RECLAMATION AND FERTILIZATION OF *APHANOMYCES*-INFESTED SUGARBEET FIELDS AMENDED WITH INDUSTRIAL WASTE LIME

Carol E. Windels[1], Albert L. Sims[1], Jason R. Brantner[1], and Carl A. Bradley[2]
[1]University of Minnesota, Northwest Research and Outreach Center, Crookston
and [2]North Dakota State University, Fargo

Aphanomyces cochlioides (= *A. cochlioides*) is a serious economic pathogen of sugarbeet and infests over 50% of acres planted in the Red River Valley (RRV) and 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. Since 1993, the region has experienced wet summers and Aphanomyces diseases have increased in prevalence and severity. American Crystal estimates that since 1997, Aphanomyces root rot has cost their growers direct losses of $20 million annually from abandonment of fields, reduced yields, and storage losses. *A. cochlioides* persists in soil for over 20 years in the absence of a sugarbeet crop. Unfortunately, management strategies include options of limited effectiveness: a seed treatment fungicide (Tachigaren), early planting, and varieties with partial resistance. Even when best management practices are implemented, wet seasons can lead to severe disease and fields either are abandoned or yield poorly. This chronic situation has generated interest in finding effective, alternative methods of Aphanomyces control.

Recent evidence suggests waste lime from sugarbeet factories suppresses Aphanomyces root rot and/or increases yield. This observation was reached serendipitously when waste lime was applied in sugarbeet fields in southern Minnesota to increase soil pH and reduce carry-over of the herbicides Pursuit (imazethapyr) and Raptor (imazamox) (1), and Aphanomyces root rot also was reduced. Subsequent trials by Bresnahan et al. (2) showed application of waste lime in a field with an initial soil pH of 6, reduced Aphanomyces root rot and increased sugarbeet yields for 4 years compared to a non-limed control; in another field with an initial pH of 7.9, disease was not affected but yields increased compared to non-limed soil. No data are available on amounts of lime needed to reduce disease, duration of suppression, or the mechanisms (biological, chemical, physical) involved.

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 an industrial waste. Seven factories in the RRV and southern Minnesota generate 500,000 dry tons of waste lime annually and some has been stockpiled for 20 years. In Europe, waste lime produced in sugarbeet factories is considered an industrial by-product and not an industrial waste (7) so its regulation is much different than in the United States. The majority of waste lime in Europe is returned to land as a soil pH amendment and to supply nutrients. In Great Britain, it is marketed and sold as LimeX to conventional farmers as well as organic growers.

Waste lime contains a wide range of nutrients utilized by plants, e.g., nitrogen (N), phosphorus (P), potassium, magnesium, and boron. Some of these nutrients also have been identified as potential problems for the environment if not managed correctly. Phosphorus is a major contaminant of the Red River and Lake Winnipeg in Canada. Current estimates are 40% of P and 28% of N entering Lake Winnipeg via the Red River are from the United States. Laboratory analysis of waste lime has shown variable P concentrations ranging from 0.25 to 0.62% (dry weight), depending on the factory and when the sample was collected. Availability of P to a growing crop and its effect on soil test P levels is unknown since waste lime may chemically bind P into insoluble compounds.

OBJECTIVES

Short-term (first year) goals of this research were: 1) to determine baseline Aphanomyces soil index values in two field experiment sites before application of waste lime – and after application of waste lime, to measure 2) Aphanomyces soil index values and soil pH, 3) populations of soil microorganisms, and 4) crop response during 2004.

The long-term (5 year) goals of this research are to develop best management practices for application of waste lime to suppress Aphanomyces root rot, elucidate underlying mechanisms of disease suppression, recycle nutrients in an economic and environmentally sound manner, and reduce storage of waste lime at sugarbeet processing factories.

MATERIALS AND METHODS

Field trials. Experiments were established at Hillsboro, ND (pH = 7) in mid-October, 2003 and at Breckenridge, MN (pH = 6.3) in mid-April, 2004. Both sites had histories of Aphanomyces root rot on sugarbeet. Each site was divided into four, 1-acre experiments treated with five rates of waste lime (including an untreated control) and replicated four times in a randomized block design. Treatments applied at Hillsboro were 0, 5, 10, 20, and 30 tons of waste lime per acre and at Breckenridge were 0, 5, 10, 15, and 20 tons per acre. One experiment at each site will be planted to sugarbeet each year starting in 2005. The remaining plots and field will be sown with a desired crop by the grower, who also will maintain the crop following recommended practices. This allows evaluation of waste lime applications on sugarbeet and other crops in the rotation every season through 2008. In 2004, the Hillsboro site was sown with corn (a Roundup Ready hybrid) and the Breckenridge site was planted to wheat ('Grandin').

