Phytopathologia Mediterranea (2015) 54, 1, 86–93
DOI: 10.14601/Phytopathol_Mediterr-14998

RESEARCH PAPERS

Occurrence of *Eutypella microtheca* in grapevine cankers in Mexico

Marcos PAOLINELLI-ALFONSO, Candelario SERRANO-GOMEZ and Rufina HERNANDEZ-MARTINEZ

Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Km 107 Carretera Tijuana-Ensenada, 22860. Ensenada, Baja California, México

Summary. Trunk diseases cause slow decline and loss in productivity of grapevine. In surveys carried out from 2010 to 2012 in vineyards in Baja California (Mexico), fungal isolates were obtained from Cabernet Sauvignon grapevines with cankers. Morphological studies and phylogenetic analyses, using the internal transcribed spacer (ITS) region of the rDNA and a partial sequence of the β-tubulin gene, identified two isolates as *Eutypella microtheca*. Pathogenicity tests, using grapevine green tissue, fulfilled Koch´s postulates. To our knowledge, this is the first report of *E. microtheca* occurring on grapevine in Mexico and highlights the need of considering this fungus as another potential threat to vineyards in this region.

Key words: Diatrypaceae, trunk disease fungi, *Vitis vinifera.*

Introduction

Grapevine trunk diseases are responsible for economic losses globally (Bertsch *et al*., 2013). They are caused by fungal pathogens that invade the xylem of plants primarily through pruning wounds, leading to symptoms such as slow decline, and cankers. Cankers and dieback in grapevine were for a long time attributed to the diatrypaceous fungus *Eutypa lata* (Pers.) Tul. & C. Tul. [Syn.: *E. armeniacae* Hansf. & Carter] (Moller and Kasimatis, 1978). In recent years, several species in the Botryosphaeriaceae were isolated and their pathogenicity has been broadly documented (van Niekerk *et al*., 2004; Úrbez-Torres and Gubler, 2009; Úrbez-Torres *et al*., 2009). Currently, it is well accepted that fungi in the both Botryosphaeriaceae and Diatrypaceae can independently cause damage on potted grapevines (Bertsch *et al*., 2013; Pitt *et al*., 2013). Nevertheless, different species within and between these families are frequently isolated together from the cankers, suggesting that grapevine trunk diseases are caused by complexes of several pathogens (Úrbez-Torres *et al*., 2009).

Corresponding author: R. Hernandez-Martinez
E-mail: ruhernan@cicese.mx

In addition to *E. lata*, several Diatrypaceae species have been isolated from diseased wood. In California, the presence of *Eutypa leptoplaca* (Durieu & Mont.) Rappaz (Trouillas and Gubler 2004) and *Diatrypella* spp. has been reported (Rolshausen *et al*. 2006). In Michigan, the presence of *Eutypella vitis* (Schwein.) Ellis & Everh. [Syn.: *E. aequilinearis* (Schwein.) Starbäck] was reported in Concord grapevines (Catal *et al*. 2007). In Texas, *E. vitis* and *Diatrypella* sp. were found to be slightly pathogenic to grapevine (Úrbez-Torres *et al*. 2009). Isolates from California of *Diatrype stigma* (Hoffm.) Fr., *Diatrype whitmanensis* J.D. Rogers & Glawe, *Cryptosphaeria pullmanensis* Glawe and *Cryptovalsa ampelina* (Nitschke) Fuckel were shown to colonize dormant canes causing vascular necrosis (Trouillas and Gubler, 2010). In Australia, seven species of Diatrypaceae were found to be associated with grapevine, including *Cryptovalsa rabenhorstii* (Nitschke) Sacc., *C. ampelina*, *Diatrype* sp., *Eutypella citricola* Speg., *E. lata, Diatrypella vulgaris* Trouillas, W.M. Pitt & Gubler and *Eutypella microtheca* Trouillas, W.M. Pitt & Gubler (Pitt *et al*., 2010; Trouillas *et al*., 2011). In Spain, *Anthostoma decipiens* (DC.) Nitschke, *C. ampelina*, *E. citricola* and *E. microtheca* were reported on grapevine, in addition to *E. lata* (Luque *et*

ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm

al., 2012). Pathogenicity studies showed that *E. leptoplaca*, *E. citricola, E. microtheca, D. vulgaris, Diatrype sp.* and *C. ampelina* are pathogenic on potted grapevines (Pitt *et al*., 2013).

