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Effects of the Fungal Endophyte *Acremonium coenophialum* on Nitrogen Accumulation and Metabolism in Tall Fescue

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ABSTRACT

Infection by the fungal endophyte *Acremonium coenophialum* affected the accumulation of inorganic and organic N in leaf blades and leaf sheaths of KY 31 tall fescue (*Festuca arundinacea* Schreb.) grown under greenhouse conditions. Total soluble amino acid concentrations were increased in either the blade or sheath of the leaf from infected plants. A number of amino acids were significantly increased in the sheath, but only asparagine increased in the blade. Infection resulted in higher sheath NH_4^+ concentrations, whereas NO_3^- concentrations decreased in both leaf parts. The effects on amino acid, NO_3^-, and NH_4^+ concentrations were dependent upon the level of N fertilization and were usually apparent only at the high rate (10 millimolar) of application. Administration of $^{14}CO_2$ to the leaf blades increased the accumulation of ^{14}C in their amino acid fraction but not in the sheaths of infected plants. This may indicate that infection increased amino acid synthesis in the blade but that translocation to the sheath, which is the site of fungal colonization, was not affected. Glutamine synthetase activity was greater in leaf blades of infected plants at high and low N rates of fertilization, but nitrate reductase activity was not affected in either part of the leaf. Increased activities of glutamine synthetase together with the other observed changes in N accumulation and metabolism in endophyte-infected tall fescue suggest that NH_4^+ reassimilation could also be affected in the leaf blade.

Endophytic fungal infections caused by *Acremonium coenophialum* Morgan-Jones and Gams are prevalent throughout the United States in stands of tall fescue (*Festuca arundinacea* Schreb.), a widely grown pasture, turf, and conservation grass (20). Since this association is such a widespread occurrence (20), tall fescue should be considered a symbiotic plant. Outwardly, such infections cannot be detected despite extensive intercellular growth within the plant; the fungus is not a pathogen. Initially recognized as an essential causal factor in the toxicity of tall fescue to cattle (3, 4, 9), infected stands of tall fescue may be enhanced in their ability to survive under stressful environments (2, 5, 23). Thus, the endophytic fungus has considerable agronomic and ecological significance on tall fescue. For example, endophyte infection increases tillering, reproduction and growth of the plant, relieves drought tolerance, and decreases the plant's susceptibility to insect feeding (2, 4, 5, 11, 21). Many effects similar to those in endophyte-infected tall fescue have also been demonstrated in other endophyte-infected species of fescue and in perennial ryegrass (*Lolium perenne* L.) (14, 21).

The underlying physiological basis for the various effects on tall fescue which result from this endophyte infection, and the extent to which the grass and fungus are responsible, are largely unknown. Two lines of evidence suggest that at least N metabolism is one aspect that may be affected. The first is from greenhouse studies which show that high rates of N fertilization increased the concentrations of one class of endophyte-synthesized N bases, ergot alkaloids, in endophyte-infected tall fescue (16). Second, other classes of nitrogenous metabolites that are insect feeding deterrents are associated with endophyte infection. Furthermore, the expression of cattle toxicoses has been quantitatively related to rates of N fertilization (3, 7, 21). Thus, in tall fescue, both fungal and host N metabolism may be contributing factors to various characteristics which are peculiar to the infected plant.

Because of the possible involvement of N metabolism in these important aspects of the tall fescue-endophyte association, comparative studies were made of infected and uninfected plants under greenhouse conditions to determine whether N metabolism is affected by the endophyte. Aspects considered in this study included the accumulation of inorganic and organic nitrogenous compounds, the activities of major enzymes of N assimilation, and the metabolism of amino acids. A unique characteristic of the relationship, in which the endophyte extensively colonizes the leaf sheath but is entirely excluded from the blade (5), made it possible to study N metabolism in infected and uninfected leaf tissues and compare this with N metabolism in similar tissues from uninfected grasses.

MATERIALS AND METHODS

Plant Establishment and Growth Conditions

Experimental plants were derived from four infected and four uninfected KY 31 tall fescue (*Festuca arundinacea* Schreb.) plants which had previously been established from the same infected seedlot. The infection status of these eight source plants was determined by microscopic examination of leaf sheaths stained with aniline blue (4). Experimental plants were established in the greenhouse from tillers of the source plants in a mixture of soil, sand, peat, and vermiculite, and their infection status was monitored throughout the experi-

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ment. Supplemental incandescent and fluorescent lighting was used to extend the photoperiod to 16 h.

