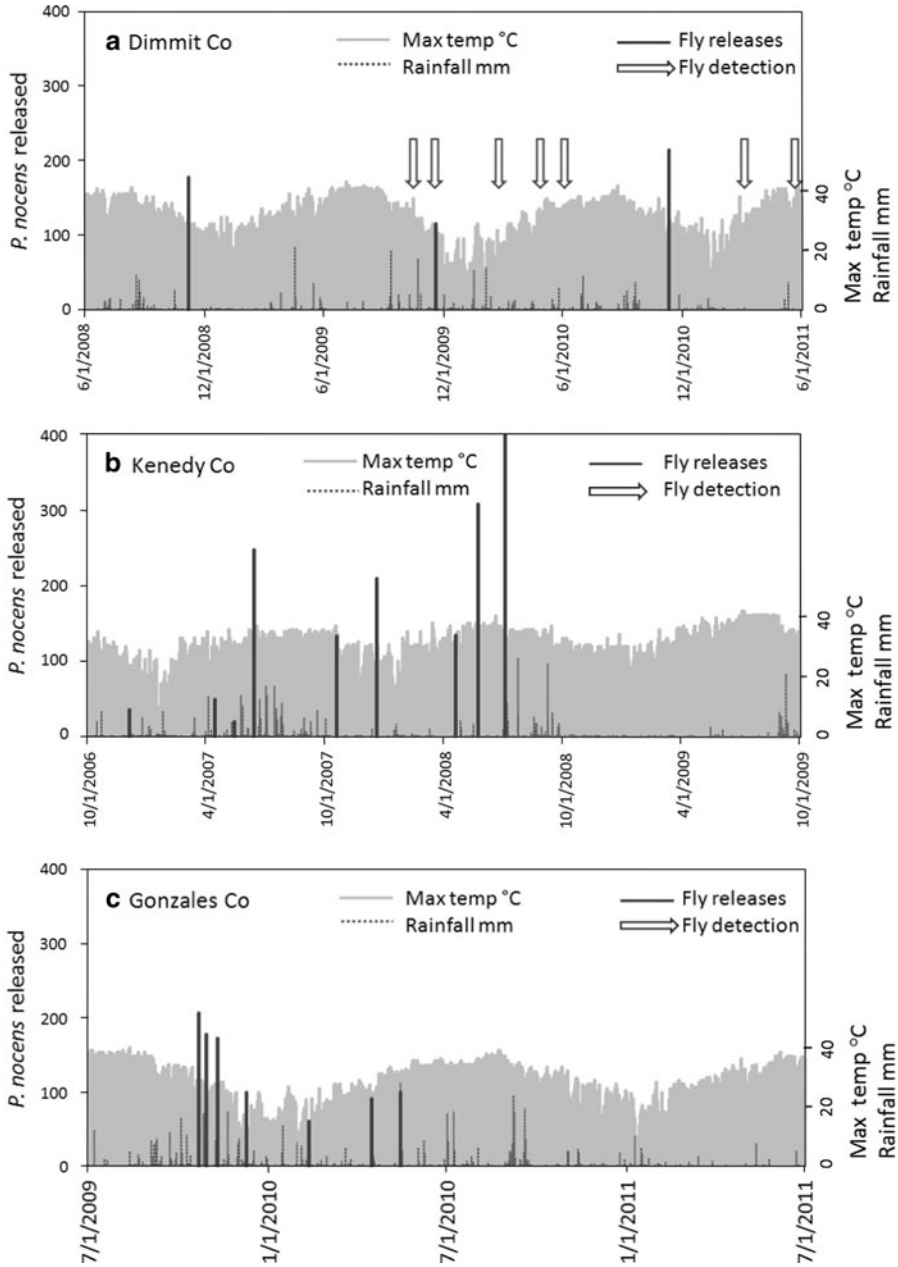

Fig. 1 Timelines of release events and weather conditions at the three release sites, **a** Dimmit Co., **b** Kenedy Co., and **c** Gonzales Co. The positive detection dates of *P. nocens* at the Dimmit Co. site are annotated with arrows

until January 2010. As described above, this culture never achieved sustained positive growth despite addition of new flies on nine occasions (Table 1; Fig. 3). The key period with positive growth occurred after the May 2009 shipment when approximately 260 female *P. nocens* produced 665 pupae (male and female). Although no conditions were changed in the subsequent time period, the following generations failed to provide positive growth.

