

Reproduction and dispersal of wing-clipped predatory stinkbugs, *Podisus nigrispinus* in cotton fields

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Abstract Release of arthropod predators holds promise for suppressing herbivorous insect populations in commercial crops below economic threshold levels, but dispersal from release sites remains a practical issue that can limit their impact. This study examined mating behavior, survival, reproduction, and dispersion of membranous wing-clipped and wing-intact adults of the predatory stinkbug *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae), a generalist predator of cotton pests in South America. After laboratory studies demonstrated that wing-clipping did not alter mating behavior or reproductive output, field studies were conducted in experimental plots. In each experiment, 50 mated females (5–10 days old) were released at a central point. After 24, 48, 72, and 96 h, dispersion was measured

via drop-cloth samples at distances up to 16 m from the release point. After 96 h, plants on each survey and release point were collected and, in the laboratory, they were inspected for egg masses. During the first 24 to 48 h after release, predators from both groups disappeared, but recovery of the wing-clipped predators was significantly greater (38.6% for wing-clipped and 17.3% for wing-intact predators) in all three releases. Importantly, oviposition rates on the release site were about three times greater for wing-clipped females (0.07 vs. 0.02 egg masses per plant), indicating that limiting flight induces females to stay and lay their eggs, hence, allowing local establishment of a new generation of predators.

Keywords Asopinae · Augmentative release · Mating behavior · Limited-flight predator · Reproductive success

This research is part of a larger project looking for improvement of natural predators into sustainable pest management conducted by the biological control and insect ecology laboratory (lead by Jorge B. Torres) of the “Universidade Federal Rural de Pernambuco (UFRPE) at Recife, PE, Brazil.

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Introduction

The importance of arthropod predators as suppressors of herbivorous insect populations on crops has long been recognized (Whitcomb 1974; Murdoch and Oaten 1975; Hagen et al. 1976). Cotton fields provide a particularly good example of such biological control by predators. Although about 250 insect species occur in the cotton ecosystem in Brazil (Silva et al. 1968), only about 27 of these are considered prone to cause damage, of which only 5–6 species reach population

levels beyond the economic threshold (Degrande 1998). However, when abundance of naturally occurring arthropod predators decreases, cotton pest populations increase (Eveleens et al. 1973; Hagerty et al. 2005). Predatory hemipterans of the genus *Podisus* (Pentatomidae, Asopinae) are commonly found in several crop ecosystems, where they feed primarily on larval stages of various herbivore pests and non-pests (McPherson 1980; De Clercq 2000; Torres et al. 2006). For over a decade, forest companies in Minas Gerais State, Southeast Brazil, have used an advanced and economical mass production system to raise *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) for augmentative releases to help protect eucalyptus plots predisposed to caterpillar attacks. More than five million predators have been released, resulting in significant reductions in insecticide use to control lepidopteran larvae (Zanuncio et al. 2002; Torres et al. 2006).

The expectation in any augmentative release program is that predator populations will increase in the targeted area and its surroundings. However, releases of predatory stinkbugs into row crop ecosystems face different conditions than those in a eucalypt forest. The row crops have a short life cycle and pest populations may suddenly surpass economic threshold before natural enemies become established among other factors. In addition, large trees in and around the eucalyptus plots receiving releases provide a dispersive barrier, whereas the area in row crops is covered by bushes that confer a poor barrier to delay predator dispersion after release.

Various practices have been tried to delay predator dispersion after releasing. Food provision such as spraying artificial honeydew has been recommended for ladybeetles and lacewings in cotton fields (Evans and Richards 1997; Mensah 2002). Companion plantings around crop fields have been used to provide nectar and shelter (Slosser et al. 2000; Olson and Wäckers 2007), especially during activities inside crop fields such as insecticide application. To enhance local residence of the coccinellid *Harmonia axyridis* (Pallas), Tourniaire et al. (2000) successfully selected adults from a normal-winged stock population with low interest in flying after five generations. For predatory hemipterans, releasing late instar nymphs has some potential mainly because they move less than adults (Sant'Ana et al. 1997; Torres et al. 2002; Grundy and Maelzer 2002). Release of late-instar