Experimental Design and Statistical Analysis

For experiments to determine effects on NO_3^-, NH_4^+, amino acid concentrations and enzyme activities, plants were arranged in a 2 × 2 × 2 factorial design in which the main effects were the endophyte, the form, and the amount of N applied. Nitrogen treatments consisted of twice-weekly applications of 100 mL per pot of Long Ashton nutrient solution (8) whose N content in either the KNO_3 or $(NH_4)_2SO_4$ form was modified to contain 0.5 mM (low N) or 10 mM (high N). These two rates of N were selected on the basis of their effect on plant growth and tillering. The form of N applied caused no apparent difference in plant growth. The eight factorial combinations were replicated four times and each replicate consisted of five plants cloned from the same source plant.

The significance of the three main effects was determined statistically by ANOV. Date for leaf sheaths and leaf blades were analyzed separately. Levels of significance for the endophyte effect and standard errors for the different treatment means are indicated in the figures and tables. Separations of the different treatment means were not conducted, since ANOV indicated, in most cases, that significant interactions occurred among the main effects.

Sample Collection and Preparation

Due to the amount of sheath and blade material necessary for NO_3^-, NH_4^+, and amino acid analyses, several sampling periods per pot were required. To minimize variation in N pools, all sampling was done when adequate regrowth had occurred and 2 d after fertilization between noon and 1600 h on cloudless days. Several tillers were removed above the soil line from the five plants making up each replicate, and the two youngest collared leaves were saved. Leaves were separated at the distal end of the ligule into sheaths and blades, immediately placed on ice, and then stored at −20°C. After the final sampling, all collected material within each replicate was combined, lyophilized, and ground in a Wiley mill to pass a 0.75 mm screen.

Amino Acid Analysis

For the analysis of free amino acids, 1 g of lyophilized sheath or blade was extracted in 80% ethanol with a Polytron homogenizer and centrifuged at 1000*g* for 5 min, and the supernatant was removed. This procedure was repeated four times, the supernatants were combined, and the ethanol was removed under vacuum at 40°C. The sample volume was adjusted to 20 mL with water and extracted twice with 20 mL of $CHCl_3$ which was discarded. The sample was dried under vacuum and the residue was dissolved in 8 mL of citrate buffer (pH 2.2) for analysis on a Beckman Automated Amino Acid Analyzer, model 121.

With the exception of serine and threonine, which coeluted with asparagine and glutamine, respectively, concentrations of the nonamide amino acids were determined directly from peak areas. Concentrations of the two amides, asparagine and glutamine, were determined from the increase in their corresponding acids, aspartic acid and glutamic acid, after a 2-mL aliquot of the amino acid sample was hydrolyzed in 1 N HCl at 100°C for 1 h. Serine and threonine concentrations were also determined in this aliquot, since hydrolysis removed the interfering amides.

Nitrate and NH_4^+ Analysis

Nitrate concentrations were determined from 20- or 200-uL aliquots from the hydrolyzed amino acid samples by the method of Wooley *et al.* (25).

For NH_4^+ determinations, 0.1 g of the lyophilized leaf sample was extracted three times with 80% methanol, and the extracts were evaporated to dryness. The residue was dissolved in 10 mL of double-distilled water, and NH_4^+ was quantitated with an Orion NH_4^+-specific electrode.

Enzyme Assays

Samples for enzyme assays were also collected on cloudless days between noon and 1600 h. Two tillers were removed from each of the five plants in a replicate; their youngest collared leaf was removed and separated into sheath and blade. Midribs were removed from each blade. Sheath and blade samples were cut into 0.5-cm segments and 1-g subsamples were removed. Samples for GS[2] assays were immediately frozen in liquid N_2 and stored at −20°C. Samples used for NR assays were conducted on freshly harvested samples.

In vitro NR activity was assayed by an infiltration technique (1). Duplicate samples were vacuum extracted and assayed in 5 mL of reaction buffer (50 mM phosphate buffer, pH 7.0; 40 mM KNO_3; 1.5% (v/v) *n*-propanol). Sheath and blade assays were terminated after 30 min and 60 min, respectively, by placing the reaction mixture in a boiling water bath for 5 min. After centrifugation at 1000*g*, NO_2^- was determined from a 1-mL aliquot of the reaction mixture by the method of Wooley *et al.* (25).

