Biol Invasions
https://doi.org/10.1007/s10530-024-03278-6

ORIGINAL PAPER

# Cigarette tobacco reduces the survival of an invasive parasite that affects Darwin's finches

**Lorraine L. Pérez-Beauchamp · Jailene Contreras · Katia Goldberg · Gabriela Mena · Alexandria Soldo · Jaime A. Chaves · Sarah A. Knutie**

Received: 8 September 2023 / Accepted: 5 February 2024


**Abstract** Invasive parasites are a major threat to biodiversity worldwide, so understanding the factors that control them is necessary to improve the health of affected host species. In the Galápagos Islands, the invasive nest ectoparasite, the avian vampire fly (*Philornis downsi*), is causing up to 100% mortality in nestling Darwin's finches. However, urban finch nests have fewer flies than non-urban finch nests. One explanation is that finches incorporate cigarette butts into their nests, which can decrease nest parasite abundance for other bird species. For our study, we exposed larval flies to cigarette tobacco-treated (concentrated or diluted) or untreated cotton, then characterized pupation success, pupal deformities and success, and adult fly eclosure success and size. The influence of moisture on the effect of tobacco treatment on fly health was also determined. Flies reared in the tobacco treatments as larvae had lower pupation success, larger pupal volume, and a higher prevalence of pupal deformities compared to control flies, regardless of moisture treatment. Furthermore, we found that tobacco-treated flies had lower eclosure success. In fact, very few tobacco-treated flies survived to adulthood. We also collected finch nests and quantified the prevalence and mass of cigarette butts and abundance of flies in the nests. Although most urban finch nests contain cigarette butts (73%), the mass of cigarette butts was very low and did not correlate with fly abundance. Compared to past studies, finch nests require ten times as many cigarette butts to affect fly survival. Although tobacco can negatively affect vampire flies, finches likely do not incorporate enough cigarette butts to affect fly fitness.

**Keywords** Ectoparasitism · Anthropogenic debris · *Philornis downsi* · Tobacco · Cigarette butts · Galápagos Islands

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10530-024-03278-6.

L. L. Pérez-Beauchamp (✉) · J. Contreras · K. Goldberg · G. Mena · A. Soldo · S. A. Knutie
Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269, USA
e-mail: lorraine.perez@uconn.edu

J. A. Chaves
Department of Biology, San Francisco State University, San Francisco, CA 94132, USA

J. A. Chaves · S. A. Knutie
Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Quito, Ecuador

S. A. Knutie
Institute for Systems Genomics, University of Connecticut, Storrs, CT 06269, USA

## Introduction

Invasive parasites and pathogens are a threat to biodiversity worldwide (Daszak et al. 2000, 2001). For example, the amphibian chytrid fungus

Published online: 02 March 2024

(*Batrachochytrium dendrobatidis)* has, in part, caused the decline of amphibian populations worldwide and the extinction of 89 species (Greenberg & Palen 2019; Scheele et al. 2019). Small island species can be particularly vulnerable to invasive parasites (Wikelski et al. 2004). In the Hawaiian Islands, approximately 17 honeycreeper species have faced extinction due, in part, to the invasive avian malarial parasite (Atkinson et al. 2000; Atkinson & LaPointe 2009). These examples demonstrate the need for understanding what variables can control invasive parasites to improve the health of naive hosts.

The Galápagos Islands have been impacted by the introduction of several invasive parasites (Wikelski et al. 2004). The avian vampire fly (*Philornis downsi*; Diptera:Muscidae) is an obligate nest ectoparasite that was accidentally introduced to the archipelago in recent decades and is responsible for population declines in endemic landbirds, such as Darwin's finches (Kleindorfer & Dudaniec 2016; McNew & Clayton 2018). Adult flies are non-parasitic but females lay their eggs in the nests of birds (Fessl et al. 2001). Once the fly eggs hatch, larvae molt through three developmental stages during which they feed on the blood from nestlings and brooding mothers (Cimadom et al. 2016; Fessl et al. 2006). Pupation occurs during the third instar larval stage, and adult fly emergence occurs approximately two weeks from pupation. The parasite can cause up to 100% mortality of nestling Darwin's finches (Koop et al. 2013; O'Connor et al. 2014) and could lead to the extinctions of several endemic bird species within the next century (Fessl et al. 2010; Koop et al. 2016). Identifying what factors control avian vampire flies are critical to protect the populations of Galápagos landbirds.

Studies have shown that birds use natural and synthetic material to reduce parasite load (Clayton & Wolfe 1993; De Roode et al. 2013). In the Galápagos, Cimadom et al. (2016) observed that Darwin's finches treat their feathers with the leaves of the endemic Galápagos guava (*Psidium galapageium*), which, in the lab, inhibits the growth of vampire fly larvae and repels adults (Martina et al. 2022). However, there is no evidence that *P. galapageium* reduces parasite survival or that the leaves are incorporated in finch nesting material. Another study found that finches can effectively reduce vampire fly abundance to near zero by incorporating at least one gram of permethrin-treated cotton into their nests (Knutie et al. 2014). Although this method of "self-fumigation" is effective, it is labor-intensive because it requires the maintenance of cotton dispensaries. In Mexico, house finches (*Haemorhous mexicanus*) and house sparrows (*Passer domesticus*) incorporate littered cigarette butts into their nests, which reduce ectoparasitic mite abundance (Suárez-Rodríguez et al. 2013; Suárez-Rodríguez & Garcia 2017); this effect is likely because smoked cigarettes contain several compounds with arthropod-killing properties. Interestingly, urban Galápagos finches also incorporate cigarette butts into their nests (Theodosopoulos & Gotanda 2018; Harvey et al. 2021). Furthermore, a recent study found that urban nests contain fewer vampire flies than non-urban nests (Knutie et al. 2024), which could be explained by the addition of cigarette butts to the nests of urban birds. Although the use of cigarette butts in nests is a potentially promising method of vampire fly control, the causal effect of cigarette tobacco on fly survival has not been explored.

