Preliminary and Regional Reports

Evaluation of Selected Fungicide Application Regimes and Biotic Agents for the Management of Basil Downy Mildew

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Summary. **Basil downy mildew (*Peronospora belbahrii*) is a destructive disease that occurs in sweet basil (*Ocimum basilicum*). Foliar fungicide treatments could reduce infection and the severity of foliar symptoms over the course of the growing season. Multiple fungicides in variable combinations, application rates, and sequences were applied to basil foliage weekly over the course of a field season in Illinois (14 July to 8 Sept. 2014), and the treatment effects on basil downy mildew were evaluated three times. The evaluated treatments included mixtures and different rotations of azoxystrobin, potassium phosphite, mandipropamid, cyazofamid, oxathiapiprolin, experimental compound A18269SE, dimethomorph, zoxamide + mancozeb, fluazinam, fluopicolide, mefenoxam + copper hydroxide, fenamidone, mancozeb, and ametoctradin + dimethomorph. Potassium phosphite, which is known to be effective against other downy mildew pathogens, was included in combination with other fungicides or in fungicide application sequences. Disease severity was rated in fungicide-treated plots (0% to 20%) and compared with the control (73% to 80%) at each evaluation time. All fungicide treatments significantly reduced the area under the disease progress curve values compared with the untreated control. Adding a nonionic surfactant did not improve the efficacy of any of the chemical treatments evaluated for reducing downy mildew. Organic basil growers need novel, effective products to minimize damage from basil downy mildew. To aid organic basil growers, two novel, effective biocontrol agents were evaluated, *Bacillus amyloliquefaciens* AS 43.3 and *Papiliotrema flavescens* OH 182.9 3C (formerly *Cryptococcus flavescens*). Greenhouse experiments were conducted with the fungicides quinoxyfen and azoxystrobin serving as negative and positive fungicide treatment controls, respectively. Azoxystrobin reduced downy mildew according to the greenhouse tests, but neither quinoxyfen nor the biocontrol agents reduced downy mildew severity compared with the untreated control. This study identified 13 fungicide regimens that resulted in less than 10% basil downy mildew disease severity. More studies are needed to identify effective control products for basil downy mildew on organic basil.**

Sweet basil is the most commonly grown and economically important species of basil in the world (Vieira and Simon, 2006). Basil downy mildew is a significant pathogen of sweet basil. Infection causes yellowing of the leaf tissue in bands delimited by large veins, which is often followed by leaf necrosis. In the United States, downy mildew was first reported in south Florida in Oct. 2007 (Roberts et al., 2009), and it has produced notable levels of disease in basil in several states every year since 2008 (Babadoost, 2010; Blomquist et al., 2009; McGrath et al., 2010; Mersha et al., 2013; Wick and Brazee, 2009; Wyenandt et al., 2015). In Canada, the disease was first reported in 2011 (Saude et al., 2013). In addition, several other countries have reported serious outbreaks of downy mildew on basil (Garibaldi et al., 2004, 2005; Khateri et al., 2007; McLeod et al., 2006; Kong et al., 2015; Ronco et al., 2009). The pathogen requires high relative humidity (>85%) or wet leaves for infection. Moderate (20 °C) rather than higher temperatures favor disease development (Wyenandt et al., 2015).

Losses due to downy mildew on basil in the United States have been estimated as tens of millions of dollars (Wyenandt et al., 2015). Management of basil downy mildew currently includes growing pathogen-free seed (Gilardi et al., 2015), reducing leaf wetness and relative humidity during plant production (Cohen and Ben-Naim, 2016), and preventative fungicide applications. Resistance to basil downy mildew, possibly from a single dominant gene, was transferred from a wild basil species to a sweet basil species (Ben-Naim et al., 2018). Therefore, it may be possible to develop elite sweet basil cultivars with genetic resistance to basil downy mildew in the near future. Registered commercial fungicides, including organic production control measures (e.g., hydrogen dioxide with peroxyacetic acid, copper salts, *Streptomyces lydicus*) and nonorganic measures (e.g., monopotassium and dipotassium salts of phosphorus acid, potassium phosphite, and azoxystrobin) have been used with varying levels of success managing downy mildew (Gilardi et al., 2013; Homa et al., 2014; Patel et al., 2016; Pintore et al., 2016). Unregistered but effective compounds include fluopicolide, mefenoxam + copper hydroxide, and oxathiapiprolin + chlorothalonil (Babadoost and DeYoung, 2012). Mefenoxam + copper hydroxide is labeled for controlling *Pythium*, not downy mildew, on basil. Fungicides with multisite effects, including dithiocarbamates (e.g., mancozeb), chloronitriles (e.g., chlorothalonil), and copper formulations, can reduce downy mildew diseases (Babadoost and DeYoung, 2013). The fungicides currently registered for use on basil for downy mildew control include azoxystrobin, fenamidone, phosphorous

acid, mandipropamid, cyzofamid, and several other organic products (Crop Data Management Systems, 2018; Wyenandt et al., 2015). Azoxystrobin and mandipropamid are registered in Illinois for use on basil (Egel et al., 2017), whereas cyzofamid is nationally registered.

