Sclerotinia head rot of sunflower: Improving the methods used to screen sunflowers for resistance and prospects for management with fungicides.

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Key outcomes:

In 2012, field trials associated with this project resulted in (1) the identification of commercial sunflower hybrids and breeding lines with elevated resistance to Sclerotinia head rot, (2) preliminary recommendations for improving the methods used to screen sunflowers for resistance to Sclerotinia head rot, and (3) a preliminary assessment of the prospects of using fungicides to manage this disease.

Introduction:

Sclerotinia head rot, caused by the fungal pathogen *Sclerotinia sclerotiorum*, is an economically important disease of sunflowers, reducing both seed yield and quality. The disease can cause complete losses for producers and presents supply and quality challenges for processors.

The goal of this multi-year project is to facilitate the development of commercial sunflower hybrids with elevated resistance to Sclerotinia head rot, to improve the screening methodologies used to evaluate the resistance of sunflowers to this disease, and to evaluate the efficacy of fungicides for control of Sclerotinia head rot.

Because resistance to Sclerotinia head rot is partial and quantitative (Bert et al. 2004) and because plants are too large to efficiently screen in growth chambers or the greenhouse, evaluations of Sclerotinia head rot resistance must be conducted in the field. Sclerotinia head rot is a frequent and severe problem in North Dakota, but the natural conditions that result in high levels of head rot incidence do not occur in every location every year. To promote high rates of infection and to maintain consistent disease pressure across hybrids and breeding lines differing in flowering times, screening methodologies have been developed in which microsprinkler misting systems are utilized to maintain high levels of moisture on sunflower heads during and after the critical bloom period and sunflowers are inoculated with laboratory-grown ascospores of *S. sclerotiorum* (Henson et al. 2001, 2003, 2004a, 2004b, 2005a and 2005b; Gulya & Schatz 2006).

When the same hybrids are screened for Sclerotinia head rot resistance across multiple locations, consistent results are not always obtained. The inoculation strategies utilized by different researchers conducting Sclerotinia head rot nurseries differ; some researchers inoculate sunflowers over multiple dates such that all plants in all entries are inoculated at the same growth stage, and others inoculate all plants across all entries on a limited number of dates such that late-maturing sunflowers are often inoculated at early bloom and early-maturing sunflowers are often

inoculated at late bloom or shortly after bloom has concluded. It is unclear whether the differences in inoculation strategies might be responsible for differences in results across screening nurseries and, if so, which inoculation strategies might result in unbiased results.

Sunflowers are known to be susceptible to Sclerotinia head rot during bloom, but it is unclear how susceptible they remain to Sclerotinia head rot after bloom. The susceptibility of sunflowers to Sclerotinia head rot during the bloom period is well documented (e.g., Bert et al. 2004, Yue et al. 2008), and sunflowers appear to be more susceptible to Sclerotinia head rot at mid-to late-bloom than at early bloom (Rashid and Seiler 2006). Field observations suggest that sunflowers may be susceptible to Sclerotinia head rot after bloom; in North Dakota, severe outbreaks of Sclerotinia head rot have been observed shortly after periods of cool, moist weather on sunflowers that completed flowering several weeks earlier and had previously exhibited little or no head rot (T. Gregoire, retired NDSU extension specialist, Tom Gulya, USDA-ARS sunflower pathologist; *personal communication*). The absence of prior disease symptoms strongly suggests that infection can occur after flowering, but these observations have not been empirically confirmed. It remains unclear whether sunflowers are equally susceptible to Sclerotinia head rot during and after bloom. Understanding the relative susceptibility of sunflowers to Sclerotinia during and after bloom is critical for (1) identifying whether inoculating some sunflower hybrids during bloom and others immediately after bloom might bias Sclerotinia head rot screening results and (2) assessing how many applications might be necessary to protect sunflowers against this disease using foliar fungicides.

The potential for managing Sclerotinia head rot on sunflowers with fungicides remains poorly understood. Fungicides are broadly employed for Sclerotinia control on other broadleaf crops (Knodel et al. 2006), but very little research has been conducted evaluating fungicides for control of Sclerotinia head rot on sunflowers. Applications of Endura 70WG (boscalid) have resulted in significant reductions in Sclerotinia head rot in field trials conducted in Manitoba (Rashid 2005) and in Langdon, ND (S. Halley 2008, *unpublished*), but fungicide efficacy testing against this disease has been limited. Additional testing is needed before recommendations can be made.

Methods:

1. *Sclerotinia head rot resistance screening nurseries.*

Locations of trials: NDSU Carrington Research Extension Center, Carrington, ND (47.5083,-99.1314); Oakes Irrigation Research Site of the NDSU Carrington Research Extension Center, Oakes, ND (46.0676,-98.0917); NDSU Langdon Research Extension Center, Langdon, ND (48.7548,-98.3385).

Randomized complete block design

Replicates: In a 31-entry trial conducted in Carrington, six replicates were conducted. In 25-entry trials conducted in Carrington, Langdon and Oakes, four replicates were conducted.

Row spacing: 30 inches / **Rows per plot:** 1

Seeded plot size: one row, 35 feet long (Carrington trials); two rows, 15 feet long (Langdon); one row, 20 feet long (Oakes)

Final plot size after alleys were cut: one row, 29 feet long (Carrington trials); two rows, 11 feet long (Langdon); one row, 17 feet long (Oakes)

Previous crop: spring wheat (Carrington), spring wheat (Langdon), spring wheat (Oakes)

Planting date: June 5, 2012 (Carrington); May 31, 2012 (Oakes); May 14, 2012 (Langdon)

Seeding rate: 2.8 seeds/linear foot of row = 49,000 seeds/ac

Final plant population: 1 plant every 10 inches of row = 21,000 plants/ac

** The final plant population was achieved by manually thinning the sunflowers at the V2 to V4 growth stage (two to four true leaves).

Irrigation: To promote disease development, overhead irrigation was applied from bloom initiation until approximately the R7 growth stage. Overhead irrigation was applied through microsprinklers established on a grid. The microsprinklers were on PVC risers that extended above the sunflower canopy.

Inoculation methods:

** Spore solutions were prepared by adding laboratory-grown ascospores of Sclerotnia sclerotiorum to water and adding a few of Tween 20. The spore solutions were adjusted such that hand-held spray bottles delivered 15,000 spores per spray, and inoculations were conducted by applying three squirts of the spray bottle (15,000 spores) to the front of each head.

** When the first heads reached R5.2 (20% of the head area flowering or already flowered), all heads that were at growth stage R5.2 or higher were inoculated, and a dot of spray paint was placed on one of the upper leaves indicating that the plant has been inoculated.

** Two to three days later, every head that was inoculated at the first inoculation date was inoculated again, and a second spray paint dot was applied to the previously marked leaves. Spores were also applied to all plants that had reached or passed the R5.2 growth stage but had not been previously inoculated, and these plants were marked with spray paint. This processed continued every one to six days until all plants had been inoculated twice during the R5 growth stage. No plants were inoculated more than twice.

** Inoculations were conducted August 8 (1:00-3:00 pm), August 10 (1:00-3:00 pm), August 15 (1:00 pm - 5:00 pm), August 17 (8:00 am - 6:00 pm), August 20 (10:00 am - 3:00 pm), August 22 (8:30 - 11:30 am), August 27 (10:00 am to 12:00 pm), and August 30 (9:30 am) in Carrington; July 27, July 30, August 1, August 6, August 8, and August 10 in Oakes; Aug. 6, Aug. 8, and Aug. 10 in Langdon.

Disease assessments: Sclerotinia head rot was assesed on at the R9 growth stage (physiological maturity) on October 17 and 19 (Carrington trial #1), October 19 (Carrington trial #2), September 28 (Langdon), and September 10 and 14 (Oakes). Each plant in each row was evaluated on a 0 to 5 scale: 0 = no Sclerotinia head rot, 1 = 1 to 25% of head exhibiting symptoms of Sclerotinia head rot, 2 = 26 to 50% of head exhibiting symptoms of Sclerotinia head rot, 3 = 51 to 75% of head exhibiting symptoms of Sclerotinia head rot, 4 = 76 to 99% of head exhibiting symptoms of Sclerotinia head rot, and 5 = 100% of head exhibiting Sclerotinia head rot. Plants exhibiting damage from sunflower midge were excluded from the analysis.

This trial was not harvested.

Statistical analysis: Data were evaluated with analysis of variance . The assumption of constant variance was assessed by plotting residuals against predicted values, and the assumption of normality was assessed with a normal probability plot. To meet these assumptions, a systematic natural-log transformation [LN(x+1)]was applied to the disease incidence and disease severity index data. Single-degree-of-freedom contrasts were performed for all pairwise comparisons of isolates; to control the Type I error rate at the level of the experiment, the Tukey multiple comparison procedure was employed. Analyses were conducted with replicate and treatment as main factor effects, and they were implemented in PROC GLM of SAS (version 9.2; SAS Institute, Cary, NC).

2. *Inoculation timing experiments.*

Locations of trials: NDSU Carrington Research Extension Center, Carrington, ND (47.508403,-99.132625); Oakes Irrigation Research Site of the NDSU Carrington Research Extension Center, Oakes, ND (46.0676,-98.0917); NDSU Langdon Research Extension Center, Langdon, ND (48.754802,-98.339215).

Experimental design – Carrington and Langdon: Completely randomized split-split-plot design; main factor: post-inoculation environment (bagged vs. unbagged heads); sub-factor: hybrid (susceptible vs. resistant); sub-sub-factor: inoculation treatment (non-inoculated; inoculated at R5, R6, R7, or R8)

Experimental design – Oakes: Completely randomized split-plot design; main factor: hybrid (susceptible vs. resistant); sub-factor: inoculation treatment (non-inoculated; inoculated at R5, R6, or R7)

Replicates: 7 replicates were utilized in Carrington, ND; 6 replicates were used in Langdon, ND; 4 replicates were used in Oakes, ND.

