The use of formic acid to control the parasitism of the introduced fly Philornis downsi on land birds nest of Galapagos: an experimental study

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Abstract

The introduced fly *Philornis downsi* is nowadays considered one of the principal threats to the survival of the land birds of Galapagos. The larvae of the fly feed on the blood and tissue of young nestlings, causing them malformations, malnutrition and death. Different control methods are being studied but preference must be given to methods that are friendlier with the environment. Formic acid is a substance naturally occurring in the Galapagos ecosystems that has been used in other parts of the world for pest management and organic meat production due to its antifungal, antibacterial, and irritant properties. A pilot study using McPhail traps indicated that a 50% formic acid solution has a significant repellent effect on *P. downsi* and other muscid flies, while direct application of the solution on finch nests showed a reduction of parasites inside them and the improvement of the health status of the nestlings that received the treatment. Further studies are recommended to determine if formic acid has also insecticides properties; however, this alternative method might be already used as a control tool to investigate other threats affecting land birds of Galapagos and thus influence conservation efforts.

Key Words: land birds of Galapagos, introduced species, *Philornis downsi*, control methods, formic acid, and conservation efforts

Introduction

The parasitic fly *Philornis downsi* Dodge and Aitken (Diptera: Muscidae) was accidentally introduced to the Galapagos Islands in the 60s, and even though is still unknown how it was introduced, a possible hypothesis states that it could be brought within the smooth-billed ani (*Crotophaga ani*) to control a tick infestation in livestock (Fessl and Tebbich 2002). However it was not until 1997 when Fessl, Couri and Tebbich (2001) reported its presence on land bird nests. The hematophagous larvae of *P. downsi* emerge from the bottom of the bird nests and attach to the skin of nestlings where they feed on their blood and tissue causing nestling malnutrition (Fessl and Tebbich 2002, Fessl et al. 2006a, Fessl et al. 2006b), beak malformations (Fessl et al. 2006a, Galligan and Kleindorfer 2009), and finally death (Fessl et al. 2006a). Nowadays, *P. downsi* has been reported to affect all 14 recognized species of Darwin finches (Kleindorfer and Dudaniec 2016), is widespread in the archipelago (Wiedenfeld et al. 2007), and is considered one of the main threats to the survival of the land bird species of the Galapagos Islands (Causton et al. 2006, Kleindorfer et al. 2014).

Even when extended methods to control the parasitism of *P. downsi* do not yet exist, several mechanisms have been tested with positive results. Fessl et al. (2006b) showed a reduction in the number of larvae per nestling and per nest after applying a 1% permethrin solution inside finch nests, while Kleindorfer et al. (2014) used fly traps to capture egg-laying *P. downsi* females. Additional research has been also done using attractants as fly pheromones; however, they did not show any effect on *P. downsi* (Doherty 2012). The need for more research on control methods is urgent since the impact of *P. downsi* in certain bird populations is severe as in the case of the mangrove finch (*Camarhynchus heliobates*) in Isabela Island with around 100 individuals in the wild (Dvorak et al. 2004), and the medium tree finch (*Camarhynchus pauper*) in Floreana Island with a declining population with less than 2500 males (O'Connor et al. 2010, Peters 2016).

Formic acid is a substance with antibacterial, antifungal properties (Revis and Waller 2004, Griggs and Jacob 2005) that is produced by ants belonging to the Formicinae subfamiliy (Formicidae: Hymenoptera). Formic acid-producer ants spread this substance from their venom gland to either defend themselves and their partners from enemies, or to submit their preys before eating them (Blum 1992). Due to its properties, formic acid has been used in pest management to treat bee hives infested with mites (Underwood and Currie 2003, Underwood 2005, Amrine and Noel 2006), to reduce Salmonella infection in broiler chickens (Iba and Junior 1995, Al-Natour and Alshawabkeh 2005), and to fight invertebrates attacking mango orchards (Peng and Christian 2005).

In Galapagos exist 8 formic acid-producer ant species that are categorized as endemic or introduced, and occupy all the islands (Herrera 2015), thus formic acid is a substance that is naturally occurring in the insular ecosystems. Fess et al. (2006b) found that finch nests containing carpenter ants inside them have none or few larvae of P. downsi inside, indicating that formic acid might be working as an insecticide or repellent substance.

