APHANOMYCES ROOT ROT MANAGEMENT IN ALFALFA

Damon L. Smith [1/] and Quinn Watson [2/]

Introduction

Alfalfa is an important crop for Wisconsin and the Midwestern, United States. Commodity records, as of February 1, 2013 report alfalfa prices reaching $265 per ton for hay (1). Nationwide, alfalfa was planted across 55.6 million acres in 2012, the 3rd field crop in terms of acreage after corn and soybean, and has an $8 billion dollar production value (15). Furthermore, alfalfa is the single largest source of protein for livestock, especially for the dairy industry (13). Wisconsin is the second largest producer of dairy in the United States, and since dairy feed is the single largest cost to the milk producer, the yield and consequent price of alfalfa is understandably important to the Wisconsin dairy industry (16).

Aphanomyces euteiches is a soil-borne oomycete that causes the disease, Aphanomyces root rot. *A. euteiches* can infect a variety of field crops worldwide, but in Wisconsin, the most important commodity is alfalfa. *A. euteiches* is most threatening in poorly drained soil conditions because it proliferates with water-motile zoospores. *A. euteiches* germinates in response to chemical signals from its host's roots during early seeding, penetrates its host, and causes stunted, chlorotic hypocotyls and cotyledons due to necrosis of the roots early after emergence (12, 13). Although this disease does not cause immediate damping off, the pathogen stunts growth and reduces alfalfa's ability to compete with weeds. This monocyclic oomycete is persistent and it is suspected that its oospores can survive as many as 30 years in soil that has not been planted with alfalfa. This suggests that *A. euteiches* can parasitize other hosts. Furthermore, *A. euteiches* has adapted to have increasingly more virulent phenotypes, beginning with race 1, race 2, and possibly now the most virulent race, race 3 (6, 12).

Currently, there exists no chemical treatment to manage *A. euteiches* infestations in alfalfa. The fungicide metalaxyl has been found ineffective against *A. euteiches* even though it effectively inhibits *Phytophthora medicaginis*, a second oomycete pathogen that frequently occurs in alfalfa fields (9). Farmers are left two management options for Aphanomyces root rot; crop rotations and planting with alfalfa cultivars that are selectively bred for resistance to specific races of *A. euteiches*. Currently, the commercial cultivar with the highest resistance available is only against race 2, which will be ineffective in prevention of *A. euteiches* of the putative race 3. Selectively breeding resistance to *A. euteiches* in alfalfa has aided in increased alfalfa yields; however breeding is a slow and costly process, especially since more virulent phenotypes than race 2 are predicted to exist (6). In addition, interest has peaked into using alfalfa varieties with the Roundup Ready trait. Anecdotal reports suggest that these Roundup Ready varieties lack the level of resistance to *A. euteiches* race 2 that exists in conventional varieties. This should be investigated further.

[1/] Assistant Professor, Dept. of Plant Pathology, 1630 Linden Drive, Univ. of Wisconsin-Madison, Madison, WI, 53706.
[2/] Undergraduate Research Assistant, Dept. of Plant Pathology, 1630 Linden Drive, Univ. of Wisconsin-Madison, Madison, WI, 53706.

Interestingly, recognition of *A. euteiches* as a growing threat to alfalfa yields in 1987 and 1990 by Delwiche, Grau, and Holub coincided with reductions of sulfate deposition through precipitation (7, 9, 14). This was a result of the Clean Air Act of 1970 that put restrictions on sulfur oxide emissions. Subsequently sulfur deficiency in soil has been reported in several alfalfa producing states that have also isolated *A. euteiches,* including Wisconsin, Iowa, and Indiana (2, 5). This development suggests a relationship between sulfur availability and alfalfa susceptibility to *A. euteiches* infection.

