Purdue University
Department of Entomology
Undergraduate Capstone
Project Summary

Name of Student:

Stephanie Hathaway

Name of Mentor:

Dr. Douglas Richmond

Project Title:

Searching for sustainable endophyte-mediated resistance to black cutworm in perennial ryegrass

Project Summary:

Fungal endophytes in the genus *Neotyphodium* have formed symbiotic relationships with many important turf and forage grasses. This relationship benefits the fungus by giving it access to plant nutrients and structural support, whereas the plant benefits from resistance to herbivory and enhanced environmental stress tolerance (Richmond & Bigelow 2009). The basis for endophyte-mediated herbivore resistance lies in the synthesis of several different defensive alkaloids, which vary in their relative activity against vertebrate and invertebrate herbivores. While resistance against insects and other invertebrate pests is a desirable characteristic for cultured turfgrasses, these same chemical defenses can cause serious physiological problems for livestock and other wildlife. Finding endophyte-infected grasses that resist insects while remaining safe for wildlife and livestock remains a major goal for plant breeders interested in developing and deploying sustainable forms of endophyte-mediated resistance

Three different classes of alkaloids have been associated with the endophyte *Neotyphodium lolii* and its perennial ryegrass host; ergot alkaloids, indole-diterpenes, and the pyrrolopyrizine alkaloid peramine. Ergot alkaloids can be very effective against insects, but they are highly toxicity to mammals, and while indole-diterpene alkaloids (lolitrems) can also reduce insect performance, they have also been known to cause neuromuscular problems in grazing animals (Keogh et. al. 1996). Alternatively, peramine has been associated with strong feeding deterrence in insects while having little to no effect on grazing mammals and wildlife (Baldauf et. al. 2011). Other unknown alkaloids associated with some novel perennial ryegrass endophytes apparently are also relatively friendly to vertebrates but have unknown effects on insects. Although factors such as temperature, season and plant genotype can influence alkaloid expression in endophyte-infected plants, the specific kinds (classes) of alkaloids associated with the symbiosis are primarily a function of endophyte haplotype (Richmond & Bigelow 2009). Identifying plant genotype x endophyte haplotype combinations (plant lines) that effectively resist insect pest without producing vertebrate toxins could enhance the safety and sustainability of anthropogenic systems by reducing the non-target effects of endophyte-mediated resistance.

Although most ecological information on black cutworms has been obtained through studies in field crop systems (Williamson et. al. 1997), this insect is also a serious pest of turfgrass. Because of its noted ability to withstand most forms of endophyte-mediated resistance and its relatively large host range among turfgrasses (Williamson et. al. 1997) black cutworm has become a model species for studies aimed at improving the sustainability of endophyte-enhanced turfgrasses. This experiment examined resistance to black cutworm among six different lines of perennial ryegrass varying in their expression of vertebrate toxins. Objectives of the study were to determine survival and biomass of 2nd and 3rd instar black cutworm larvae feeding on these grasses. This study builds on previous work examining neonate black cutworm survival and settling response on these same endophyte haplotype x grass genotype combinations.

Methods

Six lines of perennial ryegrass were established in 3” pots filled with potting soil and arranged on the greenhouse bench in a randomized complete block design (Table 1). Plants were maintained in the greenhouse using and overhead irrigation system to supply water as needed and Nitrogen fertility was supplied at a rate of 5 lbs. N/ 1000ft^2/yr using a liquid fertilizer solution. Plants were maintained at 3” by cutting with shears and were allowed to grow in the greenhouse for approximately 1 year before experiments were conducted.

Clippings from each pot were provided to four, 2nd and 3rd instar black cutworm larvae held individually in 9.0 cm (diam.) Petri dishes containing moist filter paper and a cotton dental wick moistened with 8.0 ml of water. Cutworms were allowed to feed ad libitum and fresh clippings were provided every 48 hrs. Petri dishes with fresh filter paper and dental wick were provided after 5d. Cutworm survival was examined at 5 and 10d by disturbing the caterpillars with a soft paintbrush to elicit a response. After 10d the total weight of all cutworms surviving in each treatment (x/4) was also recorded. The entire experiment was repeated a total of 6 times.

