Screening for Resistance to *Seiridium cardinale, S. cupressi,* and *S. unicorne* Isolates in Glasshouse-grown Seedlings of Cupressaceae

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Abstract

Young glasshouse grown seedlings, 18-months-old, of several species within the Cupressaceae were inoculated with the canker-causing pathogens *Seiridium cardinale*, *S. cupressi* and *S. unicorne* at two positions: position 1, 35–40 cm above the root colar and position 2, 1.5–2.0 cm a.r.c. In terms of the lengths of lesions caused on inoculated stems, *S. cardinale* proved more virulent than *S. cupressi* or *S. unicorne* on all *Cupressus* species. *S. cupressi* was more virulent than *S. unicorne* only on *C. macrocarpa*. *C. macrocarpa* was the most susceptible to *S. cardinale* (at position 1 and 2), *C. sempervirens* and *C. torulosa* were also susceptible whereas *C. arizonica* was moderately susceptible to this pathogen (position 1). In contrast, *Chamaecyparis lawsoniana* was highly resistant to *S. cardinale* (position 1 and 2) but was more damaged by *S. cupressi* at position 1 and *S. unicorne* at position 2.

Intraspecific variation in susceptibility to *S. cardinale* was found in *C. sempervirens* seedlings (inoculated at position 2) from four different seed sources which developed different canker lengths after 50 days, although this variation was lower than between species variation.

Cankers developed more rapidly when inoculations were made 35–40 cm above the root collar, where the periderm had formed recently, than when made 1.5–2.0 cm above the root collar, where rhytidome development had occurred, suggesting that older bark is more resistant than younger bark to growth of *Seiridium*.

Inoculation of seedlings of *C. sempervirens* (Krete provenance) with 8 different isolates of *S. cardinale* showed the relatively low variability in virulence within this pathogen species. Only single isolate caused lesions <10 mm in length around the inoculation points 30 days after inoculation, whereas the other 7 isolates caused lesions of approximately 30 mm in length in the same time period.

These results correlate well with those published from previous studies where inoculations were made on older trees and demonstrate the utility of this method in the early screening of *Cupressus* seedlings for resistance to *Seiridium* canker and testing the virulence of the three fungus species.

Key words: Virulence, *Seiridium*, inoculation, seedlings, *Cupressus*, *Chamaecyparis,* canker, resistance.

Introduction

Cypress canker, caused by *Seiridium cardinale* (WAG.) SUTT. & GIBS., is one of the most significant canker disease of woody plants, affecting trees in natural forests, plantations and the urban environment worldwide (GRANITI, 1998; PANCONESI, 1990). Infection by *S. cardinale* causes dieback of affected hosts, resulting in loss of form and timber quality; in highly susceptible individuals, death results when the main stem is girdled. Since the first description of the disease in California in the 1920's (WAGENER, 1928), *S. cardinale* has been reported from many regions of the world where the Cupressaceae are grown (GRASSO and PONCHET, 1979). Two other species of *Seiridium* associated with cypress canker, reported by some authors (GRANITI, 1986; PANCONESI, 1990), *S. cupressi* (GUBA) BOES. (teleomorph: *Lepeutypa cupressi* (NATRASS *et al.*) SWART) and *S. unicorne* (CKE. & ELL.) SUTT., are distinguished from *S. cardinale* based on the presence, size and orientation of apical and basal appendages (BOESEWINKEL, 1983; GRANITI, 1986). Several authors, however, have disputed the separation of *S. cardinale* into three species based on morphology (SWART, 1973; CHOU, 1989), and recent molecular evidence supports this view (VILJOEN *et al.*, 1993). The host ranges reported to be affected by these three suggested species differ, however (GRANITI, 1998).

Great variation exists in the susceptibility of different species within the Cupressaceae to *Seiridium* infection (ANDREOLI, 1979; GRASSO and PONCHET, 1979; RADDI, 1979; GRANITI, 1998). Intraspecific variation in resistance has also been reported (PONCHET and ANDREOLI, 1979; XENOPOULOS, 1990, 1991; TEISSIER DU CROS *et al.*, 1991), with marked variations within provenances and families from controlled crosses. Effective exploitation of these genetic variations may enable the replacement of stands damaged by canker with more productive selections.

Techniques for assessing susceptibility to *Seiridium* spp. have predominantly involved field or glasshouse inoculations of host trees of between 2 and 20 years old (PONCHET and ANDREOLI, 1984, 1989, 1990; XENOPOULOS, 1990, 1991; SPANOS *et al.*, 1999). These screening tests may take 3 to 4 years to complete, depending on environmental influences, including the weather, nutrition and pedology (PONCHET and ANDREOLI, 1990). The physiological age of the host tissues inoculated also appears to have an influence on the outcome of these tests (PONCHET and ANDREOLI, 1990). Methods for decreasing the time required to obtain confirmation of the susceptibility of different genotypes could reduce the costs of breeding programmes, and a number of alternative procedures have been proposed, based on *in vitro* techniques (TONON, 1994; TONON *et al.*, 1995; SPANOS and WOODWARD, 1997; SPANOS *et al.*, 1997). It is important, however, that results from such procedures are confirmed using *in vivo* tests, before being adopted on a wide scale.