Row spacing: 30 inches / **Rows per plot -** Carrington and Oakes: 1 row per plot; Langdon: 2 rows per plot

Seeded plot size: Carrington: 35 feet long x 2.5 feet (center-to-center); Langdon: 15 feet long x 5 feet (center-to-center); Oakes: 20 feet long x 2.5 feet (center-to-center)

Final plot size after alleys were cut: Carrington: 29 feet long x 2.5 feet (center-to-center); Langdon: 11 feet long x 5 feet (center-to-center); Oakes: 17 feet long x 2.5 feet (center-to-center)

Previous crop - All locations: spring wheat

Planting date - Carrington: June 5, 2012; Langdon: May 14, 2012; Oakes: May 31, 2012

Hybrids: Croplan '343 DMR HO' (resistant check) and Croplan '305 DMR NS' (susceptible check)

Seeding rate: 3.83 seeds/linear foot of row = 60,000 seeds/ac

Final plant population: 1 plant every 10 inches of row = 21,000 plants/ac

** The final plant population was achieved by manually thinning the sunflowers at the V2 to V4 growth stage (two to four true leaves) in Carrington, at the V4 growth stage in Langdon, and at the V4 to V5 growth stage in Oakes.

Irrigation: To promote disease development, overhead irrigation was applied through microsprinklers established on a grid. The microsprinklers were on PVC risers that extended above the sunflower canopy.

Inoculation methods (Carrington):

** Spore solutions were prepared by adding laboroatory-grown ascospores of Sclerotnia sclerotiorum to water and adding a few drops of Tween 20. The spore solutions were adjusted such that hand-held spray bottles delivered 5,000 spores per spray, and inoculations were conducted by applying three squirts of the spray bottle (15,000 spores) to both the front and the back of each head.

** The hybrids used in this study exhibited very similar levels of maturity, and all inoculations were conducted on both hybrids on the same dates.

** R5 inoculations were conducted at the R5.1 to R5.8 growth stage on August 9 at 8:40 to 10:30 pm and August 10 at 8:20 to 9:40 am. All plants exhibiting growth stages outside of this range were eliminated. Bags placed on heads from 8:20 to 10:20 am on Aug. 10, misters were run continuously until 5 pm, then 5 min on 15 min off for 5 days.

** R6 inoculations were conducted at 9:00 to 11:20 pm on August 26 and 8:00 to 10:00 am on August 27. Bags were placed on heads from 1:00 to 5:00 pm on Aug. 27, misters were run continuously until 8:30 pm, then 5 min on 15 off for 5 days.

** R7 inoculations were conducted at 9:00 to 10:45 am on September 13 and 8:00 to 10:00 am on September 14. Bags were placed on heads from 1:00 to 3:30 pm on Sept. 14, misters were run continuously until 8:00 pm and then 5 min on and 15 min for approx. 5 days.

** R8 inoculations were conducted at 8:30 to 9:30 am on Sept. 20 and 8:15 to 9:00 am on Sept. 21. Bags were placed on heads from 10:00 am to 12:00 pm and again from 1:15 to 2:00 pm on Sept. 21, misters were run continuously from 12:00 to 1:00 pm and 2:00 to 5:00 pm, then 5 min on 15 min off for approx. 5 days.

Inoculation methods (Langdon):

** Spore solutions were prepared by adding laboroatory-grown ascospores of Sclerotnia sclerotiorum to water and adding a few drops of Tween 20. The spore solutions were adjusted such that hand-held spray bottles delivered 5,000 spores per spray, and inoculations were conducted by applying three squirts of the spray bottle (15,000 spores) to both the front and the back of each head.

** The hybrids used in this study exhibited very similar levels of maturity, and all inoculations were conducted on both hybrids on the same dates.

** Inoculations were conducted were conducted at the R5 growth stage on July 31 and August 1.

** Inoculations were conducted were conducted at the R6 growth stage on August 8 and 9.

** Inoculations were conducted were conducted at the R7 growth stage on August 27 and 28.

** Inoculations were conducted were conducted at the R8 growth stage on September 4 and 5.

Inoculation methods (Oakes):

** Spore solutions were prepared by adding laboroatory-grown ascospores of Sclerotnia sclerotiorum to water and adding a few drops of Tween 20. The spore solutions were adjusted such that hand-held spray bottles delivered 5,000 spores per spray, and inoculations were conducted by applying three squirts of the spray bottle (15,000 spores) to both the front and the back of each head.

** Croplan 305 was inoculated at the R5 growth stage on July 27 and July 28; Croplan 343 was inoculated at the R5 growth stage on July 30 and 31. Overhead irrigation commenced on July 27 and continued for 5 minutes every half hour or hour, depending on weather conditions, until August 25.

** Both hybrids were inoculated at the R6 growth stage on August 7 and 8.

** Both hybrids were inoculated at the R7 growth stage on August 23 and 24.

Post-inoculation environment (bagged heads treatment) – Carrington: Two sheets of 12 x 16", 38# seed germination paper (Anchor Paper; Hudson, WI) were stapled together to create a bag. Bags were removed from sunflower heads three days later.

Post-inoculation environment (bagged heads treatment) – Langdon: Plastic mesh bags with fine perforations (1 mm-square perforations located 1 mm apart; bag size = 16 in. x 20 in.; Vilutis & Co., Frankfort, IL) were placed over sunflowers shortly after the first inoculation and kept on plants for 4 days. Intense misting was conducted for 4 days after each set of inoculations.

Disease assessments: Carrington - Sclerotinia head rot was assessed on October 16 to 17 (R9 growth stage). Langdon – Sclerotinia head rot was assessed on September 24, 2012 at the R9 growth stage. Oakes – Sclerotinia head rot was assessed on September 14, 2012 at the R9 growth stage. All locations: Plants exhibiting damage from sunflower midge were excluded from the analysis. On all plants not exhibiting midge damage, Sclerotinia head rot was assessed on a 0 to 5 scale: 0 = no Sclerotinia head rot, 1 = 1 to 25% of head exhibiting symptoms of Sclerotinia head rot, 2 = 26 to 50% of head exhibiting symptoms of Sclerotinia head rot, 3 = 51 to 75% of head exhibiting symptoms of Sclerotinia head rot, 4 = 76 to 99% of head exhibiting symptoms of Sclerotinia head rot, and 5 = 100% of head exhibiting Sclerotinia head rot.

These trials were not harvested, and yield was not assessed.

Statistical analysis: Data were evaluated with analysis of variance . The assumption of constant variance was assessed by plotting residuals against predicted values, and the assumption of normality was assessed with a normal probability plot. To meet these assumptions, a systematic natural-log transformation [LN(x+1)]was applied to Carrington disease incidence and severity index data. The other data met model assumptions. Single-degree-of-freedom contrasts were performed for all pairwise comparisons of isolates; to control the Type I error rate at the level of the experiment, the Tukey multiple comparison procedure was employed. Analyses were conducted with all interaction terms included in the model, and they were implemented in PROC GLM of SAS (version 9.2; SAS Institute, Cary, NC).

Results are not reported from the trial conducted in Oakes, ND. Due to the small number of replicates utilized in the trial conducted in Oakes, ND, no significant differences were observed across treatments, and the results were not informative. In future trials, a minimum of six replicates will be utilized.

3. *Fungicide efficacy trials.*

Locations of trials: NDSU Carrington Research Extension Center, Carrington, ND (47.508268,-99.130682); University of Nebraska Panhandle Research Extension Center, Scottsbluff, NE; NDSU Langdon Research Extension Center, Langdon, ND (48.754587,-98.339112).

Experimental design: Completely randomized block design with four replicates.

Hybrid: Pioneer '63N82' (an oilseed type)

Seeded plot size - Carrington: 35 feet long x 10 feet wide; Langdon: 30 ft long x 5 ft wide; Scottsbluff: 30 ft long x 5 ft wide

Harvested plot size – Carrington: approx. 29 feet x 5 feet wide; Langdon: 26 ft long x 5 ft wide; Scottsbluff: 30 ft long x 5 ft wide

Row spacing: 30 inches

Rows per plot – Carrington: treatment plots were four rows wide (fungicides were applied to four rows), but only the center two rows were assessed for Sclerotinia head rot and harvested. Langdon and Scottsbluff: treatment plots were two rows wide (fungicides were applied to two rows), and both rows were harvested.

Non-treated buffer rows – To capture spray drift, one row of sunflowers was established between treatment plots.

Previous crop - Carrington: spring wheat; Langdon: spring wheat; Scottsbluff: dry edible beans

Planting date - Carrington: June 5, 2012; Langdon: May 14, 2012; Scottsbluff: June 5, 2012

Seeding rate: 49,000 seeds/ac

Final plant population: 21,000 plants/ac (achieved by manually thinning sunflowers at the V2 to V4 growth stage in Carrington, at the V4 growth stage in Langdon, and when sunflowers were 2 ft tall in Scottsbluff).

Fungicide applications – Carrington. Application timing A: Aug. 7 at 10:30 am - 12:00 pm; sunflowers at R5.1 to R5.4 growth stage. Wind = 6.4 to 10.4 miles per hour, temperature = 71˚F to 75˚F, relative humidity = 47 to 51%. **Application timing B:** Aug. 21 at 11:00 am to 12:20 pm; sunflowers at R5.8 to R6 growth stage with R5.9-R6 predominant. Wind = 9.2 to 10.4 miles per hour, temperature = 77˚F to 81˚F, relative humidity = 41 to 47 %.

Fungicide application details - Carrington: Fungicides were applied with a 100-inch hand boom equipped with six equally spaced Spraying Systems TeeJet XR 8001VS flat-fan nozzles at a spray volume of 20 gal water/A operated at 35 psi.

Fungicide applications – Langdon. Application A: Aug. 1 at 11:30 am to 1:00 pm; sunflowers at R5.2 to R5.5 growth stage Wind = 1.5 to 3.5 miles per hour out of the Northwest, temperature = 75˚F to 78˚F, relative humidity = 55 to 64%. **Application B:** Aug. 11 at 12:00 pm to 1:30 pm; sunflowers at R6 growth stage. Wind = 2.5 to 4.0 miles per hour, temperature = 72˚F, relative humidity = 52 to 53%.

Fungicide application details - Langdon: Fungicides were applied with a 100-inch hand boom equipped with six equally spaced Spraying Systems TeeJet XR 8001VS flat-fan nozzles at a spray volume of 15 gal water/Acre operated at 35 psi.

Fungicide applications – Scottsbluff. Application A: August 22 at 1:00 pm at the early R5 growth stage (early bloom); no Sclerotinia head rot was present; calm (little wind), temperature = 90˚F, relative humidity = 20%. **Application B:** September 1 at 10:00 am at the R5 (late bloom) to early R6 (bloom completed) growth stage; calm (little wind), temperature = 84˚F, relative humidity = 25%.

Fungicide application details - Scottsbluff: Fungicides were applied in 34.85 gallons of water/ac with a backpack sprayer with a 40-inch hand boom equipped with three equally spaced Spraying Systems TeeJet 8002 flat-fan nozzles operated at 20 psi.