Classification of fungi in the Diatrypaceae has been mainly based on morphology. Characteristics of Diatrypaceae include perithecial ascomata embedded usually in black-colored stromata, long stalked asci and allantoid ascospores (Glawe and Rogers, 1984; Rappaz, 1987). However, taxonomic features often overlap complicating their classification. To overcome these conflicts, molecular markers have been used in recent years to more accurately classify members of the family (Acero et *al*., 2004, Trouillas *et al*., 2010a, 2011). In Mexico, *E. lata* has been the only member of the Diatrypaceae reported from grapevine (Téliz and Valle, 1979). The objective of the present study was to investigate the presence of Diatrypaceae in vineyards from Baja California (Mexico).

Materials and methods

Isolation of fungi and morphological characterization

Wood cankers from arms and cordons of *Vitis vinifera* cv. Cabernet Sauvignon plants approx. 10 years old, were collected from the main wine-producing region in Mexico, known as Valle de Guadalupe, and located in northwestern Baja California State. Wood was cut in several slices and surface-sterilized through a quick passage in 70% ethanol and flaming. These wood pieces were then cut in small fragments, placed on potato dextrose agar (PDA) amended with streptomycin (25 mg mL^{-1}) and incubated at 28°C for 20 d in darkness. Plates were observed daily and hyphal tips from developing colonies were transferred to fresh PDA plates and incubated in 12 h darkness 12 h fluorescent light cycles. Colony morphology was photographed using a digital camera, and microscopic features were observed using a stereomicroscope and an inverted light microscope.

Molecular identification

For the molecular analyses, a disc of fungal mycelium from each isolate grown for 3 days on a PDA plate was used to inoculate 20 mL of potato dextrose broth (PDB). Cultures were incubated at room temperature under continuous agitation at 200 rpm. After 5 d, mycelium was recovered using cheesecloth, and the genomic DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN Inc.), following manufacturer's instructions. PCR amplification of the internally transcribed spacer region of rDNA (ITS1-5.8S-ITS2) was achieved using the primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) based on the protocol of White *et al*., 1990. The PCR reaction contained a final concentration of 0.2 μM of each primer, 0.5 V of FailSafe 2X PreMix E (Epicentre®), 0.04 U μL^{-1} Taq DNA polymerase (Fermentas International Inc.) and 30 ng of DNA. The thermal cycler employed was a MJ Mini™ Gradient (BioRad®) set to the following programme: 95°C for 3 min; 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min; with a final cycle of 72°C for 10 min. A partial fragment of the β-tubulin gene, was PCR-amplified using primers T1 (AACATGCGTGAGATTGTAGTGACC (O'Donnell and Cigelnik, 1997) and Bt2b (ACCCTCAGTGTAGTGACC (Glass and Donaldson, 1995) at 0.1 μM each; 0.1 V of 10× buffer MasterAmp (Epicentre®); 200 μM of dNTPs mix (Epicentre®); 2.5 mM of $MgCl_2$ (Epicentre®); 0.04 U μL^{-1} of Taq polymerase (Epicentre®) and 30 ng of DNA as template. The PCR programme utilized was the same for ITS, but with an annealing temperature of 56°C. The amplified products were purified using the QIAquick PCR Purification Kit (Qiagen Inc.). Both strands of the ITS and β-tubulin regions were sequenced by Clemson University Genomics Institute. The sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999) to have only the regions with good sequencing quality and that showed consensus in both directions. A BLASTN analysis (Zhang *et al*., 2000) analysis (all GenBank+EMBL+DDBJ+PDB sequences but no EST, STS, GSS, environmental samples or phase 0, 1 or 2 HTGS sequences) was carried out using the consensus sequences of each gene. Sequences with the greatest similarity were retrieved from the GenBank database, and all taxa were aligned with ClustalW. Phylogenetic trees were constructed with MEGA5 software (Tamura *et al*., 2011). The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value.

To obtain the concatenated tree of b-TUB and ITS sequences, a BLASTN analysis was carried out independently for each molecular marker, and only the sequences of common strains were retrieved from the database. The independent files were fused and the resultant concatenated sequences were aligned with ClustalW, edited to remove any existing gaps and used to construct the phylogenetic tree in MEGA 5.10 using the Maximum Composite Likelihood approach.

Pathogenicity assay

Mycelium from of a 3-d-old PDA culture of *E. microtheca* was used as inoculum to perform pathogenicity assays, by inoculating five green shoots and five dormant canes of potted Cabernet Sauvignon plants. During the experiment, plants were kept in a greenhouse with temperatures ranging from 20-30°C. They were watered daily and fertilized with Hoagland solution (Hoagland and Arnon, 1950).

