

Preliminary observation of fungal colonization in a rare orchid species (*Epipactis pontica* TAUBENHEIM) in the Czech Republic

JAROSLAV JURČÁK, ALEŠ LÁTR & MARTINA ČUŘÍKOVÁ

Department of Botany, Faculty of Science, Palacký University, Šlechtitelů 11, CZ – 783 71 Olomouc – Hólice, Czech Republic; e-mail: jurcak@prfholnt.upol.cz

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Abstract: Infected and non-infected root tips of *Epipactis pontica* were examined to document morphological and anatomical organization. Histogenes (primary meristems) of the non-infected root tips were well developed. They included calypetrodermatogene, periblem and plerome. Typical organization of the histogenes of the infected root tips was not observed. The cells of the root cap were infected and lost their cellular integrity. Passage, host and digestion cells occurred at close distance from the root cap. A part of the infected cells of the calypetrodermatogene (the rhizodermis) and of the periblem (the primary cortex) appeared collapsed. Their remains together with soil particles and the hyphae are the base for the hyphal mantle, which is more or less preserved through the full length of the roots.

Keywords: root tip, anatomy, mycorrhiza, *Epipactis pontica*, *Orchidaceae*.

Introduction

Literature describing orchids of the Czech and the Slovak republic (CUDLÍN 1974, PROCHÁZKA 1980, PROCHÁZKA & VELÍSEK 1983, POTŮČEK & ČAČKO 1996, DYKYJOVÁ 2003) does not deal with the histogenes of the root tips and their mycorrhizal conditions. Only MEJSTRÍK (1970) studied the apical root tips of the

commercially utilized orchid species *Dendrobium* and more recently KOŽICHOVÁ (2002) examined some Czech terrestrial orchids.

Epipactis pontica, recently found in Slovakia, was subsequently detected in the Czech Republic, i.e. in East Moravia. According to JATIOVÁ & ŠMITÁK (1996), *E. pontica* belongs among the critically endangered species (C1). JURČÁK et al. (2005) described the anatomical and mycorrhizal characteristics of its roots. During the preparation of the root materials for that microscopic study, seven root tips were kept for further anatomical analysis.

The objectives of this study were to examine the organization of the histogenes of the root tips and to trace the mycobiont-phytobiont interactions in the apical root zones of this rare orchid species.

Material and methods

Owing to the protected status of the species, the roots of one specimen of *E. pontica* were used for the study. This specimen was taken from the locality near Bylnice in the Bílé Karpaty Mountains, the Czech Republic. The roots were carefully removed, rinsed in fresh water, fixed for 48 hours in FAA and then stored in glycerol-ethanol (1:1). The root tips were cut off following NĚMEC et al. (1962). They were dehydrated and embedded in paraffin; longitudinal transverse sections were made with a sliding microtome Meopta 115060 (their thickness about 5-7 μm), stained in haematoxylin and finally mounted in Canadian balsam. A photomicroscope (Olympus BX-40) was used to photograph the sections on Kodak Gold 100 ISO film.

Results and discussion

All seven adventitious roots of *E. pontica* were examined: four were infected and provided information of the mode of fungal colonization, three were not infected.

The organization of the primary meristems of the non-infected root tips corresponded to the histogene theory (HANSTEIN 1868, cit. ex LUXOVÁ 1974). Root caps covered the apical poles of the non-infected root tips (Fig. 1) and reached to a distance of 700-800 μm from the root apex. The histogenes of calyptrogene and dermatogene were joined to the well developed calyptrodermatogene. MEJSTRÍK (1970) described similar cellular organization in *Dendrobium cunninghamii*, as well as KOŽICHOVÁ (2002) in two Central European representatives of *Epipactis* genus. Other histogenes, i.e. periblem forming the primary cortex, and plerome from which the central cylinder with a polyarch vascular bundle differentiates through the procambium, followed the zone of apical initials. The quiescent centre was evident, as well. The histogene cells showed the typical qualities of meristematic cells, e.g. small cell size, big nuclei in comparison with protoplasts.

