The Epidemic Spread of *Seiridium cardinale* on Leyland Cypress Severely Limits Its Use in the Mediterranean

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Abstract

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Leyland cypress (*× Hesperotropsis leylandii*) is a fast-growing conifer used in most temperate regions as an ornamental tree for hedges and screens, and is one of the most commercially important trees in Europe. In recent years, severe diebacks and mortality due to cypress canker have been observed on Leyland cypress plantations in Southern Europe. This study was conducted to evaluate (i) the spread and impact of cypress canker caused by *Seiridium cardinale* in plantations of a sample area of 1,250 km² in central Italy, (ii) the response of the most commonly grown Leyland cypress varieties to artificial inoculation with *S. cardinal*, and (iii) the pathogenicity of *S. cardinale* isolates obtained from Leyland cypress. Of the 1,411 surveyed trees, 11.4% had been killed by cypress canker and 43.9% of the living trees were affected by the disease. The number of diseased or dead trees and the

percentage of cankered trunks was significantly correlated with the mean trunk diameter of the plantations. Six months after inoculation, the size of developed cankers was significantly different among the inoculated Leyland cypress cultivars but all of them showed markedly larger cankers than the *C. sempervirens* canker-resistant control clone. All of the tested *S. cardinale* isolates obtained from Leyland cypress also caused cankers on *Cupressus sempervirens* when inoculated as conidial suspensions or mycelia. Leyland cypress is highly prone to contract cypress canker in the Mediterranean due to its high susceptibility to *S. cardinale* infections, low genetic variability among the grown cultivars, and cracks which form on fast-growing trunks, favoring entry of the fungus into the inner bark and the occurrence of infections.

Leyland cypress is a fast-growing conifer of the family Cupressaceae. In view of its monocormic graceful habit, dense crown tolerant to intense pruning, adaptability to varied climatic and soil conditions, and tolerance to frost, pollution, and aerosols, it has been widely grown over the last few decades to form barriers, windbreaks, and hedges and as a shelter tree for private owners in parks and gardens (14). Leyland cypress has also been cultivated commercially as a Christmas tree and for wood products in most temperate regions (3,18). In Italy, besides being used as an ornamental tree, Leyland cypress has been extensively utilized to screen highways, main roads, and railway lines in addition to industrial buildings and infrastructures, particularly over the last 10 to 20 years.

Leyland cypress is a hybrid, first raised in 1888 from a natural cross between Monterey Cypress (*Hesperocyparis macrocarpa* Hartw. Bartel) ♂ and Nootka or Alaskan Cypress (*Callitropsis nootkatensis* (D. Don) Florin) ♀ in Welshpool (Wales), named *× Cupressocyparis leylandii* (Dall. & Jacks.) Dallimore in 1926 (19). The scientific name of Leyland cypress has changed repeatedly over the last two decades as a consequence of recurrent changes of the systematic position of both its parent species and, currently, *× Hesperotropsis leylandii* is used (1,21–23,39). However, for ease of understanding, in this article we will use the common name Leyland cypress.

Today, Leyland cypress is one of most widely sold trees in Europe. In the United Kingdom, it accounted for 10% of nursery sales in 2008 (8). Italy has a long-standing tradition of Cupressaceae production, with an estimated 2.5 million Leyland cypress plants produced each year in nurseries in the Tuscan nursery district of Pistoia (Dr. Carlo Vezzosi, nurseryman in Pistoia at Giorgio Tesi

Group, *personal communication*) for export to most Mediterranean and other European countries. More than 40 different cultivars, differing in growth rate, habit, color, and shape of the crown, have been created, although only about 10 are commonly available on the market (25). Germination of seed is rare and seedlings of the F₂ generation have been raised infrequently (2); therefore, commercial trees are mainly produced through agamic propagation of the desired genotypes by cuttings and, less frequently, by grafting (6).

Leyland cypress is known to be susceptible to cypress canker caused by *Seiridium* spp. fungal pathogens in the southeastern states of the United States (3,5,20,24,34,40,43) and in Oceania (7,18,32,42). The disease was reported to affect Leyland cypress in the nurseries in central Italy as early as 1981 (29), and, to date, this remains the only report of *Seiridium* canker on this tree in Europe.

Seiridium canker, or bark canker of cypress, is a pandemic disease due to the worldwide spread of three pathogenic fungal species—*Seiridium cardinale* (W.W. Wagener) B. Sutton & I.A.S. Gibson, *S. cupressi* (Guba) Boesew., and *S. unicorn* (Cooke & Ellis) B. Sutton—that cause stem and branch necrosis, dieback, and tree mortality in most species of Cupressaceae (9,15). *S. cardinale*, the most widespread and aggressive of the three fungi, was first reported in California in 1928 where, over a few years, it destroyed *H. macrocarpa* plantations in inland districts where this species was not native (44). In the following decades, the disease spread progressively over the five continents and gave rise to epidemics in many regions where it was introduced. In the Mediterranean, the extensive presence of susceptible hosts, in combination with climatic conditions conducive to the development of the pathogen, has promoted its rapid spread (15). Cypress canker reached an incidence as high as 70% in central Italy and Greece during the 1980s and severely affected the local cypress (*Cupressus sempervirens* L.) and other introduced cypress species such as *H. macrocarpa*. Epidemics caused the death of millions of trees, spoiled the landscape, and produced severe economic losses in woods, ornamental plantations, and nurseries (15,28). Recent studies based on the use of both simple-sequence repeat and amplified fragment length polymorphism markers have suggested that California is the most likely source of the

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Mediterranean epidemics, and probably represents the area of origin of the pathogen (12,13).