A circular wound (approx. 5 mm deep) was made in each shoot or cane using a razor blade, and a 5 mm agar plug covered with mycelium was placed in it. Each inoculated wound was covered with Parafilm® M. Plugs of non-colonized PDA were placed in wounds of control plants and a culture of *Lasiodiplodia theobromae* UC256Ma (Úrbez-Torres *et al*., 2006) was used for comparison. Evaluation on green tissue was done after 15 d, when necrotic lesions were observed; while for dormant cane tissue the evaluation was done 3 months later. Four pieces of tissue from at least 5 mm above and below the inoculation each site, with 1 cm spacings between pieces, were taken from the plant parts. The tissues were surface sterilized using alcohol and flaming, cut into small pieces, placed on PDA and incubated at 28°C.

Results

Field surveys and morphological characterization

From plants collected in Valle de Guadalupe in surveys done in 2012, two isolates were obtained from Cabernet-Sauvignon grapevine plants of around 10 years old exhibiting very poor shoot development, dead arms and wedge-shaped cankers. From the necrotic tissue cut and placed in small slices of PDA, after 10 d of observation, the only fungal colonies isolated from two different plants showed the typical white cottony mycelium reported for various Diatrypaceae spp. (Úrbez-Torres *et al*., 2009; Trouillas and Gubler, 2010). The isolates were named as BCMX01 and BCMX02. Both filled the plate in 4 days, showing white aerial mycelium that was profuse in BCMX01 and moderate in BCMX02. When colonies were exposed to light, they turned light pink or peach-coloured (Figure 1). This coloration has been reported as a characteristic to distinguish *E. microtheca* from the other members of Diatrypaceae (Trouillas *et al*., 2011). It has also been reported that *E. microtheca* produces pycnidia with age (Trouillas *et al*., 2010a). Only BCMX01, after 45 d of growth on PDA, produced irregular pycnidia from which cream-coloured masses of filiform conidia emanated (Figure 2A). The dimensions of conidia were (8.7–)11.2–11.6 × (0.3-) 0.4–0.5 μm, 10.2 ± 0.9 × 0.5 ± 0.1 μm (Minimum size, most repetitive value and maximum size, Mean ± SD for length and width of 30 conidia) (Figure 2B). With age, BCMX02 showed irregular gray to black spotting but no conidia were observed even after 45 d of growth.

Molecular identification

The consensus DNA sequences of BCMX01 and BCMX02 were deposited in the GenBank data-

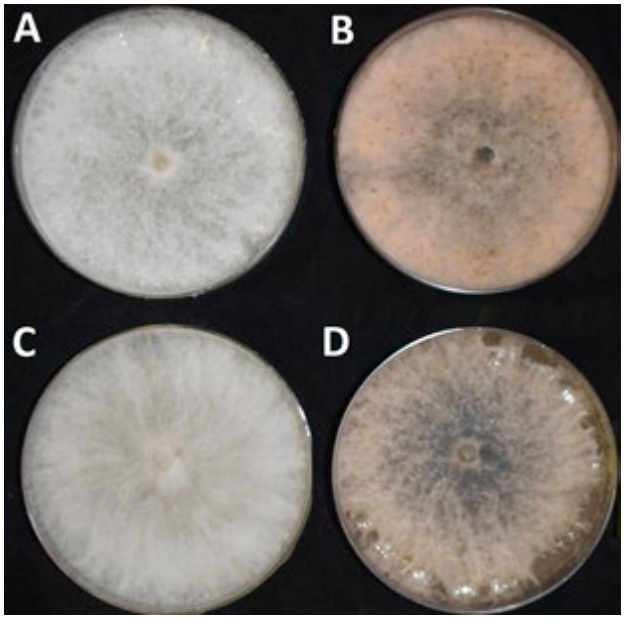

Figure 1. Isolates BCMX02 (A and B) and BCMX01 (C and D) of *Eutypella microtheca* grown on PDA at 28°C during 20 d in darkness (A and C), and with 12 h cycles of fluorescent light (B and D).

