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Root rot and damping-off of Aleppo pine seedlings caused by *Pythium* spp. in Algerian forest nurseries

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ABSTRACT: Aleppo pine (*Pinus halepensis* Miller) is a common forest tree in the Mediterranean Region. Production of Aleppo pine seedlings is hindered by damping off and root rot diseases in cooler conditions of north-western Algeria, which significantly reduces the seedling emergence. This work was carried out to evaluate the pathogenicity of different *Pythium* Pringsheim species on Aleppo pine seedlings. Seventeen isolates of *Pythium* spp. were isolated from diseased seedlings, identified to the species level based on DNA sequence from the internal transcribed spacer region and their identity was confirmed on morphological basis. The obtained isolates were pathologically characterized in Petri dish and pot assays. Germination was significantly inhibited by the different *Pythium* spp. isolates. The highest inhibition was observed with *Pythium ultimum* Trow in Petri dish and pot assays. The reduction in root growth differed significantly between different isolates. The maximum reduction in root growth (92.2%) was observed for *P. ultimum* in Petri dish assay. The various isolates of *Pythium* showed a significant influence on root length, shoot length and vigour index. The maximum disease incidence (89.7%) was caused by *P. ultimum*. Our results indicated that *Pythium* spp. is commonly found on diseased seedlings, in most containers and bare-root nurseries.

Keywords: pathogenicity; oomycetes; *Pinus halepensis*; *Pythium ultimum*; *Pythium heterothallicum*; seedling emergence

Aleppo pine (*Pinus halepensis* Miller) has an important role in the ecology and landscape of different countries around the Mediterranean Basin. This species can easily regenerate and rehabilitate in very poor and degraded soils. This tree is an essential component of reforestation strategy in arid or semi-arid climates around the Mediterranean Basin, due to its intrinsic colonizing ability as well as its effect in improvement of soil and microclimate (Quezel 1986). In Algeria, the Aleppo pine occupies a vast stands in Sidi Belabbes, Saida, Tlemcen, Tiaret and the Ouarsenis regions. It is also found in Medea and in Bibans (Mezali 2003).

Fungal diseases are amongst the most serious problems faced by forest regeneration, and can sometimes cause heavy losses due to high mortality rates. Many of the fungal pathogens are transmitted to forest nurseries through seeds and become established on seedlings. Moreover, soil-borne fungal pathogens are devastating due to their attack on young seedlings in forest nurseries (Ravishankar, Mamatha 2005; Lazreg et al. 2014).

Pre-emergence damping-off is characterized by seeds failing to germinate, or rotting of emerging shoots or radicals with associated seedling losses. Typical symptoms of infection by *Pythium* spp. include soft and decayed seed before germination, pre- or post-emergence damping-off in the seeding stage, and hypocotyl discoloration and root rot in advanced growth stages (Rosso et al. 2008).

The Aleppo pine seedlings in the Mediterranean Region have been reported to be attacked by *Pythium ultimum* Trow (Lazreg et al. 2013). The genus *Pythium* Pringsheim is widely distributed throughout the world, and around 150 species have been appropriately described (Broders 2008).

In some cases, the increased prevalence of seedling diseases such as damping-off is associated with the relative abundance of pathogenic *Pythium* species (Pankhurst et al. 1995). *Pythium* is able to colonize plant residues left on the soil from the previous crop, which leads to an inoculum build up in the seed bed. If this cycle is repeated in the course of several years, it may contribute to an increase in pathogenic *Pythium* populations in the soil (Pankhurst 1995). Once a *Pythium* sp. invades a field or greenhouse, it quickly propagates and disperses, resulting in serious yield loss.

To develop efficient disease control strategies, we need to understand the infection routes of the pathogens, monitor their presence in fields and greenhouses, and find effective ways to eliminate the pathogens (Kageyama 2014).

