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Accumulation of Ergopeptide Alkaloids in Symbiotic Tall Fescue Grown under Deficits of Soil Water and Nitrogen Fertilizer

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The fungus *Acremonium coenophialum* is endophytically associated with tall fescue (*Festuca arundinacea* Schreber). Within this symbiotum the fungus produces ergopeptide alkaloids, which are associated with livestock toxicoses. Environmental effects on the production of ergot alkaloids within the symbiotum are unknown. We conducted a greenhouse study of the effects of flooding, nitrogen rate during fertilization (11, 73, and 220 mg of N per pot weekly), nitrogen form (3.4 and 34 mg of N as NH_4^+ or NO_3^- per pot), and drought stress (−0.03, −0.05, and −0.50 MPa) on ergopeptide alkaloid concentrations in one genotype of nonsymbiotic and symbiotic tall fescue grown in plastic pots. It was determined that the concentration of ergovaline, the major type of ergopeptide alkaloid, was increased but was not as high as that in nonflooded controls. Total ergopeptide and ergovaline concentrations in plants receiving high (220 mg of N per pot) and low (11 mg of N per pot) levels of NH_4NO_3 fertilization were not affected by flooding. The form of nitrogen was important since all concentrations of NO_3^--N increased ergopeptide alkaloid content, as opposed to the effects of NH_4^+-N, which was effective only at high concentrations (34 mg of N per pot). Ergopeptide concentrations were highest in drought-stressed plants grown at −0.50 MPa and fertilized at the moderate or high N rate. The results suggest that within this genotype, ergopeptide alkaloid biosynthesis by the fungus is not appreciably affected by flooding but is greatly increased by high rates of N fertilization and moderate water deficit.

Tall fescue (*Festuca arundinacea* Schreber) is naturally associated with the fungal endophyte *Acremonium coenophialum* Morgan-Jones et Gams. This symbiotum is considered beneficial because endophyte-infected tall fescue is more drought tolerant (1, 27), produces more dry matter (6, 27), utilizes soil nitrogen more efficiently (22), deters insects (19), and is toxic to cattle (5, 17); also, infected seeds produce more vigorous seedlings than uninfected seeds do (6).

Some benefits to tall fescue derived from this association are observable only under conditions of stress. Thus, under the conditions of insect predation, ruminant grazing, or drought, symbiotic plants are more competitive than nonsymbiotic plants because of inherent defense mechanisms within the association. Although several of these benefits are the direct products of the fungus, e.g., ergot alkaloids, others may be attributed to the plant or both fungus and plant. It is not known how these defense mechanisms are affected by varying soil conditions, particularly stressful situations, within this symbiotum. It is known that enhanced soil N availability may increase or decrease the severity of the toxic response observed in cattle grazing symbiotic tall fescue (5, 25). Furthermore, soil N availability may affect concentrations of ergopeptide alkaloids (23), a class of secondary nitrogenous metabolites that may be involved in toxicosis of cattle grazing tall fescue (4, 21, 26, 30).

The objective of the work described here was to determine independently, without regard to interactions, the effects of excess soil water supply and water and N rates and deficits on accumulation of ergopeptide alkaloids in one genotype of symbiotic and nonsymbiotic Kentucky 31 tall fescue.

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MATERIALS AND METHODS

Plant culture. The plant material used in this study was Kentucky 31 tall fescue, which consisted of one symbiotic genotype and its nonsymbiotic version, whose origin was reported earlier (1). All plants were periodically checked microscopically for infection status (3). These plants were grown in 152-mm-diameter plastic pots containing a synthetic soil mixture composed of the following (wt/vol): Cecil sandy clay loam topsoil, 33.3%; sand, 16.7%; perlite, 16.7%; and vermiculite, 16.7%. Dolomitic limestone (200 g/100 kg) and concentrated superphosphate (20 g/100 kg) were also included. During the initial 60-day growth period, plants were fertilized with liquid plant food (N-P-K ratio, 12-6-6), and received two waterings per day to 100% of water-holding capacity.

Flooding stress. Ergopeptide alkaloid responses to flooding were studied in the greenhouse by using plants fertilized, prior to flooding, with a 12-6-6 nutrient solution that contained either urea-N or two concentrations of NH_4NO_3-N.

