

## A push-pull integrated pest management scheme for preventing use of parrot nest boxes by invasive Africanized honey bees

Caroline A. Efstathion,<sup>1,3</sup> Paul M. Bardunias,<sup>1,4</sup> Janice D. Boyd,<sup>2</sup> and William H. Kern Jr.<sup>1</sup>

<sup>1</sup>Department of Entomology and Nematology, Fort Lauderdale Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 3205 College Avenue, Fort Lauderdale, Florida, 33314, USA

<sup>2</sup>Schubot Exotic Bird Health Center, Department of Veterinary Pathobiology, Texas A&M University, 4467 Texas A&M University College Station, Texas, 77843-4467, USA

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**ABSTRACT.** Africanized honey bees (*Apis mellifera scutellata*) compete with endangered parrots for nest boxes and can hamper conservation efforts. We tested an integrated pest management push-pull protocol in the Atlantic Forest in São Paulo, Brazil, in an effort to prevent bee swarms from colonizing nest boxes ( $N = 30$  in the forest plus five in aviaries) meant for use by Vinaceous-breasted Amazons (*Amazona vinacea*). Fifteen parrot nest boxes were treated with a permethrin insecticide to “push” scout bees away and each parrot box was paired with a bee trap box containing a pheromone lure to “pull” bees. Over a 1-yr period (March 2013 to March 2014), 29 insect colonies moved into 18 of the 35 trap boxes. Nine Africanized honey bee, three native Jatai bee (*Tetragonisca* sp.), and 17 wasp colonies occupied trap boxes. Only one experimental push-pull pair untreated parrot box was invaded by bees and no parrot boxes in aviaries were colonized. Four of the parrot nest boxes were occupied by birds during our study. Although none were used by Vinaceous-breasted Amazons, Southern House Wrens (*Troglodytes musculus*), Green-winged Saltators (*Saltator similis*), and Plain Parakeets (*Brotogeris tirica*) nested in the boxes and all nests were successful. Although long-term studies are needed before drawing conclusions about the effectiveness of trap boxes, our results suggest that a push-pull protocol may prove useful for reducing the use of nest boxes meant for parrots and other cavity-nesting birds by Africanized honey bees and other insects.

**RESUMEN.** Un esquema integrada de alejar-atraer para el control de plagas para la prevención del uso de cajas nido para loros por las abejas de miel Africanizadas invasoras

Las abejas de miel Africanizadas (*Apis mellifera scutellata*) compiten con los loros en peligro de extinción para las cajas nido y puede impedir los esfuerzos de conservación. Probamos un método integrado para el control de plagas, el protocolo de alejar-atraer, en el Bosque Atlántico, en São Paulo, Brasil, en un esfuerzo para evitar que los enjambres de abejas colonicen las cajas nido ( $N = 30$  en el bosque, más cinco en aviarios) destinadas a ser utilizadas por el loro Amazona Vinosa (*Amazona vinacea*). Quince cajas nido fueron tratados con una insecticida permetrina para alejar las abejas exploradoras de las cajas nido. Las cajas nido también fueron emparejada con una caja trampa para abejas, que contenía una feromona para atraer las abejas. Durante un período de un año (marzo 2013 – marzo 2014), 29 colonias de insectos se trasladaron a 18 de las 35 cajas trampa para abejas. Nueve colonias abejas de miel Africanizada, tres colonias abejas nativo Jatai (*Tetragonisca* sp.) y 17 colonias de avispa ocuparon las cajas trampas. Sólo un experimento caja nido, emparejada con el método alejar-atraer, fue invadida por las abejas y ninguna de las cajas nido en aviarios fueron colonizados por abejas. Cuatro de las cajas nido estaban ocupados por aves durante nuestro estudio. Aunque ninguno fue utilizado por el loro Amazona Vinosa, los nidos fueron anidados por el Chivirín Ratón (*Troglodytes musculus*), el Pepitero Verdoso (*Saltator similis*) y la Catita Tirica (*Brotogeris tirica*) y todos los nidos fueron exitosos. Aunque se necesitan estudios a largo plazo antes de sacar conclusiones acerca de la efectividad de la caja trampa para abejas, nuestros resultados sugieren que un protocolo de alejar-atraer puede ser útil para reducir el uso de nidos de loros, y otras aves, por las abejas de miel africanizadas y otros insectos.