Disease establishment – Carrington:

** Laboratory-produced ascospores of Sclerotinia sclerotiorum were applied Aug. 8 at 9:00-11:00 am (10,000 spores delivered to the front of each head with a hand-held spray bottle), Aug. 10 at 9:00-10:00 am (10,000 spores delivered to the front of each head with a hand-held spray bottle), and Aug. 25 at 3:00-6:15 pm (10,000 spores to the front of each head and 10,000 spores to the back of each head delivered with a hand-held spray bottle). Spore solutions were adjusted to 3,333 spores/ml, and spray bottles were calibrated to deliver 1 ml per squirt.

** To facilitate disease establishment and development, microsprinklers were used to apply water to the trial 5 minutes every 30 minutes from Aug. 8 until mid-September. Microsprinklers were on long risers that extended above the sunflower heads.

Disease establishment – Langdon:

** Laboratory-produced ascospores of Sclerotinia sclerotiorum were applied on August 2, August 6, and August 8 at the R5 growth stage. In each inoculation, 10,000 spores were delivered to the front of each head with a hand-held spray bottle. Spore solutions were adjusted to 3,333 spores/ml, and spray bottles were calibrated to deliver 1 ml per squirt.

** To facilitate disease establishment and development, this trial received overhead irrigation applied via microsprinklers. Microsprinklers were on long risers that extended above the sunflower heads.

Disease establishment – Scottsbluff:

** Laboratory-produced ascospores of Sclerotinia sclerotiorum were applied August 24 and 29 at the R5 growth stage (bloom). In each inoculation, approximately 15,000 spores were delivered to heads with a backpack sprayer.

** To facilitate disease establishment and development, this trial received overhead irrigation applied via microsprinklers. Microsprinklers were on long risers that extended above the sunflower heads.

Sclerotinia head rot ratings: Sclerotinia stem rot incidence and severity were evaluated Oct. 17 at the R9 growth stage (physiological maturity) in Carrington, Sept. 24 at R9 growth stage in Langdon. A 0 to 5 scale was employed: 0 = no Sclerotinia head rot, 1 = Sclerotinia head rot affecting 1 to 25% of the head, 2 = Sclerotinia head rot affecting 26 to 50% of the head, 3 = Sclerotinia head rot affecting 51 to 75% of the head, 4 = Sclerotinia head rot affecting 76 to 99% of the head, 5 = Sclerotinia head rot affecting 100% of head. All plants in each plot were assessed (56 to 70 plants per plot).

Harvest date - Carrington: November 1, 2012; Langdon: October 15, 2012.

Statistical analysis – Carrington: Data were evaluated with analysis of variance . The assumption of constant variance was assessed by plotting residuals against predicted values, and the assumption of normality was assessed with a normal probability plot. All data met model assumptions. Single-degree-of-freedom contrasts were performed for all pairwise comparisons of isolates; to control the Type I error rate at the level of the experiment, the Tukey multiple comparison procedure was employed. Analyses were conducted with replicate and treatment as main factor effects, and they were implemented in PROC GLM of SAS (version 9.2; SAS Institute, Cary, NC).

Statistical analysis – Langdon and Scottsbluff: The disease data did not meet the assumptions of analysis of variance and were analyzed with logistic regression. Single-degree-of-freedom contrasts of all pairwise combinations of treatments were conducted with Wald $\chi 2$ tests, and the Bonferroni multiple comparison procedure was used to control the type I error rate at the level of the experiment. Analyses were implemented in PROC GENMOD of SAS (version 9.2; SAS Institute, Cary, NC). Seed yield and quality data were analyzed with analysis of variance. The assumption of constant variance was assessed by plotting residuals against predicted values, and the assumption of normality was assessed with a normal probability plot. All data met model assumptions. Single-degree-of-freedom contrasts were performed for all pairwise comparisons of isolates; to control the Type I error rate at the level of the experiment, the Tukey multiple comparison procedure was employed. Analyses were conducted with replicate and treatment as main factor effects, and they were implemented in PROC GLM of SAS (version 9.2; SAS Institute, Cary, NC).

Results and conclusions:

1. *Identification of sunflower hybrids with elevated resistance to Sclerotinia head rot.*

Field trials conducted in 2012 resulted in the identification of 10 commercial and 7 experimental sunflower hybrids from private-sector breeders that may be useful for managing Sclerotinia head rot.

- Relative to the most susceptible hybrids, the commercial oilseed hybrids Syngenta '3990 NS/CL/DM' and Seeds 2000 'Cobalt' and the experimental oilseed hybrid Mycogen 'E1013231' exhibited significantly reduced Sclerotinia head rot incidence and/or severity index ($P < 0.05$) in 4 of 4 trials in which they were tested in 2012 (Figures 1 and 2).
- Relative to the most susceptible hybrids, the commercial oilseed hybrids Syngenta 'NX24122', Syngenta 'NX24123' and Croplan '343 DMR HO' (the resistant check) and the

experimental confectionary hybrid Seeds 2000 'X3293' exhibited significant reductions in Sclerotinia head rot incidence and/or severity index ($P < 0.05$) in 3 of 4 trials in which they were tested in 2012 (Figures 1 and 2).

- Relative to the most susceptible hybrids, eight additional hybrids exhibited significant reductions in Sclerotinia incidence and/or severity index ($P < 0.05$) in 2 of 4 trials in which they were tested in 2012 (Figures 1 and 2): the commercial oil hybrids Seeds 2000 'Camaro II' and Seeds 2000 'Cobalt II'; the commercial confectionary hybrid Genosys '12GCF05'; the experimental oil hybrids Mycogen 'E1013231', Genosys 'M12-213R', Genosys 'M12-193R', and Mycogen 'E411501'; and the experimental confectionary hybrids Seeds 2000 'X2193' and Seeds 2000 'X2793'.
- Relative to the most susceptible hybrids, two additional commercial oilseed hybrids exhibited significant reductions in Sclerotinia incidence and/or severity index ($P < 0.05$) in 1 of 1 trials in which they were tested in 2012 (Figure 3): Seeds 2000 'Camaro' and Seeds 2000 'Torino'. These entries were only evaluated in a single, six-replicate field trial conducted in Carrington, ND.

2. *Improving the methods used to screen sunflowers for resistance to Sclerotinia head rot.*

Field trials conducted in 2011 and 2012 suggest that sunflowers can develop Sclerotinia head rot after bloom but that the susceptibility of sunflowers to head rot is often significantly lower at the R6 growth stage (flowering complete, ray flowers wilted) than at the R5 growth stage (bloom). In an inoculation timing trial conducted in Carrington, ND in 2011 using a pair of partially resistant and highly susceptible sunflower hybrids, a significant increase in Sclerotinia head rot ($P < 0.05$) relative to the non-inoculated control was only observed when sunflowers were inoculated during bloom (Figure 4). High levels of Sclerotinia head rot developed when sunflowers were inoculated during bloom, and very little disease developed when sunflowers were inoculated after bloom. In a parallel inoculation timing trial conducted in Carrington in 2012, sunflowers developed high levels of Sclerotinia head rot when inoculated during bloom (R5 growth stage), moderate levels of Sclerotinia head rot when inoculated immediately after bloom (R6 growth stage; flowering complete, ray flowers wilted), and low levels of Sclerotinia head rot when inoculated at the R7 (back of the head has started to turn a pale yellow color) or R8 (back of the head is yellow but bracts remain green) growth stages (Figure 5). However, sharp drops in susceptibility to Sclerotinia head rot do not always occur between the R5 and R6 or R5 and R7 growth stages. In inoculation timing trials conducted in Langdon, ND in 2011 and 2012, inoculations at R5, R6, and R7 did not result in statistically significant differences (alpha = 0.05) in Sclerotinia head rot incidence or severity index (Figure 5). However, the results from Carrington illustrate that sharp differences in susceptibility to Sclerotinia head rot can sometimes occur between the R5 and R6 growth stages. The results suggest that obtaining replicable, unbiased results from disease screening nurseries requires that inoculations be conducted such that all plants in all entries are inoculated during bloom. Because there is considerable variability in flowering date both across sunflower hybrids (due to differences in maturity) and across individuals within individual hybrids, these results suggest that obtaining replicable results from Sclerotinia head rot screening nurseries will require that all inoculations be conducted over multiple dates, such that each plant is inoculated only when it reach the target growth stage(s). To ensure that each plant receives an identical number of inoculations, plants must be marked when they are inoculated.

Results from the multi-location Sclerotinia head rot screening nurseries conducted in 2011 and 2012 support the conclusion that obtaining *replicable* results from head rot sceening nurseries requires that all sunflower plants across all entries must be inoculated at the same growth stage. In the multi-location screening nurseries conducted in 2011, Sclerotinia incidence and/or severity index results from Carrington, Langdon, Oakes and Crookston were significantly correlated with each other, while results from Sidney, MT and Morden, MB were poorly correlated both with each other and with the other screening locations (Figures 7a, 7b). In Carrington, Langdon, Oakes and Crookston, inoculations were conducted over multiple days such that all sunflower heads from all entries were inoculated either once (Oakes) or twice (Carrington, Langdon, Crookston) at the same growth stage (flowering, R5). In Sidney and Morden, all plants from all entries were inoculated on two fixed dates, irrespective of differences in growth stage across entries. In the multi-location screening nurseries conducted in 2012, Sclerotinia incidence and severity index results from Carrington and Oakes were significantly correlated with each other but not with the results from Langdon (Figure 8). At the Carrington and Oakes locations, inoculations were conducted over multiple dates such that every plant in every entry was inoculated once during early to mid-bloom and once during mid- to late bloom. At the Langdon location, inoculations over just three dates spanning five days; the early maturing hybrids were inoculated at mid- to late bloom, and the late maturing hybrids were inoculated at early to mid-bloom.