Multiple possible causes for the decline were investigated with the primary attention given to aborted larvae and mating failures. When flies were available in reasonable numbers (>ten males and females per day) we regularly observed oviposition attacks that yielded large numbers (>100 per day) of parasitized ants (Fig. 3). However, the fly larvae in these parasitized workers frequently aborted immediately prior to pupariation. These aborted larvae were

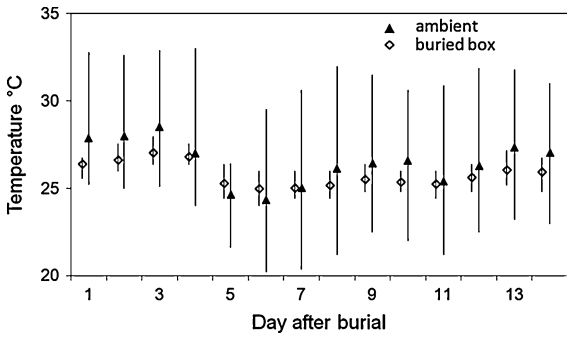

Fig. 2 Temperature in buried pupae box and ambient. Bars indicate daily temperature range (min to max)

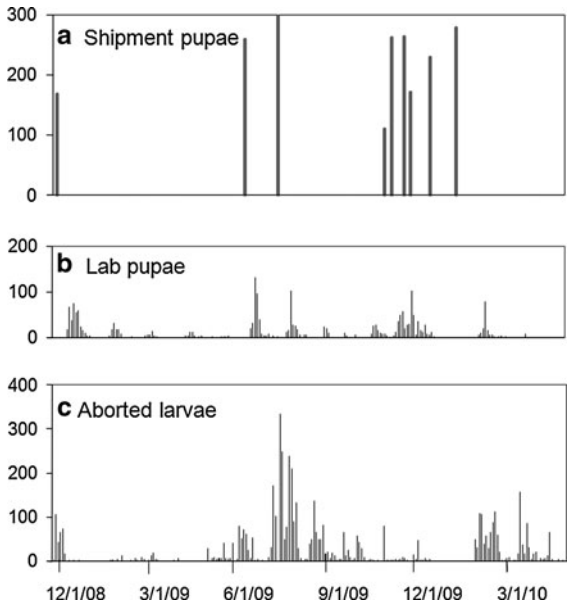

Fig. 3 Laboratory culture of *P. nocens*. **a** Number of female *P. nocens* from shipment used in lab culture, **b** number of pupae and **c** aborted larvae produced in lab culture (11–14 days post-oviposition). Peaks of aborted larvae often occurred in the week before surviving pupae were produced

observed immediately adjacent to the decapitated heads of the host worker ants. In contrast, when few flies were available (<ten males or females per day) we saw few oviposition attacks, most likely on account of mating failures.

Larvae that did not abort and were able to successfully pupate usually survived to enclose as adult flies with emergence rates around 73% for pupae raised in the lab. Emergence rates for pupae coming directly from shipments ranged from 23 to 53%, mean 38%.

Simulation model results

The simulation model showed that attempting to start a laboratory culture with a single shipment of pupae is unlikely to succeed, using typical parameters observed for *P. nocens* (Fig. 4a). The simulations were based on a log series emergence profile so that fly emergence is distributed longer than a single pulse. However with a time lag between male and female development times, asynchronous emergences may result in mating failures. The likelihood of positive population growth increased dramatically if four shipments of pupae occurred during the first generation cycle, even with a two day lag between male and female emergence. Alternately, single large shipments could result in increased absolute abundance, such that a few flies of either gender would be more likely across a longer emergence window.

As a result of these simulations, a set of closely timed shipments were made in November and December 2009. During this period the numbers of emerging males and females was adequate to provide sustained production of pupae, however, with continued high levels of larval abortion (Fig. 3), the laboratory culture was unsuccessful.

