

September 28, 2007

Dr. Thomas Armstrong
Provost and Vice Chancellor
Louisiana State University at Alexandria
8100 Highway 71 South
Alexandria, LA 71302

Dear Dr. Armstrong:

Please accept this letter as the final report on the 2006-2007 Title III Mini-Grant received to support research on the rust which is impacting soybean production in Louisiana. The funding was used to buy supplies such as chemicals, media, and Petri dishes. The supplies were used in the research projects undertaken by Malinda Holloway, Aaron Tiffée, and myself. The experiments undertaken involved isolating the fungi involved, determining the best medium for growth of the fungi, working to determine a non-toxic chemical for control of the fungi, and observing the interactions between the fungi and naturally occurring antifungal agents produced by several species of *Bacillus subtilis*. The research resulted in papers submitted by Malinda and Aaron. Copies of the papers are attached. It also resulted in a poster presentation at the Applied and Environmental Microbiology Gordon Research Conference held at Mount Holyoke College. A copy of the poster presentation is also attached. While these papers provide the results of the research, they don't completely explain all the outcomes developed. One additional outcome was the increased research capabilities of Malinda and Aaron. They worked independently and together to develop new techniques and to overcome hurdles that are not encountered in structured labs associated with classes. They also developed techniques which functioned well in the research but also would not be found in structured labs associated with classes. Both have used their results to apply for future educational goals.

The support of the Title III Mini-Grant was much appreciated and very productive.

Sincerely,

Elisabeth D. Elder
Professor of Biology

Fungal Effects of Common Food Preservatives
Sclerotinia sclerotiorum in Soybeans

Aaron S. Tiffée
May 2, 2007
Dr. E. Elder
BIOL 3990

Leaf samples from several soybean fields obtained and visually inspected for presence combined and stored together in a black paper bag for several weeks to facilitate fungal growth. On inspection, samples that were found to be infected with a fungus were transferred to malt agar. A colorimetric change from blue to a light yellow was observed. *Sclerotinia sclerotiorum* the chief cause of the disease. Colonies found within the area of the color change were cultured. Several common food preservatives were tested. The food preservatives included: Ferric Chloride, Sodium Benzoate, Sodium Acetate, and Sodium Citrate. Each food preservative was tested in drops on malt agar. Inoculation of the malt agar with the *S. sclerotiorum* was done. With the exception of Sodium Benzoate, were found to be effective. It is thought that would be unhealthy for using on soybeans. The preservatives actually facilitate the growth of the fungus.

Soybean molds and rusts have been one of the leading causes of crop failure in Louisiana as well as the entire United States. The white mold disease of soybeans, caused by the fungus *S. sclerotiorum* can cause a yield loss average of 20 percent or more. In addition to causing the white mold disease in soybeans *S. sclerotiorum* is known to cause diseases in more than 400 plant species, including: stem rot in soybeans, sclerotinia wilt and head rot of sunflowers and crown or stem rot in alfalfa.¹ These crop losses can have a significant economical impact on a community which relies on harvesting these plants. There are several commercial chemicals available to combat the plant fungi, but some of these have a maximum number of times that they may be applied. Many of the main chemicals commercially used are from the triazole group, which exhibit antifungal activity by inhibiting fungal ergosterol biosynthesis.² According to a study by the New York State Department of Health, the triazole fungicide may lead to neurotoxicity and affect the immunology and reproduction systems of rats (and possibly other toxic outcomes) when the exposure occurs during development.³ This research was initiated to generate a more environmentally friendly approach to combating fungal infections in soybeans.

¹ Stedman, Jim

² Filipov, Lawrence (2001)

³ Moser, Barone, Smialowicz, Harris, Davis, Overstreet, Mauney, and Chapin

Collection of leaf samples Leaf samples were obtained from the Dean Lee Research station near the LSU-Alexandria campus and from Louisiana Delta Plantation near Jonesville, LA. Leaves were pulled from near the bottom of the plant on the insides of the rows, a 20X hand lens was used when available to help look for possible fungal infection on the leaf, which can appear to be small white pustules. The infected leaves were enclosed in a large black, plastic bag and allowed to germinate for a period of approximately 5 weeks.

“Blue Plate” Recipe The blue plate agar was mixed using the following materials: 150 ppm oxytetracycline, 150 ppm penicillin, 150 ppm bromophenol blue, 39g of potato dextrose agar in 1 liter of deionized water. Adjust this to a pH of 4.5 with 1 Molar Chloric acid and Sodium Hydroxide. The bromophenol blue and the antibiotics should only be added when the mixture has cooled to below 50° Celsius.⁴

Inoculation of the Blue Plate Areas of the soybean leaves containing the white pustules were transferred to the blue plate agar with a sterile swab moistened with 0.9% sterile saline solution. The plates were allowed to incubate at 25° Celsius in low to zero light for 72 hours. Colorimetric change was inspected by holding the plate up to a light source.

Testing of Food Preservatives Upon appearing in the light yellow areas of the blue plate agar, each fungal colony was isolated and transferred to a beaker of sterile nutrient broth and allowed to incubate for 48 hours. 0.5 mL of the nutrient broth was transferred to malt agar and spread with a sterile “hockey stick.” Several methods of applying the preservatives were established during this step to test their various characteristics. The fungal inhibitor properties were tested by transferring 0.5 mL with an auto pipette of the chemical in concentrations of 1% and 10 % before adding the nutrient broth containing