Seiridium spp. are wound parasites, and are mainly confined to the bark cortex and secondary phloem tissues. Under certain circumstances, *S. cardinale* will penetrate the vascular cambium, medullary rays and superficial layers of sapwood (MUTTO and PANCONESI, 1987; PONCHET and ANDREOLI, 1990), developing

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intercellularly in these tissues (MUTTO and PANCONESI, 1987). Despite growing into the xylem immediately adjacent to medullary rays, the pathogen is unable to penetrate lignified cell walls (PONCHET and ANDREOLI, 1990). *Seiridium* spp. have, however, been shown to produce various plant cell wall degrading enzymes, including cellulase, xylanase and polygalacturonase (MAGRO *et al.*, 1982). *S. cardinale* also causes a rapid disorganisation in the host cytoplasm through disruption of the plasma membrane, probably via the production of toxic metabolites (SPARAPANO *et al.*, 1985; BALLIO *et al.*, 1989).

Morphologically, the responses of *Cupressus* species to *Seiridium* spp. are similar to those reported from other conifers and broad-leaved trees (PONCHET and ANDREOLI, 1990). The ability to wall out necrotic bark tissues by the formation of a boundary zone, or necrophylactic periderm (NP) is a very important reaction in conifers and broad-leaved trees (MULLICK, 1977; BIGGS, 1992; WOODWARD, 1992). This process involves the differentiation of secondary meristems and is thought to be under polygenic control, with a complex heritability (PONCHET and ANDREOLI, 1990; BIGGS, 1992). It has been suggested that the speed at which compartmentalisation of necrotic tissues occurs makes a significant contribution to the intra- and interspecific variability in resistance to *Seiridium* canker found in the genus *Cupressus* (PONCHET and ANDREOLI, 1989, 1990; SPANOS *et al.*, 1999).

The objective of this work was to make an early *in vivo* screening for resistance to *Seiridium* bark canker involving important factors (tree species, fungus, time after inoculation, host tissue). The paper reports the inoculation of 18-months-old seedlings of *Cupressus sempervirens*, *C. torulosa*, *C. arizonica*, *C. macrocarpa* and *Chamaecyparis lawsoniana* with *Seiridium cardinale*, *S. unicorne* and *S. cupressi*, and the subsequent development of lesions under glasshouse conditions.

Materials and Methods

Plant material

Seeds of *Cupressus sempervirens* were obtained from various sources. *C. sempervirens*-Thes. was collected from a plantation in Thessaloniki area, Greece; *C. sempervirens*-Kr. was collected from a natural stand in Krete island, Greece; *C. sempervirens*-x, a hybrid of two resistant to *Seiridium cardinale* clones (Σ1-22 x K-104), was supplied by the Forest Research Institute in Athens. A further seed batch of unknown origin, *C. sempervirens*-Br., was obtained from Sandeman Seeds, West Sussex, UK. Seed of *C. macrocarpa*, *C. torulosa* and *C. arizonica* was also obtained from Sandeman Seeds. *Chamaecyparis lawsoniana* seed was from Department of Forestry, University of Aberdeen, stocks.

Seeds were imbibed for 24 hours in tap water containing 0.025% Tween 20 as wetting agent, soaked in 30% v/v H_2O_2 for 30 min, rinsed three times with sterile water and stratified for four weeks at + 4° C in polyethylene bags containing moistened peat. Stratified seeds were sown (in a glasshouse) in 21.5 x 15 cm seedtrays, containing pure perlite and watered every day. Germination occurred in three weeks. Seedlings were transplanted to 8 x 6 x 6 cm plastic pots containing compost (loam/peat/sand 7:2:3 v/v/v). Before and after transplanfing, seedlings were watered with tap water containing 4 $g.l^{-1}$ Cheshunt compound (commercial product) to protect against damping off. Two months after sowing, seedlings were transplanted to 0.5 l plastic pots with the same potting medium.

Fungal material

Mycelial isolates of *Seiridium cardinale*, *S. cupressi* and *S. unicorne*, obtained from the Forest Research Institute of Athens, were maintained on 3% malt extract agar (Oxoid) at 25±2°C. These fungi were isolated from infected *C. sempervirens* in Italy (ATCC 38654), Kos (Greece – 'grey' isolate) and Portugal (SP AI86), respectively (XENOPOULOS, 1991). In addition, 8 further isolates of *S. cardinale*, isolated from infected cypress plantations (Common cypress) in central and southern Greece, were obtained from stock cultures held (for the same storage period) at the Forest Research Institute, Athens.