The goal of this study was to determine the effect of cigarette tobacco on the health and survival of larval avian vampire flies. Specifically, we conducted a $3 \times 2$ factorial experiment in which we exposed larval flies to cotton that was treated with one of three different concentrations of tobacco solution (water only, 5% tobacco solution, 3% tobacco solution) (Bahmani et al. 2012). We also manipulated the moisture of the cotton (moist or dry) since nests and associated nest material are often saturated after it rains. Once in treatment, we monitored individual larval survival, pupation success, and eclosure success. We also measured the volume of each pupa and the head width of flies as a measure of body size. Fly sex and any deformities of the pupae were noted. Because tobacco contains anti-parasitic nicotine and can affect invertebrate survival (Bahmani et al. 2012; Kasiemobi et al. 2014; Ahmed 2018; Schorderet Weber et al. 2019), we expected larvae from the tobacco treatments to have lower survival, pupation success, eclosure success, smaller pupal and adult body sizes, and higher rates of deformities compared to control larvae. Additionally, we predicted that the effects of moist tobacco treatments would have more negative effects on larvae health and survival compared to dry tobacco treatments because tobacco nicotine is highly soluble when wet and could have compounded toxicity (McBride et al. 1998). Overall, our study will

address whether the tobacco material could serve as a potential mechanism in which vampire flies are controlled by finches.

## Methods

Our study was conducted from February-May 2023 on the island of San Cristóbal (557 $km^2$) in the Galápagos archipelago. Our fieldwork was performed in three different sites that varied in their level of human activity and development, and each site was categorized as urban ($n=1$) or non-urban ($n=2$). Our urban site was within the town of Puerto Baquerizo Moreno (−0.9067715°, −89.6061678°), which is the capital of the Galápagos Islands. Puerto Baquerizo Moreno (hereinafter referred to as the "urban area") has a permanent resident population of 7199 individuals (INEC 2016) and visitation by tourists. Our non-urban sites included Jardín de las Opuntias (−0.9491651°, −89.5528454°) and Puerto Chino (−0.9259722°, −89.4298159°), which are both in the arid coastal zone. Jardín de las Opuntias and Puerto Chino are located 8.0 km and 24.7 km, respectively, from the urban site. Residents and tourists of the island visit these sites but there are no permanent populations that live in or near these areas.

We monitored the nests of small ground finches (*Geospiza fuliginosa*), medium ground finches (*Geospiza fortis*), and common cactus finches (*Geospiza scandens*) for egg-laying, hatching, and survival. In the urban area, nests are constructed with grass, leaves, native cotton, and anthropogenic material, including cigarette butts (Theodosopoulos & Gotanda 2018; Harvey et al. 2021). Nests in non-urban sites are constructed with grass, native cotton, and lichen, with little to no human material. When nestlings fledged or the nest failed, the nest was collected, dissected, and quantified total fly abundance. We then collected only live 3rd instar larvae found in the nest for the experiment because earlier instars would require blood meals to fully develop and pupate (Guimaraes & Papavero 1999). We also only used larvae from nests without cigarette butts For urban nests, we also weighed the total mass of nests and the mass of cigarette material (g). Nests from Jardín de las Opuntias ($n=28$) were collected for another study and nests from Puerto Chino ($n=1$) and Puerto Baquerizo ($n=5$) were collected opportunistically. Overall, 40 larvae from five urban nests and 270 larvae from 30 non-urban nests were collected for the study (Tables S1 and S2). Nests were also categorized as dry or not dry based on whether the nest material was moist to the touch. We included nest moisture because excessive exposure to water can affect the health and survival of larval insects (Hulthen & Clarke 2006; Li et al. 2019).

