Journal of Chemical Ecology, Vol. 26, No. 4, 2000

DIFFERENT LEVELS OF PROTECTIVE ALKALOIDS IN GRASSES WITH STROMA-FORMING AND SEED-TRANSMITTED *Epichloë/Neotyphodium* ENDOPHYTES

A. LEUCHTMANN,[1,*] D. SCHMIDT,[2,4] and L. P. BUSH[3]

[1]*Geobotanisches Institut ETH*
Zollikerstrasse 107, CH-8008 Zürich, Switzerland
[2]*Station fédérale de recherches agronomique de Changins*
CH-1260 Nyon, Switzerland
[3]*Agronomy Department, University of Kentucky*
Lexington, Kentucky 40546

(Received May 3, 1999; accepted December 7, 1999)

Abstract—The three alkaloid groups—lolines, ergopeptides, and peramine—are typically associated with endophyte infection of grasses, with the main function to protect hosts against herbivores. We determined levels of *N*-formylloline, *N*-acetylloline, ergovaline, and peramine in 18 European grasses naturally infected with seed-transmitted *Neotyphodium* endophytes or sexual *Epichloë* species. Peramine was the most common alkaloid, whereas lolines and ergovaline were only detected in *Festuca* hosts infected with *E. festucae*, *N. coenophialum*, or *N. uncinatum*. Only ten of the grass species analyzed contained detectable amounts of one or more of these alkaloids. There was a clear tendency for plants associated with stroma-forming *Epichloë* species to be free of alkaloids, and those that did produce alkaloids contained only small levels of peramine. In contrast, plants infected with seed-transmitted *Neotyphodium* endophytes often contained extremely high levels of lolines. Lolines enhance host survival through increased protection from herbivores and, thus, may be particularly favored in asexual endophytes that depend on host seed-production for their dispersal.

Key Words—Mutualistic symbiosis, herbivory, peramine, loline, ergovaline, *Epichloë*, *Neotyphodium*, European grasses.

*To whom correspondence should be addressed.
[4]Present address: Chemin de Bourgogne 11, CH-1260 Nyon, Switzerland.

0098-0331/00/0400-1025$18.00/0

INTRODUCTION

Epichloë species (Ascomycota, Clavicipitaceae) and their asexual derivatives placed in the anamorphic genus *Neotyphodium* represent a distinct group of fungal endophytes that form associations with pooid grasses (Glenn et al., 1996; Schardl, 1996). These fungi systemically colonize leaves and culms of infected hosts during the vegetative phase of plant growth, without causing disease symptoms. However, during emergence of flowering culms, *Epichloë* species form sexual structures (stromata) around developing inflorescences that partially or completely sterilize the host. Ascospores produced on the stromata are the propagules for horizontal transmission, which is mediated by infection of plants through wounds or through stigmata with subsequent seed transmission (Western and Cavett, 1959; Chung and Schardl, 1997). In contrast, asexual *Neotyphodium* species are seed-transmitted only and spend their entire life cycle within the host.

Grass endophytes can produce several groups of biologically active alkaloids that accumulate within infected plant tissues (Porter, 1994). The most important alkaloid groups include the lolines, which are active mainly against insects and perhaps small mammals (Riedell et al., 1991; Coley et al., 1995), but not cattle (Daccord et al., 1995). The alkaloid peramine is a feeding deterrent to insects (Rowan and Latch, 1994). The ergot alkaloids are active against vertebrates or in some cases insects and are implicated in livestock toxicosis (Yates et al., 1989; Bacon, 1995). Lolitrems, which cause neurotoxic disorders of mammals (Prestidge, 1993), are found mainly in *Lolium* spp. The type and amount of alkaloids accumulated in infected grasses varies considerably in different natural endophyte/grass associations, whereby the taxonomic identity of the endophyte appears to be the most important factor determining alkaloid production (Siegel et al., 1990; Bush et al., 1993; Christensen et al., 1993). However, environment and plant genotype can also influence the levels of alkaloids expressed in a particular association (Agee and Hill, 1994; Roylance et al., 1994).

