APHANOMYCES ROOT ROT AND SUGARBEET YIELDS IN THE EIGHTH GROWING SEASON AFTER A SINGLE FIELD APPLICATION OF SPENT LIME

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Aphanomyces cochlioides (= *A. cochlioides*) is an economic pathogen infesting over 90% of Township Sections planted to sugarbeet in the Red River Valley (RRV) and many fields in southern Minnesota. In 2011, the pathogen re-appeared after several years of low activity because of a late planting season combined with warm and wet soil conditions, which are conducive to Aphanomyces damping-off, root rot, and chronic root rot of older plants. *Aphanomyces cochlioides* survives in soil for years, even when sugarbeet is not planted. Recommendations for growing sugarbeet in infested fields include: early planting of partially resistant varieties treated with the fungicide Tachigaren and implementing cultural practices (e.g. cultivation and improved drainage) to avoid or reduce disease. When inoculum densities of the pathogen are high and soil is wet and warm, however, these measures are inadequate for economic yields - and fields may be abandoned or yield poorly. Indeed, Aphanomyces root rot was so severe in some fields in 2011, that they were abandoned. This chronic disease has generated interest in finding effective, alternative methods to supplement control of *A. cochlioides*.

The sugar purification process results in the by-product "spent lime". Lime (calcium carbonate) precipitates impurities in sugarbeet juice. Purified juice is further processed into crystal sugar, but spent lime (14% less acid neutralizing power than fresh lime) contains impurities and becomes a factory by-product. Seven factories in the RRV and southern Minnesota generate 500,000 tons (dry weight) of spent lime annually and some has been stockpiled for 20 years. Information on uses of sugarbeet spent lime is limited and publications usually are in government and company documents. Most spent lime generated in Europe is applied to land as an amendment to increase soil pH and supply nutrients. In Great Britain, it is marketed and sold to conventional and organic growers as "LimeX".

There is limited information in the literature on use of spent lime to reduce plant diseases. Campbell and Greathead (2) applied spent lime (2 to 4 tons A^{-1}) from a sugarbeet processing factory to fields (pH < 6.8) severely infested with the clubroot pathogen, *Plasmodiophora brassicae,* in the Salinas Valley, California. A single application gave "virtually complete control" of clubroot of crucifer crops grown repeatedly for 2 to 3 years. In other areas of the world, various forms of lime (not spent lime) have been applied for over 200 years to control clubroot of crucifers, but results have been inconsistent and little is known about how various forms of lime affect the pathogen. Sugarbeet growers in southern Minnesota apply spent lime the year before planting sugarbeet (typically every 3 years) and have observed less Aphanomyces root rot. In the RRV, application of spent lime also reduces *Aphanomyces* on sugarbeet (1, 5-10).

OBJECTIVES

Our objectives were to evaluate field trials at two locations where several rates of spent lime had been applied <u>eight growing seasons earlier</u> for: 1) long-term effects on Aphanomyces diseases and 2) sugarbeet yield and quality.

MATERIALS AND METHODS

Establishment of field trials. Trials were established in growers' fields near Hillsboro, ND (pH = 7.02) in October, 2003 and Breckenridge, MN (pH = 6.3) in April, 2004. At that time, the Hillsboro site had a history of moderate Aphanomyces root rot with a soil index value (SIV) of 48 and Breckenridge had a history of severe Aphanomyces root rot with a SIV of 98 (0 to 100 scale, 0 = no disease, 100 = potential for severe disease).

