Journal of Agricultural Extension and Rural Development Vol. 4(9), pp. 199-203, 14 May, 2012
Available online http:// academicjournals.org/JAERD
DOI: 10.5897/JAERD12.048
ISSN 2141-2154 ©2012 Academic Journals

Full Length Research Paper

Pathogenicity of some fungi isolated from cankers on *Cupressus sempervirens* var. *horizontalis* in Turkey

Lehtijärvi A.[1]*, Oskay F.[1], Doğmuş-Lehtijärvi H. T[1]. and Aday A. G.[2]

[1]Faculty of Forestry, Süleyman Demirel University, 32260 Isparta, Turkey.
[2]Yenişar Bademli Vocational School, Süleyman Demirel University, Isparta, Turkey.

Accepted 30 November, 2011

Natural stands of *Cupressus sempervirens* in Turkey are among the largest forests of this species in the world and are regarded as relicts of the centre of origin of var. *horizontalis*. In this study, we tested the pathogenicity of some of the most common fungal isolates originating from cankers on *C. sempervirens* by inoculating the isolates into the inner bark of *C. sempervirens* seedlings. The internal transcribed spacer (ITS) region of rDNA of the isolates was sequenced and compared with those in the GenBank. Among the isolates, eight ITS taxa were found. The isolates were inoculated into the inner bark of 2 year old *C. sempervirens* seedlings, on average 90 cm tall and 6 to 12 mm thick at the base. The seedlings were incubated seven weeks in a growth chamber at 70% mean relative humidity and 22.5°C mean temperature. The coaxial length of the lesion around the inoculation point on each seedling was measured. Among the eight ITS taxa *Pestalotiopsis funerea*, two other species of *Pestalotiopsis*, and two unidentified species belonging to the class Dothideomycetes caused lesions that were significantly larger than those in the controls while *Fusarium* sp., *Cytospora* sp. and an unidentified species belonging to Amphisphaeriaceae did not. In contrast to the *Pestalotiopsis* species, the two members of Dothideomycetes grew also into the sapwood of the seedlings.

Key words: *Cupressus sempervirens* var. *horizontalis*, canker, fungi.

INTRODUCTION

The natural stands of *Cupressus sempervirens* var. *horizontalis* (Mill.) Gordon in Turkey are considered among the most significant and largest natural Mediterranean cypress communities (Neyişçi, 1989; Özçelik, 2005), and regarded as relicts of the original source of *C. sempervirens* var. *horizontalis* due to the high diversity observed among the populations (Korol et al., 1997; Raddi and Sümer, 1999; Pichot et al., 1999). However, they constitute only 1392.5 ha of forests within Turkey, where more than 75% of the total is degraded (Anonymous, 2006).

In contrast to the many reports on phytopathological problems of Mediterranean cypress in areas where it has been introduced, information of the relict stands of *C. sempervirens* is available only for Greece and Cyprus (Xenopoulos and Diamandis, 1985; Tsopelas et al., 2007, 2008). The only exception is the study by Sümer (1987) reporting two pathogens in the Aegean coast of Turkey, *Seridium cardinale* (Wag.) Sutton and Gibson and *Pestalotiopsis funerea* (Desm.) Steyaert.

The aim of this study was to i) identify some of the most common fungal isolates originating from cankers on *C. sempervirens* var. *horizontalis* with the aid of the internal transcribed spacer (ITS) region sequences of their rDNA ii) to test the pathogenicity of these isolates by inoculating them into the inner bark of *C. sempervirens* seedlings.

MATERIALS AND METHODS

Fungal isolates

In a previous study, a total of 497 fungal isolates were obtained from cankers on *C. sempervirens* var. *horizontalis*. The trees were sampled during surveys in 2008 in two natural cypress stands locat-

*Corresponding author. E-mail: asko@orman.sdu.edu.tr.

Table 1. Identification and origin of the isolates used in the inoculations.