Results from the multi-location Sclerotinia head rot screening nurseries conducted in 2011 and 2012 support the conclusion that obtaining *unbiased* results from head rot sceening nurseries requires that all sunflower plants across all entries must be inoculated at the same growth stage. In the Sclerotinia head rot resistance screening trial conducted in Sidney, MT in 2011, all plants from all entries were inoculated on two fixed dates irrespective of differences in growth stage across entries; early maturing entries were inoculated at mid- to late bloom, and late maturing entries were inoculated at early bloom. The date at which sunflowers reached 50% bloom was recorded in each plot, and a strong negative correlation was observed between Sclerotinia head rot incidence and the date of 50% bloom (Figure 9). Conversely, in the Sclerotinia head rot resistance screening trial conducted in Oakes in 2012, inoculations were conducted over multiple dates such that every plant in every entry was inoculated once during early to mid-bloom and once during mid- to late bloom. The date at which sunflowers reached 50% bloom was recorded in each plot, and no correlation was observed between Sclerotinia head rot incidence and the date of 50% bloom (Figure 10). Because sunflower maturity is not expected to be linked to resistance to Sclerotinia head rot, the strong negative relationship between flowering date and susceptibility to Sclerotinia head rot observed in Sidney suggests that the head rot results observed in Sidney in 2011 were biased. The lack of a relationship between head rot incidence and flowering date in Oakes suggests that the head rot resistance results observed in Oakes in 2012 were unbiased.

3. *Assessing the potential of foliar fungicides to manage Sclerotinia head rot.*

Fungicides showed efficacy against Sclerotinia head rot in a field trial conducted in Scotttsbluff, NE but not in Langdon, ND in 2012. In Scottsbluff, three fungicides significantly reduced Sclerotinia head rot incidence and severity and four significantly reduced Sclerotinia stalk rot (Figure 11). Applied as two sequential applications at early bloom and late bloom to flowering complete, Endura 70WG (boscalid) applied at 9 oz/ac, Quash 50WDG (metconazole) applied at 3 oz/ac, and Omega 500F (fluazinam) applied at 1 pt/ac showed efficacy against

Sclerotinia head rot; Topsin 4.5FL (thiophanate-methyl) at 40 fl oz/ac, Vetisan 200EC (penthiopyrad) at 20 fl oz/ac, Omega 500F (fluazinam) applied at 1 pt/ac, and Rovral 4F (iprodione) applied at 2 pt/ac showed efficacy against Sclerotinia stalk rot. However, results from Scottsbluff should be interpreted cautiously; fungicides were applied in 35 gallons of water per acre, and fungicide coverage was likely much better than in commercial applications. In Langdon, none of the eight fungicides tested resulted in a significant reduction in Sclerotinia head rot relative to the inoculated control (Figure 12). Disease levels in a similar trial conducted in Carrington were too low to permit a rigorous assessment of fungicide efficacy (Figure 13).

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Figure 1. **Sclerotinia head rot severity of sunflower hybrids evaluated in inoculated multi-location screening nurseries conducted in North Dakota in 2012.** Overhead irrigation was utilized to facilitate disease development. Within-column means followed by different letters are significantly different ($P < 0.05$; Tukey multiple comparison procedure). *Results from the trial conducted in Langdon, ND should be treated cautiously: The inoculation protocol followed in Langdon in 2012 is likely to have produced moderately biased results. Although all plants in all entries were inoculated during bloom, entries were at different stages of bloom at the time of inoculation. Sunflowers differ in susceptibility to Sclerotinia head rot at different growth stages, and inoculating at different stages of bloom may result in misrepresentations of the true relative susceptibility of different entries.*

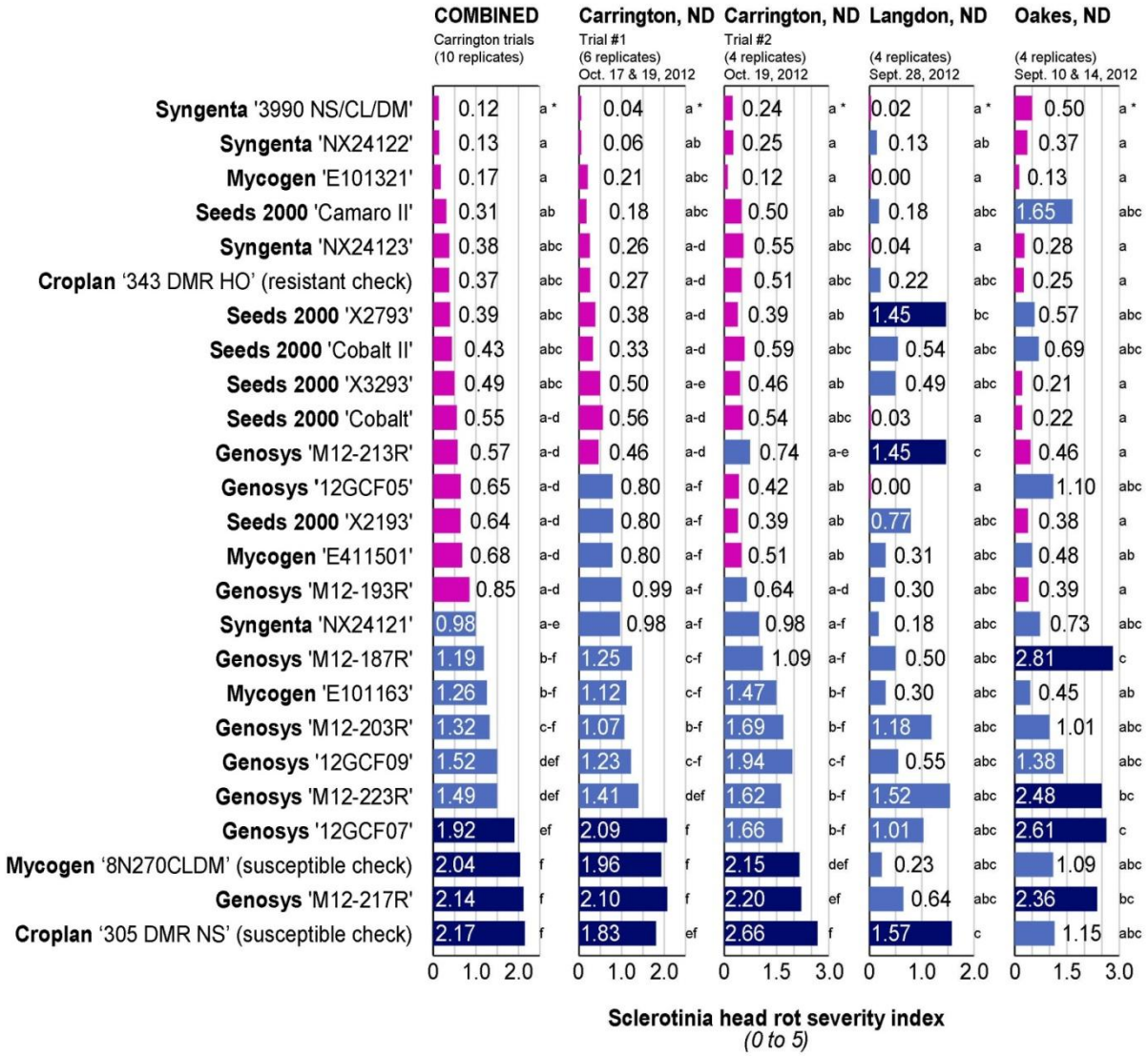

Figure 2. **Sclerotinia head rot incidence of sunflower hybrids evaluated in inoculated multi-location screening nurseries conducted in North Dakota in 2012.** Overhead irrigation was utilized to facilitate disease development. Within-column means followed by different letters are significantly different ($P < 0.05$; Tukey multiple comparison procedure). *Results from the trial conducted in Langdon, ND should be treated cautiously: The inoculation protocol followed in Langdon in 2012 is likely to have produced moderately biased results. Although all plants in all entries were inoculated during bloom, entries were at different stages of bloom at the time of inoculation. Sunflowers differ in susceptibility to Sclerotinia head rot at different growth stages, and inoculating at different stages of bloom may result in misrepresentations of the true relative susceptibility of different entries.*

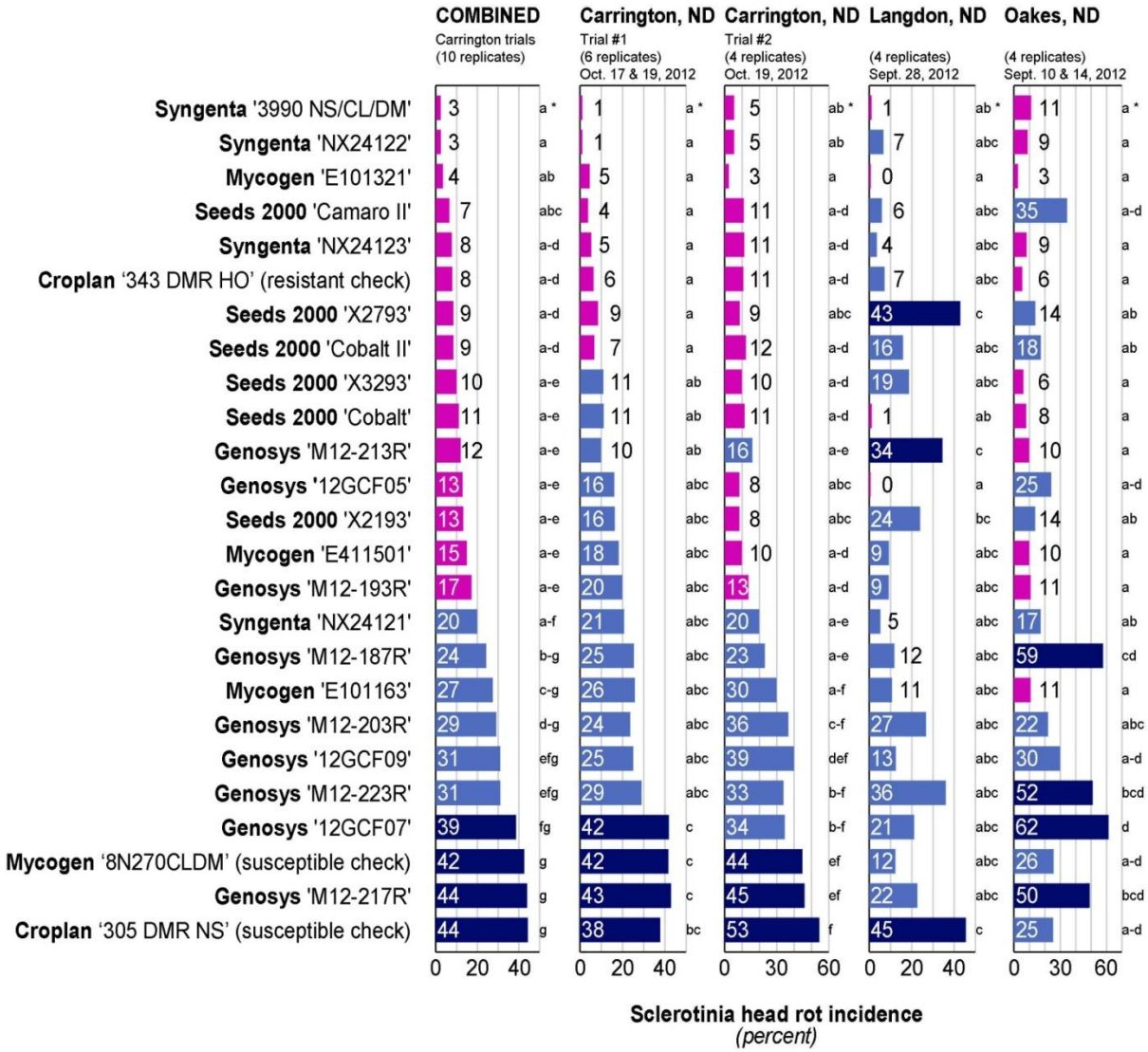

Figure 3. **Sclerotinia head rot incidence and severity index of sunflower hybrids evaluated in an inoculated six-replicate screening nursery conducted in Carrington, ND in 2012.** Overhead irrigation was utilized to facilitate disease development. Within-column means followed by different letters are significantly different ($P < 0.05$; Tukey multiple comparison procedure).

