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COMPARISON OF FOUR FUMIGANTS FOR REMOVING AVIAN LICE

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Abstract.—Fumigation chambers are commonly used to remove avian ectoparasites without harming host birds. Although several fumigants are commonly used, we know of no systematic comparison of available fumigants, and there is no agreement as to which is best. We compared the efficiency of four fumigants that have been supported in the literature: chloroform, di-ethyl ether (ether), ethyl acetate, and CO_2. We conducted two types of experiments using chewing lice (Insecta: Phthiraptera). First, fumigants were ranked according to the speed with which they immobilized lice. CO_2 immobilized lice most quickly, and ether the slowest. Second, fumigants were ranked according to the percentage of immobilized lice that detached from a suspended feather. Chloroform detached the most lice, and CO_2 the least, with ether and ethyl acetate intermediate. Third, of those lice detaching, the mean time to detachment was the quickest under CO_2. Finally, chloroform, ether, and ethyl acetate all kill lice; however CO_2 only anaesthetizes lice, and lice quickly revive when exposed to fresh air.

COMPARACIÓN DE CUATRO FUMIGANTES PARA REMOVER MALLOFAGA EN AVES

Sinopsis.—Las cámaras de fumigación son usadas comúnmente para remover ectoparásitos sin herir a las aves hospederas. Aunque hay varios fumigantes de uso comon, no conocemos de ninguna comparacion sistematica de los fumigantes disponibles, y no hay un acuerdo sobre cual es mejor. Comparamos la eficiencia de cuatro fumiganmtes que se han apoyado en la literature: cloroformo, di-etil eter (eter), acetato etilico, y CO_2. Condujimos dos tipos de experimentos utilizando Phthiraptera (Insecta). Primero se organizaron los fumigantes de acuedo a la rapidez con que inmobilizaron los Phthiraptera, El CO_2 fué el más veloz en inmobilizar los parásitos y el eter fué el más lento. En segundo lugar organizamos los fumigantes de acuerdo al porciento de Phthiraptera inmobilizado que dejó de estar suspendido de una pluma. El cloroformo removió la mayor cantidad de Phthiraptera y CO_2 los menos, con eter y acetato etílico entre medio. En tercer lugar, el tiempo promedio de los parásitos en ser removidos fué menor con CO_2. Por último, el cloroformo, el eter y el acetato etílico matan los Phthiraptera; sin embargo, el CO_2 solo anestesia los parásitos, y estos reviven rápidamente al ser expuestos al aire fresco.

Researchers who study avian ectoparasites often use fumigants to remove lice, feather mites, hippoboscid flies, and other ectoparasites (Clayton and Walther 1997). These fumigants include a range of volatile organic solvents, such as chloroform, ethyl acetate, and di-ethyl ether (also known as "ether"). These volatile solvents can be soaked in a cotton ball

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and placed in a specialized fumigation chamber that protects a bird from the noxious fumigants while the vapors are allowed to penetrate feathers and anesthetize ectoparasites. Recently, advances have been made in these techniques, including more efficient fumigation chambers (Bear 1995) and the use of CO_2 gas (H. Hoch, pers. comm.).

Presently, there is no agreement as to which fumigants are most efficient at removing parasites. Therefore, we compared the usefulness of different arthropod fumigants. In our experiments, we identify three aspects of a fumigant's "efficiency": (1) immobilization rate, (2) proportion of anesthetized lice that become detached, and (3) mean time to detachment. Additionally, we make general recommendations for adapting procedures and fumigation times so that equivalent results can be obtained with different fumigants. Finally, we discuss other aspects of the use of fumigants, including the relative dangers of low-level fumes to investigators and birds.

METHODS

Feral pigeons (*Columba livia*) were netted in Honolulu, Hawaii, and chewing lice (*Columbicola columbae,* Insecta: Phthiraptera) were manually removed from the feathers using forceps. We compared the effect of four different treatment fumigants: chloroform, di-ethyl ether, and ethyl acetate (purchased from Sigma Lab supplies; Hawaii Chemical Co.), and food-grade CO_2 gas (supplied in cylinders by GasPro Hawaii). All experiments were performed in fumigation chambers designed after Bear (1995).

The first set of experiments was conducted on white filter paper, where the toxicant's effects could be easily observed. These experiments measured how swiftly the fumigant immobilized lice. The second set of experiments was conducted on pigeon feathers to measure how efficiently the fumigant detached immobilized lice from their natural substrate.

Filter-paper experiments.—A circular Whatman 12.5-cm diameter, grade 4 (Sigma catalog #Z24 056-7) filter paper was divided into 10 sections. Ten lice were placed on the filter paper, one on each section. The filter paper was placed on the bottom of the fumigation chamber. A cotton ball saturated with 3 ml of liquid fumigant was placed on the chamber's floor, or CO_2 gas was allowed to flow constantly into the jar, maintaining positive pressure and ensuring CO_2 saturation. A lid sealed the chamber, and a timer was started. Two observers monitored the lice, and when all 10 lice were immobilized, the lice were prodded to verify anesthesia, and the time recorded (hereafter called immobilization time). These experiments were repeated three times for each fumigant, for a total of 12 trials and 120 lice. Results were analyzed with Model I single-factor ANOVA.

