Semiochemicals of the Scarabaeinae, V*: Characterization of the Defensive Secretion of the Dung Beetle *Oniticellus egregius*

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Using techniques that were developed for the concentration and analysis of airborne organic volatiles, methyl salicylate and 1,4-benzoquinone were found to be the only two compounds present in detectable quantities in the effluvium of *Oniticellus egregius*. These two compounds, previously identified in the defensive secretions of other arthropods, are presumed to be produced as a defense mechanism against predators, such as birds. Methyl salicylate and 1,4-benzoquinone have thus far not been found to occur in other Scarabaeinae.

Introduction

Depending on their nesting behaviour, dung beetles belonging to the subfamily Scarabaeinae may be divided into three groups (Halffter and Matthews, 1966; Bornemissza, 1969). Paracoprids construct their nests under or beside the dung source by excavating tunnels into which they pack dung, endocoprids excavate a chamber in the dung pat itself, forming brood balls within this chamber, and telecoprids remove a portion from the dung pat, mould it into a ball and roll it some distance away from the dung source before burying it in the soil. Of the 71 genera of dung beetles of the Scarabaeinae in Africa south of the Sahara listed by Ferreira (1972), only two genera comprising six species, are endocoprid. One of these endocoprid species, *Oniticellus egregius* Klug constructs its brood ovoids of dung in the soil immediately under the edge of the dropping, enveloping each ovoid in a soil shell before clearing the loose earth around the ovoids to produce a brood chamber (Davis, 1989). Due to the extreme temperature fluctuations imposed by the proximity of endocoprid brood chambers to the soil surface, *O. egregius* is restricted to the tropical parts of southern Africa where winters are sufficiently warm for overwintering. This species has been recorded mainly from the coarse-fibred dung of elephant, rhinoceros, and zebra (Davis, 1977).

Several protective mechanisms are utilized by *O. egregius*. The dorsal surface of this small insect (9.8 to 15.5 mm) (Davis, 1977) is metallic blue-black with a yellow border (Fig. 1a), while the ventral surface is mottled yellow and gold. If disturbed, *O. egregius* flip themselves onto their backs and exhibit thanatosis (remaining still), holding the middle and hind legs away from the body (Davis, 1977). This exposes the undersurface which is coloured similarly to the shredded fibres of rhinoceros or elephant dung and renders them inconspicuous (Fig. 1b).

If the insect is disturbed, the fore legs which are held close to the body, are suddenly released with sufficient force to lift the beetle as high as 60 cm into the air. This behaviour may serve as an escape mechanism and might conceivably frighten off vertebrate predators (Davis, 1977). At the same time an odour, reminiscent of the smell of oil of wintergreen, can be detected and is often associated with the release of a brown fluid from the lateral edge of the anterior abdominal segments just posterior to the hind legs.

In this communication the organic compounds in the defensive secretion of *O. egregius* are identified.

Materials and Methods

Gas chromatographic determinations were carried out with a Carlo Erba 4160 chromatograph

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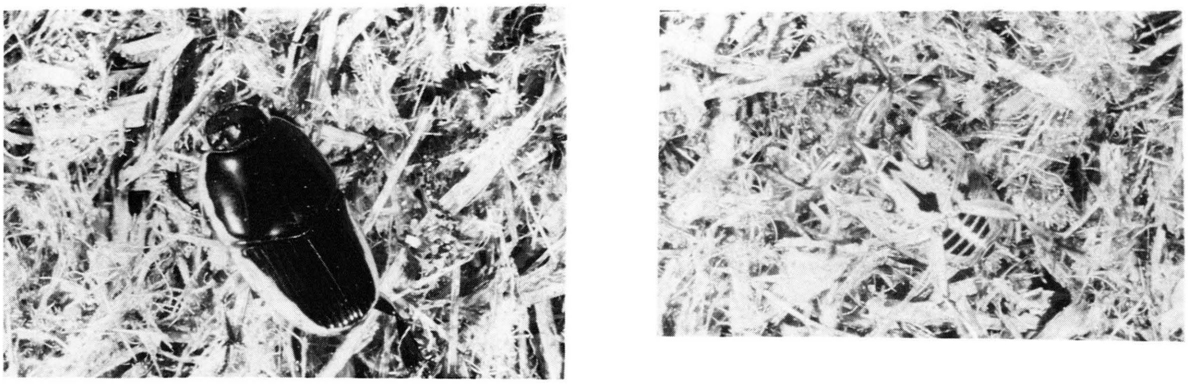

