A SINGLE FIELD APPLICATION OF SPENT LIME CONTINUES TO REDUCE APHANOMYCES ROOT ROT AND INCREASES SUGARBEET YIELDS DURING SIXTH GROWING SEASON

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Aphanomyces cochlioides (= *A. cochlioides*) is an economic pathogen infesting over 50% of acres 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. Storage of diseased roots in piles contributes to additional losses. *A. cochlioides* survives in soil for years, even when sugarbeet is not planted. Consequently, 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 infection by *A. cochlioides*. However, when inoculum densities of the pathogen are high, and soil is wet and warm, implementing these measures is 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 sugarbeet 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 less than 6.8) that were severely infested with the clubroot pathogen, *Plasmodiophora brassicae* in the Salinas Valley of 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 (1,17).

OBJECTIVES

Field experiments were evaluated for the long-term effect of a single soil application of spent lime on: 1.) Aphanomyces root rot, yield and quality of sugarbeet and 2.) yield and quality of rotation crops.

MATERIALS AND METHODS

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

Each site was divided into four, 1-acre experiments. Experiments included four rates of spent lime and an untreated control replicated four times in a randomized block design (Fig. 1). Treatments at Hillsboro were 0, 5, 10, 20 and 30 tons wet weight spent lime 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 measured 33

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

Hillsboro, ND

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

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

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 0, 5, 10, 20 and 30 tons wet weight spent lime A^{-1} and at Breckenridge with 0, 5, 10, 15 and 20 tons wet weight spent lime A^{-1}; experiments were arranged in a randomized block design and four replications (illustrated above for Breckenridge, MN). In 2004, all experiments were sown to corn at Hillsboro and wheat at Breckenridge to stabilize soil pH. In subsequent years, sugarbeet was planted in one of the experiments and other crops were sown in the other three experiments.

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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 was grown in one experiment each year from 2005 to 2008; the three other experiments were planted with the same crop grown in the field by the grower-cooperator (Fig. 1). Results have been reported from 2005 to 2008 (13-17).

2009 Sugarbeet field trials. Two Roundup Ready sugarbeet varieties, differing in susceptibility to Aphanomyces root rot, were sown in experiments last planted to sugarbeet in 2005 (Fig. 1). The susceptible variety had a disease rating of 7.3 (1-9 scale) and seed was not treated with Tachigaren; the partially resistant had a rating of 4.2 and was treated with 45 g Tachigaren per unit. Varieties were sown as subplots in lime-treated and control plots at both locations on June 1, 2009. 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 were fertilized to obtain maximum sucrose yield and quality and standard production practices were followed.

Stand counts were made three times within 40 days after planting. Plots were thinned to 6-inch spacing on July 3 at Hillsboro and July 20 at Breckenridge. Experiments were harvested on September 28 at Hillsboro and November 12 at Breckenridge (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.

2009 Rotation crop field trials. The three spent lime experiments not planted to sugarbeet were sown to soybean by the grower-cooperator at both locations. Plots were harvested at Hillsboro on November 5 with a small plot combine (Wintersteiger Seedmuch, Dimmelstrasse, Germany) by removing a 5 x 20 ft swath. Yields were adjusted to 13% moisture and calculated based on 60 lb per bushel. The experiment at Breckenridge was not harvested because of excessively wet field conditions.

2009 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 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 79 $\pm$ 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 to adjacent plants. 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*).

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

2009 Sugarbeet field trials, Hillsboro. In June, 68 months after lime was applied, soil pH was 7.2 in the non-limed control and was significantly higher (at or near pH of 8) in all limed plots (Table 1). These values were nearly identical to July, 2004, 9 months after spent lime was applied. Aphanomyces SIVs were significantly affected by lime treatment (Table 1). At 30 days after planting, stands were the same in limed and control treatments and for both varieties.

