ADDITION OF SUPPLEMENTAL SPENT LIME TO PREVIOUSLY LIMED SOILS FOR CONTROL OF APHANOMYCES ROOT ROT ON SUGARBEET

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Aphanomyces cochlioides (= *A. cochlioides*) is an economic pathogen infesting over 90% of township sections planted to sugarbeet in the Red River Valley (RRV) and many fields in southern Minnesota. The pathogen is favored by warm and wet soil conditions and can cause damping-off, root rot, and chronic root rot of older plants. *Aphanomyces cochlioides* survives in soil for years, even when sugarbeet is not planted. Recommendations for growing sugarbeet in infested fields include early planting of partially resistant varieties treated with the fungicide Tachigaren and implementing cultural practices (e.g. cultivation and improved drainage) to avoid or reduce disease. When inoculum densities of the pathogen are high and soil is wet and warm, however, these measures are inadequate for economic yields - and fields may be abandoned or yield poorly. This chronic disease has generated interest in finding effective, alternative methods to supplement control of *A. cochlioides*.

The sugar purification process results in the by-product "spent lime". Lime (calcium carbonate) precipitates impurities in sugarbeet juice. Purified juice is further processed into crystal sugar, but spent lime (14% less acid neutralizing power than fresh lime) contains impurities and becomes a factory by-product. Seven factories in the RRV and southern Minnesota generate 500,000 tons (dry weight) of spent lime annually and some has been stockpiled for 20 years. Information on uses of sugarbeet spent lime is limited and publications usually are in government and company documents. Most spent lime generated in Europe is applied to land as an amendment to increase soil pH and supply nutrients. In Great Britain, it is marketed and sold to conventional and organic growers as "LimeX".

There is limited information in the literature on use of spent lime to reduce plant diseases. Campbell and Greathead (3) applied spent lime (2 to 4 tons A^{-1}) from a sugarbeet processing factory to fields (pH < 6.8) severely infested with the clubroot pathogen, *Plasmodiophora brassicae,* in the Salinas Valley, California. A single application gave "virtually complete control" of clubroot of crucifer crops grown repeatedly for 2 to 3 years. In other areas of the world, various forms of lime (not spent lime) have been applied for over 200 years to control clubroot of crucifers, but results have been inconsistent and little is known about how various forms of lime affect the pathogen. Sugarbeet growers in southern Minnesota apply spent lime the year before planting sugarbeet (typically every 3 years) and have observed less Aphanomyces root rot. In the RRV, reduction of *Aphanomyces* on sugarbeet by addition of spent lime to soil has been well documented, even ten years after application (1,2,5).

OBJECTIVES

Our objectives were to evaluate the benefit of adding an additional 5 ton A^{-1} spent lime to a field trial where several rates of spent lime had been applied ten years earlier for: 1) effect on Aphanomyces diseases and 2) sugarbeet yield and quality.

MATERIALS AND METHODS

Establishment of field trials. Trials were originally established in a grower's field near Breckenridge, MN (pH = 6.3) in April, 2004. At that time, the Breckenridge field plot area had a history of severe Aphanomyces root rot with a soil index value (SIV) of 98 (0 to 100 scale, 0 = no disease, 100 = potential for severe disease).

The site was divided into four, 1-acre experiments. Experiments included four rates of spent lime and a non-limed control in a randomized block design of four replicates (Fig. 1). Spent lime treatments were 0, 5, 10, 15 and 20 tons fresh weight A^{-1} (= 0, 2.7, 5.3, 8, and 10.6 tons dry weight, respectively); each plot was 33 x 60 ft. To allow lime treatments to stabilize in 2004, spring wheat was sown. Sugarbeet has been grown in one experiment each year

Experiment 1

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

Experiment 2

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

Experiment 3

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

Experiment 4

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

Breckenridge, MN (illustrated above)

