A Quantitative Synthesis of the Efficacy and Profitability of Conventional and Biological Fungicides for Botrytis Fruit Rot Management on Strawberry in Florida

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Abstract

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Botrytis fruit rot (BFR) is a major disease that affects strawberry production in Florida and worldwide. BFR management relies on frequent fungicide applications. A meta-analysis was conducted on the outcomes from nine field trials to evaluate the efficacy and profitability of conventional and biological fungicides compared to a non-treated control (NTC). All trials were conducted in Florida between the 2005/06 and 2016/17 growing seasons. Fungicide treatments were applied weekly and plots were harvested twice a week for yield and BFR incidence quantification. Treatments were grouped into four categories: NTC, multi-site only ('Thiram'), 'Standard' (captan alternated with fludioxonil + cyprodinil), and '*Bacillus*'. Following primary analyses, a random effects network meta-analytical model was fitted to estimate the mean yield and BFR incidence responses for each treatment group and to compare means between pairs of groups. The 'Thiram' and the 'Standard' treatment groups increased yield by 378.8 and 502.2 kg/ha/week, respectively, compared to the NTC. The yield difference between '*Bacillus*' and NTC was not statistically significant. Besides increasing yield, 'Thiram' and 'Standard' also reduced BFR incidence by approximately 10% compared to the NTC. The mean yield responses and among-study variability from the meta-analysis were used to estimate the probability of a given yield response in a new future trial. The 'Standard' and 'Thiram' treatment groups showed higher estimated probabilities of increasing yield and resulting in a profitable return on application investments than the '*Bacillus*' group of treatments. The results from this study

provide growers with information that will aid their decision-making process regarding BFR management.

Botrytis cinerea is the causal agent of Botrytis fruit rot of strawberry (BFR), a major disease that affects strawberry production in Florida and in many other strawberry growing areas (Sutton 1998). In Florida, environmental conditions conducive for BFR development such as temperatures ranging from 15 to 25ºC during a minimum of 13 h of continuous leaf wetness are not uncommon during the growing season (Bulger et al. 1987; Mertely et al. 2018). Under such conditions, severe yield losses may occur even in well-managed fields (Legard et al. 2001).

B. cinerea inoculum is primarily introduced into Florida strawberry fields via quiescently infected transplants originating from nurseries in Canada and the northern United States (Oliveira et al. 2017). The fungus colonizes senescent leaf tissues and produces conidia that are eventually dispersed by wind, water-splash, and handling operations. Under favorable conditions, conidia germinate and infect petioles, leaves, flowers, and fruit (Braun and Sutton 1987; Mertely et al. 2018; Sutton 1998). Fruit infections usually start at flowering, since flowers are the most susceptible organs (Bristow et al. 1986; Mertely et al. 2002; Sutton 1998). Infected fruit typically develop large brown lesions, usually at the stem end. Under free moisture conditions, the fungus sporulates, covering the lesions with masses of grayish conidia and conidiophores. Diseased fruit, also referred to as gray mold because of the characteristic signs and symptoms, are unmarketable and serve as an important source of secondary inoculum (Mertely et al. 2018; Sosa-Alvarez et al. 1995).

There is great variability in susceptibility among cultivars, with the less susceptible ones having an open canopy architecture or blooming peaks during periods not favorable for disease development. However, the more tolerant cultivars may not have all the desirable agronomic and market traits. (Legard et al. 2000; Seijo et al. 2008). Cultural practices such as wider planting spacing and sanitation have been tested as strategies to suppress BFR. However, the possibility of negatively affecting yield without reducing BFR diminishes the adoption of these strategies (Legard et al. 2000; Mertely et al. 2000).

Management of BFR in Florida strongly relies on fungicide applications carried out on a calendar basis with multi-site fungicides (e.g. captan or thiram) applied weekly. If weather conditions favor BFR development during peak bloom, a site-specific fungicide (e.g. fenhexamid, cyprodinil + fludioxonil, pyraclostrobin + fluopyram, pyraclostrobin + fluxapyroxad, or isofetamid) is used instead (Legard et al. 2001; Mertely et al. 2018; Whitaker et al. 2017). Calendar-based fungicide programs are gradually being replaced by the Strawberry Advisory System (StAS) (Pavan et al. 2009). This system consists of a web-based decision support tool that times fungicide applications based on a prediction model developed by Bulger et al. (1987). Cordova et al. (2017a,b) showed that when the StAS was used, the number of fungicide applications was reduced by approximately 50% without compromising yield or BFR management.

Strategies to reduce the number of fungicide applications are essential not only to decrease costs and chemical inputs but also to aid fungicide resistance management. Oliveira et al. (2017) showed that isolates of *B. cinerea* introduced into Florida strawberry fields via transplants were resistant to several of the fungicides labeled for BFR management. Amiri et al. (2013) reported that *B. cinerea* isolates collected from 2010 to 2012 in Florida had resistance frequencies of 85.4, 86.5, 44.4, 52.7, and 59.5% to boscalid, pyraclostrobin, fenhexamid, cyprodinil, and pyrimethanil, respectively. Therefore, BFR management strategies with fewer fungicide applications and rotation of modes of action are necessary to reduce the selection of *B. cinerea*-resistant populations.