Even when formic acid has not been used to treat bird parasites, formic acid has been in direct contact with birds, mostly passerines, while they perform a behavior called "anting" where birds rub their feathers with ants (Chisholm 1959, Simmons 1985). The purpose of this behavior is still unknown but hypotheses explain that it might work as a way to reduce ectoparasites from bird feathers (Ehrlich et al. 1986), or a way that birds have to get rid of formic acid in ants to be able to eat them (Eisner and Aneshansley 2008).

The present study aims to test whether formic acid works might work as a control method to reduce the parasitism of P. downsi. The study consists of two parts where (1) the formic acid is tested in fly traps to determine repellent effect and optimal concentrations, and (2) the formic acid application direct into the land bird nests to assess their effect on *P. downsi* larvae and nestlings health. The particular characteristics of the Galapagos ecosystems prevents the broad application of conventional control and eradication methods, therefore the assessment of alternative tools to fight the negative effects of P. downsi on the emblematic land bird species of the archipelago is a priority.

Methods

Study Site
The study was conducted in San Cristobal Island (0° 54′ 9″ S, 89° 36′ 33″ W), Galapagos, Ecuador, from January to May 2015 and 2017, coinciding with the rainy season and the reproductive period of the land birds in the archipelago (Grant 1999). We were not able to collect data on 2016 due to a drought period on the islands that provoked a reduced number of *P. downsi* flies and active finch nests. The study sites were located in the lowlands within the protected area of the Galapagos National park, and close to urbanized areas. The lowlands in the Galapagos Islands are characterized by arid areas close to the coast with scarce vegetation and volcanic rocks of different size. The average monthly precipitation is between 60 to 100 mm. and the temperature oscillates between 21 to 29 Celsius degrees (Dirección del Parque Nacional Galápagos 2015).

Fly Traps Set Up and Placement
Commercial McPhail® fly traps (Fig. 1) were placed along paths with a 15 m separation between each other and close to finch active nests. Before placing the fly traps, 200 ml of papaya juice were placed at the base of the traps, which works as an attracting substance for flies. On the top of every trap, a cotton ball soaked with one treatment was placed on an aluminum foil closed compartment with 50 small holes. In 2015 we tested three treatments: (1) distilled water, (2) 12.5% formic acid solution, and (3) 50% formic acid solution. In 2017 we tested only two treatments (1) distilled water, and (2) 50% formic acid solution. Once closed, traps were hung on trees as high as it was possible. Traps were checked every third and fifth day, and data for the total number of trapped flies, number of *P. downsi* individuals, tree species, height of the trap, day of collection (Day 3 or Day 5), and shadow covering the trap were recorded.

Formic Acid Placement on Finch Nests
Active nests of the small ground finches (*Geospiza fuliginosa*) and medium ground finches (*Geospiza fortis*) were search and monitored every day from 6:00 am to 10:00 and 3:00 pm to 5:00 pm. Nests were carefully checked using an endoscope camera to observe the presence of eggs and/or nestlings. Active nests were marked using color tape placed on the tree where they were located and setting a GPS point. Nests with recently-born nestlings were sprayed 5 times on the outside with either (1) distilled water or (2) 50% formic acid solution. While treatments were left to dry for 10-15 minutes, nestlings were measured to record their weight (gr), tarsus size as a measure of height (cm), and signs of parasitism caused by P. downsi (presence/absence). Data from the number of nestlings, eggs, and species were also recorded. After measuring each nestling was marked with non-toxic paint in the toe nail. After four days, nestlings were measured again repeating the same protocol.

Once nestlings abandoned the nests, they were collected in individual Ziploc bags. The number of P. downsi larvae and pupae was determined from each nest. Larvae, pupae and flies were stored in plastic cups with 95% ethanol for preservation.

Data corresponding to the total number of flies and number of *P. downsi* individuals were log transformed to meet normality. GLMs and variance analyses were used to assess the trials made with fly traps. The total number of flies and the number of *P. downsi* individuals were used as response variables; while treatment, shadow, height, and collection day were used as independent variables. The MAM (Minimum Adequate Model) method was used to find the optimum model. Information from finch nests trials was analyzed with Kruskal-Wallis non-parametric test. Treatment was used as the only independent variable, and weight, tarsus size (height), nestlings fledged, number of parasites per nest, and number of parasites per nestling (parasitic load) were used as response variables. All the statistical analyses were done using the statistical software R 3.0.2.