Fig. 1. Dorsal (left) and ventral (right) aspects of *Oniticellus egregius* Klug.

fitted with a flame ionization detector (FID). The instrument was equipped with a 40 m x 0.3 mm glass capillary column coated with OV-1701-OH at a film thickness of 0.4 μm. Helium was used as carrier gas at a linear velocity of 28.5 cm/s at 40°C, and a temperature program of 4°C/min from 40°C to 250°C was employed. Mass spectra were recorded on a Carlo Erba QMD 1000 quadrupole mass spectrometer, using the same column and gas chromatographic conditions.

Pyrex glassware used in experiments was heated to 500°C in an annealing oven to remove any traces of organic material. Synthetic compounds for comparison were purchased from Merck.

The 1992/1993 summer was exceptionally dry in Natal and in the Mkuzi Game Reserve, during the first week of November 1992, only four *Oniticellus egregius* were collected using pitfall traps baited with horse dung (Tribe, 1976).

The secretion was collected for analysis using a headspace gas sampling technique (Burger *et al.*, 1991). A dung beetle was carefully coaxed into a 7-ml screw-cap glass vial, the vial was closed with a screw cap fitted with a PTFE-faced rubber septum (Fig. 2), and synthetic air, purified by passing it through a column of activated charcoal, was introduced into the vial through a drawn out glass capillary tube inserted through the septum. A capillary trap, coated with an apolar stationary phase at a film-thickness of 15 μm was inserted into the vial through a second pre-formed hole through the septum. A suitable length of capillary tubing was used as a restrictor to give a pre-selected flow-rate of 2.5 ml/min through the trap. This restrictor was connected to the outlet end of the trap with shrinkable PTFE tubing. The beetle was irritated by prodding it with the capillary trap to induce release of the secretion, whereafter a total volume of 10 ml of air was passed through the trap at the specified flow-rate.

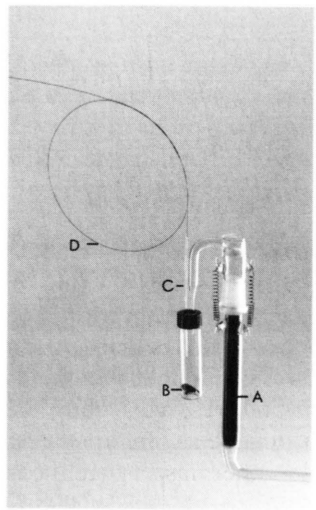

Fig. 2. Trapping organic volatile compounds from the effluvium of *Oniticellus egregius* after it has been induced to release its defensive secretion. Synthetic air is purified by passing it over a column of activated charcoal (A). The volatiles released by the dung beetle (B) are transferred to the capillary trap (C), and retained by dissolution in a thick film of apolar stationary phase. The flow through the trap is regulated by using a capillary flow restrictor (D).

The trap was disconnected from the shrinkable PTFE tube and the organic compounds retained on the trap were analyzed gas chromatographically or by GC-MS as follows: Using PTFE tubing, the trap was connected to a capillary gas chromatographic column fitted with a ferrule near its inlet end. A small polystyrene container, filled with solid CO_2 was clamped to the capillary column a few centimeters below the trap to cryofocus the volatile compounds desorbed from the trap as a sharp band on the column. With the carrier gas already turned on, the trap was inserted into the gas chromatograph's injector at a temperature of 220°C. This was done to provide a slight positive pressure at the inlet end of the trap so as to prevent volatiles escaping from the trap while it was being inserted into the hot injector. Coupling the column to the injector was accomplished in the usual manner by tightening the ferrule-retaining nut. The desorption of the volatile compounds from the trap was allowed to proceed for 15 minutes, whereafter the solid CO_2 was quickly removed from the capillary column, and the analysis started. The capillary trap was left in the injector during the analysis. The same procedure was followed in GC-MS analyses.