Fungicides with multiple or different modes of action from those currently labeled for BFR management in Florida are needed. Biological agents such as *Bacillus* spp. act in distinct ways compared to conventional fungicides; they may act by antibiosis, parasitism, or induced systemic resistance (Bargabuset al. 2002; Jacobsen and Backman 1993). In addition to the practical use, biological agents have more marketing and social appeal than the current conventional fungicides used to control BFR and other diseases. However, studies regarding the effectiveness and profitability of biological agents for managing BFR are necessary before recommending their incorporation into current management programs.

Therefore, the objectives of this study were: (i) to determine the effectiveness of *Bacillus* spp. and a multi-site conventional fungicide against BFR compared to a standard fungicide program and a non-treated control, and (ii) to estimate the profitability of the treatments tested. To accomplish these objectives, multivariate meta-analytical models (Madden and Paul 2011; Madden et al. 2016; Paul et al. 2008; Paul et al 2011) were fitted to data from nine field trials conducted from 2005 to 2017 in Florida, and overall mean differences in yield and BFR incidence were estimated as measures of efficacy. Output from the analyses were then used to estimate probabilities of profitable returns on treatment investment for multiple application cost x strawberry price scenarios.

Materials and Methods

Experiment design and data collection. Nine fungicide efficacy field trials were conducted during nine strawberry seasons from 2005/06 to 2016/17 (except 2006/07, 2008/09, 2010/11) in Florida. Trials were carried out either at the University of Florida, Gulf Coast Research and Education Center (UF/GCREC) located in Wimauma (2005/06, 2007/08, 2009/10, 2011/12, and 2012/13), or in a commercial field in Plant City, FL (2013/14 to 2016/17). Five BFR-susceptible cultivars were used; Sweet Charlie in 2005/06 and 2009/10, Strawberry Festival in 2007/08, Camino Real in 2011/12, Winter Star in 2012/13, and Radiance from 2013/14 to 2016/17.

Each experiment started with field preparation in late August by raising and covering beds with black plastic mulch. During mulching, beds were fumigated to manage weeds, and soil-borne pathogens. Beds were 0.7 m wide on 1.2-m centers and contained two staggered rows of plants. Bare-root transplants were planted in early October and overhead irrigated for approximately ten days to facilitate plant establishment. Further irrigation and fertilization were done through drip tapes. Additional operations other than BFR management followed local production standards (Whitaker et al. 2017).

In all the experiments, treatments were arranged in a randomized complete block design, with four blocks in adjacent beds. Plots (experimental units) consisted of 12 to 14 plants each to which treatments were randomly assigned. A non-treated control, a standard fungicide program, and nine other fungicides were tested throughout the study. The specific fungicide treatments varied from trial to trial regarding their trade names, but they can be grouped into four groups based on their active ingredient (a.i.) (‘Non-treated control [NTC]’, ‘Standard’, ‘*Bacillus*’, and ‘Thiram’) (Table 1).

The ‘Standard’ group consisted of weekly applications of the a.i. captan regardless of crop growth stage, except when flowers were present during periods favorable for *B. cinerea* infection (15 to 25ºC and 13 h of continuous leaf wetness), when fludioxonil + cyprodinil was applied. The '*Bacillus*' group was composed of biological treatments applied weekly with either *Bacillus amyloliquefaciens*, *B. pumilus*, or *B. subtilis* as their single a.i. Those *Bacillus* spp were grouped together because preliminary analysis of variance of individual studies did not show significant differences among them and because of their similar mode of action. The 'Thiram' group corresponded to those treatments where the multi-site fungicide thiram was applied weekly. The specific commercial fungicides used on each trial are listed on table 1.

Treatment applications were made weekly from mid-December to early-March in the experiments conducted at Wimauma (8 to 12 applications) and from late-November to early-March in Plant City (14 to 15 applications). Treatments were applied with a CO_2 back-pack sprayer, calibrated to deliver 935.4 l/ha at 413.7 kpa through a boom mounted with two hollow-cone T-Jet 8002 nozzles.

In all the experiments, plots were harvested twice a week from December to March. The number of harvests per season ranged from 20 to 26, except season 2009/10 with 16 harvests (Table 1). After each harvest, fruit from every plot were counted and classified as marketable or BFR-affected. Fruit weighing more than 12 grams and with no apparent disease signs or symptoms were considered marketable, whereas fruit with at least one BFR lesion were counted as BFR-affected fruit. Marketable fruit were weighed to determine yield, and BFR incidence was estimated as the number of BFR-affected fruit divided by total number of fruits. The total marketable fruit per trial was divide by the number of weeks of harvesting in that trial, to account for the different number of weeks or harvesting per trial, and report as kilograms per hectare per week.

