

## Observations of summit disease in *Formica rufa* LINNAEUS, 1761 (Hymenoptera: Formicidae)

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### Abstract

In coastal dunes of The Netherlands, an infection of the fungus *Pandora myrmecophaga* (TURIAN & WUEST, 1969) KELLER & PETRINI, 2005 was observed in a population of *Formica rufa* LINNAEUS, 1761. The parasitic fungus causes a behaviour in the ant that has been described as summit disease. *Pandora myrmecophaga* infections have been reported only from *Formica* species and I could not detect summit disease in other ant species. Based on field and laboratory observations, details of the behaviour of infected *Formica rufa* workers are presented, largely confirming earlier observations, but adding new insight.

**Key words:** Entomophthoraceae, fungal infection, *Pandora myrmecophaga*, summit disease, *Formica*.

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### Introduction

Many insect species are susceptible to summit disease: the infected insect seeks an elevated position where wind currents can effectively disseminate conidia (ROY & al. 2006). Summit disease is caused by several species of the fungal family Entomophthoraceae (Zygomycota, Entomophthorales), whose representatives also cause other diseases in many arthropod species. Entomopathogenic fungi produce infective spores that attach to, germinate on, and penetrate the cuticle of their hosts, ultimately killing them (ROY & al. 2006). *Pandora myrmecophaga* (TURIAN & WUEST, 1969) KELLER & PETRINI, 2005 is one of these fungi. It has exclusively been observed to infect ants of the genus *Formica*, namely *Formica rufa* LINNAEUS, 1761, *F. polyctena* FOERSTER, 1850, *F. pratensis* RETZIUS, 1783 and *F. cf. lemani* BONDROIT, 1917 (LOOS & ZIMMERMANN 1976, BALAZY & SOKOŁOWSKI 1977, TURIAN & WUEST 1977, BALAZY 1993) – note that the infected "*F. fusca* LINNAEUS, 1758" photographed by TURIAN & WUEST 1977 is probably *F. lemani*, judging by the pilosity on pronotum and gaster. The fungus is known from the former USSR, Sweden, Poland, the former Yugoslavia, the former Czechoslovakia, Switzerland, and Germany (BALAZY 1993). Infections were observed in May, August and September (LOOS & ZIMMERMANN 1976, BALAZY & SOKOŁOWSKI 1977, TURIAN & WUEST 1977). Typically, infected worker ants are found strongly attached by their mandibles and legs to distal parts of leaves of grass and other plants and fallen pine needles. The exact mechanisms leading to elevation seeking are unknown (ROY & al. 2006). The fungal mycelium grows in the head, mesosoma, gaster, antennae, and legs of the ant. Production of conidia typically occurs at intersegmental parts of the gaster (BALAZY & SOKOŁOWSKI 1977) (Fig. 1).

Here I present the first record of *P. myrmecophaga*-triggered summit disease from The Netherlands, in *Formica rufa*. I present details of the behaviour of the infected wood ants, from both the field and the laboratory.

### Methods and materials

All data were collected in an area of 1 × 1 km in the coastal dunes of Bergen, The Netherlands, 52°40' N, 04°40' E, in October 2007. This area is situated in the centre of non-calcareous dunes, 3 - 20 m above sea level. The main vegetation units in this area are: *Vaccinio-Bertuletea pubescentis*, *Quercu-Fagetea*, *Calluno-Uliceteta*, *Koelerio-Corynephoretea* and *Epilobietea angustifolii* (syntaxa according to SCHAMINÉE & al. 1996, 1998, STOTELDER & al. 1999). The wood ant population is monodomous. For 74 wood ant mounds, data were collected concerning the distribution of trees and shrubs in the habitat, the construction material of the nest, whether the mound was covered by *Carex* / *Calamagrostis* or heather, the shading of the mound, and the presence of mushrooms, as an indicator of humidity.

I searched for wood ants *F. rufa* which had attached themselves to plant leaves, both on ant mounds as well as in their surroundings. In the same area and period, I also looked for ants attached to plant leaves from nests of other ant species, particularly *Lasius meridionalis* (BONDROIT, 1920) (ca. 20 nests), *L. psammophilus* SEIFERT, 1992 (> 100 nests), *Myrmica sabuleti* MEINERT, 1861 (> 100 nests), *Formica exsecta* NYLANDER, 1846 (12 nests), and *F. fusca* (8 nests).

Twelve living ants, that were identified to be infected because of behaviour anomalies (for details see Results), were kept in 4 litre jars together with leaves of *Carex are-*



Fig. 1: *Pandora myrmecophaga* on *Formica rufa*, from coastal dunes of Bergen, The Netherlands, 4.X.2007. Photo: H. Niesen.

