Influence of temperature on alkaloid levels and fall armyworm performance in endophytic tall fescue and perennial ryegrass

Seppo O. Salminen[1,]*, Douglas S. Richmond[1], Sukhbir K. Grewal[2] & Parwinder S. Grewal[1]

[1]*Department of Entomology and* [2]*Department of Plant Pathology, The Ohio State University, OARDC, Wooster, OH 44691-4096, USA*

Accepted: 10 February 2005

Key words: endophytic fungi, *Neotyphodium coenophialum*, *Neotyphodium lolii*, Clavicipitacea, alkaloids, *Festuca arundinacea*, *Lolium perenne*, Poaceae, *Spodoptera frugiperda*, Lepidoptera, Noctuidae

Abstract

The symbiotic relationships between *Neotyphodium* endophytes (Clavicipitacea) and certain cool-season (C3) grasses result in the synthesis of several alkaloids that defend the plant against herbivory. Over a 3 month period we evaluated the effects of temperature on the expression of these alkaloids in tall fescue, *Festuca arundinacea* Schreb, and perennial ryegrass, *Lolium perenne* L. (Poaceae). Response surface regression analysis indicated that month, temperature, and their interaction had an impact on the alkaloid levels in both grasses. We aimed to identify the alkaloids most closely associated with enhanced resistance to the fall armyworm, *Spodoptera frugiperda* JE Smith (Lepidoptera: Noctuidae), and clarify the role of temperature in governing the expression of these alkaloids. The dry weights and survival of fall armyworms feeding on endophyte-infected tall fescue or perennial ryegrass were significantly lower than for those feeding on uninfected grass, whereas endophyte infection had no significant influence on survival. For tall fescue, a four-alkaloid model consisting of a plant alkaloid, perloline, and the fungal alkaloids ergonovine chanoclavine, and ergocryptine, explained 47% of the variation in fall armyworm dry weight, whereas a three-alkaloid model consisting of the plant alkaloid perloline methyl ether and the fungal alkaloids ergonovine and ergocryptine explained 70% of the variation in fall armyworm dry weight on perennial ryegrass. Although temperature had a significant influence on overall alkaloid expression in both grasses, the influence of temperature on individual alkaloids varied over time. The levels of those alkaloids most closely linked to armyworm performance increased linearly or curvilinearly with increasing temperature during the last 2 months of the study. We conclude that the growth temperature of grasses can influence the performance of fall armyworm, and that this effect may be mediated through a set of plant- and endophyte-related alkaloids.

Introduction

The cool-season grasses tall fescue, *Festuca arundinacea* Schreb, and perennial ryegrass, *Lolium perenne* L. (Poaceae), are commonly used on home lawns, recreational parks, golf courses, and pastures. These grasses are also widely infected by the fungal endophytes, *Neotyphodium coenophialum* (Morgan-Jones and Gams) Glenn, Bacon and Hanlin, and *Neotyphodium lolii* (Latch, Christensen and Samuels) Glenn, Bacon and Hanlin, respectively (Clavicipitacea). The symbiotic relationship between the fungus and the host grass results in the synthesis of fungal alkaloids that provide defense against herbivory (Porter et al., 1974; White & Cole, 1985; West et al., 1988; Hoveland, 1993).

The resistance provided by the alkaloids against insect herbivory has been well established (Siegel et al., 1990;

*Correspondence: Seppo O. Salminen, Department of Entomology, OARDC/The Ohio State University, 1680 Madison Ave., Wooster, OH 44691, USA. Tel.: +1 330 263 3643; Fax: +1 330 263 3686; E-mail: salminen.1@osu.edu

© 2005 The Netherlands Entomological Society

Rowan et al., 1990; Breen, 1993), and has generated interest in using these grasses to manage insect pests (Richmond et al., 1999). The defensive compounds associated with endophyte-infected grasses are active against a wide variety of insects (Rowan & Latch, 1994), and have been shown to influence insect development and survival. Clay & Cheplick (1989) showed that some of the commercially available alkaloids result in a reduction in the larval mass of fall armyworm, *Spodoptera frugiperda* JE Smith (Lepidoptera: Noctuidae). Decreases in fall armyworm larval weight and survival have been shown in endophytic tall fescue (Hardy et al., 1986; Bultman & Conard, 1998), and perennial ryegrass (Bultman & Ganey, 1995; Hardy et al., 1985).

The common practice of controlling insect pests with pesticides in urban environments raises concerns about possible health hazards to people and pets. As a result of the Federal Food Quality Protection Act, many frequently used pesticides, such as carbamates and organophosphates, are being removed from the market. This has limited the available options for effective and safe management of insect pests. The possibility of taking advantage of the potential of endophyte-infected grasses as biocontrol agents in turf management was raised by Clay (1989). It has now been demonstrated that levels of endophyte alkaloids can be affected by simple management practices such as mowing frequency (Salminen & Grewal, 2002), mowing height (Salminen & Grewal, 2003), nitrogen (Lyons et al., 1986), and phosphorus (Malinowski et al., 1998) applications. However, alkaloid levels may also be influenced by seasonal variations in temperature. Breen (1992) reported the effects of temperature on peramine levels and greenbug performance in perennial ryegrass. Seasonal variations in endophyte levels and resistance to Argentine stem weevil in perennial ryegrass has also been reported (Prestidge et al., 1985).