The optimal growth and germination of *S. cardinale* occurs at 25°C, and it can be considered a mesophilic fungus suited to a warm-temperate climate (28). Although reported at relatively high latitudes in both hemispheres, the pathogen has only reached epidemic proportions in regions characterized by hot summers and cool and rainy winters. Indeed, there are few and limited reports of the disease from central Europe, England, Canada, or Chile (17,27,36,41).

Since the mid-1970s, efforts were made in Central Italy to control the disease by sanitation to reduce the presence of the inoculum in the environment and also to launch a genetic improvement program aimed at selecting resistant lines of common cypress (10,11). The adopted control measures have led to cypress canker currently approaching a post-epidemic or endemic phase, with a relatively steady prevalence (mean around 22%) registered over the last few years in Tuscan woods and plantations of *C. sempervirens* (33). With the exception of particularly conducive sites, the occurrence of cypress canker caused by *S. cardinale* on *C. sempervirens* is generally milder and less frequent than in the past, although the fungus is now widespread throughout the territory.

Nevertheless, in recent years in Italy, from Lombardy and Veneto to Sicily, and especially in the central regions, severe diebacks and mortality have been observed with increasing frequency on Leyland cypress plantations disfigured by *S. cardinale* canker. Attacks rapidly develop on stems and branches, causing tree de-

cline and death within a few years. Bark-canker-like symptoms have also been observed to affect this tree in other European countries such as France, Switzerland, and Spain (G. Della Rocca and R. Danti, *unpublished data*). In the course of a 2009 campaign for collecting the fungus from cankered species of Cupressaceae, severe symptoms were also observed throughout California on Leyland cypress trees planted at a distance from the coast (G. Della Rocca and M. Garbelotto, *unpublished data*). Despite *Seiridium* canker having been reported on Leyland cypress in many regions of the world, no studies to date have been addressed to evaluating its impact in terms of incidence and the extent of damage to plantations.

The aims of this study were to (i) evaluate the incidence and impact of cypress canker disease on Leyland cypress in a sample area of Central Italy, taking into account a representative number of plants; (ii) evaluate through artificial inoculations whether there are differences in susceptibility to cypress canker caused by *S. cardinale* among the current commercially available cultivars of Leyland cypress; and (iii) verify whether there are differences in pathogenicity between *S. cardinale* isolates from Leyland cypress and common cypress.

Materials and Methods

Surveys in Leyland cypress plantations. In total, 38 nonpruned Leyland cypress plantations of various ages and sizes over an area within a radius of about 20 km from Florence (metropolitan belt of Florence) were surveyed during spring to summer 2012 (Table 1). For each tree, the diameter of the trunk at 30 cm

Table 1. List of the Leyland cypress plantations included in a 1,250-km² area around Florence and surveyed for *Seiridium cardinale* canker development^a

