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Control of white mold of dry bean and residual activity of fungicides applied by chemigation

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A B S T R A C T

Sclerotinia sclerotiorum is a necrotrophic fungal pathogen that causes white mold of dry bean (*Phaseolus vulgaris* L.). Chemigation with fungicides is used for disease control, but effectiveness of this application method and impact of irrigation level on residual fungicide activity in the plant over time under field conditions has not been well characterized. To assess the best method of application and fungicide for disease control, we conducted field studies in three field sites in São Paulo State in Brazil. Contact fungicide, fluazinam, was applied via center pivot at three irrigation levels (2.5, 5.1, 10.1 mm) at the Itaí field site in 2013. Fluazinam and procymidone (systemic) were independently applied via sprinkler at three irrigation levels (3.0, 4.5, 6.0 mm) in 2013 and four irrigation levels (2.5, 5.0, 7.5, 10.0 mm) in 2014 at the Pereiras field site. Fungicides were also applied at the Pereiras site using a backpack sprayer in 2014. Three successive fungicide applications were made at Pereiras in 2013 and two successive applications made at Pereiras in 2014. Three leaves from each treatment of the four replicated plots were collected in 2-day intervals after application, and fungicide residues assessed using a detached leaf bioassay. Lesion areas were used to estimate percent disease control. Regardless of fungicide or application method, disease control decreased over time (ANCOVA; $P < 0.05$). Area under the disease progress curve estimated from leaf lesion areas showed chemigation at the lowest irrigation level provided the best control in five of six trials of fluazinam and four out of five trials of procymidone. Ground applications were equally effective, showing no difference from chemigation at the lowest irrigation level in most comparisons. The percent reduction in number of *S. sclerotiorum* sclerotia, disease incidence and dry bean yield were evaluated at Pereiras in both years. Procymidone reduced the number of sclerotia formed. However, yield was only higher for treatments that included procymidone at Pereiras in 2013. Overall, results indicate that both lower irrigation level and ground application slow the loss of residual fungicide activity and reduce the total disease lesion area. Results from this study indicate that procymidone may be better able to reduce *S. sclerotiorum* sclerotia formation, which may be an important consideration for long-term disease management.

1. Introduction

Brazil is the world's leading producer and consumer of dry beans (*Phaseolus vulgaris* L.). More than half of dry beans are produced in three Brazilian states: Paraná, Minas Gerais, and Mato Grosso (Conab, 2016). Dry bean can be seeded year-round throughout Brazil, but in some regions there is irregular rainfall, which can be yield-limiting because dry bean is a water-sensitive crop (Guimarães et al., 2006). In some production regions of Brazil, irrigation of dry bean is used and is mandatory in the fall-winter season, during which time approximately 25% of beans are produced. Irrigation is necessary for high yield, where non-irrigated and low-input approaches (little to no fertilizer and pesticides, and locally produced seeds) yielded approximately 886 kg ha^{-1} in Brazil in the 2015/16 season (Conab, 2016).

Several pathogens cause yield-limiting diseases on dry bean in Brazil, including *Sclerotinia sclerotiorum* (Lib.) de Bary, causal agent of white mold. White mold is widespread and economically important in many countries including Canada (Bardin and Huang,

Abbreviations: DLB, detached leaf bioassay; AUDPC, area under disease progress curve or cumulative lesion area.

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2001), the USA (Bolton et al., 2006), Australia (Lethan et al., 1976) and Brazil (Lehner et al., 2015; Paula Júnior et al., 2009a; Vieira et al., 2010). Control of *S. sclerotiorum* is particularly challenging because the host range includes over 400 species of plants worldwide including important crops and numerous weeds (Boland and Hall, 1994). In addition, *S. sclerotiorum* is a necrotroph and able to survive in the soil as sclerotia for many years. Sclerotia are able to germinate myceliogenically to produce hyphae in soil, or germinate carpogenically to produce apothecia that release wind-blown ascospores that colonize injured tissue and senescing flowers, which is the primary mode of infection. Secondary infection of leaves, petioles and stems is by mycelium through direct contact with infected flowers (Abawi et al., 1975). An integrated disease management approach for *S. sclerotiorum* control is recommended, including use of certified seed, crop rotation with a non-host monocot crop, selection of upright cultivars, tilling soil, routine cleaning of agricultural implements, biological control, and fungicidal control (Harikrishnan and del Río, 2006; Lehner et al., 2015; McCreary et al., 2016; Miklas et al., 2013; Paula Júnior et al., 2009b, 2012; Vieira et al., 2003, 2010, 2012).

Due to the necrotrophic nature of this fungal plant pathogen, there are no resistant plant cultivars, resulting in greater dependency on fungicide applications that are targeted to prevent primary infection. Efficacy of fungicide applications for white mold control may be influenced by a number of factors, such as fungicide penetration of the lower canopy, timing of fungicide application (Morton and Hall, 1989), and fungicide degradation by alkaline hydrolysis (Ferrel and Aagard, 2003). Up to seven fungicides are currently registered for white mold control in dry bean, wherein fluazinam and procymidone are two of the three most frequently used by Brazilian farmers (Lehner et al., 2015).

