Evaluation of Oxathiapiprolin for the Management of Sunflower Downy Mildew

Ryan M. Humann,¹ Keith D. Johnson,² Michael J. Wunsch,³ Scott M. Meyer,⁴ James G. Jordahl,⁴ Elizabeth C. Bauske,⁴ Jessica M. Halvorson,⁴ Andrew J. Friskop,⁴ Keith A. O'Bryan,⁵ Thomas J. Gulya (retired),⁶ and Samuel G. Markell^{4†}

¹ Syngenta, Greensboro, NC 27409

² FMC Corp., Philadelphia, PA 19104

³ North Dakota State University–Carrington Research Extension Center, Carrington, ND 58421

⁴ Department of Plant Pathology, North Dakota State University, Fargo, ND 58102

⁵ Corteva Agriscience, Johnston, IA 50131

⁶ USDA-ARS Sunflower Research Unit, Fargo, ND 58102

Abstract

Downy mildew is a yield-limiting disease of sunflower, caused by the pathogen *Plasmopara halstedii*. Zoospore infection of root tissue shortly after planting results in systemic infection, causing postemergence damping off or severe stunting and head sterility. Although fungicide-applied seed treatments can be an effective management tool, the pathogen is resistant to phenylamide fungicides in many growing regions, and other available fungicides have limited efficacy. Oxathiapiprolin, the first member of the piperidinyl thiazole isoxazoline fungicides, was evaluated for efficacy on downy mildew in field trials conducted from 2011 to 2015 in North Dakota. Throughout the course of the study, the rate range was narrowed from active ingredient (a.i.) at 0.45 to 116.0 $\mu\text{g a.i. seed}^{-1}$ to an optimal effective rate of 9.37 to 18.75 $\mu\text{g a.i. seed}^{-1}$. Within that

optimal range, the downy mildew incidence of sunflower planted with oxathiapiprolin-treated seed was significantly lower than the incidence in the nontreated sunflower in all 11 trials with disease pressure. Additionally, downy mildew incidence of sunflower planted with oxathiapiprolin-treated seed was significantly lower than sunflower planted with competitive commercially available fungicide-treated seed in 10 of those 11 trials. The use of oxathiapiprolin by sunflower growers is likely to reduce disease incidence and subsequent yield loss to downy mildew.

Keywords: chemical, disease management, oilseed, oomycetes, ornamental, sunflower

Downy mildew of sunflower (*Helianthus annuus* L.), caused by *Plasmopara halstedii* (Farl.) Berl. & De Toni, is an economically important disease in the northern sunflower-producing regions of the United States. The pathogen is an obligate oomycete that is capable of overwintering as oospores in soils for up to 10 years (Humann et al. 2016b). Disease development is favored by saturated soils and cool soil temperatures (15 to 20°C) shortly after sunflower is planted. These conditions facilitate oospore germination when sunflower plants are most sensitive to infection (Gulya et al. 1997). Systemic infections occur when motile zoospores infect seedling roots. Most systemically infected plants damp-off after emergence but those that survive are stunted (Supplementary Fig. S1) and eventually die or go on to produce sterile heads (Markell et al. 2015). Secondary infection can occur when windblown zoosporangia from infected plants land on sunflower leaf tissue, causing

acute, angular leaf lesions. This type of foliar infection does not lead to systemic infection and is not considered economically important (Friskop et al. 2009; Gulya et al. 2013; Sackston 1981).

Downy mildew is a frequent problem in North Dakota, a state that tends to have cooler temperatures following planting than other sunflower-producing states (Friskop et al. 2009). Prevalence of downy mildew in North Dakota fields surveyed from 2001 to 2011 ranged from 2 to 70%, and incidence within fields was as high as 7% (Gulya et al. 2013). Surveys of sunflower downy mildew conducted in the Northern Great Plains in 2014 and 2015 have found that downy mildew occurred in over 50% of the fields in North Dakota (Gilley et al. 2015, 2016). Yield losses are most frequently observed when large water-saturated areas of the field (i.e., low spots) create conditions conducive for systemic infection, resulting in significant stand losses in concentrated areas of the field. In North Dakota, it is not uncommon for areas of fields many hectares in size to experience nearly 100% yield loss (Gulya et al. 2013).

Management of downy mildew is challenging. Crop rotation is largely ineffective due to the ability of the oospore to survive many years in the soil until conditions that are favorable for germination and infection (Gulya et al. 1997). Genetic resistance can be an effective management tool and numerous resistance genes have been incorporated into many commercial sunflower hybrids. However, frequent changes in pathogen virulence have allowed many of these resistance genes to be overcome (Gulya and McMullen 2012; Gulya et al. 2011; Markell et al. 2016). Seed treatments can be a very effective management tool because they are most active when seedlings are germinating, and systemic infection usually occurs during a short time after planting (3 to 15 days) (Gulya et al. 2013; Humann et al. 2016b). However, few available seed-treatment fungicides are efficacious on *P. halstedii*.

Throughout the 1980s and 1990s, two phenylamide (PA) fungicides (Fungicide Resistance Action Committee [FRAC] 4), metalaxyl (Allegiance FL; Bayer Crop Science, Research Triangle Park,

Former address of R. M. Humann: Department of Plant Pathology, North Dakota State University, Fargo, ND 58102.

Former address of K. D. Johnson: DuPont Field Development, Grand Forks, ND 58201.

†Corresponding author: S. G. Markell; samuel.markell@ndsu.edu

Funding: We thank DuPont Crop Protection and the National Sunflower Association for their financial support.

*The e-Xtra logo stands for “electronic extra” and indicates that two supplementary figures are published online.

The author(s) declare no conflict of interest.

Accepted for publication 16 June 2019.

