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Pathogenicity of *Fusarium* spp. associated with diseases of Aleppo-pine seedlings in Algerian forest nurseries

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ABSTRACT: In northwestern Algeria, the production of Aleppo pine (*Pinus halepensis* Mill.) seedlings in four nurseries is hindered by the damping-off disease. Results obtained indicated that *Fusarium* spp. are commonly found on diseased seedlings, in most containers and bare-root nurseries. Twenty-one isolates of *Fusarium*, belonging to seven species, were previously isolated from diseased seedlings, and identified based on their morphological and molecular characteristics and their sequences had been deposited in NCBI-Genbank. These isolates were tested for their pathogenicity to local Aleppo pine seeds. The highest inhibition was observed with *F. redolens* and *F. solani*, with 75 and 69.3%, respectively. The root growth inhibition of the Aleppo pine seedlings was significantly different for each isolate. The influence of various isolates of *Fusarium* spp. on seed germination, shoot and root length and vigour index was significantly different. The disease incidence caused by *F. redolens* and *F. solani* was 91 and 90%, respectively.

Keywords: *Pinus halepensis*; *Fusarium redolens*; *Fusarium solani*; damping-off

Aleppo pine (*Pinus halepensis* Mill.) plays an important role in the ecology and landscape of different countries around the Mediterranean basin. This pioneer and undemanding species is easily regenerated and capable of rehabilitating very poor and degraded soils. It is an essential component in reforestation strategy for limy soils in the arid or semi-arid climates around the Mediterranean basin, due to both its intrinsic ability to colonize and its effect in improving soils and microclimates (Quezel 1986). In Algeria, the Aleppo pine occupies vast stands in Sidi Belabbes, Saida, Tlemcen, Tiaret, Medea and the Ouarsenis regions (Mezali 2003).

Fungal diseases are amongst the most serious problems forest regeneration is facing and they can sometimes cause heavy losses due to high mortality rates. Many of the fungal pathogens are seed-borne and are transmitted to forest nurseries through seeds where they become established on seedlings. Apart from their seed-borne nature, soil-borne fungal pathogens are devastating by attacking young seedlings in forest nurseries (Ravishankar, Mamatha 2005).

Pre-emergence damping-off is characterized by seeds failing to germinate, or rotting of emerging shoots or radicals with the associated seedling losses. Symptoms of post-emergence damping-off include stem rotting at the soil line and subsequent toppling of the seedling shoot. Post-emergence damping-off results in damage and loss of infected seedlings after stem rotting. However, the disease can also spread by spores produced on the infected stems, and infect adjacent seedlings causing further losses (Hietala et al. 2001).

Fusarium spp. are commonly found on coniferous seedling roots and in the rhizosphere (Petrson 2008), and can behave as fungal pathogens causing damping-off and root rot of young coniferous seedlings, which result in severe crop

and economic losses in forest nurseries. This fungal genus is ubiquitous in most containers and bare root nurseries on healthy and diseased coniferous seedlings, in nursery soils, and on coniferous seeds of several species (James et al. 2000). *Fusarium* spp. can also be responsible for pre- and post-emergence damping-off upon seed germination. However, for reasons such as slow to shed the seed coat, for example, inocula contacting and infecting the emergent tissues will often cause the new shoot to rot at the soil line. Rotting of young germinated seedling at the soil line and breaking or falling over at this point typifies symptoms of post-emergence damping-off (Peterson 2008). Laboratory assays were developed to assess the pathogenicity of *Fusarium* isolates on coniferous germinants (James 1996).

Due to scarce studies on diseases of forest nurseries in Algeria, a multifaceted effort was deployed in order to alleviate the problems by approaching it from different angles. The purpose of this research was to evaluate the pathogenicity of different *Fusarium* species on Aleppo pine seedlings.