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

MATERIAL AND METHODS

Fungal material. Diseased Aleppo pine seedlings were collected from four forest nurseries in northwestern Algeria (Fig. 1). 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 2008–2010. Segments from the decayed roots and discoloured stems of about 5 mm length were disinfected, and then were rinsed three times in distilled water. The segments were dried on sterilized filter paper and then cultured on a corn meal agar (CMA) medium. *Pythium* isolates were identified to the species level on the basis of morphological observation (Plaats-Niterink, Vander 1981; Dick 1990) and an analysis of the rDNA internal transcribed spacer (ITS) sequence. The NCBI BLAST search program (Version 2, 2012) was used to search similar sequences from the NCBI GenBank sequence database for the 5.8S-ITS sequence for oomycetes. The GenBank accession number was presented (Table 1). A total of 17 isolates of *Pythium* spp. were obtained during routine isolations from Aleppo pine seedlings exhibiting disease symptoms. Isolates comprised three *Pythium* species: *P. ultimum* (14 isolates), *Pythium glomeratum* B. Paul (2 isolates) and *Pythium heterothallicum* W.A. Campbell & F.F. Hendrix (1 isolate).

Inoculum. The collected diseased tissues were cut to pieces (3–5 mm) and surface disinfected by immersing in 0.5% sodium hypochlorite for 1 min. The disinfected pieces were grown on a CMA selective medium that contained penicillin, streptomycin sulphate, pimaricin. After incubation on CMA plates for 4 days at 25°C, fungal cultures

Table 1. Isolates of *Pythium* species used in this study

No.	Code	Taxon	Nursery location	Tissue isolation	Isolation date	NCBI GenBank accession
1	U3CR	*P. ultimum* var. *ultimum* Trow	Relizane (Safa Dahra)	stem	25-12-2008	JX191921
2	U7CR			stem	20-12-2008	JX191922
3	U6RR			root	20-12-2008	JX191924
4	U8CR			stem	15-02-2009	JX191925
5	U6CR			stem	15-02-2009	JX191926
6	U14CR			stem	20-12-2009	JX191933
7	U2RR			root	15-02-2010	JX191930
8	U2CR	*P. ultimum* Trow	Relizane (Safa Dahra)	stem	15-03-2009	JX191929
9	U4CR			stem	15-03-2009	JX191931
10	U5RT	*P. ultimum* var. *ultimum* Trow	Tlemcen (conservation)	root	10-02-2009	JX191928
11	U12RT			root	15-03-2009	JX191932
12	U7RT			root	10-02-2009	JX191934
13	U17RT			stem	25-02-2010	JX191935
14	U1RT	*P. ultimum* Trow	Tlemcen (conservation)	root	10-02-2009	JX191927
15	U35RS	*P. glomeratum* B. Paul	SBA/Sfisef N3	root	26-12-2009	JX191936
16	U16RS			root	09-03-2009	JX191937
17	U15RS	*P. heterothallicum* W.A. Campbell & F.F. Hendrix	SBA/Sfisef N1	root	22-09-2009	JX191923