Replicate plants consisted of three pots of either nonsymbiotic or symbiotic grasses. Pots were placed in a plastic basin (550 by 700 by 300 mm), and enough tap water was added to completely cover the soil surface of the pots. Control plants were not flooded, but were watered twice a day to 100% of water-holding capacity. The duration of this experiment was 90 days; however, after 45 days all pots were removed from the basin and drained overnight, and the next morning they were again resubmerged.

Plants used in experiments designed to determine the effect of NH_4NO_3-N rates and flooding on ergot alkaloid concentration consisted of five replicate pots of both nonsymbiotic and symbiotic plants that were previously grown in total N concentrations of 11 and 200 mg of N per pot. The nutrient solution of Hoagland and Arnon (15) was used, and

the N content was supplied as NH_4NO_3. Plants were fertilized once a week for 2 months at these N fertilizer rates before being submerged in water for 90 days. As before, at day 45 plants were removed from the basin and drained overnight, and the next morning they received the same N treatment they had been assigned before.

Nitrogen form. Effects of the N source on ergot alkaloid accumulation were determined by using plants fertilized with a modified Long Ashton nutrient solution (13). The N source in this solution was either KNO_3 or $(NH_4)_2SO_4$. The concentration was either 34 mg of N per liter (for the high rate) or 3.4 mg of N per liter (for the low rate). Symbiotic and nonsymbiotic plants were separated into groups, and three replicates of each group were randomly assigned to a treatment. They were fertilized once a week for 60 days; each pot in a group received 1 liter of either NO_3^--N or NH_4^+-N fertilizer solution each time. Plants were watered twice daily except for the day before each fertilization, when the afternoon watering was omitted. Plants were fertilized the next morning, and normal watering was resumed the following day. This procedure allowed the 1 liter of nutrient solution to be taken up completely with a minimum of loss.

Nitrogen rates. The nutrient solution of Hoagland and Arnon (15) was used in studies of the effect of total N rates on ergot alkaloid accumulation. Plants used in this experiment consisted of nonsymbiotic and symbiotic ramets that were separated into individual tillers with three or four leaves each and then cut to a height of 200 mm. Three tillers were then placed in each pot, and four replicated pots were randomly assigned three N rates. Each pot received either 220 (high level), 73 (medium), or 11 (low) mg of N per liter. Plants were fertilized once a week for 160 days, receiving 1 liter of fertilizer solution applied each time as described above. These pots were separated into nonflooded controls and treatment groups which were submerged in water as indicated above under flooding stress.

Drought stress. Nonsymbiotic and symbiotic ramets were separated into individual tillers as described above and planted in 1,130 g of soil per pot. The Cecil topsoil was sieved through a 2-mm screen, and its water release curve was determined (1). Three symbiotic tillers were placed in each pot and watered twice daily. These plants were fertilized weekly at three N levels (220, 73, and 11 mg of N per pot) for 90 days. After this growth period, plants were subjected to drought stress. Drought stress was imposed on certain treatments by maintaining a soil water content corresponding to the desired matric potential according to the previously determined water release curve (1). Water content was calculated by using the weight of the plant, soil (1,130 g), and pot (60 g). The matric potential levels imposed were −0.03, −0.05, and −0.5 MPa. Details of this procedure were reported earlier (1).

After 20 days, one replicate from each treatment was randomly chosen. The plants were washed free of soil, blotted dry, and weighed for adjustments of soil water content due to increase in plant weight. After 40 days of drought stress, plants were harvested and samples were prepared for alkaloid analysis as described below.

Sample preparation. Grasses in all experiments were manually harvested to approximately 20 mm above the soil surface. Replicates were individually treated. Samples were then stored in plastic bags and refrigerated at 4°C. All grass samples were either dried in an oven at 65 to 70°C for 24 h or placed in a lyophilizer for 24 h until they were dry enough to be ground. Samples were ground in a Wiley mill with a 40-mesh screen. Individual samples were then stored at −20°C in glass bottles until analyzed for ergot alkaloids.

TABLE 1. Effects of 90 days of flooding stress and nitrogen rates on the alkaloid concentrations in symbiotic and nonsymbiotic tall fescue

Treatment	Total concn (μg/mg dry wt)[a] of:		
	Ergovaline	Ergopeptide	Ergotamine
Symbiotic grasses			
Urea-fertilized grasses[b]			
Before flooding	0.68 a	1.10 a	0.10
After flooding	1.10 b	1.50 a	ND[c]
Nonflooded controls	2.80 c	4.20 b	ND
NH_4NO_3-fertilized grasses[d]			
Before flooding	0.07	0.14	ND
After flooding			
11 mg of N/pot	0.12 a	0.20 a	ND
220 mg of N/pot	1.30 b	2.10 b	ND
Nonflooded controls			
11 mg of N/pot	0.31 a	0.78 a	ND
220 mg of N/pot	2.00 b	2.41 b	ND
Nonsymbiotic grasses			
After flooding			
220 mg of N/pot	ND	ND	ND
Nonflooded controls			
220 mg of N/pot	ND	ND	ND

[a] Values in each column within an experiment not having the same letter differ significantly ($P < 0.05$).

