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DETECTION OF HYPOGEOUS FUNGI BY TASMANIAN BETTONG (*Bettongia gaimardi:* MARSUPIALIA; MACROPODOIDEA)

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Abstract—The ability of Tasmanian bettongs (*Bettongia gaimardi*) to locate hypogeous fungi (their main diet) was tested in a controlled laboratory situation. Bettongs dug directly over buried fungi significantly more often than they did over buried glass marbles or over disturbed soil. This ability was not enhanced as they gained experience. Bettongs dug more often over buried filter paper onto which fungus extract was absorbed than over control papers, and showed no discrimination between the outer and inner layers of the fungi. They preferred the odor of whole fungi to individual volatile compounds. They showed no reaction to the odor of the steroid ergosterol.

Key Words—hypogeous fungus, olfactory, bettong, *Bettongia gaimardi*, rat-kangaroo, *Mesophellia*, Marsupialia.

INTRODUCTION

Many hypogeous fungi form symbiotic mycorrhizal associations with the rootlets of vascular plants forming underground fruiting bodies known as sporocarps or truffles (Trappe, 1962; Maser et al., 1988). Such fungi depend on animals to consume the fruiting bodies and disperse the spores (Kotter and Farentinos, 1984). As spores mature within a sporocarp, the odor of the fungus intensifies and with it the chance of detection by mycophagous mammals (Claus et al., 1981; Maser et al., 1988). This study was undertaken to determine whether the odor of underground fungi is utilized by *Bettongia gaimardi*, the Tasmanian rat-kangaroo or bettong, for fungus location, as has been suggested but not proven

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(Burbidge, 1983; Delroy et al., 1986). The bettong's diet consists almost exclusively of hypogeous fungi (Taylor, 1992). Confirmation of the bettong's ability to locate fungi by odor cues is a necessary precursor to investigations on the semiochemical activity of volatile and nonvolatile compounds present in the fungus. In this study we concentrate on truffles of the genus *Mesophellia*, a particularly favored food of the Tasmanian bettong (C. Johnson, personal communication).

METHODS AND MATERIALS

Fifteen bettongs, born in captivity and naive to hypogeous fungi at the initiation of testing, were used as experimental subjects. They were maintained in outdoor pens, with several individuals in each pen. At all times they were supplied with water, fruit, bread, and cereals, a standard captivity diet for this species. Although there was no departure from a 1 : 1 sex ratio in the group of subjects, no attempt was made to balance the sex ratio in each experimental replicate, following the application of a χ^2 heterogeneity test to the results of a preliminary study. Three bettongs were used in each replicate. Truffles were collected from around the bases of trees of the genus *Eucalyptus* in the southeastern part of Tasmania.

In the first part of the study we investigated whether bettongs use odor for truffle location. Plywood trays measuring 75 × 60 × 10 cm deep, partitioned into 20 cells, each 15 × 15 × 10 cm deep, were filled with dry sandy loam, which was regularly replaced to prevent its contamination by truffle odor. Each of the cells was subjected to one of three treatments. Five glass marbles and five small *Mesophellia* sp. truffles were distributed at random in holes, 2 cm deep, made in the center of each cell. Although it is recorded that *Mesophellia* sp. sometimes occurs at depths in excess of 40 cm (Claridge et al., 1992), our field observations indicated that they frequently occur very close to the surface. Ten cells were left untreated as controls, and all holes were filled in. The digging and filling of holes in the untreated cells ensured that disturbance of the soil was not a variable. Marbles represented a nonfood item of a similar size and shape to truffles, for the purpose of investigating whether bettongs show exploratory digging behavior towards buried nonfood items as has been shown in some other small mammals (Holling, 1958; Howard et al., 1968). The trays were placed in the outdoor enclosures.

Before the trials, the criterion for a "dig" was established as being any excavation that reached a depth greater than 2 cm. Data were recorded by direct observation and a trial ended when five cells experienced a dig. Two trials were performed each evening for five consecutive nights in each of four bettong enclosures. Data were analyzed by χ^2.

In the second part of the study several species of hypogeous fungi were analyzed chemically. A sample of air saturated with volatiles from a fresh fungus was manually injected into a Hewlett Packard 5980 gas chromatograph (GC) attached to a 5970 HP mass selector (MS). The samples were frozen onto an HP5 (polymethyl siloxane) column (film thickness 0.52 μm, ID 0.32 mm, length 25 m). Peaks present in each sample were identified by comparison of their spectra with reference spectra from the NIST mass spectral data base. GC-MS analyses were repeated on two or three samples of truffles.

