17 Cypress Canker

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17.1 Pathogens, Significance and Distribution

Bark canker of cypress (*Cupressus* and related genera) is a pandemic disease due to the worldwide spread of the fungal pathogen *Seiridium cardinale* (W.W. Wagener) B. Sutton & I.A.S. Gibson and of two other similar species, *S. cupressi* (Guba) Boesew. (teleomorph: *Lepteutypa cupressi* (Nattrass, C. Booth & B. Sutton) H.J. Swart) and *S. unicorne* (Cooke & Ellis) B. Sutton. *S. cardinale* is the most widespread and aggressive of the three species and has caused epidemics in many regions into which it was introduced, especially during the second half of the 19th century (Panconesi, 1990; Graniti, 1998).

Several other pathogenic fungi are known to be responsible for cankers on cypress as well. Among these, *Diplodia cupressi* A.J.L. Phillips & A. Alves, *Kabatina thujae* R. Schneid. & Arx, *Pestalotiopsis funerea* (Desm.) Steyaert, *Phomopsis occulta* (Sacc.) Traverso and *P. juniperivora* G. Hahn are the most common. This chapter, however, will take into consideration only Seiridium canker of cypress because of its spread at a global level and the serious damage and economic losses that it causes to many *Cupressaceae* in forests, windbreaks and ornamental plantings.

Cypress has suffered attacks of bark canker for more than 80 years. The first epidemic of *S. cardinale* was reported in California in 1928 on Monterey cypress (*Hesperocyparis macrocarpa* (Hartw.) Bartel), which over a period of only a few years was completely destroyed in the plantations located in inland districts (Wagener, 1939). Monterey cypress, which is extremely susceptible to canker and widely traded for ornamental purposes, had a major role in spreading the disease to other host species worldwide. In the course of the following decades, the disease spread progressively over the five continents: to New Zealand, France and Chile; to Italy, Argentina and Greece (Birch, 1933; Barthelet and Vinot, 1944; Grasso, 1951; Saravi Cisneros, 1953; Anastassiadis, 1963; Mujica *et al.*, 1980) and, subsequently, to the entire Mediterranean basin and to other countries in central and northern Europe; and to Canada, north and South Africa and Australia (Torres, 1969; Strouts, 1970; Sutton and Gibson, 1972; Funk, 1974; Caetano, 1980; Solel *et al.*, 1983; Wingfield and Swart, 1988; Danti *et al.*, 2009). *S. cardinale* canker has been reported on species of *Cupressus*, *Hesperocyparis*, *Chamaecyparis*, *Cryptomeria*, *Juniperus*, *Thuja*, *Callitropsis ×leylandii* (A.B. Jacks. & Dallim.) D.P. Little

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(*nootkatensis* × *macrocarpa*) (also known as Leyland cypress and ×*Cupressocyparis*) and *Callitropsis*.

The impact caused by epidemics of *S. cardinale* has been particularly serious in the Mediterranean area, where disease incidence in the 1980s reached 70% and 90% in some wooded and ornamental plantations of central Italy and Greece, respectively (Xenopoulos and Diamandis, 1985; Panconesi and Raddi, 1991). In this area, the Italian cypress (*Cupressus sempervirens* L.) has been grown as a domestic and multi-purpose tree since antiquity, mainly for its ornamental and symbolic role, but additionally for the production of timber, for reforestation and for barriers and hedges. In several regions, e.g. in Tuscany, where Italian cypress is a typical feature of the landscape and an important element of gardens and formations of artistic, cultural and historic interest, *S. cardinale* has represented a serious threat. Even today, the cypress canker represents a factor in the deterioration of cypress, thus limiting its planting and cultivation both in the Mediterranean basin and many other regions of the world, especially in areas where the climatic conditions are particularly favourable to the pathogen.

S. cupressi was reported for the first time in Kenya in 1942 (Wimbush, 1944) in badly damaged plantations of Monterey cypress and cedar-of-Goa (*Hesperocyparis lusitanica* (Mill.) Bartel). The current distribution area includes east and South Africa, Australia (Cunnington, 2007) and New Zealand (Chou, 1989). The host genera, *Cupressus*, *Hesperocyparis*, *Callitropsis* ×*leylandii*, *Chaemacyparis* and *Juniperus*, belong to the *Cupressaceae*. In 1984, the fungus was also reported on a few Italian cypress trees on the island of Kos in Greece (Graniti, 1986).

The geographical distribution of *S. unicorne* seems to include North America, New Zealand, Australia, Japan and Europe (Fuller and Newhook, 1954; Sutton, 1980; Van der Werff, 1984; Chou, 1989; Caetano *et al.*, 1991; Tabata, 1991; Tisserat *et al.*, 1991; Della Rocca *et al.*, 2011b), and its reported host range includes eight different families. According to Chou (1990), *S. unicorne* is unable to cause infection on species that do not belong to the *Cupressaceae* and many reports of the fungus on hosts of other families that have been made in the past may have been misidentifications of other similar fungi. New Zealand is the only country where the three *Seiridium* spp. are known to occur simultaneously (Boesewinkel, 1983).

17.2 Diagnosis

The diagnosis of Seiridium canker of cypress is based on observation of symptoms on affected trees, on the morphological and cultural characteristics of fungi, and on the use of molecular techniques based on analysis of specific genomic sequences. The symptoms on trees produced by the three *Seiridium* spp. are very similar, though *S. unicorne* generally gives rise to less virulent cankers and to less severe damage than do the other two species.