Inoculation method

Seedlings were inoculated at 18–20 months-old, when the stem diameter ranged from 5 to 10 mm and the plants were approximately 100 to 125 cm in height. Air temperature in the glasshouse during inoculation ranged from 18 to 25°C, with a relative humidity of approximately 60%. Supplementary lighting was supplied by six sodium vapour lamps giving a light intensity 6–7 $w.m^{-2}$, extending the daylength to 18 hours.

A bark plug of approximately 3 mm diameter was removed aseptically from the stem and a mycelium plug of the same diameter, taken from the edge of 2–3 week-old colonies of *S. cardinale*, *S. cupressi* or *S. unicorne* on malt extract agar, inverted onto the wound, such that the hyphae were in contact with the vascular cambium. Inoculation points were covered with autoclave tape to prevent drying out of the inoculum. Control wounds, on separate seedlings, were inoculated with plugs of sterile 3% malt extract-agar. Inoculated plants were watered with tap water every third day.

Plants were inoculated with one of the three *Seiridium* species at two positions on the main stem:

Position 1, approximately 1/3 of the total plant height (35–40 cm) above the compost surface, where periderm formation had occurred. Twenty plants each of *C. sempervirens*-Thes., *C. sempervirens*-Kr., *C. macrocarpa, C. torulosa, C. arizonica* and *Cham. lawsoniana* were inoculated with *Seiridium cardinale*, *S. cupressi* and *S. unicorne* in this treatment.

Position 2, at the base of the stem, 1.5–2.0 cm above the compost surface, where development of rhytidome by the periderm was apparent. Twenty plants each of *C. sempervirens*-Br., *C. sempervirens*-Thes., *C. sempervirens*-Kr., *C. sempervirens*-x, *C. macrocarpa* and *Cham. lawsoniana* were inoculated with *Seiridium cardinale* in this treatment. *C. sempervirens*-Br., *C. sempervirens*-Thes., *C. sempervirens*-Kr., and *Cham. lawsoniana* were inoculated with *S. cupressi* and *S. unicorne* only, due to insufficient numbers of seedlings of the other *Cupressus* selections and species being available.

Canker length was recorded as the extent of necrosis (using a lens x 5 times magn.) on the main stem of the plant and was measured as the vertical extent of the lesion. Cankers were measured 10, 20 and 30 days after inoculation for seedlings inoculated at position 1, and 20, 30, 40 and 50 days after inoculation for position 2.

In addition to these tests, the 8 further isolates of *S. cardinale* were inoculated at position 1 on the stems of *Cupressus sempervirens*-Kr. seedlings. Seedlings with similar stem diameters, heights and vigour were selected for these inoculations.

In addition to recording canker lengths, the extent of stem girdling was estimated visually, along with observations related to the health of the crown, including wilting, discoloration and necrosis. Discoloration of the crown was defined as a change from the normal green colour to light green, green-yellow, yellow-brown, brown or bronze. Crown symptoms were compared with the healthy crowns of control seedlings.

Experimental design and statistical analysis

Twenty replicate seedlings for each tree species and for each fungus species or isolate were inoculated using the method

described above. Replicate seedlings were completely randomised on the glasshouse bench. Analysis of variance (ANOVA) was used for statistical analysis of the data. The Least Significant Test (LSD) was used for the comparison of means of various treatments.

Results

Seedlings inoculated on the lower 1/3 of the main stem (Position 1)

The first symptoms, observed 7–l0 days after inoculation were a colour change to light-green or bronze-brown of the needle tips on terminal shoots, and shoot-tip wilting. Two weeks after inoculation, bark tissues were collapsing around inoculation points, with lesions extending more rapidly longitudinally than laterally. Acervuli were observed after approximately 20 days and were very common on seedlings inoculated with *S. cardinale*. Sporulation was observed under the bark surface in most *Cupressus* seedlings, but rarely in *Cham. lawsoniana*.

Thirty days alter inoculation, most of the seedlings and particularly those of *Cupressus macrocarpa* and *C. sempervirens* showed symptoms over more than 50% of the crown and most seedlings were girdled completely by *S. cardinale* (data not presented). Seedlings inoculated with *S. cupressi* had more limited crown symptoms (0–50%) and stem girdling (1/2 to 2/3 of the stem). Seedlings inoculated with *S. unicorne* showed a high percentage (more than 50%) of distinctive crown symptoms characterised by discoloration rather than wilting, and less stem girdling (1/2–2/3) compared to seedlings inoculated with *S. cardinale*.

Development of cankers caused by *Seiridium* sp. on the tested tree species are presented in *Table 1*. *S. cardinale* caused large lesions on all *Cupressus* species but much smaller on *Cham. lawsoniana* in all recordings. In all *Cupressus* species the size of the lesions caused by the pathogen increased significantly (P<0.01) from 10 to 30 days, after inoculation. In contrast, canker size on *Cham. lawsoniana* increased only slightly from day 10 to day 30.