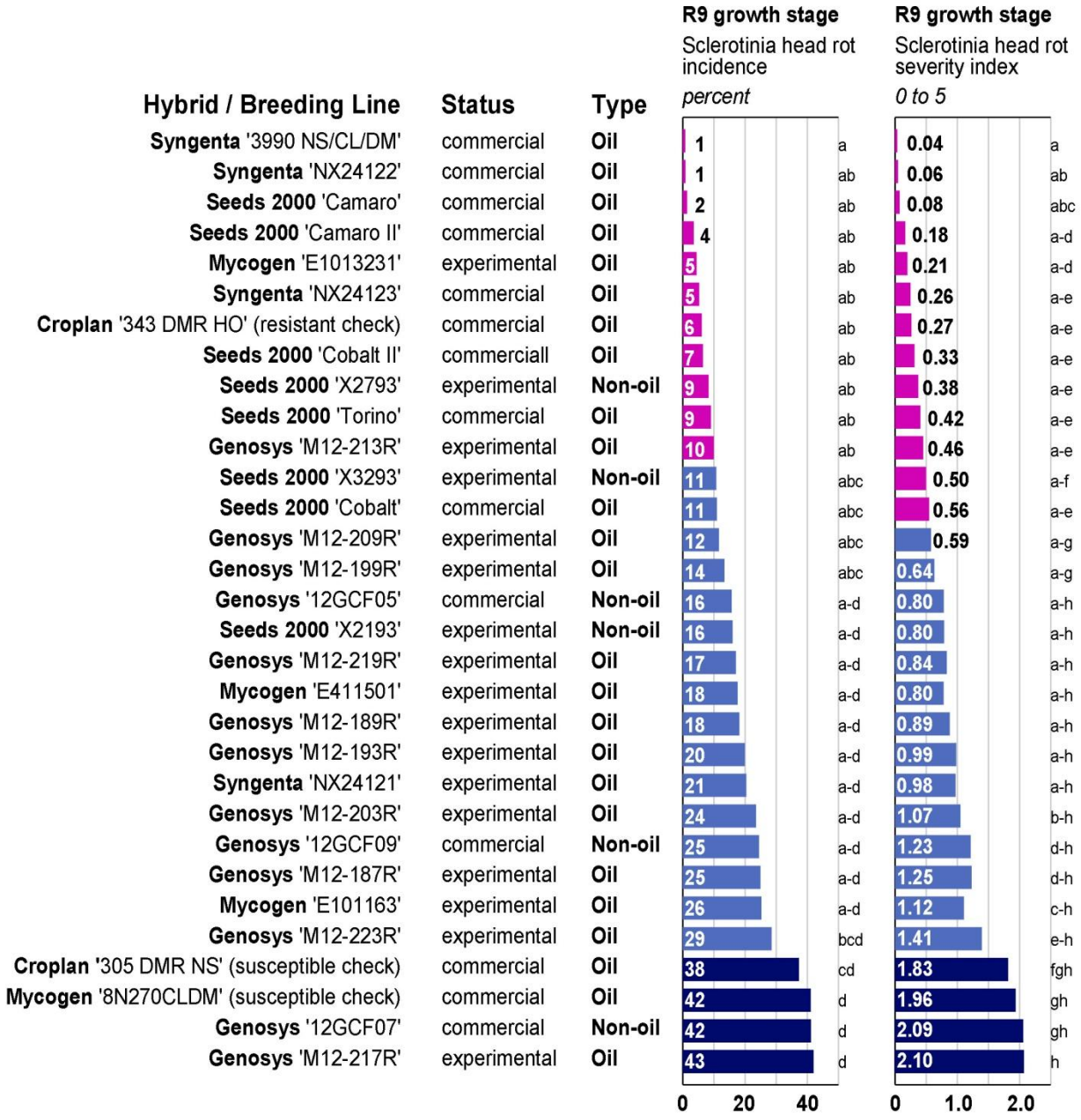

Figure 4. **Results of an irrigated inoculation timing experiment conducted in Carrington, ND in 2011.** Inoculations were conducted at the R5 (bloom), R6 (flowering complete, ray flowers wilting), R7 (back of the head has started to turn a pale yellow color), or R8 (back of the head is yellow but bracts remain green) growth stages on both a partially resistant and a highly susceptible sunflower hybrid. After inoculations, sunflower heads were either bagged for three days or left unbagged; the bagged head treatment was evaluated to test the susceptibility to head rot under elevated relative humidity. Within-column means followed by different letters are significantly different ($P < 0.05$).

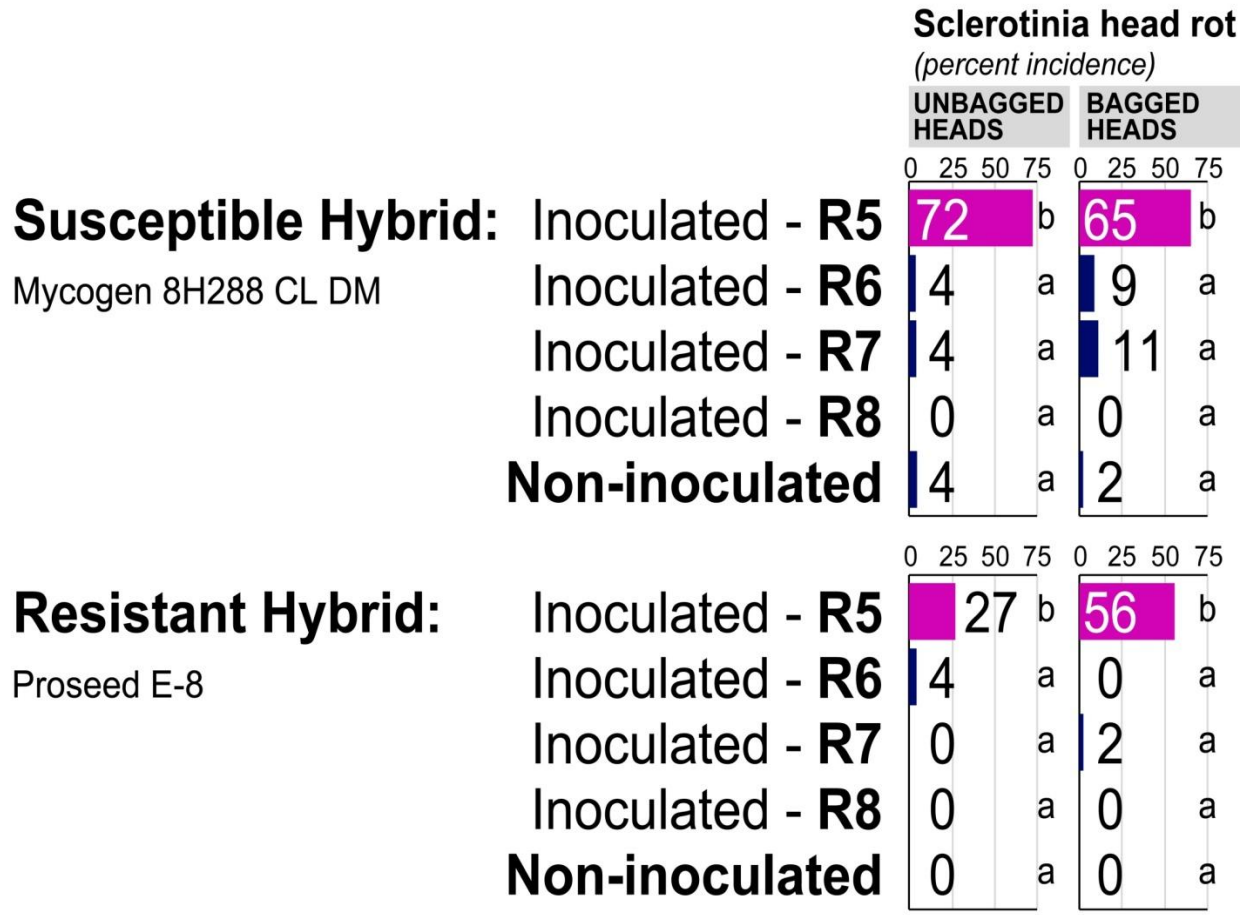

Figure 5. **Results of an irrigated inoculation timing experiment conducted in Carrington, ND in 2012.** Inoculations were conducted at the R5 (bloom), R6 (flowering complete, ray flowers wilting), R7 (back of the head has started to turn a pale yellow color), or R8 (back of the head is yellow but bracts remain green) growth stages on both a partially resistant and a highly susceptible sunflower hybrid. After inoculations, sunflower heads were either bagged for three days or left unbagged; the bagged head treatment was evaluated to test the susceptibility to head rot under elevated relative humidity. Within-column means followed by different letters are significantly different ($P < 0.05$).

