LONG-TERM EFFECTS OF A SINGLE APPLICATION OF SPENT LIME ON SUGARBEET, APHANOMYCES ROOT ROT, ROTATON CROPS, AND ANTAGONISTIC MICROORGANISMS

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Aphanomyces cochlioides (= *A. cochlioides*) is a serious economic pathogen and infests 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 root rot of older plants. Storage of diseased roots in piles contributes to additional losses. *A. cochlioides* persists in soil for years. Consequently, growing sugarbeet in infested fields requires all available control options including early planting of partially resistant varieties treated with the fungicide Tachigaren and various cultural practices (e.g., cultivation and improved drainage) to avoid or lessen infections by *A. cochlioides*. However, when inoculum levels of the pathogen are high and soil is wet, implementation of these measures is inadequate for economic yields and fields often are abandoned or yield poorly. This chronic situation has generated interest in finding effective, alternative methods to control *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 sugarbeet industry 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. Literature on use 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 other nutrients. In Great Britain, it is marketed and sold as LimeX to conventional and organic growers. In the late 1970s, Campbell and Greathead (3) applied spent lime from a near-by sugarbeet processing factory at 2 to 4.5 tons A^{-1} in fields (pH less than 6.8) in the Salinas Valley of California that were severely infested with the clubroot pathogen, *Plasmodiophora brassicae*. A single application of spent lime gave "virtually complete control" of clubroot of crucifer crops 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.

Growers in southern Minnesota started applying spent lime (4 to 8 tons wet weight A^{-1}) to sugarbeet fields in the late 1990s to increase soil pH and reduce carryover of the soybean herbicides Pursuit and Raptor (1), which persist in soil and are toxic to sugarbeet. Spent lime increased sugarbeet yields in fields with and without herbicide carry-over (1) - and less Aphanomyces root rot was observed. Consequently, growers have continued to apply spent lime the year before planting sugarbeet (typically every 3 years). Recently, growers in the RRV also have been applying spent lime to their sugarbeet fields. In earlier trials in the RRV, spent lime (3 and 10 tons wet weight A^{-1}) was applied in two *Aphanomyces*-infested fields (baseline pH values of 5.9 and 7.8) and within 1 year, there were significant reductions in Aphanomyces root rot and increases in sucrose yields compared to the non-limed control (2). In 2003, a producer in Breckenridge, MN observed healthy sugarbeet roots in a 5-acre portion of a field where spent lime (20 to 25 tons wet weight A^{-1}) had been applied 7 years earlier - while the remainder of the field had poor stand, stunted growth, and severe Aphanomyces root rot.

It is unknown why spent lime reduces Aphanomyces root rot and increases sugarbeet yields. It contains a wide variety of macro- and micro-nutrients and organic compounds obtained from the sucrose extraction process that may alter the soil and the rhizosphere (area around roots of intense microbial activity stimulated by root exudates) environments. Various types of amendments reduce some soilborne diseases (4) because they result in complex interactions among biological, chemical, and physical factors in the soil. These interactions alter nutrient uptake by plants, improve physical condition of soil, and increase beneficial microorganisms in the soil and rhizosphere (4, 6, 10). Soils suppressive to some soilborne pathogens are sources of biological control agents, which can be increased in the laboratory and applied as seed treatments or soil amendments (10). Types of biological control agents include general bacterial populations or specific groups, such as fluorescent pseudomonads and streptomyces. No data are available on amounts of spent lime needed to reduce disease, duration of disease suppression and/or yield benefits, or the mechanisms (biological, chemical, and physical) involved.

OBJECTIVES

In 2007, objectives included measuring effects of spent lime applications made October, 2003/April, 2004 on: 1.) Aphanomyces root rot, yield, and quality of sugarbeet; 2.) root rot, growth, and yield of rotation crops; 3.) Aphanomyces soil index values; and 4.) populations of microorganisms in sugarbeet plots and rhizosphere soil.

Long-term goals of this research are to develop management practices for application of spent lime (amounts needed to reduce Aphanomyces root rot, duration of disease suppressive effects), elucidate underlying mechanisms of disease suppression, recycle nutrients in an economic and environmentally sound manner, and reduce storage of spent lime at sugarbeet processing factories.

MATERIALS AND METHODS

Establishment of field trials. Experiments were established at Hillsboro, ND (pH = 7.02) in mid October, 2003 and at Breckenridge, MN (pH = 6.3) in mid April, 2004. The Hillsboro site has a history of moderate root rot and the Aphanomyces soil index value (SIV) averaged 48 (0 to 100 scale, 0 = no disease, 100 = disease severe). Breckenridge has a history of severe root rot and the Aphanomyces SIV averaged 98. Each site was divided into four, 1-acre experiments; each experiment included four rates of spent lime and an untreated control and was replicated four times in a randomized block design. Treatments at Hillsboro were 0, 5, 10, 20 and 30 tons wet weight of spent lime A^{-1} (= 0, 3.3, 6.5, 13, and 19.5 tons dry weight A^{-1}, 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 A^{-1}, respectively). Each treatment plot measures 33 x 60 ft. Four experiments were established per location so sugarbeet could be sown in one experiment each year from 2005 to 2008; the three experiments not sown with sugarbeet in these years are sown with the same crop as grown in the field and maintained by the grower-cooperator. This approach allows evaluation of spent lime applications on sugarbeet and other crops in the rotation every growing season through 2008. To allow lime treatments to stabilize in 2004, corn 'DeKalb 3551RR' was sown across the four experiments at Hillsboro and wheat 'Grandin' was sown at Breckenridge (12). Sugarbeet was planted in one of the four experiments at both locations in 2005 and 2006 and results have been reported (13, 14).