Each site was divided into four, 1-acre experiments. Experiments included four rates of spent lime and a non-limed control in a randomized block design of four replicates (Fig. 1). Spent lime treatments at Hillsboro were 0, 5, 10, 20

Experiment 1						Experiment 2						Experiment 3						Experiment 4				
5	0	10	20	15		0	15	20	10	5		10	15	0	5	20		15	5	20	0	10
10	5	20	15	0		20	5	0	10	15		15	5	20	0	10		5	0	10	15	20
10	15	20	0	5		5	10	15	20	0		20	10	15	0	5		20	0	15	5	10
0	5	10	15	20		0	5	10	15	20		0	5	10	15	20		0	5	10	15	20

Hillsboro, ND		**Experiment 1**	**Experiment 2**	**Experiment 3**	**Experiment 4**
Crop sown/yr:	2004	corn	corn	corn	corn
	2005	**sugarbeet**	fallow	fallow	fallow
	2006	corn	**sugarbeet**	corn	corn
	2007	soybean	soybean	**sugarbeet**	soybean
	2008	wheat	wheat	wheat	**sugarbeet**
	2009	**sugarbeet**	soybean	soybean	soybean
	2010	soybean	**sugarbeet**	soybean	Soybean
	2011	sugarbeet	sugarbeet	**sugarbeet**	sugarbeet

Breckenridge, MN (illustrated above)					
Crop sown/yr:	2004	wheat	wheat	wheat	wheat
	2005	**sugarbeet**	wheat	wheat	wheat
	2006	soybean	**sugarbeet**	soybean	soybean
	2007	wheat	wheat	**sugarbeet**	wheat
	2008	corn	corn	corn	**sugarbeet**
	2009	**sugarbeet**	soybean	soybean	soybean
	2010	corn	**sugarbeet**	corn	corn
	2011	soybean	soybean	**sugarbeet**	soybean

Fig. 1. Four experiments were established at Hillsboro, ND in October, 2003 and at Breckenridge, MN in April 2004. At Hillsboro, each experiment was treated with spent lime at 0, 5, 10, 20 and 30 tons wet weight A^{-1} and at Breckenridge with 0, 5, 10, 15 and 20 tons wet weight A^{-1}; experiments were arranged in a randomized block design and four replications (illustrated above for Breckenridge, MN). Sugarbeet plots are noted in **bold** the year when planted in an experiment; in 2011, sugarbeet test trials were planted in Experiment 3.

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and 30 tons wet weight A^{-1} (= 0, 3.3, 6.5, 13 and 19.5 tons dry weight, respectively) and at Breckenridge were 0, 5, 10, 15 and 20 tons wet weight A^{-1} (= 0, 2.7, 5.3, 8, and 10.6 tons dry weight, respectively); each plot was 33 x 60 ft. To allow lime treatments to stabilize in 2004, corn was sown across the four experiments at Hillsboro and spring wheat was sown at Breckenridge. Sugarbeet has been grown in one experiment each year from 2005 to 2011; the three other experiments were planted with the same crop grown in the field by the grower-cooperator as noted in Fig. 1. Results have been reported from 2004 to 2010 (4-10).

2011 Sugarbeet field trials. Two Roundup Ready sugarbeet varieties were sown in Experiment 3 at both locations (last planted to sugarbeet in 2007, Fig. 1). The *Aphanomyces*- susceptible variety had a disease rating of 6.92 (1-9 scale) and seed was not treated with Tachigaren; the partially resistant variety had a rating of 4.14 and was treated with 45 g Tachigaren per unit. Varieties were sown as subplots in limed and non-limed control plots at Breckenridge on May 6 and at Hillsboro on June 7. Seed was sown every 4 inches in rows 60-feet long and 22-inches apart (six rows of each variety centered within plots). Experiments followed standard fertility and production practices to obtain maximum sucrose yield and quality. Data were not collected on rotation crops at either site in 2011 because of lack of time.

Stand counts were made about 5 to 6 weeks after planting at both locations when soil was sufficiently dry to walk in plots. Sugarbeets were harvested September 26 at both locations (two middle rows per subplot). Twenty roots per subplot were rated for Aphanomyces root rot (0 to 7 scale, 0=healthy and 7=root completely rotted and foliage dead). Ten roots were randomly selected and analyzed for yield and sucrose quality by the American Crystal Sugar Co. Quality Laboratory, East Grand Forks, MN.

