Effects of Endophytic Fungus, Nutrient Level, and Plant Damage on Performance of Fall Armyworm (Lepidoptera: Noctuidae)

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ABSTRACT   We tested the effects of soil nutrient level, endophyte infection status, and prior herbivory on the performance (survival, growth, and assimilation) of fall armyworm, *Spodoptera frugiperda* (J.E. Smith). Using a 3-factor design, we fed larvae all possible combinations of the following treatments: endophyte-infected or uninfected tall fescue, *Festuca arundinacea* von Schreber, fescue receiving high or low nutrient levels, and fescue that was either previously damaged or not previously damaged with a fabric tracing wheel. Low nutrient treatments showed a consistent negative effect on 8-d larval mass, pupal mass, development time, assimilation efficiency, and survival of fall armyworm larvae. Previous damage negatively affected larval mass, development time, and assimilation efficiency, but did not affect pupal mass or survival. Infection status also gave mixed results; infection negatively affected pupal mass, but accelerated development time, positively affected larval mass and did not affect survival or assimilation. In general, interactions among nutrient level, wounding damage, and infection status did not affect insect performance. However, we did find that the influence of endophyte on insects depended upon nutrient level supplied to the plants. The adverse effect of endophyte on pupal mass was most pronounced when plants received low fertilizer applications; under high fertilizer applications the effect of endophyte on pupal mass was negligible. Our results show that the interaction among endophytic fungi, their host grasses, and insect herbivores is complex and can be influenced by environmental factors, such as nutrient availability.

KEY WORDS   *Spodoptera frugiperda*, endophytic fungi, induced response, insect performance, tall fescue

MANY GRASSES ARE infected with fungal endophytes (Ascomycetes: Clavicipitaceae) (White 1987). The endophytes grow intercellularly within stems, leaf sheaths, and seeds of grasses and appear to confer enhanced fitness to their hosts (Shelby and Dalrymple 1987, 1993; Clay 1990). One benefit of endophytes is that infected plants are often more resistant to insect herbivores (Popay and Rowan 1993, Breen 1994). The resistance is apparently mediated by several toxic alkaloids that are produced by the fungi (Dahlman et al. 1991). However, the hypothesis that grass endophytes form a defensive mutualism with their hosts (Clay 1988) has not consistently been supported. For example, black field crickets, Tasmanian grass grubs, and several hemipteran species were unaffected by the endophyte, *Neotyphodium lolii* (Latch Christensen and Samuels), in perennial ryegrass, *Lolium perenne* L. (Prestidge and Ball 1993). Similarly, growth of the southern armyworm, *Spodoptera eridania* (Stoll), was not affected by grass endophytes (Johnson et al. 1985, Dubis et al. 1992). Furthermore, Lopez et al. (1995) found Arizona fescue, *Festuca arizonica* Vasey, was not protected from redlegged grasshoppers, *Melanoplus femurrubrum* (De Geev), by the endophyte *Neoty-phodium starii* (White & Morgan-Jones) and, in fact, the endophyte increased assimilation by the grasshoppers.

An extension of the defensive mutualism hypothesis that might account for some of the variability in effects of grass endophytes on insect herbivores is that, in addition to constitutive resistance, endophytes also provide their hosts with inducible resistance. Increased insect resistance is often observed following plant wounding (Karban and Baldwin 1997), and may occur in endophyte-infected grasses if damage to grasses stimulates endophytes to produce higher levels of alkaloids. Plant defense theory is consistent with this expectation in that it predicts fast-growing herbaceous plants, like grasses, should benefit from resistance mechanisms that can be initiated once wounding has taken place (in contrast to constitutive resistance which exists even in the absence of herbivory) (Bryant et al. 1983, Coley et al. 1985). The induced resistance hypothesis is further supported by the finding that tall fescue, *Festuca arundinacea* von Schreber, infected with *Neotyphodium* had elevated levels of alkaloids (Eichenseer et al. 1991). Indeed, 2 recent studies have uncovered evidence for endophyte-mediated induced resistance to a lepidopteran pest of grasses (Bultman and Ganey 1995, Boning and Bultman 1996). In both cases, damage to plants (tall

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fescue and perennial ryegrass) was simulated by clipping.

