SCIENTIFIC NOTE

Drying techniques differentially affect bark beetle weight change

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Insects are commonly subjected to various drying techniques for various reasons, including testing the effects of a particular diet (i.e., host effect; Nozzolillo *et al.* 1997; Watanabe and Kitagawa 2000; Martin, 2004; Kopper *et al.* 2005; Erbilgin *et al.* 2014; Brzozowski *et al.* 2019; Guevara-Rozo *et al.* 2019) or a particular treatment effect (i.e., toxicity; Moretti *e al.* 2002; Nukenine *et al.* 2013), comparing the effects of drying on the nutritional value of edible insects (Fombong *et al.* 2017; Kröncke *et al.* 2019), or simply preserving insect specimens for long-term storage in museum collections (Flaschka and Floyd 1969). The two most common techniques used for drying insects in entomology are oven-drying and freeze-drying (Johnston and Cunjak 1999; Fombong *et al.* 2017; Kröncke *et al.* 2019; Melgar−Lalanne *et al.* 2019; Bezanson and Floate 2020). Freeze-drying also known as lyophilisation is a low-temperature (-50 °C) dehydration process that involves freezing the specimen, lowering pressure within the freeze chamber, and then removing the ice by sublimation. For oven-drying, low-temperature (range 50-60 °C) convection or forced air ovens are typically used, primarily in laboratory settings. Specimens are placed inside a drying oven to slowly and evenly remove moisture. Although the literature indicates that both techniques are commonly used, it was unknown how each treatment affects insect specimens, including bark beetles. This study is designed to investigate the effects of oven-drying and freeze-drying on the weights of specimens of mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Curculionidae, Scolytinae).

Our experimental approach included two phases. In phase one, we took 80 recently emerged adult beetles and placed them in a freezer for 24 hours at -80 °C. We then randomly grouped the specimens into 16 groups, each consisting of five specimens. We determined the weight of each group to the nearest 0.01 mg (XPE105 Delta Range®; Mettler Toledo, Columbus, Ohio, United States of America). We then split these groups evenly, with each group then undergoing two different drying treatments. The groups were either placed in an oven at 50 °C for 48 hours or in a freeze-dryer (Labconco Corp., Kansas City, Missouri, United States of America) for 48 hours (n = 8 for each treatment), with eight randomly selected groups assigned to each treatment. After the initial drying treatments, we weighed each group again and recorded their weights.

In phase 2, we further selected four groups from each of the oven-drying and freeze-drying treatments and placed them in the opposing treatment (e.g., groups from the initial oven-drying treatment underwent freeze-drying for their second

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treatment; n = 4). The remaining four groups in each treatment group were further treated in the same treatment they had been treated with initially (e.g., groups from the initial oven-drying treatment underwent the oven-drying treatment a second time). Drying conditions were the same as before. After 48 hours, we weighed the beetles in each group separately.

We compared the weight of the beetles before and after each treatment in phase one and phase two using general linear mixed models (R package nlme, version 3.1-140; Bates *et al.* 2015). We used beetle as a random factor and drying treatment as a fixed factor. We transformed data to meet model assumptions of normality and homoscedasticity. We determined the significance of the fixed effect in the model using Wald chi-squared tests. Significant models were followed by *post-hoc* Tukey's honestly significance difference tests (R package multcomp, version 1.4-10). All analyses were conducted in R, version 3.5.0. To test percent change in the weight of the beetles before and after each treatment, we used a chi-squared test. The significance of the *P* values was assessed through the Holm's adjustment method for an experiment-wise error rate of 0.05.

The initial weights of the beetles in each treatment (oven-drying *versus* freeze-drying) in phase one did not differ (Table 1). As expected, drying treatments significantly reduced the weight of beetles (Table 1). Furthermore, the percent reduction in specimen weight differed between the two treatments, with the freeze-drying treatment (28.49%) resulting in a larger reduction in beetle weight than the oven-drying treatment did (22.85%; Table 1).