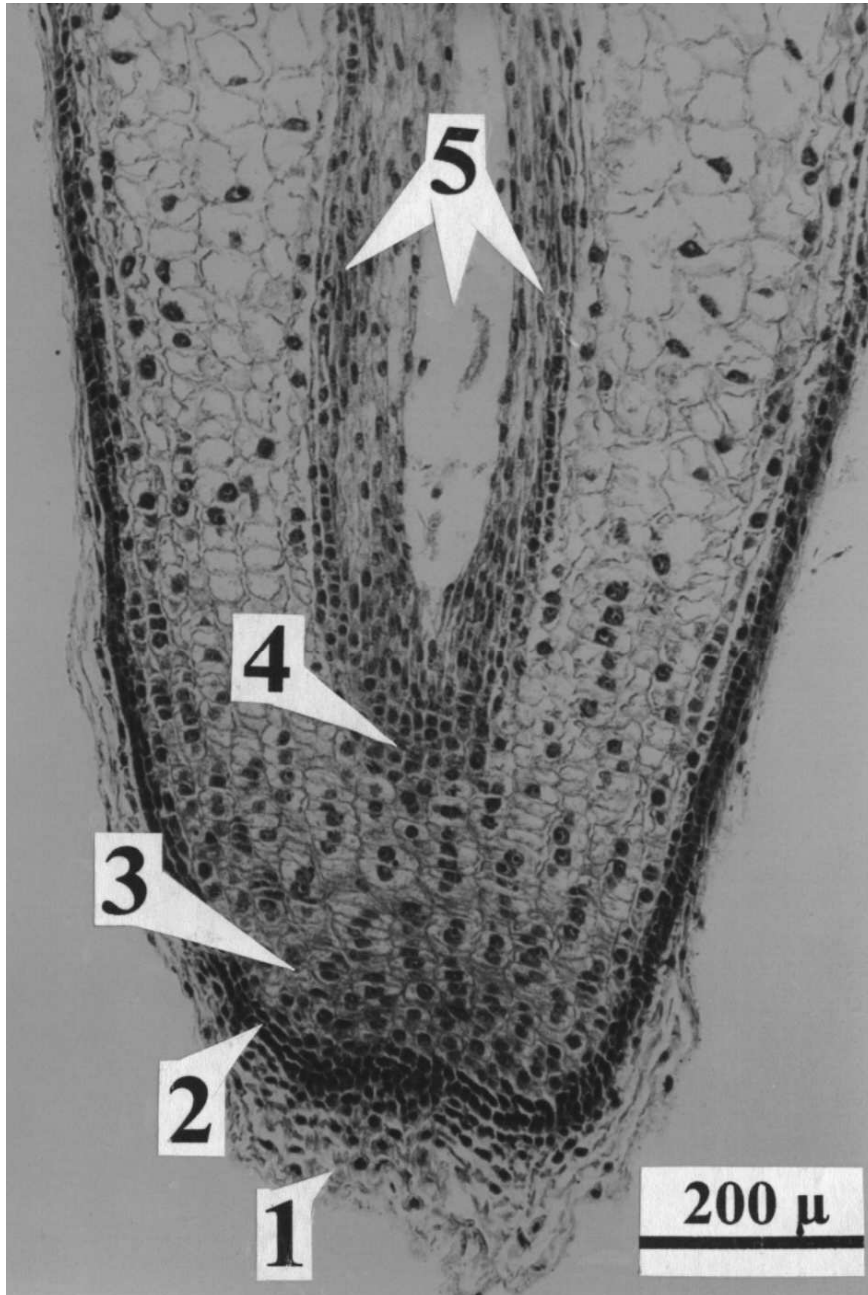


Fig. 1. Longitudinal section of *Epipactis pontica* root tip without mycobiont; 1 root cap, 2 calyptra, 3 periblem, 4 plerome, 5 central cylinder.