In this study, we tested the influence of temperature on the expression of nine different plant/endophyte alkaloids using tall fescue and perennial ryegrass. Because we anticipated that alkaloid expression may vary over time, we observed alkaloid levels over a 3 month period. Furthermore, we investigated how temperature-induced changes in alkaloid expression translated into defense against fall armyworm, and determined which alkaloids were most important in this regard. We hypothesized that the effects of temperature would vary among alkaloids and that defense against fall armyworm would vary as a result.

Methods and materials

Endophyte infection

The level of infection – 71% ± 4% (mean ± SE) for tall fescue and 82% ± 4% for perennial ryegrass – was well established in the field, and further confirmed in the core samples taken using a rapid staining method (Saha et al., 1988).

Grass management

Triplicate 15 cm (diameter) turf and soil cores of endophytic and non-endophytic tall fescue, *F. arundinacea*, and perennial ryegrass, *L. perenne*, were taken from field plots established in 1999 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Nine endophytic and nine non-endophytic cores of each grass were placed separately in 15 cm pots, and three pots of each type were placed in each of three environmental growth chambers (Conviron BDW 80, Winnipeg, Canada) maintained at 15 °C, 21 °C, or 25 °C under a L16:D8 photoperiod. The plants were cut weekly to 5 cm height from the soil surface and fertilized weekly with Peters Professional fertilizer (20-20-20 N-P-K) (O.M. Scott Co., Maysville, OH). The plants were grown under these conditions for 5 weeks before the bioassays and sampling were initiated.

Sampling

One sample (ca. 4 g) from each pot in each growth chamber was collected once a month (30 days) for 3 months by cutting the grass at the soil level, giving a total of nine endophytic and nine non-endophytic samples of each grass at each sampling date. The samples were frozen in liquid nitrogen and lyophilized. The samples were then ground in a Cyclotech 1093 mill and stored in closed glass vials until analysis.

Alkaloid extraction

The modified method was used, based on the methods of Hill et al. (1993) and described by Salminen & Grewal (2003). Samples of 200 mg and 200 ng of ergotamine as an internal standard were extracted with 9 ml of $CHCl_3$ and 1 ml of 0.01 N NaOH in a scintillation vial agitated in a Burrel wrist action shaker (Pittsburgh, PA) for 30 min. After filtering the sample through a Whatman 1 PS filter paper, the sample was loaded onto a 5 ml syringe, as described by Hill et al. (1993). The column had 1 g of Na_2SO_4 on top and 0.5 g of Ergosil (Analtech, Newark, DE) on the bottom, with Whatman no. 41 filter paper disks on the top and bottom and between the two layers.

Pigments were removed with 5 ml of acetone/chloroform (75 : 25) in an aspirated manifold, and 1 ml of diethyl ether was used to remove residual acetone/chloroform from the column. A detachable Nylon 66 (22 μm) syringe filter was attached and the alkaloid extracted by plunging 2 ml of methanol through it. The sample was evaporated to dryness under N_2 and stored in a freezer.

Quantification of alkaloids

The samples were taken in 100 µl of methanol and analyzed with a Waters LC/MS system as described in Salminen & Grewal (2003). The positive ion electrospray spectra were recorded on a Waters ZQ instrument in total ion and single ion modes.

Bioassay

Ten neonate fall armyworms were placed on moist Whatman no. 1 filter paper in 9 cm (diameter) Petri plates kept at 21 °C. Three plates for each pot were used for the first month and five plates per pot for the subsequent months. Fresh grass clippings were provided daily for 6 days, after which the survival was recorded and fresh and dry weights were determined.

Data analysis

The quantification of alkaloids was based on the ergotamine internal standard. The data for each alkaloid and for fall armyworm performance was subjected to backward step-wise regression to find appropriate the response surfaces to month and temperature. First a full quadratic model was fitted and second order variables that did not account for at least 10% of the remaining variation in the independent variable were removed. A final model was that which remained when no more second order variables could be removed using this criterion. For a dependent variable that had all the second order predictive variables removed, a similar process was used removing month or temperature. If a model could not be fitted using both, then simple linear regression models were tested (SAS, SAS Institute Inc. Cary, NC). Multiple regression was employed to determine which plant or endophyte-mediated alkaloids were the best predictors of fall armyworm biomass. Best subsets regression was performed and Mallow's Cp was used for the initial selection of the best subset of predictors ($Cp = P + 1$) and backward step-wise regression was used for confirmation purposes. To avoid the inclusion of collinear terms, variance inflation factors (VIF) for all terms included in the subset of predictors were examined according to the criteria set out by Freund & Littel (1986).

Results

Effect of growth temperature on alkaloids in tall fescue

Response surface regression analysis indicated that there was a month and temperature interaction in chanoclavine, ergonovine, and ergocryptine (Table 1, Figure 1A, B, E). Both month and temperature were predictors of alkaloid levels. Both had linear components with month indicating a quadratic function (Table 1, Figure 1A, H). Ergonovine, chanoclavine, unknown A [pseudomolecular ion $(M + H)^+ = 537$] and ergocristine had high Adj. R^2 values, especially the first two (Table 1). Adj. $R^2 = 0.62$ from the response surface regression analysis summarized the variability in the alkaloids.

Effect of growth temperature on alkaloids in perennial ryegrass

Both month and temperature were predictors of all alkaloids. Only chanoclavine and unknown, putative alkaloid B [pseudomolecular ion $(M + H)^+ = 571$] failed to show month–temperature interactions (Table 2, Figure 2A, C). Ergonovine showed quadratic responses to month and temperature (Table 2, Figure 2B). High Adj. R^2 values were seen for ergonovine, ergocristine, perloline methyl ether, and ergovaline (Table 2). Adj. $R^2 = 0.70$ from the Principal Components Analysis summarized the variability in the alkaloids.

