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FUNGAL ENDOPHYTE-INFECTED GRASSES: ALKALOID ACCUMULATION AND APHID RESPONSE

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Abstract—The occurrence of the alkaloids *N*-formyl and *N*-acetyl loline, peramine, lolitrem B, and ergovaline and the response of aphids to plants containing these compounds were determined in species and cultivars of *Festuca*, *Lolium*, and other grass genera infected with fungal endophytes (*Acremonium* spp., and *Epichloe typhina*). Twenty-nine of 34 host–fungus associations produced one or more of the alkaloids, most frequently peramine or ergovaline. Three alkaloids (lolines, peramine, and ergovaline) were found in tall fescue and in perennial ryegrass infected with *A. coenophialum*, while peramine, lolitrem B, and ergovaline were present in perennial ryegrass and in tall fescue infected with *A. lolii* and in *F. longifolia* infected with *E. typhina*. While *A. coenophialum* and *A. lolii* produced similar patterns of alkaloids regardless of the species or cultivar of grass they infected, isolates of *E. typhina* produced either no alkaloids or only one or two different alkaloids in

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the grasses tested. Aphid bioassays indicated that *Rhopalosiphum padi* and *Schizaphis graminum* did not survive on grasses containing loline alkaloids and that *S. graminum* did not survive on peramine-containing grasses. Ergovaline-containing grasses did not affect either aphid.

Key Words—Endophyte-infected grasses, *Acremonium*, endophytes, *Epichloe typhina*, grass alkaloids, lolines, ergovaline, peramine, lolitrem B, aphid responses, plant resistance to herbivory, *Rhopalosiphum padi*, *Schizaphis graminum*, Homoptera, Aphididae.

INTRODUCTION

Fungal endophytes of grasses, *Acremonium* spp. and *Epichloe typhina* (Pers.) Tul., are distributed worldwide (Latch et al., 1987; White, 1987). *Lolium* and *Festuca* species infected with *Acremonium* spp. endophytes are reported to have enhanced resistance to herbivory (Siegel et al., 1987; Clay, 1989; Dahlman et al., 1990). Resistance to herbivory is but one aspect of the mutualistic symbiotic relationship reported to occur between host and fungus (Siegel et al., 1987; Clay, 1988). Ingestion of endophyte-infected grasses has been shown to be associated with a number of animal toxicoses. Sheep and cattle grazing on tall fescue (*Festuca arundinacea* Schreb.) infected with *A. coenophialum* Morgan-Jones et Gams exhibit fescue toxicity (Stuedemann and Hoveland, 1988), while livestock grazing perennial ryegrass (*Lolium perenne* L.) infected with *A. lolii* Latch, Christensen et Samuels, may suffer from ryegrass staggers toxicity (Mortimer and di Menna, 1985). The closely related endophytes isolated from these and other grasses have been classified as imperfect fungi in the section *Albolanosa* in the genus *Acremonium* (Morgan-Jones and Gams, 1982). The teleomorphic state of *A. typhinum* Morgan-Jones et Gams is *Epichloe typhina* (Morgan-Jones and Gams, 1982), a clavicipitaceous fungus in the tribe Balansieae (Diehl, 1950). *E. typhina* prevents flowering by producing a stroma that surrounds the emerging flower panicle (choke disease). *Acremonium* species and certain biotypes of *E. typhina* spend their entire life cycle growing intercellularly within the grass and are disseminated only through the seed (Siegel, et al., 1987; White, 1988; Clay, 1988).

Endophyte-infected tall fescue and perennial ryegrass contain a number of alkaloids that are active against insect herbivores (Siegel et al., 1987; Dahlman et al., 1990). These include, in infected tall fescue, *N*-formyl and *N*-acetyl loline (Bush et al., 1982), peramine (Fannin et al., 1990), and ergot alkaloids (Yates et al., 1985); in infected perennial ryegrass, they include peramine (Rowan and Gaynor, 1986), lolitrems (Gallagher et al., 1985), and ergot alkaloids (Rowan and Shaw, 1987). Peramine has been reported to be only a feeding deterrent and not directly toxic to Argentine stem weevil (*Listronotus bonar-*

iensis Kuchel). Other of the alkaloids have been reported to be directly toxic to insects. These include lolines (Yates et al., 1989; Dahlman et al., 1990), lolitrems (Prestidge and Gallagher, 1985), and ergopeptine alkaloids, other than ergovaline (Yates et al., 1989). The objective of this study was to determine the distribution of the four types of alkaloids known from endophyte-infected grasses. In addition, the short-term survival of two species of aphids was tested in relation to the occurrence of these alkaloids.