In alfalfa, sulfur deficiency may interfere with sulfur-induced resistance against fungal pathogens (8). Sulfur deficiency is exhibited in alfalfa by uniform yellowing of leaves and stems. Each cutting of alfalfa removes 5-7 lbs of sulfur per ton of alfalfa hay. Alfalfa requires up to 25-50 lbs/acre actual sulfur during the seeding year, especially in deficient areas (11). The most inexpensive source of supplemental sulfur is $CaSO_4{\cdot}2H_20$ at 20 ppm of actual sulfur applied to soil. Calcium is typically not an element considered to be limited in Wisconsin soils; however $CaSO_4$ supplies sufficient levels of sulfur at a semi-soluble rate, allowing for useable sulfur over multiple growing seasons (10). The research presented here investigated other forms of sulfur fertilizers in addition to the standard. This included K_2SO_4, $(NH_4)_2SO_4$, and elemental sulfur, and at different soil concentrations to determine which fertilizer and concentration most effectively improved alfalfa health and/or inhibited *A. euteiches* growth. K_2SO_4 and $(NH_4)_2SO_4$ have additional nutritional benefits because they provide K and NH_4 respectively, are more soil-soluble, and are more readily absorbed by alfalfa. Elemental sulfur is an inaccessible form of sulfur for alfalfa; however it has been documented to increase spore dormancy and have other inhibiting effects on certain fungal pathogens (8), but there is no data on the potential relationship between elemental sulfur and *A. euteiches* or other oomycetes. This could be because there is no proven detrimental effect of sulfur on these particular plant pathogens (3). Additionally resistance to the three races of *A. euteiches* was investigated.

The objectives of this research were as follows:

1. Evaluate the effect of $CaSO_4{\cdot}2H_20$, K_2SO_4, $(NH_4)_2SO_4$, and elemental sulfur at 10, 20, 30, or 40 ppm, or no sulfur, on the growth of different isolates of *A. euteiches* in culture.

2. Investigate the development of Aphanomyces root rot in alfalfa seeded to growth medium amended with $CaSO_4{\cdot}2H_20$, K_2SO_4, $(NH_4)_2SO_4$, and elemental sulfur at 10, 20, 30, or 40 ppm.

3. Determine the level of resistance to all races of *A. euteiches* in select Roundup Ready alfalfa varieties.

Methods

In order to test sulfur toxicity from different sulfur sources on *A. euteiches,* isolates of *A. euteiches* from race 1 and 2 were incubated on Petri plates with varying sulfur concentration. The *A. euteiches* specimens included the isolates Larsen MF-1 (race 1) and Larsen NC-1 (race 2). One 5-mm diameter plug of each isolate was transferred from Petri plates with cornmeal agar to five different Petri plates with cornmeal agar amended with different levels of sulfur: a no sulfur treatment, and 10, 20, 30, and 40 ppm sulfur. Four different sulfur sources were used and include K_2SO_4 (potassium sulfate), Ca_2SO_4 (calcium sulfate), $(NH_4)_2SO_4$ (ammonium sulfate), and elemental sulfur, with which each isolate was incubated. Thus the experiment was a factorial study conducted using a completely randomized experimental design with three replicates. The experiment was maintained on a benchtop at approximately 24°C. The diameter of mycelial growth was measured using two radii for each plate, for each day up to 10 days after transfer to the Petri plate. Measurements commenced three days after transfer and performed using a digital caliper. The entire experiment was repeated twice. Time course data were converted to area under the growth curve (AUGC) and analyzed using analysis of variance to investigate the sulfur effect on *A. euteiches* growth. All analyses were conducted using PROC GLIMMIX of the SAS statistical package.