Crop sown/yr:					
	2004	wheat	wheat	wheat	wheat
	2005	**sugarbeet**	wheat	wheat	wheat
	2006	soybean	**sugarbeet**	soybean	soybean
	2007	wheat	wheat	**sugarbeet**	wheat
	2008	corn	corn	corn	**sugarbeet**
	2009	**sugarbeet**	soybean	soybean	soybean
	2010	corn	**sugarbeet**	corn	corn
	2011	soybean	soybean	**sugarbeet**	soybean
	2012	wheat	wheat	wheat	**sugarbeet**
	2013	**sugarbeet**	soybean	soybean	soybean
	2014	corn	**sugarbeet**	soybean	corn
	2015	soybean	soybean	**sugarbeet**	soybean

Fig. 1. Four experiments were established at Breckenridge, MN in April 2004. Each experiment was treated with spent lime at 0, 5, 10, 15 and 20 tons wet weight A^{-1}; experiments were arranged in a randomized complete block design with four replications (illustrated above). Sugarbeet plots are noted in **bold** the year when planted in an experiment; in 2015, sugarbeet test trials were planted in Experiment 3.

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from 2005 to 2014; two other experiments were planted with the same crop grown in the field by the grower-cooperator as noted in Fig. 1. In 2014, experiment 3 was planted to soybean. On October 31, 2014, original lime plots were split in half and 5 ton A^{-1} fresh weight (3.5 tons dry weight) spent lime from the Minn-Dak Farmers' Cooperative sugar factory in Wahpeton, ND were spread in half of each plot. The lime was worked into the soil by a chisel plow on November 11.

2015 Sugarbeet field trial. The Roundup Ready, Aphanomyces-susceptible sugarbeet variety 'Crystal 765RR' was sown in Experiment 3 (last planted to sugarbeet in 2011, Fig. 1) on May 22. This was the third time Experiment 3 had been planted to sugarbeet since the original application of lime. The variety had a two-year Aphanomyces disease rating of 5.9 (1-9 scale) (4) and seed was treated with Kabina ST (14 g a.i./unit) but not with Tachigaren. Approval as an Aphanomyces specialty variety requires a 2-year rating of <= 4.4 (4). Seed was sown every 4.7 inches in rows 60-feet long and 22-inches apart (six rows of each variety centered within split plots). Twenty-foot alleys were cut later leaving 40-ft plots. Experiments followed standard fertility and production practices to obtain maximum sucrose yield and quality.

Stand counts were made 2, 4, and 7 weeks after planting. Sugarbeet roots were harvested September 21 (two middle rows per subplot). Twenty roots per subplot were rated for Aphanomyces root rot (0 to 7 scale, 0=healthy and 7=root completely rotted and foliage dead). Ten roots were randomly selected and analyzed for yield and sucrose quality by the American Crystal Sugar Company Quality Laboratory, East Grand Forks, MN.

2014/2015 Soil pH and Aphanomyces soil index values (SIVs). Soil samples were collected from main plots on October 23, 2014 prior to spreading additional lime and again from subplots of supplemental lime and original lime on March 31, 2015. Five samples (6-inch depth) were collected randomly across each plot, combined, screened through 0.25-inch hardware cloth, and assayed for Aphanomyces soil index value. Subsamples were sent to Agvise Laboratories, Northwood, ND for pH, calcium, and other nutrient level determination.

Bioassays to determine Aphanomyces soil index values (SIVs, which indicate potential for *Aphanomyces* activity and populations under warm and wet conditions) were conducted by filling four (4 x 4 x 4-inch) plastic pots with soil from each sample. Then 25 seed of sugarbeet 'Crystal 985' were sown per pot to "bait" *A. cochlioides* from soil. Pots were placed in a growth chamber and arranged in a randomized block design at 70 $\pm$ 2 °F for 1 week. Temperature then was increased to 77 $\pm$ 2°F (14-hour photoperiod) and soil was kept moist to favor infection by *A. cochlioides*. Stand counts were made three times per week (beginning at emergence) and dying seedlings were removed to prevent disease spread, assayed in the laboratory, and examined microscopically to verify infection with *A. cochlioides*. At 4 weeks after planting, an Aphanomyces SIV was calculated (0 to 100 scale, 0 = *Aphanomyces*-free and 100 = all seedlings dead and soil severely infested with *A. cochlioides*) for each pot.

