The olfactory response of the reindeer nose bot fly, Cephenemyia trompe (Oestridae), to components from interdigital pheromone gland and urine from the host reindeer, Rangifer tarandus

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Summary. The linked gas chromatographical/electroantennogram (GC/EAG) technique revealed that the parasitic reindeer nose bot fly is able to specifically sense components produced by the interdigital pheromone gland of reindeer. The head-space extraction technique, with Porapak Q as the collecting polymer, was used to trap pheromone gland and urine components used to assess fly responses. One component from reindeer urine also was a potent stimulus for the sensory neurons of the fly. These components can be important chemical signals to the flies for long distance orientation towards host animals. This is the first report on EAG in Oestridae.

Key words. endoparasite – pheromone – host attraction – Diptera – Cephenemyia trompe – Rangifer tarandus

Introduction

Two parasitic flies extensively infest reindeer, Rangifer tarandus (L.), in Norway, Sweden, Finland, Russia and North America (Zumpt 1965; Wood 1987). The reindeer nose bot fly, Cephenemyia trompe (Modeer) (Diptera: Oestridae), infests the nasal passages and throat, whereas the reindeer warble fly, Hypoderma tarandi (L.), infests the body (Zumpt 1965; Anderson & Nilssen 1990). Both species are common where reindeer are infested, these parasitic flies must cope with long distance in finding their hosts.

These oestrids have a limited life span and do not feed as adults. Even so, they must have the capacity for flying long distances as well as efficient mechanisms to locate their hosts which may be dispersed over vast areas. Anderson & Olkowski (1968) found that carbon dioxide attracted North American Cephenemyia females, and Anderson & Nilssen (1985; unpubl.) later found that carbon dioxide also attracted the reindeer nose bot fly C. trompe under field conditions in northern Norway.

As C. trompe and H. tarandi almost exclusively attack reindeer (Zumpt 1965), more host specific attractants are likely. Field observations (Nilssen & Anderson unpubl.) indicate that these flies are able to find reindeer from long distances and after many hours (e.g. one night at least). For obvious reasons, simultaneous emittance of volatiles from reindeer is unlikely to be functional chemical signal over long distances, after many hours and in varying wind directions. Therefore we hypothesized that more lasting cues, i.e. compounds deposited on the ground by the reindeer, might function as attractants. Likely candidates were thought to be the reindeer interdigital glands between the hoofs and deposits of urine and/or feces on the ground. Components produced by the interdigital gland are partly known, with the major ones functioning as reindeer pheromones (Brundin & Anderson 1979; Andersson et al. 1979).

For other Diptera, host finding and host attractants have been studied most extensively for tsetse flies (Glossina spp.). Carbon dioxide, acetone and 1-octen-3-ol, identified as components in cattle odour (Hall et al. 1984), have elicited positive responses both at the receptor neuron level (Den Otter & Van der Goes Van Naters 1990) and in behavioural studies (Vale & Hall 1985a,b; Vale et al. 1985; Warnes 1989; KyorKu et al. 1990), whereas para-cresol and 3-n-propylphenol have been the most attractive phenols isolated from bovine urine (Bursell et al. 1988; Ogawa et al. 1988).

The aim of the present study was to test the hypothesis that compounds from both the interdigital pheromone gland and reindeer urine are active in the behavioural decisions leading C. trompe to a host. The
method used to test the olfactory responses of the antenna to specific compounds from the host was the recording of electroantennograms (EAG). This is the first report on EAG in Oestrinae. The test compounds used included both synthetic compounds, and extracts from reindeer interdigital pheromone gland and urine fractionated by gas chromatography (GC).

Materials and methods

Insects

The Cephenemyia trompe (Modeer) used originated from the county of Finnmark, Northern Norway. Some flies (females) were caught wild on white reindeer hides in the field, whereas others were laboratory reared from larvae dropped from reindeer kept in captivity. As adults live only 2–4 weeks, a supply of living insects was secured over a two month period by holding larvae/pupae in chambers of different temperatures.