Another factor that could cause variable impacts of endophytes on insect herbivores is nutrient availability to plants. Because fertilization of endophyte-infected grasses results in elevated production of at least some alkaloids (Lyons et al. 1986, Bush et al. 1993, Arechavaleta et al. 1992, Latch 1993), effects of grass endophytes on insects should vary with differing nutrient availability to the grasses. We tested if a form of simulated herbivory, namely pricking small holes in leaf blades, elicits resistance to fall armyworm, *Spodoptera frugiperda* (J. E. Smith), in endophyte-infected tall fescue, and if nutrient availability to the plants alters the plant-endophyte-insect interaction.

Materials and Methods

Tall fescue plants ('KY-31') infected with and lacking the endophyte *Neotyphodium coenophialum* (Morgan-Jones & Gams) were grown from seed (provided by H. Fribourg at the University of Tennessee) in plastic root masters filled with Pro-mix potting soil. Plants were fertilized weekly with liquid fertilizer at 1 of 2 rates: those in the high fertilizer treatment received fertilizer (20:10:20 [N:P:K] with micronutrients) at a rate of 150 ppm N, whereas those in the low fertilizer treatment received one-third that concentration. Microscopic inspection of seed revealed infection levels were 90% for infected seeds and 0% for uninfected seeds. We assumed loss of infection from germinated seeds was negligible and did not confirm infection in plants before feeding them to insects. At 4 wk after germination, a 3rd treatment variable, plant damage, was added in a factorial design producing 8 treatment combinations (infection status × fertilizer × damage; each with 2 levels). Leaf blades of damaged plants were punctured with a fabric tracing wheel (Ohnmeiss and Baldwin 1994) for a distance of 9 cm longitudinally aligned along the blades. All blades on plants were damaged with 1-3 (the number depended upon blade width) rows of punctures. This mode of damage produced small holes within leaf blades, but did not remove leaf tissue. Undamaged plants served as controls. An advantage of this mode of damage is that wounding does not remove portions of infected plants containing low concentrations of fungal hyphae. In contrast, clipping grass removes the distal portions of leaf blades and it is these portions that contain lower concentrations of fungal hyphae and at least some alkaloids (Gallagher et al. 1984, Lyons et al. 1986, Keogh et al. 1996). Hence, clipping experiments have the disadvantage of producing results consistent with the inducible resistance hypothesis but that may instead be caused by removal of low alkaloid containing plant tissues (Bultman and Ganey 1995).

Four days after damage, plants were harvested and fed to individual neonate fall armyworm larvae housed in separate petri dishes. Fall armyworm is a generalist herbivore that prefers grasses (Luginbill 1928) and can be a serious pest of tall fescue in the United States

(Bair et al. 1991). Larvae were housed in an environmental chamber at 26°C, 50-60% RH, and a photoperiod of 14:10 (L:D) h. Larvae received fresh plant tissue (leaf blades and stems) every 2-3 d from 1 of the 8 treatments (45 insects per treatment) and were reared to adult emergence. Plant tissue presented to insects, insect feces and plant tissue not eaten were all oven-dried and weighed to determine dietary assimilation by insects. Additional plants in each treatment group were grown, weighed, dried, and reweighed to allow conversion of plant mass supplied to insects to its dry mass equivalent. Response variables measured were: 8-d larval mass, pupal mass, development time to pupation, survival, and dietary assimilation efficiency. Assimilation efficiency was calculated as dry weight of food ingested − dry weight of insect feces (Waldbauer 1968). Because data were not normally distributed, pupal mass and development time were rank transformed (Conover and Iman 1981), while the square root of larval mass was used before comparisons by analysis of variance (ANOVA). Insect survival was analyzed by contingency table analysis using the G^2 test (Sokal and Rohlf 1981).

Results

Fertilizer had a strong effect on performance of fall armyworm (Table 1). Larval mass was reduced for insects fed plants from the low versus high fertilizer treatment [29.6 ± 2.0 mg versus 81.4 ± 2.6 mg, respectively (means ± SE)]; the same was true for pupal mass (169.3 ± 2.4 mg versus 198.1 ± 5.5 mg). Furthermore, insects took longer to develop when fed plants from the low (390.9 ± 3.3 h) versus the high (341.6 ± 4.7 h) fertilizer treatments. Assimilation by insects also differed between treatments; those fed plants receiving low fertilizer exhibited lower assimilation efficiency (0.0634 ± 0.013 mg) compared with those fed plants in the high (0.324 ± 0.008 mg) fertilizer group ($F = 103.4$; df = 1, 80; $P < 0.0001$). Fertilizer level also affected insect survival; 93% of insects survived in treatment groups fed plants from high fertilizer groups, whereas only 66% survived in the treatment groups fed plants receiving low fertilizer ($G^2 = 41.5$, df = 1, $P < 0.001$).

