RESEARCH

CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

EVALUATION OF *Trichoderma* spp. AND *Clonostachys* spp. STRAINS TO CONTROL *Fusarium circinatum* IN *Pinus radiata* SEEDLINGS

Priscila Moraga-Suazo[1], Alex Opazo[1], Salomé Zaldúa[1], Gastón González[2], and Eugenio Sanfuentes[1*]

The fungus *Fusarium circinatum* Nirenberg & O'Donnell causes pine pitch canker, an important disease for conifers worldwide. *F. circinatum* was first detected in Chile in 2001 and to date is present in nurseries and clonal hedges from Libertador General Bernardo O'Higgins Region to Los Ríos Region. The purpose of this study was to evaluate the potential of *Trichoderma* spp. and *Clonostachys* spp. strains to control *F. circinatum* in *Pinus radiata* D. Don seedlings in the absence of other effective control methods. Eighty-one *Trichoderma* spp. and *Clonostachys* spp. strains were evaluated through *in vitro* assays to determine their ability to act as antagonists of *F. circinatum* and 21 strains were tested for their ability to reduce post-emergence mortality and increase *P. radiata* survival under greenhouse conditions. During *in vitro* experiments, 15 strains of *Trichoderma* inhibited mycelial growth of the pathogen by more than 60% and one strain of *Clonostachys* showed parasitism of *F. circinatum* hyphae. Greenhouse experiments showed no control of the disease when the antagonists were added to substrate after the pathogen. However, when the antagonists were added before the pathogen, four strains (*Clonostachys* UDC-32 and UDC-222 and *Trichoderma* UDC-23 and UDC-408) reduced post-emergence mortality between 80 and 100%. Among these strains, only *Clonostachys* UDC-222 significantly increased the survival of *P. radiata* seedlings. These results showed that *Clonostachys* UDC-222 has the potential to be used as a biocontrol agent against *F. circinatum* in the production of *P. radiata* plants.

Key words*:* Antagonists, biological control, seedling death, pitch canker.

Fusarium circinatum Nirenberg & O'Donnell is a necrotrophic fungus that causes pitch canker disease, which affects pine species (Coutinho *et al*., 2007; Wingfield *et al*., 2008; Quesada *et al*., 2010) and *Pseudotsuga menziesii* (Gordon *et al*., 2006). Among the affected pine species, *Pinus radiata* D. Don is considered one of the most susceptible to this pathogen (Gordon *et al*., 2001).

Pine pitch canker was first detected in south-eastern United States and then in Mexico (Schweigkofler *et al*., 2004), where it likely originated (Wikler and Gordon, 2000). More recently, this disease has been reported in California, South Africa, Haiti, Japan, and Spain (Storer *et al*., 2002). In Chile, the presence of the pathogen was reported in late 2001 at *P. radiata* nurseries located between Libertador General Bernardo O'Higgins Region and Los Ríos Region, but rarely in 1 to 4-yr-old plantations, where secondary dissemination has not been reported (Wingfield *et al*., 2002). The behavior of the disease in Chile is similar to that observed in South Africa with *P. patula* Schltdl. & Cham., where the pathogen mostly attacked nursery seedlings and was not associated with pitch canker, the typical symptom in plantations. However, the first outbreaks of pitch canker in *P. patula* plantations have recently been reported in South Africa (Coutinho *et al*., 2007). The absence of field infections in Chile has been attributed to the low frequency of insect vectors, unfavorable climatic conditions for the disease development (Wingfield *et al*., 2002), and low inoculum concentration in the environment.

The practices used to control this disease in Chilean forestry nurseries include cultural measures, such as early detection of symptoms and signs of *F. circinatum* and removal of diseased seedlings, environmental management, pathogen population monitoring in nursery soil and bark substrate, container sterilization, pathogen spread prevention to uninfected areas, and detection of asymptomatic seedlings (González, 2005; Rotella, 2005).