METHODS AND MATERIALS

Sources of Endophytes and Growth of Plants. Infected plants and seed (Table 1) were either donated, collected in Kentucky or New Zealand, or plants were artificially infected with endophytes via meristem wounding of 1-week-old seedlings using the method of Latch and Christensen (1985).

Endophytes, isolated on potato dextrose agar, were identified as *Acremonium* spp. by the presence of single conidia on conidiophores in the typical T configuration (Latch et al., 1984). In addition, all plants were tested prior to alkaloid and aphid analysis by ELISA for the presence of *Acremonium* spp. (Johnson et al., 1985b).

Plants were grown in 10-cm plastic pots in the greenhouse (winter 17–21°C, summer 24–28°C) and maintained in growth condition by dividing older plants and repotting them in a mixture of one part soil (Maury silt loan) and three parts PRO-MIX BR every four to six months. Most, but not all, plants were routinely trimmed to reduce excess foliage. Plants were fertilized biweekly in the winter and weekly in the summer with a dilute solution of soluble fertilizer (317 mg/liter of N–P–K).

Aphid Assay. The oat-birdcherry *Rhopalosiphum padi* L. and greenbug *Schizaphis graminum* Rondani aphids were used to assay for the presence of toxins in endophyte-infected grasses because they were shown previously to be sensitive to endophyte-containing grasses (Johnson et al., 1985a). The aphid cultures were maintained in high numbers by weekly transfers to 1-week-old barley plants (cv. Barsoy). In order to prevent contamination, each aphid species was maintained on plants in screened cages in separate rooms under fluorescent light (50–70 μE/sec/m^2, 14-hr day).

Plant tissue for aphid bioassay consisted of individual test treatment stems (pseudostems containing whorls of leaf sheaths and blades) of tall fescue or multiple stems of perennial ryegrass (three to five stems), fine fescues (six to nine stems) and other grass genera listed in Table 1. The stems were cut from plants at the soil line, stripped of dead tissue, and placed in a 14.8-ml polystyrene cup (containing 11 ml of water and 1 μg/ml of kinetin) by passing the stem(s) through a hole (ca. 2–4 mm diam.) in a waxed paper cap (Figure 1).