NC) and mefenoxam (MEF) (Apron XL; Syngenta Crop Protection, Greensboro, NC), were widely used by sunflower growers in the United States (Markell et al. 2016). Both of these compounds had excellent efficacy until the 1990s, when PA-insensitive *P. halstedii* isolates were identified. By 1999, 91% of isolates collected and screened in North Dakota were insensitive to the PAs (Gulya et al. 1999). No efficacious fungicides were available for use until the 2000s, when two quinone outside inhibitors (QoIs) (FRAC 11) were labeled for use; azoxystrobin (AZO) (Dynasty; Syngenta Crop Protection) and fenamidone (FEN) (Idol; Bayer Crop Science) (Gulya et al. 2013). FEN was only available for several years but AZO was widely adopted by growers. However, AZO is only labeled for suppression and is not as efficacious as FRAC 4 fungicides prior to resistance development. Additionally, QoIs such as AZO target a single site of action and are considered a high risk for fungicide resistance development (Brent and Hollomon 2007). In order to better manage downy mildew with seed treatments, and to prolong the life of new host resistance genes and available fungicides, it is necessary to find other fungicides with efficacy on *P. halstedii*.

Oxathiapiprolin (OXA), the first member of the piperidinyl thiazole isoxazoline fungicides (FRAC 49), was recently discovered and developed by DuPont Crop Protection (Pasteris et al. 2016). OXA has shown efficacy on oomycete pathogens in other crops (Ji et al. 2014; Kness et al. 2016; Patel et al. 2015). This fungicide has a different mode of action than fungicide seed treatments currently available on sunflower and could be an additional tool for management of downy mildew. The objective of this study was to evaluate the efficacy of the novel fungicide compound OXA for the management of downy mildew on sunflower.

Materials and Methods

Locations and experimental design. Between 2011 and 2015, 17 field trials were conducted at three locations in North Dakota; the Fargo Agricultural Experiment Station in Fargo, ND; the DuPont Agricultural Research Farm in Thompson, ND; and the North Dakota State University Carrington Research Extension Center in Carrington, ND (Table 1). All treatments within trials were evaluated in a randomized complete block with four to six replicates. All locations were dryland; however, Fargo and Carrington had temporary drip-irrigation systems in place during artificial inoculations to provide

supplemental water to facilitate infection. Single-row plots were planted in all locations, with the exception of Carrington 2011 and 2012, where two-row plots were established. A cone-type planter (Almaco, Nevada, IA, U.S.A.) was used for planting in the Fargo and Carrington locations and a vacuum-type planter (Monosem, Edwardsville, KS, U.S.A.) was used at the Thompson location. Row width was 76.2 cm at all locations. Seed spacing, plot length, planting date, inoculation date, and incidence assessments varied by location and years (Table 1). Recommended agronomic practices for sunflower production were followed for all trials (Berglund 2007).

Pathogen and inoculation. To facilitate infection, all plots were inoculated with *P. halstedii* zoospores. Pathogen isolates selected for this study were originally collected from North Dakota and virulence phenotyped on an internationally recognized set of differentials to determine their race nomenclature (Tourville de Labrouhe et al. 2000). A bulk mixture of isolates of races 714 and 734, which are commonly found in North Dakota, were used in all trials. Additionally, all isolates included were insensitive to metalaxyl and MEF.

Fresh inoculum of the isolate mixture was used to inoculate each field trial. Inoculum was increased by germinating the susceptible hybrids Mycogen 270 and 8D310 (Dow AgroSciences, Indianapolis, IN) in a germination chamber at 21°C for 3 days or until radicles grew to a length of 3 cm. *P. halstedii* zoospores were suspended in a 10-mM CaCl₂ solution at a concentration of approximately 4.0×10^4 zoospores ml⁻¹. Germinated seedlings were soaked in this zoospore suspension in a 15°C dark room for 3 to 4 h, then hand planted into a mixture of sand and perlite (3:2 ratio). The seedlings were grown in a greenhouse at 20 to 22°C under a 14-h diurnal photoperiod. After 10 to 11 days, the infected seedlings were moved into chambers in a 16 to 20°C dark room at 100% relative humidity and allowed to sporulate for 16 to 24 h (Gulya 1996). Cotyledons covered with zoospores were dried for 4 h, harvested into plastic bags, and stored at 4°C until they were used for the field inoculation (up to 10 days).

Field trials were inoculated when sunflower radicles reached a length of 1 to 3 cm, which was approximately 3 to 12 days after planting the trial, depending on soil temperature and moisture. Immediately before inoculation, zoospores on the harvested seedlings were washed off by shaking them vigorously in distilled water. The solution was then poured through a strainer to remove the plant debris, leaving only the zoospores suspended in distilled water. The inoculum concentrations for trials ranged from 5.2×10^5 to

Table 1. Location, agronomic, and evaluation parameters in fungicide trials for management of sunflower downy mildew in North Dakota from 2011 to 2015

Location	Planting day/year	Row width (cm)	Plot width (cm)	Plot length (m)	Seed spacing (cm)	DAP inoc ^a	VE-V4 (DAP) ^b	V8-V12 (DAP) ^b	V14-R1 (DAP) ^b	Trmt ^c	Disease pressure ^d
Carrington	13 May 2011	76.2	152.4	5.8	6.8	10	31	46	60	10	Yes
Fargo	18 May 2011	76.2	76.2	7.6	15.2	7	26	37	51	10	Yes
Thompson	16 May 2011	76.2	76.2	7.6	15.2	7	36	54	...	10	Yes
Carrington	15 May 2012	76.2	152.4	5.8	7.6	10	37	48	...	9	Yes
Fargo 1	17 May 2012	76.2	76.2	7.6	15.2	12	...	...	...	9	No
Fargo 2	5 July 2012	76.2	76.2	7.6	15.2	5	...	...	...	9	No
Thompson	14 May 2012	76.2	76.2	7.6	15.2	7	45	52	...	9	Yes
Carrington	24 May 2013	76.2	76.2	5.8	7.6	7	34	48	59	8	Yes
Fargo 1	28 May 2013	76.2	76.2	7.6	15.2	7	23	38	52	8	Yes
Fargo 2	25 July 2013	76.2	76.2	7.6	15.2	7	28	40	61	8	Yes
Thompson	16 May 2013	76.2	76.2	7.6	15.2	7	27	35	46	8	Yes
Carrington	15 May 2014	76.2	76.2	6.4	13.3	8	23	39	57	5	Yes
Fargo	9 June 2014	76.2	76.2	7.6	15.2	7	24	35	49	5	Yes
Thompson	27 May 2014	76.2	76.2	7.6	13.4	3	...	...	...	5	No
Carrington	12 May 2015	76.2	76.2	4.5	13.3	9	...	...	...	8	No
Fargo	5 June 2015	76.2	76.2	7.6	15.2	5	...	...	...	8	No
Thompson	22 May 2015	76.2	76.2	7.6	15.2	5	...	...	...	8	No

^a DAP = days after planting that plot was inoculated.

