Journal of Chemical Ecology, Vol. 25, No. 5, 1999

Brief Communication

PROBLEMS ENCOUNTERED IN FEEDING MICROENCAPSULATED OILS TO A FOLIVOROUS MARSUPIAL, THE COMMON RINGTAIL POSSUM (*Pseudocheirus peregrinus*)

IVAN R. LAWLER,* and WILLIAM J. FOLEY

Division of Botany and Zoology
Australian National University
0200, Canberra, Australian Capital Territory

(Received June 5, 1998; accepted December 30, 1998)

Abstract—In our study of interactions between marsupial folivores and *Eucalyptus*, we have examined the role of terpenes, including the use of bioassay experiments where terpenes are added to an artificial diet. In pursuit of greater ecological realism in these experiments, we have sought means of packaging terpenes more realistically, by using microencapsulation to simulate oil glands of leaves. We report here on a preliminary experiment with microencapsulated olive oil (intended for use as an experimental control) in which the food intake of the animals was substantially reduced, to the point that starvation appeared imminent and we aborted the experiment. We discuss why this occurred and recommend caution to others intending to use microencapsulated terpenes (or other oils) in herbivore diets.

Key Words—Microencapsulation, terpene, plant–herbivore interaction, possum, *Eucalyptus*.

INTRODUCTION

Terpenes are volatile plant secondary metabolites that have been the subject of a number of studies of plant-herbivore interactions (Foley et al., 1987;

*To whom correspondence should be addressed.

0098-0331/99/0500-1195$16.00/0

Edwards *et al.*, 1993; Hume & Esson, 1993; Duncan et al., 1994; Lawler & Foley, 1999). Because they are found in varying concentrations between and within species, have distinct tastes and odors, and are toxic to some animals in small doses (McLean & Foley, 1997), they are often suspected to be deterrents to feeding by both mammalian and insect folivores. Several studies have investigated their effect on mammalian feeding by using correlative analysis of the relationship between food intake and concentration of terpenes in the foliage (Hume and Esson, 1993; Lawler et al., 1998a). However, such correlative studies do not show cause and effect, and bioassay studies, in which isolated terpenes are added to artificial diets, are required to ascribe a definite role to the terpenes (Lawler et al., 1998a).

The presentation of terpenes in artificial diets in an ecologically realistic manner is problematic. In plants, terpenes in the foliage typically are contained in microscopic glands. In contrast, most bioassay experiments with terpenes are conducted with the liquid terpenes added freely to the diet or adsorbed onto a dry component of the diet (Reichardt et al., 1990; Lawler et al., 1998a, 1999). Because terpenes are highly volatile and odoriferous, this presentation results in an artificial diet that presents much stronger taste and olfactory cues to the animal, even thought the total terpene concentration may be similar to that in foliage. If these aspects are important (Lawler et al., 1999), then results obtained with artificial diets may not be comparable to those obtained with foliage diets. Stronger signals would likely exaggerate the importance of terpenes in artificial diets. As a consequence, we have sought means of packaging terpenes for addition to diets in a realistic manner, one of which has been microencapsulation (Clancy et al., 1992). The microencapsulation technique used was coacervation of gelatin and acacia resin in an emulsion of the target oil in water. Manipulation of the pH causes the gelatin and acacia to bind at the oil–water interface of each oil droplet to form the capsule. The technique has been widely used in a variety of applications, such as feeding of marine larvae (Southgate and Lou, 1995), as well in testing, similar to ours, on the effects of terpenes on insect feeding (Clancy et al., 1992). The size of the capsules can be controlled by the speed at which the emulsion is blended, producing capsules of sizes similar to the oil glands found in leaves.

METHODS AND MATERIALS

Preparation of Capsules. Several attempts were made to produce the capsules in our laboratory by using the technique described in Southgate and Lou (1995). We were unsuccessful, as terpenes are much less viscous than the oils commonly used in this procedure. Further, we required the capsules to be in powder form for addition to the diet, which required hardening of the capsules with glutaraldehyde (Clancy et al., 1992) before drying. Finally, we contracted a commercial manufacturer to produce capsules containing a terpene (1,8-cineole) and a separate batch containing olive oil, intended for use as experimental controls (as we had previously established that addition of free olive oil to the diet had no effect on food intake). The capsules that were supplied were approximately 12.5% oil by weight.

