SPENT LIME EFFECTS ON SUGARBEET, ROOT ROT, MICROORGANISMS, AND ROTATON CROPS

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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 requires all available control options including early planting of 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 in the Salinas Valley of California, spent lime from a near-by sugarbeet processing factory was applied at 2 to 4.5 tons per acre in fields (pH less than 6.8) severely infested with the clubroot pathogen, *Plasmodiophora brassicae* (3). 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. Despite long-term application of lime to control clubroot, little is known how various forms of lime affect the pathogen.

Growers in southern Minnesota started applying spent lime (4 to 8 tons wet weight per acre) 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). In the last couple of years, growers in the RRV also have been applying spent lime to their sugarbeet fields. In trials in the RRV, spent lime (3 and 10 tons wet weight per acre) 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 per acre) 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. Since *A. cochlioides* causes severe root rot of sugarbeet over a range of soil pH values from 5.5 to 8, benefits of spent lime treatments are more complicated than increasing soil pH. Spent lime contains a wide variety of macro- and micro-nutrients and organic compounds obtained during 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). Sources of biological control agents include general bacterial populations or specific groups, such as fluorescent pseudomonads and streptomyces. These microorganisms often are antagonistic to soilborne pathogens, compete with the pathogen for nutrients, or induce plant resistance (4, 9, 10, 11). No data are available on amounts of spent lime needed to reduce disease, duration of suppression, or the mechanisms (biological, chemical, and physical) involved.

OBJECTIVES

In 2006, research 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 = 7.4) in mid October, 2003 and at Breckenridge, MN (pH = 6.5) in mid April, 2004. The Hillsboro site has a history of moderate root rot and the Aphanomyces soil index value (SIV) averaged 48 (0 – 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 applied at Hillsboro were 0, 5, 10, 20 and 30 tons wet weight of spent lime per acre (= 0, 3.3, 6.5, 13, and 19.5 tons dry weight per acre, respectively) and at Breckenridge were 0, 5, 10, 15 and 20 tons wet weight per acre (= 0, 2.7, 5.3, 8, and 10.6 tons dry weight per acre, respectively). Each treatment plot measures 33 x 60 ft. The four experiments were established 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 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. Sugarbeet was sown in lime experiments at both locations for the first time in 2005 and results have been reported (13).

2006 Sugarbeet field trials. Sugarbeet was sown in one experiment (non-limed and limed plots, replicated four times) at Hillsboro on May 5 and at Breckenridge on May 9, 2006. Varieties Seedex Alpine (partially resistant to *Aphanomyces*) and Hilleshog 2467RZ (susceptible and treated with 45 g of Tachigaren per unit of seed) were sown as subplots within lime treatment 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. per acre) was incorporated into soil and the insecticide Counter 15G (12 lb product per acre) 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 + UpBeet + Stinger + Select + MSO (8.7 fl oz + 0.125-0.5 oz + 1.3 fl oz + 0-2 oz + 1.5% per acre, respectively) were applied on May 29 and June 3, 12, and 20 at Hillsboro; Betanex (16 fl oz per acre) was substituted for Progress on the last application date. The same microrate mix was applied at Breckenridge on May 29 and June 12 and 20, but rates varied slightly from those used at Hillsboro. Plants were hand-thinned to a 6-inch spacing on June 6 at Hillsboro and to a 4-inch spacing (because of considerable early-season Aphanomyces root rot) on June 9 at Breckenridge. Plots at both locations were cultivated on June 14. Cercospora leaf spot was controlled by application of Eminent (13 oz per acre) and Headline (9 fl oz per acre) on August 21 and September 5, respectively, at Hillsboro and on July 27 and August 17, respectively, at Breckenridge (20 gpa at 100 psi). Alleys separating replicates were rototilled throughout the season.

Data were collected on seedling stand at 2 and 4 weeks after planting and shortly after thinning at both locations. Experiments were harvested at Hillsboro on October 10 and Breckenridge on October 9 on the two middle rows of each variety per treatment. Ten roots were randomly selected and analyzed for yield and sucrose quality by the American Crystal Sugar Company Quality Laboratory, East Grand Forks, MN. On October 13, 20 roots were randomly selected from each subplot and rated for Aphanomyces root rot (0 – 7 scale, 0 = healthy and 7 = root completely rotted and foliage dead).