Meta-analysis of fungicide effect on yield and BFR incidence. Two-stage network meta-analysis was performance on yield and BFR incidence data, following the methodology described by Madden et al. (2016). In the first stage of the analysis, treatments were grouped based on their a.i. (Table 1), hereafter referred to by treatment group. The experimental design was considered as generalized randomized block design, to account for the unbalance number of experimental units per treatment group per block. Each individual experiment and response variable (yield and BFR incidence) was analyzed separately by fitting linear mixed models with treatment group as fixed and block as random effects using the PROC MIXED procedure in the SAS software. To obtain the estimated least square means for yield and BFR incidence for each treatment group (LS-means), the *lsmeans* statement in PROC MIXED was used.

In the second stage, LS-means for each treatment group in each individual trial were then used in an unconditional network meta-analysis, considering trial as a random effect, second-order multiplicative structure, and using maximum likelihood to estimate model parameters (model R5 of Madden et al. 2016). Mean difference in marketable yield (YieldD) and BFR incidence (BFRD) for the four treatment groups relative to the baseline NTC, and their respective standard error, confidence interval and level of significance were estimated by *estimate* statements in PROC MIXED. Contrasts were also used to compare means between treatment groups.

Yield projection and risk analysis. Growers and decision-makers are interested in knowing the chance or likelihood of a given fungicide program resulting in yield increases and profitability, as well as their magnitude. To estimate the probability of a certain treatment group mean being higher than the NTC, we used the estimated mean yield difference YieldD and between trial standard deviation ($\hat{\sigma}_{\mathrm{D}}$) from the meta-analysis. As described by Paul et al. (2008), $\hat{\sigma}_{\mathrm{D}}$ can be calculated based on the variance-covariance matrix, using the follow equation:

$$\hat{\sigma}_{\mathrm{D}} = \sqrt{\hat{\sigma}^2_{\mathrm{TG}} + \hat{\sigma}^2_{\mathrm{NTC}} - 2\hat{\sigma}_{\mathrm{TG,\,NTC}}}$$

Where $\hat{\sigma}^2_{\mathrm{TG}}$ is the estimated variance of a treatment group; $\hat{\sigma}^2_{\mathrm{NTC}}$ is the variance of non-treated control; and $2\hat{\sigma}_{\mathrm{TG,\,NTC}}$ is the covariance between the treatment group and non-treated control (Paul et al. 2008). The probabilities of YieldD being greater than a given constant (*C*) is given by the follow equation:

$$P^{c}_{+} = \Phi\left(\frac{C - \mathrm{YieldD}}{\hat{\sigma}_{\mathrm{D}}}\right)$$

Where Φ(.) is the cumulative standard-normal function (Paul et al. 2008; Sylvester et al. 2018), and the other terms are as defined above. The probability of yield increasing (YieldD be greater than *C*) was calculated for each treatment group, ranging from 0 to 2,500 kg ha^{-1}. Using the values of *C* and strawberry prices that represent scenarios with low (\$2.00 kg^{-1}), medium (\$5.00 kg^{-1}), and high (\$7.00 kg^{-1}) crop value, we calculated the probability of profitability by the use of the treatment groups in comparison to not use any pesticide (NTC). The range of profitability calculated ranged from \$0 to \$4,500 week^{-1}.

Results

Fungicide effect on BFR incidence. Across the nine trials, the incidence of BFR for the NTC ranged from 1.9% to 26.6%, with a median of 5.4%. For the 'Standard' treatment group, incidence ranged from 1.2% to 8.4%, with a median of 2.5%. The 'Thiram' treatment group had a median disease incidence of 1.5%, and values ranging from 0.5 to 12.6%. For the '*Bacillus*' treatment group, BFR incidence ranged from 4.2% to 28.6%, and the median was 4.8% (Fig. 1A).

BFRD was significantly different from zero for all treatment groups contrasted with the NTC, indicating a significative reduction of BFR incidence in comparison to the NTC. However, the magnitude of the effect was greater for conventional fungicide groups than for the biological control group. For instance, on a percentage scale, BFRD for the 'Standard' and 'Thiram' treatment groups contrasted with the NTC were -9.79% (P <.0001) and -9.98% (P <.0001), with confidence limits of -13.95% to -5.64% and -13.33% to -6.65%, respectively. The BFRD for '*Bacillus* vs NTC' was -3.5% (P = 0.047), with a confidence limit of -6.97% to -0.046%. The BFRD for 'Standard' vs 'Thiram' was not statistically significant (P = 0.912). Both conventional fungicide treatment groups were significantly more effective reducing BFR incidence than the *Bacillus* treatment group, with an estimated BFRD of approximately -6.5% (Table 2).