[b] All grasses were fertilized with liquid 12-6-6 fertilizer once weekly for 120 days before initiation of the 90-day flooding experiment; nitrogen was supplied in urea.

[c] ND, not detected.

[d] All grasses were fertilized with Hoagland and Arnon fertilizer for 60 days before initiation of the experiments; nitrogen was supplied in NH_4NO_3.

Ergot alkaloid analysis. Before initiation of each of the experiments described above, some symbiotic and nonsymbiotic plants were randomly chosen and harvested for evaluation of ergot alkaloid concentration prior to each treatment. Ergopeptide alkaloids were extracted, identified, and quantitated on a 1-mg equivalent of an extract prepared by the procedure of Plattner et al. (26), using a Finnigan 4535/TSQ quadropole mass spectrometer in the negative chemical ionization mode. This procedure identifies all known ergopeptide alkaloids in tall fescue and is sensitive to the picogram level, with a linear response from 1 pg to 66 ng. Data were reported as ergovaline and total ergopeptide alkaloids. Results of ergot alkaloid analyses within experiments were analyzed by a two-sample (unpaired) *t* test.

RESULTS

Grasses fertilized with urea-N and grown for 120 days in greenhouse culture, although pot bound and not vigorously growing, contained ergopeptide alkaloids. The initial ergovaline concentration in grasses increased approximately twofold after flooding, but this was 40% less than the ergovaline concentration in the nonflooded controls at the conclusion of the experiment (Table 1). Ergotamine was found in grasses initially, but this alkaloid was not detected in any of the treatment groups during the experimental period. The concentration of total ergopeptide alkaloids remained essentially the same before (1.10 μg/mg) and after (1.50 μg/mg) flooding. Nonflooded controls had approxi-

TABLE 2. Effects of nitrogen form and total rate on the alkaloid concentrations in symbiotic and nonsymbiotic tall fescue

Treatment	Total concn (μg/mg dry wt)[a] of:	
	Ergovaline	Ergopeptide
Symbiotic grasses		
Initial alkaloid content	0.07	0.14
Nitrogen form[b]		
After fertilization		
NH_4^+ 3.4 mg of N/pot	0.05 a	0.20 a
34.0 mg of N/pot	0.47 b	0.55 b
NO_3^- 3.4 mg N/pot	0.39 b	0.63 b
34.0 mg of N/pot	0.52 b	0.60 b
Nitrogen rate[c]		
After NH_4NO_3 fertilization		
11 mg of N/pot	ND[d]	ND
73 mg of N/pot	0.25 b	0.43 b
220 mg of N/pot	0.41 b	0.57 b
Nonsymbiotic grasses[e]		
After fertilization		
220 mg of N/pot	ND	ND

[a] Values in a column within an experiment not followed by the same letter differ significantly ($P < 0.05$).
[b] All plants were grown for 60 days and fertilized weekly with the indicated N form.
[c] All plants were grown for 160 days and fertilized weekly at the indicated N rate.
[d] ND, not detected.
[e] Nonsymbiotic grasses (controls) were fertilized with NH_4NO_3.

TABLE 3. Effects of a 40-day drought stress period on the alkaloid contents of symbiotic and nonsymbiotic plants grown at different nitrogen rates (NH_4NO_3) for 90 days

Treatment	Total concn (μg/mg dry wt)[a] of:	
	Ergovaline	Ergopeptide
Symbiotic grasses		
Before treatment[b]	0.07	0.14
After treatment		
−0.03 MPa		
11 mg of N/pot	0.26 a	0.53 a
73 mg of N/pot	0.26 a	0.35 a
220 mg of N/pot	0.48 a	0.66 a
−0.05 MPa		
11 mg of N/pot	1.20 a	2.30 a
73 mg of N/pot	0.36 b	0.50 b
220 mg of N/pot	0.77 b	1.00 b
−0.50 MPa		
11 mg of N/pot	0.30 a	0.58 a
73 mg of N/pot	1.10 b	1.80 b
220 mg of N/pot	1.80 c	2.60 c
Nonsymbiotic grasses		
After treatment		
−0.03 MPa 220 mg of N/pot	ND[c]	ND
−0.05 MPa 220 mg of N/pot	ND	ND
−0.50 MPa 220 mg of N/pot	ND	ND