SBA – Sidi Bel Abbes, N1 – nursery 1, N3 – nursery 3

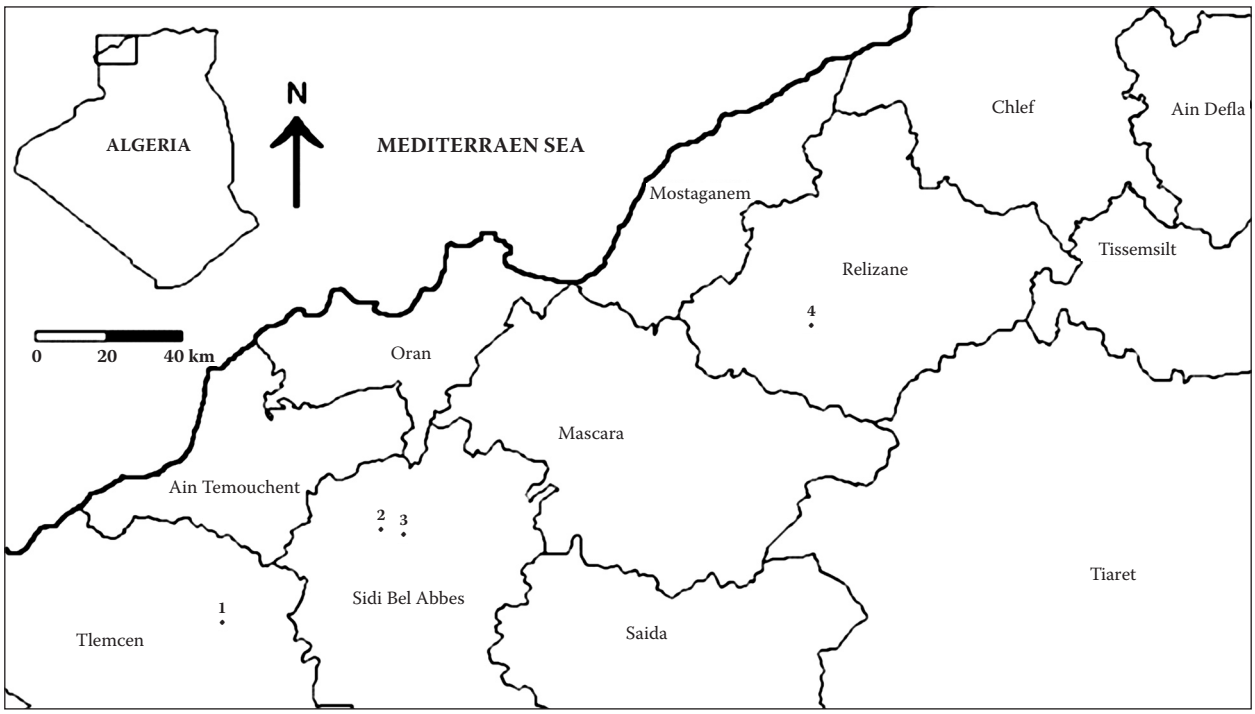

Fig. 1. The location of nurseries from which the isolates of *Pythium* Pringsheim species were obtained

1 – Tlemcen (conservation), 2 – Sidi Bel Abbes/Sfisef N1, 3 – Sidi Bel Abbes/Sfisef N3, 4 – Relizane (Safa Dahra)

were transferred to plates containing 2% water agar (Pahlavani et al. 2009). These plates were kept at room temperature for 24 h and the pathogen was purified by a single hyphal tip isolation technique. Liquid cultures of fungi were prepared by adding inoculum discs and grass blades to sterile water in a Petri dish at 25°C.

Inoculum was produced as described earlier (Kirkpatrick et al. 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 inoculum evenly.

Seed germination. Aleppo pine seeds were surface disinfected by agitation in 2% NaOCl solution on a shaker at 120 rpm for 25 min, rinsed four times in sterile distilled water and dried on sterile filter paper. Seeds were wrapped in moistened, sterile cheesecloth, enclosed in Petri dishes of 12 cm diameter, and incubated at 25°C for 3 weeks (Lazreg et al. 2014).

Pathogenicity test. The effect of *Pythium* isolates on seed germination and root development was tested in two different assays (Petri dish assay and pot assay) (Zhang, Yang 2000). For Petri dish assay each *Pythium* isolate was grown on CMA for 4 days and ten seeds were placed on each colony. The Petri dishes were incubated at 22°C. Seeds placed on CMA media without inoculum served as control.

The pathogenicity of the isolates was estimated as the percentage of inhibition of seed germination and the inhibition of young seedling's root development (Broders 2008).

For pot assays, the inoculum from each isolate was transferred to three 500 ml pots, and ten Aleppo pine seeds were sown in each pot with three replicates for each isolate (Broders 2008). Four weeks later, seedlings were carefully removed from the pots, and excess soil was gently removed from roots (the remaining soil was considered rhizosphere soil). Roots were then washed under running tap water for 2 min. Shoot and root length was recorded for each seedling according to Menzies et al. (2005). Symptoms on roots were rated as follows: (*i*) no symptom, (*ii*) slight root rot and discoloration, (*iii*) moderate root rot, (*iv*) extensive root rot. Flasks was inoculated as described by Teymoori et al. (2012). The disease incidence was calculated using Eq. 1 (Song et al. 2004):

$$\frac{\sum \text{scale} \times \text{number of infected seedlings} \times 100}{\text{highest scale} \times \text{total number of seedlings}} \quad (1)$$

The germination rate, root length inhibition, and the shoot growth inhibition were calculated. The

seedling vigour index in each treatment was calculated by Eq. 2 (Maisuria, Patel 2009):

$$\text{Seedling vigour index} = (\text{root length} + \text{shoot length}) \times \times \text{seed germination (\%)} \quad (2)$$

Koch's postulates were performed for all isolates tested.

Statistical analysis. Differences between the means were compared by one-way ANOVA at a 5% level of significance. All statistical analyses were performed using the SAS software (Version 8.1, 2000).