Isolate group	Isolate code	Origin of the isolate	Identification (Id)
PF	Pf-K1	Köprülü Kanyon, Antalya	*Pestalotiopsis funerea*
	Pf-K2	Köprülü Kanyon, Antalya	*Pestalotiopsis funerea*
	Pf-K3	Köprülü Kanyon, Antalya	*Pestalotiopsis* sp. 1
	Pf-K4	Köprülü Kanyon, Antalya	*Pestalotiopsis funerea*
	Pf-K5	Köprülü Kanyon, Antalya	*Pestalotiopsis* sp. 2
	Pf-K6	Köprülü Kanyon, Antalya	*Pestalotiopsis funerea*
SE	SE-K1	Köprülü Kanyon, Antalya	Mitosporic Amphisphaeriaceae
PO	Po-K1	Köprülü Kanyon, Antalya	Unidentified Dothideomycetes sp. 1
	Po-K2	Köprülü Kanyon, Antalya	Unidentified Dothideomycetes sp. 1
	Po-K6	Köprülü Kanyon, Antalya	Unidentified Dothideomycetes sp. 1
	Po-M1	Aydıncık, Mersin	Unidentified Dothideomycetes sp. 1
	Po-M2	Aydıncık, Mersin	Unidentified Dothideomycetes sp. 1
	Po-M3	Aydıncık, Mersin	Unidentified Dothideomycetes sp. 1
	Po-M4	Aydıncık, Mersin	Unidentified Dothideomycetes sp. 1
	Po-M5	Aydıncık, Mersin	Unidentified Dothideomycetes sp. 1
A	A-K1	Köprülü Kanyon, Antalya	Unidentified Dothideomycetes sp. 2
	A-M1	Aydıncık, Mersin	Unidentified Dothideomycetes sp. 2
CY	Cy-K1	Köprülü Kanyon, Antalya	*Cytospora* sp.
	Cy-M1	Aydıncık, Mersin	*Cytospora* sp.
FS	Fs-M1	Aydıncık, Mersin	*Fusarium* sp.

ed in the Köprülü Kanyon National Park, Antalya and in Aydıncık, Mersin, in the Mediterranean region of Turkey (Lehtijärvi et al., 2009). For the present study, 27 isolates, mainly representing the most common fungi isolated from the canker tissues were selected (Table 1).

DNA extraction

The isolates were cultured on cellophane membranes placed on either potato dextrose agar (PDA) with additional agar (20 g/l PDA, 20 g/l agar; Merck, Germany) or ground cypress needle amended PDA (CN-PDA; 20 g/l PDA, 20 g/l ground cypress needles). Cypress needles were used in order to stimulate the mycelial growth of some relatively slowly growing isolates (morphotypes PO and A). The cultures were incubated at 25°C until the mycelia had covered the cellophane membranes. The mycelia were harvested from the membranes and ground with mortar and pestle in liquid nitrogen. Immediately after grinding, the genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Amplification of the ITS region and DNA sequence analyses

Polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region (ITS1, 5·8S and ITS2) of the rRNA genes of the isolates and the sequencing of the PCR products in both directions was performed by a commercial laboratory (IonTek, Istanbul, Turkey) using the primer set ITS1 (5'–TCCGTAGGTGAACCTGCGG–3') and ITS4 (5'–TCCTCCGCTTATTGATATGC–3') (White et al., 1990). The sequences were determined using an ABI PRISM automated sequencer.

The sequences were compared with those in GenBank database using the BLAST algorithm and the putative taxa of the isolates determined. The sequences showing a similarity above 95% with the query sequence were considered. Additionally, another online BLAST program by UNITE analysis (http://unite.ut.ee/analysis.php; Kõljalg et al., 2005) was used for comparisons. This molecular database for identification of fungi, provides only sequences from the ITS region and allows to search in the International Nucleotide Sequence Database (INSD), which contains all GenBank, EMBL and DDJB data.

Inoculation experiments

Cypress (*C. sempervirens* var. *horizontalis*) seedlings, 1+1 years old, were obtained from the state forest nursery in Denizli, Turkey. The plants were raised from seeds collected from urban amenity trees within the Denizli province. The height of the seedlings ranged from 56 to 136 cm with a mean of 90.6 cm (±0.7 SD). The average ground level and inoculation point diameters were 8.7 (±0.7 SD; range from 6-12 mm) and 6.9 mm (±0.1 SD; 4-11 mm), respectively. The seedlings were placed into a growth chamber and kept under controlled conditions prior to inoculation and during incubation. The mean daily temperature and relative humidity during incubation were 22.5°C and 70.7%, respectively. The seedlings were irrigated with two days intervals.