Variation in black cutworm performance parameters was examined using multivariate ANOVA. Post-hoc means comparison was performed using Fishers LSD test (α=0.05)

Results

Plant line had no significant influence on black cutworm larval survival at 5d or 10d ($F \leq 1.4$; df=5, 25; $P \geq 0.26$). However, black cutworm biomass varied significantly among plant lines (F=3.5; df=5, 25; P=0.02) (Fig. 1). Black cutworm biomass was highest on the endophyte-free plant line (#6=control), but larval biomass on the plant line expressing both ergots and lolitrems (#5) and one of the plant lines expressing unknown (undescribed) alkaoids was not significantly different from the endophyte-free control. While larval biomass on all remaining plant lines expressing only peramine or the previously referred to unknown alkaloids were not significantly reduced compared to the plant line producing the vertebrate toxins (#5), larval biomass on all but one of these plant lines (#3) was significantly reduced compared to the endophyte-free control.

Discussion

In order to enhance the sustainability of endophyte-mediated resistance and reduce non-target effects against wildlife and livestock, it is essential to evaluate plant genotype × endophyte haplotype combinations that express only those toxins that are active against insects. In the current study, three of the six plant lines caused a decrease in insect biomass. The three lines that resulted in a significant decrease in BCW biomass compared to the control produced only friendly alkaloids (peramine or udescribed), which have little to no effect on vertebrate herbivores. The "Slow growth – high mortalilty hypothesis" implies that decreases in insect biomass may lead to longer development times and, in turn, increase insect susceptibility to natural enemies (Clancy 1987). These grasses could potentially be used by breeders to develop more sustainable forms of endophyte-enhanced resistance. Such grasses could play an important role in reducing the use of insecticides in turfgrass systems.

Previous experiments with these same plant lines indicated reduced settling response of neonate BCW on 2 of the 3 lines resulting in reduced biomass in the current study (lines 1 & 4) This is important because resistance against neonates does not always reflect activity against later larval instars. This is particularly true of BCW due to the relatively high mobility and propensity for dispersal among later instar larvae. Black cutworm larvae can move great distances at night and may move from reservoir populations in surrounding grassy areas into more valuable high maintenance turf (Williamson 1997). To achieve effective management of black cutworms through endophyte-mediated resistance grass lines that affect both neonate and later instar black cutworms would be benficial. Through this work, we have identified 2 plant lines infected by "friendly endophytes" that could potentially be used in breeding programs aimed at improving the sustainability and durability of endophyte-mediated resistance.

References

Ball, O.J.P., G.M. Barker, R.A. Prestidge, and J.M. Sprosen. 1997. Distribution and accumulation of the mycotoxin lolitrem B in *Neotyphodium loii*-infected perennial ryegrass. Journal of Chemical Ecology 23: 1435–1449

Baldauf, M.W., W. J. Mace, and D.S. Richmond. 2011. Endophyte-mediated resistance to black cutworm as a function of plant cultivar and endophyte strain in tall fescue. Environmental Entomology 40: 639-647.

Bultman, T.L., K.L. Borowicz, R.M. Schneble, T.A. Coudron, and L.P. Bush. 1997. Effect of a fungal endophyte on the growth and survival of two euplectrus parasitoids. Oikos 78: 170-176.

Clancy, K.M. and P.W. Price. 1987. Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. Ecology 68: 736-738

Keogh, R. G., B.A. Tapper, and R.H. Fletcher. 1996. Distributions of the fungal endophyte *Acremonium lolii*, and of the alkaloids lolitrem B and peramine, within perennial ryegrass. New Zealand Journal of Agricultural Research 39:121-127.

Richmond, D.S and C.A. Bigelow. 2009. Variation in endophyte–plant associations influence black cutworm (Lepidoptera: Noctuidae) performance and susceptibility to the parasitic nematode *Steinernema carpocapsae*. Environmental Entomology 38: 996-1004.

Williamson, C.R. and D.A. Potier. 1997. Nocturnal activity and movement of black cutworms (Lepidoptera: Noctuidae) and response to cultural manipulations on golf course putting greens. Journal of Economic Entomology 90: 1283-1289.

Williamson, C.R. and D.A. Potter. (1997) Turfgrass Species and Endophyte Effects on Survival, Development, and Feeding Preference of Black Cutworms (Lepidoptera: Noctuidae). Jounral of Economic Entomology. 90(5): 1290-1299

Young, C.A., B.A. Tapper, K. May, C.D. Moon, C.L. Schardl, and B. Scott. 2009. Indole-diterpene biosynthetic capability of *Epichloë* endophytes as predicted by *ltm* gene analysis. Applied and Environmental Microbiology 75: 2200-2211.