Once larvae were collected from nests, they were each placed individually in a ventilated 2 mL snap top tube with cotton from their respective treatment. For the tobacco treatment, we used Marlboro Red cigarettes; the tobacco from one cigarette weighed an average of 0.92 g and contained 20.30 mg of nicotine per gram of tobacco (Lawler et al. 2017). Marlboro Red cigarettes were chosen due to their availability at local stores (LLP, pers. obs.). We first removed the filter, then collected the tobacco within the cigarette paper. One gram of tobacco was weighed and mixed with 20 mL of boiling drinking water (100 °C) to facilitate the breakdown of tobacco (Forster et al. 2015). Because individual smoking behavior can influence the chemical composition of cigarette smoke (Soleimani et al. 2022), and thus cigarette butts, we chose to standardize tobacco exposure by extracting chemicals from the tobacco by mixing it with boiling water. The water and tobacco mixture was held for five minutes, which is the average duration of cigarette smoking (NIDA 2021). The solution was then poured through a strainer to remove the solid tobacco pieces and the liquid was used as our concentrated tobacco solution (4.76% tobacco; see Supplemental Methods for calculations). To create the diluted tobacco solution, we mixed 10 mL of boiling water with 10 mL of the concentrated tobacco solution for a final concentration of 3.33% tobacco. Boiled water without tobacco was used for the control treatment. Square cotton pads (57.55 mm × 50.85 mm) were saturated with 5 mL of their respective treatments (Fig. S1A). Once dry, cotton squares were cut into rectangular pieces that were approximately 27 mm × 12 mm. Within seven days of preparation (mean ± SE = 4.81 ± 0.48 days), the cotton was used in the experiment by placing one piece in a 2 mL tube with one larva (Fig. S1B). For the moisture treatment, cotton was sprayed with approximately one pump of drinking water (0.12 mL) using a travel-sized spray bottle at the start of the experiment. All experimental treatments received the same water.

Suárez-Rodríguez et al. (2013) found that house finches (*Haemorhous mexicanus*) incorporate an average of 2.45 g (or approximately 12 cigarette butts) into their nests, or a total of 84 mg of nicotine (7.00 mg of nicotine per butt; Green et al. 2014). Cigarette butts comprised 7% of total nest material and therefore the nest contained approximately 6 mg of nicotine per gram of nest material. Since our experiment directly exposed larvae to treated material, we reduced the nicotine exposure by a magnitude of 10 (0.60 mg of nicotine per cotton pad). We also included a treatment for which we diluted the concentrated tobacco solution to determine whether a lower concentration had lethal or sublethal effects as well. Larvae were monitored every other day for survival until pupation. Larval mortality was noted when they stopped moving, turned gray in color, and shriveled in size. The length and width of surviving pupae were measured to calculate volume ($mm^3$), which was calculated using the formula for a cylinder (Knutie et al. 2016). Pupae were also classified as "deformed" or "not deformed" based on the condition of the puparium (Fig. S2A-D). Non-deformed pupae have smooth abdominal segments and rounded posterior spiracles (Skidmore 1985; Guimaraes & Papavero 1999; Fessl et al. 2001) (Fig. S2A-B). In contrast, deformed pupae have rugged abdominal segments and shriveled posterior spiracles (Fig. 2C–D). Pupae were exposed to their respective cotton treatment for a total of 5–7 days post-pupation and then the cotton was removed to facilitate adult fly eclosure. After one week post-pupation, pupae were checked daily for eclosure; fly eclosure occurs 10–12 days from pupation (Kleindorfer et al. 2014). If the fly eclosed, their head width (mm) was measured using dial calipers and sex was determined. Eclosure failure was noted if the fly did not emerge within 15 days.

Analyses were conducted in R (2021, version 4.0.4) and RStudio (2021, version 1.4.1103). All figures were created in Prism (2023, version 9.5.0). We used generalized linear mixed effects models (GLMMs) with binomial distributions to determine the main effect of, and interaction between, tobacco and moisture treatment on binomial response variables, such as pupation success, pupal deformities, and eclosure success. For variables with continuous data (pupal and adult head size) we used a Shapiro–Wilk test to test for normality. Adult fly head width and pupal length and width were normally distributed ($P > 0.05$ for all measurements), but pupal volume was not normally distributed ($W = 0.96$, $P < 0.0001$). Since adult size and pupal length and width were normally distributed, we used GLMMs with Gaussian distributions to determine the main effect of, and interaction between, tobacco and moisture treatment on these response variables. Since pupal volume was not normally distributed, we used a GLMM with Gamma distribution for this analysis. We also used GLMMs with Gamma distribution and binomial distribution to determine whether pupal size and fly eclosure success, respectively, differed between deformed and non-deformed pupae. Lastly, we used a GLM to determine the effect of cigarette butt mass on fly abundance in urban nests.

Covariates were included in the analyses (location, Julian day, nest moisture, bird species, fly sex [for analyses on adults], and number of days the cotton was treated) and were removed if they did not contribute significantly to the model. All covariates were removed for models that included pupal volume and deformities, fly eclosure success, and adult fly head width. For the effect of deformities on pupal volume, Julian day was included as a covariate. When we found a significant effect of tobacco treatment on a response variable, we used a pairwise t-test with a Bonferroni correction to determine which treatments were significantly different from each other. When we found a significant interaction between tobacco treatment and moisture, the function emmeans was used for the post-hoc tests (Garofalo et al. 2022) using the *emmeans* package (Lenth 2021). The GLM and GLMMs were conducted using the glm (GLM), lmer (GLMM), and glmer (GLMM) function with the *lme4* package (Bates et al. 2015). Probability values ($X^2$) were calculated using log-likelihood ratio tests using the Anova function in the *car* package (Fox & Weisberg 2018).

## Results

In the urban area, 11/15 (73.33%) nests contained cigarette butt material, which ranged from 0.01 to 0.25 g ($0.09 \pm 0.02$ g; 0.03–0.70% of total nest mass). In these nests, parasite abundance ranged from 0 to 40 ($7.87 \pm 3.51$ parasites), but did not correlate with the mass of cigarette material ($\chi^2 = 1.86$, $df = 1$, $P = 0.17$).