nymphs has its problems, however. A major one is the low survival of nymphs after release, based on low recovery rates relative to the number of nymphs released. Another is that late instars will soon complete their development and disperse just like released adults. An additional justification for investigating adult release is the anticipated multiplier effect; after releasing adults into an early succession crop field, it is reasonable to expect that they will lay eggs over the field and start a local population. The instability of prey availability early in the crop ecosystem can be the major cause of failure of natural enemies to colonize a field. However, adults of *P. nigrispinus* can live nearly 4 weeks on cotton plants in the absence of target or alternative prey items, while nymphs do not molt to the next stadium (Evangelista Junior et al. 2004). Also, adults are less susceptible to entomopathogens (França et al. 2006). Nonetheless, predatory stinkbug adults are very dispersive (Yang 2000), and the initial releases to colonize crop fields have a high likelihood of failure.

Studies using wing-clipped natural enemies at the axillary region were conducted with the larval parasitoid, *Cotesia flavicincta* (Ashmead), and the predators, *Hippodamia convergens* Guérin-Ménéville and *Podisus maculiventris* (Say) by Ignoffo et al. (1977) and Lambdin and Baker (1986). The former authors found in the laboratory and in experimental plots (ca. 24 soybean plants) larger parasitism and predation rates for wing-clipped individuals compared to winged ones. With the overall goal of limiting the flight of newly released adult *P. nigrispinus* so that they stay and initiate a new generation in the cotton field, this study was undertaken to determine how flight limitation might influence this predatory pentatomid's life history and field residence after releasing. We had two major objectives: (i) to compare the mating behavior and reproductive output of wing-clipped females and males of *P. nigrispinus* with that of wing-intact individuals; and (ii) to study the time of residence of adults in cotton fields by recapturing individuals and evaluating their oviposition at the release sites.

Materials and methods

Predatory stinkbug production

Adults of the predatory stinkbug used in the experiments were reared in the Biological Control

Laboratory of the UFRPE at $25.3 \pm 1.2^{\circ}\text{C}$, and 13L:11D photoperiod. Briefly, nymphs from second instar (first instar does not feed on prey) to adult emergence were maintained in Plexiglas[®] cages of $25 \times 20 \times 40$ cm with circular openings on both sides closed with nylon mesh to improve ventilation inside the cages. Each cage contained a free upper cover that fit externally to the upper border of the cage. Nymphs were fed yellow mealworm (*Tenebrio molitor* L.) pupae *ad libitum*. Additionally, they were offered green bean pods and provided with moisture through soaked cotton inside a plastic container of 5 ml. Wrinkled paper towels were placed inside the cages as substrate. Every 3–4 days, depending on prey consumption along nymphal development, mealworm pupae and bean pods were replaced and more water was added to the cotton ball. Under these laboratory conditions, development from second instar to adult took about 16 days.

Mating behavior and reproductive output

To investigate the effects of flight limitation on mating behavior and reproductive output of male and female *P. nigrispinus*, adults weighing 55–65 mg were selected from rearing cages at adult emergence. After 48 h in the adult stage, each had the tip of its membranous wings cut off with scissors. These adults were maintained in isolation until the next day (ca. 72 h-old), when they were observed for normal behavior (i.e., walking, feeding, and grooming). Then, winged and limited flight adults were randomly assigned to one of four pairing conditions, each of which was replicated 20 times: (i) winged males and females; (ii) limited-flight males with winged females; (iii) winged males with limited-flight females; and (iv) limited-flight males and females. Pairings occurred in plastic cups of 500 ml volume, each containing a 1.5 ml water-filled vial plugged with a soaked cotton pad placed through the cups's lid. Two mealworm pupae were offered inside each cup as prey. At the onset of the daylight cycle (Carvalho et al. 1994), one female and one male per cup were placed together and observed to determine mating time. Only couples mating during the first 3 h after pairing were maintained; the remaining males and females were discarded. Therefore, the final sample sizes for the four pairing conditions were 11, 12, 12 and 12 pairs,

respectively. Males were discarded, but mated females were maintained in the same cups and provided with food, water, and wrinkled towel paper as an oviposition substrate. Every second day, the cups were checked, noting female mortality, collecting eggs, and replacing food. Egg masses were counted and maintained in Petri dishes to verify hatching.