Infection status had a more variable effect on insect performance. Pupae reared from infected plants weighed less (176.0 ± 2.6 mg) than those reared from uninfected plants (195.9 ± 6.3 mg). However, endophyte infection also reduced development time (368.9 ± 5.6 h versus 356.1 ± 3.8 h for uninfected and infected treatments, respectively) and increased 8-d larval mass (54.2 ± 2.9 mg versus 65.1 ± 3.5 mg) (Table 1). Survival ($G^2 = 0.06$, df = 1, NS) and assimilation ($F = 1.81$; df = 1, 185; NS) were not affected.

Plant damage reduced 8-d larval mass (65.7 ± 3.0 mg versus 53.4 ± 3.4 mg for undamaged and damaged treatments, respectively), lengthened development time (353.2 ± 3.1 h versus 372.0 ± 6.0 h for undamaged and damaged treatments, respectively), but did not affect pupal mass (183.5 ± 2.4 mg versus 188.5 ± 6.6 mg for undamaged versus damaged treatments, re-

Table 1. Comparison of larval and pupal masses, and development time (neonate to pupation) of fall armyworm subjected to diet in a 3 factorial design, with main effects being fertilizer application to plants, endophyte infection, and plant damage

Source	df	SS	MS	F	P
		Larval mass			
Fertilizer application	1	1,000.6	1,000.6	287.1	<0.001
Infection status	1	25.3	25.3	7.3	<0.01
Damage	1	117.8	117.8	33.8	<0.001
Fertilizer * Infection	1	1.6	1.6	0.5	NS
Fertilizer * Damage	1	34.8	34.8	10.0	<0.01
Infection * Damage	1	0.2	0.2	0.1	NS
Fertilizer * Infection * Damage	1	17.2	17.2	5.0	<0.05
Error	284	989.8	3.5		
Total	291	2,158.3			
		Pupal mass			
Fertilizer application	1	329,866.8	329,866.8	67.3	<0.001
Infection status	1	146,762.0	146,762.0	30.0	<0.001
Damage	1	1,753.6	1,753.6	0.4	NS
Fertilizer * Infection	1	66,287.2	66,287.2	13.5	<0.001
Fertilizer * Damage	1	7,952.1	7,952.1	1.6	NS
Infection * Damage	1	3,515.8	3,515.8	0.7	NS
Fertilizer * Infection * Damage	1	9,606.1	9,606.1	1.8	NS
Error	273	1,337,230.4	4,898.3		
Total	280	1,848,964.5			
		Development time			
Fertilizer application	1	802,909.2	802,909.2	235.9	<0.001
Infection status	1	15,406.1	15,406.2	4.5	<0.05
Damage	1	64,390.4	64,390.4	18.9	<0.001
Fertilizer * Infection	1	155.7	155.7	0.1	NS
Fertilizer * Damage	1	2,508.4	2,508.4	0.7	NS
Infection * Damage	1	2,538.5	2,538.5	0.8	NS
Fertilizer * Infection * Damage	1	3,824.0	3,824.0	1.1	NS
Error	273	929,261.8	3,403.9		
Total	280	1,819,679.0			

a NS, not significant

spectively) (Table 1) or survival ($G^2 = 0.6$, df = 1, NS). Assimilation by insects was, however, reduced by damaging plants (0.147 ± 0.02 mg versus 0.172 ± 0.02 mg for insects fed damaged and undamaged plants, respectively; $F = 9.66$; df = 1, 185; $P < 0.01$).

Interactions among the main effects of fertilizer, damage, and infection status generally did not affect insect performance (Table 1). We found no interaction between infection status and damage, contrary to what would be predicted if endophytes mediate induced resistance to fall armyworm. However, pupal mass of insects reared on plants receiving low fertilizer was lower than that of insects reared on plants receiving high fertilizer (Fig. 1).

Discussion

Our findings mirror results of several other experiments with grass endophytes and fall armyworm. For example, Boning and Bultman (1996) found that tall fescue endophyte's influence on fall armyworm growth varied from positive to negative depending on the particular insect performance parameter measured. Likewise, Clay et al. (1985) found somewhat variable effects of tall fescue endophyte on fall armyworm.