Discussion

The establishment of *Pseudacteon nocens* in North America is an important milestone in the research program of potential biocontrol agents for *Solenopsis invicta*. *P. nocens* is widely distributed in their native range, occurring in a variety of continental and arid zones. *P. nocens* is often a co-dominant with *P. obtusus*, *P. tricuspis*, *P. curvatus* and *P. litoralis* (Calcaterra et al. 2005; Folgarait et al. 2007b), other species that have been introduced into North America. *P. nocens* is crepuscular and active during periods of high ant activity. Behaviorally, they are an aggressive species that elicit a strong behavioral response in attacked ants. While *P. nocens* prefer large workers ($\sim$0.6 mm head width), they develop successfully in a wide range of worker sizes resulting in a corresponding range of fly adult body sizes. Cumulatively, these factors have made *P. nocens* an important candidate biocontrol agent.

At the site in which they were established in Dimmit County, Texas, in November 2008, *P. nocens*

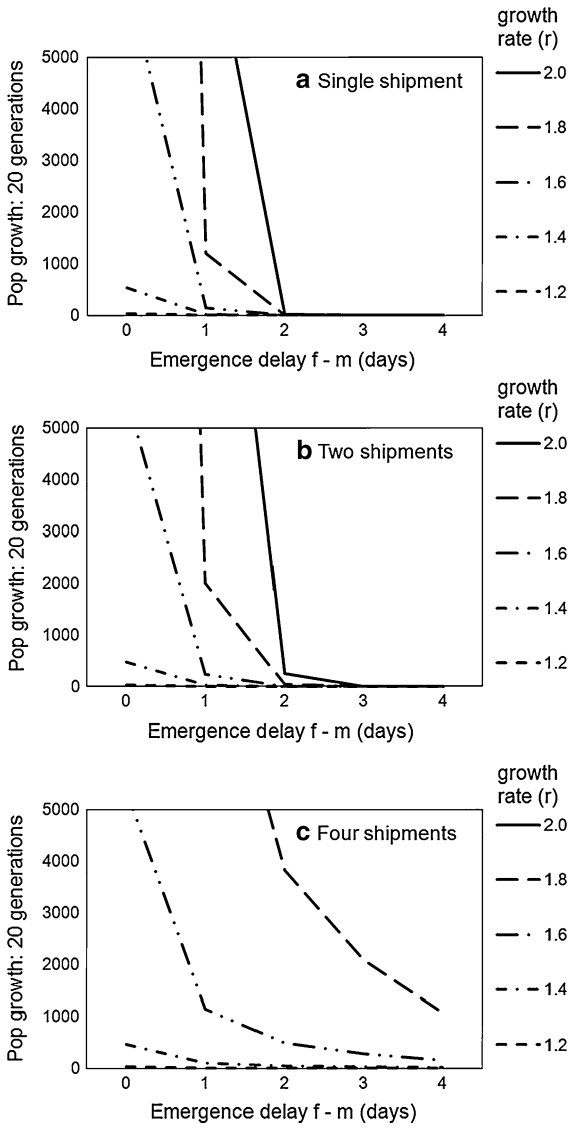

Fig. 4 Simulation model of population growth comparing strategies of **a** one, **b** two and **c** four shipments during the first generation cycle. Each chart shows the population growth after 20 generations (y-axis) for five cases when the female per capita replacement rates (r) were set at increments between 1.2 and 2.0. The lag between female and male emergence (x-axis) was varied from zero to four days (actual male-female development delay is usually two days)

co-occurs with three other introduced *Pseudacteon* species (*P. obtusus*, *P. curvatus* and *P. tricuspis*). Although an ideal release site for new founding species would be free of other competing species, this site was selected in a river corridor for year-round warmth, high soil moisture and high densities of fire ants to help start the founding population. The continued low densities of *P. nocens* at this site may in part be attributable to the presence of competing species (LeBrun et al. 2009), but may also reflect a phenomenon noted among other *Pseudacteon* species of having a long latency period prior to population growth and spread (LeBrun et al. 2008; Porter et al. 2004). Nearby in South Texas, several releases of *P. tricuspis* at Brownsville, Laredo and Kingsville around 1999–2001 were all assessed as having failed, but each population was found to have survived when flies were detected after 2007 (RMP, LEG unpublished). Similarly *P. litoralis* in Alabama was also considered to have a weak founding population, remaining in small numbers near the release site since 2005 until they were recently found to have expanded (Porter et al. 2011). There is some possibility that the other two *P. nocens* release sites may later yield viable populations, especially the Gonzales County site where substantial releases were made, while the Kenedy Co. site has been frequently monitored with no fly detection and was subject to widespread flooding during the release period.