Tobacco treatment affected pupation success ($\chi^2 = 10.02$, $df = 2$, $P = 0.007$). Larvae from the control treatment had higher pupation success compared to larvae from the diluted ($P = 0.02$) and concentrated treatments ($P = 0.001$) (Fig. 1A, Table 1). The main effect of moisture and the interaction between the moisture and tobacco treatment did not significantly affect pupation success (moisture: $\chi^2 = 1.54$, $df = 1$, $P = 0.22$, interaction: $\chi^2 = 1.53$, $df = 2$, $P = 0.47$).

Tobacco treatment affected pupal volume ($\chi^2 = 11.45$, $df = 2$, $P = 0.003$). Control pupae were smaller than pupae from the diluted ($P < 0.0001$) and concentrated tobacco treatments ($P = 0.0001$) (Fig. 1B, Table 1). Differences in pupal volume across treatment were driven by pupal width ($\chi^2 = 36.38$, $df = 2$, $P < 0.0001$) and not length ($\chi^2 = 1.08$, $df = 2$, $P = 0.58$). The main effect of moisture and the interaction between the moisture and tobacco treatment

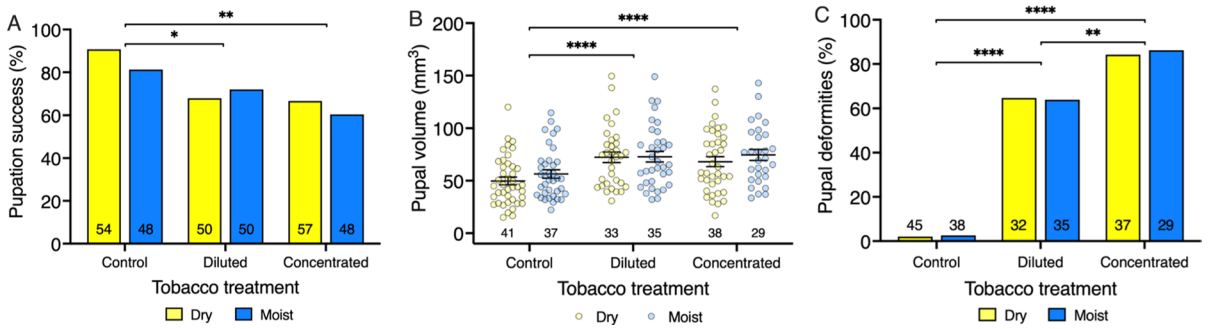


**Fig. 1** Effect of tobacco and moisture treatment on **A** % pupation success, **B** pupal volume, and **C** % pupal deformities. The yellow and blue values indicate the dry and moist treatments, respectively, for each tobacco treatment. Sample sizes for each treatment are above the x-axes. $*P < 0.05$, $**P < 0.01$, $****P < 0.0001$

**Table 1** Results and sample sizes for the effect of tobacco and moisture treatments on larval health and survival

| Fly trait | Control | | Diluted | | Concentrated | |
|---|---|---|---|---|---|---|
| | Dry | Moist | Dry | Moist | Dry | Moist |
| Total # of larvae/# nests | 54 larvae/20 nests | 48 larvae/25 nests | 50 larvae/22 nests | 50 larvae/22 nests | 57 larvae/24 nests | 48 larvae/25 nests |
| Pupation success (# pupated/total # of larvae; [%]) | 49/54 (90.74%) | 39/48 (81.25%) | 34/50 (68.00%) | 36/50 (72.00%) | 38/57 (66.67%) | 29/48 (60.41%) |
| Mean ± SE pupal volume | 49.71 ± 3.63 $mm^3$ (41 pupae) | 56.40 ± 3.93 $mm^3$ (37 pupae) | 72.26 ± 5.07 $mm^3$ (33 pupae) | 72.82 ± 4.85 $mm^3$ (35 pupae) | 68.11 ± 4.90 $mm^3$ (38 pupae) | 74.57 ± 5.22 $mm^3$ (29 pupae) |
| Pupal deformity prevalence (# deformed/total # of pupae; %) | 1/45 (2.22%) | 1/38 (2.63%) | 22/32 (68.75%) | 23/35 (65.71%) | 32/37 (86.49%) | 25/29 (86.21%) |
| Eclosure success(# eclosed/total # of pupae; %) | 29/40 (72.50%) | 30/36 (83.33%) | 9/31 (26.47%) | 14/35 (38.89%) | 5/38 (13.16%) | 1/29 (3.44%) |
| Mean (± SE) adult head width (# of flies) | 2.81 ± 0.07 mm (26) | 2.72 ± 0.09 mm (26) | 2.76 ± 0.09 mm (9) | 2.86 ± 0.10 mm (12) | 2.86 ± 0.09 mm (5) | 3.20 ± 0.00 mm (1) |

did not significantly affect pupal volume (moisture: $\chi^2=0.76$, $df=1$, $P=0.38$, interaction: $\chi^2=0.89$, $df=2$, $P=0.64$).