Feather experiments.—A flight feather was clipped from the original pigeon host, and 30 lice, all collected from the same host, were allowed to crawl onto and settle on the feather. The feather was suspended from the lid of the fumigation chamber by a string, so that it hung 5 cm above the

TABLE 1. Comparison of the effectiveness of four fumigants for immobilizing and detaching avian lice.

Fumigant	Filter-paper experiment Average immobilization time (s) [standard deviation]	Feather experiment	
		Average time to detachment (s) [standard deviation]	Percent lice detached
CO_2	60.7 [5.87]	181.24 [112.76]	21.7%
Chloroform	122.3 [10.69]	192.47 [9.72]	75.6%
Ether	200.7 [17.56]	293.43 [38.34]	55.8%
Ethyl acetate	198.0 [28.58]	307.3 [53.42]	32.5%

floor of the chamber. A cotton ball was saturated with 3 ml of liquid fumigant and placed on the chamber floor, or CO_2 gas was piped in with a constant flow rate as described above. Clean white filter paper was placed on the floor of the fumigation chamber, so lice could clearly be seen when they detached from the feather. The chamber was sealed, and a timer was started. As the fumigant anesthetized the lice, they detached from the feathers and dropped onto the filter paper. The time was recorded each time a louse detached from the feather (hereafter called detachment time). The number of lice detaching from the feather within 17 min was counted (hereafter called detachment number). The feather was then taken from the chamber and the lice remaining on the feather were removed and counted. We made no attempt to blow, fluff, or otherwise mechanically detach anesthetized lice. We conducted at least three replicates with each fumigant. More than three replicates were performed for fumigants that detached few lice. This increased the total number of individuals that detached and allowed us to more accurately estimate a mean detachment time for each fumigant.

Rather than using ANOVA, which is not robust when variances are highly unequal, the detachment time data were analyzed using *t*-tests assuming unequal variances.

RESULTS

Filter-paper experiments.—There was a strong difference ($F_{3,8} = 42.556$, $P < 0.001$) among the fumigants in the time it took to immobilize all 10 lice (Table 1). CO_2 gas worked quickest, chloroform took about twice as long, and ethyl acetate and ether both took about three times as long.

Feather experiments.—Data for the feather experiments were more complex. First, we compared the number of parasites detached from each feather when exposed to the fumigants. There was a significant difference among the fumigants in the percentage of lice detaching from the feather ($F_{13,3} = 24.39$, $P < 0.001$, Table 1). Lice that failed to detach were easily removed from the feathers with minimal blowing or ruffling of the feathers.

Detachment times varied significantly among fumigants. CO_2 showed the most rapid mean detachment time (Fig. 1) for those lice that did detach, however CO_2 detached fewer than 25% of total lice on the feather. Although CO_2 appeared to quickly anesthetize the lice, they retained their grip by clamping their mandibles on the barbules. A small number of lice continued to detach as the experiment progressed. The cumulative lice detachment curves (Fig. 1) for both chloroform and ether were smooth *S*-shaped curves. Chloroform had shorter mean detachment times than ether and cumulative detachment under chloroform asymptoted both earlier and with a higher percent detached, showing that chloroform more quickly and more efficiently detached the lice than ether. Ethyl acetate failed to detach many lice, and did not reach its asymptote until after 10 min (Fig. 1).

The variance in detachment time differed greatly among fumigants, especially considering how similar the means were. We found that mean detachment times with CO_2 were not significantly shorter than for chloroform (one-tailed *t*-test assuming unequal variances, df = 49, $t = 0.09$, $P > 0.5$). However, chloroform detachment times were significantly shorter than those for ether (two-tailed *t*-test assuming unequal variances, df = 126, $t = 3.49$, $P < .001$).

DISCUSSION

Although no single fumigant performed best in all three measures of efficiency, CO_2 most rapidly immobilized and detached lice. Chloroform ranked second in the above measures and it also detached the greatest number of lice.

We conclude that CO_2 is the most effective fumigant overall. Rapid anesthesia reduces processing time for each bird, reducing stress to it and allowing the investigator to process more birds per unit time. Furthermore, we found CO_2 to be the least offensive of the fumigants for both researchers and birds. Even in outdoor conditions the noxious vapors of chloroform, ether and ethyl acetate can cause investigators to have headaches, especially after several hours of low-level exposure. These vapors can also diffuse through the hood of the fumigant jar and cause smaller, fragile birds to become drowsy or anesthetized, requiring termination of the fumigation and extra time for reviving and nursing the bird.

The effect of CO_2 on the lice differed greatly from that of chloroform, ether, or ethyl acetate. The latter three fumigants killed lice, whereas lice treated with CO_2 were only temporarily anesthetized and began moving within 5 min after the CO_2 ceased flowing. Thus, CO_2 fumigation is advantageous for DNA analysis because living lice can be starved prior to their preservation. This causes them to excrete ingested host materials, which may interfere with the DNA analysis. Furthermore, CO_2 fumigation may be useful for researchers that require live ectoparasites for pathogen screening or other experiments.

