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# Testing the effect of routine fungicide application on ectomycorrhiza formation on *Pinus halepensis* seedlings in a nursery

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## Summary

The fungicides benomyl, captan, hymexazol, iprodione, propamocarb hydrochloride and thiram were applied in a combined schedule to *Pinus halepensis* seedlings grown under nursery conditions to study their effect on mycorrhiza formation and plant growth. The inoculated fungi *Pisolithus tinctorius* (strains 3SR and Mx) and *Lactarius deliciosus* formed mycorrhizas when fungicides were applied. However, fungicide application led to a significant decrease of mycorrhizal plants with *Pisolithus tinctorius* strain 3SR (from 62% without fungicides to 35% with fungicides). This effect could be attributed to a direct effect of fungicides on mycelial growth. The morphometric and nutritional parameters were very similar in plants treated with or without fungicides, and no differences on sanitary status of plants were noted. The use of preventive fungicide treatments in plant production, which may have a harmful effect on the environment, is therefore questioned.

## 1 Introduction

Fungicide treatments are frequently and routinely applied in most forest nurseries in order to improve high-quality plant production. Fungicides may inactivate pathogens and/or prevent the infection of healthy tissues. Nonetheless in practice, fungicides rarely act as erradicants as their main role is prophylaxis. It is normal in preventive treatments to mix or alternate several products to avoid resistances and to control a broad spectrum of fungi (De Liñan 2005).

However, fungicides might be expected to affect soil microorganisms, particularly the mycorrhizal fungi. Previous studies reported how fungicides influenced ectomycorrhizal (ECM) development in pine seedlings in nurseries, but these results are diverse and even contradictory. The use of benomyl neither affected nor even enhanced the formation of ectomycorrhizas (Kais et al. 1981; Marx and Rowan 1981; Pawuk and Barnett 1981; Trappe et al. 1984) or decreased the number of ECM (O'Neill and Mitchell 2000). Captan had also little effect (Trappe et al. 1984; Landis et al. 1990; O'Neill and Mitchell 2000) or stimulated ECM formation (Pawuk et al. 1980; Marx and Rowan 1981). Other studies reported reduction of ECM infection with thiram (Landis et al. 1990), propiconazole (Manninen et al. 1998), dithane (Cudlin et al. 1983), thiphanate methyl (Teste et al. 2006) and triadimefon (Pagedumroese et al. 1996).

The effect of several fungicides used in combination as usually applied in commercial nurseries has not been previously tested. Indeed, no previous information exists about this topic on *Pinus halepensis*, a pine widely used in reforestation in Mediterranean ecosystems.

The assessment of the effect of fungicides on ECM development would be convenient for the elaboration of inoculation programmes with selected species such as edible fungi like *Lactarius* spp., or fungi used for reforestation like *Pisolithus tinctorius.* The effect of several pesticides on the *in vitro* growth of *Lactarius deliciosus* and *Pisolithus tinctorius* was assessed, where hymexazol and iprodione were the most damaging fungicides, benomyl, captan and thiram were compatible with mycelial growth at low doses and propamocarb hydrochloride was the most tolerated fungicide (Díaz et al. 2003).

The aim of this study was to investigate the effects of fungicide application in a combined drench on *Pinus halepensis* under routine nursery conditions, in terms of mycorrhiza formation by selected fungal species and plant growth responses.

## 2 Materials and methods

The experiment was conducted under standard production conditions (seeds, potting substrate, watering, fertilization, fungicides, growth conditions) used in the forest nursery at the Centro Nacional de Mejora Genética Forestal El Serranillo, Guadalajara, Spain.

Seeds of *Pinus halepensis* provenience Maestrazgo-Los Serranos, Teruel, Spain, were disinfected in 30% $H_2O_2$ for 20 min, rinsed in distilled water and sown in polystyrene containers [Poliforest® tray with 25 individual cells (Poliex, Alicante, Spain), each containing a 350-ml, plastic pot with a lid], containing unsterile Sphagnum peat VAPO® BS (Kekkilä, Jyväskylä, Finland; pH 5.3). After germination, seedlings were thinned to one per cell.

Plants were grown outside for one growing season (9 months) under natural conditions, watered with mini-diffusers (Mycron Plus®, Irrimon, Valencia, Spain) when necessary and fertilized 10 times every 2 weeks with Peter's Professional® (Scotts, Tarragona, Spain) fertilizer, with a total supply of 35-27-61 mg NPK/plant.

The effects of three mycorrhiza-forming fungi and a fungicide treatment on mycorrhiza formation, growth performance and nutrient acquisition were studied using a randomized block design as experimental set-up. One hundred seedlings (four

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