Tobacco treatment affected the prevalence of pupal deformities ($\chi^2=236.54$, $df=2$, $P<0.0001$). Few pupae from the control treatment were deformed (Fig. 1C, Table 1). Pupae from both the diluted and concentrated treatments had a higher prevalence of deformities, but pupae from the concentrated treatment had more deformed pupae than the diluted treatment ($P<0.05$ for all pairwise comparisons). The main effect of moisture and the interaction between the moisture and tobacco treatment did not significantly affect prevalence of pupal deformities (moisture: $\chi^2=0.00$, $df=1$, $P=0.98$, interaction: $\chi^2=0.13$, $df=2$, $P=0.94$). Deformed pupae were larger in volume than non-deformed pupae ($\chi^2=13.19$, $df=1$, $P<0.001$), which was because deformed pupae had a longer width ($\chi^2=30.74$, $df=1$, $P<0.0001$) but not length ($\chi^2=3.31$, $df=1$, $P=0.07$).

Tobacco treatment affected fly eclosure success ($\chi^2=43.68$, $df=2$, $P<0.0001$). Pupae from the control treatment had higher eclosure success compared to pupae from the diluted ($P=0.02$) and concentrated treatment ($P=0.001$) (Fig. 2A, Table 1). Pupae from the control treatment had the highest rate of eclosure (67%), followed by pupae from the diluted treatment (33%); only seven flies eclosed from the concentrated treatment (10%; $P<0.0001$ for all pairwise comparisons). The main effect of moisture and the interaction between the moisture and tobacco treatment did not significantly affect eclosure success (moisture: $\chi^2=0.52$, $df=1$, $P=0.47$, interaction: $\chi^2=2.57$, $df=2$, $P=0.28$). Deformed pupae were less likely to eclose than non-deformed pupae ($\chi^2=255.00$, $df=1$, $P<0.0001$). More female flies ($n=36$) than male flies ($n=22$) were found in the control treatment, but more male flies ($n=14$) than female flies ($n=9$) were found in the diluted treatment (Table. S3).

Because so few flies eclosed from the concentrated tobacco treatment, we excluded these data from our analysis on adult head width. Overall, flies reared in the diluted tobacco treatment were larger than flies reared in the control treatment ($\chi^2=5.39$, $df=1$, $P=0.02$) (Fig. 2B, Table 1). Head width differed within the interaction between tobacco and moisture treatments ($\chi^2=5.43$, $df=1$, $P=0.02$). Moisture treatment had opposing effects on head width across tobacco treatments. Within the control treatment, head width was larger for flies in the dry treatment compared to the wet treatment. In contrast, within the diluted tobacco treatment, head width was larger for flies in the wet treatment compared to the dry treatment. Moisture alone did not affect fly head width ($\chi^2=3.64$, $df=1$, $P=0.06$).

## Discussion

Our study determined the experimental effects of tobacco and moisture on avian vampire fly health and survival. We found that flies reared in the tobacco treatment had lower pupation success, larger pupal volume, and a higher rate of pupal deformities compared to control flies. Furthermore, larvae reared in the concentrated tobacco treatment were more likely to be deformed as pupae compared to larvae reared in the diluted treatment. Deformed pupae were wider, which likely led to the larger pupal volume in the tobacco treatments. Furthermore, flies reared in the tobacco treatment had lower eclosure success compared to control flies. In fact, very few flies from the

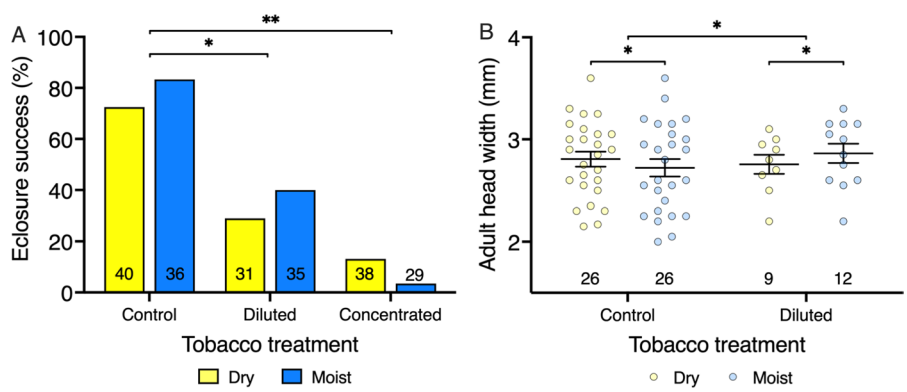


**Fig. 2** Effect of tobacco treatment on **A** % eclosure success and **B** head width of adult flies. The yellow and blue values indicate the dry and moist treatments, respectively, for each tobacco treatment. Samples sizes for each treatment are indicated above the x-axes. *$P<0.05$, **$P<0.01$, ****$P<0.0001$

concentrated treatment eclosed. These effects of treatment were likely because tobacco was directly toxic to larvae and disrupted metamorphosis. Interestingly, we found that moisture influenced the lasting effect of tobacco on adult head width. Flies reared in the dry control treatment were larger than control flies reared in the moist treatment and vice versa for the tobacco flies. Most urban finch nests contained cigarette butts but in very small amounts and did not correlate with fly abundance. Thus, although tobacco exposure affects fly survival, finches do not incorporate enough cigarette butts in their nests to reduce the number of flies.