^b Disease incidence evaluations of percent disease incidence and phytotoxicity were made at three growth stages (VE to V4, V8 to V12, and V14 to R1). Percent incidence was calculated as incidence (%) = (number of systemically infected plants/total number of plants) × 100 and phytotoxicity = observation for any sign of chemical damage to fungicide treated seedlings. DAP are presented for each evaluation time.

^c Number of treatments evaluated in each trial, including a nontreated control and a nontreated noninoculated control.

^d Signs and symptoms of downy mildew were visually observed, including plant stunting, leaf chlorosis, and masses of white zoospores on undersides of chlorotic leaf tissue.

6.9×10^7 zoospores per linear meter, with a combined average across all locations of 1.5×10^7 zoospores per linear meter.

Two equally effective techniques were used for artificially inoculating the field trials. Technique one introduced the spores to the plots via a drip tape system. Drip tape (T-tape 510-08-340; John Deere Water, San Marcos, CA) was placed over every plot row (76.2-cm intervals) and connected to a header hose (3.8 cm in diameter) at the front of the trial. A chemical injection system was used to inject the spore solution into the header hose of the drip tape system. The second technique introduced the zoospore suspension via a 7.6-liter watering can, where 7.6 liters of the spore solution was poured overhead onto each row within the trial. Technique one was used for the five Carrington test years 2011 through 2015 and three Fargo test years 2011 through 2012, while technique two was used for the four Fargo trials from 2013 through 2015 and five Thompson site trials from 2011 through 2015.

Fungicide efficacy. The number and combination of fungicide treatments in trials evaluated varied among years but remained consistent across locations within the same year. Fungicides evaluated included MEF (Apron XL), a PA fungicide (FRAC 4); AZO (Dynasty), a QoI fungicide (FRAC 11); FEN (Idol), a QoI fungicide (FRAC 11); fludioxonil (FLU) (Maxim; Syngenta Crop Protection), a phenylpyrrole fungicide (FRAC 12); acibenzolar-S-methyl (ACI) (BION; Syngenta Crop Protection), a benzo-thiadiazole fungicide (FRAC P1); and OXA (Lumisena; DuPont Crop Protection, Wilmington, DE, U.S.A.), a piperidinyl thiazole isoxazoline fungicide (FRAC 49). Additionally, AZO and OXA were combined with FLU and MEF to form combination treatments, each with three different modes of action (Friskop et al. 2017). Inoculated, nontreated controls (NTC) and noninoculated, nontreated controls (NI NTC) were included in all trials. All treatments at all locations in all years had a neonicotinoid insecticide, thiamethoxam (Cruiser; Syngenta Crop Protection), applied with active ingredient (a.i.) at a rate of 250 μg a.i. seed⁻¹ to protect from insect pests (Knodel et al. 2014). All combinations of fungicides and insecticides were applied as a seed treatment by Incotec Coating and Seed Technology (Salinas, CA, U.S.A.) and supplied by DuPont Crop Protection.

Data collection. Disease evaluations were conducted visually by examining all plants within a treatment plot for signs and symptoms consistent with systemic infection by *P. halstedii*, including chlorosis bordering veins or on an entire leaf, masses of white zoospores on undersides of chlorotic leaves, and stunting. Percent incidence was calculated as incidence (%) = (number of systemically infected plants/total number of plants) $\times$ 100. Additionally, stand counts (plants per hectare) and phytotoxicity were assessed while rating incidence. Phytotoxicity was assessed four to six times by visually examining plants for necrosis, bronzing, distorted or misshapen plant

parts, death, and any other potential phytotoxic reactions (Berglund 2007). Phytotoxicity was not observed at any rating in any trial and, as such, only negative data (no symptoms present) were recorded. Two to three disease evaluations were conducted when sunflower plants were in early vegetative stages (VE to V4), midvegetative stages (V8 to V12), late vegetative stages, or early reproductive stages (V14 to R1), depending on time of symptom onset and development (Berglund 2007). Incidence evaluations conducted on the first date were considered the most accurate for three reasons: (i) preemergence damping off is very rare, (ii) seed emergence of confectionary sunflower is very consistent, and (iii) severely infected plants frequently damp off between the first and second or second and third emergence evaluation, creating an artificial reduction in disease incidence. Yield data were not collected for any of the trials.

Statistical analysis. SAS (version 9.4; SAS Institute, Cary, NC, U.S.A.) was used to conduct Levene's test of homogeneity to determine whether incidence data from trials conducted within the same year could be combined. The variances of trials conducted within the same year were not homogeneous for all years (2011 through 2014); therefore, data could not be combined. This was likely due to differences in disease development at each trial. Consequently, data were analyzed separately for each location using SAS; a general linear model procedure was used to conduct an analysis of variance (ANOVA). When analyses were significant at the 95% level of confidence, Fisher's protected least significant difference test at $\alpha = 0.05$ was used to separate treatment means.