The same isolates of *A. euteiches* used above were used as inoculum on different alfalfa cultivars planted on soilless growth medium amended with four different sulfur treatments. Three alfalfa cultivars were chosen according to resistance, WAPH-1 (expresses resistance to race 1, susceptible to race 2 of *A. euteiches)*, WAPH-5 (expresses resistance to race 1 and race 2 of *A. euteiches)*, and Vernal (is susceptible to all races of *A. euteiches)*. ConeTainers (4 cm diameter x 7.5 cm height) with punctured bottoms were filled with soilless media of known mass to the top of the container. Ninety-eight ConeTainers were placed in a rack in a shallow flat, which was filled with water for irrigation. Before inoculating, the four sulfur treatments were applied by dissolving an exact amount of fertilizer in 20 mL deionized water to make 10, 20, 30, and 40 ppm concentrations. The sulfur treated water, including the no sulfur control, were incorporated evenly into the top 3 cm of media in each cone.

During the inoculations, each flat was designated to one specific *A. euteiches* race to avoid cross contamination, but within each tub the arrangement of ConeTainers with different alfalfa cultivars was randomized. Seeds of each cultivar were planted and flooded with *A. euteiches* mycelial fragments. One flat received no inoculum and was the negative control. The experiment was repeated, with three replicates per run. Incubation time, watering schedule, and a disease rating scale will be performed according to the protocol by Fitzpatrick *et al* (5). To confirm infection Koch's Postulates was completed by baiting the *A. euteiches* cultivars upon selective media as performed by Delwiche *et al* (4). Data were analyzed using analysis of variance to investigate the sulfur effect on *A. euteiches* growth. All analyses will be conducted using PROC GLIMMIX of the SAS statistical package.

Twelve alfalfa varieties were tested for resistance to *A. euteiches* races 1, 2, and, 3. Vernal was used as a susceptible check and WAPH5 as a resistant check. The Roundup Ready varieties tested included Dekalb DKA4118 RR, WL 356 HQ RR, Pioneer 54R04, Aphatron RR, WL 350RR LH, Dekalb DKA4416 RR, and Pioneer 54R02. Three conventional varieties were also included, which were WL 354 HQ, Legacy 449 Aph2, and Pioneer 55V50. Evaluation was carried out using the standardized *A. euteiches* resistance testing protocol. Briefly, thirty seeds of each variety were placed into a single cell of a greenhouse cell-pack. After seeding, plants were inoculated with mycelial fragments of each race of *A. euteiches*. Plants were bottom watered for seven days (depth of water maintained at 2.5 cm). Plants were then flooded and maintained for seven more days in the flooded conditions. Finally, plants were rated for proportion of plant survival. A survival rating of approximately 50% would be considered a "highly resistant reaction."

Results and Discussion

Sulfur source did not significantly impact the growth of *A. euteiches* (Fig. 1; P=0.09). Rate of sulfur source did not impact the growth of *A. euteiches* either (P=0.32). Sulfur did not have an inhibitory effect on *A. euteiches* in these experiments.

Significant interaction of *A. euteiches* race, alfalfa variety, and sulfur source were identified in experiments were plants were inoculated in ConeTainers (P=0.05). For all varieties, no difference in level of disease was identified in plants treated with sulfur compounds and inoculated with the less aggressive race 1 isolate, Larsen MF-1 (data not shown). For plants inoculated with the more aggressive race 2 isolate, Larsen NC-1, application of Ca_2SO_4 (calcium sulfate) was the most consistent treatment to reduce DSI across all alfalfa varieties (Fig. 2). However, DSI was not significantly different from the non-treated control in all cases. Follow-up experiments are being performed to investigate the role of Ca_2SO_4 (calcium sulfate) in Aphanomyces root rot control.

Roundup ready varieties inoculated with *A. euteiches* race 1 typically had better survival than the susceptible check Vernal (Fig. 3). However, the only Roundup Ready variety to perform as well as WAPH5 (resistant check) was Pioneer 54R02, which was also comparable to the best conventional cultivars, Legacy 449 Aph2 and Pioneer 55V50. Only the conventional alfalfa variety Pioneer 55V50 performed as well as WAPH5 when inoculated with *A. euteiches* race 2 (Fig. 4). Most of the roundup ready varieties did not have significantly better levels of survival compared to Vernal, with the exception of Dekalb DKA4416 RR. Inoculations performed using *A. euteiches* race 3 yielded similar results as the race 2 inoculations (Fig. 5). However, all conventional varieties had higher levels of survival than all Roundup Ready varieties with the exception of Dekalb DKA4416 RR and WL 354 HQ. Pioneer 55V50 was the best performing commercial cultivar in these trials, with levels of survival comparable to WAPH5 (resistant check). In the presence of *A. euteiches* races 2 and 3, Roundup Ready varieties tested in these experiments had levels of plant survival comparable to Vernal (susceptible check).