Organic fungicides have had little success controlling basil downy mildew (Wyenandt et al., 2015); therefore, novel control measures are urgently needed for organic basil growers. Basidiomycetous yeast *Papiliotrema flavescens* [previously reported as *Cryptococcus flavescens* (Dunlap et al., 2007) strain OH 182.9 3C (NRRL Y-30216)] and *Bacillus amyloliquefaciens* AS 43.3 [NRRL B-30210 (Dunlap et al., 2013)] are antagonists of *Fusarium* head blight (FHB) (*Gibberella zeae*, anamorph: *Fusarium graminearum*) (Khan et al., 2001). These antagonists, which are isolated from anthers of flowering wheat heads (*Triticum aestivum*) (Khan et al., 2001), reduce FHB and the deoxynivalenol content of grain in greenhouse and field settings (Khan et al., 2004; Schisler et al., 2002) when assayed alone or in combination with other biocontrol agents (Kolombet et al., 2005; Yuen et al., 2010). Their influence on downy mildew is not known, but it is worth testing because a bacterium was able to produce compounds that inhibited both downy mildew and *Fusarium oxysporum* (Liang et al., 2016).

The first objective of this study was to determine the effectiveness of registered products, including azoxystrobin, phosphorous acid, mandipropamid, and cyzofamid, for managing downy mildew in the field when applied as tank mixes or in alternation with different chemical classes. Using mixtures and alternations of fungicides is advantageous for growers because they can reduce the risk of the pathogen developing fungicide resistance. The second objective of this study was to evaluate foliar applications of antagonists OH 182.9 3C and AS 43.3 to determine their efficacy for reducing downy mildew on organically produced basil under greenhouse conditions.

Materials and methods

Field trial. A field trial was conducted in Momence, IL to evaluate the efficacy of selected fungicides applied in several combinations and application sequences for reducing basil downy mildew. The experimental field was plowed in Nov. 2013, after basil had been harvested; it was tilled on 17 June 2014. The soil was sandy loam with a pH of 6.4. Plant beds were made on 2 July 2014, and seeds of sweet basil cultivar San Remo (downy mildew-susceptible) (Farahani-Kofoet et al., 2014) were sown on the same day. During the season, weeds were controlled by cultivation and hand weeding. No insecticides were applied to the plots. Nineteen treatments and one untreated control (Table 1) were included in the trial. Treatments (60 plants per treatment) were arranged in a randomized complete block with a repeated measures factorial design with four replicate blocks (15 plants per block). Each plot measured 5 × 10 ft and consisted of four rows of basil. Fungicides were applied at label rates at 60 gal/acre with a backpack sprayer, boom, and single-drop nozzle. A total of nine spray applications of fungicide were performed. Weekly applications began on 14 July 2014 and ended on 8 Sept. 2014. After 8 Sept. 2014, temperatures decreased to levels that were not conducive for the development of downy mildew.

Several fungicides with varying modes of action were tested: azoxystrobin [Quadris 2.08 SC; Syngenta Crop Protection, Greensboro, NC (FRAC code 11)], potassium phosphite [ProPhyt SC; Helena Chemical Co., Collierville, TN (FRAC code 33)], mandipropamid [Revus 2.09 SC, Syngenta Crop Protection (FRAC code 40)], cyazofamid [Ranman 400 SC; FMC Corp., Philadelphia, PA (FRAC code 21)], oxathiapiprolin [Orondis Gold 200; Syngenta Crop Protection (FRAC code 49)], A18269 SE (an experimental compound; Syngenta Crop Protection), dimethomorph [Forum 4.16 SC; BASF Corp. Research Triangle Park, NC (FRAC code 40)], zoxamide + mancozeb [Gavel; Gowan Co., Yuma, AZ (FRAC code 22 and M03)], fluazinam [Omega, Syngenta Crop Protection (FRAC code 29)], fluopicolide [Presidio; Valent BioSciences Corp., Libertyville, IL (FRAC code 43)], mefenoxam + copper hydroxide [Ridomil Gold Copper; Syngenta Crop Protection (FRAC code 4 and M01)], fenamidone [Reason 500 SC; Bayer Crop Science, Research Triangle Park, NC (FRAC code 11)], mancozeb [Mancozeb; Bonide Products, Oriskany, NY (FRAC code M03)], and ametoctradin + dimethomorph [Zampro 525F; BASF Corp. (FRAC code 45 and 40)]. A nonionic surfactant (Induce 90; Helena Chemical Co.) was used in some treatments. Rates and sequences of the products used for the various treatments are listed in Table 1. The severity of downy mildew (percentage of the total area of all leaves displaying signs or symptoms of disease) was visually assessed in the

