EVALUATION OF NITROGEN SOURCE ON THE *IN VITRO* GROWTH AND MORPHOLOGY OF *RHIZOCTONIA SOLANI* AG 2-2 LP

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INTRODUCTION

Large patch of zoysiagrass is an important disease in the transition zone that limits the utility and aesthetics of lawns and golf course fairways. Large patch is caused by the pathogen *Rhizoctonia solani* AG 2-2 LP. Patch symptoms appear as circular matted brown areas ranging from 1 to 6 meters in diameter, with bright orange margins when the disease is active. On individual infected plants, reddish-brown lesions are observed on leaf sheaths, and basal sheath rotting is present in advanced disease stages.

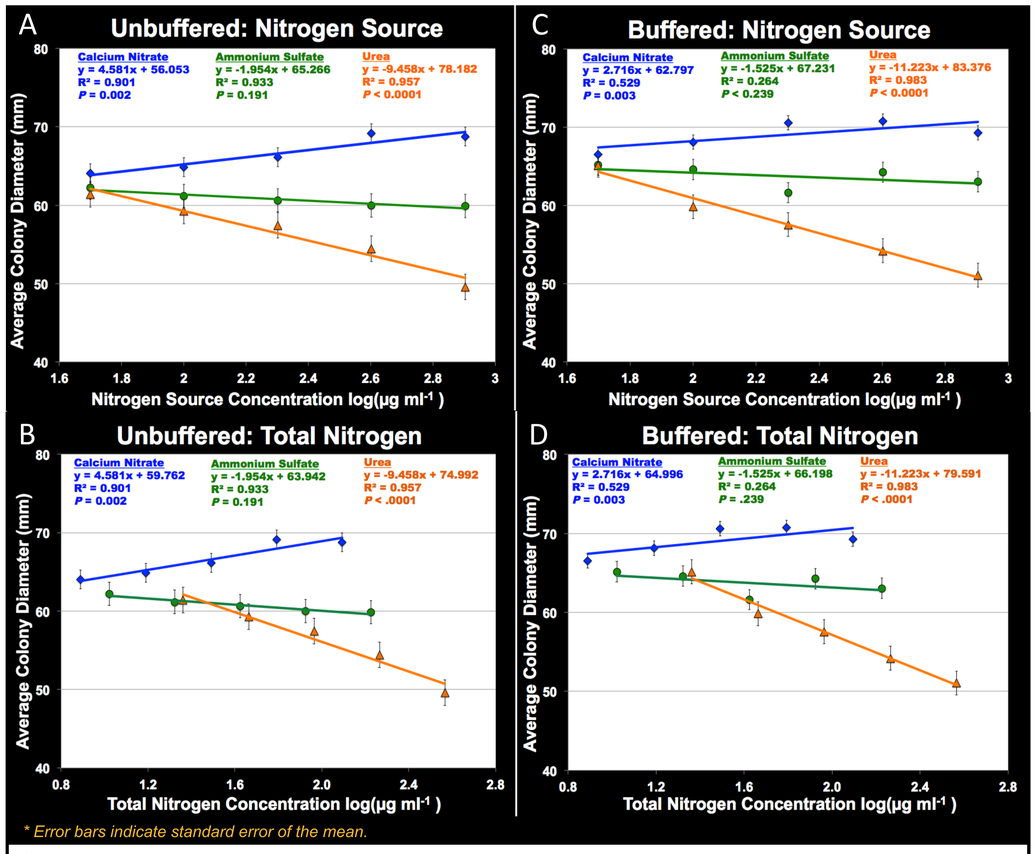
Little research has been done on the effects of cultural practices on large patch incidence. A preliminary laboratory trial in our lab demonstrated *R. solani* AG 2-2 LP grown on nitrogen amended media containing NH_4-N as a sole nitrogen source remained white, while the pathogen on PDA and NO_3-N media had brown, melanized hyphae typically associated with the wild-type. Melanin is used by plant pathogenic fungi such as *Magnaporthe grisea* and *Colletotrichum* spp. to build turgor pressure for plant host penetration (Bell and Wheeler, 1986). The impact of nitrogen source on the growth and pathogenicity of *R. solani* AG 2-2 LP is unknown.

MATERIALS AND METHODS

- Thirty-four *R. solani* AG 2-2 LP isolates from MO, IL, and KS were initially grown on PDA+++, and transferred to water agar for seven days.
- A basal medium (He and Suzuki, 2003) was amended with ammonium sulfate (AMS), calcium nitrate (CN), and urea at concentrations of 0, 50, 100, 200, 400, or 800 µg ml^{-1} as sole nitrogen sources. A second set of each concentration was amended with 200 µg ml^{-1} of fumaric acid (FA) buffer. Each medium was brought to pH 7 before autoclaving using NaOH and lactic acid.
- Agar plugs (9 mm diameter) were taken from the edge of the 7-day old colonies grown on water agar, and transferred to the middle of each amended plate. All dishes were then placed into an incubator at 25°C. After four days, radial mycelial growth was recorded as the average length of two perpendicular colony diameters. Plates were kept after measuring to observe morphology differences.
- Least squared means for mycelial growth were subjected to analysis of variance using the PROC GLIMMIX (SAS 9.3). Regression lines were calculated with PROC REG. LSMeans were separated with Fisher's Protected LSD (α = 0.05).