TABLE 1. GRASS SPECIES AND CULTIVARS AND FUNGAL ENDOPHYTES USED IN STUDY

Plant code	Infected grass species and cultivars	Fungal endophyte[a]	Origin of endophyte[b]
	Lolium perenne L.		
1	Nui	*A. lolii*	NI
2	Repel	*A. lolii*	NI
3	Ruanui	*A. coenophialum*	AI, *F. arundinacea* (KY 31)
4	Unknown cv.	*E. typhina*	NI
5	Ruanui	*E. typhina*	AI, *F. rubra commutata*[c]
6	Gator	*E. typhina*	AI, *F. rubra commutata*[c]
7	Gator	*E. typhina*	AI, *L. perenne*[d]
8	Gator	*E. typhina*	AI, *F. longifolia*
	Festuca arundinacea Schreb.		
9	G1-320 (Johnstone)	*A. coenophialum*	AI, *F. arundinacea* (KY 31)
10	G1-320 (Johnstone)	*A. coenophialum*	AI, *F. arundinacea* (Au-Triumph)
11	G1-320 (Johnstone)	*A. lolii*	AI, *L. perenne* (Nui)
12	G1-320 (Johnstone)	*A. lolii*	AI, *L. perenne* (Repel)
13	G1-320 (Johnstone)	*A. starrii*	AI, *F. arizonica*
14	G1-320 (Johnstone)	*Phialophora* sp.	AI, *F. arundinacea*[c]
15	G1-320 (Johnstone)	*E. typhina*	AI, *F. longifolia*
16	KY 31	*A. coenophialum*	NI
	F. longifolia Thuill.		
17	SR-3000	*E. typhina*	NI
18	SR-3000	*E. typhina*	AI, *F. rubra commutata*[c]
	F. glauca Vill.		
19	Carolyn blue	*E. typhina*	NI
	F. rubra commutata Guad.		
20	Longfellow	*E. typhina*	NI
21	Enjoy	*E. typhina*	NI
	F. rubra litoralis (Mey.) Auquier		
22	Dawson	*E. typhina*	AI, *F. rubra commutata*[c]
	F. rubra rubra (Gaud.) Hayek		
23	Ensylva	*E. typhina*	NI
24	Pernille	*E. typhina*	NI
25	*F. arizonica* Vasey	*A. heurfanum*	NI
26	*F. paradoxa* Desv.	*Acremonium* sp.	NI
27	*F. versuta* Beal.	*Acremonium* sp.	NI
28	*F. obtusa* Bieler	*A. starrii*	NI
29	*F. gigantea* (L.) Vill.	*Acremonium* sp.	NI
30	*Poa ampla* Merr.	*E. typhina*	NI
31	*Poa autumnalus* (Muhl.) Ell.	*A. coenophialum*	NI
32	*Bromus anomalus* Rupt.	*A. starrii*	NI
33	*Elymus canadensis* L.	*E. typhina*	NI
34	*Sitanion longifolium* Smith	*E. typhina*	NI
35	*Agrostis hiemalis* (Walt.) B.S.P.	*E. typhina*	NI

[a] Plants 4, 7, 17, 18, 22, 35 produced symptoms (stroma) of choke disease in the field.
[b] NI, naturally infected plant; AI, artificially infected strain from indicated plant species (cultivar).
[c] Isolate from unknown cultivar.
[d] Same isolate as in plant 4.

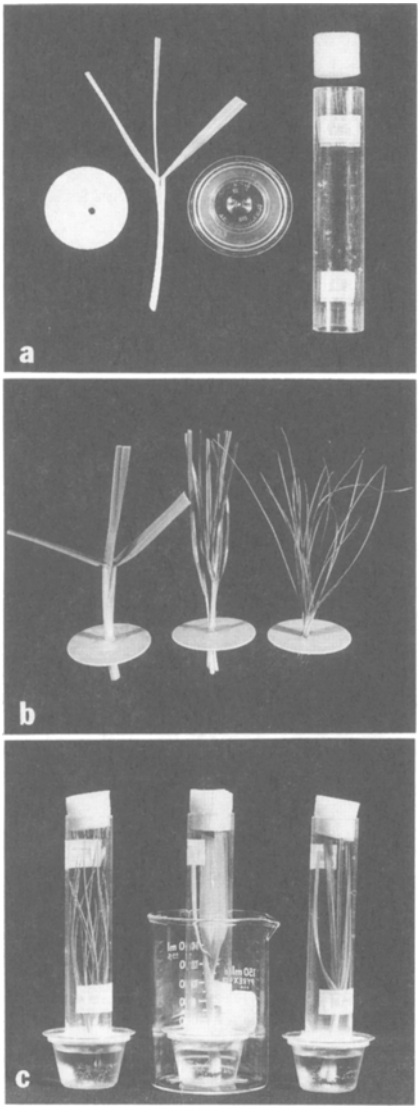

FIG. 1. Stem assay used to determine the presence or absence of chemicals toxic to *Rhopalosiphum padi* or *Schizaphis graminum*. (a) Disassembled components of assay, left to right waxed paper cap with hole to hold stem; cut tall fescue stem; polystyrene cup, Plexiglas cylinder with two fine-mesh screen-covered slits and polyurethane plug at top. (b) Partially assembled components, left to right tall fescue stem, three perennial ryegrass stems, and six *Festuca longifolia* stems. All stems were sealed above and below cap with paraffin. (c) Fully assembled components, left to right stems of *F. longifolia*, tall fescue, and perennial ryegrass. Polystyrene cups contain 11 ml of water and 1 μg/ml of kinetin. Fifty aphids were added to each cage, which was incubated under fluorescent lights for 48–72 hr.