Subzone, mean temperature and rainfall	Plantation number	Municipality	Elevation (m above sea level)	Latitude N	Longitude E	Number of trees
1, Bagno a Ripoli: 14.3°C, 975mm	1	Bagno a Ripoli	63	43°45'54"	11°20'28"	174
	2	Bagno a Ripoli	70	43°44'14"	11°17'43"	85
	3	Bagno a Ripoli	70	43°44'33"	11°17'14"	59
	4	Bagno a Ripoli	90	43°46'31"	11°21'46"	23
	5	Bagno a Ripoli	90	43°47'09"	11°22'59"	42
	26	Pontassieve	105	43°46'32"	11°26'14"	15
	27	Rignano sull'Arno	260	43°47'08"	11°23'33"	37
	28	Rignano sull'Arno	350	43°43'26"	11°23'14"	16
	29	Rignano sull'Arno	180	43°41'50"	11°24'35"	53
2, Lastra a Signa: 14.6°C, 914 mm	23	Lastra a Signa	140	43°44'41"	11°04'49"	15
	24	Lastra a Signa	140	43°44'19"	11°04'26"	19
	25	Lastra a Signa	40	43°46'01"	11°07'09"	11
	32	San Casciano	80	43°41'06"	11°08'01"	13
	33	San Casciano	100	43°40'41"	11°07'07"	9
	34	Scandicci	74	43°44'28"	11°10'30"	90
	36	Signa	36	43°47'41"	11°06'22"	82
	8	Calenzano	72	43°51'42"	11°10'23"	46
	9	Calenzano	112	43°53'50"	11°11'01"	47
3, Sesto F.no 14.5°C, 997 mm	10	Calenzano	400	43°56'47"	11°12'47"	43
	11	Campi Bisenzio	38	43°49'30"	11°07'49"	23
	35	Sesto Fiorentino	72	43°50'03"	11°12'18"	37
	6	Barberino di Mugello	270	44°00'13"	11°14'18"	15
	7	Barberino di Mugello	210	43°57'52"	11°17'37"	59
	30	Rufina	150	43°51'41"	11°31'35"	16
4, Borgo San Lorenzo: 13.4°C, 996 mm	31	Rufina	114	43°49'46"	11°29'00"	10
	37	Vaglia	347	43°52'31"	11°17'32"	37
	38	Vicchio del Mugello	200	43°53'18"	11°26'53"	19
	12	Fiesole	164	43°50'03"	11°18'31"	11
	13	Fiesole	161	43°48'10"	11°17'14"	22
	14	Firenze	41	43°46'19"	11°12'29"	4
	15	Firenze	40	43°46'39"	11°10'16"	19
	16	Firenze	40	43°46'39"	11°10'16"	72
	17	Firenze	68	43°43'11"	11°13'25"	44
5, Firenze airport: 14.6°C, 910 mm	18	Firenze	70	43°44'40"	11°17'07"	16
	19	Firenze	40	43°46'59"	11°11'20"	7
	20	Firenze	40	43°46'54"	11°11'14"	8
	21	Firenze	40	43°46'24"	11°11'59"	87
	22	Firenze	40	43°46'20"	11°11'40"	26
Total	...	...	...	...	...	1,411

^a Plantations were distributed in five subzones based on the topographical features of territory (see text). For each subzone, the mean annual temperature and the mean annual rainfall recorded in the period 2003 to 2012 in a representative site were reported, referring to data collected in the archives of Il Meteo (www.ilmeteo.it) and of the Regional Hydrologic Service (www.cfr.toscana.it).

above the ground (as semi-sum of two perpendicular diameters) and height were measured. The conditions of health of each tree were recorded, with particular reference to symptoms of *S. cardinale* attacks, taking into account the development of the disease on affected trees (i.e., the affected organs: trunk, branches, or twigs) and extent of damaged crown (in percent). The latter parameter was estimated visually, subdividing the crowns into 10 classes from 0 to 10 to 91 to 100% based on the quantity of discolored or dead crown, following the procedure described by Stone et al. (35) for determining the extent of necrotized area of Eucalypt leaves.

Isolation and identification of the pathogen. From the Leyland cypress trees showing typical bark canker symptoms, twig, branch, or stem samples were cut and brought to the laboratory. Isolation of the fungal pathogen *S. cardinale* was carried out within 24 h by plating small (about 10 mm²) fragments of necrotic bark cut from the margins of branch or stem cankers on potato dextrose agar (PDA). White to gray or olive, cottony colonies with a salmon-orange reverse side, grown from the plated fragments after an incubation of 3 to 4 days at 25°C in the dark, were separately transferred onto 1% malt extract agar (malt extract at 10 g/liter + agar at 20 g/liter) supplemented with autoclaved cypress seed. The plates were then incubated at 18°C under mixed white and near-ultraviolet light (400 to 200 nm), set to provide 12-h light and dark cycles to induce sporulation of the fungus. For each isolate, seed-bearing acervuli were put inside sterile and hermetic plastic tubes and stored at -20°C.

The identity of the fungal isolates was determined based on the morphology of conidia suspended in water and observed under light microscope at ×400 (7,26,38,45) and confirmed by sequence analysis of the β -tubulin locus performed as described by Della Rocca et al. (12) and Barnes et al. (4). The DNA sequences of a β -tubulin portion of all 45 isolates obtained from necrotic Leyland cypress bark samples and of the *S. cardinale* American Type Culture Collection (ATCC) isolate 38654 used as reference showed from 98 to 100% homology with sequences of *S. cardinale* isolates from Italy and Greece deposited in GenBank (accessions HQ678147 and HQ678149, respectively), while they were clearly distinct from sequences deposited for an *S. cupressi* isolate from Greece (accession AF320492) and an *S. unicornis* (accession AF320482) isolate from Portugal.