Fungicide effect on yield. Mean weekly yield of marketable fruit ranged from 676.9 to 2,833.5 kg/ha/week for the NTC, with a median of 1,652.5 kg/ha/week. The median for the 'Standard' treatment group was 2,411.2 kg/ha/week, with a range of 2,034.2 to 2,712.5 kg/ha/week. A numerically lower median was observed for the 'Thiram' treatment group (1,880.6 kg/ha/week), with yield ranging from 1,029.8 to 3,073.0 kg/ha/week. The '*Bacillus*' treatment group had a similar median to the NTC (1,859.0 kg/ha/week), however, with a narrower range (1,243.7 to 2,422.5 kg/ha/week) (Fig. 1B).

Results from the network meta-analysis indicated that compared to the NTC, YieldD was significantly different from zero only for the 'Standard' and 'Thiram' treatment groups, with estimates of 502.2 (P = 0.0004) and 378.8 kg/ha/week (P <.0001), and confidence intervals from 225.5 to 778.9 and 202.4 to 555.3 kg/ha/week, respectively. YieldD were not statistically significant for differences between '*Bacillus*' and NTC' (-9.64 kg/ha/week, P = 0.888) or between 'Standard' and 'Thiram' (123.4 kg/ha/week, P = 0.4432 (Table 2).

Yield projection and risk analysis. The 'Thiram' group had 61.2% of chance of increase yield to at least 150 kg/ha/week; and decreasing to a 50% for a projection of yield increase of 378 kg/ha/week (Fig. 2A). The 'Standard' group, however, had even higher chances of yield increase compared to the 'Thiram' group. For instance, it had 70.8% and 50% likelihood of reaching a weekly yield increase of 150 and 502 kg/ha relative to the NTC, respectively. '*Bacillus*' group was the only group that did not show probabilities > 0.5 of yield increasing, as comparison, the likelihood of have an increasing 150 kg/ha/week is only 43%.

Similar trends were observed in all the profitability of application. In the scenario with low strawberry price (\$2 kg^{-1}), the 'Standard' group showed 50% likelihood of have a profit of at least \$1,000/ha/week (Fig. 2B). However, the 'Thiram' group, under the same scenario, had 50% chance of reaching a return of \$720/ha/week. In the second scenario, with \$5 kg^{-1} of fruit, the application of 'Standard' or 'Thiram' group resulted on more than 50% of chances of have a profit above \$1,800 /ha/week (Fig. 2C). In the simulation with the highest strawberry price (\$8 kg^{-1}) the chances of profitability above \$2,500/ha/week for 'Standard' and 'Thiram' were 61 and 52%, respectively (Fig. 2D). Under any of the scenarios tested, the 'Bacillus' group never showed a likelihood above 50%. For instance, the chances having a profit above \$500 /ha/week using the 'Bacillus' group are 38%, 46% and 47% for the low, medium, and high crop value scenario, respectively. Therefore, the chances of reaching, even a relative small profit, are smaller than the chances of not reaching it.

Discussion

BFR is one of the main diseases for both organic and conventional strawberry growers in Florida and worldwide (Mertely et al. 2018; Sutton 1998). The use of fungicides has been the most reliable management strategy deployed by Florida growers with calendar-based fungicide programs adding up to 24 applications per season (Legard et al. 2001). The high number of fungicide applications coupled with inherent *B. cinerea* characteristics such as its short and polycyclic disease cycle, abundant sporulation, and high genetic variability makes this a classical example of a pathosystem at high-risk for fungicide resistance development (Fernández-Ortuño et al. 2012; Veloukas et al. 2014). Several studies have been published reporting *B. cinerea* populations resistant to different classes of fungicides (Amiri et al. 2013; Baggio et al. 2018; Fernández-Ortuño et al. 2014; Grabke et al. 2013; Oliveira et al. 2017). Thus, there is a need for incorporating different modes of action that may help mitigate the selection of resistant *B. cinerea* populations. Multi-site fungicides are not easily overcome by mutations that confer resistance to pathogens and its incorporation into current BFR management programs may delay resistance selection (Brent and Hollomon 1995; Deising et al. 2008).

In this study, the effects of conventional (thiram) and biological (*Bacillus* spp.) multi-site fungicides on BFR management and marketable yield were compared to a standard fungicide program and a non-treated control. The meta-analysis of the outcomes of nine field trials shows that 'Thiram' was just as effective as the 'Standard' treatment group, providing comparable BFR suppression and yield increase relative to the NTC. Although the '*Bacillus*' treatment group reduced BFR incidence compared to the NTC, it was less effective than the 'Standard' and 'Thiram' treatment groups and did not provide yield benefit relative to the NTC.

The mean yield differences were used together with the estimated among-study variance from the meta-analysis to estimate probabilities of future outcomes and to assess economic benefits of using a certain fungicide program at different strawberry prices. We used three values of strawberry prices ($2.00, $5.00, and $8.00/Kg) in our analysis that represents possible values received by Florida growers based on the USDA National Agricultural Statistics Service database (USDA 2017). Strawberry prices fluctuate considerably within the Florida strawberry season, mainly because the peak production periods and market competition with Californian and Mexican's production which can drastically increase the market offer and consequently decrease prices.