Tobacco exposure decreased pupation success, pupal condition, and eclosure success. Other studies have found that tobacco affects the survival of larvae in house flies (*Musca domestica*) and brown dog ticks (*Rhipicephalus sanguineus*) (Schorderet Weber et al. 2019), which is likely because nicotine, the main compound in tobacco, has insecticidal properties. Consequently, nicotine has been used as a commercial insecticide against crop pests for several centuries. Specifically, nicotine is directly toxic to insects by binding to nicotinic acetylcholine receptors (nAChRs) and depleting acetylcholine (ACh) uptake (Millar & Denholm 2007). In honey bees (*Apis* spp.), ACh depletion during the larval stage is associated with slower development, higher larval mortalities (possibly via paralysis), and higher prevalence of deformities in adult bees (Grünewald & Siefert 2019). These studies suggest that our tobacco treatment had negative effects on larvae because of the effects of nicotine on nAChRs and ACh.

Because of the negative effects of cigarette tobacco on larvae, cigarette butts could also act as a repellent for female flies looking for a nest to lay their eggs. To our knowledge, studies have only examined the lethal effects, but not repelling effects, of tobacco on Muscid flies (Showler et al. 2017). However, other studies have found that tobacco can repel adult Calliphorid flies and ticks (*Hyalomma marginatum rufipes*) (Magano 2011; Bootyothee et al. 2022). Future studies are needed to evaluate whether tobacco and/or cigarette butts could act as a vampire fly deterrent when incorporated into finch nests.

The minimum amount of tobacco-derivatives required in the nest to reduce fly abundance remains unknown. Although nearly half of Darwin's finch nests in the urban site contain evidence of cigarette butts (Harvey et al. 2021), most nests only contain a small amount of cigarette material (0.01–0.25 g) with no significant correlation with fly abundance. Suárez-Rodríguez et al. (2013) also examined the relationship between cigarette butt and parasitic mite abundance in the nests of wild birds in Mexico. House finches and house sparrows (*Passer domesticus*) incorporate an average of 2.45 g (7.00% of total nest mass) and 3.06 g (9.34%) of cigarette butts into their nests, respectively, which is 10× higher than the maximum amount of cigarette butts found in Darwin's finch nests. Furthermore, approximately 3.5 g of cigarette butt cellulose was necessary to observe a decline in parasite abundance. Unfortunately, it appears that finches do not incorporate enough tobacco material into the nest to effectively reduce parasite abundance, which is either due to a lack of preference for the material or lack of availability in the environment. Future studies could explore this idea with a behavioral choice experiment and also determine the minimum concentration of tobacco required to reduce fly abundance in the nests.

Moisture did not influence the effect of tobacco treatment on larval health and survival, likely because the larval cuticle is permeable and moisture is not required to increase the absorption of the treatment. However, the moisture treatment had lasting and opposing effects on head width across tobacco treatments. For flies reared in the control dry treatment, head width was larger than flies reared in the moist treatment, and vice versa for flies reared in the tobacco treatment. In contrast, a previous study found that larval Muscid flies reared under moist conditions were larger as adults compared to drier conditions (Fatchurochim et al. 1989). Unfortunately, the mechanisms by which moisture had lasting effects on larval vampire flies is unclear. But, because fly size is correlated with lifetime fitness (Schmidt & Blume 1973; Moon 1980), this information could be important in understanding variation in fly traits across populations.

One outcome of this study is that tobacco could be a more natural solution to control flies with "self-fumigation", compared to permethrin (Knutie et al. 2014). The effects of tobacco on finch health is still needed to understand the potential costs of using this solution. For example, nicotine exposure can bind to nAChRs in juvenile songbirds and alter their song development (Asogwa et al. 2022). Suárez-Rodríguez

et al. (2014) found that nestling house finches exposed to more cigarette butts gained more mass and had higher fledging success, but had more erythrocyte damage, than nestlings that were exposed to fewer cigarette butts. Additionally, nestlings exposed to more cigarette butts had a stronger inflammatory response than nestlings that were exposed to fewer cigarette butts (Suárez-Rodríguez & Macías Garcia 2014). Other studies suggest that short-term exposure to tobacco in laboratory rabbits can have immune-enhancing effects (Lehrer et al. 1978). Because tobacco exposure can have varying effects on nestling birds, the influence of tobacco exposure on finch health and survival are needed before further exploring the potential for tobacco to be used for fly control.

**Acknowledgements** We thank the Galápagos Science Center and the Galápagos National Park for support. We also thank Sila Inanoglu for feedback on the manuscript. The work was supported by a Research Experience for Undergraduate Fellowship through a National Science Foundation Grant (DEB-1949858) to SAK. All bird handling and work was conducted according to approved University of Connecticut Institutional Animal Care and Use Committee protocols (No. A20-050). Our work was done under GNP permit PC 35-22.

**Author contributions** Conceptualization: LLP and SAK; Methodology: LLP and SAK; Analyses: LLP and SAK; Investigation: LLP, JC, KG, GM, AS, SAK; Visualization: LLP and SAK; Funding acquisition: SAK; Project administration: LLP, SAK, and JC; Supervision: SAK; Writing—original draft: LLP and SAK; Writing—review & editing: All authors.

**Data availability** Supporting information has been made available online. Data are available at FigShare (doi: available upon acceptance).