Table 1 Response surface regression analysis describing the relationship between temperature, month, and expression of different alkaloids in endophyte-infected tall fescue, *Festuca arundinacea*. First and second principal components, parameter estimates, and adjusted R^2 are given

Alkaloid	Month	Temperature	Month^2	Month × temp.	Adj. R^2
Principal comp. 1	1.045	−0.734			0.335
Principal comp. 2	−8.122	0.625	−1.844		0.501
Chanoclavine	−1.002	−0.244	0.172	0.137	0.835
Ergonovine	−0.449	−0.387		0.374	0.928
Ergovaline	0.089				0.392
Ergocristine	−0.299	0.196			0.507
Ergocryptine	−0.054	−0.050		0.021	0.270
Perloline methyl ether	−1.173				−0.011
Perloline	−3.011	1.961			0.434
Unknown A	113.72	12.706	−27.556		0.539
Unknown B	0.359				−0.012

Overall adjusted $R^2 = 0.62$ indicates how well the principal component analysis summarized the variability in the alkaloids.

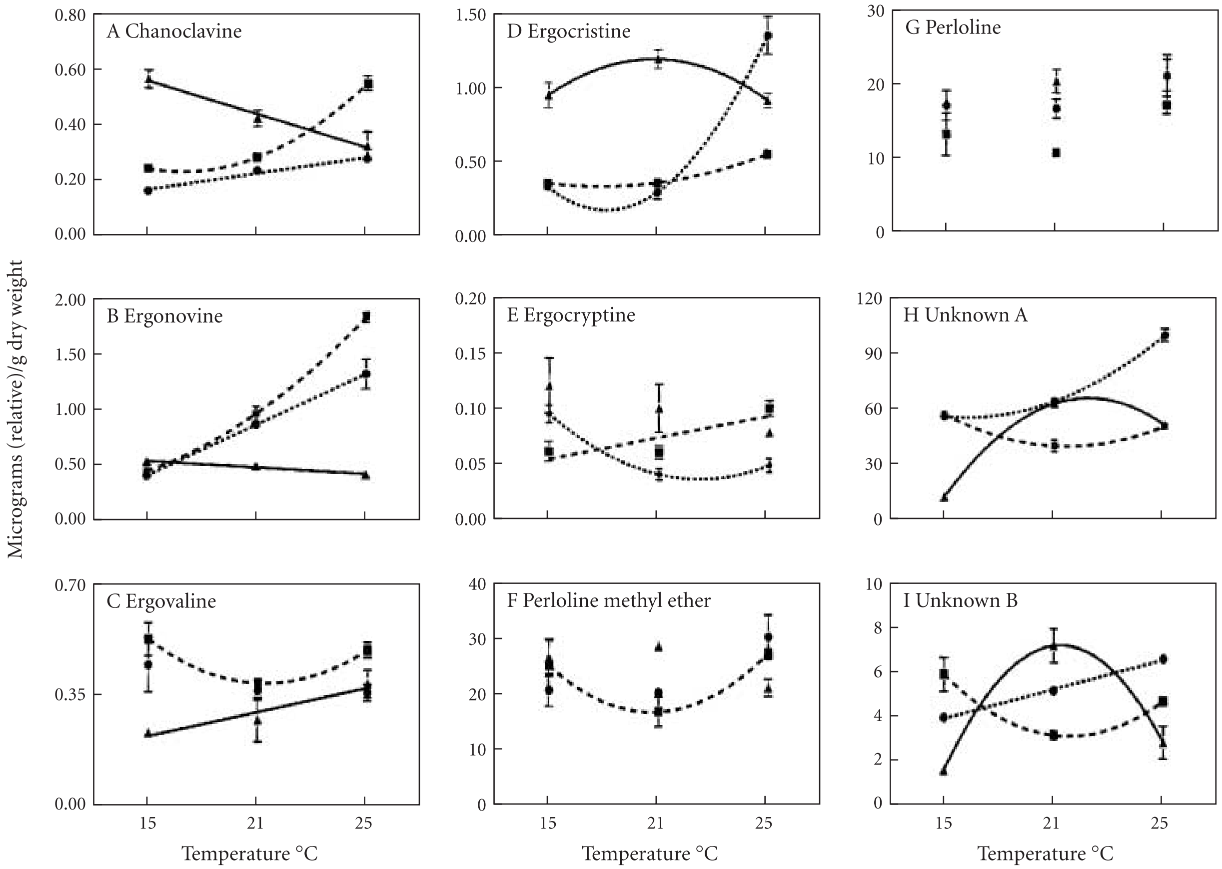

Figure 1 Mean (± SE) levels (µg g^{-1} dry wt.) of: (A) chanoclavine, (B) ergonovine, (C) ergovaline, (D) ergocristine, (E) ergocryptine, (F) perloline methyl ether, (G) perloline, (H) unknown alkaloid A, and (I) unknown alkaloid B of endophytic tall fescue, *Festuca arundinacea,* grown in the greenhouse at 15 °C (▲), 21 °C (●), and 25 °C (■). Triplicate 200 mg samples were extracted at each temperature for 3 months.

