Purdue University
Department of Entomology
Undergraduate Capstone
Project Summary

Name of Student:

Gregory McGraw

Name of Mentor:

Dr. Doug Richmond

Name of Academic Advisor:

Amanda Pendleton, Dr. Alan York

Project Title:

Evaluating Resistance to Black Cutworms in Experimental Lines of Perennial Ryegrasses.

Project Summary:
Perennial ryegrass is widely used for forage and turf purposes throughout the temperate world and most ryegrass cultivars grown for turf in the United States, Australia and New Zealand are infected with the endophytic fungus *Neotyphodium lolii* (Roberts *et al*. 2005). This seed borne fungus is transmitted through the seed of infected plants from one generation to the next. The endophytic fungus is found within the leaf and stem of the plant and does not occur in the roots like some other endophytes (Bacon & White 2000). The endophytic fungus has been found to help improve resistance to diseases, increase drought resistance, and help in nutrient uptake. The toxic nature of the alkaloids produced by the endophyte also acts as a deterrent to herbivores (Schardl 2004). However, many of the alkaloids produced are toxic to mammals as well as insects. Mammalian herbivores grazing on infected perennial ryegrass may succumb to a neuromuscular condition known as 'ryegrass staggers'. Ryegrass stagger has been observed in sheep, cattle, horses, and other wildlife. The mortality rate of ryegrass stagger for mammals is rather low, however the financial cost to an individual rancher can be considerable (Ball *et al*. 1997). To circumvent this problem, different cultivars of endophytic infected perennial ryegrasses have been developed. These plant lines have different strains of *Neotyphodium lolii* that produce alkaloids that are not toxic to mammals, but their ability to provide defense against insects (Malinowski & Belesky2006) has not been examined. To determine the ability of these new perennial ryegrass cultivars to resist insect herbivory, this experiment examined resistance to black cutworm among several plant/endophyte lines that do not produce the mammalian toxins.

Material and Methods
Individual plants from five different endophyte/plant lines were grown from seed until they reached the age of 10 weeks. These 5 plant/endophyte lines included 4 lines that lacked the mammalian toxins (X2, X3, X4 and X5) and one line that contained the mammalian toxins in addition to the known insect toxins (X1). After 10 weeks, the endophyte infection status of each plant was determined using a tissue print immunoblot technique (Agrinostics Ltd. Co.,

Watkinsville, GA). A total of two hundred forty infected plants were then selected and separated into 46.7 cm^3 square plastic pots containing Promix potting medium and allowed to grow for another 10 weeks. The pots were arranged on the greenhouse bench in a randomized complete block design with 10 replicates. Every week each plant was cut to 7.6 cm and every four weeks, 0.04g of slow release urea (46% Nitrogen by weight) was administered to maintain growth.

Bioassay

Filter paper was placed in the bottom of fifty 5.0 cm diameter Petri dishes. Each Petri dish received a moistened dental wick cut to 3.0 cm long, and 100 µm of water. Four 2.0 cm long perennial ryegrass leaf clippings were placed in each Petri dish and ten black cutworm neonates were placed in the middle of the dish. After 24 hours the Petri dishes were examined and the number of surviving black cutworms was recorded. To measure settling response, the number of black cutworms settling on and actively feeding on the clippings was also recorded. Each treatment was replicated a total of 10 times.

Results

The results from the endophyte tissue amino blot showed all plant lines to be 100% endophyte positive. Although no significant difference in black cutworm survival was observed after 24 hours (Fig. 1), settling response varied significantly between plant/endophyte lines with X1 and X4 resulting in a significantly lower settling response than X2 and X3 (Fig. 2). While X5 produced a significantly lower settling response than X2, settling response on this line was intermediate and did not differ significantly from any other line. Mean settling response was lowest on X4 and settling response on this line was not significantly different from the line producing the mammalian toxins (X1).

Conclusion

The X4 line appears to be a good candidate for further testing because although it did not produce mammalian toxic alkaloids, it resulted in the lowest settling response (numerically) and yielded reductions in black cutworm settling response that were similar to, or stronger than those produced by the mammalian toxic line (X1). The lack of any clear differences in black cutworm survival on the different lines was likely due to the short duration of the experiment.