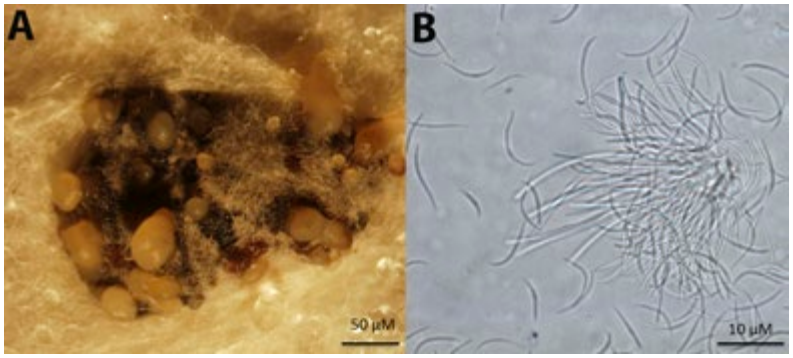

Figure 2. *Eutypella microtheca* pycnidia (A) and masses of conidia (B), grown on PDA at 28 °C, during 45 d with 12 h cycles of fluorescent light.

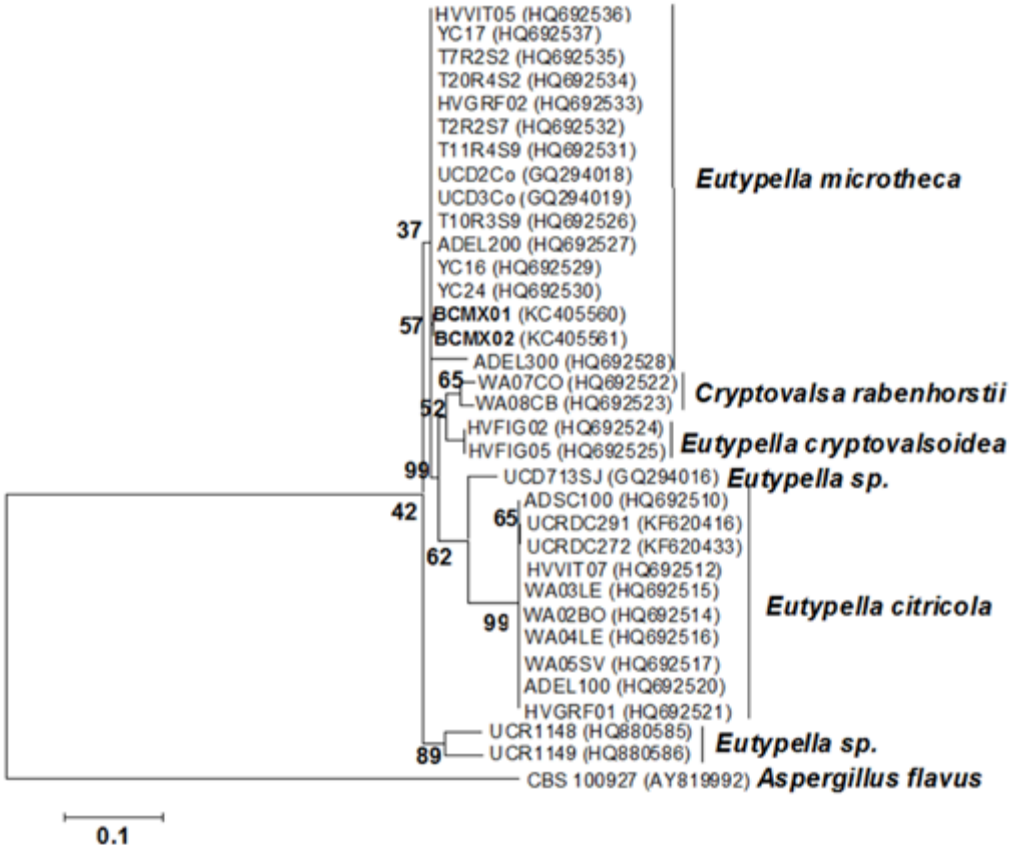

Figure 3. Phylogenetic tree with the highest log likelihood (-1414.0028) obtained from the b-tubulin-ITS concatenated dataset. Bootstrap values from 1000 replicates are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Species names are shown in bold and the accession numbers are shown between brackets.

base under the accession numbers KC405560 and KC405561 (b-tubulin), and KC405563 and KC405562 (ITS). BLASTN analyses of the ITS sequences of approx. 500 pb revealed 99 % identities with *Libertella* sp. R686I and *Libertella* sp. R699H (DQ286281, DQ286282; van Rensburg *et al*., 2006); *Eutypella* sp.