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.4047	acre(s)	ha	2.4711
29.5735	fl oz	mL	0.0338
0.0731	fl oz/acre	L·ha^{-1}	13.6840
0.3048	ft	m	3.2808
3.7854	gal	L	0.2642
9.3540	gal/acre	L·ha^{-1}	0.1069
2.54	inch(es)	cm	0.3937
1.1209	lb/acre	kg·ha^{-1}	0.8922
0.0254	mil(s)	mm	39.3701
0.0701	oz/acre	kg·ha^{-1}	14.2749
62.5000	oz/lb	g·kg^{-1}	0.0160
1.1692	pt/acre	L·ha^{-1}	0.8553
(°F − 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Table 1. Pesticide treatment combinations and timing of applications to control basil downy mildew in the field in Illinois in 2014.

Treatment	First fungicide[z]	Second fungicide[z]	Third fungicide[z]
A	15.5 fl oz azoxystrobin + 4 pt potassium phosphite + 0.125% nonionic surfactant (1, 4, 7)[y]	8 fl oz mandipropamid + 4 pt potassium phosphite + 0.125% nonionic surfactant (2, 5, 8)	2.75 fl oz cyazofamid + 4 pt potassium phosphite + 0.125% nonionic surfactant (3, 6, 9)
B	15.5 fl oz azoxystrobin + 4 pt potassium phosphite (1, 4, 7)	8 fl oz mandipropamid + 4 pt potassium phosphite (2, 5, 8)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (3, 6, 9)
C	15.5 fl oz azoxystrobin + 4 pt potassium phosphite + 0.125% nonionic surfactant (1, 5, 9)	4 pt potassium phosphite + 0.125% nonionic surfactant (2, 4, 6, 8)	8 fl oz mandipropamid + 4 pt potassium phosphite + 0.125% nonionic surfactant (3, 7)
D	15.5 fl oz azoxystrobin + 4 pt potassium phosphite + 0.125% nonionic surfactant (1, 5, 9)	4 pt potassium phosphite (2, 4, 6, 8)	8 fl oz mandipropamid + 4 pt potassium phosphite + 0.125% nonionic surfactant (3, 7)
E	15.5 fl oz azoxystrobin (1, 5, 9)	4 pt potassium phosphite (2, 4, 6, 8)	8 fl oz mandipropamid (3, 7)
F	15.5 fl oz azoxystrobin (1, 5, 9)	4 pt potassium phosphite (2, 4, 6, 8)	2.75 fl oz cyazofamid (3, 7)
G	15.5 fl oz azoxystrobin + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	9.6 fl oz oxathiapiprolin + 4 pt potassium phosphite, 4 pints (3, 6, 9)
H	8 fl oz mandipropamid + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	4.8 fl oz A18269 SE + 4 pt potassium phosphite (3, 6, 9)
I	15.5 fl oz azoxystrobin + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	6 fl oz dimethomorph + 4 pt potassium phosphite (3, 6, 9)
J	8 fl oz mandipropamid + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	6 fl oz dimethomorph + 4 pt potassium phosphite (3, 6, 9)
K	8 fl oz mandipropamid + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	2 lb zoxamide + mancozeb + 4 pt potassium phosphite (3, 6, 9)
L	15.5 fl oz azoxystrobin + 4 pt potassium phosphite (1, 4,7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	1 pt fluazinam + 4 pt potassium phosphite (3, 6, 9)
M	8 fl oz mandipropamid + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	1 pt fluazinam + 4 pt potassium phosphite (3, 6, 9)
N	8 fl oz mandipropamid + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	4 fl oz fluopicolide + 4 pt potassium phosphite (3, 6, 9)
O	8 fl oz mandipropamid + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	1 lb mefenoxam + copper hydroxide + 4 pt potassium phosphite (3, 6, 9)
P	8 fl oz mandipropamid + 4 pt potassium phosphite (1, 4, 7)	1 lb mefenoxam + copper hydroxide + 4 pt potassium phosphite (2, 5, 8)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (3, 6, 9)
Q	8 fl oz mandipropamid + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	5.5 fl oz fenamidone + 4 pt potassium phosphite (3, 6, 9)
R	8 fl oz mandipropamid + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	10 oz mancozeb + 4 pt potassium phosphite (3, 6, 9)
S	8 fl oz mandipropamid + 4 pt potassium phosphite (1, 4, 7)	2.75 fl oz cyazofamid + 4 pt potassium phosphite (2, 5, 8)	14 fl oz ametoctradin + dimethomorph + 4 pt potassium phosphite (3, 6, 9)