Field experiments

Predator release studies were conducted in 2006 and 2007 seasons. Field release in 2006 took place in three pairs of cotton fields, 30 m long and 20 m wide, that were separated by a 5 m strip planted with corn and surrounded by a large cornfield planted 2 weeks earlier. All were located in Aldeia County, PE, Brazil, between the coordinates $07^{\circ}56'25.8''$ S and $35^{\circ}01'38.9''$ W. The cotton variety used was BRS 201 donated by the seed department of Embrapa Cotton, Campina Grande, PB, Brazil. Seeds were planted manually at 30 cm within rows and 70–80 cm between rows on July 15. The seeds were treated with the fungicide Captan (Captan 750 TS) at rate of 145 g a.i. per 100 kg of seeds. Standard crop management practices were adopted during the growing season.

Females of *P. nigrispinus* (~60 mg), produced as mentioned above, were selected from rearing cages at the day of emergence and kept separately in groups of ten. Three days later, part of the selected females had their membranous wings clipped and designated to the treatment wing-clipped females. These females were paired with wing-intact males in adult Plexiglas cages until they began laying eggs (2–3 days after mating). Similarly, wing-intact females were also paired with wing-intact males. Then, wing-clipped females and wing-intact females were selected to be released at a rate of 50 females per cotton plot; each treatment had three replicates totaling 150 females to be released per treatment. In the laboratory, groups of 25 females were transferred to plastic cups of 500 ml volume for transport to the field to be released on the same day. Releases were made in the morning (~8:00 to 9:00 a.m.) by opening the plastic cups and placing them in the upper third of the central plant in each plot.

At 24, 48, 72 and 96 h after release (i.e., every morning before 9:00 a.m.), predators were surveyed

at the releasing point and at 2, 4 and 8 m from the releasing point in the four cardinal directions. Drop cloths (1 m long white canvas) were laid on the ground between two cotton rows, and plants on the adjacent rows were shaken vigorously over the cloth. Predatory stinkbugs that fell on the cloth were counted and released at the same location. After the 96 h survey, one plant per sampling point, including the releasing point, was selected for harvesting; these 13 plants per field were thoroughly inspected in the laboratory for egg masses of the predator.

During the 4 days of sampling after predator release, the air temperature was monitored with a WatchDog logger (SpectrumTM Technologies, Inc., Plainfield, IL) set to record at 30 min intervals and hung in the same central plant where the predators were released. The average temperature was 24.1°C (maximum 34.5°C, minimum 18.0°C). Rainfall and relative humidity (R.H.) data were obtained from the weather station of the “Secretaria de Recursos Hídricos do Estado de Pernambuco”; the accumulated rainfall was 75.9 mm for the period and R.H. averaged $78.1 \pm 20.1\%$.

The predator releases in 2007 were conducted at a private commercial cotton field (85 ha) in the “Fazenda Guarita”, Rondonópolis County, MT, Brazil located between the coordinates 16°34′06″ S and 54°41′05″ W. Seeds were planted at rate of 9 kg/ha on 15 December 2006 with the variety FMT 710. Fertilizers, hormones, insecticides, fungicides and herbicides were applied when needed according to the standard grower practices. However, an interval of at least 3–5 days occurred between any previous spraying and predator releases in the fields.

Two releases of predators were carried out in 2007. The first release was done on February 14 and the second release on March 2 when cotton plants were 54 and 70 day-old with mean height of 1.3 and 1.65 m, respectively. Within the 85 ha cotton field, six field plots were established by marking central points with flags attached to wood sticks set approximately 200 m apart. This distance between releasing points and sampling points was chosen so that the probability of predators moving among releasing points would be very low. All *P. nigrispinus* used were produced in the Biological Control Laboratory of the UFRPE. Wing-clipped and wing-intact females that were beginning to oviposit (ca. 5–6 days old) were caged in plastic cups of 500 ml volume at a

rate of 30 females per cup to account for possible mortality during transportation. Females were provided with mealworm pupae, green bean pods, and wrinkled towel paper as substrate. The predators were shipped using Express delivering system (Sedex[®]) to the Mato Grosso Research Foundation in Rondonópolis, MT, located about 2,500 km from UFRPE and the delivery required 2 days. At arrival, predators were checked and mortality was recorded to be around 10% (2–4 deaths per 30 individuals per cup). The next morning, at the marked points in the field, either 50 wing-clipped females or 50 wing-intact females per plot were released; thus these trials had two treatments with three replications each.