2011 Soil pH and Aphanomyces soil index values (SIVs). In June, **s**oil samples were collected in plots sown to sugarbeet. Six soil cores (2.5-inch diameter x 6-inch depth) were collected randomly across each plot, combined, screened through 0.25-inch hardware cloth, and assayed (usually within 1 month after collection). To determine pH, small quantities of soil were air-dried for 24 hours and ground into powder with a mortar and pestle. A 5 gram quantity was removed and mixed with 5 ml of deionized water. After 10 minutes, a pH probe was inserted into the mixture, gently stirred for 3 seconds, and the pH was read (Accumet® pH Meter 15, Fisher Scientific).

Bioassays to determine Aphanomyces soil index values (SIVs, which indicate potential for *Aphanomyces* activity and populations) were conducted by filling four (4 x 4 x 4-inch) plastic pots with soil from each sample. Then 25 seed of sugarbeet 'ACH 261' were sown per pot to "bait" *A. cochlioides* from soil. Pots were placed in a growth chamber and arranged in a randomized block design at 70 $\pm$ 2 ^{0}F for 1 week. Temperature then was increased to 77 $\pm$ 2^{0}F (14-hour photoperiod) and soil was kept moist to favor infection by *A. cochlioides*. Stand counts were made three times per week (beginning at emergence) and dying seedlings were removed to prevent disease spread. At 4 weeks after planting, an Aphanomyces SIV was calculated (0 to 100 scale, 0 = *Aphanomyce*s-free and 100 = all seedlings dead and soil severely infested with *A. cochlioides*) for each plot.

Statistical analysis. Data were analyzed for effect of lime rate by linear and quadratic contrasts for significance at P = 0.05, 0.01, and 0.001. Regression analyses were made for rate of lime and recoverable sucrose per acre.

RESULTS

2011 Sugarbeet field trials: Hillsboro. In June of 2010, 92 months (~ 7.7 years) after lime was applied, soil pH was 7.2 in the non-limed control; 7.7 in plots treated with 5 and 10 tons of lime; and 7.8 when treated with 20 and 30 tons of lime A^{-1} (Table 1). These values were very similar to pH values determined July, 2004, 9 months after spent lime was applied (4). Aphanomyces soil index values (SIVs) were high (ranged from 75 to 100) and were statistically the same in limed and the non-limed control (Table 1).

There were no interactions between rate of lime and sugarbeet variety, so main treatments (lime rate and variety) are presented separately in Table 1. There were no significant differences in stand among limed and non-limed treatments at 36 days after planting, nor were there differences in numbers of roots harvested. Disease ratings were highest in the non-limed control (= 3.4 where 5 to 25% of root surface has lesions or scars) and decreased linearly with increasing rates of lime. Root yields significantly increased linearly with increasing rates of lime compared to the non-limed control. Percent sucrose and pounds of sucrose per ton increased significantly in a quadratic relationship, with maximum values at 10 tons wet weight of lime A^{-1}. Recoverable sucrose significantly (P = 0.01) increased linearly by 1,513 pounds with increasing rates of lime compared to the non-limed control (Table 1, Fig. 2A). Revenue per acre also significantly increased linearly with increasingly higher rates of lime.

The sugarbeet variety with partial resistance to *Aphanomyces* (seed also was treated with Tachigaren) had significantly lower stands at 36 days after planting and at harvest compared to the susceptible variety (Table 1). The partially resistant variety, however, had significantly lower Aphanomyces root rot ratings and higher yields of roots than the susceptible variety. The susceptible variety had significantly higher pounds of sucrose per ton than the partially resistant variety. There were no significant differences between varieties for percent sucrose and pounds of recoverable sucrose, although percent sucrose tended to be higher in the susceptible variety and recoverable sucrose was higher in the partially resistant variety. Both varieties had similar gross revenue returns, but were higher in the partially resistant variety.