Diet Composition. (Lawler et al., 1998a), established that a threshold concentration of cineole of approximately 2.6% of the dry matter (DM) of the diet was required to produce any reduction in food intake by common ringtail possums. We decided to begin experiments with a concentration (approximately 8% DM) that would normally decrease intake to approximately 40% of normal intake, in order for differences between intakes of free and encapsulated diets to be statistically discernible. A preliminary experiment was conducted to test the control capsules (with olive oil) before commencing a full experiment comparing the effects of free and encapsulated cineole. However, we found that the diet had to be substantially modified to make it palatable to the animals. The capsules were hydrophobic and did not mix well with the diet, requiring the addition of substantially more fruit to make it cohesive and palatable to the animals. The final diet contained approximately 60% capsules (DM) in order to reach an olive oil concentration of 8% (DM) (equivalent to that desired for terpenes in the subsequent experiments). Due to the failure of this initial experiment (see Results), it was considered unsafe to continue with further experiments.

RESULTS

All of the 12 adult ringtail possums offered the diet with microcapsules containing olive oil on the first night showed an unexpected reduction in food intake (Figure 1). Only two were given the capsule-containing diet again on the second night while the remainder were returned to the normal basal diet. Again, the intakes were low. The two animals given capsules for a second night had extremely low intakes and, surprisingly, those not given capsules (which had eaten them the previous night) reduced intakes even further than they had when given the capsules. At this point the experiment was discontinued and after another night of similarly low intakes, we began to offer the animals several alternative foods to induce them to feed. These included the basal diet, highly palatable foliage from two species of *Eucalyptus*, and honeyed water. Intakes gradually recovered, and the animals were again weaned off honeyed water and foliage and back onto the artificial diet. It was not until 12 days after first giving the animals the microcapsules that intakes of all individuals had reached levels similar to those recorded previously.

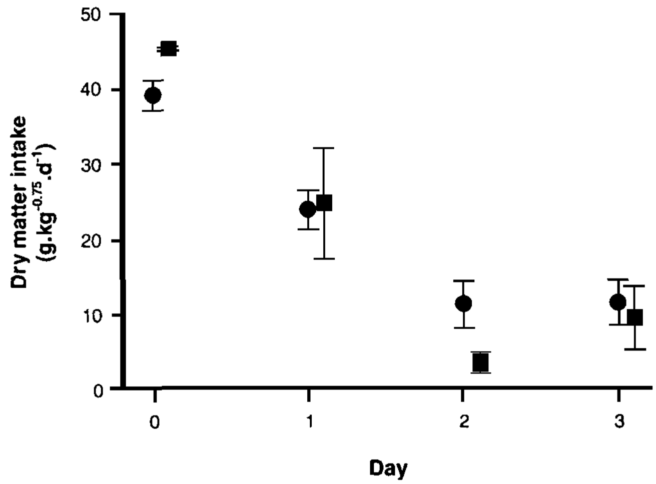

FIG 1. The effect of microcapsules containing olive oil on food intake by common ringtail possums. Day 0 represents the day before the experiment when animals were given the basal diet only. Circles represent animals given microcapsules on day 1 only and basal diet on subsequent days. Squares represent animals given microcapsules on days 1 and 2. Values are means ± standard error.

DISCUSSION

The use of microcapsules to present terpenes to folivorous marsupials in artificial diets in an ecologically realistic fashion is theoretically sound. However, the data presented here show that in practice the technique is more complex. This study was conducted as part of a larger project studying the effect of deterrent compounds on the interaction between marsupial folivores and *Eucalyptus*. It is now becoming clear that a specific group of compounds other than terpenes is the major determinant of deterrency (Lawler et al., 1998a, 1999), and hence the work required to investigate fully the problems encountered here is not warranted. However, terpenes have been suggested to be important in a number of other interactions, such as between deer and spruce (Duncan et al., 1994) and between leaf-chewing insects and *Eucalyptus* (Edwards et al., 1993). It is likely that others will attempt to adopt microencapsulation in their studies, and hence this note is written as a caution. Below we discuss possible reasons for the problems encountered.