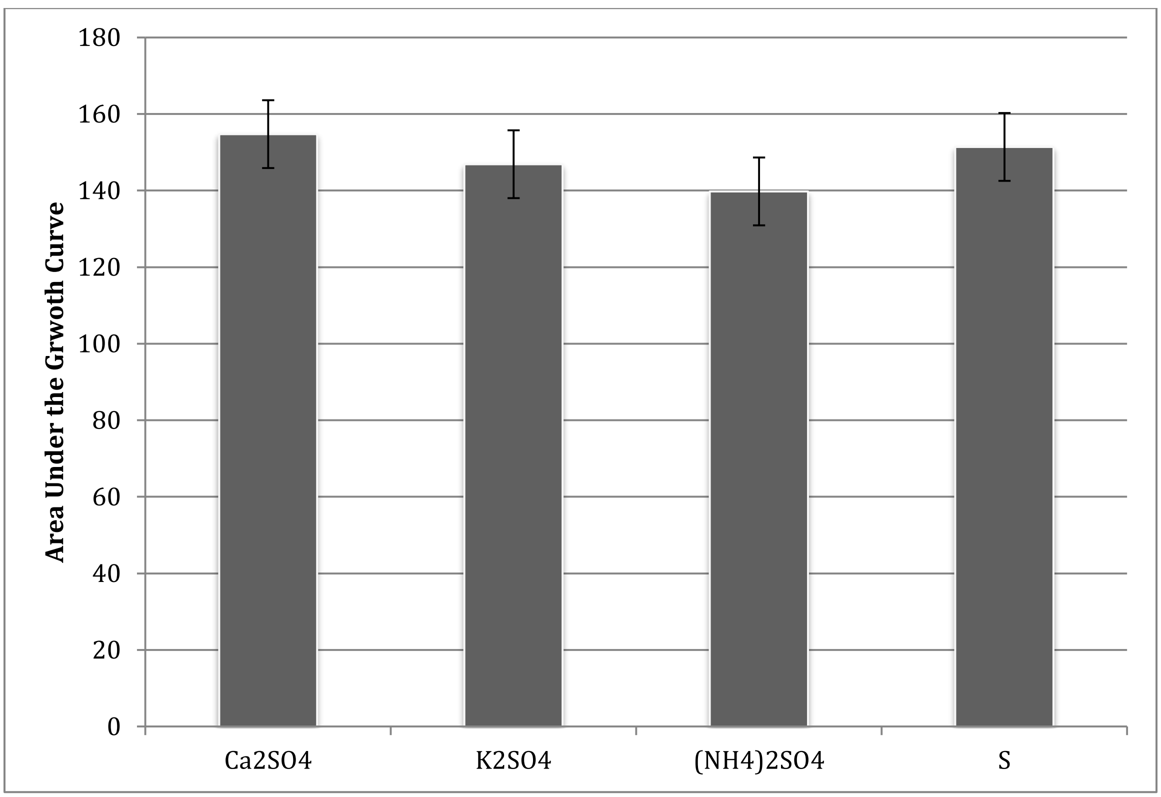

Figure 1. Area under the growth curve (AUGC) ratings for *A. euteiches* in culture in the presence of different sources of sulfur, which include K_2SO_4 (potassium sulfate), Ca_2SO_4 (calcium sulfate), $(NH_4)_2SO_4$ (ammonium sulfate), and S (elemental sulfur). Bars indicate 95% confidence intervals.