Over the following 4 days, predators were surveyed using the same procedure adopted for the 2006 release for time intervals and sampling methods. Only the distances from the releasing point were modified. In 2007, predators were surveyed at the release point and at distances of 2, 8 and 16 m away. At the conclusion of the 96 h survey, one plant per sampling point, including the releasing point, was selected. These 13 plants per field were harvested and completely inspected in the laboratory for egg masses.

Statistical analyses

Data from laboratory experiments regarding the reproductive behavior, mating time, number of eggs and nymphs produced per female were tested for normality (Kolmogorov D: normal test) and homogeneity of variance (Bartlett’s test), with data transformed when needed (but untransformed data are presented in tables and figures). Data analysis used the PROC ANOVA of SAS (SAS Institute 1999–2001). On variables with significant results by ANOVA (Fisher’s test), a Tukey HSD test was performed to separate the means of the four pairing conditions studied. On field release data, comparisons between releasing strategies were conducted on percentage of predators recovered on the drop cloth out of 50 predators released per field plot. Release strategy (i.e., wing-clipped versus wing-intact females) and distance of sample from release point were set as main factors for repeated measures ANOVA run with time after releasing (24, 48, 72 and 96 h) as a blocking factor according to the profile

written by Cody and Smith (1997). Because the samples were taken considering the four geographic coordinates around the releasing point, the data from the four coordinates were averaged per distances 2, 4 and 8 m (2006) or 2, 8 and 16 m (2007) to be comparable with the releasing point (one per field). When modeling the proportion of predators recovered over time from fields receiving limited-flight females versus wing-intact females, we disregarded the sampling points. Instead, the total numbers of predators recovered from all sampled points over time were regressed as a function of time after release, using the PROC REG of SAS (SAS Institute 1999–2001). All parameters of the regressions were subject to a 0.05 level of significance. To test the hypothesis that predators with limited flight spend more time at the release site, the linear slopes of fitted models for percentage of predators recovered over time were compared for limited-flight and wing-intact females, using PROC MIXED to test the equality of linear slopes (SAS Institute 1999–2001). To compare numbers of egg masses recovered and total numbers of eggs per field (based on 13-plant samples) between plots receiving releases of limited-flight and plots receiving wing-intact females, Wilcoxon exact test was used through PROC NPARIWAY of SAS because the data did not meet the assumptions for a parametric analysis even after data transformation.

Results

Mating behavior and reproductive output of limited-flight predatory stinkbugs

Cutting off the tip of the membranous wings of predators of either sex did not interfere with mating behavior as measured through the proportion of successful mating (55% for intact winged and 60% for wing-clipped). Likewise, clipping wings did not affect duration of copulation ($F_{df=3,43} = 0.92$, $P = 0.4401$). On average, all pairings showed similar mating duration (521.7–638.3 min). In addition, mating duration did not show any significant relationships with number of nymphs produced ($P = 0.106$ by females across all four pairing conditions). Among the adult characteristics evaluated, only the age of first oviposition differed significantly ($F_{3,43} = 3.63$; $P = 0.0403$). On average, wing-clipped females

paired with wing-intact males initiated oviposition 2 days earlier than wing-intact females paired with either wing-intact or wing-clipped males (Table 1). All other characteristics regarding female reproductive output were similar across all four pairing conditions (Table 1).

Egg hatching rates were similar for females under different pairing conditions ($P > 0.05$), suggesting equal amounts of spermatozoa being transferred to the females. Egg hatching rates can also be similar because of no reproductive cost of wing clipping. For all pairing conditions, egg hatching rates were above 70% during the first 2 weeks and decreased to rates between 50% to 70% from the 2nd to the 4th week.

Field experiments

The percentage of predators recovered through drop cloth sampling differed significantly according to the predators' ability to fly ($F_{1,2} = 26.82$; $P = 0.0035$), their distance from the releasing point ($F_{3,6} = 7.29$; $P = 0.0200$), and the elapsed time since release ($F_{3,6} = 56.60$; $P < 0.0001$). Regardless of flight ability, greater numbers of predators dispersed during the first 24 h period after release, followed by 24–48 h after releasing (Fig. 1—left) and from the releasing point (Fig. 1—right). This early disappearance resulted in significant regression second order models with negative linear coefficients fitted on the proportion of predator recovery over time for both limited-flight and winged predators (Fig. 1—left). An exception was the second release in Rondonópolis in 2007 that exhibited a significant regression first order model with a negative linear coefficient.