The well-documented antiherbivore effects of alkaloids, particularly the lolines and peramine, contribute to the increased persistence of endophyte infected compared to uninfected grasses and are, in many cases, the basis of mutualism between host and endophyte (Clay, 1988; Siegel et al., 1990; Siegel and Bush, 1996). Other fitness enhancements that may be provided by endophytes include growth stimulation, increased drought tolerance, and protection from pathogen attack (Latch et al., 1985; Kimmons et al., 1990; Gwinn and Gavin, 1992; Elbersen and West, 1996). From an evolutionary point of view, increased survival of host grasses may be especially relevant for purely seed-transmitted endophytes, which are permanently associated with their host lineages. Therefore, the levels of alkaloids may be related to the life history strategy of grass and endophyte. There is a tendency for asexual, seed-transmitted endo-

phytes to express higher levels of alkaloids compared to horizontally transmitted sexual endophytes (Siegel et al., 1990; Miles et al., 1996).

In this study, we present additional data of alkaloid levels produced in natural associations of European grasses with seed-transmitted or sexual *Epichloë/Neotyphodium* endophytes. From two of the host grasses, different samples infected with stroma-forming or asexual forms of *Epichloë* were included.

METHODS AND MATERIALS

Sources and Harvest of Plants. Most infected plants originated from natural sites in Switzerland and were transplanted to either field plots at the Research Station Changins or to pots kept in a greenhouse at Changins and on an open terrace at the Botanical Garden in Zürich (Table 1). Some plants of *D. glomerata* and *F. arundinacea*, and all plants of *F. pratensis*, were breeding lines from breeding programs at Changins. In addition, fresh plant material for alkaloid analyses was directly collected at natural sites in the field from infected plants of some host grasses (Table 1).

Transplanted plants were maintained for one to several years before harvest. Field plots in Changins established within farmland were fertilized once a year in late fall with solid long-term fertilizer (100 kg/ha P, 300 kg/ha K) and during the growing season after the cuts (usually four times a year) with 60 kg/ha N. Plants kept in the greenhouse or on the open terrace were grown in 18-cm plastic pots in commercial potting mix (40% bark peat, 35% regular peat, 20% expanded Lecca clay, 5% clay) and fertilized weekly during the growing season with a dilute solution of soluble fertilizer (200 mg/liter N, 200 mg/liter P, 160 mg/liter K, and trace elements). During the winter months, pots from the open terrace were brought to an experimental garden and immersed in the soil, while pots in Changins were left in the cold greenhouse. Fertilization was reduced or omitted in the winter.

Plant harvest was during the growing season between May and October in 1993 and between May and August in 1994. Nine additional samples of *Bp. sylvaticum* were collected in July 1996. To allow for detection of possible seasonal changes in alkaloid contents, several plants were sampled twice, usually before or at the time of flowering in spring, and later after flowering in summer (Table 2). A third sample of one *F. rubra* plant and one *F. arundinacea* plant was collected in October 1993. Each sample usually included stems, leaves, and, when present, fungal stromata of an individual plant (clone). In the few cases that single plants yielded too little material, tissue from more than one plant was combined for the analysis. Sample material was cleaned from soil debris and dead tissue, then lyophilized, sealed in Ziploc bags, and immediately sent to the University of Kentucky for alkaloid analysis.

