Video Article

A Push-pull Protocol to Reduce Colonization of Bird Nest Boxes by Honey Bees

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Abstract

Introduction of the invasive Africanized honey bee (AHB) into the Neotropics is a serious problem for many cavity nesting birds, specifically parrots. These bees select cavities that are suitable nest sites for birds, resulting in competition. The difficulty of removing bees and their defensive behavior makes a prevention protocol necessary. Here, we describe a push-pull integrated pest management protocol to deter bees from inhabiting bird boxes by applying a bird safe insecticide, permethrin, to repel bees from nest boxes, while simultaneously attracting them to pheromone-baited swarm traps. Shown here is an example experiment using Barn Owl nest boxes. This protocol successfully reduced colonization of Barn Owl nest boxes by Africanized honey bees. This protocol is flexible, allowing adjustments to accommodate a wide range of bird species and habitats. This protocol could benefit conservation efforts where AHB are located.

Video Link

The video component of this article can be found at https://www.jove.com/video/53950/

Introduction

Since the accidental introduction of more than two dozen African honey bee queens into Sao Paulo, Brazil in 1957[1], these insects have spread throughout South and Central America and up into the southern United States, usurping and replacing the less well adapted European honey bees (EHB) in the feral population. These hybrid Africanized honey bees (AHB) have traits that put them in conflict with both humans and wildlife. They exhibit highly defensive behavior, reproduce very fast, and are less selective about where they make a home[1,2].

Although nest boxes have long been used by ornithologists to enhance breeding by cavity-nesting birds[3,4], their use has been shown to be especially effective for enhancing the breeding of threatened and endangered parrots in the tropics[5,6]. Unfortunately, Africanized honey bees often choose these same nest boxes – sometimes even killing the occupants. Additionally, these AHB pose a significant threat to biologists when they are conducting nest studies. In areas of South America where AHB have been established, several parrot conservation projects have seen their artificial nest boxes occupied by AHB[7-10]. Competition for nest boxes with honey bees is widespread and is not confined to parrots. Many other bird species both in the New and Old World are affected[11-15]. Without efforts to mitigate the threat of these invasive AHB, recent successes in parrot conservation could be lost.

Current practices for managing competition from bees includes the use of repeated, high doses of permethrin[7], manually removing swarms, closing nest entrances immediately after breeding season[8], or the application of chemicals to the outsides of nest boxes[15]. The labor involved and the danger to biologists render these strategies less than optimal. Development of a prevention protocol is warranted.

Honey bee colonies multiply via a process known as swarming, when the old queen leaves the colony with up to three fourths of the worker bees[16]. Africanized honey bees' high reproductive rate allows them to swarm more frequently (four to eight times per year) than EHB (who swarm once or twice per year)[17]. Moreover, AHB may swarm throughout the year rather than during a particular season like EHB. Consequently, AHB can quickly occupy most of the suitable nesting sites in a given area.

Honey bee house hunting behavior can be utilized to influence their choice of nest site. A small fraction of bees in a swarm fly off to locate and inspect new potential nest sites. Several potential nest sites are scouted for simultaneously. These scout bees return to the swarm and perform dances to indicate the location of the site. The stronger the dancing is for a site, the faster the number of scout bees builds up at that site[18]. Therefore, bees dance stronger for better quality sites, compared to sites that are mediocre or poor. Once a quorum threshold is reached, the swarm takes off and flies to the new chosen home site[18].

Sub-lethal contact doses of the insecticide permethrin hamper a scout bee's ability to orient and reduce its activity level[19,20]. An application of permethrin inside nest boxes at first should be repellant to scout bees[21], and overtime as the chemical degrades, scout bees exposed to permethrin during their assessment of the box, should be rendered less able to perform strong recruitment dances, decreasing the amount of scout bees recruited to the site. A more suitable (ideal cavity volume, small entrance hole located at the bottom, and a pheromone attractant)

alternate nest site is provided in the form of a trap box for the bees. This excellent trap site should reach a quorum faster than the insecticide treated bird box leading the swarm to choose the trap box over the bird box.