The first evidence of infection produced by *S. cardinale* is the appearance on the bark of a reddish brown necrotic spot around the entry point of the pathogen, often associated with resin emission. The necrosis spreads out progressively and becomes fusiform and slightly depressed. Longitudinal cracks subsequently appear, from which resin flows more or less abundantly, depending on the genotype and on the reactivity of the host (Plate 21). In young plants, the canker may even girdle the stem or branches in a relatively short time, whereas in adult trees canker development requires more time and, in addition, depends on the individual reaction of the host. Large cankered organs often appear to be greatly deformed and depressed, with bark cracks and resin emission as a result of the action of the pathogen and the host's reaction. When a canker has girdled a branch or a stem, the portion above the canker dies; its foliage turns first pale green, then yellow, brownish red and, lastly, ash grey. Within a few months, the foliage falls off and the affected branch (or stem) becomes completely bare. Both early symptoms (yellow crowns) and advanced development of symptoms (bare branches) can frequently be observed on the same tree (Plate 22).

Infections can occur in any part of the crown and the trunk, but are generally more frequent on the outer branches and twigs, which are more exposed to the fungal inoculum and also have a less thickened periderm. A useful diagnostic trait is represented by the acervuli, the asexual fruiting bodies of the fungus that develop on the surface of the necrotic bark and on cones during the spring and autumn. Acervuli appear as black sub-epidermal or sub-peridermal pustules (0.2–3 mm in size) that open, when mature, with the breakage of the upper tissues. However, similar acervuli are produced by other fungal pathogens of cypress as well, e.g. *P. funerea*.

In the genus *Seiridium*, the conidia (the spores produced by the acervuli) are five euseptate, fusiform, and with four brown median cells and conic, hyaline terminal cells. The main distinctive trait among the three species of *Seiridium* causing canker of cypress trees is the morphology of the terminal (apical and basal) cells of the conidia. *S. cardinale* has very short appendages (1 μm long), sometimes absent, whereas *S. cupressi* and *S. unicorne* have appendages up to 13 μm in length (Fig. 17.1), although in *S. cupressi* they generally follow the curve of the conidia, and in *S. unicorne* the apical appendage frequently forms a right angle with the axis of the conidium (Guba, 1961; Boesewinkel, 1983). Sutton (1980) and Chou (1989) considered the orientation of the conidial appendages a too variable trait, and treated *S. cupressi* and *S. unicorne* as a single species.

Some cultural differences have been pointed out among the three species in colony colour and morphology (off-white with grey-green shades and with a salmon orange reverse in *S. cardinale*; salmon or apricot with grey and off-white areas in *S. cupressi*; olive green or dark green in *S. unicorne*) and in radial growth, which, measured after 6 days at 26°C, was higher in *S. cardinale* (31 mm diameter) than in *S. cupressi* (13 mm diameter) and *S. unicorne* (18 mm diameter) (Boesewinkel, 1983). Graniti (1986) also observed that in *S. cupressi* the acervuli are formed close to the margin of the cankered area and are slightly larger (up to 2 mm) than in *S. cardinale* (up to 1.5 mm), which forms acervuli over the entire surface of the canker.

Molecular studies based on the sequencing of the genes coding for structural proteins (histones and β-tubulins) have supported the separation into three distinct species of the fungi responsible for cypress canker (Barnes *et al.*, 2001). Analysis of sequence data showed that *Seiridium* isolates resided in two major clades: one accommodated *S. unicorne* and the other consisted in two sub-clades, which included the other two species separately. Existence of three species was confirmed by the restriction fragment length polymorphism (RFLP) technique, based on the digestion of β-tubulin amplicons with a single restrictive

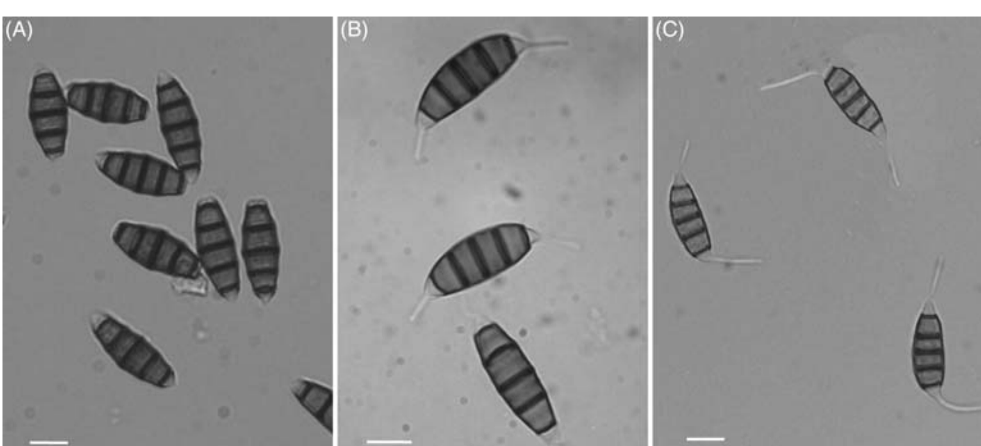

Fig. 17.1. Conidia of *Seiridium* spp. observed under a light microscope. (A) *Seiridium cardinale* (W.W. Wagener) B. Sutton & I.A.S. Gibson, size 21–30 × 8–10 μm; (B) *S. cupressi* (Guba) Boesew., size 27–32 × 7.5–10 μm; and (C) *S. unicorne* (Cooke & Ellis) B. Sutton, size 25–27 × 7.5–10 μm. Bars, 10 μm.

endonuclease (Krokene *et al.*, 2004). Studies on the *in vitro* production of toxins seem to support the separation of the fungi affecting cypress into three distinct species too. The differentiated production of toxins exhibited by the three fungi suggests the possible use of this chemotaxonomic means for their identification. It should be noted here that as *S. cupressi* has been often misidentified as *S. unicorne* on the basis of morphological traits (Graniti, 1986; Cunnington, 2007), the real nature of epidemics attributed to *S. unicorne* in some countries should be verified by the use of suitable diagnostic techniques.

17.3 Infection Biology

The biology, epidemiology and pathogenicity of *S. cardinale* have been widely studied, whereas relatively few studies have involved *S. cupressi* and *S. unicorne*.