Aphanomyces soil index values and soil pH. Soil cores (6-inch depth) were collected at random across each plot at Hillsboro and Breckenridge (80 plots per location) to determine baseline populations of *A. cochlioides*. Waste lime treatments were applied later on the same day. In 2004, all plots were sampled again at Hillsboro in July and at Breckenridge in September (9 and 4 months after application of waste lime, respectively).

Soil samples were screened and assayed to determine an Aphanomyces disease index value (= inoculum density of *A. cochlioides*). Since the pathogen cannot be directly quantified, 25 seed of sugarbeet 'ACH 261' were sown per pot (4 pots/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 and then at 79 $\pm$ 2 ^{0}F (14 hour photoperiod) to favor disease. Soil was watered daily to keep moist. Stand counts were made two times per week starting at emergence. Dying seedlings were removed at each stand count to prevent disease spread to adjacent seedlings. Four dying seedings per treatment were cultured in the laboratory to confirm infection by *A. cochlioides*. They were washed free of soil, surface-treated in 0.5% NaOCl for 15 sec, rinsed twice in sterile deionized water (SDW), placed in 5 ml SDW, and examined microscopically 24 to 48 hours later. Four weeks after planting, surviving seedlings were rated for disease and a root rot index (0 to 100 scale, 0 = healthy, 100 = all seedlings dead or severely diseased) was calculated (12).

To determine soil pH (5), a small quantity of soil from all plots (as collected in 2004) in both locations was oven-dried overnight at 86 ^{0}F and then ground with a mortar and pestle. A 5 gram (g) quantity was removed and mixed with 5 ml of deionized water. After 10 minutes, a pH probe was inserted into the mixture with gentle stirring for 3 seconds and the pH was read (Accumet® pH meter 15, Fisher Scientific).

Soil microorganisms. Soil samples collected in the 2004 growing season at Hillsboro and Breckenridge, as previously described, were placed in a walk-in cooler until assayed for microorganisms. To date, soil collected in one of the four experiments at Breckenridge has been assayed to enumerate various groups of microorganisms. For each soil sample, the equivalent of 10 g of oven-dry soil (based on previously determined moisture content) was placed in a flask containing 100 ml of 0.15% water agar, 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 soil suspension was pipetted from a dilution flask onto each of three standard-sized Petri dishes containing various culture media: 1/10-strength tryptic soy agar (TSA) for isolation of total culturable bacteria, Kings B medium for fluorescent psuedomonads, and peptone rose bengal agar for total culturable fungi (6). For isolation of *Bacillus* species, 10 ml of soil suspension was removed from the 10^{-4} dilution flask, placed in an oven, and held at 176 ^{0}F for 30 minutes to kill heat-sensitive organisms. One milliliter of suspension was added to each of three Petri dishes containing 1/10-strength TSA. To quantify populations of *Streptomyces* species, soil samples were air-dried overnight and ground with a mortar and pestle. For each soil sample, the equivalent of 5 g of oven-dry soil (based on previously determined moisture content) was placed in a flask containing 50 ml of 0.15% water agar, agitated on a rotary shaker for 30 minutes, serially diluted, and pipetted onto STR medium (4), as previously described. For all groups of microorganisms, culturing of serial dilutions on appropriate media were "bracketed" to ensure a reasonable number of colonies to count. Plates were incubated for recommended times and temperatures before counting (3, 6).

2004 Crop measurements. When wheat reached the soft dough stage, whole plant samples were harvested from two quadrants per plot (each 2 ft [4 rows] by 3 ft) by cutting plants to leave a 1.5- to 2-inch stubble. Both samples were combined and weighed. Subsamples were removed, weighed in the field, transported to the NWROC where they were dried at 140 ^{0}F about 3 days (until weight did not change), and reweighed. These weights were used to determine moisture and dry matter content. Subsamples were ground to a coarse powder in a Wiley mill and will be analyzed in the laboratory during the winter for concentration of P.

