EFFECTS OF LIME AMENDMENT ON REDUCING *APHANOMYCES COCHLIOIDES* INFECTION OF SUGARBEET OVER A RANGE OF SOIL pH

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The soilborne pathogen *Aphanomyces cochlioides* (= *A. cochlioides*) continues to be a major concern in the sugarbeet growing areas of Minnesota and North Dakota. Warm, wet soil conditions promote the growth of *A. cochlioides* which causes early season damping-off and root rot in older sugarbeet. Current methods used to reduce *Aphanomyces* infection on sugarbeet include planting of partially resistant cultivars, treating seed with Tachigaren, cultivating, and draining wet fields (6). Amending fields with spent lime, a byproduct of the sugarbeet purification process, has also shown promise in reducing *Aphanomyces* infection and increasing sugarbeet yield. Brantner et al. reported that an application of lime to a grower's field in 2004 continued to show significant reduction in *Aphanomyces* as well as an increase in sugarbeet yield eight years after the application (1).

The first report of the effects of spent lime on sugarbeet yield came from a trial in which lime was used to raise the pH of soil in hopes of reducing herbicide carryover (2). Trials were then established in 2003 to monitor the effects spent lime had on sugarbeet yield and on reducing *Aphanomyces* infections in low and high pH soils (3). While increases in yield and recoverable sucrose per acre were seen in both the low and high pH soils (pH = 6.0 and 7.9, respectively), reduction of *Aphanomyces* infections was only reported in the low pH soil (3). Similarly in multiple-year lime trials, lime amendment eight growing seasons prior continued to show increased yields and reduction of *Aphanomyces* infections in a field where lime was added to low pH (pH = 6.3-6.5) soil, but yield differences were less, sometimes even insignificant, and disease pressure was low in a field where lime was applied to high pH (pH = 7.0-7.4) soil (7).

Spent lime is known to increase soil pH and has shown potential to reduce *Aphanomyces* infections in soils amended with lime, but there is limited information on these effects over a range of soil pH. Furthermore, there is a lack of knowledge of spent lime effects on soils from a range of pH values with high *Aphanomyces* populations. It is also unclear whether the benefits of amending fields with spent lime come from its ability to raise soil pH or are derived from other interactions such as the increase in concentration of a macronutrient comprising lime, i.e. calcium. Olsson et al. reported a negative correlation between soil calcium concentration and Aphanomyces disease severity index and suggested a threshold of 250 mg/100 g (2,500 ppm) for reduced risk of Aphanomyces problems in sugarbeet (5).

OBJECTIVES

The objectives of this trial were to 1) observe whether the addition of spent lime could reduce Aphanomyces damping-off of sugarbeet in soils across a range of pH values and 2) to observe any relationship between calcium or other soil factors and Aphanomyces soil index values (SIVs) in lime-amended and non-amended soils.

MATERIALS AND METHODS

Soils that represented a range of pH values and also had high populations of *A. cochlioides* were collected from fields in sugarbeet growing areas of Minnesota and North Dakota (Table 1). A total of fifteen fields were sampled in Grant, Renville, and Wilkin counties in Minnesota, as well as in Cass, Richland, and Traill counties in North Dakota. Soils were screened through 0.25-inch hardware cloth and stored at 35-40°F until beginning the trial.

Soil pH values were measured after the samples were screened but prior to lime amendment. A small amount of soil taken from each sample was set out to dry at room-temperature. After 24 hours, the dry soils were ground into powder with a mortar and pestle. A 5 g sample was removed, and with a glass stirring rod, was mixed vigorously with 5 ml of deionized water for 5 seconds. After 10 minutes of incubation, a pH probe was inserted into the soil/water slurry and gentle stirring was provided until the pH meter stabilized (~3 sec) and the pH value was recorded (Accumet® pH Meter 15, Fisher Scientific).

Table 1. Fifteen soil samples with a range of pH values and high *Aphanomyces cochlioides* populations collected from fields in sugarbeet growing areas of Minnesota and North Dakota used in a soil assay to determine effect of lime amendment on Aphanomyces damping-off.