The occurrence of *S. cardinale* infections is favoured by the presence of small wounds in the periderm of stems and branches through which the conidia or mycelium enter the inner bark. Although it acts mainly as a wound pathogen, the fungus can also reach the living bark through natural openings, such as stomata and lenticels, when the temperature and relative humidity (RH) are optimal for the germination of conidia and mycelial growth (25°C and 100% RH, respectively) (Panconesi, 1990). Young trunks and branches are particularly exposed to infections, because they are more greatly subjected to bark injuries due to cold, hail, or to forced growth by fertilizers, insects, rodents, etc.

During milder and more humid periods, such as the spring and autumn, the acervuli develop on the surface of the necrotic bark and on maturing cones, which are easily colonized by the fungus (Fig. 17.2). The conidia are disseminated by rainwater that flows downwards on to the surface of stems and branches, by windborne raindrops, by insects, and presumably by birds and small mammals, and can give rise to new infections on the same trees or on nearby trees. The progressive occurrence and development of a series of infections on a tree can cause its death in a relatively short time, depending on the age, susceptibility and environmental conditions.

In addition it has been observed that the fungus can remain viable for several years after the death of the infected tissues, and can give rise to subsequent sporulations in previously formed acervular craters, thereby contributing to the maintenance of a high amount of inoculum. Forms in which the fungus has been conserved have been observed in dead tissues, and include resting mycelium, chlamydospores and sclerotia (Panconesi, 1990).

Well-known vectors of the pathogen are bark beetles of the genus *Phloeosinus* (*P. aubei* (Perris), *P. thujae* (Perris) and *P. armatus* Reitter). During their life cycles, these insects can carry the inoculum of the fungus from the cankered trees, where the insects reproduce, to healthy trees, where they feed, thus contributing to the spread of the pathogen. Other cone-spermophagous insects, e.g. *Orsillus maculatus* (Fieber) and *Cydia cupressana* Kearfott, are known to carry the conidia of the fungus even over long distances. A potential role in the spread of *S. cardinale* is likely for some other cone insects too (Roques and Battisti, 1999).

The conidia of *S. cardinale* germinate at temperatures ranging from 5°C to 35°C, with an optimum at 25°C. Germination of the conidia is greatly influenced by the RH: when the RH drops from 100% to 80%, the percentage of germinated conidia decreases drastically (from 98% to 60%), and germination is completely prevented when the RH drops below 50% (Panconesi, 1990). *S. cardinale* is more thermophilic than either *S. cupressi* or *S. unicorne*, and can cause infections at a temperature up to 30°C. In contrast, *S. cupressi* shows a maximum pathogenicity between 20°C and 25°C, while infections develop more slowly at 30°C, whereas for *S. unicorne in vitro* germination and growth are higher at 20°C, and infective capacity is maximum between 25°C and 30°C (Graniti, 1998).

Based on the results of artificial inoculations on different host species, *S. cardinale* is more aggressive than the other two species under Mediterranean climatic conditions. However, the bark necrosis caused by *S. cupressi* grows constantly during the summer, whereas

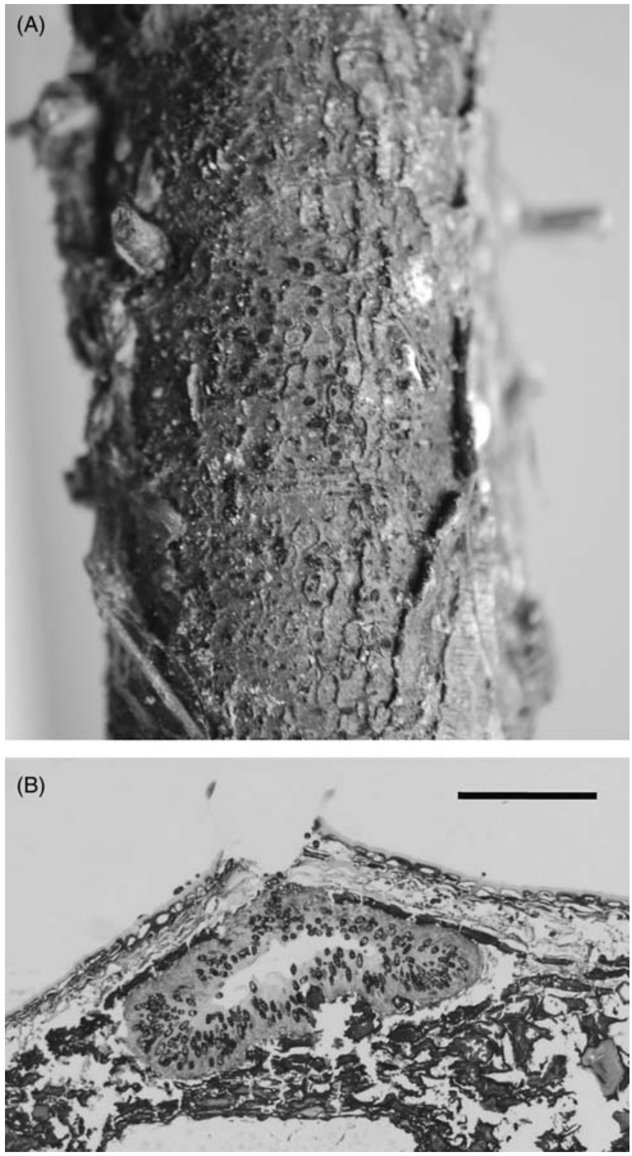

Fig. 17.2. (A) Acervuli of *Seiridium cardinale* (W.W. Wagener) B. Sutton & I.A.S. Gibson developed on the surface of a cankered stem; (B) section of a sub-epidermal erumpent acervulum of *S. cardinale* observed by light microscopy. Bar, 30 µm.

the development of the *S. cardinale* necrosis slows down during the warmest months. The necrotrophic action of *S. cupressi* thus takes advantage of the host summer dormancy. In the same Mediterranean climate, *S. unicorne* has shown a reduced virulence compared with the other two *Seiridium* spp. (Ponchet *et al.*, 1990; Xenopoulos, 1991).

