Crop Protection 94 (2017) 192–202

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

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

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ARTICLE INFO

*Article history:*
Received 1 September 2016
Received in revised form
21 December 2016
Accepted 23 December 2016
Available online 13 January 2017

*Keywords:*
Fluazinam
Procymidone
Detached leaf bioassay
*Phaseolus vulgaris*
*Sclerotinia sclerotiorum*

ABSTRACT

*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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http://dx.doi.org/10.1016/j.cropro.2016.12.023
0261-2194/

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

irrigated with sprinklers limited to movement through a 180° area (half circle), with a radius of 6 m, totaling approximately 56.5 $m^2$ and irrigation level controlled by adjusting the total volume of water applied to each of the four replicated plots. Fields were irrigated as needed to maintain optimal plant health and vigor. In 2013, from seed sow to harvest, Pereiras received 268 mm rainfall and 105 mm irrigation, whereas in 2014, rainfall was 76 mm and irrigation was 120 mm. Temperature, rainfall, and total irrigation were not recorded at the Itaí field site. Average temperature during plant development at Pereiras ranged from 8.0 to 20.8 °C in 2013 and from 11.0 to 19.8 °C in 2014. In both years the average temperature was 16 °C.

### 2.2. Fungicide applications

Applications of fluazinam and procymidone at different irrigation levels were made in 2013 and 2014. In 2013, at the Itaí field site, fluazinam was applied once via irrigation at three irrigation levels (2.5, 5.1, and 10.1 mm), whereas at the Pereiras field site, both fluazinam and procymidone were applied independently three times with differing irrigation levels (3.0, 4.5, and 6.0 mm). In 2014, two applications of fluazinam and procymidone were made independently at four irrigation levels (2.5, 5.0, 7.5, and 10.0). In addition, application was made with a backpack sprayer to simulate tractor driven ground application.

Plots at the Itaí field site were eight rows wide and 5.5 m long, each totaling 22 $m^2$ in area. The label rate of fluazinam (1 L $ha^{-1}$ commercial product) was applied at four replicated plots at three irrigation levels: 2.5, 5.1 and 10.1 mm water applications in randomized design. Only a single fungicide application was made at 50% full bloom. The control treatment was no fungicide and no additional water, which was achieved by covering plots with plastic during chemigation in randomly selected locations. Fungicide was injected into the center pivot using an Injeferd (Solomaq, Uberaba, MG), which is an instrument used to inject solid and water-soluble agrochemicals into the center pivot pipeline during irrigation. This instrument has an advanced dilution and dosing system that allows controlled application into flowing water; which can apply about 25 kg $min^{-1}$ of the product. During the course of the experiment, only a single fungicide application was needed due to lack of disease.

Plots at the Pereiras field site were eight rows wide and 7 m long, totaling 28 $m^2$. There were 7 treatments in 2013 and 11 treatments in 2014; each applied in four replicated plots. Experimental design in the field used randomized blocks with treatments distributed in factorial $2 \times 3 + 1$ in 2013 and $2 \times 5 + 1$ in 2014. Two fungicides (fluazinam and procymidone) were applied at three irrigation levels in 2013 (3.0, 4.5 and 6.0 mm) and four irrigation levels in 2014 (2.5, 5.0, 7.5, and 10.0 mm). In 2014, an additional treatment of ground fungicide application using a backpack sprayer was included, applied in four replicated plots. Fungicide applications were made at the maximum label rate for fluazinam (1.5 L $ha^{-1}$ in irrigation and 1.5 L $ha^{-1}$ by ground application) and procymidone (2.0 kg $ha^{-1}$ in irrigation and 1.5 kg $ha^{-1}$ by ground application). Control plots were separate from chemigated plots, where neither fungicides nor additional irrigation water were applied. Total additional water received by chemigated plots at the highest irrigation level was minimal, representing a seasonal total increase of no more than 4.83% (18 mm) at Pereiras-2013 and no more than 10.20% (20 mm) at Pereiras-2014. This difference was not compensated for because it represented typical field conditions created by chemigation at different irrigation levels.

The number of fungicide applications made at Pereiras was determined based on disease pressure. Disease pressure was high in 2013, requiring three fungicide applications beginning at 50% full bloom with subsequent applications at intervals of approximately 15 days thereafter. In 2014, disease pressure was low, requiring only two fungicide applications, with the first application at 50% full bloom and the second application 16 days later. Fungicides applied via chemigation used sprinklers connected by 12.7 mm hoses to a tank of 2000 L. The hose was connected to the tractor's power take-off set to 540 rpm with pressure gauge set to around 300,000 Pa. For each treatment, the commercial product and water were added to the tank in the amount needed to make four applications. Ground application was made using a backpack sprayer pressurized with $CO_2$ and equipped with a 2.5 m long spray boom with single flat fan nozzles (TeeJet XR 11004, Spraying Systems Co., Glendale Heights, IL). Nozzles were spaced 0.5 m apart with a spray volume rate of 1000 L $ha^{-1}$, which is the label rate recommended for white mold control.

### 2.3. Detached leaf bioassay

The youngest and fully expanded trifoliate leaves in each treatment were collected from the upper plant canopy and immediately transported to the laboratory. Residual fungicidal activity was determined using a detached leaf bioassay, in which three leaves were collected after fungicide application from each of four replicated plots in 2-day intervals after fungicide application. At the Itaí field site, a total of six leaf collections were made over the 11 day post-application period and resulted in a total of 288 trifoliate leaves subjected to the bioassay. At the Pereiras field site in 2013, five leaf collections were made in each of three 10-day post-application periods that amounted to 180 trifoliate leaf collections from each of the seven treatments, for a total of 1260 trifoliate leaves subjected to the bioassay. In 2014, there were eight collections in each of two 16-day post-application periods that resulted in 192 trifoliate leaves collected in each of the 11 treatments, for a total of 2112 leaves subjected to bioassay. A grand total of 3660 leaves were collected and subjected to the detached leaf bioassay.