A robust statistical analysis for all fungicide treatments across all location-years was not completed because the experimental protocol changed yearly. However, three fungicide treatments (OXA at 9.37 μg a.i. seed⁻¹, OXA at 18.75 μg a.i. seed⁻¹, and AZO + FLU + MEF at 100.0 + 2.5 + 29.0 μg a.i. seed⁻¹) were evaluated in eight location-years and a combined analysis using a generalized linear mixed model (PROC GLIMMIX) was conducted in SAS. Fungicide treatment was treated as a fixed effect. Random effects included location-year and the nested variable of replication within location-year. The dependent variable disease control [1 - (incidence value of fungicide treatment/incidence value of nontreated control)] was used to evaluate fungicide efficacy. Least squares disease control means were constructed for each fungicide treatment and pairwise comparisons were used to make statistical inferences.

Results

In 2011, three trials were conducted: one each at Fargo, Thompson, and Carrington (Table 2). In total, 10 treatments were evaluated, including five OXA treatments (at rates of 0.45, 1.8, 7.25, 29.0, and 116.0 μg a.i. seed⁻¹), MEF (at 29.0 μg a.i. seed⁻¹), AZO (at 50.0 μg

Table 2. Effects of fungicide treatments on percent sunflower downy mildew incidence at several growth stages in three North Dakota locations in 2011

Fungicide ^b	Rate ^c	Incidence (%) ^a							
		Carrington 2011			Fargo 2011			Thompson 2011	
		VE-V4	V8-V12	V14-R1	VE-V4	V8-V12	V14-R1	VE-V4	V8-V12
OXA	0.45	30.9	15.3	29.1	33.0	58.2	38.5	27.6	30.9
OXA	1.8	10.4	13.7	18.3	11.2	19.8	15.9	10.9	11.8
OXA	7.25	3.0	3.0	10.8	4.6	11.1	8.0	1.7	2.5
OXA	29	2.9	4.2	9.5	0.0	0.6	1.3	0.8	0.8
OXA	116	1.9	1.1	8.3	0.0	0.6	3.5	0.0	0.9
MEF	29	59.5	49.3	52.8	60.9	74.1	48.7	35.3	38.2
AZO	50	34.2	15.8	31.0	45.8	64.0	41.6	24.7	25.4
FEN	188.9	10.5	12.1	23.1	3.4	9.1	11.5	19.8	22.8
NI NTC	...	0.9	5.4	7.6	73.4	82.1	72.2	0.0	0.0
NTC	...	65.7	44.0	39.0	79.9	83.2	55.2	36.1	36.8
LSD ^d	...	7.0	10.9	12.7	21.7	17.4	22.2	9.4	9.1

^a Percent incidence was calculated as incidence (%) = (number of systemically infected plants/total number of plants) $\times$ 100.

^b All treatments had thiamethoxam (active ingredient [a.i.]) applied at a rate of 250 μg a.i. seed⁻¹. OXA = oxathiapiprolin; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = noninoculated, nontreated control; NTC = nontreated control; and LSD = least significant difference ($\alpha = 0.05$).

^c Micrograms of active ingredient per seed.

^d Incidence from all treatments at all ratings and locations was significant ($P < 0.0001$).

a.i. seed⁻¹), FEN (at 188.9 µg a.i. seed⁻¹), NI NTC, and NTC. All three trials had measurable disease pressure. The incidence values of the NTC at the first evaluation rating for Carrington, Fargo, and Thompson, were 65.7, 79.9, and 36.1%, respectively. ANOVA indicated that fungicide treatments had a significant effect on incidence for all ratings at all locations ($P < 0.0001$). All treatments had lower incidence than the NTC in at least one location, except MEF. For all trials, the four highest rates of OXA (at 1.8, 7.25, 29.0, and 116.0 µg a.i. seed⁻¹) had lower incidence than the NTC and MEF. The three highest rates of OXA (at 7.25, 29.0, and 116.0 µg a.i. seed⁻¹) had the same incidence values, and all had lower incidence values than the AZO standard. Additionally, the three highest OXA rates had lower incidence levels than FEN at the Carrington and Thompson locations. Overall, the three highest rates of OXA consistently had the lowest incidence levels for all three trials. No phytotoxicity was observed in any of the treatments containing fungicide.

In 2012, four trials were conducted: two at Fargo and one each at Thompson and Carrington (Table 3). In total, nine treatments were tested, two of which were treatments with a combination of three fungicides. The treatments included four OXA treatments (at rates of

4.69, 9.37, 18.75, and 37.5 µg a.i. seed⁻¹), OXA + FLU + MEF (9.37 + 2.5 + 29.0 µg a.i. seed⁻¹), AZO + FLU + MEF (100.0 + 2.5 + 29.0 µg a.i. seed⁻¹), FEN (188.9 µg a.i. seed⁻¹), NI NTC, and NTC. Downy mildew developed in both the Carrington and Thompson locations but disease failed to develop in both Fargo trials (data not presented). The disease pressure was higher at Thompson than at Carrington, with incidence values of the NTC at the first rating of 62.9 and 11.7%, respectively. According to the ANOVAs, fungicide treatments had a significant effect on incidence for both ratings at the Thompson ($P < 0.0001$) and Carrington ($P = 0.0301$ and $P = 0.112$) locations. For the Thompson location, all fungicide treatments had lower incidence than the NTC. All treatments containing OXA had statistically lower incidence than all other treatments at the first evaluation, and all other treatments but FEN at the second evaluation. Low disease pressure at the Carrington location resulted in all seven treatments containing a fungicide performing equally, and all treatments containing OXA and FEN resulted in lower incidence than the NTC. No phytotoxicity was observed in any of the treatments containing fungicides.

In 2013, four trials were conducted: two at Fargo and one each at Thompson and Carrington (Table 4). The same treatments from 2012 were used in the 2013 trials, except that the 4.69-µg a.i. seed⁻¹ rate of OXA was not tested. All four trials had measurable disease pressure and were among the highest at all location-years in the study (Supplementary Fig. S2). The incidence values of the NTC at the first rating for Carrington, Fargo 1, Fargo 2, and Thompson were 67.1, 91.5, 45.0, and 58.0%, respectively. All fungicide treatments had a significant effect on the incidence for all ratings at all four locations ($P < 0.0001$). All treatments containing a fungicide had lower disease incidence compared with the NTC. All treatments containing OXA or FEN had incidence values that were the same but lower than the AZO + FLU + MEF treatment. Overall, all treatments performed consistently at each of the four locations. No phytotoxicity was observed in any of the treatments.

