Sensitivity and Efficacy of Boscalid, Fluazinam, and Thiophanate-Methyl for White Mold Control in Snap Bean in New York

M. S. Lehner, School of Integrative Plant Science, Plant Pathology & Plant-Microbe Biology Section, Cornell University, Geneva, NY 14456; E. M. Del Ponte, Departamento de Fitopatologia, Universidade Federal de Viçosa, MG, 36570-900, Brazil; B. K. Gugino, Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, PA 16802; J. R. Kikkert, Cornell Cooperative Extension, Canandaigua, NY 14424; and S. J. Pethybridge, School of Integrative Plant Science, Plant Pathology & Plant-Microbe Biology Section, Cornell University, Geneva, NY 14456

Abstract

White mold (*Sclerotinia sclerotiorum*) of leguminous crops in New York is generally managed with preventive applications of fungicides. However, no research has been conducted during the last decade to assess the sensitivity of the *S. sclerotiorum* population to fungicides or compare their performance under field conditions. The sensitivity of *S. sclerotiorum* to boscalid, fluazinam, and thiophanate-methyl was assessed in 151 isolates from 15 fields across New York using an agar dilution method with discriminatory concentrations. In addition, the effective concentration at which mycelial growth is reduced by 50% (EC₅₀) was estimated for one representative isolate from each field. The efficacy of commercial formulations of each fungicide on white mold incidence in plants and pods was also tested in two field trials (2015 and 2016). The EC₅₀ values ranged from 0.068 to 0.219, 0.001 to 0.002, and 1.23 to 2.15 µg/ml for boscalid, fluazinam, and thiophanate-methyl, respectively. Evidence of resistance was not found using the discriminatory concentration tests. The mycelial growth inhibition relative to the control ranged from 56

to 83%, 66 to 84%, and 53 to 83% at discriminatory concentrations of boscalid (5 µg a.i./ml), fluazinam (0.05 µg a.i./ml), and thiophanate-methyl (5 µg a.i./ml), respectively. Fourteen isolates with mycelial growth inhibition lower than 60% at 5 µg/ml of thiophanate-methyl, did not exhibit point mutations within a partial sequence of the β-tubulin gene. In the field trials, fungicides effectively reduced white mold incidence on plants by 75% (2015) and 93% (2016) and on pods by 81% (2015) and 87% (2016), both relative to the nontreated plots. However, fungicide applications led to significant increases in pod yield, relative to the nontreated plots, only in 2015 when the incidence of white mold on plants and pods were higher (85 and 49.2%) than in 2016 (31.3 and 10.3%). Although fungicide resistance was not detected, and thus control failures reported by New York snap bean growers may be due to other factors, further monitoring of sensitivity within the *S. sclerotiorum* population is encouraged as well as the use of rational systems to base their judicious and economic use.

Beans are an important component of rotational cropping systems in New York. Snap and dry beans (*Phaseolus vulgaris* L.) are grown annually on approximately 27,925 and 9,884 acres, respectively. Processing baby lima beans are also becoming popular with a production area that expanded four-fold in the last 4 years, reaching 1,976 acres in 2015. Soybean production also continues to expand with 69,893 acres harvested in 2012 (USDA-NASS 2012).

White mold caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is a persistent problem for beans and other crops in regions with an environment favorable for the disease, especially due to the presence of long-lived sclerotia produced by the pathogen and the absence of high levels of resistance in commercial cultivars (Boland and Hall 1994; Bolton et al. 2006; Peltier et al. 2012; Schwartz and Singh 2013). Ascospores released by apothecia are the primary inoculum for white mold epidemics (Abawi and Grogan 1979). Infected and senesced flowers fall onto the leaves and stems and contribute to disease spread within the plant and, more rarely, between plants (Natti 1971). At high incidence, white mold may lead to severe reductions in the number of marketable pods because of premature pod abscission, fungal growth on the pods that remain attached to the plant, and canopy collapse due to lodging making mechanical harvest problematic and exacerbating economic losses from the disease (Boland and Hall 1987; Jones et al. 2011a).