The detached leaf bioassay was described previously by Leone and Tonneijck (1990) and is similar to methods used with *S. sclerotiorum* resistance studies in soybean (Kim et al., 2000; Kull et al., 2004) and dry bean (Kull et al., 2003). Leaves were first prepared for inoculations. To prevent wilting, the petiole of each leaf was pushed through a test tube (12 mm × 75 mm) filled with tap water and with a bung lid that had a central hole to allow the entrance of the leaf petiole. Four paper towels were placed in the bottom of each aluminum pan "D100" (WYDA, Sorocaba, São Paulo) (515 mm × 355 mm x 73 mm) that would eventually serve as a moist chamber. In each pan there were four glass petri dishes (100 mm × 15 mm) placed upside down to serve as platforms for each leaf. Four trifoliate leaves were placed in each aluminum pan with the middle leaflet on top of the glass petri dish platform.

Leaflet inoculations using *S. sclerotiorum* mycelium were designed to simulate natural infection resulting from direct contact infection. The isolate used for inoculations was originally collected from dry bean at Pereiras field site in 2012. Sclerotia from storage were first grown on water agar and subsequently transferred to potato dextrose agar (Becton, Dickinson and Company Sparks, MD). For inoculations, an actively growing culture was transferred from water agar to PDA and, after 48 h, a 6-mm diameter agar plug containing actively growing mycelium was aseptically placed onto each middle trifoliate leaflet with mycelia in contact with the leaf. After inoculations, 300 mL of water was added to each aluminum pan and, to maintain humidity, enclosed by stretchable PVC film that was 450 mm wide (Alpes − Indústria e Comércio de Plásticos Ltda., São Paulo, SP). Moist chambers were maintained at ambient room temperature (25 ± 2 °C) and, after 48 h, evaluations were performed. To estimate necrotic lesion area, a digital image of each

leaf was analyzed with software Image J (Wayne Rasband National Institutes of Health, USA). Actual size of each lesion was estimated by pre-calibration of the software on a grid of a known size (1 $cm^2$) that was included in each photo (Fig. 1).

### 2.4. Disease incidence, yield, and residual sclerotia

Estimates of disease incidence, yield, and residual *S. sclerotiorum* sclerotia were evaluated at the Pereiras site in 2013 and 2014. Disease incidence of white mold was evaluated 80 days after emergence, estimated as the percentage of plants with visible white mold symptoms. In 2013, disease incidence was estimated using a visual assessment (Mahoney et al., 2014; McCreary et al., 2016) of all plants in the field within each plot (28 $m^2$). In 2014, a more thorough approach was used to estimate disease incidence in which visual estimation was performed on each plant within an area of 16 $m^2$ (2 m × 8 m) and averaged to provide the total estimated disease incidence. To estimate yield, plants were harvested when 90% of the pods were dry. Dry bean plants were uprooted manually in an area of 28 $m^2$ (4 m × 7 m length) and air-dried in the field for about 4 days. Dry beans were threshed and stored in labeled paper bags. The weight of beans was measured using an electronic balance and water content was measured with a portable device (Moisture Match, Deere & Company Moline, Illinois). Weights were adjusted to 13% relative moisture and converted to kg $ha^{-1}$. Quantification of *S. sclerotiorum* sclerotia on plants and in soil was estimated for each treatment. After dry bean harvest, all *S. sclerotiorum* sclerotia on plants in an area of 28 $m^2$ in each plot were manually collected and weighed. *S. sclerotiorum* sclerotia in soil in a 0.25 $m^2$ plot to a 5 cm depth (approx. 12.5 L) were manually counted.

### 2.5. Statistical analysis

Percent disease control was estimated as the difference in lesion area of the control and treatment, divided by lesion area of the control and expressed as a percent. As described above, percent disease control over time was estimated for each treatment using the average lesion area of 12 leaflets from four replicated plots harvested in 2-day intervals. Average percent disease control over time was fit with linear regression and comparisons were performed using an analysis of covariance (ANCOVA) with PROC REG and PROC MIXED in SAS (version 9.4, Institute Inc., Cary, NC). This analysis was used to determine if there was a significant difference in estimated parameters (slope or intercept) and whether there was an interaction between fungicide treatment and irrigation level.

Lesion area data from each treatment was also used to calculate Area Under Disease Progress Curve (AUDPC) (Shaner and Finney, 1977) and was averaged among a total of 12 leaves from the four replicated plots in each treatment. AUDPC from each treatment were compared in an analysis of variance (ANOVA), with Tukey's post-hoc test, performed using PROC GLIMMIX in SAS. Percent disease control in days after application (slope) and AUDPC of lesion area were each compared in pairwise combination between repeated applications of each fungicide using a paired *t*-test (PROC TTEST).

Percent reduction in number of sclerotia collected in soil and weight of sclerotia after dry bean harvest were calculated by taking the difference between control and treatment, divided by the control, and quotient multiplied by 100. Comparisons of yield, percent reduction of *S. sclerotiorum* sclerotia (number and weight), and disease incidence were made using an analysis of variance (ANOVA) using PROC GLIMMIX, followed by a Tukey's post-hoc test. For comparisons involving number of sclerotia a negative binomial distribution was specified and for comparisons involving weight of sclerotia and disease incidence, a beta distribution was specified. A random-effects model was applied for analysis of data from the Pereiras site, and interaction tested between fungicide and irrigation level (2013) or application method (2014).

## 3. Results

Lesion areas estimated using a detached leaf bioassay showed that fungicide applications reduced lesion development when compared to the control treatment of no fungicide application. Thus, percent disease control was always a positive value and, regardless of fungicide or application method, decreased over time. At the first assessment (1–2 days after application), percent disease control among all fungicide treatments averaged 85.7%, demonstrating full residual activity of the fungicide. By the end of the assessment period, after a single fungicide application, percent disease control averaged 10.4%, 10–16 days (Figs. 2–4).