S. cardinale shows its necrotrophic action in the bark tissues located between the outer xylem and the outer bark, which become brown-purplish in colour (hence the name *S. cardinale*) and also resin soaked. Based on ultrastructural observations, the hyphae of the fungus are concentrated particularly in the phloem, in the parenchyma, in the cambium and in the medullary rays, including the fibres (Mutto Accordi and Panconesi, 1987). The hyphae develop mostly in the intercellular spaces, colonizing and degrading the cell walls through the production of cell wall degrading enzymes (CWDEs), such as cellulase, xylanase and polygalacturonase (Magro *et al.*, 1982).

The longitudinal growth of the fungus is favoured by the structure of the sieve plates of the phloem through which the hyphae are able to pass easily. Wagener (1939), Moriondo (1972), Grasso *et al.* (1979) and Solel *et al.* (1983) described resin exudation and the formation of resin ducts as a result of the infection process. In the genus *Cupressus*, and in related genera, no constitutive resin ducts are present in the xylem and the phloem but the local formation of resin ducts is induced as a defence response in the phloem of the Italian cypress as a result of *S. cardinale* infections or mechanical wounds (Moriondo, 1972).

The death of the distal portion of a cankered twig, branch or stem generally occurs when the necrotic process has girdled the bark of an affected organ. It has been observed that this is due to the occlusion of the pit membranes of the tracheids by metabolites produced by the fungus and by the response of the tree to the pathogen, which blocks the sap flow in the xylem vessels. The wilting cannot be attributed to the sparse hyphal colonization of the sapwood, or to the occasional accumulation of tannins, or to tylosis or resin production, which have not been observed in the xylem (Madar *et al.*, 1990). Even in dead trees or seriously affected trees, the wood is not decayed by the fungus and the timber is marketable.

The necrosis of the host cells that occurs before colonization by the fungus, and the development of foliar symptoms on branches and twigs that are not yet girdled, suggest that toxins produced by the fungus take part in the pathogenesis, and that these toxins diffuse to the adjacent tissues and possibly move to the leaves with transpiration. Nine non-host-specific toxins produced *in vitro* have been purified and characterized (Graniti, 1998). Each of the three *Seiridium* spp. produces, *in vitro*, at least one major toxin plus other secondary toxins. *S. cardinale* mainly produces seiridin and isoseiridin. *S. cupressi* mainly produces cyclopaldic acid and, secondarily, sericuprolide, in addition to small amounts of seiridin, isoseiridin and seiricardin. *S. unicorne* generally shows a lower production of toxins, among which are seiridin, isoseiridin and seiricardin-c. Among the three species, *S. cupressi* is able to produce a wider range of toxins (Graniti, 1998). In several bioassays, the application of seiridin and cyclopaldic acid induced symptoms that were similar to those observed in nature, and were more severe than symptoms induced by other toxins (Sparapano and Evidente, 1995).

Inoculations with toxin-deficient mutants of *S. cupressi* produced a milder symptomatology in leaves and branches than did inoculations of wild isolates, even if the development of cankers remained unchanged. The virulence of *S. cardinale* isolates was correlated with the amount of toxins (seiridin, isoseiridin) produced *in vitro*. Most isolates of *S. cardinale* produced both seiridin and isoseiridin, but in variable proportions, and 5% of the isolates did not produce any toxin, whereas other isolates produced either only seiridin or only isoseiridin (Sparapano and Evidente, 1995; Graniti, 1998). Toxins may, therefore, be virulence factors for the three species of *Seiridium* that are pathogenic on cypresses. This hypothesis finds confirmation in the fact that *S. unicorne*, the least aggressive species of the three, which is unable to produce cyclopaldic acid or any significant amount of seiridin, causes cankers in trunks and branches, but does not induce systemic symptoms on the crown as do the other two species (Graniti, 1998).

17.4 Epidemiology

For almost 80 years, cypress has suffered from destructive bark canker epidemics that have arisen as a result of the introduction of the various causal pathogens to different regions of the world. Epidemics were favoured by climatic conditions suited to the pathogens and by the density and continuity of a susceptible host over a vast area.

The Mediterranean climate, which is characterized by a marked thermal range between summer and winter, and by mild, rainy and humid seasons (usually the spring and autumn), is an essential requirement for epidemic development of *S. cardinale* canker. The humid and rainy seasons favour the sporulation and germination of the conidia, which can retain their germinability and pathogenicity for several months, even under unfavourable conditions (Panconesi *et al.*, 1993). In the northern part of the Mediterranean, cypress trees are predisposed to infections by the winter cold and by frequent late frost, which favour the occurrence of bark lesions. Generally, a clear increase of new infections can be observed during the course of particularly humid and rainy springs that have been preceded by very cold winters. As both the sporulation and germination of *S. cardinale* conidia take place within a temperature range of 5°C to 30°C, infections in the Mediterranean environment may occur for most of the year, if RH conditions are suitable.

The lack of one or more favourable factors can prevent the spread of the fungus. Microclimatic conditions may play a major role in the development of the disease and explain the presence of nuclei of the host that have escaped the disease not far from areas that have been severely affected.

In Greece, the severity of the *S. cardinale* attacks that have occurred over recent decades seems to be closely connected with the climatic conditions in the different districts of the country. Higher incidences, from 25% to 50%, have been observed along the western coast, which is exposed to more humid winds, whereas along the drier eastern coast, cypress canker has occurred only sporadically. The most serious attacks were reported in several isolated sites with a particularly favourable microclimatic conditions, such as the small valleys of Megalopolis (in the Peloponnese) and Karystos (on the island of Eubea), where incidences of 80% and 95% were recorded, respectively, at the end of the 1980s. At Karystos, 80% of the infected trees died (Xenopolous and Diamandis, 1985).