[z]The rate per acre is listed before each pesticide. The concentration of the nonionic surfactant is given as a percentage (by volume): 1 fl oz/acre = 0.0731 L·ha^{-1}; 1 pt/acre = 1.1692 L·ha^{-1}; 1 lb/acre = 1.1209 kg·ha^{-1}; 1 oz/acre = 0.0701 kg·ha^{-1}.

[y]The dates of applications are listed in parentheses; the following numbers refer to the actual date: 1 = 14 July; 2 = 21 July; 3 = 28 July; 4 = 4 Aug.; 5 = 11 Aug.; 6 = 18 Aug.; 7 = 25 Aug.; 8 = 1 Sept.; 9 = 8 Sept.

middle two rows of each plot on 18 Aug. 2014, 27 Aug. 2014, and 16 Sept. 2014. The area under the disease progress curve (AUDPC) values were calculated according to Shaner and Finney (1977).

Severity data were arcsine-transformed before analysis of variance (ANOVA), which was conducted using PROC MIX in SAS (version 9.3; SAS Institute, Cary, NC). Treatment means were calculated, and the mean separation was determined at $P \leq 0.05$ with Bonferroni adjustment. AUDPC values were determined using PROC GLM, and means were separated using Fisher's protected least significant difference (FPLSD) at $P \leq 0.05$.

Greenhouse trial. Seeds of *Fusarium*-resistant basil hybrid AROMA 2 OG F1 (Johnny's Selected Seeds, Winslow, ME) were stored at 4 °C. Seeds were sown in six 13- × 13-cm punnets containing 3 × 4 cells per punnet. Punnets were contained within a 25- × 50-cm open flat tray. Three seeds per well were sown onto pasteurized (60 °C air/steam for 30 min after the product reached temperature) propagation mix (Redi-Earth plug and seedling mix; Sun Gro Horticulture, Seba Beach, AB, Canada) supplemented with 0.5 $g \cdot kg^{-1}$ micronutrients (Micromax; ICL Specialty Fertilizers, Dublin, OH) and 3.0 $g \cdot kg^{-1}$ macronutrients [15N–6.6P–12.5K (Osmocote; Everris NA, Dublin, OH)]. Water was added to the open flat tray daily as needed. Basil plants in the tray were grown in a plant growth chamber with a photoperiod of 13 $h \cdot d^{-1}$, light intensity of 600 $\mu E \cdot m^{-2} \cdot s^{-1}$, relative humidity of 60% to 80%, and temperatures of 23 °C (day) and 18 °C (night). After cotyledons of basil seedlings expanded, seedlings were thinned to ≈12 plants per punnet and grown for a total of 3 to 4 weeks.

Maintenance of basil downy mildew inoculum. The isolate of basil downy mildew used in these studies was obtained from a symptomatic basil plant that was collected in Danville, IL in 2014. To maintain the isolate for experimental use, sporangia from the infected basil plants were washed from infected leaves and then used to inoculate 3- to 4-week-old basil seedlings. Seedlings were sprayed until runoff with an aqueous suspension of sporangia at $\approx 5 \times 10^4$ sporangia/mL. Inoculated plants in trays were then watered to soil saturation and placed in a 50- × 76-cm 4-mil poly bags (Uline, Chicago, IL). Bags were then sealed to create relative humidity of 95% to 100% within the bag, and the bagged trays were incubated in a plant growth chamber with light and temperature conditions as described previously. Sporangia developed after ≈7 d, and these sporangia were used to inoculate new basil plants every 10 to 14 d to maintain a constant source of basil downy mildew sporangial inoculum.