C. macrocarpa was significantly more susceptible (P<0.01) than the other *Cupressus* species to *S. cardinale*, with mean canker lengths of 20.1±1.1, 35.0±1.1 and 42.8±1.4 mm, at 10, 20 and 30 days from inoculation, respectively. *C. sempervirens* (Thes., Kr.), and *C. torulosa* were also susceptible to *S. cardinale*. By 30 days post-inoculation, lesions caused on *C. arizonica* were significantly smaller (P<0.01) than those on other *Cupressus* species. *Chamaecyparis lawsoniana* seedlings were resistant to *S. cardinale* and *S. unicorne* with mean canker lengths of 8.3±0.3 mm and 8.7±0.3 mm respectively, 30 days after inoculation, the smallest canker size (P<0.01) among the tested tree species.

Lesion sizes produced by *S. cupressi* on the main stem of all tested *Cupressus* spp. increased significantly from day 0 to day 30 (P<0.01) (*Table 1*). On *Cham. lawsoniana* canker size increased slightly from day 0 (5.5±0.2 mm) to day 20 (6.6±0.2 mm), but increased significantly (P<0.01) from day 20 to 30 (11.8±0.7 mm). Cankers produced by *S. cupressi* on *Cupressus* species were significantly smaller (P<0.01) than those induced by *S. cardinale* in all recordings. However, while canker lengths induced by *S. cupressi* on *Cham. lawsoniana* were almost equal (P>0.01) to those caused by *S. cardinale* 10

Table 1. – Lengths of cankers caused by *Seiridium* species following inoculation 35–40 cm above the compost surface (position 1), of 18-month old cypress seedlings.

	Fungus species		
Tree species	*S. cardinale*	*S. cupressi*	*S. unicorne*
Length of canker (mm) 10 days after inoculation [1, 2]			
Cupressus sempervirens-Thes.	16.5±0.5^{b}	8.0±0.3^{ef}	10.7±0.4^{h}
Cupressus sempervirens-Kr.	15.4±0.5^{bc}	8.6±0.2^{efg}	10.1±0.4g^{h}
Cupressus macrocarpa	20.1±1.1^{a}	7.9±0.5^{e}	9.7±0.5^{fgh}
Cupressus torulosa	14.0±1.0^{c}	7.9±0.3^{de}	6.5±0.4^{de}
Cupressus arizonica	15.4±0.5^{bc}	5.6±0.2^{d}	5.1±0.2^{d}
Chamaecyparis lawsoniana	5.9±0.1^{d}	5.5±0.2^{d}	5.4±0.1^{d}
ANOVA Test [3]	***	***	***
Length of canker 20 days after inoculation			
Cupressus sempervirens-Thes.	28.4±1.1^{k}	17.3±0.7^{n}	16.3±0.9^{np}
Cupressus sempervirens-Kr.	24.5±0.5^{l}	12.4±0.4^{o}	16.6±0.4^{n}
Cupressus macrocarpa	35.0±1.1^{j}	18.8±0.6^{n}	17.7±1.0^{n}
Cupressus torulosa	27.1±1.5^{kl}	13.9±0.7^{op}	8.3±0.4^{m}
Cupressus arizonica	26.7±0.6^{kl}	13.6±0.8^{op}	8.5±0.5^{m}
Chamaecyparis lawsoniana	6.6±0.2^{m}	6.6±0.2^{m}	6.6±0.2^{m}
ANOVA Test [3]	***	***	***
Length of canker 30 days after inoculation			
Cupressus sempervirens-Thes.	35.2±1.0^{s}	26.7±1.0^{tv}	25.7±1.0^{v}
Cupressus sempervirens-Kr.	32.4±0.9^{s}	21.4±1.1^{w}	22.2±0.9^{w}
Cupressus macrocarpa	42.8±1.4^{r}	26.1±0.8^{v}	20.4±0.8^{w}
Cupressus torulosa	33.1±0.8^{s}	18.3±0.8^{x}	16.6±0.4^{xy}
Cupressus arizonica	29.3±0.7^{t}	16.5±0.5^{xy}	14.0±0.5^{yz}
Chamaecyparis lawsoniana	8.3±0.3^{u}	11.8±0.7^{z}	8.7±0.3^{u}
ANOVA Test [3]	***	***	***

[1]) Means followed by the same superscript letter (for the same recording period) do not differ significantly (LSD test, P=0.01).
[2]) Data presented are the means of 20 replicate inoculated plants ± standard errors.
[3]) Significant difference (P<0.001).

and 20 days after inoculation, after this time cankers induced by this pathogen were significantly longer (P<0.01) than those on of *S. cardinale*-inoculated plants.