In 2014, three trials were conducted: one each at Fargo, Thompson, and Carrington (Table 5). The number of fungicide treatments tested was reduced to three and included two OXA treatments (9.37 and 18.75 µg a.i. seed⁻¹) and AZO + FLU + MEF (100.0 + 2.5 + 29.0 µg a.i. seed⁻¹). Measurable disease pressure developed at both the Fargo and Carrington locations but disease failed to develop in Thompson (data not presented). The disease incidence for the first evaluation was higher at Carrington than at Fargo, with incidence values of the NTC at the first rating of 83.4 and 36.5%, respectively. Fungicide treatments had a significant effect on incidence for all ratings at both locations at the 95% level of confidence. In Fargo, all fungicide treatments had significantly lower incidence levels than the NTC. Both rates of OXA had lower incidence levels than the AZO + FLU + MEF combination treatment. In Carrington, all

Table 3. Effect of fungicide treatments on percent sunflower downy mildew incidence at two growth stages in two North Dakota locations in 2012

Fungicide ^b	Rate ^c	Incidence (%) ^a			
		Carrington 2012		Thompson 2012	
		VE-V4	V8-V12	VE-V4	V8-V12
OXA	4.69	3.9	5.3	18.7	16.2
OXA	9.37	2.7	2.7	12.8	10.4
OXA	18.75	2.4	2.8	19.9	18.4
OXA	37.5	1.6	1.0	8.4	7.3
OXA + FLU + MEF	9.37 + 2.5 + 29	1.4	1.4	7.2	4.5
AZO + FLU + MEF	100 + 2.5 + 29	6.3	6.5	40.1	37.7
FEN	188.9	1.9	2.5	31.1	25.3
NI NTC	...	0.0	0.0	1.7	1.3
NTC	...	11.7	11.8	62.9	54.9
LSD ($\alpha = 0.05$)	...	6.3	5.8	7.1	7.7
P	...	0.0301	0.0112	<0.0001	<0.0001

^a Percent incidence was calculated as incidence (%) = (number of systemically infected plants/total number of plants) × 100.

^b All treatments had thiamethoxam (active ingredient [a.i.]) applied at a rate of 250 µg a.i. seed⁻¹. OXA = oxathiapiprolin; FLU = fludioxonil; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = noninoculated, nontreated control; NTC = nontreated control; and LSD = least significant difference.

^c Micrograms of active ingredient per seed.

Table 4. Effects of fungicide treatments on percent sunflower downy mildew incidence at three growth stages in four North Dakota locations in 2013

Fungicide ^b	Rate ^c	Incidence (%) ^a											
		Carrington 2013			Fargo 1 2013			Fargo 2 2013			Thompson 2013		
		VE-V4	V8-V12	V14-R1	VE-V4	V8-V12	V14-R1	VE-V4	V8-V12	V14-R1	VE-V4	V8-V12	V14-R1
OXA	9.37	4.0	3.8	3.1	0.0	0.4	2.0	0.0	0.0	0.0	0.7	3.0	3.5
OXA	18.75	1.9	2.4	2.2	0.0	0.0	0.4	0.0	1.1	1.1	1.2	3.5	3.5
OXA	37.5	0.0	0.0	0.0	0.4	0.0	0.8	2.1	2.2	0.0	0.7	1.7	1.1
OXA + FLU + MEF	9.37 + 2.5 + 29	0.8	0.6	0.5	0.0	0.0	1.2	0.8	0.8	0.0	0.3	2.2	2.6
AZO + FLU + MEF	100 + 2.5 + 29	20.0	23.3	18.7	38.9	54.3	51.4	28.4	32.2	27.8	12.8	23.5	27.5
FEN	188.9	1.3	1.3	0.7	0.4	1.2	3.1	1.8	5.3	6.1	0.4	2.4	3.3
NI NTC	...	67.7	67.3	62.2	60.9	60.9	63.7	1.8	1.9	0.0	8.5	9.6	8.2
NTC	...	67.3	68.5	65.0	91.5	87.3	87.6	45.0	38.7	41.2	58.0	55.8	52.3
LSD ^d	...	5.3	7.0	6.7	14.5	14.9	16.9	7.2	8.9	10.9	7.3	8.4	9.5

^a Percent incidence was calculated as incidence (%) = (number of systemically infected plants/total number of plants) × 100.

^b All treatments had thiamethoxam (active ingredient [a.i.]) applied at a rate of 250 µg a.i. seed⁻¹. OXA = oxathiapiprolin; FLU = fludioxonil; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = noninoculated, nontreated control; NTC = nontreated control; and LSD = least significant difference ($\alpha = 0.05$).

^c Micrograms of active ingredient per seed.

^d Incidence from all treatments at all ratings and locations was significant ($P < 0.0001$).

treatments with fungicides had lower incidence levels than the NTC in the first rating ($P < 0.0001$). A notable increase in disease pressure was observed in the second rating, although all treatments with fungicides still had lower incidence levels than the NTC ($P < 0.0001$). At the third rating, only the two OXA treatments had lower incidence than the NTC ($P = 0.0004$). Additionally, both treatments of OXA had lower incidence levels than the AZO + FLU + MEF treatment. No phytotoxicity was observed in any of the treatments containing fungicides.

