Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz
Journal of Plant Diseases and Protection
110 (5), 437–443, 2003, ISSN 0340-8159
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Fusarium species on seeds of *Pinus taeda* L. and *Pinus elliottii* Engelm. in Argentina

Fusarium-Arten auf den Samen von *Pinus taeda* und *Pinus elliottii* Engelm. in Argentinien

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Received 9 October 2002; accepted 25 June 2003

Summary

The quality of planted seeds has a critical influence on the ability of crops to become established and to realize their full potential of yield. Loblolly pine (*Pinus taeda* L.) and slash pine (*Pinus elliottii* Engelm.) are forest trees species, both native to the southern United States of America, mostly planted in Northeastern Argentina for site restoration and forest management due to their fast growth on diverse sites. Numerous diseases attack these two pine species in forest nurseries: damping-off and root rot. Many *Fusarium* species present on seeds were found to be associated with damping-off and root diseases in loblolly and slash pine seedlings. Seed of *Pinus elliottii* and *P. taeda* were collected from Northeastern Argentina and analyzed for seed-borne *Fusarium.* Seed treatments as running water for 24 and 48 h were tested for effectiveness in changing *Fusarium* populations on seeds. Seven different species of *Fusarium* were isolated from the seed lots assayed: *F. solani, F. verticillioides (= F. moniliforme), F. oxysporum, F. proliferatum, F. incarnatum (= F. pallidoroseum = F. semitectum), F. equiseti* and *F. acuminatum. Fusarium* populations detected on seeds changed after running water treatments for 24 and 48 h but this changes varied among seedlots. This is the first report on *Fusarium* species associated with *P. elliottii* and *P. taeda* seeds in Argentina.

Key words: *Fusarium* species; seedborne fungi; conifer seeds; forest seeds; seed quality

Zusammenfassung

Die Qualität ausgesäter Samen hat einen entscheidenden Einfluss auf die Fähigkeit der Pflanzen, sich zu etablieren und ihr volles Ertragspotential zu realisieren. Sumpfkiefer *(Pinus taeda)* und Karibische Kiefer (*Pinus elliottii* Engelm.) sind Arten von Forstbäumen, die beide im Süden der USA heimisch sind. Wegen ihres schnellen Wachstums werden sie im Nordosten Argentiniens zur Aufforstung und in der Forstwirtschaft angepflanzt. Zahlreiche Krankheiten befallen diese Kiefern in der Baumschule, z. B. Umfallkrankheit und Wurzelfäule. Für viele auf den Samen vorhandene *Fusarium*-Arten wurde eine Assoziation mit der Umfallkrankheit und den Wurzelkrankheiten an den Jungpflanzen der Sumpf- und Karibischen Kiefer festgestellt. An Samen von *Pinus elliottii* und *P. taeda* aus dem Nordwesten Argentiniens erfolgte eine Untersuchung auf samenbürtige Fusarien. Dabei sollte auch festgestellt werden, ob eine 24- bzw. 48-stündige Behandlung mit fließendem Wasser zu Veränderungen der Populationen von *Fusarium*-Arten auf den Samen führte. Sieben verschiedene *Fusarium*-Arten konnten von den untersuchten Samenproben isoliert werden: *F. solani, F. verticillioides (= F. moniliforme),*

F. oxysporum, F. proliferatum, F. incarnatum (= F. pallidoroseum = F. semitectum), F. equiseti und *F. acuminatum.* Die auf den Samen gefundenen *Fusarium*-Populationen veränderten sich nach 24- bzw. 48-stündiger Behandlung mit fließendem Wasser, aber zwischen den Samenproben traten Unterschiede auf. Hiermit wird zum ersten Mal über das Vorkommen von *Fusarium*-Arten auf Samen von *P. elliottii* und *P. taeda* in Argentinien berichtet.

Stichwörter: *Fusarium*-Spezies; samenbürtige Pilze; Koniferensamen; Samenqualität

1 Introduction

Loblolly pine (*Pinus taeda* L.) and slash pine (*Pinus elliottii* Engelm.) are ideal tree species for site restoration and forest management due to their fast growth on diverse sites. They are both native to the Southern United States of America but their adaptability and potential for culture in other parts of the world has long been recognized (Schultz 1997). They were first introduced into South America in 1948 and are presently the leading pine species in the northeastern part of Argentina (Golfari 1970). Both species are grown on 15- to 25-year rotations for both fiber and solid wood products. More than 170.000 ha in the Misiones and Corrientes Provinces of northeastern Argentina next to southern Paraguay and southern Brazil (approximate latitudes 27° to 29° S) are planted with both loblolly and slash pines (Crotto 1987).