Table 1. Hillsboro, ND: Soil pH, Aphanomyces soil index values, stands, root rot ratings, and harvest data for sugarbeet sown June 7, 2011, 92 months (= 7.7 years) after several rates of spent lime were applied in a field naturally infested with *Aphanomyces cochlioides.*

Main treatments	Soil pH	Aph SIV (0-100)[x]	Stand@ 36 DAP (plants/100 ft)[y]	No. roots harvested/ 100 ft	Aph RRR (0-7)[z]	Yield (ton/A)	Sucrose %	Sucrose lb/ton	Sucrose lb/recov/A	Gross revenue ($/A)
Lime (ton/A)[u]										
Wet wt. Dry wt.										
0 0	7.2	100	175	146	3.4	15.5	18.2	333	5167	903
5 3.3	7.7	86	175	151	2.9	18.1	18.6	343	6210	1116
10 6.5	7.7	83	198	182	2.3	18.0	19.3	357	6410	1191
20 13.0	7.8	86	191	171	2.6	17.5	18.9	350	6116	1118
30 19.5	7.8	75	182	165	2.6	19.8	18.4	337	6680	1180
Linear[w]	**	NS	NS	NS	*	**	NS	NS	**	*
Quadratic[w]	*	NS	NS	NS	NS	NS	**	**	NS	NS
Variety[v]										
Var. 1 + no Tach (S)	-	-	215	180	3.2	16.8	18.8	349	5887	1076
Var. 2 + 45 g Tach (PR)	-	-	154	146	2.2	18.7	18.5	339	6347	1127
P-value[w]	-	-	***	***	***	*	NS	*	NS	NS
Lime x Var (linear)[w]			NS	NS	NS	NS	NS	NS	NS	NS

[U] Spent lime was applied in October, 2003 in a randomized block design of four replicates per experiment (total of four experiments) and incorporated by cultivation. In 2011, sugarbeet was sown on June 7, 92 months (= 7.7 years) after spent lime had been applied; each value in this portion of the table is averaged across two sugarbeet varieties.

[V] Two Roundup Ready sugarbeet varieties were sown as subplots within each plot; one was susceptible (S, rating of 6.92) to *Aphanomyces* and not treated with Tachigaren (Tach) and the other was partially resistant (PR, rating of 4.14) and treated with 45 g Tach per unit of seed. Plots were harvested on September 26, 2011. Each value in this portion of the table is averaged across all lime treatments.

[W] * = significant at P = 0.05, ** = significant at P = 0.01, *** = significant at P = 0.001, NS = not significant.

[X] Aphanomyces SIV = soil index value (determined in a 4-week greenhouse assay of soil); 0-100 scale where 0 = soil *Aphanomyces*-free, 100 = all seedlings dead by 4 weeks after planting and soil severely infested with *Aphanomyces*.

[Y] DAP = days after planting; plots were sown at 70,180 seeds per acre (seed sown about 4 inches apart in 22-inch rows).

[Z] RRR = Aphanomyces root rot rating, 0-7 scale (0 = roots healthy; 7 = root completely rotted and foliage dead).

2011 Sugarbeet field trials, Breckenridge. In June, 85 months (~ 7.1 years) after lime was applied, soil pH was 6.4 in the non-limed control and increased by 1.3 points as lime rates increased to 15 and 20 tons per acre (Table 2). The pH values remained nearly identical to those measured 6 months after lime was applied in 2004 (4). Maximum Aphanomyces SIVs of 100 occurred across nearly all treatments (Table 2).

There were no interactions between rate of lime and sugarbeet variety, so main treatments (lime rate and variety) are presented separately in Table 2. Seedling stands at 45 days after planting were not significantly different among rates of lime and the non-limed control. Although the trial was not thinned, there was a significant loss in plant populations during the season. By harvest, numbers of lifted roots were significantly higher for all rates of lime compared to the non-limed control and increased in a highly significant (P = 0.001) linear relationship. Aphanomyces root rot was extremely severe and roots in all plots had high disease ratings. Disease severity did decrease as rates of lime increased, but even the highest rates of lime had unacceptable levels of root rot (a value of 4 means 25 to 50% of the root surface is rotted and/or scarred). Low numbers of roots and severe Aphanomyces root rot in all treatments resulted in very poor yields, although increasing rates of lime increased root and sucrose yields (Table 2, Fig. 2B). Revenue per acre significantly increased linearly with increasing rates of lime, but economic returns were extremely low and unacceptable.