RESULTS

Effect of *Pythium* spp. on pine seed germination and seedling development

All tested *Pythium* species were isolated from diseased pine seedlings with stand establishment problems. The germination and root development of pine seedlings were significantly affected by *P. ultimum*, *P. glomeratum* and *P. heterothallicum*. Furthermore, the germination of pine seeds inoculated with different species of *Pythium* varied considerably. Petri dish assay demonstrated that the highest inhibition percentages of seed germination (87.6 and 65.38%) were recorded for *P. ultimum* (isolates U7CR, U3CR, U14CR, U2CR, U7RT, U1RT). Similarly, *P. heterothallicum* showed 65.38% of seed inhibition and 86.6% of root development reduction. The root growth was also significantly affected by the *Pythium* species. The latter resulted in a significant root growth reduction from 92.2 to 44.87%. The seed germination in pot assay was reduced significantly by different species of *Pythium* (Table 2). The inhibition of germination varied from 72 to 28% and of the root growth for the *P. ultimum* isolates varied from 68.38 to 32.85%. All species of *P. ultimum* were significantly ($P < 0.001$) more pathogenic as compared to the control. However, there was also a significant reduction in the root growth ($P < 0.001$). This confirms that this isolate has a negative impact on germination and seedling root development. The two tested *P. glomeratum* isolates (U35RS and U16RS) showed lower pathogenicity on Aleppo pine seedlings.

Effect of *Pythium* spp. on growth parameters and pathogenicity of Aleppo pine seedlings

Pythium species were able to successfully infect pine seedlings in soil experiments. All species of *Pythium* significantly reduced growth parameters of pine seedlings as compared to the control. The *Pythium* species caused necrotic lesions on the roots.

Table 2. Effect of *Pythium* isolates on seed germination and root development of Aleppo pine seedlings in Petri dish and pot assays

No.	Code	Germination inhibition (%)		Root growth reduction (%)	
		Petri dish	pot	Petri dish	pot
1	U3CR	73.09 ± 3.85^{ab}	72 ± 4.00^{a}	83.90 ± 3.90^{ab}	68.38 ± 2.98^{a}
2	U7CR	87.61 ± 3.84^{a}	60 ± 4.00^{ab}	92.2 ± 1.58^{a}	50.87 ± 2.58^{bcd}
3	U6RR	38.45 ± 10.17^{efg}	40 ± 0.00^{cdef}	74.87 ± 0.98^{abcd}	45.11 ± 6.21^{cde}
4	U8CR	53.84 ± 6.66^{cdef}	44 ± 4.00^{bcde}	69.14 ± 2.23^{bcdef}	41.96 ± 1.57^{def}
5	U6CR	57.68 ± 3.84^{bcde}	32 ± 10.58^{def}	76.65 ± 1.28^{bcd}	46.14 ± 2.93^{cde}
6	U14CR	73.07 ± 3.84^{ab}	44 ± 8.00^{bcde}	80.63 ± 3.82^{abc}	42.40 ± 2.58^{def}
7	U2RR	61.53 ± 3.84^{def}	52 ± 12.00^{bc}	75.66 ± 4.97^{abcd}	51.55 ± 4.09^{bcd}
8	U2CR	73.07 ± 10.17^{ab}	32 ± 4.00^{def}	85.36 ± 2.50^{ab}	47.53 ± 2.11^{bcde}
9	U4CR	49.99 ± 3.84^{cde}	28 ± 6.92^{ef}	84.17 ± 1.09^{ab}	38.23 ± 4.62^{efg}
10	U5RT	30.76 ± 6.65^{g}	44 ± 4.00^{bcde}	44.87 ± 4.33^{hg}	46.74 ± 8.07^{cde}
11	U12RT	57.68 ± 3.84^{bcde}	60 ± 4.00^{ab}	77.72 ± 3.30^{abc}	57.25 ± 2.69^{b}
12	U7RT	65.38 ± 6.66^{abc}	40 ± 0.00^{cdef}	84.70 ± 2.37^{ab}	39.15 ± 2.05^{efg}
13	U17RT	46.14 ± 3.84^{def}	32 ± 4.00^{def}	62.26 ± 4.21^{bcdef}	32.85 ± 1.16^{fg}
14	U1RT	69.22 ± 3.84^{abc}	56 ± 4.00^{abc}	79.61 ± 1.83^{abc}	54.65 ± 2.68^{bc}
15	U35RS	30.76 ± 6.66^{g}	23 ± 8.00^{def}	47.96 ± 1.52^{fg}	29.25 ± 1.53^{g}
16	U16RS	38.45 ± 3.84^{fg}	48 ± 4.00^{bcd}	46.92 ± 8.25^{fg}	46.94 ± 2.74^{cde}
17	U15RS	65.38 ± 6.66^{abc}	48 ± 4.00^{bcd}	86.60 ± 2.02^{ab}	47.72 ± 2.35^{bcde}

means compared by one-way ANOVA, values in a column followed by different letters are significantly different from the control treatment at $P > 0.05$