Results of lesion area were converted to percent disease control, averaged among replicates for each day, and fitted with a linear regression (Figs. 2–4). Estimated slope, intercept, and $r^2$ corresponding to each regression are reported in Table 1 for treatments with fluazinam and Table 2 for procymidone. Fit of linear regression

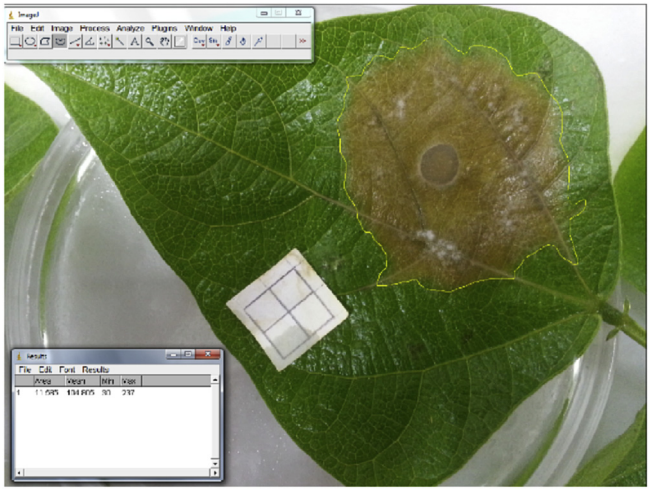

**Fig. 1.** Necrotic lesion area analyzed with software Image J 48 h after the *S. sclerotiorum* inoculation. Area of the outlined lesion in this example is 11.585 $cm^2$.

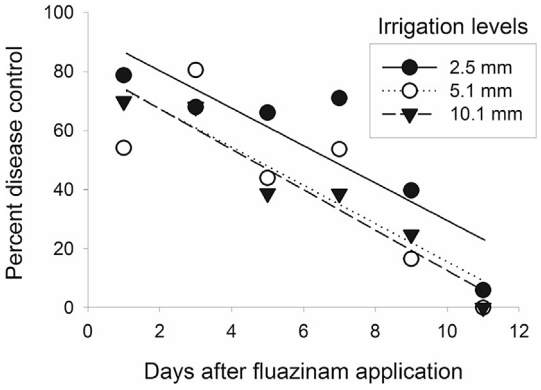


**Fig. 2.** Percent disease control of white mold in dry bean by fluazinam in days after a single application at three chemigation levels (2.5, 5.1, and 10.1 mm) at the Itaí field site in 2013; points are fitted to linear regression, in which each point represents the average of 12 bioassay leaf lesions obtained from four replicated plots.

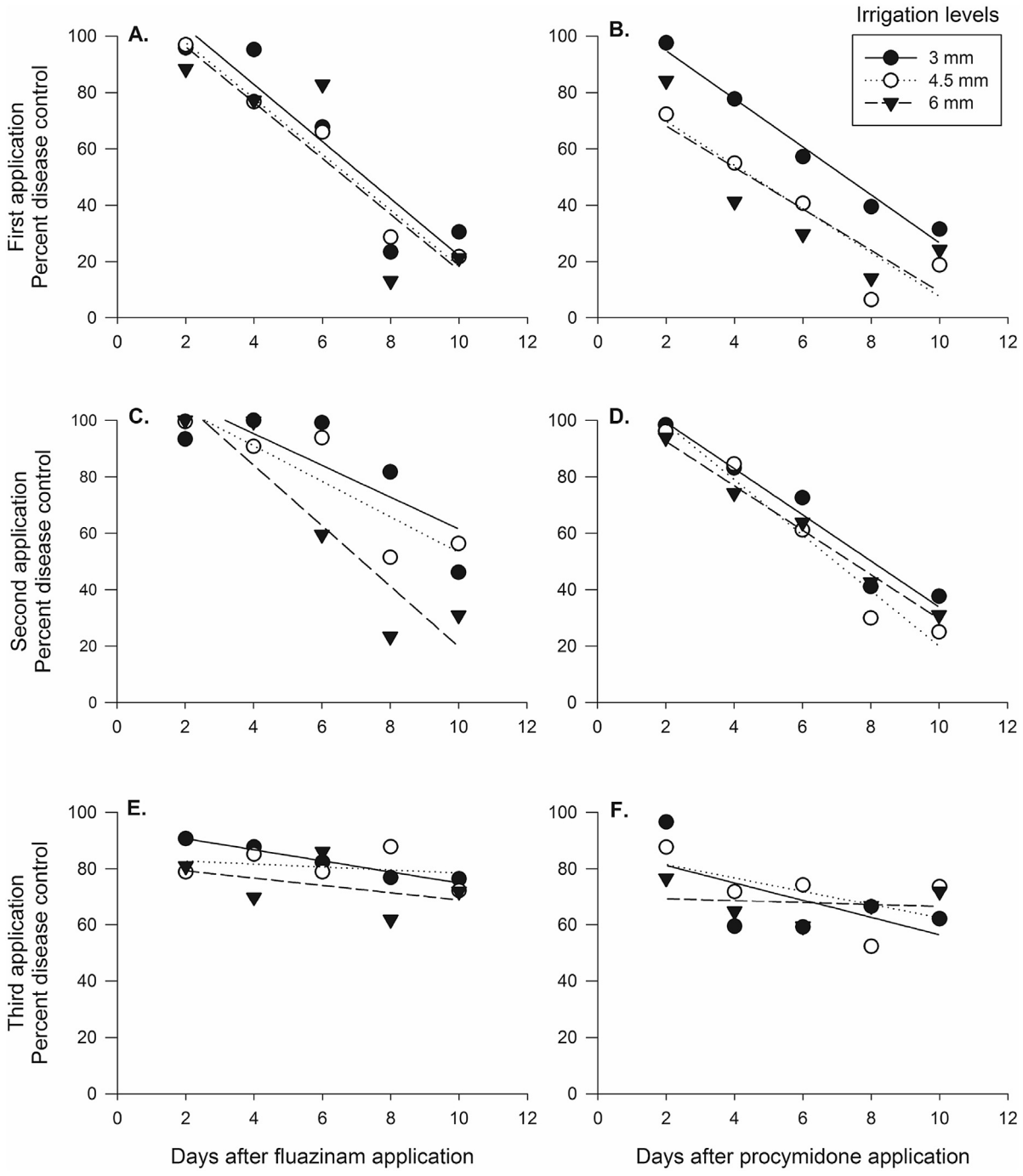


**Fig. 3.** Percent disease control of white mold in dry bean by fluazinam (A, C, and E) and procymidone (B, D, and F) applied at three chemigation levels (3.0, 4.5, and 6.0 mm) in days after three successive applications (first applications were A and B, second applications were C and D, and third applications were E and F) at the Pereiras field site in 2013; points are fitted to linear regression, in which each point represents the average of 12 bioassay leaf lesions obtained from four replicated plots.