2007 Sugarbeet field trials. Sugarbeet was sown in one of the four experiments at Hillsboro on May 10 and Breckenridge on May 16. 'Seedex Alpine' (partially resistant to *Aphanomyces* + 45 g of Tachigaren per unit of seed) and 'Hilleshog 2467RZ' (susceptible, no Tachigaren) were sown as subplots within lime-treated and control plots. Seed was sown every 2 inches in rows 60-feet long and 22-inches apart (six rows of each variety centered within each plot). A pre-plant application of the herbicide Nortron (3.75 lb a.i. A^{-1}) was incorporated into soil and the insecticide Counter 15G (12 lb product A^{-1}) was applied modified in-furrow at planting. After sugarbeet seedlings emerged, 10 feet of row was cut from the front and back of each plot, resulting in rows 40 feet long. Microrates of Progress (or Betamix or Betanex at 9-12 fl oz or Betanex at 12-16 fl oz A^{-1}, respectively) + UpBeet + Stinger + Select + MSO (5.7 fl oz + 0.125–0.5 oz + 1.3 fl oz + 2-6 fl oz + 1.5% A^{-1}, respectively) were applied at Hillsboro on June 2 and 27 and July 2 and 9 and at Breckenridge on June 20 and 27 and July 5. Plants were hand-thinned to a 6-nch spacing on June 25 at Hillsboro and to a 4-inch spacing (because of considerable early-season stand loss caused by *Aphanomyces*) on June 28 at Breckenridge. Plots at Breckenridge were cultivated on June 14. At Hillsboro, Cercospora leaf spot was controlled with one application of Eminent (13 oz A^{-1}) on August 2 and at Breckenridge, with Eminent (13 fl oz A^{-1}) and Headline (9 fl oz A^{-1}) on August 2 and 16, respectively (20 gpa at 100 psi). Alleys separating replicates were rototilled throughout the season.

Stand counts were made at 21 to 34 days after planting and after thinning at both locations. Experiments were harvested on September 19 and 25 at Hillsboro and Breckenridge, respectively (two middle rows of each variety per treatment). Twenty roots were randomly selected from each subplot and 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.

2007 Rotation crop field trials. The three spent lime experiments at Hillsboro not planted to sugarbeet were sown with soybean 'Wensman 2090' in rows 22 inches apart on May 14 by the grower-cooperator. Plots were harvested with a small plot combine (Wintersteiger Seedmuch, Dimmelstrasse, Germany) on October 15 by removing a 5 x 20

ft swath. Yields were adjusted to 13% moisture and calculated based on 60 lb per bushel; protein and oil content were determined with an Infratec 1229 grain analyzer.

At Breckenridge, the three spent lime experiments not sown to sugarbeet were planted to wheat 'Agri-Pro Freyr' on May 1 by the grower-cooperator. Plant densities were determined indirectly by counting numbers of tillers per square meter on August 1. Twenty-five plants also were removed per plot and subcrown internodes were rated for common root rot (caused primarily by *Bipolaris sorokiniana* and *Fusarium graminearum*). Roots were rated on a scale of 0 to 4 (0 = no lesions, 4 = more than 66% of surface with lesions (13). Plots were harvested with a small plot combine (Wintersteiger Seedmuch, Dimmelstrasse, Germany) on August 1 by removing a 5 x 25 ft swath; yields were adjusted to 13.5% moisture and calculated based on 60 pounds per bushel.

Aphanomyces soil index values (SIVs). Soil samples were collected from plots (including subplots where two sugarbeet varieties were grown in 2006) at Hillsboro on May 23, and Breckenridge on June 20 (total of 100 plots per location). Six soil cores (2.5-inch diameter x 6-inch depth) were collected randomly across each plot and combined. Soil samples were screened through 0.25-inch hardware cloth to remove debris and improve consistency and then stored in a walk-in cooler until assayed (usually within 1 month after collection).

Soil samples were assayed to determine Aphanomyces soil index values (SIVs), which indicate potential for Aphanomyces diseases and populations of *A. cochlioides*. Twenty-five sugarbeet seed of 'ACH 261' were sown per pot (four pots per soil sample) to "bait" *A. cochlioides* from soil. Pots were placed in a controlled environment chamber in a randomized block design at 70 $\pm$ 2^0F for 1 week for optimal emergence. Temperatures then were increased to 79 $\pm$ 2^0F (14 hour photoperiod) and soil was kept moist to favor infection and disease development. Stand counts were made three times weekly starting at emergence. Dying seedlings were removed at each stand count to prevent disease from spreading to adjacent plants. At 4 weeks after planting, surviving seedlings were rated for disease on a 0 to 3 scale (0 = healthy, 3 = stem and root brown, constricted, and plant dead). Disease ratings and numbers of dead seedlings during the 4-week assay were used to calculate an Aphanomyces SIV (0 to 100 scale, 0 = *Aphanomyce*s-free and 100 = soil severely infested with *A. cochlioides*).