TABLE 1. ORIGIN AND SITE OF HARVEST OF INFECTED PLANTS USED IN THIS STUDY

Grass species	Origin	Site of harvest[a]	Plant code
Agrostis tenuis Sibth.	Changins, Ct. Vaud	F	At1, At2
	Seealpsee, Ct. Appenzell	PG	At3
Anthoxanthum odoratum L.	Aubonne, Kt. VD	F	Ao1
Brachypodium pinnatum (L.) P.B.	Zumikon, Ct. Zurich	N	Bp1
	Gingins, Ct. Vaud	PG	Bp2
Brachypodium sylvaticum	Albisgüetli, Ct. Zurich	N	Bs1-Bs8
(Huds.) P.B.	Sihlwald, Ct. Zurich	P	Bs9, Bs10
Bromus benekenii (Lange)	Albisgüetli, Ct. Zurich	N	Bb1, Bb2
Trimen	La Combe du Faoug, Ct. Vaud	F, PT	Bb3
Bromus erectus Huds.	Vesancy, France	PG	Be1
	Bois de Chêne, Ct. VD	F	Be2
Bromus ramosus Huds.	Albisgüetli, Ct. Zurich	N	Br1, Br2
Dactylis glomerata L.	Changins, breeding lines	F	Dg1-Dg6
	La Tourne, Ct. Neuchâtel	F	Dg7
	Gingins, Ct. Vaud	F	Dg8
	Vesancy, France	F	Dg9
Elymus europaeus L.	Albisgüetli, Ct. Zurich	PT	Ee1-Ee4
Festuca arundinacea Schreb.	Changings, breeding lines	F	Fa1-Fa3
	Nyon, Ct. VD	F	Fa4-Fa6
	St. Jean, Pyrenee, Spain	F	Fa7
Festuca gigantea (L.) Vill.	Albisgütli, Ct. Zurich	N	Fg1, Fg2
		PT	Fg3
Festuca pratensis Huds.	Changins, breeding lines	F	Fp1-Fp6
Festuca rubra L.	La Ferrière, Ct. Bern	F	Fr1
	Lens, Ct. Valais	PG	Fr2, Fr3
	Rougement, Ct. Vaud	F	Fr4
	Vesancy, France	F	Fr5
Holcus lanatus L.	Aubonne, Ct. Vaud	F	H11
	La Rippe, Ct. Vaud	F	H12
	Soglio, Ct. Grisons	PG	H13
Lolium perenne L.	Nyon, Ct. Vaud	F	Lp1
Phleum pratense L.	Bex, Ct. Vaud	F	Ph1, Ph2
	St. Triphon, Ct. Vaud	F	Ph3
Poa nemoralis L.	Aubonne, Kt. VD	PG	Pn1
Poa trivialis L.	Rueyres, Ct. Fribourg	F	Pt1
	Nyon, Ct. Vaud	PG	Pt2
	St. Triphon, Ct. Vaud	PG	Pt3
	Gingins, Ct. Vaud	PG	Pt4
		N	Pt5
	Vesancy, France	PG	Pt6

[a] F = field plot, Changins; N = natural site of origin; PG = pots kept in greenhouse, Changins; PT = pots kept on open terrace, Zürich.

TABLE 2. ALKALOID LEVELS PRODUCED IN INFECTED PLANTS AT DIFFERENT TIMES OF HARVEST IN 1994

Grass species/ plant code	Alkaloid (μg/g dry weight tissue; ppm)			Harvest	
	Lolines[a]	Ergovaline[b]	Peramine	Date	Site[c]
Bromus benekenii					
Bb1	0	0	2.57	June 21	N
Bb2	0	0	3.55	June 21	N
Bb3	0	0	0	May 27	F
Bb3	0	0	1.59	June 21	PT
Bb3	0	0	4.52	Aug 12	F
B. ramosus					
Br1	0	0	1.68	June 21	N
Br2	0	0	0	June 21	N
Festuca rubra					
Fr1	0	0	2.98	May 27	F
Fr1	0	0.58	1.94	Aug 12	F
Fr1	0	1.40	15.8	Oct.[d]	F
F. arundinacea					
Fa1	70	0	0	May 9	F
Fa1	1025	0	2.27	Aug 12	F
Fa2	219	0	0	May 9	F
Fa2	1540	0	0	Aug 12	F
Fa3	98	0	0	May 12	F
Fa3	1319	1.03	3.10	Aug 12	F
Fa4	347	0.71	0	May 12	F
Fa4	625	0	0.62	Aug 12	F
Fa4	1252	0	0	Oct.[d]	F
Lolium perenne					
Lp1	0	0	1.35	May 3	F
Lp1	0	0	0	Aug 12	F

[a] *N*-formylloline and *N*-acetylloline are combined.
[b] Values for ergovaline include those of its isomer ergovalinine.
[c] F = field plot, Changins; N = natural site of origin; PT = pots kept on open terrace, Zürich.
[d] Harvested in 1993.

Determination of Alkaloids. The lolines were measured by a modification of the gas chromatographic methods of Kennedy and Bush (1983) and Yates et al. (1990). To 1 g dry, powdered plant tissue was added 0.3 g $NaHCO_3$ and 2 ml water, and the mixture was ground in a mortar. The resultant paste was suspended in 8 ml CH_2Cl_2–methanol (19 : 1, v/v) containing 4-phenylmorpholine as internal standard. After 15 min, the suspension was centrifuged, and 1 μl of the supernatant injected into a gas chromatograph equipped with a 15-m × 0.53-mm ID poly(dimethylsiloxane) (0.5-μm film) column and a FID. The oven temperature

program was 80°C (2 min hold) to 212°C at 4°C/min. Alkaloids were quantified by the internal standard method. *N*-Formylloline and *N*-acetylloline were synthesized by the methods of Petroski et al. (1989), and the response factors were determined in calibration studies. The limit of detection was estimated at 2 μg/g.

