CHAPTER THREE

MOWING HEIGHT AND NITROGEN FERTILIZATION EFFECTS ON LARVAL BLACK CUTWORM HERBIVORY IN ENDOPHYTIC TURF-TYPE TALL FESCUE

Abstract

Endophytic grasses have been shown to be more resistant to biotic stresses, such as insect herbivory, due to the presence of alkaloids produced by the endophyte. Interactions between cultural practices have not received much attention in terms of their affects on endophyte expression and ultimately insect performance. The objective of this study was to examine how two mowing heights (5 and 9 cm) and three N fertility programs (0, 99, and 196 kg N ha\(^{-1}\) yr\(^{-1}\)) affect black cutworm performance and which plant parameters are correlated with overall insect performance in endophytic turf-type tall fescue. Twenty-five black cutworm larvae were placed into Petri dishes and fed four clippings from each field plot maintained at either five or nine centimeters and fertilized annually with either 0, 99, or 196 kg N ha\(^{-1}\). Neonate settling response (number feeding on clippings) was measured on day two and mortality was recorded every other day. Time was a significant factor on black cutworm performance for all three trials. Mortality was greatest for trail one (21 June through 10 July, 2006) than for the other two trials which occurred during the fall (2 Sept. through 21 Sept., 2006 and 10 Oct. through 18 Oct., 2006). In addition, black cutworm performance in regards to initial settling response and mortality for all three trials was correlated to several plant parameters. Settling response was positively correlated with sugar concentrations in which the percentage of black cutworm actively feeding on clippings increased as sugar concentrations increased. Black cutworm mortality at day 7 and 14 was negatively correlated with leaf tissue N content. As N content increased, black cutworm mortality decreased. Mortality was negatively correlated to protein and amino-N concentrations for days 1, 7, and 14 in
which mortality decreased protein and amino-N concentrations increased. Mortality was positively correlated to alkaloid concentrations on day one and seven. Black cutworm mortality increased as alkaloid concentrations increased. Cultural practices used to maintain turfgrass did not effect overall insect performance as much as plant parameters measured in this study. However, future research should evaluate how cultural practices affect endophyte expression and other physiological characteristics.
Introduction

Tall fescue (Schedonorus phoenix (Scop.) Holub; formally know as Festuca arundinacea Schreb.) is frequently infected with the endophytic fungus Neotyphodium coenophialum [Morgan-Jones and Gams] Glenn, Bacon, and Hanlin) that lives in the stem and leaves of the grass plant. Endophytes and the grasses they infect have a mutualistic, symbiotic association with one another in which the plant provides the fungi with water, nutrients, and structural refuge and in return the endophyte provides several benefits such as enhanced competitiveness (Hill et al., 1991, 1998; Malinowski et al., 1999; Marks et al., 1991) more efficient water utilization (Arachevaleta et al., 1989), and enhanced environmental stress tolerance (Elmi and West, 1995; Elbersen and West, 1996; Ravel et al., 1995). More specifically in tall fescue, N. coenophialum increases tillering (Clay, 1987) and root growth, resistance to drought stress (Arachavaleta et al., 1989), fungal pathogens (Gwinn and Gavin, 1992), nematodes (Kimmons et al., 1990), mammalian herbivores (Bacon et al., 1977) and provides protection against insect herbivory (Rowan, 1994). These benefits have made the use of endophytes in turf an attractive alternative to the chemical inputs, such as insecticides, to control insects and to enhance overall turfgrass performance (Funk et al., 1985, 1992; Sun et al., 1990; Clarke et al., 2006).