($r^2$) were similar for both fungicides, in which 13 of 22 fluazinam treatments had a $r^2 \geq 0.70$ and 11 of 19 procymidone treatments had $r^2 \geq 0.73$.

Lines fit to percent disease control decreased each day after application. Average rate of decay in disease control (slope) after a single application via irrigation for fluazinam was −6.55% $day^{-1}$ at Itaí-2013, -9.98% $day^{-1}$ at Pereiras-2013, and -5.97% $day^{-1}$ at Pereiras-2014 (Table 1). Subsequent applications of fluazinam at the Pereiras site resulted in rates of decay in disease control (slope) closer to zero, in which averages (excluding estimates with $r^2 < 0.60$) of the second and third applications in 2013 were −7.56% $day^{-1}$ and -1.97% $day^{-1}$, and the second application in 2014 was −2.69% $day^{-1}$. The rate of decay in disease control after a single chemigated application of procymidone was similar to fluazinam, in which disease control at Pereiras-2013 decreased faster (−7.87% $day^{-1}$) than at Pereiras-2014 (−5.56% $day^{-1}$). The second application of procymidone at Pereiras-2013 resulted in a slightly faster rate of decay in disease control (−8.64% $day^{-1}$); the third application data at Pereiras-2013 and the second application data at Pereiras-2014 did not fit a linear model ($r^2 < 0.60$).

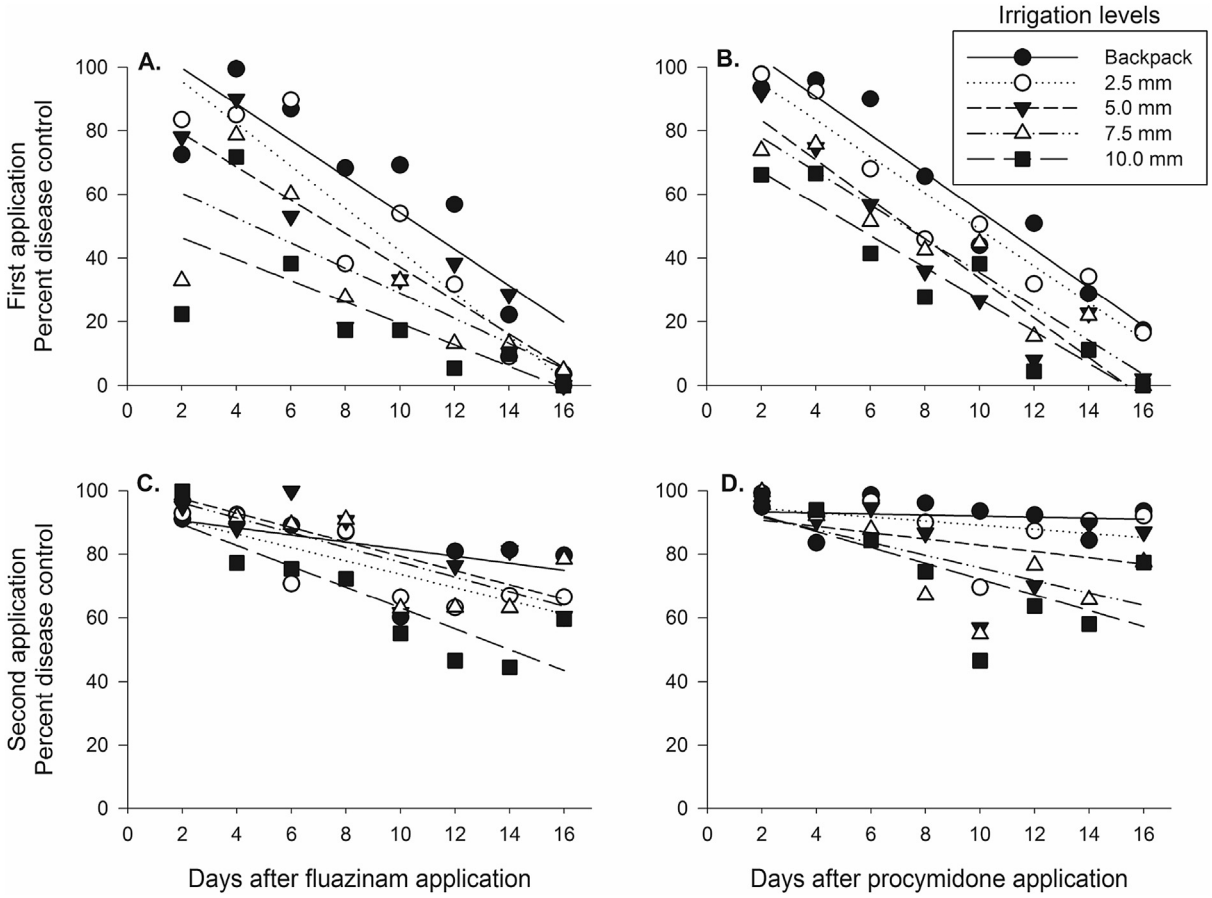


**Fig. 4.** Percent disease control of white mold in dry bean by fluazinam (A and C) and procymidone (B and D) treatment applied at four chemigation levels (2.5, 5.0, 7.5, and 10.0 mm) and also by backpack ground application, in days after two successive applications (first application A and B, second application C and D) at the Pereiras field site in 2014; points are fitted to linear regression, in which each point represents the average of 12 bioassay leaf lesions obtained from four replicated plots.

Comparisons of lines within each treatment using ANCOVA showed a significant difference (Tables 1 and 2) according to day after application for all treatments, excluding the third application of fluazinam at Pereiras in 2013 ($P = 0.08$) and the third application of procymidone at Pereiras in 2013 ($P = 0.10$). There was no statistical support for an interaction between time (days) and irrigation level ($P > 0.05$). Comparisons according to irrigation level showed most were not significantly different within each treatment (Tables 1 and 2). Exceptions were the first applications at Pereiras in 2014, where significant differences between irrigation levels of both fluazinam ($P = 0.0254$) and procymidone ($P = 0.0475$) were found.