2006 Rotation crop field trials. The three spent lime experiments at Hillsboro not sown to sugarbeet were planted to corn 'DKC35-02 (RR2/YGCB)' in rows 22 inches apart on April 22 by the grower-cooperator. Population densities were determined on June 15 (V-3 growth stage) by counting the number of plants in 1-m lengths of row in two areas of each plot. Roots were assessed for root rot on June 22 (V-4 growth stage) by removing 10 consecutive plants from the middle of each plot, which were washed and rated on a 0 to 4 scale (0 = no visible lesions, 4 = more than 66% of roots with lesions). Plots were hand-harvested on September 26 by removing ears from all plants within a 20-foot row in the middle of each plot; grain moisture (200 g sample) was measured with a hand moisture tester (Dickey-John Corp., Auburn, Il); yield was adjusted to 15.5% moisture and was based on 56 pounds per bushel.

At Breckenridge, the three spent lime experiments not sown to sugarbeet were planted to soybean 'Pioneer 90M91' in rows 6 inches apart on May 22 by the grower-cooperator. Plant densities were determined on June 15 (V-3 growth stage) by counting the number of plants in 1-m lengths of row in six areas per plot. Ten consecutive soybean plants were removed from the middle of plots on June 20 (V-3 to V-4 growth stage), washed, and the length of taproot discolored (or with lesions) was measured. Plots were harvested with a small plot combine on October 2 by removing a 5 x 20 ft swath; yields were adjusted to 13% moisture and based on 60 pounds per bushel.

Aphanomyces soil index values (SIVs). **S**oil samples were collected from plots (including subplots where two sugarbeet varieties were grown in 2005) at Hillsboro on May 17 and 23, and Breckenridge on April 25 to 27 (total of 100 soil samples 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 seed of sugarbeet 'ACH 261' were sown per pot (4 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 ^{0}F for 1 week for optimal emergence. Then temperatures were increased to 79 $\pm$ 2 ^{0}F (14 hour photoperiod) and soil was kept moist to favor infection and disease development. Stand counts were made twice weekly starting at emergence. Dying seedlings were removed at each stand count to prevent disease from spreading to adjacent plants. Four weeks after planting, surviving seedlings were rated for disease with a 0 to 3 scale (0 = healthy and 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 April and May, 2006 were oven-dried overnight at 86 ^{0}F 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).

Rhizosphere microorganisms. Sugarbeet root and soil samples were collected from Breckenridge on August 22 and Hillsboro on September 28, 2006, to determine populations of microorganisms. Five roots of Seedex Alpine were removed from the non-limed control and plots treated with 10 tons of spent lime per acre (non-harvested rows) for each of four replicates. Soil samples were collected between rows, in 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 from along the lateral root grooves (which also are 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 and contained root plus adhering soil and a shallow layer of lateral (secondary roots). This procedure was followed to collect samples along both lateral root grooves for each of five roots per plot. Thus, a total of 30 cores were collected per treatment plot (about 40 cm^2) and combined.

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 an aluminum cup, which was placed in an oven at 105 ^{0}C 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.

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

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Table 1. Hillsboro, ND: Soil pH, stands, root rot ratings, and harvest data of sugarbeet sown on May 5, 2006, 31 months after several rates of spent lime were applied in a field naturally infested with moderate inoculum densities of *Aphanomyces cochlioides*.

Main treatments		Soil pH	No. plants/80-ft row (Days after planting)[X]			No. roots harvested/ 80 ft row	RRR 0 - 7[Y]	Yield (Ton /A)	Sucrose			Gross return ($/A)
			12	27	Post-thinning				%	lb/T	lb recov./A	
Lime (Ton/A)[V] Wet wt.	Dry wt.											
0	0	7.11 a	261	327	158	148	1.9	31.9	17.2	313	9893 a	1147 a
5	3.3	7.66 b	268	322	155	148	1.9	32.5	18.0	330	10683 b	1318 bc
10	6.5	7.69 b	259	330	158	151	1.9	34.5	17.1	310	10588 b	1217 ab
20	13.0	7.77 bc	252	333	159	152	2.0	33.3	17.5	318	10559 b	1254 abc
30	19.5	7.83 c	275	326	157	149	2.0	33.4	18.2	335	11169 b	1400 c
LSD (P = 0.05)[Z]		0.13	NS	NS	NS	NS	NS	NS	NS	NS	628	169
Variety[W]												

HM 2467RZ + 45 g Tach	-	230 a	325	158	148	1.9	30.5 a	18.1 a	330 a	10012 a	1234 a
Seedex Alpine (0 Tach)	-	296 b	330	157	151	2.0	35.8 b	17.1 b	312 b	11145 b	1300 b
LSD (P = 0.05)[Z]		11	NS	NS	NS	NS	1.1	0.3	5	374	56

[V] Spent lime was applied in October, 2003 in a randomized block design of four replicates per experiment (total of four experiments) and incorporated by chisel plow. In 2004, the four experiments were sown with corn; in 2005, one experiment was sown with sugarbeet and the other three experiments were left fallow. In 2006, one experiment was sown with two sugarbeet varieties and the other three experiments were sown with corn (Table 3 in this report). Each value in this portion of the table is averaged across both sugarbeet varieties sown in one experiment in 2006.