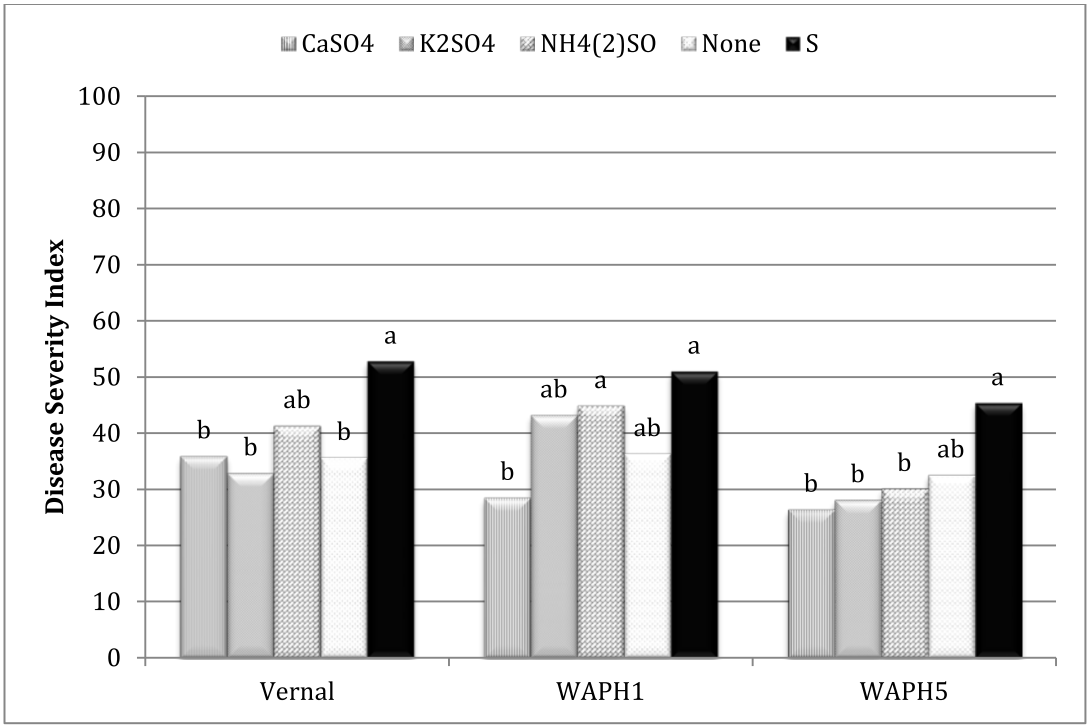

Figure 2. Disease severity index for three alfalfa cultivars inoculated with *A. euteiches* race 2 (Larsen NC-1) and fertilized with four sulfur-containing compounds, which include K_2SO_4 (potassium sulfate), Ca_2SO_4 (calcium sulfate), $(NH_4)_2SO_4$ (ammonium sulfate), and S (elemental sulfur). Bars with the same letter are not significantly different (α=0.05).

