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NOTE

New records of microcyclic conidiogenesis in some powdery mildew fungi

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Abstract Microcyclic conidiogenesis (MC) was recently described in several species of powdery mildew fungi. This process, defined as the production of conidia on a fungal spore without any, or only a minimal, involvement of hyphal growth, was observed on powdery mildew conidia that have already germinated on host plant surfaces and have been attached to the epidermal cells. Most probably, MC contributes to a quick propagation of young powdery mildew colonies because new conidia are sometimes produced in a shorter time on microcyclic conidiophores than on the hyphae of the young mycelium. This article reports MC in *Erysiphe necator* ex grapevine, *Podosphaera leucotricha* ex apple, *Golovinomyces orontii* ex tobacco, and *Neoerysiphe galeopsidis* ex *Lamium purpureum* based on light and low-temperature scanning electron microscopic studies.

Keywords Conidial germination · Erysiphales · Erysiphaceae · Microcyclic conidiation

Microcyclic conidiogenesis (MC) was defined as the production of conidia on a fungal spore without any, or only a minimal, involvement of hyphal growth (for a review, see Hanlin 1994). This process was described in several fungal species, such as *Aspergillus* spp. (Anderson and Smith 1971; Ahearn et al. 2007), *Epichloe typhina* (Bacon and Hinton 1991), and *Cercospora zea-maydis* (Lapaire and Dunkle 2003), and has also been studied from a practical point of view to ensure the synchronized production of large amounts of conidia of different strains in the shortest possible time for industrial or other mass production purposes (e.g., Krasniewski et al. 2006; Cascino et al. 1990; Bosch and Yantorno 1999). In fact, there are considerable morpho-physiological differences among the various processes described as MC. In *Aspergillus niger*, for example, where this process was described for the first time (Anderson and Smith 1971), conidia simply swelled, and became two to three times bigger than their original size, before producing sporulating conidiophores directly on their surfaces. Thus, in this case, there was an obvious uptake of water and/or nutrients through the cell wall before conidiogenesis occurred. In *Neurospora crassa*, which produces two types of vegetative spores, uninucleate microconidia and multinucleate macroconidia, the so-called microcycle microconidiation occurred when the germinated macroconidia produced lateral protuberances on their germ tubes and these developed directly into microconidia (Maheshwari 1999). In *C. zea-maydis*, both germ tubes and conidiophores were produced on the septate conidia (Lapaire and Dunkle 2003); thus, there was some hyphal development occurring simultaneously with MC. Clearly, different types and subtypes of MC could be distinguished in the fungal world, but these have not been categorized in the literature to date.

Recently, it was shown that several species of powdery mildew fungi (Erysiphales), in addition to producing conidia on conidiophores developed on the hyphae, can generate functional conidiophores directly on the surfaces of

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the already germinated conidia (Kiss et al. 2008, 2010). This process, described as a type of MC, might favor a quick propagation of young powdery mildew colonies because new conidia are sometimes produced in a shorter time than on the hyphae of the young mycelium (Fig. 1). Before producing a microcyclic conidiophore, powdery mildew conidia exhibiting MC had always produced one or more germ tubes, and these have subsequently penetrated the epidermal cells of the host plants to ensure the biotrophic uptake of nutrients by the young, developing mycelium. Thus, conidia bearing one, or sometimes two, microcyclic conidiophores have already become a part of the young mycelium on the leaf surface. In some cases MC was clearly a very early process of the colonization of host plant surfaces by a powdery mildew fungus, being completed before conidiogenesis started on the young hyphae (Kiss et al. 2010). In these cases conidia originating from MC were among the first produced by the young mycelium, thus ensuring a rapid propagation of the colonies. In other cases, however, conidiogenesis had already started on the hyphae before MC occurred, so the germinated conidium, as an integral part of the already formed mycelium, simply served as a site for conidiogenesis similar to other cells of the hyphae (Kiss et al. 2010).

In the Erysiphales, MC was first described in *Oidium longipes* (Kiss et al. 2008) and then documented in seven other, phylogenetically distinct powdery mildew species (Kiss et al. 2010). It was also shown to occur in a newly described species, *Oidium aloysiae* (Takamatsu et al. 2008). These reports suggested that MC is apparently rare in powdery mildews because only up to 5% of germinated conidia exhibited MC. Similar low values were reported for other fungi, such as *Aspergillus* spp. (Ahearn et al. 2007), which might be the reason why MC was not observed earlier in powdery mildew fungi in spite of detailed studies on their conidial germination (Braun 1987, Green et al. 2002; Bolay 2005; Cook and Braun 2009).