MATERIALS AND METHODS

Origin of Fusarium isolates. Diseased Aleppo pine seedlings were collected from four forest nurseries in northwestern Algeria. Information on the origin and host tissue of each isolate used is listed in Table 1. Sampling was carried out during the winter and spring of 2009–2010. Surface disinfected segments from the decayed roots and discoloured stems of about 5 mm length were cultured on PDA medium. A total of 21 isolates were obtained during routine isolations from Aleppo pine seedlings exhibiting disease symptoms. Isolates comprised seven *Fusarium* species [*F. acuminatum* (4 isolates), *F. chlamydosporum* (2 isolates), *F. equiseti* (2 isolates), *F. oxysporum* (3 isolates), *F. redolens* (5 isolates), *F. solani* (2 isolates) and *F. tricinctum* (3 isolates)], identified earlier on the basis of morphological characters (Leslie, Summerell 2006).

Seed stratification. Aleppo pine seeds were surface disinfected by agitation in 2% NaOCl solution on a shaker at 120 rpm for 25 minutes, rinsed four times in sterile distilled water and dried on sterile filter pa-

Table 1. Isolates of the *Fusarium* species used in this study

No.	Taxon	Isolate code	Tissue isolation	Nursery location	Collection date	GenBank Accessions
1	*F. acuminatum*	F12SS1	stem	SBA/Sfisef N1	09-03-2009	JX114788
2	*F. acuminatum*	F30SS3	stem	SBA/Sfisef N3	20-03-2009	JX114782
3	*F. acuminatum*	F25RS3	root	SBA/Sfisef N3	20-03-2009	JX114790
4	*F. acuminatum*	F14SS3	stem	SBA/Sfisef N3	20-03-2009	JX114785
5	*F. chlamydosporum*	F12RR	root	Relizane (safa dahra)	20-12-2009	JX114795
6	*F. chlamydosporum*	F4RS1	root	SBA/Sfisef N1	09-03-2009	JX114789
7	*F. equiseti*	F3RS1	root	SBA/Sfisef N1	20-03-2009	JX114784
8	*F. equiseti*	F19RS1	root	SBA/Sfisef N1	22-09-2009	JX114791
9	*F. oxysporum*	F16RS3	root	SBA/Sfisef N3	09-03-2009	JX114787
10	*F. oxysporum*	F3PRT	root	Tlemcen (conservation)	10-02-2010	JX114794
11	*F. oxysporum*	F6RS3	root	SBA/Sfisef N3	22-02-2009	JX114792
12	*F. redolens*	F5RS3	root	SBA/Sfisef N3	26-12-2009	JX051323
13	*F. redolens*	F8RS3	root	SBA/Sfisef N3	26-12-2009	JX051326
14	*F. redolens*	F09SS1	stem	SBA/Sfisef N1	26-12-2009	JX114783
15	*F. redolens*	F91SR	stem	Relizane (safa dahra)	20-12-2009	JX051324
16	*F. redolens*	F55RS1	root	SBA/Sfisef N1	26-12-2009	JX051325
17	*F. solani*	F20ST	stem	Tlemcen (conservation)	10-02-2010	JX114796
18	*F. solani*	F12RT	root	Tlemcen (conservation)	10-02-2010	JX114793
19	*F. tricinctum*	F2RR	root	Relizane (safa dahra)	20-12-2009	JX114797
20	*F. tricinctum*	F44SS3	stem	SBA/Sfisef N3	26-12-2009	JX114781
21	*F. tricinctum*	F39SS3	stem	SBA/Sfisef N3	26-12-2009	JX114786

N1 – nursery 1, N3 – nursery 3

per. Seeds were wrapped in moistened, sterile cheesecloth, enclosed in Petri dishes of 12 cm in diameter, and incubated at an ambient temperature of about 25°C for 3 weeks (Ocamb et al. 2002).

In-vitro effect of isolates on seed germination and root development. *Fusarium* isolates were tested for their effect on seed germination and root development. A Petri dish assay was used to evaluate the pathogenicity of *Fusarium* spp., as was described by El Androuse (2006); Zhang and Yang (2000). Each isolate was grown on PDA for 4 days and ten stratified seeds were placed on each colony. The Petri dishes were incubated again at 22°C (El Androusse 2006). The pathogenicity of *Fusarium* isolates was estimated as the percentage of inhibition of seed germination and the inhibition of young seedling root development (Kirk 2008).