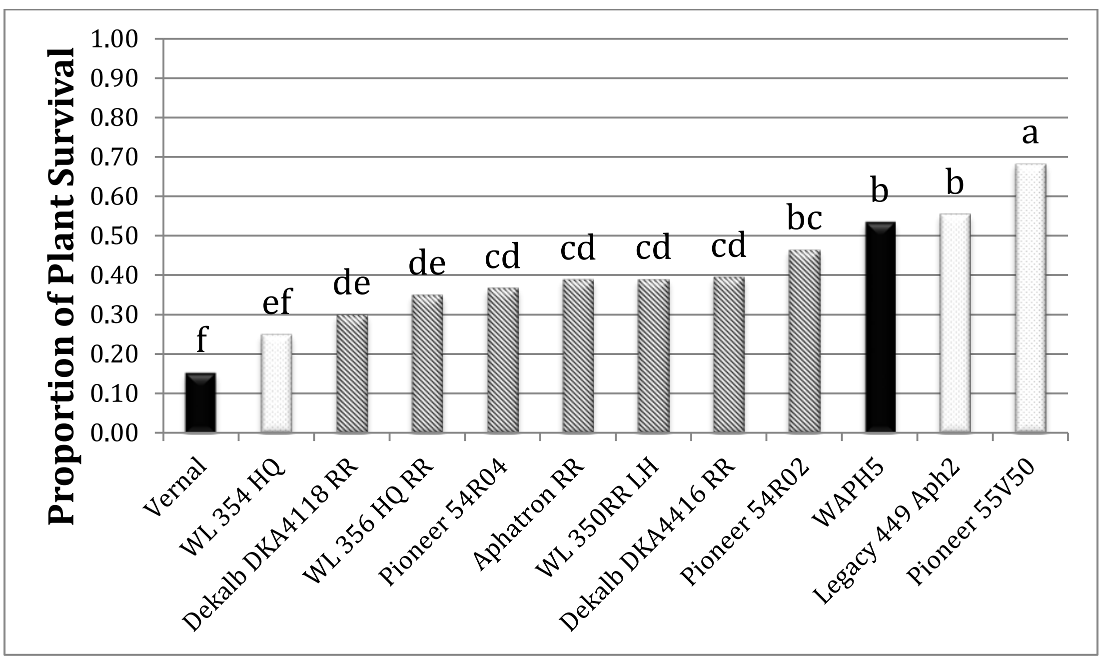

Figure 3. Proportion of alfalfa seedling survival after inoculation with *A. euteiches* race 1. Bars with the same letter are not significantly different (α=0.05). Solid bars indicate "check" varieties. Light bars indicate conventional varieties. Bars with diagonal hashes are Roundup Ready varieties.