Glutamine synthetase activity was measured as the synthesis of γ-glutamyl hydroxamate. Samples were ground to a powder in liquid N_2 with a mortar and pestle and quickly homogenized in 3 mL of extraction buffer (0.05 M Tris HCl, pH 7.4; 1.0 mM 2-mercaptoethanol; 0.5 mM sodium EDTA; 10% [v/v] ethylene glycol) with the aid of a small amount of acid-washed sand. The homogenate was filtered through cheesecloth and centrifuged for 10 min at 20,000*g*. All extraction steps were carried out at 4°C. The supernatant was immediately assayed in 1 mL of reaction buffer (0.2 M Tris HCl, pH 7.8; 0.1 M L-glutamate; 0.05 M $MgSO_4$; 0.01 M ATP; 0.01 M hydroxylamine) by the method of Rhodes *et al.* (19). Either 100 μL or 200 μL of the supernatant were used for blade and sheath samples, respectively. The reaction was initiated by addition of ATP and terminated after 30 min by addition of 100 μL of $FeCl_3$ solution (4% [w/v] TCA and 3.2% [w/v] $FeCl_3$ in 0.5 N HCl). After centrifugation for 10 min at 2000*g*, the absorbance was measured at 500 nm. Zero time reaction mixtures were used as controls, and results were expressed as total enzyme activity in μmol hydroxamate $min^{-1}g^{-1}$.

[2] Abbreviations: GS, glutamine synthetase, NR, nitrate reductase.

Protein Determination

The soluble protein was determined from a 10% (w/v, final concentration) TCA precipitate of the supernatant used in the GS assay. The precipitate was rinsed with ethanol, dissolved in 0.5 M NaOH, and assayed for protein by the method of Lowry *et al.* (15).

$^{14}CO_2$ Assimilation

For these experiments, plants were grown in the greenhouse as described above but were fertilized weekly with a complete Long Ashton nutrient solution. Assimilation of $^{14}CO_2$ into sugars, organic acids, and amino acids by blades and transport of assimilates to sheaths were determined in four separate paired experiments comparing infected and uninfected plants. Experimental plants comprised three tillers from which all but the youngest collared leaf had been removed. So that only blades were exposed to $^{14}CO_2$, each potted plant was placed in a 10 × 10 × 14-cm clear plastic container, and a hole was placed in the top of this container through which a leaf blade extended outward. The hole surrounding the leaf and the seam of the lid of the plastic container were sealed with clay. Two infected and two uninfected plants, enclosed in plastic containers as described, were placed in a 10-L glass chromatography tank. The lid of this tank was sealed with clay, and 200 μCi of $^{14}CO_2$ was released into the chamber as described by Smith *et al.* (22). The air within the chamber was not forcefully circulated. Plants were illuminated with overhead fluorescent tubes and an incandescent lamp which produced a total of about 150 $\mu E\ m^{-2}\ s^{-1}$ (as measured with a Li-Cor Quantum Meter, Lincoln, NE). During the labeling period, the temperature of this chamber was 30 ± 2°C. The concentration of CO_2 in the chamber was 710 $\mu L\ CO_2\ L^{-1}$, which was approximately two times the concentration of laboratory atmospheric CO_2. The amount of CO_2 remaining after the experiment averaged less than 2% of the initial value. Preliminary experiments indicated that under these conditions, 1 h was required for maximum labeling. After 1 h, excess $^{14}CO_2$ in the tank was trapped by injection of 5 mL of ethanolamine-glycol (10) into a dish, and the plants were removed after 15 min. Tillers were immediately excised at the base and placed on ice, and leaves were separated into sheath and blade. These were frozen in liquid N_2 and stored at −20°C until analysis.

Weighed leaf samples were cut into small segments, placed in a mortar, and ground to a powder in liquid N_2. The powder was homogenized with 5 mL of 80% ethanol, and the homogenate was transferred to a 50-mL centrifuge tube. The mortar was rinsed with an additional 5 mL of 80% ethanol and this was combined with the homogenate. The homogenate was centrifuged 5 min at 2000*g*, and the supernatant was transferred to a round bottom flask. The cell residue from the homogenate was resuspended with 5 mL of 80% ethanol and centrifuged. This was repeated three times, and the supernatants were transferred to the round bottom flask. The ethanolic extract was evaporated to dryness, and the resulting residue was dissolved in 12 mL of water and extracted with 20 mL of $CHCl_3$, which was discarded. The aqueous sample was evaporated to dryness.

The sample residue was taken up in 2 mL of water and transferred to a 1 × 5 cm Dowex 50 column (H^+ form). The column was rinsed with 10 mL of water, and amino acids were eluted with 15 mL of NH_4OH. The aqueous rinse from the Dowex 50 column was transferred to a 1 × 5-cm Dowex 1 column (Cl^- form), and neutral sugars were rinsed from this column with 20 mL of water. Organic acids were eluted from the Dowex 1 column with 20 mL of 4 N formic acid. Radioactivity in the amino acid, sugar, and organic acid fractions and cell residue was determined from aliquots of each fraction in Bray's solution (6) on a Packard liquid scintillation counter, model 3255. Data from the four experiments were analyzed statistically as a single paired difference experiment.