Effect of isolates on seed germination and root development in pots assays. The inoculum was produced as described earlier by Kirkpatrick (2006) by adding 5-mm plugs of each isolate to a previously sterilized 500-ml flask containing 237.5 g of sand, 12.5 g of cornmeal and 80 ml of deionised water. Isolates were allowed to grow on the medium for 9 days, and the flasks were shaken every other day to disperse the inocula evenly. Inocula of each isolate were then transferred to three 500 ml pots, and 10 Aleppo pine seeds were sown in each pot with three replications for each isolate (Kirk 2008). Four weeks later, seedlings were carefully removed from the pots and excess soil was gently removed from roots (the remaining soil was considered as the rhizosphere soil). Roots were then washed under running tap water for 2 min. Shoot and root length was recorded for each seedling. Root systems were rated on 1–5 rating scale (Okamb et al. 2002). Disease incidence was calculated (Song et al. 2004). The germination rate, root length inhibition, shoot growth inhibition and the seedling vigour index were also calculated following the method described by Maisuria and Patel (2009). Koch's postulates were performed for all isolates tested.

Statistical analysis. Differences between the means were compared by Duncan's Multiple Range Test at a 5% level of significance. All statistical analyses were performed using SAS 8.1 software (SAS Institute, Cary, USA).

RESULTS

Effect of *Fusarium* spp. on pine seedling test

All isolates of *Fusarium* recovered from diseased pine seedlings with stand establishment problems are presented in Table 2. *F. redolens*, *F. acuminatum*, *F. solani*, *F. oxysporum* and *F. chlamydosporum* strongly affected the germination and the root growth of pine seedlings. The results presented in Table 2 show that the germination of pine seeds inoculated with different species of *Fusarium* varied considerably. The pine seeds were more sensitive to infection where the germination and root growth inhibition were more important.

The highest percentages of seed germination inhibition were recorded for *F. redolens*, *F. solani*, *F. acuminatum* and *F. tricinctum* with 74.9, 69.28, 53.84 and 49.9%, respectively. Seed germination was reduced significantly ($P = 0.49$) by the different species of *Fusarium*. Additionally, there was a significant effect of the different *Fusarium* species ($P = 0.04$) on the root growth (Table 2). The root growth was inhibited by *F. redolens*, *F. solani*, *F. acuminatum* and *F. tricinctum* at 89.9, 85.8, 76 and 63.4%, respectively (Table 2).

Pathogenicity of *Fusarium* spp. in *in-vivo* assay

The *Fusarium* species were able to successfully infect pine seedlings in infested soil experiments. All species of *Fusarium* were able to significantly ($P < 0.0001$) reduce the germination of pine seeds compared to the control, where the germination percentage was 86%. The *Fusarium* species also caused necrotic lesions on the roots. The lesions were brown and ranged from small elongated to large coalesced lesions and, in several instances, the entire root system was colonized and decayed or the seed did not germinate at all. These species significantly ($P < 0.0001$) decreased the seedling root lengths (Table 3). Parallelly, the length of the pine seedling shoots resulting from the inoculated seeds was significantly ($P < 0.0001$) reduced. The influence of various species of *Fusarium* on seed germination, seedling shoot and root length can be estimated from the vigour index. The vigour index of isolates was significantly different ($P < 0.0001$).

DISCUSSION

This study was able to demonstrate that there are many *Fusarium* species present in the northwestern Algerian forest nurseries and these species were implicated in pine seedling damping-off disease. The exception was *F. redolens* and *F. solani* that appeared to be important pathogens. *Fusarium* spp. are often found on the surface and within coniferous seeds, including loblolly pine (*Pinus taeda* L.), longleaf pine

Table 2. Effects of different *Fusarium* isolates on seed germination and root development of Aleppo pine seedlings in Petri dishes and pot essays