Statistical analysis. Data were analyzed for effect of supplemental lime by analysis of variance and for effect of original lime rate and supplemental lime by original lime interactions using linear and quadratic contrasts for significance at P = 0.05, 0.01, and 0.001. Regression analyses were made for recoverable sucrose per acre and Aphanomyces root rot rating vs. original rate of lime.

RESULTS

2015 Sugarbeet field trial. There were significant (P = 0.05) interactions between supplemental lime and rate of original lime for early season stand and all harvest parameters, indicating that supplemental lime was not equally beneficial when added to plots with different original lime rates. As a result, t-tests were made to determine whether there were significant differences from supplemental lime at each rate of original lime. In addition, the effect of original lime rate in the absence of supplemental lime was evaluated.

The supplemental lime by original lime rate interaction and results of t-tests are illustrated in Fig. 1 for early season stand, Aphanomyces root rot, root yield, and recoverable sucrose A^{-1}, and shown in Table 1 for number of harvested roots, percent sucrose, recoverable sucrose ton^{-1}, and soil pH and calcium levels. For stand at 7 weeks after planting, addition of 5 ton supplemental lime A^{-1} resulted in increased stand compared to plots without supplemental lime where original lime rates were 0 or 5 ton A^{-1}, but not where original lime rates were 10-20 ton A^{-1} (Fig. 1A). At harvest, addition of supplemental lime resulted in significantly lower Aphanomyces root rot ratings and significantly higher root yield and recoverable sucrose A^{-1} only where lime had not been originally applied, but not where plots had received original lime amendment at 5, 10, 15, or 20 ton A^{-1} (Fig. 1 B-D). Similarly, the number of harvested roots was significantly higher where supplemental lime was applied to plots that had not received original lime, but not where plots had received original lime at 5-20 ton A^{-1} (Table 1). Supplemental lime significantly increased percent sucrose in plots that had received 10 ton A^{-1} original lime, but not in plots that had received other rates of original lime (Table 1). Recoverable sucrose ton^{-1} was not significantly affected by addition of supplemental lime at any rate of original lime (Table 1).

Eleven years after application, original lime was still highly effective in reducing Aphanomyces root rot and increasing sugar beet yield. In plots without supplemental lime application, there was significant (P = 0.05) linear effect of original lime rate on all early season stand counts, Aphanomyces root rot ratings (Fig. 2B), and all harvest parameters (illustrated for recoverable sucrose A^{-1} in Fig. 2A).

2014/2015 Soil pH and Aphanomyces soil index values (SIVs). In soil samples collected March 31, 2015, 11 years after original lime was applied and 5 months after additional lime was applied to half of each original lime plot, there was no significant original lime by supplemental lime interaction for Aphanomyces SIV. Aphanomyces SIVs were high in soil samples from all plots, but there was a significant (P = 0.05) linear response to original rate of lime. Aphanomyces SIVs averaged 99, 98, 97, 93, and 94 in plots originally treated with 0, 5, 10, 15, and 20 ton lime A^{-1}, respectively (data not shown). Addition of 5 ton A^{-1} supplemental lime also significantly reduced Aphanomyces SIV from 99 in soil samples without supplemental lime to 94 in soil samples with supplemental lime (data not shown).