As an example, we describe an experiment to reduce colonization of Barn Owl artificial nest boxes by AHB in South Florida sugarcane fields. Our objective was to test the effectiveness of using a push-pull method that will "push" bees away from owl nest boxes treated with an insecticide and "pull" them toward pheromone baited swarm traps.

Protocol

1. Construction of Swarm Trap Boxes

NOTE: There are two alternate protocols for swarm trap construction included as steps 1.1 and 1.2. There are several additional ways trap boxes could be constructed. The following two methods are presented here because we have tested them in experiments and they have been successful. For either protocol, use 1.3 cm to 2 cm thick non-pressure treated plywood.

1. Construction of Swarm Trap Protocol Option 1: Building a Trap Box
    1. Cut six pieces of plywood 30 cm x 30 cm.
    2. Attach the four sides of the box together with nails or screws.
    3. Attach the bottom piece by nails or screws.
    4. Attach the top piece with screws or staples.
        1. Do not use nails to secure the top. The top must be removable to get bees out and clean the box.
    5. Drill a 2.5 cm hole in the middle of one face of the trap box 2.5 cm from the bottom edge.
    6. Apply primer, sealant, or paint to the outside of the trap box to protect wood from weather.
        1. Do not apply any paint or sealant to the inside of the trap box. This could be repellant or toxic to the bees.

2. Construction of Swarm Trap Box Protocol Option 2: Use of a Standard Deep Hive Body
    1. Obtain a standard 10 frame deep hive body from a local bee supply store.
    2. Cut a top and bottom from plywood each measuring 50.8 cm long by 41.3 cm wide.
    3. Attach the bottom piece by nails or screws.
    4. Attach the top piece with screws or staples.
        1. Do not use nails to secure the top. The top must be removable to get bees out and clean the box.
    5. Drill a 2.5 cm hole in the middle on one side of the trap box 2.5 cm from the bottom edge.
    6. Apply primer, sealant, or paint to the outside of the trap box to protect wood from weather.
        1. Do not apply any paint or sealant to the inside of the trap box. This could be repellant or toxic to the bees.

2. Preparation of the Pheromone Lure

1. Prepare a mixture of citral and geraniol at a 2:1 ratio.
2. Place 1 ml of this mixture into a snap-top 1.5 ml polypropylene microcentrifugation tube.
3. Wrap lures in aluminum foil to protect them from light.
   Note: The cap of the centrifugation tube is punctured with a push pin when it is placed in the mounted swarm trap.
   Note: Lures can be stored in a dark place at RT or for longer storage in a -10 °C freezer.

3. Installation of Swarm Trap Boxes

NOTE: There are two alternate protocols for the installation of swarm trap boxes presented in steps 3.1 and 3.2.

1. Installation of Swarm Trap Box Protocol Option 1: Installation on a Post
    1. Place a 10 cm by 10 cm pressure treated wood fence post into the ground 10 m to 15 m from where the bird nest box is located.
    2. Construct a hanging board of 1.3 cm thick pressure treated wood measuring 20 cm by 20 cm.
    3. Align the hanging board in the middle of one side of the trap box, with 10 cm above the top of the box. Secure the board to the box with at least two nails or screws on either side of the board.
    4. Place the trap box on the post at a height (1.5 m to 3 m) that is safe and easy for removal when the trap box is colonized by bees. Screw the box to the post by attaching the hanging board. Place a screw approximately 5 cm below the top of the hanging board and screw in place.
        1. Do not use nails because they are difficult to remove.

2. Installation of Swarm Trap Box Protocol Option 2: Installation on a Tree
    1. Drill a 5 mm hole in the upper back corner of each side of the box.
    2. Thread a wire through the holes.
       Note: If using this protocol it will be easier to put the wire through the holes prior to securing the top of the box.
    3. Select a tree approximately 10 m to 15 m away from where the bird nest box is located.

1. Do not place trap boxes in the same trees as bird boxes because when someone checks on the status of the bird box, trap boxes colonized by bees may be disturbed causing bees to become defensive.