No.	Isolates	Germination inhibition		Root growth inhibition	
		Petri dish	pot	Petri dish	pot
1	*F. acuminatum*	51.91 ± 3.54 ^{EBDFC}	44.00 ± 4 ^{EDCF}	69.10 ± 2.16 ^{EDFC}	51.44 ± 8.67 ^{BDC}
2	*F. acuminatum*	40.38 ± 3.84 ^{EDF}	60.00 ± 4 ^{EBDAC}	60.62 ± 4.16 ^{EF}	64.25 ± 1.24 ^{BDAC}
3	*F. acuminatum*	53.84 ± 6.66 ^{EBDFC}	56.00 ± 4 ^{EBDACF}	64.32 ± 6.08 ^{EDFC}	64.593 ± 4.49 ^{BDAC}
4	*F. acuminatum*	53.84 ± 5.95 ^{EBDFC}	48.00 ± 8 ^{EBDCF}	76.03 ± 5.92 ^{EBDAC}	42.40 ± 3.38 ^{DC}
5	*F. chlamydosporum*	38.45 ± 5.70 ^{EF}	52.00± 0 ^{EBDCF}	42.72 ±5.33 ^{G}	50.70 ± 2.05 ^{BDC}
6	*F. chlamydosporum*	44.22 ± 5.50 ^{EDFC}	52.00 ± 6.92 ^{EBDCF}	64.38 ± 2.20 ^{EDFC}	51.94 ± 2.68 ^{BDAC}
7	*F. equiseti*	55.76 ± 4.63 ^{EBDAC}	32.00 ± 4 ^{F}	68.18 ± 5.53 ^{EDFC}	38.87 ± 1.96 ^{D}
8	*F. equiseti*	55.76 ± 4.63 ^{EBDAC}	40.00 ± 6.92 ^{EDF}	76.9 ± 3.231 ^{EBDAC}	44.61 ± 2.03 ^{DC}
9	*F. oxysporum*	34.61 ± 4.86 ^{F}	32.00 ± 4 ^{F}	21.95 ± 4.75 ^{H}	42.26 ± 6.99 ^{DC}
10	*F. oxysporum*	44.22 ± 5.50 ^{EDFC}	44.00 ± 4 ^{EDCF}	62.02 ± 7.85 ^{ED}	42.26 ± 4.11 ^{DC}
11	*F. oxysporum*	48.07 ± 5.76 ^{EDFC}	40.00 ± 6.92 ^{EDF}	58.55 ± 8.31^{GF}	45.65 ± 2.57 ^{DC}
12	*F. redolens*	74.99 ± 5.70 ^{A}	80.000 ± 4 ^{A}	89.98 ± 2.76 ^{A}	77.46 ± 3.78 ^{BA}
13	*F. redolens*	61.53 ± 4.86 ^{BAC}	44.00 ± 4 ^{EDCF}	78.00 ± 5.36 ^{BDAC}	47.31 ± 0.49 ^{DC}
14	*F. redelons*	44.22 ± 5.50 ^{EDFC}	48.00 ± 4 ^{EBDCF}	70.83 ± 5.19 ^{EBDFC}	52.90 ± 9.25 ^{BDAC}
15	*F. redolens*	34.610 ± 4.86 ^{F}	44.00 ± 4 ^{EDCF}	55.694 ± 4.09 ^{GF}	47.31 ± 0.49 ^{DC}
16	*F. redolens*	57.68 ± 3.84 ^{EBDAC}	60.00 ± 8 ^{EBDAC}	69.54 ± 5.09 ^{EBDFC}	55.95 ± 7.23 ^{BDAC}
17	*F. solani*	69.28 ± 2.43 ^{BA}	72.00 ± 4 ^{BA}	85.81 ± 3.06 ^{BA}	78.76 ± 4.58 ^{A}
18	*F. solani*	44.22 ± 5.50 ^{EDFC}	36.00 ± 4 ^{EF}	65.46 ± 1.80 ^{EDFC}	44.36 ± 2.32 ^{DC}
19	*F. tricinctum*	59.61 ± 4.94 $^{BD\ AC}$	64.00 ± 6.92 ^{BDAC}	79.19 ± 4.71 ^{BAC}	45.67 ± 0.88 ^{DC}
20	*F. tricinctum*	49.99 ± 5.70 ^{EBDFC}	44.00± 4 ^{EDCF}	65.96 ± 4.37 ^{EDFC}	51.25 ± 7.70 ^{BDC}
21	*F. tricinctum*	49.99 ± 3.84 ^{EBDFC}	44.00 ± 4 ^{EDCF}	63.42 ± 5.39 ^{EDFC}	51.31 ± 4.85 ^{BDC}

means compared by one-way ANOVA, numbers followed by the same letters are not significantly different ($P > 0.05$)