The interface between the paper cap and the stems was sealed with paraffin. The leaves were clipped to accommodate a 11.0 × 2.3-cm Plexiglas cylinder, the diameter of which fitted snugly on the top of the paper cap within the inner rim of the plastic cup. Air exchange in the cylinder occurred through two fine-mesh screen-covered slits in the sides and the polyurethane plug in the top. Fifty apterous (nonwinged) aphids (ca. 3–7 days old) were introduced into the cage by the removal of the plug. The bioassay was run at ambient temperatures under fluorescent light (14 : 10 day–night). There were six test treatment stems for each infected grass species or cultivar. A noninfected control consisting of six stems was present unless otherwise stated. Each sample stem originated from a different infected or noninfected plant used previously for the analysis of alkaloids. The *R. padi* aphids were caged with the stems(s) for 72 hr, while the *S. graminum* aphid test lasted 48 hr. The number of live aphids on the stems and live wandering aphids in the cage were counted. A *t* test, assuming unequal variances, was used to determine if the percent of live aphids on the infected stems was significantly different ($P < 0.05$) from the percent of live aphids on the noninfected stems. After the assay, the stems were tested by ELISA (Johnson et al., 1985b), to verify the presence or absence of endophyte.

Determination of Alkaloids. Harvest of tissue for analysis of alkaloids varied from one to four weeks after the aphid assay. A composite sample of tissue (stems and leaves) from infected plants used in the aphid assay was harvested from three to six plants and cleaned to remove soil debris and dead tissue. The tissue was lyophilized and ground in a Wiley mill to pass through a 40 mesh screen. Samples were stored in a freezer (−20°C) with silica gel. Loline alkaloids were determined, on a 1-g dry wt. sample, by gas chromatography (Kennedy and Bush, 1983).

Peramine was extracted from a 0.1-g sample and the concentration determined by HPLC as described by Tapper et al. (1989).

Lolitrem B was measured by a modification of the procedure of Gallagher et al. (1985). Three-milliliter portions of the lipophilic upper phase from the two-phase extraction used for the peramine HPLC analysis were aspirated through silica columns (Analytichem BondElut, 0.5 g) such that lolitrem B and much of the pigments were retained on the silica. The columns were rinsed with 1 ml of dichloromethane before eluting the lolitrem-containing fraction with 2 ml of dichloromethane–acetonitrile, 4 : 1 v/v. Amounts of lolitrem were estimated by comparison of peak heights with those obtained using a pure sample (gift of Dr. R. Gallagher).

Ergot alkaloids were determined from specific fungi grown in liquid culture and plant tissue. For liquid culture analysis, fungi were isolated and maintained on corn–malt-extract agar and then grown in larger quantities in liquid culture and screened for total alkaloids as described by Bacon (1988).

For plant analyses, ergovaline and its isomer ergovalinine were determined

as an indicator of ergot alkaloid synthesis (Yates et al., 1985; Rowan and Shaw, 1987). Ground samples (0.5 g) were extracted with 5 ml of 80% methanol for 1.5 hr. The mixture was centrifuged at 3000*g* for 15 min and supernatant (2 ml) extracted three times with 10 ml petroleum ether. The petroleum ether was discarded and the extract was diluted with one volume of 80% methanol containing 40 μg/ml of dihydroergocristine methane sulfonate (internal standard). Ergovaline and ergovalinine were quantified by HPLC using a modification of the method of Yates and Powell (1988). The modification involved use of a shorter column, elimination of the silica gel precolumn, and doubling the pump rate. Samples (20 μl) were analyzed on either a Varian 5000 liquid chromatograph using a Phenomenex Ultracarb 5, ODS 30 column (4.6 × 150 mm) and an Upchurch C-130B ground column filled with du Pont Permaphase ODS or a Dynamax microsorb C181 (5 μm) axial compression column (4.6 × 250 mm) with a 4.6 × 15-mm ground module. Fluorescence was monitored on a Kratos Spectroflow 980 programmable fluorescence detector with excitation of 310 nm and detection above a 370-nm long-pass emission filter. Ergovaline and ergovalinine values were combined and the total given as ergovaline.