The founding population of *P. nocens* in Dimmit County was supplemented by additional shipments during the 2010 drought. Once the *P. nocens* population at this site is well-established and spreading, it could be used as a source from which to infect ant colonies for release elsewhere.

The weather conditions at each site (Fig. 1) may have played a role in the success or failure outcome as observed during the establishment of *P. obtusus* (Plowes et al. 2011). Habitat conditions and host ant status are likely to reflect recent rainfall, while fly mortality may be high during extremely hot or cold episodes. Although the successful Dimmit County site had lower overall rainfall, the site conditions remained somewhat moist and warm given its location along the deeply wooded Nueces River.

Techniques for field collections and shipments from Argentina were perfected such that large numbers (>3,000 pupae per shipment, Table 1) of *P. nocens* pupae were regularly imported and used for laboratory cultures or field releases. Given the prior failures by University of Texas, Austin and USDA-ARS to initiate laboratory cultures, the decision was made in 2006 to attempt a novel release method by installing pupae into buried boxes from which they could emerge as prompted by local environmental cues. This method was shown to be successful in a test

box at Brackenridge Field Laboratory with temperature and humidity maintained at levels necessary for successful development and eclosion of *P. tricuspis*, and was also proven to work with *P. nocens* at the Dimmit site (Table 1).

Mass rearing of myrmecophagous parasitoids has only been attempted among *Pseudacteon* species and, given the flies' complex life histories in association with their eusocial hosts, such mass rearing efforts are likely to face considerable challenges over and above those encountered when rearing parasitoids of non-social insects (van Lenteren 2003). The rearing protocols we used for *P. nocens* were based on the methods used for other *Pseudacteon* (Pesquero et al. 1995; Porter et al. 1997; Vogt et al. 2003) and included close regulation of conditions for larval and pupal development, eclosion, mating, foraging, host-location and oviposition. Our attempts to maintain a laboratory culture may have failed for several reasons. At low per capita female replacement rates, the small cultured population was vulnerable to a range of stochastic events. Furthermore, the two day lag in development and emergence between male and female flies resulted in many occasions when emerging females lacked mating partners. Our model supported the notion that at least three shipments of pupae were needed in a short period during the first generation cycle to provide a flow of emerging flies to overcome these problems. In some cases, mating failure was suspected to arise from lack of stimuli and cues such as lighting intervals, light quality, and lack of suitable mating substrate and vegetation structure. We found that once a female was mated, she was able to sustain a high attack rate. However, we consider that the most important negative impact on population growth was the high level of larval abortion that frequently occurred immediately prior to pupariation (Fig. 3). On many days, over 70–90% of developing larvae aborted. The cause of this sustained larval abortion is still unknown but may be environmental or host related. Low humidity during pupation is a critical factor resulting in pupal mortality, but our cultures were maintained at >90% humidity so this can be discounted as a possible cause of larval abortion. Host quality problems are likely to be the cause of aborted larvae, possibly based on mismatched biotypes, nutritional deficiencies, pathogen infection status or ant immune responses. The source of these *P. nocens* pupae was from Santiago del Estero province which is over 500 km distant from the likely source of the introduced fire ants in Formosa province (Caldera et al. 2008). This is not considered to be a root cause of the aborted larvae problem since on many days the fly development proceeded with few aborted larvae and other species of *Pseudacteon* can be raised successfully on host ants derived from either region, and the USDA imports that also failed were from the Formosa area.