Ergot alkaloids were extracted from 0.5 g ground sample, and levels of ergovaline and its isomer ergovalinine were quantified by HPLC as described by Siegel et al. (1990). Sample extraction was done by shaking the ground sample in 5 ml of 80% methanol for 1.5 hr at 22°C. The suspension was centrifuged, and the supernatant was filtered through a 0.45-μm nylon membrane filter. The internal standard, dihydroergocristine methane sulfonate was added, and 20 μl was injected onto the HPLC column (3.9 × 300 mm, Bondapak C18 Waters column; 5 μm particle size) attached to a Varian 5000 liquid chromatograph with an Applied Biosystems 980 programmable fluorescence detector. Excitation was done at 310 nm, and detection was above a 370-nm long-pass emission filter. Ergovaline and ergovalinine values were combined, and the total was reported as ergovaline. The detection limit was 0.03 μg/g. During method development, retention times for ergovaline and ergovalinine were determined with standard compounds, and the amounts of ergovaline in plant samples were determined from calibration standards by external standards and confirmed by tandem mass spectrometry.

Peramine was extracted from 150 mg samples and concentration was determined by TLC as described by Fannin et al. (1990). To the sample in a microfuge tube was added 1200 μl of 80% ethanol, and then the sample was mixed, sonicated, and shaken for 1 hr. After centrifugation, 500 μl of supernatant was removed and taken to dryness in a stream of nitrogen. The residue was dissolved in 50 μl, and 10 μl was applied to a reverse-phase TLC plate. The spot was placed about 2.5 cm from one edge of the plate. Development was done in two directions. The edge of the plate opposite the origin was immersed in 0.1% H_3PO_4 at pH 7.25 and 60% methanol, and the solvent was allowed to migrate to the top of the plate. The plate was removed, and a strip of adsorbent, about 14 mm wide from the edge to the plate towards the origin, was removed. After drying, the plate was developed in 0.6 M NaCl in 50% methanol. The plate was removed from the tank and sprayed with van Urk's reagent. After the plate was thoroughly dry, it was scanned in a Shimadzu high-speed thin-layer chromato-scanner (model CS-920) at 600 nm in the reflectance mode. Amounts present were determined from a standard calibration curve with authentic peramine. Detection limit was 0.3 μg/g.

RESULTS

Content of the alkaloids *N*-formylloline, *N*-acetylloline, ergovaline, and peramine varied widely among different endophyte infected grass species (Table 3). Peramine was the most common alkaloid and was found in nine of 18 host grasses infected with either *Epichloë* or asexual *Neotyphodium* species. Ergova-

TABLE 3. ALKALOIDS PRODUCED IN *Epichloë/Neotyphodium*-INFECTED GRASSES

Fungal endophyte	Grass species	Plant symptom[a]	No. of samples	Alkaloid (μg/g dry weight tissue; ppm)			
				N-Formylloline	*N*-Acetylloline	Ergovaline[b]	Peramine
E. typhina	*Anthoxanthum odoratum*	S	1	0	0	0	0
	Brachypodium pinnatum	S	2	0	0	0	0
	Dactylis glomerata	S	9	0	0	0	0
	Phleum pratense	S	6	0	0	0	0
	Poa nemoralis	S	1	0	0	0	0
	Poa trivialis	S	6	0	0	0	4.5–12.2
E. clarkii	*Holcus lanatus*	S	8	0	0	0	0–5.0
E. baconii	*Agrostis tenuis*	S	4	0	0	0	0
E. bromicola	*Bromus erectus*	S	3	0	0	0	0–6.2
	Bromus benekenii	NS	5	0	0	0	0–4.5
	Bromus ramosus	NS	2	0	0	0	0–1.7
E. sylvatica	*Brachypodium sylvaticum*	S	5	0	0	0	0
		NS	5	0	0	0	0
E. festucae	*Festuca gigantea*	NS	3	224–347	0–36	0	2.8–5.2
	Festuca rubra	S	3	0	0	0	0
		NS	4	0	0	0–1.4	0–15.8
N. coenophialum	*Festuca arundinacea*	NS	12	70–1539	0–2286	0–2.0	0–3.1
N. uncinatum	*Festuca pratensis*	NS	6	3260–5459	1021–1354	0	0
N. lolii	*Lolium perenne*	NS	2	0	0	0	0–1.4
Neotyphodium sp.	*Elymus europaeus*	NS	4	0	0	0	0