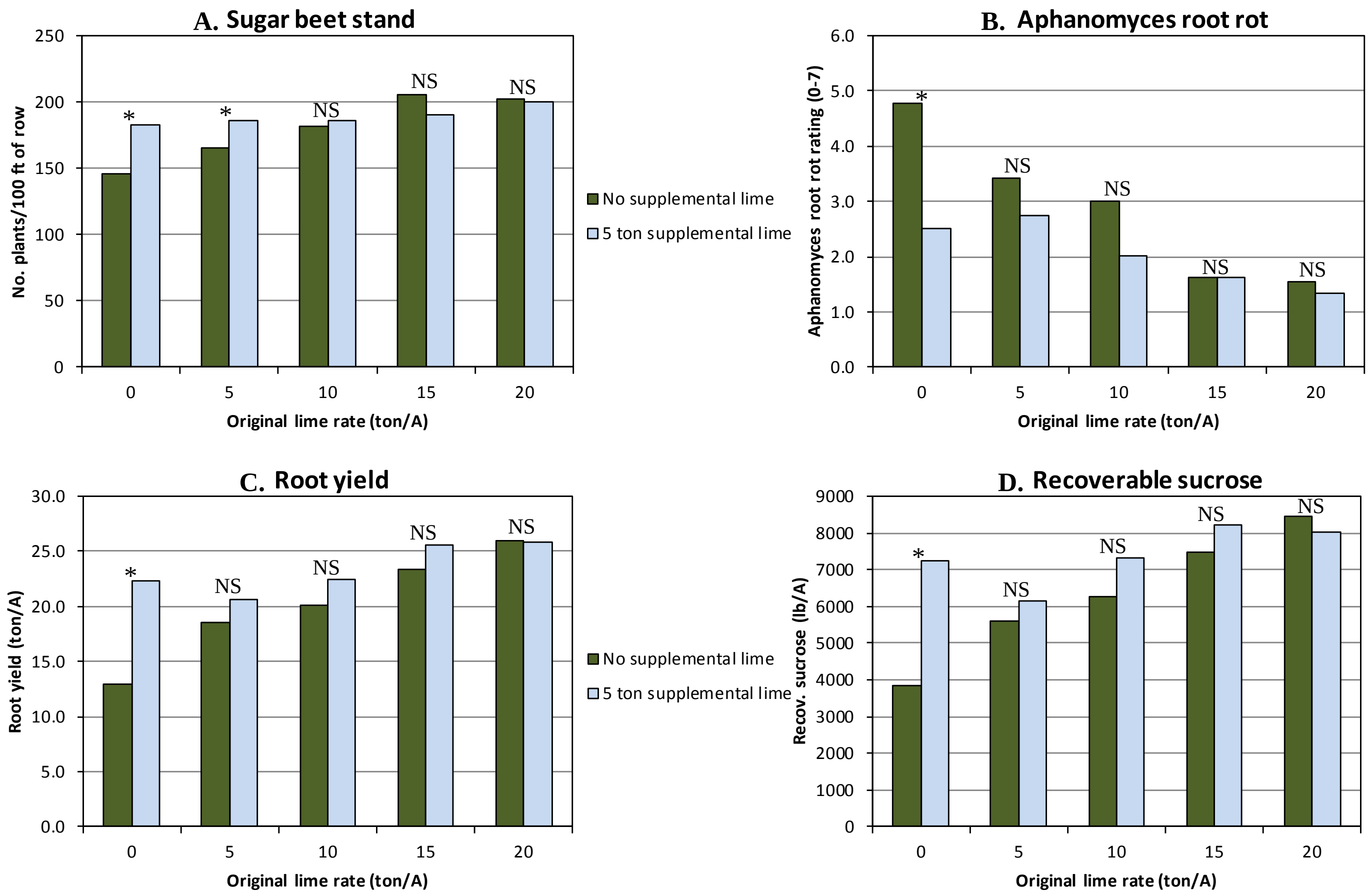

Fig. 1. A) Stand, B) Aphanomyces root rot, C) root yield, and D) recoverable sucrose of sugar beet grown during 2015 in a field severely infested with *Aphanomyces cochlioides* in plots amended in 2004 with 0, 5, 10, 15, or 20 ton fresh weight lime per acre (original lime rate) with and without supplemental 5 ton fresh weight lime per acre added on October 31, 2014. For all variables, there was significant ($P = 0.05$) interaction among original lime rate and supplemental lime, so significance of supplemental lime was tested at each rate of original lime; for each original lime rate, * = significant ($P = 0.05$) difference with supplemental lime, NS = not significantly different. Aphanomyces root rot rating, 0-7 scale (0 = roots healthy; 7 = root completely rotted and foliage dead)