Twenty-seven day old isolates grown on CN-PDA were used for inoculations (Table 1). The seedlings were inoculated 10 cm above root collar. A cork borer (4 mm diameter) was used to remove the bark and expose the cambium for inoculation and to obtain mycelial plugs from growing cultures. A mycelial plug was placed into each wound with the mycelium surface facing the xylem. After inoculation, the wound was covered with Parafilm® M (Alcan inc.), to prevent contamination and desiccation of the wound and the inoculum. Each isolate was inoculated onto ten seedlings. In addition, ten seedlings were inoculated with sterile CN-PDA to serve as controls.

After seven weeks, the seedlings were harvested, the outer bark around the inoculation point was removed with a sterile scalpel, and the lesion lengths on the seedlings were measured. Reisolations were made from three randomly selected replicate seedlings per isolate. Three small pieces of tissues containing both necrotic and healthy tissues were taken from the lesion edges and placed onto PDA petri plates.

The SPSS GLM procedure (SPSS Inc., Chicago, IL, USA) was used to analyse the data. Duncan's multiple range test was used to determine the differences among mean lesion lenghts. Correlations between lesion lenghts and seedling size was calculated using Pearson's product moment correlation coefficient test.

RESULTS AND DISCUSSION

Molecular identification

When compared with sequences in GenBank, the ITS sequences of the isolates belonging to the PF group, with two exceptions, most closely matched those of *P. funerea*. The ITS sequence similarity of PF-K1, PF-K2, PF-K4 and PF-K6 isolates with *P. funerea* was greater than 95%, and therefore determined to belong to that species. The isolate PF-K5, in contrast, had a similarity percentage lower than 95% with *P. funerea*, and therefore identified only to be a member of the genus *Pestalotiopsis*. The isolate PF-K3, in turn, had higher similarity with *P. yunnanensis* J.G. Wei and T. Xu than with *P. funerea*.

The SE-K1 isolate, with cultural characteristics similar to those of the PF isolates, matched also with Pestalotioid fungi. However, the range of matching genera was larger than for the PF isolates, including *Pestalotiopsis*, *Sarcostroma* and *Truncatella*. Therefore, the isolate could only be identified to the family level as a member of Amphisphaeriaceae.

A comparison of ITS sequences of the isolates in the PO group with sequence data in GenBank resulted in only matches with low similarity, such as *Spencermartinsia* sp., *Diplodia medicaginis* Golovin, *Neofusicoccum parvum* (Pennycook and Samuels) Crous, Slippers and A.J.L. Phillips, and *Botryosphaeria parva* Pennycook and Samuels, with 86 to 89% similarity. The best match, 93% sequence similarity with *Septoria pinithunbergii* S. Kaneko, was obtained with a BLAST search in UNITE database. As none of the matching ITS sequences showed similarity high enough, the identity of the PO group isolates could not be determined. Nevertheless, the PO isolates may represent a fungal taxon within the Pezizomycotina, class Dothideomycetes.

The A group isolates had 98% similarity with *Dothideomycetes* sp. However, the fungi in GenBank under the name Dothideomycetes sp. represent the fungal class Dothideomycetes, not a genus. The identity of the CY group isolates could be determined only to genus level as *Cytospora* sp., although they had high sequence similarity with *C. cedri* Syd., P. Syd. and E.J. Butler. Similarly, the single FS isolate was determined to be *Fusarium* sp. showing the highest sequence similarity with *F. equiseti* (Corda) Sacc. and *F. chlamydosporum* Wollenw. & Reinking.

In summary, the fungal isolates subjected to molecular identification were grouped into eight different taxa based on their ITS sequence, while grouping based only on the colony morphology resulted in six taxa. The PF group isolates were found to contain three *Pestalotiopsis* species: *P. funerea* and two unidentified ones.