*Key words:* *Amazona vinacea*, *Apis mellifera scutellata*, nasonov, nest competition, permethrin, swarm-founding wasp

<sup>3</sup>Corresponding author. Email: cefstathion@ufl.edu

<sup>4</sup>Current address: Department of Environmental and Forest Biology, SUNY College of Environmental Science and Forestry, 1 Forestry Drive, Syracuse, New York 13210, USA.

Almost 20% of bird species roost and nest in cavities, and populations of ~120 of these species are currently in decline (Eadie et al. 1998). Many cavity nesters are in decline because of the loss of mature trees with suitable cavities. To mitigate nest-site limitation, artificial nest boxes are often used

as a conservation tool to increase reproduction rates (Newton 1994, Eadie et al. 1998), and this has contributed to the recovery of several species of birds (Carlile et al. 2003, Proudfoot et al. 2006).

Artificial nest boxes often attract non-target species (Delnicki and Bolen 1977, Ingold 1998). This competition can negatively impact recovery efforts for the intended bird species (Pell and Tidemann 1997). In addition to other bird species, common competitors for nest boxes include honey bees (Coelho and Sullivan 1994, Oldroyd et al. 1994, Jensen et al. 1995, Prange and Nelson 2007, Madeiros et al. 2012, Veiga et al. 2013), most notably the Africanized hybrid. Since their accidental introduction in São Paulo, Brazil, in 1957 (Winston 1992), African queen bees (*Apis mellifera scutellata*) have spread throughout South and Central America and into the southern United States (Schneider et al. 2004). Rather than creating a true hybrid, African genes persist and dominate, eventually replacing those of the European honey bee (*Apis mellifera*; EHB), creating a purer line of African bees after several generations (Hall and Muralidharan 1989, Diniz-Filho and Malaspina 1995, Quezada-Euán 2000). Invasive Africanized honey bees (hereafter AHB) present a challenge to endemic wildlife because they are highly defensive (Winston 1992), reproduce quickly (Winston 1992), and are less selective about where they nest (Schmidt and Hurley 1995).

Throughout the Neotropics, AHB compete with many endangered species of parrots for nest boxes (Synder et al. 2000, U. S. Fish and Wildlife Service 2008, WCS Guatemala Program 2011, Berkunsky et al. 2012), and are listed as one of the threats affecting the future release of the nearly extinct Spix Macaw (*Cyanopsitta spixii*; ICMBio 2012). This competition results in birds being unable to nest in these boxes and, when established nests are invaded, nestlings are stung to death by the bees. These bees also present a hazard for biologists who monitor nest cavities.

There is currently no consensus on a protocol for managing honey bee colonization of nest boxes. Current practices include use of repeated, high doses of permethrin (Bjork and McNab Balas, pers. comm.) to keep bees out, manually removing swarms multiple times a week (Wittkoff, pers. comm.), and closing up

nest boxes immediately after the breeding season (U.S. Fish and Wildlife Service 2008). Because these methods are unsustainable due to the labor involved and the danger posed to biologists, a prevention protocol is needed.

Integrated pest management (IPM) employs multiple methods to reduce the incidence of an insect pest and focuses on preventive rather than remedial methods (Kogan 1998). One strategy for use in IPM programs is the push-pull method (Pyke et al. 1987). This method simultaneously employs a repellent (push) to deter insects from a resource and an attractant (pull) to bring insects to another area, such as a trap. This method modifies the behavior of an insect by directing their movement (Cook et al. 2007). Development of an IPM protocol could reduce the incidence of honey bee colonization in nest boxes of birds and improve the success of conservation efforts.