Yield projection and risk analysis can be used by growers and decision-makers to adjust management strategies throughout the season. These types of analyses have been used by various researchers to estimate whether yield increases brought about by certain fungicide programs were sufficient to offset application costs (Paul et al. 2011; Sylvester et al. 2018; Willyerd et al. 2015). In our study, because of the high value of the crop and relatively low cost of a fungicide application, the likelihood of application costs being offset by yield increases is very high. For instance, considering local application costs of \$64.00 for the 'Standard', \$42.00 for the 'Thiram', and \$82.00 ha^{-1} for the '*Bacillus*' treatment groups, the chances of at least breakeven were respectively 76.7%, 67.2%, and 47.2% for strawberry prices of \$2.00 ha^{-1}; 77.6%, 67.8%, and 48.5% for \$5.00 ha^{-1}; and 77.9%, 67.9%, and 48.9% for \$8.00 ha^{-1} (Fig. 2). For all the strawberry prices x application costs evaluated, probabilities of offsetting the cost of applying the '*Bacillus*' treatment group were all less than 0.5. The minimal profit expected at 0.5 probability for the 'Standard' and 'Thiram' treatment groups were respectively \$1,000 and \$720/ha/week; \$2,500 and \$1,800/ha/week; and \$4,050 and \$2,900/ha/week in the three strawberry price scenarios evaluated. This confirms their economic advantage over the '*Bacillus*' treatment group (Fig. 2).

The 'Standard' treatment group consisted of weekly fungicide applications. Cyprodinil + fludioxonil was only applied during bloom if weather conditions favored BFR development, and captan was applied otherwise. Captan is a phthalimide with multi-site contact activity, whereas cyprodinil and fludioxonil are respectively anilino-pyrimidines and phenylpyrroles with single-site modes of action. The first acts on methionine biosynthesis, and the second on MAP/Histidine-Kinase in osmotic signal transduction (FRAC 2018). This program has been a standard and effective treatment for BFR management in Florida for decades and its success strongly relies on the efficacy of the single-site fungicides used (Cordova et al. 2017a; Legard et al. 2001). However, high frequencies (>50%) of cyprodinil resistant *B. cinerea* populations have been reported on the southeastern United States. Thus, most of the control efficacy is probably due to the fludioxonil activity, although populations with reduced sensitivity have been found, including isolates recovered from nursery transplants (Amiri et al. 2013; Fernández-Ortuño et al. 2014; Oliveira et al 2017). The overuse of standard programs such as described above as well as use of the single-site materials at nurseries might put the BFR management at risk, justifying the need for incorporating other multi-site fungicides such as thiram and *Bacillus* spp. into disease management programs.

Thiram (tetramethylthiuram disulfide) acts by contact inhibiting spore germination and mycelial growth. It belongs to the dithiocarbamates and relatives (electrophiles) group (FRAC code M3) and has been in commercial use since 1925. It is used as seed coating and/or as spray application to protect crops against fungus infection pre- and post-harvest (FRAC 2018; Sharma et al. 2003). Here, we quantified the effectiveness of weekly thiram applications for managing *B. cinerea* and increasing yield. Its effectiveness for managing *B. cinerea* in strawberry and other crops (Brook 1956; Cox and Hayslip 1956; Legard et al 2001; Presley and Maude 1980; Sharma et al 2003) has been well documented. Because its multi-site mode of action and its similar effectiveness to the 'Standard' treatment group in this study, weekly thiram applications or its incorporation into the 'Standard' program are good alternatives to manage BFR while aiding fungicide resistance management.

Bacillus spp. are currently classified by the Fungicide Resistance Action Committee (FRAC) as microbial disrupters of pathogen cell membranes (FRAC code 44) (FRAC 2018). However, they may also act by antibiosis, induced systemic host resistance (strains QST713 and FZB24), and lipopeptides production (Jacobsen et al. 2004). The use of biological compounds such as strains of *Bacillus* spp. to manage strawberry and vegetable diseases has increased in Florida over the past years. Among many factors that may have contributed to this increase, the social interest for a more environmentally sound agriculture, the need for alternative strategies to manage fungicide resistance issues, and the increase of organic production can be listed. For instance, Florida strawberry organic acreage in 2017 almost doubled the 182 acres harvested on 2015 (USDA 2017; Smith and Vallad 2017). *Bacillus* spp. effectiveness in inhibiting *B. cinerea* mycelial growth has been reported by Hang et al. (2005) and Toure et al. (2004). Hang et al. (2005) not only reported the effectiveness of the biocontrol agent *in vitro*, but also showed that application of *B. subtilis* S1-0210 reduced BFR incidence of strawberry in greenhouse. Similar, but not as effective results were found in this study with weekly *Bacillus* spp. applications; however, no yield benefit was achieved compared to the NTC. Contrary to Hang et al. (2005) studies, ours were conducted on open fields, a non-controlled environment with a wide range of microflora interactions that might have prevented the '*Bacillus*' treatment group to perform better.