Concerning infection status, we obtained the counterintuitive result that infection depressed pupal mass, but elevated larval mass (see Results). This reversal

did not exist when comparing weights of larvae and pupae between fertilizer or damage levels. The result might occur if infection accelerated development to the point of reducing the size of pupae. Contrary to this hypothesis, our results showed infection slowed larval development (see Results). An alternative explanation is that infection has a strongly negative effect on performance of late instars, but the opposite
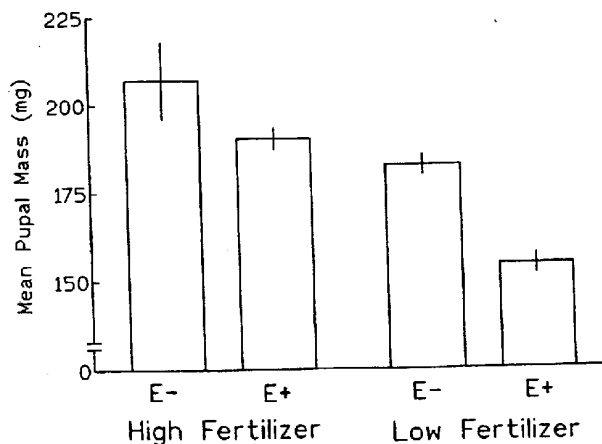

Fig. 1. Fertilizer and infection status interacted in a significant way ($F = 13.5$; df = 1, 280; $P < 0.001$) to affect mass (mean $\pm$ SE) of fall armyworm pupae. E− = endophyte-free and E+ = endophyte-infected.

effect on early instars. Yet another possible explanation is that pupae reared from infected plants lost water more readily than those reared from uninfected plants. Reversals in larval versus pupal weight of fall armyworm that were associated with *Neotyphodium* infection have been observed previously and the reversals have even exhibited trends opposite in direction from one another (Clay et al. 1985, Boning and Bultman 1996). It is clear that fall armyworm is influenced by endophyte infection, and that the patterns can be quite variable.

The effect of fertilizer applied to plants was far more conclusive. Well-fertilized plants, irrespective of infection status or damage, resulted in greater assimilation and better growth and survival by insects. Such a result is not uncommon for herbivorous insects, which are often nitrogen-limited (Scriber and Slansky 1981) and has previously been shown for fall armyworm reared on tall fescue (Davidson and Potter 1995). Of interest, however, was an interaction between fertilizer level and infection status. Pupal mass of insects reared from endophyte-free, low fertilizer treatment plants was much greater than it was for insects reared from endophyte-infected plants that also received low levels of fertilizer (Fig. 1). The disparity was less pronounced for insects reared on high fertilizer treatment plants. One explanation for this result may be that in nutrient poor soils the fungus becomes an important nitrogen sink and thus reduces the suitability of the grass for fall armyworm. This hypothesis gains support from studies that have uncovered the "cost of endophyte infection" to grasses when grown in nitrogen-limiting soils (Cheplick et al. 1989, Kelrick et al. 1995). Furthermore, there is some evidence that endophyte infection tends to reduce the amount of nitrate in tall fescue leaf blades and sheaths (Lyons et al. 1990). Under this hypothesis, fungal nitrogen is less accessible (indeed, some is used to manufacture alkaloids (Dahlman et al. 1991]) to S. *frugiperda* than is nitrogen in the plant. Chemical analyses of the states of nitrogen in tissues of the fungus and plant and its transfer between the symbionts will be required to further test this hypothesis.

Our study failed to demonstrate induced resistance in endophyte-infected tall fescue; we found no interaction between infection status and plant damage for any measured indices of fall armyworm performance. This is in contrast to previous work with tall fescue (Boning and Bultman 1996) and perennial ryegrass (Bultman and Ganey 1995) in which plants were wounded by clipping. Mediation by grass endophytes of induced resistance, if it occurs, may depend upon the type of damage. Pricking small holes in leaf blades, as was done in the present study, appears insufficient to elicit any differential response by endophyte-infected tall fescue. Interestingly, the wounding treatment did have a negative effect on larval mass and development time of insects, regardless of infection status. The many small holes created in the leafblades by the wounding treatment may have altered water content of the blades and caused the observed negative effects. A 2nd difference between the present

experiment and the previous wounding studies with tall fescue is that plant tissue was fed to *Spodoptera* just 4 d following damage by pricking; previous work used plants 4–17 d after damage. It may be that induced responses that are mediated by grass endophytes require longer than the 4 d allowed in the present study. Alternatively, the occurrence of induced resistance mediated by grass endophytes may be inconsistent. Although our study does not support the hypothesis that fungal endophytes mediate induced resistance, our results suggest the defensive mutualism between fall armyworm and its tall fescue endophyte is variable and can be influenced by other factors, such as nutrient availability.

Acknowledgments

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