Susceptibility of Leyland cypress cultivars to *S. cardinale*. The susceptibility to *S. cardinale* infections of five of the Leyland cypress cultivars most widely grown by Italian nurseries was evaluated through artificial inoculations on stems. The assessed cultivars were 'Leighton green' (common Leylandii), 'Castlewellan Gold', 'Variegatus', 'Gold Rider', and 'Piramidalis 2001'. For each cultivar, 10 3-year-old plants obtained from rooted cuttings and grown in 15-liter pots containing a mixture of peat compost and perlite (3:1:1, vol/vol/vol) were inoculated in April 2012. The plants had a mean trunk diameter of 3.2 cm at collar level and a mean height of 1.75 m. Inoculations were carried out on stems where the diameter was about 1.5 cm using the *S. cardinale* standard isolate ATCC 38654 by removing a 3-mm-diameter circular plug of bark using a cork borer and replacing it with a plug of the same size taken from the margin of a colony of the fungus grown on PDA for 2 weeks at 25°C in the dark. The inoculation site was covered with cotton and wrapped with tape, both of which were removed after 1 week. Ten grafted plants of the same age as the *C. sempervirens* clone 'Agrimid n.1', patented as resistant to cypress canker caused by *S. cardinale*, were also inoculated as described above and used as a control sample. After being inoculated, the plants were kept in a tunnel for 6 months under 50% shade netting and were regularly irrigated to field capacity three times a week. The development of cankers on inoculated stems was evaluated in October 2012, 6 months after inoculation. The cankered portions of stems were cut, put separately into plastic bags, and processed in the laboratory within 24 h. The outer bark was removed in order to expose the margin of the cankers and to measure the length and width of the necrotic area. Isolation of the inoculated fungus was performed from a sample of induced cankers.

Pathogenicity of *S. cardinale* isolates from Leyland cypress.

Artificial inoculations were carried out on *C. sempervirens* trees to assess the ability of *S. cardinale* isolates obtained from Leyland cypress to cause cankers on common cypress. Ten isolates obtained from heavily cankered Leyland cypress plantations were used in this trial along with *S. cardinale* ATCC 38654 and Sc 404 (Istituto per la Protezione delle Piante [IPP]–Consiglio Nazionale delle Ricerche culture collection) from *C. sempervirens* used as a reference. Inoculations were executed in April 2012 following the procedure described in the previous paragraph, on branches of 17-year-old *C. sempervirens* grafted trees growing in the field at the IPP farm situated in Antella (Florence). Four branches located in the lower-mid portion of the crown of each tree were separately inoculated where the diameter was around 1.5 to 2 cm. For each isolate, 10 branches randomly selected among 15 trees were inoculated.

Inoculations were also carried out using conidial suspensions of the same isolates on stems of 3-year-old *C. sempervirens* grafted saplings growing in 3.5-liter pots containing a mixture of peat compost and perlite (3:1:1, vol/vol/vol). For each isolate, conidia were obtained as described above ('Isolation of the pathogen'). Conidia were suspended in 0.3% water agar (WA) inside 1.5-ml microcentrifuge tubes, adjusting the concentration to 1×10^5 conidia ml⁻¹ by using a hemocytometer (Burker chamber). For each plant, two 5-mm-diameter wounds extending into the cambium made on stems with a cork borer (at a distance of at least 20 cm from each other) were inoculated in April 2012 with 100 μ l of conidial suspension prepared for all the assessed isolates. The inoculation site was covered with cotton and parafilm, which was then removed after 2 weeks. Ten plants were inoculated for each isolate. On 10 further control plants, 100 μ l of sterile WA was applied to the wounds, which were then covered with cotton and parafilm as described above. After inoculation, the plants were kept in a tunnel under 50% shade netting and were regularly watered to field capacity three times a week. Assessment of inoculations that resulted in infections on the inoculated stems were carried out after 60 days.

Statistics. Plantations in the surveyed area were assigned to five geographical subzones, reflecting the morphology (topographical features) of the territory. The southeastern subzone located east of Florence, south of the Arno river (subzone 1); the southwestern subzone located south of Florence between the Arno river and the valley of the Pesa river (subzone 2); the subzone located on the northwest slopes of the plane of Florence (subzone 3); the subzone located north of Florence in the valley of the Sieve river and separated from the city by a mountain range with elevation of 1,000 m (subzone 4); and the subzone comprising the plane of Florence (subzone 5). For each subzone, the plantations were also subdivided into five classes based on the mean trunk diameter to evaluate the effect of the size of plantations on the frequency of diseased trees (Table 2). Percentages of diseased trees were calculated for each of the five subzones and each class of trunk diameter. Differences among subzones and trunk diameter classes were separately evaluated through the χ^2 test based on five-by-two contingency tables in which the proportion between diseased and healthy trees was considered.

The Pearson correlation coefficient was calculated to explore the relationship between elevation and the frequency of diseased trees in each plantation. Correlations between the mean trunk diameter of each plantation and the frequency (in percent) of diseased trees, the frequency of trees with cankered trunks, the frequency of dead trees, and the mean extent of crown damage were also studied.

The differences in size of the cankers developed on the inoculated Leyland cypress cultivars were calculated through analysis of variance and the post-hoc honestly significant difference (HSD) Tukey test. Percent values of necrotized trunk circumference were subjected to angular transformation using the formula $y = \arcsin \sqrt{(p/100)}$, where p is the percentage of necrotized trunk circumference, before being analyzed. Analysis of variance and the post-hoc HSD Tukey test were also used to explore differences in size

of the cankers developed on *C. sempervirens* trees inoculated with *S. cardinale* isolates derived from Leyland cypress and common cypress (analysis of variance and post hoc tests were performed using the software package STATISTICA 7.0).