Table 1. Results of the linear mixed model, showing the comparison of weights of mountain pine beetle, *Dendroctonus ponderosae*, after freeze-drying and oven-drying. Beetle was considered a random factor and drying treatment was considered a fixed factor. Results with different letters differed statistically, as indicated by Tukey's honestly significant difference test at $\alpha = 0.05$. Upper case and small case letters following the means (±SE) indicate differences within a row and between rows, respectively.

Treatments	Mean (SE) weight pre-treatment (n=5)	Mean (SE) weight post-treatment (n=10)	Statistics	Mean (SE) percent difference in weight
Oven-dry	26.8 (±2.1) A	20.0 (±1.1) B	$F_{1,7} = 8.2$ $P = 0.02$	22.9 (±2.7) a
Freeze-dry	30.0 (±2.9) a	21.0 (±1.0) B	$F_{1,7} = 14.8$ $P = 0.006$	28.5 (±2.4) b
	$F_{1,7} = 0.6$ $P = 0.5$	$F_{1,7} = 0.3$ $P = 0.4$		$\chi_7 = 14.5$ $P = 0.04$

Interestingly, in phase two of the experiment, we also found significant differences in weight occurred among the four treatments where beetles from the oven-drying or freeze-drying treatments were further subjected to an additional drying treatment (Figure 1). Specifically, when beetles were treated in the oven first and then freeze-dried, the percent difference in specimen weight was the highest (2.66%), followed by the freeze-dry–freeze-dry (0.70%) and the oven-dry–oven-dry (0.34%) combination treatments. The lowest percent reduction in weight occurred in the freeze-dry–oven-dry combination (0.08%).

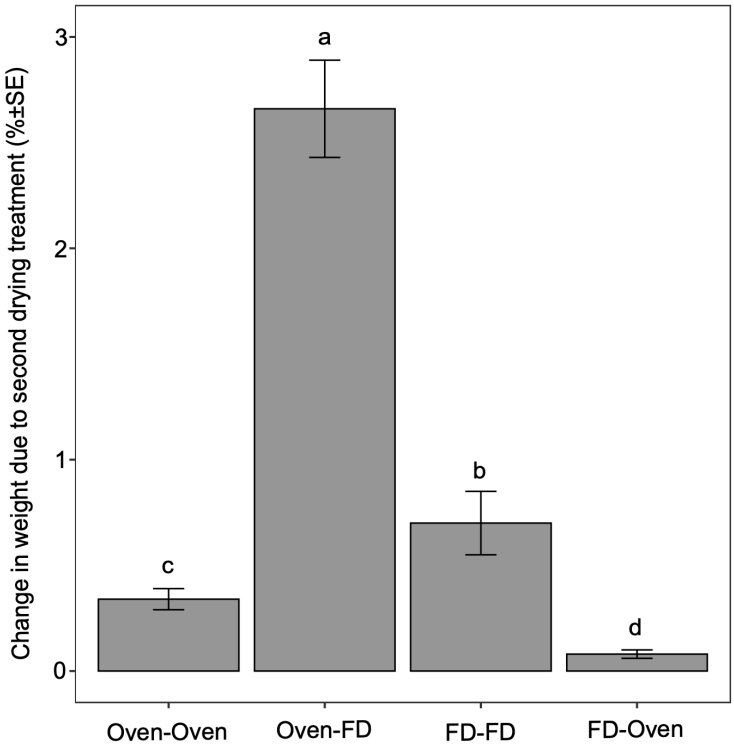

Figure 1. Effect of a second drying treatment on the mean weight of mountain pine beetles, *Dendroctonus ponderosae*. Beetles were dried in an oven (Oven) or freeze-dryer (FD), and then subjected to a second drying treatment (see text). In the linear mixed model beetle was considered a random factor and drying treatment was considered a fixed factor (see text). Bars with different letters are statistically significant at $P = \leq 0.05$. Error bars are ±SE.