Colonization of nest cavities by bees can occur through a reproductive process called swarming. A swarm occurs when approximately half the workers and the old queen leave the original nest site and cluster at a nearby location (Schmidt 1994). Over several hours to days, scout bees fly off to locate a new nest site. When a site is found, scouts walk around the inner surface to gauge the size and quality of the cavity (Seeley 1977) and then fly back to the swarm and communicate the location to other bees by dancing with excitement relative to the quality of the site (Seeley and Visscher 2003). Eventually all the bees fly to one site, rejecting the others (Seeley and Visscher 2004).

One method to discourage a swarm from occupying a potential nest site is to spray it with a repellent insecticide, such as permethrin. Permethrin is a synthetic pyrethroid insecticide that is nontoxic for birds (Mineau et al. 2001), but toxic (Danka et al. 1986) and repellent (Rieth and Levin 1988) to honey bees. By applying permethrin to nest boxes, we can initially "push" bees away by repelling them. Then, as the chemical degrades to non-repellant concentrations over time, scout bees exposed during their cavity assessment walks will be less effective at performing recruitment dances because sub-lethal contact doses of permethrin reduce a bee's ability to orient and hamper their activity level (Taylor et al. 1987).

More attractive nest sites are then provided to "pull" bees toward these nest sites. Bee swarms

tend to make the “right” decision when choosing a new home (Seeley and Buhrman 2001). Therefore making swarm traps as attractive as possible will increase the probability of being chosen instead of nest boxes. Africanized honey bees show little preference for particular cavity volumes (Schmidt and Hurley 1995) or shapes (Schmidt and Thoenes 1992), but the size and position of the entrance hole may make a box more attractive. Small entrance openings are easier for bees to defend and also help maintain the microclimate within the nest cavity, and bottom positions help aid nest hygiene by easing debris removal (Seeley and Morse 1978). Additionally, small differences (entrance direction, height, or cavity volume) could enhance the attractiveness of trap boxes. However, pheromone lures are considered the main element for attracting bee swarms (Schmidt and Thoenes 1992, Schmidt 1994). Nasonov is a pheromone produced by worker bees that is used for orientation and scouts seeking nest sites (Schmidt 1999). Synthetic versions of this pheromone have been used to attract bee colonies to trap boxes (Schmidt and Thoenes 1992, Schmidt 1994).

In the North Atlantic Forest of southeastern Brazil, four nest boxes were installed in October 2012 to encourage nesting by endangered Vinaceous-breasted Amazons (*Amazona vinacea*). During November and December 2012, three of the boxes were colonized by AHB and the fourth colonized by wasps in February 2013. With all four nest boxes colonized by insects, there was no chance for Vinaceous-breasted Amazons to breed during the 2012–2013 breeding season. Additionally, since 2008, nest boxes in aviaries of captive-bred parrots at this site have been colonized by AHB (Wittkoff, pers. comm.). Thus, our objective was to examine the use of a push-pull IPM protocol that might deter AHB from colonizing nest boxes treated with permethrin (push), while simultaneously attracting them to pheromone-baited swarm traps (pull).

## METHODS

Our study was conducted in Jquitiba, São Paulo, Brazil, in the Atlantic Forest (23.9319°S, 47.0686°W) from March 2013 to March 2014. Average annual rainfall in this region is 1913 mm and average temperatures range from a low of 13°C to a high of 29°C and an average

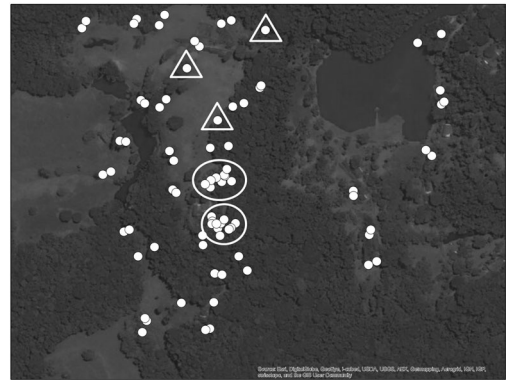


Fig. 1. Locations within the Lymington Farm in Jquitiba, São Paulo, Brazil, where 30 experimental push-pull pairs of parrot boxes and bee traps were placed (paired dots). The locations of the combined 15 captive breeding parrot boxes and associated five traps are circled and the three nest boxes for Vinaceous Amazons are within triangles.

of 18°C. The wet season is from September to March. The nesting season for Vinaceous Amazons in this region is October through February (Wittkoff, pers. comm.).