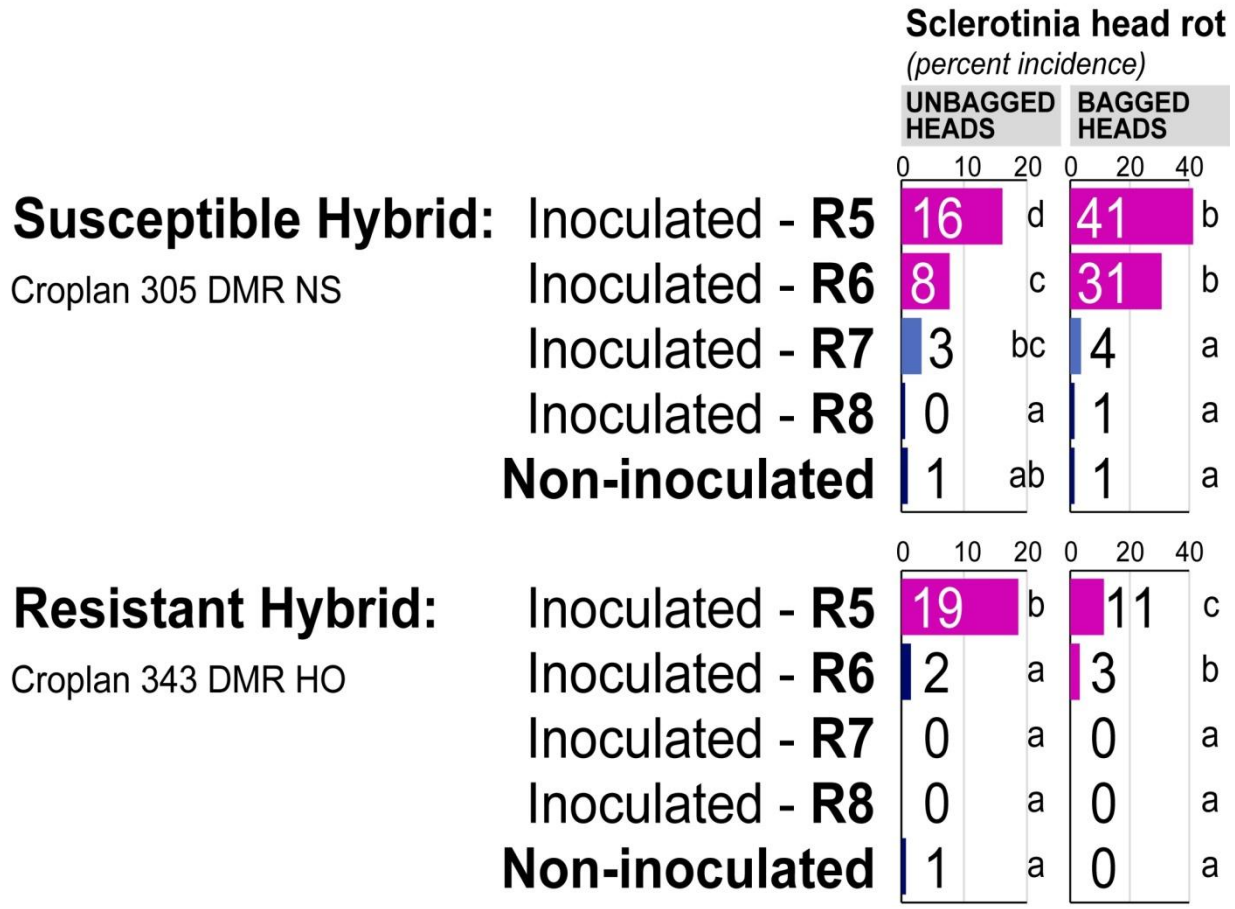

Figure 6. **Results of irrigated inoculation timing experiments conducted in Langdon, ND in 2011 and 2012.** Inoculations were conducted at the R5 (bloom), R6 (flowering complete, ray flowers wilting), R7 (back of the head has started to turn a pale yellow color), or R8 (back of the head is yellow but bracts remain green) growth stages on both a partially resistant and a highly susceptible sunflower hybrid. After inoculations, sunflower heads were either bagged for three days or left unbagged; the bagged head treatment was evaluated to test the susceptibility to head rot under elevated relative humidity. No interaction effects were observed between Sclerotinia head rot incidence and bagging of heads, data from the bagged and unbagged heads treatment were combined. Within-column means followed by different letters are significantly different ($P < 0.05$).

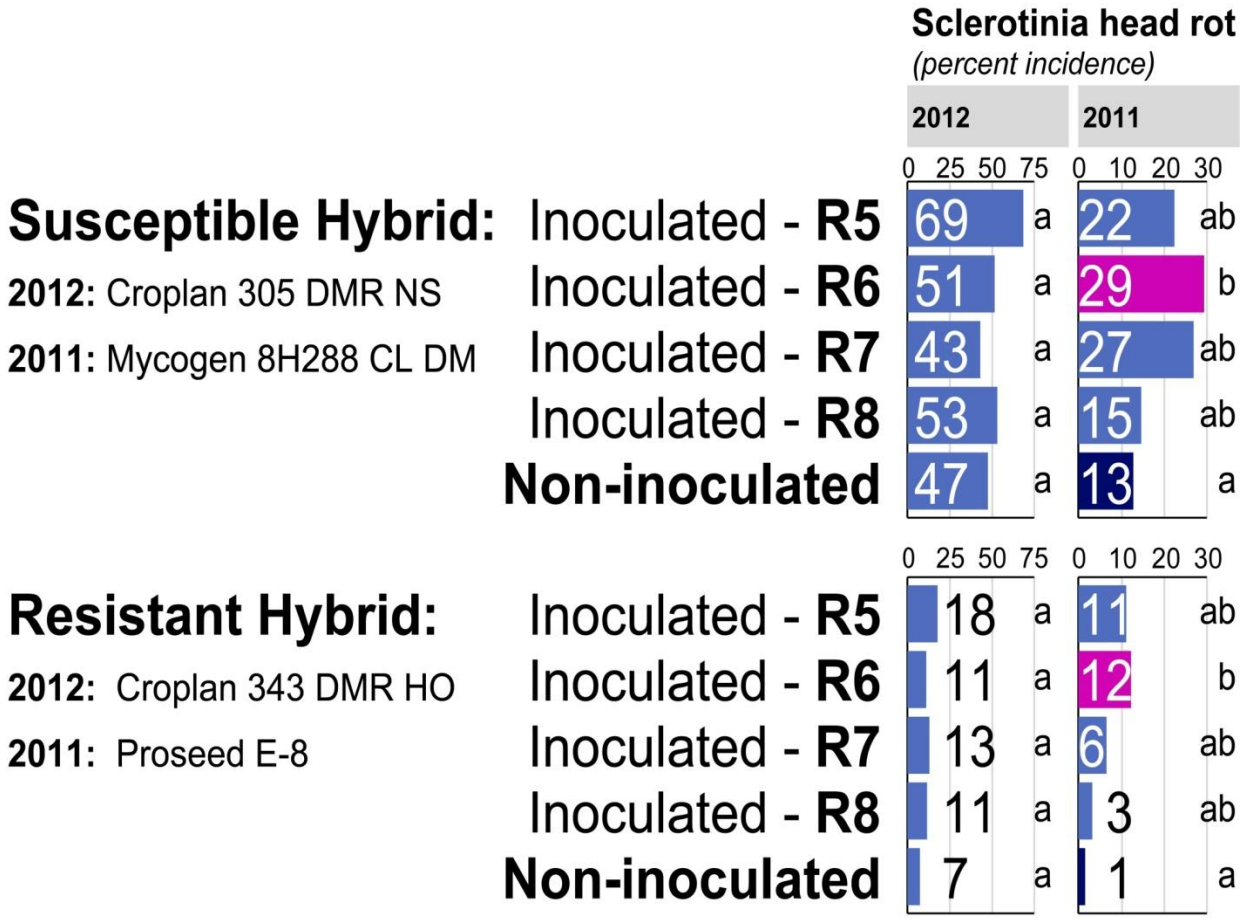

Figure 7a. **Correlation in the sunflower Sclerotinia head rot disease severity index results across multi-location nursery screening locations in 2011.** The Pearson Correlation coefficient is denoted below the axis; the P-value associated with the correlation coefficient is denoted above the axis. *In the screening trials conducted in Carrington, Crookston, Oakes, and Langdon, inoculations were conducted over multiple days such that every plant in each entry was inoculated once (Oakes) or twice (Carrington, Crookston, Langdon) during bloom. In the screening trials conducted in Morden and Sidney, all plants across all entries were inoculated on two fixed dates.*

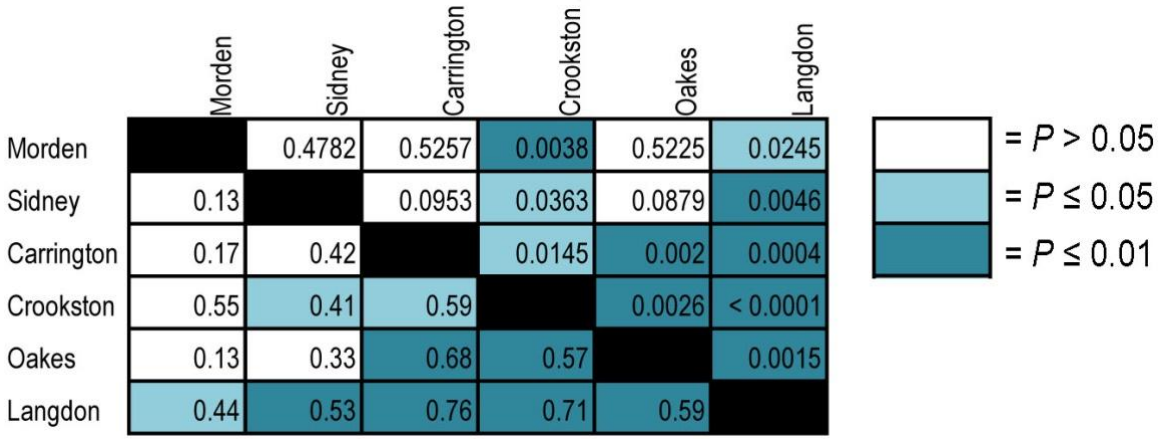

Figure 7b. **Correlation in the sunflower Sclerotinia head rot disease incidence results across multi-location nursery screening locations in 2011.** The Pearson Correlation coefficient is denoted below the axis; the P-value associated with the correlation coefficient is denoted above the axis. *In the screening trials conducted in Carrington, Crookston, Oakes, and Langdon, inoculations were conducted over multiple days such that every plant in each entry was inoculated once (Oakes) or twice (Carrington, Crookston, Langdon) during bloom. In the screening trials conducted in Morden and Sidney, all plants across all entries were inoculated on two fixed dates. Due to high Sclerotinia head rot disease pressure, it was difficult to differentiate the susceptibility of hybrids at the Carrington location; in Carrington, disease incidence was 89 to 100% across all entries at physiological maturity.*