Table 2 Response surface regression analysis describing the relationship between temperature, month, and expression of different alkaloids in endophyte-infected perennial rye grass, *Lolium perenne*. First and second principal components, parameter estimates, and adjusted R^2 are given

Alkaloid	Month	Temp	Month^2	Temp.^{2}	Month × temp.	Adj. R^2
Principal comp. 1	6.951	2.406	−1.737		−0.922	0.862
Principal comp. 2	−9.703	0.625	1.661		1.083	0.792
Chanoclavine	1.439	−0.244				0.549
Ergonovine	173.998	−6.727	45.624	9.725	−11.815	0.932
Ergovaline	−1.220	−0.228	0.246		0.125	0.634
Ergocristine	1.498	4.275			−1.216	0.715
Ergocryptine	−0.849	−0.216	0.142		0.100	0.298
Perloline methyl ether	−93.273	−15.448	21.379		8.908	0.651
Unknown A	−154.233	−63.964	28.118		28.976	0.473
Unknown B	10.763	−1.946				0.577

The results for perloline were not significant. Overall adjusted $R^2 = 0.70$ indicates how well the principal component analysis summarized the variability in the alkaloids.

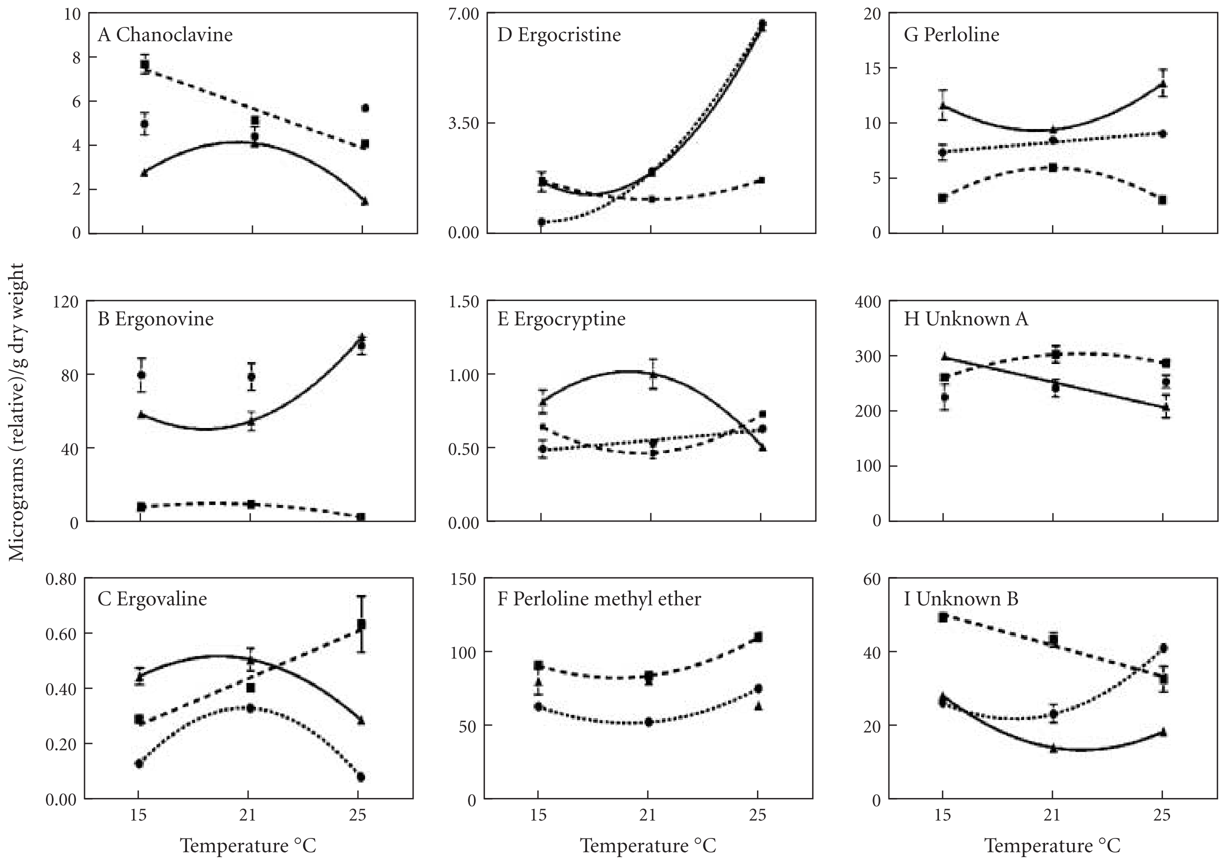

Figure 2 Mean (± SE) levels (µg g^{-1} dry wt.) of: (A) chanoclavine, (B) ergonovine, (C) ergovaline, (D) ergocristine, (E) ergocryptine, (F) perloline methyl ether, (G) perloline, (H) unknown alkaloid A, and (I) unknown alkaloid B of endophytic perennial ryegrass, *Lolium perenne,* grown in the greenhouse at 15 °C (▲), 21 °C (●), and 25 °C (■). Triplicate 200 mg samples were extracted at each temperature for 3 months.

Effect of tall fescue growth temperature on fall armyworm

Temperature was a significant predictor of fall armyworm dry weight in both endophytic and non-endophytic tall fescue (Table 3, Figure 3A, B). The fall armyworms feeding on the endophytic grass had lower dry weights than those feeding on non-endophytic grass. The dry weights on both grasses grown at 15 °C were high in the first month, but declined subsequently (Figure 3A, B). Both linear and quadratic models described the effect of temperature on the dry weights of fall armyworms where increasing temperature was associated with decreasing dry weights (Figure 3A, B). Temperature was a predictor of the survival of fall armyworm feeding on endophytic or non-endophytic tall fescue only at month 3 in a linear function (Table 3, Figure 3C, D).