Observed 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 decreased the seedling root length (Table 3). On the other hand, the length of the pine seedling shoots resulting from the inoculated seeds was significantly reduced. The influence of various *Pythium* species on seedling shoot and root length can be appreciated from the vigour index. The vigour index of isolates was significantly different. Our results showed that *P. ultimum* isolates were highly pathogenic to Aleppo pine seeds when compared to *P. glomeratum* and *P. heterothallicum*. Moreover, *P. ultimum* was able to cause lesions on seedling roots. Brown lesions developed on seedling roots as well as those on the root tips that restricted root growth. This species was successfully recovered from the diseased tissue upon re-isolation.

Root discoloration increased rapidly and exceeded the inoculated plants, whereas all roots of non-inoculated control plants remained white. The incidence of colonization of inoculated plant root segments by *P. ultimum* was higher than in the control at all sampling times. Following conventional destructive harvesting, the estimation of the shoot and root development was significantly lower in the inoculated plants as compared with those in the control (Table 3). The shoots and roots were shortened in length and discoloured. Roots of the control grew continuously during the experiment, whereas in the inoculated plants, the length of the longest root decreased over time. The root and shoot length was significantly shorter.

DISCUSSION

The present work was aimed to evaluate the pathogenicity of different *Pythium* species on Aleppo pine seedlings from Algerian forest nurseries. The ability of each *Pythium* isolate to cause damping-off (either pre- or post-emergence) was assessed by recording the number of surviving seedlings in each replicate Petri dish and pot weekly for 4 weeks and the counts were compared with the negative control. Our results revealed the presence of several *Pythium* species in the forest nurseries of north-western Algeria. These species were implicated in pine seedling damping-off and root rot disease. Numerous *Pythium* species have already been associated with conifers, conifer seedlings, and forest nursery soils (Weber et al. 2004). Of these, *Pythium aphanidermatum* (Edson) Fitzpatrick, *Pythium debaryanum* R. Hesse, *Pythium irregulare* Buisman, *Pythium mamillatum* Meurs and *P. ultimum* were reported to be the most common pathogenic species of conifer seedlings (Weiland et al. 2013). *P. ultimum* was reported for first time in Algeria and the Mediterranean Basin as the causal organism of Aleppo pine seedling damping off (Lazreg et al. 2013).

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

No.	Code	Shoot growth reduction (%)	Incidence (%)	Seedling vigour index
1	U3CR	66.39 ± 2.98^{a}	89.67 ± 10.33^{a}	800 ± 125.94^{j}
2	U7CR	41.31 ± 8.83^{cd}	77.78 ± 11.11^{abc}	1,850 ± 152.75^{ghi}
3	U6RR	38.78 ± 4.15^{cd}	57.78 ± 4.44^{defg}	2,990 ± 245.83^{cdef}
4	U8CR	34.97 ± 1.41^{cd}	56.11 ± 12.18^{defg}	2,987 ± 183.33^{cdef}
5	U6CR	30.97 ± 3.02^{d}	51.06 ± 2.35^{efgh}	3,757 ± 638.61^{bcd}
6	U14CR	40.04 ± 3.73^{cd}	60.74 ± 3.22^{cdefg}	2,873 ± 503.01^{cdef}
7	U2RR	44.21 ± 3.37^{bc}	70.37 ± 3.70^{bcd}	2,240 ± 710.86^{fghi}
8	U2CR	35.57 ± 4.54^{cd}	59.26 ± 4.89^{defg}	3,457 ± 219.42^{bcde}
9	U4CR	37.51 ± 7.44^{cd}	44 ± 4.44^{h}	3,840 ± 376.44^{bc}
10	U5RT	41.31 ± 5.85^{cd}	64.82 ± 1.85^{bcde}	2,810 ± 400.55^{defg}
11	U12RT	54.52 ± 2.93^{ab}	79.63 ± 6.67^{ab}	1,527 ± 138.60^{ij}
12	U7RT	31.22 ± 5.33^{d}	48.89 ± 1.11^{efgh}	3,377 ± 147.69^{bcde}
13	U17RT	30.19 ± 5.00^{de}	46 ± 6.66^{fgh}	4,040 ± 158.22^{b}
14	U1RT	54.71 ± 2.09^{ab}	72.22 ± 5.55^{bcd}	1,730 ± 194.25^{hij}
15	U35RS	17.51 ± 0.60^{e}	34.92 ± 1.58^{h}	5,070 ± 575.02^{a}
16	U16RS	35.98 ± 5.28^{cd}	56.66 ± 1.66^{defg}	2,640 ± 160.73^{efgh}
17	U15RS	40.51 ± 0.14^{cd}	62.50 ± 4.16^{bcdef}	2,716 ± 190.42^{efgh}