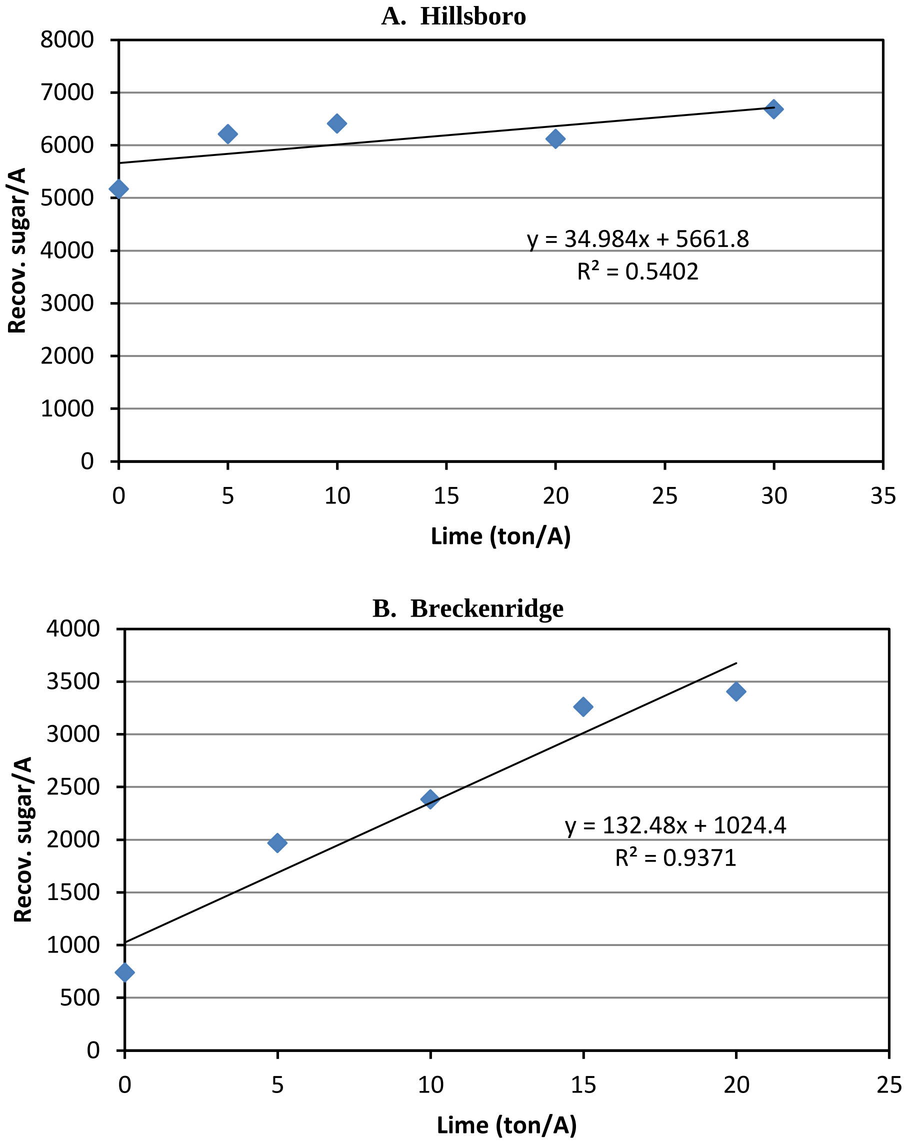

Fig. 2 Regression of recoverable sucrose vs. rates of lime per acre: **A.)** Hillsboro, ND and **B.)** Breckenridge, MN. Data is averaged across two varieties (a partially resistant variety with Tachigaren (45 g/unit) and a susceptible variety without Tachigaren). There was no significant lime rate x variety interaction.