[W] Sugarbeet varieties Hilleshog 2467 RZ (susceptible to *Aphanomyces* and treated with 45 g of Tachigaren (Tach) per unit of seed) and Seedex Alpine (partially resistant to *Aphanomyces*) were sown as subplots within each spent lime treatment plot. Plots were harvested on October 10, 2006. Each value in this portion of the table is averaged across all lime treatments.

[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 6. Post-thinning stand counts were made on June 8.

[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.

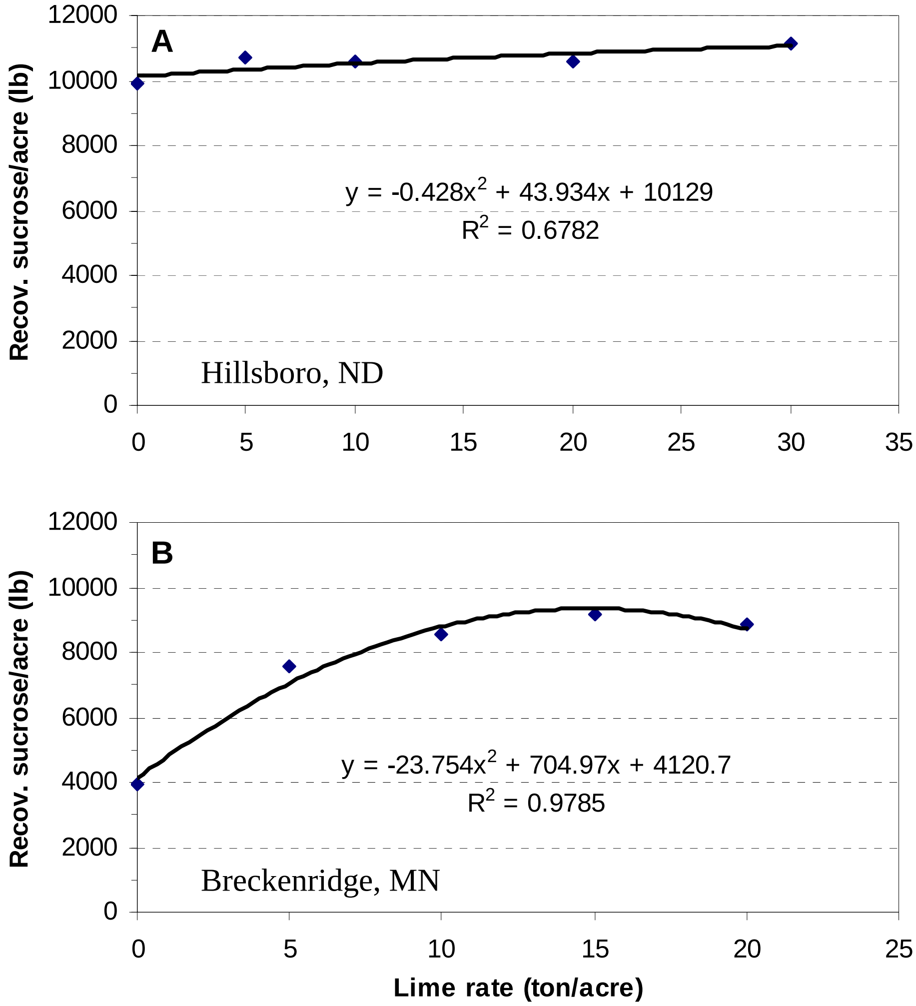

Figure 1. Regression analyses of recoverable sucrose per acre in 2006 verses rate of spent lime (wet weight per acre) at **A.)** Hillsboro (applied October, 2003; no significant difference) and **B.)** Breckenridge, MN (applied May, 2004; significant at P = 0.01).