4. Secure the wire around the tree 1.5 m to 3 m off the ground.
   Note: To protect the tree, a cambium saver can be used around the wire.
   1. Make sure the box is level and secure so that it does not tip over. The box can be rested on a tree branch for added stability if available.

4. Addition of the Pheromone Lure

1. Take a previously prepared lure from section 2 and puncture the top of the tube with a push pin.
2. Place the lure inside the trap box on the bottom near the entrance after installation to ensure it does not fall out while installing the box.

5. Treatment of Bird Nest Box with Permethrin

1. Select a permethrin product that is labeled for use in poultry or caged birds only.
2. Follow label instructions for mixing and application
   CAUTION: follow any personal protection guidelines on label recommendations.
3. Spray the inside of the nest box entirely, until the point that the liquid runs off the sides of the box using a 3.7 L hand pump sprayer.
   Note: Nest boxes can be treated prior to or after installation.

6. Removal of a Trap Box Occupied by Honey Bees

1. Plug the entrance hole with a small towel.
   CAUTION: wear proper protective garments to prevent stings from honey bees.
   1. If swarm is to be killed, do so as soon as discovered. If swarm is to be utilized, allow bees to fully colonize trap boxes before attempting to remove them (usually about 2 weeks). If possible, remove occupied trap boxes at night (when all the bees are inside). The trap entrance can be plugged at night and then the box removed the following morning in daylight.
      Note: Use a red filtered light when working around bees at night.

2. Unscrew the box from the post or cut the wire holding the box to the tree.
3. Move the box to its new location (at least 4 km away) and pull the towel out from the entrance hole.

7. Destruction of Bee Colony in Trap Box

Note: In areas where it is illegal to keep Africanized honey bees, destruction of the colony may be warranted. If so, two protocols are presented here.

1. Solarizing the Colony
   1. Plug the entrance hole with a small towel.
      CAUTION: wear proper protective garments to prevent stings from honey bees.
   2. Unscrew the box from the post or cut the wire holding the box to the tree.
   3. Enclose and seal the trap box completely in a plastic bag and leave in full sun for several days
      CAUTION: wear proper protective garments to prevent stings from honey bees.
   4. Clean out the box by removing dead bees and comb.

2. Use of an Insecticide Spray
   1. Use an insecticide spray labeled for use to kill bees to spray inside the trap box.
      CAUTION: follow label instructions on insecticide and use appropriate personal protection equipment to prevent stings from honey bees.
   2. Plug the entrance and leave the box for 24 hr.
   3. Clean out the box by removing dead bees and comb.
      Note: To reuse the trap box clean the inside with a soapy water solution to remove residual insecticide. Rinse well and allow to dry completely before reinstalling.

3. Use of an insecticide foam
   1. Fill the entire cavity of the occupied trap with a detergent-based foam. (CAUTION: follow label instructions on insecticide and use appropriate personal protection equipment and wear proper protective garments to prevent stings from honey bees).
      Note: The foam can be applied with a commercial pest control foam generator or a compressed air fire extinguisher.
      Note: For using a fire extinguisher: Place 3.8 L of water into a 9.5 L fire extinguisher, add 190 ml of foaming agent (or dish washing detergent), seal the extinguisher, and pressurize it to 100-125 pounds/sq. inch (psi). Just prior to use, agitate vigorously, and then apply the foam until the trap is overflowing.
      Note: Foam kills the bees within 60 sec by entering the spiracles and blocking the trachea.
   2. Plug the entrance and leave the box for 24 hr.
   3. Clean out the box by removing dead bees and comb.

Note: To reuse the trap box clean the inside with a soapy water solution to remove residual insecticide. Rinse well and allow to dry completely before reinstalling.