The control of white mold in snap bean in New York is based on preventive applications of fungicides aimed at protecting flowers from infection by ascospores (Shah et al. 2002). Until the early 2000s, vinclozolin (Ronilan; BASF, Florham Park, NJ) and benomyl

(Benlate; DuPont, Wilmington, DE) were the most commonly used fungicides for white mold control and other pod diseases of bean in the region (Shah et al. 2002). However, in 2001, the registration of benomyl was cancelled because of human health and environmental concerns. Four years later, vinclozolin was also withdrawn for similar reasons. These fungicides were replaced in 2006 by thiophanate-methyl, which rapidly became the most commonly used fungicide due to its relatively low cost (Shah et al. 2002).

In 2014, dry, snap, and lima bean growers and industry stakeholders across New York reported suboptimal control of white mold from the use of thiophanate-methyl. One potential hypothesis is the development of fungicide resistance within the *S. sclerotiorum* population. Thiophanate-methyl is a site-specific benzimidazole fungicide and a member of the Fungicide Resistance Action Committee (FRAC) group 1 (Fungicide Resistance Action Committee 2013). This kind of fungicide is classified as a high risk for resistance development and consequently control failures in the field may occur (Brent and Hollomon 2007). Resistance to benzimidazoles in *S. sclerotiorum* has been reported in China for carbendazim (Shi et al. 2000) and in Brazil for thiophanate-methyl (Lehner et al. 2015), respectively. No information is available on the sensitivity of the *S. sclerotiorum* populations from New York to benzimidazole fungicides.

Boscalid and fluazinam are among the most efficacious fungicides for white mold control in leguminous crops (Mahoney et al. 2014; McCreary et al. 2016). Boscalid is a site-specific fungicide from the succinate dehydrogenase inhibitors (SDHIs) class (FRAC group 7). The risk of resistance development to this fungicide in *S. sclerotiorum* has been suggested as low to moderate (Wang et al. 2015). There has been a single report of resistance to boscalid in a field isolate of *S. sclerotiorum* from France (Stammler et al. 2010). However, laboratory-based studies have demonstrated that selection for resistant isolates can be achieved (Wang et al. 2015). Boscalid was first registered for use on dry and succulent beans in New York in 2005, but its adoption has been limited because of cost implications on profit margins.

Corresponding author: S. J. Pethybridge, E-mail: sjp277@cornell.edu

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Fluazinam is a multisite phenylpyridinamine fungicide (FRAC 29; Guo et al. 1991), which has rapidly become the industry standard for white mold control in many parts of the United States and worldwide (Mahoney et al. 2014; McCreary et al. 2016; Pynenburg et al. 2011; Varner and Terpstra 2007). The risk of resistance development to fluazinam is low because of its multiple site mode of action. In field studies, resistance to fluazinam has not yet been reported in plant pathogenic fungi, but laboratory-induced resistant isolates of *Ustilago maydis* were obtained after treatment with N-methyl-N-nitro-N-nitrosoguanidine (Vitoratos 2014). Fluazinam was registered in New York in 2015 and became available for use in snap, dry, lima, and soybean the following year. Its recent registration in the region provides an opportunity to establish the baseline sensitivity to this fungicide, which will be useful for further monitoring of the temporal shifts in the sensitivity of *S. sclerotiorum* (Russell 2004).

The objectives of this study were to (i) determine the sensitivity to boscalid and thiophanate-methyl in the *S. sclerotiorum* population from New York; (ii) establish the baseline sensitivity within the *S. sclerotiorum* population to fluazinam; and (iii) evaluate the efficacy of boscalid, fluazinam, and thiophanate-methyl for white mold control in snap bean under field conditions. Integration of this information will be vital in the design of management strategies for white mold control in snap bean and other leguminous crops in New York as well as in the surrounding production regions.