Wheat was harvested on August 12 with a small plot combine. An area 25 ft long by 5 ft wide was harvested per plot. Harvested grain was dried over night with forced air at 129 ^{o}F, cleaned, and weighed. A subsample was removed to determine moisture content and protein concentration (Tecator Infratec 1229 grain analyzer).

Near maturity (after an early killing frost) at the Hillsboro site, corn ears were hand-harvested from two, adjacent rows (10 ft long per plot). Ears were placed in a gunny sack, air-dried for 3 days with forced air at 129 ^{0}F, and weighed. Grain was removed by a single-ear, hand-fed sheller. After weighing, a subsample was placed in an Tecator Infratec 1229 grain analyzer to determine moisture content. The day after ears were harvested, all stover (stalks, leaves, and ear husks) within the harvested area was cut with machetes, leaving 2- to 3-inch of stubble. Stover was fed into a hammer mill-style wood chipper that deposited ground material in a container. A subsample of stover was collected, weighed, dried for 3 days with forced air at 129 ^{o}F, dried at 140 ^{o}F for 2 days, and weighed. A subsample of grain and stover was saved and will be ground to a fine powder and analyzed in the laboratory during the winter for concentration of P.

RESULTS

Aphanomyces soil index values and soil pH. Baseline soil index values at Hillsboro (before waste lime was applied) were statistically ($P \leq 0.05$) the same across the entire experiment (Table 1) and averaged a moderate index value of 48 (maximum index value is 100). Nine months after application, all rates of waste lime provided equal and statistically lower soil index values (average = 20) compared to the non-limed control (= 45). All rates of lime also statistically increased soil pH compared to the non-limed control (Table 1). The pH of soil treated with the highest rate of lime (30 ton per acre) was statistically higher than soil treated with the lowest (5 ton) rate and was intermediate for 10 and 20 tons (Table 1).

Baseline soil index values at Breckenridge (before waste lime application) were statistically ($P \leq 0.05$) the same across the entire experiment (Table 2) and averaged a high index value of 98. Four months after application of waste lime, all rates statistically reduced soil index values compared to the non-limed control (Table 2). The soil

Table 1. Hillsboro, ND location: Baseline Aphanomyces soil index values in mid October, 2003 and soil index values and soil pH 9 months (July, 2004) after application of waste lime.

Lime (Tons/acre)[W]	Aphanomyces soil index value: Before lime (October, 2003)[XY]	Aphanomyces soil index value: After lime (9 mo) (July, 2004)[XY]	Change[X]	Soil pH (July, 2004)[XY]
0	53	45 a	-8	7.02 a
5	49	26 b	-23	7.64 b
10	45	17 b	-28	7.74 bc
20	42	21 b	-21	7.75 bc
30	52	17 b	-35	7.84 c
LSD ($P \leq 0.05$)[Z]	NS	13.5		0.13

[W] Wet weight of lime when applied and incorporated in mid-October, 2003

[X] Each value based on an average of 16 plots. Six soil cores were collected to a 6-inch depth per plot, combined, and sown with 25 sugarbeet seed of "ACH 261' per pot (4 pots per plot). Four weeks after planting, index values were determined on a 0-100 scale (0 = plants healthy, 100 = all plants dead or severely rotted).

[Y] For each column, values followed by the same letter are not significantly different; NS = not statistically different.

[Z] LSD = Least Significant Difference, $P \leq 0.05$.

Table 2. Breckenridge, MN location: Baseline Aphanomyces soil index values in mid April, 2004 and soil index values and soil pH 5 months (September, 2004) after application of waste lime.

Lime (Tons/acre)W	Aphanomyces soil index value: Before lime (April, 2004)[XY]	Aphanomyces soil index value: After lime (5 mo) (Sept, 2004)[XY]	Change[X]	Soil pH (July, 2004)[XY]
0	98	100 a	1	6.30 a
5	97	89 b	- 8	7.42 b
10	97	87 bc	-11	7.58 c
15	98	74 c	-24	7.70 d
20	99	78 cd	-21	7.72 d
LSD ($P \leq 0.05$)[Z]	NS	9.5		0.11

[W] Wet weight of lime when applied and incorporated in mid-April, 2004.

[X] Each value based on an average of 16 plots. Six soil cores were collected to a 6-inch depth per plot, combined, and sown with 25 sugarbeet seed of "ACH 261' per pot (4 pots per plot). Four weeks after planting, index values were determined on a 0-100 scale (0 = plants healthy, 100 = all plants dead or severely rotted).