RESULTS

Alkaloid Production in Endophyte-Infected Grasses. The levels of lolines, peramine, lolitrem, and ergovaline produced singly or in combination varied greatly in the infected grass species and cultivars (Table 2). The most widely distributed alkaloids were peramine and the ergot alkaloid ergovaline. These alkaloids were recovered from the majority of plants infected with *Acremonium* spp. and *E. typhina*. Of the 35 host–fungus combinations studied, peramine was detected in 23 (66%) and ergovaline in 21 (60%). Since ergovaline and other ergot alkaloids are of fungal origin (Bacon, 1988), selected isolates also were grown in liquid culture and analyzed colorimetrically for total ergot alkaloid content. Of the eight isolates grown in artificial media, five produced ergot alkaloids in culture as well as in infected plants. One isolate (from plant 27) did not produce alkaloids in culture or in the infected plants. Two isolates produced ergovaline in the plant (plants 22 and 23) but ergot alkaloids were not found in culture. This lack of synthesis in culture may reflect the age of the isolate used, as continuous artificial culture of isolates apparently results in reduced synthesis of ergot alkaloids (Bacon, 1988).

Loline alkaloids were found in only six (17%) of the host–fungus combinations (plants 3, 9, 10, 16, 29, 31); however, five of these were associated with the five *A. coenophialum*-infected plants examined and the sixth with an unidentified *Acremonium* sp. Not only were lolines identified in naturally infected tall fescue (plants 9, 10, 16) but also in perennial ryegrass (plant 3)

TABLE 2. PRESENCE OF ALKALOIDS AND APHID RESPONSE IN ENDOPHYTE-INFECTED GRASSES

Plant Code[a]	Alkaloid, μg/g dry wt tissue (ppm)[b]				Live aphids on infected stems (%)[d]	
	Lolines	Peramine	Lolitrem B	Ergovaline[c]	*R. padi*	*S. graminum*
1	0	42	10	5.4 (50)	109	4*
2	0	30	5	2.3	93	6*
3	1028	29	0	2.5	25*	1*
4	0	56	0	0	112	1*
5	0	0	0	0	100	111
6	0	0	0	0	98	101
7	0	53	0	0	94	1*
8	0	10	0	1.1	92	8*
9	1800	10	0	2.0	4*	16*
10	1544	4	0	6.2	8*	2*
11	0	4	2	1.1	87	14*
12	0	33	44	1.3	92	9*
13	0	0	0	0	89	112
14	0	0	0	0	102	98
15	0	3	0	0	110	15*
16	1109	10	0	1.5	3*	2*
17	0	22	4	0.9 (38)	100	2*
18	0	0	0	1.8	89	85
19	0	5	0	0.8 (19)	98	13*
20	0	18	0	0.5 (9)	98	1*
21	0	16	0	0	ND[e]	25*
22	0	0	0	0.8 (0)	109	104
23	0	0	0	1.3 (0)	84	67*
24	0	0	0	1.1	ND	100[f]
25	0	0	0	0	ND	104[f]
26	0	132	0	0	ND	15*
27	0	0	2	0 (0)	ND	2[f]
28	0	0	0	0	ND	104[f]
29	2205	0	0	0.7	2*	3*
30	0	32	0	0	101	8[f]
31	972	2	0	0	21*	13*
32	0	8	0	0.3 (29)	ND	10[f]
33	0	12	0	0	ND	ND
34	0	74	0	0.6	ND	1[f]
35	0	16	0	0.4	ND	ND
Number containing alkaloids	6	23	6	20		
% distribution	17	66	17	60		

[a] See Table 1 for identifications of plant code numbers.

[b] Alkaloids were determined from composite tissue samples (3-6 plants) from which stems had been used for aphid assay.