Representative Results

A previous small pilot push-pull study conducted in Brazil showed swarm traps were effective and reduced bee occupation of parrot nest boxes[22]. To determine if the push-pull method can be effective at a larger scale to reduce colonization of bird nest boxes by AHB, we conducted a field study in sugarcane fields in southern Florida, USA. Sixty Barn Owl nest boxes were installed and each was paired with a swarm trap located 15 m away. Every swarm trap received a pheromone lure. Odd numbered owl boxes were treated with a microencapsulated permethrin spray insecticide every 60 days and even numbered nest boxes were left as untreated controls. Nest boxes and swarm traps were checked weekly for the presence or absence of 1) scout bees and 2) bee colonies.

Boxes and traps were monitored from March 2014 to July 2015. Of the 77 swarms caught, 75 were in swarm traps (**Figure 1**). Only two owl nest boxes were colonized during this experiment; both were untreated. We allowed bees to remain in trap boxes for a minimum of two weeks before we relocated them 4 km away to an apiary. To reduce the chances of bees absconding (leaving the trap box) we let them stay in the trap box for an additional two weeks before they were transferred to a new hive box. Honey comb and debris was scraped out of the trap box, it was remounted in the experimental site and a new lure was placed inside. Significantly more bees chose swarm traps over untreated owl boxes ($X^2_{(df=1)}$ = 29.2, P <0.0001) and treated owl boxes ($X^2_{(df=1)}$ = 36.1, P <0.0001) as determined by Pearson's chi-square test with Yates' continuity correction. Several trap boxes were colonized by bees more than once, 12 trap boxes were occupied twice by bees, five boxes were occupied three times and two boxes were occupied four times. For statistical analysis, occupancy per trap box was only counted once. Scents from previous bee colonies in a nest site can attract new colonies[23] so likely previously occupied trap boxes were more attractive than unoccupied boxes. Although several treated owl nest boxes were scouted by honey bees, no swarms moved into these boxes. At a nearby control site (containing 30 Barn owl boxes) honey bees colonized 63% of boxes. We found that the use of swarm traps greatly reduced the number of bee colonies occupying owl nest boxes. No treated nest boxes were colonized, suggesting that permethrin is effective at keeping AHB from colonizing nest boxes. However, swarm traps alone were effective, therefore application of permethrin could be used only at critical time periods (*i.e.,* bee swarm season and just prior to birds' breeding season) rather than applying throughout the year.

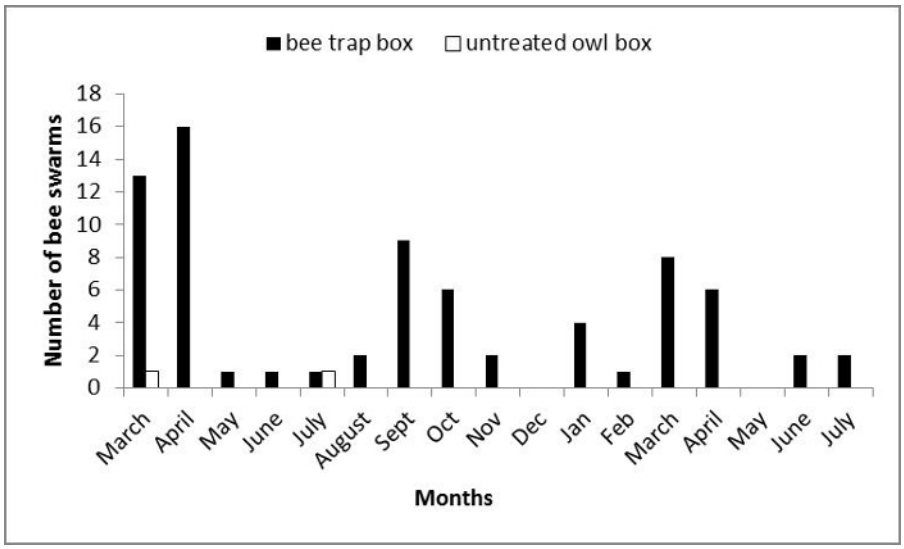

Figure 1. Number of boxes colonized by honey bees. Number of Africanized honey bee swarms that colonized either trap boxes or untreated barn owl nest boxes over a 17 month time period (March of 2014 to July of 2015) in sugarcane fields of southern Florida. Please click here to view a larger version of this figure.