C. macrocarpa and *C. sempervirens*-Thes. inoculated with *S. cupressi* had significantly longer (P<0.01) lesions at day 30 (26.1±0.8 mm and 26.7±1.0 mm, respectively) than did *C. sempervirens*-Kr., *C. torulosa* or *C. arizonica* (21.4±1.1 mm, 18.3±0.8 mm and 16.5±0.5 mm, respectively). Amongst the tested species, *Cham. lawsoniana* was the least susceptible (P<0.01) to *S. cupressi*, with canker lengths of 5.5±0.2 mm, 6.6±0.2 mm, and 11.8±0.7 mm at 10, 20 and 30 days, respectively.

Cankers produced by *S. unicorne* on the main stem of *Cupressus macrocarpa*, *C. sempervirens*-Thes., *C. sempervirens*-Kr., *C. torulosa* and *C. arizonica* increased significantly in size between day 10 and 30 (*Table 1*). On *Cham. lawsoniana* seedlings, however, the increase in canker size produced by *S. unicorne* between days 10 and 30 was much smaller (5.4±0.1 mm → 8.7±0.3 mm).

Cankers produced by *S. unicorne* and *S. cupressi* on the tested *Cupressus* species were significantly smaller (P<0.01) than those induced by *S. cardinale*. *S. unicorne* was less virulent (day 30: P<0.01) on *C. macrocarpa* (canker length 20.4±0.8 mm) than on *C. sempervirens*-Thes (canker length 25.7±1.0 mm). In comparison with other *Cupressus* spp., *C. torulosa* and *C. arizonica* were significantly less susceptible to *S. unicorne* (*Table 1*, P<0.01). Cankers induced by *S. unicorne* on *Cham. lawsoniana* (position 1) were almost equal in size (P>0.01) to those caused by *S. cardinale* on this host, but significantly smaller than those caused by *S. cupressi*, 30 days after inoculation (P<0.01).

Seedlings inoculated at the base of the main stem (Position 2)

Production of acervuli on *S. cardinale*-inoculated plants was observed 30 days after inoculation and was very common on all *Cupressus* species. Fifty days after inoculation, more than 50% of *C. macrocarpa* and *C. sempervirens* (Thes., Kr.) seedlings were girdled completely by *S. cardinale*, with wilting and discoloration of 30–70% of the crown observed on more than 50% of plants (data not presented). *S. cupressi* and *S. unicorne* caused less stem girdling (1/2 to 2/3 of the stem) on most plants. *S. unicorne* caused crown necrosis, with a distinctive colour change to bronze in most seedlings.

Cankers caused by *S. cardinale* increased in size markedly up to 40 days after inoculation, on all *Cupressus* species tested (*Table 2*). After 40 days, rates of increase in canker size declined for all *Cupressus* spp.. Seedlings of *Cham. lawsoniana* were more resistant to *S. cardinale* from the start of recording (20 days); an insignificant increase in canker length was recorded with time.

Seiridium cardinale produced significantly different canker sizes on the various host species tested (P<0.001), with *C. macrocarpa* the most susceptible (P<0.01) to this pathogen (*Table 2*). Seedlings of *C. sempervirens*-Thes., *C. sempervirens*-Kr. and *C. sempervirens*-Br. were also very susceptible to *S. cardinale*, and developed significantly longer cankers (P<0.01) than those on *C. sempervirens*-x. *Cham. lawsoniana* developed the smallest cankers among the tree species tested (P<0.01).

Progress of cankers caused by *S. cupressi* and *S. unicorne* on the main stems of tested seedlings is shown in *Table 3*. The length of the cankers caused by *S. cupressi* on *C. sempervirens* seedlings (Thes., Kr. and Br.) increased significantly from 20 to 40 days after inoculation (P<0.01). The length of the canker induced on *Cham. lawsoniana* by *S. cupressi* increased slightly up to 40 days, but no further development occurred before day 50. Cankers of approximately equal size (P>0.01) developed on seedlings of *C. sempervirens* (Thes., Kr. and Br.) in all recordings. On *Cham. lawsoniana*, however, cankers were significantly smaller (P<0.01) than those on *C. sempervirens*.

Cankers produced by *S. unicorne* on all *C. sempervirens* seedlings increased in size significantly up to 40 days after inoculation, but after this time, the rate of increase declined. On *Cham. lawsoniana*, the lengths of cankers caused by *S. unicorne* increased in size only slightly between 20 and 30 days after inoculation. After this time the size of the cankers increased significantly (P<0.01).

