Phytopathologia Mediterranea (2015) 54, 1, 128−132
DOI: 10.14601/Phytopathol_Mediterr-14686

NEW AND UNUSUAL DISEASE REPORT

Diplodia bulgarica, as a new pathogen and potential threat to the apple industry in Iran

Jafar ABDOLLAHZADEH

Department of Plant Protection, Faculty of Agriculture, University of Kurdistan, P.O. Box 416, Sanandaj, Iran

Summary. *Diplodia bulgarica* has been recently identified on apple trees in Bulgaria and Iran. This fungus was isolated from apple trees in the west of Iran, showing canker, gummosis, dieback and twig blight symptoms. To determine its significance as a pathogen on apple, Koch's postulates were fulfilled by stem inoculation of 2-year-old apple trees (cv. Golden Delicious) under greenhouse and field conditions. Results confirmed *D. bulgarica* as a highly virulent pathogen on apple under greenhouse and field conditions. This is the first record of *D. bulgarica* as a pathogen of apple, emphasizing the need to study biological and genetic aspects of this new pathogen.

Key words: apple, Botryosphaeriaceae, canker, dieback, gummosis, pathogenicity.

Introduction

Apple (*Malus domestica*), is an important economically horticultural crop cultivated throughout Iran. In 2011, apple production was ≈2.9 million t in Iran as the most important fruit crop after grapevine and citrus (http://www.maj.ir). Apple is damaged by several abiotic and biotic stresses. Amongst the fungal pathogens, Botryosphaeriaceae are common pathogens associated with various diseases on different woody plants around the world (Slippers and Wingfield, 2007; Phillips *et al.*, 2013). Recently, 17 genera have been recognized in this family including *Diplodia* (Phillips *et al.*, 2013). Species of *Diplodia* have been found in association with different disease symptoms such as canker, gummosis, fruit rot, dieback and twig blight (Lazzizera *et al.*, 2008; Phillips *et al.*, 2012; Abdollahzadeh *et al.*, 2013). Some *Diplodia* species, namely *D. corticola*, *D. mutila*, *D. pinea* and *D. seriata*, are well-known pathogens of woody plants (Phillips *et al.*, 2012). Five authentic *Diplodia* species have been characterized on apples (Phillips *et al.*, 2012; Farr and Rossman, 2014). *Diplodia mutila* and *Diplodia seriata* are two frequent species worldwide that cause black rot and canker of apple (Stevens, 1933; Laundon, 1973; Brown and Britton 1986; Brown-Rytlewski and McManus, 2000; Trapman *et al.*, 2008). Pathogenicity and virulence of these species have been considered on various trees including apple in different countries (Stevens, 1933; Laundon, 1973; Brown and Britton, 1986; Brown-Rytlewski and McManus, 2000; Úrbez-Torres, 2011).

In an extensive survey of the taxonomy and phylogeny of the Botryosphaeriaceae in Iran, seven isolates resembling Botryosphaeriaceae members were found on apple trees showing canker, gummosis, dieback and twig blight symptoms in Gahvareh, Kermanshah Province, West of Iran. In ISSR and rep-PCR fingerprinting profile analyses all of these isolates were grouped together (Abdollahzadeh, 2009; Zolfaghari, 2012). Recent phylogenetic analyses based on ITS and EF1-α confirmed these representative Iranian isolates, together with three isolates obtained from *M. sylvestris* in Bulgaria, as a new species named *Diplodia bulgarica* (Phillips *et al.*, 2012). Later, this pathogen was found again on apple in West Azarbaijan Province, Iran, and identified

Corresponding author: J. Abdollahzadeh
E-mail: J.abdollahzadeh@yahoo.com

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

as *Diplodia* sp. (Arzanlou and Bakhshi, 2012) and *D. bulgarica* (Ketabchi and Ghosta, 2013; Nourian *et al.*, 2013). The purpose of the present study was to evaluate the pathogenicity and virulence of *D. bulgarica* strains to *M. domestica* in Iran.