AUDPC were smallest among chemigation treatments at the lowest irrigation level within each consecutive application of fungicides. For example, the lowest irrigation level resulted in the smallest AUDPC in five of six groups treated with fluazinam (Table 1) and four out of five groups treated with procymidone (Table 2). However, in both fungicide treatments, many of these were not significantly different within each group and several failed to show a significant difference from the next highest irrigation level. Notable exceptions were both of the first applications of procymidone at Pereiras-2013 (*AUDPC* = 15.20 $cm^2$ cumulative lesion area) and Pereiras-2014 (*AUDPC* = 60.68 $cm^2$ cumulative lesion area).

Average AUDPC of lesion area also showed a general trend of decreasing lesion area with successive application of fungicides. For example, average AUDPC at Pereiras-2013 after the first, second, and third application of fluazinam were 15.67, 8.92, and 7.21 $cm^2$ cumulative lesion area (Table 1). This trend was also observed at Pereiras-2014, which showed AUDPC after the first fluazinam chemigated application was greater than after the second (*AUDPC* = 84.26 and 28.55 $cm^2$ cumulative lesion area). A decrease in cumulative lesion area in successive fungicide applications was also observed for procymidone (Table 2). Average AUDPC for each of three procymidone applications at Pereiras-2013 were *AUDPC* = 21.93, 14.89, and 11.20 $cm^2$ cumulative lesion area, whereas at Pereiras-2014 the average AUDPC in two successive chemigated applications were 78.91 and 23.63 $mm^2$ cumulative lesion area. Due to the cumulative nature of the AUDPC measure and difference in length of assessment periods, direct comparison of AUDPC across years was not practical.

At the Pereiras field site in 2014, application using a backpack sprayer was also included as a treatment for both fungicides in order to simulate ground application typically made using a tractor. Cumulative lesion area (AUDPC) estimated from the first application of fluazinam showed ground application controlled disease better (48.78 $cm^2$ cumulative lesion area; Table 1) than chemigation at all irrigation levels. However, the second ground application (21.08 $cm^2$ cumulative lesion area), was no different from chemigation at all except the highest irrigation level (10 mm irrigation). Both the first and second ground application of procymidone (Table 2) resulted in lower cumulative lesion area (*AUDPC* = 50.97 and 9.97 $cm^2$) than chemigation at the lowest irrigation level (2.5 mm irrigation *AUDPC* = 60.68 and 13.45 $cm^2$),

**Table 1**
Linear regression of percent disease control for white mold in days after application of fluazinam in a bioassay following different chemigation levels, by backpack ground application, and with up to three successive applications at two locations, the Itaí field site in 2013 and Pereiras field site in 2013 and 2014, with corresponding cumulative lesion area under each treatment (AUDPC).

| Site | Application | Irrigation level | Linear regression | | | (p-value) | | AUDPC of Lesion area ($cm^2$) ± SE |
|---|---|---|---|---|---|---|---|---|
| | | | Slope | Intercept | $r^2$ | Day | Treatment | |
| Itaí-2013 | First | 2.5 mm | −6.33 | 92.9 | 0.75 | <0.0001 | 0.7007 | 25.62 ± 3.37B[a] |
| | | 5.1 mm | −6.47 | 80.3 | 0.70 | | | 34.02 ± 2.13 AB |
| | | 10.1 mm | −6.84 | 81.0 | 0.93 | | | 35.26 ± 2.38 A |
| Pereiras-2013 | First | 3.0 mm | −10.11 | 123.2 | 0.86 | <0.0001 | 0.9464 | 14.38 ± 1.46 |
| | | 4.5 mm | −9.91 | 117.5 | 0.96 | | | 15.95 ± 0.93 |
| | | 6.0 mm | −9.91 | 116.0 | 0.75 | | | 16.68 ± 1.21 |
| | Second | 3.0 mm | −5.64 | 117.9 | 0.63 | 0.0003 | 0.8684 | 4.85 ± 0.90 B |
| | | 4.5 mm | −6.30 | 116.2 | 0.77 | | | 7.64 ± 1.13 B |
| | | 6.0 mm | −10.73 | 127 | 0.86 | | | 14.26 ± 2.47 A |
| | Third | 3.0 mm | −1.97 | 94.6 | 0.96 | 0.0797 | 0.4490 | 6.00 ± 1.01 |
| | | 4.5 mm | −0.53 | 83.7 | 0.07 | | | 6.50 ± 1.57 |
| | | 6.0 mm | −1.29 | 81.8 | 0.18 | | | 9.12 ± 1.67 |
| Pereiras-2014 | First | 2.5 mm | −6.68 | 109 | 0.87 | <0.0001 | 0.0254 | 65.80 ± 4.01 C |
| | | 5.0 mm | −5.25 | 89.7 | 0.73 | | | 77.53 ± 4.17 BC |
| | | 7.5 mm | −3.94 | 68.3 | 0.59 | | | 88.88 ± 3.27 B |
| | | 10.0 mm | −3.36 | 53 | 0.51 | | | 104.84 ± 3.17 A |
| | | Ground[b] | −5.71 | 111.2 | 0.76 | <0.0001 | 0.0113 | 48.78 ± 3.72 D |
| | Second | 2.5 mm | −2.10 | 94.6 | 0.66 | <0.0001 | 0.8877 | 27.64 ± 2.58 AB |
| | | 5.0 mm | −2.24 | 101.7 | 0.56 | | | 21.30 ± 2.00 B |
| | | 7.5 mm | −2.31 | 100.5 | 0.58 | | | 24.59 ± 3.80 B |
| | | 10.0 mm | −3.27 | 95.7 | 0.75 | | | 40.66 ± 4.18 A |
| | | Ground | −1.1 | 92.6 | 0.28 | <0.0001 | 0.8962 | 21.08 ± 3.46 B |

[a] Treatments with the same letters are not significantly different, determined using a Tukey's post-hoc test ($\alpha = 0.05$).
[b] Backpack ground application with pressure $CO_2$ at the label rate.

but did not represent a significant difference ($P > 0.05$).