Lesions caused by *S. unicorne* on seedlings of all *C. sempervirens* seed sources were significantly larger (P<0.01) than those caused by *S. cupressi*, 20 and 30 days after inoculation. *S. unicorne* produced similar sized cankers to *S. cupressi* (P>0.01) on *Cham. lawsoniana* in 20 and 30 days after inoculation. After this time, however, *S. unicorne* cankers were markedly longer (P<0.01) than *S. cupressi* cankers on this host species. All *C. sempervirens* seed sources showed almost equal susceptibility (P>0.01) to *S. unicorne* in all recordings. Seedlings of *Cham. lawsoniana* inoculated with *S. unicorne* developed smaller (P<0.01) cankers compared to those of *C. sempervirens* up to day 40. By 50 days after inoculation, however, the size of canker on this host was not significantly different from those induced by *S. unicorne* on the three seed sources of *C. sempervirens*.

Table 2. – Lengths of cankers caused by *Seiridium* cardinale following inoculation 1.5-2.0 cm above the compost surface (position 2) of 18-month old *Cupressus sempervirens*-Br., *C. sempervirens*-Thes., *C. sempervirens*-Kr., *C. sempervirens-x, C. macrocarpa* and *Chamaecyparis lawsoniana* seedlings.

	Length of canker (mm) caused by *S. cardinale* [1, 2]			
	Days after inoculation			
Tree species	20 days	30 days	40 days	50 days
C. sempervirens -Br.	18.0±0.8 ^{abc}	25.1±0.9 ^{b}	31.0±1.0 ^{b}	33.0±0.6 ^{b}
C. sempervirens -Thes.	18.4±0.6 ^{ab}	23.7±0.6 ^{b}	30.4±0.9 ^{b}	32.1±1.0 ^{b}
C. sempervirens -Kr.	17.3±0.6 ^{bc}	23.8±0.8 ^{b}	28.4±0.8 ^{b}	31.1±0.8 ^{b}
C. sempervirens -x	15.8±0.5 ^{c}	20.3±0.6 ^{c}	23.4±0.6 ^{c}	25.2±0.5 ^{c}
C. macrocarpa	19.8±1.0 ^{a}	28.6±0.9 ^{a}	35.0±1.2 ^{a}	39.6±1.2 ^{a}
Cham. lawsoniana	6.1±0.2 ^{d}	6.8±0.2 ^{d}	7.3±0.2 ^{d}	7.8±0.3 ^{d}
ANOVA Test [3]	***	***	***	***

[1]) Means followed by the same superscript letter (in the same column) do not differ significantly (LSD test; P=0.01).
[2]) Data presented are the means of 20 replicate inoculated plants ± standard errors.
[3]) Significant difference (P<0.001).

Table 3. – Lengths of cankers caused by *S. cupressi* and *S. unicorne* following inoculation 1.5-2.0 cm above the compost surface (position 2) of 18-month-old seedlings of *Cupressus sempervirens*-Br., *C. sempervirens*-Thes., *C. sempervirens*-Kr. and *Chamaecyparis lawsoniana*.

	Fungus species	
Tree species	*S. cupressi*	*S. unicorne*
Length of canker (mm) 20 days after inoculation [1, 2]		
Cupressus sempervirens-Br.	6.9±0.3 [a]	13.3±0.6 [b]
Cupressus sempervirens-Thes.	6.8±0.4 [a]	12.0±0.6 [b]
Cupressus sempervirens-Kr.	6.9±0.2 [a]	12.4±0.7 [b]
Chamaecyparis lawsoniana	5.4±0.1 [a]	6.1±0.1 [a]
ANOVA Test [3]	***	***
Length of canker 30 days after inoculation		
Cupressus sempervirens-Br.	9.8±0.7 [ac]	15.6±0.7 [b]
Cupressus sempervirens-Thes.	9.9±0.7 [ac]	14.7±0.7 [b]
Cupressus sempervirens-Kr.	10.2±0.6 [a]	13.8±0.5 [b]
Chamaecyparis lawsoniana	6.6±0.4 [d]	7.7±0.3 [cd]
ANOVA Test [3]	***	***
Length of canker 40 days after inoculation		
Cupressus sempervirens-Br.	13.8±1.2 [ac]	18.2±0.9 [b]
Cupressus sempervirens-Thes.	14.2±1.1 [ac]	16.8±0.6 [ab]
Cupressus sempervirens-Kr.	13.8±1.1 [ac]	16.5±0.8 [ab]
Chamaecyparis lawsoniana	9.0±0.9 [d]	12.8±0.4 [c]
ANOVA Test [3]	***	***
Length of canker 50 days after inoculation		
Cupressus sempervirens-Br.	14.7±1.5 [ab]	18.4±0.9 [b]
Cupressus sempervirens-Thes.	14.4±1.5 [a]	18.1±0.9 [ab]
Cupressus sempervirens-Kr.	15.4±1.3 [ab]	17.8±0.9 [ab]
Chamaecyparis lawsoniana	9.0±0.7 [c]	16.5±0.7 [ab]
ANOVA Test [3]	***	***

[1]) Means followed by the same superscript letter (for the same recording period) do not differ significantly (LSD test; P=0.01).
[2]) Data presented are the means of 20 replicate inoculated plants ± standard errors.
[3]) Significant difference (P<0.001).

