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EVALUATION OF SUSCEPTIBILITY OF SOME WOODY TREES AND GRAPEVINE CULTIVARS TO *Neofusicoccum parvum* AND *Diplodia seriata* ASSOCIATED WITH GRAPEVINE DECLINE IN IRAN

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Abstract

Ten grapevine cultivars include of "Yaghuti Siah", "Askari Zarghan", "Khalil Bavanat", "Bidane Ghermez Ghazvin", "Bidane Sefid Ghazvin", "Yaghuti Sefid", "Rish Baba", "Rotabi", "Siah Sisakht", "Siah" and 14 trees include of pistachio, almond, peach, apple, apricot, willow, sycamore, fig, service, elm, acacia, mulberry, ash and pomegranate were evaluated to infection by *Neofusicoccum parvum* and *Diplodia seriata* which were isolated from grapevines showing decline symptoms in Iran. Artificial inoculations were made on rooted cuttings of grapevine and trees branches in the greenhouse and field conditions respectively. Data were collected by recording the external symptoms and length of the resulting necrosis 4 months later. Based on the pathogenicity tests on grapevine cultivars both species gave the longest and the smallest lesions on "Yaghuti Siah" and "Siah" cultivars respectively. Based on the pathogenicity tests on the trees in the field, *N. parvum* and *D. seriata* gave the least lesions on pomegranate. In this regards *N. parvum* produced the longest lesions on pistachio while *D. seriata* caused the longest lesions on apple. Generally, this study indicated that the presence of trunk pathogens such as *D. seriata* and *N. parvum* in native vegetation and orchards planted in close proximity to vineyards, as an alternative hosts and potential source of inoculum, may affect the health of the vineyards.

Keywords: Botryosphaeriaceae, Pathogenicity test, Vine, Woody plants.

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Introduction

Botryosphaeriaceae Theiss. & P. Syd species are known to be very cosmopolitan, inhabiting the wood of many monocotyledonous, dicotyledonous and gymnosperm hosts (Wingfield *et al.* 2001; Van Niekerk *et al.* 2004; Burgess *et al.* 2005). The genus *Botryosphaeria* Ces. & De Not (Ascomycota, Dothideomycetidae, Dothideales, *Botyrosphaeriaceae*) was first introduced in 1863, with *Botryosphaeria dothidea* (Moug. Fr.) Ces. & De Not. as type species and includes of many species with a worldwide distribution (Crous *et al.* 2006). Botryosphaeriaceae members are major pathogens of grapevines worldwide (Taylor *et al*. 2005). In grapevine, *Botryosphaeria* spp. have often been regarded as weak pathogens (Phillips 2002; Van Niekerk *et al.* 2006) but many species have been shown to cause severe symptoms on hosts (Slippers and Wingfield 2007). Although the pathogenicity of Botryosphaeriaceae members on grapevines has not yet been fully clarified, different symptoms associated with the species has been reported on grapevine worldwide. Members of this family occur on a large number of hosts (Denman *et al*. 2000) but some well characterized species are clearly specialized on certain host genera or specific plant families in a defined area, e.g. *Saccharata protea* and *Neofusicoccum protearum* (Denman & Crous) Crous, Slippers & AJL Phillips, on Proteaceae (Denman *et al*. 2003), *Neofusicoccum eucalyptorum* (Crous, H. Smith & M.J. Wingf.) Crous, Slippers & AJL Phillips (Smith *et al*. 2001) and *Neofusicoccum eucalypticola* (Slippers Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, on Eucalyptus (Swart *et al*. 2000; Slippers *et al*. 2004) and *Diplodia pinea* (Desm.) J.J. Kickx and *Diplodia scrobiculata* J. de Wet, Slippers & M.J. Wingf. on *Pinus* (Burgess *et al*. 2004a,b). *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips is the dominant species infecting Eucalyptus, as well as 11 other native and non-native hosts in western Australia (Burgess *et al*. 2006). A specific host can also be infected by a diverse community of Botryosphaeriaceae, as has emerged in a recent South African study where eight species were isolated as endophytes of *Syzygium cordatum*, often co-occurring on the same plant (Pavlic *et al*. 2007). In Iran, *Diplodia seriata* De Not. has been reported on *Malus pomila* (Viennot-Bourgin *et al*. 1970) and also as *Sphaeropsis malorum* (Berk.) Berk from pine trees in Golestan province (East Gorgan forests) (Nasrollah-Nejad *et al*. 1998). During a survey in Iran two fungi with diplodia-like anamorphs namely *Barriopsis iraniana* Abdollahzadeh, Zare & A.J.L. Phillips and *Phaeobotryon cupressi* Abdollahzadeh, Zare & A.J.L. Phillips were isolated from various trees (Abdollahzadeh *et al*. 2009). Six species of *Lasiodiplodia* have been recently isolated and reported from a range of woody hosts in this country (Abdollahzadeh *et al*. 2010) but role of *Botryosphaeria* spp. on grapevines in Iran has remained largely unknown. Based on morphological and molecular studies, two species of *D. seriata* and *Neofusicuccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips were recently isolated and identified from grapevine, showing decline symptoms, in Iran (Mohammadi *et al*. 2013) but the susceptibility of grapevine cultivars and possibility of these fungi occurring on non-grapevine woody trees, has yet to be explored in this country. Thus, the objective of this study was to evaluate some grapevine cultivars, most widely planted in Iran, and non-grapevine woody trees susceptibility to *N. parvum* and *D. seriata* associated with grapevine decline.