Materials and Methods

Isolate collection. In 2014, 151 *S. sclerotiorum* isolates were collected from 15 fields in the western and central regions of New York (Fig. 1). The isolates were collected from fields cultivated with dry bean ($n = 8$ fields), snap bean ($n = 4$), soybean ($n = 2$), and lima bean ($n = 1$). These fields were selected based on reports from growers and extension educators of suboptimal control despite the application of thiophanate-methyl. The number of isolates collected within each field varied from five to 15. One sclerotium from each sampled site within the fields was surface-sterilized using standard procedures (Lehner et al. 2015) and transferred to Petri dishes (90 mm diameter) containing potato dextrose agar (PDA; Hardy Diagnostics, Santa Maria, CA). The dishes were incubated at 23°C for the germination of sclerotia and mycelial growth. Mycelial discs (5 mm diameter) from the colonies resulting from germination of sclerotia were transferred to water agar (WA; Hardy Diagnostics, Santa Maria, CA) and incubated at 23°C for 24 h. Individual hyphal tips were removed using a scalpel and transferred to freshly prepared PDA plates using a dissecting microscope. To ensure genetic uniformity, only hyphal-tipped isolates were used in the experiments (Lehner et al. 2016).

Fig. 1. Geographical location of bean fields across New York in which suboptimal control of white mold from thiophanate-methyl application was reported and where *Sclerotinia sclerotiorum* isolates were collected for fungicide sensitivity testing.

Sensitivity to boscalid, fluazinam, and thiophanate-methyl using EC₅₀. One *S. sclerotiorum* isolate from each field was randomly selected to estimate the effective concentration of fungicide at which mycelial growth was reduced by 50% (EC₅₀). The following commercial fungicide formulations were used in all assays: Endura 70 WG (50% boscalid, BASF Corporation, Florham Park, NJ); Omega 500 F (40% fluazinam, Syngenta Crop Protection, Greensboro, NC); and Topsin 4.5 FL (45% thiophanate-methyl, United Phosphorus, Inc., King of Prussia, PA). The fungicides were dissolved in dimethyl sulfoxide (DMSO) to obtain 100 mg of a.i./ml for the stock solution. To obtain the desired concentrations of each fungicide, serial dilutions were prepared from each of the stock solutions. The concentration of DMSO did not exceed 0.1% of the testing solution (fungicide-amended medium). Fungicides were added to cooled (42 to 50°C) PDA. The concentrations used for each fungicide were: 0 (PDA + DMSO), 0.03, 0.1, 0.3, 1.0, and 5 µg of boscalid/ml; 0, 0.0025, 0.005, 0.01, 0.05, and 0.1 µg of fluazinam/ml, and 0, 0.5, 1.0, 2.5, 5.0, and 10 µg of thiophanate-methyl/ml.

Mycelial plugs (5 mm diameter) from a 2-day-old culture of each of the 15 isolates were placed in the center of Petri dishes (6 cm diameter) containing 10 ml of PDA amended with the fungicides at each of the above concentrations. The colony diameter of each isolate was measured in two perpendicular directions with 24 h of incubation at 23°C in the dark. A completely randomized design with four replicates for each concentration of fungicide was used. A Petri dish with a mycelial plug was considered as an experimental unit. The experiment was performed twice.

The EC₅₀ values were calculated by fitting a log-logistic model to the data on the relationship between colony diameter and the tested concentrations. A three-parameter log-logistic model, with estimation of parameters based on maximum likelihood, was fitted using the *drm()* function within the *drc* package for dose-response analysis of R (Ritz et al. 2015). As the variances of EC₅₀ values in the two experiments were homogeneous, data were combined and reanalyzed considering eight replicates.

Sensitivity to boscalid, fluazinam, and thiophanate-methyl using discriminatory concentrations. The sensitivity of 151 *S. sclerotiorum* isolates to boscalid, fluazinam, and thiophanate-methyl was quantified using discriminatory concentrations. For fluazinam and thiophanate-methyl, the concentrations of 0.05 and 5 µg/ml, respectively, were selected as outlined by Lehner et al. (2015). For boscalid, the discriminatory concentration was established as 5 µg/ml as this concentration caused more than 75% of mycelial growth inhibition in the *S. sclerotiorum* isolates used in the previous experiment (Lehner et al. 2015). The percent of mycelial growth inhibition was calculated for each fungicide as: $1 - (\text{colony diameter in fungicide-amended} / \text{colony diameter in non-amended control treatment}) \times 100$. Isolates were considered resistant if the percent inhibition induced by each discriminatory concentration was less than 50% relative to control, otherwise they were considered sensitive (Lehner et al. 2015). Mycelial plugs (5 mm diameter) from a 2-day-old culture of each isolate were placed in the center of Petri dishes (6 cm diameter) containing 10 ml PDA amended with the discriminatory concentrations of each fungicide. The plates were incubated for 24 h at 23°C in the dark. Colony diameter of each isolate was measured in two perpendicular directions using a digital caliper. The experimental design was completely randomized. For each isolate, four replicates with and without fungicides were used. The experiment was performed twice.