*naria* L., to study their behaviour. About 45 dead infected ants attached to leaves were collected, to study the nature of the attachment.

The identification of the fungus was confirmed by S. Bałazy. He made microscopic slides of the fungus to examine the conidia morphology by transmitted-light microscopy. The collected infected ants have been deposited in the private collection of the author and the collection of S. Bałazy, Research Centre for Agricultural and Forest Environment, Poznan, Poland. Images of the fungus were made with an Olympus motorized stereomicroscope SZX12, at magnifications of 50×, using AnalySIS Extended Focal Imaging Software.

To find possible preferences of *P. myrmecophaga*, characteristics of infected nest mounds were subjected to G tests for goodness of fit with Williams' correction for small sample (SOKAL & ROHLF 1995). The distribution of characteristics in all nest mounds was taken as the "expected values" to detect possible significant deviations in the infected nest mounds.

## Results

*Formica rufa* wood ants displaying summit disease were found on 16 (= 22 %) of the 74 investigated wood ant mounds between 4 and 23 October 2007 (including seven mounds without any vegetation within a radius of 1 metre). These wood ants were almost exclusively found on the vegetation on the mounds. This vegetation consisted mainly of *Carex arenaria* (Fig. 2), sometimes *Calama-*



Fig. 2: One of the *Formica rufa* mounds where dozens of *F. rufa* workers infected by *Pandora myrmecophaga* were found. The mound is partly overgrown by *Carex arenaria*. It is situated in a coniferous wood, bordering a birch-oak wood, in the coastal dunes of Bergen, The Netherlands. Photo: P. Boer.

*grostis epigejos* (L.) ROTH and once *Empetrum nigrum* L. One infected wood ant was found on a dead twig of *Populus tremula* L. above a mound.

The sixteen mounds with *P. myrmecophaga* infections were distributed over the whole research area, without any significant preference of association with mound construction material (birch, oak, pine, spruce, heather;  $G = 1.66$ ,  $p = 0.65$ ), degree of covering by *Carex* / *Calamagrostis* ( $G = 0.08$ ,  $p = 0.96$ ), degree of covering by heather ( $G = 0.62$ ,  $p = 0.73$ ), presence of mushrooms ( $G = 0.02$ ,  $p = 0.80$ ) or degree to which the mound was exposed to sunlight ( $G = 5.06$ ,  $p = 0.28$ ). However, the distribution of the vegetation (in terms of the dominant trees or shrubs) surrounding the infected nest mounds deviated significantly from the value for all nest mounds ( $G = 8.68$ ,  $p < 0.05$ ): *P. myrmecophaga* was found significantly more often than expected in proximity to deciduous trees.

By means of several observations in the field and in laboratory conditions, a description can be made of the phases of the disease in the infected ant until sporulation of the fungus.

(1) Approximately a day before the ant dies, she becomes sluggish, with uncoordinated movements.

(2) The ant climbs to a plant on or around the nest. The movement on the leaf is uncertain, and uncoordinated; the antennae are stretched along both sides of the leaf and the normally very agile legs regularly slide off the leaf. The mandibles are continuously opened and closed. In an anthropocentric analogy, the movements resemble those of a drunken person, with arms and legs swaying.

(3) When the ant reaches the top of the leaf, she becomes very unsteady, as if frightened of falling. Then she descends approximately 8 cm, turns and goes up again. This behaviour continues repeatedly. The ant does not return to the ground or the wood ant mound.

(4) At a certain moment the ant attaches herself with her jaws around the plant leaf, usually with the head upwards, some centimetres below the top (Fig. 3). Within some

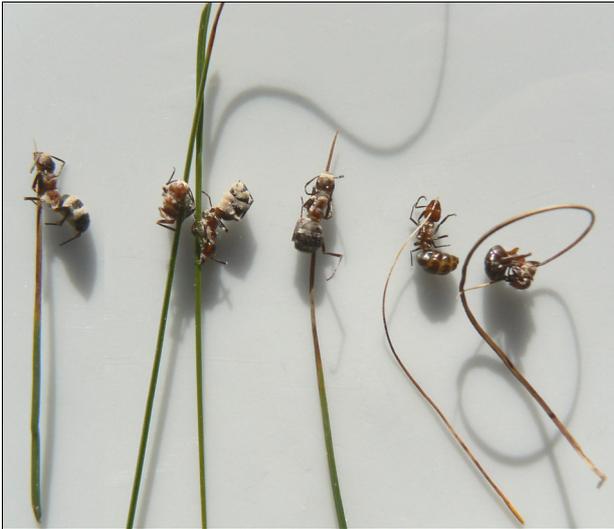


Fig. 3: Dead infected *Formica rufa* workers attached to the distal parts of leaves, mostly of *Carex arenaria*. Photo: P. Boer.

hours she dies; I took an ant for dead when all movements stopped and when touching triggered no reaction.