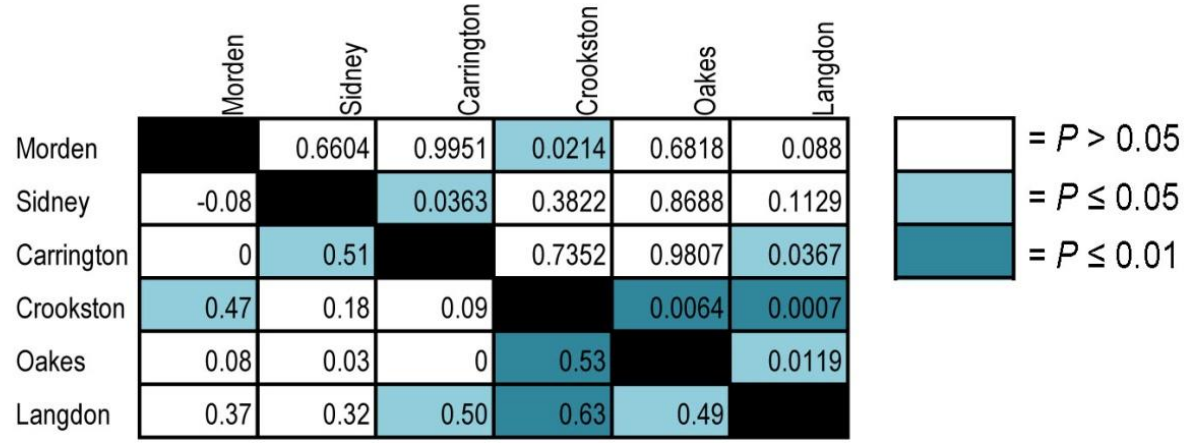

Figure 8. **Correlation in the sunflower Sclerotinia head rot results across multi-location nursery screening locations in 2012.** The Pearson Correlation coefficient is denoted below the axis; the P-value associated with the correlation coefficient is denoted above the axis. *In all trials, all plants across all entries were inoculated twice during bloom. At the Carrington and Oakes locations, inoculations were conducted over multiple days such that every plant was inoculated at early to mid-bloom and again at mid- to late bloom. In Langdon, inoculations were conducted over three days, and early maturing hybrids were inoculated at mid- to late bloom and the late maturing hybrids were inoculated at early to mid-bloom.*

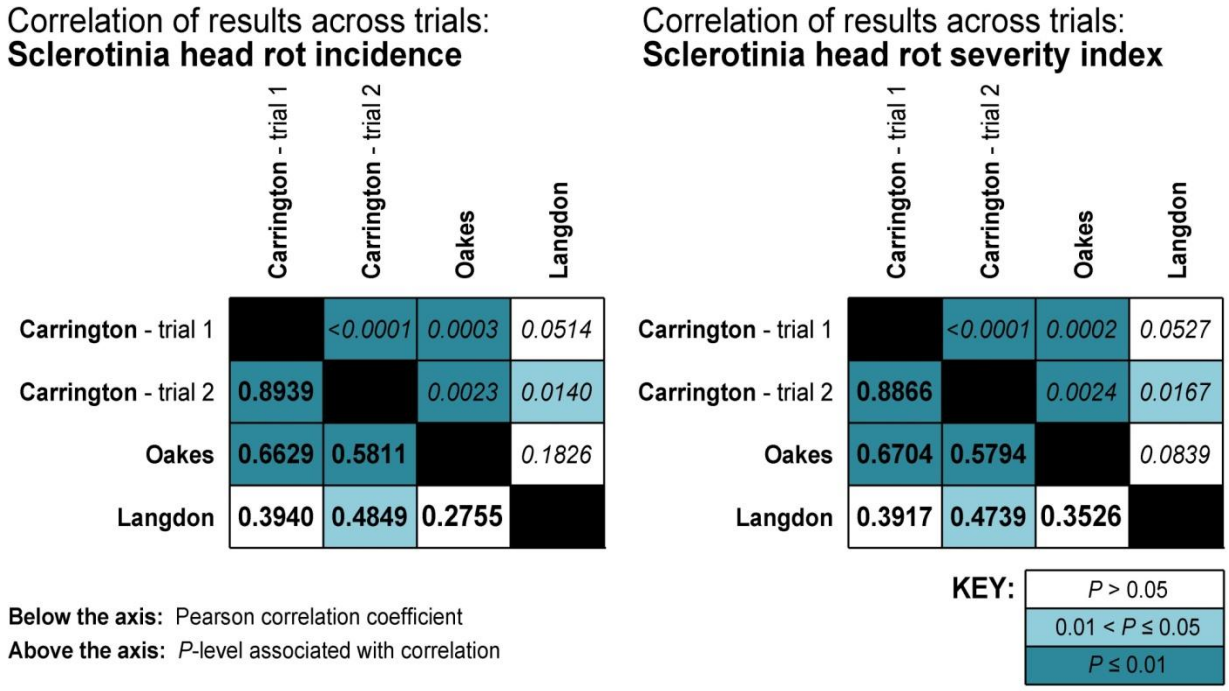

Figure 9. **Relationship between Sclerotinia head rot incidence and sunflower maturity in the Sclerotinia head rot screening trial conducted in Sidney, MT in 2011.** All plants across all entries were inoculated on two fixed dates (Aug. 2-3 and Aug. 8), irrespective of growth stage. The strong negative correlation between disease incidence and sunflower maturity suggests that this inoculation strategy resulted in biased Sclerotinia head rot resistance results.

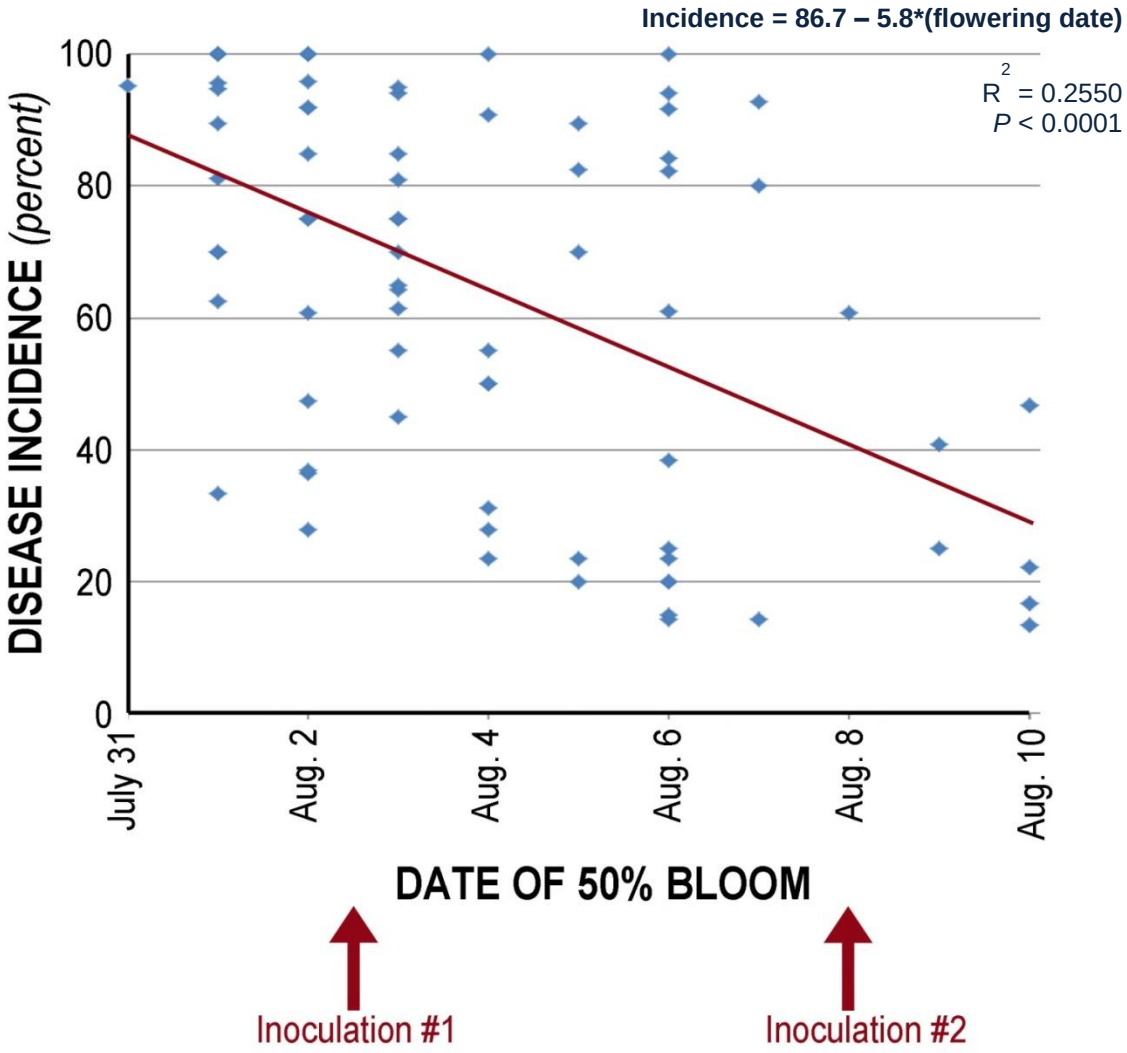

Figure 10. **Relationship between Sclerotinia head rot incidence and sunflower maturity in the Sclerotinia head rot screening trial conducted in Oakes, ND in 2012.** Inoculations were conducted over multiple dates such that all plants across all entries were inoculated twice: once at early to mid-bloom and once at mid- to late bloom. Inoculations were conducted over six different dates, but each sunflower plant was inoculated only twice. The lack of a correlation between disease incidence and sunflower maturity suggests that this inoculation strategy resulted in unbiased Sclerotinia head rot resistance results.

