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Using Automated Flight Mills to Manipulate Fat Reserves in Douglas-fir Beetles (Coleoptera: Curculionidae)

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ABSTRACT Because current techniques for quantifying fat, the main fuel used for flight in insects, are destructive, researchers are limited to only one direct measure of fat per specimen. This limitation is problematic for studies aimed at assessing whether fat loss through flight influences subsequent behavioral activity. To overcome this problem, we used body volume, body mass, emergence day, and brood density as parameters in a multiple regression model to predict initial fat levels in female Douglas-fir beetles, *Dendroctonus pseudotsugae* Hopkins, on emergence from the host. The model explained 54% of the variation in fat reserves as determined by Soxhlet extraction with petroleum ether. Treatments of 30–1,380 min of flight on rotary flight mills were used to establish the relationship between flight and fat reserves. Using a model that incorporated estimated initial fat levels, as well as time spent in flight and time in nonflight activities on the flight mills, we found that 6 h of flight decreased fat by ≈50%. Flight activity and nonflight activity did not differ significantly in terms of their effect on fat reserves. Individual beetles with high initial fat content flew longer and faster on flight mills than beetles with low initial fat reserves. Our study shows how researchers can manipulate fat levels in bark beetles and other insects through flight, thereby opening the door to using these manipulations in behavioral studies.

KEY WORDS *Dendroctonus pseudotsugae*, Douglas-fir beetle, fat reserves, insect flight, Soxhlet extraction

Most insects rely on flight for dispersing, finding mates, and searching for hosts or prey. Determining the rate at which fat, the most efficient source of energy for flight muscles in insects (Candy et al. 1997, Canavoso et al. 2001), is used during flight can provide valuable information about an insect's dispersal capacity. The information can also be used to examine how fat, an index of physiological condition, influences behavioral decisions (Beenakkers 1969, Sundström 1995, Ellers et al. 1998, Elkin and Reid 2005). However, quantifying fat in insects requires killing the subjects (Atkins 1969, Botterweg 1983, Kinn et al. 1994). Destructive techniques limit fat sampling to only one direct measure per specimen (i.e., pre- or postflight), which is clearly problematic for studies aimed at assessing whether fat loss through flight influences subsequent behavioral activity (e.g., host selection, mate choice).

To bypass the problem of destructive sampling when estimating fat loss from flight, researchers have typically used a multigroup approach whereby one group of insects is randomly assigned as the control, and one or several other groups are flown for set periods of time (Atkins 1966, 1969, Thompson and Bennett 1971, Kinn et al. 1994). After flight, mean fat levels for the various treatment groups are compared with the mean fat level of the nonflight control group, with the difference in fat representing mean fat loss from flight. The multigroup approach, while useful for establishing basic relationships between flight and fat loss, provides no information about fat loss for specific individuals, and it precludes subsequent behavior assays because specimens are killed by the procedure.

An alternative to the multigroup approach is to establish the relationship between an insect's actual fat reserves, quantified through standard destructive methods, and some measure of the living insect's body condition (see Jakob et al. 1996). Once an insect's fat reserves have been estimated, the subject can be flown, killed, and assayed for fat, thereby providing researchers the information needed to estimate fat loss as a function of flight. This relationship between fat loss and flight can then be applied to additional beetles without destructive sampling. An advantage of this approach is that estimates of fat reserves can be made before and after flight on the same individuals, and these individuals can be used in follow-up experiments. A potential weakness of the approach is that it relies on the strength of the correlation between actual and estimated initial fat contents.

As a prelude to a study that examined how fat reserves after flight influence host choice decisions by

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