In February 2013, 30 pairs of parrot nest boxes and bee trap boxes were placed throughout the study site (Fig. 1). Parrot boxes were numbered from 1 to 30 and bee trap boxes from 31 to 60. An additional five bee trap boxes (61–65) were placed near the captive bred parrot aviaries to protect those 15 nest boxes. Both types of boxes were constructed from exterior CD grade 1.3-cm non-pressure-treated pine plywood. Dimensions of the parrot boxes were 20 cm wide by 40 cm long by 20 cm high with an 8-cm-diameter entry hole located at the top right corner on the front (2.5 cm from the top and side). Bee trap box dimensions were 30 cm × 30 cm × 30 cm with a 2.5-cm entrance hole located in the middle on the front face 2.5 cm from the bottom edge. Cavity volume of the parrot boxes was 16 l and bee trap boxes had a volume of 27 l. Outside surfaces of both types of boxes were sealed with two coats of a water repellent sealer.

Pairs of boxes (one parrot box with one bee trap box) were placed throughout the study site near trails for ease of location. Parrot boxes were placed ~9 m high in various tree species. They were secured to trees with two 61-cm plastic cable ties hooked together and threaded

through holes ~1 cm in diameter drilled into the upper back corner of each side of the box. Bee trap boxes were placed ~2 m above ground and no more than 10 m laterally from parrot boxes. Placement distance of the bee trap box from the parrot box depended on availability of trees with desirable diameters and accessibility. Boxes were secured to trees with 16-gauge steel galvanized wire threaded through two 4-mm holes drilled into the upper back corner of each side of the box. Bee trap boxes and parrot boxes were placed in different trees as a safety precaution to reduce the chance that a trap box occupied with bees would become agitated and attack nesting birds or biologists checking on the status of a nest. Trap boxes were placed lower to the ground for ease of observation and for safer removal despite evidence that honey bees prefer higher nest sites (Seeley and Morse 1978). Higher placement would likely make the boxes even more attractive to AHB, but we decided that safety for the person removing the swarm was more important and practical.

An attractant pheromone was placed inside each bee trap box to act as a lure (Schmidt 1994). The attractant used was a synthetically prepared nasonov pheromone, consisting of citral and geraniol in a 2:1 ratio. We placed 1 ml of this pheromone in a 1.5-ml Eppendorf tube with a pin hole punched in the center of the lid. The tube was then placed on the bottom of the boxes toward the back edge prior to the boxes being mounted on trees. The inside surfaces of odd numbered parrot boxes ( $N = 15$ ) and all captive-bred parrot nest boxes ( $N = 15$ ) in the aviary were sprayed liberally with an emulsifiable concentrate (EC) formulation of permethrin diluted to 0.25% (Permetrina 384 CE, Fersol Indústria e Comércio S.A., Mairinque, Sao Paulo, Brazil) until the liquid began to run off the surfaces before being mounted on a tree. Even-numbered parrot boxes ( $N = 15$ ) were not sprayed. Treated parrot boxes were re-sprayed approximately every 3 mo (February, May, August, and November 2013 and February 2014).

Boxes were checked every 14 d for insects. Once occupied, the entrance hole was plugged with a cloth, and the box removed from the tree. If occupied by AHB, boxes were transferred to a bee keeper located 10 km away. Trap boxes containing native Jatai bees were removed and relocated to the interior of the forest. Because wasps are considered pests (Wittkoff,

pers. comm.), they were killed by plugging the entrance hole and left in the sun for 2–3 d. After cleaning, trap boxes received a new lure and were mounted back on the tree. Lures remained effective at attracting swarms after 6 mo in preliminary experiments (Efstathion, unpubl. data) and, therefore, we replaced them after 6 mo.