In contrast to the difficulty encountered with maintaining a laboratory culture, even when supplemented by numerous inputs of flies, burial releases at one of the three field release sites resulted in a successful outcome. The initial population appears to have started from a large shipment of 6,485 pupae in November 2008, with flies detected again in October 2009 before supplemental pupae were introduced. This release was equivalent to a single shipment into the laboratory (Fig. 4a) and the successful establishment implies a per capita replacement rate >1.7 which is not unrealistic but was not sustained in laboratory cultures. The size of the shipment and the outdoor weather conditions were probably important factors in the success of this founding population. The use of large numbers of buried pupae (Table 1) ensured that sufficient flies emerged over an extended period, while variable temperatures of the outdoor environment compared to laboratory conditions may have broadened the range of developmental periods, both leading to establishment of overlapping generations. This implies that laboratory populations could have benefited from exposure to a wider range of temperatures to alter the developmental periods, but would also need to be numerically larger. Furthermore, field emerging flies may have better prospects than laboratory cultures if in the field they select preferred ant colonies and microhabitats, with potentially positive fitness consequences.

Overall, the establishment of *P. nocens* in North America is considered to be a noteworthy achievement for the fire ant biological control research program. This is an important species to include in the biocontrol program given their relatively high abundance and wide distribution in the native range. Considerable time and resources were invested in this introduction and establishment effort in comparison to the relative ease with which other phorid species have been raised in laboratory cultures and established in the field. *P. nocens* differs in several important ways from its congeners and future research will examine the co-occurrence of these new assemblages and the potential functional impacts on fire ants.

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References

Azzimonti G, Folgarait PJ, Gilbert LE (2004) Ritmo circadiano y circanual de fóridos parasitoides de hormigas de fuego en Santiago del Estero. II Reunión Binacional de Ecologia, Argentina

Calcaterra LA, Porter SD, Briano JA (2005) Distribution and abundance of fire ant decapitating flies (Diptera: Phoridae: *Pseudacteon*) in three regions of Southern South America. Ann Entomol Soc Am 98:85–95

Calcaterra LA, Delgado A, Tsutsui ND (2008) Activity patterns and parasitism rates of fire ant-decapitating flies (Diptera: Phoridae: *Pseudacteon* spp.) in their native Argentina. Ann Entomol Soc Am 101:539–550

Caldera EJ, Ross KG, DeHeer CJ, Shoemaker DD (2008) Putative native source of the invasive fire ant *Solenopsis invicta* in the USA. Biol Invasions 10:1457–1479

Disney RHL (1994) Scuttle flies: the phoridae. Chapman & Hall, London, p 467

Estrada C, Patrock RJW, Folgarait PJ, Gilbert LE (2006) Host specificity of four *Pseudacteon* spp (Diptera: Phoridae), parasitoids of fire ants in Argentina (Hymenoptera: Formicidae). Florida Entomol 89:462–468

Feener DH Jr, Brown BV (1992) Reduced foraging of *Solenopsis geminata* (Hymenoptera: Formicidae) in the presence of parasitic *Pseudacteon* spp (Diptera: Phoridae). Ann Entomol Soc Am 85:80–84

Folgarait PJ, Gilbert LE (1999) Phorid parasitoids affect foraging activity of *Solenopsis richteri* under different availability of food in Argentina. Ecol Entomol 24:163–173

Folgarait PJ, Bruzzone OA, Patrock RJW, Gilbert LE (2002) Developmental rates and host specificity for *Pseudacteon* parasitoids (Diptera: Phoridae) of fire ants (Hymenoptera: Formicidae) in Argentina. J Econ Entomol 95:1151–1158

Folgarait PJ, Bruzzone O, Porter SD, Pesquero MA, Gilbert LE (2005) Biogeography and macroecology of phorid flies that attack fire ants in south-eastern Brazil and Argentina. J Biogeogr 32:353–367

Folgarait PJ, Patrock RJ, Gilbert LE (2006) Development of *Pseudacteon nocens* (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* fire ants (Hymenoptera: Formicidae). J Econ Entomol 99:295–307

Folgarait PJ, Patrock RJW, Gilbert LE (2007a) Associations of fire ant phorids and microhabitats. Environ Entomol 36:731–742

Folgarait PJ, Patrock RJW, Gilbert LE (2007b) The influence of ambient conditions and space on the phenological patterns of a *Solenopsis* phorid guild in an arid environment. Biol Control 42:262–273