Effect of perennial ryegrass growth temperature on fall armyworm

Temperature as a predictor of the dry weight of fall armyworm feeding on endophytic or non-endophytic perennial ryegrass had linear and quadratic components for all months (Table 4, Figure 4A, B). The dry weights of fall armyworm feeding on endophytic grass were lower than those on non-endophytic grass (Figure 4A, B). The data suggested an increased survival of the fall armyworm in the absence of the endophyte, but this was not significant (Table 4, Figure 4C, D). In the case of the endophytic grass, temperature was a significant predictor of survival only at month 3, whereas a significant relationship was seen at months 2 and 3 with the fall armyworms feeding on non-endophytic grass.

Relationship between fall armyworm dry weight and alkaloid levels in tall fescue

In an attempt to assess what combination of alkaloids would best predict the fall armyworm dry weights, the data were subjected to a multiple regression analysis. For tall fescue a four-component model consisting of a plant alkaloid, perloline, and the fungal alkaloids ergonovine, chanoclavine, and ergocryptine best described the data

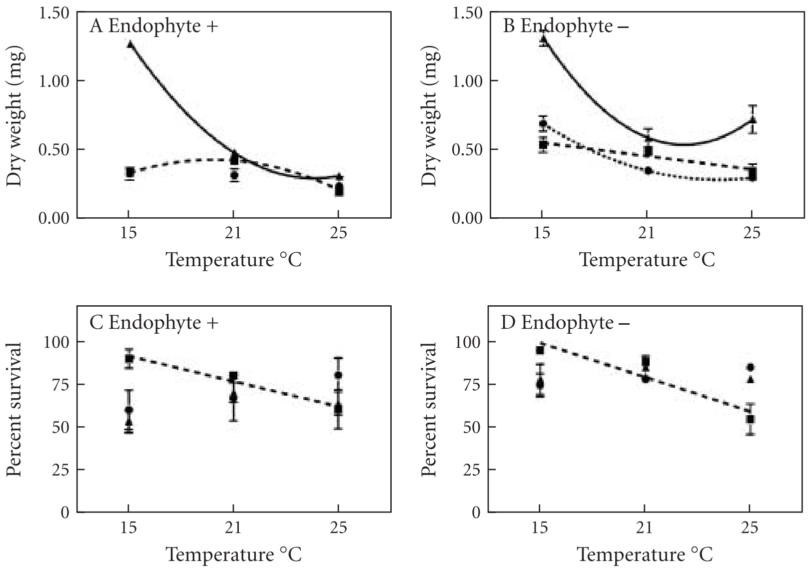

Figure 3 Mean (± SE) dry weights (mg/worm) of fall armyworm, *Spodoptera frugiperda,* feeding on (A) endophytic and (B) non-endophytic tall fescue, and percent larval survival on (C) endophytic and (D) non-endophytic tall fescue grown in the greenhouse at 15 °C (▲), 21 °C (●), and 25 °C (■). Three Petri plates containing five neonate larvae for each pot were used for the first month and 10 plates per pot for the subsequent months.

Table 3 Regression parameters and performance values for regression models describing the relationship between temperature and dry weight and temperature and percent survival of fall armyworms (*Spodoptera frugiperda*) feeding on endophytic and non-endophytic tall fescue, *Festuca arundinacea*. Parameter estimates and adjusted R^2 are given

Determination	Month	Temperature	Temp.^{2}	Adj. R^2
Dry weight				
Endophytic	1	−0.463	0.009	0.973
	2			
	3	0.286	−0.008	0.708
Non-endophytic	1	−0.682	−0.016	0.861
	2	−0.024	0.221	0.868
	3	−0.019		0.541
Percentage survival				
Endophytic	1			
	2			
	3	−0.041		0.556
Non-endophytic	1			
	2			
	3	−0.051		0.667

Empty spaces denote non-significant differences.

Table 4 Regression parameters and performance values for regression models describing the relationship between temperature and dry weight and temperature and percentage survival of fall armyworms (*Spodoptera frugiperda*) feeding on endophytic and non-endophytic perennial ryegrass, *Lolium perenne*. Parameter estimates and adjusted R^2 are given

		Linear	Quadratic	
Determination	Month	Temp.	Temp.^{2}	Adj. R^2
Dry weight				
Endophytic	1	−0.594	0.019	0.663
	2	0.273	−0.012	0.558
	3	0.557	−0.009	0.880
Non-endophytic	1	−0.629	0.022	0.744
	2	−0.277	0.014	0.635
	3	−0.036		0.747
Percent survival				
Endophytic	1			
	2			
	3	−3.954		0.756
Non-endophytic	1			
	2	2.409		0.344
	3	−3.163		0.572

Empty spaces denote non-significant differences.

(Table 5). The adjusted R^2 indicated that 47% of the variation could be explained by these four alkaloids.

Relationship between fall armyworm dry weight and alkaloid levels in perennial ryegrass

Multiple regression analysis indicated that three predictors, viz., the plant alkaloid perloline methyl ether and the fungal alkaloids ergonovine and ergocryptine, described the relationship between fall armyworm dry weight and alkaloid levels in perennial ryegrass (Table 6). The model accounted for 70% of the variation in the results.