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RESULTS

2006 Sugarbeet field trials. ***Hillsboro.*** Soil pH in non-limed plots averaged 7.1 (Table 1). All rates of spent lime significantly increased soil pH and there were small increases in pH values with increasing rates of lime (Table 1). Soil pH levels for samples collected in May, 2006 were nearly identical to 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). *A. cochlioides* was inactive because of dry weather throughout the growing season. There were no significant differences in seedling stands for limed and non-limed plots. Plant populations were uniform shortly after thinning and at harvest for all plot treatments; only 5% of stand was lost during this interval. At harvest, root rot ratings were negligible and averaged a rating of 2 (= root is large and superficial scarring affects less than 5% of the root surface). Yield (tons of roots per acre), percent sucrose, and pounds of sucrose per ton were not significantly different among limed and non-limed plots, but sometimes were higher as rates of lime increased. An accumulative affect of these factors resulted in significantly higher and equal amounts of recoverable sucrose per acre in limed plots (all rates) compared to the non-limed control. Regression analysis revealed no significant relationship between amount of spent lime applied and yield of recoverable sucrose per acre (Figure 1A). In 2006, 5 tons wet weight of spent lime per acre was sufficient to significantly increase pounds of recoverable sucrose per acre. Furthermore, plots treated with 5 and 30 tons of spent lime resulted in significantly more gross dollars per acre compared to the non-limed control; the other lime treatments had intermediate economic returns.

Table 2. Breckenridge, MN: Soil pH, stands, root rot ratings, and harvest data of sugarbeet sown on May 9, 2006, 25 months after several rates of spent lime were applied in a field naturally infested with high inoculum densities of *Aphanomyces cochlioides.*

Main Treatments Lime (Ton/A)[V]		Soil pH	No. plants/80-ft row (Days after planting)[X]			No. roots harvested/ 80 ft row	RRR 0-7[Y]	Yield (Ton/A)	Sucrose			Gross return ($/A)
Wet wt.	Dry wt.		13	28	Post-thinning				%	lb/T	lb recov./A	
0	0	6.53 a	253	242 b	133	76 a	4.7 a	14.3 a	15.2 a	270 a	3911 a	388 a
5	2.7	7.51 b	245	231 a	125	85 b	3.6 b	26.0 b	16.3 b	292 b	7550 b	812 b
10	5.3	7.61 b	252	245 bc	139	103 d	3.3 b	30.7 bc	16.2 b	289 b	8858 bc	942 b
15	8.0	7.78 c	243	254 c	145	106 d	3.3 b	31.5 c	16.4 b	291 b	9168 c	987 b
20	10.6	7.79 c	228	246 bc	134	96 c	3.3 b	30.5 bc	16.3 b	290 b	8849 bc	949 b
LSD (P = 0.05)[Z]		0.16	NS	10	NS	6	0.5	5.1	0.6	14	1523	181
Variety[W]												
HM 2467RZ + 45 g Tach		-	230 a	243	137	93	3.7	22.8 a	16.2	288	6604 a	707 a
Seedex Alpine (0 Tach)		-	258 b	244	134	93	3.6	30.4 b	16.0	285	8731 b	924 b
LSD (P = 0.05)[Z]			14	NS	NS	NS	NS	1.6	NS	NS	457	57

[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 experiments were sown with wheat. In 2006, one experiment was sown with two sugarbeet varieties and the other three experiments were sown with soybean (Table 4 in this report). Each value in this portion of the table is averaged across both sugarbeet varieties sown in one experiment in 2006.

[W] Sugarbeet varieties Hilleshog 2467 RZ (susceptible to *Aphanomyces* and treated with 45 g of Tachigaren [Tach] per unit of seed) and

Seedex Alpine (partially resistant to *Aphanomyces*) were sown as subplots within each spent lime treatment plot. Plots were harvested on

October 9, 2006. Each value in this portion of the table is averaged across all lime treatments.

[X] Plots were sown at 142,560 seeds per acre (seed every 2 inches in row 22 inches apart) and hand-thinned to a 4-inch spacing on June 9 (34 days after planting). Post-thinning stand counts were made on June 12 (3 days after thinning).

[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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The sugarbeet variety with partial resistance to *A. cochlioides* (Seedex Alpine) had significantly higher stands than the susceptible variety at 12 days after planting, but there were no significant differences in stand between these varieties for the remainder of the season (Table 1). Seedex Alpine yielded significantly lower percent sucrose and pounds of sucrose per ton compared to the susceptible variety. However, Seedex Alpine resulted in significantly higher tons of roots, pounds of recoverable sucrose, and gross economic return per acre compared to the *Aphanomyces*-susceptible variety. This illustrates the excellent yield potential of an *Aphanomyces*-resistant variety grown in the absence of disease pressure.

Breckenridge. Soil pH in non-limed plots averaged 6.5 and all rates of spent lime significantly increased soil pH (Table 2). Soil pH levels of samples collected in April, 2006 were slightly higher compared to measurements made in September, 2004 (12), 6 months after spent lime was applied (soil pH likely had not yet stabilized).