Endophyte-infected grasses are often more vigorous and resistant to herbivory than uninfected grasses (Clay et al., 1985, Bacon et al., 1986; Read and Camp, 1986; Clay, 1990). Previous research conducted on insect herbivory in endophyte-infected grass include: fall armyworms (Clay and Cheplick 1993; Davidson and Potter, 1995; Boning and Bultman, 1996; Braman et al., 2002; Richmond et al., 2004), sod webworms (Murphy et al., 1993; Richmond, 2007), bluegrass webworms, (Richmond and Shetlar, 1999,) billbugs (Murphy et al., 1993; Richmond et al., 2000), black cutworm (Richmond and Shetlar, 2001), chinch bugs (Richmond and Shetlar, 2000; Anderson et al., 2006), and aphids (Davidson and Potter, 1995; de Sassi et al., 2005; Hunt and Newman, 2005; Meister et al., 2005; Krauss et al., 2007).

Protection against herbivory comes in the form of defensive alkaloids that are mediated by the endophyte. Alkaloids are poisonous or distasteful to many insects and
may deter insect feeding (antixenosis), or reduce insect performance (antibiosis) (Braman et al., 2002; Bacon et al., 1997; Breen, 1994) on grasses containing endophytes. Four classes of alkaloids have been associated with endophytes: ergots (clavines, lysergic acid, ergopeptides), indoleterpenes (paxilline, paxitriol(s), lolitriol, lolitrem(s), pyrrolopyrazine (peramine), and saturated aminopyrrolizidines (lolines). All four classes of alkaloids provide resistance to insect herbivory (Porter, 1994; Rowan, 1993) but the most potent are the ergots (particularly ergovaline), peramine and the lolines (Dahlman et al., 1991; Popay and Bonos, 2005; Wilkinson et al., 2000; Siegel and Bush, 1996). As a result, endophyte-infected grasses are more competitive, than non-infected grasses when herbivores are present (Marks et al., 1991; Bacon and Hill, 1996; Hill et al., 1998).

However, alkaloid concentrations within endophyte-infected grasses can vary with plant species and genotypes (Hiatt and Hill, 1997; Rowan and Latch 1993; Roylance et al., 1994), between plant parts such as leaf blades and sheaths (Lane et al., 2000; Ball et al., 1997; Siegel and Bush, 1996), and with plant age (Lyons et al., 1986). However, there is a need for research to determine how cultural practices such as nitrogen (N) fertilization and mowing height influence expression of endophyte-mediated resistance.

Leaf N content can affect the feeding behavior of insect herbivores (Slansky and Wheeler, 1992). According to Mattson (1980), insects feeding on plant tissue containing low amounts of N must consume more plant tissue to obtain the amount of N required for growth and development, likewise, previous research has shown that N fertilization can influence alkaloid concentration in endophyte-infected grasses. Richardson et al. (1999) found that concentrations of the alkaloid ergovaline in Chewings fescue were higher when fertilized with ammonium N sources whereas N-form had no effect on peramine. Ergopeptine alkaloid concentrations were increased by high rates of N fertilizer in a controlled environment (Lyons and Bacon, 1984) and in a field experiment with endophyte infected tall fescue (Belesky et al., 1988). Belesky et al. (1988) found that increasing N fertilization from 134 to 334 kg ha\(^{-1}\) increased total ergopeptine alkaloid concentration by 60 to 80 %. Arachavaleta et al. (1992) found that the concentration of ergot alkaloids in ‘Kentucky-31’ tall fescue increased due to increased in response to elevated NH\(_4\)-N.
Of all the cultural practices used to maintain turf, none directly affects plant growth and physiology, and the ability to tolerate biotic and abiotic stresses, to the degree that mowing does (Fry and Huang, 2004). Clipping removal has been shown to reduce alkaloid content in tall fescue presumably due to increased allocation to structural components (Belesky and Hill, 1997). Salminen et al., (2003) found that decreased mowing frequency (biweekly vs. weekly mowing) enhanced alkaloid production in tall fescue mowed at 5 cm. This is important because a small increase in alkaloid content could have a major impact on herbivore performance. Bigelow and Richmond (2006) showed that after a 48 h settling period, newly hatched black cutworm (Agrotis ipsilom, Hufnagel) larvae showed a stronger preference for clippings mowed at 5 cm than 9 cm for three of four turf-type cultivars including ‘Da Vinci’. Black cutworm survival was also reduced by the 9 cm mowing height on two of four cultivars during a five-day feeding study. Bigelow and Richmond (2006) also reported that black cutworm larva survival was higher on endophyte-infected ‘Plantation’ and ‘2nd Millennium’ turf-type tall fescue clippings mowed at 5 cm and higher on clippings from ‘2nd Millennium’ fertilized with urea without a nitrification inhibitor.