[c] Values for ergovaline include those of its isomer ergovalinine. Numbers in parentheses are for total ergot alkaloids present in liquid culture (mg/liter).

[d] Each assay consisted of 50 aphids each on six test treatment stem(s) of infected or noninfected grasses in screened cages. *Rhopalosliphum padi* (oat birdcherry) aphid, assay for 72 hr. *Schizaphis graminum* (greenburg) aphid, assay for 48 hr; *Significant difference ($P < 0.05$) between the percent live aphids on endophyte-infected stems, as compared to the percent on noninfected stems determined by a t test, assuming unequal variance.

[e] ND, not determined.

[f] No noninfected control available, % alive based on total of 50 aphids.

when artificially infected with the endophyte from tall fescue. Loline alkaloids were not detected in perennial ryegrass infected with its natural endophyte (*A. lolii*) (plants 1 and 2).

Lolitrem B was found in only six host–fungus combinations, four of which were in the four *A. lolii*-infected plants examined. Lolitrem B was present in naturally infected perennial ryegrass (plants 1 and 2) and in tall fescue artificially infected with *A. lolii* (plants 11 and 12). In addition, lolitrem B was recovered from *F. longifolia* infected with *E. typhina* (plant 17) and *F. versuta* (plant 27) infected with an unidentified *Acremonium* sp.

The classes of alkaloids listed in Table 2 were detected singly, in various combinations, or not detected at all (Table 3). Host–fungal associations of *A. coenophialum* in tall fescue cultivars (plants 9, 10, 16) and in one perennial ryegrass cultivar (plant 3) produced peramine, lolines, and ergovaline; *A. lolii* in two perennial ryegrass cultivars (plants 1 and 2) and in two tall fescue cultivars (plants 11 and 12) produced peramine, lolitrem B, and ergovaline. *E. typhina* in *F. longifolia* (plant 17) produced the same alkaloids as found in *A. lolii*-infected plants.

There was a great diversity in the number of different endophyte–host interactions in which only one or two of the alkaloids classes were detected. In eight different plant species–fungal interactions, various combinations of two alkaloid classes were found, while 10 different combinations of fungus and plant

TABLE 3. COMBINATION OF ALKALOIDS IN GRASS–FUNGUS ASSOCIATIONS

Associations (plant code)[a]	Alkaloids[b]			
	Peramine	Lolitrem	Lolines	Ergovaline
No alkaloids detected				
5, 6, 13, 14, 25, 28				
Single alkaloid detected				
4, 7, 15, 21, 26, 30, 33	+			
27		+		
18, 22, 23, 24				+
Two alkaloids detected				
8, 19, 20, 32, 34, 35	+			+
29			+	+
31	+		+	
Three alkaloids detected				
1, 2, 11, 12, 17	+	+		+
3, 9, 10, 16	+		+	+

[a] See Table 1 for identifications of plant code numbers.
[b] +, detection of alkaloid.

produced only one alkaloid. Lolines were always found associated with other alkaloids but never with lolitrem. In six different endophyte-infected grasses, no alkaloids were detected.

Several endophyte taxa were capable of producing similar patterns of alkaloids regardless of the species or cultivar of grass they infected. As previously mentioned, the tall fescue endophyte, *A. coenophialum*, and the perennial ryegrass endophyte, *A. lolii*, produced the same three alkaloids in their naturally infected host grasses as well as when artificially introduced into other grasses. An isolate of *E. typhina* obtained from perennial ryegrass (plant 4) and introduced into a different cultivar (plant 7) produced the same alkaloid, peramine.

However, several associations show that isolates of apparently the same endophyte can produce different alkaloids depending upon the host grass. Of the three *A. starrii*-plant species combinations analyzed, two did not produce any alkaloids in their host grasses (plant 13 artificially infected and plant 28 naturally infected), but the one from *Bromus anomalus* (plant 32) produced two alkaloids in its host. Likewise, three isolates of *E. typhina* from different cultivars of *F. rubra commutata* produced different alkaloid patterns in infected grasses. One of the *E. typhina* isolates, from an unidentified New Zealand cultivar, introduced into two perennial ryegrass cultivars (plants 5 and 6) produced no alkaloids. When this *E. typhina* isolate was introduced into *F. longifolia* (plant 18) and *F. rubra litoralis* (plant 22), ergovaline was present. Unfortunately, the naturally infected host grass was not available for analysis. The other two *E. typhina* endophytes in their own hosts, Longfellow (plant 20) and Enjoy (plant 21), produced peramine in both grasses but in Longfellow ergovaline was identified also.