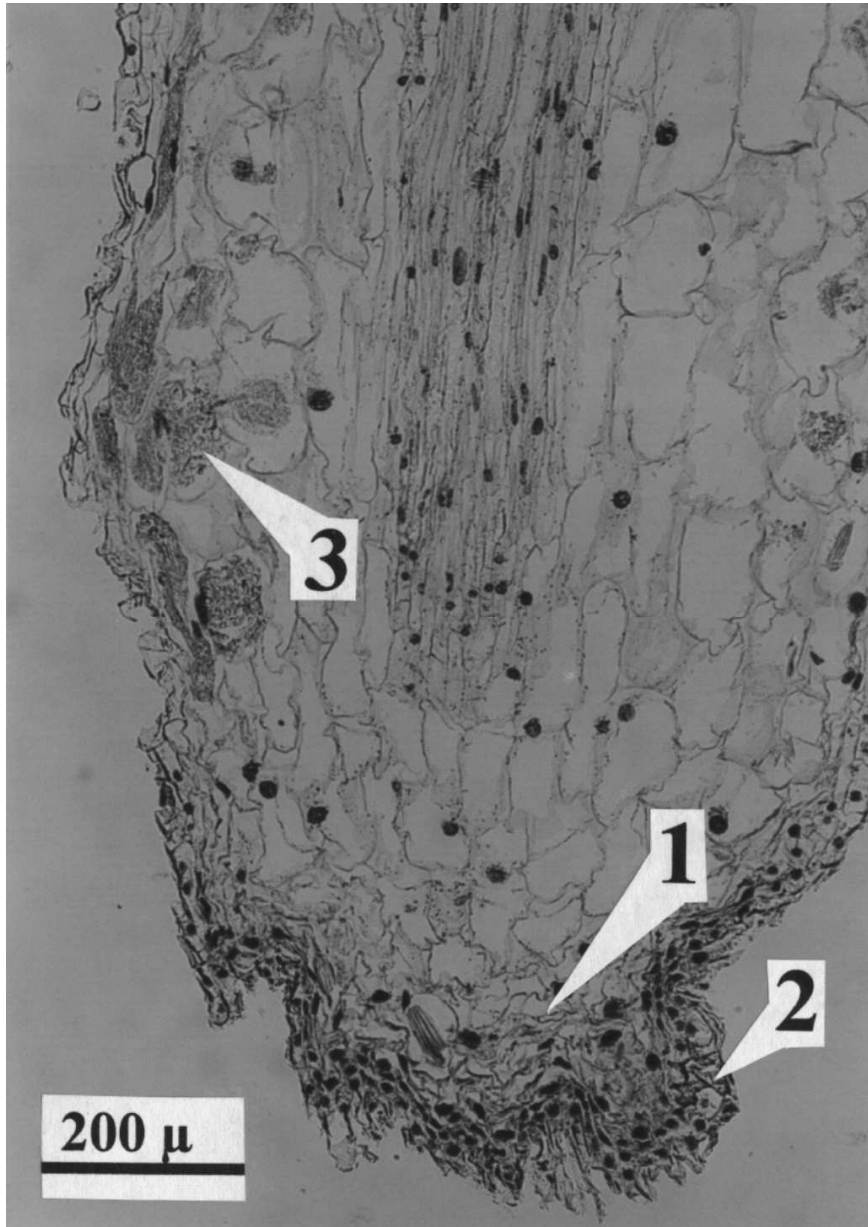


Fig. 2. Longitudinal sections of infected *Epipactis pontica* root tips; 1 destroyed zone of primary meristems, 2 root cap infected by fungal hyphae, 3 zone of the mycorrhizal cells.