Materials and methods

Plant material and fungal isolates

In this study 10 grapevine (*Vitis vinifera*) cultivars (Table 1) from vineyards at Shahid Bahonar University and 14 tree species close to these vineyards (Table 2) were evaluated to infection with *N. parvum* (IRN1) and *D. seriata* (IRB2). These species were isolated from grapevines exhibiting decline symptoms in Bavanat location of Fars province in 2006 and identified based on morphological and cultural characters along with molecular analysis [partial sequences of the nuclear ribosomal internal transcribed spacer (ITS), beta-tubulin (BT) and elongation factor 1-α (EF)]. They were kept in 15% glycerol solution at –20°C for long term preserving. Active cultures were obtained on potato dextrose agar (PDA, Merk, Germany, supplemented with 100 mg/L streptomycin sulfate) at 25°C for seven days prior to inoculation.

Table 1. Mean lesion length, re-isolation frequencies and symptoms caused by *Neofusicoccum parvum* and *Diplodia seriata* on rooted grapevine cuttings after 4 months.

Grapevine cultivars	Fungal isolates									
	Neofusicoccum parvum [a]					*Diplodia seriata* [b]				
	Mean lesion [c] length (mm)	Re-isolation [d] frequencies%	Pycnidium formation	Internal [e] Symptoms	External [f] Symptoms	Mean lesion [c] length (mm)	Re-isolation [d] frequencies%	Pycnidium formation	Internal [e] Symptoms	External Symptoms
Yaghuti Siah	36.80 **a**	100	+	TWS	PLD-LDO	22.40 **a**	60	+	TWS	—
Askari Zarghan	30.10 **b**	80	+	TWS, CWN	PLD -LDO	19.70 **b**	50	—	SNL	—
Bidane Ghermez Ghazvin	26.80 **b**	80	+	TWS, CWN	PLD	20.00 **b**	70	—	TWS	—
Khalil Bavanat	25.80 **b**	100	+	SWS	PLD	20.80 **ab**	50	+	SWS, CWN	—
Bidane Sefid Ghazvin	20.00 **c**	80	+	SWS	—	10.80 **d**	80	—	CWN	—
Yaghuti Sefid	18.70 **c**	80	+	SWS, SNL	PLD	13.30 **c**	60	—	CWN	—
Rish Baba	16.70 **cd**	100	+	SWS, CWN	—	10.90 **d**	60	—	TWS	—
Siah Sisakht	15.50 **cd**	100	+	SWS, CWN	—	14.70 **c**	70	—	TWS	—
Rotabi	13.10 **d**	100	—	SWS	PLD	9.70 **d**	60	—	SNL	—
Siah	5.90 **e**	40	—	SWS, SNL	—	5.70 **e**	20	—	SNL	—

a= IRN1 isolate, GenBank accession numbers include of GU121836, GU121891 and GU121863 for beta tubulin (BT), internal transcribed spacer (ITS) and elongation factor (EF1-α) respectively

b= IRB2 isolate, GenBank accession numbers include of GU121822, GU121877 and GU121849 for beta tubulin (BT), internal transcribed spacer (ITS) and elongation factor (EF1-α) respectively

c= Means followed by the same letter do not differ significantly at $P \leq 0.05$ according to Tukey's test

d= Number of samples from which the fungus was re–isolated out of 10 samples inoculated

e= SWS= Small wedge shape, CWN= Central wood necroses, TWS= Typical wedge shape, SNL= Small necrotic lesion

f= PLD= Petioles and leaves dieback, LDO= Leaves dried out.

Table 2. Mean lesion length, re-isolation frequencies and symptoms caused by *Neofusicoccum parvum* and *Diplodia seriata* on trees in the field condition after 4 months.