Partial sequence of β -tubulin gene. A partial sequence of the β -tubulin gene usually associated with point mutations in fungi resistant to benzimidazoles was analyzed in 14 *S. sclerotiorum* isolates with mycelial growth inhibition close to 50% (52 to 59%) at 5 µg/ml of thiophanate-methyl (Fig. 2). The partial sequence of the β -tubulin gene of two sensitive isolates that were inhibited by 80% at 5 µg/ml of thiophanate-methyl was also analyzed for comparative purposes. Isolates were grown in potato dextrose broth at 23°C for 7 days. Mycelia were then washed with distilled water, transferred to filter paper to dry, and DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI) following the manufacturer's instructions.

DNA integrity was analyzed on an agarose gel (1% w/v agarose in Tris-acetate-EDTA) amended with 0.5× (v/v) nucleic acid stain GelRed (Biotium Inc., Fremont, CA) and concentration was estimated in a spectrophotometer (Nanodrop 2000; Thermo Scientific, Waltham, MA).

PCR (50 µl volume) contained 1× Standard PCR buffer (New England Biolabs Inc., Ipswich, MA), 1.5 mM MgCl₂ (New England Biolabs Inc.), 0.1 mM dNTPs (New England Biolabs Inc.), 0.1 µM of each of the forward and reverse primers, 0.7 U Taq polymerase (New England Biolabs Inc.), and 75 ng of template DNA. The primer pair used was TUB-F1 (5'-GCTTTTGATCTCCAAGATCCG-3') and TUB-R1 (5'-CTGGTCAAAGGAGCAAATCC-3'). PCR was conducted in a C1000 Thermal Cycler (Bio-Rad, Hercules, CA). PCR conditions were an initial denaturation for 5 min at 95°C, followed by 36 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 68°C for 30 s; and a final extension at 68°C for 5 min. Amplification was confirmed by using electrophoresis in a 1% agarose gel and Tris-borate-EDTA (TBE) and viewed under UV light after staining with GelRed (Biotium Inc., Fremont, CA). PCR products were purified using ExoSAP-IT according to the manufacturer's instructions (Affymetrix, Santa Clara, CA) and sequenced at the Cornell University Institute of Biotechnology Genomic Diversity Facility, Ithaca, NY. The nucleotide sequences were edited using Geneious software (Geneious v. 7.1.8, <http://www.geneious.com>; Kearse et al. 2012) and aligned using MEGA 5.0 (Tamura et al. 2011).

Field experiments. Two small-plot, replicated field trials were conducted in 2015 and 2016 to evaluate the efficacy of boscalid, fluazinam, and thiophanate-methyl for white mold control in snap bean in Geneva, New York. Trials in both years were established using the variety 'Huntington' (Seneca Foods) at an in-row plant density of 28.5 seeds/m, and rows separated by 0.76 m. The snap bean seeds were planted on 17 July 2015 and 31 May 2016. Fertilizer (336 kg/ha 15 N:5 P:10 K) was banded at planting, and the pre-emergent herbicide, Dual Magnum (0.34 liter/ha; Syngenta Corporation; Greensboro, NC; 83.7% s-metolachlor) was applied within 24 h of planting for weed control. At 35 days after planting (DAP), 56.1 kg/ha 22% nitrogen was applied within the rows in both trials. The experimental area was irrigated at a rate of approximately 5 mm/h at five- and two-day intervals in 2015 and 2016, respectively.