The partially resistant variety treated with Tachigaren resulted in significantly lower seedling stands than the susceptible variety but numbers of harvested roots were statistically the same (Table 2). Aphanomyces root rot was significantly less severe and root, sucrose, and economic returns were significantly higher in the partially resistant variety than in the susceptible variety. Sucrose yields and economic returns, however, were unacceptable for the partially resistant variety.

Table 2. Breckenridge, MN: Soil pH, Aphanomyces soil index values, stands, root rot ratings, and harvest data for sugarbeet sown May 6, 2011, 85 months (= 7.1 years) after several rates of spent lime were applied in a field naturally infested with *Aphanomyces cochlioides.*

Main treatments		Soil pH	Aph SIV (0-100)[x]	Stand@ 45 DAP (plants/100 ft)[y]	No. root harvested/ 100 ft	Aph RRR (0-7)[z]	Yield (ton/A)	Sucrose %	Sucrose lb/ton	Sucrose lb/recov/A	Gross revenue ($/A)
Lime (ton/A)[u]											
Wet wt.	Dry wt.										
0	0	6.4	100	178	33	5.6	2.5	15.5	243	738	111
5	2.7	7.1	100	177	77	5.1	6.5	16.4	299	1966	311
10	5.3	7.4	100	176	95	4.8	7.8	16.6	303	2380	378
15	8.0	7.7	100	186	126	4.2	10.4	17.0	312	3258	537
20	10.6	7.7	99	180	133	4.3	10.9	17.0	312	3404	557
Linear[w]		***	**	NS	***	***	***	***	*	***	***
Quadratic[w]		**	*	NS	NS	*	NS	NS	NS	NS	NS
Variety[v]											
Var. 1 + no Tach (S)		-	-	216	90	5.2	6.7	16.3	284	2048	328
Var. 2 + 45 g Tach (PR)		-	-	143	95	4.4	8.6	16.7	304	2650	430
P-value[w]		-	-	***	NS	***	**	**	NS	**	**
Lime x Var (linear)[w]				NS	NS	NS	NS	NS	NS	NS	NS

U Spent lime was applied in April, 2004 in a randomized block design of four replicates per experiment (total of four experiments) and incorporated by cultivation. In 2011, sugarbeet was sown on May 6, 85 months (= 7.1 years) after spent lime had been applied; each value in this portion of the table is averaged across two sugarbeet varieties.

V Two Roundup Ready sugarbeet varieties were sown as subplots within each plot; one was susceptible (S, rating of 6.92) to *Aphanomyces* and not treated with Tachigaren (Tach) and the other was partially resistant (PR, rating of 4.14) and treated with 45 g Tach per unit of seed. Plots were harvested on September 26, 2011. Each value in this portion of the table is averaged across all lime treatments.

w * = significant at $P = 0.05$, ** = significant at $P = 0.01$, *** = significant at P=0.001, NS = not significant.

x Aphanomyces SIV = soil index value (determined in a 4-week greenhouse assay of soil); 0-100 scale where 0 = soil *Aphanomyces*-free, 100 = all seedlings dead by 4 weeks after planting and soil severely infested with *Aphanomyces*.

y DAP = days after planting; plots were sown at 70,180 seeds per acre (seed sown about 4 inches apart in 22-inch rows).

z RRR = Aphanomyces root rot rating, 0-7 scale (0 = roots healthy; 7 = root completely rotted and foliage dead).

DISCUSSION

In 2011, both field trials had environmental conditions favorable for Aphanomyces diseases and benefited from applications of lime, however, the overall yield results were much better at Hillsboro than at Breckenridge. These differences are due to interactions of environmental conditions (especially moisture) and inoculum density (population) of *A. cochlioides* in soil, which resulted in overwhelming disease pressure at Breckenridge.

Both trials had similar stands at 5 weeks after planting in Hillsboro and 6.5 weeks in Breckenridge, despite Hillsboro being planting on June 7 and Breckenridge on May 6. The fact that there were no significant differences among limed and non-limed treatments at either location at this time indicates that Aphanomyces damping-off was not occurring. Even the susceptible variety had a higher stand than the partially resistant variety treated Tachigaren seed treatment at both locations. This also suggests a potential difference in seed germination between the two varieties in the absence of Aphanomyces disease pressure (under conditions favorable for Aphanomyces infection, Tachigaren would have benefited seedling stands).