Table 1. Plant genotype (Cultivar) x endophyte haplotype (Endophyte) combinations and alkaloid profiles (Alkaloids) represented by six plant lines used to characterize endophyte-mediated resistance to 2nd and 3rd instar black cutworms in perennial ryegrass.

Plant Line	Cultivar	Endophyte	Alkaloids
1	AGRLP-135	AR1	Peramine
2	AGRLP-140	AR1	Peramine
3	AGRLP-141	AR47	Unknown friendly alkaloids
4	AGRLP-142	AR48	Unknown friendly alkaloids
5	FLp-322	HE toxic	Ergots, Lolitrems, Peramine
6	BG24T	None	None

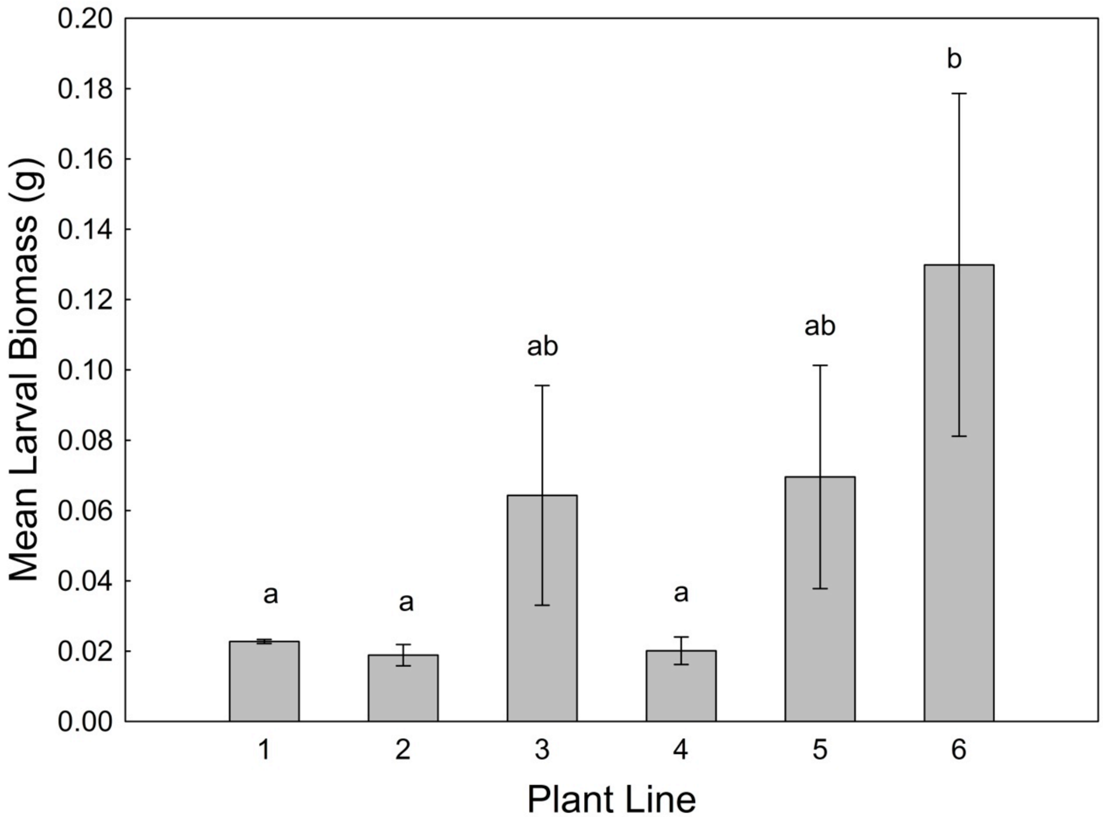

Figure 1. Biomass of 3rd instar black cutworms *Agrotis ipsilon* Hufnagel feeding on six different lines of endophyte-infected (1-5) or uninfected (6) perennial ryegrass *Lolium perenne* L., expressing different alkaloid profiles. Lines 1-4 express peramine or unknown friendly alkaloids only. Line 5 expressed peramine, ergots, and lolitrems.