In this work, to continue the study of MC in powdery mildew species, we examined more than 500 samples of powdery mildew-infected leaves representing the following fungal and plant species: *Erysiphe necator* ex grapevine, *Podosphaera leucotricha* ex apple, *Golovinomyces orontii* ex tobacco, and *Neoerysiphe galeopsidis* ex *Lamium purpureum*. All the samples were freshly collected from the field, or, in the case of tobacco, from the greenhouse, brought to the laboratory, and immediately examined using light and/or low-temperature scanning electron microscopy (LT-SEM). To prepare the samples for light microscopy, small pieces of powdery mildew-infected leaf tissues were cut from the leaves and boiled, with the powdery mildew mycelium downward, in a drop of lactic acid on a slide as described by Shin and La (1993). After boiling, the mycelium was scraped off the leaf and mounted in either lactic acid or cotton blue in lactic acid for light microscopy

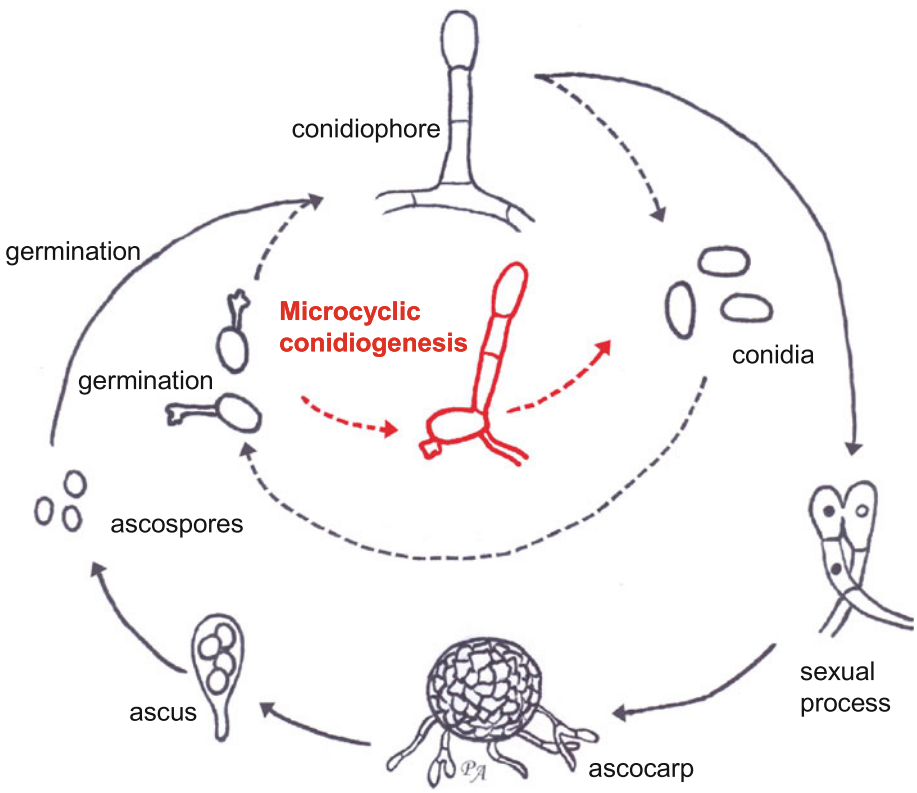

Fig. 1 The life cycle of the powdery mildew fungi supplemented with the newly described microcyclic conidiogenesis. (Redrawn from Braun 1987)

using bright-field, phase-contrast, and differential interference contrast (DIC) optics. Samples of *E. necator* infecting grapevine were also prepared for LT-SEM studies as described by Rügner et al. (2002) and observed with a Philips XL 30 ESEM electron microscope connected with a Gatan Alto 2500 Cryo unit.

In all the four species studied, germinated conidia bearing microcyclic conidiophores were identified in up to 15% of the samples. LT-SEM documented MC in *E. necator* (Fig. 2), a species with a pseudoidium-type anamorph representing *Oidium* subgen. *Pseudoidium*, while the same process was detected in the other three species using light microscopy (Fig. 3A–C). The latter three species, *P. leucotricha*, *G. orontii*, and *N. galeopsidis*, represent different genera of the Erysiphales with distinct chain-forming anamorphs belonging to subgenera *Fibroidium*, *Reticuloidium*, and *Striatoidium*, respectively, of the anamorphic genus *Oidium*. In all these four species, both developing and mature microcyclic conidiophores were detected on the germinated conidia (Figs. 2, 3), and these have undoubtedly contributed to the conidial production of the newly formed colonies.