Soil sample	pH	Location (Co., St.)	Soil type	Aph. SIV (no-lime)
RCM	5.3	Richland, ND	Gardena Silt Loam	100
Mang	5.9	Renville, MN	Nicollet Clay Loam	100
PK2	6.0	Traill, ND	Fargo Silty Clay/Fargo Silty Clay Loam/Swenoda Loam	83
Palm	6.0	Renville, MN	Clarion Loam	86
Larry	6.5	Grant, MN	Formdale Clay Loam	100
Hend	6.7	Richland, ND	Wyndmere Loam	100
Breck	6.7	Wilkin, MN	Doran Clay Loam/Lindaas Silt Loam	99
PK3	6.7	Traill, ND	Fargo Silty Clay/Fargo-Hegne Silty Clays	92
JB2	7.5	Traill, ND	Fargo-Hegne Silty Clays	52
PK1	7.6	Traill, ND	Glyndon Silt Loam/Overly-Fargo Complex/Embden Fine Sandy Loam	14
JB1	7.7	Traill, ND	Fargo-Hegne Silty Clays/Fargo-Enloe Complex	99
Kind	7.9	Cass, ND	Fargo-Hegne Silty Clays/Fargo Silty Clay	92
RD1	8.0	Traill, ND	Bearden-Lindaas Silty Clay Loams	77
Aber	8.0	Richland, ND	Mantador-Delamere-Wyndmere Fine Sandy Loam	4
Drive	8.1	Renville, MN	Harps Clay Loam	14

Approximately 10 L of each soil sample were mixed thoroughly before being split into two 5 L samples, one labeled "no-lime" and the other "lime". The "no-lime" samples were mixed again before being measured into 4 x 4 x 4-inch plastic pots, seven replicate pots per soil. Approximately 700 cc of soil were measured into each pot. Each "lime" sample was amended with spent lime at a rate of 10 tons wet weight A^{-1} (= 5.4 tons dry weight). The lime was measured out (162 g/5 L soil) and thoroughly mixed into each soil sample before the soil was measured into pots. As the amount of soil taken from the fifteen fields was not uniform, four of the fifteen samples had less than 10 L of total soil volume. Thus, one soil's "lime" and "no-lime" portions were divided into five replicates each while three additional samples had soil enough for three replicates. The amount of lime added to these samples was adjusted to account for the smaller volumes of soil. Pots were randomized within replicates and were incubated at 77 ± 2°F under minimal light for 4 weeks. Pots were watered once on the day of amending and then 2-3 times per week thereafter during the 4-week incubation.

After 4 weeks of incubation, pots were sown with 25 seed per pot of sugar beet cultivar 'Crystal 985RR'. Soil (250 cc) was removed from the top of each pot, seed was planted into the remaining soil, and then the 250 cc was returned to cover the seed. Six replicate pots were planted per soil treatment for eleven of the fifteen samples. Three replicates were planted for each of the three smallest samples and four replicates were planted for the fifteenth sample. Pots were again randomized within replicates and incubated at 70 ± 2°F for one week followed by 77 ± 2°F (with a 14-hour photoperiod) for the remaining 3 weeks of the assay. Pots were watered daily to provide high soil moisture favorable for infection by *A. cochlioides*.

At the same time as the soil assays were planted, soil samples (500 g) from the extra replicates, replicates 5 or 7, were taken to Agvise Laboratories in Northwood, ND for analysis. For soils with less than four replicate pots, samples were not sent to Agvise Laboratories until after the soil assay was complete. The soil from the extra replicates, both limed and non-limed, was also used for at-plant pH measurements using the procedure described above.

Stand counts were conducted three times weekly beginning seven days after planting. Dead and dying seedlings were removed from pots, ~2-cm portions of infected hypocotyls were excised, surface treated with 0.5% sodium hypochlorite, rinsed twice in sterile deionized water, and placed in quad-divided petri dishes containing sterile water. After 48 hours, hypocotyls were examined under the microscope for growth of *A. cochlioides*. At 4 weeks after planting, remaining seedlings were removed from pots, washed, and rated on a scale of 0-3 (0 = no disease; 3 = all tissue necrotic, seedling dead). Soil index values (SIVs, 0-100 scale, 0 = no disease, 100 = all plants died during

the 4-week assay) were calculated based on these ratings along with the numbers of emerged and dead seedlings per pot.