There were no significant interactions between rate of lime and sugarbeet variety for nearly all data collected at Breckenridge, so results are presented separately for these main effects (Table 2). Within 2 weeks after planting, weather was too dry for *A. cochlioides* to infect seedlings and there were no differences in stands among limed and non-limed control plots. Rainfall from about mid May through mid June resulted in considerable activity of *A. cochlioides.* At 28 days after planting, there were significant differences in stand among treatments. Stands were statistically lower in plots treated with 5 tons of lime compared to the other limed plots and control; stands were highest in plots treated with 15 tons of spent lime (Table 2). These results are explained by a significant interaction ($P = 0.016$) in non-limed control plots where stand was significantly higher for the *Aphanomyces*-susceptible

Table 3. Hillsboro, ND: Plant populations, severity of root disease, and yield of corn sown on April 22, 2006, 30 months after several rates of spent lime were applied in a field naturally infested with moderate inoculum densities of *Aphanomyces cochlioides*.

Lime (Ton/A)[V]		Plant population	Root disease	Yield
Wet weight	Dry weight	(No. plants/m^2)[W]	(0-4)[X]	(bu/A)[Y]
0	0	8.3	1.0	132
5	3.3	7.0	1.1	132
10	6.5	7.2	1.0	138
20	13.0	8.1	1.0	152
30	19.5	7.8	1.0	148
LSD ($P = 0.05$)[Z]		NS	NS	NS

V 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 2004, the four experiments were sown with wheat; in 2005, one experiment was sown with sugarbeet and the other three experiments were left fallow. In 2006, one experiment was sown with sugarbeet (Table 1 in this report) and the other three experiments were sown with corn 'DKC35-02 (RR2/YGCB)'.

[W] Population densities of corn were measured on June 15, 2006 by counting numbers of plants in a 1-m length of row in two areas of each plot. Each value is an average of 12 plots.

[X] Ten consecutive plants were dug from the middle of each plot on June 22, 2006. Roots were washed and disease severity was rated on a 0 to 4 scale, where 0 = no lesions and 4 = more than 66% of roots with lesions (8). Each value is an average of 120 plants.

[Y] Corn plots were hand-harvested on September 27, 2006 by removing all ears from a 20-foot length of row in the center of each plot. Yields were adjusted to 15.5% moisture and based on 56 pounds per bushel. Each value is an average of 12 plots.

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

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variety HM 2467RZ treated with 45 g of Tachigaren than for the *Aphanomyces*-resistant variety Seedex Alpine with no Tachigaren (data not shown); this interaction did not occur in limed plots (data not shown). Thus, the benefit of sowing Tachigaren-treated seed of a susceptible variety in non-limed plots was so effective, it obscured the positive effect of spent lime on maintaining seedling stands of both varieties.

Sugarbeet stands were the same across all plots soon after thinning but considerable stand loss occurred over the rest of the season (Table 2). At harvest, all rates of spent lime resulted in significantly higher stands than the non-limed control. Among spent lime treatments, stands were significantly highest and equal in plots treated with 10 and 15 tons of lime, lowest at 5 tons, and intermediate at 20 tons. In the non-limed control, Aphanomyces root rot ratings averaged 4.7 (=50 to 75% of the root surface was constricted, rotted, and/or scarred) and were significantly higher than in limed plots which averaged a rating of 3.4 (=25% of root surface was affected by disease). Among lime treatments, there were no significant differences in Aphanomyces root rot ratings but the 5 ton rate resulted in somewhat more root rot than the higher rates of spent lime.

Sugarbeet yield (tons of roots per acre) were significantly higher for all rates of spent lime compared to the control; among lime treatments, yields were significantly higher in the 15 ton plots compared to 5 tons and were intermediate for 10 and 20 tons (Table 2). All rates of spent lime resulted in significant and equal increases in percent sucrose, pounds of sucrose per ton, and gross return per acre compared to the control. Although all rates of lime significantly increased pounds of recoverable sucrose per acre compared to the non-limed control, there were differences among lime treatments. A significantly higher amount of sucrose was recovered from plots treated with 15 tons of spent lime compared to 5 tons; amounts were intermediate in the 10 and 20 ton plots. Regression analysis confirmed significantly highest recoverable sucrose in plots treated with 15 tons wet weight of lime per acre (Figure 1B).

The *Aphanomyces*-resistant variety (Seedex Alpine) resulted in significantly higher stands at 13 days after planting than the susceptible variety (Hilleshog 2467RZ) but there were no differences in stand or Aphanomyces root rot between the two varieties for the rest of the season (Table 2).

Yet, Seedex Alpine resulted in significantly higher tons of roots, pounds of recoverable sucrose, and gross return per acre than the susceptible variety.

