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First record of a plasmodiophorid parasite in grapevine

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Summary

In the context of an interdisciplinary project on grape pests and pathogens in Rheingau (Germany), the fine root system of grafted rootstocks has been screened for pathogenic fungi associated with root galls induced by grape phylloxera (*Daktulosphaira vitifoliae* (Fitch)). In several insect-induced galls, masses of resting spores of a plasmodiophorid could be seen. An additional selective screening revealed the occurrence of the plasmodiophorid parasite also in samples of gall-free rootlets: cortical cells of small necrotic areas were crowded with resting spores or other developmental stages of its life cycle. According to current taxonomic concepts, this plasmodiophorid could be identified as a member of the genus *Sorosphaera* Schroeter, resembling *S. veronicae* Schroeter. This is the first record of a plasmodiophorid parasite in grapevine.

Key words: Plant pathogen, soil, sporosori, *Sorosphaera*, *Vitis*.

Abbreviations: AFE - glacial acetic acid: formol 37 %: ethanol 70 % = 5: 5: 90; DAPI - 4'6-diamidino-2-phenylindole; SEM - scanning electron microscope.

Introduction

Plasmodiophorids are zoosporic eukaryotes of uncertain phylogenetic affinities that have been classified as true fungi or protists, although results of recent phylogenetic analyses of rDNA-sequences point at a closer relationship to protozoans (Castlebury and Domier 1998; Cavalier-Smith 2000; Bulman *et al*. 2001; Down *et al*. 2002), their taxonomic ranking remains unclear. Therefore, the informal term "plasmodiophorid", as suggested by Braselton (1995), is used in this report. Plasmodiophorids are found in soil, fresh water or marine habitats and are obligate endobionts of plants (*e.g.* flowering plants and green algae) and stramenopiles (*e.g.* brown algae, diatoms, and oomycetes). Thus, their distribution follows that of their hosts; no member conclusively has been shown to complete a life cycle in the absence of host cells (Dylewski 1990; Braselton 2001). A generalised life cycle for members of the plasmodiophorids is characterised by two distinct developmental phases: in the sporogenic phase (i) mature, multinucleate plasmodia within host cells cleave into uninucleate cells which subsequently differentiate into thick-walled, presumably haploid resting spores. Depending on the genus, resting spores may occur singly or in aggregations called sporosori. Upon germination, such a resting spore – in soils it may remain viable for many years – releases a single, biflagellated, free-swimming, primary zoospore (Kole and Gielink 1962; MacFarlane 1970; terminology according to Braselton 2001). Encountering the wall of a potential host cell, the zoospore encysts, retracts its flagellae, and injects its protoplast into the host cytoplasm (for a full description of this complicated process see Keskin and Fuchs 1969 and Aist and Williams 1971). Thereafter, growth of the protoplast accompanied by cruciform nuclear divisions initialises the sporangial phase (ii) which terminates in the production of thin-walled sporangial lobes (zoosporangia), each of them containing 4 or more secondary zoospores. Completing the life cycle, discharged zoospores encyst on new host cells and attack them in the same way as primary zoospores.

Material and Methods

In the context of an interdisciplinary project on grape pests and pathogenes, phylloxera-infested root samples from a vineyard near Kiedrich (50° N; 8° E; 160 m asl) in Rheingau (Germany) were screened for fungal colonisation. The vineyard was planted in 1994 with cv. Pinot Noir on SO 4 (*V. berlandieri* x *V. riparia*). The emphasis of this screening was to examine the hypothesis that phylloxera-induced root galls (nodosities), constitute, because of their fissured surface, a preferential target for fungal invaders. For this purpose, root samples were taken monthly from May to October 2000 and again in June 2002 from 10 randomly chosen rootstocks. Roots from the upper 25 cm soil horizon were sampled with a spade 10 - 15 cm to the left and to the right of trunks underneath the row (Porten and Huber 2003). Roots were washed by hand with tap water. Nodosities were fixed in AFE, dehydrated in an ascending ethanol series and embedded in paraffin wax. Semithin sections (10-30 µm) were made using a Leitz 1208 microtome. Light micrographs (Normarski differential interference contrast) were taken with a Leitz Diaplan. For fluorescence microscopy the sections were placed on a glass slide and paraffin was removed in an ascending xylene series and mounted in Mowiol containing 0.05 % (w/v) DAPI. Micrographs were taken using a Leitz Axioplan fluorescence microscope at an excitation wavelength of 365 nm. For scanning electron microscopy (SEM) the semithin sections were placed on specimen mounts, paraffin was removed in an ascending xylene series and sputtered with gold. SEM micrographs were taken using a Philips