Among the fungicides used to control *F. circinatum* are prochloraz, tebuconazole, and propamocarb (TPCP, 2002). In studies conducted by Mitchell *et al*. (2005), the first two fungicides were the most effective. In Chile, difenoconazole, tebuconazole, and fludioxinil inhibited mycelial growth of the pathogen during *in vitro* tests (González, 2005). Nevertheless, little is known about the most suitable fungicides for controlling *F. circinatum*.

Due to more stringent environmental restrictions on fungicides, the search for alternative methods of disease control has increased, such as biological control. The use of microorganisms, individually or in combination

[1]Universidad de Concepción, Facultad de Ciencias Forestales, Casilla 160-C, Concepción, Chile.
[*]Corresponding author (esanfuen@udec.cl).
[2]BIOCAF S.A., km 18 Camino a Coronel, Chile.
Received: 21 January 2011.
Accepted: 7 June 2011.

with other measures, that reduce the use of pesticides has become increasingly important. Antagonistic microorganisms, including fungi and bacteria, applied as treatments for seeds provide unique and beneficial opportunities for the production of different crops, especially protection against soil-borne pathogenic fungi (Mao *et al*., 1997).

Several studies have used fungi and bacteria to control diseases caused by *Fusarium* species affecting different crops (Mao *et al*., 1997; Mao *et al*., 1998; Bacon *et al*., 2001), including some non-pathogenic strains of *Fusarium* spp. (Paulitz *et al*., 1987; Postma and Rattink., 1992; Larkin and Fravel, 2002; Silva and Bettiol, 2005). Nonetheless, few studies have been done on biological control of *F. circinatum*, among them the use of *Trichoderma harzianum* strains to control the disease in *P. patula* seedlings (Mitchell *et al*., 2005).

Despite the importance of the disease, no effective methods for its control have been found, no chemical products have been registered and no biological agents are being marketed. This limits disease management options in affected nurseries. Due to current and potential damage arising from the presence of *F. circinatum*, the secondary dissemination risk in plantations and the lack of efficient control methods, the objective of this study was to select and evaluate strains of *Trichoderma* spp. and *Clonostachys* spp. for the control of *F. circinatum* in *P. radiata* seedlings.

MATERIALS AND METHODS

Antagonists and pathogen strains

Strains of *Trichoderma* (71 strains) and *Clonostachys* (10 strains) were selected for the assays from the Forest Pathology Laboratory collection from the Universidad de Concepción. These strains have shown activity as antagonists against other pathogens in previous studies (Grandón, 2006; Mellado, 2006; Molina *et al*., 2006). One *F. circinatum* strain (Pr44-4641) was also included, isolated from symptomatic *P. radiata* hedges that had more aggressive behavior in previous pathogenicity tests (González, 2005). Antagonistic fungi and pathogens were stored in tubes containing potato dextrose agar (PDA) as culture medium at 4 ºC. Fungal strains were replicated in Petri dishes containing PDA and incubated at 25 ºC for 4 d (*Trichoderma*) and 7 d (*Clonostachys* and *F. circinatum*).

In vitro antagonistic activity

For the *in vitro* antagonism test, a modified version of the dual culture technique described by Bell *et al*. (1982) was used. A 6 mm diameter plug, containing expanding colonies of *F. circinatum*, was removed from the edge of actively growing culture and placed approximately 1 cm from the edge of a Petri dish containing PDA diluted to 50%. Similarly, using the antagonist strains available, a 6 mm diameter round plug was removed from the actively growing culture and placed directly opposite to the *F. circinatum* sample, approximately 1 cm from the edge of the Petri dish. As control, a 6 mm diameter plug of *F. circinatum* strain was placed onto a separate Petri dish to compare their uninhibited growth with paired cultures. The paired cultures were incubated at 25 °C for 7 d before being assessed. The pathogen plug was inoculated 24 h before the antagonist plug on each Petri dish. After the assay, the mycelial growth inhibition (MGI) of *F. circinatum* was determined along with the presence of parasitism in the pathogen hyphal.

