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CYPRESS CANKER: A Pandemic in Progress

Antonio Graniti
Dipartimento di Patologia vegetale, University of Bari, I-70126 Bari, Italy;
e-mail: granitia@agr.uniba.it

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ABSTRACT

Over the past 70 years a destructive blight of *Cupressus macrocarpa* and other Cupressaceae, caused by *Seiridium cardinale*, has spread worldwide from California, devastating forests, plantations, and ornamental cypresses. The epidemic has been particularly severe in the Mediterranean region, on *C. sempervirens*. A similar destructive blight induced by *Lepteutypa cupressi*, which caused serious losses to Monterey cypresses in East Africa in the 1940s, has now also spread to distant continents, albeit to a lesser extent. There is yet a third wave of canker disease induced by *S. unicorne*, although this is a milder type.

This review deals with problems related to identification of the pathogens, their taxonomy, pathogenesis and role of fungal toxins, and early screening of cypress clones or hybrids for resistance to the pathogens and tolerance to their toxins.

INTRODUCTION

Over the past half century, pandemics of destructive diseases have devastated various species of trees, such as elm and chestnut, that are important in the agriculture, forestry, and landscape of many countries, or which are widely used as ornamental plants. This devastation was attributable to the accidental introduction of Dutch elm and chestnut pathogens into North America and Europe. These epidemics resulted in the loss of millions of elm trees, the virtual elimination of American chestnut stands, and severely reduced production of fruit and timber from the European chestnut.

A similar deadly scenario is unfolding for another group of the world's most important forest and ornamental species, the cypress trees. Epidemics of

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cypress canker have resulted from the introduction of one or a few pathogenic fungal species or strains from one part of our planet to another, not unlike elm wilt and chestnut blight. At least one of these pathogens has adapted to hosts and ecological conditions prevalent in temperate regions, breaking down the centuries-old immunity of the cypress to destructive or fatal diseases.

Cypress canker has been the focus of intensive research over the past 20 years, and an extensive literature has been published (25, 31, 44, 48, 53). This review discusses some taxonomic, biological, and toxicological aspects of the causal agents that are significant for diagnosis and appraisal of different types of canker, beginning with a brief discussion of the two species of cypress that are particularly threatened.

In the Mediterranean region, the epidemic of cypress blight is so advanced that it threatens to become another ecological disaster (22). The devastation of planted and ornamental cypress trees threatens not merely serious economic losses for this area, but other unanticipated but grim consequences for the environment and social life, including tourism. This holds true wherever cypress is not only a key component of the landscape and an irreplaceable decoration for monuments and historical places, but an integral part of the nation's history and traditions.

THE HOSTS

The Cypress Tree: From Here to Eternity

THE ITALIAN CYPRESS, A SACRED TREE[1] If western civilization was born on the eastern shores of the Mediterranean, then its cradle stood in the shade of a sacred tree: the cypress.

The tall, statuesque, fastigiate, or narrow-columnar Italian cypress, *Cupressus sempervirens* L. var. *stricta*, has long been a symbol of Mediterranean civilization. Its flamed-shaped canopy once adorned the Persian temples of the fire worshippers, and watched over the endeavors, rites, artistic expressions, and daily life of the inhabitants of the region.

Cypresses became part of the Mediterranean landscape, spreading either naturally from their centers of origin or through cultivation by migrants. In biblical times in Asia Minor, cypresses covered the mountain slopes and were characterized as the emblem of wisdom (*I was exalted like . . . a cypress tree on Mount Zion.* Eccles. 24:17) and of moral righteousness, e.g. the just man who stands *as a cypress tree rearing itself on high* (Eccles. 50:11). In Roman times, the cypress continued to be regarded as a holy tree in the *luci* (the sacred

[1]Reproduced in part from Reference 22 (with permission).

woods) and represented immortality and transcendence on altars, in temples, and on funerary monuments.

The Italian cypress, like other Afro-Mediterranean cypress species such as *C. dupreziana* Camus, is extremely long-lived (up to about 2000 years). Its wood is resistant to woodworms, rot, and deterioration: *cariem vetustatemque non sentium cupressus* (cypress trees do not suffer from wood rot and old age: Pliny, *Naturalis Historia* 5:16). Cypress wood was therefore considered to be indestructible and was widely used in buildings exposed to harsh climatic conditions and in shipbuilding (see Homer's *Odyssey*, Solomon's *Canticle of Canticles*). The portals of the temple of Diana in Ephesus and those of St. Peter's in Rome (removed after about 1000 years), the tables of the Athenian Public Law, the gates of Constantinople, and the statue of Jupiter on the Capitol were all made of cypress wood; apart from the legendary Noah's ark (*make yourself an ark of gopher wood*, Genesis 6:14), so were Alexander's fleet, and Caligola's ships, found intact at the bottom of Lake Nemi some 1960 years later. Cypress, with its fine texture and slightly resinous quality, used to be the wood of choice for trousseau chests. One of the earliest reported uses of the now common agricultural practice of seed treatment dates back to A.D. 60, when Pliny suggested using wine and crushed cypress leaves to protect seeds from storage pests.

Cypress trees are easily distinguishable in some of the world's oldest paintings thanks to their characteristic symmetrical beauty, slender outline, and austere dark-green appearance. In the Renaissance, the cypress was depicted in the works of the greatest masters, from Fra Angelico and Paolo Uccello to Leonardo da Vinci and Domenico Ghirlandaio. In Roman and medieval times, the cypress was widely used in gardens, reaching its most splendid expression in Renaissance parks and in landscape gardening of the seventeenth and eighteenth centuries. Today, cypress trees are still a major feature of the countryside in many areas of the Mediterranean region, where they also embellish historical sites, gardens, villas, and roads. There is a flourishing nursery trade in Italian and other species of cypress for use as ornamental trees. Local people and visitors alike regard cypress trees as plant monuments, and many of these trees have witnessed the ebbs and tides of human activities for hundreds of human generations.

Cypress trees grow easily in poor, arid soils, and thus are almost irreplaceable in replanting degraded hilly areas. Cypress groves produce a timber that is so highly valued that they used to be called "the daughter's dowry" (planting a small cypress wood is still a tradition for men in the Peloponnesian countryside on the birth of a daughter). Cypresses are also widely used as efficient windbreaks for citrus and other subtropical crops. Finally, oils are extracted from cypress seeds and leaves for use as pharmaceuticals.