Results

Even though in 2017 the reproductive period of land bird occurred normally, the number of *P. downsi* individuals remained low during the first months of the year. For this reason, the data from both fly trap and finch nests trials in 2015 and 2017 was analyzed separately.

McPhail Traps Trails

In 2015, the interaction between the day of collection and formic acid concentration showed significant results on the total number of flies collected ($\chi^2_{2,225} = 17.49$, $p < 0.001$). At day 3 after the application of treatments, as formic acid concentration increases the number of collected flies decreases; however, at day 5 after application the effect of formic acid is not significant (Fig. 1). The variable shadow also showed significant differences ($\chi^2_{2,225} = 9.94$, $p < 0.01$) on the total number of flies collected. As shadow covering the trap increases, the number of flies decreases. In the same line, the interaction between the factors day of collection and formic acid concentration showed significant results for the number of *P. downsi* flies ($\chi^2_{2,225} = 7.09$, $p < 0.05$). As formic acid concentration increases the number of *P. downsi* flies decreases at Day 3 of collection; however, at Day 5 this pattern is not significant (Fig. 2).

In 2017 data confirmed the results from 2015. There were significant differences between the formic acid treatments on the total number of flies ($\chi^2_{1,278} = 15.90$, $p < 0.0001$), finding less flies in the traps that receive a formic acid solution (Fig. 3). The factor day of collection also indicated significant differences ($\chi^2_{1,278} = 18.96$, $p < 0.0001$), being less flies trapped at Day 3 compared to Day 5. Finally, the variable shadow also showed significant differences ($\chi^2_{2,278} = 13.80$, $p < 0.001$), finding less flies as shadow increases. In the same way, the number of *P. downsi* individuals also varied significantly among the formic acid treatments ($\chi^2_{1,278} = 10.19$, $p < 0.001$), finding less *P. downsi* flies in traps treated with the formic acid solution (Fig. 4). The variable shadow also indicated significant differences ($\chi^2_{2,278} = 13.06$, $p < 0.001$), finding less *P. downsi* flies in the traps as shadow increases.

Formic Acid Application in Finch Nests

Between 2015 and 2017 a total of 30 finch nests were used in the study: 4 nest of medium ground finch, and 26 nests of small ground finch. Analyses were done using data from small-ground finch nests only. There were not significant differences in clutch size between years ($\chi^2_{1,30}$ =0.26, *p*=0.61), neither for the survival of nestlings (number of fledged nestlings) between years ($\chi^2_{1,30}$ =0.67, *p*=0.41). The number of parasites per nest ($\chi^2_{1,24}$ =6.34, *p*<0.05) and per nestling ($\chi^2_{1,24}$ =7.06, *p*<0.01) showed significant differences between 2015 and 2017.

In 2015, the analyses did not show a significant effect of formic acid on the nestlings morphologic measurements: weight gained ($\chi^2_{1,10}$ =1.05, *p*=0.30), height gained ($\chi^2_{1,10}$ =0.85, *p*=0.35). However, the data showed greater weight and height gained on the nestlings that received the formic acid treatment (Figure 5a and 5b). The formic acid solution did not show effect on the number of parasites per nest ($\chi^2_{1,10}$ =1.05, *p*=0.30), or the parasitic load of nestlings ($\chi^2_{1,10}$ =2.92, *p*=0.08) as well, nevertheless the data indicates lower numbers of parasites per nest and nestling in the nest treated with formic acid (Figure 5c and 5d). The number of fledged nestlings did not differ along the treatments ($\chi^2_{1,10}$ =1.37, *p*=0.24), but also showed a tendency of more fledged nestlings on the nests with the formic acid treatment.

In 2017, formic acid solution had a significant effect on the nestlings height gained ($\chi^2_{1,16}$ =8.06, *p*<0.01), but not in weight gained ($\chi^2_{1,16}$ =1.10, *p*=0.29) (Figure 5a and 5b). Formic acid also showed an effect on the number of parasites per nest ($\chi^2_{1,16}$ =5.10, *p*<0.05), and per nestling expressed ($\chi^2_{1,16}$ =5.10, *p*<0.05) (Figure 5c and 5d). Formic acid had not significant effect on nestling survival (nestlings fledged) ($\chi^2_{1,16}$ =2.27, *p*=0.13); however the data indicated that the nests that received the formic acid solution, had higher survival.