If the overall goal of turfgrass managers is to produce a persistent, high quality turf with the fewest chemical inputs, more information is needed regarding the effects varying cultural practices on endophyte-mediated insect resistance. Therefore, the objective of this study was to examine how two mowing heights (5 and 9 cm) and three N fertility programs (0, 99, and 196 kg N ha⁻¹ yr⁻¹) affect black cutworm settling response and mortality on E+ turf-type tall fescue. Plant parameters that were associated with overall insect performance in endophytic turf-type tall fescue were also examined.

Materials and Methods

A field experiment was conducted on a mature stand of turf-type tall fescue from May 2006 through November 2007 at the Purdue University, W. H. Daniel Turfgrass Research and Diagnostic Center, West Lafayette, Indiana. The soil was a Stark silt-loam (fine-silty mixed mesic Aeric Ochraqualfs) with a pH of 7.4, 67 kg P ha⁻¹, 147 kg K ha⁻¹, and 42 g kg⁻¹ organic matter. Prior to planting, the entire study area (17 x 9 m) was
treated with glyphosate, N-(phosphonomethyl) glycine, to eradicate the existing turf. On
the day of seeding, the entire study area was verticut to facilitate seed to soil contact.
Endophytic turf-type tall fescue (‘Da Vinci’) was seeded 28 Aug., 2005 at 391 kg ha\(^{-1}\)
(Lebanon Seed Co., Lebanon, IN) using a drop spreader. The cultivar was selected based
on its high (84%) endophyte seed infection level (Mohr et al., 2002). After seeding, the
entire study area received an application of 73 kg P ha\(^{-1}\) from 6-24-24 (N-P\(_2\)O\(_5\)-K\(_2\)O) and
was covered with an Agrofabric Pro17 germination blanket (American Agrifabrics,
Alpharetta, GA) to conserve moisture and promote germination. The study area was
frequently irrigated via an overhead sprinkler system to keep the soil moist and promote
germination and seedling establishment.

Nitrogen fertilizer programs were initiated on 29 May 2006. Three nitrogen
fertility programs were evaluated, which varied in annual N, ranging from 0, 99, and 196
kg N ha\(^{-1}\) yr\(^{-1}\), and in application timing. For the 99 kg N ha\(^{-1}\) yr\(^{-1}\) treatment, 25 kg N ha\(^{-1}\)
was applied in May and Sept and 49 kg N ha\(^{-1}\) was applied in Nov. For the 196 kg N ha\(^{-1}\)
yr\(^{-1}\) treatment, 49 kg N ha\(^{-1}\) was applied in May, July, Sept., and Nov. Nitrogen was
supplied as urea alone in Nov., and in May, July, and Sept. sulfur coated urea (SCU) was
applied alone. Nitrogen fertilizer was applied on 8 May, 5 July, 3 Sept., and 22 Nov.,
2006. In addition, plots were mowed at either 5 or 9 cm with a rotary mower and
clippings were returned.

General Plot Maintenance

The study site was located in full-sun and in the absence of significant rainfall (12
mm per week), overhead irrigation was applied every two days (approximately 5 mm) to
promote growth.