MONTEREY CYPRESS, A BEST-SELLING ORNAMENTAL TREE According to palaeobotanists, in the early Pleistocene, Californian forests of Monterey cypress, *Cupressus macrocarpa* Hartw, and other closed-cone conifers covered a 1400-km-long coastal area stretching from northern Marin County southward into northern Baja California. Fossil deposits found at Costa Mesa indicate that one million years ago both the ecology and climate were similar to those now prevailing at Monterey, 560 km north, where *C. macrocarpa* is presently restricted to a narrow, two-mile-long coastal belt (3). In Monterey, the climate is mild, with no frosts in winter and moderate temperatures in summer, and strong moist sea breezes that keep the area humid most of the year. These are ideal climatic conditions for an epidemic outbreak of a plant pathogen such as *Seiridium cardinale* treated here. Moreover, the remote settlement of a highly susceptible, autochthonous cypress species in its center of origin suggests that the pathogen in question was introduced.

In certain respects, features of Italian and Monterey cypresses are the antithesis of each other. The compact, severe, nearly ascetic appearance of the former (the so-called plant priest) contrasts with the earthly, thrifty, effuse crown of the latter, which looks joyous and free when disheveled by ocean winds and its trunk bent downward as if trying to cling to the ground. The relatively fast growth of young trees and the undoubted beauty of Monterey cypress has made it a very popular and widely appreciated ornamental tree.

Thus, *C. macrocarpa*, and to a lesser extent other American species such as *C. arizonica* Greene, *C. lusitanica* Mill., and *C. glabra* Sudw. have spread to faraway continents as part of a flourishing trade that, however, has not been without risk of spreading diseases.

THE PATHOGENS

Several Ascomycetes or mitosporic fungi, e.g. species of *Botryosphaeria*, *Sphaeropsis*, or *Diplodia*, produce cankers on wild and cultivated species of *Cupressus* and related conifers, sometimes causing serious losses. However, discussion here is restricted to a small group of fungi, with anamorphs in the genus *Seiridium*, that are especially destructive and widely dispersed geographically.

A Fungus Cardinal and Its Court of Killers

The anamorph genus *Seiridium* Nees : Fr. is described in detail by Sutton (72–74) and Nag Ray (40).

Most species of *Seiridium* live on the leaves or bark of woody plants (20, 40). Three species are presently responsible for losses to cypress plantations in the Mediterranean and other ecological regions, although these vary in incidence and severity.

SEIRIDIUM CARDINALE The outbreak of a destructive cypress blight was first reported from northern California in 1927 (85), but the pathogen had probably been introduced into the area 12 years earlier (84). The main effect of this epidemic was on the highly susceptible Monterey cypress, which also sustained the greatest damage and, to a lesser extent, on Italian cypress and other species of American and exotic Cupressaceae. Canker disease subsequently spread along the California coast, inland across the United States and into South America, then was transported east and west across the Atlantic and Pacific Oceans to New Zealand, Europe, Asia Minor, and South Africa; the disease became established in the boreal latitudes between 30° and 40°N and in some austral areas between 30° and 50°S (30, 43, 75). The fungus responsible for the disease was first described as a new species, *Coryneum cardinale* Wag. (1939) (84). The epithet *cardinale* may refer to the purplish cast imparted to the resin-infiltrated, inner tissues of the cankered bark. The species was later reassigned to the genus *Seiridium* (75) as *S. cardinale* (Wag.) Sutton et Gibson (1972).

The subperidermal acervular conidiomata of the fungus appear as minute, black pustular bodies scattered or clustered on infected stems, branches, and cones of affected trees, and dehisce by rupture of the upper wall.

Conidia of *S. cardinale* are distinguishable from those of other cypress-infecting congeneric species by very short (approximately 1 μm long) or nonexistent appendages. Conidia (Figure 1*a*) are oblong-fusiform, 17–34 (mostly 21–26) × 7–12 (mostly 8–10) μm (length/width ratio: 2.5–3), straight, sometimes slightly curved, 5-distoseptate; the four median cells are of the same brown or dark brown color, slightly collapsed when conidia are not fully turgid, the end cells are hyaline, the apical cell is campanulate, and the basal cell truncate. Transient production of hyaline, filiform spermatia may occur within the acervuli. Other morphological, physiological, and cultural characters have also been used to define this taxon (21, 26, 40, 73, 75).

A *Leptosphaeria*-like teleomorph (*Leptosphaeria* does not seem consistent with the current view of the relationships of either *Leptosphaeria* or *Seiridium*) has been observed in north-central California on dead branches of *C. macrocarpa* as well as in mixed cultures of heterothallic isolates (33). However, it has not been described, nor has it been further reported either from this region or elsewhere.

Of the three species of *Seiridium* that cause canker diseases on cypress, *S. cardinale* is the most thermophilic. Conidia can germinate, and colonies can grow in vitro, up to 35°C. The disease, however, may occur in temperatures up to 30°C, although infection is optimal about 25°C (26). Growth of *S. cardinale* in host tissues is slow or is even arrested during the hottest months of the year (43, 52).

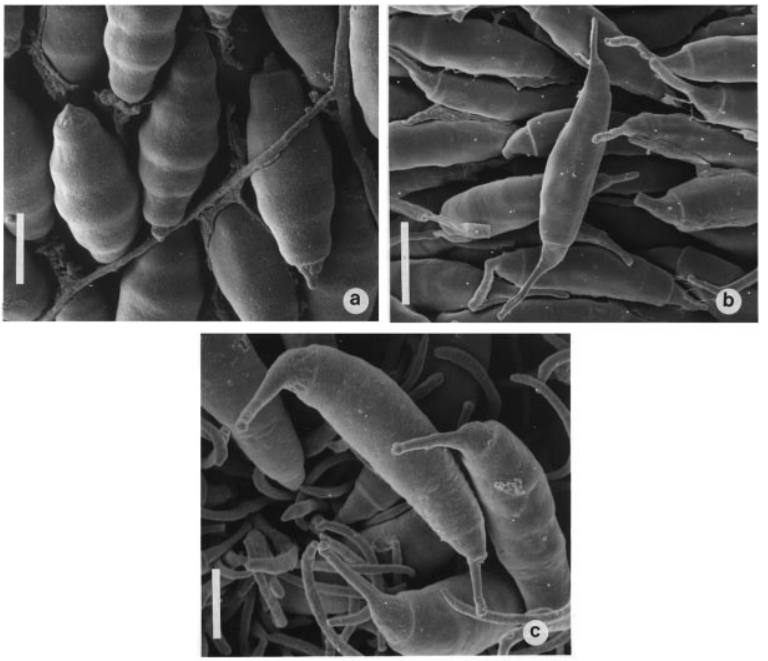

Figure 1 Conidia of *Seiridium cardinale* (*a*), *S. cupressi* (*b*), and *S. unicorne* (*c*, with spermatia) at SEM. Bars = 10 μm.