Trees	Fungal isolates									
	Neofusicoccum parvum					*Diplodia seriata*				
	Mean lesion [a] length (mm)	Re-isolation [b] frequencies%	Pycnidium formation	Internal [c] Symptoms	External Symptoms	Mean lesion [a] length (mm)	Re-isolation [b] frequencies%	Pycnidium formation	Internal [c] Symptoms	External Symptoms
Pistachio *Pistacia vera* cv. *Ohadi*	48.62 **a**	100	+	TWS, CWN	—	7.62 **b**	40	+	SWS, SNL	—
Almond *Prunus dulcis* cv. *Sahand*	22.25 **b**	70	—	TWS, CWN,	Brown gum exudation	7.37 **b**	20	+	CWN, TWS	Yellow gum exudation
Peach *Prunus persica* cv. *Elberta*	20.37 **bc**	90	—	TWS	Red and black gum exudation	4.75 **ef**	40	—	TWS, SNL	Red and black gum exudation
Apple *Malus domestic* acv. *Golab*	20.00 **bc**	100	+	WN	Red gum exudation , Leaf yellowing and dieback	11.87 **a**	90	+	SNL	Black gum exudation , Leaf yellowing and dieback,
Apricot *Peronus armenica* cv. *Shahroodi*	18.50 **cd**	90	+	TWS, SLN	Brown and black gum exudation	3.12 **g**	50	—	SNL	Red and black gum exudation
Willow *Salix babylonica*	16.75 **cde**	70	—	SWN, TWS	Black gum exudation	6.00 **cde**	50	—	SNL	—
Sycamore *Platanus orientalis*	15.87 **def**	100	—	SLN	Red gum exudation	2.75 **g**	80	—	SNL	—

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Fig *Ficus carica*	13.12 **efg**	40	+	SWN, SLN	—	6.37 **bcd**	30	—	SNL	—
Service *Eleagnus angustifolia*	13.12 **efg**	30	—	TWS, WN	—	5.37 **de**	60	—	SNL,	—
Elm *Ulmus campestre*	12.75 **fg**	100	—	SWS, SNL	Yellow gum exudation	6.75 **bc**	40	—	SNL	—
Acacia *Robinia pseudoacaria*	11.25 **g**	80	—	SWN, SLN	—	4.75 **ef**	90	—	SNL	—
Mulberry *Morus alba*	7.62 **h**	80	+	SWN, SLN	White gum exudation	3.50 **fg**	50	—	SWS, SNL	White gum exudation
Ash *Fraxinus excelsior*	4.62 **hi**	40	+	SLN	—	3.37 **g**	30	—	SWS	—
Pomegranate *Punica granatum* cv. *Malas*	2.37 **i**	20	—	SLN	—	2.50 **g**	10	—	SLN	—

a= Means followed by the same letter do not differ significantly at $P \leq 0.05$ according to Tukey's test
b= Number of samples from which the fungus was re–isolated out of 10 samples inoculated
c= SWS= Small wedge shape necroses, CWN= Central wood necroses, TWS= Typical wedge shape, SNL= Small necrotic lesion, WN= Wood necroses.

Pathogenicity studies

Inoculation of grapevine cultivars under greenhouse conditions

Pathogenicity test was conducted on rooted cuttings of 10 grapevine cultivars include of "Yaghuti Siah", "Askari Zarghan", "Khalil Bavanat", "Bidane Ghermez Ghazvin", "Bidane Sefid Ghazvin", "Yaghuti Sefid", "Rish Baba", "Rotabi", "Siah Sisakht" and "Siah" which were cut from healthy mature grapevines in some vineyards at Shahid Bahonar University, Kerman, Iran. In all, 150 cuttings of approximately 30 cm in length and 8-10 mm in diameter from each cultivar were cut from vines showing neither any foliar symptoms nor any wood deterioration. In order to rooting, cuttings were planted into sterilized sand and maintained at room temperature. After rooting, cuttings were surface sterilized with 70 % ethanol, wounded at the uppermost internode with a 4-mm cork borer and a 4 mm diameter inoculum plug of a 7-d-old isolate on PDA was then placed into each hole and was covered with Parafilm®. Five cuttings per fungal isolate were used for each cultivar. Five cuttings of each cultivar were inoculated with 4-mm noncolonized PDA agar plugs as negative control. Inoculated cuttings were planted in individual pots and maintained in a glasshouse at approximately 25°C. Plants were arranged in a completely randomized design. After four months, the inoculated shoots were collected, sectioned longitudinally and extent of vascular discoloration was measured upward and downward from the point of inoculation. Wood pieces (5×5 mm) from the margin between necrotic and apparently healthy tissue of each lesion were surface sterilized (1 min in 5% NaOCl) and placed on potato-dextrose agar in an attempt to recover the inoculated fungus and complete Koch's postulates. Fungal identity was verified by its colony and conidial morphology.