In this study, both drying techniques significantly reduced the weight of beetles. However, when the results of the two drying techniques used were compared, freeze-drying led to greater loss of specimen weight than the oven-drying did under the conditions tested. In particular, the percent difference between the initial and post-treatment weights of beetles was higher in the freeze-drying treatment. Particularly, freeze-drying reduced beetle weight about 6% further than oven-drying did. Furthermore, when beetles dried in the oven were then placed in the freeze-dryer, the percent reduction (2.66%) in beetle's dried weight was several times higher than any other combination treatment tested. In contrast, beetle specimens treated in the freeze-dryer followed by the drying oven had the lowest percent difference (0.08%) between initial and post-treatment weights.

The differences between each drying technique can be explained by how each method operates. Briefly, freeze-drying first freezes insect specimens at sub-zero temperature (-50 °C in our case) through the application of partial vacuuming (of air), which reduces the pressure inside the chamber where the samples are stored. This freezing process produces ice crystals within the specimens and promotes faster removal of water vapour during sublimation by heating the samples under

low pressure. Because a low temperature is used in freeze-drying and is retained in the specimens while the water is still being removed, other temperature-sensitive chemicals such as volatiles can be mostly retained in the specimens, and water is most likely affected by freeze-drying process. Also, because the water is removed while the specimens are in a frozen state, the specimens' cell structure remains intact (Ratti 2001). In contrast, a typical laboratory drying oven causes specimens to dry through evaporation. Drying ovens use convection heating, with air currents heating the specimens. Water escapes from specimens into the air, and the oven discharges the warm moist air, thereby allowing the specimens to dry rapidly. Oven-drying can cause significant shrinkage in samples due to the high temperatures used and may damage the cell structure (Del Valle *et al.* 1998). The results of the present study suggest that freeze-drying appears to be more successful for removing moisture from the specimens under the conditions tested.

Other studies have similarly reported that freeze-drying is known to be more effective for preserving plant samples compared to other drying techniques. For example, the freeze-drying process solidifies the water inside plant tissues, which protects the basic structure and quality of the physical, chemical, and nutritional product through less volume reduction (Ratti 2001). Gümüşay *et al.* (2015) tested the effects of freeze-drying, sun-drying, vacuum oven-during, and oven-drying on the phenolic contents of tomatoes and observed that freeze-drying removed less of the tomatoes' phenolic contents compared to other drying methods tested. Similarly, Asami *et al.* (2003) reported that freeze-drying preserved the amount of phenolic compounds within corn, strawberries, and marionberries better than air-drying did.

It is currently unknown how each drying method affects other heat-sensitive metabolites that are stored in insects, including carbohydrates, fats (fatty acids), proteins, essential amino acids, inorganic salts, vitamins, and sterols. However, Fombong *et al.* (2017) investigated the effects of two drying methods (freeze-drying and oven-drying) on the fatty acids, amino acids, and mineral composition of a grass hopper, *Ruspolia differens* (Serville) (Orthoptera: Tettigonidae), and found no difference between the two methods.

Unlike plants, insects contain very few volatile organic compounds that are affected by heat. Senthilkumar *et al.* (2012) reported several volatile organic compounds released by two species of adult beetles, *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae), including methyl-1,4-benzoquinone, ethyl-1,4-benzoquinone, and 1-tridecene. These compounds are mainly pheromones of beetles. Because the beetles in the present study were frozen at –80 °C before they underwent the treatments, any volatile organic compounds stored inside beetles likely were released during treatments. However, considering the miniscule amount of pheromones released by mountain pine beetle (Erbilgin *et al.* 2014), this amount is much smaller than the amount of water stored in the beetles.

This study includes a couple of limitations. First, for simplicity, we oven-dried the beetles at a single temperature setting and duration (at 50 °C for 48 hours). Even though the duration seems adequate (i.e., the difference between the single and double oven treatments is negligible), we suspect that using different drying temperatures likely would yield different results. Second, in the present study, we tested our objective using beetle specimens; it is unknown if we would

see similar results for plant tissue samples because they contain a large amount of moisture and volatile organic compounds.

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