Inoculation tests on several species of *Cupressus* indicated that *S. cardinale* is more pathogenic than *S. cupressi* and considerably more so than *S. unicorne*. Under the climatic conditions prevailing in the mildest areas of the Mediterranean region, i.e. not excessively hot and dry, *S. cardinale* is by far the commonest, most destructive, and widespread canker-inducing fungus (26, 52). *C. macrocarpa* is more susceptible than *C. sempervirens*, whereas *C. arizonica* and especially *C. glabra*, *C. torulosa* Don., and other exotic species show a range of resistance (1, 2, 71).

The pathogen affects several species of *Cupressus*, *Chamaecyparis*, *Cryptomeria*, *Cupressocyparis*, *Juniperus*, *Thuja*, and related genera (and hybrids) of Cupressaceae. No subspecific entity or forma specialis of the fungus has been reported.

LEPTEUTYPA CUPRESSI The outbreak of a previously unknown canker disease caused serious losses to cypress plantations in East Africa (Kenya) in the 1940s (12, 42). The Monterey cypress was the most severely affected, whereas other

species, especially the Mexican cypress *C. lusitanica*, were less affected. The cause was initially classified as *Monochaetia unicornis* (Cooke et Ellis) Sacc. and then controversially placed in the genus *Cryptostictis* (32) as a new species, *C. cupressi* Guba (1961), but the pathogen was eventually referred to the genus *Seiridium* as *S. cupressi* (Guba) Boesew., anamorph of *Lepteutypa cupressi* (Nattrass et al) Swart (6, 7, 41, 76).

The fungus was subsequently identified in New Zealand (6, 32) and, in 1984, on the Greek island of Kos in a natural forest of *C. sempervirens* (21, 87). It has also been reported from other parts of the world (7), but some of these records may be misidentifications of *S. unicorne* (see below).

Conidia of *L. cupressi* (Figure 1*b*) are elongate-fusoid, unequally fusoid or sublanceolate, straight or crescent-shaped; 26–37 (mean 32) × 6–9 (mean 7) μm, length/width ratio is 4:8 (i.e. thinner than those of *S. unicorne*), and the four median cells are brown or pale brown. The apical cell is hyaline, conic, and extended in a single, 3–11 μm long, uniformly tubular appendage, sometimes shortly bifid at the apex; the basal cell bears a simple, slightly longer (6–15 μm) appendage. Appendages, especially the apical appendage, are usually straight or obliquely projected at an angle that normally follows the curve of the conidium. Curved, filiform, hyaline spermatia may also be produced.

Ascostromata of the teleomorph are immersed in the bark, and penetrate the periderm by their stout necks. Ascospores are broadly oblong to ellipsoid, brown at maturity, four-celled, with obtuse or rounded ends, 16–19 μm long, and 6–7.5 μm thick (7, 41).

In culture, the fungus does not grow well on certain media (e.g. on Czapek-Dox's medium) (26). For germination and the growth of colonies and infection, conidia require temperatures in the range of 10–30°C, with an optimum of 25°C.

Inoculation experiments with *L. cupressi* on susceptible hosts showed highest pathogenicity at 20°C to 25°C. At 30°C the pathogen produces little disease (26). Field experiments conducted in three European countries showed that after infection *S. cupresssi* progresses slowly during winter and faster during spring and early summer, with little or no growth at 35°C (52). The necrotic process initiated by *L. cupressi* in its hosts continues even in the hottest months of the year, whereas that of *S. cardinale* is slowed. Hence, *L. cupressi* could potentially become established in the warmest Mediterranean areas (43).

C. sempervirens is more susceptible than are *C. macrocarpa* and *C. arizonica*, whereas *C. lusitanica* is relatively resistant. In East Africa, where the first epidemics of this type of canker occurred, planting of susceptible Monterey cypress was abandoned in favor of Mexican cypress (7).

The host range of *L. cupressi* appears to be restricted to Cupressaceae and includes several species of *Cupressus*, *Cupressocyparis*, *Chamaecyparis*, and *Juniperus*. Although *S. cupressi* poses no serious threat to clones of

C. sempervirens selected in Europe for resistance to *C. cardinale*, this is not true of clones carrying an intermediate type of resistance (52).

SEIRIDIUM UNICORNE This is an old species, described on *Chamaecyparis thyoides* (L.) B. S. P. from New Jersey in 1878 as *Pestalozzia unicornis* Cooke et Ellis, and reassigned as *S. unicorne* (Cooke et Ellis) Sutton almost a century later (73).

Conidia of *S. unicorne* (Figure 1*c*) are fusiform, mostly slightly curved and shorter than those of *L. cupressi*, 22–31 (mostly 25–27) × 7–9 μm (length/width ratio: 3.2–3.9), with median thick-walled cells, olivaceous-brown to dark brown, and colorless, sometimes yellowish, end cells. The apical cell is broadly conic and gradually extends into a subulate or beak-like, simple cellular appendage, oblique or turned to one side, whereas the basal cell is obconic with a narrow truncate base and bears an attenuated, straight or oblique appendage. Both appendages are comparable in length (3–5 up to 12–13 μm). Colorless, filiform spermatia may be produced.

The temperature requirements of *S. unicorne* for conidial germination, growth in vitro, and infection are relatively low (optimum about 20°C; maximum 25°C to 30°C) compared to the other two species of *Seiridium*. Further, most isolates tested in the climatic conditions of the Mediterranean area are not highly virulent (26, 52).