The MGI was obtained by dividing the pathogen colony area into different dual cultures facing the antagonists by the pathogen colony area from the control treatment. Colony surface areas were measured with a dot grid of 0.5 × 0.5 cm.

Parasitism was determined under microscope observation at 100X and 400X, observing the interaction area between colonies confronting each other in the dual cultures. The occurrence of coiled hyphae was considered to be evidence of hyphal parasitism. When no interactions were clearly observed among hyphae, new pairings were made on slides coated with a thin film of agar-water (AW) and incubated at 25 °C for 2 to 3 d.

Control of *F. circinatum* in *P. radiata* seedlings

Eighteen strains of *Trichoderma* and three of *Clonostachys* were used for this study. These strains were selected during an earlier *in vitro* antagonism test; since they showed either higher pathogen mycelial inhibition or hyphal parasitism, and some of these selected strains have also been used to control other pathogens such as *Macrophomina phaseolina* and *Botrytis cinerea* (Grandón, 2006; Molina *et al*., 2006). The experiments were performed in the greenhouse propriety CPF (Controladora de Plagas Forestales) and authorized by the government agency Servicio Agrícola y Ganadero (SAG) for quarantine tests with *F. circinatum*.

Pinus radiata seeds provided by Forestal Mininco S.A, previously surface disinfected for 15 min with hydrogen peroxide, were sown in trays containing pine bark compost previously sterilized by autoclaving twice at 121 °C for 30 min, with an interval of 24 h. Two assays were established simultaneously: in the first assay, the antagonists were applied 7 d before *F. circinatum* inoculation, and in the second assay, the pathogen was inoculated 48 h before antagonists. The substrate was sprayed with separate suspensions containing each antagonist (10^7 to 10^8 conidia mL^{-1}) and pathogen (10^3 conidia mL^{-1}) in sterile distilled water.

Two comparative treatments were used in both assays. In the first treatment, only the pathogen was applied and in the second one, only water was applied as a control. The assays were maintained for 60 d after seedling emergence and two variables were evaluated, post-emergence mortality and seedling survival.

Post-emergence mortality was defined as those seedlings having the symptoms described for damping-off. The post-emergence mortality rate was obtained by dividing the number of dead seedlings by the total seedlings emerged (%). Emerged seedlings were considered as those with cotyledons above the substrate surface. All dead seedlings were collected, surface disinfected with sodium hypochlorite (5% v/v) for 3 min, and then transferred to Petri dishes with PDA medium to determine infection by *F. circinatum*.

The seedling survival rate was obtained by dividing the number of symptom-free *P. radiata* seedlings at the end of the assay by the number of seeds sown (%).

Experimental design and data analysis

The *in vitro* studies used a completely randomized design with four replicates for each strain plus a control (*F. circinatum* only). The experimental unit consisted of one Petri dish. Greenhouse experiments also used a completely randomized design with four replicates, and the experimental unit was composed of five plastic containers with one seedling each.

Statistical data analysis was performed by ANOVA with a significance level of 0.05. All the data were subjected to analysis of homogeneity of variance and normality assumptions and pooled accordingly. Multiple comparisons were made using the Tukey test. Analyses were performed with Statistical Analysis System sofware (SAS Institute, 2000).

RESULTS AND DISCUSSION

***In vitro* antagonistic activity**

The *in vitro* assays showed mainly an inhibitory effect of the antagonistic strains on the growth of *F. circinatum* colony (Figure 1). Many of the *Trichoderma* strains colonized a large area of the culture medium in the plates due to the speed of their mycelial growth, which was higher than that of *F. circinatum*. On the contrary, *Clonostachys* strains normally had slower growth rates than the pathogen. Furthermore, it was also noted that several *Trichoderma* strains sporulated abundantly when growing over the pathogenic colony, thereby indicating that they can be highly competitive for space and nutrients (Figure 1B). Only *Clonostachys* UDC-222 (Figure 1C) was capable of parasitizing *F. circinatum* hyphae (Figures 2A and 2B). None of the tested strains showed antagonism by antibiosis.