To determine soil pH, small quantities of soil from all plots collected in May and June, 2007 were air-dried at least 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).

Microorganisms. Sugarbeet root and soil samples were collected from Breckenridge and Hillsboro near harvest in 2006 and 2007 and assayed to determine populations of bacteria and whether they were antagonistic to *A. cochlioides*. Roots of Seedex Alpine were removed from the non-limed control and plots treated with 10 tons of spent lime A^{-1} in four replicates. Soil also was collected between rows of the non-limed and 10 ton plots (six soil cores, 1-inch diameter x 6-inch depth, and combined). Root and soil samples were stored at 39 ^{0}F until assayed.

Sugarbeet root surfaces were smooth and had little adhering soil, so rhizosphere samples were collected along the lateral root grooves (also sites for infection by *A. cochlioides*). A cork borer (1.3-cm diameter) was inserted about 1-2 mm deep into the lateral root zone at approximately 1-, 2.5-, and 4-inch depths below the soil surface. Each core was gently removed (root surface with a shallow layer of lateral, secondary roots plus adhering soil). Five roots per plot were sampled in this matter and combined (total of 30 cores or about 40 cm^2). Rhizosphere cores were placed in a flask containing 100 ml of 0.15% sterile water and 50 g of glass beads, agitated on a rotary shaker for 30 minutes, and serially diluted at 10-fold increments in flasks containing 0.15% water agar. Then, 1 ml of suspension was pipetted onto each of three Petri dishes containing various culture media: 1/10-strength tryptic soy agar (TSA) for isolation of cultureable bacteria, Kings B medium for fluorescent pseudomonads, and STR medium for streptomyces. Serial dilutions on appropriate media were "bracketed" to ensure reasonable populations for counting. Plates were incubated at recommended times and temperatures before counting. Oven-dry weights of rhizosphere samples were determined by pipetting 50 ml of suspension from the first flask of each dilution series into aluminum cups, which were placed in an oven at 221 ^{0}F for 1 day and then re-weighed.

For each soil sample, microorganisms were quantified by placing the equivalent of 10 g of oven-dry soil (based on previously determined moisture content) in a flask containing 100 ml of 0.15% water agar. Samples then were diluted and cultured for groups of bacteria, as previously described for rhizosphere samples.

Antagonism to *A. cochlioides*. From 24 to 36 cultures of bacteria (streptomyces and fluorescent pseudomonads) were randomly selected from rhizosphere and soil assays of the 0 and 10 ton lime samples at both locations (6 to 9 cultures/replicate/treatment/location) in 2006 and 2007. In 2006, a total of 267 cultures of streptomyces and 252 fluorescent pseudomonads were tested for antagonism to *A. cochlioides* with a dual-culture technique on potato dextrose agar (PDA, 20 ml/9-cm diameter Petri plate). A small piece (5-mm diameter) of *A. cochliodes* actively growing on PDA was placed about 1-cm inside the edge of the Petri dish. Then, a streak (~ 4 cm long) of an actively growing culture of streptomyces (growing on PDA) or fluorescent pseudomonad (growing on PDA) was made on the opposite side of the plate, about 5 cm from *A. cochlioides*. Assays for each bacterial culture were replicated three times. The dual plate assays were incubated at 70 ±2 ^{0}F for 7 days and then measured for antibiosis against *A. cochlioides* using a 1 to 5 scale. A rating of 1 = zone of inhibition between bacterium and *A. cochlioides* was more than 5 mm, 2 = 1.5 mm zone of inhibition, 3 = *A. cochlioides* and bacteria touch, 4 = *A. cochlioides* overgrows bacterial streak but does not cover entire plate, and 5 = *A. cochlioides* grows over bacterium and covers entire plate. Bacteria with a score of 1 were ranked as antagonistic to *A. cochlioides*. Populations of bacteria antagonistic to *A. cochlioides* then were calculated as a percent of the total number isolated per treatment. Cultures collected in 2007 have not yet been assayed for antagonism.

Data analysis. Field data were transformed when appropriate, and subjected to analysis of variance. If significant (P = 0.05), means were separated by Least Significant Difference (LSD). Regression analyses also were calculated to determine the rate of spent lime needed to maximize pounds of sucrose recovered per acre.

RESULTS

2007 Sugarbeet field trials: *Hillsboro*. Soil pH in non-limed plots averaged 7.55 (Table 1). All rates of spent lime resulted in significantly higher pH values compared to the control and there were small increases in pH values with increasing rates of lime (Table 1). Soil pH levels in all limed and non-limed plots in 2007 were slightly higher than levels recorded in July, 2004, 9 months after spent lime was applied (12).