Results

Survey on Leyland cypress plantation. In total 1,411 Leyland cypress trees belonging to 38 plantations were surveyed for symptoms of bark canker (Table 1). Acervuli of the fungus were observed on the bark of most cankered stems and branches along with abundant resin exudation. Of the living trees, 43.9% were affected by cypress canker due to *S. cardinale* and 11.4% of the original planted trees had died as consequence of the attacks by the pathogen (Table 2).

Significant differences in the frequency of diseased trees were observed among the five subzones: the highest value (54.5%) was registered for subzone 1 and the lowest (9.7%) for subzone 3 ($\chi^2 = 188.1$; df 4; $P < 0.01$). The frequency of diseased trees was also significantly different among the five diametric classes to which the plantations were assigned by mean trunk diameter ($\chi^2 = 84.7$; gdl 4; $P < 0.01$). More specifically, a clear increase in the percentage of affected trees (from 32.6 to 69.6%) was associated with an increase in diameter of the trunk.

No significant correlation was observed between the percentage of affected trees and the elevation of each plantation ($r = 0.16$; $P > 0.05$). Instead, the mean diameter of trunk of each plantation was positively and significantly related to the percentage of diseased trees ($r = 0.622$; $P < 0.01$), the percentage of trees with cankered trunks ($r = 0.403$; $P < 0.05$), the percentage of dead trees ($r = 0.309$; $P < 0.01$), and the extent of crown damage ($r = 0.363$; $P < 0.05$) (Fig. 1).

Susceptibility of some Leyland cypress cultivars to *S. cardinale* infections. The size of cankers developed 6 months after inoculation with *S. cardinale* mycelium on stems of various Leyland cypress cultivars is reported in Table 3. Both the mean length and width of resultant cankers varied significantly ($P < 0.01$) among the Leyland cypress cultivars considered. In detail, longer cankers were observed on Variegatus and Leighton green whereas, on Castlewellan Gold, cankers were not significantly longer than those observed on the resistant control cypress clone Agrimed n.1. Cankers developed on all Leyland cypress cultivars were markedly wider than those developed on the resistant control ($P < 0.01$). Wider cankers ($P < 0.01$) were observed on Gold Rider, Leighton green, and Variegatus compared with the Castlewellan Gold, while Piramidalis 2001 showed an intermediate width of cankers.

Table 2. Frequency of diseased trees (referred to number of trees affected by *Seiridium cardinale* canker on the number of living trees) was displayed for each of the five subzones and for each of the five diametric classes to which plantations were assigned according to their mean trunk diameter measured 30 cm above the ground^y

Subzone	Frequency of diseased trees per diametric class					Diseased (%)	Dead (%)
	5–10 cm	11–15 cm	16–20 cm	21–25 cm	>25 cm		
1	103/180	10/35	14/33	100/174	9/11	54.5	14.0
2	25/117	—/—	7/13	10/26	56/89	42.4	6.4
3	16/164	—/—	—/—	—/—	—/—	9.7	5.2
4	—/—	17/44	28/67	11/33	—/—	38.8	13.7
5	37/93	11/22	19/54	7/7	63/88	51.9	13.4
Diseased (%)	32.6	39.4	39.6	55.2	69.6	43.9 ^z	11.4 ^z

^y Percentages of both diseased and dead trees (the latter referred to the total number of trees of the original plantation) were also reported for each subzone.

^z Overall.

Fig. 1. Correlation between mean diameter of trunk of each of the surveyed Leyland cypress plantation and **A**, number of *Seiridium cardinale*-diseased trees as percentage of the living trees; **B**, number of trees with cankered trunk as percentage of the diseased trees; **C**, number of killed trees as percentage of the total number of trees; and **D**, extent of damaged crown in percent (as mean of the values registered on the diseased trees of each plantation).

In terms of the percentage of necrotized trunk circumference (the ratio between the width of cankers and the trunk circumference), a clear and significant difference emerged between the resistant control clone (36.7%) and all the Leyland cypress cultivars considered (from 76.7% of Castlewella Gold to 98.0% of Leighton green; Table 3). A significant difference was also observed when comparing Variegatus and Leighton green, which showed values close to 100% (which means complete girdling of stem) with Castlewella Gold.

Pathogenicity of *S. cardinale* isolates from Leyland cypress. All artificial inoculations with mycelium of isolates from Leyland cypress successfully caused cankers on branches of *C. sempervirens*. Significant differences were observed 6 months after inoculation in both the length and width of cankers among isolates from Leyland cypress, whereas cankers generated by the reference isolate ATCC 38654 from *C. sempervirens* were not significantly different from those produced by isolates from Leyland cypress (Table 4).