Based on our results, thiram can be recommended as an effective fungicide to be incorporated into programs to manage BFR in Florida. Thiram was not only effective at reducing disease incidence but also showed high probabilities in offsetting application costs and delivering economic benefits, even at relatively low strawberry prices. Besides the biological and economic benefits, the inclusion of a multi-site fungicide such as thiram into a program may aid fungicide resistance management, but further studies on the subject are needed. Even though the '*Bacillus*' treatment group had low probabilities of bringing economic benefits and did not result in yield increment relative to the NTC, it was effective at reducing BFR incidence compared to the NTC. If manufacturers were able to reduce the cost of *Bacillus*-based products, they could become a viable alternative to be tank-mixed with more effective single-site fungicides and contribute to fungicide resistance management by virtue of their multi-site activity. Another application to *Bacillus*-based products could be its incorporation into strawberry nursery disease management programs. Since fruit yield is not a concern for nurseries, the use of *Bacillus* spp. could decrease the selection pressure of *B. cinerea* resistant populations and save the single-sites for use in strawberry commercial fields.

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Table 1. Treatment group description with fungicide brand names, season, strawberry field location, and number of harvests and fungicide applications conducted on each of the nine trials.

Treatment group[a]	Active ingredient	Brand name and formulation[b]	Season	Location	Number of applications[c]	Number of harvests (weeks)[d]	Trial
Non-treated control	NA	NA	05_06	Wimauma/FL	-	23 (12)	1
			07_08	Wimauma/FL	-	20 (11)	2
			09_10	Wimauma/FL	-	16 (9)	3
			11_12	Wimauma/FL	-	21 (11)	4
			12_13	Wimauma/FL	-	23 (12)	5
			13_14	Plant City/FL	-	26 (13)	6
			14_15	Plant City/FL	-	23 (12)	7
			15_16	Plant City/FL	-	25 (14)	8
			16_17	Plant City/FL	-	25 (14)	9
Standard	Captan / fludioxonil + cyprodinil	Captan 80WDG / Switch 62.5WG	12_13	Wimauma/FL	4 / 5	23 (12)	5
			13_14	Plant City/FL	8 / 7	26 (13)	6
			14_15	Plant City/FL	8 / 6	23 (12)	7
			15_16	Plant City/FL	10 / 5	25 (14)	8
			16_17	Plant City/FL	10 / 5	25 (14)	9

463 **Table 1.** Continued

Treatment group	Active ingredient	Brand name and formulation	Season	Location	Number of applications	Number of harvests (weeks)	Trial
Thiram	Thiram	Thiram Granuflo 75WDG	05_06	Wimauma/FL	12	23 (12)	1
		Thiram Granuflo 75WDG	07_08	Wimauma/FL	12	20 (11)	2
		Thiram Granuflo 75WDG	09_10	Wimauma/FL	10	16 (9)	3
		Thiram Granuflo 75WDG	11_12	Wimauma/FL	11	21 (11)	4
		Thiram 24/7	14_15	Plant City/FL	14	23 (12)	7
		Thiram 24/7	15_16	Plant City/FL	15	25 (14)	8
		Thiram 24/7	16_17	Plant City/FL	15	25 (14)	9
Bacillus	*B. pumilus*	Sonata	05_06	Wimauma/FL	12	23 (12)	1
	B. amyloliquefaciens	Double Nickel	14_15	Plant City/FL	14	23 (12)	7
	B. subitilis	Serenade Max	05_06	Wimauma/FL	12	23 (12)	1
	B. subitilis	Serenade Max	12_13	Wimauma/FL	8	23 (12)	5
	B. subitilis	Serenade Optimum	13_14	Plant City/FL	14	26 (13)	6
	B. subitilis	Serenade Optimum	14_15	Plant City/FL	14	23 (12)	7
	B. subitilis	Serenade Optimum	15_16	Plant City/FL	15	25 (14)	8
	B. subitilis	Companion	15_16	Plant City/FL	15	25 (14)	8

464 [a] Treatments were grouped according to the similarities of their active ingredient.
465 [b] Brand name and formulation of the fungicide used on each individual trial.
466 [c] Number of times each active ingredient was applied during an individual trial.
467 [d] Number of times and weeks that fruit were harvest on each individual trial.

Table 2. Mean difference of yield (YieldD) and Botrytis fruit rot (BFRD) and correspondent statistics estimated by network meta-analysis of fungicide treatments trials for management of Botrytis fruit rot of strawberry from field trials conducted in Florida from 2005 to 2017.