Significantly greater proportions of predators were recovered in the plots after releases of wing-clipped females in Aldeia in 2006 (PROC MIXED for equality of linear coefficient: $t_{df=20} = 2.58$; $P = 0.0189$) and after the first 2007 release in Rondonópolis ($t_{20} = 3.67$; $P = 0.0015$). However, the results for the second 2007 release in Rondonópolis showed only marginal differences ($t_{20} = 1.92$; $P = 0.0708$), probably because a relatively lower number of both classes of predators were recovered as compared to the results from Aldeia and from the first release in Rondonópolis (Fig. 1).

During the 96 h survey across all releases and fields, from a total of 450 released predators of each

Table 1 Reproductive characteristics for various mating combinations of wing-clipped (WC) and wing-intact (WI) *P. nigrispinus*

Characteristics	♀WI × ♂WI (n = 11)	♀WI × ♂WC (n = 12)	♀WC × ♂WI (n = 12)	♀WC × ♂WC (n = 12)	Statistics
Age at 1st oviposition (days) ^b	8.4 a (7.2–9.5)	8.2 ab (6.7–9.6)	6.2 b (5.5–7.0)	7.0 ab (5.8–8.2)	$F_{3,43} = 3.63^a$
Number of eggs per female	562.7 (162–1,095)	517.4 (275–883)	450.5 (79–946)	354.2 (77–778)	$F_{3,43} = 1.97^{ns}$
No. of nymphs per female	266.9 (201.7–463.3)	364.9 (237.3–481.2)	275.2 (216.1–477.6)	224.3 (132.2–316.4)	$F_{3,43} = 1.36^{ns}$
Female longevity (days)	72.5 (50.4–94.7)	55.5 (39.8–71.2)	64.9 (39.2–90.7)	50.1 (39.7–67.1)	$F_{3,43} = 1.29^{ns}$

Values are means with 95% confidence intervals in parentheses

^a $P < 0.05$ by ANOVA Fisher test, hence, treatment means are followed by different letters ranked by Tukey HSD test. ^{ns} $P > 0.05$ by ANOVA Fisher test