[a] Values in each column within an experiment not having the same letter differ significantly ($P < 0.05$).
[b] Samples in this treatment also contained ergotamine (0.50 μg/mg, dry wt).
[c] ND, not detected.

mately a fourfold increase over time in both total ergopeptide and ergovaline alkaloids.

When grasses were divided, transplanted, and fertilized at two rates of NH_4NO_3 for 60 days before the 90-day flood treatment was imposed, there was a response to the fertilizer rate (Table 1). These plants were growing vigorously before flooding. There were no significant differences in ergovaline and total ergot alkaloid concentrations before or after flooding at the lower NH_4NO_3 application rate. Symbiotic grasses fertilized for 60 days with NH_4NO_3 initially contained a lower concentration of ergopeptide alkaloids than did the 120-day-old group of the earlier experiment (Table 1). This may be a result of fertilization of grasses in this later experiment with a different type of N. There was approximately a twofold increase in total ergopeptide and ergovaline concentrations after the 90-day flooding period at the high N rate. However, at the low N rate there were no significant differences ($P < 0.05$) in ergot alkaloid concentration. Total ergopeptide concentrations of flooded (compared with nonflooded) grasses were reduced by 75 and 12.5% at low and high rates of N fertilization, respectively. No ergopeptide alkaloids were produced in noninfected plants.

Grasses grown with NH_4^+-N contained increased concentrations of both total ergopeptide and ergovaline at the higher NH_4^+-N rate (Table 2). This increase did not occur when NO_3^--N was used as the source. Overall, grasses fertilized at the low NO_3^--N rate contained higher concentrations of ergopeptide alkaloids than did plants receiving NH_4^+-N.

Total ergopeptide and ergovaline concentrations in all water treatments and N rates increased from the initial concentrations (Table 3). Nonsymbiotic grasses were negative for ergot alkaloids. The total ergopeptide alkaloid concentration increased as the soil matric potential decreased from −0.03 to −0.05 MPa. Alkaloid responses to N levels were inconsistent at the lower matric potentials; however, at −0.50 MPa, concentrations of both ergovaline and total ergopeptide increased with N fertilization.

Herbage yield and tiller number are increased by the endophyte (1), and they can be expected to reflect the quantity of ergot alkaloid produced. Herbage yield of symbiotic tall fescue obtained under the three moisture levels of experiment II indicated that there was a relationship between soil moisture and N rate (Fig. 1). At each soil moisture level, herbage yield increased with higher N rates. At the lowest N rate soil moisture level had no effect on herbage yield, whereas at the two higher rates herbage yields were highest with the least moisture stress (−0.03 MPa).

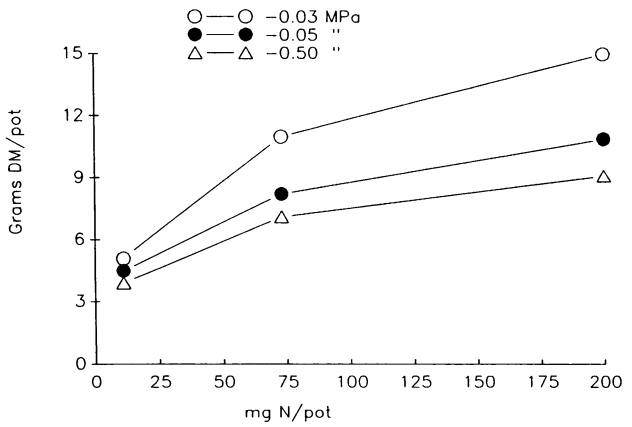

FIG. 1. Herbage yield, in grams of dry matter (DM) per pot, of endophyte-infected tall fescue as affected by NH_4NO_3-N rate and soil water matric potential (in megapascals).