Unlike *S. cardinale* and *L. cupressi*, the reported host range of *S. unicorne* includes members of several botanical families (6, 32, 34). The geographical distribution of *S. unicorne* is reported to be widespread worldwide as a plurivorous fungus. However, variability among populations of *S. unicorne* appears to be very high, hence records of its occurrence on hosts or locations could have been based on morphologically similar species.

Recent reports restrict the host range of populations of *S. unicorne* living on cypress to Cupressaceae in countries such as New Zealand and Japan (77, 81). Inoculation of a cypress strain of *S. unicorne* from Portugal on various conifers and angiosperms was negative on hosts other than species of *Cupressus* (26). Although host specificity has not been found in isolates from several Cupressaceae in the United States (78), the existence of races or ecotypes cannot be ruled out.

Destructive epidemics of *S. unicorne* have not been reported. However, an epidemic blight of Hinoki cypress, *Chamaecyparis obtusa* (Sieb. et Zucc.) Endl., was caused by *S. unicorne* in the 1970s in northern Japan and has subsequently spread into central and southern parts of the country on several Cupressaceae, with serious losses to young plantations (77). In the Mediterranean area, cypress canker caused by *S. unicorne* is common, but not serious, only under certain ecological conditions in Portugal (8, 43).

How Many Species?

The taxonomy of the three fungi reported to cause cypress canker has been controversial and is not yet resolved.

Based primarily on conidial morphology, the asexual state of *L. cupressi* has been regarded as a distinct anamorph-species (current name: *S. cupressi*) (6, 7, 21, 26, 32) or as a subspecific taxon (41) or a variant (76), and as such a synonym of *S. unicorne* (20, 40, 74).

A study based on length of conidia and cultural characteristics of over 50 isolates from New Zealand considered to be *S. unicorne* according to Sutton's criteria (74) suggested that variability of these strains in culture was too extensive to justify designation of separate species (10). Other authors have recognized three species among these strains based on their criteria (see below).

S. cardinale is distinguished from *S. unicorne* or *S. cupressi* on the basis of morphological traits (21, 40, 73, 74), and this has been confirmed by physiological (26), toxicological (28), and enzymatic polymorphism (55) data. However, Swart (76) argued that differences in conidial morphology of the three anamorphs may be extreme variations of one single species. Following Swart's view, other authors (82) have concluded from the analysis of sequence data of the variable ITS1 region of ribosomal DNA of two New Zealand and nine South African isolates tentatively classified as *S. unicorne*, *S. cupressi*, and *S. cardinale*, and three authenticated isolates of the same species from Portugal, Greece, and Italy, that the first two species are synonyms of *S. cardinale*. According to this conclusion, only one species of variable morphology (and that would have been *S. unicorne* for priority reasons) would induce three types of cypress canker. This assumption, however, despite substantial evidence to the contrary, seems to be based on the interpretation that a small fragment of a rDNA spacer region, ITS1, can reveal sequence homology among isolates, which may not be sufficient to infer identity at the species level of phylogenetically related taxa. On the other hand, extensive data from morphological, cultural, pathogenic, toxicological, and physiological studies indicate that three distinct species, *S. cardinale*, *L. cupressi*, and *S. unicorne*, are the causal agents of three types of cypress canker (6, 21, 26, 28, 52). This view was corroborated recently by the preliminary results of a phylogenetic analysis of about 60 isolates of *Seiridium* from various sources and geographical origins, which demonstrated great sequence divergence among the three species of *Seiridium*. This comparison was based on the highly conserved 5.8S ribosomal gene and the two hypervariable spacer regions, ITS1 and ITS2, of rDNA (38).

Additionally, careful examination of fresh canker material, several herbarium exsiccata including type specimens, and living cultures from various countries,

indicated that the "Australian strain" studied by Swart (76) and referred to as *Seiridium* state of *L. cupressi*, is a distinct taxonomic entity, for which the binomial *S. swartii*, anamorph of *L. swartii*, was proposed (23, 27).

When strains of a pathogen are used in studies on the features and variability of the species, accurate identification at the species level is crucial if reliable results are to be obtained. Thus, recognition of the taxonomic rank of related plant pathogenic fungi is not merely an academic exercise, but may indeed have practical implications for the correct diagnosis of relevant diseases, interpretation of experimental results, and control efforts. In this case, certain New Zealand strains that should have been regarded as different species were used in comparative studies on fungal population analysis (10), pathogenicity, and host reactions (11, 52) as if belonging to one species, *S. unicorne*, inevitably leading to the conflicting interpretation cited.

THE DISEASE

The first evidence of cypress blight caused by *S. cardinale* is a browning or a reddening of the live bark around the point of entry of the pathogen. This is followed by a slight depression in the infected area, formation of longitudinal cracks, and a resinous exudation. Subsequently, lentiform or elongated cankers develop on the bark around the infection sites, where a necrosis of the infected tissues occurs, and these may girdle the small branches or the stem of young plants. The expansion of cankers, however, is a slow process on the large branches or main stems of adult trees. Often, continuous flows of resin exude from cracks or fissures formed on the cankers, which may extend to the infected stem or branches. This exudation may contribute to a localization of cankers. Generally, sectors of the tree on the side of the cankers decline and die.

A diffuse yellowing or reddening first appears on the foliage of twigs, branches, and apical parts of the infected trees, subsequently turning to brown or reddish-brown as the dieback progresses. The spread of one, several, or many infections on a single tree can kill the whole tree within a relatively short time, depending on its age, susceptibility, and the environment. The most conspicuous symptoms of the disease, i.e. the fading and dieback of twigs, branches, and tree tops, are noticeable at a distance, and these traits may facilitate a disease survey.

L. cupressi produces symptoms somewhat similar to those induced by *S. cardinale* on the same hosts. However, some differences are detectable in the larger size of conidiomata and appearance of cankers that are associated with a profuse resin flow (12, 56). In the conditions prevalent in the Mediterranean region (Portugal), *S. unicorne* produces slow-growing cankers and mild symptoms (52).