There were no significant interactions between rate of lime and sugarbeet variety, so results are presented separately for these main effects (Table 1). Some seedlings were infected by *A. cochlioides* early in the season but there were no significant differences in stands in limed and non-limed plots. There was a trend, however, for higher stands in limed plots than in the control. Plant populations were uniform after thinning. By harvest, stands had declined about 15% and there were no significant differences among treatments. Root rot ratings were negligible and averaged 1.8 (a rating of 2 = root is large with superficial scarring on less than 5% of root surface). Yield (tons of roots A^{-1}), percent sucrose, pounds of sucrose ton^{-1}, pounds of recoverable sucrose A^{-1}, and gross economic return A^{-1} were not significantly different among limed and non-limed plots, but tended to increase as rates of lime increased. Regression analysis revealed a significant (P = 0.05) linear relationship between amount of spent lime applied and yield of recoverable sucrose A^{-1} (Fig. 1A).

The sugarbeet variety with partial resistance to *A. cochlioides* (Seedex Alpine) had significantly higher stands than the susceptible variety (HM 2467RZ) at 21 and 34 days after planting and at harvest (Table 1). Seedex Alpine also had significantly lower Aphanomyces root rot ratings than the susceptible variety, although disease ratings were low. Seedex Alpine had significantly higher yields (tons of roots A^{-1}) but significantly lower percent sucrose, and pounds of sucrose ton^{-1} than the susceptible variety. Yet, Seedex Alpine resulted in significantly higher pounds of recoverable sucrose A^{-1} and gross economic return compared to the susceptible variety.

Breckenridge. Soil pH in non-limed plots averaged 6.38 and all rates of spent lime significantly increased soil pH (Table 2). Soil pH levels of samples collected in 2007 were nearly identical to measurements made in September, 2004 (12), 5 months after spent lime was applied.

Analysis of variance indicated significant main effects of lime rate and variety for most stand and yield variables measured. There also were significant interactions between rate of spent lime and sugarbeet variety for most data, so to illustrate interactions, results are presented separately for each variety (Table 2). Weather was very favorable for Aphanomyces during the early part of the growing season. By 35 days after planting, stands were higher for all

Table 1. Hillsboro, ND: Soil pH and stands, root rot ratings, and harvest data for sugarbeet sown on May 10, 2007 (43 months after several rates of spent lime were applied in October, 2003) in a field naturally infested with a moderate population density of *Aphanomyces cochlioides*. The trial was harvested on September 19, 2007.

Main treatments		Soil pH	No. plants/80-ft row (Days after planting)[X]			No. roots Harvested/	RRR	Yield	Sucrose			Gross return
			21	34	Post-thinning	80 ft row	0-7[Y]	(Ton/A)	%	lb/T	lb recov./A	($/A)
Lime (Ton/A)[V]												
Wet wt.	Dry wt.											
0	0	7.55 a	233	252	136	108	1.9	17.5	16.2	300	5215	556
5	3.3	7.88 b	247	274	138	111	1.8	16.7	16.2	301	4992	535
10	6.5	8.03 bc	250	267	138	114	1.7	19.3	16.4	304	5849	637
20	13.0	8.06 bc	247	270	136	117	1.7	19.2	16.5	306	5831	637
30	19.5	8.09 c	237	263	142	120	1.7	20.4	16.8	312	6335	707
LSD ($P = 0.05$)[Z]		0.18	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Variety[W]												
HM 2467RZ (0 Tach)		-	233 a	248 a	138	108 a	1.9 a	16.2 a	17.1 a	318 a	5156 a	591
Seedex Alpine + 45 g Tach		-	252 b	283 b	138	120 b	1.7 b	21.1 b	15.7 b	291 b	6132 b	638
LSD ($P = 0.05$)[Z]			10.2	7.9	NS	5.2	0.14	1.9	0.4	9.1	584	NS

[V] Spent lime was applied in October, 2003 in a randomized block design of four replicates and incorporated by cultivation. This trial was sown with wheat in 2004, fallowed in 2005, and planted to corn in 2006. On May 10, 2007, the trial was sown with two sugarbeet varieties. For rates of lime, each value is averaged across both sugarbeet varieties.

[W] Sugarbeet varieties HM 2467RZ (susceptible to *Aphanomyces*) and Seedex Alpine (partially resistant to *Aphanomyces* and treated with 45 g of Tachigaren [Tach] per unit of seed) were sown as subplots within each spent lime treatment plot. For variety, each value is averaged across all lime treatments and the non-limed control.

[X] Plots were sown at 142,560 seeds per acre (seed every 2 inches in rows 22 inches apart) and hand-thinned to a 6-inch spacing on June 25, 2007; post-thinning stand counts were made on June 29.

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

[Z] LSD = Least significant difference, $P = 0.05$; for each column, values followed by the same letter are not significantly different; NS = not significantly different.

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rates of lime compared to the non-limed control for both varieties, but the susceptible variety (HM 2467RZ without Tachigaren) had much lower stands in the non-limed control. Stands for the susceptible variety were so low in the control that after thinning, they still were lower than all limed plots. Stands after thinning were similar across all lime rates for the resistant variety (Seedex Alpine = 45g Tachigaren). At harvest, stands in non-limed plots of the susceptible variety were very low and were two- to three-times higher in limed plots. Stands in the non-limed control of the resistant variety were as high as in limed plots of the susceptible variety.

Aphanomyces root rot ratings were considerably higher in the non-limed control plots of the susceptible variety (6.1 root rot rating = >75% of root rotted) than the resistant variety (= 3.8) (Table 2). Lime reduced root rot ratings of both varieties.