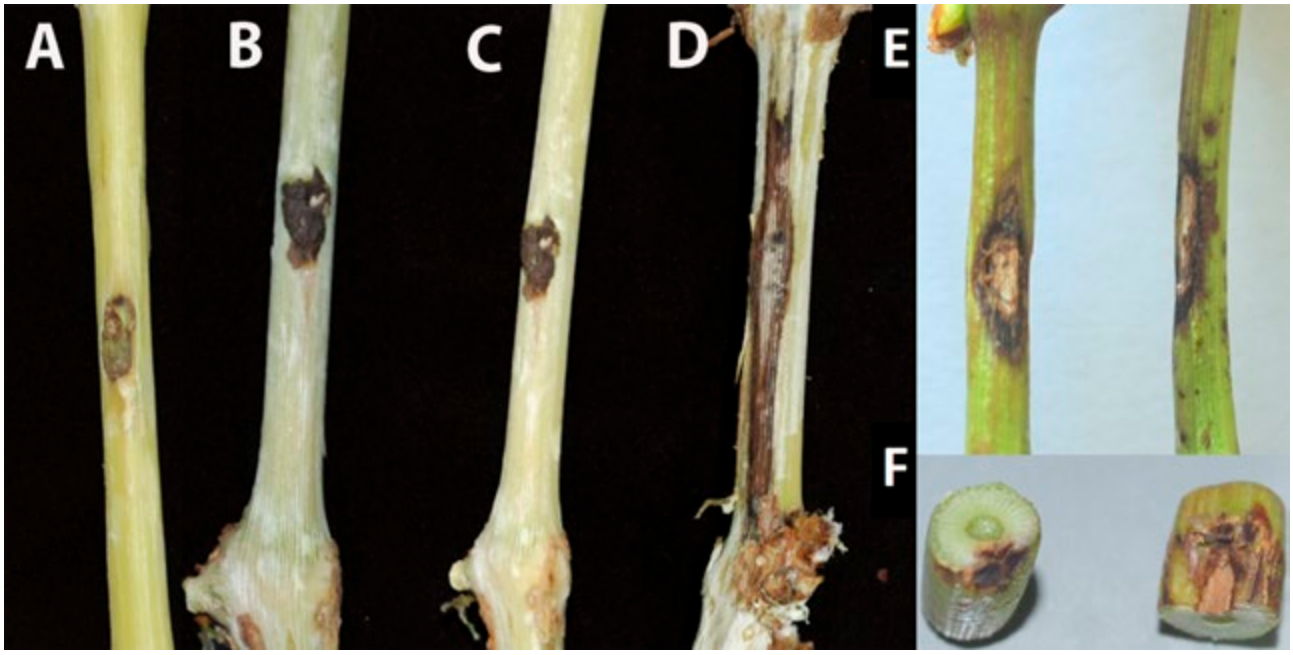

Figure 4. A-D: Grapevine dormant canes showing dark-brown lesions at 3 months after inoculation with: A, uncolonised plug of PDA as control; B, *Eutypella microtheca* isolate BCMX01; C, isolate BCMX02 and D, *Lasidiplodia theobromae* UCD-256Ma. E-F, green shoots of dormant canes showing necrotic tissue 15 d after inoculation with isolates BCMX01 and BCMX02.

UCR1149 (HQ880582; Adesemoye and Eskalen, 2011), UCD2Co (GQ293958; Trouillas *et al*., 2010a), and several isolates of *E. microtheca* (Trouillas *et al*., 2011; Luque *et al*., 2012) BLASTN analysis of the β-tubulin partial gene sequence for both isolates, showed 99% identity to *Eutypella* sp. FPT-4 strains UCD3Co (GQ293957, Trouillas *et al*., 2010a) and UCD2Co (GQ293958, Trouillas *et al*., 2010a); and to *E. microtheca* isolates YC17 (HQ692562; Trouillas *et al*., 2011), HVVIT05 (HQ692572; Trouillas *et al*., 2011), HVGRF02 (HQ692569; Trouillas et al., 2011), T11R4S9 (HQ692570; Trouillas *et al*., 2011), YC24 (HQ692565; Trouillas *et al*., 2011), YC16 (HQ692565; Trouillas *et al*., 2011), ADEL200 (HQ692559; Trouillas *et al*., 2011) and T10R3S9 (HQ692526; Trouillas *et al*., 2011).