All five tested *S. cardinale* isolates from Leyland cypress produced necrotic lesions when inoculated through conidial suspensions on stems of 3-year-old saplings of *C. sempervirens* (Table 5).

Discussion

Observations carried out from the north to the south of Italy, and an in-depth survey conducted over a restricted area in central Italy, on a wide sample of Leyland cypress plantations has demonstrated that this tree is prone to attack and disfigurement by cypress canker due to *S. cardinale*. Of the 1,411 surveyed trees, 11.4% had been killed by cypress canker and 43.9% of the living trees were affected by the disease. Of 38 surveyed plantations, 12 exhibited a prevalence of diseased trees greater than 50% and, in some cases, the frequency of diseased trees was as high as 80 or 90%. This high percentage of attacked trees, along with the severe damage to crowns reported in most plantations, confirms that Leyland cypress is highly vulnerable to *S. cardinale*. The fungus may easily colonize an infected tree and spread rapidly to other trees; thus, *S. cardinale* represents a limiting factor for cultivation in the climatic conditions of central Italy.

The susceptibility of Leyland cypress to *Seiridium* canker was already known, because cankers and severe dieback were reported in the Great Plains states of the south-central and southeastern United States (Georgia, Florida, Arkansas, Texas, and North and South Carolina) in addition to California and New Zealand (3,18,20,24,34,40,42,44).

Despite the fact that *Seiridium* cankers were reported as severely affecting Leyland cypress in various regions of the world, to date, no studies have focused on evaluating the distribution of the disease and quantifying the extent of damage over a specific territory in order to define the real impact of the disease on this tree. This work aims to evaluate the distribution and development of *S. cardinale* attacks, and the extent of damage to plantations over an area of 1,250 km² around the city of Florence. Over the past few decades, this tree has been extensively planted in this region and, since the 1970s, the pathogen has caused serious epidemics on *C. sempervirens*. The occurrence of virulent and spreading infection of Leyland cypress is in contrast to the endemic (or post-epidemic) phase that *S. cardinale* canker is currently exhibiting in the Italian

cypress in the surveyed territory and over central Italy. Furthermore, excluding some sites with microclimatic conditions that prove to be particularly conducive to the spread of the pathogen, attacks on *C. sempervirens* are generally sporadic in this region (22% mean prevalence of the disease in Tuscany; 33).

A different prevalence of diseased trees was revealed among the five subzones into which the surveyed area was divided, and a clear rise in the frequency of diseased trees was also observed with the increase of the mean diameter of plantations. Unfortunately, the trunk diameter classes into which the plantations were grouped were not evenly distributed among the five subzones (e.g., all plantations in subzone 3 belonged to the smaller diametric class) and, for this reason, it was difficult to separate the effect of site (local climate) from the effect of the size of plantations. The collected climatic data showed that both the mean temperature and the mean annual rainfall of the five subzones are very similar and, even though a site effect cannot be ruled out, differences in the percentage of affected trees observed within the studied area can be ascribed mostly to the size of plantations in the subzones. The absence of a relationship between elevation and the percentage of

Table 4. Mean development of necrotic lesions on branches of 17-year-old *Cupressus sempervirens* trees measured 6 months after artificial inoculation with *Seiridium cardinale* isolates obtained from Leyland cypress^z

Isolate	Host of origin	Canker length (mm)	Canker width (mm)
Sc ATCC 38654	<i>C. sempervirens</i>	25.8 ab	11.4 ab
Sc 404	<i>C. sempervirens</i>	33.0 ab	10.8 ab
Sc 519	Leyland cypress	26.2 ab	9.3 a
Sc 520	Leyland cypress	23.1 ab	10.5 ab
Sc 526	Leyland cypress	33.7 ab	13.7 b
Sc 527	Leyland cypress	17.4 a	9.1 a
Sc 529	Leyland cypress	17.6 a	13.5 b
Sc 530	Leyland cypress	32.5 ab	12.0 ab
Sc 533	Leyland cypress	38.0 b	12.5 ab
Sc 535	Leyland cypress	23.9 ab	10.9 ab
Sc 536	Leyland cypress	27.8 ab	11.0 ab
Sc 537	Leyland cypress	30.2 ab	12.1 ab

^z Isolates ATCC 38654 and Sc 404 from *C. sempervirens* were included as reference. Means not sharing same letters within each column were significantly different for $P < 0.05$ according to Tukey's honestly significant difference test.

Table 5. Number of positive infections (out of 20 inoculations) developed 60 days after stem inoculation of 3-year-old *Cupressus sempervirens* plants using conidial suspensions of *Seiridium cardinale* isolates obtained from Leyland cypress^z

Isolate	Host of origin	Number of positive infections
ATCC 38654	<i>C. sempervirens</i>	16/20
Sc 526	Leyland cypress	14/20
Sc 527	Leyland cypress	20/20
Sc 533	Leyland cypress	16/20
Sc 535	Leyland cypress	12/20
Sc 536	Leyland cypress	14/20
Control	...	0/20

^z Isolate ATCC 38654 derived from common cypress was included as reference. Control = sterile agar.