Inoculation of trees under field conditions

In addition a pathogenicity test was conducted under field conditions on 14 trees include of pistachio, almond, peach, apple, apricot, willow, sycamore, fig, service, elm, acacia, mulberry, ash and pomegranate (Table 2). Plants showing neither any foliar symptoms nor any wood deterioration were selected and inoculated in April 2011. For each isolate, 4 branches in each tree were randomly chosen and the outer bark at the inoculation area cleaned and sprayed with 70% ethanol. Inoculation was made as described above by placing a 4 mm diameter mycelial plug into artificial wounds (1 cm deep holes drilled radially with an ethanol-disinfected borer into a branch). Four branches were similarly treated but received a 4 mm diameter plug of PDA as control plants. After four months, inoculated branches were collected and immediately taken to the laboratory. Inoculated branches were split lengthwise through the inoculation site to reveal the xylem and wood regions for lesion measurement. Fungal re–isolations were made as previously described.

Statistical analyses

One-way analyses of variance (ANOVA) in SAS (SAS System, version 9.1; SAS Institute) was performed in order to assess differences in the extent of vascular discoloration induced by *N. parvum* and *D. seriata* for pathogenicity tests in grapevine cultivars and trees tested. The Tukey's test was used for comparison of treatment means at $P < 0.05$. A correlation between lesion length and re-isolation frequency was determined by calculating the correlation coefficient for every treatment to determine interaction between the lesion lengths and establishment of isolates within the host.

Results

Grapevine cultivars inoculation

Mean lengths of the extent of vascular discolorations caused by *N. parvum* and *D. seriata* isolates on inoculated rooted grapevine cuttings are shown in Table 1 and Fig. 2. There were significant differences in the mean lesion lengths produced by the botryosphaeriaceous species isolates and control ($P < 0.0001$). Based on the analysis of variance there were significant differences in the pathogenicity of *N. parvum* and *D. seriata* isolates (F=815.52; $P< 0.0001$), susceptibility of cultivars (F=65.69; $P< 0.0001$) and interaction between variety and pathogen species (F= 21.05; $P< 0.0001$, ANOVA not shown). *N. parvum* and *D. seriata* isolates used in this study were pathogenic and caused longer basipetal than acropetal lesions in all treatments (Fig. 2). Mean lesion lengths for *N. parvum*, *D. seriata* and the control were 39.20, frequency of 40% to 100% and 20% to 80