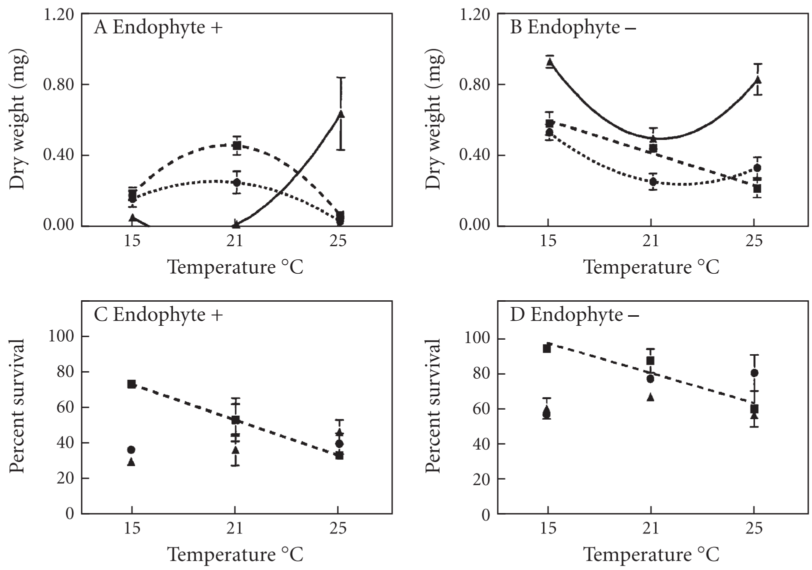

Figure 4 Mean (± SE) dry weights (mg/worm) of fall armyworms, *Spodoptera frugiperda*, feeding on: (A) endophytic and (B) non-endophytic perennial ryegrass, *Lolium perenne*, and percentage larval survival on (C) endophytic and (D) non-endophytic perennial ryegrass grown in the greenhouse at 15 °C (▲), 21 °C (●), and 25 °C (■). Three Petri plates containing five neonate larvae for each pot were used for the first month and 10 plates per pot for the subsequent months.

Table 5 Model parameters, variance inflation factors (VIF), and performance values for the multiple regression model describing fall armyworm (*Spodoptera frugiperda*) biomass as a function of alkaloids produced by endophyte-infected tall fescue, *Festuca arundinacea*

Effect	VIF	R^2	Parameter estimate	SE	T	P
Intercept			0.679521	0.111828	6.07651	0.000008
Perloline	1.33	0.25	−0.010578	0.003916	−2.70116	0.014154
Ergonovine	1.51	0.34	−0.154037	0.038296	−4.02229	0.000728
Chanoclavine	1.61	0.38	0.387217	0.162171	2.38771	0.027498
Ergovaline	1.22	0.18	−0.420639	0.165271	−2.54514	0.019761

Adjusted R^2 = 0.47; variance inflation threshold = 1.87. Second principal component proved to be a moderately useful predictor of the dry weight.

Table 6 Model parameters, variance inflation factors (VIF), and performance values for the multiple regression model describing fall armyworm (*Spodoptera frugiperda*) biomass as a function of alkaloids produced by endophyte-infected perennial ryegrass, *Lolium perenne*

Effect	VIF	R^2	Parameter estimate	SE	T	P
Intercept			1.022550	0.138029	7.40825	0.000000
Perloline methyl ether	2.73	0.638	−0.005564	0.001490	−3.73430	0.001086
Ergonovine	2.47	0.596	−0.003880	0.000552	−5.86460	0.000006
Ergocryptine	1.17	0.142	−0.387149	0.091751	−4.21957	0.000326

Adjusted R^2 = 0.70; variance inflation threshold = 3.30. Principal Components Analysis did not help in estimating the dry weight.

Discussion

Response surface regression analysis indicated that in tall fescue, temperature and month had a significant impact on seven of the nine alkaloids measured. Only perloline methyl ether and unidentified, presumptive alkaloid B seemed to be unaffected. It was obvious that different alkaloids responded differently to temperature, and that the levels fluctuated from month to month even under the relatively controlled conditions of the growth chamber. For instance, in tall fescue at month 1, chanoclavine and ergonovine levels showed a negative relationship with temperature, whereas ergovaline levels increased. The highest relative amounts were found with the two unidentified presumptive alkaloids and two plant alkaloids, perloline and its methyl ether. Perloline, a diazaphenanthrene alkaloid, was first isolated from perennial ryegrass (Grimmet & Melville, 1943) and shortly afterward from tall fescue (Grimmet & Waters, 1943). It has been shown to inhibit the in vitro activity of rumen bacteria (Bush et al., 1970), as well as protein and cellulose digestion in lambs (Boling et al., 1975). The methyl ester was found to be less effective than perloline in cellulose digestion (Bush et al., 1976). Perloline and its methyl ether are present in both endophytic and non-endophytic tall fescue (Hovin & Buckner, 1983). Our results indicate that both of these plant alkaloids are involved in fall armyworm performance.

The effect of temperature on alkaloid levels in perennial ryegrass differed from that seen in tall fescue, as indicated by the higher Adj. R^2 values. Response surface regression analysis indicated that all nine of the alkaloids were significantly influenced by temperature and month. Again, no generalizations on the effect of temperature on the alkaloid levels could be made. High levels of perloline, its methyl ether, and unknowns A and B were seen, in the perennial ryegrass.