Black Cutworm Resistance

Black cutworm eggs (Benzon Research, Carlisle, PA) were surface sterilized upon
arrival with a 1% bleach solution. Eggs were then triple rinsed with deionized water and
kept at room temperature until they hatched. Twenty-five black cutworm larvae were
placed into a 90 mm Petri dish lined with moistened filter paper (0.5 ml deionized water).
Four E+ clippings and a cotton dental wick (containing 1 mL deionized water) to maintain moisture was placed into the Petri dish and larvae were allowed to feed for 24 h. after which, settling response was recorded by counting the number of larvae on the clippings. Fresh clippings were provided at 24 hrs. and every two days thereafter. Every two days, the number of larvae surviving in each Petri dish was recorded and at the end of each trial, surviving larvae from each dish were weighed and mean larval weight was recorded. Independent trials were conducted on three dates: 20 June through 10 July, 1 Sept. through 21 Sept., and 9 Oct. through 18 Oct., in 2006.

Endophyte Infection

Approximately two weeks after fertilization, twenty tillers were randomly selected and cut at the soil surface from each plot (1.5 x 1.5 m) to determine stem endophyte infection. Tillers were wrapped in a damp towel and placed into a cooler of ice for transport to the lab where they were stored overnight at 4°C. The next day two, 2-mm cross sections of each stem, 0.3 cm above the base, were analyzed for endophyte infection using commercial tissue print-immunoblot (TPIB) test kits (Agrinostics Ltd. Co., Watkinsville, GA) (Gwinn et al., 1991). Percent endophyte infection was determined by the number of infected tillers divided by the total number of tillers sampled per plot. For additional information on this procedure, refer to Appendix A.

Plant Response to N Programs

Turf response to the N programs was measured through leaf tissue N, ergot alkaloid, sugar, protein, and amino-N concentrations and visual ratings (Appendix B). Fresh clippings (approximately 5 g) were randomly harvested from the entire plot by hand every two days during the black cutworm feeding trials. Clippings were placed on dry ice in the field until they could be placed in a -20°C freezer. Clippings were then lyophilized, and ground in a UDY Mill (UDY Corp., Ft. Collins, CO) to pass through a 0.5 mm screen. Approximately 0.05 g from each plot was analyzed for tissue N content using the LECO CHN-2000 analyzer (LECO Corp., St. Joseph, MI).
Alkaloid Concentration

Clippings were analyzed for ergot alkaloid concentration (Hill and Agee, 1994) using a commercial enzyme-linked immunosorbent assay (ELISA) test kit (Agrinostics Ltd. Co., Watkinsville, GA). For additional information on this procedure, refer to Appendix A.

Total Sugars, Protein, and Amino-N Analyses

Total sugars were extracted from approximately 40 mg of lyophilized, ground tissue in 1 mL of water in 1.5 mL microfuge tubes. Microfuge tubes were vortexed to suspend tissues, agitated for 10 min on a horizontal shaker, centrifuged at 14,000 x g for 10 min, and the supernate retained. This process was done a series of three times, and the combined three supernates were diluted to 10 mL with water. Sugar concentrations in 200 μL aliquots from the water extracts was determined using an anthrone assay (Koehler, 1952) using fructose as a standard and absorbance read using a spectrophotometer at 625 nm.

Buffer-soluble proteins were extracted from approximately 50 mg of lyophilized, ground tissue with 1 mL of 0.1 M sodium phosphate buffer (pH 6.8) containing 1 mM phenylmethyl sulfonyl fluoride (PMSF) and 10 mM 2-mercaptoethanol in 1.5 mL microfuge tubes. Microfuge tubes were vortexed 15 s then set in ice (4°C) a series of four times, centrifuged at 14,000 x g for 10 min, and three 100 μL aliquots of diluted supernate (1:10) were removed for protein quantification using the Bradford assay (Bradford, 1976). Buffer-soluble protein concentration was determined using bovine serum albumin as a standard and absorbance using a spectrophotometer was read at 595 nm. Amino-N in the supernatant was determined using ninhydrin with glycine as a standard, and spectrophotometer absorbance was read at 570 nm (Rosen, 1957).

