

NEW OR UNUSUAL DISEASE REPORTS

Grapevine decline in Italy caused by *Lasiodiplodia theobromae*

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Summary. The first report of a dieback of grapevine caused by *Lasiodiplodia theobromae* in Sicily (Italy) is given. About twelve per cent of the vines in the cv. Insolia vineyard surveyed, showed spur dieback, retarded growth and wood necrosis. Isolation trials and pathogenicity tests are briefly reported, together with morphological, cultural and molecular characters on which identification was based.

Keywords: *Botryosphaeria rhodina*, Botryosphaeriaceae, wood canker, trunk diseases, *Vitis vinifera*.

Species in the Botryosphaeriaceae, including *Botryosphaeria*, *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* (Crous *et al.*, 2006), have been reported with increasing frequency as the cause of various decline symptoms in grapevines (*Vitis vinifera*) (Luque *et al.*, 2006; Van Niekerk *et al.*, 2006). These symptoms include cankers, wood necrosis, spur dieback and retarded growth (Luque *et al.*, 2005; Taylor *et al.*, 2005; Ûrbez-Torres *et al.*, 2006; van Niekerk *et al.*, 2006; Ûrbez-Torres *et al.*, 2008). Some authors also report Botryosphaeriaceae as associated with foliar chlorosis and necrosis (Lehoczy, 1974; Cristinzio, 1978; Rovesti and Montermini, 1985; Lehoczy, 1988; Larignon *et al.*, 2001;

Auger *et al.*, 2004), or with fruit rot symptoms (van Niekerk *et al.*, 2006; Trapman *et al.*, 2007).

Even if the epidemiology of the diseases caused by these fungi in grapevines is at present poorly understood, their occurrence shows a regional variability, apparently linked also to climatic differences. Among other species *Lasiodiplodia theobromae* is reported (usually as the teleomorph stage “*Botryosphaeria*” *rhodina*) as a tropical and sub-tropical pathogen on many different tree species (Mohali *et al.*, 2005). It is frequently isolated from grapevines in warmer grape-growing areas such as California, Arizona and Mexico (Ûrbez-Torres *et al.*, 2006, 2008), Egypt (El-Goorani and El Meleigi, 1972) and some of the warmer areas of Australia (Taylor *et al.*, 2005).

During a three-year study of esca disease in Sicilian vineyards (2004–2007), an unusual decline was observed in one vineyard of 13-year-old cv. Insolia grafted on 140R rootstock in western Sicily (Marsala, Trapani) (Fig. 1). The first samples examined in Oc-

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tober 2006 showed in cross section internal brown wood necrosis, often with a wedge shape (Fig. 2A and B). In addition to the necrosis, discolouration of the wood in 2-year-old and older shoots, canes and trunks, accompanied by small, brown-black spots or arc-shaped discolourations in the vascular tissue was also seen (Fig. 2B and C). The discoloured area was associated with cortical lesions.

In the spring of 2007, 8.8% of the 500 grapevines surveyed in the same vineyard showed bud mortality and delayed growth (Fig. 1). During the summer,

the severity of the disease increased greatly: the infected vines showed progressive spur, cane and arm dieback, with depressed bark and cracks and re-vegetation at the base, while 20 plants died. At the same time the number of symptomatic vines increased, so that by the end of the season a total of 12.4% of the 500 vines surveyed had died or were showing decline symptoms. In the survey carried out in August 2007, 43% of the declining vines also showed a paler colour along the margins and in the interveinal area of the leaves (Fig. 3).



Fig. 1. Retarded growth and spur dieback in 13-year-old vines cv. Insolia infected with *Lasiodiplodia theobromae*.



Fig. 3. Mild chlorosis in the marginal and interveinal leaves of cv. Insolia. All vines showing foliar chlorosis sampled were infected with *L. theobromae*.

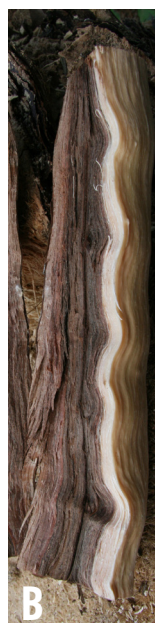


Fig. 2. Brown necrosis caused by *L. theobromae* extending along a large part of the trunk: A, cross section; B, longitudinal section; C, black spots.

Sampling the declining vines for internal wood symptoms revealed a wide dark brown necrosis which often covered more than half of the section of the trunk and when the bark was peeled off appeared as a dark longitudinal band that, in a few fully analyzed vines, extended back to the rootstock. Wood necrosis and occasionally brown-black spots could also be noticed, as described above (Fig. 2).

Some wood disks and sections of canes and trunks (2–5 cm²) with sub-cortical discolourations or sectorial necrosis were flamed briefly and placed in moist chambers. Other disks and sections were surface-sterilised for 1 minute with 70% ethanol, followed by brief flaming. Wood fragments were dissected and placed on 90 mm diam. Petri dishes containing 2% malt-extract agar (MA) and incubated at 20–25°C. Hyphae growing out of the wood in moist chambers were also transferred to MA Petri dishes.

On samples of canes and trunks maintained in moist chambers for 2 weeks, black, globose, smooth and ostiolate pycnidia 0.5–1.5 mm diam. developed that later became erumpent. The same fungal colony was isolated consistently from the sectorial discolouration in the wood samples that had been kept in moist-chambers and from the isolations on MA. All isolated colonies were initially white, becoming dark-grey to black with dense aerial mycelium, not producing a yellow pigment in agar. In all colonies black and ostiolate pycnidia (1 mm diam.) formed after 25–30 days.