The effects of endophyte and growth temperatures on dry weights of fall armyworms were seen with both tall fescue and perennial ryegrass. The effect of the endophyte was more pronounced with perennial ryegrass. An effect of temperature on peramine levels and greenbug antixenosis in perennial ryegrass has been demonstrated (Breen, 1992). The high dry weights in both endophytic and non-endophytic grass at 15 °C, seen with tall fescue at month 1, could indicate that the 5 weeks in the growth chamber prior to the first harvest had not been sufficient for this grass to reach a steady state at this temperature. The closer agreement of the dry weights seen in the rest of the data at all temperatures would be consistent with this idea. The differences in the dry weights did not directly translate to survival in the 6 days of the assay, although a non-significant trend for greater survival with non-endophytic grass, especially with perennial ryegrass, could be discerned. An effect of temperature on survival was seen at month 3 with both grasses regardless of the presence of the endophyte.

Month turned out to be an unexpected predictor of the alkaloid levels. Watering could be a possible source of monthly variation. Water requirements differed with the growth temperature, and it is likely that, in spite of our best efforts, there was enough variation in the water status of the pots to account for the monthly fluctuation in the alkaloid levels (Arechavaleta et al., 1992).

Different alkaloids will vary in their effectiveness against herbivory. As a single alkaloid does not adequately explain the fall armyworm data, a combination of alkaloids could result in additive or synergistic interactions. A synergistic effect of perloline and ergocryptine on the large milkweed bug, *Oncopeltus fasciatus*, in tall fescue has previously been reported (Yates et al., 1989). Thus, we tried to arrive at a multiple regression model for fall armyworm dry weight and the alkaloids, in order to draw out those alkaloids having the greatest impact on the larval dry weight. With tall fescue, a four-component model consisting of perloline, ergonovine, chanoclavine, and ergovaline explained 47% of the effect. Three of the components, viz., ergonovine, chanoclavine, and perloline, were significantly influenced by temperature, whereas ergovaline was not. This suggests that at ambient temperatures near 25 °C, tall fescue would have increased resistance against insect herbivory, at least against fall armyworm, thus decreasing the need for insecticide application. The fact that temperature did not have a significant impact on ergovaline could indicate the presence of some constant level of resistance against herbivory offered by this alkaloid. Fall armyworms have shown a preference for non-endophytic over endophytic grass (Hardy et al., 1986). This difference can be influenced by fertilizer application (Lyons et al., 1986; Malinowski et al., 1998). A negative effect of endophyte on the pupal mass of fall armyworm feeding on tall fescue can be negated by high fertilizer application (Bultman & Conard, 1998). In a three-component model, which explained 70% of the response in perennial ryegrass, perloline methyl ether and ergonovine increased with temperature, whereas ergocryptine did not. The dry weights of fall armyworm feeding on perennial ryegrass showed a pronounced difference between endophytic and non-endophytic grass. The reduced dry weights of fall armyworm feeding on endophytic over non-endophytic perennial ryegrass agree with the results of Hardy et al. (1985) and Bultman & Ganey (1995). We attribute the decrease in fall armyworm dry weight to the alkaloids, in light of the results of Clay & Cheplick (1989) who showed a decrease in fall armyworm weight in response to alkaloids.

In spite of the different responses of individual alkaloids to the temperature and the monthly fluctuations in fall armyworm dry weight, it was possible to describe predictive models for fall armyworm dry weights for both grasses. It was noteworthy that the endophyte-independent, plant synthesized perloline or its methyl ester were components in both models. It was clear from the comparison of the performance of fall armyworm feeding on endophytic and non-endophytic grass that these plant alkaloids by themselves did not provide a complete deterrence against herbivory. It remains to be established whether these same predictors would also have the biggest impact under field conditions. It also remains to be established whether these same alkaloids would emerge as major indicators of performance with other insect herbivores. Once it has been established which alkaloids are major predictors affecting insect performance, future management practices should be directed towards increasing the levels of those alkaloids, assuming that any benefits from the other alkaloids for plant growth would not be diminished.

Acknowledgements

This study was funded by an OARDC interdisciplinary grant. We wish to thank Bert Bishop, OARDC Senior Statistician, for his expert guidance in the data analysis.

References

Arechavaleta M, Bacon CW, Plattner RD, Hoveland CS & Radcliffe DE (1992) Accumulation of ergopeptide alkaloids in symbiotic tall fescue grown under deficits of soil water and nitrogen fertilizer. Applied and Environmetal Microbiology 58: 857–861.

Boling JA, Bush LP, Buckner RC, Pendlum LC, Burrus PB, Yates SG, Rogovin SP & Tookey HL (1975) Nutrient digestibility and metabolism in lambs fed added perloline. Journal of Animal Science 40: 972–976.

Breen JP (1992) Temperature and seasonal effects on expression of *Acremonium* endophyte-enhanced resistance to *Schizaphis graminum* (Homoptera: Aphididae). Environmental Entomology 21: 68–74.

Breen JP (1993) Enhanced resistance to three species of aphids in *Acremonium* endophyte-infected turfgrasses. Journal of Economic Entomology 84: 1279–1286.

Bultman TL & Conard NJ (1998) Effects of endophytic fungus, nutrient level, and plant damage on performance of fall armyworm (Lepidoptera: Noctuidae). Environmental Entomology 27: 631–635.

Bultman TL & Ganey DT (1995) Induced resistance to fall armyworm (Lepidoptera: Noctuidae) mediated by fungal endophyte. Environmental Entomology 24: 1196–1200.