(*P. palustris* Mill.), slash pine (*P. elliotti* Engelm), spruce (*Picea* sp.), and true fir (*Abies* sp.) (Hoefnagels 1999). Young germinant or seedling root infections, resulting from roots growing in the close proximity of germinating chlamydospores, can lead to stem rotting at the soil line, which typifies post-emergence damping-off (Perterson 2008). In previous reports, *Pinus* species yielded *F. acuminatum*, *F. avenaceum*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, *F. roseum*, *F. sambucinum*, *F. solani*, *F. subglutinans* or *circinatum*, and *F. tricinctum* in seed and seedling isolations (Anderson 1986; Lazreg et al. 2013a–d).

The *in vitro* contaminatin combined with the *in vivo* assay provided an evaluation of the probable role of *F. redolens* and *F. solani* as damping-off pathogens of pine seedlings confirming previous findings. More important is that *F. redolens* was found in the diseased pine seedlings (Lazreg et al. 2013b). Symptomatic roots from problematic white pine nursery fields were examined for *Fusarium* species and the predominant species isolated included *F. proliferatum*, *F. oxysporum* and *F. oxysporum* var. *redolens* (Ocamb, Juzwik 1995).

F. oxysporum and *F. redolens* contained isolates causing a wide range of diseases including wilts, seedling damping-off, and cortical rot; several of these show host specificity (Booth 1971). *F. redolens* is associated with the root rot disease of a large number of plant species (Baayen et al. 2000). *F. solani* causing basal rot and root rot is the predominant pathogen of greenhouse-grown seedlings (Chen, Chung 2008; Chen et al. 2006, 2009). *F. solani* was the most commonly found species in bare roots of *P. radiata* nurseries, and was recovered from both seedlings and soil (Dick, Dobbie 2002). Results from previous studies and information from this study confirmed that *F. redolens* and *F. solani* are pathogenic to pine seedlings.

The Petri plate pathogenicity assay is an effective method for determining the pathogenic capability of Fusarium species whereas the laboratory assays were developed to assess and confirm the pathoge-

Table 3. Growth parameters of Aleppo pine seedlings as influenced by different species of *Fusarium*