[a] S = plants produced symptoms (stroma) of choke disease; NS = no stromata produced.
[b] Values for ergovaline include those of its isomer ergovalinine.

line was detected only in *Festuca arundinacea* and in one plant (Fr1) of *F. rubra*, whereas the two loline alkaloids occurred in three *Festuca* hosts infected with *E. festucae* Leuchtmann, Schardl & Siegel, *N. coenophialum* Morgan-Jones & Gams, or *N. uncinatum* Gams, Petrini & Schmidt. In general, a particular alkaloid or combination of alkaloids appeared to be more consistently associated with a host species than with naturally occurring endophyte taxa. For example, *F. gigantea* and *F. rubra*, both naturally infected with *E. festucae*, had different combinations of alkaloids, and peramine was found in only one of the six hosts of *E. typhina*. There also seemed to be a relationship between asymptomatic associations and the tendency to produce alkaloids. Alkaloids were found in seven of nine host grasses naturally associated with non-stroma-forming endophyte strains compared to only three of 11 stroma-forming associations. In *Festuca rubra*, a grass host where symptom expression is variable, stromata-forming plants contained no alkaloids, whereas in samples of an asymptomatic plant (Fr1) ergovaline and peramine was present (Table 3). However, no alkaloids were detected in a second infected plant (Fr4) that never formed stromata.

The level of alkaloids was not only dependant on a particular plant–fungus association but sometimes also on the time of harvest (Table 2). Concentration of peramine in an infected plant of *B. benekenii* (Bb3) was below the detectable level in May, and highest in August with an intermediate level in June. In plant Fr1 of *F. rubra*, the yield of peramine was higher in the early harvest than in August and was more than five times higher in October of the previous year compared to that in May. In addition, this plant contained low levels of ergovaline in August and October. All four plants of *F. arundinacea* yielded more alkaloids in August than in May, whereas in *L. perenne*, peramine was found only in May. Multiple harvests at different times of *Ph. pratense* and *A. tenuis* gave identical negative results for all alkaloids tested (data not shown).

DISCUSSION

The three alkaloid groups studied—lolines, ergot alkaloids, and peramine—are typically associated with endophyte infection of grasses (Siegel and Bush, 1996). In our study, only 10 of the 18 naturally infected grass species analyzed contained detectable amounts of one or more of these alkaloids. Although previous studies have occasionally found host–fungus associations where no alkaloids were produced (Siegel et al., 1990; TePaske et al., 1993), the high number of grasses apparently lacking any of the alkaloids here is unexpected. Since we used routine assays and small amounts of tissue for alkaloid analyses, sample measurements have inherent detection limits. Thus, alkaloids could be present in these grasses but below the limit of detection.

It is possible that in some associations the time when samples were collected

was not appropriate to yield detectable amounts of alkaloids. Levels of alkaloids may show seasonal variation (Belesky et al., 1987; Roylance et al., 1994; Ball et al., 1995) or depend on specific growth or environmental conditions (Siegel et al., 1990; Huizing et al., 1991). However, at least for the host grasses *Ph. pratense* and *A. tenuis* this seems unlikely, since infected samples from multiple harvests in May, June, and August were all negative. In addition, seed samples of infected *Bp. sylvaticum* collected in October at six different sites contained none of the alkaloids (data not shown), conforming with the results of vegetative tissues of this host grass. Furthermore, our extractions were made from samples containing all above-ground plant parts and, thus, represented average levels of alkaloids present in the plant. It is known that accumulation and distribution of alkaloids can vary considerably in different types of host tissues (Ball et al., 1997; Justus et al., 1997).