Table 3. Mean development of necrotic lesions on stems of 3-year-old plants of Leyland cypress and Common cypress as control measured 6 months after artificial inoculation with the ATCC 38654 isolate of *Seiridium cardinale*^z

Cultivar, species	Length (cm)	Width (cm)	Necrotized trunk circumference (%)
<i>Cupressus sempervirens</i> 'Agrimed n.1'	8.44 ab	2.45 a	36.7 a
Leyland cypress			
'Castlewella gold'	6.24 a	5.17 b	76.7 b
'Pyramidalis 2001'	11.33 b	5.42 bc	86.5 bc
'Gold rider'	9.18 ab	5.86 c	84.6 bc
'Leighton Green'	11.80 b	6.82 c	98.8 c
'Variegatus'	11.24 b	7.09 c	95.2 c

^z Means not sharing same letters within each column were significantly different for $P < 0.01$ according to Tukey's honestly significant difference test.

diseased trees in the study area failed to confirm any potential effect of climate variation at a local level on the spread of cypress canker in Leyland cypress. Furthermore, Leyland cypress plantations currently appear to be severely affected by bark canker throughout Italy, in both the north and south, where climatic conditions are markedly different.

The study also revealed a significant positive correlation between the mean percentage of damaged crown (within diseased trees), the percentage of cankered trunks and dead trees, and the size of Leyland cypress plantations. The younger plantations (2 to 4 m tall) were generally mildly affected by *S. cardinale*. This is not consistent with the higher susceptibility to *S. cardinale* infections that is reported in literature for young cypress trees or on twigs and small branches (28). *S. cardinale* infections generally occur more frequently on young twigs or on small plants that have a thin outer bark and are more subject to microlesions (due to cold, insects, hail, and so on) through which the pathogen may enter the inner bark and begin the infection process. We have observed that the outer bark of Leyland cypress remains smooth on small trunks and branches; however, the very rapid diametric growth of this tree causes the occurrence of visible cracks on trunks when they reach a diameter of 8 to 10 cm. The bark is probably subjected to excessive tension due to the intense growth of the xylem and, consequently, tends to crack and fissure. Frequently, trunks exceeding 10 to 15 cm in diameter are characterized by visible cracks that expose the inner bark. This can favor the entrance of the pathogen into the host tissues, and can explain the higher number of cankered trunks and the more severe damage observed in older plantations in the present survey.

As an intergeneric and sterile hybrid, raised in relatively recent times (19th century), this tree has theoretically not experienced co-evolution with any pathogens or other stress factors and, at the same time, has inherited the susceptibility to *S. cardinale* from at least one of its parents. Nothing is known about the response of Nootka cypress to *S. cardinale* infections. This species, due to its Northern range along the Pacific coast from northern California to Alaska, has never been reported as host of the pathogen, which is ecologically suited to warm-temperate regions. However, Nootka cypress could be susceptible to *S. cardinale* when cultivated outside its native area in regions adapted to the development of the fungus, which has a wide range of hosts among species of Cupressaceae. The other parent species, Monterey cypress (*H. macrocarpa*), while not attacked by *S. cardinale* in its native habitat (a restricted area facing the Pacific coast near Monterey in California), is known for its extreme susceptibility to the fungus when grown in warm-temperate regions. Monterey cypress plantations have been reported to be severely affected or compromised by *Seiridium* cankers since the first half of the last century in inland California, and in the Mediterranean, New Zealand, Australia, and South Africa (28,37,38,45,46). Additionally, in terms of the response of Monterey cypress to *S. cardinale* infections, artificial inoculation campaigns conducted in Italy and France also revealed a substantial lack of intraspecific variability in the resistance response to the pathogen. All young stems were completely girdled by the induced cankers, and trees were not able to survive the inoculation (16,30,31).

It is likely that bark cracks that appear on trunks when they exceed a certain diameter represent a limiting factor for the growth of this tree in regions where the climatic conditions are favorable to *S. cardinale*. Although most of the surveyed plantations were located several kilometers away from one another, the high density of susceptible hosts and the long-established presence of the fungus in the studied area could have favored the spread of the disease in Leyland cypress, as observed in the survey. Furthermore, Leyland cypress has also recently appeared to be severely affected by *S. cardinale* throughout inland California, although this tree and other susceptible members of the family Cupressaceae (i.e., Monterey cypress and Italian cypress) are sparsely distributed in the territory, mainly as ornamentals in parks and gardens (G. Della Rocca and M. Garbelotto, unpublished data). We have observed that there was

no apparent relationship between the number of trees and the percentage of diseased trees in each plantation (*data not shown*). This seems to corroborate the marked propensity of Leyland cypress to contract bark canker, even when the level of the inoculum is relatively low, provided that climatic conditions are favorable to the pathogen.