Ascopore suspensions were used to inoculate *S. sclerotiorum* onto plants within the entire plot area in both trials. The suspensions were prepared using ascospores stored on filter paper discs at -20°C as described by Pethybridge et al. (2015). The ascospores were scraped from the discs using a sterile scalpel and immersed in sterile distilled water amended with Tween-20 at 0.01%. The ascospore suspensions were sprayed two times over the flowering period using a backpack sprayer. In 2015, the first inoculation occurred at 37 DAP, when approximately 10% of plants had at least one open flower (10% flowering). The second inoculation occurred at 39 DAP, when 100% of plants had at least one open flower (100% flowering). In 2016, ascospore inoculations occurred at 44 (10% flowering) and 46 DAP (100% flowering). The concentrations of ascospores used in the first and second inoculations in 2015 were 5.6×10^5 and 6.5×10^5 ascospores/ml, respectively. In 2016, the concentrations of ascospores used in the first and second inoculations were 7.4×10^5 and 3.5×10^5 ascospores/ml, respectively. The viability of the ascospores was quantified prior to each application by assessing the germination of 100 ascospores following placement of 50 µl of ascospore suspension on three replicates of 2% WA. An ascospore was considered germinated if the germ tube was at least two times the length of the ascospore.

In the 2015 trial, each plot had two 3.96-m-long rows. In the 2016 trial, plots had two 3.05-m-long rows. The plots between blocks were separated by four nontreated and noninoculated rows and by 1.2 m within rows. The treatments tested in both trials were: control (nontreated); boscalid as Endura (126.2 g/ha), fluazinam as Omega 500 F (162.8 ml/ha), and thiophanate-methyl as Topsin 4.5 FL (35.9 ml/ha). The fungicides were applied twice in both trials using a carbon dioxide-pressurized backpack sprayer with four TeeJet 8002VS flat fan nozzles at volumes of 202.9 and 261.9 liters/ha in 2015 and 2016, respectively. The fungicide applications

occurred at 35 DAP (10% flowering) and 40 DAP (100% flowering). The experimental design in both years was a completely randomized block with five replications.

The incidence of white mold on plants and pods, the total yield of the healthy pods per 1-m row section, the average weight of individual healthy pods and the total number of plants per 1-m row were assessed in each trial at harvest (67 to 69 DAP). Plants were removed from two 1-m row lengths (total = 2 m/plot). Pods were separated from the plants by hand and sorted into either healthy or diseased and counted. Diseased pods either had white mold symptoms and/or had signs of *S. sclerotiorum* (mycelia and/or sclerotia). Data from the two trials were analyzed separately due to differences in disease

Fig. 2. Percentage reduction in mycelial growth relative to the growth in the nonamended media of the 151 *Sclerotinia sclerotiorum* isolates at discriminatory concentrations of boscalid, fluazinam, and thiophanate-methyl. Each dot in the plot represents an isolate.

incidence between the years. Analysis of variance was performed using Genstat Version 17.1 (Hemel Hempstead; United Kingdom) and least square means were calculated. Treatments means were separated using Fisher's protected least significant difference test (LSD) at $P < 0.05$.

Results

Sensitivity to boscalid, fluazinam, and thiophanate-methyl using EC₅₀. The nonlinear model provided a good fit to the data. On average, the estimated EC₅₀ values were lowest for fluazinam (0.0014 µg/ml), intermediate for boscalid (0.116 µg/ml), and highest for thiophanate-methyl (1.69 µg/ml). There was no trend for an association of EC₅₀ values for any of the fungicides with host of origin or location given the low heterogeneity in the EC₅₀ values (Table 1).

Sensitivity to boscalid, fluazinam, and thiophanate-methyl using discriminatory concentrations and partial DNA sequences of the β-tubulin gene. Although all isolates were able to grow at the discriminatory concentrations of each fungicide, evidence of resistance was not found. For boscalid and thiophanate-methyl, in most cases the mycelial growth inhibition ranged from 60 to 80%. For fluazinam, the mycelial growth inhibition was mostly between 75 and 85% (Fig. 2). Analysis of the partial sequence of the β-tubulin gene from the selected isolates based on the response to thiophanate-methyl did not identify any mutations in comparison with the sensitive isolates.