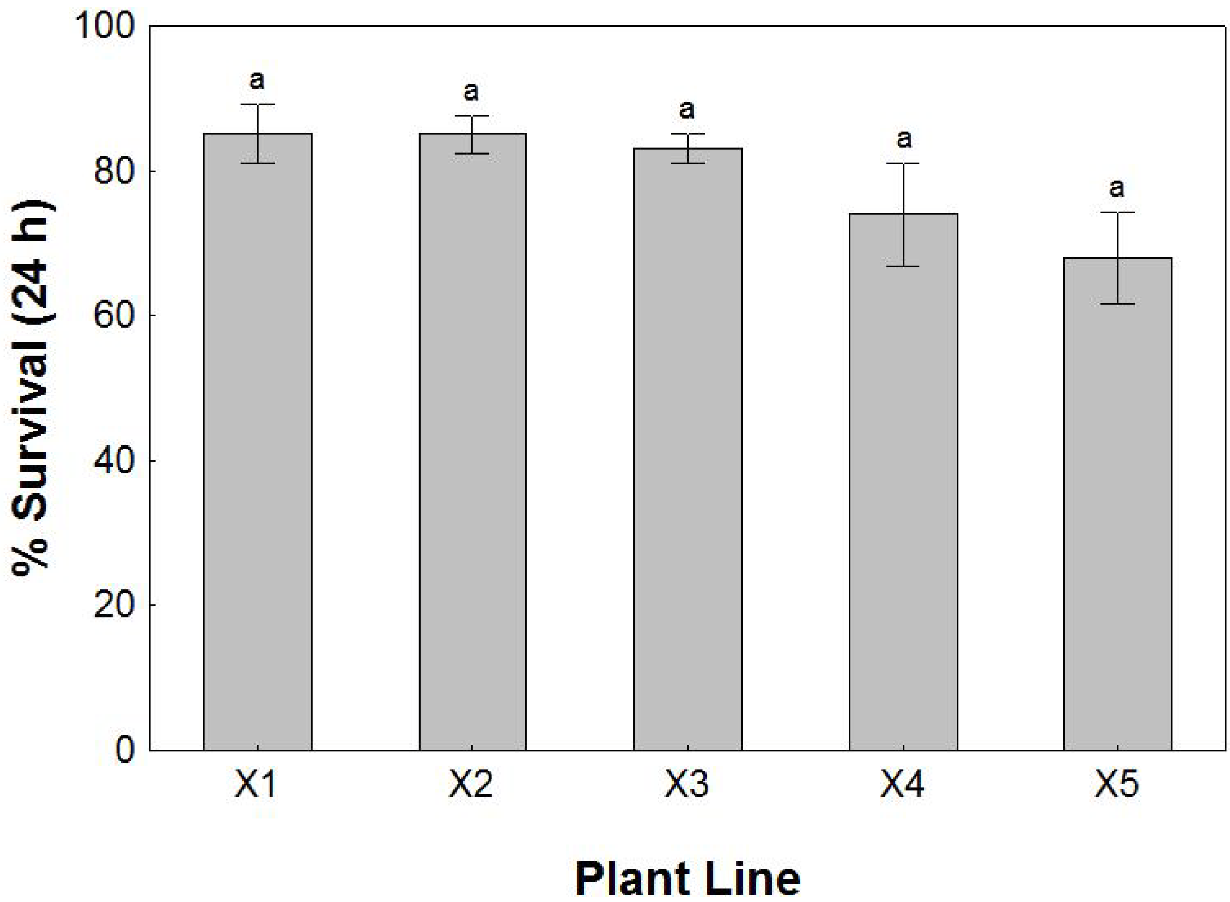

Figure 1. Survival of neonate black cutworms after 24 hours on five different lines of endophyte infected perennial ryegrass.