Sporocarps of *Mesophellia* sp. were also extracted with dichloromethane and analyzed by thin-layer chromatography (TLC) and GC-MS to identify the less volatile truffle compounds. Elution times of the truffle fractions were compared with those of known plant oils and steroids. GC-MS analysis of extracts from several species of fungi involved injection of extracts into the GC-MS equipped with an HP1 column (film thickness 0.17 μm, ID 0.32 mm, length 25 m), using an autosampler. Sporocarp peridia and glebae of *Mesophellia* sp. were extracted separately and analyzed to determine whether the two parts of the truffle contained the same compounds.

In the third part of the study several fractions of *Mesophellia* sp. truffles were individually tested for bioactivity, using the testing procedures outlined above. The fractions were dissolved in vegetable oil and applied to pieces of filter paper that were buried in the cells. Fractions included extract and residue of whole truffles, extracts of peridia and glebae, and the individual compounds identified from *Mesophellia* sp. headspace analyses. The concentration of all compounds was 5×10^{-6} mol in 0.05 ml of vegetable oil solution. This concentration was arbitrarily low, but based on levels of fungal volatiles that have been reported to be stimulatory to insects (Bengtsson et al., 1991) and because natural concentrations of compounds in mixtures of fungal volatiles are reported as "low" (Hutchinson, 1971), but without quantification. Separate trials were run for each component and for the vegetable oil vehicle, using the same experimental testing procedures as before.

RESULTS

Heterogeneity χ^2 tests showed that the results from all trials were homogeneous ($\chi^2 = 4.3$, $P > 0.05$). Digging by bettongs was not random ($\chi^2 = 430.5$, $P \ll 0.005$) with significantly more digs in truffle-containing cells than in either marble-containing cells or untreated cells (Figure 1). There was no significant improvement in the accuracy of bettongs to locate truffles following first exposure (Figure 2, upper trace) or any reduction in the time taken by bettongs to complete a trial following experience (Figure 2, lower trace).

Classes of compounds present in truffle volatile mixtures were simple alco-

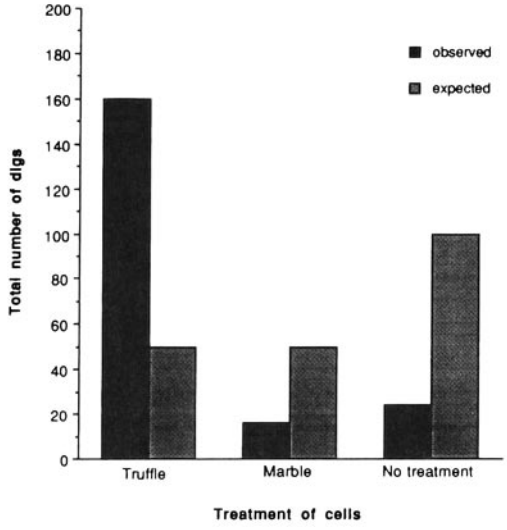

FIG. 1. Effect of buried truffles, buried glass marbles, or no treatment on foraging behavior by bettongs. The first five digs from 40 trials was recorded.

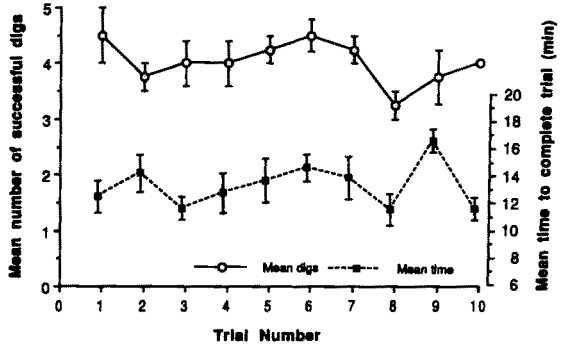

FIG. 2. Effect of experience on the accuracy of bettongs when locating buried truffles (upper trace), and effect of experience on the time taken for bettongs to complete a foraging trial, i.e., completion of five digs (lower trace) (mean ± 1 SE, for four replicates combined).

hols, aldehydes, ketones, alkenes, and esters. No single compound or group of compounds identified from the chromatographs was common to all species of hypogeous fungi tested. In all chromatographs, many minor components were present in concentrations too low for accurate mass spectral identification. The

GC-MS analyses of *Mesophellia* sp. volatiles revealed many compounds; most of the identifiable ones were esters. Both peridia and glebae of fresh *Mesophellia* sp. truffles contained the same volatile compounds. TLC and GC-MS analyses of truffle involatile extracts revealed that all extracts of both peridia and glebae consist of long-chain fatty acids (C_{16}–C_{24}) and the fungal steroid ergosterol.