In 2015, field trials were planted at Fargo, Thompson, and Carrington, where six fungicide treatments were tested. Three treatments were combinations of two or three fungicides, including ACI, which hadn't been included in previous year tests. The fungicide treatments were two OXA treatments (at rates of 9.37 and 18.75 $\mu\text{g a.i. seed}^{-1}$), ACI (25.0 $\mu\text{g a.i. seed}^{-1}$), OXA + ACI (9.37 + 25.0 $\mu\text{g a.i. seed}^{-1}$), OXA + ACI (18.75 + 25.0 $\mu\text{g a.i. seed}^{-1}$), and AZO + FLU + MEF (100 + 2.5 + 29.0 $\mu\text{g a.i. seed}^{-1}$). No measurable disease pressure developed at any of the three locations (data not presented). No phytotoxicity was observed in any of the treatments containing fungicides.

Efficacy of three treatments evaluated in multiple locations from 2012 to 2014 were calculated as percent disease control (Fig. 1). Percent disease control of OXA treatments at two rates (9.37 $\mu\text{g a.i. seed}^{-1}$ and 18.75 $\mu\text{g a.i. seed}^{-1}$) was the same, and statistically greater than the AZO + FLU + MEF (100.0 + 2.5 + 29.0 $\mu\text{g a.i. seed}^{-1}$) treatment ($P < 0.0001$).

Discussion

The results of this study demonstrate that the new fungicide compound, OXA, significantly and consistently reduced downy mildew incidence under a wide range of disease pressure. During this study, the rate range of OXA tested was narrowed from 0.45 to 116.0 $\mu\text{g a.i. seed}^{-1}$ to an optimal effective rate of 9.37 to 18.75 $\mu\text{g a.i. seed}^{-1}$. Within the optimum range, OXA is more effective than any other fungicide currently available to sunflower producers in the United States. Throughout this study, OXA at rates of both 9.37 and 18.75 $\mu\text{g a.i. seed}^{-1}$ provided 91 and 88% disease control, respectively, whereas AZO, the market standard, provided only 43% disease control. Additionally, OXA outperformed AZO (solo and in combination) in 10 of the 11 trials with measurable disease pressure. The only exception was in Carrington 2012, where OXA performed the same as the AZO treatment. This was likely an artifact of low disease pressure in the Carrington 2012 location, where the incidence value observed for the NTC was 11.7%, which was the lowest value for an NTC in all 11 trials where disease developed. Additionally, sunflower treated with OXA showed no symptoms of phytotoxicity in any of the 17 trial locations, even when applied at the highest rate of 116.0 $\mu\text{g a.i. seed}^{-1}$.

Within the optimal OXA rate range, incidence remained consistent across all rating dates in all trials, with the exception of Carrington 2014. In that trial, downy mildew incidence over the three ratings increased from 1.8% to 21.6 to 30.7% with seed treated with OXA at 9.37 $\mu\text{g a.i. seed}^{-1}$, and from 1.7% to 17.8 to 40.5% with seed treated

with OXA at 18.75 $\mu\text{g a.i. seed}^{-1}$. This may be explained by frequent measurable rainfall events occurring several weeks after planting, which could facilitate infection from natural inoculum or (rarely) secondary systemic infection as the seed treatment is losing residual efficacy. Between planting and VE-V4 at the Carrington location, measurable rain occurred on 6 days and totaled 16 mm; between VE-V4 and V8-V12, measurable rain occurred on 9 days and totaled 64 mm; and between V8 to V12 and V14 to R1, measurable rainfall occurred on 9 days and totaled 41 mm (data not presented). During those same time periods in Fargo 2014, days of measurable rain occurred on 12 (totaling 128 mm), 2 (totaling 15 mm), and 2 (totaling 16 mm) days. Although increasing levels of disease within the optimal rate range of OXA were only observed once in this study, a reduced level of disease control may be expected during a prolonged environment favorable for infection.

OXA is the first member of the piperidinyl thiazole isoxazoline class of fungicides, a new class of fungicides discovered and developed by DuPont (Pasteris et al. 2016). It targets organisms' oxysterol-binding complex and has been classified within the FRAC group 49 (Pasteris et al. 2016). OXA's mode of action is novel and, therefore, is being evaluated in many other crops to determine its efficacy on other economically important pathogens. Efficacy has been demonstrated on multiple oomycete pathogens on diverse crops, ranging from pod rot of lima bean caused by *Phytophthora capsici* Leonian, downy mildew in basil caused by *Peronospora belbahrii* Thines, and black shank on tobacco caused by *Phytophthora nicotianae* Breda de Haan (Ji et al. 2014; Kness et al. 2016; Patel et al. 2015). Additionally, OXA has shown efficacy when applied as a seed

Fig. 1. Percent downy mildew control ($P > 0.0001$) of three sunflower seed treatments in combined analysis of eight location-years, where; OXA 1 = active ingredient (a.i.) of oxathiapiprolin (OXA) at 9.37 $\mu\text{g a.i. seed}^{-1}$, OXA 2 = 18.75 $\mu\text{g a.i. seed}^{-1}$ oxathiapiprolin, and AZO + FLU + MEF = azoxystrobin, fludioxonil, and mefenoxam, respectively, at 100 + 2.5 + 29.0 $\mu\text{g a.i. seed}^{-1}$.

Table 5. Effect of fungicide treatment on percent sunflower downy mildew incidence at three growth stages in two North Dakota locations in 2014

Fungicide ^b	Rate ^c	Incidence (%) ^a					
		Carrington 2014			Fargo 2014		
		VE-V4	V8-V12	V14-R1	VE-V4	V8-V12	V14-R1
OXA	9.37	1.8	21.6	30.7	1.2	1.9	1.8
OXA	18.75	1.7	17.8	40.5	1.4	1.4	1.4
AZO + FLU + MEF	100 + 2.5 + 29	21.9	62.9	59.6	9.7	22.1	24.7
NI NTC	...	0.0	13.8	26.7	26.8	34.0	25.0
NTC	...	83.4	90.3	72.2	36.5	49.8	44.1
LSD ($\alpha = 0.05$)	...	8.9	8.7	21.3	16.0	18.8	15.7
P	...	<0.0001	<0.0001	0.0009	0.0004	<0.0001	<0.0001

^a Percent incidence was calculated as incidence (%) = (number of systemically infected plants/total number of plants) $\times$ 100.