(5) Within hours after attachment, the rhizoids of *Pandora* appear out of the intersegmental parts of the basisternum and laterocervical plates and attach the ant to the leaf (Fig. 4). The ant is now firmly fastened to the substrate. The viscous rhizoids glue the ant to the stem and harden very quickly on exposure to air.

(6) Within one or two days fur-like fungus appears out of the intersegmental parts of the mesosoma and gaster, mainly from the dorsal parts (Fig. 1), and somewhat later also near the bases of the mandibles and the antennal insertions.

### Discussion

Although I have been monitoring the *Formica rufa* population of the coastal dunes of Bergen for years, I have never observed the infection before, possibly due to failure to detect it. In line with previous reports of *P. myrmecophaga* to be host specific to the *Formica* subgenera, *Formica* s. str. and *Serviformica*, I could not find any sign of summit disease at or around nests of the other ant species present in the habitat. MARIKOWSKY (1962) attributed his observations of summit disease in *Formica* wood ants to the fungus *Alternaria tenuis* NEES (1817). Possibly, though, also those ants had primarily been infected by *Pandora myrmecophaga* and *A. tenuis* had been acquired only as a secondary infection (S. Bałazy, pers. comm.).

My behavioural observations largely agree with those of MARIKOWSKY (1962) and LOOS-FRANK & ZIMMERMANN (1976). An important difference between my observations and those of LOOS-FRANK & ZIMMERMANN (1976) is that they generally observed that first *Pandora* attaches to and twists around the leaf, the ant still being alive, and only then does the wood ant start holding onto the leaf with her mandibles. I did not observe fungal rhizoids on collected wood ants in phases 1 through 4, and neither did MARIKOWSKY (1962). ROY & al. (2006) consider the active attachment to the substrate by the insect host through use of mandibles to be limited to Coleoptera (Cantharidae) in-



Fig. 4: *Pandora myrmecophaga* rhizoids growing out of the intersegmental parts of the basisternum and laterocervical plates of a *Formica rufa* worker that had died of the infection. Magnification 50×. Photo: C. van Achterberg.

fectured with Entomophthorales. The host attachment process reported here, a combination of first, attachment by mandibles and second, appearance of fungal rhizoids more firmly attaching the ant, could thus be unique among Entomophthorales fungi.

MARIKOWSKY (1962) stated that infected ants leave their nest only in the evening, before attaching themselves. This is in accord with a wide range of other studies documenting that entomophthorean fungi kill insect hosts during the late afternoon or evening (ROY & al. 2006). I did, however, observe this behaviour over the whole day.

I did not observe that apparently healthy workers of the colony tried to stimulate infected workers to move away from the nest, which one might have expected as a behaviour evolved by the ants to reduce pathogen transmission. Rather, the infected ants invariably moved to elevated positions above and around the nest, i.e., positions optimal for the fungus for sporulating and reinfection. MARIKOWSKY (1962) noticed that workers removed the dead ants (in pieces) from the leaves, carried them to the nest and used them for food (except dead ants covered with conidia). I repeatedly observed workers touch the dead ants with their antennae, including the ones with conidia, but I never observed that they removed them.

*Pandora myrmecophaga* does not seem to be a fungus species which threatens local wood ant populations. Given the number of ants collected per mound and size of the mound population, I estimate that the number of victims was below 0.1 % of the population at any given time in October 2007, assuming that all infected ants can be observed outside the nest. Note, though, that infected ants are easy prey for predators such as spiders, robber flies and tits and some could thus have been removed prior to detection by the researcher.

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### Zusammenfassung

In Küstendünen der Niederlande wurde eine Infektion des Pilzes *Pandora myrmecophaga* (TURIAN & WUEST, 1969) KELLER & PETRINI, 2005 in einer Population von *Formica rufa* LINNAEUS, 1761 beobachtet. Der Pilz verursacht bei seinen Wirtsameisen die "Wipfelkrankheit" mit charakteristischem Verhalten. *Pandora myrmecophaga*-Infektionen waren bisher nur von *Formica*-Arten bekannt. Auch ich konnte die Infektion nicht bei anderen Ameisenarten feststellen. Anhand von Feld- und Laborbeobachtungen schildere ich Details des Verhaltens der infizierten *Formica rufa*-Arbeiterinnen. Großteils stimmen diese Beobachtungen mit solchen früheren Datums überein, aber ich füge neue Details hinzu.

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