The cultural conditions of the plants sampled for alkaloid analysis differed among plants (Table 1) and may have influenced alkaloid accumulation. High levels of soil nitrogen and phosphorus can increase the production of ergopeptide alkaloids (Arechavaleta et al., 1992; Malinowski et al., 1998; Richardson et al., 1999). Here, the majority of the plants representing all host grasses, except *B. ramosus*, were grown in a well-fertilized, experimental environment (pots or field plots). Thus, the negative results, at least for ergot alkaloids, are likely to be meaningful under a wide range of soil fertilities.

There was a clear tendency for stroma-forming plants to be free of alkaloids, and those that did produce alkaloids contained only small levels of peramine (Table 3). This observation parallels to some extent previous findings in several other *Epichloë* infected grasses that often showed higher levels of peramine and in some cases also traces of ergovaline (Siegel et al., 1990). Peramine acts as a feeding deterrent to the Argentine stem weevil, but most other insects seem to be insensitive to this alkaloid (Rowan and Latch, 1994). By contrast, loline alkaloids, particularly those from infected *Festuca* species, are known for their insecticidal activities both as contact and metabolic toxins to a broad range of insect species (Bush et al., 1997).

The absence of alkaloids in many stroma-forming grass–endophyte associations may have something to do with the fact that for sexual reproduction *Epichloë* species depend on symbiotic flies of the genus *Botanophila* (referred to *Phorbia* by some authors) (White and Bultman, 1987). These insects appear to be the main vector of spermatia that need to be transferred among self-incompatible stromata of the same species. Female flies lay their eggs on young conidial stromata and actively fertilize the fungus in the process (Bultman and White, 1988; Bultman et al., 1998). The hatching insect larvae then feed on the fungal tissues as their exclusive food source until pupation. High levels of alkaloids in the tissues would be detrimental to the larvae. Alternatively, *Botanophila* flies may not be sensitive to alkaloids if present, which could be the reason for this

association. In any case, the interaction of insect and fungus appears to be a mutualistic relationship.

Plants infected with seed-transmitted *Neotyphodium* endophytes often contained extremely high levels of lolines compared to those infected with *Epichloë* species, particularly *Festuca* hosts (Table 3). The metabolic cost to accumulate high alkaloid amounts, which for loline might be comparable to the total endophyte biomass in the host plant, must be considerable. This finding is not unprecedented in grass endophytes (Siegel et al., 1990; Bush et al., 1993) and must be viewed in an ecological context. Lolines are known to provide increased protection from insects and perhaps other herbivores (Siegel and Bush, 1996) and, thus, can enhance host survival. This may be relevant from an evolutionary point of view, because asexual endophytes, unlike ascospore producing *Epichloë* species, depend on host fitness and seed-production for their dispersal. Under such circumstances, natural selection will favor endophytes (and their hosts) that invest resources into products that enhance host survival.

Acknowledgments—We thank Stefan Kellenberger, Changins, for technical assistance, and C. L. Schardl for helpful comments on the draft manuscript.

REFERENCES

AGEE, C. S., and HILL, N. S. 1994. Ergovaline variability in *Acremonium*-infected tall fescue due to environment and plant genotype. *Crop Sci.* 34:221–226.

ARECHAVALETA, M., BACON, C. W., PLATTNER, R. D., HOVELAND, C. S., and RADCLIFFE, D. E. 1992. Accumulation of ergopeptide alkaloids in symbiotic tall fescue grown under deficits of soil water and nitrogen fertilizer. *Appl. Environ. Microbiol.* 58:857–861.

BACON, C. W. 1995. Toxic endophyte-infected tall fescue and range grasses: Historic perspectives. *J. Anim. Sci.* 73:861–870.

BALL, O. J. P., PRESTIDGE, R. A., and SPROSEN, J. M. 1995. Interrelationships between *Acremonium lolii*, Peramine, and Lolitrem B in perennial ryegrass. *Appl. Environ. Microbiol.* 61:1527–1533.

BALL, O. J. P., BARKER, G. M., PRESTIDGE, R. A., and LAUREN, D. R. 1997. Distribution and accumulation of the alkaloid peramine in *Neotyphodium lolii*-infected perennial ryegrass. *J. Chem. Ecol.* 23:1419–1434.

BELESKY, D. P., ROBBINS, J. D., STUEDEMANN, J. A., WILKINSON, S. R., and DEVINE, O. J. 1987. Fungal endophyte infection—loline derivative alkaloid concentration of grazed tall fescue. *Agron. J.* 79:217–220.