PATHOGENESIS

Infection usually occurs through wounds produced by various agents (wind, frost, insects), although penetration through natural openings may also occur. Relative humidity (RH) close to saturation is required for infection (at 80% RH about half the conidia of *S. cardinale* are unable to germinate), whereas the temperature requirements for the production and germination of conidia are wider, with the range dependent on the species of *Seiridium*.

After penetration, *S. cardinale* produces a necrotic lesion of the bark. The pathogen spreads relatively rapidly in the cortical parenchymas, but more slowly in the phloem; eventually, all tissues including the cambium turn brown and die. Cell necrosis of the cankered bark progresses steadily, with some seasonal variation, until the branches or stem are girdled. Relatively large amounts of resin are produced by actively growing cankers, i.e. until they are able to enlarge.

In resistant cypress trees, reaction to infection takes the form of separation of living tissue from the diseased bark by the formation of a new periderm through neophellogenic activity. This activity can be estimated by the thickness of the constitutive phelloderm. Potentially resistant cypress clones may be characterized by phelloderm thicker than 100 μm (50). Compartmentalization tends to restrict and to isolate the tissues invaded by the pathogen, thus preventing its further spread, usually followed by restoration of the bark. The reaction involves a series of processes that can be detected histologically, allowing differentiation of resistant or tolerant species or clones from susceptible ones (51).

The outer layers of the sapwood adjacent to cankers, as well as the medullar rays, may be colonized both by *S. cardinale* (36, 47, 50, 51) and *S. cupressi* (12, 56). *S. cardinale* can survive for a long period in the woody tissues of cypress without loss of pathogenicity. When fungal inoculum is placed deep into the stem, it can spread to the bark and give rise to cankers (41, 47). Disorganization of xylem elements and occlusion of vessel pits by electron-dense materials have been demonstrated in histological and SEM observations of xylem tissues from branches of cypress trees inoculated with *S. cardinale*. The effect of this was first thought to reduce the movement of water within the plant and to induce mortality in branches (36). However, since the xylem forms an integrated system, these phenomena, if not too extensive, should not produce such dramatic effects, since impairment of the transpiration stream in one sector of the cypress stem or branch could be compensated by redistribution of the water potential by the functioning parts of the xylem.

Resinosis as well as occlusions of the xylem elements most likely play a role in the plant's defense against a pathogen that is invading the bark with potentially systemic activity. Water stress, wilting, and other deleterious effects on the leaves and other tissues of affected trees could be induced by diffusion

and translocation to the transpiring plant organs of toxic metabolites produced by the pathogen in the invaded bark or wood.

An Arsenal of Weapons: Bows and Poisoned Arrows

Leaf symptoms may develop on the branches of affected trees, regardless of the girdling effect of the cankers, e.g. foliage distal from where the fungus can be isolated. In the developing cankers, outgrowths of bark tissues or other histological abnormalities, as well as plant cell necrosis may occur in advance of the invading hyphae (50, 51). This suggests that some extracellular metabolites produced by the fungus, other than those involved in breaking down the apoplastic structures of the host, e.g. pectolytic and cellulolytic enzymes (37), play a role in pathogenesis.

It has been suggested but not yet demonstrated that the pathogen may produce substances that regulate plant growth. If so, this may contribute or account for hypertrophic outgrowths at the margin or around the cankers. Also, some metabolites excreted by the pathogen (see below), have been shown to promote plant cell and tissue growth at low concentration. However, it appears that fungal toxins may play a key role in producing disease symptoms.

Fungal Toxins as Virulence Factors

The appearance of symptoms caused by infection of *Seiridium* species on their hosts, as well as the type of actual damage to the infected tissues (necrosis), suggest that toxins may be produced in the cypress bark or wood colonized by the fungus; these may be subsequently diffused to adjacent tissues, and eventually translocated to leaves via the transpiration stream. Current research into the activity of phytotoxic metabolites produced in culture by *S. cardinale*, *S. cupressi*, and *S. unicorne* is under way.

Nine nonhost-selective toxins produced in vitro by the pathogens have been purified and characterized including four butenolides, seiridin (SE), *iso*seiridin (ISE), and two hydroxyseiridins (7′HSE and 7′HISE); one 14-macrolide, seiricuprolide (SCU); one aromatic *ortho*-dialdehyde, cyclopaldic acid (CA); and three cyclic sesquiterpenes, seiricardines (SCA-A, -B, and -C) (Figure 2).

Each species of *Seiridium* produced at least one major toxin as well as several minor phytotoxic metabolites in vitro (Table 1). *S. cardinale* produced the most seiridins; *S. cupressi* produced two toxins, CA and SCU, which were not excreted by the other two species, and relatively high amounts of SCA-A, -B, and -C. *S. unicorne* produced relatively low amounts of SE, ISE, and SCA-C (70). These results suggest the possibility of a complementary, chemiotaxonomic means of identifying *Seiridium* species both in vitro and in vivo.

Symptoms produced by the individual toxins on cypress or on herbaceous test plants suggested different modes of action (25, 28, 70). In bioassays on host

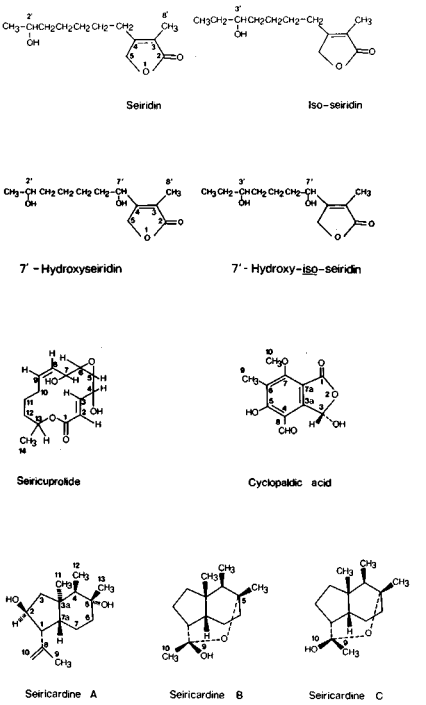

Figure 2 Structures of the main toxic metabolites produced in vitro by the species of *Seiridium* infecting cypress (from L Sparapano, A Graniti, A Evidente, 1994. See Reference 70).

and nonhost plants, effects of seiridin or cyclopaldic acid were more severe than those caused by seiricuprolide or seiricardins. Moreover, some *Seiridium* metabolites affected plant cell growth and induced cell proliferation of cypress bark.

