SDU Faculty of Forestry Journal
Serial: A, Number: Special Issue, Year: 2009, ISSN: 1302-7085, Page: 200-205

PATHOGENICITY OF *Fusarium circinatum* NIREMBERG & O'DONNELL ON SEEDS AND SEEDLINGS OF RADIATA PINE

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ABSTRACT

Pathogenicity of seven *Fusarium circinatum* isolates from Northern Spain was evaluated on Monterey pine (*Pinus radiata*) seeds and seedlings. The objectives of our study were also to investigate emergence and post-emergence damping-off damage, and to observe differences in pathogenicity among the isolates.

The effect of *F. circinatum* on seed emergence was approximately 10 to 19% lower than the control treatment. However, all *F. circinatum* isolates severely affected pine seedlings, causing 63% to 90% mortality of plants 30-days post inoculation. Sixty days after inoculation, isolate FcCa7 killed all the seedlings, while the less aggressive FcCa2 affected 79% of the plants. We believe this homogeneity in aggressiveness among *Fusarium* isolates may possibly be attributed to the recent introduction of the pathogen in this region.

Key words. Pitch Canker, Biological control, *Pinus radiata*, Nurseries, Plant health care

1. INTRODUCTION

Fusarium circinatum is a pathogenic fungi with great virulence in species of the genus *Pinus*, causing a disease called pitch canker. It was first discovered as a pathogen in California during 1986 (Mccain et al., 1987). Since then, *F. circinatum* was also found in Mexico (Rodriguez, 1989), South Africa (Viljoen et al., 1994; Nirenberg and O'Donnell, 1998; Crous et al., 2000; Steenkamp et al., 2002; Coutinho et al., 2007), Japan (Aoki et al., 2001; Kobayashi, 2007), Chile

(Wingfield et al., 2002; Jacobs et al., 2007), Korea (Cho and Shin, 2004), Italy (Carlucci et al., 2007) and Spain (Landeras et al., 2005; Perez-Sierra et al., 2007).

One of the most important actions to control the disease is to have a better understanding about the populations of the fungus, and it behaviour over plants host. Thus, the aim of this work is to investigate the effect of *F. circinatum* over seeds and seedlings of *Pinus radiata*, and to observe the differences in pathogenicity between isolates.

2. MATERIAL AND METHODS

2.1. Fungal material

Seven different isolates of *Fusarium circinatum* obtained from *Pinus radiata* plantations of the Autonomous Community of Cantabria, in the northern Spain, were used for this study. Malt extract media (20 g/l) was prepared to achieve the spore dissolution of the fungus used in the inoculation (50 ml of autoclaved media in a Erlenmeyer flask). Once esterilized, four pieces of fungal mycelium grown in PDA-S (potato-dextrose-agar with 0.5 g/l of streptomycin sulfate) were placed inside the flasks. Production of spores was induced by using an orbital shaker. After that, the media was filtered in order to collect only spores in the dissolution. In order to obtain the fitted concentration (10^6 spores/ml), we used a Thoma counting chamber.

2.2. Plant material

A total of 672 seeds were sown to observe the effect of the fungus over the plant material. Before sowing them, seeds were washed with sterile distilled water repeatedly and kept there for twelve hours. After that, seeds were maintained in hydrogen peroxide (3%) for 30 minutes. Finally they were washed twice with sterile distilled water to remove the remaining hydrogen peroxide permeating the seeds.

2.3. Substrate. A mixture of peat and vermiculite at 50% were used for the experiment. Before filling the nursery trays, the substrates were autoclaved twice during one hour at 120 ºC.

2.4. Seeds sowing

Seeds were grown in nursery trays, placing four seeds in each hole. Twenty one holes were used for each isolate. After sowing, trays were covered with a transparent plastic paper to prevent the aerial contaminations. The assay was developed in controlled conditions of temperature (20 ºC) and photoperiod (16/8) inside a growth chamber (Figure 1). Seedlings were watered once a week, with twenty millilitres of sterile distilled water, and checked for the progress of the assay.

Figure 1: Growth chamber with the trays inside.

2.5. Data capture

Seed germination was measured once a week. Futhermore dead seedlings were counted ten weeks after the sowing. At the end of the experiment, attempts to reisolate the pathogen from the seedlings were made to verify it presence in the necrotic lesions.

2.6. Statistical analysis

A Kruskal-Wallis test was performed with Statgraphics Plus 5.1. to find differences between germination and mortality taxes of the different isolates.

3. RESULTS AND DISCUSION

3.1. Germination

Despite genus *Fusarium* is considered as one of the most important causes of pre-emergence and post-emergence damping-off (Machon et al., 2006; Pinto et al., 2006), in our present assay fungus did not cause great damages over seeds but, decreasing slightly germination rates. As it can be observed in the Figure 2, CONTROL was the treatment where seeds were more germinated. There were significant differences between this and the others treatments in which *F. circinatum* was present. On the other hand, no differences were observed among the seven isolates of the fungus used in the assay.

