

## *Short Report*

# FIRST REPORT of *DIPLODIA SERIATA* FROM *ZELKOVA CARPINIFOLIA* WITH CANKER SYMPTOMS IN IRAN

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### Abstract

*Zelkova carpinifolia* is native to the Alborz mountains in Iran. It is a deciduous tree growing 20-35 m tall, with a trunk of up to 2 m in diameter. During the winter of 2011 cankers and dieback of branches were most commonly seen on zelkova trees in the Daland forest in Golestsn province. Cankers were enlarged and severely led to decline in March. Distinctive symptoms, assumed to be caused by a fungal infection, were observed in bark tissue of infected stems and branches trees.

Cankered twigs, branches and stem were noticeable when dieback occurred. When the bark was removed, the wood beneath the bark was discolored brown to reddish-brown instead of white. In some cases cankers appeared sunken and darkened and in some cases were surrounded and contained by callus wound wood, particularly on larger branches and trunks. The bark of the canker's area was peeled and dropped. To isolate the causal agent, surface-sterilized wood pieces of the infected tissue were plated on potato dextrose agar (PDA), and malt extract agar (MA). Hyphal tip isolates were obtained from these colonies. Isolates were cultured on half strength PDA and incubated at 25°C for determination of cultural characters. Pycnidial formation was stimulated by growing the isolates on 2% water agar that included pieces of autoclaved zelkova twigs and incubated at 25°C under daylight condition.

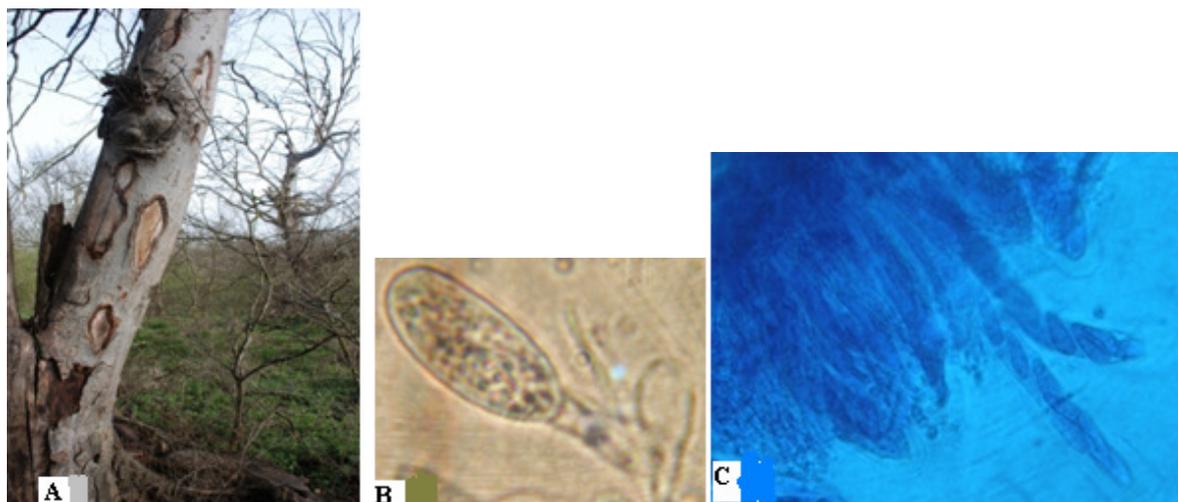
A representative isolate was deposited at the CBS-KNAW Fungal Biodiversity Centre, The CBS accession number assigned to the strain is CBS 134016.

For molecular identification, this isolate was subjected to amplification and sequencing of the internal transcribed spacer (ITS) regions of the nrRNA gene operon.

Pathogenicity tests were performed by inoculation of 2-year-old zelkova plants; two isolates were used and for every isolate four plants were wounded and mycelium plugs from actively-growing cultures on PDA were placed on the wounds and sealed with Para film. Two control plants were inoculated with sterile PDA plugs.

Fungal colonies on PDA were dark olive to black after 7-10 d. Pycnidia were globose, solitary, stromatic, up to 450µm diam. Conidiogenous cells were hyaline, thin-walled, smooth, cylindrical, producing a single conidium at the tip. Conidia were ovoid with truncated or rounded base and obtuse apex which were initially hyaline but became brown before they were released from the pycnidia and remained aseptate. On the basis of these morphological characters, the fungal species was identified as *Diplodia seriata* de Not. (Phillips *et al.* 2007).

The sexual morph *Botryosphaeria obtusa* (Schwein.) Shoemaker, was produced on the edge of the zelkova canker embedded within the cortical tissue, solitary, dark brown to black. Asci were clavate interspersed amongst filiform paraphyses, 8-spored, bitunicate. Ascospores were hyaline, unicellular, fusoid, wider around the mid region.



**Fig. 1.** symptoms of Zelkova cankers caused by *Diplodia seriata* (A), conidiogenous cell and conidium (B), asci and ascospores of *Botryosphaeria obtusa* were formed in Zelkova infected tissues(C).

The ITS sequence results indicated that the sequence generated in this study (GenBank KC461297) was identical over 569 nucleotides to *Diplodia seriata* (GenBank JQ659282, strain UCR1653) and over 574 nucleotides to *Botryosphaeria obtusa*, (Schwein.) Shoemaker (GenBank HQ629955, strain SDZ-02).

After 2 months past inoculation, brown necrosis was observed under the bark and *D. seriata* was constantly reisolated from the inoculated plants and thus fulfilled Koch's postulates and confirmed the pathogenicity of *D. seriata* on zelkova as causal agent of zelkova cankers. Control plants showed no symptoms of the disease.

*D. seriata* is an important pathogen has been recognized as the cause of branch and stem canker of several trees and shrubs (Phillips *et al.* 2007), also on at least 34 different hosts (Punithalingam and Walker 1973). To my knowledge; this is the first report of *Botryosphaeria obtusa* (Schwein.) Shoemaker, (*D. seriata*), as causal agent of *Zelkova carpinifolia*

canker in Iran. The disease could represent a serious threat for old zelkova trees throughout Alborz mountain forests. *Botryosphaeria* fungi are not host-specific but can cause disease on many plant species. The causal agent can thus be regarded as a cosmopolitan, plurivorous fungus and can colonize plant tissue through wounds, growth cracks, leaf scars, and lenticels. Many environmental stress factors, such as heat, drought, freeze injury, and compacted soil, can predispose trees and shrubs to infection and colonization by this agent.

#### Acknowledgements

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#### References

PHILLIPS, A.J.L., CROUS, P.W. AND ALVES. A. 2007. *Diplodia seriata*, the anamorph of "*Botryosphaeria*" *obtusa*. Fungal Diversity 25: 141-155.

PUNITHALINGAM E and WALKER JM. 1973. *Botryosphaeria obtusa*. Descriptions of pathogenic fungi and bacteria, No. 394. Commonwealth Mycological institute, Kew, Surrey, England.