Short communication

CASE OF WILLOW RUST SPORES (*MELAMPSORA* SPP.) COLLECTED BY HONEY BEES

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Abstract

Honey bee workers (*Apis mellifera* L.) collect nectar, honeydew and pollen from plants in orderto provide the colony with, among others, carbohydrates and protein. Whenever these sources are unavailable in the environment, bees turn to alternatives. An example of this are fungi spores collected accidentally or on purpose. This last phenomenon is the aim of this study, in which we have shown that worker bees can collect willow rust (*Melampsora* spp.) spores. We observed as the bees obtained spores and placed them in pollen baskets. The presence of spores was demonstrated with the use of a scanning electron microscope (SEM). This observation indicates that the honey bee may use alternative sources as a potential supplement. There are few studies in this field and it requires a deeper analysis.

Key words: fungi spores, honey bee workers, SEM

INTRODUCTION

Honey bee workers collect nectar, pollen, honeydew and propolis. They were also observed collecting wax in pollen baskets (Olszewski et al., 2022) and fungal spores (Bushnell, 1972; Renzi et al., 2016a; Rutkowski et al., 2023). The carbohydrates derived from nectar or honeydew are sources of energy. The availability of protein from pollen is related to the functioning of the bee colony, including the longevity of the bees or the production of royal jelly (Human et al., 2007). Honey bee workers, when they have limited access to pollen, seek alternative sources of protein, and one such source may be fungal spores (Shaw, 1990). Simone-Finstrom et al. (2016) indicate that fungi affect bee foraging, development, and pathogen interactions, however, there is evidence that some fungi can be bees symbiotics, but knowledge about this phenomenon is still scarce (Rutkowski et al., 2023). While low quality and shortage of pollen have been raised as hypotheses for fungal spore collection, the nutritional value of fungal spores for honey bees is poorly understood (Renzi et al., 2016b). Fungal spores are also considered as sources of many lipids important in honey bee survival (Bushnell, 1972).

Willow rusts are fungal diseases that appear on willows (*Salix*), and clusters of spores (*Melampsora* spp.) in the form of uredinia appear on the underside of the leaves. The genus *Melampsora* belongs to the order Pucciniales, commonly known as the rust fungi. Within the family Melampsoraceae, the genus *Melampsora* encompasses a diverse group of rust fungi, approximately 100 described species. More than half of these species have been found

and described on plants within the *Salicaceae* family (Ciszewska-Marciniak & Jędryczka, 2011). As willow rust develops, the tree loses its leaves, which causes severe defoliation (Bubner et al., 2014). In our work, we want to show the interesting behavior of worker honey bees which collect the spores (uredinia) of willow rusts in their pollen baskets on their legs. This is the first documented observation regarding willow rust spores (*Melampsora* spp.).

MATERIAL AND METHODS

At the Arboretum Wojsławice (Poland, 50°43' N, 16°50' E), working bees were observed visiting the underside of infected *Salix* x *wimmeriana* leaves (Fig. 1), where there are clusters of spores, called urediniospores. Rust spores were collected from the leaves of *Salix* x *wimmeriana*, which is an interspecific hybrid introduced to Poland. The third pair of legs with the pollen basket, willow rust spores (*Melampsora* spp.) on the leaves and isolated spores, were fixed in 2% glutaraldehyde in a cacodylate buffer (pH=7.2), for 24 hours in 4°C. Then, the samples were washed in cacodylate buffer and next run in an ascending alcohol series (30%, 50%, 70%, 90%, 96%, 99.8%) - each alcohol for 10' (minutes). Scanning Electron Microscope (SEM) Auriga 60 Zeiss (Germany) was used to image the sample in SmartSEM V05.06 software.

RESULTS AND DISCUSSION

Sample observation with a microscope confirms the presence of *Melampsora* spp. spores in the pollen baskets of honey bee workers (Fig. 2). Previously, the consumption of fungi or their spores by bees were believed to be mainly associated with accidental ingestion or limited to yeast (Canché-Collí et al., 2021). Parish et al., (2020) show that honey bees can also eat spores of *Botrytis cinerea*, *Cladosporium spp.*, or *Colletotrichum acutatum.* In our study, we showed that honey bee workers actively collected spores of *Melampsora* spp.

SUMMARY

The presented observations clearly indicate that the *Melampsora* spp. spores are collected by honey bee foragers. The behavior associated with taking up fungal spores by honey bees is poorly understood. It would be worth deepening knowledge in this field and considering fungi for microbiological tests and learning whether bee workers collect fungal spores as a substitute for pollen when its sources are insufficient, and what nutritional value *Melampsora* spores have.