Pairwise comparisons of percent disease control per day (slope) between consecutive fungicide applications (Figs. 3 and 4) were performed using a paired *t*-test. With three consecutive fungicide applications made in 2013 (Fig. 3), three possible comparisons were made (first-to-second, second-to-third, and first-to-third), using data from all irrigation levels. For fluazinam, only the first-to-third applications showed a significant difference ($P = 0.0017$). Procymidone showed a significant difference in the first-to-third application ($P = 0.0079$) and second-to-third application ($P = 0.0140$),

**Table 2**
Linear regression of percent disease control for white mold in days after application of procymidone in a bioassay following different chemigation levels, by backpack ground application, and with up to three successive applications at the Pereiras field site in 2013 and 2014, with corresponding cumulative lesion area under each treatment (AUDPC).

| Site | Application | Irrigation level | Linear regression | | | (p-value) | | AUDPC of lesion area ($cm^2$) ± SE |
|---|---|---|---|---|---|---|---|---|
| | | | Slope | Intercept | $r^2$ | Day | Treatment | |
| Pereiras-2013 | First | 3.0 mm | −8.51 | 111.8 | 0.98 | <0.0001 | 0.2441 | 15.20 ± 1.22 B[a] |
| | | 4.5 mm | −7.76 | 85.2 | 0.85 | | | 25.33 ± 1.20 A |
| | | 6.0 mm | −7.35 | 82.8 | 0.73 | | | 25.25 ± 1.01 A |
| | Second | 3.0 mm | −8.19 | 115.7 | 0.95 | <0.0001 | 0.5214 | 13.01 ± 1.52 |
| | | 4.5 mm | −9.85 | 118.4 | 0.96 | | | 15.93 ± 1.39 |
| | | 6.0 mm | −7.87 | 108.3 | 0.99 | | | 15.73 ± 1.78 |
| | Third | 3.0 mm | −3.10 | 87.3 | 0.38 | 0.0980 | 0.5516 | 11.47 ± 1.65 |
| | | 4.5 mm | −2.38 | 86.2 | 0.35 | | | 10.33 ± 1.28 |
| | | 6.0 mm | −0.37 | 70.1 | 0.03 | | | 11.81 ± 0.82 |
| Pereiras-2014 | First | 2.5 mm | −5.74 | 106.3 | 0.93 | <0.0001 | 0.0475 | 60.68 ± 4.73B |
| | | 5.0 mm | −6.21 | 95.7 | 0.91 | | | 82.01 ± 2.50 A |
| | | 7.5 mm | −5.31 | 88.4 | 0.93 | | | 79.95 ± 3.93 A |
| | | 10.0 mm | −5.01 | 77 | 0.89 | | | 93.01 ± 3.92 A |
| | | Ground[b] | −6 | 114.7 | 0.93 | <0.0001 | 0.0056 | 50.97 ± 3.29 B |
| | Second | 2.5 mm | −0.65 | 95.5 | 0.13 | 0.0040 | 0.9901 | 13.45 ± 1.76 CD |
| | | 5.0 mm | −0.98 | 92.5 | 0.13 | | | 20.07 ± 2.00 BC |
| | | 7.5 mm | −1.98 | 95.5 | 0.42 | | | 28.91 ± 3.46 AB |
| | | 10.0 mm | −2.49 | 97 | 0.47 | | | 32.10 ± 2.55 A |
| | | Ground | −0.16 | 93.5 | 0.02 | 0.0033 | 0.9968 | 9.97 ± 2.16 D |

[a] Treatments with the same letters are not significantly different, determined using a Tukey's post-hoc test ($\alpha = 0.05$).
[b] Backpack ground application with pressure $CO_2$ at the label rate.

**Table 3**
Comparison of fungicide treatments and irrigation rates on percent reduction in number of *Sclerotinia sclerotiorum* sclerotia in soil compared to the control, percent reduction in weight of sclerotia (g) collected after dry bean harvest in 28 $m^2$ compared to the control, disease incidence (%), and yield (kg $ha^{-1}$) in Pereiras, SP.

| | | Reduction (%) | | Disease Incidence (%) | Yield (kg $ha^{-1}$) |
|---|---|---|---|---|---|
| | | Number of Sclerotia | Weight of Sclerotia | | |
| **Pereiras-2013** | | | | | |
| Control vs fungicide-treated | | **[a] | ** | ** | ** |
| Control | | 27.3[b] A[c] | 16.5[b] A | 71.9 A | 1274 B |
| Fungicide-treated (pooled) | | 8.7[b] B | 6.9[b] B | 16.9 B | 2387 A |
| Fungicide | | ** | ** | ** | ** |
| Fluazinam | Irrig. level[d] | | | | |
| | 3.0 mm | 60.1[e] a | 48.5[e] | 27.4 a | 2297 |
| | 4.5 mm | 49.0 ab | 41.2 | 16.4 b | 2188 |
| | 6.0 mm | 31.4 b | 32.8 | 19.9 b | 2108 |
| | Average | 46.8 B | 40.8 B | 21.3 A | 2198 B |
| Procymidone | 3.0 mm | 84.2 | 78.1 | 10.1 b | 2606 |
| | 4.5 mm | 86.8 | 78.9 | 10.0 b | 2512 |
| | 6.0 mm | 94.2 | 83.9 | 17.4 a | 2609 |
| | Average | 88.4 A | 80.3 A | 12.5 B | 2576 A |
| **Pereiras-2014** | | | | | |
| Control vs fungicide-treated | | ** | ** | ** | ** |
| Control | | 8.0[b] A | 5.9[b] A | 6.6 A | 2649 B |
| Fungicide-treated (pooled) | | 0.8[b] B | 1.1[b] B | 0.8 B | 3183 A |
| Fungicide | | ns | ** | ns | * |
| Fluazinam | Irrig. level[d] | | | | |
| | 2.5 mm | 90.6[e] | 67.5[e] ab | 1.0 a | 3286 |
| | 5.0 mm | 96.9 | 69.9 ab | 1.0 a | 3208 |
| | 7.5 mm | 90.6 | 59.7 b | 1.2 a | 3218 |
| | 10.0 mm | 84.4 | 66.9 ab | 1.4 a | 3448 |
| | Ground[f] | 84.3 | 79.9 a | 0.2 b | 3219 |
| | Average | 89.4 | 68.8 B | 1.0 | 3276 A |
| Procymidone | 2.5 mm | 100.0 | 94.7 | 0.2 c | 2695 c |
| | 5.0 mm | 93.8 | 94.3 | 0.4 bc | 3129 ab |
| | 7.5 mm | 81.2 | 92.4 | 0.9 ab | 3248 ab |
| | 10.0 mm | 90.6 | 88.0 | 1.5 a | 3373 a |
| | Ground[f] | 87.4 | 93.3 | 0.2 c | 3004 bc |
| | Average | 90.6 | 92.5 A | 0.6 | 3090 B |