DISCUSSION

Temporary flooding of sections of tall fescue pastures is common in many areas. Our research indicates that a period of 90 days of soil inundation, although exceptionally long, will not eliminate the endophyte or decrease production of toxic ergot alkaloids. Tall fescue is considered reasonably tolerant to flooding and low soil oxygen tension (7). Generally, flood-stressed plants accumulate primary and secondary metabolites, while photosynthesis and translocation are reduced, primarily because of anaerobiosis in roots (16). These alterations in plant metabolism might be expected to limit metabolism of the endophyte. This was the case in our study since ergovaline and total ergopeptide alkaloid concentrations were lower than in the nonflooded controls at the end of the experiment. The reduction in accumulation of ergot alkaloids resulting from flooding was N dependent. For example, tall fescue fertilized with NH_4NO_3 and flooded for 90 days produced 93% more total ergopeptide than was initially found in these plants. Therefore, the endophyte of tall fescue should not be considered latent under flooding conditions.

The occurrence of ergopeptide alkaloids in symbiotic tall fescue was first reported by Yates et al. (30) and Lyons (21). These studies indicated that ergovaline, which is considered toxic (8), was the major ergot alkaloid isolated from symbiotic tall fescue. The same results were obtained in this study, but we also report that ergotamine was found in 120-day-old grasses fertilized with liquid fertilizer. The cooccurrence of ergotamine and ergovaline can be accounted for by the fact that ergotamine and ergopeptide alkaloids, including ergovaline, are derived from a common precursor (8). This is the first report of production of ergotamine in large amounts by a fungus other than *Claviceps* spp.

Increases in soil N availability and cool air seem to amplify signs of fescue toxicosis in cattle (4, 5). This effect may be due to an increase in forage intake or in ergot alkaloid concentration. In addition to extrinsic factors (1, 4, 12, 23, 25), ergot alkaloid concentrations are affected by intrinsic factors, i.e., the genetic makeup of the grass and fungus (14). However, since only one genotype of symbiotic tall fescue was used in this study, our results are not due to genetic variation.

The total ergopeptide concentration increased with increasing N rate except in grasses fertilized with NO_3^--N. In this case a slight decrease was observed for ergopeptide alkaloids with an increased NO_3^--N rate. These results agree with those of Lyons et al. (23) and Belesky et al. (4), who concluded that higher rates of N increased the total concentration of ergopeptide alkaloids. However, Lyons et al. (23) stated that the N source had no effect on ergopeptide concentration. Our study indicates that the source is important but is concentration dependent. That is, high NO_3^--N and NH_4^+-N concentrations are equally effective in producing similar concentrations of ergot alkaloids, but NH_4^+-N is effective only at a high rate, a concentration not used by Lyons et al. (22). A similar observation was reported by Marten et al. (24) for reed canary grass (*Phalaris arundinacea* L.), which presumably did not involve a fungal endophyte. In their study, the alkaloid concentration was increased 56% by the N source but only 31% by the N rate (24). Other nutrients are expected to interact with soil N and ergot alkaloid production which has been reported for grasses parasitized by *Claviceps* species (20).

The relationship of water supply to ergot alkaloid accumulation in tall fescue is related to an interaction between plant growth and N uptake. The endophyte is considered to derive its nutrients from the apoplasm (2), and therefore the responsible mechanism for driving ergot alkaloid synthesis should involve the manner in which the apoplasm receives nutrients from the symplasm. Plant roots under drought stress show a low permeability to N (10), resulting in low total N (9, 11). Therefore, under drought conditions, soil N availability is not expected to affect the total accumulation of nitrogen. In our study, plants grown at −0.5 MPa, a severe drought condition, had high concentrations of ergot alkaloids. Plants grown under drought conditions contain higher concentrations of nutrients in both the apoplasm and symplasm than do non-drought-stressed grasses (10). Furthermore, there is considerable, although circumstantial, evidence that plants under drought stress often produce organic solutes as a survival mechanism (18, 29), and the basis for this phenomenon in symbiotic tall fescue may involve the accumulation of specific carbohydrate compounds (28). Possibly the increased ergot alkaloid concentrations in drought-stressed tall fescue reflect this increase in carbohydrate solutes, some of which may accumulate in the apoplasm and serve as precursors for alkaloid synthesis.

In no way do we mean to imply that ergot alkaloids are stress metabolites or indicators of stress. These defensive biologically active compounds, as well as others, are probably constitutive defense compounds whose regulation and interactions with the environment are complex. Further experimental work on environmental interactions with in situ production of ergot alkaloid in genotypes other than the one used here, and in mixed populations, is necessary before a detailed and unequivocal explanation of their significance can be made.

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