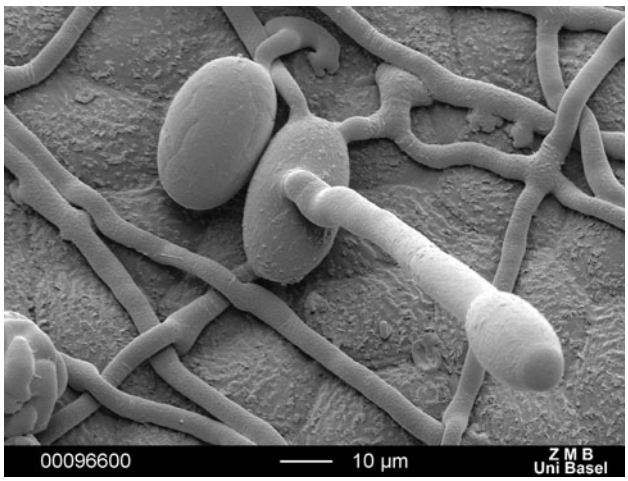

Fig. 2 A mature microcyclic conidiophore arising from a germinated conidium of *Erysiphe necator* on a grapevine leaf. Another conidium, germinated later than the one bearing the microcyclic conidiophore, is also shown on the picture taken using a low-temperature scanning electron microscope (LT-SEM) (ZMB, University of Basel, Switzerland). *Bar* 10 µm

MC was recognized in some groups of filamentous fungi as a specific process that leads to a rapid formation of new asexual spores following 'regular' conidiogenesis (Hanlin 1994). In fact, the production of basidiospores from teliospores of rust and smut fungi is a similar process, although it leads to sexually formed spores, following meiosis, and thus cannot be classified as MC. In powdery mildew fungi, MC clearly differs from some of the similar conidiation mechanisms described in various fungal groups and resembles the process described, for example, in *C. zea-maydis*, where conidia germinated with germ tubes and have also produced microcyclic conidiophores on their bodies (Lapaire and Dunkle 2003). This study showed that it commonly occurs in different species of the Erysiphales, contributes to some extent to the rapid propagation of the newly formed, or older, colonies, and should not be neglected when considering conidial germination of powdery mildew fungi on host plant surfaces.

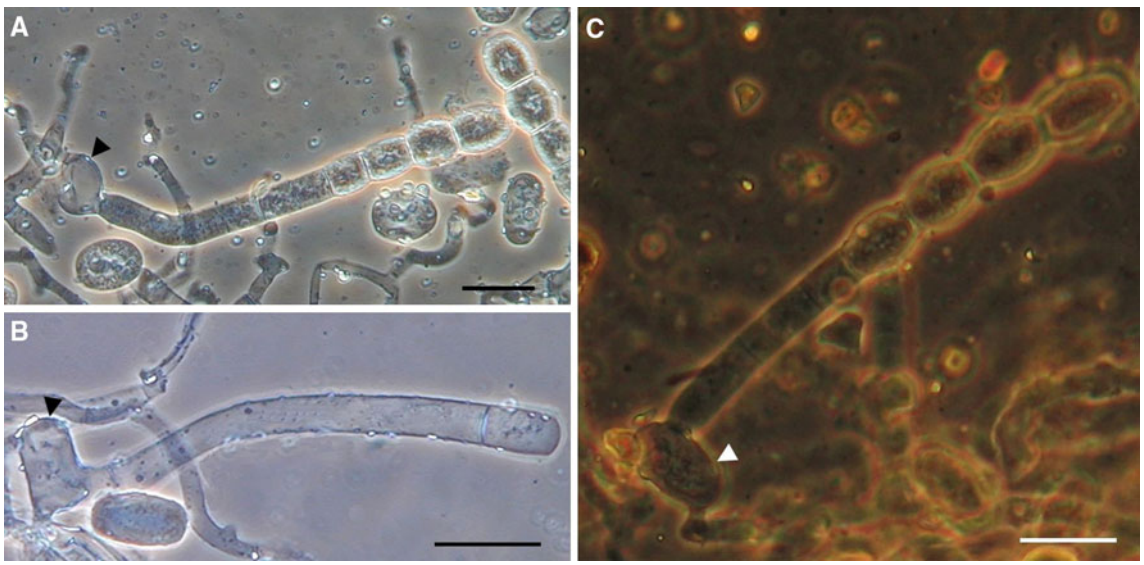

Fig. 3 Microcyclic conidiogenesis in three powdery mildew species. **A** *Podosphaera leucotricha* on apple. **B** *Golovinomyces orontii* on tobacco. **C** *Neoerysiphe galeopsidis* on *Lamium purpureum*. Conidia bearing microcyclic conidiophores are shown by *arrows*. *Bars* **A–C** 30 µm

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