[a] Significance indicated with ** at the $\alpha = 0.01$ level, * at $\alpha = 0.05$, and ns = not significant.
[b] Average number/weight of sclerotia not expressed as percent reduction.
[c] Treatments with the same letters are not significantly different, determined using Tukey's post-hoc test ($\alpha = 0.05$).
[d] Irrig. level = irrigation level.
[e] Percent reduction determined as the difference between control and fungicide treatments, divided by the control, and multiplied by 100.
[f] Backpack ground application at label rate (1000 L $ha^{-1}$).

but no difference in first-to-second application ($P = 0.3928$). Since only two fungicide applications were made in 2014 (Fig. 4), there was only one possible comparison (first-to-second), where only procymidone application showed a significant decrease ($P = 0.0090$). Using data from all irrigation levels, similar comparisons of cumulative lesion areas (AUDPC) were also made using a paired *t*-test, (Tables 1 and 2). Significant differences in 2013 were observed for fluazinam in a comparison to the first-to-second and first-to-third applications ($P < 0.0001$), but showed no difference in the second-to-third application ($P = 0.1052$). Procymidone showed a significant difference for all possible comparisons ($P < 0.05$). In 2014, comparisons of AUDPC from first-to-second applications for both fungicides showed a significant difference ($P < 0.0001$).

Results on the effect of treatments on reduction of number of *S. sclerotiorum* sclerotia collected from soil, reduction in weight of sclerotia after dry bean harvest, disease incidence, and dry bean yield at the Pereiras field site are presented in Table 3. Fungicide treatments were significantly more effective than the control treatment (no fungicide) in both years. A major difference between years is that disease incidence in 2013 (71.9%) with no fungicide application was greater than in 2014 (6.6%). However, differences in the method for disease incidence assessment may have overestimated disease in 2013. In both years, there was significant difference between irrigation levels within each fungicide, but only procymidone showed higher disease incidence with higher irrigation levels. Yield in the control was also considerably lower in 2013 (1274 kg $ha^{-1}$) than in 2014 (2649 kg $ha^{-1}$). Comparison of fluazinam to procymidone treatments showed yields were significantly different in both years. In 2013, yield of plots treated with procymidone was higher than those treated with fluazinam, and there were greater reduction in the number and weight of *S. sclerotiorum* sclerotia, and reduced disease incidence (Table 3). There was also a significant difference between irrigation levels with fluazinam in 2013 on reduction in number of sclerotia in soil. Reduction in weight of residual sclerotia were also significantly different between irrigation levels of fluazinam 2014, with greatest reduction observed with backpack application. In 2014, there was no significant difference between fungicide treatments with respect to reduction in number of *S. sclerotiorum* sclerotia in the soil or in disease incidence, but percent reduction in weight of sclerotia was significantly lower with fluazinam treatment. Yield was significantly higher with fluazinam treatment (Table 3).

## 4. Discussion

Our results showed that irrigation level had little effect on percent disease control over time. For example, comparisons of lines estimated from percent disease control over time using ANCOVA showed only the first applications of fluazinam and procymidone in 2014 resulted in significant differences (Tables 1 and 2). However, a limitation of ANCOVA is that small differences between treatments may not be detected, but may have a greater cumulative effect. For that reason, we also made comparisons using cumulative lesion areas (AUDPC), which showed in almost every chemigation treatment (5 of 6 fluazinam; 4 of 5 procymidone) that the lowest irrigation level resulted in the lowest AUDPC (Tables 1 and 2). Although not all pairwise comparisons of AUDPC according to irrigation level were significant, these results support a conclusion that application of fungicides at lower irrigation levels enables greater disease control.

The effect of consecutive applications was tested at Pereiras, where a second fungicide application was made in 2013 and 2014, and third application in 2013. Consecutive fungicide applications resulted in disease control that decreased more slowly, with estimated slopes closer to zero. In both cases, these data more often had poor fit with linear regression ($r^2 < 0.7$), which was likely the result of greater variance in small lesion areas when disease control was high. For both fluazinam and procymidone, percent disease control remained highest after the final successive application, which was not lower than 55% control after the third application in 2013 (Fig. 3) and not less than 40% control after the second application in 2014 (Fig. 4). Greater sustained disease control observed with successive fungicide applications may be related to increasing plant age over the duration of the study (Augusto and Brenneman, 2012). For example, the first DLB performed at Pereiras in 2013 and 2014 was more than 36-days prior to the final DLB. However, such an effect is thought to be minimal because percent disease control was calculated relative to untreated plants that were the same age as fungicide-treated plants, and leaves selected for the DLB were the youngest fully expanded trifoliate leaves. Thus, results in the present study are more likely due to accumulating fungicide residue and not due to greater resistance of older dry bean plants, which was evidenced by reduced cumulative lesion areas (AUDPC, Tables 1 and 2).