Pathogenicity tests

All isolates used in the inoculation trial induced lesions on the *C. sempervirens* seedlings (Table 2). The mean lesion lengths ranged from 6.0 to 37.0 mm. Short lesions were formed also on the mock-inoculated control seedlings. The lesions in the inoculated seedlings resembled the cankers observed in the field. Furthermore, all fungal inoculations resulted in more or less necrotic needles around the inoculation point. Contrary to our field observations, no resin exudation was observed on the seedlings inoculated with any of the isolates tested.

Cankers resulting from inoculations with the PO (*Dothideomycetes* sp. 1) and A (*Dothideomycetes* sp. 2) isolates tended to have more distinct margins than those resulting from inoculations with the PF (*Pestalotiopsis* spp.), CY (*Cytospora* sp.), FS (*Fusarium* sp.) and SE-K1 isolates. Moreover, the PO isolates caused a remarkable discoloration in the sapwood in contrast to the PF isolates indicating a different ability to grow in sapwood.

The differences between the isolate groups in their ability to induce lesions on cypress seedlings were statistically significant ($P<0.01$). In general, the isolates in PO and the PF groups were virulent with mean lesion lengths of 17.2 (SE ± 0.5) and 17.1 (SE ± 0.8) mm, respectively. However, the isolates PF-K1, PF-K2 and PO-K3 from Köprülü Kanyon and PF-M1 from Mersin induced shorter lesions which did not differ statistically from those of the controls ($P<0.01$). Isolates belonging to all other groups (SE, FS, CY, A) were regarded as avirulent as they did not differ significantly in mean lesion length from the controls, with the exception of the A-M2 isolate.

Among the *Pestalotiopsis* species, the PF-K5 and PF-K6 isolates induced significantly longer lesions than the PF-K1, PF-K2, PF-K4, and PF-M1 isolates ($P<0.01$). The PF-K5 isolate, which was identified to be a species distinct from *P. funerea* within the genus *Pestalotiopsis*

Table 2. Mean lesion lengths produced by isolates.

Isolate group	ITS taxon	isolate	n	Mean lesion length (mm) ± SE	range (mm)
PF	*Pestalotiopsis* sp. 2	PF-K5	10	24.4 ± 2.4^{a}	13.0 – 37.0
	Pestalotiopsis funerea	PF-K6	10	21.8 ± 2.2^{abc}	10.0 – 36.0
	Pestalotiopsis sp. 1	PF-K3	10	18.6 ± 2.2^{abcd}	8.0 – 31.0
	Pestalotiopsis funerea	PF-K4	10	14.9 ± 1.1^{cdefg}	11.0 – 21.0
	Pestalotiopsis funerea	PF-K1	10	13.6 ± 1.5^{defgh}	8.0 – 23.0
	Pestalotiopsis funerea	PF-K2	10	13.2 ± 0.8^{defgh}	8.0 – 16.0
	Pestalotiopsis funerea	PF-M1	10	13.1 ± 2.0^{defgh}	9.0 – 28.0
SE	Mitosporic Amphisphaeriaceae	SE-K1	10	8.0 ± 0.4^{gh}	6.0 – 11.0
FS	*Fusarium* sp.	FS-M1	10	12.5 ± 1.0^{defgh}	8.0 – 18.0
CY	*Cytospora* sp.	CY-M1	10	9.5 ± 0.4^{efgh}	7.0 – 11.0
	Cytospora sp.	CY-K1	10	8.7 ± 0.2^{gh}	8.0 – 10.0
A	Unidentified Dothideomycetes sp. 2	A-M2	10	14.3 ± 1.7^{defg}	9.0 – 25.0
	Unidentified Dothideomycetes sp. 2	A-K3	10	10.5 ± 1.8^{efgh}	8.0 – 26.0
	Unidentified Dothideomycetes sp. 2	A-M1	10	10.0 ± 1.8^{efgh}	7.0 – 25.0
	Unidentified Dothideomycetes sp. 2	A-K2	10	9.0 ± 0.8^{fgh}	7.0 – 15.0
	Unidentified Dothideomycetes sp. 2	A-M3	10	8.6 ± 0.4^{gh}	7.0 – 11.0
	Unidentified Dothideomycetes sp. 2	A-K1	10	8.1 ± 0.4^{gh}	7.0 – 11.0
PO	Unidentified Dothideomycetes sp. 1	PO-M3	10	22.8 ± 1.2^{ab}	19.0 – 30.0
	Unidentified Dothideomycetes sp. 1	PO-K6	10	19.6 ± 1.4^{abcd}	13.0 – 25.0
	Unidentified Dothideomycetes sp. 1	PO-K4	10	19.3 ± 1.3^{abcd}	13.0 – 26.0
	Unidentified Dothideomycetes sp. 1	PO-M5	10	18.7 ± 1.3^{abcd}	11.0 – 24.0
	Unidentified Dothideomycetes sp. 1	PO-M4	10	18.3 ± 1.4^{abcd}	13.0 – 25.0
	Unidentified Dothideomycetes sp. 1	PO-M2	10	16.5 ± 2.0^{bcde}	6.0 – 28.0
	Unidentified Dothideomycetes sp. 1	PO-M1	10	16.0 ± 0.8^{bcdef}	13.0 – 22.0
	Unidentified Dothideomycetes sp. 1	PO-K1	10	15.2 ± 1.2^{cdefg}	11.0 – 25.0
	Unidentified Dothideomycetes sp. 1	PO-K2	10	14.1 ± 1.1^{defg}	10.0 – 21.0
	Unidentified Dothideomycetes sp. 1	PO-K3	10	11.0 ± 1.0^{efgh}	8.0 – 17.0
CONTROL			10	6.7 ± 0.3^{h}	5.0 – 8.0