Discussion

As a highly successful invasive insect[17], AHB constitute a major competitor for cavity-nesting birds throughout their range. The protocol presented here is designed to reduce the colonization rates of bird nest boxes by AHB. The main components of this design include 1) the use of a repellant and bird-safe insecticide, permethrin; 2) The provision of an alternate nesting site for bees; and 3) the use of an attractant pheromone inside trap boxes. Bees usually make the "right" decision when choosing a new nest site[18]. Making trap boxes more attractive than bird nest boxes will increase the likelihood of the former being chosen. Specifically, trap boxes should have a smaller entrance hole located closer to the floor than the ceiling[23]. Finally, higher boxes tend to be preferred by honey bees. However, because AHB are far less choosey about nest sites than EHB[2], the pheromone is the most important component of attractiveness[24].

This protocol can be adapted to fit a variety of situations. For areas with a very high density of honey bees, one can install two traps per nest box. For cliff nesting birds, traps can be hoisted into place parallel to a nest cavity or above and below. Trap boxes can be placed on pulley systems for easier removal in areas that are hard to reach. Africanized honey bees have a swarming season and an absconding season that varies with location. Swarming usually coincides with the peak blooming period of flowers. Absconding occurs when the entire colony of bees abandon a nest site, usually due to a dearth or deterioration of the nest site. It is important to predict when these seasons are. Because the

timing and frequency of swarming and absconding varies and is often not reported in the literature, one should contact local beekeepers or agricultural agencies for information. This will allow for more targeted application of permethrin and/or lures. Additionally, a management plan is essential for the success of this protocol. Occupied trap boxes need to be monitored regularly (at least every three weeks) and relocated or destroyed. The maintenance of available trap boxes for bees to colonize is important for the protocol to be effective. The overall goal is not to reduce the population of bees in an area, but rather to give them alternative nest sites.

Safety should always be a priority. For example, even though bees generally prefer higher nest sites, we tend to place trap boxes lower to the ground to facilitate their safe removal. Trap boxes should be placed in locations that will not place a biologist checking the status of a bird nest box in danger. Personal protective equipment (bee suit or jacket and bee gloves) should be worn by anyone removing a trap box with bees inside.

We used a microencapsulated permethrin spray formulation in this study because it is effective for 60 days. Other formulations are emulsified concentrates (EC) and dusts. Dust formulations are difficult to apply to tops and sides of boxes, where scout bees spend the majority of time performing assessment walks. Although EC formulations are common and easy to apply, they remain effective for only two weeks. The use of the natural product pyrethrum is not recommended as it is photosensitive and is only effective for a few days. Permethrin is the insecticide of choice because is it relatively non-toxic for birds and humans. It does not persist in the environment, is repellant to bees and lasts longer than the natural derived chemical. For these reasons permethrin spray formulation is recommended.

Attempts to keep AHB out of areas has failed throughout the Americas. Trapping near ports in Florida, delayed the arrival of AHB, but by 2007 they were established throughout the southern part of the state. Countries that are dominated by AHB, have simply given up and resorted to learning how to manage AHB. In areas where managing AHB is not illegal, bees that move into trap boxes can easily be removed and utilized for beekeeping. They can be relocated to an apiary for management or given to local beekeepers. Incorporating a local beekeeper to assist in the project by removing colonized boxes can benefit both parties. The beekeeper gets free bees and the biologists have one less task to concern themselves with.

This push-pull method greatly reduced colonization of Barn Owl nest boxes by AHB. The defensive nature of AHB and the difficulty of removing swarms from nest boxes makes a prevention protocol essential. This protocol can be used for any cavity nesting bird as well as cavity nesting mammals. It could prove to be an important conservation tool in the recovery of many endangered parrots throughout the Caribbean, South America, Africa and even Australia where the recently introduced Asian honey bee (*Apis cerana*) is quickly spreading.

Disclosures

The authors have nothing to disclose.

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