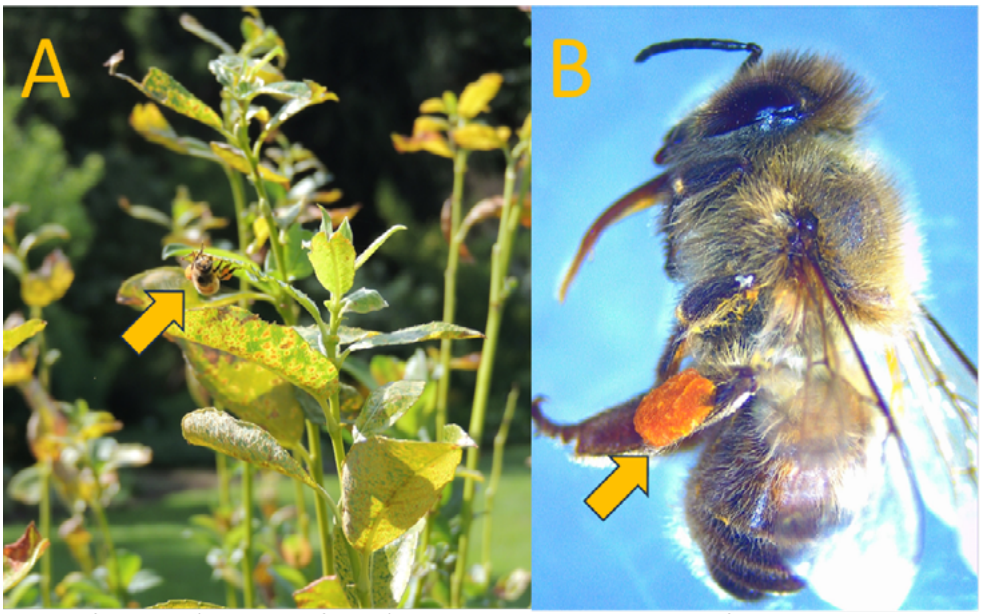

Fig. 1. A) Honey bee foragers (*Apis mellifera* L.) working on willow leaves infected with willow rust B) The worker bee with visible pollen basket filled with willow rust spores.

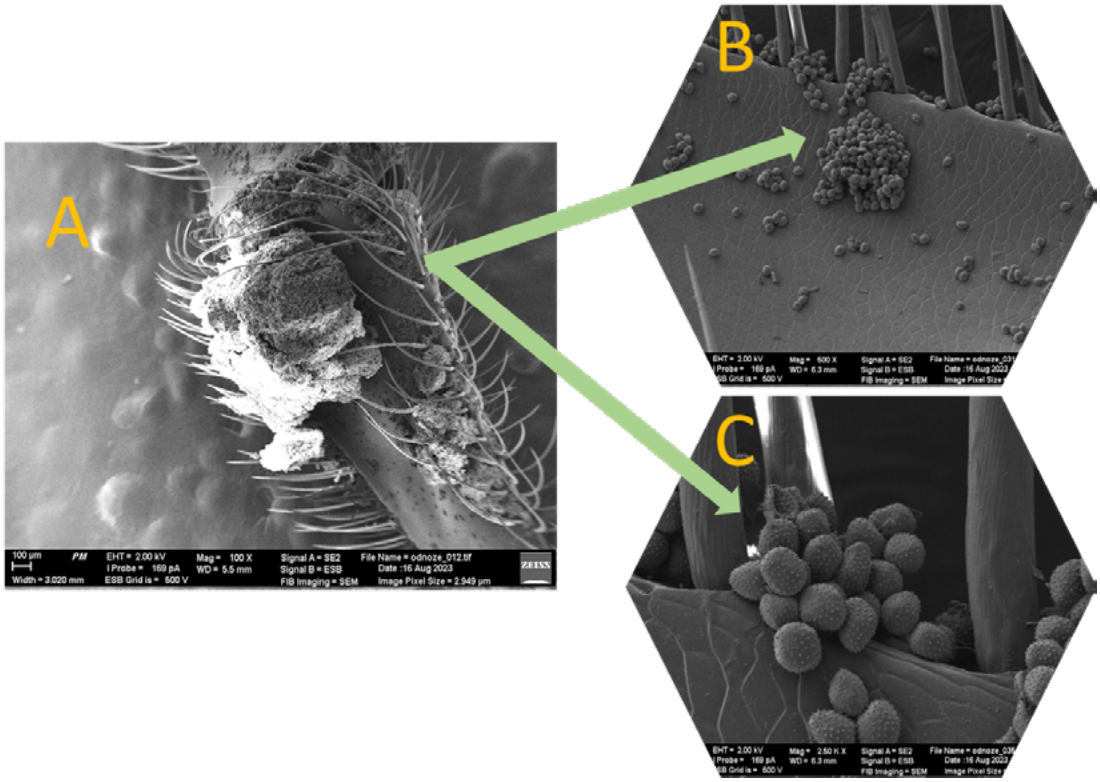

Fig. 2. *Melampsora* spp. spores collected in baskets (A, B). A cluster of *Melampsora* spp. spores around a pollen basket hair (C).

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