

# **Quantification of Yield Loss from *Rhizopus* Head Rot in Sunflower**

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## **Introduction**

*Rhizopus* head rot (RHR) has traditionally been considered a minor problem to sunflower production in North America (Gulya, et al., 1991, Gulya et al., 1997). However, recent NSA surveys have identified and found it was among the most prevalent diseases in Colorado, Kansas and Nebraska (Central High Plains). The disease is caused by several species of the fungus *Rhizopus* which are known to commonly occur in soils. Under conditions of high humidity, the pathogen can invade heads through wounds created by birds, hail, or insects, which initiates infection (Klisiewicz, 1979, Rogers et al., 1978, Yang et al. 1979). RHR has been a consistent constraint in the Central High Plains, particularly in Nebraska (Harveson, 2013), and in some cases has caused complete crop failures. In 2016, the disease was responsible for substantial economic losses to growers in Minnesota, North Dakota, and South Dakota. Furthermore, NSA survey data documented an average of 40% prevalence in the fields monitored during the 2015 and 2017 growing seasons.

We began this study with the purpose of investigating and understanding any negative implications to sunflower production caused by this disease. Our objectives were to successfully induce disease within plots and to document the extent of potential damage to both oil and confectionary sunflower yields under field conditions from multiple geographically and environmentally different locations within sunflower production areas of the Great Plains. There have been a few prior efforts made years ago in Israel (Shtienberg, 1997) and Australia (Middleton, 1977), but to our knowledge no research has ever focused on quantifying yield losses in Canada or the U.S. Information gained through this study could be useful for future screening of sunflower breeding lines for resistance. This is a final report for the completion of a three-year study begun in 2017.

## **Methodology**

### **2017**

Plots were established at 4 sites in the high plains: North Dakota (1 site), South Dakota (1 site), and Nebraska (2 sites). The SDSU site was planted in late April, 2017 and those from North Dakota and Nebraska were planted in mid-May. Plots consisted of 4 rows, 30 inches apart and were 25 ft in length. The Nebraska sites were planted with confectionary sunflowers and were sprinkler irrigated. The NDSU and SDSU sites utilized oil types and were rain-fed.

Preliminary studies from the Panhandle REC in Scottsbluff, NE as well as published techniques (Yang and Thomas 1981) have demonstrated effective methods for creating infections in the field and greenhouse. We employed a modification of these previous techniques utilizing two isolates of *Rhizopus oryzae*. Five healthy plants from each of the two middle rows were chosen for each plot and flagged with spray paint and subjected to 5 treatments. Treatments consisted of 1) control with no wounding or inoculation; 2) wounding on back of head with a ball-peen hammer; 3) wounding with a hammer and inoculation of wound with a small plug of agar containing pathogen mycelial growth and spores; 4) wounding with a cork borer; and 5) wounding with a cork borer and inoculation with pathogen as previously described.

Inoculations were performed in early August for SD and ND, and mid-August in Nebraska. Disease ratings were performed at least once at each site. Disease ratings were based on a scale that estimates the percentage of inoculated heads showing rotting symptoms: 0 = no rot, 1 = rot limited to the site of inoculation injuries, 2 = rot < 25% of the head, 3 = rot < 50% of the head, 4 = rot < 75% of the head and 5 = > 75 % rot or rot encircles the peduncle. Plots were harvested in late September for SD and ND and late-October in Nebraska. Only the 10 inoculated heads from each plot were harvested.

## Results

**North Dakota** – Disease did not develop in the North Dakota location. Only four total heads were infected with *Rhizopus* in the entire study (data not presented). Unfortunately, other diseases developed late in the season, which confounded the trial. Notably, this included *Sclerotinia* head rot, which directly impacted yield in heads that were inoculated with *R. oryzae*. Consequently, the trial was not harvested. It is likely that disease by *Rhizopus* did not develop due to unfavorable temperatures that were too cool; which also benefited disease development and progress from *Sclerotinia* head rot, which is favored by cool temperatures.

**South Dakota** – Disease was successfully created from some of the treatments (ball-peen hammer + inoculum being the most effective) (Table 1). Plots were harvested in late September. Unfortunately, no significant differences were seen among treatments concerning the yields. However, it was demonstrated that a 30% reduction of yield was observed for one of the treatments compared with the un-wounded, un-inoculated control (Table 1). Seed yields were obtained and presented in Table 1 as average for the total weights in grams of the inoculated heads.

**Nebraska** – Disease was successfully induced at both sites although field one had higher disease incidence and severity (note the differences in disease in untreated controls for each site) (tables 2 and 3). That particular field was subjected to a severe hailstorm in late August, which presumably explains the greater levels of disease. For both sites, few differences were seen among non-control treatments however, all were significantly different than the untreated controls (Tables 2 and 3). Although the two sites were different in degree of infection, both showed roughly a 40% reduction in ratio of control treatment yields and highest inoculation

treatment for each of the two sites. Ratings were recorded only once in October, due to late onset of disease with harvest occurring in late October. Cool temperatures during September delayed plant growth and senescence as well as disease progress. We additionally had some disease problems from *Sclerotinia* head rot, but not as severely as that from both North and South Dakota. Yields are presented in tables 2 and 3 as pounds per plot. Due to the lateness of harvest, we took the weights of entire diseased heads rather than threshing heads and getting seed weights.