From the 81 strains assayed *in vitro*, 76 significantly reduced *F. circinatum* mycelial growth. Of these, 49 inhibited the pathogen by 40 to 60% and 15 by 60 to 68%. All strains inhibiting the mycelial growth belonged to the genus *Trichoderma*. Strains of *Clonostachys* showed lower levels of pathogen inhibition (1-23%), among them *Clonostachys* UDC-A32 had the highest value (Figure 3).

These results are consistent with previous studies

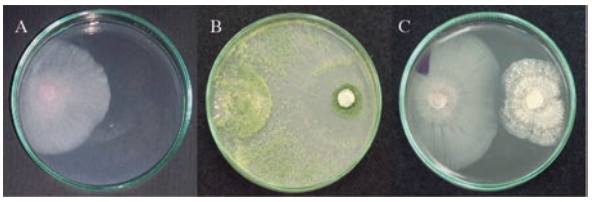


Figure 1. *In vitro* assays to detect antagonistic activity of fungal strains against *Fusarium circinatum*. (A) Control (only *F. circinatum*). (B) *Trichoderma* UDC-412 (right) with a marked inhibition of the mycelial growth of the pathogen (left). (C) *Clonostachys rosea* UDC-222 (right), which showed hyphal parasitism on *F. circinatum* (left).

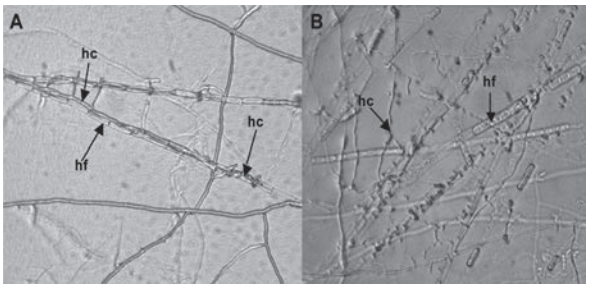


Figure 2. Signs of parasitism of *Clonostachys* UDC-222 (hc) on *Fusarium circinatum* hyphae (hf). Antagonistic hyphae growing along with *F. circinatum* hyphae, where coiling points were observed (A). Hyphae of the pathogen showing numerous points of contact with hyphae of the antagonist (B).

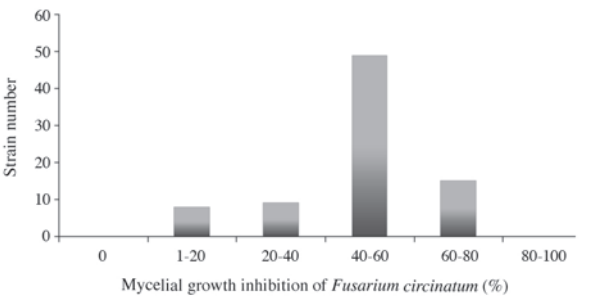


Figure 3. *In vitro* inhibition of the mycelial growth of *Fusarium circinatum* obtained by the antagonistic strains.

in which *Trichoderma* strains have been reported to be agents that inhibit pathogen growth as a biocontrol mechanism (Benítez *et al*., 2004; Vinale *et al*., 2008). In this study, the main mechanism of antagonism detected was space and nutrients competition, expressed as MGI. This mechanism, used by *Trichoderma* spp., has also been found against other *Fusarium* species such as *F. solani* and *F. oxysporum* (Herrera, 2005). In a study with *F. circinatum*, a strain of *T. harzianum* was observed to grow rapidly on the culture medium, restricting the growth of the pathogen and even causing the collapse of the hyphae after 7 d (Mitchell *et al*., 2005).