Bush LP, Burton H & Boling JA (1976) Activity of tall fescue alkaloids and analogues in vitro rumen fermentation. Journal of Agricultural and Food Chemistry 24: 869–872.

Bush LP, Streeter C & Buckner RC (1970) Perloline inhibition of *in vitro* ruminal cellulose digestion. Crop Science 10: 108–109.

Clay K (1989) Clavicipitaceous endophytes of grasses: their potential as biocontrol agents. Mycological Research 92: 1–12.

Clay K & Cheplick GP (1989) Effect of ergot alkaloids from fungal endophyte-infected grasses on fall armyworm (*Spodoptera frugiperda*). Journal of Chemical Ecology 15: 169–182.

Freund RJ & Littell RC (1986) SAS System for Regression, 1986 edn. SAS Institute Inc., Cary, IN.

Grimmett RE & Melville J (1943) A fluorescent alkaloid in ryegrass (*Lolium perenne* L.) I. Introduction. New Zealand Journal of Science and Technology 24: 149B.

Grimmett RE & Waters DF (1943) A fluorescent alkaloid in ryegrass (*Lolium perenne* L.) II. Extraction from fresh ryegass and separation from other bases. New Zealand Journal of Science and Technology 24: 151B.

Hardy TN, Clay K & Hammond AM Jr (1985) Fall armyworm (Lepidoptera: Noctuidae): a laboratory bioassay and larval prefernce study for the fungal endophyte of perennial ryegrass. Journal of Economic Entomology 78: 571–575.

Hardy TN, Clay K & Hammond Jr AM (1986) Leaf age and related factors affecting endophyte-mediated resistance to fall armyworm (Lepidoptera: Noctuidae) in tall fescue. Environmental Entomology 15: 1083–1089.

Hill NS, Rottinghaus GE, Agee GS & Schultz LM (1993) Simplified sample preparation for HPLC analysis of ergovaline in tall fescue. Crop Science 33: 331–333.

Hoveland CS (1993) Importance and economic significance of the *Acremonium* endophytes to performance of animals and grass plants. Agricultural Ecosystems and Environment 44: 2–12.

Hovin AW & Buckner RC (1983) Alkaloids in tall fescue and reed canary grass. Handbook of Naturally Occurring Food Toxicants (ed. by M Reichel), pp. 241–247. CRC Press, Boca Raton, FL.

Lyons PC, Plattner RD & Bacon CW (1986) Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. Science 232: 487–489.

Malinowski DP, Belesky DP, Hill NS, Baligar VC & Fedders JM (1998) Influence of phosphorus on the growth and ergot alkaloid content of *Neotyphodium coenophialum*-infected tall fescue (*Festuca arundinacea*). Plant and Soil 198: 53–61.

Porter JK, Bacon CW & Robbins JD (1974) Major alkaloids of a *Claviceps* isolated from toxic. Bermuda Grass Journal of Agricultural and Food Chemistry 22: 838–841.

Prestidge RA, diMenna ME & Van Der Zijpp S (1985) Minimum *Acremonium loliae* levels needed to maintain Argentine stem weevil resistance in infected ryegrasses and in cultures. Proceedings, 4th Australasian Conference of Grassland Invertebrate Ecology. Lincoln College, University of Agriculture, Canterbury, NZ, 13–17 May 1985, pp. 332–337. Department Entomology, Lincoln College, Canterbury, NZ.

Richmond DS & Shetlar DJ (1999) Larval survival and movement of bluegrass webworm in mixed stands of endophytic perennial ryegrass and Kentucky bluegrass. Journal of Economic Entomology 93: 1662–1668.

Rowan DD, Dymock JJ & Bimble MA (1990) Effect of fungal metabolite peramine and analogs on feeding and development of Argentine stem weevil (*Listronotus bonariensis*). Journal of Chemical Ecology 16: 1683–1695.

Rowan DD & Latch GCM (1994) Utilization of endophyte-infected perennial ryegrass for increased insect resistance. Biotechnology of Endophytic Fungi of Grasses (ed. by CW Bacon & JF White Jr.), pp. 169–183. CRC Press, Boca Raton, FL.

Saha DC, Jackson MA & Johnson-Cicalese JM (1988) A rapid staining method for detection of endophytic fungi. Phytopathology 78: 237–239.

Salminen SO & Grewal PS (2002) Does decreased mowing frequency enhance alkaloid production in endophytic tall fescue and perennial ryegrass? Journal of Chemical Ecology 28: 931–942.

Salminen SO & Grewal PS (2003) Does mowing height influence alkaloid production in endophytic tall fescue and perennial ryegrass? Journal of Chemical Ecology. 29: 1295–1304.

Siegel MR, Latch CCM, Bush LP, Tannin NF, Rowan DD, Tapper RA, Bacon CW & Johnson MC (1990) Alkaloids and aphid response in grasses infected with fungal endophytes. Journal of Chemical Ecology 16: 3301–3315.

West CP, Izekor DM, Oosterhuis DM & Robbins RT (1988) The effect of *Acremonium coenophialum* on growth and nematode infestation of tall fescue. Plant and Soil 112: 3–6.

White Jr JF & Cole GT (1985) Endophyte–host associations in forage grasses. III. In vitro inhibition of fungi by *Acremonium coenophialum*. Mycologia 82: 218–226.

Yates SG, Fenster JC & Bartelt RJ (1989) Assay of tall fescue seed extracts, fractions, and alkaloids using the large milkweed bug. Journal of Agriculture and Food Chemistry 37: 354–357.