BULTMAN, T. L., and WHITE, J. F., JR. 1988. "Pollination" of a fungus by a fly. *Oecologia* 75:317–319.

BULTMAN, T. L., WHITE, J. F., JR., BOWDISH, T. I., and WELCH, A. M. 1998. A new kind of mutualism between fungi and insects. *Mycol. Res.* 102:235–238.

BUSH, L. P., FANNIN, F. F., SIEGEL, M. R., DAHLMAN, D. L., and BURTON, H. R. 1993. Chemistry, occurrence and biological effects of saturated pyrrolizidine alkaloids associated with endophyte-grass interactions. *Agric. Ecosyst. Environ.* 44:81–102.

BUSH, L. P., WILKINSON, H. H., and SCHARDL, C. L. 1997. Bioprotective alkaloids of grass fungal endophyte symbioses. *Plant Physiol.* 114:1–7.

CHRISTENSEN, M. J., LEUCHTMANN, A., ROWAN, D. D., and TAPPER, B. A. 1993. Taxonomy of *Acre-*

monium endophytes of tall fescue (*Festuca arundinacea*), meadow fescue (*Festuca pratensis*), and perennial rye-grass (*Lolium perenne*). *Mycol. Res.* 97:1083–1092.

CHUNG, K.-R., and SCHARDL, C. L. 1997. Sexual cycle and horizontal transmission of the grass symbiont, *Epichloë typhina. Mycol. Res.* 101:295–301.

CLAY, K. 1988. Fungal endophytes of grasses: A defensive mutualism between plants and fungi. *Ecology* 69:10–16.

COLEY, A. B., FRIBOURG, H. A., PELTON, M. R., and GWINN, K. D. 1995. Effects of tall fescue endophyte infestation on relative abundance of small mammals. *J. Environ. Qual.* 24:472–475.

DACCORD, R., ARRIGO, Y., GUTZWILLER, A., and SCHMIDT, D. 1995. Les endophytes: Un facteur limitant les performances du ruminant? *Rev. Suisse Agric.* 27:197–199.

ELBERSEN, H. W., and WEST, C. P. 1996. Growth and water relations of field grown tall fescue as influenced by drought and endophyte. *Grass Forage Sci.* 51:333–342.

FANNIN, F. F., BUSH, L. P., SIEGEL, M. R., and ROWAN, D. D. 1990. Analysis of peramine in fungal endophyte-infected grasses by reversed-phase thin-layer chromatography. *J. Chromatogr.* 503:288–292.

GLENN, A. E., BACON, C. W., PRICE, R., and HANLIN, R. T. 1996. Molecular phylogeny of *Acremonium* and its taxonomic implications. *Mycologia* 88:369–383.

GWINN, K. D., and GAVIN, A. M. 1992. Relationship between endophyte infestation level of tall fescue seed lots and *Rhizoctonia zeae* seedling disease. *Plant Dis.* 76:911–914.

HUIZING, H. J., VAN DER MOLEN, W., KLOEK, W., and DEN NIJS, A. P. M. 1991. Detection of lolines in endophyte-containing meadow fescue in The Netherlands and the effect of elevated temperature on induction of lolines in endophyte-infected perennial ryegrass. *Grass Forage Sci.* 46:441–445.

JUSTUS, M., WITTE, L., and HARTMANN, T. 1997. Levels and tissue distribution of loline alkaloids in endophyte infected *Festuca pratensis. Phytochemistry* 44:51–57.

KENNEDY, C. W., and BUSH, L. P. 1983. Effect of environmental and management factors on the accumulation of *N*-acetyl and *N*-formyl loline alkaloids in tall fescue. *Crop Sci.* 23:547–552.

KIMMONS, C. A., GWINN, K. D., and BERNARD, E. C. 1990. Nematode reproduction on endophyte-infected and endophyte-free tall fescue. *Plant Dis.* 74:757–761.

LATCH, G. C. M., HUNT, W. F., and MUSGRAVE, D. R. 1985. Endophytic fungi affect growth of perennial ryegrass. *N.Z. J. Agric. Res.* 28:165–168.

MALINOWSKI, D. P., BELESKY, D. P., HILL, N. S., BALIGAR, V. C., and FEDDERS, J. M. 1998. Influence of phosphorus on the growth and ergot alkaloid content of *Neotyphodium coenophialum*-infected tall fescue (*Festuca arundinacea* Schreb.). *Plant Soil* 198:53–61.