Discussion

Results from 2015 and 2017 analyses suggest a potential repellent effect of formic acid on flies from the Muscidae family and specifically on *Philornis downsi*. Since the function of the formic acid has been described as an irritant alarm substance produced by Formicinae ants when they are attacking their prey or for self-defense (Fujiwara-Tsujii et al. 2006), avoidance of this substance from other insects is an expected behavior in order to protect themselves and their offspring. For example, in nature larvae of several insects are common preys of ants (Way and Khoo 1992, Waseloh 1989). Even though there are no specific studies on the use of formic acid as a repellent itself, its insecticide efficacy has been proved for killing mite larvae inside bee hives (Amrine and Noel 2001, Elzen et al. 2004); and the direct use of formic acid-producer ants resulted to be a powerful tool for controlling different mango pests that include Hemiptera, Thysanoptera, Lepidoptera, Coleoptera and Diptera insects (Peng et al. 2007). The formic acid properties and the ant behavior could explain why Fessl et al. (2006b) found few or none *P. downsi* larvae in finch nests that contained carpenter ants.

A small concentration of formic acid (12.5%) worked at repelling Muscidae flies; however the effect of formic acid to repel *P. downsi* was only noticeable when its concentration was high (50%). This agrees with the concentration of formic acid used to treat bee hives without reporting negative effects on bees (Amrine and Noel 2006). Even when 60% is the concentration of formic acid in ant discharges in nature (Lofqvist 1976), this study considered a

50% formic acid concentration for treating the finch nests. No harmful signs where observed on the finches within the nests that were treated with formic acid.

Day of collection also showed significant results on the number of total flies and *P. downsi* individuals collected in the traps. At day 3 the repellent effect of formic acid is clear, while at day 5 their effectiveness seems to decrease (Fig. 1, 2, 3 and 4). Formic acid is a substance classified as medium evaporating with a value of 1.0 within the evaporation scale where values above 3.0 are considered fast evaporating substances that indicate high health or explosion risk (PubChem Compound Database 2017). Calderone and Nasr (1999) and Elzen et al. (2004) indicate that evaporation rates of formic acid are greater during the first week of application. After four weeks, soaked pads with formic acid already lost 68.2% of their weight in warm environments and thus their efficiency for killing parasites (Elzen et al. 2004). This might suggest that more than one application would be needed within the entire nestling period, which lasts around 15 days for Darwin's finches (Grant and Grant 1980).

Shadow covering the traps had an independent effect on the total number of flies and *P. downsi* individuals. As shadow above traps decreases, the number of muscid and *P. downsi* flies increases. According to Randolph (2004) free-living stages of parasites are more abundant in scarce canopy, hot and drier environments.

Impact of P. downsi on Darwin Finches

There was a significant variation in the parasitic load per nest and nestling between years 2015 and 2017. Even when studies indicate that parasitism of *P. downsi* in nests has increased over the past decade (Kleindorfer et al. 2014), the findings of this project showed less *P. downsi* parasites in 2017 compared to 2015. The reason behind this could be the drought that Galapagos Islands experienced in 2016 which delayed the early appearance of *P. downsi* flies in 2017 during the reproductive period of land birds. However it is important to mention that all nestlings in the study had signs of *P. downsi* infestation as enlarged narines, and/or blood spots in their bellies (Galligan and Kleindorfer 2009).

Formic acid seems to influence the weight and height by nestlings, allowing them to gain more mass and height in presence of reduced *P. downsi* larvae. Fess et al. (2006b) showed that a single application of the insecticide permethrin in finch nests produced more weight and height gained in nestlings. This is important because body mass is a key factor for fledging and post-fledging success (Tinbergen and Boerlijst 1990, and Magrath1991), thus when parasites as *P. downsi* influence this characteristic the later recruitment in land bird populations might be severely affected.