Production of antagonist inoculum. Biocontrol strains *B. amyloliquefaciens* AS 43.3 and *P. flavescens* OH 182.9 3C were used in all studies. Strains AS 43.3 and OH 182.9 3C were stored at -80 °C in 10% glycerol, streaked on 1/5 tryptic soy broth agar [TSBA/5 (pH 6.8; Difco Laboratories, Detroit, MI)] to determine purity, restreaked on TSBA/5, and then grown for 24 h at 25 °C to produce cells used to inoculate precultures. Precultures containing 25 mL of semi-defined complete medium (SDCL) (Slininger et al., 2010) in 100-mL erlenmeyer flasks were incubated for 24 h and then used to inoculate test cultures containing 50 mL of SDCL in 200-mL unbaffled erlenmeyer flasks to an initial absorbance (OD_{620}) of 0.1. Then, test cultures were incubated in a shaker incubator at 250 rpm, 2.0-cm eccentricity, and 25 °C for 24 h before use. Final concentrations of the inoculum tested in bioassays were $\approx 5 \times 10^8$ and 3×10^7 colony forming units (cfu)/mL for bacteria and yeast strains, respectively, as determined by plating serial dilutions of each colonized liquid medium onto TSBA/5 (Schisler et al., 2002).

Greenhouse assay of biocontrol agents and fungicides against basil downy mildew: Expt. 1. Studies were conducted in Peoria in a climate-controlled greenhouse where temperatures ranged from 15 to 20 °C at night and 23 to 28 °C during the day. High-pressure sodium lights supplemented natural sunlight for 14 $h \cdot d^{-1}$. Treatments were biocontrol agents *B. amyloliquefaciens* AS 43.3 ($\approx 1.1 \times 10^{14}$ cfu/ha), *P. flavescens* OH 182.9 3C ($\approx 6.8 \times 10^{12}$ cfu/ha), the fungicide quinoxyfen (FRAC code 13), as a negative control because it is registered for use on powdery mildew (such as *Sphaerotheca, Leveillula, Podosphaera*, and *Erysiphe*), the fungicide azoxystrobin (a positive control) (Gilardi et al., 2013)], and an untreated control. All products were applied in a volume equivalent to 469 $L \cdot ha^{-1}$. For the biological control agents, this corresponded to 2.4×10^8 cfu/mL and 1.4×10^7 cfu/mL applied until runoff for *B. amyloliquefaciens* AS 43.3 and *P. flavescens* OH 182.9 3C, respectively. One day after fungicides and bioagents were applied, the unifoliate leaves of basil plants were inoculated with basil downy mildew by spraying an aqueous suspension of sporangia at $\approx 5 \times 10^5$ sporangia/mL onto seedlings at 469 $L \cdot ha^{-1}$.

Inoculated plants in trays were watered to soil saturation and placed in a 50- × 76-cm 4-mil poly bag (Uline, Chicago, IL). The bags were sealed to create relative humidity of 95% to 100% within the bag, and the bagged trays were incubated in the greenhouse as described. After 7 d, plastic bags were opened while keeping the punnets inside of the bags until the plants were rated. Punnets were watered daily to keep the potting medium at saturation levels. Disease severity was rated 14 d after inoculation by visually estimating the percent area of the unifoliate leaves exhibiting signs and symptoms of basil downy mildew. The experiment was performed as a completely randomized design with four replications, and it was performed three different times during the same year. Data were arcsine-transformed before ANOVA using the general linear model procedure (PROC GLM) in SAS (version 9.3). Because Brown and Forsythe's F test indicated homogenous variance between the replicated trials ($P \leq 0.05$), data from all trials were combined for the final analysis. Treatment means were separated using FPLSD ($P \leq 0.05$).

Expt. 2. A second set of replicated experiments were conducted using methods identical to those of greenhouse Expt. 1, except that after the application of treatments and pathogens, additional applications of the treatments were performed 1 and 2 weeks later, for a total of three treatment applications. Disease incidence and severity were rated 1 week after initial treatment application, and then weekly for 2 additional weeks. Greenhouse Expt. 2 was a five-fungicide × 3-week factorial with three replications arranged in a completely randomized design. The experiment was performed

on two different dates during the same year. Data from repeated experiments were pooled after statistical analysis demonstrated homogenous variance between the replicated trials ($P \leq 0.05$). Disease severity and AUDPC data were normalized using the arcsine transformation before ANOVA. Means were separated using FPLSD ($P \leq 0.05$).