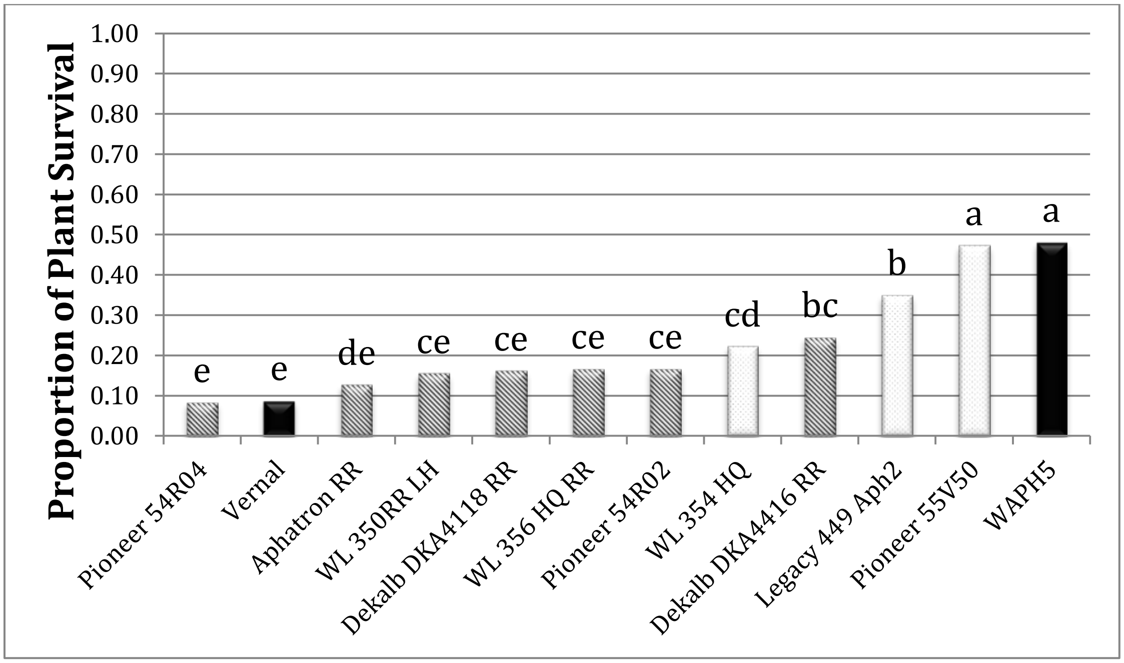

Figure 4. Proportion of alfalfa seedling survival after inoculation with *A. euteiches* race 2. Bars with the same letter are not significantly different (α=0.05). Solid bars indicate "check" varieties. Light bars indicate conventional varieties. Bars with diagonal hashes are Roundup Ready varieties.

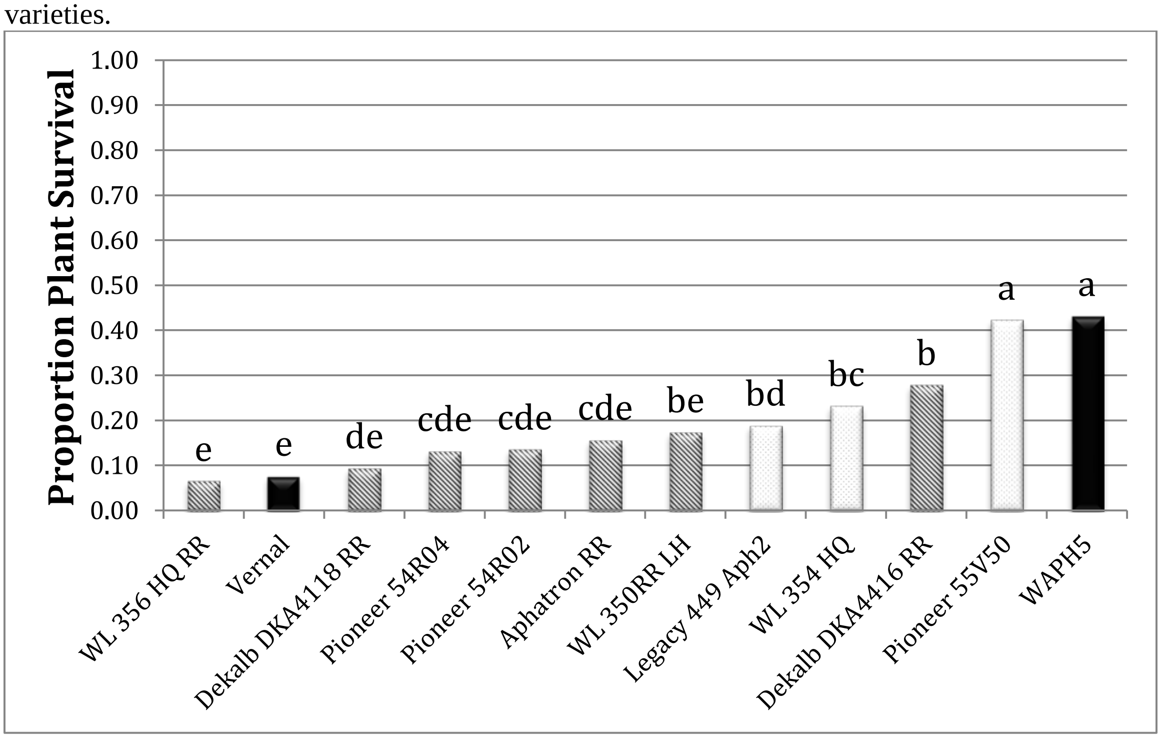

Figure 5. Proportion of alfalfa seedling survival after inoculation with *A. euteiches* race 3. Bars with the same letter are not significantly different (α=0.05). Solid bars indicate "check" varieties. Light bars indicate conventional varieties. Bars with diagonal hashes are Roundup Ready varieties.