No.	Isolates	Shoot growth inhibition (%)	Vigour index	Disease index (%)
1	*F. acuminatum*	42.62 ± 6.17 ^{BC}	2,650.0 ± 507.18 ^{BDAC}	53.33 ± 1.00 ^{G}
2	*F. acuminatum*	55.72 ± 0.34 ^{BA}	1,413.3 ± 163.33 ^{EDFC}	63.33 ± 4.33 ^{FBEDC}
3	*F. acuminatum*	57.57 ± 5.18 ^{BA}	1,540.0 ± 305.66 ^{EDFC}	60.00 ± 3.88 ^{FGEDC}
4	*F. acuminatum*	39.64 ± 3.32 ^{BC}	2,666.7 ± 391.93 ^{BDAC}	56.66 ± 4.88 ^{FGE}
5	*F. chlamydosporum*	35.63 ± 4.58 ^{BC}	2,793.3 ± 114.64 ^{BDAC}	65.66 ± 3.17 ^{FGED}
6	*F. chlamydosporum*	47.24 ± 3.03 ^{BC}	2,116.7 ± 294.75 ^{EBDFC}	77 ± 0.77 ^{FGEDC}
7	*F. equiseti*	26.99 ± 3.08 ^{C}	4033.3 ± 268.59 ^{A}	59 ± 4.84 ^{G}
8	*F. equiseti*	37.40 ± 1.16 ^{BC}	3116.7 ± 399.05 ^{BAC}	65 ± 2.58 ^{G}
9	*F. oxysporum*	26.59 ± 4.47 ^{C}	3710.0 ± 434.66 ^{BA}	54.00 ± 4.61 ^{G}
10	*F. oxysporum*	37.76 ± 1.76 ^{BC}	2,970.0 ± 263.12 ^{BDAC}	60.33 ± 2.60 ^{FGEDC}
11	*F. oxysporum*	36.04 ± 1.36 ^{BC}	3,116.7 ± 345.89 ^{BAC}	54.00 ± 3.05 ^{FG}
12	*F. redolens*	72.65 ± 1.69 ^{A}	455.8 ± 123.57 ^{F}	91.00 ± 3.33 ^{A}
13	*F. redelons*	42.62 ± 2.73 ^{BC}	2,710.0 ± 244.40 ^{BDAC}	53 ± 3.60 ^{FGE}
14	*F. redolens*	44.12 ± 10.04 ^{BC}	2,381.9 ± 526.65 ^{EBDAC}	61.66 ± 4.40 ^{FGEDC}
15	*F. redelons*	42.62 ± 2.73 ^{BC}	2,710.0 ± 244.40 ^{BDAC}	57.00 ± 3.60 ^{FGE}
16	*F. redolens*	46.96 ± 3.73 ^{BC}	1,750.0 ± 464. 57 ^{EDFC}	73.33 ± 1.66 ^{BEDC}
17	*F. solani*	70.04 ± 4.59 ^{A}	660.2 ± 200.68 ^{EF}	90.00 ± 1.92 ^{BA}
18	*F. solani*	35.25± 3.88 ^{BC}	3,632.2 ± 463.36 ^{BA}	50.44 ± 1.55 ^{G}
19	*F. tricinctum*	35.75 ± 3.11 ^{BC}	1,887.8 ± 385.47 ^{EBDF}	77.77 ± 2.22 ^{BAC}
20	*F. tricinctum*	41.30 ± 1.86 ^{BC}	2,678.6 ± 396.45 ^{BDAC}	63.33 ± 1.66 ^{FGED}
21	*F. tricinctum*	42.57 ± 4.96 ^{BC}	2,400.9 ± 140.97 ^{EBDA}	58.88 ± 4.84 ^{FGEDC}

means compared by one-way ANOVA, numbers followed by the same letters are not significantly different ($P > 0.05$)

nicity of *Fusarium* spp. isolates on coniferous germinants (James 1996).

The germination and the root growth were decreased in seeds contaminated by *F. redolens* and *F. solani.* Similarly, Zehhar et al. (2006) found that *F. solani* reduces the germination and the roots growth of rice. The *in vivo* assay using *F. redolens*, *F. solani* and *F. acuminatum* affected the germination, root growth, shoot growth and the index vigour (Lazreg et al. 2013a). Rajput et al. (2008) found a maximum reduction in root and shoot length in *Dalbergia sissoo* Roxb., inoculated by injecting the spore suspension of *F. solani* as compared to the soil amended with spore suspension of the fungus or plants sprayed with spore suspension. Root and shoot weights were also decreased when the spore suspension of *F. solani* was injected into stems followed by the soil amended with spore suspension and plants sprayed with spore suspension.

In-vivo assay also demonstrated a wide range of pathogenicity among these different species, where Fusarium such as *F. redolens* and *F. solani* were able to infect the entire root system. However, even species with low levels of pathogenicity had the ability to cause lesions, most generally on the roots. *F. acuminatum* and *F. equiseti* were reported as damping-off causal organisms of Aleppo pine seedlings in Algeria (Lazreg et al. 2013a,c).

Evidences are now available on the occurrence of *F. solani* and *F. redolens* as serious root rot pathogens that could be important sources of inoculum (Bienapfl et al. 2010; Hamini et al. 2010). A wide range of variation in pathogenicity and mycotoxin production between isolates was also reported (Jestoi 2008; Vogelgsang et al. 2008).

The results of this study indicate that *F. redolens* and *F. solani* may be the most important pathogens causing damping-off of pine seedlings in Algeria.

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