Lastly, the *E. typhina* from *F. longifolia* (plant 17) was shown to produce peramine, lolitrem, and ergovaline when in its own naturally infected host; only peramine and ergovaline were found when introduced into perennial ryegrass (plant 8), and only peramine was found when introduced into tall fescue (plant 15).

Aphid Response to Endophyte-Infected Grasses. The data in Table 2 indicate that the *R. padi* aphid is only sensitive to those infected plants containing loline alkaloids (plants 3, 9, 10, 16, 29, 31). These plants were all infected with *A. coenophialum*, except for *F. gigantea* (plant 29), which was infected with an unidentified *Acremonium* sp. The specificity of the toxic effect of loline alkaloids is supported by the survival of the *R. padi* aphids on plants that contained no lolines but that have other alkaloids, singly or in various combinations (e.g., plants 1, 4, 17, 30).

The *S. granimum* aphid showed reduced survival on plant material containing peramine only (plants 4, 7, 15, 21, 26, 30) and on plants containing both peramine and loline alkaloids (plants 3, 9, 10, 16, 31). Survival of *S. graminum* aphid did not appear sensitive to ergovaline, as when this alkaloid

occurred by itself (plants 18, 22, 24) the percentage of live aphids on infected and noninfected stems was not significantly different ($P < 0.05$). However plant 23, infected with *E. typhina*, produced ergovaline only and caused a significant ($P < 0.05$) reduction (33%) in the percentage of live aphids. However, since plants 18, 22, and 24 also contained ergovaline only and were not toxic to *S. graminum*, some other factor(s) must be responsible for the effects noted for plant 23. Plant 29 contained both ergovaline and loline alkaloids, and the toxicity of this plant to *S. graminum* may be attributed to the loline alkaloids alone. Lolitrem usually occurred with peramine (plants 1, 2, 11, 12, 17) and consequently it was not possible to determine if the presence this alkaloid alone correlated with aphid toxicity. *S. graminum* did not survive on plant 27, which contained only lolitrem; however, a suitable noninfected control was not available for this combination.

Six plants (plants 5, 6, 13, 14, 25, 28) produced no detectable levels of the four classes of alkaloids and were not toxic to either of the aphids. In this group, plant 14 was infected with a seed-disseminated non-*Acremonium* endophyte, a *Phialophora*-like species (Latch et al., 1984).

DISCUSSION

The concentrations of the alkaloids lolitrem B, lolines, peramine, and ergovaline were analyzed in different grass species and cultivars infected with *Acremonium* spp. and *E. typhina* endophytes. The lolines and lolitrem alkaloids were found in relatively few of the host–fungus combinations and were primarily associated with grasses infected with *A. coenophialum* and *A. lolii*, respectively. The most common alkaloids were peramine and ergovaline, detected in 66% and 60% of the infected grasses, respectively. Various combinations of the four classes of alkaloids were found in 83% of the infected grasses. None of the alkaloids was detected in five host–fungus associations involving different *Acremonium* spp., *E. typhina*, and a *Phialophora*-like sp.

A number of interacting factors are probably responsible for the variations in amounts and numbers of alkaloids present in infected plants. Levels of lolines (Kennedy and Bush, 1983; Belesky et al., 1987) and total ergot alkaloids (Belesky et al., 1988) show seasonal variations. In addition, loline levels in infected plants were increased under greenhouse conditions by repeated cutting of plant tissue (Bush, unpublished data), and levels of lolitrem B and peramine were affected by temperature and plant nutrition (Latch and Tapper, unpublished). In addition, it was assumed that ergovaline and its isomer ergovalinine were the primary ergot alkaloids present in *Acremonium*-infected grasses. However, this is yet to be proven for grasses other than infected tall fescue and perennial ryegrass where they are 40–60% of the total present (Yates et al., 1985; Rowan and Shaw, 1987). Even though the detection method can deter-

mine levels as low as 0.1 ppm, a lack of ergovaline does not completely preclude the presence of other ergot alkaloids. In culture, *Balansia* spp. produced ergot alkaloids that did not contain ergovaline (Bacon et al., 1986).

