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Association of a second phase of mortality in cucumber seedlings with a rapid rate of metalaxyl biodegradation in greenhouse soils

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Abstract

A study was undertaken from 2004 to 2007 to investigate factors associated with decreased efficacy of metalaxyl to manage damping-off of cucumber in Oman. A survey over six growing seasons showed that growers lost up to 14.6% of seedlings following application of metalaxyl. No resistance to metalaxyl was found among *Pythium* isolates. Damping-off disease in the surveyed greenhouses followed two patterns. In most (69%) greenhouses, seedling mortality was found to occur shortly after transplanting and decrease thereafter (Phase-I). However, a second phase of seedling mortality (Phase-II) appeared 9–14 d after transplanting in about 31% of the surveyed greenhouses. Analysis of the rate of biodegradation of metalaxyl in six greenhouses indicated a significant increase in the rate of metalaxyl biodegradation in greenhouses, which encountered Phase-II damping-off. The half-life of metalaxyl dropped from 93 d in soil, which received no previous metalaxyl treatment to 14 d in soil, which received metalaxyl for eight consecutive seasons, indicating an enhanced rate of metalaxyl biodegradation after repeated use. Multiple applications of metalaxyl helped reduce the appearance of Phase-II damping-off. This appears to be the first report of rapid biodegradation of metalaxyl in greenhouse soils and the first report of its association with appearance of a second phase of mortality in cucumber seedlings.

Keywords: HPLC; ITS rDNA; Oomycetes; *P. aphanidermatum*; *P. spinosum*; Ridomil

1. Introduction

Cucumber is an important crop worldwide. In Oman, greenhouse soil-based cucumber production is one of the most important vegetable sectors. However, post-emergence damping-off disease is one of the biotic factors most limiting cucumber production in soil-based greenhouses, killing up to 70–80% of cucumber seedlings in the most affected greenhouses (Stanghellini and Phillips, 1975; Al-Kiyumi, 2006). Although, some reports indicated association of some *Rhizoctonia* and *Fusarium* spp. with damping-off of cucumber (Lida et al., 1983; Howard et al., 1994), *Pythium* species have been frequently reported as the main causal agents of this disease (Stanghellini and Phillips, 1975; Al-Sa'di et al., 2007).

Chemical control through application of the phenylamide fungicide metalaxyl as pre- and post-transplanting treatments is a common method for damping-off control in different parts of the world (Al-Kiyumi, 2006; Hickman and Michailides, 1998). In Oman, the use of metalaxyl increased rapidly from 549 kg a.i. in 2001 to 2741 kg a.i. in 2002 and 3155 kg a.i. in 2003 (Al-Toobi et al., 2005) as a result of an increase in *Pythium*-induced diseases of cucurbits (Al-Sa'di et al., 2007, 2008d).

After initial good disease control, a reduction in the efficacy of metalaxyl to control damping-off of cucumber in Oman was reported in the early 2000s, with mortality after metalaxyl application still reaching up to 15% within 2 weeks after transplanting seedlings into greenhouses (Deadman et al., 2002). In a subsequent study, Al-Kiyumi (2006) reported no significant differences in incidence of damping-off between greenhouses where metalaxyl was utilized and greenhouses where no control measures were

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practiced. This raised questions concerning factors involved in reducing the efficacy of metalaxyl for damping-off control under greenhouse conditions.

Failure to control Oomycete plant pathogens using metalaxyl is usually due to two main causes. Firstly, several *Pythium* and *Phytophthora* species have been reported to develop resistance to metalaxyl, resulting in failures of disease control (Davidse et al., 1981; Sanders, 1984; Moorman et al., 2002). Secondly, continual use of metalaxyl in open fields has been reported to lead to enhanced biodegradation in soil, resulting in a reduction of its half-life from 12 weeks to as short as 1–2 weeks (Droby and Coffey, 1991; Davison and McKay, 1999).

Previous studies have established that the *Pythium* populations in Oman had not developed resistance to metalaxyl (Al-Sa'di et al., 2008a, b). However, there is a lack of studies on persistence of metalaxyl in soil under greenhouse conditions following repeated use. Therefore, the overall objective of this study was to characterize factors associated with increased incidence of damping-off in greenhouse grown cucumbers following the use of metalaxyl. The specific questions we are addressing in this paper include: (1) does the continuous use of metalaxyl give rise to an increase in incidence of damping-off in cucumber; (2) is the increase in disease incidence due to the build up of resistance to metalaxyl (3) is the increase in disease incidence due to rapid biodegradation of metalaxyl in greenhouse soils, (4) does applying multiple doses of metalaxyl give better control than a once only application. Testing these hypotheses may resolve some of the reasons behind the decrease in control of damping-off and provide insight into the future efficacy of metalaxyl use in greenhouse systems.