Table 2. Breckenridge, MN: Soil pH and stands, root rot ratings, and harvest data for two sugarbeet varieties sown on May 16, 2007 (37 months after several rates of spent lime were applied in April, 2004) in a field naturally infested with a high population density of *Aphanomyces cochlioides*. The trial was harvested on September 25, 2007.

Treatments		Soil pH	No. plants/80-ft row (Days after planting)[Y] 35	No. plants/80-ft row (Days after planting)[Y] Post-thinning	No. roots harvested/ 80-ft row	RRR 0-7[Z]	Yield (Ton/A)	Sucrose %	Sucrose lb/T	Sucrose lb recov./A	Gross return ($/A)
Lime rate p-value		<0.001	<0.001	0.015	0.002	<0.001	<0.001	0.044	0.079	<0.001	0.002
Variety p-value		-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.339
Interaction p-value		-	0.015	0.004	0.056	<0.001	0.004	0.083	0.105	0.006	0.016
HM 2467RZ (0 Tach)[W]											
Lime (Ton/A)[X]											
Wet wt.	Dry wt.										
0	0	6.38 a	35	34	29	6.1	6.4	14.4	251	1633	140
5	2.7	7.31 b	121	86	73	4.0	17.3	15.4	270	4693	446
10	5.3	7.54 bc	137	86	82	3.3	18.0	16.5	293	5294	555
15	8.0	7.65 c	156	94	83	3.3	19.2	16.0	281	5407	539
20	10.6	7.61 c	167	103	99	3.0	20.3	16.2	290	5877	608
Seedex Alpine + 45 g Tach [W]											
Lime (Ton/A)[X]											
Wet wt.	Dry wt.										
0	0	6.38 a	150	112	87	3.8	15.6	14.4	255	4021	353
5	2.7	7.31 b	186	111	94	3.2	19.3	14.8	261	5021	447
10	5.3	7.54 bc	220	116	111	3.0	21.6	15.3	270	5793	542
15	8.0	7.65 c	206	119	105	2.9	20.4	15.0	263	5361	486
20	10.6	7.61 c	213	128	116	2.7	22.5	15.2	271	6083	576

[W] Sugarbeet varieties HM 2467RZ (susceptible to *Aphanomyces*) and Seedex Alpine (partially resistant to *Aphanomyces* and treated with 45 g of Tachigaren [Tach] per unit of seed) were sown as subplots within each spent lime treatment and control plot. Each value is an average of four replicates.

[X] Spent lime was applied in April, 2004 in a randomized block design of four replicates and incorporated by cultivation. The trial was sown with wheat in 2004 and 2005 and with soybean in 2006.

[Y] Plots were sown on May 16, 2007 at 142,560 seeds per acre (seed every 2 inches in rows 22 inches apart) and hand-thinned to 4-inch spacing on June 26, 2007; post-thinning stand counts were made on June 29.

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

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Similarly, harvest variables including yield (ton A^{-1}), pounds of recoverable sucrose A^{-1}, and gross return A^{-1} were much lower in non-limed plots of the susceptible variety than in the non-limed and limed plots of the resistant variety. Yield variables of both varieties increased with lime rates, but the susceptible variety showed greater increases because the non-lime control was so poor. Percent sucrose increased in both varieties grown in limed plots, although the susceptible variety tended to have a higher percent sugar and thus, more pounds of sucrose ton^{-1}. Regression analyses between amount of spent lime applied and yield of recoverable sucrose A^{-1} (Fig. 1B) illustrate the different responses of the two varieties to rates of lime. The susceptible variety had a much steeper response to lime primarily because sucrose yields were much lower in the non-limed control.

2007 Rotation crop field trials. At Hillsboro, there were no significant effects of spent lime on soybean yields or on percent protein or oil compared to the non-limed control (Table 3). Similarly, at Breckenridge application of spent lime had no significant effect on wheat, as measured by number of tillers, root rot rating, or yield compared to the control (Table 4).