Field experiments. The viability of *S. sclerotiorum* ascospores was at least 98% across inoculations and years, resulting in 85.6 and 49.2%

of plants in the nontreated control plots with white mold symptoms in 2015 and 2016, respectively. The effect of fungicides on the number of plants per m (both trials) and on total pod yield in the trial conducted in 2016 was not significant (Table 2). Fungicides significantly affected the incidence of white mold on plants in 2015 ($F = 24.37$; $P < 0.001$) and 2016 ($F = 61.68$; $P < 0.001$). The mean white mold incidence on plants ranged from 14.2 to 85.6% in 2015, and from 0.6 to 49.2% in 2016 (Table 2). In both trials, there was no significant difference in white mold incidence on plants among the fungicides. Fungicides also significantly affected the incidence of white mold on pods in 2015 ($F = 32.31$; $P < 0.001$) and in 2016 ($F = 17.79$; $P < 0.001$). The mean white mold incidence on pods ranged from 2.8 to 31.3% in 2015, and 0.3 to 10.3% in 2016. The effect of fungicides on the total pod yield per 1-m row was significant in 2015 ($F = 4.35$; $P = 0.029$), as well as the effect on the weight of individual pods ($F = 21.6$; $P < 0.001$). In plots receiving thiophanate-methyl, total pod yield was significantly higher compared with the nontreated control and no difference was identified among plots receiving thiophanate-methyl, boscalid, and fluazinam. The weight of individual pods in the nontreated control plots was significantly lower than in fungicide-treated plots, which were not significantly different between each other (Table 2).

Discussion

Growers are often reliant upon the prophylactic application of fungicides for the control of white mold in snap bean and other leguminous crops in New York. This is as a direct result of the substantial crop loss incurred from white mold epidemics on beans (del Río et al. 2004;

Table 1. Concentrations of the fungicides boscalid, fluazinam, and thiophanate-methyl that resulted in 50% mycelial growth inhibition (EC₅₀) and their respective 95% confidence intervals (95% CI)

Isolate code	Host	EC ₅₀ (µg/ml) (95% CI)		
		Boscalid	Fluazinam	Thiophanate-methyl
1	Dry bean	0.079 (0.059 to 0.100)	0.0016 (0.0012 to 0.0019)	1.99 (1.65 to 2.35)
2	Dry bean	0.193 (0.134 to 0.252)	0.0016 (0.0013 to 0.0020)	1.31 (1.04 to 1.58)
3	Dry bean	0.148 (0.111 to 0.186)	0.0015 (0.0011 to 0.0019)	2.02 (1.68 to 2.37)
4	Dry bean	0.103 (0.074 to 0.132)	0.0015 (0.0011 to 0.0019)	1.51 (1.22 to 1.79)
5	Snap bean	0.076 (0.054 to 0.097)	0.0015 (0.0011 to 0.0018)	2.14 (1.72 to 2.58)
6	Snap bean	0.068 (0.048 to 0.088)	0.0013 (0.0010 to 0.0017)	1.55 (1.27 to 1.83)
7	Snap bean	0.129 (0.094 to 0.165)	0.0014 (0.0010 to 0.0017)	2.15 (1.75 to 2.54)
8	Snap bean	0.117 (0.085 to 0.150)	0.0016 (0.0012 to 0.0019)	1.46 (1.21 to 1.71)
9	Dry bean	0.219 (0.169 to 0.268)	0.0011 (0.0009 to 0.0014)	1.55 (1.23 to 1.87)
10	Lima bean	0.132 (0.099 to 0.164)	0.0015 (0.0012 to 0.0018)	2.12 (1.78 to 2.47)
11	Dry bean	0.102 (0.073 to 0.132)	0.0019 (0.0015 to 0.0023)	2.06 (1.74 to 2.37)
12	Dry bean	0.124 (0.082 to 0.166)	0.0020 (0.0017 to 0.0023)	1.70 (1.42 to 1.99)
13	Dry bean	0.093 (0.069 to 0.117)	0.0015 (0.0012 to 0.0018)	1.23 (1.03 to 1.44)
14	Soybean	0.173 (0.128 to 0.217)	0.0014 (0.0011 to 0.0017)	1.45 (1.09 to 1.81)
15	Soybean	0.074 (0.056 to 0.093)	0.0010 (0.0007 to 0.0013)	1.48 (1.25 to 1.72)
Mean	-	0.116 (0.106 to 0.125)	0.0014 (0.0013 to 0.0016)	1.69 (1.59 to 1.78)