Preparation and electrophysiological recordings

Flies were immobilized in a plexiglass holder, and antennal movement was prevented by securing the base segment to the head by water-based Tipp-ex® fluid. The antenna was cut at the distal end and a recording glass capillary electrode filled with insect ringer solution was slipped over the tip of the antenna to make contact. The indifferent glass electrode was inserted near the base of the antenna. In this species the control of the smallest movement and the prevention of leakage of electrode ringer solution were extremely important for long lasting (2–3 h) successful EAG-recordings with sustainable baseline for evaluation of antennal responses.

Linking gas-chromatograph to electroantennogram recordings (GC/EAG) (Guerrin et al. 1983). A Carlo Erba GC connected to a LDC Milton Roy integrator and printer, which continuously displayed the retention time, was used. The GC was equipped with a FID-detector and a S.G.E. 25 m fused silica BP 5 capillary column, film thickness 0.5 µm. The capillary column was split at the end using a Chrompack quick-seal splitter. Half of the effluent was led through a deactivated fused silica tube to the detector while the other part was led in the same way out of the GC-oven in a temperature controlled heated transfer line into an airstream continuously blowing over the antenna at the rate of 500 ml/min (cf. Tommerås & Mustaparta 1987).

Test compounds

The synthetic compounds were provided by G. Andersson, Forsvarets Forskningsanstalt in Umeå, Sweden (cf. Andersson et al. 1979). The compounds, previously found to be the three main components of the interdigital pheromone gland of reindeer (Andersson et al. 1979), were: 1-hydroxy-7-methyl-3-octanone, 7-methyl-1-octen-3-one and 7-methyl-3-octanone. 1-octen-3-ol, known as a common volatile from many bovines and other ruminants (Hall et al. 1984; Bursell 1984), was bought commercially from Merck Company, FRG. Another substance known to be produced in mammalian urine, para-cresol (Merck Company), was also tested. In addition, CO₂ was qualitatively tested by the use of dry ice inside a syringe and blown over the insect antenna. The following alcohols were on the stimulation program to broaden the knowledge of the olfactory receptor system: propanol, butanol, pentanol, hexanol, heptanol and octanol.

Extracts

Extracts were made both from reindeer urine (1 year old males) and from interdigital pheromone glands (males and females partly from captive animals fed on lichens and partly wild reindeer from Rondane, a central mountain area in southern Norway). Fresh urine and freshly cut glands from killed reindeer were immediately frozen and stored at –20°C. One type of extracts was obtained by a straight forward extraction procedure using 4 ml dichloromethane directly into 8 ml urine or pieces of 6–8 interdigital glands, and concentrated to 100 µl using N₂. The headspace procedure, using Porapak Q as collecting polymer, was the second way used for trapping volatiles. Twelve interdigital glands cut into 4–6 pieces or 200 ml of urine were kept in a glass flask sucking charcoal cleaned air from the room (200 ml/min) for at least two days at room temperature. The Porapak (0.6–0.8 g in each trap) was cleaned by pentane, using soxhlet for 6 h and then conditioned by N₂; after extraction the Porapak was washed out with 2 ml pentane. The extracts were then concentrated to about 100 µl using N₂.

Experimental procedure

Synthetic compounds applied from a “syringe olfactometer” (Kafka 1970) were tested by the stimulation procedure described by Mustaparta et al. (1980). The synthetic compounds were tested at six concentration steps, from the lowest to the highest. A syringe containing 0.01, 0.1, ..., 1000 µg of the compound inserted onto a filterpaper, was used.

GC-stimulation

After obtaining as stable a baseline as possible for the recordings, a screening test using the synthetic compounds was performed. Then the extracts were injected “on column” in the GC for stimulation via the GC-column. The GC was programmed as follows: 1 min standby at 32°C, then 10°C/min up to 72, 5°C/min to 122°C and 10°C/min up to 200°C with a final isothermal period lasting 3 min. Both the chromatograms and the EAG-level were printed out on printers using the same chart speed. A controlled reset procedure was used to have the possibility for exact comparison of the chromatography data and EAG-level both during and after the stimulation runs.

The same extract was injected as many times as possible to obtain repetition in order to discriminate real responses from uncontrolled shifts in baseline.

Results

Seven females were tested in the EAG-studies using synthetic compounds by the “syringe olfactometer” stimulation technique. In all experiments, CO₂, a piece of a reindeer interdigital gland inside a syringe, and the extracts inserted onto a filterpaper inside a syringe, and the extracts inserted onto a filterpaper were used.

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