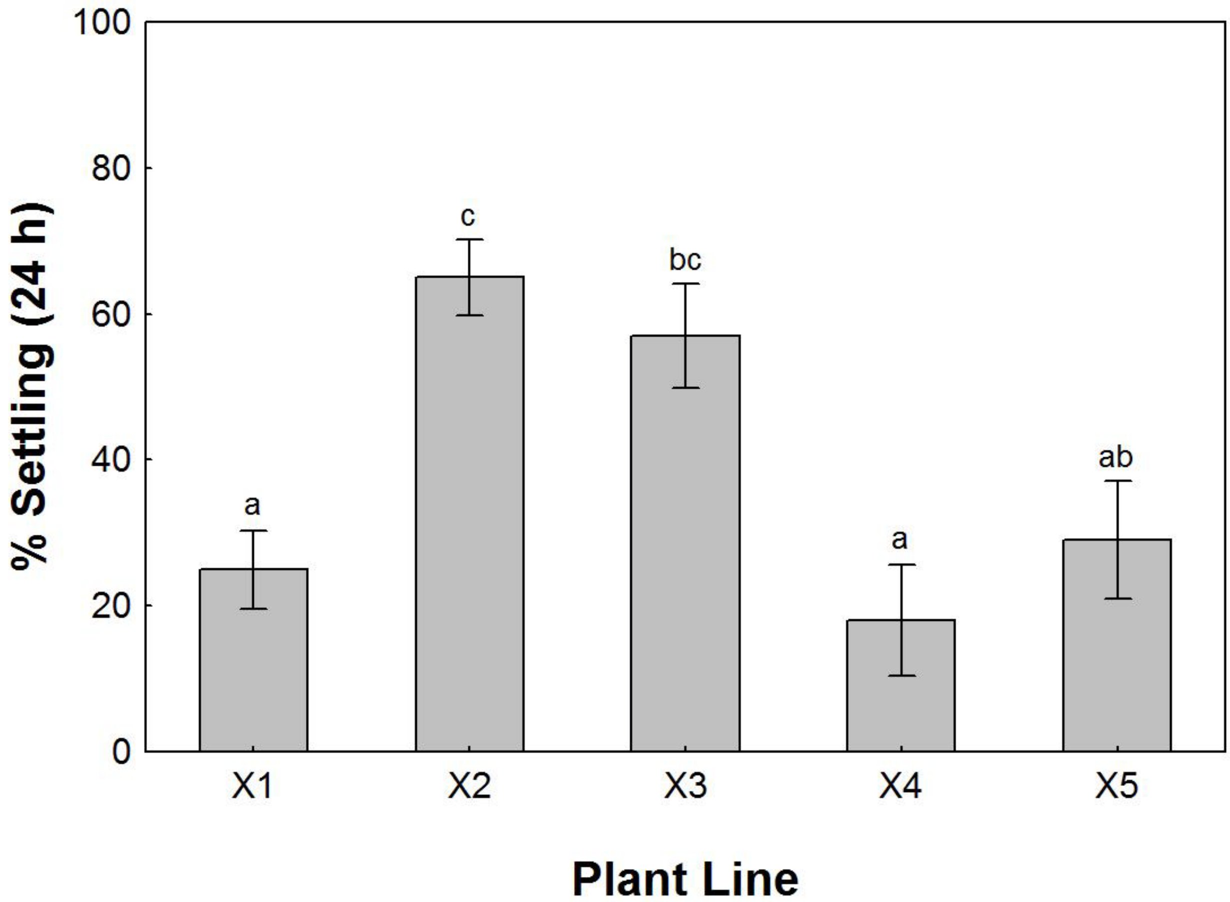

Figure 2. Twenty four hour settling response of neonate black cutworms on five different lines of endophyte infected perennial ryegrass

References

Bacon CW, White JF (2000) Microbial Endophytes. Marcel Dekker, New York.

Ball OJP, Barker GM, Prestidge RA, Sprosen JM. (1997) Distribution and accumulation of the mycotoxin lolitrem B in *Neotyphodium loii*-infected perennial ryegrass. *J. Chem. Ecol.* 23: 1435–1449

Malinowski DP, Belesky DP (2006) Ecological importance of *Neotyphodium* spp. grass endophytes in agroecosystems. *Grassland Science* 52: 1–14.

Roberts CA West CP Spiers DE (2005) *Neotyphodium in Cool-Season Grasses.*

Schardl CL, Leuchtmann A, Spiering M. (2004) Symbioses of grasses with seed borne fungal endophytes. *Annu. Rev. Plant Biol.* 55: 315–340.