Table 2. Effect of fungicides on the incidence of white mold, and pod yield components in two small plot, replicated trials conducted in Geneva, New York, in 2015 and 2016

Active ingredient ^w	White mold incidence on plants (%) ^x		White mold incidence on pods (%)		Total pod yield (g/1-m row)		Average weight of individual pods (g)	
	2015	2016	2015	2016	2015	2016	2015	2016
Boscalid	26.9 b	1.9 b	8.1 b	0.9 b	590 b	1,177	4.5 a	8.5
Thiophanate-methyl	25.2 b	0.6 b	6.7 b	0.3 b	863 a	870	4.5 a	7.9
Fluazinam	14.2 b	7.6 b	2.8 b	2.8 b	713 ab	875	4.7 a	8
Nontreated	85.6 a	49.2 a	31.3 a	10.3 a	448 b	817	3.1 b	7.9
<i>Statistics</i>								
LSD ^y	20.9	9.1	7.3	3.4	-	-	0.5	-
P-value	<0.001	<0.001	<0.001	<0.001	0.029	0.19 (ns)	<0.001	0.863 (ns)
F	24.37	61.68	32.31	17.79	4.35	1.86	21.6	0.25
CV (%) ^z	39.1	24.6	43.7	26	25.9	11.8	11.8	6.1

^w Boscalid applied as Endura 70 WG (126.2 g/ha), fluazinam as Omega 500 F (162.8 ml/ha), and thiophanate-methyl as Topsin 4.5 FL (35.9 ml/ha).

^x Means followed by the same letter are not significantly different ($P > 0.05$).

^y LSD = least significant difference ($P = 0.05$).

^z CV (%) = coefficient of variation.

Lehner et al. 2017; Peltier et al. 2012), which drives risk averse behavior. Cultural methods of white mold control such as reduced plant density, lower rates of nitrogen fertilizer and irrigation to manipulate green leaf area of the canopy, and use of upright cultivars may be insufficient when used alone and often are inversely correlated with high yield (Grau and Hartman 1999; Grau and Radke 1984; Peltier et al. 2012; Vieira et al. 2010). Thus, the use of fungicides is a more reliable and cost-effective strategy to reduce the risk of substantial crop loss.

An effective and durable fungicide-based management strategy relies upon the implementation of the fungicide resistance guidelines, including monitoring of the sensitivity of the pathogen and alternation of products with different modes of action, such as boscalid, fluazinam, and thiophanate-methyl. The exposure of the *S. sclerotiorum* population from New York to boscalid and thiophanate-methyl for at least 10 years limits the establishment of a baseline sensitivity for these fungicides. Nevertheless, knowledge of the current sensitivity status to these fungicides within the *S. sclerotiorum* populations remains valuable information to guide effective disease management strategies.

In this study, there was no evidence of reduced sensitivity to boscalid. The average EC₅₀ for boscalid found here was within the range of values found in populations from China (Liu et al. 2009) and Australia (Jones et al. 2011b), where no resistance was also detected. To date, there has been only a single report of resistance to boscalid in a *S. sclerotiorum* isolate from France and associated with the SdhD-H134R mutation (Stammler et al. 2010). However, in *Botrytis cinerea*, a related necrotrophic fungal pathogen from the family Sclerotiniaceae, resistance to this fungicide has been more frequently reported (Veloukas et al. 2011; Yin et al. 2011). Moreover, boscalid-resistant isolates of *S. sclerotiorum* have been induced in the laboratory by repeated fungicide exposure (Wang et al. 2015), which depicts the risk of resistance development under field conditions. Therefore, the use of sublethal doses and consecutive sprays of boscalid should be avoided to preserve the efficacy of this fungicide over time.