χ^2 analysis of the data of bioactivity of truffle fractions revealed that bettongs significantly preferred cells that contained any truffle extract to cells that contained only soil ($\chi^2 = 12.8$, $P < 0.005$). Bettongs did not show a significant discrimination between extracts of peridia and glebae ($\chi^2 = 0.0$, $P > 0.05$). Bettongs were significantly attracted to the seven compounds (acetaldehyde, ethyl acetate, *n*-propyl acetate, isobutyl acetate, ethyl isobutanoate, ethyl butanoate, and ethyl propanoate) that dominate the *Mesophellia* sp. headspace chromatograph ($0.025 < P < 0.05$ for ethyl propanoate and $P < 0.005$ for the others). However, bettongs preferred the odor of the entire truffle to the odors of any of the individual compounds ($0.005 < P < 0.01$ for ethyl acetate and ethyl butanoate and $P < 0.005$ for the others). Bettongs did not respond to the separated components of truffle involatile compound extract (long-chain fatty acids and ergosterol) ($\chi^2 = 2.56$, $P > 0.05$ for both cases).

DISCUSSION

The observations reported here suggest that bettongs dig much more frequently in cells that contain truffles or pieces of filter paper onto which truffle volatile compounds have been applied than in cells that contain marbles or nothing other than soil. This suggests that bettongs respond to olfactory cues emanating from the fungi. We further suggest that bettongs do not need to learn the characteristics of truffle odor, since naive subjects were able to find hidden truffles as readily as experienced subjects. This conclusion is further supported by the observation that bettongs show no reduction in the time taken to locate truffles as they become more familiar with truffle odor. When truffles were located, they were eaten with great speed and apparent relish equally by experienced and inexperienced subjects. In this respect, bettongs differ from small rodents that have been reported to have to learn the characteristics of novel food items and exploit them with increasing efficiency (Holling, 1958). This is an interesting observation that requires further study.

Our observations reveal that while bettongs are significantly attracted to a range of volatile compounds found in truffles, they were more strongly attracted to the odor of whole truffles than to a single compound. This suggests that the cue that triggers digging behavior is chemically complex. Recent studies on European black truffles (*Tuber melanosporum*), however, have indicated that dimethyl sulfide is the compound that attracts not only dogs and pigs, but also

mycetophilous insects (Talou et al., 1990; Pacioni et al., 1991). In the experiments of Talou et al. (1990), one experienced pig and four dogs trained to truffle odor were used in a field-based series of location trials. Only dimethyl sulfide was detected on every occasion by both the pig and the dogs. It has previously been reported that pigs are attracted to a steroidal compound, 3α-hydroxy-5α-androst-16-ene, present in European truffle odor and also found as a sex pheromone in male pig saliva (Claus et al., 1981). Talou et al. (1990) found that, when presented alone, their test pig could not locate the buried source of this odor with the same accuracy and repeatability as it could locate either buried whole truffle odor or methyl disulfide alone. In our study with the Tasmanian bettong, we found no response to the odor of buried ergosterol, a steroid compound identified in *Mesophellia*, even though this compound has a strong odor to the human nose.

Tasmanian bettongs occur in dry forested areas characterized by poor quality soils, where the availability of plant and invertebrate food is generally low (Taylor, 1992). In such forests even epigeous fungi are uncommon. The detection of hypogeous fungi is therefore critically important for survival in this type of environment. A number of other species of rat-kangaroos also feed on hypogeous fungi (e.g., brush-tailed bettong, *B. penicillata*; rufous bettong, *Aepyprymnus rufescens*; long-nosed potoroo, *Potorous tridactylus*; and long-footed potoroo, *P. longipes*), but whether these species utilize odors in the same way as we have shown the Tasmanian bettong to do is not known. As all rat-kangaroos are endangered or vulnerable (Ride and Wilson, 1982), further research into this important aspect of bettong feeding ecology is clearly needed.

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