Means are averages of N measurements and those followed by the same letter are not significantly different from each other at P < 0.01 significance level according to Duncan's multiple range test. ITS, Internal transcribed spacer.

based on its ITS sequence was found to be the most aggressive isolate among all isolates (24.4±2.4 mm). The PF-M1 isolate which was not subjected to molecular identification but accounted to be *P. funerea* based on its cultural and conidial similarities especially to those of PF-K6 produced the smallest lesions (13.1±2.0 mm).

Within each isolate group, the differences in mean lesion length between isolates originating from Mersin and Köprülü Kanyon were statistically insignificant. There was no correlation between the seedling size and lesion length. However, there was a negative correlation between the inoculation point diameter and lesion length produced by PF isolates ($r=0.302$, $p<0.05$).

Fusarium sp. could not be reisolated from the inoculated seedlings. However, the reisolation frequencies of the PO and A isolates were also lower than those of the PF isolates. Interestingly, *P. funerea* was isolated from nearly all seedlings, regardless of which fungal isolate they were inoculated. In addition, some fungal isolates resembling the A group isolates were isolated from seedlings which were inoculated with fungi other than the A isolates. This indicates that *P. funerea* could be an endophytic species occurring frequently in *C. sempervirens* (Panconesi et al., 1999; Santini and Di Lonardo, 2000). This fungal species is considered a weak pathogen of a wide range of conifer hosts including *Cupressus*, *Pinus*, *Juniperus* and *Thuja* spp (Madar et al., 1991; Sinclair et al., 1993; Santamaria et al., 2007). This species is endemic in Europe and also present in the native areas of cypress, and therefore considered to have co-evolved with *C. sempervirens.* Common moulds, such as *Alternaria* spp., *Cladosporium* spp., and *Penicillium* spp., were also isolated from the inoculated seedlings.

Conclusions

The most virulent isolates were found among the PF and PO groups. The PF group consists of three different *Pestalotiopsis* species. *Pestalotiopsis funerea* may belong to normal endophytic flora in the bark of *C. sempervirens* in natural stands without causing any signi-

ficant damage unless the trees are weakened (Panconesi et al., 1999; Santini and Di Lonardo, 2000). Drought stress could increase the susceptibility of the trees (Madar et al., 1991), but that was not tested in the present study. The PO group consists of isolates of unidentified Dothideomycetes sp. 1, which may be an opportunistic wound parasite.

ACKNOWLEDGEMENT

Financial support from the TUBITAK (Project No. TOVAG-108 O 287) is gratefully acknowledged.

REFERENCES

Anonymous (2006). Orman Varlığımız [Forest Asset]. T. C. Orman Bakanlığı, Orman Genel Müdürlüğü, Ankara.

Kõljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjoller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vralstad T, Ursing BM (2005). UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol. 166: 1063-1068

Korol L, Kara N, Işık K, Schiller G (1997). Genetic differentiation among and within natural and planted *Cupressus sempervirens* L. Eastern Mediterranean populations. Silvae Genet. 46: 151-155.

Lehtijärvi A, Doğmuş-Lehtijärvi HT, Oskay F, Aday AG (2009). Preliminary results of mycoflora associated with cankers on *Cupressus sempervirens* var. *horizontalis* (Mill.) Gordon in Turkey. In: Proceedings of the Conference of IUFRO Working Party 7.02.02. Süleyman Demirel University, Faculty of Forestry Journal Serial: A, Special Issue: 141-149.

Madar Z, Solel Z, Kimchi M (1991). *Pestalotiopsis* canker of cypress in Israel, Phytoparasitica 19: 79-81.

Neyişçi T (1989). Beşkonak Saf Servi (*Cupressus sempervirens* L.) Ormanında Ekolojik Araştırmalar [Ecological investigations in Beşkonak Cypress (*Cupressus sempervirens* L.) forests]. Ormancılık Araştırma Enstitüsü Yayınları, Teknik Raporlar Serisi 43: 49-76.

Özçelik R (2005). Aydıncık Yöresi Doğal Dallı Servi (*Cupressus sempervirens* L. var. *horizontalis* (Mill) Gord) Meşcerelerinde Homojenlik Durumunun ve Gövde Niteliklerinin Değerlendirilmesi [Evaluation of stand Homogeneity and Stem Quality in natural Mediterranean Cypress (*Cupressus sempervirens* L. var. *horizontalis* (Mill) Gord) Stands in Aydıncık District.]. Batı Akdeniz Ormancılık Araştırma Müdürlüğü Dergisi, Sayı 6: 29-42.

Panconesi A, Raddi P, Andréoli C, Ramos P, Xenopoulos S, Caetano F, Pinto-Ganhão J (1999). Diseases. In: Teissier du Cros E, Ducrey M, Barthélémy D, Pichot C, Giannini R, Raddi P, Roques A, Sales Luis J, Thibaut B (Eds.), Cypress. A Practical Handbook, Studio Leonardo, Florence: 54-73.

Pichot C, Xenopoulos S, Raddi P, Papageorgiou A, Fady B, Andréoli C, Panconesi A (1999). Choice of reproductive material. In: Teissier du Cros E, Ducrey M, Barthélémy D, Pichot C, Giannini R, Raddi P, Roques A, Sales Luis J, Thibaut B (Eds.), Cypress. A Practical Handbook. Studio Leonardo, Florence: 35-44.

Raddi S, Sümer S (1999). Genetic diversity in natural *Cupressus sempervirens* L. populations in Turkey. Biochem. Syst. Ecol. 27: 799-814.

Santamaría O, Bajo J, Pajares JA, Diez JJ (2007). Susceptibility of *Cupressus arizonica* and *Pinus pinea* to *Pestalotiopsis funerea* Isolates. Acta Silv. Lign. Hung., Spec. Edition, p. 283.

Santini A, Di Lonardo V (2000). Genetic variability of the 'bark canker resistance' character in several natural provenances of *Cupressus sempervirens*. For. Pathol. 30: 87-96.

Sinclair WA, Lyon HH, Johnson WT (1993). Diseases of Trees and Shrubs. New York: Cornell University Press.

Sümer S (1987). The distribution of cypress (*Cupressus* L.) in Turkey and the current status in its pests and diseases, especially cypress canker disease. Istanbul Univ. Orman Fakültesi Dergisi 37: 56-66.

Tsopelas P, Angelopoulos A, Nikolaou K (2008). *Seiridium cardinale* is a new threat to cypress trees in Cyprus. Plant Pathol. 57: 784.

Tsopelas P, Barnes I, Wingfield MJ, Xenopoulos S (2007). *Seiridium cardinale* on *Juniperus* species in Greece. For. Pathol. 37: 338–347.

White T, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds.) PCR Protocols, a Guide to Methods and Applications. Academic Press, New York.

Xenopoulos S, Diamandis S (1985). A Distribution map for *Seiridium cardinale* causing the cypress canker disease in Greece, Eur. J. For. Pathol. 15: 223-226.