## RESULTS

Out of 35 bee trap boxes placed throughout the study cite, 18 were occupied by an insect colony at least once over the year period. Fifteen of these trap boxes were part of the push-pull pair and three were set up near the captive parrot aviaries. Seven traps were occupied by insect colonies more than once, with two traps being occupied three different times. In total, 29 insect colonies occupied trap boxes over the study period, including nine by AHB, three by native Jatai bees (*Tetragonisca* sp.), and 17 by wasps. Two species of wasps were found in the trap boxes, with one box was occupied by *Polybia ignobilis* and 16 by *Agelaia pallipes*.

Out of the 30 experimental “push-pull” parrot nest boxes, only one was occupied by an insect colony (an untreated nest box occupied by AHB). Additionally, none of the four specially constructed Vinaceous-breasted Amazon boxes or any of the 15 nest boxes in parrot aviaries were occupied by insects during our study. Four of the experimental parrot boxes were occupied by birds during our study. Although none were used by Vinaceous-breasted Amazons, Southern House Wrens (*Troglodytes musculus*), Green-winged Saltators (*Saltator similis*), and Plain Parakeets (*Brotogeris tirica*) nested in the boxes and all nests were successful.

Of the 29 insect colonies, 26 moved into trap boxes during the wet season. During the nesting season, 14 insect colonies moved into trap boxes, including six boxes with AHB, six with wasps, and two with Jatai bees. Seven of the nine AHB colonies moved into boxes during the absconding season and two during the swarm season.

## DISCUSSION

Insects in our study established more colonies in trap boxes than in parrot nest boxes. Because only one untreated parrot box was occupied by bees, we were unable to determine if permethrin

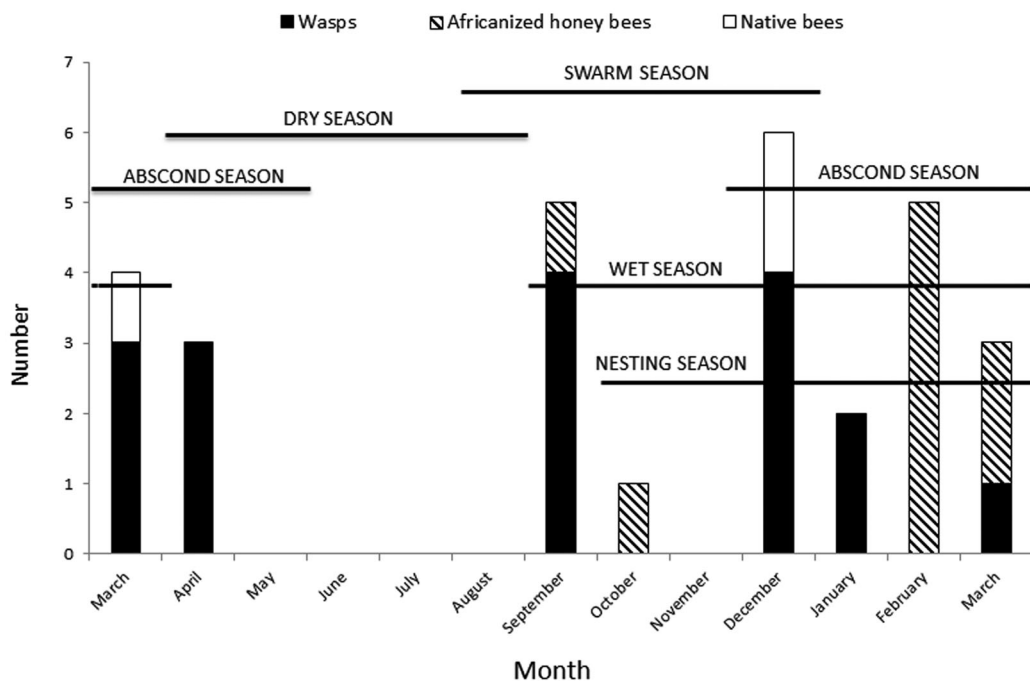


Fig. 2. Number of trap boxes that were colonized by different insects for each month of the study conducted in Juquitiba, São Paulo, Brazil, from March 2013 to March 2014. Wet and dry seasons along with the swarming and absconding seasons of honey bees in this region are indicated by the horizontal lines. The nesting season for Vinaceous Amazons is also shown.

was an effective repellent to bees and wasps. However, the trap boxes may have reduced the likelihood of insects moving into parrot boxes because, in the year prior to our study, all four Amazon boxes as well as aviary nest boxes were colonized by insects. None of these nest boxes were colonized during our study.