In Tuscany (Italy), the first epidemics of *S. cardinale* canker developed in areas characterized by greater humidity, such as the plain of Florence. Cypress canker, which was reported here in 1951, spread rapidly as a typical polyetic disease (i.e. the disease occurred over several growing seasons, with the inoculum produced in one season carried over to the next, leading to a build-up of an inoculum over the years) during the following decades; by the end of the 1980s, it was in many forests and plantations, with more than 50% of the trees affected, and peaks of disease occurrence of 70–80% (Panconesi and Raddi, 1991). In the other provinces, the disease incidence increased subsequently. According to the regional monitoring network of forest health (META Service), the mean incidence in the region for the 2006–2009 period was 22–23%, with marked differences between the various provincial districts due to the variation in the microclimatic conditions in a very heterogeneous territory, and also to the effect of the scattered sanitation interventions that have been carried out so far.

In Italy as a whole, cypress canker is reported throughout the territory (Panconesi, 1990), even if in most regions the sporadic presence of cypresses has contributed to limiting its spread. The incidence of the disease rose only in districts where the host population is more dense and climatic conditions are suitable for the pathogen, such as around the Garda Lake (from 14% to 17%), in the province of Trento (from 9% to 53%) (Santini *et al.*, 1997a) and in Sicily, where scattered peaks exceeding 50% were reported in plantations of Monterey cypress (Sidoti *et al.*, 2007). Currently, serious damage is frequently recorded in north and central Italy on Leyland cypress, which was widely planted in barriers during the last two decades because of its rapid growth, but is very susceptible to *S. cardinale* canker.

The spread and severity of attacks of canker has been maintained rather low in the warmer and drier regions of north Africa, where the disease has also been reported (Panconesi, 1990; Danti *et al.*, 2009).

The absence of epidemics involving native populations of Italian cypress can be attributed to geographic and climatic barriers that limit the spread of the pathogen. The native populations of Italian cypress are mostly residual, often located in areas with limiting pedo-climatic conditions, e.g. in Crete. In these environments, *S. cardinale* presumably has less adaptive capacity than its host, which slows down its development, thus causing only sporadic and limited infections. In addition, it has been observed that in natural areas of Italian cypress, the number of *S. cardinale* conidia vectored by insects has markedly decreased (Santini and Di Lonardo, 2000).

Similarly, in California, *S. cardinale* canker heavily damaged the inland plantations of Monterey cypress, whereas the coastal native populations remained mostly unaffected (Wagener, 1939). In Monterey cypress, the absence of the disease in native populations does not seem to be due to a form of genetic resistance, given the marked susceptibility shown by this species to artificial inoculations with *S. cardinale* (Grasso *et al.*, 1979; Raddi, 1979). In its restricted native range along the coast of the Monterey Bay, Monterey cypress is subjected to the influence of the cold ocean winds that even during the summer prevent the temperatures from rising to values that are more favourable to fungal growth and sporulation. A few kilometres inland though, the climatic conditions change drastically and become similar to those typical of the Mediterranean, with marked differences between summer and winter, and with humid and rainy seasons, so that the epidemic spread of the pathogen is favoured.

Climatic conditions favourable to the pathogen have also been found in the state of Victoria in south-eastern Australia (Purnell, 1960; Swart, 1973), where Monterey cypress has been severely damaged, so that its use in new plantations has been discouraged. Here, cypress canker seems to be caused especially by *S. cupressi*, and to a lesser extent by *S. cardinale* and *S. unicorne* (Cunnington, 2007). In New Zealand, where the disease was reported in 1933 (Birch, 1933), Seiridium canker is now widespread throughout the territory on various *Cupressaceae*, with the greatest incidence of the disease observed in the northern part of the country, around Auckland, where the temperatures are warmer (Van der Werff, 1984).

17.5 Management Strategies and Tactics

17.5.1 Avoidance

S. cardinale is a primary pathogen that has exhibited greater aggressiveness on plants growing fast in non-limiting edaphic conditions, e.g. on fertile and deep soils (Panconesi, 1990). Cultural measures aimed at modifying the environmental conditions favourable to the occurrence of new infections and to the development of the disease may include: (i) locating nurseries or new plantations in very sunny and airy sites, far from diseased adult cypresses and other inoculum sources of the pathogen; (ii) avoiding valley-bottom zones that are subject to stagnation of humidity and thermal inversion; (iii) not making overly dense sowings/plantings; and (iv) maintaining sufficiently large spaces between the plants, both between the rows and along the rows, in order to facilitate air flow among the crowns.

17.5.2 Exclusion

Today, globalization (transport, trade, tourism, etc.) and the opening up of new borders (as in the European Union) increase the risk of introduction of new plant diseases. The introduction to new countries and regions of cypress plants or other propagation materials may be a cause of the spread of bark canker if they are not subjected to sanitary inspections.

S. cardinale has now attained a pandemic distribution and has been reported in many temperate regions that are suitable for the cultivation of *Cupressaceae*. Phytosanitary measures may be useful for preventing further spread of the pathogen in territories that are potentially favourable to its development, e.g. Japan, where until now only the presence of *S. unicorne* (*sensu* Sutton) has been reported (Tabata, 1991).

Furthermore, the introduction of new isolates characterized by greater virulence into a given region may favour the outbreak of new epidemics, to the detriment of plants that have escaped the previous waves of the disease. The introduction of isolates of a complementary mating type can allow sexual reproduction to occur in the pathogen population, thereby promoting a greater variability in the fungus and conferring on it a greater evolutionary potential in terms of virulence. The *S. cardinale* teleomorph has been reported only once in California by Hansen (1956), as *Leptosphaeria* sp., although it was not officially recognized because it was incompletely described. Therefore, studying the variability, reproduction and distribution of the pathogen may help to prevent both its introduction into new regions and the introduction of new genotypes into regions where the pathogen is already present.