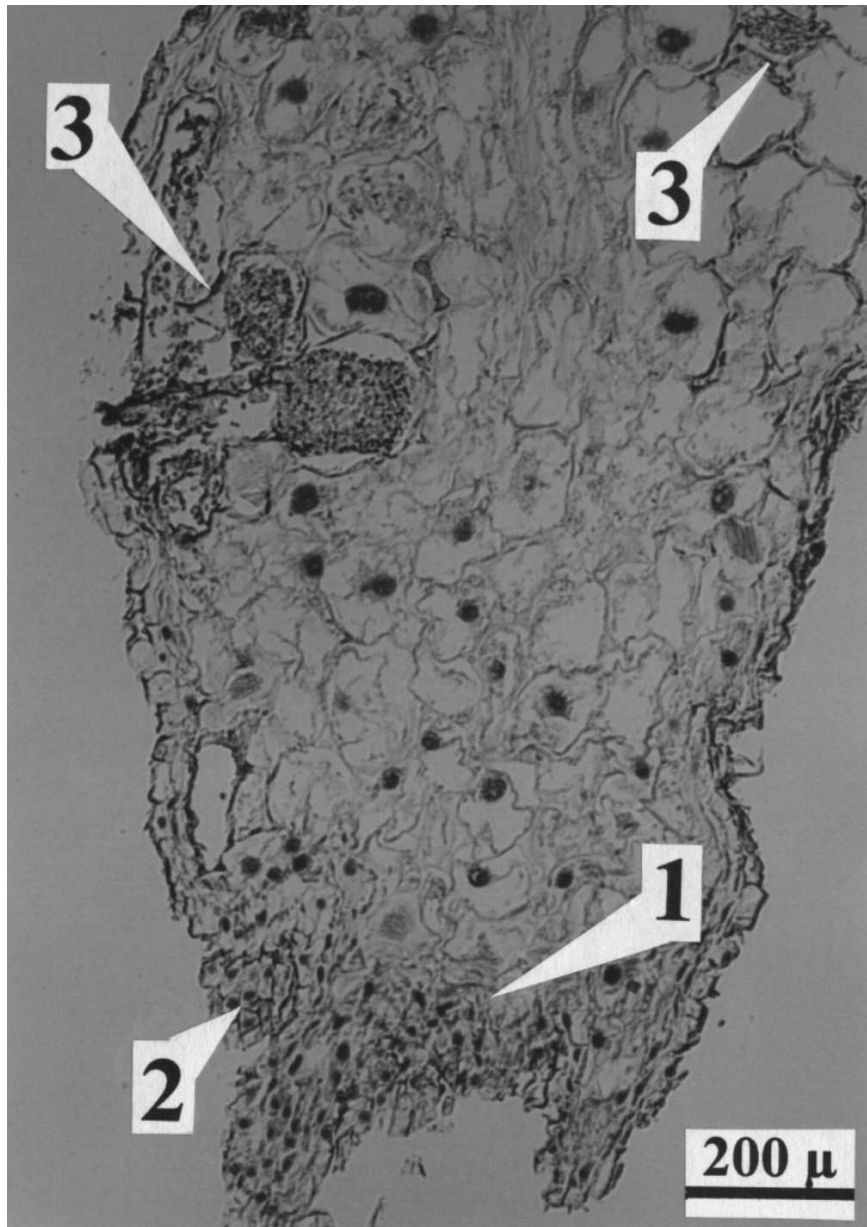


Fig. 3. Longitudinal sections of infected *Epipactis pontica* root tips; 1 destroyed zone of primary meristems, 2 root cap infected by fungal hyphae, 3 zone of the mycorrhizal cells.