There was significant original lime by supplemental lime interaction for soil pH and extractable calcium levels. Soil pH was significantly increased by addition of supplemental lime where rate of original lime was 0 or 5 ton A^{-1}, and soil calcium was increased by addition of supplemental lime for all original lime rates (Table 1). In soil from plots without supplemental lime, there was a significant linear effect of original lime rate on soil pH and extractable calcium (Table 1).

DISCUSSION

Conditions at the site near Breckenridge, MN were conducive for early-season damping-off and root rot through the early part of the growing season. Planting was late (May 22) and rainfall during the following week at the Wahpeton North Dakota Agricultural Weather Network station was 2.5 inches. This resulted in warm, moist, favorable soils for infection by *A. cochlioides* that continued for some time after emergence. Stand in plots with no

Table 1. Number of harvested roots, sucrose, soil pH and extractable calcium data for sugarbeet plots amended with several rates of spent lime in April, 2004, then split and either amended with an additional 5 ton A^{-1} spent lime on October 31, 2014 or non-amended in a field near Breckenridge, MN naturally infested with *Aphanomyces cochlioides.*

Lime[X] (ton A^{-1})	No. roots harv./100 ft		Percent sucrose		Recov. sucrose (lb ton^{-1})		Soil pH[Z]		Soil extractable Ca[Z]	
	None[Y]	5 ton[Y]	None[Y]	5 ton[Y]	None[Y]	5 ton[Y]	None[Y]	5 ton[Y]	None[Y]	5 ton[Y]
0	94	162*	16.2	17.7	294	325	7.0	7.7*	2977	4845*
5	138	151	16.5	16.5	301	298	7.3	7.8*	3402	4768*
10	145	163	16.9	17.7*	309	326	7.6	7.8	3598	5090*
15	179	189	17.4	17.6	318	321	7.9	8.0	4085	5224*
20	195	186	17.6	17.1	325	312	7.9	8.0	4314	5403*

X Original 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 2015, sugarbeet was sown on May 22, 11 years after original spent lime had been applied. Plots were harvested on September 21, 2015.

Y On October 31, 2014, original lime plots were split and either amended with a supplemental 5 ton A^{-1} or non-amended. There was significant original lime rate by supplemental lime interaction, so data is shown separately for subplots with no supplemental lime (None) and subplots with 5 ton A^{-1} supplemental lime (5 ton). Within original lime rates, values for 5 ton supplemental lime marked with an asterisk were significantly higher ($P = 0.05$) than corresponding no supplemental lime value.

Z Soil pH and extractable calcium (Ca) values from Agvise Laboratories, Northwood, ND.