Formic acid is significantly reducing the number of *Philornis downsi* parasites inside finch nests and nestlings. The alarm and repelling properties of formic acid might be persuading female flies to oviposit in nests; nevertheless more studies are needed to determine if formic acid is also having an insecticide effect on larvae and pupae of *P. downsi.*

Control methods contribute to improve nestling survival. Studies made with parasite-controlled nests indicated a decrease in finch nestling mortality from 66% to 14% (Fessl et al. 2006b), and from 96% to 67% (Koop at al. 2011). Even when there was not a significant effect of formic acid on nestling survival, there were more nestlings fledged on nests that received the formic acid treatment.

Implications for Conservation

Even though it is not the only one, the parasite *Philornis downsi* have become one of the most serious threats to the welfare on Darwin's finches populations along with invasive species predation (O'Connor et al. 2010, Cimadom et al. 2014), and habitat modification (O'Connor et al. 2010). The reported presence of *P. downsi* in most of the islands of Galapagos (Weidenfeld et al. 2007), and the still scarce information about the ecological and reproductive behavior of this parasite, make the control and eradication of *Philornis downsi* a difficult and expensive task. For this reason the study of short-term solutions is imperative while other more complex and long-lasting solutions are developed.

The repellent function of formic acid on fly traps, plus its possible insecticide action on the finch nests suggests that formic acid could be an alternative tool for a short-term control of *Philornis downsi* in Galapagos. However, further studies must be performed in order to determine the specific effects of formic acid on *Philornis* larvae and eggs. Special attention must be also given to monitor nestlings in order to check possible negative effects of formic acid.

While some finch populations are already declining, alternative methods that intent to use substances already occurring on the natural environment are suitable options in Galapagos due to the fragile particularities of its ecosystems. Conservation strategies that are friendlier with nature might be applied more promptly compared to other methods, and could be also replied in the future in other places around the world.

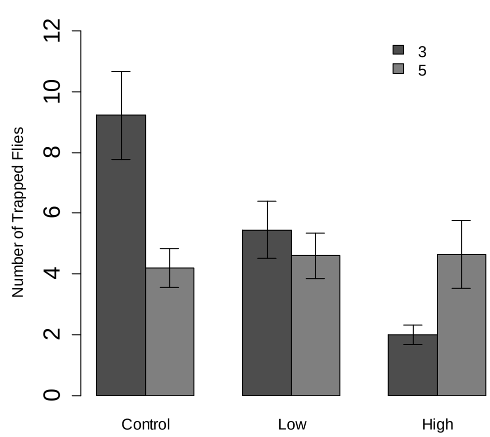

Figure 1. Number of Muscidae Flies trapped along different treatments of Formic Acid at Day 3 and Day 5.

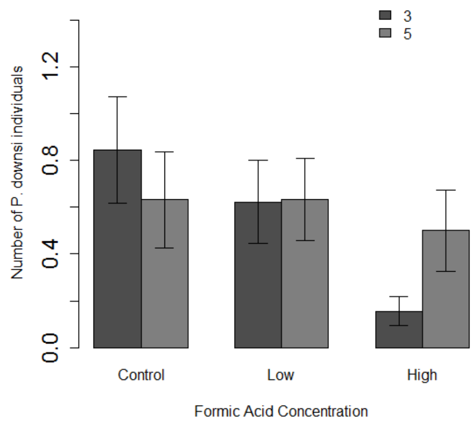

Figure 2. Number of *P. downsi* individuals trapped along different treatments of Formic Acid at Day 3 and Day 5.

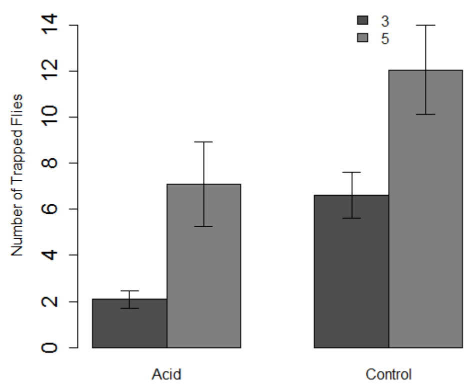

Figure 3. Number of Muscidae Flies trapped along different treatments of Formic Acid at Day 3 and Day 5.