Statistical analysis. The soil samples Aber, Drive, and PK1 were not included in statistical analysis as the Aphanomyces SIVs of their no-lime samples were very low indicating low *A. cochlioides* populations. Correlation analysis of the change in Aphanomyces SIV upon addition of lime versus the original soil pH was performed to determine if reduction of Aphanomyces damping-off by lime amendment is dependent on soil pH. Additional correlation analyses were performed to determine if other soil characteristics from the Agvise analyses were related to Aphanomyces SIV. In addition, percent stand during the 4-week assay and Aphanomyces SIV for each lime-amended versus non-amended soil was compared by analysis of variance ($P = 0.05$).

RESULTS

There was no correlation between the original soil pH and reduction in Aphanomyces SIV upon amendment with lime (Fig. 1A). Reduction of Aphanomyces SIVs by lime amendment was not dependent on original soil pH value (whether it was low or high) but instead occurred in most soils regardless of original pH value (Table 2). Moreover, the addition of lime raised the pH value of each soil sample (Table 2). Soil samples with the lowest no-lime pH values saw the greatest increase in pH upon amendment with lime, but magnitude of pH change was not correlated with reduction in Aphanomyces SIV (Fig. 1B). Instead, the magnitude of the change in calcium concentration was significantly correlated with reduction in Aphanomyces SIV for the 12 pairs of lime-amended and non-amended soils (Table 2 and Fig. 1C). In addition, soil calcium concentration was negatively correlated with Aphanomyces SIV across all 24 soil samples (lime-amended and non-amended, Fig. 1D). The correlation coefficient of calcium concentration and Aphanomyces SIV was -0.5165 (22 df) which is significant at $P = 0.01$. Among other soil characteristics tested by Agvise Laboratories, SIV was significantly correlated with sulfur and cation exchange capacity (CEC) at $P = 0.01$, and with pH, magnesium, sodium, iron, and calcium carbonate equivalent (CCE) at $P = 0.05$ (data not shown).

Stand loss caused by Aphanomyces damping-off was reduced in most soils by the addition of spent lime. Lime amendment significantly ($P = 0.05$) increased seedling stand over that of non-amended soil in nine of twelve fields analyzed (Fig. 2). Even with the addition of lime, however, most seedlings in four samples (RCM, Mang, Larry, and Hend) did not survive the 4-week assay. This is most likely due to very severe infestation by *A. cochlioides*.

Table 2. Soil pH, Aphanomyces soil index values (SIVs), and calcium concentrations of soils collected from Minnesota and North Dakota to represent a wide range of pHs with high populations of *Aphanomyces cochlioides*.

	pH			Aphanomyces SIV			$[Ca^{2+}]$ (ppm)		
Soil sample	No-lime	Lime[X]	Δ pH[Y]	No-lime	Lime[X]	Δ SIV[YZ]	No-lime	Lime[X]	Δ $[Ca^{2+}]$[Y]
RCM	5.3	7.8	2.5	100	100	-0.2	935	2900	1965
Mang	5.9	7.9	2.0	100	98	-2.3	3232	5784	2552
PK2	6.0	7.9	1.9	83	31	-51.9**	2299	4929	2630
Palm	6.0	8.0	2.0	86	14	-71.9**	2960	6895	3935
Larry	6.5	8.0	1.5	100	98	-1.8	3047	6451	3404
Hend	6.7	8.0	1.3	100	91	-8.2	1588	3288	1700
Breck	6.7	8.0	1.3	99	77	-22.6*	3660	6779	3119
PK3	6.7	8.0	1.3	92	10	-81.9***	3392	6632	3240
JB2	7.5	8.1	0.6	52	14	-37.8*	2963	5527	2564
JB1	7.7	8.1	0.4	99	70	-29.8*	4302	6480	2178
Kind	7.9	8.2	0.3	92	56	-35.3	5642	8372	2730
RD1	8.0	8.3	0.3	77	72	-4.7	4430	6083	1653

[X] Soil amended with 10 ton A^{-1} fresh weight lime.