The tree obtained using the ITS sequences showed a cluster made of the isolates BCMX01, BCMX02, *Libertella* sp. R686I and R699H and *Eutypella* sp. UCR1149 and UCR1148. This cluster has a bootstrap of 57% and emerged as a subgroup from the *E. microtheca* group (data not shown). In the tree obtained for the b-tubulin sequences both BCMX01 and BCMX02 isolates clustered together (bootstrap of 91%) with all the *E. microtheca* isolates (data not shown).

A phylogenetic tree constructed using the concatenated dataset of ITS and b-tubulin showed that BCMX01 and BCMX02 clustered with *E. microtheca* isolates, and clearly were differentiated from the *E. citricola*, *E. cryptovalsoidea* and *C. rabenhorstii*. Despite this clear association with *E. microtheca*, Mexican isolates showed a slight difference which is designated as a subcluster with a bootstrap value of 57% (Figure 3).

Pathogenicity

In the greenhouse assay, inoculated green shoots developed lesions up to 5 mm around the inoculation site (Figures 4E and 4F), but there were no symptoms observed in the leaves. Both isolates were recovered from the margins of the necrotic tissue of all inoculated shoots but not from the control plants. On dormant canes assessed 3 months after inoculation, necrotic tissue was visible around the inoculation points, slightly different than in the control plants,

in which the restored tissue looked greener (Figures 4A, 4B and 4C). Only BCMX02 was recovered at 7 mm away from the inoculation sites, from one of five inoculated dormant canes. The typical growth and characteristics of *E. microtheca* (Úrbez-Torres *et al*., 2009; Trouillas and Gubler, 2010) were observed from tissue pieces at 6 d after incubation at 28 ºC on PDA plates. No lesions were observed and nothing was recovered from the uninoculated control plants.

Discussion

Grapevine cankers are frequently found in Mexican vineyards; in Baja California they have been associated with Botryosphaeriaceae spp. (Úrbez-Torres *et al*., 2008, Candolfi-Arballo *et al*., 2010). In the present research, we isolated Diatrypaceae fungi from a symptomatic grapevine. The phylogenetic analysis, using ITS, showed 99% homology among these isolates, and with a *Libertella* sp. R686I and R699H, isolated from rooibos (*Aspalathus linearis*) by van Rensburg *et al*. (2006) as well as with *Eutypella* sp. UCR1149 and UCR1148 forming a subgroup. *Libertella* sp. is a common anamorph of diatrypaceous fungi, including *Eutypella*, although no teleomorphic state associated with this species was found on host material or induced in culture (van Rensburg *et al*., 2006). In contrast, in the phylogenetic tree obtained with β-tubulin sequences, BCMX01 and BCMX02 clustered with *E. microtheca* isolates (91 % of bootstrap value), but were different to UCR1149 and UCR1148, that clearly clustered in a different branch apart from the rest of the *E. microtheca* isolates. This was also noticed in the phylogenetic analysis using the concatenated datasets (Figure 3). Since it is well accepted that phylogenetic trees obtained from a concatenated datasets present greater resolution than trees obtained with the sequences of the molecular markers in an independent analysis (Gadagkar *et al*., 2005), Mexican isolates BCMX01 and BCMX02 belong to *E. microtheca*.

Ascomata typical of *E. microtheca*, were not observed either on pruning debris or in the collected plant. It has been suggested that the precipitation regime influences ascomata production in *E. lata*, since perithecia have been observed only in grape production zones were annual rainfall is greater than 350 mm (Carter, 1991, Trouillas and Gubler 2010). The annual rainfall in Valle de Guadalupe is approx. 300 mm (Ramírez and González, 2013), which could explain the absence of ascomata in the collected samples. Furthermore, it may take several years for ascomata to develop. For example in Spain only one of four *E. microtheca* isolates was obtained from ascomata pruning debris, indicating that perithecia are not commonly produced by this fungus on grapevine (Luque *et. al*., 2012).

According to Trouillas *et al*. (2011), colonies of *E. microtheca* are typically light pink, producing cottony mycelium that aggregates to produce pycnidia after 1 month of incubation in the dark at 25°C. Here, only the isolate BCMX02 produced conidia after 45 d of growth on PDA, and the sizes of the conidia were similar to that reported in previous studies (Trouillas *et al*., 2010a, Trouillas *et al*. 2011). In contrast to this description, using the same conditions, we were unable to find conidia in BCMX01. Similarly, in a survey done in California, Trouillas *et al*. (2010a) did not observe conidial production in *E. microtheca* (under *Eutypella* sp. UCD2Co and UCD3Co), reflecting that the asexual spores are not commonly produced in *E. microtheca* when cultured on PDA.