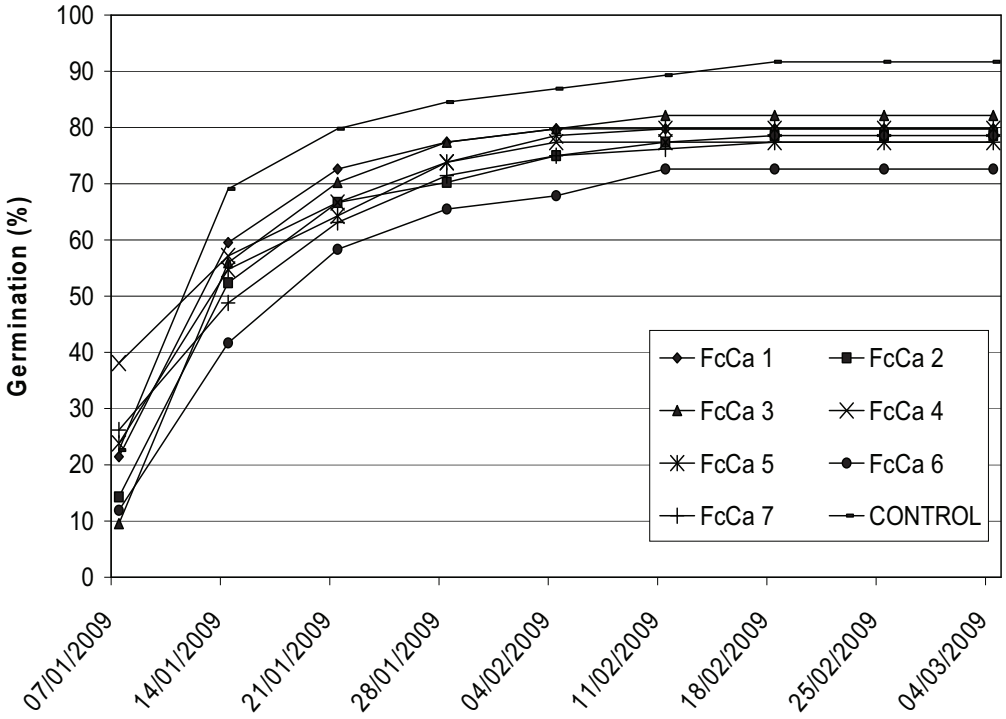

Figure 2: Rates of germination of the seeds depending on the isolate of *Fusarium circinatum* used

3.2. Virulence

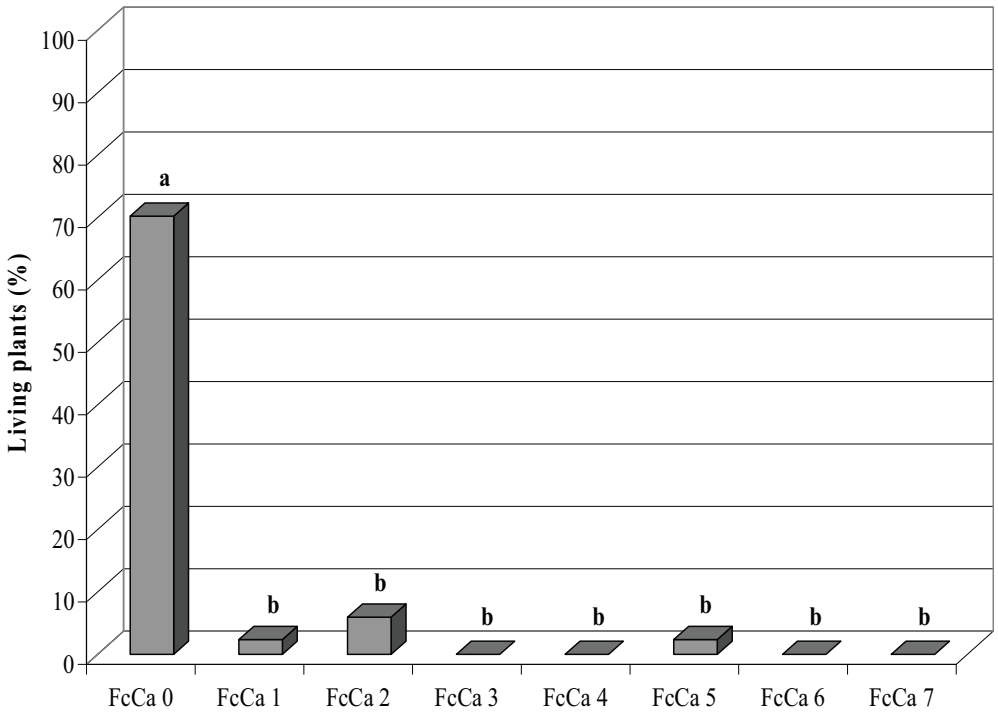

Figure 3: Percentage of living plants ten weeks after the inoculation according to the treatment used

All isolates of *F. circinatum* were highly virulent and significant differences among them were not observed. Aegerter and Gordon (2006) obtained the rates of seedling mortality ranging from 3.5 to 52%, however in our investigation most of the seedlings died after ten weeks of the inoculation. On the other hand, 70% of seedlings were still alive in case of the treatment where the fungus was not present (p-value < 0.001).

4. CONCLUSIONS

1. The effect of *Fusarium circinatum* over seed germination was small, reducing the rate of germinated seeds in between 10 and 20%. No differences were seen in germination among the seven isolates used.

2. Mortality of *Pinus radiata* seedlings inoculated with the *Fusarium circinatum* was high for the seven isolates. Four isolates killed all the *P. radiata* seedlings.

3. No big differences were found in virulence among the seven isolates.

5. ACKNOWLEDGEMENTS

We thank the Government of the Autonomous Community of Cantabria, the Ministry of Agriculture, and the Ministry of Environment (INIA-CIFOR) for financial support. We also want to thank Mohammed Masum Ul Haque for the revision of the article.

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