Leyland cypress can be used successfully in regions where *Seiridium* inoculum is absent or very scarce, such as in north-central Europe, where the climate is relatively cold and unsuited to the spread of the pathogen. However, in the Mediterranean, Leyland cypress growth can be severely conditioned by attacks of the fungus, even where the disease has become endemic on the local common cypress. Indeed, many of the older plantations of Leyland cypress included in the survey appeared devastated, and their ornamental or screening functions substantially negated.

Artificial inoculation with the *S. cardinale* isolate ATCC 38654 showed a greater development of necrotic lesions on all the tested Leyland cypress varieties than on the *C. sempervirens* canker-resistant control clone. Significant differences were observed in both the length and width of necrosis among the various Leyland cypress cultivars. In particular necrosis was significantly less developed on Castellellan Gold compared with all the other tested cultivars. Greater differences were observed in the width (and percentage of necrotized trunk circumference) rather than the length of cankers among the various Leyland cypress cultivars and the common cypress resistant clone. The pathogen showed rapid lateral growth on Leyland cypress and, 6 months after inoculation, 76.7 to 98.8% of the trunk circumference was affected by the necrosis whereas, on the *C. sempervirens*-resistant clone, only 36.7% of the trunk circumference was necrotized. The rapid lateral spread of the fungus in the bark tissues observed in artificially inoculated Leyland cypress trees means that trunks and branches can be easily girdled by cankers, explaining the severity of damage observed in most plantations. In summary, none of the tested varieties of Leyland cypress seemed to show some form of resistance against cypress canker caused by *S. cardinale*; however, a proper assessment of their response would require longer-term observations.

All of the assayed *S. cardinale* isolates obtained from Leyland cypresses showed the ability to produce necrotic lesions when inoculated on *C. sempervirens*. No significant differences were noted 6 months after mycelial inoculation in regard to the size of the necrosis between the Leyland cypress isolates and the two *S. cardinale* isolates obtained from *C. sempervirens*. This seems to exclude any kind of host specialization or differentiation in pathogenicity among isolates from common and Leyland cypress. Furthermore, inoculations made with conidial suspensions of five isolates from Leyland cypress gave rise to necrotic processes in the stems of 3-year-old *C. sempervirens* plants. Hence, the inoculum of the fungus that is progressively increasing in the diseased Leyland cypress plantations, and the development of fruit bodies frequently observed on cankers, might favor the spread of the disease to neighboring common cypress trees and foster new outbreaks. Nevertheless, in the surveyed area, the disease currently appears to spread preferentially to Leyland cypress, whereas common cypress is only sporadically affected. It is possible that the prolonged contact between *S. cardinale* and *C. sempervirens* which occurred during epidemics in previous decades in central Italy has induced the common cypress to develop defenses, probably through a form of selection, in conjunction with the adopted sanitation measures that have removed many affected and susceptible trees throughout the territory. Leyland cypress trees are vulnerable due to their marked susceptibility to cypress canker and genetic uniformity, which is perpetuated by the agamic propagation of few genotypes.

In the south-central and southeastern United States, the disease is reported to be caused by *S. unicorne* (and, in Florida, by *S. cupressi*), and its occurrence is considered to be favored by prolonged drought periods which predispose Leyland cypress to infections and canker development (3,24,40). According to Martinez et al. (24), *Seiridium* cankers enlarge up to three times faster on drought-stressed trees than on adequately irrigated trees. Among the three

Seiridium fungal species causing cypress canker, *S. unicorne* is considered to be the least aggressive, often acting as an opportunistic pathogen, which can increase its impact on stressed Leyland cypress trees, ecologically unsuited to the hot and dry seasons typical of these regions of the United States. *S. cardinale* is considered a primary pathogen, causing cankers which generally show a greater development on healthy and fast-growing trees (growing in deep, fertile, and well-irrigated soils; 9,28). In our survey, new discolorations due to girdling and developing cankers were frequently observed during humid springs. Moreover, severe *S. cardinale* attacks occurred in plantations that showed good crown conditions in terms of growth, full color, and density, or in indirectly watered plantations (located in private gardens with meadow) without any sign of decline due to other reported stress factors (sparse foliage, stunted growth, chlorosis, and so on).

This small-scale survey allowed us to define and quantify the actual amount of damage observed on Leyland cypress on a larger scale in most regions of Italy, and underlines the fact that Leyland cypress is unsuited to areas where conditions are favorable to the development of *S. cardinale* because of its high susceptibility to attack by this pathogen. In these areas, the use of this tree should be discouraged, whereas it can be used successfully in cooler climates where the inoculum of the fungus is absent or scarce. For areas with a Mediterranean climate, some patented canker-resistant *C. sempervirens* clones available on the market can be used to replace Leyland cypress, particularly the clone Agrimed n.1, which has a shape suitable for barriers and windbreaks and is known for its vigor and its adaptation to Mediterranean climatic conditions and to different types of soil.

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