A recent publication reported that *E. microtheca* isolates from Australia had equivalent virulence to *E. lata*, after 9 months of incubation in the trunk of grapevines, and were only slightly less virulent at 18 months (Pitt *et al*., 2013). The pathogenicity assay in the present study showed that both isolates can cause necrosis on green tissue, but they did not cause major symptoms in dormant canes at 3 months after inoculation. The absence of lesions and the low recovery on grapevines below and above the inoculation points in dormant canes could be explained by the short time of the pathogenicity assay and the fact that the inoculation sites were excluded during the recovery of the fungus. Further experiments with incubation of least 9 months after inoculation are required to confirm the pathogenicity of the Mexican isolates, since the observation of this low virulence is not in accordance with the marked symptoms (canker) found in the plants from where BCMX01 and BCMX02 were isolated. Also, as occurs with *E. lata*, *Eutypella* pathogenicity may be triggered when host plants are under stress (Sosnowski, *et al*., 2011). To test this, an evaluation of the pathogenicity of *E. microtheca* in vines under stress is advised.

In the phylogenetic analyses (Figure 3), Mexican isolates clustered with several Californian isolates of *E. microtheca*, including the type strain. The geographical closeness of California with Baja Califor-

nia, and the fact that the cuttings were obtained from a Californian nursery could explain the similarities of the isolates at both sides of the border. This result highlights the importance of combining efforts with USA to deal with grapevine diseases in vineyards from the shared region.

Acknowledgements

Professor Douglas Gubler from UCDavis provided the isolate of *L. theobromae* UCD256Ma. This research was support by CONACYT Basic Science project grant 104356.

Literature cited

Acero F.J., V. González, J. Sánchez-Ballesteros, V. Rubio, J. Checa, G.F. Bills, O. Salazar, G. Platas and F. Peláez, 2004. Molecular phylogenetic studies on the Diatrypaceae based on rDNA-ITS sequences. *Mycologia* 96, 249–259.

Adesemoye A.O. and A. Eskalen, 2011. First Report of *Eutypella* spp. Associated with Branch Canker of Citrus in California. *Plant Disease* 95, 1187–1187.

Bertsch C., M. Ramírez-Suero, M. Magnin-Robert, P. Larignon, J. Chong, E. Abou-Mansour and F. Fontaine, 2013. Grapevine trunk diseases: complex and still poorly understood. *Plant Pathology* 62, 243–265.

Candolfi-Arballo O., C. Valenzuela-Solano, W.D. Gubler and R. Hernández-Martínez, 2010. Botryosphaeriaceae species associated with grapevine decline in Mexico. *Phytopathologia Mediterranea* 49, 105–106.

Carter M.V., 1991. The status of *Eutypa lata* as a pathogen. In: *Monograph Phytopathological Paper No 32*. Commonwealth Agricultural Bureau, International Mycological Institute, UK.

Catal M., S.A. Jordan, S.C Butterworth and A.M.C. Schilder, 2007. Detection of *Eutypa lata* and *Eutypella vitis* in grapevine by nested multiplex polymerase chain reaction. *Phytopathology* 97, 737–747.

Gadagkar S.R., M.S. Rosenberg and S. Kumar, 2005. Inferring species phylogenies from multiple genes: concatenated sequence tree versus consensus gene tree. *Journal of Experimental Zoology (Molecular and Developmental Evolution)* 304, 64–74.

Glass N.L. and G.C. Donaldson, 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61, 1323–1330.

Glawe D.A. and J.D. Rogers, 1984. Diatrypaceae in the Pacific Northwest. *Mycotaxon* 20, 401–460.

Hall T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series* 41, 95–98.

Hoagland D.R. and D.I. Arnon, 1950. *The water-culture method for growing plants without soil.* California Agricultural Experiment Station Circular 347. Berkeley, CA, USA, 32 pp.

Luque J., F. García-Figuéres, F.J. Legorburu, A. Muruamendiaraz, J. Armengol and F.P. Trouillas, 2012. Species of Diatrypaceae associated with grapevine trunk diseases in Eastern Spain. *Phytopathologia Mediterranea* 51, 528−540.

Moller W.J. and A.N. Kasimatis, 1978. Dieback of grapevine caused by *Eutypa armeniacae*. *Plant Disease Reporter* 62, 254–258.

O'Donnell K. and E. Cigelnik, 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7, 103–116.