Mature conidia, oozing out as a black mass from mature pycnidia, were oval, 16–23×8–11 µm, dark-brown, medianly one-septate with longitudinal striations on the conidial wall. Immature conidia were hyaline, aseptate and without striations, with a similar shape and size to the mature ones. Paraphysis were septate. The macro- and microscopic features of pycnidia, paraphyses and conidia (Burgess *et al.*, 2006; Alves *et al.*, 2008) identified the fungus as *Lasiodiplodia theobromae*, one of the species reported as causing various diseases on more than 100 genera of host plants (Zhou and Stanosz, 2001; van Niekerk *et al.*, 2006), especially in tropical and subtropical climates (Mohali *et al.*, 2005), and often viewed as one of the most virulent on grapevines (Van Nierk *et al.*, 2006; Ūrbez-Torres *et al.*, 2008).

Isolations from brown-black spots or arc-shaped discolourations never yielded any of the wood pathogens that commonly occur in grapevine wood as fungal agents of esca (Mostert *et al.*, 2006; Surico *et*

al., 2006), but only strains of *L. theobromae* (100% positive isolation). Furthermore no typical esca foliar symptoms (Mugnai *et al.*, 1999; Surico *et al.*, 2006) had been recorded in any part of the vineyard.

A representative isolate, PvPa 1, was deposited in the collection of the Dipartimento S.En.Fi.Mi. Zo, Sezione di Patologia Vegetale e Microbiologia Agraria, Università degli Studi di Palermo, and was grown in Malt extract liquid culture and DNA extracted using the DNeasy[®] Plant Miny Kit protocol (Qiagen, Hiden, Germany) following manufacturer's instructions. The ITS cluster was amplified with the universal primers ITS5 and ITS4 (White *et al.*, 1990). A BLAST search of the sequence of the amplicon obtained (~600 bp) (GenBank accession No. FJ210791) showed the highest homology (99%) with *Lasiodiplodia theobromae*.

In September 2006, isolate PvPa 1 of *L. theobromae* was inoculated on 2-year-old grapevines cv. Insolia in a greenhouse and in the field (seven vines in each replicate). A slit 1–1.5 cm long and 5 mm deep was made in the wood of the trunk, 10 cm above soil level. A 6-mm diam. mycelial plug was applied to the wound and covered with a plastic bag for 48 h to maintain high humidity. Three control plants were inoculated with sterile MA plugs. Two months after inoculation all inoculated trunks showed internal necrosis (Table 1) extending about 2.7 cm away from the infection site (2±0.16 cm down and 0.7±0.13 cm up), while only a 1–2 mm long discolouration was observed in the control vines. *Lasiodiplodia theobromae* was always re-isolated from the discoloured tissues.

This is the first report of a canker of grapevine caused by a Botryosphaeriaceae species in Sicily, and the first report of infections caused by *L. theobromae* on grapevine in Italy. Bark cankers and dieback on grapevine associated with Botryosphaeriaceae species have rarely been described in Italy. Cristinzio (1978) and Rovesti and Montermini (1987) described dieback, necrosis and also some foliar necrosis of grapevine, which were reported to be associated with strains of *Sphaeropsis malorum* (= *Diplodia seriata*), as the causal agents of the dieback. The present paper reports the occurrence of a different species that is normally linked to a tropical or sub-tropical climate (Mohali *et al.*, 2005; Haggag *et al.*, 2007) such as on grapevines in Egypt (El-Goorani and El Meleigi, 1972) or Mexico (Ūrbez-Torres *et al.*, 2008). This fits very

well with the semiarid climate, with mild winters and hot summers, that are typical in Sicily. In this way it is highly probable that the pathogen could establish itself easily within the area and become a significant, problem, especially as it is reportedly a virulent species on grapevine (van Niekerk *et al.*, 2006; Urbez-Torres *et al.*, 2008). Special attention must therefore be paid henceforth to distinguishing the dieback associated with *L. theobromae* from the dieback associated with other wood disease and decline agents such as esca-associated fungi. This is all the more important since the spread of the pathogen in the vineyard sampled was quite rapid in the season investigated, and may represent a threat for the vine-growing area where it was first isolated, and indeed for the whole of Sicily. The occurrence of an aspecific chlorotic pattern in the leaves of several of the declining vines surveyed in the present report will also need to be better evaluated bearing in mind that the diagnosis of trunk diseases is often made more difficult by the coexistence of several fungal pathogens in the same vine. Furthermore all the reports cited of dieback of grapevine due to *L. theobromae* (= *B. rhodina*) only described wood necrosis, and no foliar symptoms except stunted leaves. This aspect will also need to be evaluated more clearly in view of recent findings about the production of EPS (β -glucan) by isolates of *L. theobromae* (as *B. rhodina*) (Corradi da Silva *et al.*, 2005) as well as other species in the Botryosphaeriaceae (Martos *et al.*, 2008). These EPSs may accumulate in the leaves, as observed in the case of esca (Bruno and Sparapano, 2007; Andolfi *et al.*, 2008), where they produce phytotoxic effects such as chlorosis of the leaves. Future surveys will better define the occurrence and importance of the decline reported and of the symptoms found to be associated with it.

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