2006 Rotation crop field trials. ***Hillsboro.*** Effects of spent lime on corn are shown in Table 3. There were no differences among limed plots and the non-limed control for plant populations, root disease ratings (which were very low for all treatments), or yield.

Table 4. **Breckenridge, MN:** Plant populations, severity of root disease, and yield of soybean 'Pioneer 90M91' sown on May 22, 2006, 25 months after several rates of spent lime were applied in a field naturally infested with high inoculum densities of *Aphanomyces cochlioides.*

Lime (Ton/A)[V]		Plant populations (No. plants/m^2)[W]	Length of lesion on taproot (mm)[X]	Yield (bu/A)[Y]
Wet weight	Dry weight			
0	0	47	30	44 a
5	2.7	45	30	49 b
10	5.3	48	30	49 b
15	8.0	48	30	48 b
20	10.6	53	30	53 b
LSD (P = 0.05)[Z]		NS	NS	4

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 chisel plow. In 2004, the four experiments were sown with wheat; in 2005, one experiment was sown with sugarbeet and the other three experiments were sown with wheat. In 2006, one experiment was sown with sugarbeet (Table 2 in this report) and the other three experiments were sown with soybean 'Pioneer 90M91' on May 22.

W Population densities of soybean were measured on June 15, 2006 by counting numbers of plants in a 1-m length of row in six areas of each plot (rows 6 inches apart). Each value is an average of 12 plots.

X Ten consecutive plants were dug from the middle of each plot on June 20, 2006. Roots were washed and disease severity was determined by measuring the length of taproot discolored or with lesions. Each value is an average 120 plants.

Y Soybeans were harvested on October 2, 2006 with a small plot combine in a 5 x 20 foot swath per plot. Yields were adjusted to 13% moisture and based on 60 pounds per bushel. Each value is an average of 12 plots.

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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Breckenridge. Effects of spent lime on soybean are shown in Table 4. There were no differences in plant populations in limed plots and the non-limed control. Overall, disease ratings were moderate and the same across all treatments. Soybean yields were significantly higher and equal in all plots treated with spent lime compared to the non-limed control.

Aphanomyces soil index values (SIVs). ***Hillsboro.*** For soil samples collected in May, 2006, Aphanomyces SIVs varied depending on 2005 crop history (data not shown). For instance, 2006 SIVs were very high in plots sown to sugarbeet in 2005 and averaged 95. In soil samples left fallow in 2005, Aphanomyces SIVs in 2006 were the same for limed and non-limed plots and averaged 61. The 2006 Aphanomyces SIVs increased compared to 2004. In 2004, 9 months after spent lime was applied, the Aphanomyces SIV in the non-limed control was 45 and across limed plots averaged 20.

Breckenridge. Aphanomyces SIVs were extremely high and averaged nearly 100 (data not shown) for soil samples collected in April, 2006, regardless of 2005 cropping history (sugarbeet or wheat). In 2004, 5 months after spent lime was applied, Aphanomyces SIVs in the non-limed control averaged 100 and across limed plots averaged 82.

Rhizosphere microorganisms. Populations of bacteria in soil and in the rhizosphere of sugarbeet roots in the control and plots treated with 10 tons of lime differed dramatically, depending upon field location. For instance, populations of total cultureable bacteria were low in soils collected from the control and plots treated with 10 tons of lime at Breckenridge and Hillsboro (Figure 1A). Although these microorganisms increased in the rhizosphere of roots from control and limed plots at both locations, the overall increase was considerably higher at Breckenridge than at Hillsboro.

Populations of fluorescent pseudomonad bacteria were low in soils collected from the control and plots treated with 10 tons of lime at both locations (Figure 1B). Although these microorganisms increased in the rhizosphere of roots in the control and limed plots at both locations, the overall increase was significantly higher at Breckenridge than at Hillsboro. Furthermore, at Breckenridge, the population of fluorescent pseudomonads in the rhizosphere of sugarbeet roots in plots treated with 10 tons of spent lime was significantly higher than in the rhizosphere of roots in the non-limed control.