Pitt W.M., R. Huang and F. Trouillas, 2010. Evidence that *Eutypa lata* and other diatrypaceous species occur in New South Wales vineyards. *Australasian Plant Pathology* 39, 97–106.

Pitt W.M., F.P. Trouillas, W.D. Gubler, S. Savocchia and M.R. Sosnowski, 2013. Pathogenicity of Diatrypaceous Fungi on Grapevines in Australia. *Plant Disease* 97, 749–756.

Ramírez J.G. and R.V. González, 2013. Modeling of the Water Table Level Response Due to Extraordinary Precipitation Events: The Case of the Guadalupe Valley Aquifer. *International Journal of Geosciences* 4, 950–958.

Rappaz F., 1987. Taxonomie et nomenclature des Diatrypacées à asques octosporées. *Mycologia Helvetica* 2, 285–648.

Rolshausen P.E., N.E. Mahoney, R.J. Molyneux, and W.D. Gubler, 2006. A Reassessment of the Species Concept in *Eutypa lata*, the Causal Agent of Eutypa Dieback of Grapevine. *Phytopathology* 96, 369–377.

Sosnowski M., J. Luque, A.P. Loschiavo, S. Martos, F. Garcia-Figueres, T.J. Wicks and E.S. Scott, 2011. Studies on the effect of water and temperature stress on grapevines inoculated with *Eutypa lata*. *Phytopathologia Mediterranea* 50, 127–138.

Tamura K. and M. Nei, 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10, 512-526.

Tamura K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.

Téliz D. and P. Valle, 1979. Eutypa dieback in Mexican vineyards. *Plant Disease Reporter* 63, 312–314.

Trouillas F.P. and W. D. Gubler, 2004. Identification and characterization of *Eutypa leptoplaca*, a new pathogen of grapevine in Northern California. *Mycological Research* 108, 1195-1204.

Trouillas F.P. and W.D. Gubler, 2010. Pathogenicity of Diatrypaceae Species in Grapevines in California. *Plant Disease* 94, 867–872.

Trouillas F.P., J.R. Úrbez-Torres and W.D. Gubler, 2010a. Diversity of diatrypaceous fungi associated with grapevine canker diseases in California. *Mycologia* 102, 319–336.

Trouillas F.P., M.R. Sosnowski and W.D. Gubler, 2010b. Two new species of Diatrypaceae from coastal wattle in Coorong National Park, South Australia. *Mycosphere* 1, 183–188.

Trouillas F.P., M. Wayne, M.R. Pitt, R.H. Sosnowski, F. Peduto,

A. Loschiavo, S. Savocchia, E.S., Scott and W.D. Gubler, 2011. Taxonomy and DNA phylogeny of Diatrypaceae associated with *Vitis vinifera* and other woody plants in Australia. *Fungal Diversity* 49, 203–223.

Úrbez-Torres J.R. and W.D. Gubler, 2009. Pathogenicity of Botryosphaeriaceae species isolated from grapevine cankers in California. *Plant Disease* 93, 584–592.

Úrbez-Torres J.R., G.M. Leavitt, T.M. Voegel and W.D. Gubler, 2006. Identification and distribution of Botryosphaeria spp. associated with grapevine cankers in California. *Plant Disease* 90, 1490–1503.

Úrbez-Torres J.R., G.M Leavitt, J.C. Guerrero, J. Guevara, and W.D. Gubler, 2008. Identification and Pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata*, the Causal Agents of Bot Canker Disease of Grapevines in Mexico. *Plant Disease* 92, 519–529.

Úrbez-Torres J.R., P. Adams, J. Kamas and W.D. Gubler, 2009. Identification, incidence, and pathogenicity of fungal species associated with grapevine dieback in Texas. *Vitis* 4, 497–507.

van Niekerk J.M., P.W. Crous, J.Z.E. Groenewald, P.H. Fourie, and F. Halleen, 2004. DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. *Mycologia* 96, 781–798.

van Rensburg J.C.J., S.C. Lamprecht, J.Z. Groenewald, L.A. Castlebury and P.W. Crous, 2006. Characterisation of *Phomopsis spp*. associated with dieback of rooibos (*Aspalathus linearis*) in South Africa. *Studies in mycology* 55, 65–74.

White T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: A Guide to Methods and Applications* (M. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White, ed.), Academic Press, San Diego, CA, USA, 315–322.

Zhang Z., S. Schwartz, L. Wagner and W. Miller, 2000. A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7, 203–214.

Accepted for publication: October 18, 2014
Published online: April 14, 2015