means compared by one-way ANOVA, values in a column followed by the same letters are not significantly different from the control treatment at $P > 0.05$

Regarding the pathogenicity of *Pythium* species on Aleppo pine seedlings, the studied species were characterized as weakly or highly virulent pathogens depending on their ability to reduce seedling survival and to cause root lesions. Seedlings inoculated with *Pythium*, regardless of the species, were always more likely to have root lesions than those from the negative control treatment. The higher percentage of lesions on inoculated seedlings indicates the potential for all of the *Pythium* species tested to affect root health and to be considered as pathogens. Most damage by damping-off pathogens in forest nurseries occurs in a few weeks following germination when the root system is small and the root tissues are still relatively young and succulent (James 2012). *Pythium* species are frequently considered root "nibblers" (Sutherland, Dennis 1992), and the presence of root lesions could adversely affect seedling health. Findings we report in the present study expand the number of known *Pythium* species that are pathogenic to seedlings of Aleppo pine. The isolates of *P. ultimum* var. *ultimum* Trow were characterized as a highly virulent pathogen based on their ability to reduce seedling survival and cause root lesions. The results of the inoculation experiments illustrate the interspecific variation in pathogenicity of *Pythium* spp.

Weiland (2014) tested 16 *Pythium* species on Douglas-fir seedlings, although these species were classified into virulent to weakly virulent; eight species (*P. mamillatum*, *Pythium rostratifingens* de Cock & Lévesque, *Pythium* aff. *oopapillum* Bala, de Cock & Lévesque, *Pythium dissotocum* Drechsler, *Pythium sylvaticum* W.A. Campbell & F.F. Hendrix, *P. ultimum*, *Pythium* aff. *macroporum* Vaartaja & Plaats-Niterink, and *P. irregulare*) reduced the survival of Douglas-fir seedlings by at least 25% and were considered highly virulent species. Although the remaining species reduced seedling survival by less than 25%, these species did cause significantly more root lesions than were observed on the non-inoculated seedlings, and were therefore considered weakly virulent species.

CONCLUSIONS

Results of this study indicated that *P. ultimum* and *P. heterothallicum* may be the most important pathogens causing damping-off and root rot of pine seedlings in Algeria. Evidences are now available on the occurrence of these soil-borne pathogens as serious root rot pathogens that could be important sources of inoculum. The information obtained will serve as basic knowledge regarding pathogenicity on *Pythium* species and will facilitate the development of pathogen specific disease control practices.

References

Broders K.D. (2008): Seed and seedling disease of corn and soybean in Ohio: The role of *Fusarium graminearum*, *Pythium* species diversity, fungicide sensitivity, *Pythium* community composition, and soil properties in disease severity. [Ph.D. Thesis.]. Columbus, Ohio State University: 204.

Dick M.W. (1990): Keys to *Pythium*. Reading, University of Reading: 64.

James R.L. (2012): Damping-off. In: Cram M.M., Frank M.S., Mallams K.M. (eds): Forest Nursery Pests. Agriculture Handbook No. 680. Washington, DC, USDA Forest Service: 115–116.

Kageyama K. (2014): Molecular taxonomy and its application to ecological studies of *Pythium* species. Journal of Genetic and Plant Pathology, 80: 314–326.