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

Ahmed KM (2018) The effect of aqueous plant extracts of tobacco on Third larvae of house fly (*Musca domestica L.*, (Diptera: Muscide). J Entomol Zool Stud 6:403–408

Asogwa NC, Toji N, He Z, Shao C, Shibata Y, Tatsumoto S, Ishikawa H, Go Y, Wada K (2022) Nicotinic acetylcholine receptors in a songbird brain. J Comp Neurol 530:1966–1991

Atkinson CT, LaPointe DA (2009) Introduced avian diseases, climate change, and the future of Hawaiian honeycreepers. J Avian Med Surg 23:53–63

Atkinson CT, Dusek RJ, Woods KL, Iko WM (2000) Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. J Wildl Dis 36:197–201

Bahmani M, Farkhondeh T, Sadighara P (2012) The anti-parasitic effects of *Nicotina tabacum* on leeches. Comp Clin Pathol 21:357–359

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw. https://doi.org/10.48550/arXiv.1406.5823

Bootyothee K, Aunpromma S, Artchayasawat A, Pitaksakulrat O, Boonjaraspinyo S, Boonmars T (2022) Screening of natural product extracts for fly repellent and larvicide. Vet Integr Sci 20:25–40. https://doi.org/10.12982/VIS.2022.003

Cimadom A, Causton C, Damiens D, Fessl B, Hood-Nowotny R, Lincango P, Mieles AE, Nemeth E, Semler EM, Teale SA, Tebbich S (2016) Darwin's finches treat their feathers with a natural repellent. Sci Rep 6:34559

Clayton DH, Wolfe ND (1993) The adaptive significance of self-medication. Trends Ecol Evol 8:60–63

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife-threats to biodiversity and human health. Science 287:443–449

Daszak P, Cunningham AA, Hyatt AD (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop 78:103–116

De Roode JC, Lefèvre T, Hunter MD (2013) Self-medication in animals. Science 340:150–151

Fatchurochim S, Geden CJ, Axtell RC (1989) Filth fly (Diptera) oviposition and larval development in poultry manure of various moisture level. J Entomol Sci 24:224–231

Fessl B, Couri MS, Tebbich S (2001) *Philornis downsi* Dodge & Aitken, new to the Galapagos Islands (Diptera, Muscidae). Stud Dipterol 8:317–322

Fessl B, Sinclair BJ, Kleindorfer S (2006) The life-cycle of *Philornis downsi* (Diptera: Muscidae) parasitizing Darwin's finches and its impacts on nestling survival. Parasitology 133:739

Fessl B, Young GH, Young RP, Rodríguez-Matamoros J, Dvorak M, Tebbich S, Fa JE (2010) How to save the rarest Darwin's finch from extinction: the mangrove finch on Isabela Island. Philosophical Transactions of the Royal Society B: Biological Sciences 365(1543):1019–1030. https://doi.org/10.1098/rstb.2009.0288

Forster M, Liu C, Duke MG, McAdam KG, Proctor CJ (2015) An experimental method to study emissions from heated tobacco between 100–200°C Abstract. Chemistry Central Journal 9(1):1–10. https://doi.org/10.1186/s13065-015-0096-1

Fox J, Weisberg S (2018) An R companion to applied regression. SAGE Publications, Thousand Oaks

Garofalo S, Giovagnoli S, Orsoni M, Starita F, Benassi M (2022) Interaction effect: are you doing the right thing? PLoS ONE 17:e0271668. https://doi.org/10.1371/journal.pone.0271668

Green ALR, Putschew A, Nehls T (2014) Littered cigarette butts as a source of nicotine in urban waters. J Hydrol 519:3466–3474

Greenberg DA, Palen WJ (2019) A deadly amphibian disease goes global. Science 363:1386–1388

Grünewald B, Siefert P (2019) Acetylcholine and its receptors in honeybees: involvement in development and impairments by neonicotinoids. InSects 10:420

Guimaraes JH, Papavero N (1999) Myiasis in man and animals in the Neotropical region. Pleiade/FAPESP, Sao Paulo

Harvey JA, Chernicky K, Simons SR, Verrett TB, Chaves JA, Knutie SA (2021) Urban living influences the nesting success of Darwin's finches in the Galápagos Islands. Ecol Evol 11:5038–5048

Hulthen AD, Clarke AR (2006) The influence of soil type and moisture on pupal survival of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). Aust J Entomol 45:16–19

Instituto Nacional de Estadistica y Censos (INEC). (2016). Censo de Población y Vivienda Galápagos, 2015. INEC, Quito, Ecuador. https://www.ecuadorencifras.gob.ec/galapagostiene-25-244-habitantes-segun-censo-2015/

Kasiemobi O, Mauel B, Membere, (2014) Larvicidal effect of aqueous leaf extract of tobacco (*Nicotiana tabaum*) on the third instar larvae of *Musca domestica*. IOSR J Agric Vet Sci 7:35–40

Kleindorfer S, Dudaniec RY (2016) Host-parasite ecology, behavior and genetics: a review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. BMC Zool 1:1

Kleindorfer S, Peters KJ, Custance G, Dudaniec RY, O'Connor JA (2014) Changes in Philornis infestation behavior threaten Darwin's finch survival. Curr Zool 60:542–550

Knutie SA, McNew SM, Bartlow AW, Vargas DA, Clayton DH (2014) Darwin's finches combat introduced nest parasites with fumigated cotton. Curr Biol 24:R355–R356