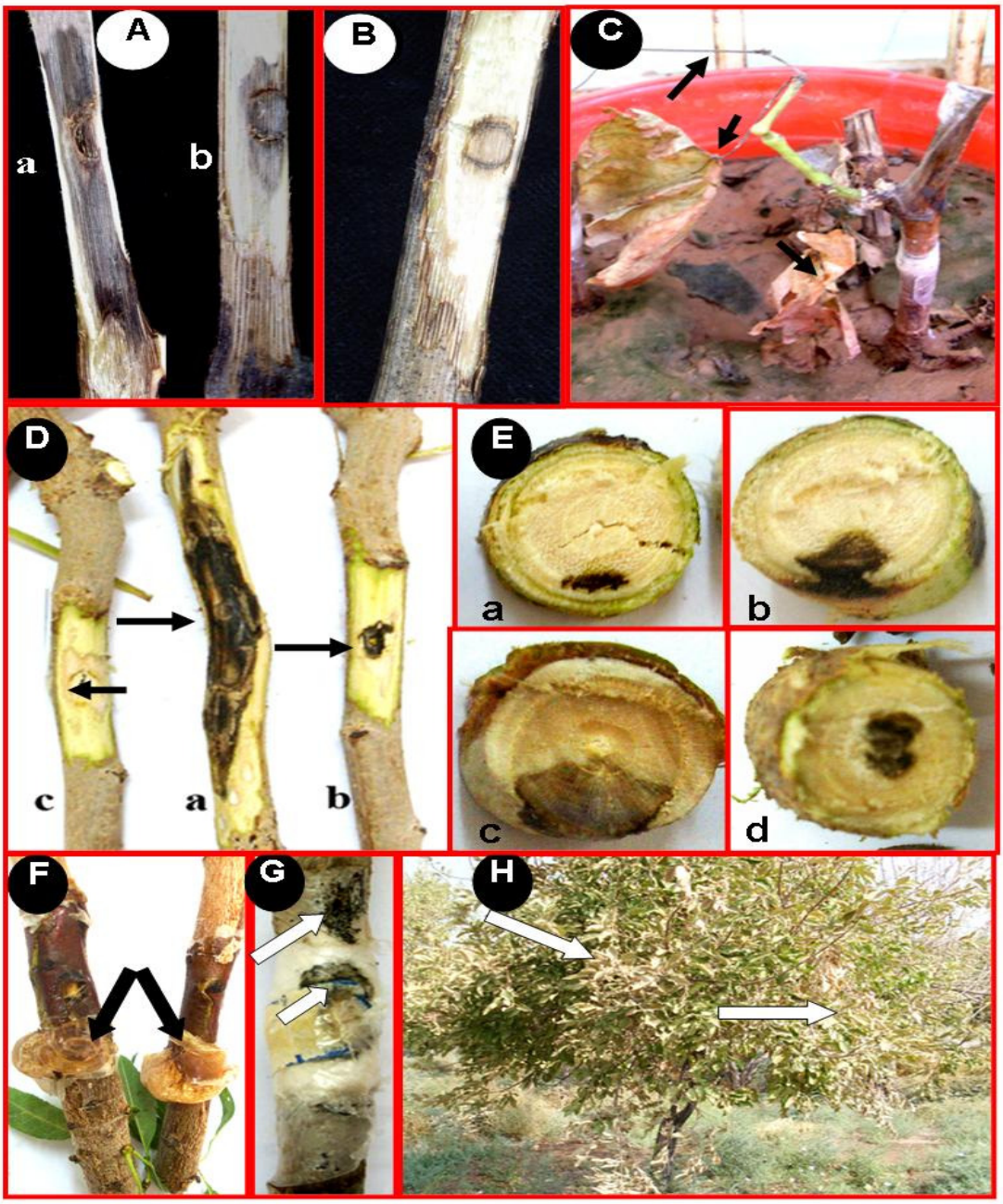

Fig. 1. Pathogenicity of *Neofusicoccum parvum and Diplodia seriata* on inoculated grapevines and trees. A: Rooted cuttings of "Yaghuti Siah" cultivar, 4 months after inoculation with *N. parvum* (a), *D. seriata* (b) compared with control (B)- C-Petioles and leaves dieback symptoms caused by *N. parvum* on rooted cuttings of "Yaghuti Siah" cultivar, 4 months after inoculation. D- Lesions caused by *N. parvum* (a) and *D. seriata* (b) on pistachio twigs 4 months after inoculation under field conditions (c= control), arrows indicate points of inoculation, E-Cross sections of inoculated twigs of trees 4 months after inoculation under field conditions, a- Small necrotic lesion caused by *D. seriata* on willow, b- Small wedge shape necrosis caused by *D. seriata* on acaria, c- Typical wedge shape necrosis caused by *N. parvum* on apricot d- Central wood necrosis caused by *N. parvum* on acaria. F- Most copious gummosis on peach twigs (showed by black arrows) caused by *N. parvum* 4 months after inoculation under field conditions. G- Pycnidia of *N. parvum* formed on mulberry twigs 4 months after inoculation (showed by white arrows). H-External symptoms as leaf yellowing on a 20-year-old apple tree inoculated by *D. seriata* 4 months after inoculation.