Numerous diseases attack these pines species in seedling nurseries. Seeds are infested with pathogenic micro-organisms which may be present within seeds or on seed coats. Some of the fungi present on the outside of the seeds as well as systemic growing ones such as *Caloscypha fulgens* and *Sphaeropsis sapinea* can cause new germinants to damp off before emerging (pre-emergence damping-off). Furthermore, several fungal genera such as *Fusarium, Pythium, Curvularia, Phomopsis, Pestalotia,* and *Aspergillus* can cause post-emergence damping-off (Hamm et al. 1990). Internal fungi can develop during the different physiological phases in the seed production field (McGee 1995) or through cracks in the seed coat, whereas external fungi can develop on the seed at any time after the seed coat is formed (Anderson et al. 1984).

The effects of seed-borne *Fusarium* on the quality and quantity of germination of seeds of conifers has been the subject of much debate (Mittal et al. 1990; Mittal and Wang 1993; Richardson 1979, 1981). Many *Fusarium* species present on seeds were found to be associated with damping-off and root diseases in loblolly and slash pine seedlings (Mason and van Arsdel 1978; Huang and Kuhlman 1990). In addition, seed-borne *Fusarium circinatum* Nirenberg & O'Donnell (= *F. subglutinans* f. sp. *pini*), the causal agent of pitch canker, cause an economically important disease of planted loblolly and slash pines in the USA, mainly shoot dieback in the upper crown, bleeding resinous cankers on the trunk and larger branches which can lead to tree mortality (Dwinell and Phelps 1977; Dwinell et al. 1985). The latter disease causes a reduction in the quality and quantity of viable seeds, and mortality and deterioration of internal seed tissues (Anderson 1986; Schultz 1997). Viljoen and Wingfield (1994) reported the presence of *F. circinatum* for the first time in the Southern Hemisphere, as causing root diseases of *P. patula* seedlings in forest nurseries in South Africa. Recently, Wingfield et al. (2002) reported this fungus on *P. radiata* seedlings in forest nurseries in Chile. Although the latter disease has not yet been found on pine trees in plantations in Argentina, it is currently regarded as one of the most serious diseases of pines in the world as *F. circinatum* also occurs in the USA, Japan and Mexico (Muramoto and Dwinell 1990; Viljoen and Wingfield 1994; Wingfield et al. 2002) and it can be introduced into new areas on seed and infected plant material (Britz et al. 2001). Considering that all these diseases can be transmitted by infected seeds, the primary aim of this study was to identify seed-borne *Fusarium* taxa associated with seeds of *P. taeda* and *P. elliottii* in Argentina as well as the effects of running water soak on Fusaria population.

2 Materials and methods

2.1 Seed samples

Two seedlots of loblolly pine and two seedlots of slash pine were selected for this study. Slash pine seedlots were collected from the provinces of (i) Corrientes (Subtropical Region) Latitude: 27° 30' S;

Longitude: 58° 30' W; Elevation: 50 m and (ii) Buenos Aires (Temperate Region) Latitude: 35° S; Longitude: 58° W; Elevation: 50 m. One of the loblolly pine seedlots was collected in the province of Misiones (Subtropical Region) Latitude: 26° 30' S; Longitude: 54° 30' W; Elevation: 300 m, Argentina. All these three seedlots were harvested (current's year collection) from orchards within the forest nurseries. The other loblolly pine seedlot came from Alabama (USA) and was used to compare the mycoflora present.

Seeds were surface-dried to a moisture content between 10 and 11 %, placed in double plastic bags and stored at 2–3 °C in order to prevent mold development, which may develop under high humidity conditions during extended stratification (Campbell and Landis 1990), until processed.