[Y] Δ = change in pH, SIV, and calcium concentration (Ca^{2+}) upon amendment with lime.

[Z] Based on *p*-value from ANOVA for no-lime versus lime SIVs, * = significant at $P = 0.05$, ** = significant at $P = 0.01$, *** = significant at $P = 0.001$.

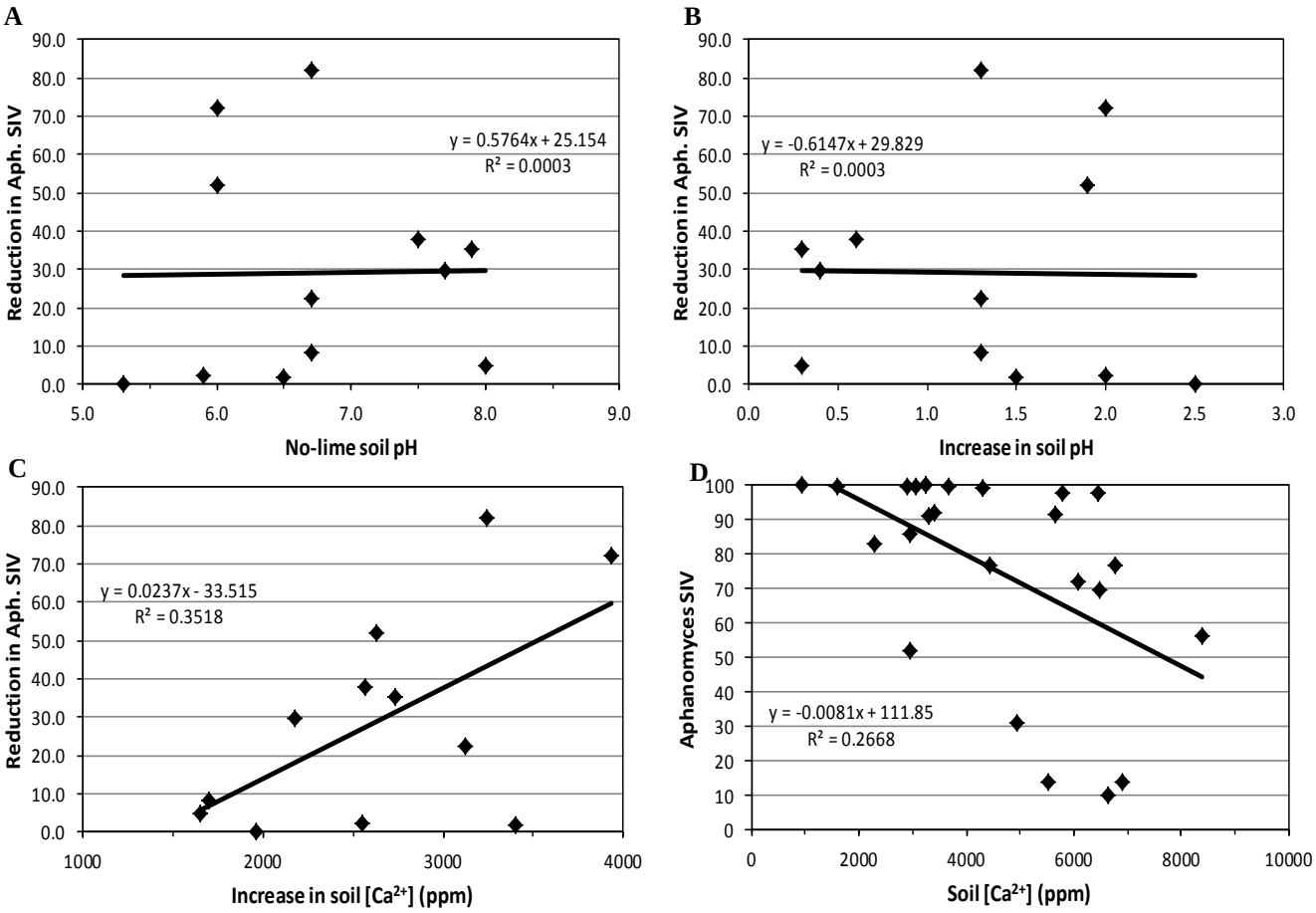

Fig. 1. Correlation between **A)** reduction in Aphanomyces soil index values (SIVs) upon amendment with 10 tons fresh weight A^{-1} lime and pH of the original non-amended soil samples, **B)** reduction in Aphanomyces SIV upon lime amendment and increase in soil pH, **C)** reduction in Aphanomyces SIV and increase in soil calcium concentration (ppm), and **D)** Aphanomyces SIV and calcium concentration (ppm) for all soil samples both lime-amended and non-amended.

DISCUSSION

Most soils tested showed a reduction in Aphanomyces SIV and/or decrease in stand loss from Aphanomyces damping-off upon the addition of spent lime. Starting soil pH values did not influence the benefit of lime amendment. Lime amendment also increased the pH and calcium concentration in all soils. In a study of the effects of calcium on reducing *Aphanomyces* infections of pea in Sweden, Heyman et al. reported that calcium carbonate raised soil pH and reduced *Aphanomyces* infections while the addition of calcium sulfate had little effect on soil pH but was better able to reduce *Aphanomyces* (4). Furthermore, the addition of sodium bicarbonate reduced the amount of soluble calcium and raised soil pH. With less calcium available, Aphanomyces disease severity increased. The effects of calcium sulfate and sodium bicarbonate led the authors to suggest that high soil pH may not play an important role in disease suppression (4). Alternatively, they proposed that calcium ions play a major role in reducing *Aphanomyces* infections (4). In a second study completed in Sweden on sugarbeet, Olsson et al. found a negative correlation between soil calcium concentration and Aphanomyces disease severity index and suggested that a calcium threshold of 250 mg Ca/100 g soil (=2,500 ppm Ca) can be used to asses soils at risk for *Aphanomyces* (5). They reported that soils falling below this calcium threshold are at an increased risk for *Aphanomyces* infections (5). Similar to these findings, the soil samples in our study from Minnesota and North Dakota had a significant negative correlation between calcium concentration and Aphanomyces SIV. The significant correlations between SIV and other soil characteristics tested by Agvise are most likely due to close association with soil pH. Additionally in our study, a correlation was observed between the increase in calcium