Experimental Design and Statistical Analysis

Each treatment was replicated three times and plots (1.5 x 1.5 m with 0.3 m borders) were arranged in a randomized complete-block design. Settling response and black cutworm mortality was subjected to analysis of variance (ANOVA) using Statistica.
7.0 (Statsoft Inc., Tulsa, OK, USA). In addition, a repeated measure MANOVA was used to examine patterns in black cutworm mortality over time. Correlation matrices were also used to identify which plant parameters, ergot alkaloids, leaf tissue N, sugars, proteins, and amino-N, were associated with overall black cutworm performance. Black cutworm settling response and mortality as well as plant parameter data was initially pooled for all three trials (Table 3-1). If trial was found to be significant (p<0.05) each trial period was ran separately (Table 3-2 through Table 3-4).

Results

Multivariate analysis of variance (MANOVA) conducted on the entire data set, trials one through three, indicated significant variation in black cutworm response variables (settling response and mortality) among trials (F=98.2; df=2, 64; p=0.00). Therefore, statistical analysis was conducted on each trial separately.

After being exposed to clippings for one day, black cutworm mortality was greater than 20% in trial one, whereas mortality was less than 15% for trials two and three. After three days of exposure, mortality was greater than 50% for trial one, whereas mortality in trials two and three were less than 30%. After 6 days of exposure, black cutworm mortality was greater than 80% in trial one, whereas mortality in trials two and three was less than 40%.

For trial one, black cutworm mortality on day seven was higher on clippings from plants maintained at 9 cm compared plants maintained at 5 cm, but neither mowing height nor N fertility alone were significant predictors of black cutworm performance in either of the other two trials. For trial two, repeated measures analysis of variance test indicated a time by N treatment interaction (F=2.7; df=6, 54; p= 0.02) in which black cutworm mortality was lower on days 12 and 14 when fertilized with high N compared to low N and the unfertilized control.

When data from all trials was pooled, black cutworm settling response and mortality was correlated with several plant parameters (Table 3-1). Settling response was positively correlated with leaf sugar concentrations (r=0.70; p=0.001) (Figure 3-1). Black cutworm mortality at day 7 (r=-0.49; p=0.04) and 14 (r=-0.47; p=0.05) was negatively
correlated leaf tissue N content (Figure 3-2) and mortality day 1, 7, and 14 was negatively correlated with leaf protein and amino-N concentrations (Figures 3-3 and 3-4). However, mortality was positively correlated with ergot alkaloid concentrations on day one (r= 0.66; p=0.003) and seven (r=0.53; p=0.02) (Figure 3-5). Black cutworm performance was not correlated with overall endophyte infection rates (data not shown). When correlations were ran on trial two (Table 3-3), black cutworm performance was positively correlated to sugars (r=0.87, p=0.02) (Figure 3-6) and was negatively correlated to proteins (r=-0.97, p=0.002) (Figure 3-7), amino-N (r=-0.96, p=0.002) (Figure 3-8), and ergot alkaloids (r=-0.84, p=0.04) (Figures 3-9).

Discussion

Time of year seemed to be an overriding factor in determining black cutworm performance with the affect of mowing height and N fertility varying with time of year. Black cutworm mortality was the highest during the first trial implying that the time of year clippings were collected and fed to black cutworm may have an important influence on black cutworm performance.