Effect size[a]	Contrast[b]	Mean[c]	SE	t Value	Pr > |t|	95% Conf. Int.
YieldD	***Bacillus versus* NTC**	**-9.64**	**68.48**	**-0.14**	**0.888**	**-143.88 ↔ 124.60**
	Standard *versus* NTC	502.25	141.16	3.56	0.0004	225.54 ↔ 778.95
	Thiram *versus* NTC	378.84	90.00	4.21	<.0001	202.41 ↔ 555.26
	Standard *versus* Thiram	123.41	160.92	0.77	0.4432	-192.04 ↔ 438.85
BFRD	***Bacillus versus* NTC**	**-0.03511**	**0.018**	**-1.99**	**0.0471**	**-0.0698 ↔ -0.0005**
	Standard *versus* NTC	-0.09796	0.021	-4.62	<.0001	-0.1395 ↔ -0.0564
	Thiram *versus* NTC	-0.09987	0.017	-5.86	<.0001	-0.1333 ↔ -0.0665
	Standard *versus* Thiram	0.001918	0.017	0.11	0.9122	-0.0322 ↔ 0.0360
	Thiram *versus Bacillus*	-0.06476	0.023	-2.8	0.0051	-0.1101 ↔ -0.0194
	Standard *versus Bacillus*	-0.06284	0.027	-2.36	0.0185	-0.1151 ↔ -0.0106

[a] YieldD and BFRD corresponds to the yield and Botrytis fruit rot incidence difference between the fungicide programs tested and the non-treated control (NTC).
[b] Contrast between fungicide groups and the NTC conducted in the multivariate meta-analysis. *Bacillus* group was treated with weekly applications of *Bacillus* formulations; Thiram treatments were applied weekly with the a.i. thiram; and the Standard group was treated weekly with captan in the absence of flowers, and applications of fludioxonil + cyprodinil were made if flowers were present during periods favorable for *B. cinerea* infection.
[c] Mean= Estimated effect size. SE= standard error of the estimated effect size. t value= the t statistic from the meta=analysis. Pr>|t|= the probability value or significance level for the effect of treatment in yield and BFR incidence.

Figure 1. Box plots of Botrytis fruit rot incidence (**1A**) and yield (**1B**) of strawberry plants treated with three different fungicide programs. *Bacillus* group was treated with weekly applications of *Bacillus* formulations; Thiram treatments were weekly applied with the a.i. thiram; and the Standard group was treated weekly with captan in the absence of flowers, and applications of fludioxonil + cyprodinil if flowers were present during periods favorable for *B. cinerea* infection. The 75th and 25th percentiles of the data are respectively represented by the top and bottom lines of the boxes. The average and median are represented by the dashed and solid lines inside the boxes. Outliers are represented by the empty circles.

Figure 2. Estimated probability of fungicide programs resulting in **A**, yield increase of 150 to 2000 kg/ha/week relative to the non-treated control; and **B-D** assessment of application return relative to the non-treated control in $/ha/week at strawberry prices of $2.00, $5.00, and $8.00/kg. Probability estimations were based on the mean effect size and among-study variance obtained from the network meta-analysis from nine field trials conducted in Florida from 2005 to 2017 on the management of Botrytis fruit rot of strawberry. Fungicide programs included: a non-treated control; *Bacillus*, which corresponded to weekly applications of *Bacillus* formulations; Thiram treatments, which were treated weekly with the a.i. thiram; and the Standard group was treated weekly with captan during absence of bloom, and applications of fludioxonil + cyprodinil during bloom and suitable conditions for *B. cinera* infection.

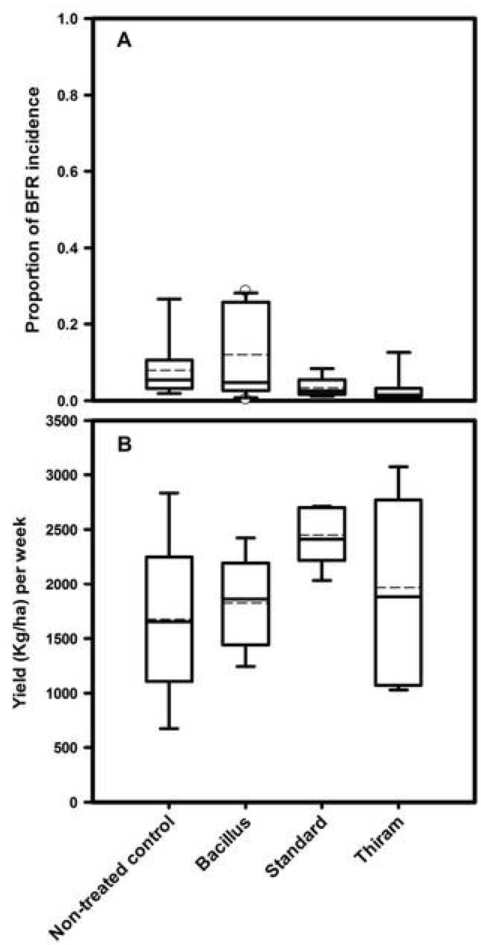

Fig. 1. Box plots of Botrytis fruit rot incidence (1A) and yield (1B) of strawberry plants treated with three different fungicide programs. Bacillus group was treated with weekly applications of Bacillus formulations; Thiram treatments were weekly applied with the a.i. thiram; and the Standard group was treated weekly with captan in the absence of flowers, and applications of fludioxonil + cyprodinil if flowers were present during periods favorable for B. cinerea infection. The 75th and 25th percentiles of the data are respectively represented by the top and bottom lines of the boxes. The average and median are represented by the dashed and solid lines inside the boxes. Outliers are represented by the empty circles.