Materials and methods

Symptoms and fungal isolation

In October 2007 during a survey on the Botryosphaeriaceae species associated with woody plants, various symptoms including gummosis (Figure 1c-d), canker, dieback and twig blight were observed in seven investigated apple orchards of Gahvareh village in Kermanshah Province. Superficial or partially immersed pycnidia were found on the trunks and dead branches of trees (Figure 1a-b). Pycnidia contained ellipsoid to ovoid, hyaline and aseptate or dark brown and septate conidia. Seven isolates were obtained by transferring single conidia to potato-dextrose agar (PDA) supplemented with chloramphenicol (100 mg/L). Representative isolates were deposited at the Iranian Research Institute of Plant Protection (IRAN, Tehran, Iran).

Pathogenicity trials

Pathogenicity tests were conducted in 2012 (May to July) with two Iranian isolates (IRAN1532C,

Figure 1. Disease symptoms observed in orchards (a-d), greenhouse (e-i) and field trials (j-l). Vascular discoloration of infected shoots (m-n). Septate and aseptate conidia of *Diplodia bulgarica* (o), and a 2-wk old colony of the fungus on PDA (p). Bar = 10μm.

IRAN1548C) on 2-year-old apple trees (cv. Golden Delicious) under greenhouse (20–30°C, RH = 50–80%) and field conditions. To expose the cambium of test plants, wounds were made on the internodes with a 5 mm cork borer. Wounds were each inoculated with an agar plug from 5-d-old cultures and sealed with Parafilm to prevent desiccation. Sterile agar plugs instead of mycelium plugs were used as experimental controls. Three replicates were used for each isolate and the control treatment. The bark was removed and lesion lengths were recorded after 4 weeks in the greenhouse test, and 6 weeks under field conditions. To confirm Koch's postulates, the pathogen was recovered by transferring fragments from the margins of infected tissues to PDA.

Statistical analyses

The experiments were conducted based on randomized complete block designs with three replications both in greenhouse and field trials. After ANOVA, lesion length means were compared with Duncan's test (P = 0.05) using SPSS (ver. 18) software.

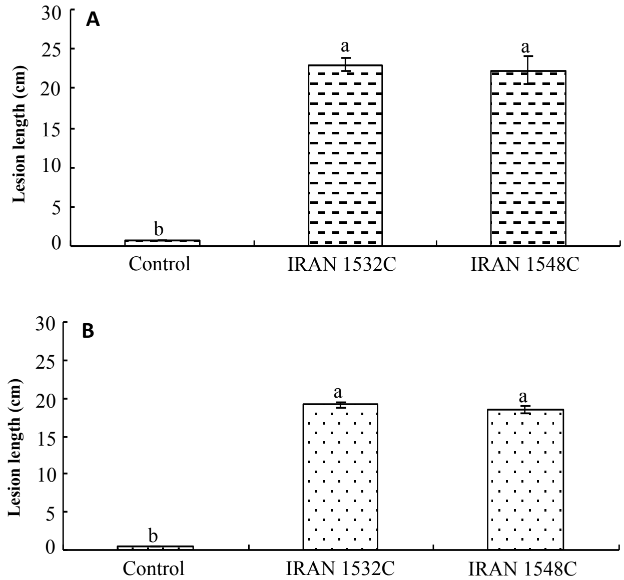

Figure 2. Mean lesion lengths (cm) on apple plants inoculated with *Diplodia bulgarica* under A) field and B) greenhouse conditions based on Duncan's test. Different letters show significant difference at P = 0.01. Bars represent standard errors of means (n = 3).