[Y] For each column, values followed by the same letter are not significantly different; NS = not statistically different.

[Z] LSD = Least Significant Difference, $P \leq 0.05$.

Table 3. Populations of various soil microorganisms 5 months after application and incorporation of waste lime in plots at Breckenridge, MN.

Lime (Tons/acre)[X]	No. of various microorganisms/g of oven-dry soil (x 10,000)[Y] Total culturable bacteria	*Streptomyces* species	Fluorescent pseudomonads	*Bacillus* species	Total culturable fungi
0	1,730	136	9	21	16
5	2,270	152	6	13	17
10	2,610	122	10	18	16
15	2,610	238	4	14	13
20	8,590	178	14	13	16
LSD ($P \leq 0.05$)[Z]	NS	NS	NS	NS	NS

[X] Wet weight of lime when applied and incorporated in mid-April, 2004.

[Y] Each value based on counts from an average of nine petri plates (three per soil treatment, three replicates); total culturable bacteria on 1/10th strength tryptic soy agar (TSA); fluorescent psuedomonads on Kings B medium; *Bacillus* species on 1/10th strength TSA (after heat treatment at 176 ^{0}F for 30 min); total culturable fungi on peptone rose bengal agar (6); and *Streptomyce*s species on STR medium (4).

[Z] LSD = Least Significant Difference, $P \leq 0.05$; NS = not statistically different.

index value was statistically lowest with the highest (20 ton per acre) rate of waste lime compared to the lowest (5 ton) rate and indices were intermediate for 10 and 15 tons (Table 2). All rates of waste lime also statistically increased soil pH compared to the non-limed control (Table 2). The 15 and 20 ton per acre rates resulted in statistically equal and higher soil pH values than 10 tons, which in turn had a pH value statistically higher than the 5 ton rate.

Soil microorganisms. Populations of total culturable bacteria, *Streptomyces* species, fluorescent psuedomonads, *Bacillus* species, and total culturable fungi were not statistically affected by application of waste lime compared to non-limed control plots in one experiment evaluated for the Breckenridge trial (Table 3). There were trends, however, indicating an increase in populations of total culturable bacteria and *Streptomyces* species after application of lime compared to the non-limed control.

2004 Crop measurements. There were no statistical differences among waste lime treatments and the control for grain yield variables measured on wheat and corn (data not shown). At Breckenridge, average wheat grain yields were 64.2 bushels per acre, grain protein was 14.8%, and total biomass production was 9,728 pounds per acre. At Hillsboro, corn yields averaged 138.5 bushels per acre and stover dry matter averaged 8,380 pounds per acre.

DISCUSSION

Early results from trials at both locations show that within a few months after application of waste lime, there was an increase in soil pH, a corresponding reduction in Aphanomyces soil index values, and no effects on wheat or corn compared to non-limed control plots. A reduction in Aphanomyces soil index values (determined in controlled environment chambers) after only a few months contrasts with an earlier report by Bresnahan et al. (2). They reported a significant decrease in Aphanomyces root rot and an increase in sugarbeet yield within one year after application of waste lime compared to a non-limed control, but Aphanomyces soil index values were unaffected until 3 years later. These results suggest it is harder to measure affects of waste lime on *A. cochlioides* by measuring soil index values than it is by rating Aphanomyces root rot in the field (2). If so, the results reported for our trials look promising for sugarbeet crops sown at both locations in 2005.

Severity of Aphanomyces root rot is not directly affected by soil pH (9) and the disease occurs in fields over a wide range of pH values (5 to 8) in Minnesota and North Dakota. Inorganic and organic nutrients in waste lime, as well as changes in soil pH that alter nutrient availability, may increase certain groups of beneficial soil microorganisms (that interfere with survival or infection by the pathogen) and enhance sugarbeet growth. Waste lime also may alter physical properties of the soil (e.g., improve water drainage) that reduce Aphanomyces root rot. Although our initial

bioassay of soil did not indicate statistical increases in microorganisms, trends indicated higher populations of total culturable bacteria and *Streptomyces*. Species of *Streptomyces* are known for antagonistic activity against soilborne plant pathogens (6, 11). It may be too early to expect significant changes in populations of microorganisms. As more soil samples are assayed over a longer period of time, it will be apparent if microbe populations and activities are affected by waste lime. Also, assays conducted by Dr. John Weiland (USDA-ARS, Crops Research Laboratory, Fargo) for microbial DNA in soil samples collected at Hillsboro and Breckenridge will supplement our direct quantification assays and detect microorganisms that do not grow on standard media (8, 10).