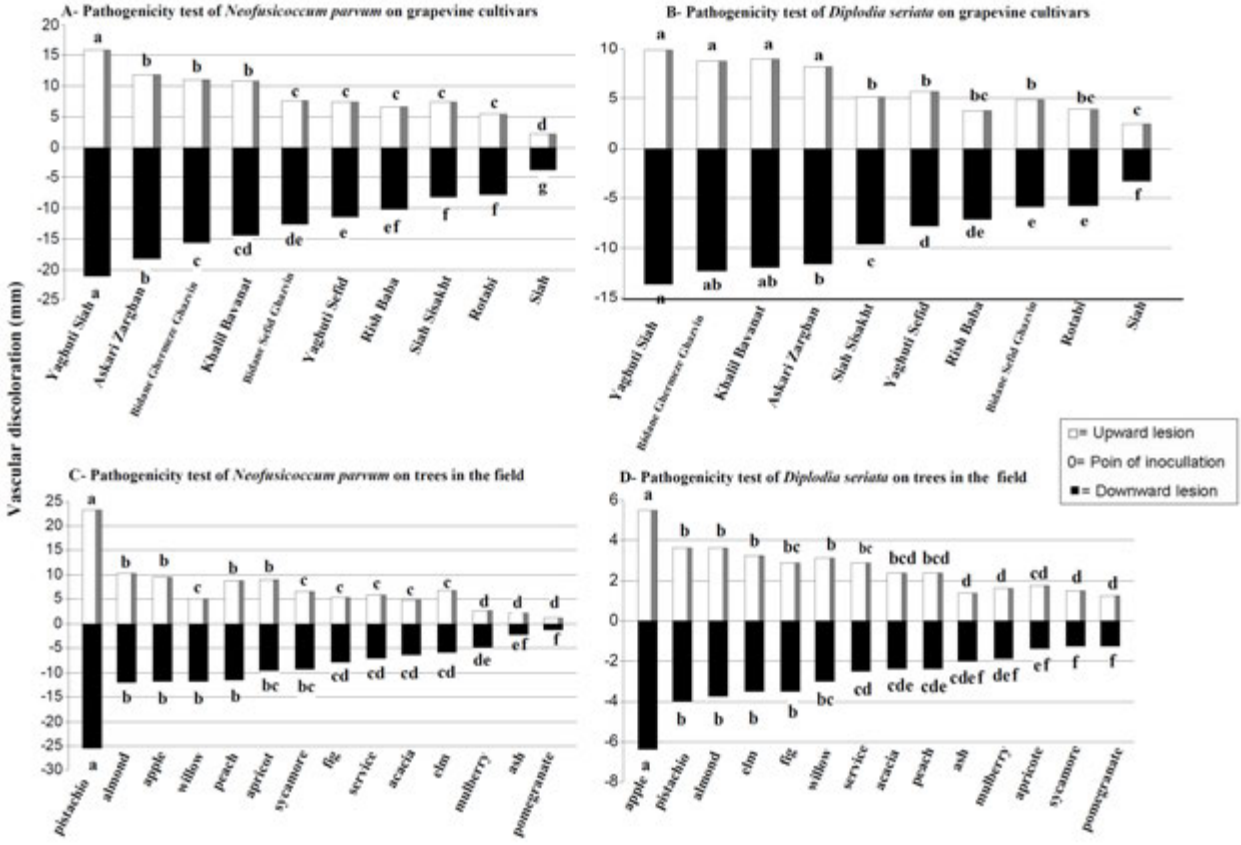

Fig. 2. Pathogenicity study of *Neofusicoccum parvum* and *Diplodia seriata* in grapevine rooted cuttings under greenhouse (A and B) and trees in the field (C and D) conditions. Mean lesion length is based on 5 and 4 replicates per isolate for grapevine rooted cuttings and trees respectively. Means followed by different letters differ significantly ($P < 0.05$) according to Tukey's test.

26.96 and 2.68 mm respectively. *D. seriata* produced smaller lesions than those caused by *N. parvum* in all inoculated rooted cuttings but still differed significantly from the control. Different internal symptoms include of typical wedge shape necrosis, small wedge shape necrosis, central wood necrosis and small necrotic lesion produced by both species were observed when cross sections were made in shoots. "Yaghuti Siah", "Askari Zarghan", "Bidane Ghermez Ghazvin", "Khalil Bavanat", "Yaghuti Sefid" and "Rotabi" cuttings inoculated with *N. parvum* showed petioles, and leaves died back and sometimes dried out (Fig.1-C) while all rooted cuttings grapevine inoculated with *D. seriata* isolates developed healthy without any foliar symptoms which did not differ from the control. *N. parvum* produced pycnidia on shoot surfaces of all cultivars except of "Rotabi" and "Siah" while *D. seriata* isolate only produced pycnidia on shoot surfaces in "Yaghuti Siah" and "Khalil Bavanat" cultivars. *N. parvum* caused the longest lesions on "Yaghuti Siah", followed by "Askari Zarghan", "Bidane Ghermez Ghazvin", "Khalil Bavanat", "Bidane Sefid Ghazvin", "Yaghuti Sefid", "Rish Baba", "Rotabi", "Siah Sisakht" and "Siah". *D. seriata* caused the longest lesions on "Yaghuti Siah" followed by "Bidane Ghermez Ghazvin", "Khalil Bavanat", "Askari Zarghan", "Yaghuti Sefid", "Siah Sisakht", "Bidane Sefid Ghazvin", "Rish Baba", "Rotabi" and "Siah". On "Yaghuti Siah" *N. parvum* (36.80 mm) and *D. seriata* (22.40 mm) gave the longest lesions and could be considered the most susceptible cultivar to these species. On "Siah" *N. parvum* (5.90 mm) and *D. seriata* (5.70 mm) gave the smallest lesions and could be considered the lowest susceptible cultivar to these pathogens. *N. parvum* and *D. seriata* were re-isolated from the inoculated plants with a

respectively. No *Botryosphaeriaceae* fungi were re–isolated from the control treatments. The correlation between lesion length and re-isolation for *N. parvum* and *D. seriata* were 43.14%, and 27.45% respectively.