Inoculation of *C. sempervirens*-Kr. seedlings at position 1 with 7 different isolates of *S. cardinale* (1 and 3 to 8) induced similar sized cankers (P>0.01) at all recording times (*Table 4*). Isolate 2 produced significantly smaller cankers (P<0.01), however, than the other isolates. The cankers caused by isolates 1 and 3 to 8 increased in size significantly with time and most seedlings were girdled by the pathogens 40 days after inoculation, whereas the size of lesions induced by isolate 2 increased slightly from 10 to 30 days. Three months after inoculation with isolate 2, inoculation points were in the process of healing.

Discussion

Artificial inoculations of 18–20 month old cypress seedlings with *Seiridium* spp. demonstrated that, amongst the *Cupressus* species tested, *C. macrocarpa* was the most susceptible to *S.*

Table 4. – Lengths of cankers produced by eight isolates of *Seiridium cardinale* on the main stem of *Cupressus sempervirens*-Kr. seedlings following artificial inoculation 35 cm to 40 cm above the compost surface (position 1).

	Length of canker (mm)[1, 2]		
	Days after inoculation		
Isolate No. [3]	10 days	20 days	30 days
1	15.4±0.3 [a]	24.9±0.4 [a]	31.8±0.6 [a]
2	6.9±0.2 [b]	7.8±0.3 [b]	10.0±0.4 [b]
3	15.3±0.2 [a]	24.8±0.4 [a]	30.6±0.8 [a]
4	15.2±0.3 [a]	24.8±0.4 [a]	30.5±0.9 [a]
5	15.5±0.3 [a]	25.2±0.5 [a]	31.9±1.0 [a]
6	15.2±0.2 [a]	24.0±0.3 [a]	30.0±0.8 [a]
7	15.2±0.2 [a]	24.0±0.5 [a]	31.2±0.7 [a]
8	15.6±0.3 [a]	24.1±0.3 [a]	31.0±1.1 [a]
ANOVA Test [4]	***	***	***

[1]) Means followed by the same superscript letter (for the same column) do not differ significantly (LSD test; P=0.01).
[2]) Data presented are the means of 20 replicate inoculated plants ± standard errors.
[3]) Isolates were obtained from the Forest Research Institute in Athens.
[4]) Significant difference (P<0.001).

cardinale whereas *C. arizonica* was moderately susceptible. *Chamaecyparis lawsoniana* proved to be the least susceptible to *S. cardinale* among the tested tree species, developing the smallest cankers around points of inoculation.

These results, from inoculations on young glasshouse-grown plants, support the findings of many authors using older material. In field and glasshouse resistance tests using plants with stem diameters >8–10 mm, it has been found that *Cupressus macrocarpa* is the most susceptible species to *Seiridium* canker in the Cupressaceae (WIMBUSH, 1944; WAGENER, 1948; JONES, 1954b; GILMOUR, 1966; OLEMBO, 1969; STROUTS, 1973; BERESFORD and MULHOLLAND, 1982; CHOU, 1989). *C. sempervirens* is considered susceptible to moderately susceptible to *S. cardinale*, depending upon the seed source of the host (WAGENER, 1939; RADDI and PANCONESI, 1981; SMITH *et al.*, 1988). *C. torulosa* and *C. arizonica* are considered resistant or moderately susceptible to *Seiridium* attack depending upon variety and seed source (WAGENER, 1948; FULLER and NEWHOOK, 1954; NEWHOOK, 1962; GILMOUR, 1966; BERESFORD and MULHOLLAND, 1982; RADDI *et al.*, 1990; ANDREOLI and PONCHET, 1991). Similar responses were also reported by SMITH *et al.* (1988) who found that various degrees of susceptibility to *S. cardinale* are shown by different species of *Cupressus*, ranging from the highly susceptible *C. macrocarpa* and other North American species, through the moderately susceptible *C. sempervirens* to the relatively resistent northern Californian and Arizona cypresses, *C. bakeri* and *C. glabra. Chamaecyparis lawsoniana* has been reported to posses a high level of resistance to *S. cardinale* canker (WAGENER, 1939; STROUTS, 1973; VAN DER WERFF, 1988; CHOU, 1989), but it is susceptible to *S. unicorne* (FULLER and NEWHOOK, 1954; NEWHOOK, 1962; GILMOUR, 1966; CHOU, 1990) in agreement with the results of this study (inoculation at position 2).