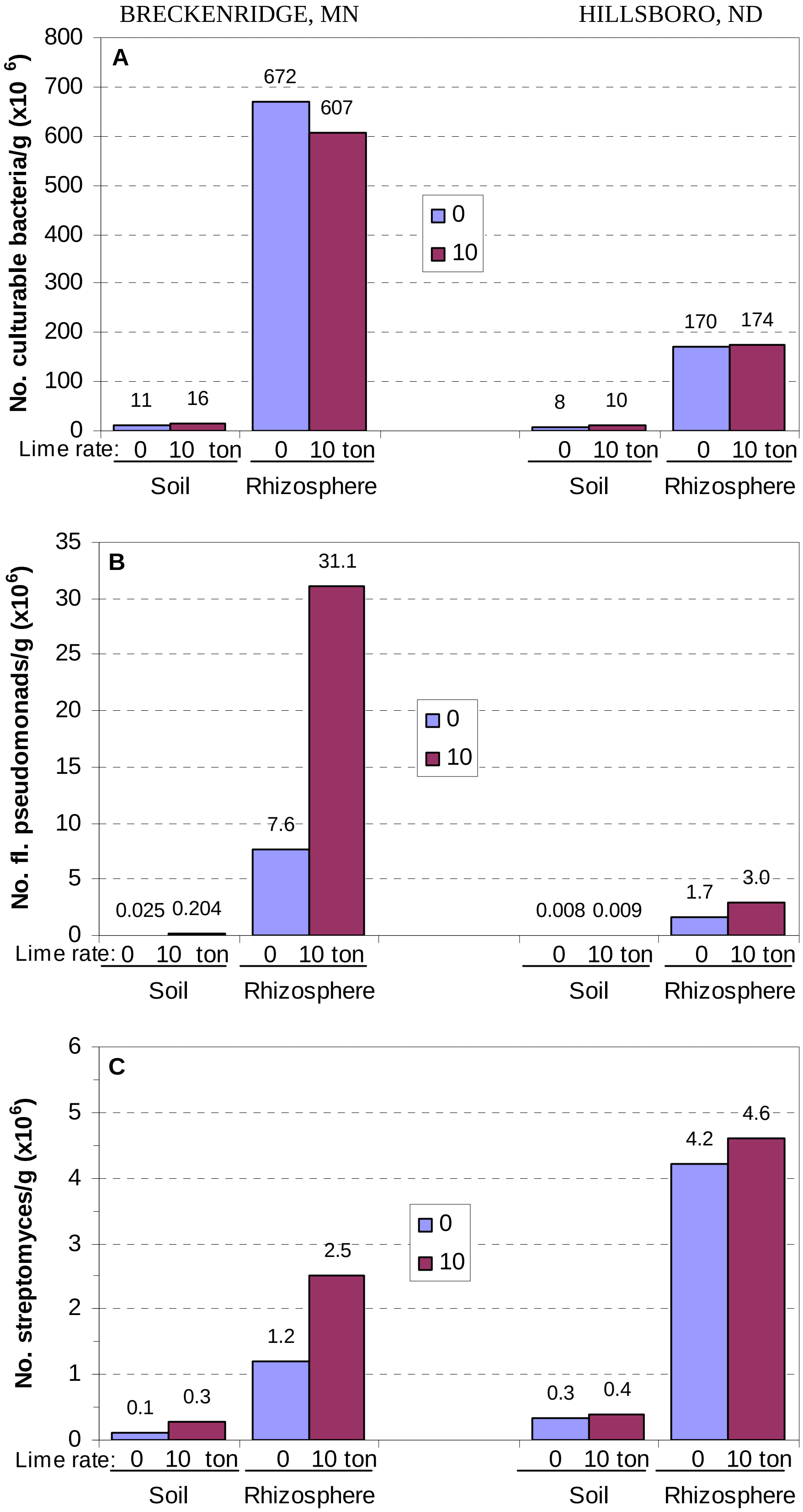

Figure 2. Populations of bacteria per gram (g) of oven-dry field soil compared to sugarbeet rhizosphere soil of variety Seedex Alpine collected near harvest in 2006 from the (non-limed) control and plots where 10 tons (wet weight) of spent lime were applied per acre in May, 2004 at Breckenridge, MN and October, 2003 at Hillsboro, ND for:

A.) cultureable bacteria, **B.)** fluorescent (fl) pseudomonads, and **C.)** streptomyces (x 10^6 = 1 million). Each bar is an average of four replications.

Populations of streptomyces bacteria were relatively low in soils collected from the control and plots treated with 10 tons of lime at both locations (Figure 1C). Although these microorganism increased in the rhizospheres of roots in control and limed plots at both locations, the overall increase was much higher at Hillsboro than at Breckenridge.

DISCUSSION

Application of spent lime two growing seasons before planting sugarbeet in 2006 significantly increased sucrose yields and economic returns at both locations, despite no Aphanomyces disease pressure at Hillsboro and severe Aphanomyces root rot at Breckenridge. Similar results were report in 2005, one growing season after spent lime was applied (13). Although soil index values (SIVs) at both locations indicated high potential for disease in 2006, soil moisture was low at Hillsboro, so *A. cochlioides* was inactive. On the other hand, *A. cochlioides* was active early in the growing season at Breckenridge and wet soil conditions occurred intermittently until harvest. In 2006, the *Aphanomyces*-resistant variety was superior to the susceptible variety for most harvest data measured at both locations. In 2005, these trends were developing but were not statistically established (13).