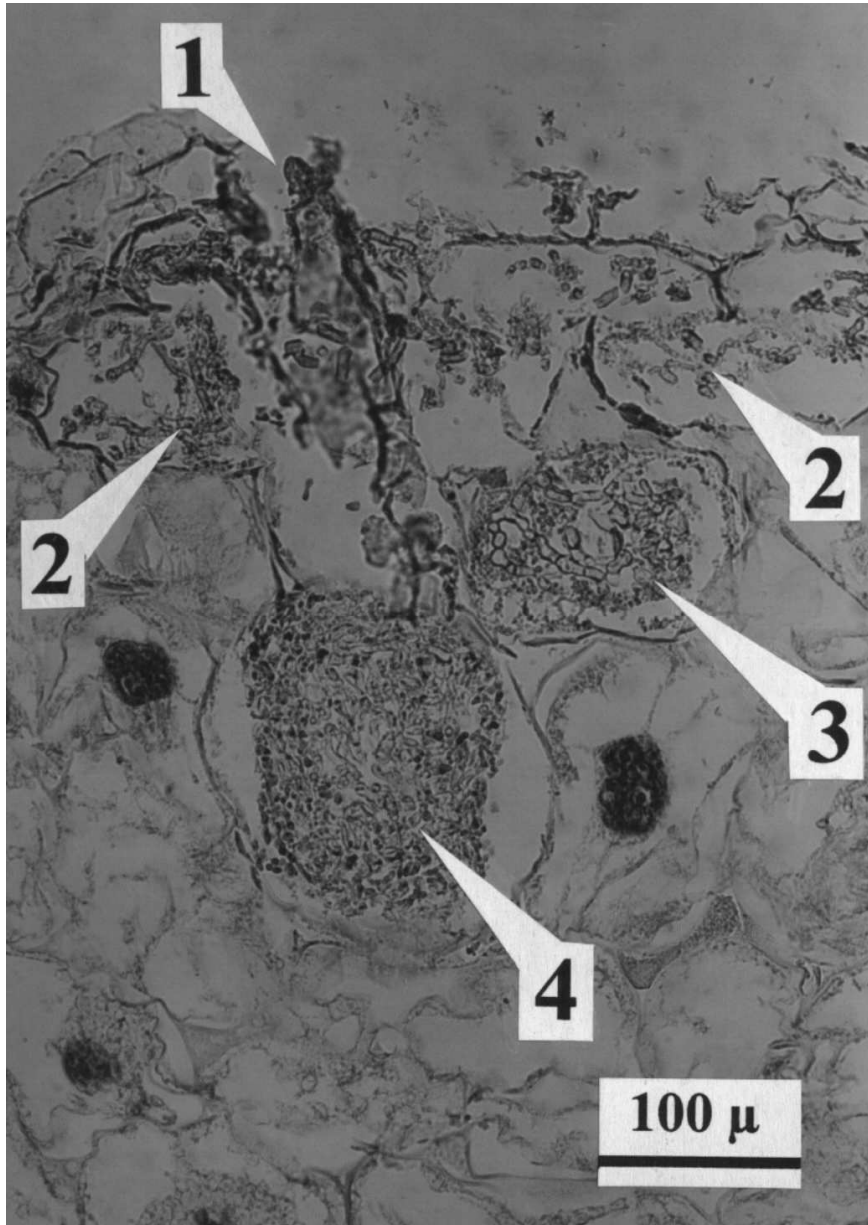


Fig. 4. Detail of mycorrhizal zone of *Epipactis pontica* root tip; 1 remains of damaged dermatogenes and rhizodermis, 2 passage cell, 3 host cell, 4 digestion cell.

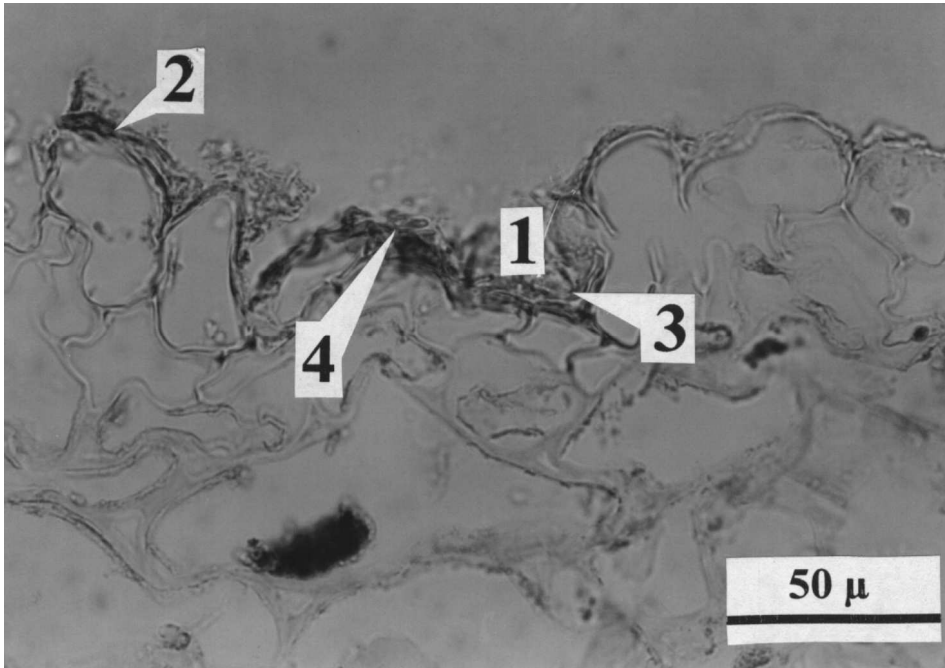


Fig. 5. Detail of damaged rhizodermis of *Epipactis pontica* root tip; 1 decayed rhizodermal cells, 2, 3, 4 fungal hyphae