Results and Discussion

The secretion is at first readily released by *O. egregius* but after about the third discharge no further liquid is secreted, either because of conditioning or because the reservoir is empty. This does not seem to be unique as similar observations have been made in other insects (*e.g.* Burger *et al.*, 1986). Because *O. egregius* beetles were captured in pitfall traps together with other dung beetle species, some of which such as *Pachylomerus femoralis* (Kirby) are quite aggressive when confined in large numbers in the small space of the trap, the supply of defensive secretion was found to be depleted. It was therefore not possible to collect the secretion in bulk and a technique developed for the determination of airborne organic compounds was used to filter the volatile organic compounds from the effluvium of the insects. This method entails the concentration or trapping of the volatile compounds by their dissolution in a thick film of an apolar stationary phase on the inside wall of a glass capillary (Burger *et al.*, 1991), a method yielding excellent results. A typical reconstructed total ion chromatogram of material desorbed from the trap is shown in Fig. 3. The most notable feature of this chromatogram is that it is exceptionally free from background peaks. This is partly due to the fact that the insects apparently still released relatively large quantities of the secretion, but the use of purified air in the experiment also contributed to the elimination of background peaks of compounds present in laboratory air. Another factor contributing to the absence of contaminants is that *O. egregius* nests in very fibrous dung which does not adhere to the insect's body. The two constituents trapped from the effluvium of the insect were identified as methyl salicylate (1) and 1,4-benzoquinone (2) by

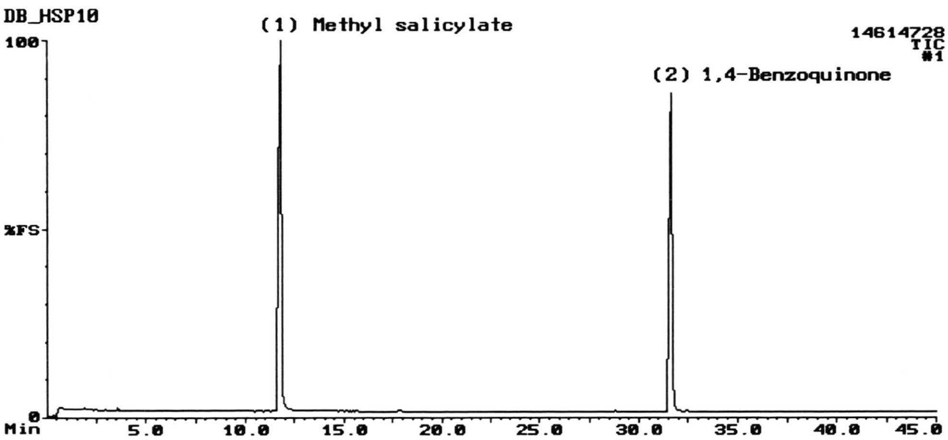

Fig. 3. Total ion chromatogram (TIC) of volatile organic compounds trapped from the effluvium of *Oniticellus egregius* on a capillary trap coated with cross-linked polydimethylsiloxane stationary phase having a film thickness of 15 μm. Glass capillary column coated with OV 1701-OH (40 m x 0.3 mm i.d., film thickness 0.4 μm); temperature programmed from 40 to 250°C at 4°C/min.

mass-spectral and retention-time comparison with authentic synthetic compounds.

A vast amount of information has been accumulated on the subject of arthropod defensive secretions (Blum, 1981). 1,4-Benzoquinone and its derivatives have been identified in several orders of the Diplopoda and Insecta and these compounds appear to be relatively common in the defensive secretions of the Coleoptera (*e.g.* Blum, 1981; Moore and Wallbank, 1968; Gnanasunderam, *et al.*, 1985). The esters of salicylic acid seem to be much less well represented, and methyl salicylate has been found only in the secretion of a carabid beetle (Schildknecht *et al.*, 1968). To the best of our knowledge, very little information on the existence (Davis, 1977), and none on the chemical composition of defensive secretions in the Scarabaeinae has so far been published. The results reported here appear therefore to be the only chemical evidence for the existence of chemical defense mechanisms in this sub-family as yet.

This research also demonstrates the elegance with which qualitative analyses of organic compounds in the effluvia of small organisms can be carried out by using techniques developed for headspace gas analysis.

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