Results

Field experiment. After six foliar applications of fungicides of each treatment during the time from 14 July 2014 to 6 Aug. 2014, symptoms of downy mildew were seen on basil plants in all plots. Disease severity in all plots sprayed with the 19 fungicide treatments was significantly lower than that of untreated plots (see Table 2). There was no significant difference in disease severity among 19 fungicide treatments on 18 Aug. 2014, 35 d after the application (DAA) of the fungicide treatments started. Similar results were determined on 27 Aug. 2014 (46 DAA) compared with the untreated control, but disease severity was greater in the plots with treatment N than in plots with treatment P (Table 2).

At 64 DAA, all treatments continued to show lower disease severity than the untreated control, and there were instances of differences in disease severity among the 19 fungicide treatments (Table 2). Disease severity in the plots with treatment O was significantly higher than that in plots with treatments B, C, G, and H (Table 2). Thirteen of the treatments (A-D, G-M, R, S) resulted in statistically similar disease severity of less than 10%.

All fungicide treatment combinations also reduced AUDPC values calculated for the treatments compared with the control (Table 2). There were differences in AUDPC values among fungicide treatment regimes, with the highest AUDPC (529) resulting from fungicide treatment N and the lowest AUDPC (157) resulting from fungicide treatment B (Table 2). Sixteen of the treatments (all except treatments E, N, and O) resulted in statistically similar AUDPC values. Adding nonionic surfactant (Induce 90) did not improve the efficacy of the fungicide for controlling basil downy mildew.

Greenhouse experiments. During greenhouse Expt. 1, symptoms appeared on basil seedlings by 7 DAA. By 14 DAA, downy mildew severity was more than 50% for the untreated control, both biocontrol treatments, and quinoxyfen (the negative fungicide treatment control). Only azoxystrobin significantly reduced downy mildew disease to 0% (Table 3). Biocontrol treatments did not reduce disease severity compared with the control.

During greenhouse Expt. 2, treatment with biocontrol strains AS43.3 and OH182.9 did not modify disease severity compared with the untreated control at 7, 14, and 21 DAA (Table 4). The negative fungicide control (quinoxyfen) did not reduce downy mildew; however, the positive fungicide control (azoxystrobin) reduced disease to undetectable levels at every time point evaluated. Cumulative disease development, as measured by AUDPC, reached undetectable levels with the weekly azoxystrobin applications, but downy mildew disease was not reduced by weekly applications of biocontrol strains AS 43.3 and OH 182.9 or by quinoxyfen compared with the untreated control (Table 4).

Table 2. Evaluating the efficacy of selected pesticides for controlling basil downy mildew in the field in Illinois in 2014.

Treatment[z]	Disease severity on 18 Aug. (%)	Disease severity on 27 Aug. (%)	Disease severity on 16 Sept. (%)	AUDPC[y]
Untreated control	80.0 a[x]	80.0 a[x]	72.5 a[x]	3,330 a[w]
A	10.0 b	5.0 bc	7.5 bcd	312 cde
B	7.5 b	6.2 bc	0.0 d	157 e
C	11.2 b	3.9 bc	3.8 cd	233 de
D	8.8 b	5.0 bc	6.2 bcd	276 cde
E	7.5 b	10.0 bc	15.0 bc	512 bc
F	8.8 b	6.2 bc	12.5 bc	419 bcde
G	7.5 b	5.0 bc	3.8 cd	216 de
H	3.0 b	5.0 bc	3.8 cd	176 cde
I	10.0 b	6.2 bc	5.0 bcd	280 cde
J	7.5 b	8.8 bc	6.2 bcd	319 bcde
K	6.8 b	6.2 bc	6.2 bcd	275 cde
L	8.0 b	8.8 bc	8.8 bcd	376 bcde
M	8.8 b	6.2 bc	8.7 bcd	343 bcde
N	8.8 b	13.8 b	12.5 bc	529 b
O	8.8 b	3.8 bc	20.0 b	534 bcd
P	3.0 b	1.2 c	5.0 bc	144 cde
Q	8.8 b	5.0 bc	10.0 bc	352 bcde
R	6.2 b	5.0 bc	7.5 bcd	278 cde
S	8.0 b	7.5 bc	7.5 bcd	331 bcde

[z]See Table 1 for treatment rates and application timing.
[y]Area under disease progress curve.
[x]Within a column, means not followed by the same letter are significantly different ($P \leq 0.05$, Bonferroni mean separation).
[w]Within a column, means not followed by the same letter are significantly different ($P \leq 0.05$, Fisher's protected least significant difference).

Table 3. Evaluation of the efficacy of a single treatment with selected biocontrol agents to manage basil downy mildew.