To date, field-application of spent lime in our experiments has had no adverse effects on, or increased yields of, rotation crops. For instance, there were no effects on corn sown in limed and non-limed plots at Hillsboro in 2006. When soybean was sown at Breckenridge in 2006, lime did not affect plant population densities but resulted in a significantly higher and equal increase in yields in all limed plots compared to the control. Smith et al. (8) also reported an increase in soybean yields at some rates of spent lime. On the other hand, when wheat was sown at Breckenridge in 2005, there were significantly higher population densities in limed than in non-limed plots (13); unfortunately, plots were not harvested. 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 lime is applied, as well as differences in soil types and associated soil characteristics.

The pH of lime-amended plots increased compared to non-limed controls at both locations, although pH has not changed at Hillsboro since 2004 and at Breckenridge has increased only slightly since 2004 (12). Severe Aphanomyces root rot, however, occurs naturally in fields over a wide range of pH values (5 to 8) in Minnesota and North Dakota. Improved production of sugarbeet and rotation crops by soil-application of spent lime may be caused by increases in soil pH, which alters 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 directly fertilize crops. Additionally, spent lime alters physical properties of the soil, e.g., improving water drainage, which results in less Aphanomyces root rot.

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 1000-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*).

Aphanomyces SIVs were surprisingly high in all limed and non-limed plots at both locations in June, 2006, despite SIVs dropping within a few months after spent lime was applied in 2004. In 2005, SIVs remained low in limed plots - except where sugarbeet was sown, where they returned to pre-limed levels. It is unknown why growing sugarbeet (and in 2006, growing rotation crops) negated earlier suppression of Aphanomyces SIVs. Perhaps lime suppresses germination of oospores (survival spores that produce infective zoospores) of *A. cochlioides* and this inhibition is overcome when crop roots release exudates into soil (including rotation crops which are non-hosts of *A. cochlioides*). This theory, however, does not explain why planting sugarbeet and rotation crops in limed soil returned SIVs to pre-limed levels at Breckenridge and to higher than pre-limed levels at Hillsboro, yet yields of sugarbeet increased at both locations. Aphanomyces SIVs in fields also may vary over time because of changing environmental conditions and their effects on survival structures of *A. cochlioides.*

Increases in total culturable bacteria, fluorescent pseudomonads, and streptomyces in the rhizosphere of sugarbeet may represent increases in microorganisms antagonistic to *A. cochlioides* or that benefit plants in other ways. Fluorescent pseudomonads and streptomyces often are antagonistic to soilborne pathogens (10, 11) and also are known to compete with the pathogens for nutrients, or induce plant resistance (9). About 260 cultures of fluorescent pseudomonads and 280 of streptomyces from 2006 assays are preserved in our collection for screening *in vitro* antibiosis against *A. cochlioides*. This will allow us to determine the proportion of fluorescent pseudomonad and streptomyces bacteria that are antagonistic to *A. cochlioides*.

SUMMARY AND CONCLUSIONS

1. Application of spent lime two growing seasons before planting sugarbeet in 2006 significantly increased recoverable sucrose and economic return at two field locations, despite no Aphanomyces disease pressure at Hillsboro and severe Aphanomyces root rot at Breckenridge.

2. When *A. cochlioides* was active, there was a significant reduction in root rot. Under conditions of Aphanomyces disease pressure, increasing rates of lime tended to decrease root rot and increase sugarbeet yields; 15 tons wet weight (= 8 ton dry weight) spent lime per acre was optimal; 10 tons wet weight gave better results than 5 ton wet weight per acre.

3. When *A. cochlioides* was inactive, sucrose yields significantly improved with a lime application of 5 tons wet weight per acre (= 3.3 tons dry weight) or higher compared to the non-limed control.

4. To date, field-application of spent lime in our experiments either has increased yields of, or had no adverse effects on, rotation crops.

5. Within months after spent lime was applied, Aphanomyces soil index values (SIVs) decreased compared to non-limed controls. Two growing seasons later, SIVs in all plots (limed and non-limed) increased to pre-limed levels or higher in plots sown to sugarbeet as well as rotation crops.

6. Various microoganisms isolated from soil and sugarbeet rhizospheres in limed and non-limed field plots are being screened to determine their activity against *A. cochlioides*. This research may identify underlying effects of spent lime in suppression of Aphanomyces root rot.

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