^b Time from adult emergence to initial oviposition

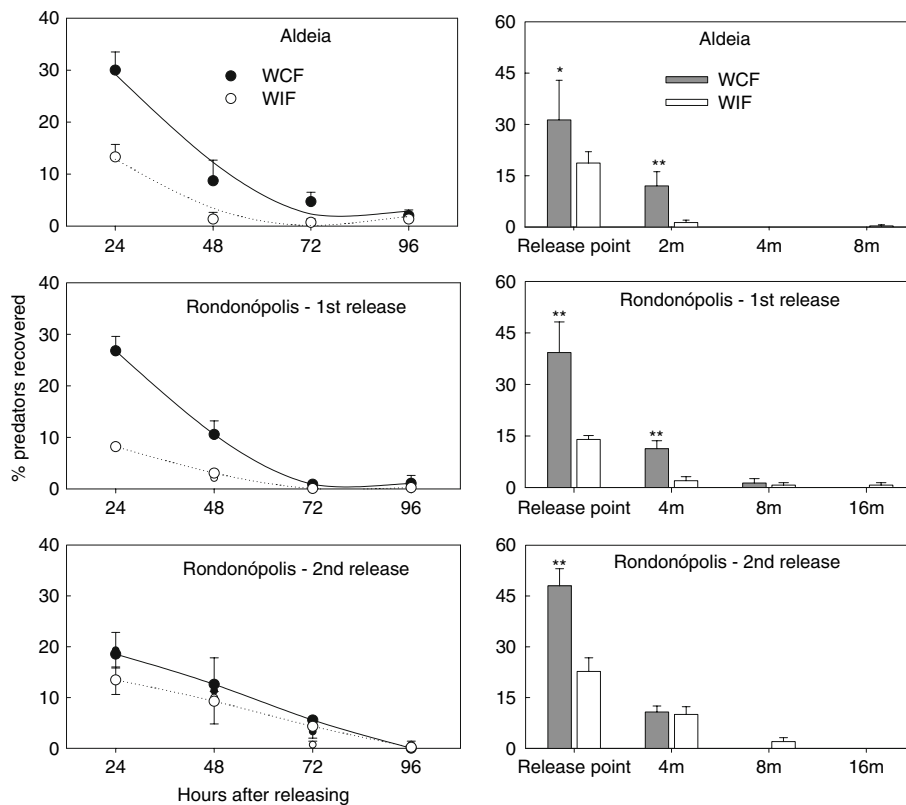


Fig. 1 Left: numbers of wing-clipped females (WCF) and wing-intact females (WIF) recovered (mean + SE) by drop cloth samples over time at the three plots (Aldeia WCF: $y = 56.66 - 1.33x + 0.008x^2$; $R^2 = 0.86$; $F_{2,9} = 27.16$; $P = 0.0002$; Aldeia WIF: $y = 29.17 - 0.41x + 0.0027x^2$; $R^2 = 0.83$; $F_{2,9} = 22.07$; $P = 0.0003$; Rondonópolis 1st release, WCF: $y = 52.87 - 1.27x + 0.007x^2$; $R^2 = 0.91$; $F_{2,9} = 48.74$; $P < 0.0001$; Rondonópolis 1st release, WIF: $y = 16.49 -$

$0.40x + 0.002x^2$; $R^2 = 0.89$; $F_{2,9} = 40.38$; $P < 0.0001$; Rondonópolis 2nd release, WCF: $y = 25.0 - 0.27x$; $R^2 = 0.64$; $F_{1,10} = 17.97$; $P = 0.0017$; Rondonópolis 2nd release, WIF: $y = 18.0 - 0.19x$; $R^2 = 0.77$; $F_{1,10} = 33.74$; $P = 0.0002$). Right: the average (+SE) percentage of female *P. nigrispinus* captured at different sampling distances from the release point in the different study sites

flight type, $38.6 \pm 5.9\%$ (mean \pm SD, $n = 174$) of the limited-flight predators and $17.3 \pm 7.0\%$ ($n = 78$) of the winged predators were recollected. These recovery rates were lowest in the field plots with releases of wing-intact females: 13%, 8.4% and 13.3%, respectively (Fig. 1). Many of these wing-intact females were observed flying from the site at the moment of release. It is likely that they continued to disperse during the following intervals. On the other hand, higher proportions of wing-clipped females were recovered. From 50 wing-clipped females released per field, 30%, 20.6%, and 19.3% were recollected in Aldeia, Rondonópolis 1st and 2nd release, respectively.

The numbers of egg masses and eggs recovered were highly variable, so that the difference between fields receiving wing-clipped females or winged females was not statistically significant ($\chi^2_{df=1} = 0.201$; $P = 0.654$). However, approximately three times more egg masses and eggs were found per 13-plant sample from the fields with limited flight-females (average \pm SD, 0.7 ± 1.3 egg masses and 9.0 ± 12.3 eggs per 13 plants) in comparison to fields with winged females (0.22 ± 0.44 egg masses and 3.11 ± 5.44 eggs per 13 plants).

Discussion

The wing-clipped male and wing-clipped female bugs exhibited courting behavior in a manner similar to winged male and female pairs as described in Carvalho et al. (1994). Thus, other factors may have been responsible for later oviposition in the wing-clipped female combination (Table 1) such as the within-population variation. Overall, *P. nigrispinus* starts laying eggs 1 day after mating (ca. 4–5 days into the adult stage); a female produces on average 400–600 eggs and lives around 4 weeks (De Clercq 2000; Torres et al. 2006). However, in experimental populations, quite frequently one may find females taking more than 1 week for first oviposition, laying up to 1,200 eggs and living more than 100 days (JBT, personal observation).

Missing the tip of their membranous wings and being limited in flight did not interfere with mating behavior and reproductive output of *P. nigrispinus* females in the laboratory. Based on prey scarcity studies with *P. nigrispinus* (De Clercq and Degheele

1992; Molina-Rugama et al. 2001; Oliveira et al. 2002), one prey item consumed fully can sustain a *P. nigrispinus* female for several days, and those females holding fertilized eggs will lay them in the following days. Based on this information, it seems reasonable to hypothesize that local colonization can be initiated by releasing mated females that will lay their eggs on nearby plants when flight limitation slows their dispersion from the release sites.