Results

Disease symptoms on apple trees including necrosis, gummosis, canker, vascular discoloration of the wood and dieback were observed (Figure 1e-n), and all inoculated trees were dead after 6 weeks under greenhouse and 8 weeks under field conditions. Brown necrotic lesions extended both upwards and downwards from the inoculation sites (Figure 1f-g, k-l). The mean lesion lengths were not significantly different (P>0.05) between isolates but showed significant differences (P<0.05) with the controls in both greenhouse and field trials (Table 1, Figure 2). Pycnidia formed abundantly on the bark of the necrotic lesions. To confirm Koch's postulates the pathogen was successfully re-isolated from the edge of the necrotic lesions (Figure 1p). Pycnidia formed on dead tissues were crushed and mounted in 100% lactic acid and conidia were observed with a microscope to confirm species identification (Figure 1o). Control plants remained symptomless and no fungi were re-isolated.

Table 1. Analysis of variance on the basis of randomized complete block design for lesion length measured in pathogenicity tests of *Diplodia bulgarica* on apple in greenhouse and field conditions.

Source of Variation	df	Mean Square	
		Greenhouse	Field
Replication	2	0.310^{ns}	3.640^{ns}
Strain	2	331.510^{**}	477.790^{**}
Error	4	0.535	0.940
CV%		5.74	6.31

** P< 0.01; ns P> 0.05.

Discussion

The results of this study revealed that *D. bulgarica* is a virulent pathogen to apple trees in Kermanshah Province, Iran. Moreover, pathogenicity tests conducted on detached shoots of healthy Golden Delicious apple and some other fruit trees under laboratory conditions confirmed pathogenicity and virulence of other *D. bulgarica* strains collected from West Azarbaijan Province (Arzanlou and Bakhshi, 2012;

Ketabchi and Ghosta, 2013; Nourian *et al.*, 2013). As discussed by Phillips *et al.* (2012), the pathogenicity and virulence of some *Diplodia* species is controversial. In the case of *D. seriata*, conflicting pathogenicity results have been obtained on various hosts in different geographic regions using different strains (Stevens, 1933; Laundon, 1973; Brown and Britton, 1986; Phillips, 1998, 2000; Brown-Rytlewski and McManus, 2000; Larignon *et al.*, 2001; van Niekerk *et al.*, 2004; Savocchia *et al.*, 2007; Epstein *et al.*, 2008; Laveau *et al.*, 2009). Our findings together with those obtained in other studies (Arzanlou and Bakhshi, 2012; Ketabchi and Ghosta, 2013; Nourian *et al.*, 2013) have confirmed *D. bulgarica* as a highly virulent pathogen of apple in Iran, thus representing a serious threat to the apple industry in this country. To have provide more information on pathogenicity, virulence and host range of *D. bulgarica*, further studies are urgently required with isolates from different geographic regions on all apple cultivars and other woody plants, especially under field condition. This is the first report regarding *D. bulgarica* as a pathogen of *Malus domestica* in greenhouse and field conditions.

Acknowledgements

This work was financed by the University of Kurdistan. Dr. M. Majdi, Department of Agronomy, University of Kurdistan gave advice on statistical analyses.

Literature cited

Abdollahzadeh J., 2009. *Taxonomy and phylogeny of the Botryosphaeriaceae in Iran*. PhD thesis, Tarbiat Modares University, Tehran, Iran, 200 pp.

Abdollahzadeh J., F. Hosseini and A. Javadi, 2013. New records from Botryosphaeriaceae for mycobiota of Iran. *Mycologia Iranica* 1, 34–41.

Arzanlou M. and M. Bakhshi, 2012. ITS-rDNA sequence differentiates a new lineage of *Diplodia* associated with canker disease of apple in Iran. *Plant Pathology and Quarantine* 2, 132–141.

Brown II E.A. and K.O. Britton, 1986. *Botryosphaeria* diseases of apple and peach in the southeastern United States. *Plant Disease* 70, 480–484.

Brown-Rytlewski D.E. and P.S. McManus, 2000. Virulence of *Botryosphaeria dothidea* and *Botryosphaeria obtusa* on apple and management of stem cankers with fungicides. *Plant Disease* 84, 1031–1037.