^b All treatments had thiamethoxam (active ingredient [a.i.]) applied at a rate of 250 $\mu\text{g a.i. seed}^{-1}$. OXA = oxathiapiprolin; AZO = azoxystrobin; FLU = fludioxonil; MEF = mefenoxam; NI NTC = noninoculated, nontreated control; NTC = nontreated control; and LSD = least significant difference.

^c Micrograms of active ingredient per seed.

treatment or foliar spray (Ji et al. 2014; Kness et al. 2016; Patel et al. 2015). Efficacy and flexibility of application suggest that OXA could be a useful disease management tool in many oomycete pathosystems.

The results of this current study may help explain why incidence of downy mildew remains high in North Dakota (Gilley et al. 2015, 2016), even though nearly all sunflower growers use a fungicide seed treatment to manage the disease. Prior to the availability of OXA, AZO was considered the market standard for seed treatment fungicides. In our study, when AZO was applied alone, it was often overwhelmed by downy mildew in high disease pressure situations. Additionally, even when the highest rate of 100 µg a.i. seed⁻¹ was applied, disease incidence was only moderately (although statistically) reduced when compared with the NTC. This insufficient efficacy is well known to growers because of the ease of downy mildew identification (even from a distance). Consequently, it is likely that sunflower growers will quickly adopt OXA into their management programs, if they have not done so already.

The development of fungicide resistance by *Plasmopara halstedii* is a concern, and fungicide resistance should be considered as OXA is made available to growers. The pathogen is highly variable and notorious for quickly overcoming both fungicides and resistance genes, especially when only one of these management strategies is utilized (Gulya et al. 1999, 2011, 2013; Markell et al. 2016). AZO was labeled in 2003, was adopted by growers very quickly, and is still currently being heavily relied on by many growers (Gulya et al. 2013). This is placing a heavy selection pressure on the *P. halstedii* population. Evidence of insensitivity to AZO in the *P. halstedii* population in the Northern Great Plains was not found when 42 *P. halstedii* isolates collected in 2014 and 2015 were tested (Gilley 2017). However, development of insensitivity to the fungicide in the future is possible with continued use of AZO. Resistance to the single-site-of-action QoI fungicides such as AZO has been well documented in other crops (Pasche et al. 2004; Wise et al. 2009; Wong and Wilcox 2000). OXA targets a different site of action than metalaxyl (FRAC 4), MEF (FRAC 4), and AZO (FRAC 11), which means that OXA will be able to effectively manage *P. halstedii* populations that are resistant to metalaxyl and MEF or which may become resistant to AZO in the future. It also means that a viable resistance management strategy may be to rotate or blend OXA with AZO. Rotation of FRAC groups is critical for fungicide longevity, and the presumed availability of two products (OXA and AZO) could conceivably extend both of their effective lifespans.

Interestingly, ACI, which is a compound believed to induce resistance, has also been tested in sunflower and was found to have efficacy on downy mildew. In 2015, our study attempted to test this compound in combination with OXA but disease did not develop at any of the three locations. However, other research conducted in a 2012 field trial found that ACI significantly lowered downy mildew incidence when compared with both the NTC and the treatment containing AZO (Humann et al. 2014). Efficacy is further supported by a separate study in 2015 that showed that, when ACI was applied alone, it resulted in significantly lower disease incidence compared with the NTC in three separate field trials (Humann et al. 2016a). These results suggest that ACI could be another compound of a different mode of action that could complement either OXA or AZO if applied in combination.

To keep OXA viable for many years to come, a few good management practices need to be followed. The first is to not rely solely on fungicides for management of downy mildew. This can be accomplished by pairing genetically resistant varieties with the fungicidal seed treatments. This practice may help prolong the life of the fungicide and help slow selection of virulent races. The second good management practice would be to apply OXA with another fungicide compound within a different FRAC group, such as AZO, or with a compound that works entirely differently, such as ACI.

The discovery, development, and availability of OXA presents a very important advance in downy mildew management for sunflower growers in the United States. OXA is now the new market standard for seed treatment efficacy and appears to be at least as effective as

metalaxyl and MEF were before the development of fungicide resistance by *P. halstedii*. Possibly more important, multiple efficacious fungicides with different modes of action have not been available to United States sunflower growers simultaneously before. With potential widespread adoption of OXA and a careful resistance management plan, it is possible that the use of OXA will help mitigate the yield loss from downy mildew for many years to come.

Acknowledgments

We thank Mycogen Seeds for their generous contribution of seed for pathogen increase and field trial establishment; M. Gilley for contributing *P. halstedii* isolates; the staff at the North Dakota Agricultural Experiment station in Fargo, ND, the Carrington Research Extension Center, and the DuPont Agricultural Research Farm in Thompson, ND for their support, including B. Kraft, S. Kallis, M. Schaefer, A. Streifel, and the many undergraduate students who helped with the experiments; and M. McMullen for critical review of this manuscript.