Gilbert LE, Patrock RJW (2002) Phorid flies for the biological suppression of imported fire ant in Texas: region specific challenges, recent advances and future prospects. Southwest Entomol 25:7–17

Gilbert LE, Barr CL, Calixto AA, Cook JL, Drees BM, LeBrun EG, Patrock RJW, Plowes RM, Porter SD, Puckett RT (2008) Introducing phorid fly parasitoids of red imported fire ant workers from South America to Texas: outcomes vary by region and by *Pseudacteon* species released. Southwest Entomol 33:15–29

LeBrun EG, Plowes RM, Gilbert LE (2008) Dynamic expansion in recently introduced populations of fire ant parasitoids (Diptera: Phoridae). Biol Invasions 10:989–999

LeBrun EG, Plowes RM, Gilbert LE (2009) Indirect competition facilitates widespread displacement of one naturalized parasitoid of imported fire ants by another. Ecology 90:1184–1194

Morrison LW, Porter SD (2005) Phenology and parasitism rates in introduced populations of *Pseudacteon tricuspis*, a parasitoid of *Solenopsis invicta*. BioControl 50:127–141

Morrison LW, Porter SD, Gilbert LE (1999) Sex ratio variation as function of host size in *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). Biol J Linn Soc 66:257–267

Orr MR, Seike SH, Benson WW, Gilbert LE (1995) Flies suppress fire ants. Nature 373:292–293

Patrock RJ, Porter SD, Gilbert LE, Folgarait PJ (2009) Distributional patterns of *Pseudacteon* associated with the *Solenopsis saevissima* complex in South America. J Insect Sci 9:1–17

Pesquero MA, Porter SD, Fowler HG, Campiolo S (1995) Rearing of *Pseudacteon* spp. (Diptera: Phoridae), parasitoids of fire ants (Solenopsis spp.) (Hymenoptera: Formicidae). J Appl Entomol 119:677–678

Plowes RM, LeBrun EG, Gilbert LE (2011) Introduction of the fire ant decapitating fly *Pseudacteon obtusus* in the United States: factors influencing establishment in Texas. BioControl 56:295–304

Porter SD (1998) Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). Florida Entomol 81:292–309

Porter SD, Briano JA (2000) Parasitoid-host matching between the little decapitating fly *Pseudacteon curvatus* from Las Flores, Argentina and the black fire ant *Solenopsis richteri*. Florida Entomol 83:422–427

Porter SD, Gilbert LE (2004) Assessing host specificity and field release potential of fire ant decapitating flies (Phoridae: *Pseudacteon*). In: van Driesche RG, Reardon R (eds) Assessing host ranges for parasitoids and predators used for classical biological control: a guide to best practice. FHTET-2004-03. USDA Forest Service, Morgantown, USA, pp 152–176

Porter SD, Williams DF, Patterson RS (1997) Rearing the decapitating fly *Pseudacteon tricuspis* (Diptera: Phoridae) in imported fire ants (Hymenoptera: Formicidae: Solenopsis) from the United States. J Econ Entomol 90:135–138

Porter SD, Nogueira de Sá LA, Morrison LW (2004) Establishment and dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* in North Florida. Biol Control 29:179–188

Porter SD, Graham LC, Johnson SJ, Thead LG, Briano JA (2011) The large decapitating fly *Pseudacteon litoralis* (Diptera: Phoridae): successfully established on fire ant populations in Alabama. Florida Entomol 94:208–213

Taylor C, Hastings A (2005) Allee effects in biological invasions. Ecol Lett 8:895–908

Valles S, Porter S (2007) *Pseudacteon* decapitating flies: Potential vectors of a fire ant virus? Florida Entomol 90:268–270

van Lenteren JC (ed) (2003) Quality control and production of biological control agents: theory and testing procedures. CABI Publishing, Wallingford, UK

Vogt JT, Porter SD, Nordlund DA, Smith R (2003) A modified rearing system for production of *Pseudacteon curvatus* (Diptera: Phoridae), a parasitoid of imported fire ants. Biol Control 28:346–353

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