MILES, C. O., LANE, G. A., DIMENNA, M. E., GARTHWAITE, I., PIPER, E. L., BALL, O. J. P., LATCH, G. C. M., ALLEN, J. M., HUNT, M. B., BUSH, L. P., MIN, F. K., FLETCHER, I., and HARRIS, P. S. 1996. High levels of ergonovine and lysergic acid amide in toxic *Achnatherum inebrians* accompany infection by an *Acremonium* like endophytic fungus. *J. Agric. Food Chem.* 44:1285–1290.

PETROSKI, R. J., YATES, S. G., WEISLEDER, D., and POWELL, R. G. 1989. Isolation, semisynthesis, and NMR spectral studies of loline alkaloids. *J. Nat. Prod.* 52:810–817.

PORTER, J. K. 1994. Chemical constituents of grass endophytes, pp. 103–123, *in* C. W. Bacon, and J. F. White, Jr. (eds.). Biotechnology of Endophytic Fungi of Grasses. CRC Press, Boca Raton, Florida.

PRESTIDGE, R. A. 1993. Causes and control of perennial ryegrass staggers in New Zealand. *Agric. Ecosyst. Environ.* 44:283–300.

RICHARDSON, M. D., CABRERA, R. I., MURPHY, J. A., and ZAUROV, D. E. 1999. Nitrogen-form and endophyte-infection effects on growth, nitrogen uptake, and alkaloid content of Chewings fescue turfgrass. *J. Plant Nutr.* 22:67–79.

RIEDELL, W. E., KIECKHEFER, R. E., PETROSKI, R. J., and POWELL, R. G. 1991. Naturally occurring and synthetic loline alkaloid derivatives: insect feeding behavior modification and toxicity. *J. Entomol. Sci.* 26:122–129.

ROWAN, D. D., and LATCH, G. C. M. 1994. Utilization of endophyte-infected perennial ryegrasses for increased insect resistance, pp. 169–183, *in* C. W. Bacon, and J. F. White, Jr. (eds.). Biotechnology of Endophytic Fungi of Grasses. CRC Press, Boca Raton, Florida.

ROYLANCE, J. T., HILL, N. S., and AGEE, C. S. 1994. Ergovaline and peramine production in endophyte-infected tall fescue: Independent regulation and effects of plant and endophyte genotype. *J. Chem. Ecol.* 20:2171–2183.

SCHARDL, C. L. 1996. *Epichloë* species: fungal symbionts of grasses. *Annu. Rev. Phytopathol.* 34:109–130.

SIEGEL, M. R., and BUSH, L. P. 1996. Defensive chemicals in grass-fungal endophyte associations. *Recent Adv. Phytochem.* 30:81–119.

SIEGEL, M. R., LATCH, G. C. M., BUSH, L. P., FANNIN, F. F., ROWAN, D. D., TAPPER, B. A., BACON, C. W., and JOHNSON, M. C. 1990. Fungal endophyte-infected grasses: Alkaloid accumulation and aphid response. *J. Chem. Ecol.* 16:3301–3315.

TEPASKE, M. R., POWELL, R. G., and CLEMENT, S. L. 1993. Analysis of selected endophyte-infected grasses for the presence of loline-type and ergot-type alkaloids. *J. Agric. Food Chem.* 41:2299–2303.

WESTERN, J. H., and CAVETT, J. J. 1959. The choke disease of cocksfoot (*Dactylis glomerata*) caused by *Epichloë typhina. Trans. Br. Mycol. Soc.* 42:298–307.

WHITE, J. F., JR., and BULTMAN, T. L. 1987. Endophyte-host associations in forage grasses. VIII. Heterothallism in *Epichloë typhina. Am. J. Bot.* 74:1716–1721.

YATES, S. G., FENSTER, J. C., and BARTELT, R. J. 1989. Assay of tall fescue seed extracts, fractions, and alkaloids using the large milkweed bug. *J. Agric. Food Chem.* 37:354–357.

YATES, S. G., PETROSKI, R. J., and POWELL, R. G. 1990. Analysis of loline alkaloids in endophyte-infected tall fescue by capillary gas chromatography. *J. Agric. Food Chem.* 38:182–185.