SEVENTH GROWING SEASON AFTER A SINGLE FIELD APPLICATION OF SPENT LIME: EFFECTS ON APHANOMYCES ROOT ROT AND SUGARBEET YIELDS

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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 most acres in southern Minnesota. When soil is warm and wet, *A. cochlioides* causes damping-off of seedlings and chronic root rot of older plants, which also affects storability of roots. *A. cochlioides* survives in soil for years, even when sugarbeet is not planted. Recommendations for growing sugarbeet in infested fields includes 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. This chronic situation 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 of 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 erratic 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 (1,8-13).

OBJECTIVES

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

Experiment 1

5	0	10	20	15
10	5	20	15	0
10	15	20	0	5
0	5	10	15	20

Experiment 2

0	15	20	10	5
20	5	0	10	15
5	10	15	20	0
0	5	10	15	20

Experiment 3

10	15	0	5	20
15	5	20	0	10
20	10	15	0	5
0	5	10	15	20

Experiment 4

15	5	20	0	10
5	0	10	15	20
20	0	15	5	10
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
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

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 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).

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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 2010; 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 2009 (8-13).

2010 Sugarbeet field trials. Two Roundup Ready sugarbeet varieties were sown in Experiment 2 at both locations (last planted to sugarbeet in 2006, 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 June 1 and at Hillsboro on June 3. Seed was sown every 2.5 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 2010 because of lack of time.

Stand counts were made about 4 weeks after planting at both locations. Plots were thinned to 6-inch spacing on July 8 at Hillsboro and July 9 at Breckenridge. Sugarbeets were harvested September 30 at Breckenridge and October 7 at Hillsboro (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.

2010 Soil pH and Aphanomyces soil index values (SIVs). In June, soil 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

2010 Sugarbeet field trials: Hillsboro. In June of 2010, 80 months after lime was applied, soil pH was 7.4 in the non-limed control and increased significantly with all rates of lime (Table 1). These values were very similar to pH values determined July, 2004, 9 months after spent lime was applied (8). Aphanomyces soil index values (SIVs) were high (ranged from 56 to 83) and were statistically the same in limed plots and the non-limed control (Table 1).

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Table 1. Hillsboro, ND: Soil pH, Aphanomyces soil index values, stands, root rot ratings, and harvest data for sugarbeet sown June 3, 2010, 80 months 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@ 28 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	7.38	82	351	153	1.9	25.0	18.2	338	8423	1398
5	3.3	7.75	76	343	158	1.7	25.4	18.4	342	8659	1454
10	6.5	7.80	83	339	152	1.6	26.7	18.0	333	8862	1449
20	13.0	7.87	56	341	156	1.5	26.3	18.4	340	8931	1492
30	19.5	7.88	80	348	167	1.5	25.5	18.6	348	8855	1509
Linear[w]		***	NS	NS	NS	*	NS	NS	NS	NS	*
Quadratic[w]		*	NS	NS	NS	NS	NS	NS	NS	NS	NS
Variety[v]											
Var. 1 + 0 Tach (S)		-	-	318	153	1.73	26.3	18.5	345	9047	1528
Var. 2 + 45 g Tach (PR)		-	-	371	161	1.57	25.2	18.2	336	8445	1392
P-value[w]		-	-	***	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 2010, sugarbeet was sown on June 3, 80 months after spent lime had been applied; each value in this portion of the table is averaged across two sugarbeet varieties. Plots were harvested on October 7, 2010.

V Two Roundup Ready sugarbeet varieties were sown as subplots within each plot; one was susceptible (S) to *Aphanomyces* and not treated with Tachigaren (Tach) and the other was partially resistant (PR) and treated with 45 g Tach per unit of seed. 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 114,048 seeds per acre (seed every 2.5 inches in rows 22 inches apart) and thinned to a 6-inch spacing on July 8.

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

Table 2. Breckenridge, MN: Soil pH, Aphanomyces soil index values, stands, root rot ratings, and harvest data for sugarbeet sown June 1, 2010, 74 months 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@ 30 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	6.53	100	255	93	4.5	9.5	15.3	279	2675	364
5	2.7	7.41	99	255	109	3.9	16.4	15.8	290	4792	685
10	5.3	7.54	96	284	120	3.7	20.6	16.2	298	6156	904
15	8.0	7.70	80	282	122	3.2	23.8	15.9	290	6913	980
20	10.6	7.79	87	286	123	3.0	23.4	15.8	288	6737	949
Linear[w]		***	*	NS	***	***	***	NS	NS	***	***
Quadratic[w]		**	NS	NS	*	NS	***	*	*	***	***
Variety[v]											
Var. 1 + 0 Tach (S)		-	-	251	98	4.4	16.4	15.8	288	4793	684
Var. 2 + 45 g Tach (PR)		-	-	294	128	2.9	21.1	15.8	290	6116	869
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 2010, sugarbeet was sown on June 1, 74 months after spent lime had been applied; each value in this portion of the table is averaged across two sugarbeet varieties. Plots were harvested on September 30, 2010.