## **2018 season**

### **Methodology**

Plots were established at 3 sites in the high plains in 2018, one from each of the 3 states involved: North Dakota, South Dakota, and Nebraska. All methods employed in 2017 were repeated in 2018 with the SDSU site planted in late April and mid-May in North Dakota and Nebraska. Plots at all sites again consisted of 4 rows, 30 inches apart and were 25 ft in length, with confectionary sunflowers planted and irrigated with overhead sprinkler irrigations in Nebraska, while the NDSU and SDSU sites were oil types and rain-fed.

In 2018, we employed a modification of the techniques developed by Yang and Thomas (Yang and Thomas 1981). Based on the positive results in 2017, used only a single isolate of *Rhizopus oryzae*. As before, five healthy plants from each of the two middle rows were chosen for each plot, flagged with a ribbon tied around the stem below the head, and the same 5 treatments from the previous year were utilized in 2018 as previously described.

Inoculations were performed in early August for SD and ND, and mid-August in Nebraska. Disease ratings were performed twice at SD and ND sites, with only one rating from Nebraska shortly before harvest. Ratings were based on the same scale with an estimate of the percentage of inoculated heads exhibiting rotting symptoms as described above.

### **Results**

**North Dakota** – As in 2017, disease did not develop well in the North Dakota location. Only two total heads were infected with *R. oryzae* in the entire study (data not presented). Consequently, the trial again was not harvested. We presume that disease did not develop due to unfavorable temperatures for the pathogen that were too cool for optimal infection.

**South Dakota** – Very little disease was achieved from the site in 2018. No differences were observed with the treatments compared with the control (Table 4). Plots were harvested in late September. Similarly, no significant differences were seen among treatments concerning the yields from this site during 2018.

**Nebraska** – Disease was successfully induced at the Scottsbluff site (Table 5), although it was less than what was obtained from the same location in 2017. That particular field in 2017 was

subjected to a severe hailstorm in late August. This presumably explains the greater levels of disease from that field, whereas in 2018, no storms occurred at any time during the trial.

Nevertheless, there were still differences among the treatments. All treatments resulted in significantly higher levels of disease compared with the untreated controls (Table 5). However, the treatments resulting in the highest yield reductions were the two that included both wounding and inoculation with the fungus. One of them showed a 60% reduction in yield compared with control treatment. Ratings were recorded once in October, due to late onset of disease with harvest occurring in late October. Cool temperatures arriving in September delayed plant growth and senescence as well as disease progress. Yields are presented in Table 5 as pounds per plot for both infected heads and seed weights.

## **2019 Season**

### **Methodology**

Due to lack of disease development from the North Dakota sites in both prior years of the study (2017-2018), it was not included in the study in 2019. Therefore, plots were established at only 2 sites in 2019, one from South Dakota, and one from Nebraska. All methods employed in the previous two seasons were repeated in 2019, with the same sized plots at each site. The SDSU field was planted in late April with an oil-type hybrid with dry-land cultivation and in Nebraska planting occurred in mid-May with a confectionary hybrid under sprinkler irrigation.

### **Results**

**South Dakota** – Disease development was successfully created within plots, but it was not as dramatic as in 2017. Nevertheless, the yields of the two inoculated treatments were still significantly poorer than the same two wounding treatments without inoculation.

**Nebraska** – Due to 3 very severe hailstorms within 4 days of each other, fields were severely damaged with yields that were very poor. However, we still made ratings and plots were harvested with yields consisting of total weight of heads. Despite the low yields, we observed that all treatments were significantly reduced compared with the uninoculated control.

### **Conclusions 2017-2019**

The objectives of this study were to induce infection by the fungal pathogen, *Rhizopus oryzae*, monitor disease development, and quantify the degree of damage to sunflower yields due to the pathogen. We were able to successfully initiate infections at six of the nine site years in the three states, however results consistently identifying the most effective method for creating disease were inconclusive. This concept has been difficult to experimentally document, due to the nature of the disease in the field.

We assume that the failure to produce disease in North Dakota in 2017-2018 and South Dakota in 2018, was due to cooler environmental conditions than those in Nebraska. Warm and moist weather is required for optimal growth of the fungal pathogen and those conditions were not adequately produced from those disease-free sites. In order for infection and disease severity to occur, there must also be some form of wound to heads (storms), followed by a warm and wet climate.

In South Dakota and Nebraska, the wounding and inoculation consistently resulted in higher disease levels and lower yields as would be expected. In fact, yield reductions ranging from 30-60% were observed from plots with inoculated or wounded plants compared to the untreated controls.