Perhaps the most significant factors in determining if this will be a viable technique are the ratio of oil (or terpene) to capsule wall (payload) and the concentration required in the diet to produce the desired effect. In their study of the effects of individual terpenes on performance of insect herbivores of Douglas fir

(*Pseudotsuga menziesii*), Clancy et al. (1992) had similar payloads of terpenes but required dry matter concentrations of not more than 0.8% and generally less than 0.4%. In contrast, we required a minimum of 7% dry matter, some 10 times higher. Microencapsulation may be a more viable technique for dietary studies in those species with lower thresholds for the encapsulated compound. It is now known that ringtail possums can tolerate dietary terpenes at levels higher than those found in most resistant *Eucalyptus* foliage (Lawler et al., 1999), and hence the use of microencapsulated diets will not be viable unless the terpene payload can be substantially increased. It should be noted, however, that others have used microencapsulated oils (corn oil) in mammalian diets at high concentrations (approximately 3% of dry matter) without encountering such problems (M. D. Dearing, personal communication).

Given that similar amounts of microcapsules of similar composition have been fed successfully to other mammals, the effect of these capsules on intake of common ringtail possums is difficult to explain. The effect of the capsules on the nitrogen content of the diet is an unlikely explanation, because the diet with capsules was 1.7% N (dry matter) (basal diet was 1.9% N), while common ringtail possums can easily maintain themselves on diets substantially lower in nitrogen. It is also unlikely that the olive oil itself caused the effects seen, as similar doses (7.5% DM) added freely to the diet had no such effect on intake (Lawler, unpublished data).

We consider it likely that the particular digestive physiology of common ringtail possums is a factor in the problems encountered here. Ringtail possums cope with a fibrous diet by selective retention of fine particles in the cecum where fermentative digestion takes place (Cork and Foley, 1991). The capsules are of appropriate size to be retained, so one explanation may be that the capsules have allowed the oil to be carried through to the cecum, rather than absorbed earlier as is usually the case. This may, in turn, result in disruption of fermentation, perhaps through effects on the gut flora. If capsules are not ruptured or broken down previously, then perhaps the olive oil is released into the cecum, or it may be a result of fermentation of the capsule wall material itself, if capsules are previously ruptured in the stomach. Animals often become sick if the site of digestion of diet components changes. McLean et al. (1995) found that when ringtail possums were dosed by gastric lavage with citronellal, they showed no ill effect, but when the same compound was dosed in peanut oil, animals stopped feeding for four to five days, and one died. In that case, the peanut oil may have protected the citronellal from acid rearrangements in the stomach, causing unchanged citronellal to be absorbed from the small intestine. Cheeke and Patton (1980) also concluded that similar symptoms shown in rabbits eating grain-rich diets were due to the soluble carbohydrates being digested in the cecum and not the small intestine.

Unfortunately, it is not possible to assess the likely effects of the capsule material itself as proprietary arrangements with the supplying company prevent our revealing the detailed nature of the capsules. Capsules produced by coacervation are frequently used in animal feeds for a wide variety of taxa (e.g. Clancy et al., 1992; Southgate and Lou, 1995), and the capsules used here were approved by the United States Food and Drug Agency for human consumption. Hence, we assumed they would be safe for this purpose, but cannot eliminate the possibility that the unforseen problems are a result of the much higher concentrations given to these animals, relative to any human application.

In conclusion, for anyone who intends to use this technique for a similar purpose, there are several problems to address. Firstly, it is important to determine both the active dose of the compound required and the achievable payload, so that some assessment can be made of whether the diet's appearance and palatability will be fundamentally changed in order to reach this concentration. Secondly, it is important to consider the likely effect of the capsules themselves, to prevent or allow for the problems encountered here. Finally, the question of cost is significant. In this instance, the capsules cost over US$3000 and required the use of over five times the amount of terpene required to be encapsulated. This cost will vary depending on the physical and chemical properties of the compound to be encapsulated, but may in many cases prove to be the limiting factor.