2. Materials and methods

2.1. Incidence of damping-off following the use of metalaxyl

A total of 189 greenhouses were surveyed in five cucumber-growing regions in Oman (Batinah, Interior, Muscat, Buraimi and Sharqiya) from 2004 to 2006 in order to determine the incidence of damping-off disease following the use of metalaxyl. Surveys conducted in 2004 and 2005 attempted only to determine the overall incidence of damping-off in greenhouses in which metalaxyl was applied to control the disease. This was achieved through questioning growers utilizing metalaxyl about the percentage of seedling mortality in their greenhouses that occurred within 3 weeks of transplanting. However, a more detailed three-season survey was conducted in 2006 to investigate the incidence of damping-off disease over time after transplanting. This was achieved by assessing the percent seedlings showing mortality on a daily basis for 3 weeks after transplanting. Over the course of the surveys conducted in 2006, information was also collected from most of the surveyed greenhouses on the amount of metalaxyl applied.

2.2. Pathogens associated with damping-off of cucumber

Pathogens associated with cucumber seedlings expressing damping-off symptoms following application of metalaxyl were identified over the course of the surveys by taking random samples from affected greenhouses. This was done to eliminate the possibility of association of *Rhizoctonia* and *Fusarium* spp. with seedling mortality following the use of the oomycete-selective fungicide, metalaxyl. Isolations from root and stem bases of cucumber seedlings were done in corn meal agar (CMA, BBL Microbiology Systems, Cockeysville, Maryland, USA) amended with 5 $\mu g\,ml^{-1}$ pimaricin, 250 $\mu g\,ml^{-1}$ ampicillin and 10 $\mu g\,ml^{-1}$ rifampicin (Jeffers and Martin, 1986) as well as on potato dextrose agar (PDA, Difco Laboratories, Detroit, Michigan, USA) as described by Al-Sa'di et al. (2007).

Identification of *Pythium* to species level was achieved using sequences of the internal transcribed spacers (ITS1, 5.8S, ITS2) of the ribosomal DNA. DNA was extracted from 70 mg freeze-dried mycelium using a modified protocol of Lee and Taylor (1990) as described by Al-Sa'di et al. (2007). Using the universal primers ITS1 and ITS4 (White et al., 1990), the polymerase chain reaction (PCR) mixture and PCR conditions were as per Al-Sa'di et al. (2007), except for the use of *Taq* polymerase (Fisher Biotec, Perth, Australia) in the PCR mixture. After cleanup of the PCR products using the UltraClean PCR Clean-up kit (Mo-Bio Laboratories, Carlsbad, California, USA), samples were submitted to the Australian Genomic Research Facility (AGRF) in Brisbane (Australia) for sequencing using BigDye V3.1 (Applied Biosystems, Foster City, California, USA). Sequencing was performed in both directions using the ITS1 and ITS4 primers. The resulting ITS sequences were compared with sequences deposited at the National Center for Biotechnology Information (NCBI) and with sequences generated by Al-Sa'di et al. (2007) for reference isolates deposited in the Centraalbureau voor Schimmelcultures (CBS) using the program Clustal W (fast) (Thompson et al., 1994).

2.3. Metalaxyl sensitivity of Pythium isolates

To confirm that reduction in metalaxyl activity is not associated with build up of resistance among *Pythium* isolates to metalaxyl, sensitivity of the obtained *Pythium* isolates to metalaxyl was determined in CMA amended with 0, 0.1, 0.5, 1.0, 5.0 and 20.0 $\mu g\,ml^{-1}$ a.i. metalaxyl (Novartis, Basel, Switzerland) as described by Al-Sa'di et al. (2008a). A 5-mm-diameter mycelium/agar plug was transferred from the margin of an axenic 3-d-old *Pythium* culture to the edge of metalaxyl amended CMA culture plates, which were incubated at 25 °C. Four replicate plates were used for each treatment and each isolate was tested twice. Linear growth of colonies was measured daily up to 4 d. Metalaxyl concentration resulting in 50% growth