S. cupressi is widespread in different continents, but at present it has not been reported in Europe. The pathogen was found in the 1980s on Kos (Greece) on several cankered Italian cypresses (Graniti, 1986), which were promptly felled in order to prevent the disease from spreading further. The possible introduction of *S. cupressi* into the Mediterranean area, which has been already devastated by the epidemic of *S. cardinale*, would represent a serious danger for cypress trees, and would also complicate the genetic improvement of cypress for resistance to *S. cardinale* canker that has been developed over the past three decades. As has already been suggested by Graniti (1986), *S. cupressi* should be included in the European and Mediterranean Plant Protection Organisation (EPPO) A1 list in order to adopt all the sanitary measures necessary for preventing its introduction into the European countries and its possible spread. The threat represented by the possible introduction of *S. cupressi* should be seriously considered both because of the virulence exhibited by the fungus in other regions, and because of the theoretical possibility that the onset of new epidemics may be favoured by the spread of ascospores produced by the teleomorph and their distribution by the wind (Moricca *et al*., 2000).

S. unicorne is less aggressive than the other two species of *Seiridium*, and has never been associated with epidemics. Compared with the other two species, the possible introduction of *S. unicorne* into new regions is less worrisome.

17.5.3 Eradication

Sanitation is the main method for controlling cypress canker, and this is aimed at eliminating or reducing the inoculum sources of the pathogen in a territory, or in more or less restricted areas – in forest stands, in ornamental plantations, or in single trees. This is an extinction control strategy that involves the mechanical removal, by means of the felling and/or pruning of the infected trees or parts of the crown, and providing for all resulting infected material to be destroyed by burning.

In the forest stands, all cypress trees affected by the disease should be felled, because cutting off the infected organs would involve obvious operational difficulties in entering such locations with the proper vehicles, e.g. elevated platforms. The procedure is carried out in the same way in the nursery owing to the need to eliminate definitively every source of infection. In adult plantations, where it is possible to use an elevated platform, and particularly in plantations that have an ornamental or historic role, or are monuments (rows, groups, hedges, single trees), the procedure is different, with all cypresses compromised by canker being felled, while crowns that are affected to a limited extent are subjected to pruning. This is done in an attempt to recover some of the affected trees.

By reducing the inoculum of the pathogen in the environment, sanitation makes it possible to attain a series of objectives: (i) reduce pathogen spread and control the epidemic in a given area; (ii) safeguard the cypresses from deterioration due to the uncontrolled action of bark canker; (iii) recover diseased trees through a well-performed pruning, which currently is the only therapeutic method available; (iv) increase the genetic value of seed stands through the elimination of susceptible trees; and (v) reduce the populations of bark beetles, which are important vectors of the pathogen.

The effectiveness of sanitation is linked to the prompt, careful and swift execution of cuttings/fellings, as well as to the extent of the area that it is essential to treat, with the overall aim of reducing the amount of inoculum in the environment as much as possible, and thereby limiting the destructive potential of the disease. In most cases, i.e. when confined to the peripheral portions of the crown, infections are easy to control with a prompt pruning of the affected organs. Interventions will become gradually more difficult and will have more uncertain results when the disease has involved larger branches and the stem.

In California, as a result of the epidemic of *S. cardinale* in the extensive plantations of Monterey cypress, it was possible to adopt drastic measures for eradicating the inoculum through the elimination of all infected trees and by discouraging the use of susceptible hosts in sensitive areas of the inland districts (Wagener, 1939). In Italy, in contrast, the use of such drastic measures would have radically changed the features of the landscape by removing one of its main characterizing elements.

17.5.4 Protection

Chemical control

Trials of chemical treatments for protecting cypresses from *S. cardinale* canker have been promoted basically by the need to support a considerable nursery production of *Cupressaceae*, as this has significant economic weight in the Mediterranean regions (Della Rocca *et al.*, 2011a).

A range of studies was undertaken in the past to evaluate the preventive and curative effectiveness of various fungicides for controlling *S. cardinale* canker. These studies revealed the effectiveness of systemic benzimidazoles and thiophanate-methyl for the preventive control of infections. Good effectiveness was also exhibited by several contact fungicides (i.e. chlorothalonil, dichochlofuanide, mancozeb), whereas limited results were obtained with copper products (Panconesi and Parrini, 1979; Govi and Deserti, 1980; McCain, 1984; Panconesi and Raddi, 1986).

In recent years, the European Directive 2009/128/EC (European Parliament and Council of the European Union, 2009) required a drastic reduction in chemicals used in agriculture, including benzimidazole and many contact fungicides that have been shown to be effective against *S. cardinale* canker. A recent experiment was carried out in order to test several compounds never evaluated before for the protection of cypress against canker (Della Rocca *et al.*, 2011a). This study provided evidence of good results for azoxystrobin, which was as effective as thiophanate-methyl in reducing canker development when sprayed on to plants before test inoculations. Boscalid was found to be able to prevent *in vitro* conidial germination and mycelial growth of *S. cardinale*, and appeared to be a promising contact fungicide for the prevention of cypress canker infections. Azoxystrobin and boscalid are both included in a less hazardous class than thiophanate-methyl, and have fewer risks for the environment and for users.

Chemical control constitutes an irreplaceable method for controlling bark canker of cypress in the nursery, where fertilization, watering and a high plant density may increase the predisposition of plants to *S. cardinale* infections, but it is characterized by important limitations. While it is particularly suitable for young plants in nurseries or in plantations, it can only seldom be proposed for adult trees that have a particular landscape value, or value as a monument or of historic interest; even then, spraying the upper and inner part of the crowns properly is technically difficult and time-consuming. Chemical treatments cannot really be advised for the protection of adult plantations either, especially in urban sites, or in forest stands, owing to the unavoidable dispersal of the sprayed products into the environment and to the difficulties of executing such an exercise.