The biosynthetic origin of the alkaloids (fungal and/or fungal–plant) and the genetics of the symbionts also may affect the concentrations and numbers of alkaloids present. The ergopeptine alkaloids (Bacon, 1988) and peramine (Rowan and Gaynor, 1986) are of fungal origin. Lolitrem B has not been found in culture but paxilline, an obvious biosynthetic intermediate of the lolitrems, has been found when *A. lolii* is cultured and also in infected seeds (Gallagher et al., 1985; Weedon and Mantle, 1987). Lolines have not yet been found in liquid cultures of *A. coenophialum* (Bush, unpublished data). Regardless of how the alkaloids are synthesized, the interaction of symbionts does affect the alkaloids present in the infected plants. Some endophytes produce the same pattern of alkaloids in naturally and in artificially infected grass species and cultivars (i.e., the *A. lolii* and *A. coenophialum* isolates in their own and reciprocal hosts). However, other endophytes do not produce similar patterns of alkaloids in new hosts. This was best illustrated in the case of the *E. typhina* from *F. longifolia* (plant 17). This association produced the same three alkaloids (lolitrem, peramine, and ergovaline) found in perennial ryegrass infected with *A. lolii*. When *E. typhina* from *F. longifolia* was artificially introduced to perennial ryegrass, only peramine and ergovaline were detected, and when inoculated into tall fescue, only peramine. The plants infected with *E. typhina* from *F. rubra commutata* also demonstrated differences in the distribution of peramine and ergovaline. Depending on the source of the endophyte, either no alkaloids were detected, or peramine alone or in combination with ergovaline was found. The dissimilar patterns of alkaloid production may relate to host–fungus compatibility and the eventual survival of the endophyte. The density of mycelium was greater in the naturally infected plant and less in the artificially infected grasses. In addition, approximately 8–12 months after alkaloid analysis it was difficult to find substantial amounts of mycelium in the greenhouse-grown infected perennial ryegrass and tall fescue plants.

Poor performance by two aphid species on endophyte-infected grasses was correlated with the presence of specific alkaloids. Only plants containing lolines, regardless of the presence of peramine and/or ergovaline, affected survival of *R. padi*. Consequently, this aphid species can be used for routine detection of endophytes in those associations known to produce loline alkaloids. Survival of *S. graminum* was reduced by grasses containing a greater number of alkaloids (lolines, peramine, and/or lolitrems). Because of the short duration of the assays, neither chronic toxicity could be studied nor could it be determined if alkaloids in the infected stems were directly toxic or feeding deterants to the aphids.

If these four classes of alkaloids are indeed important in determining herbivory, then the associations that have the largest number of toxic chemicals

should have the greatest spectrum of biological activity. Conversely, endophyte–grass associations lacking these alkaloids should not be biologically active, as was shown in this study. Mammalian or insect toxicity would be further increased if two or more of the alkaloids acted synergistically (Yates et al., 1989). A multiplicity of compounds with differing modes of action would be of great value as it increases the spectrum of activity against insect herbivores as well as reducing the potential for the development of insect resistance to these alkaloids.

Not all grass–endophyte associations confer insect resistance, and increased insect resistance is not the only factor in determining the superiority of infected grasses. Resistance to abiotic stresses, such as drought (Barker et al., 1985; Read and Camp, 1986; Arachevaleta et al., 1989), and improved growth and persistence (Funk et al., 1985; Latch et al., 1985; Hill et al., 1990) has been demonstrated and may play just as important a role in plant survival as does resistance to herbivory. Consequently, all factors need to be considered when plant breeders select endophyte-infected grasses for improved pasture and turf production.

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