2.2 Seed treatments

In all treatments, 200 seeds were used and after treatment, 10 seeds/plate were plated onto a modified Nash and Snyder medium (Nash and Snyder 1962; Lori and Wolcan 1996). The following treatments were performed for both pine species either to speed germination and to reduce fungal contamination or allow growth of the fungi present in the seed coats: 1-Seeds rinsed under running water for 24 h with periodic gentle agitation and 2-Seeds rinsed under running water for 48 h with periodic gentle agitation. Seeds rinsed with sterile distilled water for a few minutes were used as the untreated control seeds. All treatments were set up at the same time. The dishes were incubated at 22–25 °C in the darkness and monitored for the presence of fungi after 7, 14, 21 and 30 days. Macroscopical examination of the seeds did not reveal visible fungal mycelium, prior to initiating the experiments.

2.3 *Fusarium* identification

For taxonomic identification, the colonies were transferred to potato-dextrose agar (PDA); single spores were then isolated from each colony, plated on PDA medium and incubated 7–14 days at 22 °C under fluorescent lamps supplemented with NUV light during 12 h photoperiod. The micromorphology and culture features were examined on PDA, carnation leaf agar (CLA) and ClK agar medium (Fischer et al. 1983). The isolates were identified according to the system of Booth (1971) and Nelson et al. (1983).

2.4 Statistical analysis

The data were subjected to analysis of variance and the means were compared using LSD test ($P < 0.05$). To correct for heterogenity of variance, the percentage data were arcsin-transformed prior to analysis. The analysis was performed using the STATGRAPHICS program (7.0).

3 Results

3.1 *Fusarium* spp. isolated from *Pinus elliottii* seedlots

Both lots yielded *Fusarium* species but there were differences in occurrence (Table 1). The following species were isolated from either sources: *F. oxysporum* Schlechtend.: Fr., *F. solani* (Mart.) Sacc., *F. equiseti* (Corda) Sacc., *F. acuminatum* Ellis & Everh., *F. incarnatum* (Rob.) Sacc. (= *F. pallidoroseum* Cooke) Sacc. = *F. semitectum* Berk. and Rav.), *F. proliferatum* (T. Matsushima) Nirenberg. and *F. verticillioides* (Sacc.) Nirenberg (= *F. moniliforme* Sheldon).

Six *Fusarium* species were isolated on seeds from Buenos Aires. Total *Fusarium* population decreased after the running water rinse treatment. *F. solani* increased and *F. acuminatum* was recovered after water rinse. There were significant differences ($P < 0.05$) on total *Fusarium* levels between treated and untreated seeds (Table 1).

Seeds collected at Corrientes yielded five species. Water rinse increased levels of *F. oxysporum, F. solani* and *F. equiseti* whereas this treatment decreased levels of *F. proliferatum* and *F. verticillioides.* Significant differences ($P < 0.05$) were observed on total Fusaria between the treatments (Table 1).

Table 1. *Fusarium* species isolated from *Pinus elliottii* and *Pinus taeda* seeds collected from different places

Fusarium spp. (%)	*Pinus elliottii* Corrientes			*Pinus elliottii* Buenos Aires			*Pinus taeda* Misiones			*Pinus taeda* Alabama (USA)		
	C[1]	WR 24 h	WR 48 h	C	WR 24 h	WR 48 h	C	WR 24 h	WR 48 h	C	WR 24 h	WR 48 h
F. oxysporum	5	10	28	2	0	0	24	5	0	3	6	0
F. solani	0	0	5	3	4	6	26	50	48	0	0	5
F. equiseti	0	3	4	9	0	2	0	5	0	2	0	0
F. acuminatum	0	0	0	0	2	1	0	0	0	2	0	0
F. incarnatum	0	0	0	11	2	1	20	0	10	4	0	0
F. proliferatum	11	0	0	0	0	0	6	19	12	0	5	7
F. verticillioides	18	4	0	7	4	4	24	20	26	5	9	8
Total Fusaria	34b[2]	17cd	37b	32b	12d	14d	100a	99a	96a	16cd	20c	20c

[1] C: untreated seed control; WR: seeds rinsed for 24 and 48 h
[2] Values followed by common letters are not significantly different at $P < 0.05$.