Many insect feeding studies use clippings from plants grown in greenhouses. This study is different in the fact that clippings were actually taken from field plots which were subjected to seasonal variation in abiotic stresses such as temperature and day length. Endophytic grasses are known to be more tolerant of environmental stresses than E- grasses and therefore E+ grasses are often more competitive than E- grasses. However, this field and laboratory study was conducted during three distinct periods within the growing season for cool-season turfgrasses. The first trial was conducted from 20 June through 10 July, 2006 at the peak of summer stress. During the summer, top growth slows or essentially stops due to the high air and soil temperatures, and rooting depth, are only able to obtain water and nutrients from the top few centimeters of soil. During this period, respiration in the plant was greater than photosynthesis which greatly decreases the rate of shoot and root growth and therefore sugars (carbohydrates) concentrations are lower (Christians, 1998; Carrow et al., 2001). Trail two and three were conducted from 1 Sept. through 21 Sept. and 9 Oct. through 18 Oct., in 2006 during early fall. During this
period, air and soil temperatures decrease, shoot and root growth are initiated again but as the plant nears winter dormancy shoot and root growth start to taper off. During the fall months the turfgrass plant accumulates sugars (carbohydrates) for winter storage in which photosynthesis is greater than respiration (Christians, 1998; Carrow et al., 2001). Mortality of black cutworms for trials two and three occurred at a much slower rate than trial one and at lower percentages. Observed differences between trials are likely due to seasonal differences in plant stress and resource allocation.

Although inconsistent, mowing height affected black cutworm mortality during one of the three trials with black cutworm mortality being higher on clippings taken from plants maintained at 9 cm compared to clippings maintained at 5 cm. These findings were similar to those found by Bigelow and Richmond (2006) and are supported by the findings of Salminen et al. (2003) in which increasing mowing height also increased alkaloid concentrations. They found that after a 48 h settling period, newly hatched black cutworm larvae showed a stronger preference for clippings mowed at 5 cm than 9 cm for three of the four turf-type cultivars in the study including ‘Da Vinci’. Black cutworm survival was reduced by the 9 cm mowing height on two of the four cultivars during a five-day feeding study.

While N fertility did not directly affect overall black cutworm performance, N fertility has been shown to affect physiological characteristics of the turfgrass plant which can then ultimately affect overall insect performance. In this study, leaf tissue N content was negatively correlated with black cutworm on days 7 and 14 in which black cutworm mortality decreased as N content increased. Previous research has shown that leaf tissue N content can affect the feeding behavior of insect herbivores (Slansky and Wheeler, 1992). Davidson and Potter (1995) found that fall armyworms, greenbugs, and bird cherry-oat aphids developed faster on fertilized (150-300 kg N ha\(^{-1}\)) tall fescue. However, greenbugs and bird cherry-oat aphids preferred endophyte-free tall fescue, whereas fall armyworms were not affected. In this study, N fertility (99-196 kg N ha\(^{-1}\)) increased leaf tissue N content and as N content increased black cutworm mortality decreased. Previous research has shown that insect development is enhanced when plants are grown with additional fertilizer (Honek, 1991; Davidson and Potter, 1995) which could result in a
conflicting situation where insect growth rates are enhanced by increased fertilization, but reduced through higher concentrations of higher toxic alkaloid levels (Krauss et al., 2007).

Previous research has shown that N fertilization can also influence alkaloid concentration in endophyte-infected grasses in which additional N could also increase the production of alkaloids in endophyte-infected plants. Since N is a key component of alkaloids, it is expected that N additions would increase alkaloid concentrations in endophyte-infected plants (Lyons et al., 1986; Marks et al., 1991; Latch, 1993; Faeth and Fegan; 2002). In this study, ergot alkaloid concentrations also affected black cutworm mortality on day one and seven in which black cutworm mortality increased as ergot alkaloid concentrations increased. Lyons and Bacon, (1984) showed that ergopeptine alkaloid concentration was increased by high rates of N fertilizer in a controlled environment and in a field experiment with endophyte-infected tall fescue (Belesky et al., 1988). Belesky et al. (1988) found that increasing N fertilization from 134 to 334 kg ha⁻¹ increased total ergopeptine alkaloid concentration by 60 to 80 %, depending on the year. Latch (1993) showed that peramine and loliterm B concentrations were higher in fertilized perennial ryegrass plants. Krauss et al. (2007) showed that peramine concentration in perennial ryegrass was enhanced by addition of a balanced fertilizer. However, endophyte infection had no negative effect on aphid or parasitoid abundances.