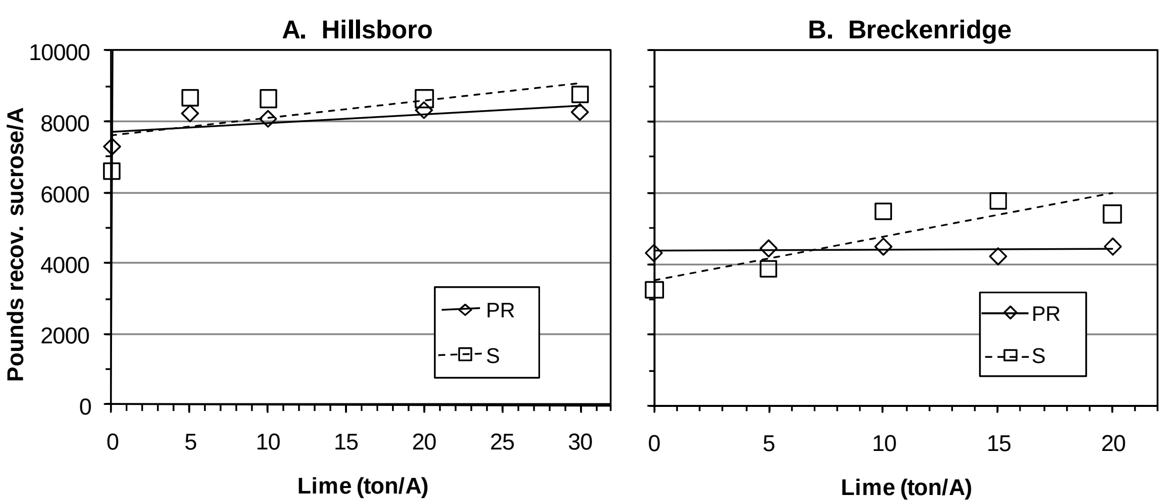

Fig. 2. Relationship of recoverable sucrose per acre of two sugarbeet varieties (partially resistant [PR] and susceptible [S] to *Aphanomyces cochlioides*) in limed and non-limed plots where there was a significant interaction ($P \leq 0.05$) between variety and rate of lime in 2009 at **A.)** Hillsboro (R^2 = 0.4601 for the PR variety, R^2 = 0.3983 for the S variety) and **B.)** Breckenridge (R^2 = 0.0317 for the PR variety, R^2 = 0.7673 for the S variety).

Table 1. **Hillsboro, ND:** Soil pH, Aphanomyces soil index values, stands, root rot ratings, and harvest data for sugarbeet sown June 1, 2009, 68 months after several rates of spent lime were applied in a field naturally infested with *Aphanomyces.*

Main treatments		Soil pH	Aph SIV (0-100)[x]	Stand@ 30 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	251	124	2.3	24.6	15.5	282	6935	778
5	3.3	7.8	79	251	131	1.8	29.0	15.9	292	8453	991
10	6.5	7.9	52	245	126	1.8	28.7	15.9	292	8367	982
20	13.0	8.0	88	239	126	1.9	29.0	16.0	293	8493	1002
30	19.5	7.9	78	253	131	1.8	29.4	15.8	289	8516	990
Linear[w]		***	NS	NS	NS	*	**	NS	NS	**	*
Quadratic[w]		**	*	NS	NS	*	*	NS	NS	*	*
Variety[v]											
Variety 1 + 0 Tach (susceptible)		-	-	199	128	2.0	28.2	15.9	292	8261	975
Variety 2 + 45 g Tach (partially resistant)		-	-	198	128	1.8	28.1	15.7	287	8045	922
P-value[w]		-	-	NS	NS	*	NS	*	*	NS	*
Lime x Var (linear)[w]				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 2009, sugarbeet was sown on June 1, 68 months 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 to *Aphanomyces* and not treated with Tachigaren (Tach) and the other was partially resistant and treated with 45 g Tach per unit of seed. Plots were harvested on September 28, 2009. 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 3.

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

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At harvest, there were no significant interactions between rate of lime and sugarbeet variety for most variables measured, so main treatments will be presented, along with interactions when they occur. Overall, Aphanomyces root rot ratings were low, but nevertheless, were lowest in all limed plots compared to the control (Table 1). Lime treatment did not significantly affect number of harvested roots, percent sucrose, or pounds of sucrose per ton. Yields of roots and recoverable sucrose per acre were significantly affected by treatment with lime. There was a significant (P = 0.01) linear relationship between rate of lime and pounds of recoverable sucrose per acre (Table 1), where increasing rates of lime increased yield (Fig. 2A). There also was an interaction between rate of lime and variety for recoverable sucrose (Table 1) where the susceptible variety produced more sucrose per acre with increasing rates of lime than the partially resistant variety (Fig. 2A). This interaction was further reflected in gross revenue per acre (Table 1).