Acknowledgments—We thank Dr. Paul Southgate for his assistance in our initial attempts to produce microcapsules. This research was supported by a grant from the Australian Research Council to W.J.F.

REFERENCES

CHEEKE, P. R., and PATTON, N. M. 1980. Carbohydrate overload of the hindgut—a probable cause of enteritis. *J. Appl. Rabbit Res.* 3:20–230.

CLANCY, K. M., FOUST, R. D., HUNTSBERGER, T. G., WHITAKER, J. G., and WHITAKER, D. M. 1992. Technique for using microencapsulated terpenes in lepidopteran artificial diets. *J. Chem. Ecol.* 18:543–560.

CORK, S. J., and FOLEY, W. J. 1991. Digestive and metabolic strategies of arboreal mammalian folivores in relation to chemical defenses in temperate and tropical forests, pp. 133–166, *in* R. T. Palo and C. T. Robbins (eds.). Plant defenses Against Mammalian Herbivory. CRC Press, Boca Raton, Florida.

DUNCAN, A. J., HARTLEY, S. E., and IASON, G. R. 1994. The effect of monoterpene concentrations in Sitka spruce (*Picea sitchensis*) on the browsing behaviour of red deer (*Cervus elaphus*). *Can. J. Zool.* 72:1715–1720.

EDWARDS, P. B., WANJURA, W. J. and BROWN, W. V. 1993. Selective herbivory by Christmas beetles in response to intraspecific variation in eucalyptus terpenoids. *Oecologia* 95:551–557.

FOLEY, W. J., LASSAK, E. V., and BROPHY, J. 1987. Digestion and absorption of *Eucalyptus* essential oils in greater glider (*Petauroides volans*) and brushtail possum (*Trichosurus vulpecula*). *J. Chem. Ecol.* 13:2115–2130.

HUME, I. D., and ESSON, C. 1993. Nutrients, antinutrients and leaf selection by captive koalas (*Phascolarctos cinereus*). *Aust. J. Zool.* 41:379–392.

LAWLER, I. R., and FOLEY, W. J. 1999. Chemical ecology of herbivory in *Eucalyptus:* Interactions between insect and mammalian herbivores and plant essential oils. *In* J. Coppen (ed.). Medicinal and Aromatic Plants—Industrial Profiles: *Eucalyptus.* Harwood Academic Press, UK.

LAWLER, I. R., FOLEY, W. J., ESCHLER, B., PASS, D. M., and HANDASYDE, K. 1998a. Intraspecific variation in *Eucalyptus* secondary metabolites determines food intake by folivorous marsupials. *Oecologia* 116:160–169.

LAWLER, I. R., STAPLEY, J., FOLEY, W. J., and ESCHLER, B. M. 1999. An ecological example of a conditioned flavor aversion in plant–herbivore interactions: The effects of terpenes of *Eucalyptus* leaves on feeding by common ringtail and brushtail possums. *J. Chem. Ecol.* 25:401–415.

MCLEAN, S., and FOLEY, W. J. 1997. Metabolism of *Eucalyptus* terpenes by herbivorous marsupials. *Drug Metab. Rev.* 29:213–218.

MCLEAN, S., FOLEY, W. J., DAVIES, N. W., BRANDON, S., and PEACOCK, E. J. 1995. A novel metabolite of citronellal formed in arboreal marsupials fed *Eucalyptus citriodora.* Meeting of the Australasian Pharmaceutical Sciences Association (APSA), Auckland, December 1994. *N.Z. Pharm. Suppl.* **15**(3): 8 abstract.

REICHARDT, P. B., BRYANT, J. P., MATTES, B. R., CLAUSEN, T. P., CHAPIN, F. S. I., and MEYER, M. 1990. Winter chemical defense of Alaskan balsam poplar against snowshoe hares. *J. Chem. Ecol.* 16:1941–1959.

SOUTHGATE, P. C., and LOU, D. C. 1995. Improving the *n*-3 HUFA composition of *Artemia* using microcapsules containing marine oils. *Aquaculture* 134:91–99.