Biological control

In one of the few studies conducted on this topic to date (Magro *et al.*, 1984), volatile and non-volatile metabolites of *Trichoderma viride* Pers. were capable *in vitro* of inhibiting germination and mycelial growth of *S. cardinale. T. viride*, which is commonly found as an epiphyte in the phylloplane of cypresses, has revealed a potential usefulness for the control of *S. cardinale*. However, no experiment has ever been carried out aimed at verifying the antagonist action of *T. viride in planta*, or at studying an effective form of application.

A recent study showed that the bacterium *Pseudomonas chlororaphis* subsp. *aureofaciens* prevented both the conidial germination and mycelial growth of *S. cardinale in vitro* through the production of phenazine-1-carboxylic acid (Raio *et al.*, 2011). Furthermore, when applied *in planta* before test inoculations, it also prevented the development of cankers on Italian cypress stems. Further, the bacterium was able to colonize the cypress phylloplane of Italian cypress as an epiphyte and appears to be a promising means for the control of cypress canker, to be eventually used within an integrated disease control strategy.

Fertilization

Cultural measures aimed at promoting the vigour of plants may promote the development of cankers. In order to reduce the predisposition of plants to infections, it is advisable not to apply excessive watering and fertilization, especially in the nursery. In particular, the excessive use of nitrogen promotes plant growth, slowing down the lignification of the tissues. The abundance of parenchymal tissues that results predisposes the bark to lesions due to forced growth and to cold, thus favouring the occurrence of infections and their subsequent development.

17.5.5 Resistance

Among the cypress species, a high interspecific variability in response to *S. cardinale* inoculations has been demonstrated. North American species phylogenetically close to Monterey cypress were found to be extremely susceptible, whereas the American species belonging to the *Hesperocyparis arizonica* (Greene) Bartel group were found to be more or less susceptible, as well as Italian cypress. Instead, the Asiatic species (*Cupressus chengiana* S.Y. Hu, *C. duclouxiana* Hickel, *C. funebris* Endl., *C. torulosa* D. Don) and North African species (*Cupressus sempervirens* L. var. *dupreziana* (A. Camus) Silba, *C. sempervirens* L. var. *atlantica* (Gaussen) Silba), in addition to a couple of North American species (*H. glabra* (Sudw.) Bartel and *H. bakeri* (Jeps.) Bartel) showed good resistance to *S. cardinale* (Allemand, 1979; Andréoli and Ponchet, 1991).

Variability of the pathogen

Several trials, based on artificial inoculations of Italian cypress clones with some Mediterranean *S. cardinale* isolates, revealed significant differences among isolates (in terms of canker size) only on tolerant and susceptible clones, but not on resistant clones. No host specificity at species or clone level have ever been revealed for any *S. cardinale* isolate (Raddi and Panconesi, 1984; Ponchet *et al.*, 1990). The absence of the sexual reproduction has contributed to lowering the variability of virulence within the Mediterranean *S. cardinale* population. The substantial evenness of the fungus has favoured the execution of the genetic improvement programme for cypress, and the criteria for selecting resistant genotypes that have been followed for years are currently still effective.

A low variability among *S. cardinale* isolates was also revealed by a molecular approach, but to date most studies have been based on an analysis of conserved DNA regions, which are more suited to the discrimination of taxa at species or subspecies level (the genes for the internal transcribed spacer regions ITS1 and ITS2, 5.8S, histone and β-tubulin) (Viljoen *et al.*, 1993; Moricca *et al.*, 2000; Barnes *et al.*, 2001; Krokene *et al.*, 2004). The use of random amplification of polymorphic DNA (RAPD) did not reveal any informative polymorphism among 77 isolates of *S. cardinale* from northern Italy (Pedron *et al.*, 2007). Recently, eight specific microsatellites have been developed for *S. cardinale* (Della Rocca *et al.*, 2009), and these have been employed for studies on the population genetics of 98 isolates from Europe and California. This has revealed the presence of a number of multi-locus genotypes and a higher genotypic diversity in the California population (Della Rocca *et al.*, 2011c). The absence of linkage disequilibrium calculated for the *S. cardinale* population in California also supported the potential occurrence of sexual reproduction of the fungus in this region. A better understanding of the dynamics of the pathogen might be derived from a comparison of the populations that have settled on the different continents.

The mechanisms of resistance

Inoculation tests have demonstrated that no immunity to *S. cardinale* infections exists within the host populations, and that in resistant species most plants are able to recover completely from the canker by healing the necrotic lesion, whereas in susceptible species this happens only in a few plants. A detailed histological study of the host reaction processes induced as a result of infection with *S. cardinale* was carried out by Ponchet and Andréoli (1990). The tree defence response, based on the formation of necrophylactic periderm (also called neoperiderm), is activated by the host to compartmentalize the damaged tissues, as in the case of any sort of wound, and is not a specific response to *S. cardinale* infection. The vigour of this reaction determines the resistance of a cypress tree to bark canker, because only plants that are able to set up effective barriers and then compartmentalize the infected tissues can then succeed in repairing the lesion. In inoculated plants, the development of necrophylactic periderm is influenced in timing (a delay) and intensity by the pathogen (Madar and Liphschitz, 1989).

In susceptible plants, necrophylactic periderm is not formed, or it is discontinuous and not very thick. The reaction is weak, so compartmentalization is ineffective and does not prevent the mycelium of the fungus from continuing its development in the bark tissues. In resistant plants, necrophylactic periderm is multilayered, thickened and well integrated with the constitutive periderm. The necrosis is thus confined by a solid barrier and the healthy tissues can start a repairing process through the annual production of new layers of phloem and xylem, until the wound is completely healed. After several (usually 4–5) years, the stem has recovered, and only a vertical scar can be seen on the bark.