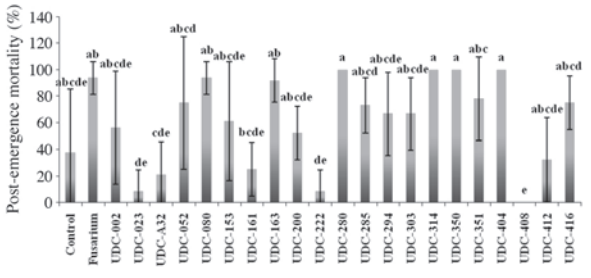
Parasitism of the hyphae was found to be the form of antagonism used in the case of *Clonostachys* UDC-222, identified as *Clonostachys rosea*. The ability of *C. rosea* for mycoparasitism of hyphae, sclerotia, and other fruiting bodies in various types of fungi is well known (Sutton *et al*., 1997). The species also produces fungal inhibitors and

enzymes (glucanases) that degrade cell walls, inducing the loss of turgor and causing lysis of pathogenic hyphae (Papavizas, 1985; Sutton *et al*., 1997).

Control of *F. circinatum* in *P. radiata* seedlings

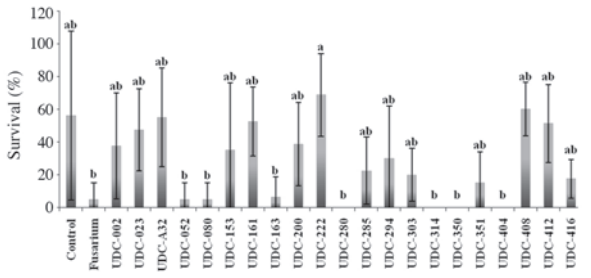
When the pathogen was added to substrate 48 h before antagonists, none of the variables evaluated differed significantly between treatments, with post-emergence mortality of seedlings ranging from 25% to 100%. Only three *Trichoderma* strains (UDC-280, UDC-351, UDC-404) showed less than 50% seedlings mortality. However, these had low survival, indicating that much of the mortality occurred before the seedlings emerged. In the control treatment (without the application of the pathogen), the post-emergence mortality was high (89%). This was attributed to the presence of the inoculant in the greenhouse environment, which allowed the pathogen to colonize the treatment, mainly targeting the aerial parts of plants through the cotyledons (data not shown).

When the antagonists were added to the substrate before adding the pathogen, significant differences were observed in post-emergence mortality and seedling survival (Figures 4 and 5). The application of *Trichoderma* strains UDC-23 and UDC-408 significantly reduced post-emergence mortality, with values of 8.3% and 0%, respectively, compared to 94% mortality obtained when applying *F. circinatum* only. On the other hand, *Clonostachys* strains UDC-A32 and UDC-222 reduced post-emergence mortality to 20.8% and 8.3%, respectively (Figure 4).



Different letters indicate significant differences according to Tukey Test, $\alpha = 0.05$.

Figure 4. Effect on the post-emergence mortality (%) of *Pinus radiata* seedlings of incorporating the antagonists to the substrate prior to the application of *Fusarium circinatum*.



Different letters indicate significant differences according to Tukey Test, $\alpha = 0.05$.

Figure 5. Effect on the survival (%) of *Pinus radiata* seedlings of incorporating the antagonists to the substrate prior to the application of *Fusarium circinatum*.

Regarding the survival of seedlings, the four strains able to reduce post-emergence mortality were the same ones that had the highest values of seedling survival. However, a significant increase was achieved in this variable (69%) by applying *C. rosea* UDC-222. Treatments in which only the pathogen was added had seedling survival rates of about 5% (Figure 5).