Table 2. Breckenridge, MN: Soil pH, Aphanomyces soil index values, stands, root rot ratings, and harvest data for sugarbeet sown June 1, 2009, 62 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 RRI (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	6.7	100	220	124	3.9	15.0	13.8	252	3798	359
5	2.7	7.3	100	251	125	3.7	15.4	14.6	270	4146	435
10	5.3	7.7	100	254	140	3.4	18.3	14.7	272	4983	533
15	8.0	7.8	100	281	138	3.4	18.5	14.6	270	4986	523
20	10.6	7.8	100	281	154	3.2	18.4	14.6	270	4948	519
Linear[w]		***	NS	***	*	NS	NS	*	**	NS	*
Quadratic[w]		**	NS	NS	NS	NS	NS	*	**	NS	NS
Variety[v]											
Variety 1 + 0 Tach (susceptible)		-	-	238	129	3.7	17.7	14.5	269	4753	500
Variety 2 + 45 g Tach (partially resistant)		-	-	278	144	3.3	16.6	14.3	265	4391	447
P-value[w]		-	-	***	*	*	NS	*	NS	NS	*
Lime x Var (linear)[w]				***	*	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 2009, sugarbeet was sown on June 1, 62 months 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 to *Aphanomyces* and not treated with Tachigaren (Tach) and the other was partially resistant and treated with 45 g Tach per unit of seed. Plots were harvested on November 12, 2009. 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 20.

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

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2009 Sugarbeet field trials, Breckenridge. In June (62 months after lime was applied), soil pH was 6.7 in the non-limed control and was significantly affected by lime treatment (Table 2); pH values remained nearly identical to those measured 6 months after lime was applied. There were significant linear (P=0.001) and quadratic (P=0.01) relationships between soil pH and rate of lime where increasing rates of lime (5, 10 and 15 ton A^{-1}) increased soil pH. Aphanomyces SIVs, however, were equally high across all treatments.

Early in the season, *Aphanomyces* was active, and seedling stands were significantly affected by lime treatment (Table 2). Furthermore, there was a significant interaction between variety and rate of lime. Stands of the susceptible variety responded to treatment with lime much more than the resistant variety (Fig. 3). This effect on plant populations continued through harvest (Table 1).

Harvest was delayed until November 12 because of extremely wet soil conditions that necessitated hand harvesting. All taproots were malformed and sprangled, indicating most root development occurred in very wet soil. Attempts to rate roots for rot were compromised by root deformities, which sometimes also are caused by *Aphanomyces*. Disease ratings were unaffected by rate of lime but were significantly lower for the partially resistant variety than the susceptible variety (Table 2).

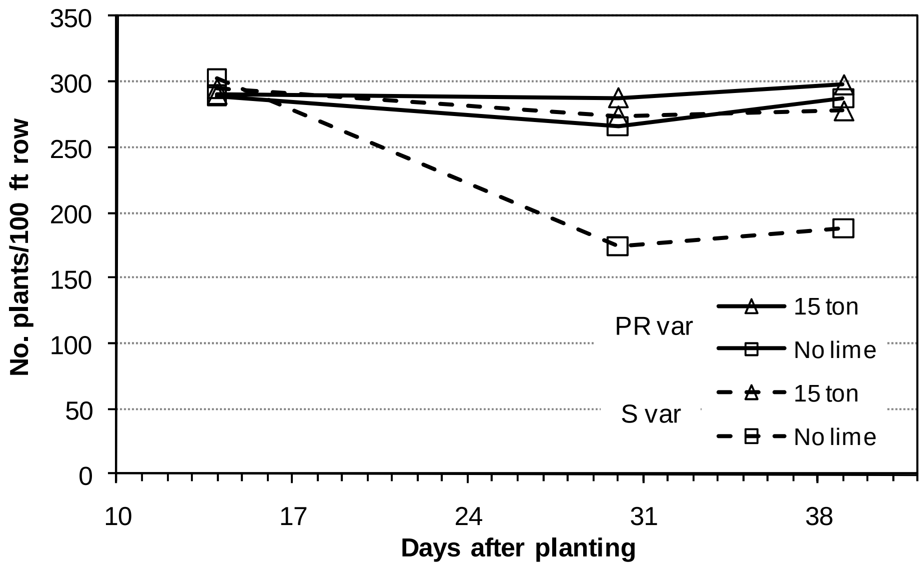

Fig. 3. Seedling stand of two sugarbeet varieties (partially resistant [PR] and susceptible [S] to *Aphanomyces cochlioides*) in limed and non-limed plots where there was a significant interaction ($P \leq 0.001$) between variety and rate of lime (illustrated for 15 ton wet weight spent lime compared to a non-limed control) at Breckenridge, MN in 2009.