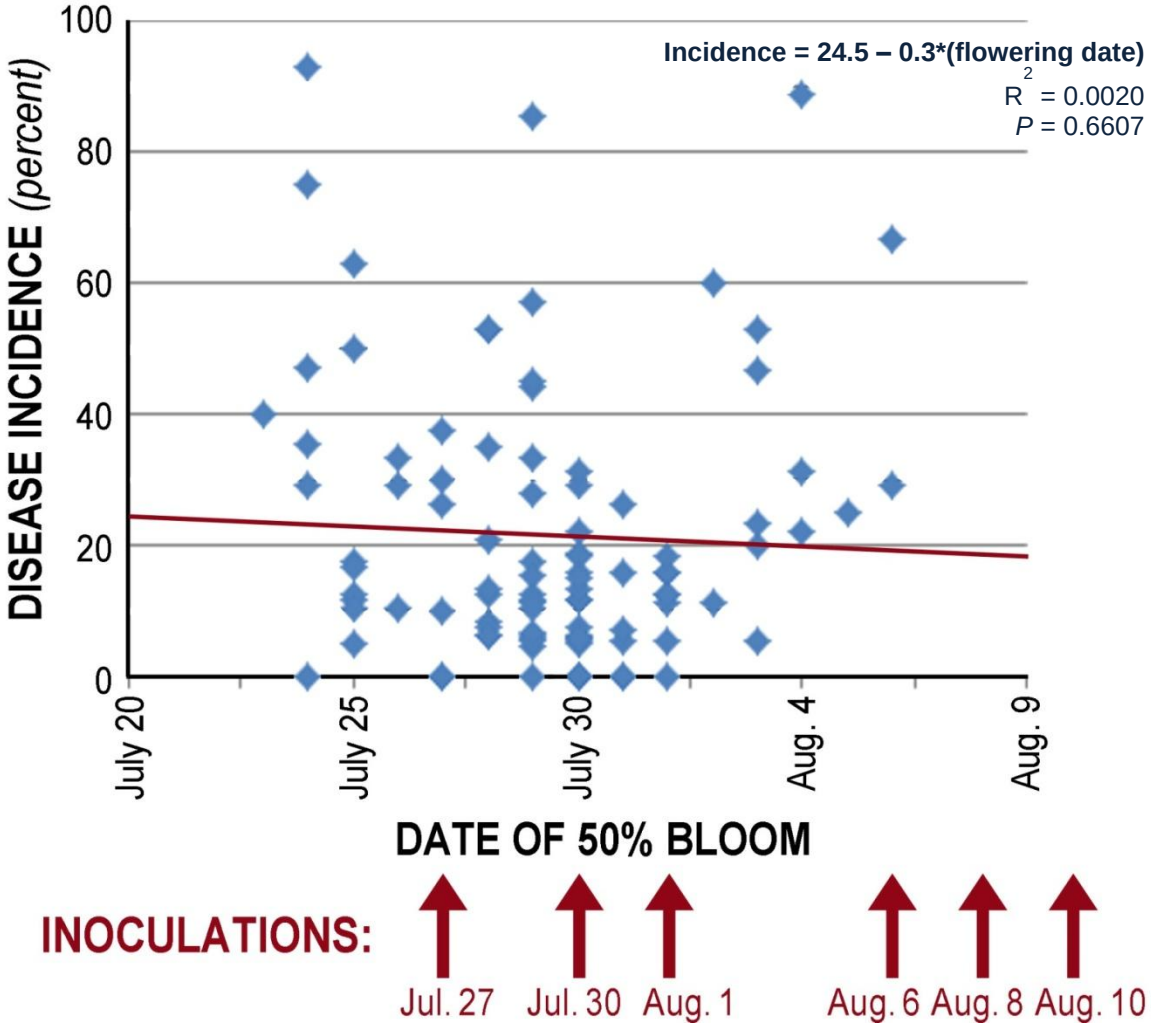

Figure 11. **Field evaluation of foliar fungicides for control of Sclerotinia head rot and stalk rot of sunflowers; Scottsbluff, NE (2012).** Fungicides were applied at 20 psi in 35 gallons of water/ac on August 22 at early bloom (application timing A) and on Sept. 1 at late bloom to flowering complete (application timing B). The oilseed hybrid Pioneer '63N82' was seeded at 49,000 seeds/ac on June 1 and thinned to 21,000 plants/ac when sunflowers were 2 ft tall.

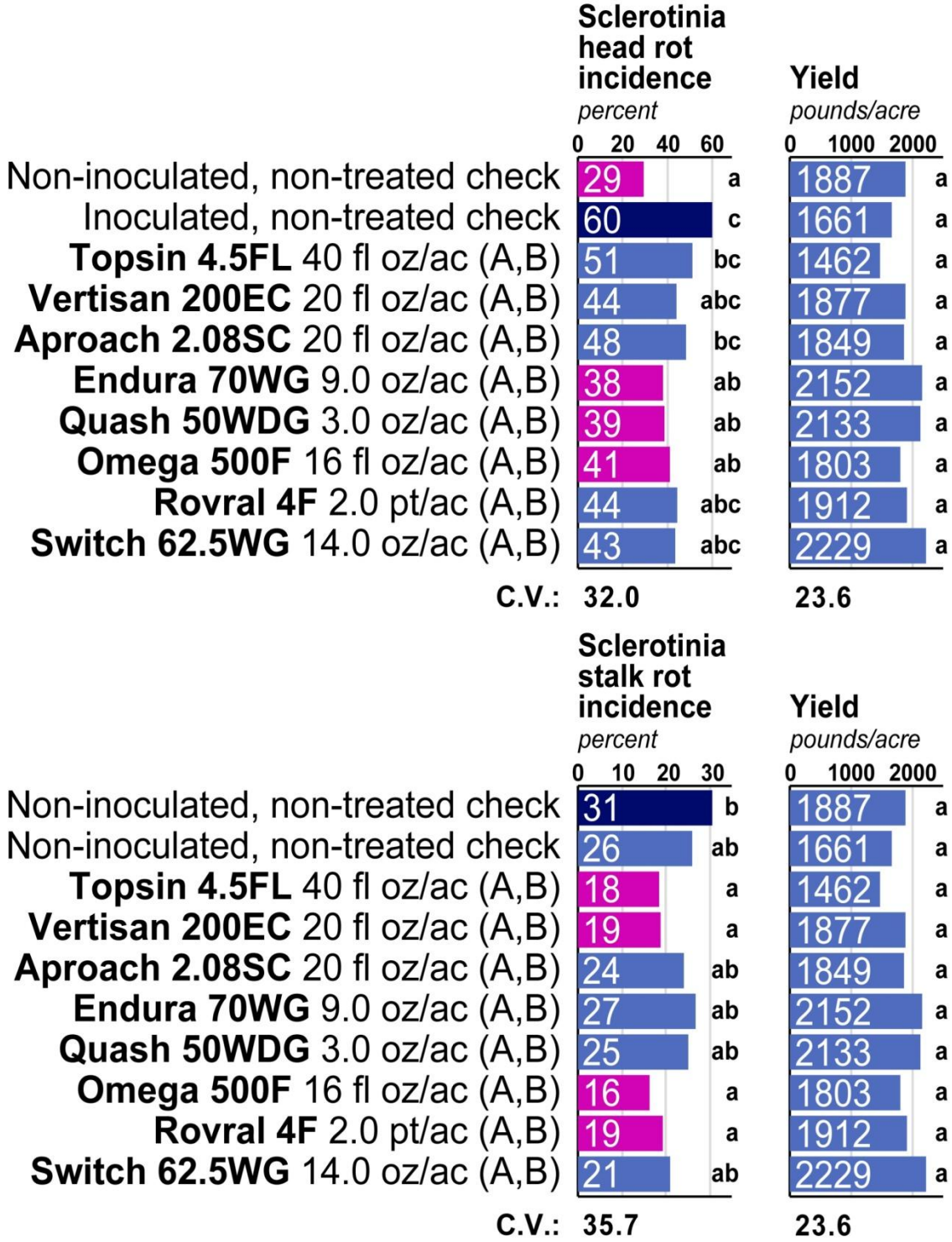

Figure 12. **Field evaluation of foliar fungicides for control of Sclerotinia head rot of sunflowers; Langdon, ND (2012).** Fungicides were applied at 35 psi in 15 gallons of water/ac on August 1 at early bloom (application timing A) and on Aug. 11 shortly after flowering ended (application timing B). The oilseed hybrid Pioneer '63N82'was seeded at 49,000 seeds/ac on May 14 and thinned to 21,000 plants/ac at the V4 growth stage.

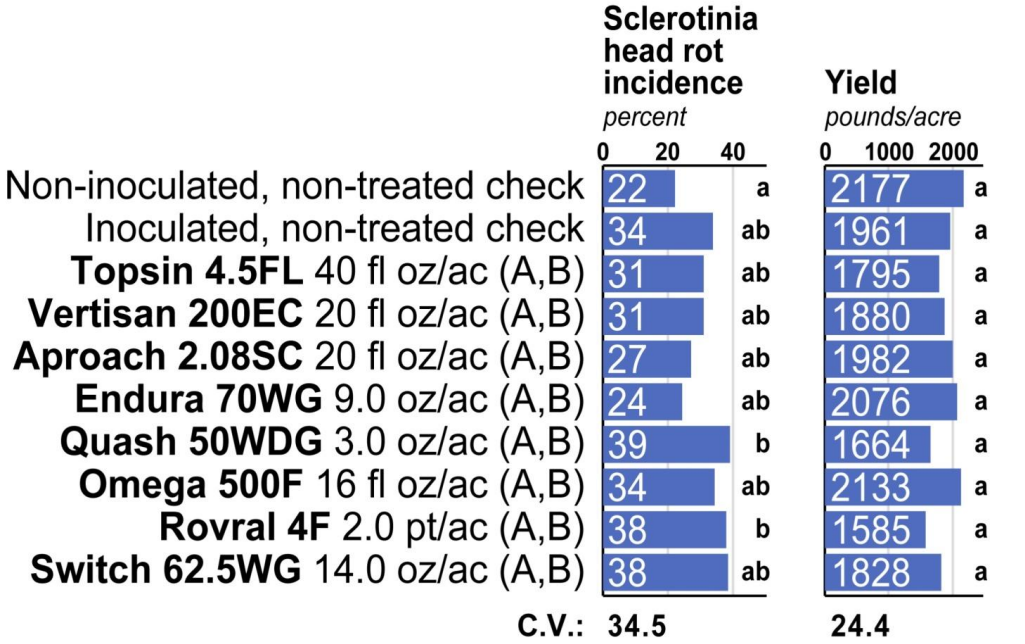

Figure 13. **Field evaluation of foliar fungicides for control of Sclerotinia head rot of sunflowers; Carrington, ND (2012).** Fungicides were applied at 35 psi in 20 gallons of water/ac on August 7 at early bloom (application timing A) and on Aug. 21 at late bloom (application timing B). The oilseed hybrid Pioneer '63N82'was seeded at 49,000 seeds/ac on May 14 and thinned to 21,000 plants/ac at the V2 to V4 growth stage.