Inoculations at the base of the main stem suggested that different seed sources of *C. sempervirens* express variability in resistance to *S. cardinale*. Seedlings of *Cupressus sempervirens*-Br., *C. sempervirens*-Thes., and *C. sempervirens*-Kr. were found to be more susceptible to *S. cardinale* than were seedlings of the hybrid between two *C. sempervirens* clones showing resistance to *S. cardinale*. In general, the virulence of all three *Seiridium* sp. was significantly lower at the base of the seedlings, compared with inoculations made closer to the crown in *Cham. lawsoniana* and *C. sempervirens* (*Table 1, 2* and *3*). It has been reported previously that disease development is faster in young than in mature tissues (VALDIVIESO and LUISI, 1987), in agreement with this finding. The reaction of the host may be more robust near the base of the stem, due to the thicker bark, higher amounts of lignified tissue, and increased concentrations of inhibitory secondary metabolites.

The pathogens *S. cupressi* and *S. unicorne* induced much smaller cankers than *S. cardinale* on all *Cupressus* species tested in this study. Cankers on *Cham. lawsoniana* caused by *S. cupressi* were significantly longer compared to those caused by *S. cardinale*. On *C. macrocarpa*, *C. torulosa* and *C. arizonica*, *S. unicorne* was less virulent, whereas on *C. sempervirens* (Br., Thes., Kr.) this pathogen caused slightly longer cankers than those resulting from *S. cupressi* inoculation. On *Cham. lawsoniana*, *S. unicorne* was more virulent than either *S. cardinale* or *S. cupressi* (*Table 2* and *3*).

The virulence of the tested *Seiridium* species is comparable to findings reported by other authors, with some differences possibly due to the different plant and fungal material utilised here, particularly the younger ages of the inoculated plants, and different methods used for recording symptoms. For example, BERESFORD and MULHOLLAND (1982) reported that *S. cardinale* caused more damage than *S. unicorne* on all cypress species tested. Artificial inoculation tests with isolates of *S. cardinale* and *S. unicorne* on *Cupressus macrocarpa* and *C. lusitanica* carried out by CHOU (1990) in New Zealand, showed that the symptoms caused by the two fungi were similar, but all isolates of *S. cardinale* were of higher virulence than those of *S. unicorne*. Stem inoculations with isolates of *S. cardinale*, *S. cupressi* and *S. unicorne* of various origins, reported by XENOPOULOS (1991) using 3-year-old plants of *C. sempervirens* under glasshouse conditions, demonstrated that all isolates of *S. unicorne* were weak parasites, inducing the development of small cankers which healed after six months. *S. cardinale*-induced cankers were much larger than those caused by *S. cupressi*, six months after inoculation, but cankers on plants inoculated with *S. cupressi* developed more quickly during the following year, when those of *S. cardinale* were in the process of healing. This delay in expression of virulence of *S. cupressi* (XENOPOULOS, 1991) requires further study.

S. cardinale is considered more virulent than *S. unicorne* and *S. cupressi* on *Cupressus* species, but the host range and variability in virulence reported for *S. unicorne* are wider than those of *S. cardinale* and *S. cupressi* (NATTRASS and CICCARONE, 1947; JONES, 1953, 1954a; SASAKI and KOBAYASHI, 1975; TISSERAT *et al.*, 1991; GRANITI, 1998). The wide host range and variability of *S. unicorne* may explain the higher virulence of this fungus on *Cham. lawsoniana.* In this work the isolate of *S. unicorne* (from Portugal) used was clearly a virulent strain since it produced active cankers on all cypress species tested.

No significant variations in virulence of *S. cardinale* isolates have been found by other authors (RADDI and PANCONESI, 1981, 1984; CHOU, 1990). However, in the inoculations reported here of *C. sempervirens*-Kr. with different isolates of *S. cardinale*, one isolate caused significantly smaller cankers on the tested seedlings, in comparison to the other isolates tested. All cankers caused by this isolate were healed by three months from inoculation. Thus, isolate 2 proved to be of low virulence on *C. sempervirens.*

Artificial inoculations of young seedlings (less than 2 years old) in the Cupressaceae proved to be useful in determining the relative virulence of the three *Seiridium* species, expressed as size of the canker induced by the pathogens on the main stem (30–50 days postinoculation). These results support the distinction between the 3 species, although it is necessary to use further isolates of each species in parallel trials to ensure reproducibility of results. The similar levels of virulence displayed by the seven (7) different isolates of *S. cardinale* suggest that the degree of disease caused by different isolates of this species may not vary greatly.

Inoculations of cypress seedlings with *Seiridium* spp. demonstrated significant variability in resistance related to maturation of the bark. Young bark tissue, close to the crown, is significantly more susceptible to *Seiridium* infection than the more developed bark found close to the base of the main stem. The reasons for this variability are unclear and require further research to determine possible biochemical mechanisms conferring resistance to *Seiridium* canker in species of *Cupressus*.

Conclusively for a routine screening test and good resistance results we recommend inoculations to be applied at position 2 and results recorded 30 to 50 days after inoculation.

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