Inoculation of trees in the field

Both spices of *N. parvum* and *D. seriata* were pathogenic and produced lesions on inoculated trees twigs under field conditions. Mean lengths of the extent of wood discolorations caused by *N. parvum* and *D. seriata* isolates on the tree branches are shown in Table 2 and Fig. 2. Analyses of variance of the lesion length data indicated a significant treatment effect ($P < 0.0001$, ANOVA tables not shown). Results showed significant differences in the pathogenicity of *N. parvum* and *D. seriata* isolates (F=1235.41; $P< 0.0001$), susceptibility of trees (F=92.43; $P< 0.0001$) and interaction between trees and pathogen species (F= 68.26; $P< .0001$, ANOVA not shown). Mean lesion lengths for *N. parvum*, *D. seriata* and the control were 29.30, 7.71 and 3.16 mm respectively. Based on the results, *D. seriata* produced smaller lesions than those caused by *N. parvum* in all inoculated branches but still differed significantly from the control treatments. Both species caused longer basipetal than acropetal lesions in all trees tested (Fig. 2). *N. parvum*, on pistachio (48.62 mm) and pomegranate (2.37 mm) gave the longest and least lesions respectively. *D. seriata* produced the longest and lowest lesions on apple (11.87 mm) and pomegranate (2.50 mm) trees respectively. *N. parvum* produced pycnidia on shoot surfaces of six trees include of pistachio, apple, apricot, fig, mulberry (Fig. 1-G) and ash while *D. seriata* isolate only produced pycnidia on shoot surfaces in apple, almond and pistachio trees. Different internal symptoms include of typical and small wedge shape necrosis, small necrotic lesion and wood discoloration produced by both species were observed when cross sections were made in inoculated shoots. No foliar symptoms were observed on inoculated tress after four months except for apple which showed leaf yellowing and shoot dieback cased by both species (Fig. 1-H) while controls did not show shoot and foliar symptoms in any of the replicates. Based on the results, *N. parvum* were the most virulent, and caused the largest lesions and most copious gummosis on all inoculated trees except pistachio, fig, service, acacia, ash and pomegranate, while *D. seriata* caused much less gum production (on almond, peach and apricot and mulberry) and the smaller lesions on inoculated trees. Percentage of inoculations from which the pathogen was recovered varied from 20% to 100 for shoots inoculated with *N. parvum* and 10% to 90% for shoots inoculated with *D. seriata*. While the twigs in the control set remained non-symptomatic and did not yield the pathogens. The correlation between lesion length and re-isolation for *N. parvum* and *D. seriata* were 57.40%, and 25.22% respectively.

Discussion

This study is the first comprehensive work that investigates the pathogenicity of two Botryosphaeriaceae species as *N. parvum* and *D. seriata* on grapevine cultivars and different trees in Iran. Pathogenicity test on 10 grapevine cultivars presented in this paper give an indication of the existence of tolerant and susceptible cultivars. The results (Tables 1 and 2) showed a variation in length of lesions formed and re-isolation frequencies between *N. parvum* and *D. seriata* in all inoculated treatments. There have been some reports about the virulence of *N. parvum* and *D. seriata*. Although Botryosphaeriaceae members have often been regarded as weak pathogens on grapevine (Phillips 2002; Van Niekerk *et al.* 2006) but some species have been shown to be pathogenic and extremely virulent. According to the results obtained in the pathogenicity tests *D. seriata* and *N. parvum* can be considered potentially pathogenic to grapevine cultivars and trees tested. In this study *D. seriata* isolate from Iran was much less pathogenic than those of *N. parvum* but still differed significantly in symptom expression from the control treatments. These results agree with previous pathogenicity studies conducted by some researchers. Auger *et al*. (2004) concluded *Botryosphaeria obtusa* (Schwein.) Shoemaker, to be a pathogen of grapevines in Chile after inoculation of rooted cuttings of "Red Globe". Van Niekerk *et al*. (2004) reported this species produce significantly larger lesions on cultivar "Periquita" than in controls in South Africa. Larignon *et al*. (2001) also reported *B. obtusa* to be responsible for dark streaks in one-year old canes of "Cabernet Sauvignon" in France. Similar results were recorded by Castillo-Pando *et al*. (2001) on one-

month-old "Chardonnay" plantlets and potted "Chardonnay" grapevines in the New South Wales region in Australia. Urbez-Torres *et al.* (2008) found *D. seriata* pathogenic on rooted cuttings and green shoots of "Thompson" seedless and "Chardonnay" cultivars. In contrast, *D. seriata* isolates from grapevines in Western Australia were found to be nonpathogenic (Taylor *et al*. 2005). Different researchers from a range of countries have observed differences in virulence among Botryosphaeriaceae species. These differences may have been due to different factors such as variations in susceptibility of the local grapevine varieties, fungal isolates, environmental conditions, inoculation methods, or incubation times. Among 10 grapevine cultivars, "Siah" has proved to be the most tolerant cultivar while cv. "Yaghuti Siah" the most susceptible to both species under our conditions, when statistical analysis was based on the measurements of the lesion length. Based on the results, other cultivars were significantly less susceptible than "Yaghuti Siah". "Siah" cultivar was the most resistant cultivar in a 2006 trial performed in Iran (Mohammadi and Banihashemi 2010). Rooted cuttings inoculated by *N. parvum* showed petioles and leaves died back and dried out while those inoculated *D. seriata* did not show any foliar symptoms after 4 months. This agrees with previous studies conducted in Portugal (Phillips 1998), California (Úrbez-Torres *et al*. 2006) and Mexico (Úrbez-Torres *et al*. 2008) which found that there were no foliar symptoms associated with *D. seriata*. Our results showed that non-grapevine woody trees can be colonized by two Botryosphaeriaceae species isolated from grapevine. In fact four different internal symptom types including small wedge shaped, central wood necrosis, typical wedge shaped necrosis and small necrotic lesions, similar to those occurring in grapevine, were identified in different trees inoculated by both species. Small necrotic lesion and wedge-shape canker symptoms were observed on Thompson seedless green shoots and 1-year-old Chardonnay cutting respectively inoculated by *D. seriata* (Urbez-Torres *et al.* 2008). Wedge-shaped necrosis and wood discoloration is commonly associated with the presence of Botryosphaeriaceae spp. on grapevine (Castillo-Pando *et al*., 2001; Phillips, 2002; Savocchia *et al*., 2007). Some species of *Phaeoacremonium* and *Botryosphaeriaceae* were also mostly isolated from wedge shaped and brown internal necrotic lesions of apple and pear trees (Cloete *et al*. 2011).