Epstein L., K. Sukhwinder and J.S. VanderGheynst, 2008. *Botryosphaeria*-related dieback and control investigated in noncoastal California grapevines. *California Agriculture* 62, 161–166.

Farr D.F. and A.Y. Rossman, 2014. *Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA*. Retrieved February, 2014, <nt.ars-grin. gov/fungaldatabases/>

Ketabchi M. and Y. Ghosta, 2013. Host range of *Diplodia bulgarica*, the causal agent of canker and bark gumosis of apple trees in West Azarbaijan Province. In: *Abstracts, 1th Iranian Mycological Congress*, September 3–5, 2013, University of Guilan, Rasht, Iran, 108 (abstract).

Larignon P., R. Fulchic, L. Cere and B. Dubos, 2001. Observation on black dead arm in French vineyards. *Phytopathologia Mediterranea* 40, S336–S342.

Laundon G.F., 1973. *Botryosphaeria obtusa*, *B. stevensii* and *Otthia spiraeae* in New Zealand. *Transactions of the British Mycological Society* 6, 369–374.

Laveau C., A. Letouze, G. Louvet, S. Bastien and L. Guerin-Dubrana, 2009. Differential aggressiveness of fungi implicated in esca and associated diseases of grapevine in France. *Phytopathologia Mediterranea* 48, 32–46.

Lazzizera C., S. Frisullo, A. Alves, J. Lopes and A.J.L. Phillips, 2008. Phylogeny and morphology of *Diplodia* species on olives in southern Italy and description of *Diplodia olivarum*. *Fungal Diversity* 31, 63–71.

Nourian A., N. Safaie and J. Abdollahzadeh, 2013. Report of *Diplodia bulgarica* and *Neoscytalidium dimidiatum*, the causal agents of apple canker in Western Azerbaijan and Isfahan provinces. In: *Abstracts, 1th Iranian Mycological Congress*, September 3–5, 2013, University of Guilan, Rasht, Iran, 119 (abstract).

Phillips A.J.L., 1998. *Botryosphaeria dothidea* and other fungi associated with excoriose and dieback of grapevines in Portugal. *Journal of Phytopathology* 146, 327–332.

Phillips A.J.L., 2000. Excoriose, cane blight and related diseases of grapevines: a taxonomic review of the pathogens. *Phytopathologia Mediterranea* 39, 341–356.

Phillips A.J.L., J. Lopes, J. Abdollahzadeh, S. Bobev and A. Alves, 2012. Resolving the *Diplodia* complex on apple and other Rosaceae hosts. *Persoonia* 29, 29–38.

Phillips A.J.L., A. Alves, J. Abdollahzadeh, B. Slippers, M.J. Wingfield, J.Z. Groenewald and P.W. Crous, 2013. The Botryosphaeriaceae: genera and species known from culture. *Studies in Mycology* 76, 51–167.

Savocchia S., C.C. Steel, B.J. Stodart and A. Somers, 2007. Pathogenicity of *Botryosphaeria* species isolated from declining grapevines in subtropical regions of Eastern Australia. *Vitis* 46, 27–32.

Slippers B. and M.J. Wingfield, 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* 21, 90–106.

Stevens N.E., 1933. Two apple black rot fungi in the United States. *Mycologia* 25, 536–548.

Trapman M., P. Maxin and R.W. Weber, 2008. *Diplodia seriata*, cause of black fruit rot in organically grown apples in Holland, Belgium and Northern Germany. In: *Abstracts, Ecofruit-13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing*, February 18–20, 2008, at Weinsberg, Germany, 177–181.

Urbez-Torres J.R., 2011. The status of Botryosphaeriaceae species infecting grapevines. *Phytopathologia Mediterranea* 50, S5-S45.

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

Zolfaghari S., 2012. *Differentiation of Botryosphaeriaceae species in Iran, using rep-PCR fingerprinting technique*. MSc thesis, University of Kurdistan, Sanandaj, Iran, 70 pp.

Accepted for publication: September 28, 2014
Published online: April 14, 2015