The phenyl-pyridinamine fungicide fluazinam is one of the most effective fungicides for *S. sclerotiorum* control (Mahoney et al., 2014; Matherom and Porchas, 2004; McCreary et al., 2016; Vieira et al., 2012). Fluazinam is considered a protectant (Lemay et al., 2002; Vitoratos, 2014) and must be applied prior to disease onset for best results, which typically coincides with first bloom and an additional application may be necessary if the favorable conditions to white mold continues (Paula Júnior et al., 2009b). The mode of action of fluazinam is uncoupling of mitochondrial oxidative phosphorylation, consequently halting synthesis of ATP without affecting the respiratory chain and ATP synthesis (Guo et al., 1991; Vitoratos, 2014). With activity at multiple sites, fluazinam is considered to have a low risk of resistance development (Lehner et al., 2015).

Procymidone is another effective and commonly used dicarboximide fungicide for white mold control in Brazil. Unlike fluazinam, procymidone can be used as both a preventive and curative fungicide, with moderate systemic activity (Chen et al., 2010). Procymidone in the soil may be absorbed by roots, and translocated to leaves and flowers (Chen et al., 2010), which makes it particularly effective (Ma et al., 2009). The target site of this fungicide is cytochrome *c* of the mitochondrial oxidative pathway. Due to the site-specific mode of action, dicarboximide fungicides are considered to be at high risk of resistance development (Ma et al., 2009).

Fungicide application in dry bean for white mold control is typically made by ground application using a tractor-mounted sprayer, self-propelled sprayer or by chemigation using sprinklers or a center pivot. The labeled rate of fungicide application using each of these methods is the same for fluazinam (1.0–1.5 L ha^{-1}). However, labeled rate of procymidone application by chemigation is greater than for ground application (2.0 kg ha^{-1} compared to 1.0–1.5 kg ha^{-1} by ground application). There are no consistent recommendation for water usage in chemigation and ground application, so the amount of water used for chemigation will vary and results in differences in final fungicide concentrations. Such differences can be significant because large volumes of irrigation water are used during chemigation, at least 25,000 L ha^{-1} as compared with 200 to 1000 L ha^{-1} for ground application. Consequently, as compared with application via irrigation, ground application results in higher initial fungicide residue levels because the dilution effect is reduced (Hamm and Clough, 1999).

Chemigation has been shown to be effective for foliar disease control of angular leaf spot, alternaria spot, and rust in dry bean (Cunha et al., 2001; Pinto and Costa, 1999). For white mold disease control in dry bean, some studies suggest chemigation facilitates better ground penetration and reduces apothecial development (Venegas and Saad, 2010) and is equivalent to fluazinam applied directly to the soil (Vieira et al., 2003). For farmers with an irrigation system already in place, application of fungicides via chemigation can save time. Most farmers will apply fungicides at the label rate and run the center pivot at maximum speed to reduce the amount of irrigation water and increase the final fungicide concentration. However, larger water volumes during chemigation may improve ground penetration and absorption of systemic fungicides by increasing the duration of soil saturation. No previous studies have characterized the effect of varying irrigation levels on disease control of white mold.

Assessment of fungicide activity in the plant can be determined using an agrochemical residue analysis performed using analytical techniques such as gas chromatography and high performance liquid chromatography or using assays such as a detached leaf bioassay. In the latter method, plants are treated with fungicides, and leaves are harvested for inoculation with the pathogen under controlled-environment conditions, allowing quantification of necrotic lesion formation. The detached leaf bioassay has been used previously to compare fungicide treatments applied in the greenhouse (Mueller et al., 2002) and to assess translocation of fungicide within peanut plants in the field (Augusto and Brenneman, 2012). No previous studies have used this method to assess residual fungicidal activity in dry bean to assess disease control over time and after successive applications under field conditions. Thus, our objective was to use the detached leaf bioassay to characterize the residual effect of two fungicides with different modes of action and movement, fluazinam (contact fungicide) and procymidone (systemic fungicide), applied at label rate via chemigation, using different levels of irrigation, and assess the effect on dry bean yield, disease incidence and percent reduction in number of *S. sclerotiorum* sclerotia produced on plants and in soil. Collectively, the results from this study will provide new information on effective use of chemigation for white mold disease control and enable grower recommendations for optimal disease suppression.

2. Materials and methods

2.1. Field sites

One field site was located at the farmer-cooperator managed Cercadinho Farm in Itaí, São Paulo, Brazil, and the other field site was located at the Agricultural Research and Development Center (CPDA) at Arysta LifeScience in Pereiras, São Paulo, Brazil. Experiments were conducted at both field site locations in 2013, and at the Pereiras field site in 2014.

Fields were planted with the dry bean of seed class Carioca, cultivars 'Pérola' in Pereiras and 'Bola Cheia' in Itaí, with 0.5 m between rows. Itaí field was center pivot-irrigated using Naandan 435, 12.7 mm sprinklers, with a total irrigated area of 59.7 ha and irrigation level applied in each treatment was controlled by adjusting the speed of the center pivot (2.5 mm irrigation level was achieved at the highest speed). Pereiras fields were sprinkler