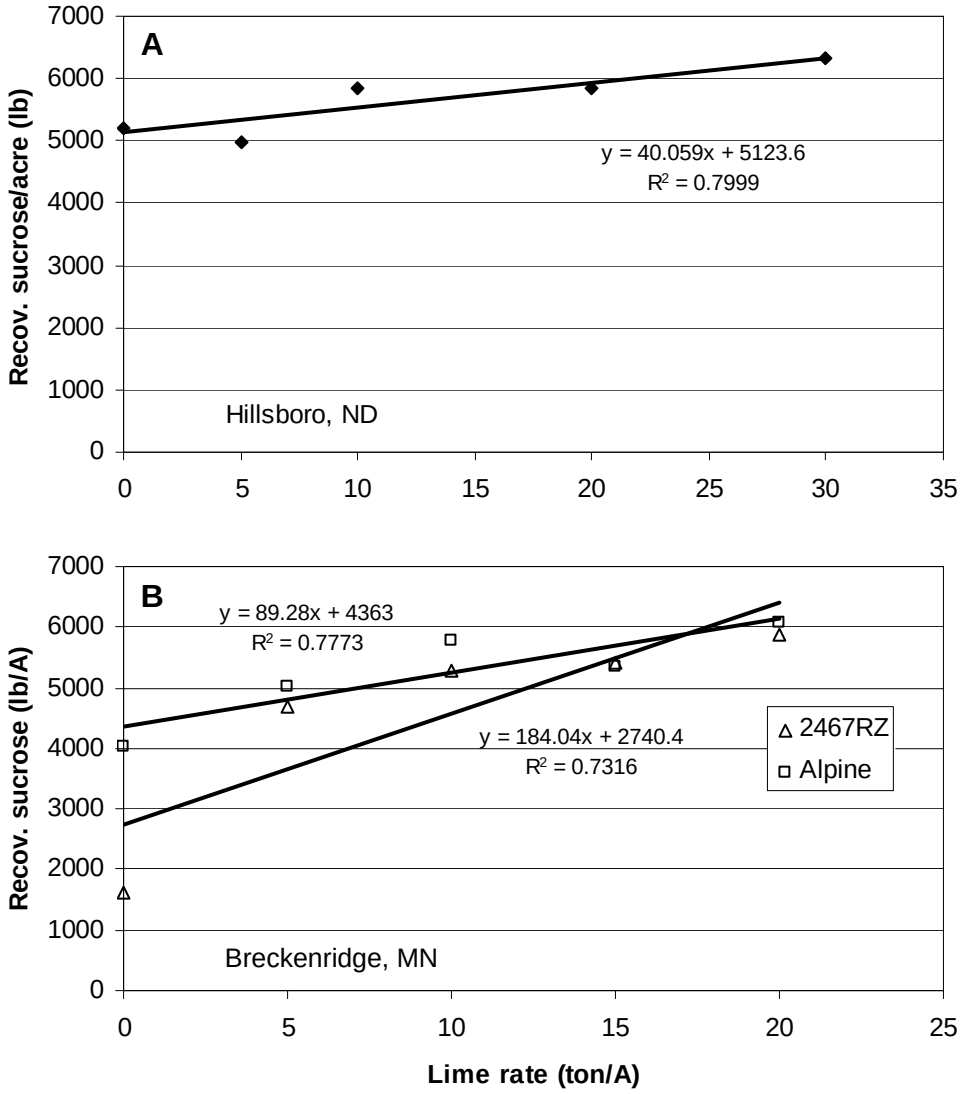

Fig. 1. Regression analysis of recoverable sucrose per acre in 2007 verses rate of spent lime (wet weight per acre) at **A.)** Hillsboro, ND (applied October, 2003; significant at $P = 0.05$); there were no lime rate by variety interactions so data are averaged for both varieties (HM 2467RZ and Seedex Alpine) and **B.)** Breckenridge, MN (applied May, 2004); there was a significant lime rate by variety interaction so varieties are shown separately; HM 2467RZ at $P = 0.065$; Seedex Alpine significant at $P = 0.05$.

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Aphanomyces soil index values (SIVs). When averaged across the four experiments at Hillsboro, soil treated with 0, 5, 10, 20, 30 tons of spent lime A^{-1} had SIVs of 54, 44, 40, 44, and 35, respectively (data not shown). Although there was a tendency for lower SIVs as rates of lime increased, there were no significant differences among limed or non-limed treatments. At Breckenridge, SIVs were extremely high across the four experiments. For 0, 5, 10, 15 and 20 tons of lime A^{-1} SIVs averaged 99, 96, 92, 84, and 78, respectively (data not shown). Aphanomyces SIVs were lower as rates of lime increased and also were significantly different at 15 and 20 tons of lime compared to lower rates and the control.

Table 3. Hillsboro, ND: Yield and quality of soybean 'Wensman 2090' sown on May 14, 2007 (43 months after several rates of spent lime were applied in October, 2003) in a field naturally infested with a moderate population density of *Aphanomyces cochlioides*. Plots were harvested on October 15, 2007.

Lime (Ton/A)[W]		Yield	Quality[Y]	
Wet weight	Dry weight	(bu/A)[Y]	Protein (%)	Oil (%)
0	0	48	32.1	18.3
5	3.3	50	32.3	18.3
10	6.5	49	32.2	18.2
20	13.0	47	32.5	18.1
30	19.5	47	32.4	18.1
LSD ($P = 0.05$)[Z]		NS	NS	NS

[W] 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. The four experiments were sown with wheat in 2004; one experiment was sown with sugarbeet and the other three were fallowed in 2005; one experiment was sown with sugarbeet and the other three with corn in 2006. In 2007, plots were sown with soybean 'Wensman 2090'.

[X] Yields were adjusted to 13% moisture and based on 60 pounds per bushel.

[Y] Protein and oil were determined with an Infratec 1229 grain analyzer.

[Z] LSD = Least significant difference, $P = 0.05$; NS = not significantly different.

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Table 4. Breckenridge, MN: Plant populations, root rot ratings, and yield of wheat 'Agri-Pro Freyr' sown on May 1, 2007 (37 months after several rates of spent lime were applied in April, 2004) in a field naturally infested with a high population density of *Aphanomyces cochlioides*. Plots were harvested on August 1, 2007.