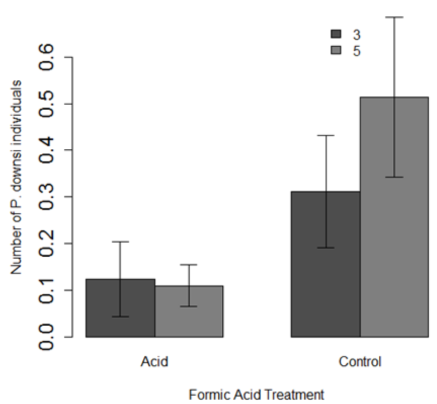

Figure 4. Number of *P. downsi* individuals trapped along different treatments of Formic Acid at Day 3 and Day 5.

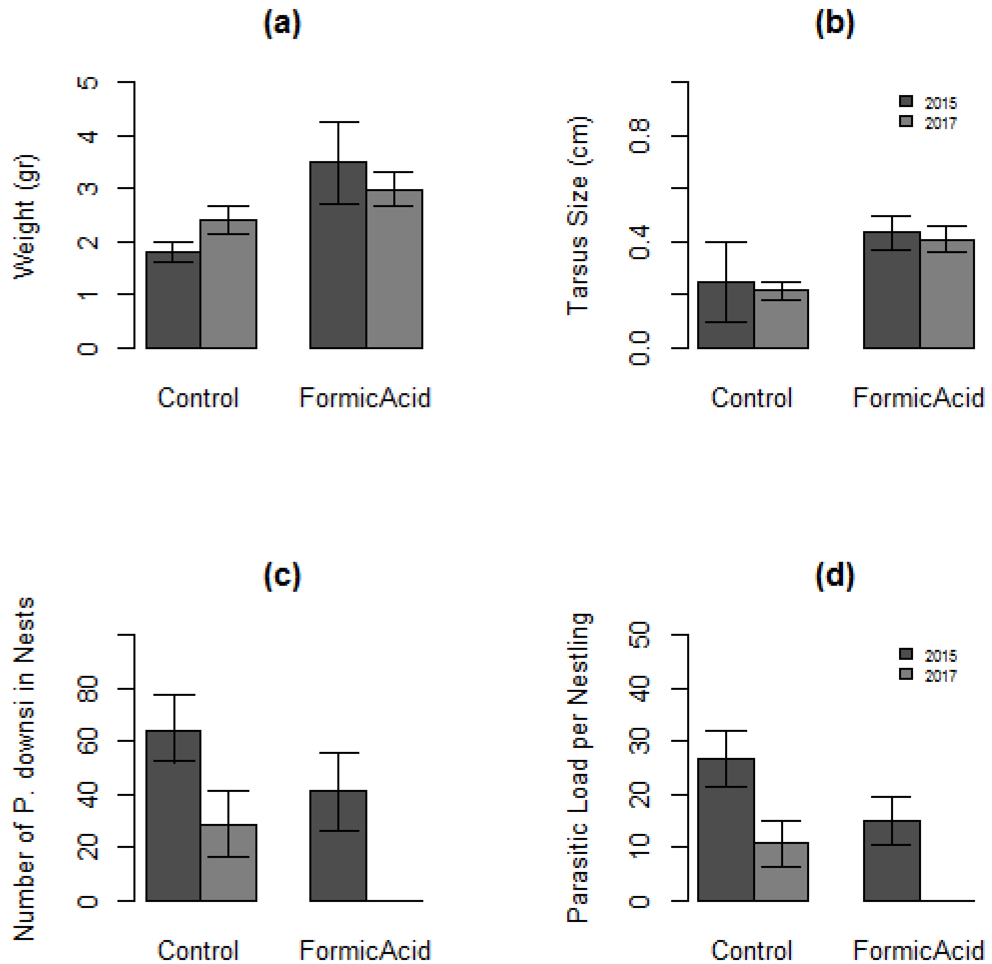

Figure 5. Effect of Formic Acid on (a) Nestlings Gained Weight, (b) Nestling Tarsus Size as a measure of Height, (c) Number of *P. downsi* parasites per nest, (d) Parasitic Load of *P. downsi* per nestling.

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Annexes

Checking McPhail fly traps and counting trapped flies

Finch Nest.

Left: Taking out the nestlings to apply formic acid solution. Right: Finch Nestlings.

Left: Nestling with enlarged narines caused by *Philornis downsi.* Right: Taking measurements of nestlings

Examining abandoned finch nest for larvae and pupae

Philornis downsi pupae