Previous research on how N fertility affects other plant parameters such as sugar, protein, and amino-N concentrations in endophytic grasses is less extensive. However, Belesky et al. (1991) found that the endophyte did not affect carbohydrate accumulation in field grown tall fescue grown at either 134 or 336 kg N ha⁻¹. Rasmussen et al. (2007) studied how N fertilization interacted with water-soluble carbohydrate content, soluble protein, and amino acid content in two cultivars of E+ perennial ryegrass in which one of the cultivars, ‘AberDove’, produced high concentrations of water-soluble carbohydrates. Rasmussen et al. (2007) found that sugar concentrations (high and low molecular weight sugars) were reduced by high N (17 mg g⁻¹) treatment compared with low N (34 mg g⁻¹) treatment in endophyte-infected perennial ryegrass. Whereas, soluble protein and total amino acid concentrations were higher under high N (81 and 21 mg g⁻¹) than under low N (70 and 10 mg g⁻¹) treatments, respectively.
Sugar concentrations were positively correlated to black cutworm settling response in this study (Figure 3-1). This data suggests that black cutworm were initially attracted to the clippings solely on the basis of sugar content since none of the other plant parameters were correlated to settling response. Ravindran and Xavier (1997) suggested that higher cotton aphid populations were associated with increases in total sugars. Slosser et al. (2004) reported a negative linear relationship was observed between change in aphid numbers and sugar ratio. With aphids, overall population growth was limited by high levels of sugars in cotton leaves. However, in our study, sugar content did not have any effect on black cutworm mortality. It was previously mentioned that Rasmussen et al. (2007) found that sugar concentrations (high and low molecular weight sugars) were reduced by high N (17 mg g\(^{-1}\)) treatment compared with low N (34 mg g\(^{-1}\)) treatment in endophyte-infected perennial ryegrass. Future research, should explore the potential negative effects of excess N application on sugar content and ultimately insect performance. The effect protein and amino-N concentrations on insect mortality are less known. In this study, black cutworm mortality was affected by protein and amino-N concentrations on all three days data was collected indicating a strong relationship.

In summary, cultural practices such as mowing height and N fertility, used to maintain turfgrasses did not generally affect overall insect performance as much as plant parameters measured in this study. Black cutworm settling response was influenced by sugar concentrations and mortality was influenced by protein, amino-N, alkaloids, and N content. Therefore, future research should evaluate how mowing height and N fertility affect endophyte expression and other physiological characteristics (refer to Chapter 4).
References


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Table 3-1. Black cutworm performance (settling response and mortality on day 1, 7, and 14) correlations relative to sugar, nitrogen (N) content, protein, amino-N, and ergot alkaloid concentrations for all three feeding trials.

<table>
<thead>
<tr>
<th>Leaf characteristics</th>
<th>Sugars</th>
<th>N content</th>
<th>Proteins</th>
<th>Amino-N</th>
<th>Ergot alkaloids</th>
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<tbody>
<tr>
<td>Trial</td>
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<td>-0.09</td>
<td>0.34</td>
<td>0.68</td>
<td>-0.24</td>
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<tr>
<td>Settling response</td>
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<td>-0.40</td>
<td>0.02</td>
<td>0.21</td>
<td>0.09</td>
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<tr>
<td>Mortality day 1</td>
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<td>-0.65</td>
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<td>Mortality day 7</td>
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<td>-0.47</td>
<td>-0.71</td>
<td>-0.90</td>
<td>0.53</td>
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<tr>
<td>Mortality day 14</td>
<td>-0.05</td>
<td>-0.38</td>
<td>-0.67</td>
<td>-0.91</td>
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</table>

Bold faced correlations indicate significance at p<0.05.
Table 3-2. Black cutworm performance (settling response and mortality on day 1, 7, and 14) correlations relative to sugar, nitrogen (N) content, protein, amino-N, and ergot alkaloid concentrations for trial 1.