References:

1. Barnett, K. "Weekly Hay Market Demand and Price Report for the Upper Midwest." *UWExtension*. UW Extension, 1 Feb 2013. Web. 7 Feb 2013. <http://www.uwex.edu/ces/forage/pubs/hay_market_report.htm>.
2. Camberato, J., Maloney, S., Casteel, S., and Johnson, K. "Soil Fertility Update." *Purdue Agriculture: Department of Agronomy*. Purdue University Department of Agronomy, 03 May 2012.
3. Cooper, R. M., & Williams, J. S. (2004). Elemental sulfur as an induced antifungal substance in plant defense. *Journal of Experimental Botany*, *55*(404), 1947-1953.
4. Delwiche, P.A., Grau, C.R., Holub, E.B., Perry, J.B. 1987. Characterization of *Aphanomyces euteiches* Isolates Recovered from Alfalfa in Wisconsin. Plant Disease 71:155-161.
5. Fitzpatrick, S., Brummer, J., Hudelson, B., Malvick, D., and Grau, C.R. 1998. Aphanomyces root rot resistance (Races 1 and 2). Page D-2 in: Standard Tests Bull. N. Am. Alfalfa Improvement Conference.
6. Gibbs, A. 2009. The prevalence and geospatial distribution of *Aphanomyces euteiches* (Race 1 and 2) and *Phytophthora medicaginis* in Wisconsin and Southwestern Minnesota alfalfa fields. M.S. Thesis, University of Wisconsin, 49 Pages.
7. Grau, C.R., ed. *Status of Aphanomyces Root Rot in Wisconsin*. Madison: University of Wisconsin-Extension, 2000. B3. Web. 7 Feb. 2013. <http://www.soils.wisc.edu/extension/wcmc/proceedings/3B.grau.pdf>.
8. Haneklaus, S., Elke, B., and Ewald, S. "Sulfur and Plant Disease." Trans. Array *Mineral Nutrition and Plant Disease*. Lawrence E. Datnoff, Wade H. Elmer and Don M. Huber. 1st ed. St. Paul, Minnesota: The American Phytopathological Society, 2007. 101-105. Print.
9. Holub, E.B. and Grau, C.R. 1990a. Ability of *Aphanomyces euteiches* to cause diseases of Seedling Alfalfa compared with *Phytophthora megasperma* f. sp. *medicagnis*. Phytopathology 80:331-335.
10. Kelling, K.A., and E.E. Shulte. "Soil and Applied Calcium."*Understanding Plant Nutrients*. University of Wisconsin-Extension. Web. 7 Feb 2013. <http://learningstore.uwex.edu/Assets/pdfs/A2523.pdf>.
11. Kelling, K.A., and E.E. Shulte. "Soil and Applied Potassium." *Understanding Plant Nutrients*. University ow Wisconsin-Extension. Web. 7 Feb 2013. <http://learningstore.uwex.edu/Assets/pdfs/A2521.pd
12. Malvick, D.K. and Grau, C.R. 2001. Characteristics and frequency of *Aphanomyces euteiches* races 1 and 2 associated with alfalfa in the Midwestern United States. Plant Dis. 74, 716-718.
13. Parke, J.L., and C.R. Grau. "Aphanomyces." *Methods for Research on Soilborne Phytopathogenic Fungi*. St. Paul, Minnesota: 1992
14. United States. Environmental Protection Agency. *Clean Air Act*. Washington D.C.:, 2012. Print. <http://www.epa.gov/air/caa/>.
15. United States. United States Department of Agriculture.*National Agricultural Statistics Service*. Washington D.C.: , 2013. Print. <http://www.nass.usda.gov/Statistics_by_Subject/result.php?010991CB-E24E-3ED1-8B85-0EEAB631EC29§or=CROPS&group=Field CROPS&comm=HAY>.
16. United States. USDA. *Report of the Dairy Industry Advisory Committee*. Washington D.C.: , 2011. Print.