The performance of *C. rosea* UDC-222 in reducing post-emergence mortality and increasing seedling survival indicates that this fungus is able to protect seedlings from the beginning of the development of (pre-emergence stage), producing a protective effect on the seed. The better capacity of *C. rosea* UDC-222 to act as a biocontrol agent could be explained in part by its use of two forms of antagonism: competition for space and nutrients and, more importantly, parasitism of the hyphae. Considering that both the pathogen and the antagonist presented similar growth rates, this form of antagonism collapsed pathogenic hyphae, removing it from the substrate and previously colonized tissues (Sutton *et al*., 1997).

Another hypothesis that could explain the success of this strain is its ability to colonize. Some *Clonostachys* strains are known to act as non-pathogenic endophytes, colonizing host tissues without causing alterations in the plant and thereby eliminating the potential development and sporulation of pathogenic fungal agents (Sutton *et al*., 2002). In this case, *C. rosea* UDC-222 could have colonized some seedling tissues before the arrival of the pathogen, allowing a reduction in the incidence of the disease.

This study has demonstrated the importance of timing in the application of biocontrol agents: good results were obtained only when the antagonists were previously incorporated in the substrate. This strategy was also demonstrated in studies with raspberries, where *C. rosea* suppressed sporulation and interfered with infection and colonization of *B. cinerea* most effectively when applied before or when the pathogen was introduced (Yu and Sutton, 1997).

CONCLUSION

These results indicate that *C. rosea* UDC-222 has the ability to reduce the incidence of *F. circinatum* in *P. radiata* seedlings, establishing the potential use of antagonists for the control of this pathogen in forestry nurseries. However, initial results indicate that control is only possible before the pathogen reaches the substrate.

ACKNOWLEDGEMENTS

The authors acknowledge the SAG-funded project "*Fusarium circinatum* Nirenberg & O'Donnell: Conocimiento del patógeno y establecimiento de bases

para su control en *Pinus radiata*", and the CPF for facilitating the greenhouses authorized for work with *F. circinatum*.

Evaluación de cepas de *Trichoderma* spp. y *Clonostachys* spp. para controlar *Fusarium circinatum* en plántulas de *Pinus radiata*. *Fusarium circinatum* Nirenberg & O'Donnell es el hongo que causa el cancro resinoso del pino, una enfermedad de importancia mundial en coníferas. En Chile, *F. cicirnatum* fue detectado por primera vez el año 2001 y a la fecha se encuentra presente en algunos viveros y huertos clonales desde la Región del Libertador General Bernardo O'Higgins hasta la Región de Los Ríos. Debido a la ausencia de métodos eficientes para controlar este patógeno, el propósito de este estudio fue evaluar el potencial uso de cepas de *Trichoderma* y *Clonostachys* como control de *F. circinatum* en plántulas de *Pinus radiata* D. Don. Ochenta y un cepas de *Trichoderma* spp. y *Clonostachys* spp. fueron evaluadas a través de ensayos *in vitro* para determinar su habilidad para actuar como antagonistas de *F. circinatum* y 21 cepas fueron probadas por su habilidad para reducir la mortalidad post-emergencia e incrementar la supervivencia de *P. radiata* bajo condiciones de invernadero. Durante los experimentos *in vitro*, 15 cepas de *Trichoderma* inhibieron el crecimiento del patógeno más de un 60% y una cepa de *Clonostachys* mostró parasitismo de hifas de *F. circinatum*. En experimentos en invernadero, los antagonistas no controlaron la enfermedad cuando fueron agregados después de la inoculación del patógeno. Sin embargo, cuando los antagonistas fueron sembrados antes del patógeno, cuatro cepas (*Clonostachys* UDC-32 y UDC-222, *Trichoderma* UDC-23 y UDC-408) redujeron la mortalidad post-emergencia entre 80 y 100%. Entre estas cepas, sólo *Clonostachys* UDC-222 incrementó significativamente la supervivencia de plántulas de *P. radiata*. Estos resultados demuestran que *Clonostachys* UDC-222 posee gran potencial para ser empleado como agente de biocontrol contra *F. circinatum* en la producción de plantas de *P. radiata*.