Knutie SA, Owen JP, McNew SM, Bartlow AW, Arriero E, Herman JM, DiBlasi E, Thompson M, Koop JAH, Clayton DH (2016) Galápagos mockingbirds tolerate introduced parasites that affect Darwin's finches. Ecology 97:940–950

Knutie SA, Webster CN, Vaziri GJ, Albert L, Harvey JA, LaRue M, Verrett TB, Soldo A, Koop JAH, Chaves JA, Wegrzyn JL (2024) Urban living can rescue Darwin's finches from the lethal effects of invasive vampire flies. Glob Change Biol 30(1):e17145

Koop JAH, Owen JP, Knutie SA, Aguilar MA, Clayton DH (2013) Experimental demonstration of a parasite-induced immune response in wild birds: Darwin's finches and introduced nest flies. Ecol Evol 3:2514–2523

Koop JAH, Kim PS, Knutie SA, Adler F, Clayton DH (2016) An introduced parasitic fly may lead to local extinction of Darwin's finch populations. J Appl Ecol 53:511–518

Lawler TS, Stanfill SB, DeCastro RB, Lisko JG, Duncan BW, Richter P, Watson CH (2017) Surveillance of nicotine and pH in cigarette and cigar filler. Tob Regul Sci 3:101–116

Lehrer S, Wilson M, Salvaggio J (1978) Immunogenic properties of tobacco smoke. J Allergy Clin Immunol 62:368–370

Lenth RV (2021) emmeans: Estimated Marginal Means, aka Least-Squares Means. Available from https://CRAN.R-project.org/package=emmeans.

Li Z, Chambi C, Du T, Huang C, Wang F, Zhang G, Li C, Juma Kayeke M (2019) Effects of water immersion and soil moisture content on larval and pupal survival of *Bactrocera minax* (Diptera: Tephritidae). InSects 10:138

Magano SR (2011) Repellent properties of *Nicotiana tabacum* and *Eucalyptus globoidea* against adults of *Hyalomma marginatum rufipes*. Afr J Microbiol Res 3:1–5

Martina C et al (2022) Evaluating volatile plant compounds of *Psidium galapageium* (Myrtales: Myrtaceae) as repellents against invasive parasitic Diptera in the Galapagos Islands. J Med Entomol 59:89–98

McBride JS, Altman DG, Klein M, White W (1998) Green tobacco sickness. Tob Control 7:294–298

McNew SM, Clayton DH (2018) Alien invasion: biology of *Philornis* flies highlighting *Philornis downsi,* an introduced parasite of Galápagos birds. Annu Rev Entomol 63:369–387

Millar NS, Denholm I (2007) Nicotinic acetylcholine receptors: targets for commercially important insecticides. Invertebr Neurosci 7:53–66

Moon RD (1980) Effects of larval competition on face fly. Environ Entomol 9:325–330

NIDA (2021) How does tobacco deliver its effects? https://nida.nih.gov/publications/research-reports/tobacco-nicotine-e-cigarettes/how-does-tobacco-deliver-its-effects

O'Connor JA, Robertson J, Kleindorfer S (2014) Darwin's finch begging intensity does not honestly signal need in parasitised nests. Ethology 120:228–237

Scheele BC et al (2019) Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363:1459–1463

Schmidt CD, Blume RR (1973) Laboratory-reared horn flies: relationships between width of head and weight of pupa in both sexes and between these measurements and number of ovarioles in females. Ann Entomol Soc Am 66:1307–1308

Schorderet Weber S, Kaminski KP, Perret J-L, Leroy P, Mazurov A, Peitsch MC, Ivanov NV, Hoeng J (2019) Antiparasitic properties of leaf extracts derived from selected Nicotiana species and *Nicotiana tabacum* varieties. Food Chem Toxicol 132:110660

Showler AT (2017) Botanically based repellent and insecticidal effects against horn flies and stable flies (Diptera: Muscidae). J Integr Pest Manag 8:15

Skidmore P (1985) The biology of the Muscidae of the world. Junk Publishers

Soleimani F, Dobaradaran S, De-la-Torre GE, Schmidt TC, Saeedi R (2022) Content of toxic components of cigarette, cigarette smoke vs cigarette butts: a comprehensive systematic review. Sci Total Environ 813:152667

Suárez-Rodríguez M, Garcia CM (2017) An experimental demonstration that house finches add cigarette butts in response to ectoparasites. J Avian Biol 48:1316–1321

Suárez-Rodríguez M, Macías Garcia C (2014) There is no such a thing as a free cigarette; lining nests with discarded butts brings short-term benefits, but causes toxic damage. J Evol Biol 27:2719–2726

Suárez-Rodríguez M, López-Rull I, Macías Garcia C (2013) Incorporation of cigarette butts into nests reduces nest ectoparasite load in urban birds: new ingredients for an old recipe? Biol Let 9:20120931

Theodosopoulos AN, Gotanda KM (2018) Death of a Darwin's Finch: a consequence of human-made debris? Wilson J Ornithol 130:1023

Wikelski M, Foufopoulos J, Vargas H, Snell H (2004) Galápagos birds and diseases: invasive pathogens as threats for island species. Ecol Soc 9(1):10