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The only yield variables positively affected by lime were percent sucrose, pounds of sucrose per ton, and gross revenue per acre (Table 2). There was a significant interaction between rate of lime and variety for number of harvested roots, root yields, recoverable sucrose, and gross revenue. For these factors, the *Aphanomyce*s-susceptible variable performed more favorably than the partially resistant variety. The interaction between variety and rate of lime is illustrated for recoverable sucrose per acre in Fig. 2B; the susceptible variety had a strong positive response to increasing rates of lime, while the partially resistant variety showed no response to any treatment.

Rotation Crop. Soybeans harvested from the three experiments at Hillsboro were unaffected by lime compared to the non-limed control and averaged 42.3 bushels per acre, 33.3% protein, and 17.3% oil (data not shown). Soybeans at the Breckenridge experiment were not harvested because of excessively wet soil.

DISCUSSION

Single soil applications of spent lime continued to have long-term, beneficial effects on sugarbeet in 2009, the sixth growing season since lime initially was applied. Intensity of response differed with location in 2009 and in comparison to previous years. At Hillsboro in 2009, root and sucrose yields were significantly higher in limed than in non-limed control plots. At this site in 2008, yields showed a trend in being higher in limed verses non-limed plots (17). At Breckenridge in 2009, there was a trend for higher yields in limed plots compared to the non-limed control. This is in contrast to results every year from 2005 to 2008, where there were significant increases in sucrose yield and quality and reductions in Aphanomyces root rot in limed plots compared to non-limed plots (17). Sucrose yields were very good at Hillsboro and marginal at Breckenridge despite both sites being planted on June 1, 2009. Differences in yield likely are attributable to favorable weather for sugarbeet growth at Hillsboro and excessively wet conditions for most of the growing season at Breckenridge.

In 2009, Aphanomyces SIV's at Hillsboro were higher in all plots than in previous years and this reflects a trend for increasingly higher SIVs with each consecutive year of sugarbeet production (despite 3 years between sugarbeet crops). At Breckenridge, SIVs have remained at or near 100 since lime was applied to soil in 2004 (17). Overall, SIVs equal to or greater than 80 indicate high potential for Aphanomyces root rot when soil is wet and warm. Aphanomyces root rot, however, was mild at Hillsboro because of the cool growing season and the susceptible variety had higher yields than the partially resistant variety. This is because under low (or no) disease pressure from

Aphanomyces, this particular susceptible variety out-yields the partially resistant variety (6). At Breckenridge, roots likely were rated too high for Aphanomyces root rot because disease and excessive soil moisture both distort roots and their effects cannot be visually separated. Furthermore, the susceptible variety had higher yields and quality than the partially resistant variety, which indicates Aphanomyces disease pressure was low in a season distinguished by high moisture and low temperatures.

It is unknown why spent lime reduces Aphanomyces root rot and/or increases sugarbeet yields. The amendment contains nitrogen, phosphorus, potassium, and other inorganic and organic nutrients (7) obtained from the sucrose extraction process. These nutrients may alter the soil environment and the rhizosphere (area around roots of intense microbial activity stimulated by root exudates). Various types of soil amendments reduce some soilborne diseases (3,5,9,12) because they create complex interactions among biological, chemical, and physical factors. These interactions alter nutrient uptake by plants, improve physical condition of soil (e.g. improving water drainage, which results in less root disease), increase beneficial microorganisms in the soil and rhizosphere, and induce plant resistance (11,12). Spent lime also may provide excess calcium ions (8) that interfere with production, motility and infectivity of infective zoospores produced by *Aphanomyces* and other oospore-forming pathogens (4,10).

Application of spent lime at both sites increased soil pH within a few months (13), and these values continued to remain relatively stable through 2009. *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 increased soil pH after application of spent lime involves changes in availability of micronutrients to the root, increases of beneficial microorganisms in the rhizosphere and/or other factors.

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 2009, the sixth growing season after the amendment was applied.

2. Yield and quality of a soybean rotation crop was the same in limed and non-limed plots.

3. Application of spent lime at Hillsboro (October, 2003) and Hillsboro (April, 2004) increased soil pH within a few months, and has remained stable into 2009 (baseline pH is 7.0 at Hillsboro and 6.3 at Breckenridge)

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

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