Palabras clave: antagonistas, control biológico, muerte de plántulas, pitch canker.

LITERATURE CITED

Bacon, J., I. Yates, D. Hinton, and M. Filmore. 2001. Biological control of *Fusarium moniliforme* in maize. Environmental Health Perspectives 109:325-332.

Bell, D., H. Wells, and C. Marham. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology 72:379-382.

Benítez, T., A. Rincón, M. Limón, and A. Codón. 2004. Biocontrol mechanisms of *Trichoderma* strains. International Microbiology 7(4):249-260.

Coutinho, T., E. Steenkamp, K. Mongwaketzi, M. Wilmot, and M. Wingfield. 2007. First outbreak of pitch canker in South African pine plantation. Australasian Plant Pathology 36:256-261.

González, G. 2005. Resultados preliminares del estudio *Fusarium circinatum*: conocimiento del patógeno y establecimiento de bases para su control. *In* Resúmenes de la XX Silvotecna: Sanidad forestal en un mundo globalizado. Sesión Nº 13. 7-8 November 2005. CORMA Bío Bío, Concepción, Chile.

Gordon, T.R., S.C. Kirkpatrick, B.J. Aegerter, D.L. Wood, and A.J. Storer. 2006. Susceptibility of Douglas fir (*Pseudotsuga menziesii*) to pitch canker, caused by *Gibberella circinata*. Plant Pathology 55:231-237.

Gordon, T.R., A.J. Storer, and D.J. Wood. 2001. The pitch canker epidemic in California. Plant Disease 85:1128-39.

Grandón, G. 2006. Selección *in vitro* de hongos antagonistas a *Macrophomina phaseolina* (Tassi) Goid. Tesis Ingeniero Forestal. Universidad de Concepción, Facultad de Ciencias Forestales, Concepción, Chile.

Herrera, R. 2005. Control biológico de *Rhizoctonia solani*, *Fusarium oxysporum f*. sp. *lycopersici* y *Fusarium solani* en tomates bajo invernaderos. Universidad de Chile Facultad de Ciencias Agronómicas, Santiago, Chile. Available at http://mazinger.sisib.uchile.cl/repositorio/lb/ciencias_agronomicas/montealegre_j/14.html (accessed 16 September 2009).

Larkin, R., and D. Fravel. 2002. Effects of varying environmental conditions on biological control of *Fusarium* wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 92:1160-1166.

Mao, W., J. Lewis, P. Hebber, and R. Lumsden. 1997. Seed treatment with a fungal or a bacterial antagonist for reducing corn damping-off caused by species of *Pythium* and *Fusarium*. Plant Disease 81:450-454.

Mao, W., R.D. Lumsden, J.A. Lewis, and P.K. Hebbar. 1998. Seed treatment using pre-infiltration and biocontrol agents to reduce damping off corn caused by species of *Pythium* and *Fusarium*. Plant Disease 83:294-299.

Mellado, C. 2006. Control biológico de la pudrición carbonosa de la raíz (*Macrophomina phaseolina* (Tassi) Goid). en viveros forestales mediante hongos antagonistas. Tesis Magister en Ciencias, mención Microbiología. Universidad de Concepción, Facultad de Ciencias Biológicas, Concepción, Chile.

Mitchell, G., N. Jones, and T. Coutinho. 2005. Alternatives to benomyl fungicide in controlling *Fusarium circinatum:* results from *in vitro* studies. Forest Shaw Research Centre, SAPPI. Document 3. p. 1-14.

Molina, G., S. Zaldúa, G. González, y E. Sanfuentes. 2006. Selección de hongos antagonistas para el control biológico de *Botrytis cinerea* en viveros forestales en Chile. Bosque 27(2):126-134.

Papavizas, G. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biological control. Annual Review of Phytopathology 23:23-54.

