

# Endophytic Occurrence of *Sphaceloma murrayae* on White Willow (*Salix alba*) in Italy

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## ABSTRACT

Despite being considered a common disease in countries such as New Zealand, Argentina and the United States, the occurrence in Europe of grey scab of willows incited by *Sphaceloma murrayae* has been reported quite recently, and to date no observation concerns Italy. An endophytic isolate of this foliar pathogen was recovered from the secondary branch of a white willow tree (*Salix alba*) in Basilicata. This finding represents the first documented report of *S. murrayae* from Italy and of its endophytic occurrence in *S. alba*.

**Keywords:** fungal endophytes, grey scab, *Salix* spp., *Sphaceloma populi*

**Abbreviations:** PDA, potato dextrose agar

Endophytic fungi are the subject of an increasing number of studies concerning their ability to produce bioactive secondary metabolites of pharmaceutical interest (Tan and Zou 2001; Schulz *et al.* 2002; Strobel *et al.* 2004). Fungal endophytes from several forest plants are being collected in our laboratory for screening based on the fungitoxic and cytostatic properties of their culture filtrates. Isolations are carried out from secondary branches by which 5 cm-cuttings are taken and sterilized through a standard protocol (Nicoletti *et al.* 2008); fragments of subcortical tissues are then excised and placed on potato dextrose agar (PDA) plates amended with streptomycin sulphate (200 ppm, Sigma), and incubated in the dark at 25°C. Fungal colonies emerging from the plant fragments are readily transferred to fresh PDA plates. By this procedure, a quite slow-growing fungus (isolate E30SP) was recovered from a willow tree (*Salix alba*) in a holt near Picerno, in Basilicata, southern Italy. Colonies on PDA attained a diameter of about 10 mm in 15 days (Fig. 1); they were pulvinate, showed a peculiar brown-reddish coloration of the reverse, and produced prominent conidiomata with hyaline aseptate conidia which were elliptical in size ( $4.5 \div 7 \times 3 \mu\text{m}$ ). A specimen sent to the CBS-Fungal Biodiversity Centre (Utrecht, the Netherlands) for a more accurate examination was identified as *Sphaceloma murrayae* Jenk. and Grods. (de Cock, pers. comm.). This species was described as a pathogen of willows causing grey leaf spots (grey scab), and reported to be of quite common occurrence in New Zealand, Argentina and the United States (Jenkins and Grodsinsky 1943). It has been recorded on a number of willow species including *S. alba*, *S. babylonica*, *S. caprea*, *S. fragilis*, *S. hippophaefolia*, *S. lasiolepis*, *S. matsudana*, *S. nigricans*, *S. purpurea*, *S. triandra* and *S. viminalis* (Grodsinsky and Jenkins 1943; Spiers and Hopcroft 1992). The first documented report from Europe was published a few years ago after its recovery from *S. fragilis* and *S. purpurea* in several regions of Germany (Butin and Kehr 2004). After examining herbarium specimens collected in 1959, these authors supposed the pathogen to be present in Germany since at least 50 years, while they consider unreliable a previous identification from *S. viminalis* in Latvia as mentioned by Jenkins and Grodsinsky (1943). These remarks, together with the existence of a former undetailed report of the species from France (Morelet 1967),



Fig. 1 Isolate E30SP after 3 weeks culturing on PDA in a 50 mm-diameter Petri dish.

indicate that its occurrence in Europe is possibly more widespread and not derived from accidental introduction. Great Britain is also likely to be included in the European distribution of *S. murrayae*. In fact, in 2002, the occurrence of *Sphaceloma populi* (Sacc.) Jenk. on *S. fragilis* leaves was reported in Surrey (Punithalingam and Spooner 2002). *S. populi* is a foliar pathogen of poplars (*Populus* spp.) and, considering both its morphological affinity with *S. murrayae* and the close taxonomic relationship between *Salix* and *Populus*, these authors observed that there is little justification for keeping two distinct species. However, in order to eventually accept this presumed identity, a more accurate morphological examination and investigations on DNA homology of a consistent number of isolates are needed. Isolate E30SP has been deposited at the CBS collection (reference CBS 128291), and is available for a more circumstantial study to this regard. Should synonymy be proved, then the species is to be considered indigenous in Italy, where *S. populi* was originally described as *Hadrotrichum populi* by Saccardo (1878). Whatever the conclusion, this appears to be the first report of an endophytic occurrence of *S. murrayae* in *S. alba*. Previous investigations concerning endophytes of *S. fragilis* and *S. caprea* did not detect this species (Petri and Fisher 1990; Likar and Reg-

var 2009). Considering that many pathogens of forest plants regularly develop endophytically and induce disease as a consequence of environmental stress (Sieber 2007; Slippers and Wingfield 2007), if confirmed by further findings the endophytic aptitude may be indicative that primarily *S. murrayae* establishes a compatible interaction with willow trees. As a matter of fact, no plant at the holt where isolate E30SP was recovered showed grey scab symptoms, nor have we so far happened to find symptomatic willows during on-spot surveys at several locations in other Italian regions, such as Campania, Basilicata, Abrutium, Latium, Tuscany and north-western Piedmont. A further more systematic investigation will be carried out on both *Salix* and *Populus* spp. to achieve additional data on endophytism of *S. murrayae* and its relatedness with *S. populi*.

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