SEIRIDINS In tests with 100 isolates of *S. cardinale* from Italy, 95 were toxigenic (71). *S. cardinale* produces SE and ISE as major toxins that also exhibited antibacterial activity (66, 67). At low concentrations, seiridins enhanced plant cell growth. For example, in media devoid of other plant growth regulators, SE sustained growth of cypress callus and cell cultures. A comparison with kinetin and 2,4-D indicated that SE (50 μM) can replace kinetin in the medium (62).

Seiridins, however, are phytotoxic at higher concentrations. Assays at 150 μM on various hosts and nonhost plants, SE, and to a lesser extent ISE, resulted in leaf chlorosis and necrosis and also induced leakage of electrolytes from

Table 1 Toxins produced in culture by three species of *Seiridium* (mg 1^{-1})[a]

Toxins	*S. cardinale*	*S. cupressi*	*S. unicorne*
$D^{\alpha,\beta}$-Butenolides			
Seiridin	**93.5**	36.4	8.2
iso-seiridin	**142.7**	23.2	4.1
7′-Hydroxyseiridin	0.8	<0.1	<0.1
7′-Hydroxy-*iso*-seiridin	0.2	<0.1	<0.1
14-Macrolides			
Seiricuprolide	0	**1.7**	0
Aromatic *ortho*-dialdehydes	0	**171.0**	0
Cyclopaldic acid			
Cyclic Sesquiterpenes			
Seiricardine A	1.1	**3.1**	0.8
Seiricardine B	1.0	**2.8**	0.9
Seiricardine C	0.5	**1.7**	1.4

[a]Values are the mean yield of 20 fermentations. Figures in bold type indicate the highest yield of that metabolite produced by the three species of *Seiridium*.

cypress tissues (66). Similar symptoms were caused by 7′HSE and 7′ISE at 100 μM (19).

Subperidermal injections of 2 ml of a 0.1 mg ml^{-1} solution of SEI into the stem of susceptible cypress seedlings induced leaf chlorosis as well as hypertrophic reactions and bark cracking at the point of injection. At higher concentrations (0.2–0.3 mg ml^{-1}), injections caused extensive dieback and death of the seedlings within 6–8 months (66, 69, 70). These symptoms are reminiscent of those shown by *Seiridium*-infected seedlings. However, although traces of seiridin have been detected in cankered tissues of cypress trees naturally infected by *S. cardinale*, no evidence is yet available on the occurrence of phytotoxic concentrations of seiridins at the initial stages of the disease, nor has translocation of the toxins from cankers to the symptomatic parts of the tree been shown. Serological assays could facilitate the detection of seiridins, but they are not currently available.

The susceptibility of species of *Cupressus* to *S. cardinale* correlated with their sensitivity to seiridins. Inoculations with highly toxigenic isolates of *S. cardinale* killed only 5% of *C. arizonica* seedlings within four months compared to 30% of *C. sempervirens* seedlings and 75% of *C. macrocarpa* seedlings (71).

CYCLOPALDIC ACID In addition to its antimicrobial activity, cyclopaldic acid, the major toxin of *S. cupressi*, induced severe leaf chlorosis and necrosis in test plants when assayed at 50 μM (29). A single subepidermal injection of 3 ml

toxin solution (0.1 mg ml^{-1}) into the stem of seedlings of the most susceptible cypress species (*C. sempervirens*) caused stem yellowing and browning within two months, followed by necrosis and dieback of distal parts. Symptoms were slower to appear on young trees of the less susceptible *C. macrocarpa* and of the moderately resistant *C. arizonica* (29, 70).

CA has been shown to reproduce the systemic symptoms of the disease. It has also been detected by ELISA in physiological concentrations in shoot tissues of cypress seedlings stem-inoculated with *S. cupressi*, using a specific anti-CA polyclonal antibody (16, 65). CA was detected in tissues of all three cypress species as early as one month after inoculation of the pathogen, even before the less susceptible *C. macrocarpa* and *C. arizonica* showed any symptoms.

When two toxin-deficient mutants of *S. cupressi* were inoculated into seedlings of three cypress species in a comparison with a wild type of the pathogen, relatively mild symptoms were visible on leaves and branches, but the ability of mutants to produce stem cankers remained unchanged (63). This demonstrates that toxins are virulence factors for species of *Seiridium* pathogenic to cypress. On the other hand, the Mediterranean strains of *S. unicorne*, the less toxigenic species of *Seiridium* that is unable to produce CA or relatively high amounts of seiridins, cause cankers without inducing severe systemic symptoms on the crown of the affected trees. With one exception (*iso*-CA), seven analogues of CA showed little or no toxicity to cypress cuttings. Two analogues induced cuttings to root profusely (65, 68). This finding may have practical applications for accelerating propagation of cypress, which is normally not easy to root and is usually reproduced by seed (35).

SEIRICUPROLIDE AND SEIRICARDINES Seiricuprolide is a metabolite selectively produced by *S. cupressi* (5). Like seiricardines, it appears to be a minor toxin of the pathogen. Nevertheless, as a component of an array of fungal metabolites, it may contribute to the overall toxicity of the pathogen.

Seiricuprolide (0.4 mg ml^{-1}) and seiricardines (0.1 mg ml^{-1}) caused leaf chlorosis and necrosis when absorbed by cuttings of test plants. When injected (3 ml) into the stem of young cypress trees, SCA-A and -B induced hypertrophic reactions of bark tissues, longitudinal lesions on stems, and a reddish discoloration of distant leaves. All seircardines showed fungistatic activity in vitro (4, 5, 18).

DIFFERENTIAL RESPONSES OF CYPRESS TISSUE OR CALLUS TO TOXINS Susceptibility to Seiridium blight varies among and within the species of Cupressaceae. Usually the degree of susceptibility or resistance of the host to a particular species or strain of *Seiridium* is assessed by inoculating cypress seedlings in the greenhouse or young trees in the open field. These tests, however, may

take 1–2 years and up to 8 years, respectively, for reliable results to be obtained (43).