Africanized honey bees disperse in two ways, reproductive swarming and absconding (McNally and Schneider 1992, Hepburn 2006). Absconding occurs when an entire colony and queen leave, usually due to unfavorable nest cavity conditions or resource dearth (Winston et al. 1979). In Brazil, seasonal absconding occurs during the wet season when resources are scarce (Cosenza and Silva 1972, Winston et al. 1979, Pereira et al. 2010; Fig. 2). Most AHB in our study moved into trap boxes during the absconding season (7) rather than the swarm season (2). At our study site, the bee swarm season begins 2 mo before the parrot nesting season (Fig 2). Thus, to prevent swarming bees or wasps from colonizing nest boxes and to keep them available

for nesting birds, application of permethrin or other repellents should take place during the wet season (when absconding is most likely) and, at our study site, during the 2 mo preceding the parrot nesting season when bees are swarming.

Although we focused on preventing AHB from taking over parrot nest boxes, we discovered that swarm-founding wasps also found trap boxes suitable for colonization. In all but one of 17 cases, *A. pallipes*, a Neotropical swarm-founding wasp in the tribe Epiponini, was the wasp species inhabiting trap boxes. Swarm-founding wasps move to new nest sites in a coordinated synchronous movement (Jeanne 1991) facilitated by trail pheromones (Jeanne 1980, 1981). These swarms are made up of a large number of workers and a small number of queens (Jeanne 1991). *A. pallipes* are commonly found in honey bee trap boxes and prefer nests close to the ground (Noll, pers. comm.). This could partly explain the high colonization rate of trap boxes in our study. However, prior to our study, one of the nest boxes was infested with

wasps and a couple of colonies took up residence in chimneys and roofs of buildings on the study site (Wittkoff, pers. comm.). These wasps are becoming an increasing issue for nesting birds in our study area; prior to 2013, no nest boxes or buildings were occupied by wasps (Wittkoff, pers. comm.).

As a repellent, we used an EC formulation of permethrin obtained at a local hardware store. We would have preferred to use a microencapsulated (MC) form that persists longer, but were unable to obtain it in Brazil. PermaCap CS<sup>®</sup> (MC; BASF, Florham Park, NJ) was tested with nestling European Starlings (*Sturnus vulgaris*) and was found to prevent mite infestations for at least 60 d (Efstathion, unpubl. data). Pilot studies showed that bees exposed to EC-treated boxes after 21 d showed no ill effects from the insecticide (Efstathion, unpubl. data), suggesting that EC formulations may not be effective at repelling or intoxicating honey bees scouts after 3 weeks. Therefore, use of an MC permethrin would likely extend the duration of protection to nest boxes against AHB to 60 d or longer.

Bee swarms prefer cavities previously occupied by another bee colony, likely due to the scent of residual beeswax or propolis (Visscher et al. 1985). Scout bees release nasonov pheromone to mark potential sites of high quality for other scouts. This is why synthetic nasonov pheromones are often used to attract bee swarms. Scent attraction would explain the multiple hits of traps in our study including the one parrot box previously occupied by birds. Bird boxes colonized by AHB once are more likely to be re-invaded if they are not replaced or adequately cleaned. This was the case at our study site, i.e., prior to our study, one Hyacinth Macaw (*Anodorhynchus hyacinthinus*) nest box was invaded three times by AHB and two Golden Parakeet (*Guaruba guarouba*) nest boxes were each invaded twice by AHB (Wittkoff, pers. comm.).

Our results suggest that bee trap boxes may be effective at reducing the number of bird nest boxes colonized by AHB and wasps. However, long-term studies are needed before drawing conclusions about the effectiveness of trap boxes. Additional study is also needed to determine if permethrin is effective at preventing bee colonization of nest boxes. However, our push-pull protocol may prove useful for reducing the likelihood of AHB and other insects using nest

boxes meant for use by parrots and other cavity-nesting birds.

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