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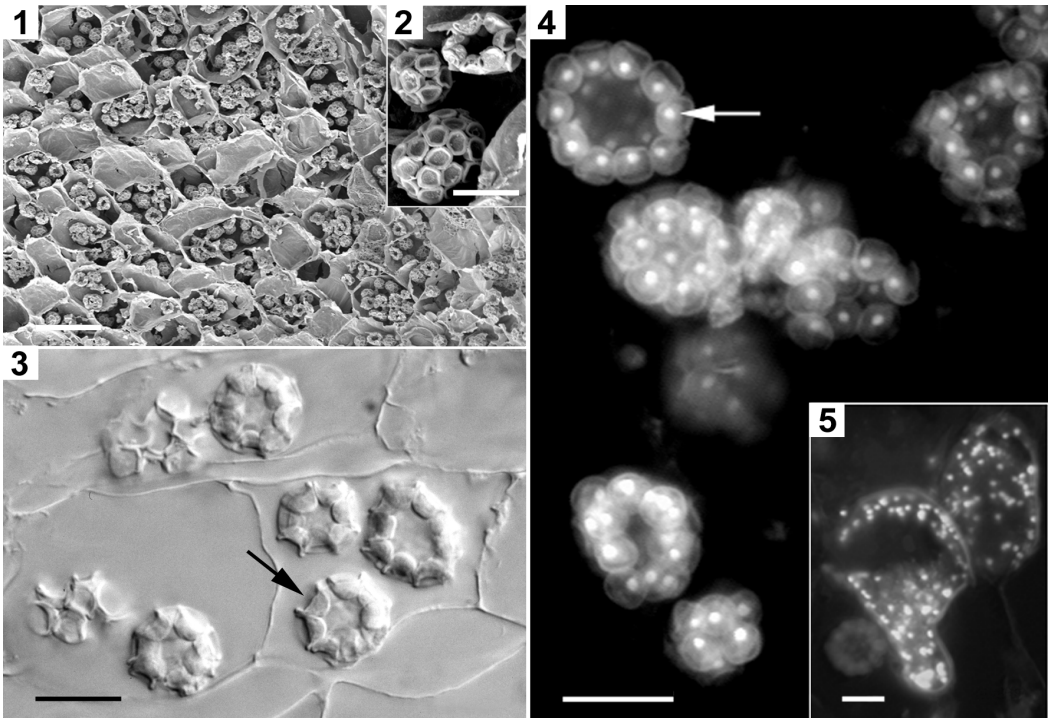
ESEM scanning electron microscope. In the cortical tissue of 5.4 % out of 129 isolated root galls, masses of conspicuously structured plasmodiophorid sporosori were found. The spherical sporosori with an average diameter of 12 µm are formed by 15 - 50 peripherically arranged resting spores leaving a hollow centre (Figs 1-3). The individual resting spores are thick-walled, saucer shaped, roundish to somewhat polygonal in plan view, measuring 4-5 µm in diameter and 2.5-3 µm in total height (side view). In an additional selective screening in June 2002, in 8 randomly chosen rootstocks the parasite could be observed once in fresh root segments without phylloxera-induced hypertrophies: cortical cells within small necrotic brownings were crowded as well with sporosori or contained plasmodial stages of the parasite (Figs 4 and 5).

According to current taxonomic concepts, major taxonomic characteristics for the distinction of plasmodiophorids are size and shape of resting spores and sporangia, but primarily the specific host (DYLEWSKI 1990). Since *Sorosphaera* Schroeter is so far the only known genus forming hollow, spherical sporosori, the newly discovered grape plasmodiophorid could be clearly identified as a member of this genus. It resembles *S. veronicae* Schroeter but differs from it in some important characters: (i) *Sorosphaera veronicae* infects exclusively speedwell species (*Veronica spp.,* Scrophulariaceae) (KARLING 1975; PREECE 2002) where sporosori are produced only in parasite-induced galls on the shoot system, (ii) the size of resting spores is remarkably different: they are at least twice as long (total height 8-9 µm; SCHROETER 1885) in *S. veronicae*. These differences strongly suggest that the grape *Sorosphaera* represents an undescribed species.

Results and Discussion

Although the parasite was found in stunted grapevines only, no evident disease pattern, with the exception of root necroses, could be correlated with the infection. However, many plasmodiophorids are parasitic on food plants as, *e.g. Plasmodiophora brassicae* Woronin (club root disease of cabbage and other crucifers) or *Spongospora subterranea* (Walr.) Lagerheim var. *subterranea* (powdery scab disease of potatoes). In this context, it might be even more important that some plasmodiophorids cause economic losses by transmission of plant viruses. Among them, several cereal viruses, for instance the barley mild mosaic bymovirus (BaMMV), or the oat mosaic bymovirus (OMV), are transmitted by *Polymyxa graminis* Ledingham. *Spongospora nasturtii* M.W. Dick is the vector of the unclassified watercress yellow spot virus (WYSV, ROCHON *et al*. 2004). Considering the fact that grapevines also suffer from different viruses and that some of their organismic vectors are still unknown (BOVEY *et al*. 1980; FRISON and IKIN 1991), the new grape plasmodiophorid (*Sorosphaera sp.*) might represent a promising target for future research. Viruses transmitted by *Polymyxa graminis* and other plasmodiophorids show certain physical and biochemical properties, like non-enveloped filamentous virions with single stranded RNA genomes, which are similar to the characteristics of grapevine closteroviruses. WYSV probably belongs to the same family (Tombusviridae, CLAY and WALSH 1997) like the Grapevine Algerian latent virus (GALV) and the Petunia asteroid mosaic virus (PeAMV). Plasmodiophorid vectors aquire the virus in vivo; this means the virus is present in resting spores and in zoospores. Although particles of Bymoviruses have



Figs 1-5: *Sorosphaera sp.* in roots of grapevine. **1**: SEM micrograph of cortical cells in phylloxera-induced root galls crowded with sporosori. **2**: Detail of sporosori. **3**: Light micrograph of hollow sporosori formed by peripherically arranged, saucer-shaped resting spores (arrow). **4**: DAPI-stained sporosori in different devolopmental stages found in a root segment without hypertrophies. Mature resting spores are uninucleate (arrow). **5**: Multinucleate plasmodia filling out two cortical cells. Bar = 50 µm in Fig. 1, 10 µm in Figs. 2-5.

been found in zoospores and sporangia the underlying mechanisms of uptake are not known but certain evidence suggests that specific sites on the capsid and the plasmodiophorid zoospore are involved (for a more detailed description see KANYUKA *et al*. 2003 and ROCHON *et al*. 2004).

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