Lime (Ton/A)[V]		Plant populations	Root rot rating	Yield
Wet weight	Dry weight	(No. tillers/m^2)[W]	(0-3)[X]	(bu/A)[Y]
0	0	426	2.0	49.5
5	3.3	467	1.8	46.8
10	6.5	444	1.8	50.8
20	13.0	436	1.9	47.7
30	19.5	448	1.9	49.3
LSD ($P = 0.05$)[Z]		NS	NS	NS

[V] 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 2004, the four experiments were sown with wheat; in 2005, one experiment was sown with sugarbeet and the other three with wheat; and in 2006, one experiment was sown with sugarbeet and the other three with soybean. In 2007, experiments were sown with wheat 'Agri-Pro Freyr'.

[W] Population densities of wheat were measured on August 1, 2007 by counting the number of tillers in a square meter. Each value is an average of 12 plots.

[X] Subcrown internodes from 25 random plants per plot were rated on August 1, 2007 with a 0-3 scale; 0 = clean, 3 = >50% of subcrown internodes dark-brown (necrotic). Each value is an average of 12 plots.

[Y] Wheat plots were harvested on August 1, 2007. Yields were adjusted to 13.5% moisture and based on 60 pounds per bushel. Each value is an average of 12 plots.

[Z] LSD = Least significant difference, $P = 0.05$; NS = not significantly different.

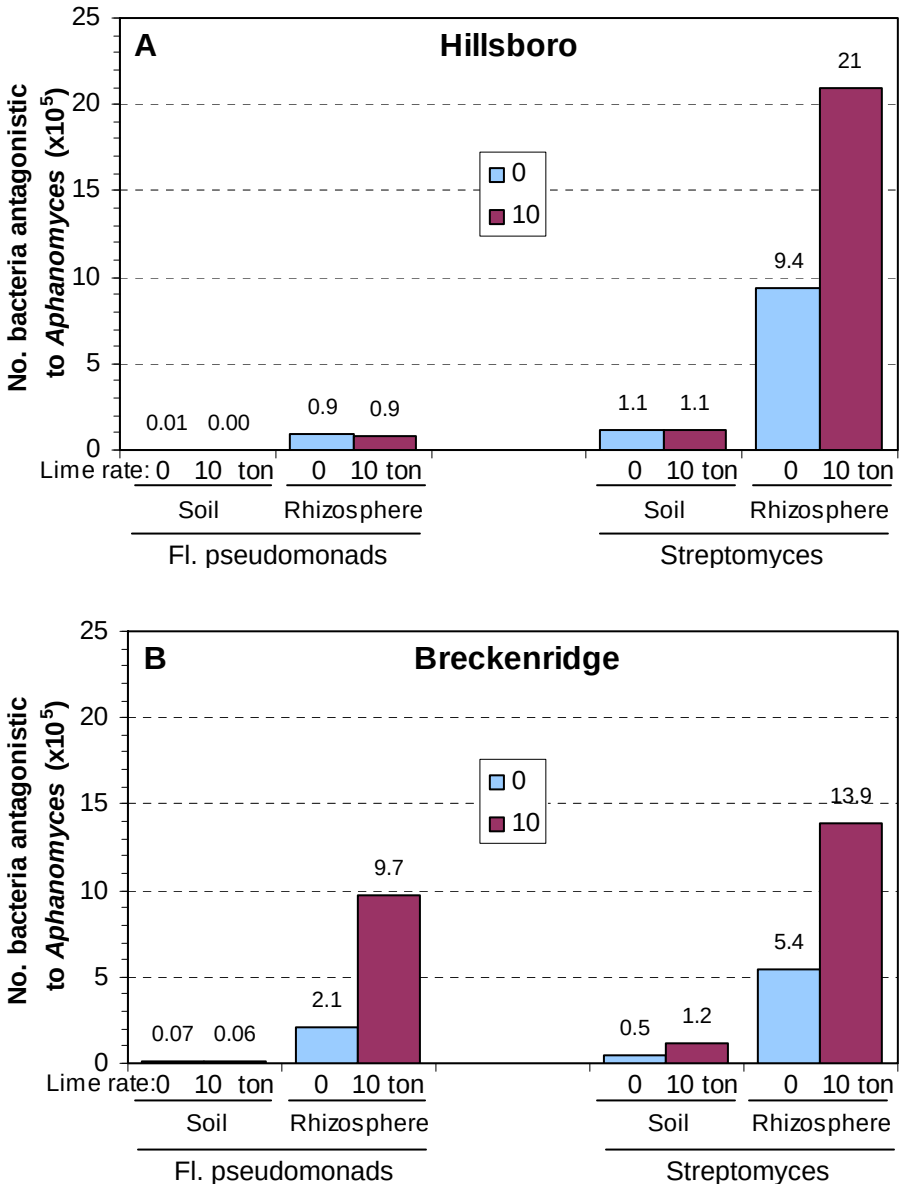

Fig. 2. Populations (value x 10^5 = value x 100,100) of fluorescent (Fl) pseudomonads and streptomyces bacteria (per gram oven-dry soil) that were antagonistic to *Aphanomyces cochlioides* based on a dual-culture assay. Cultures were collected in 2006 from the sugarbeet rhizosphere of 'Seedex Alpine' and soil of plots treated with 10 tons of spent lime A^{-1} and a non-limed control at **A.)** Hillsboro, ND and **B.)** Breckenridge, MN. Plots were treated with spent lime in October, 2003 at Hillsboro, ND and May, 2004 at Breckenridge, MN.

