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# Smoke-water Controls *Pythium* Damping-off in Papaya Seedling

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***Abstract.*** **The antifungal efficacy of smoke-water on damping-off caused by *Pythium* sp. was evaluated both in vitro and in vivo. Smoke-water was generated by burning plant material and bubbling the smoke through water; its effect on the morphology of *Pythium* sp. was investigated by scanning electron microscope (SEM). Mycelia growth and oospore production of the fungus were significantly inhibited when cultured on water agar amended with smoke-water at 0%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, or 5%. In growth chamber experiments with potted seedlings growing in peatmoss, papaya plants treated with smoke-water exhibited reduced symptoms of damping-off when compared with control plants treated with water. Furthermore, the pots treated with 1.5% or higher smoke-water did not show any symptoms of damping-off disease. Plant height significantly increased with treatment by higher concentrations of smoke-water. Maximum plant height was observed with treatment of 1% smoke-water or higher concentrations. One day after smoke-water application, SEM observations of the *Pythium* sp. revealed loss of structural integrity, abnormal degradation, deformation, abnormal lysis, cytoplasmic leakage, and hyphal slimming. This study showed that the addition of smoke-water to soil exerted significant disease suppression against *Pythium* sp., leading to improved growth of papaya seedlings.**

Papaya is one of the most important tropical fruit crops with annual production of ≈9.1 million tones and economic value of U.S. ≈$6097 million (FAOSTAT, 2012). Damping-off is a major disease of papaya (*Carica papaya* L.) seedling in nurseries, glasshouses, gardens, fields, and forests (Agrios, 2005) and can be caused by several species of fungi, including *Rhizoctonia solani*, *Pythium* spp., *Phytophthora* spp., *Sclerotinia* spp., and *Fusarium* spp. (Stephens et al., 1982). These diseases are serious problems and have caused economic losses in commercial greenhouse seedling production.

Damping-off disease caused by *Pythium* spp. usually begins as a root rot. *Pythium* survives in the soil as oospores that germinate and attack root hairs and root tips of seedlings, causing progressive deterioration of the root systems. The seed may fail to germinate because it rots in the ground or the seedling may wilt before aboveground lesions are evident (Franklin, 2001). Conditions for the development of this disease are high temperature, high humidity, high soil moisture, poor aeration, high levels of nitrogen fertilizer, and closely sown seed (Agrios, 2005).

Strategies to control and/or manage crop diseases effectively use a combination of cultural, biological, and chemical tools. Control of damping-off diseases is difficult. Damping-off must be anticipated and prevented by using seed and transplant treatments before the seed or plants are put in the field (Franklin, 2001). Prevention practices include providing good soil drainage and good air circulation among plants, planting when temperatures are favorable for fast plant growth, avoiding application of excessive amounts of nitrate in nitrogen fertilizers, and practicing crop rotation (Agrios, 2005). Fungicides can currently be used but do present several problems. The use of fungicides has resulted in environmental pollution resulting from the accumulation of residual toxicity and has changed the profile of microorganisms in the soil (Muthukumar et al., 2010). Furthermore, the potential for undesired residues in the plant (Vanachter et al., 1983) and fungicide resistance in phytopathogenic fungi are limiting factors in using fungicides in crop protection (Brent, 1995). Resistance of the target pathogens to chemical a.i. curtails the efficacy and useful lifetime of fungicides, which must then be further developed at increasingly higher costs (Ma and Michailides, 2005).

Smoke-water, which is generated by burning plant material and bubbling the smoke through water, has been shown to enhance germination, improve growth, and promote the production of healthier plants (Light and Van Staden, 2004). It has the potential to be used in the horticultural and agricultural industries, particularly by farmers without the resources to purchase costly pesticides. Smoke-water contains a highly active compound, butenolide [3-methyl-2*H*-furo(2,3-*c*) pyran-2-one], derived from the burnt plant material (Van Staden et al., 2004) and cellulose. Butenolide has been shown to stimulate germination of lettuce at concentrations as low as $10^{-9}$ M (Flematti et al., 2004) and to promote seed germination in many other species (Soos et al., 2009). In addition, smoke-water protects against microbial attack (De Groot, 1996). The phenolic compounds of smoke-water extract show antimicrobial activity that reduces the growth of microorganisms. Numerous studies have shown that phenolic compounds can inhibit the growth of soilborne pathogens (Muthukumar et al., 2010; Yangui et al., 2008).

To our knowledge, no studies have been reported on using smoke-water to manage the papaya seedling damping-off disease caused by *Pythium* sp. The objectives of this study were 1) to evaluate the antifungal properties of smoke-water in vitro and in vivo for efficacy against damping-off caused by a soilborne *Pythium* sp. isolated from papaya; and 2) to investigate the effect of smoke-water on the morphology of *Pythium* sp. using SEM observation.

## Materials and Methods

*Smoke-water production.* A smoke-water solution was prepared by igniting 5 kg of dry rice straw (*Oryza sativa* L.) material in a 20-L stainless steel barrel. Using compressed air, the smoke was continuously bubbled through a 500-mL graduated cylinder filled with distilled water for 45 min. Solutions of this aqueous smoke extract (500 mL) were filtered through Whatman No.1 filter paper and used as the stock solution. A similar method of preparing smoke-water with different plant material has been described by Boucher and Meets (2004). The apparatus for producing smoke-saturated water has been illustrated

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