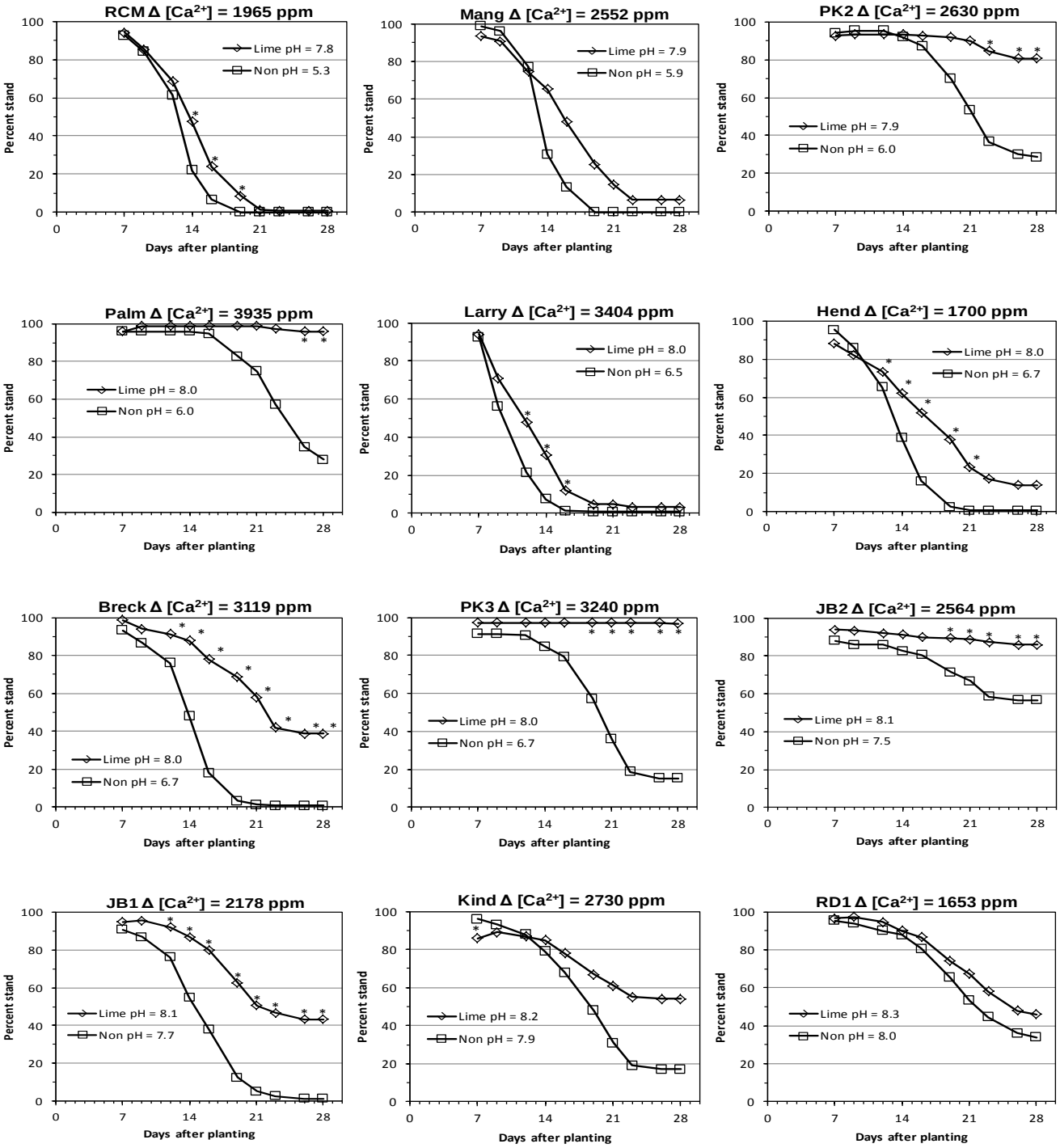

Fig. 2. Percent stand over a 4-week assay for sugarbeet sown in lime-amended (10 ton fresh weight A^{-1}) (Lime) compared to non-amended soils (Non) representing a range of soil pH (from 5.3 to 8.0 in non-amended soils) with high populations of *Aphanomyces cochlioides*. Δ [Ca^{2+}] = the change in soil calcium concentration for each soil upon amendment with lime. For each stand count date, presence of an asterisk indicates a significant difference (P = 0.05) between percent stands of lime-amended and non-amended soils.

concentration with lime amendment and reduction in Aphanomyces SIV. Calcium concentrations reported in our study (performed by Agvise) are based on ammonium acetate extractions, while the papers from Sweden used ammonium lactate extractions. It is not known how closely the calcium concentrations obtained by these two methods correlate. Only three of the twelve soil samples used for statistical analysis fell below the 2,500 ppm calcium threshold prior to lime amendment. The remaining nine initially had calcium concentrations above the 2,500 ppm threshold yet still had high Aphanomyces SIVs. Perhaps the calcium threshold is different in Minnesota and North Dakota soils or less relevant than the increase in calcium concentration with spent lime amendment. High levels of calcium do not always cause complete suppression of *Aphanomyces* as many other factors are involved including *Aphanomyces* population levels (4). Similarly, in our study, Aphanomyces SIV in a few soil samples with very high *A. cochlioides* populations was still very high after amendment with lime. While increasing soil calcium concentration seems to be important in reducing Aphanomyces damping-off, pathogen population and other soil factors are likely involved.

SUMMARY AND CONCLUSIONS

1. Spent lime application reduced *A. cochlioides* infection in soils ranging from pH 5.3-7.7.

2. Reduction of Aphanomyces soil index value by lime amendment was correlated with both the increase in soil calcium concentration in lime-amended soils and the concentration of calcium across lime-amended and non-amended soils.

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