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#### **Review**

# Carotenoid pigments in rust fungi: Extraction, separation, quantification and characterisation

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#### ABSTRACT

Diseases caused by rust fungi represent critical constraints to global plant production. A characteristic feature of rust pathogens is the striking pigments they produce in one or more spore forms, which give them a rusty appearance. Here, we review the literature published to date on the extraction, separation, quantification and characterisation of carotenoid pigments in rust fungi. These pigments are thought to protect rust fungi against UV radiation and oxidative stress, and possibly act as virulence factors. The yelloworange colour of some rust species is due to carotenoid pigments. Four carotenoids have been found in rust fungi: phytoene, lycopene,  $\gamma$ -carotene and  $\beta$ -carotene, but their relative contributions to biological functions are largely unknown. Different pre-processes and storage of spore materials, as well as different extraction processes, have been applied in a wide range of investigations on rust spore pigments. We find that the value of the current literature on rust carotenoids for taxonomic diagnostics in understanding the evolution of pigment biosynthesis and in assessing their role in pathogenesis is limited. Reinvestigation of rust carotenoid composition using modern analytical technologies is therefore critical to further these fields of research. Our review includes detailed guidance on choice of techniques for rust carotenoid experimental analyses.

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#### 1. Introduction

The rusts are a group of pathogenic microfungi comprising the order Pucciniales (formerly, Uredinales). The common name "rust" was coined to reflect the rusty appearance of the spores produced by most species of the Pucciniales. Rust species parasitise a wide range of cultivated and wild plants; those that attack the grass cereals such wheat and oat, shrubs such as coffee, and trees such as pine and poplar, are among the most destructive of plant pathogens. The rust diseases of cereals have featured in human history; for example, the Romans practised the Robigalia on April 25 around 500BC to appease Robigus, the corn or rust God and spare their crops from failure (McIntosh *et al.*, 1995).

Currently, there are thought to be over 7,000 species of rust fungi in 168 genera (Gautam and Avasthi, 2017). The colour of rust spores produced by different rust species varies considerably and, within a species, may vary with the type of spore produced. Many rust species are heteroecious, which means that two unrelated hosts are required to complete the life

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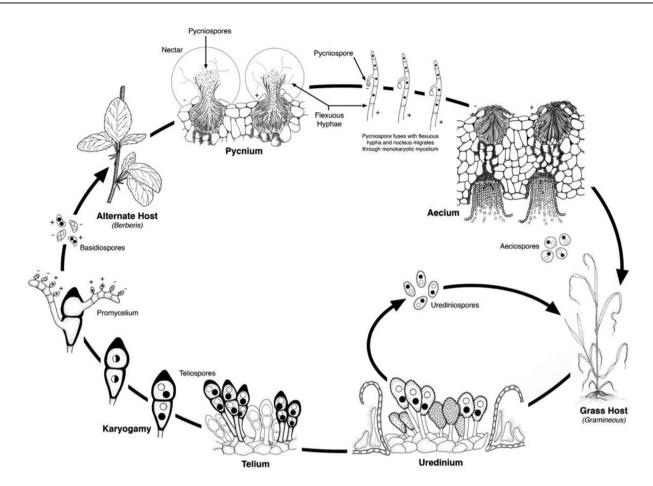


Fig. 1 – Life cycle and five spore states of Puccinia graminis f. sp. tritici. Figure from Leonard and Szabo (2005) (with permission from John Wiley and Sons).

cycle. Wheat stem rust, *Puccinia graminis* f. sp. tritici, for example (Fig. 1), requires not only wheat (the primary host) but also barberry (*Berberis vulgaris*) as a common alternate host (Leonard and Szabo, 2005). In this case, both the urediniospores (produced on wheat), and the aeciospores (produced on barberry), are rusty in appearance and are presumed to contain carotenoid pigments. The teliospores of *P. graminis* f. sp. tritici (produced on wheat), are black and give rise to the term 'black rust', one of the common names of the disease it causes.

Depending on the rust species, up to five spore types may feature during the obligate parasitic life cycles of rust fungi, including pycniospores (0), aeciospores (I), urediniospores (II), teliospores (III) and basidiospores (IV) (Park, 2000). Among the five spore types of rust fungi, the urediniospore (II) (a dikaryon<sup>1</sup>) is the most abundant in many rust species and the most important in terms of pathogenesis, as it can reinfect the host plant on which it was produced. Usually, urediniospores are pigmented, but colour differences both between and within species do exist. The urediniospores of the fern rust pathogen *Uredinopsis pteridis* are white (McTaggart *et al.*, 2014), those of the wheat stripe rust pathogen *Puccinia*  striiformis f. sp. tritici are typically yellow-orange, and those of the wheat stem rust pathogen *P. graminis* f. sp. tritici are typically reddish brown. Isolates of *P. graminis* f. sp. tritici with urediniospores have been described with antique brown, white, grey-brown and orange colour (Bush, 1967).

For the economically important rusts of winter cereals, teliospores are typically produced after urediniospores, often coinciding with host senescence at the end of the growing season. Typical rust teliospores are black-coloured, with thick spore walls. Teliospores are also known as Winter spores, as they can be dormant and overwinter, and then, in the following Spring, germinate to form four basidiospores (IV) (haploid). The appearance of basidiospores varies from colourless to yellow, and for most cereal rusts, these spores only infect rust alternate hosts. For many autoecious rust species (e.g. flax rust), all five spore forms are associated with a single host. Basidiospores germinate to form a mycelium that grows inside the host plant tissue and forms pycnia, on which haploid pycniospores (0) and receptive hyphae are produced. Pycniospores (spermatia) are usually small, hyaline and single-celled (Hiratsuka and Sato 1982). Once a haploid pycniospore fertilises a receptive hypha (in some cases with the help of insects), the dikaryotic pycniospore grows into an aecium on the adaxial side of the leaf of the alternate host and produces aeciospores (I) (dikaryon). Aeciospores are

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<sup>&</sup>lt;sup>1</sup> A dikaryon is a pair of unfused haploid nuclei of opposite mating type in a cell or spore which divide simultaneously when the cell divides.

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