Longitudinal extension or size of cankers on stem or branches, and rating by visual scales of leaf damage and severity of dieback, are commonly used for disease assessment. A more exact appraisal by which to compare either the response of cypress clones or the pathogenicity of *Seiridium* strains is through the size of the necrotic lesion at cambium level, as measured on decorticated stems of inoculated seedlings (49).

Early screening methods to assay cypress species, cultivars, clones, or progenies for resistance to the pathogens or low sensitivity to their toxins are urgently needed. Information to date indicates that either cypress explanta or callus cultures could be used to screen cypress genotypes prior to field evaluation.

Two recently proposed methods involve either direct inoculation of *S. cardinale* on callus cultures from micropropagated shoots of cypress or inhibition of fungal growth in dual cultures. Callus from resistant species or clones of *Cupressus* supported significantly less surface growth of the pathogen than did callus of susceptible hosts (61). Treating explanta or tissue cultures of *C. sempervirens* clones with culture filtrate of *S. cardinale* and determining the effects on ion leakage or ethylene evolution may provide information about the sensitivity of clones to the metabolites excreted by the pathogen (79, 80). A more precise assessment may be obtained using purified preparations of the key toxins of each pathogen. Results obtained so far with cypress seedlings, cuttings, or explanta correlated with known field resistance to the pathogen of the tested cypress species (71) and even with resistance of newly selected cypress clones (L Sparapano, unpublished data). For example, seiridin-induced loss of electrolytes from stem tissues of three species of *Cupressus* correlated with their resistance to *S. cardinale* (66).

The response of both callus and cell cultures to either seiridin or cyclopaldic acid could be used to screen cypress germplasm in vitro. In tissue culture assays, the addition of 0.01–0.03 mg ml^{-1} SE to the nutrient medium promoted growth of callus tissues of both susceptible (*C. macrocarpa*) and moderately resistant (*C. arizonica*) cypresses; however, *C. arizonica* calluses showed moderate browning. When assayed at tenfold higher concentrations, SE inhibited growth of both callus types; growth inhibition was higher in the susceptible cypress species, whereas callus tissues of *C. arizonica* turned deep brown (63, 66).

Cell alteration and browning, which were also shown by callus tissues of *C. arizonica* grown on media containing 0.1 mg ml^{-1} CA, were expected to follow elicitation of a hypersensitive-like reaction (69, 70). The assumption that some antifungal metabolites are produced by the resistant species of *Cupressus* was supported by the results of in vitro experiments with cell suspensions of *C. arizonica* challenged by 100 μM SE. Substances released by the cypress cells

in the culture medium caused a selective reduction of growth and sporulation of *S. cupressi* and induced the fungus to form chlamydospores (64).

EPIDEMIOLOGY AND IMPACT

Where Did the Enemy Come From?

The origin of *S. cardinale* in producing the first epidemics remains uncertain. Some of the native species of Cupressaceae in California or in New Zealand, where the disease was first recorded, may have exerted selection pressure on a population of saprobic or weakly pathogenic fungi existing in the area giving rise to a virulent strain able to severely affect susceptible cypress trees (85). Although a teleomorph is unknown for *S. cardinale*, heterokaryosis is a common feature in this group of mitosporic fungi, and no evidence of vegetative incompatibility was found among strains (57); consequently, the occurrence of natural variants of the pathogen cannot be ruled out. However, current data do not support a condition of high variability in *S. cardinale* (43).

An alternative and most likely explanation is that the first epidemics originated from accidental introduction of the pathogen into California on imported nursery stocks of ornamental trees. A similar means of spread is likely to have occurred with the further distribution of the fungus in other parts of the world. The first epidemic of *L. cupressi* in Kenya was thought to have originated from a single focus, either by a mutant strain of the fungus parasitic on a wild juniper host or following introduction of the pathogen from abroad (56).

Dissemination: "The Wind Cannot Read", Nor Can Insects and Birds; Man Could but Won't

The abundant production of conidia by *S. cardinale* during spring and autumn, made possible by the Mediterranean climate, may assure the availability of fresh inoculum year round. Moreover, these conidia of *S. cardinale* substantially retain their germinability and pathogenicity for more than one year (45, 46). Under moist conditions, conidiomata of the three species of *Seiridium* open wide on the surface of the cankers, thus exposing cirrhi or slimy conidial masses that when dry, can be released into the environment by strong winds. Generally, however, conidia extruded from the acervuli are dispersed by rain over short distances, mostly in a downward direction, then spread laterally by windborne conidia-laden droplets.

Conidiomata are produced on cypress galbuli. In a survey in Italy, 0.5% to 70% of cypress seeds were either contaminated or infected by *S. cardinale*, even when collected from healthy-looking trees (60).

Infection in *L. cupressi* is usually by conidia but occasionally by ascospores, which are extruded in slimy masses and usually are disseminated by rain, within a limited airborne range (56).

Long-distance spread of the pathogens, even to isolated areas, would be rare without the help of vectors. The thick growth and dense foliage of the Italian cypress provide an ideal refuge for birds (9). Though not proven experimentally, birds carrying inoculum may well spread conidia and ascospores and thus contaminate the tree tops.

Insects, especially cork-borers, are highly efficient vectors. Twig-mining beetles such as *Ploeosinus aubei* Perris, *P. thujae* Perris, and *P. armatus* Reitter are common in the Mediterranean region, and they can spread the disease either by carrying the inoculum into shoots or by opening wounds in the cypress bark through which rain-carried conidia enter and initiate infection (14, 15, 84).

Finally, the commonest vehicle for the worldwide diffusion of all three types of canker disease on cypress has been through the international trade in infected nursery stock. Ironically, however, the species of *Seiridium* pathogenic to cypress are not included in the lists of quarantine organisms (17).

Impact and Losses

Infection of susceptible cypress trees by *S. cardinale* or *S. cupressi* under favorable environmental conditions is fatal. Death of the tree may take up to a few months or even years, depending on the species, clone, age, and environmental factors.

On relatively resistant clones of *C. sempervirens*, on *C. arizonica*, and even more on resistant species such as *C. torulosa*, *C. glabra*, and *C. lusitanica*, infection can develop slowly. With resistant hosts, eventually the cankers can be compartmentalized and sealed off by the defense reactions (50, 51).