The lower recovery of wing-clipped females during the second release in Rondonópolis at the 24 h-period after release compared to previous releases (ca. Aldeia and Rondonópolis 1st release) (Fig. 1) suggests a greater death rate after release. This explanation seems plausible since on 19 March, only 3 days before the stinkbugs were released, the insecticide endosulfan was applied by the grower to control an early infestation of boll weevil. Endosulfan is a neurotoxic organochlorine insecticide certified for longer residue than 3 days on cotton foliage (Wilson 1989) and toxic to pentatomids (Gazzoni 1998).

The apparent disappearance of many wing-clipped females might have been due to events such as predators falling from the plants onto the ground, moving to nearby weeds or dying. However, the dispersion of wing-clipped females compares favorably to that of late nymphal stages of predatory bugs. Waddill and Shepard (1975) recovered only 9.8% and 11% of *P. maculiventris* nymphs during three consecutive surveys. In another study, Grundy and Maelzer (2002) found that only about 40% of third instar nymphs of the assassin bug, *Pristhesancus plagipennis* Walker remained in the release site after being individually released by hand onto the terminal shoots of soybean, sunflower and cotton.

In a number of closed indoor crop systems, released natural enemies have been effective for pest management (van Lenteren 2000). However, attaining similar practical biological control outdoors with predatory arthropods has been more problematic. Predator dispersion from release sites seems to be the main cause of failure. Once satiated, generalist predators may disperse or rest. Another factor potentially related to the short persistence of released predators in our fields was the low infestation levels of the common lepidopteran pests of cotton in the study sites. Only soybean looper larvae were caught in the drop cloth survey at the end of the season.

Other cotton pests and preferred prey of *P. nigrispinus* such as *Spodoptera* spp., *Alabama argillacea* (Hübner) and *Heliothis virescens* (Fab.) were absent.

Despite of the greater number of wing-clipped females residing in the release sites and a trend for more oviposition than winged females in these sites, there are several remaining questions such as: how many wing-clipped female *P. nigrispinus* have to be released for initiating a new generation in the field successfully and is it profitable? To clip one or two membranous wings of one *P. nigrispinus* female takes around 1 min. Therefore, 1 day labor can prepare around 500 females to be released on the next day. Despite of the costs to raise and to clip the wings of the bugs to release, the methodology can be feasible for small areas and organic farming. The Brazilian Northeast cotton region is characterized predominantly by family agriculture planting from 1 to 3 hectares, absence of machinery and, hence, intensive hand labor in cultivation practices. The small farmers, however, planted 76,891 hectares of cotton in this region during the 2007 season (IBGE 2007). In the Northeast, the cotton leafworm is a key pest (Ramalho 1994) and the predatory stinkbug has been addressed for its control. Therefore, releasing wing-clipped *P. nigrispinus* might be a valuable strategy for this low input agricultural system, despite the time needed for wing clipping. Considering that one *P. nigrispinus* female caged on single cotton plants and fed only every 6 days produced more than 100 viable eggs over 3 weeks (Oliveira et al. 2002), one wing-clipped female well fed and released during the oviposition peak (ca. 5–10 days old) will be laying eggs in the release site for some time. It is reasonable to expect that such oviposition would produce a sufficient number of nymphs to initiate a local population. These nymphs will take around 2 weeks to complete nymphal development and will be preying on target pests. Furthermore, farmers in the low input agricultural system of the Brazilian northeast are faced with lepidopteran pests in the other crops that they grow and these may also be attacked by *P. nigrispinus* (Torres et al. 2006). Therefore, a successful release procedure for this predator when established in these small grower diversified fields will be useful beyond the cotton crop.

Augmentative releases of advanced nymphal stages or wing-intact females require a well-planned large scale production of the predator, whereas the

release of a limited number of wing-clipped females at the peak of their oviposition rather aims to introduce eggs and hence nymphs in the field prior to pest outbreak. Ongoing experiments are incorporating enhanced prey levels (simulating an early generation of *A. argillacea*), sex pheromone lures and field insectaries with wing-intact females to determine whether post-release predator residence times and oviposition rates in the field can be increased.

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