52x67mm (300 x 300 DPI)

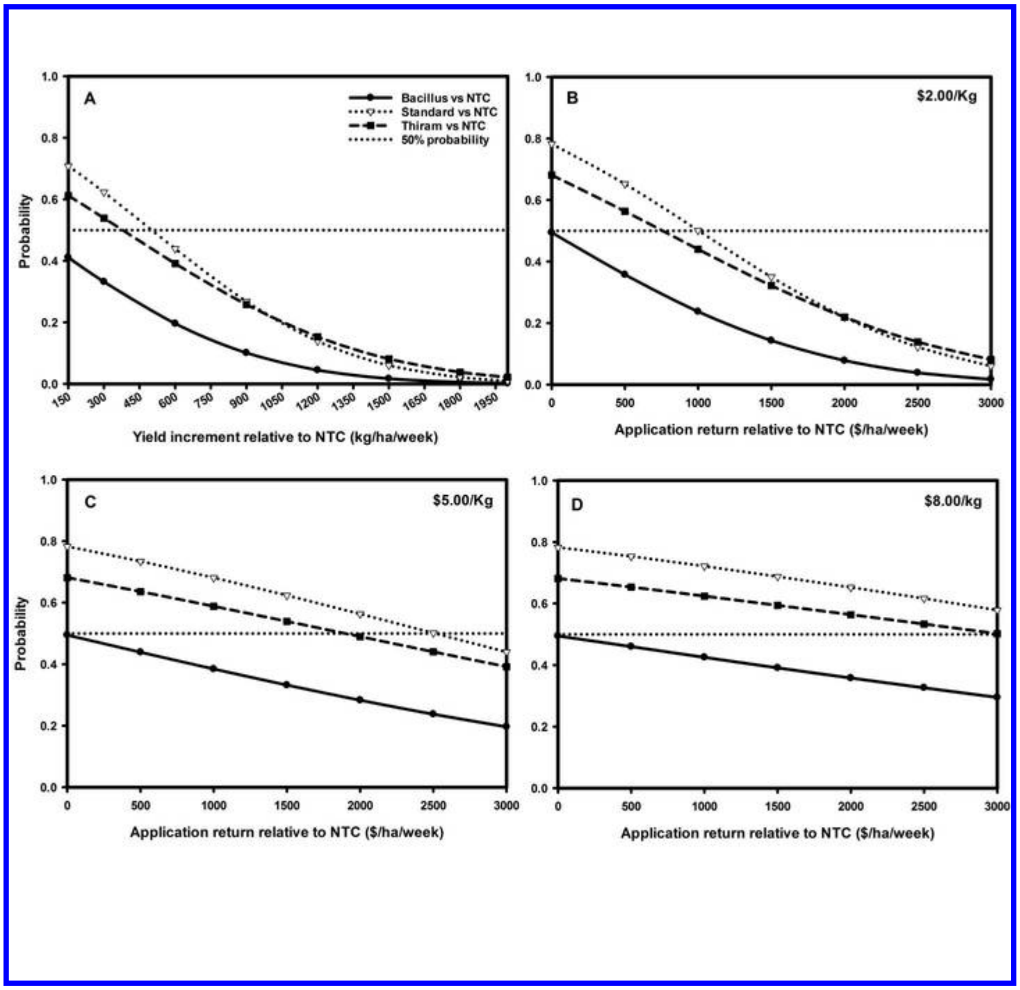

Fig. 2. Estimated probability of fungicide programs resulting in A, yield increase of 150 to 2000 kg/ha/week relative to the non-treated control; and B-D assessment of application return relative to the non-treated control in $/ha/week at strawberry prices of $2.00, $5.00, and $8.00/kg. Probability estimations were based on the mean effect size and among-study variance obtained from the network meta-analysis from nine field trials conducted in Florida from 2005 to 2017 on the management of Botrytis fruit rot of strawberry. Fungicide programs included: a non-treated control; Bacillus, which corresponded to weekly applications of Bacillus formulations; Thiram treatments, which were treated weekly with the a.i. thiram; and the Standard group was treated weekly with captan during absence of bloom, and applications of fludioxonil + cyprodinil during bloom and suitable conditions for Botrytis cinera infection.

60x58mm (300 x 300 DPI)