Between susceptibility and resistance, a range of intermediate responses reveals that the defence reaction of cypress to *S. cardinale* infections is a gradual process. An intermediate response can tend towards susceptibility or resistance, depending also on the environmental conditions; it is defined as tolerance when plants are able to maintain their normal growth rate even when affected by several active cankers. This suggests that the mechanism that governs the reaction of cypress to *S. cardinale* infections is a quantitative trait under polygenic control and that its phenotypic expression is influenced by the genotype and by the environment. So most of the clones showed a change to their response to canker when inoculated in different environments, and the host–pathogen–environment interaction was significant for most of the tested genotypes. Often, a resistant clone may need a longer time to heal the canker completely in more humid and cooler sites than it needs in sites with mild winters and droughty summers (Santini *et al.*, 1997b). It is, therefore, necessary that candidates are evaluated on different sites to identify the clones with the highest level of resistance for each environment.

Patented varieties and other selections

Starting in 1986, a series of canker-resistant clones of Italian cypress were patented by the Plant Protection Institute of the Italian National Research Council (CNR) and put on sale. Several of these clones, such as 'Bolgheri', 'Italico', 'Mediterraneo', 'Le Crete 1' and 'Le Crete 2' (Panconesi and Raddi 1990a,b; Danti *et al.*, 2006), are characterized by a fastigiate and columnar habit and are particularly suitable for ornamental plantings. In particular, 'Le Crete 2' was selected for its tolerance to clayey substrates, and is propagated by rooted cuttings. 'Agrimed n. 1' is particularly suitable for windbreaks, by virtue of its typical crown architecture, whereas 'Florentia' and 'Etruria' are characterized by a wider crown habit, and are suitable for soil protective plantings as well as for hedges. In France, a multi-clonal variety, called 'Mistral', consisting of five canker-resistant clones, has been patented for windbreak use, whereas the 'Sancorey' clone with its fastigiate crown shape is appreciated as an ornamental.

The constant effort of the breeder is to enlarge the genetic base of resistance as much as possible. Today, the wide availability of cypress germplasm selected by IPP-CNR for resistance to *S. cardinale* canker makes it possible to successfully use cypress to cope with a series of current and emerging problems in the Mediterranean countries. In the past decade, a number of experimental field plantings have been set up in various Mediterranean countries for the evaluation of 100 Italian cypress canker-resistant clones for the protection of crops from strong winds, the production of quality timber, the mitigation of fire risks through cypress barriers, and also for combating desertification, protecting soils from erosion and screening buildings and infrastructures.

Through a complex programme of controlled crosses on 1500 mother plants, the (narrow sense) heritability of *S. cardinale* canker resistance estimated for Italian cypress was found to be a little lower than 0.2. Thus, the general combining ability (GCA) of clones was evaluated with the aim of selecting the best parents to be assigned to the large-scale production of improved seed in clonal orchards. The first cypress clonal orchard has recently been realized in Italy, using genotypes selected from the local seed stand.

17.5.6 Therapy

The only possibility of assisting a cankered tree to recover consists of the mechanical removal of the parts affected by the infection. In the crown, this can be done through proper pruning, as previously described. When a canker is located in the lower portion of a large-sized trunk and its width does not exceed 30–40% of the trunk's circumference, it is possible to attempt therapy through a delicate conservation intervention, aimed at removing only the cankered tissues. The intervention consists first of delimiting the borders of the necrotic area by means of appropriate debarking of the outer bark. Subsequently, at a distance of very few centimetres from the border of the necrosis, the healthy bark is cut down to the wood below and the canker is removed with a scalpel. Lastly, a protective mastic is applied to the wound, e.g. vinyl glue, to which a fungicide may be added. This operation, which requires the operators to have a certain technical background, is not easy to perform, especially on trunks with an irregular cross-section or inside dense crowns. It is justified, therefore, only for trees of particular aesthetic value, or with value as monuments.

None of the fungicides evaluated to date has ever shown a therapeutic effect on active growing cankers. Several systemic products, such as benzimidazoles, thiophanate-methyl and recently, azoxystrobin (see under Section 17.5.4, Protection, *Chemical control*), have been shown only to slow down the growth of the canker during to the first colonization phases of the bark by the fungus (Panconesi and Parrini, 1979; Della Rocca *et al.*, 2011a).

17.5.7 Integrated disease management

The control of cypress canker has been based on variable integration of the different methods of disease control, depending on the context in which the trees to be protected are located, e.g. plantations with a landscape or historic value, or a value as monuments, seed stands and other types of wooded formations, plants in gardens, nurseries, etc.

The protection of plantations that have an ornamental and landscape value, or that are a part of historic villas and gardens, is based on the eradication of the inoculum sources of the disease through sanitation. This will also have a therapeutic aim due to the need to recover affected cypresses that are not yet compromised by canker. As part of the proper measures, sanitation should be extended to the adjacent areas as well, so as to create a (sort of) protective belt, and canker-resistant varieties should be used to replace all the trees that have been removed. In addition, for particularly valuable plantations, it should be possible to carry out a selection for resistance in order to use the original selected genotypes for replacements, through the creation of experimental fields assigned to the collection of germplasm. This strategy is currently used for the preservation of cypress trees that are monuments at historic villas, and in the boulevards and gardens of Tuscany. In the case of cypresses in private or public gardens, in addition to sanitation and to the use of resistant varieties, chemical prevention can be adopted to protect young plants during the seasons that are most favourable to the occurrence of infections. The execution of chemical treatments is even easier on hedges that are regularly pruned.

The protection of cypresses in forest stands (either seed stands or protective stands) is based on the removal of all infected trees (sanitation), irrespective of how diseased they are. In nurseries, the control of cypress canker is based on the adoption of cultural practices aimed at reducing the risk of new infections and on chemical prevention by means of spring and autumn treatments, whose frequency will depends on weather conditions. The prompt elimination of symptomatic trees and the sanitation of cypresses situated in the surrounding area will help to eradicate inoculum of the pathogen. For new plantings, the use of varieties selected for resistance is advisable. For ornamental purposes and windbreaks, it is possible to use the patented varieties available on the market that come from Italy and France.

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