Paulitz, T.C., C.S. Park, and R. Baker. 1987. Biological control of Fusarium wilt of cucumber with nonpathogenic isolates of *Fusarium oxysporum*. Canadian Journal of Microbiology 33:349-353.

Postma, J., and H. Rattink. 1992. Biological control of *Fusarium* wilt of carnation with a non-pathogenic isolate of *Fusarium oxysporum*. Canadian Journal of Botany 70:1199-1205.

Quesada, T., V. Gopal, P. Cumbie, A. Eckert, J. Wegrzyn, D. Neale, *et al*. 2010. Association mapping of quantitative disease resistance in a natural population of loblolly pine (*Pinus taeda* L.). Genetics 186:677-686.

Rotella, A. 2005. Manejo del hongo *Fusarium circinatum* en viveros forestales. *In* Resúmenes de la XX Silvotecna: Sanidad forestal en un mundo globalizado. Sesión Nº 12. 7-8 November 2005. CORMA Bío Bío, Concepción, Chile.

SAS Institute. 2000. User's guide statistic. Version 8.1. 646 p. SAS Institute, Cary, North Carolina, USA.

Schweigkofler, W., K. O`Donnell, and M. Garbelotto. 2004. Detection and quantification of airborne conidia of *Fusarium circinatum*, the causal agent of pine pitch canker, from two California sites by using a real-time PCR approach combined with a simple spore trapping method. Applied and Environmental Microbiology 70:3512-3520.

Silva, J., and W. Bettiol. 2005. Potencial of non-pathogenic *Fusarium oxysporum* isolates for control of *Fusarium* kilt of tomato. Fitopatologia Brasileira 30:409-412.

Storer, A.J., D.L. Wood, and T.R. Gordon. 2002. The epidemiology

of pitch canker of Monterrey pine in California. Forest Science 48:694-700.
Sutton, J., D.W. Li, G. Peng, H. Yu, P. Zhang, and R. Valdebenito-Sanhueza. 1997. *Gliocladium roseum*, a versatile adversary of *Botrytis cinerea* in crops. Plant Disease 81:316-328.
Sutton, J., W. Liu, R. Huang, and N. Owen-Going. 2002. Ability of *Clonostachys rosea* to establish and suppress sporulation potential of *Botrytis cinerea* in deleafed stems of hydroponic greenhouse tomatoes. Biocontrol Science and Technology 12:413-425.
TPCP. 2002. *Fusarium circinatum* in pine nurseries: A guide to appropriate management strategies. Tree Pathology Cooperative Programme (TPCP), University of Pretoria, Pretoria, South Africa. Available at http://www.fabinet.up.ac.za/tpcpweb/pamphlets/fusarium.pdf (accessed 16 September 2009).
Vinale, F., K. Sivasithamparam, E. Ghisalberti, R. Marra, S. Woo, and M. Lorito. 2008. Trichoderma-plant-pathogen interactions. Soil Biology and Biochemistry 40:1-10.
Wikler, K., and T.R. Gordon. 2000. An initial assessment of genetic relationships among populations of *Fusarium circinatum* in different parts of the world. Canadian Journal of Botany 78:709-717.
Wingfield, M.J., A. Hammerbacher, R.J. Ganley, E.T. Steenkamp, T.R. Gordon, B.D. Wingfield, and T.A. Coutinho. 2008. Pitch canker caused by *Fusarium circinatum* - a growing threat to pine plantations and forests worldwide. Australasian Plant Pathology 37:319-334.
Wingfield, M.J., A. Jacobs, T.A. Coutinho, R. Ahumada, and B.D. 2002. First report of the pitch canker fungus, *Fusarium circinatum*, on pines in Chile. Plant Pathology 51:397.
Yu, H., and J. Sutton. 1997. Morphological development and interactions of *Gliocladium roseum* and *Botrytis cinerea* in raspberry. Canadian Journal of Plant Pathology 19:237-246.