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Antagonism of microorganisms to *A. cochlioides*. At Hillsboro, streptomyces antagonistic to *A. cochlioides* were more common in the sugarbeet rhizosphere than in soil, especially in plots treated with 10 tons of spent lime A^{-1} compared to the non-limed control (Fig. 2A). Populations of antagonistic fluorescent pseudomonads were low but overall, were higher in the rhizosphere than in soil, but were similar for plots treated with no lime or 10 tons (Fig. 2A).

At Breckenridge, fluorescent pseudomonads and streptomyces antagonistic to *A. cochlioides* were more abundant in the sugarbeet rhizosphere than in soil, particularly when treated with 10 tons of spent lime A^{-1} compared to no lime Fig. 2B). Antagonistic populations of both bacteria were low in soil treated with 10 tons of lime or no lime (Fig. 2B).

DISCUSSION

Initial soil applications of spent lime continued to have long-term, beneficial effects in 2007sugarbeet trials, the fourth growing season after the amendment was applied. All rates of spent lime reduced Aphanomyces root rot and increased sugarbeet yields at Breckenridge where disease pressure was severe, especially for the susceptible variety without Tachigaren which was not a harvestable crop without lime. At the Hillsboro site, disease pressure from *A. cochlioides* was negligible, and although there were no significant differences among limed and non-limed treatments, increasing rates of lime resulted in increasingly higher yields. The 2007 results are consistent with results for sugarbeet sown at both experimental sites in 2005 (13) and 2006 (14), 2 and 3 growing seasons after initial application of spent lime, respectively. No other reports on the long-term effects of spent lime on sugarbeet yields or on reduction of Aphanomyces root are available, so these results continue to be encouraging.

To date, a single soil-application of spent lime in our experiments at Hillsboro and Breckenridge (2004-2007) have had no effect on rotation crops including spring wheat, corn, and soybean, except for increased soybean yields at Breckenridge in 2006 (third year after lime applied, 14). Smith et al. (8) also reported an increase in soybean yields at some rates of spent lime. Giles and Cattanach (5), reported variable effects of spent lime applications on wheat, with yields increasing or decreasing compared to the non-limed control. It is unknown why variable responses occur on rotation crops but may be associated with low rates of lime, inadequate time for soil to stabilize after application, production practices, as well as differences in soil types and associated soil characteristics.

Application of spent lime at both sites increased soil pH within a few months (12), and these values continued to remain stable and relatively unchanged in 2007. *Aphanomyces cochlioides* 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 and/or favors increases of beneficial microorganisms in the rhizosphere. Spent lime also contains nitrogen, phosphorus, potassium, and other inorganic and organic nutrients (7) that fertilize crops. Spent lime also alters physical properties of soil, e.g., improving water drainage, which results in less root disease.

Constituents within spent lime also may directly affect *A. cochlioides*. In preliminary studies, we evaluated soil extracts from field plots treated with 20 tons of spent lime per acre for direct effects on structures of *A. cochlioides.* Soil extracts diluted 10-, 100-, and 1,000-fold prevented production of sporangia (structures originating from oospores or hypha that produce infective zoospores). Water controls, adjusted to pH values corresponding to diluted spent lime extracts, resulted in production of zoosporangia, which released motile zoospore inoculum (*unpublished*). Fluctuations in Aphanomyces SIVs from year to year (12,13,14) suggest that population densities are not reduced, but that infections are somehow suppressed/reduced.

In our assays, the sugarbeet rhizosphere proved to be an abundant source of streptomyces antagonistic to *A. cochlioides*, especially when soil was treated with 10 tons of spent lime A^{-1} compared to the non-limed control. Streptomyces often produce antibiotics that are antifungal in activity. Fluorescent pseudomonads were abundant in the sugarbeet rhizosphere in 2006 (14), but only a low proportion of the population was antagonistic to *A. cochlioides*. On the other hand, the laboratory assay favored antibiotic-producing bacteria and some bacteria are biologically active in other ways, e.g., altering availability of soil nutrients, competing with pathogens for nutrients, or stimulating plant resistance (4, 9, 10, 11). Cultures of streptomyces and fluorescent pseudomonads isolated form sugarbeet rhizospheres and soil at the Hillsboro and Breckenridge locations in 2007 also will be assayed for antagonism to *A. cochlioides*. Our research continues to explore other underlying roles of soil microorganisms in the effects of spent lime in suppression of Aphanomyces root rot.

SUMMARY AND CONCLUSIONS

1. Initial soil applications of spent lime continued to have long-term effects in reducing Aphanomyces root rot and/or increasing sugarbeet yields in 2007, the fourth growing season after the amendment was applied.

2. To date, field-application of spent lime in our experiments has had no adverse effects on rotation crops.

3. Application of spent lime at both sites increased soil pH within a few months, and these values remained stable and relatively unchanged in 2007.

4. Populations of *A. cochlioides* in soil were not significantly reduced by application of spent lime.

5. The sugarbeet rhizosphere was an abundant source of streptomyces antagonistic to *A. cochlioides*, especially in plots treated with 10 tons of spent lime A^{-1} compared to the non-limed control. The significance of this relationship will be further explored.

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