CO_2 may be difficult and sometimes impossible to obtain in remote field localities. Transporting unwieldy, heavy and dangerous high pressure

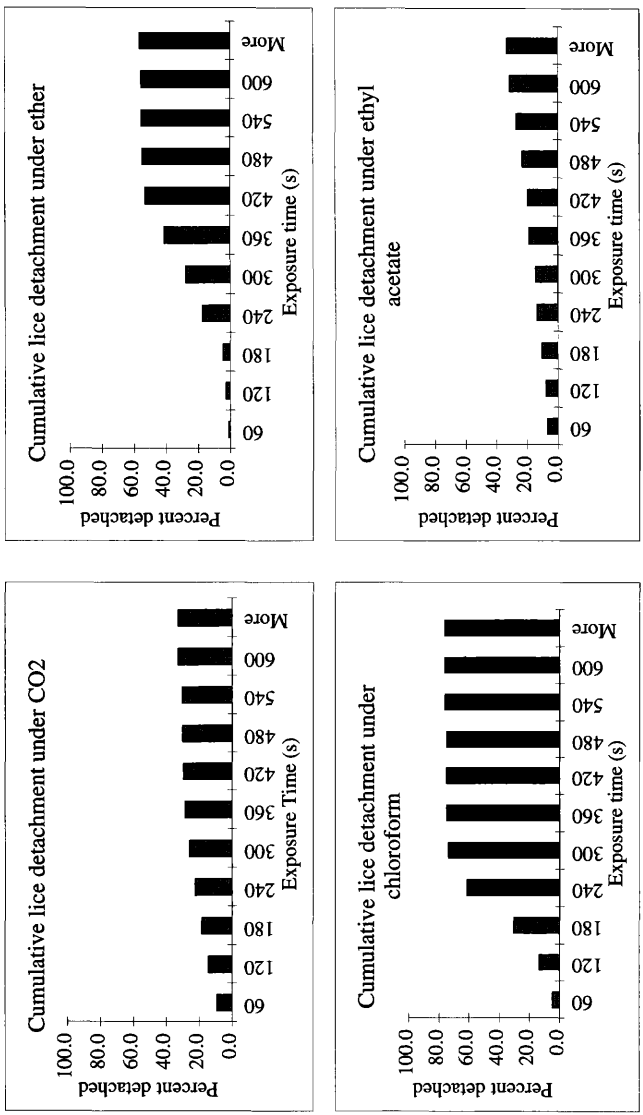

FIGURE 1. Cumulative histograms of percent of lice detached in 60-s intervals during the feather experiments.

CO_2 cylinders into remote localities presents an assortment of logistical problems. If CO_2 is not an option, chloroform would be our second choice fumigant. Chloroform consistently out-performed ether and ethyl acetate in each of the experiments. Chloroform may also be preferred because it is not as flammable or as reactive as ether or ethyl acetate. Therefore, to remove avian lice effectively, CO_2 should be used for a minimum of 2–3 min, assuming complete saturation of plumage, and should be accompanied by vigorous feather ruffling or blowing (Bear 1995). Chloroform should be used for at least twice the time that CO_2 is used. Assuming variation in susceptibility between parasite species and incomplete saturation of plumage we strongly recommend a minimum of ten minutes exposure with strong blowing and ruffling in the fumigation chambers to immobilize and remove ectoparasites.

We have used both CO_2 and chloroform in remote field stations in Papua New Guinea (Dumbacher 1997). Additional field trials with other field-caught arthropods (ants, spiders, and moths) showed that CO_2 also anesthetized these animals more rapidly than chloroform (Visnak and Dumbacher, unpubl. data). In our field studies, we found that CO_2 and chloroform effectively removed lice, feather mites, and hippoboscid flies. Avian ectoparasites that attach to the host via their mouthparts or by burrowing beneath the skin cannot be recovered with these fumigants, regardless of whether they were anesthetized. Note however, that the entire populations of lice and feather mites may not be recovered using the fumigation jar. Immature forms and tenacious lice and feather mites may remain trapped between feather barbules or otherwise attached to the host. Killing the avian host is necessary if the researcher wishes to investigate the complete ectoparasite load found on a bird.

The investigator must take special care while working with chloroform, ether, and ethyl acetate, to reduce exposure and provide adequate ventilation for the birds and researchers. If the procedure is performed carefully, thoroughly, and consistently, we believe parasite recoveries should closely approximate total parasite loads, and at least be highly correlated with parasite loads. Thus, in situations where it is not acceptable to collect birds, field fumigations using either CO_2 or chloroform should allow adequate quantification of parasite loads, providing that adequate host sample sizes are surveyed. Clayton's dust-ruffling technique using pyrethrin has also proven effective for ectoparasite retrieval where birds cannot be collected, and although it is more labor intensive, it requires less equipment than fumigation chambers (Clayton and Walther 1997).

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