According to Amponsah *et al*. (2009) *Botryosphaeria* species, including *Botryosphaeria parva* Pennycook & Samuels from infected grapevines and other woody hosts, produce symptomatic infections on green shoots of grapevine. Species of Botryosphaeriaceae generally have the ability to colonize a wide range of woody hosts in various environments throughout the world. The fungus *N. parvum*, previously known as *B. parva*, has been reported as a pathogen of pome and stone fruit trees (Slippers *et al*. 2007). In addition, this pathogen has been isolated from many other hosts, such as blueberry (Espinoza *et al*. 2009), *Syzygium cordatum* (Pavlic *et al*. 2009), avocado (McDonald *et al*. 2009), grapevine (Laveau *et al.* 2009), eucalyptus (Smith *et al*. 2001) and walnut. This species also was isolated from blighted shoots of pistachio in Greece (Inderbitzin *et al*. 2010). Based on the studies, some *Diplodia* species such as *D. seriata* has wide host ranges (Phillips *et al*. 2007) but some species such as *D. scrobiculata* and *D. pinea* (= *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton) occur only on conifers. The results of this study demonstrated that fruit trees and other non-grapevine woody hosts tested here are susceptible to *N. parvum* and *D. seriata*. In Iran non-grapevine woody trees were found close to vineyards; therefore, different infected trees could act as a source of inoculum and alternative hosts in the absence of grapevine plants. On the other hand, different plant species infected by *N. parvum* and *D. seriata* can serve as inoculum sources for infections of grapevines. Slippers *et al*. (2007) reported six species of Botryosphaeriaceae on pome and stone fruit trees namely *Neofusicoccum ribis* Grossenb. & Duggar, *N. parvum, N. australe, B. dothidea, D. mutila* and "*Botryosphaeria*" *obtusa* in South Africa. *D. seriata* has been found to be the dominant species of Botryosphaeriaceae on pome fruit trees (Slippers *et al.* 2007), stone fruit trees (Damm *et al.* 2007) and grapevine (Van Niekerk *et al*. 2004, 2010) in South Africa. Recently, several fungal isolates including *Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai, *Phaeoacremonium iranianum* L. Mostert, Gra¨f., W. Gams & Crous, *Phaeoacremonium mortoniae* Crous & W. Gams, *Phaeoacremonium viticola* J. Dupont, *Neofusicoccum vitifusiforme* (Van Niekerk & Crous) Crous, Slippers & A.J.L.

Phillips, *N. australe*, *D. seriata* and *Eutypa lata* (Pers.: Fr.) Tul et C. Tul., which were isolated from grapevine, were found associated with apple and pear trees in South Africa. Based on pathogenicity tests on apples and pear, *N. australe* caused the longest lesions while *D. seriata* caused lesions that were significantly longer than the control inoculations (Cloete *et al*. 2011). During an investigation by Abdollahzadeh *et al.* (2009) two new species of Botryosphaeriaceae, namely *B. iraniana* on *Citrus*, *Mangifera* and *Olea*, and *P. cupressi* on *Cupressus sempervirens* were reported from Iran. More recently, six species of *Lasiodiplodia* also have been isolated and reported from different woody hosts in this country from which four of them including *Lasiodiplodia iraniensis* Abdollahzadeh, Zare & A.J.L. Phillips, *Lasiodiplodia hormozganensis* Abdollahzadeh, Zare & A.J.L. Phillips, *Lasiodiplodia gilanensis* Abdollahzadeh, Javadi & A.J.L. Phillips and *Lasiodiplodia citricola* Abdollahzadeh, Javadi & A.J.L. Phillips, have been described as new species (Abdollahzadeh *et al.* 2010), but the exact host range of all these species is still unknown. Therefore, more investigation on different trees, especially trees which are often planted as windbreaks of vineyards, should be done to clarify the possibly relationship between trunk pathogens of grapevine and other trees in Iran.

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