3.2 *Fusarium* spp. isolated from *Pinus taeda* seeds

Six *Fusarium* species were found on *P. taeda* seeds collected from Misiones. The species isolated were *F. oxysporum, F. solani, F. equiseti, F. incarnatum, F. proliferatum* and *F. verticillioides.* The same six species were identified in *P. taeda* seeds from USA and in addition *F. acuminatum* was detected. After rinsing the seeds with running water either for 24 or 48 h, levels of *F. oxysporum* decreased whereas levels of *F. solani* greatly increased. After treatments with either running water, *F. verticillioides* and *F. proliferatum* had almost the same counts.

Slash pine seed lot from Alabama, USA, showed relatively lower counts compared to the Argentinian seedlot. Several *Fusarium* species were removed after the running water rinse treatment. Levels of *F. proliferatum* and *F. verticillioides* slightly increased after rinsing the seeds with running water.

Common saprophytic seed inhabitants including *Penicillium* spp., *Trichoderma* spp. and *Mucor* sp. were also commonly isolated.

4 Discussion

Information about disease organisms that damage pine seeds from subtropical pine species in Argentina (Southern Hemisphere) is lacking. This is the first report on the *Fusarium* population associated with *P. elliottii* and *P. taeda* seed in Argentina. All *Fusarium* species isolated from both loblolly and slash pine seedlots either from the provinces of Misiones, Corrientes and/or Buenos Aires are common seed-borne fungi reported previously on *Pinus* spp. in the United States (Fraedrich and Miller 1995; Huang and Kuhlman 1990; Oak et al. 1999). Furthermore, *F. oxysporum, F. incarnatum* and *F. verticillioides* were previously isolated from *P. elliottii* and *P. taeda* species in Brazil (Homechin et al. 1986).

Five species *(F. oxysporum, F. equiseti, F. incarnatum, F. proliferatum,* and *F. verticillioides)* have been previously isolated from seeds of *Pinus ponderosa* and *Pseudotsuga menziesii* [Douglas-fir (Mirb.) Franco] in the Sub Antarctic region of Argentina (Lori and Salerno 2002). Two species *(F. acuminatum* and *F. solani)* were isolated for the first time from seeds of pine species in this country.

Fusarium species isolated from either loblolly pine or slash pine seed did not vary whereas *Fusarium* levels did vary and reflect initial differences between both pine species.

Fusaria taxa associated with *P. elliottii* seeds show differences among seedlots collected either in the Subtropical region or the Pampean region. The levels of *Fusarium* spp. recovered from untreated seed were also different. The results demonstrate that seed contamination may vary with physiographic regions, climatic factors, tree conditions and stand conditions, on the condition of equal seed treatment within the tested samples.

F. oxysporum, F. incarnatum, F. proliferatum and *F. verticillioides* were the most abundant species recovered from slash pine seeds collected from the Subtropical Region. Similarly, Fraedrich and Miller (1995) found *F. proliferatum* as *Fusarium* species most often associated with slash pine *(P. elliottii* var. *elliottii)* seeds. The latter authors also found *F. incarnatum* associated with *P. elliottii* seedlots but this *Fusarium* species was not recovered from seed collected in the Subtropical region. In our study, *F. incarnatum* was only isolated from seed collected in the Pampean region. Furthermore, Anderson et al. (1984) reported *F. verticillioides* among the known pathogens recovered from *P. elliottii* seeds. In this sense, several conifer seedling diseases have been attributed either to *F. proliferatum* and/or *F. verticillioides* (Chakravarty and Unestam 1986; Fraedrich and Miller 1995; Huang and Kuhlman 1990). These three species were also main species along with *F. solani* isolated from *P. taeda* seed collected from Misiones.