This type of research has seldom been attempted because *Rhizopus* head rot is a difficult pathosystem to work with due to a requirement for disease to occur on senescing plants. Furthermore, the results can often be confounded by other diseases such as *Sclerotinia* head rot (SHR). This is a similar but distinct disease which can mimic and be confused with the symptoms of *Rhizopus* head rot. Nevertheless, these studies have still helped us to better understand this disease and demonstrate how it can substantially reduce yield on sunflowers in this High Plains of the U.S.

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Table 1. Results of South Dakota Field Study (2017) – *Rhizopus* Head Rot

	Disease Rating 1	Disease Rating 2	Yield (g)
Control	18.7b	35.7a	904.8a
Hammer	15.2b	26.8a	925.5a
Hammer + Inoculum	35.7a	40.2a	820.9a
Cork Borer	17.8b	27.7a	949.4a
Cork Borer + Inoculum	13.4b	33.0a	648.4a

Disease ratings made two and three weeks after inoculation (8/4/17). Disease ratings are based on a scale of 0-5. 0 = no rot, 1 = rot limited to the site of inoculation injuries, 2 = rot < 25% of the head, 3 = rot < 50% of the head, 4 = rot < 75% of the head and 5 = > 75 % rot.

Table 2. Results of Nebraska Rhizopus head Rot Study - Field 1 (2017)

	Disease Rating	Yield (lbs)
Control	33.0c	9.3a
Hammer	78.5a	5.5b
Hammer + Inoc	75.5ab	6.2b
Cork borer	63.5b	6.4b
Cork borer + Inoc	84.0a	5.5b

Disease ratings made 10/15/17. Inoculation of plots (8/14/17). Disease ratings are based on a scale of 0-5. 0 = no rot, 1 = rot limited to the site of inoculation injuries, 2 = rot < 25% of the head, 3 = rot < 50% of the head, 4 = rot < 75% of the head and 5 = > 75 % rot.

Table 3. Results of Nebraska Rhizopus Head Rot Study - Field 2 (2017)

	Disease Rating	Yield (lbs)
Control	19.0b	7.3a
Hammer	65.9a	6.7ab
Hammer + Inoc	59.5a	4.4c
Cork borer	51.5a	4.9bc
Cork borer + Inoc	59.7a	5.3bc

Disease ratings made 10/20/17. Inoculation of plots (8/14/17). Disease ratings are based on a scale of 0-5. 0 = no rot, 1 = rot limited to the site of inoculation injuries, 2 = rot < 25% of the head, 3 = rot < 50% of the head, 4 = rot < 75% of the head and 5 = > 75 % rot.

Table 4. Results of South Dakota Field Study – Rhizopus Head Rot (2018)

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	Disease	Seed Yield (lbs)
Control	15.7a	1.9a
Hammer	13.8a	1.7a
Hammer + Inoc	13.0a	1.9a
Cork borer	10.2a	1.7a
Cork borer + Inoc	15.1a	1.8a

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Disease ratings made two and three weeks after inoculation (8/7/18). Disease ratings are based on a scale of 0-5. 0 = no rot, 1 = rot limited to the site of inoculation injuries, 2 = rot < 25% of the head, 3 = rot < 50% of the head, 4 = rot < 75% of the head and 5 = > 75 % rot.

Table 5. Results of Nebraska Rhizopus head Rot Study – (2018)

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	Disease	Seed (lbs)	Head (lbs)
Control	5.7c	2.4b	9.0c
Hammer	20.1b	2.3b	10.2c
Hammer + Inoc	32.5a	1.0a	3.8a
Cork borer	18.7b	2.3b	10.0c
Cork borer + Inoc	31.5a	1.7a	5.5b

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Disease ratings made 10/27/18. Inoculation of plots (8/19/18). Disease ratings are based on a scale of 0-5. 0 = no rot, 1 = rot limited to the site of inoculation injuries, 2 = rot < 25% of the head, 3 = rot < 50% of the head, 4 = rot < 75% of the head and 5 = > 75 % rot.



Table 6. Results of South Dakota Rhizopus Head Rot Study – 2019

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	Disease	Yield (g)
Control	76a	423.3bc
Hammer	66a	546.0ab
Hammer + Inoc	67a	291.1c
Cork borer	62a	604.1a
Cork borer + Inoc	79a	356.5c

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Disease ratings made two and three weeks after inoculation (8/8/19). Disease ratings are based on a scale of 0-5. 0 = no rot, 1 = rot limited to the site of inoculation injuries, 2 = rot < 25% of the head, 3 = rot < 50% of the head, 4 = rot < 75% of the head and 5 = > 75 % rot.

Table 7. Results of Nebraska Rhizopus head Rot Study – 2019

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	Disease	Yield (lbs)
Control	14.7b	1.1a
Hammer	22.0a	0.9bc
Hammer + Inoc	25.2a	0.8c
Cork borer	26.4a	0.9bc
Cork borer + Inoc	24.0a	0.9bc

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Disease ratings made 9/29/19. Inoculation of plots (8/17/19). Disease ratings are based on a scale of 0-5. 0 = no rot, 1 = rot limited to the site of inoculation injuries, 2 = rot < 25% of the head, 3 = rot < 50% of the head, 4 = rot < 75% of the head and 5 = > 75 % rot.