V Two Roundup Ready sugarbeet varieties were sown as subplots within each plot; one was susceptible (S) to *Aphanomyces* and not treated with Tachigaren (Tach) and the other was partially resistant (PR) and treated with 45 g Tach per unit of seed. 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 114,048 seeds per acre (seed every 2.5 inches in rows 22 inches apart) and thinned to a 6-inch spacing on July 9.

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

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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 between limed and non-limed treatments at 28 days after planting, nor were there differences in numbers of roots harvested. Potential for Aphanomyces diseases was high because of high soil index values but soil conditions were fairly dry throughout the season and disease was minimal. Despite low disease pressure from *Aphanomyces*, root rot ratings were significantly lower as rates of lime increased compared to the non-limed control. Yields of root and sucrose tended to be higher in limed than non-limed plots, but were not statistically significant. Revenue per acre significantly increased linearly with increasingly higher rates of lime.

The sugarbeet variety with partial resistance to *Aphanomyces* (and seed also was treated with Tachigaren) had significantly higher stands than the susceptible variety at 28 days after planting (Table 1). There were no differences between varieties, however, for number of harvested roots. The partially resistant variety had significantly lower Aphanomyces root rot ratings than the susceptible variety, but the ratings were low for both varieties and indicated minimal disease pressure from the pathogen. The susceptible variety, however, resulted in significantly higher root and sucrose yields and revenue than the partially resistant variety because of low disease pressure and because it had higher yield potential when disease pressure is very low or absent.

2010 Sugarbeet field trials, Breckenridge. In June (74 months after lime was applied), soil pH was 6.5 in the non-limed control and increased as lime rates increased (Table 2). The pH values remained nearly identical to those measured 6 months after lime was applied in 2004 (8). Aphanomyces SIVs were high across all treatments, but were lower as lime rates increased in a significant linear relationship (Table 2).

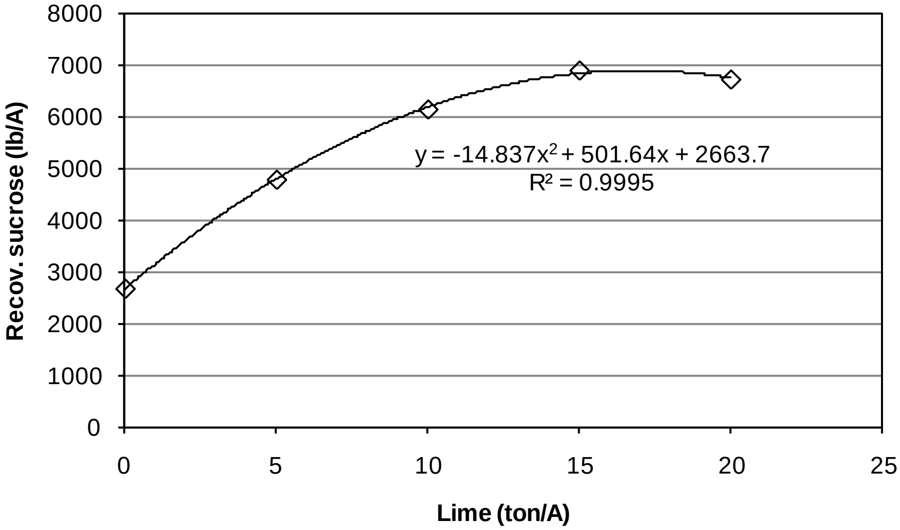

Fig. 1 Regression of recoverable sucrose A^{-1} vs. lime rate at Breckenridge, MN. Data is averaged across two varieties, a partially resistant variety with Tachigaren (45 g/unit) and a susceptible variety without Tachigaren. There was no significant lime rate x variety interaction.