F. circinatum, responsible for the pitch canker disease in pine species, was not present neither in slash pine or loblolly pine seeds. Miller and Bramlett (1979) isolated earlier this fungus from gametophyte and embryo tissue of both slash and loblolly pine seeds. Subsequent reports also cited this pathogen on both pine tree seeds (Huang and Kuhlman 1990). The pitch canker fungus *F. circinatum* has been found causing damage to *Pinus radiata* plants in Chilean nurseries (Wingfield et al. 2002), next to the Argentine border, but this pathogen has, as yet not been found on trees in plantations in Chile. The absence of this air-borne pathogen in Chilean plantations (Wingfield et al. 2002) could be the reason why *F. circinatum* has not been found infesting pine seeds in the Subtropical area in Argentina. Inoculum spread to our country is likely to result from sources such as wind, irrigation and insects acting as wounding agents thus contributing to the disease spread in any region. Current research is targeted at determining if the pathogen is largely on the seed surface or if it infects the internal tissues (Dwinell and Fraedrich 1997). In the late 1980s, Dwinell (unpublished) isolated the pitch canker fungus from seeds of Monterey pine in areas where the disease was prevalent, but not from seeds collected at locations in which the disease was absent. At present, the relationship between the surface contamination of pine seeds by *F. circinatum* and pre- and post-emergence damping-off as well as disease in older seedlings has not been fully elucidated (Dwinell et al. 2001).

Seed-borne *Fusarium* taxa as well as diversity in *Fusarium* population changed following running water rinse. The results showed that some *Fusarium* species were removed; this behavior may be due to the fact that running water imbibition removes *Fusarium* species present externally. On the other hand, we have found that other *Fusarium* species were increased which may have resulted from accelerated seed germination and proliferation of internal seed-borne fungi (James 1986). The build-up of *Fusarium* population levels varied between pine species and within seedlots. This behavior was mainly observed on slash pine seeds contaminated with *F. solani* and collected from Misiones. Other *Fusarium* species such as *F. oxysporum,* isolated from seed of *P. elliottii* collected in Corrientes, also increased following the running water rinse treatment. Infected seeds may serve as an inoculum source for damping-off and root diseases in nursery beds caused by *F. oxysporum* considering that this species has been mainly isolated from diseased pine species (Lori and Salerno 2000). Black root rot disease caused by *F. oxysporum* may also destroy loblolly pine roots of older stock which can be a severe problem in nurseries, mainly on survival of seedlings when outplanted in the field (Schultz 1997).

F. equiseti has not been reported earlier as a seed-borne fungus of loblolly pine and slash pine; nevertheless, it has been found pathogenic to loblolly pine when it enters a wound and it is well described as seed-borne on many other tree seed species including *P. caribaea, P. merkusii, P. oocarpa, P. pseudostrobus* and *P. roxburghii.* This fungus is also pathogenic to young loblolly seedlings, but not to older stock (Schultz 1997).

Genetically superior seed orchards have been established earlier in many locations in Argentina, based on seed source trials which provide an adequate amount of reasonably good, locally grown, southern US pine seeds which supplement nowadays available improved seeds. However, *Pinus taeda* seeds collected in Northeastern Argentina were very much contaminated compared to seeds imported from the Southeastern USA. Slash pine seeds coming from the USA are reported to be very often contaminated with *Fusarium* spp. Low levels of *Fusarium* species on loblolly seeds collected from Alabama show that this region continues providing with excellent bulk seeds that produce fast-growing trees (Schultz 1997). Among the *Fusarium* species obtained, *F. acuminatum* was the only species reported previously on imported seeds collected in Alabama, USA (Fraedrich and Miller 1995).

In conclusion, the quality of planted seeds has a critical influence on the ability of crops to become established and their full potential of yield and value be maximized. Significant changes have occurred in the global seed industry that includes the greater importance of seed-transmitted pathogens. Our results show the natural occurrence of diverse *Fusarium* species associated with *P. elliottii* and *P. taeda* seeds in Argentina. Seed-borne *Fusarium* may provide inoculum sources available for pre- and post-emergence damping-off diseases in forest nurseries of both loblolly and slash pine species. Additional work is needed to determine the ability of each *Fusarium* spp. present on seeds to cause disease considering that the mere presence of fungi does not necessarily harm seeds. Nevertheless, it is generally recognized that, as pathogen counts increase, the viability of seed decreases. It has been shown that cultural controls, such as water rinse soaks, which are generally recommended to nursery growers to reduce the possibility of fungal contamination, may not represent the best insurance to minimize *Fusarium* spp. infection and provide an adequate source of high-quality seeds and prevent potential losses from damping-off and root rot diseases of newly germinated seedling.

Acknowledgments

We thank Alicia Bohren (University of Misiones) for supplying the loblolly pine and slash pine seeds used in this study. We thank Mónica Murace and Paula Morelli for assistance in laboratory work.

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