The average EC₅₀ calculated for fluazinam in this study was similar to that found in an *S. sclerotiorum* population from China that had not been exposed to this fungicide (Liang et al. 2015). In contrast, another non-exposed population from China exhibited average EC₅₀ values approximately four times higher than reported herein (Wang et al. 2016). It is difficult to explain this disparity of results. This may be due to different methods used for sensitivity testing. The average EC₅₀ found for fluazinam in our study was three times lower than that found in Brazil using an identical method to estimate sensitivity (Lehner et al. 2015). In Brazil, fluazinam has been broadly adopted for white mold control in dry bean and the *S. sclerotiorum* isolates assessed by Lehner et al. (2015) had been previously exposed to this fungicide. Indirectly, this provides evidence that loss of sensitivity to fluazinam can be occurring due to higher EC₅₀ values found in the population previously exposed to this fungicide. Further monitoring of the sensitivity of the *S. sclerotiorum* population is required to robustly address this issue.

The reported EC₅₀ values associated with resistance to benzimidazole fungicides in *S. sclerotiorum* are usually greater than 5 µg/ml (Lehner et al. 2015; Ma et al. 2009). Moreover, resistance to benzimidazole fungicides is usually associated with point mutations within the β-tubulin gene altering the amino acid sequences at the benzimidazole-binding site (Ma and Michailides 2005). In our study, in a sample of 15 isolates, the EC₅₀ values for thiophanate-methyl were lower than 2.5 µg/ml. However, when sensitivity of the entire population was assessed, some isolates had mycelial growth almost 50% relative to the control at 5 µg/ml of thiophanate-methyl. The absence of mutations within the β-tubulin gene in these isolates suggests the presence of reduced sensitivity phenotypes but not resistance. Therefore, judicious use of thiophanate-methyl and adherence to best practices with rotation to different active ingredients and the inclusion of multisite active ingredients is recommended to avoid or delay the thiophanate-methyl resistance development. Resistance to thiophanate-methyl within the *S. sclerotiorum* population probably was not the primary cause of the suboptimal control of white mold experienced by growers in 2014.

Boscalid, fluazinam, and thiophanate-methyl were equally effective in reducing the white mold incidence on plants and pods in the field trials conducted in both years. This result aligns with earlier studies, in which a larger set of fungicides for white mold control in dry bean were evaluated (Mahoney et al. 2014; McCreary et al. 2016). Average white mold incidence across treatments was lower in 2016 than the previous year because of drought conditions resulting in higher summer temperatures and less rainfall. In 2015, when the white mold incidence on control plots was relatively higher than in 2016, the average disease control provided by the fungicides was 75 and 81% in plants and pods, respectively. In 2016, when the disease incidence was moderate, the reduction in disease incidence provided by the fungicides was 93 and 87% on plants and pods, respectively. Therefore, alternate application of these products for the management of white mold in snap bean is unlikely to significantly affect disease control and would promote adherence to resistance management guidelines.

No significant effect of the fungicides was found on total pod yield in the trial conducted in 2016. The lack of a yield response to the fungicides may have resulted from the relatively lower disease incidence on pods, which may not have been sufficient to result in a measurable difference in the number of healthy pods among the treatments. Alternatively, in 2015 when the incidence of white mold on pods was three times higher than in 2016, the effect of fungicides was significant. A similar effect may have occurred for the average weight of individual pods, in which a significant effect of the fungicides was only observed when high white mold incidence on pods occurred. These results suggest that in seasons when environmental conditions are not conducive to epidemics, considerable cost savings may occur from the judicious use of fungicides. Moreover, if growers reduce the frequency of false positive decisions (unwarranted use of fungicide), the exposure of the *S. sclerotiorum* population to fungicides would be decreased. This would reduce the chance of selecting for resistant isolates which may complicate the control of white mold in the future. Further studies to quantify site-specific risk may also assist in reducing the occurrence of false positive decisions including examination of the potential utility of white mold forecasting models (Bom and Boland 2000; Clarkson et al. 2004; Jones et al. 2011a; Koch et al. 2007).

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