The progress of epidemics of cypress canker disease is governed by environmental factors and fluctuates from year to year. The development of cankers on even the same cypress clone may vary according to local conditions (45, 57, 58).

Adaptation of *S. cardinale* to the Mediterranean environment was facilitated both by the presence of susceptible hosts and by the density and contiguity of the cypress groves and plantations. The high variability and instability of the Mediterranean climate (13) has certainly favored the establishment of the pathogen and the spread of the disease.

The susceptibility of *C. sempervirens* populations to *S. cardinale* in the Mediterranean region is relatively high, even where the Italian cypress shows the highest variability, for example in natural woods of the Aegean Islands, where most of the present forests or plantations were established with wind-pollinated seed produced by wild or domesticated trees (45, 86–88).

The incidence and severity of cypress blight by *S. cardinale* may be high or even very high in areas where climatic factors, particularly rain and high relative humidity during the infection season (autumn through spring), favor the production and dissemination of inoculum, and where frost or strong winds produce wounds and lesions on trees.

In central Italy, the average incidence of canker on residual cypress plantations is currently about 25%, but it may reach 75% in some groves around Florence (44). In Greece, the highest incidences were recorded in the areas around Kyrgia (70%), in the valley of Megalopolis, western Peloponnese (90%), and around Karistos (98%), a windy valley of Euboia island, where cypresses are used extensively as windbreaks (45, 87, 88). Some cypress plantations close to a devastated areas of high incidence may escape the disease for lack of just one predisposing factor, e.g. strong winds or high humidity.

A 1978 survey in central Italy (district of Florence) showed that some 720,000 of the 4 million cypress trees in the area (i.e. 18%) were either dead or severely affected by the disease (45). This figure would have been even higher (probably close to 1 million trees), if all diseased trees with only light infections, which would die subsequently, had been considered. Further assessments were not made in the same area for 20 years; meanwhile, the disease has progressed. A 7200-tree cypress grove near Florence was sampled to assess disease incidence during the period 1981–1991. The relative figures were 31.3% and 48.3% incidence, representing a 17% increase in ten years (45). The annual increase of the disease in some stands in the Peloponnese (Greece), with an initial attack of 20%, ranged from 5% to 20% (87).

By contrast, the spread and severity of canker caused by *S. cardinale* have been low or virtually negligible in the warmest areas such as North Africa; however, *S. cupressi* represents a potential threat in this region.

Virulence of the population of *S. cardinale*, a fungus with no ascigerous state, has not decreased significantly during the past ten years or more (45). No evidence has been presented thus far to associate a decrease of *S. cardinale* blight epidemics, as recorded in some restricted areas of the Mediterranean, with a change in the pathogen's virulence. If low virulent strains do indeed exist, they have not been able to build up and spread or to lower the mean aggressiveness of the entire population of the pathogen.

Economic losses caused by *Seiridium* blight over the past 50 years have been very serious, especially for the ornamental cypress trade. Agricultural losses also occurred after cypress windbreaks were destroyed. These windbreaks had provided highly effective protection for citrus and other subtropical crops in many southern areas. The largest impact of the aftereffects of disease on cypress forests and plantations has been felt primarily in declining timber production, caused by the loss of millions trees, and also in soil erosion of

the depopulated hills. Moreover, it was not possible to replant the devastated groves with resistant clones, since they were not then available.

CONCLUSIONS

Experience with cypress blight emphasizes the need for diagnosis of plant diseases and proper identification of the pathogen in determining actions to be taken. Identification of cypress blight does not present a problem for an experienced plant pathologist working in areas where the same species or even the same clones of cypress are grown, and where one pathogenic species of *Seiridium*, e.g. *S. cardinale*, is dominant. Cypress trees showing characteristic symptoms can be identified at a distance. Potential losses from disease in a cypress forest can be assessed from the air. The flow of resin exuding from the cankers makes it easy to localize stem cankers hidden by the dense and compact foliage of *C. sempervirens*. When several species of Cupressaceae, often showing nonspecific symptoms, are mixed in groves or parks, or when more than one pathogen is present, identification is more difficult and diagnosis is usually based both on microscopic examination of conidiomata or reproductive structures formed in vivo, and by isolation and determination of the pathogen in culture. This is not always easy because the presence of the pathogen can be masked by other fungi (e.g. species of *Pestalopsiopsis*) colonizing cankers, or because of the existing diverse views on the taxonomy of cypress-infecting species of *Seiridium*. Usually, only a few selected characters are needed for diagnostic purposes, and these should be easy to detect by microscopic, cultural, serological, chemical, or other methods, as is the case with cypress canker.

The sensitivity of cypress species and clones to the toxins produced by the species of *Seiridium*, as well as in vitro screening methods with the pathogen, can be used to accelerate the long-term selection of cypress clones for resistance to the specific pathogen and for tolerance or insensitivity to the toxigenic species or strains of *Seiridium*.

Early diagnosis of the disease and the pathogen makes it possibile to take timely action. In the current situation of cypress canker in the Mediterranean region, the epidemics of cypress blight would have been even more destructive had naturally resistant or tolerant cypress trees, either native or cultivated, not been present in the area. Nevertheless, stringent effort is needed to prevent the cypress, once regarded as a symbol of immortality, from becoming a symbol of death. It is still possible to control the epidemics through sanitation and other preventive measures and to replace dead trees with the resistant clones now available, thanks to local research and international co-operation (46, 53, 54, 59).

Although late in the day, we urge an end to indecision and for the plant pathology community "to take arms against a sea of troubles"; as soon consciousness of the problem becomes common, exit Hamlet.

Devastation by disease of tree species like the cypresses can only be regarded as a natural calamity, not only because of the economic, agricultural, and environmental losses, but other societal factors as well. In October 1997, an earthquake in Italy caused the loss of Cimabue's frescos in St. Francis' basilica, Assisi. The loss to the world if the cypresses vanish from the hills of Florence, the stadium of Olympia, the temples of Delphi, and other sites depicted by artists and sung about by poets, would be as great as if famous masterpieces were removed from the Uffizi, the Louvre, or from other world museums. These artworks of nature are just as much part of our heritage as are the works of human genius. We cannot stand idly by and witness the demise of the Italian cypress in its homeland through our inaction or lack of concern.

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