Kirkpatrick M.T., Rothrock C.S., Rupe J.C., Gbur E.E. (2006): The effect of *Pythium ultimum* and soil flooding on two soybean cultivars. Plant Disease, 90: 597–602.

Lazreg F., Belabid L., Sanchez J., Gallego E., Bayaa B. (2014): Pathogenicity of *Fusarium* spp. associated with diseases of Aleppo pine seedlings in Algerian forest nurseries. Journal of Forest Science, 60: 115–120.

Lazreg F., Belabid L., Sanchez J., Gallego E., Garrido-Cardinas J.A., Elhaitoum A. (2013): First report of *Globisporangium ultimum* causing *Pythium* damping-off on Aleppo pine in Algeria, Africa and the Mediterranean region. Plant Disease, 97: 1111.

Maisuria K.M., Patel S.T. (2009): Seed germinability, root and shoot length and vigor index of soybean as influenced by rhizosphere fungi. Journal of Agricultural Sciences, 22: 1120–1122.

Menzies J.G., Ehret D.L., Koch C., Hall J.W., Seifert K.A., Bissett J., Barr D.J.S. (2005): Fungi associated with roots of cucumber grown in different greenhouse root substrates. Canadian Journal of Botanic, 83: 80–92.

Mezali M. (2003): Rapport sur le secteur forestier en Algérie. Available at: http://www.un.org/esa/forests/pdf/national_reports/unff4/algeria.pdf

Pahlavani S., Razavi E., Kavusi F., Hasanpoor M. (2009): Influence of temperature and genotype on *Pythium* damping-off in safflower. Journal of Plant Breeding and Crop Science, 1: 1–7.

Pankhurst C.E., McDonald H.J., Hawke B.G.H. (1995): Influence of tillage and crop rotation on the epidemiology of *Pythium* infections of wheat in a red-brown earth of South Australia. Soil Biology and Biochemistry, 27: 1065–1073.

Plaats-Niterink A., Vander J. (1981): Monograph on the Genus *Pythium*. Studies in Mycology. Baarn, Centraalbureau voor Schimmelcultures: 242.

Quezel P. (1986): Les pins du groupe «*Halepensis*»: Ecologie, végétation, écophysiologie. Options Méditerranéennes, Series B, 86: 11–25.

Ravishankar R.V., Mamatha T. (2005): Seedling diseases of some important forest trees and their management. In: Sutherland J.R., Procházková Z. (eds): Proceedings of IUFRO Meeting on Diseases and Insects in Forest Nurseries, Uherské Hradiště, Sept 10–15, 2005: 51–63.

Rosso M.L., Rupe J.C., Chen P., Mozzoni L.A. (2008): Inheritance and genetic mapping of resistance to *Pythium* damping-off caused by *Pythium aphanidermatum* in 'Archer' soybean. Crop Science, 48: 2215–2222.

Song W., Zhou L., Yang C., Cao X., Zhang L., Liu X. (2004): Tomato *Fusarium* and its chemical control strategies in hydroponic system. Crop Protection, 23: 243–247.

Sutherland J., Dennis J. (1992): *Pythium* water mould in British Columbia forest nurseries. Seed Seedling Extension Topics, 5: 9–10.

Teymoori S., Shahri M.H., Rahnama K., Afzali H. (2012): Identification and pathogenicity of *Pythium* species on Cantaloupe in Khorasan Razavi province of Iran. Journal of Crop Protection, 1: 239–247.

Weber R.W.S., Sulzer F.L., Haarhaus M. (2004): *Pythium undulatum*, cause of root rot of *Abies procera* Christmas trees and *Pseudotsuga menziesii* in northern Germany. Mycological Progress, 3: 179–188.

Weiland J.E. (2014): Understanding the pathology of Douglas-fir seedlings in Pacific Northwest nurseries. In: Wilkinson K.M., Haase D.L., Pinto J.R. (eds): RMRS-P-72: National Proceedings: Forest and Conservation Nursery Associations, Lafayette, July 22–25, 2013: 38–40.

Weiland J.E., Beck B.R., Davis A. (2013): Pathogenicity and virulence of *Pythium* species obtained from forest nursery soils on Douglas-fir seedlings. Plant Disease, 97: 744–748.

Zhang B.Q., Yang X.B. (2000): Pathogenicity of *Pythium* populations from corn soybean rotation fields. Plant Disease, 84: 94–99.

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