<table>
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<th>Proteins</th>
<th>Amino-N</th>
<th>Ergot alkaloids</th>
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<td></td>
<td></td>
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<td>Leaf characteristics</td>
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<tr>
<td>Settling response</td>
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<td>Mortality day 7</td>
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<td>-0.73</td>
<td>-0.76</td>
<td>-0.11</td>
</tr>
<tr>
<td>Mortality day 14</td>
<td>0.66</td>
<td>-0.77</td>
<td>-0.72</td>
<td>-0.64</td>
<td>-0.13</td>
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</table>

Bold faced correlations indicate significance at p<0.05.
Table 3-3. Black cutworm performance (settling response and mortality on day 1, 7, and 14) correlations relative to sugar, nitrogen (N) content, protein, amino-N, and ergot alkaloid concentrations for trial 2.

<table>
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<td>Settling response</td>
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<td>-0.58</td>
<td>-0.74</td>
</tr>
<tr>
<td>Mortality day 14</td>
<td><strong>0.87</strong></td>
<td>-0.74</td>
<td><strong>-0.97</strong></td>
<td><strong>-0.96</strong></td>
<td><strong>-0.84</strong></td>
</tr>
</tbody>
</table>

Bold faced correlations indicate significance at p<0.05.
Table 3-4. Black cutworm performance (settling response and mortality) correlations relative to sugar, nitrogen (N) content, protein, amino-N, and ergot alkaloid concentrations for trial 3.

<table>
<thead>
<tr>
<th>Leaf characteristics</th>
<th>Sugars</th>
<th>N content</th>
<th>Proteins</th>
<th>Amino-N</th>
<th>Ergot alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Settling response</td>
<td>-0.37</td>
<td>0.47</td>
<td>0.35</td>
<td>0.40</td>
<td>-0.18</td>
</tr>
<tr>
<td>Mortality day 1</td>
<td>0.14</td>
<td>-0.35</td>
<td>-0.28</td>
<td>-0.35</td>
<td>0.18</td>
</tr>
<tr>
<td>Mortality day 7</td>
<td>0.41</td>
<td>-0.41</td>
<td>-0.34</td>
<td>-0.46</td>
<td>-0.37</td>
</tr>
<tr>
<td>Mortality day 14</td>
<td>0.50</td>
<td>-0.40</td>
<td>-0.33</td>
<td>-0.44</td>
<td>-0.37</td>
</tr>
</tbody>
</table>

Bold faced correlations indicate significance at p<0.05.
Figure 3-1. Correlation of settling response (percentage of black cutworm feeding on leaf tissue after 1 day) to sugar concentration in endophyte-infected turf-type tall fescue for all three trials.
Figure 3-2. Response of black cutworm mortality, as a percentage, to leaf tissue N concentration in endophyte-infected turf-type tall fescue for all three trials on day 1 and 7.
Figure 3-3. Response of black cutworm mortality as a percentage to protein concentration in endophyte-infected turf-type tall fescue for all three trials on day 1, 7, and 14.
Figure 3-4. Response of black cutworm mortality as a percentage to amino-N concentration in endophyte-infected turf-type tall fescue for all three trials on day 1, 7, and 14.
Figure 3-5. Response of black cutworm mortality as a percentage to ergot alkaloid concentration in endophyte-infected turf-type tall fescue for all three trials on day 1 and 7.
Figure 3-6. Response of black cutworm mortality as a percentage to sugar concentration in endophyte-infected turf-type tall fescue for trial 2 on day 14.
Figure 3-7. Response of black cutworm mortality as a percentage to protein concentration in endophyte-infected turf-type tall fescue for trial 2 on day 14.
Figure 3-8. Response of black cutworm mortality as a percentage to amino-N concentration in endophyte-infected turf-type tall fescue for trial 2 on day 14.
Figure 3-9. Response of black cutworm mortality as a percentage to ergot alkaloid concentration in endophyte-infected turf-type tall fescue for trial 2 on day 14.