The infected root tips displayed significant differences (Fig. 2, 3). Their root caps were collapsed; they showed signs of collapses due to the presence of fungal hyphae. From the primary meristems, a hint of calypetrodermatogene was evident only. In contrast to non-infected root tips, the zones of periblem and plerome were not typically developed. Their cells displayed the characters of the permanent, thus specialized tissues (parenchyma). The cells were relatively big and isodiametric, i.e. they have not passed through the elongation period. The fungus extensively colonized the tissues, even at a distance of 600-800 μm from the root apex. The passage, host and digestion cells occurred in the infected portions of the root (Fig. 4). The colonized rhizodermal cells often lost their cellular integrity (Fig. 5). From the passage cells, the fungal hyphae spread into deeper cell layers of the primary cortex (to the zone of periblem). Invading hyphae passed directly from cell to cell without occupying the intercellular spaces, often making use of existing pit fields in the cell walls. In the stage of host cell, the intracellular hyphae formed dense coils (known as pelotons) that occupied major parts of the cell lumen (Fig. 4). After a build-up period, the peloton was broken down to an aggregated clump, i.e. was lysed, which is the stage of digestion cell (Fig. 4) (ANDERSEN & RASMUSSEN, 1996). No hyphae occurred in the endodermis and the central cylinder.

A part of the infected cells of the primary cortex appeared collapsed due to the fungal infection as well. The cell destruction might not be considered as artifacts caused by the handling with the plant material during the preparation of the permanent dissections because the collapsed cells regularly occurred next to the undamaged cells. According to RASMUSSEN (1995), some orchid fungi are able to synthesize various wall-degrading enzymes in pure culture, which suggests that these enzymes could be responsible for the breakdown of cell walls (PAIS & BARROSO, 1990).

The remains of the rhizodermal cells infected by the fungal hyphae as well as the cells situated beneath them were preserved as detritus elements and together with soil particles formed substrate through which passed the fungal hyphae. Thus, these structures made the hyphal mantle. Isles of the hyphal mantle persist on the roots during the elongation period and are preserved even on the oldest parts of the roots (Fig. 6). The hyphal mantle is a source of the hyphae for the root colonization and enables contact between the root environs and inner root structures.

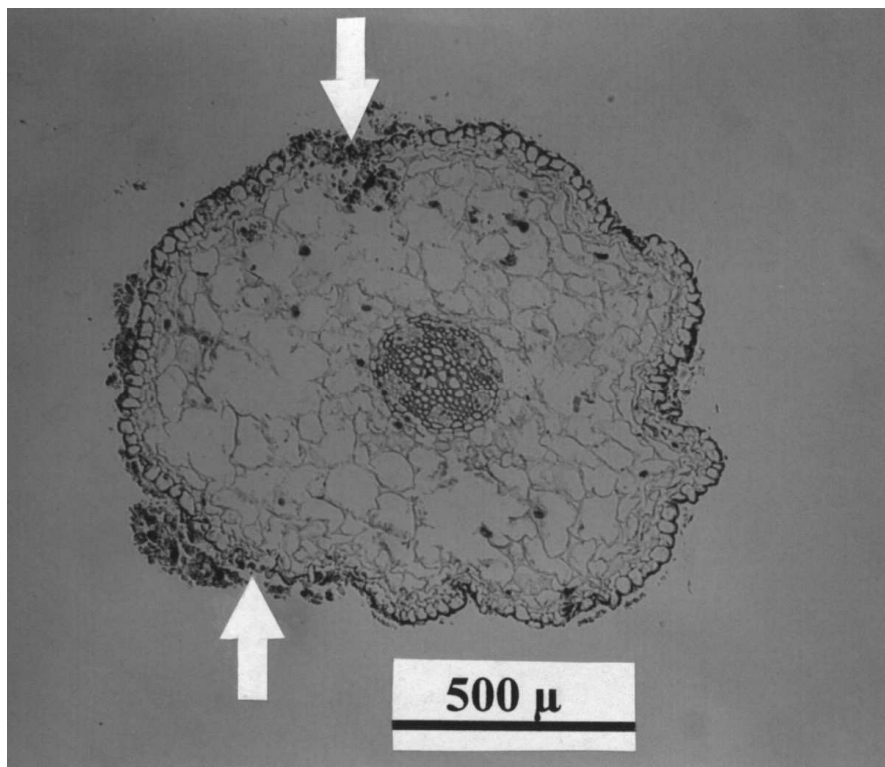


Fig. 6. Adventitious root of *Epipactis pontica*, 5 mm from the rhizome (transverse section); arrows show isles of hyphal mantle.

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