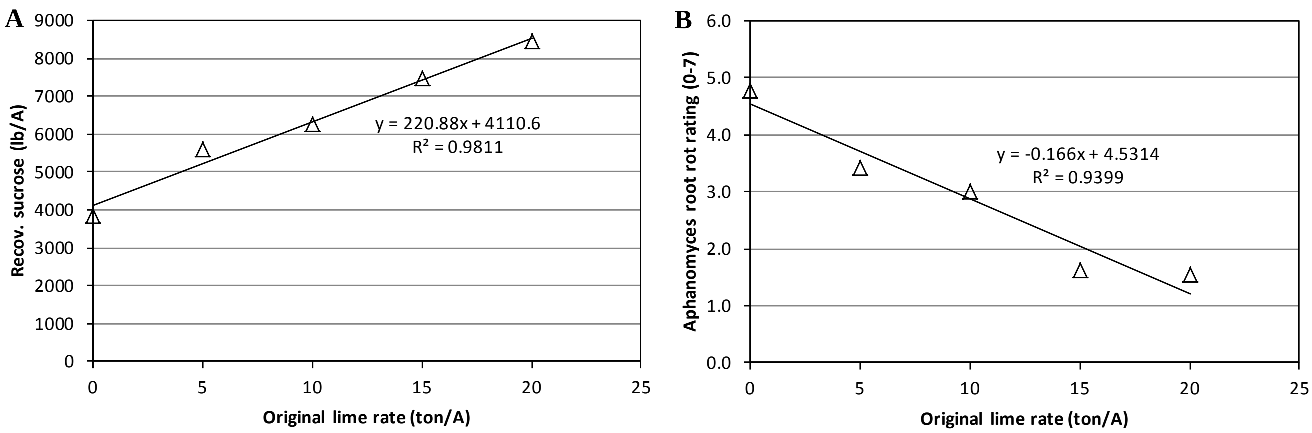

Fig. 2 Regression of **A)** recoverable sucrose and **B)** Aphanomyces root rot rating vs. original rate of lime per acre (with no supplemental lime) in a grower's field near Breckenridge, MN, naturally infested with *Aphanomyces cochlioides*. Plots were amended with original lime in April, 2004. Data is from sugarbeet sown May 22, 2015 (11 years after spent lime application) and harvested September 21, 2015.

original or supplemental lime application declined from 203 plants per 100 feet of row on June 4 to 146 plants per 100 feet of row on July 9 (a 28% reduction), indicating high disease pressure (data not shown). Rainfall in June, July, August, and September was 2.4, 2.8, 1.0, and 0.4 inches, respectively, lower than normal. As a result, plants that didn't die were able to recover and produce good yields, especially as lime rates increased (Fig. 1). Root rot ratings at harvest were based on scarring and malformed roots, indicative of earlier infections from which roots had tried to recover.

Results from this trial did not justify application of supplemental lime to fields that have received at least 10 ton A^{-1} spent lime in the past, even up to ten years earlier. However, it should be noted that this is one year's worth of data, during a season with high early-season but low late-season disease pressure. In a wet season with high soil moisture during a larger part of the growing season, results may have been different. In addition, use of the highly susceptible variety without Tachigaren seed treatment in this trial was intended to increase the likelihood of seeing differences from lime treatments. Data from previous trials over several years, with various levels of disease severity, utilizing both a resistant variety treated with Tachigaren and a susceptible variety without Tachigaren indicate better results with a resistant variety treated with Tachigaren. Application of lime cannot be expected to protect a sugarbeet variety with a low level of resistance to Aphanomyces, especially under favorable disease conditions.

Previously at the Breckenridge site, original rates of lime were applied in April, 2004 with sugarbeet not planted until the spring of 2005, a full year later. Supplemental lime application in this trial was applied in late fall (October 31, 2014) with sugarbeet planted the following spring (May 22, 2015). The excellent results observed in the 2015 sugarbeet crop indicate that spent lime application can be made the fall preceding sugarbeet planting.

SUMMARY AND CONCLUSIONS

1. Where lime had not previously been applied, application of spent lime at 5 ton A^{-1} in the fall of 2014 resulted in significant reduction of Aphanomyces and increased sugarbeet yield the following season.
2. Where lime had previously been applied at 5 ton A^{-1}, benefits from adding 5 ton A^{-1} supplemental lime were significant for early season stand protection, but not sugarbeet yield.
3. Where lime had previously been applied at 10, 15, or 20 ton A^{-1}, there were no significant benefits from adding 5 ton A^{-1} supplemental lime.
4. Eleven years after application, original spent lime rates with no supplemental lime significantly reduced Aphanomyces root rot and increased sugarbeet stand, yield, and quality.

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