Treatment[z]	Rate[y]	Disease severity at 14 d (%)[x]
Untreated control	-	58.7 a[w]
Bacillus amyloliquefaciens AS43.3	227 L·ha^{-1}	58.5 a
Papiliotrema flavescens OH182.9 3C	227 L·ha^{-1}	58.1 a
quinoxyfen (negative fungicide control)	0.28 kg·ha^{-1}	55.6 a
azoxystrobin (positive fungicide control)	0.09 kg·ha^{-1}	0 b

[z]Basil plants were treated at 21–28 d after planting with cells of a biocontrol agent or with fungicide controls and with inoculum of basil downy mildew 1 d later.
[y]*B. amyloliquefaciens* AS 43.3 was applied at 227 L·ha^{-1} and 5×10^{11} colony forming units (cfu)/L, whereas *P. flavescens* OH 182.9 3C was applied at 227 L·ha^{-1} and 2×10^{10} cfu/L. Fungicides were applied at the listed amount. One day later, an aqueous suspension of 5×10^{8} sporangia/L of basil downy mildew was applied: 1 L·ha^{-1} = 0.1069 gal/acre; 1 kg·ha^{-1} = 0.8922 lb/acre; 1 cfu/L = 3.7854 cfu/gal; and 1 sporangium/L = 3.7854 sporangia/gal.
[x]Pooled results from three experiments are presented.
[w]Within a column, means not followed by the same letter are significantly different (Fisher's protected least significant difference, $P \leq 0.05$).

Table 4. Evaluation of the efficacy of three consecutive weekly treatments with selected biocontrol agents to manage basil downy mildew.

Treatment[z]	Rate[y]	Disease severity at 7 d (%)	Disease severity at 14 d (%)	Disease severity at 21 d (%)	AUDPC[x]
Untreated control	-	24.3 a[w]	37.7 a	89.2 a	661.2 a
Bacillus amyloliquefaciens AS43.3	227 L·ha^{-1}	25.0 a	36.6 a	90.5 a	660.8 a
Papiliotrema flavescens OH182.93C	227 L·ha^{-1}	29.1 a	36.8 a	88.3 a	668.5 a
quinoxyfen (negative fungicide control)	0.28 kg·ha^{-1}	26.1 a	37.7 a	85.0 a	652.8 a
azoxystrobin (positive fungicide control)	0.09 kg·ha^{-1}	0 b	0 b	0 b	0 b

[z]Basil plants were treated at 21–28 d after planting with either cells of a biocontrol agent or a fungicide and with inoculum of basil downy mildew 1 d later.
[y]*B. amyloliquefaciens* AS 43.3 was applied at 227 L·ha^{-1} and 5×10^{11} colony forming units (cfu)/L, whereas *P. flavescens* OH 182.9 3C was applied at 227 L·ha^{-1} and 2×10^{10} cfu/L. Fungicides were applied at the listed amount. One day later, an aqueous suspension of 5×10^{8} sporangia/L of basil downy mildew was applied; 1 L·ha^{-1} = 0.1069 gal/acre, 1 kg·ha^{-1} = 0.8922 lb/acre, 1 cfu/L = 3.7854 cfu/gal, 1 sporangium/L = 3.7854 sporangia/gal.
[x]Area under the disease progress curve; pooled results from two experiments are presented.
[w]Within a column, means not followed by the same letter are significantly different (Fisher's protected least significant difference, $P \leq 0.05$).