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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 30 days after planting were not significantly different among rates of lime and the non-limed control, although stands tended to be highest at 10 to 20 wet weight lime A^{-1}. Numbers of harvested roots, however, were significantly higher for all rates of lime compared to the non-limed control. These results were related to severity of Aphanomyces root rot where disease was most severe in the non-limed control and increasingly less severe as rates of lime increased. Low numbers of roots and severe Aphanomyces root rot in the non-limed control also resulted in significantly lower root and sucrose yields compared to all rates of lime. Recoverable sucrose per acre was related to rate of lime and was optimal at 15 tons of wet weight lime A^{-1} (Fig. 1), when averaged across both varieties. Revenue per acre significantly increased linearly with increasing rates of lime (Table 2).

The partially resistant variety treated with Tachigaren resulted in significantly higher seedling stands, harvested roots, and root and sucrose yields compared to the susceptible variety (Table 2). The superior performance of the partially resistant variety was because of severe Aphanomyces disease pressure. Disease ratings were significantly higher for the susceptible variety (= 4.4, which averaged about 50% of each root diseased) compared to the partially resistant variety (= 2.9, which averaged about 15% of each root diseased).

DISCUSSION

Single soil applications of spent lime continued to have long-term, beneficial effects on sugarbeet in 2010, the seventh growing season since lime initially was applied, but intensity of response differed with location. At Hillsboro, Aphanomyces disease pressure was very mild and benefits of lime were weak but there was a trend toward improved yields in limed plots compared to the non-limed control. On the other hand, Aphanomyces was active at Breckenridge and all rates of lime significantly increased numbers of plants and root and sucrose yields compared to the non-limed control; optimal sucrose yields occurred at 15 ton wet weight lime A^{-1}. The partially resistant variety had lower Aphanomyces root rot ratings compared to the susceptible variety at both locations, even when disease pressure was mild at Hillsboro and severe disease at Breckenridge.

Currently, Aphanomyces soil index values (SIV) are high at both locations. Before lime was applied at Hillsboro, SIVs were moderate (average SIV = 48) and they initially declined with application of all rates of lime (8). Over the years, however, the SIVs at Hillsboro have gradually increased in all plots (12,13) and remained stable in 2010 (SIV = 74). Overall, SIVs equal to or greater than 75 indicate high potential for Aphanomyces root rot when soil is wet and warm. At Breckenridge, SIVs have been high (average SIV = 98) and they also initially declined with application of all rates of lime (8). Nearly 18 months later, SIVs in all plots (limed and non-limed) at Breckenridge had SIVs near 100 (12) and have remained high. The biggest difference between the two locations is the weather, which has a profound effect on disease severity. Hillsboro has been quite dry and unfavorable for Aphanomyces diseases every year since the trial was established, while Breckenridge has been wet and favorable for disease. Under these circumstances, it is surprising that soil index values have increased so quickly at Hillsboro. Apparently, even when sugarbeets are grown in a 3-year rotation and environmental conditions are not particularly favorable for *Aphanomyces* activity, enough infections occur (albeit at a low level) to increase concentration of inoculum of the pathogen in soil.

Application of spent lime increased soil pH at both locations within a few months (8), and these values have continued to remain relatively stable through 2010. *Aphanomyces* causes severe root rot of sugarbeet over a range of soil pH values from 5.5 to 8, so benefits of spent lime treatments are more complicated than increasing soil pH. More likely, the effect of spent lime and increased soil pH involves changes in the physical, chemical and biological environment of the soil and around roots (rhizosphere). Spent lime may affect nutrient uptake by plants, improve physical condition of soil (e.g. improve water drainage, which results in less root disease), increase beneficial microorganisms in the soil and rhizosphere, and/or induce plant resistance (6,7). Spent lime also may provide excess calcium ions (4) that interfere with production, motility and infectivity of infective zoospores produced by *Aphanomyces* and other oospore-forming pathogens (3,5).

Research will continue to explore the effects of spent lime on sugarbeet and rotation crops as well as the complex interactions among biological, chemical, and physical factors in lime-amended soil.

SUMMARY AND CONCLUSIONS

1. A single soil application of spent lime continued to have long-term effects in reducing Aphanomyces root rot and/or increasing sugarbeet yields in 2010, the seventh growing season after application.

2. Application of spent lime at Hillsboro (October, 2003) and Breckenridge (April, 2004) increased soil pH within a few months, and has remained stable into 2010 (baseline pH is 7.0 at Hillsboro and 6.3 at Breckenridge)

3. Aphanomyces SIV are increasing at Hillsboro with successive sugarbeet crops and remain high at Breckenridge.

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