OBJECTIVES

This study aimed to evaluate the effects of three commonly used nitrogen sources with and without a pH buffer on the radial growth and morphology of *R. solani* AG 2-2 LP.



* Error bars indicate standard error of the mean.

Figure 1. Growth of large patch isolates on nitrogen amended media.

- Radial growth versus **(A)** log (nitrogen source concentration) and **(B)** log (total nitrogen concentration) on unbuffered media.
- Radial growth versus **(C)** log (nitrogen source concentration) and **(D)** log (total nitrogen concentration) on buffered media.

RESULTS

Radial Growth

- Radial growth was highest on CN amended media, AMS had intermediate growth, and urea had the lowest amount of growth ($P < 0.0001$).
- Increasing concentration had a positive effect on growth on CN amended media, a negative effect on growth on urea amended media, and no effect on growth on AMS amended media (**Fig. 1**).
- Fumaric acid increased growth on all nitrogen sources when compared to unbuffered media ($P < 0.0001$). Growth trends remained the same with and without fumaric acid, however, with no interaction between the two independent variables (P > 0.05) (**Fig. 1**).

Culture Melanization

- At concentrations of 200 µg ml^{-1} AMS and above, isolates grown on unbuffered media remained white and did not melanize. On fumaric acid buffered media, isolates melanized at 200 µg ml^{-1} AMS, but did not melanize at 400 or 800 µg ml^{-1} AMS (**Fig. 2**).
- All isolates grown on buffered or non-buffered CN and urea amended media melanized similar to the wild type.

CONCLUSIONS

The findings of this study suggest *R. solani* AG 2-2 LP has a preference of NO_3-N for growth compared to NH_4-N and urea. Although the radial growth of the concentrations varied with increasing nitrogen concentration, visual density increased with increasing nitrogen. Future trials will use liquid media to assess density differences of growth and evaluate the impact of nitrogen source on pathogenicity of *R. solani* AG 2-2 LP.

Only ammonium sulfate at 200 µg ml^{-1} and above prevented melanization of tested isolates (**Fig. 2B**). This result may be related to pH. As AMS concentrations increased, pH of the media decreased (**Fig. 2C**). This was not noted in CN or urea media. Fumaric acid buffered this change in pH up to 200 µg ml^{-1} AMS, but 400 and 800 µg ml AMS still experienced a large drop in pH and isolates grown at these concentrations failed to melanize. This study provides evidence that the acidifying nature of ammonium sulfate fertilizers may have an impact on the pathogenicity of *R. solani* AG2-2 LP, and their use has the potential to reduce large patch severity in the field.

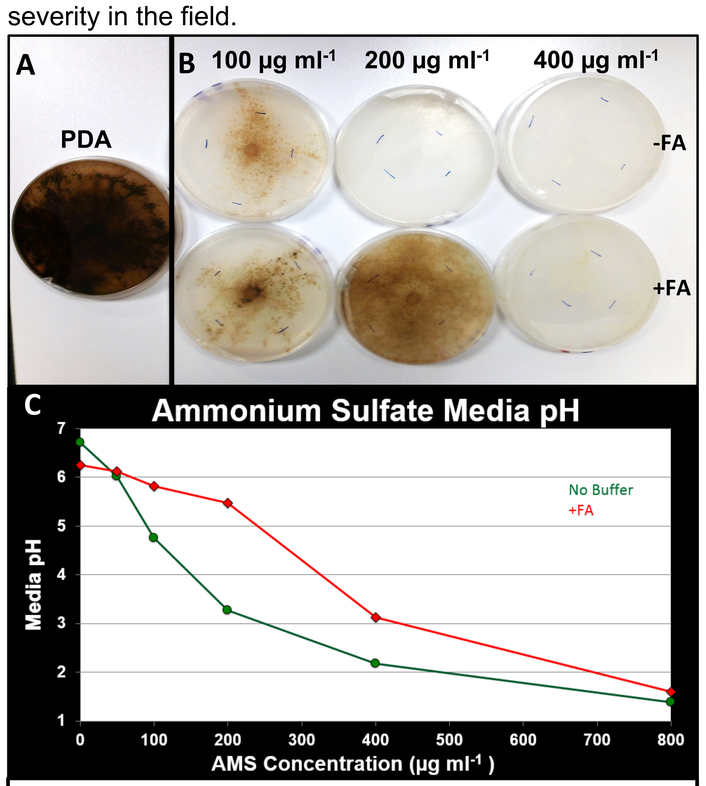


Figure 2. Colony morphology
A. Rhizoctonia solani AG 2-2 LP grown on potato dextrose agar.
B. Morphology of *R. solani* AG 2-2 LP on AMS amended media. Top row is without buffer (-FA), bottom row is with buffer (+FA).
C. Change in pH of AMS amended media after 10 d isolate growth.

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