We hypothesized that disease control with fluazinam would decrease more rapidly than procymidone because this is a systemic fungicide that is able to be absorbed through plant roots and translocate to leaves (Chen et al., 2010). This was evidenced in paired *t*-test comparison of rate of percent disease control (slope) between successive applications, in which no significant difference suggested a lack of accumulation of residual fungicide activity from one application to the next. Indeed, the rate of percent disease control was significantly different from first-to-third and second-to-third applications of procymidone in 2013 (15-day intervals), and first-to-second application in 2014 (16-day intervals). For fluazinam, rate of percent disease control was not significantly different, regardless of application, in which only second order comparisons of percent disease control were significantly different (i.e. first-to-third in 2013). Lesion areas were also compared between consecutive applications using a paired *t*-test and showed more pairwise differences for procymidone treatments than for fluazinam treatments. Differences in each successive procymidone application were observed in 2013 and 2014, suggesting an accumulation of fungicide in the plant leaves. In comparison, fluazinam did not yield a significant difference from second-to-third applications in 2013. Taken together, these results suggest that procymidone had a longer lasting residual activity compared to fluazinam. However, some authors suggest that systemic activity of fungicides in younger plants may not correlate with that in older plants due to increased thickness of plant membranes, waxes, distance of translocation, velocity of transpiration stream in xylem, chemical composition, and flow rate of phloem (Augusto and Brenneman, 2012). Thus, such an accumulation of fungicide after additional successive applications may not continue to increase over time and increasing plant age may be one reason this occurs.

We also evaluated fungicide chemigation level on yield and reduction of *S. sclerotiorum* sclerotia at Pereiras in 2013 and 2014. Overall, results showed procymidone treatments resulted in greater reduction in *S. sclerotiorum* sclerotia than fluazinam in both 2013 and 2014 (Table 3), although differences were marginal in 2014 due to low disease pressure. This is in concordance with a publication that showed procymidone was more effective than fluazinam in reducing the number of sclerotia produced on infected plants (Berger-Neto et al., 2017). In 2013, procymidone showed greater reduction in weight of residual sclerotia and greater reduction in number of sclerotia collected in soil irrigated at the highest level (6 mm). Our results are consistent with a previous study using procymidone that compared chemigation (5.5 and 11 mm) with ground application and showed chemigation at the highest level resulted in the best disease control, fewer apothecia and lower weight of residual sclerotia at harvest (Venegas and Saad, 2010). In our study, however, reduction in number of sclerotia collected from soil after fluazinam treatment was greater when irrigation levels were lower in 2013. Moreover, greater sclerotial weight reduction was observed for fluazinam ground application. This is consistent with a previous study in which fluazinam application by backpack ground application (667 L $ha^{-1}$) was compared to chemigation (3.5 mm) and showed that ground application reduced the weight and number of *S. sclerotiorum* sclerotia collected after dry bean harvest (Vieira et al., 2003). The same authors also reported that fluazinam can control white mold when applied directly to the soil surface and is superior to benomyl (systemic) in reducing incidence and severity. However, these fungicides are not presently labeled for direct soil application for dry bean and soybean.

Although ground application resulted in significantly smaller AUDPC of lesion area than chemigation applied at the highest irrigation level, ground application only once resulted in the lowest total lesion area via bioassay when compared to the first chemigation of fluazinam at the lowest irrigation level (Table 1). Nevertheless, ground-applied fungicides resulted in the lowest disease incidence among all treatments in 2014, which suggests additional irrigation applied via chemigation may have an affect on microclimatic conditions within the plant canopy to promote disease development. Indeed, greater irrigation levels (without fungicide application) have been shown previously to increase white mold disease incidence and severity (Napoleão et al., 2005). Despite having the lowest disease incidence, ground applied plots did not have the highest yields. Highest yield was obtained with chemigation at 10.0 mm irrigation, which suggests there was an interaction between increased irrigation level and plant productivity, as has been shown previously (Napoleão et al., 2005). However, comparison within chemigated plots showed no difference in yield, with the exception of procymidone applied at Pereiras-2014. This is consistent with previous work that showed that yield of soybean with ground application of fluazinam was no different from treatments applied via irrigation (by Vieira et al., 2001). Thus, a significant difference in yield among treatments with procymidone application in Pereiras-2014 may be related to the overall low disease pressure in 2014 and additional water supplied with the highest chemigation irrigation level. These results may not remain the same under high disease pressure and more data are needed before conclusions can be made.

As a whole, our results indicate that both lower irrigation level and ground application are methods that slow the loss of residual fungicide activity and lessen total lesion area development. These results are similar to a previous study that showed application of fluazinam and benomyl to dry bean using either chemigation at 3.5 mm or ground application provided similar levels of white mold control (Vieira et al., 2003). Additionally, our results are in concordance with previous studies showing fungicide efficacy decreases with increasing irrigation level. This is especially evident at levels above 8 mm (Vieira and Sumner, 1999), in which some have suggested that the mechanism is related to an inverse relationship between irrigation level and deposition efficiency of the fungicide onto foliage (Geary et al., 2004). However, it is important to consider that results from the present and previous studies were based on leaves harvested from the upper plant canopy, whereas leaves from the lower or inner canopy may have shown greater differences due to dependence of contact fungicides on canopy penetration for effectiveness. Harvesting leaves from different portions of the plant canopy was not considered in the present study because leaves from the lower and inner canopy represent leaves of different ages, and the age of a leaf affects severity of disease development (Augusto and Brenneman, 2012). Future research is needed to fully characterize the effect of fungicide application via irrigation on formation and development of *S. sclerotiorum* apothecia. For example, no previous studies have examined the effect of chemigation on apothecial development, which may be more indicative of disease control ability because ascospores are the primary mode of plant infection. In addition, future work should examine residual fungicide activity in the lower and inner plant canopy, where systemic fungicides may play a more important role in disease control and secondary spread.

## Acknowledgements

Acknowledged is financial support provided to T. J. J. Miorini by Coordination for the Improvement of Higher Education Personnel. The authors also thank Cercadinho Farm and Agricultural Research and Development Center (CPDA) for use of research plots, with financial support for research also provided by Arysta LifeScience. Special thanks to those who assisted with the project, including Ângelo Stasievski, João Miyasaki and Dorival Boer Júnior, also to Lucky Mehra for advice with statistical analyses, and Harald Scherm for thoughtful review of this manuscript during preparation.

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