The lack of effect of waste lime on yields of wheat and corn the first growing season after application is only a partial indicator of plant response. Nutrient uptake in grain and plant biomass samples will be conducted in the laboratory during the winter in 2005. There may be a change in nutrient availability in soil and uptake by crop plants when soil pH changes. Waste lime is mostly calcium carbonate so it should be effective in raising the pH of acidic soils. Most soils in the sugarbeet growing areas of Minnesota and North Dakota, however, are not acidic and in fact, many are alkaline because of naturally occurring calcium carbonates.

Overall, early results from plots at Hillsboro and Breckenridge show a favorable response to lime (increased soil pH, lower Aphanomyces soil index values, no deleterious effects of wheat and corn as rotation crops). Research will continue in these plots until at least 2008. Crops in rotation with sugarbeet at both locations will be assessed for diseases, as needed. Additional research will be conducted to analyze waste lime off production lines at each factory for P, N, calcium, magnesium, potassium and sodium. Extracts of waste lime from various factories also will be evaluated for direct effects on structures and growth of *A. cochlioides*. In the long term, this research is expected to clarify amounts and duration of waste lime needed to suppress Aphanomyces root rot and mechanisms of Aphanomyces root rot suppression. We also will know the contribution and duration of waste lime on P soil test levels and uptake by crops in the rotation, effects on other chemical characteristics in the soil, and variation in chemical characteristics of waste lime among factories and throughout the processing season.

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LITERATURE CITED

1. Bresnahan, G.A., A.G. Dexter, and W.C. Koskinen. 1998. The effect of soil pH on sugarbeet yield and herbicide degradation. Sugareet Res. Ext. Rept. 29:82-88.

2. Bresnahan, G.A., A.G. Dexter, C.E. Windels, J.R. Branter and J.L. Luecke. 2003. The effect of spent lime on sugarbeet yield and *Aphanomyces cochlioides* suppression. Sugarbeet Res. Ext. Rept. 33:273-276.

3. Bulluck III, L.R., J.B. Ristaino. 2002. Effect of synthetic and organic soil fertility amendments on Southern blight, soil microbial communities, and yield of processing tomatoes. Phytopathology 92:181-189.

4. Conn, K.L., E. Leci, G. Dritzman, and G. Lazarovits. 1998. A quantitative method for determining soil populations of *Streptomyces* and differentiating potential scab-inducing strains. Plant Disease 82:631-638.

5. Dahnke, W.C. (Ed.). 1980. Recommended chemical soil test procedures for the North Central Region. North Central Regional Publ. 221 (Rev.). North Dakota Agr. Expt. Stat., North Dakota State Univ., Fargo. 33 pp.

6. Dhingra, O.D., and J.B. Sinclair. 1985. Basic Plant Pathology Methods. CRC Press, Inc., Boca Raton, Florida. 355p.

7. Gendebien, A, R. Ferguson, J. Brink, H. Horth, M. Sullivan, and R. Davis (WRc) H. Brunet, F. Dalimier,B Landrea, D. Krack, and J. Perot (SEDE) and C. Orsi (REI). 2001. Survey of wastes spread on land - final report. Report No. CO4953-2. WRc Medmenham, Henley Road, Medmenham, Marlow, Bucks, SL7 2HD., UK.

8. Kennedy, N., and N. Clipson. 2003. Fingerprinting the fungal community. Mycologist. 17:158-164.

9. Papavizas, G.C., and W.A. Ayers. 1974. Aphanomyces Species and Their Root Diseases in Pea and Sugarbeet. Tech. Bull. No. 1485. United States Dept. Agric., Agric. Res. Serv., Washington, DC. 158 pp.

10. Ritchie, N.J., M.E. Schutter, R.P. Dick, and D.D. Myrold. 2000. Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Appl. Environ. Microbiol. 66:1668-1675.

11. Weller, D.M., J.M. Raaijmakers, B. B. McSpadden Gardener, and L.S. Thomashow. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu. Rev. Phytopathol. 40:309-348.

12. Windels, C.E., and D.J. Nabben-Schindler. 1996. Limitations of a greenhouse assay for determining potential Aphanomyces root rot in sugarbeet fields. J. Sugar Beet Res. 33:1-13.