Literature Cited

- Berglund, D. R. 2007. Sunflower production guide. N. D. State Univ. Coop. Ext. Serv. Publ. A-1331. Fargo, ND, U.S.A.
- Brent, K. J., and Hollomon, D. W. 2007. Fungicide Resistance: The Assessment of Risk, 2nd ed. Fungicide Resistance Action Committee, Brussels, Belgium.
- Friskop, A., Markell, S., and Gulya, T. 2009. Downy mildew of sunflower. N. D. State Univ. Coop. Ext. Serv. Publ. PP-1402. Fargo, ND, U.S.A.
- Friskop, A., Markell, S. G., Khan, M., Gudmestad, N., and Secor, G. 2017. 2018 North Dakota Field crop plant disease management guide. N. D. State Univ. Coop. Ext. Serv. Publ. PP-622. Fargo, ND, U.S.A.
- Gilley, M., Markell, S., Gulya, T., and Misar, C. 2016. Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in sunflowers. In: Proc. 38th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND, U.S.A.
- Gilley, M. A. 2017. Downy mildew of sunflower: Establishment of baseline sensitivity to azoxystrobin and monitoring for the development of fungicide resistance and *Plasmopara halstedii* virulence phenotype changes. Masters thesis, North Dakota State University.
- Gilley, M. A., Markell, S. G., Gulya, T. J., and Misar, C. G. 2015. Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in sunflowers in 2014. In: Proc. 37th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND, U.S.A.
- Gulya, T., Draper, M., Harbour, J., Holen, C., Knodel, J., Lamey, A., and Mason, P. 1999. Metalaxyl resistance in sunflower downy mildew in North America. In: Proc. 21st Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND, U.S.A.
- Gulya, T., Kandel, H., McMullen, M., Knodel, J., Berglund, D., Mathew, F., Lamey, H. A., Nowatzki, J., and Markell, S. 2013. Prevalence and incidence of sunflower downy mildew in North Dakota between 2001 and 2011. Plant Health Prog. doi:10.1094/PHP-2013-0522-01-RS.
- Gulya, T., and McMullen, M. 2012. New virulent races of downy mildew: What was new in 2011. In: Proc. 34th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND, U.S.A.
- Gulya, T., Rashid, K. Y., and Masirevic, S. M. 1997. Sunflower diseases. Pages 263-379 in: Sunflower Technology and Production. A. A. Schneiter, ed. American Society of Agronomy, Madison, WI, U.S.A.
- Gulya, T. J. 1996. Everything you should know about downy mildew testing but were afraid to ask. In: Proc. 18th Sunflower Res. Workshop. National Sunflower Association, Mandan, ND, U.S.A.
- Gulya, T. J., Markell, S. G., McMullen, M. V., Harveson, R. M., and Osborne, L. E. 2011. Emergence of new virulent races of *Plasmopara halstedii* inciting downy mildew on sunflower in the United States. (Abstr.) Phytopathology 101:S2.3.
- Humann, R., Belles, D., Gilley, M., Halvorson, J., and Markell, S. 2016a. Evaluation of fungicide seed treatment efficacy against three *Plasmopara halstedii* (downy mildew) races in inoculated field trials. In: Proc. 38th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND, U.S.A.
- Humann, R., Gulya, T. J., and Markell, S. G. 2016b. Downy mildew. Pages 18-20 in: Compendium of Sunflower Diseases. R. M. Harveson, S. G. Markell, C. C. Block, and T. J. Gulya, eds. American Phytopathological Society, St. Paul, MN, U.S.A.
- Humann, R., Wunsch, M., Gulya, T., Kraft, B., Schaefer, M., Friskop, A., and Markell, S. 2014. Evaluation of sunflower seed treatment efficacy for downy mildew (*Plasmopara halstedii*) in North Dakota, 2012. Plant Dis. Manage. Rep. 8:ST026.
- Ji, P., Csinos, A. S., Hickman, L. L., and Hargett, U. 2014. Efficacy and application methods of oxathiapiprolin for management of black shank on tobacco. Plant Dis. 98:1551-1554.
- Kness, A. A., Johnson, G., Everts, K. L., Evans, T. A., Donofrio, N. M., and Ernest, E. G. 2016. Managing pod rot of lima bean caused by *Phytophthora capsici* with fungicides in the Mid-Atlantic region. Plant Health Prog. 17: 130-132.

- Knodel, J. J., Beauzay, P., and Boetel, M. 2014. 2015 North Dakota field crop insect management guide. N. D. State Univ. Coop. Ext. Serv. Publ. E-1143. Fargo, ND, U.S.A.
- Markell, S. G., Harveson, R. M., Block, C. C., and Gulya, T. J. 2015. Sunflower Diseases. Pages 93-128 in: Sunflower: Chemistry, Production, Processing and Utilization. E. Martínez-Force, N. T. Dunford, and J. J. Salas, eds. AOCS Press, Urbana, IL, U.S.A.
- Markell, S. G., Humann, R. H., Gilley, M., and Gulya, T. J. 2016. Downy mildew pathogen: *Plasmopara halstedii*. Pages 115-117 in: Compendium of Sunflower Diseases. R. M. Harveson, S. G. Markell, C. C. Block, and T. J. Gulya, eds. American Phytopathological Society, St. Paul, MN, U.S.A.
- Pasche, J. S., Wharam, C. M., and Gudmestad, N. C. 2004. Shift in sensitivity of *Alternaria solani* in response to QoI fungicides. Plant Dis. 88:181-187.
- Pasteris, R. J., Hanagan, M. A., Bisaha, J. J., Finkelstein, B. L., Hoffman, L. E., Gregory, V., Andreassi, J. L., Sweigard, J. A., Klyaschitsky, B. A., Henry, Y. T., and Berger, R. A. 2016. Discovery of oxathiapiprolin, a new oomycete fungicide that targets an oxysterol binding protein. Bioorg. Med. Chem. 24: 354-361.
- Patel, J. S., Costa de Novaes, M. L., and Zhang, S. 2015. Evaluation of the new compound oxathiapiprolin for control of downy mildew in basil. Plant Health Prog. 16:165-172.
- Sackston, W. E. 1981. Downy mildew of sunflower. Pages 545-576 in: The Downy Mildews. D. M. Spencer, ed. Academic Press, London, England.
- Tourvieille de Labrouhe, D., Gulya, T. J., Masirevic, S., Penaud, A., Rashid, K., and Viranyi, F. 2000. New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). In: Proc. 15th Int. Sunflower Conf. International Sunflower Association, Toulouse, France.
- Wise, K. A., Bradley, C. A., Pasche, J. S., and Gudmestad, N. C. 2009. Resistance of QoI fungicides in *Ascochyta rabiei* from chickpea in the Northern Great Plain. Plant Dis. 93:528-536.
- Wong, F. P., and Wilcox, W. F. 2000. Distribution of baseline sensitivities to azoxystrobin among isolates of *Plasmopara viticola*. Plant Dis. 84:275-281.