By harvest, plant populations had decreased for all treatments at both locations (plots were not thinned). Plant population losses at Hillsboro, however, were typical and averaged about 15%. Assay of soil from the Hillsboro trials indicated a high potential for Aphanomyces root rot developing under warm, wet soil conditions. Indeed, there was relatively high precipitation (10.97 inches) in Hillsboro from about late June through mid August (from 103 to 147% above normal). Correspondingly, Aphanomyces root rot ratings were higher than in previous years (4-10) and lime applications resulted in significantly lower root rot ratings and higher yields. The partially resistant variety outperformed the susceptible variety in root and sucrose yields and economic return (although the susceptible variety had a significantly higher number of harvestable roots). Under conditions of significant Aphanomyces disease pressure, partially resistant varieties outperform susceptible varieties. On the other hand, susceptible varieties have higher yields than partially resistant varieties when sown in fields without *Aphanomyces* or when environmental conditions do not favor development of Aphanomyces root rot. Overall, the higher rates of lime resulted in yields similar to the factory average at Hillsboro (6,427 pounds of recoverable sucrose per acre).

Meanwhile, precipitation at Breckenridge also was above normal (9.17 inches) from mid-July to mid-August (from 101 to 145% above normal). Assays of soil from this site have consistently had SIV's of about 100. Similar SIVs have developed recently at Hillsboro (9-10), however, seedlings in soil from Breckenridge die sooner and in greater abundance than in soils from Hillsboro. These results indicate a much higher population of *A. cochlioides* in soil at Breckenridge than at Hillsboro. Typically, the higher the population density of inoculum in soil, the sooner infections occur, even under marginal environmental conditions. Thus, it was not unexpected to see more Aphanomyces root rot at Breckenridge than at Hillsboro. By harvest, plant populations decreased 81% in the non-limed control, and for 5, 10, 15, and 20 tons of lime decreased by 56, 46, 31, and 26%, respectively. However, the severity of root rot at Breckenridge, among all limed and non-limed plots, was higher than in previous years (4-10), and while limed plots had less severe root rot than the non-limed control, disease was not reduced to economically acceptable levels by any of the treatments. At harvest, all roots were scarred – and root scarring occurs when soils dry out and roots "slough off" diseased areas. Apparently, the especially high rainfall from mid-July to mid-August created unusually favorable and <u>persistent soil wetness</u>, which resulted in continuing re-infection of roots and disease development for over a month. As the soil dried from mid-August to late September, plants "recovered", but roots were too small and severely diseased to produce sugar and economic yields. These results are similar to some fields in the Fargo-Moorhead area that were abandoned because of severe Aphanomyces root rot.

Under prolonged environmental conditions highly favorable for disease, especially in soils with high populations of *Aphanomyces*, the best management strategies (plant resistance, Tachigaren seed treatment, good soil drainage, early planting, application of factory lime) fail. Under these conditions, the pathogen predominates and overrides plant resistance and all other management strategies – and is a "pathogen dominant" situation (3). In a more typical season, the same practices are effective, economic strategies. In 2011, fields with exceptionally high population densities of *A. cochlioides* and prolonged, wet soil conditions experienced Aphanomyces root rot as a "pathogen dominant" disease.

SUMMARY AND CONCLUSIONS

1. In the eighth growing season after application of spent lime, there was a significant reduction in Aphanomyces root rot and increased yields at Hillsboro, where disease pressure was moderate.

2. At Breckenridge, lime also reduced Aphanomyces root rot and increased yields compared to the non-limed control, but disease pressure was very intense for a prolonged time (several weeks) and even best management practices resulted in insufficient disease control and non-economic yields.

3. Soil pH levels initially increased with application of spent lime and have remained relatively stable; soil index values (SIVs) are high at both locations, regardless of rate of spent lime applied to soil.

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