Discussion

In the field experiment, a wide variety of fungicides, combinations of fungicides, and application sequences were applied weekly. As determined by individual disease ratings and the AUDPC calculated from data obtained from 3 weeks of disease ratings, these consistently reduced downy mildew (Table 2). The decision to select these fungicides for testing was based on their effectiveness controlling downy mildew disease of basil and other crops. Alternating fungicide applications are necessary to manage fungicide resistance of pathogens. Most of these fungicides should not be applied more than two consecutive times before alternating with fungicides with different modes of action. Azoxystrobin was a component in each of the three treatments that resulted in the lowest AUDPC values. Fungicides that show promise controlling basil downy mildew and are approved, or are nearing approval, for use against downy mildew on basil are increasing. These include, but are not limited to, compounds used in the current field study, such as azoxystrobin, potassium phosphite, mandipropamid, cyazofamid, oxathiapiprolin, fluopicolide, mefenoxam + copper hydroxide, and fenamidone (Wyenandt et al., 2015). In a previous field experiment (Babadoost and DeYoung, 2012), potassium phosphite, mandipropamid, cyazofamid, and fluopicolide reduced disease severity for basil cultivar Esmeralda (sensitive to basil downy mildew) when applied singly with a nonionic surfactant (Induce 90) at weekly intervals during the growing season. In a field study conducted the following year (Babadoost and DeYoung, 2013), azoxystrobin was added to the list of fungicides tested, and many of the compounds, combinations, and sequences of fungicides tested were repeated in the present study. As in the present study, all combinations and sequences of fungicides that included azoxystrobin also reduced disease severity for basil cultivar Esmeralda in the presence or absence of nonionic surfactant [Induce 90 (Babadoost and DeYoung, 2013)]. Results of the current field study using sweet basil cultivar San Remo agreed with previous efficacy studies (Babadoost and DeYoung, 2012, 2013) in which the best treatment combinations (described previously) reduced disease severity at the end of the growing season to less than 5%. Additionally, results of the current study demonstrated that a nonionic surfactant (Induce 90) at 0.125% did not enhance the effectiveness of the fungicide combinations tested when included in fungicide tank mixes (Table 2) (Babadoost and DeYoung, 2013). The fungicides used in the current study are systemic, and surfactants are more effective with contact fungicides. Other studies have tested some of these fungicides (McGrath, 2016a, 2016b; McGrath and Sexton, 2017; Raid et al., 2017), including glasshouse studies that demonstrated that fungicides mefenoxam + copper hydroxide, mandipropanid, and azoxystrobin also reduced disease (Gilardi et al., 2013). Field studies by Homa et al. (2014) also showed that mandipropamid, cyazofamid, and phosphorous acid were consistently effective, whereas azoxystrobin and fluopicolide were only effective in one of two trials.

Repeated applications of a fungicide with a single mode of action may result in the development of resistance to the compound by the pathogen, as has been reported regarding mefenoxam-resistant strains of basil downy mildew (Cohen et al., 2013b, 2017; Pintore et al., 2016). Strains of other pathogens that are resistant to fungicides such as azoxystrobin and mandipropamid have been detected (Hagerty et al., 2017; Sawant et al., 2017), suggesting that basil downy mildew resistance to these fungicides may also develop from the exclusive and frequent use of these compounds against the pathogen. During the field experiment, some of the fungicide products used were composed of two fungicides with differing modes of action (e.g., ametoctradin + dimethomorph and zoxamide + mancozeb). Results of the current study indicated that multiple combinations of compounds and sequences of compounds can substantially reduce basil downy mildew. The use of these compounds and sequences in the field as part of an integrated management approach against basil downy mildew may have the added benefit of reducing the likelihood of the development of pathogen resistance against the individual products used.

In both greenhouse experiments, biocontrol strains were not effective for reducing downy mildew; however, azoxystrobin, as in the field study, was effective (Tables 3 and 4). As anticipated, quinoxyfen, a fungicide with known efficacy against powdery mildews caused by ascomycetous fungi (Nelson et al., 2014), was ineffective for controlling downy mildew of basil. *B. amyloliquefaciens* AS 43.3 and *P. flavescens* OH 182.9 3C were both originally isolated from wheat anthers and were effective for reducing FHB of wheat during field applications (Khan et al., 2004; Schisler et al.,

2015). Increasing the dosage of these biocontrol agents to levels used during field trials against FHB on wheat may increase the effectiveness of these treatments against basil downy mildew. However, biocontrol organisms are known to vary in their competence in colonizing plant tissues depending on the species of plant inoculated (Blouin Bankhead et al., 2016). It is possible that the biocontrol strains tested in the present study were not able to effectively colonize basil leaves; therefore, they failed to reduce disease. In another study, treatment with *Bacillus subtiilis* QST 713 was also ineffective for reducing basil downy mildew, as was thyme oil [from *Thymus vulgaris* (Gilardi et al., 2013)].

Since the introduction of downy mildew in the United States, the list of fungicides labeled for the management of basil downy mildew has increased, but indications from other pathosystems suggest that the development of fungicide-resistant strains of basil downy mildew is a valid concern. Therefore, the evaluation of new fungicide products against basil downy mildew is warranted. Organic production of basil remains difficult due to a lack of highly effective compounds for reducing downy mildew on basil to commercially acceptable levels, but the development of resistant cultivars, pathogen-free seed, moving air to reduce leaf wetness and humidity (Cohen and Ben-Naim, 2016), and light management to reduce sporulation (Cohen et al., 2013a) can be helpful. It appears that continued research with an emphasis on the integrated control of downy mildew has the most promise for organic and traditional agricultural production of basil (Pintore et al., 2016).

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