Chromatography and Autoradiography of ^{14}C-Amino Acids

Amino acid fractions from leaf blades of the infected and uninfected plants were separated by two-dimensional chromatography on mixed cellulose/silica gel thin-layer plates prepared according to the procedure of Turner and Redgwell (23). The solvent system in the first direction was phenol/water (80:20, w/v) and in the second direction it was *n*-butanol/acetic acid/water (5:1:4, v/v, upper phase). Samples were chromatographed in the second direction, the plates dried, and then they were rechromatographed in the second direction again. ^{14}C-labeled amino acids on the chromatograms were identified by autoradiography.

RESULTS

NO_3^-, NH_4^+, and Soluble Proteins

Mean NO_3^- concentrations were decreased in leaf sheaths of infected as compared to uninfected plants with all treatment combinations (Table I). Similarly, this effect was observed in leaf blades with all combinations except those at the low rate of KNO_3 fertilization when NO_3^- concentrations were below detectable limits (25 $\mu g\ NO_3^-/g$ dry weight) in both infected and uninfected plants. ANOVA indicated that the endophyte effect on NO_3^- accumulation was significant in both the sheath and blade. Significant interactions occurred between the effects of the endophyte and the rate and form of N fertilization in both parts of the leaf, indicating that the extent to which the endophyte affected NO_3^- concentrations in the plant was dependent upon these two additional factors. Overall, the effect of the endophyte was greatest in plants treated with the high rate of $(NH_4)_2SO_4$ and least in those treatments receiving the low rate of N. The fact that NO_3^- accumulated at all in plants fertilized exclusively with $(NH_4)_2SO_4$ indicates that substantial conversion from NH_4^+ to NO_3^- occurred in the soil due to nitrification. No effort was made to inhibit this conversion since nitrification is a normal phenomenon under field conditions as well.

Ammonium concentrations were also significantly affected by infection but only in the leaf sheath where, in contrast to NO_3^-, concentrations increased rather than decreased (Table I). This effect was also interactive with both the rate and the form of N fertilization. The greatest effect of the endophyte on NH_4^+ concentrations was observed at the high rate of

Table I. *Effects of Fungal Endophyte, A. coenophialum, on Nitrate and Ammonia Concentrations in Leaves of KY 31 Tall Fescue*

Values (± SE) are mean nitrate or ammonia concentrations in leaf blades and leaf sheaths of endophyte-infected (+) or uninfected (−) tall fescue fertilized at the low (0.5 mM) or high (10 mM) N rate with KNO_3 or $(NH_4)_2SO_4$. Endophyte infection significantly affected nitrate concentrations in both blade and sheath, and ammonia concentrations in sheaths (P = 0.01).

Leaf Part	Infection Status	Nitrate Concentration				Ammonia Concentration			
		Nitrate fertilized		Ammonia fertilized		Nitrate fertilized		Ammonia fertilized	
		Low N	High N	Low N	High N	Low N	High N	Low N	High N
		μg N/g dry wt							
Blade	+	<25	2552 ± 119	44 ± 30	357 ± 98	48 ± 15	71 ± 14	38 ± 18	54 ± 6
	−	<25	3150 ± 480	91 ± 23	1498 ± 338	42 ± 11	67 ± 3	33 ± 5	55 ± 17
Sheath	+	71 ± 29	4788 ± 289	48 ± 23	1120 ± 245	29 ± 4	126 ± 14	41 ± 16	231 ± 29
	−	106 ± 28	6868 ± 868	71 ± 23	3235 ± 658	25 ± 28	76 ± 8	46 ± 23	84 ± 24

$(NH_4)_2SO_4$ fertilization. There was no apparent endophyte effect at low rates of N fertilization.

The mean total soluble protein concentration (mg/g fresh wt) for all treatments was 14.6 in blades, which was approximately 3-fold higher than that in sheaths. Protein concentrations were not significantly affected by the endophyte under any of the N treatment combinations.

Amino Acids

The total amino acid concentrations were significantly increased in infected sheaths and blades (Table II) under the high rates of N fertilization. In the leaf sheath, the endophyte effect was dependent upon the rate of N fertilization but not in the blade.

Concentrations of the individual amino acids present in excess of 1.0 μmol/g fresh weight for any of the N fertilization treatments are shown in Figures 1 and 2. Those amino acids significantly affected by endophyte infection are indicated in both figures. For most of the amino acids affected by the endophyte, this effect was dependent upon the rate and, in some cases, the form of N applied. When the amino acid data for infected and uninfected fertilization are compared among the different N fertilization treatments, it can be seen that the endophyte effect usually resulted in increased concentrations only at the high rates of fertilization.

The most notable effect of the endophyte on amino acid concentrations were observed in the sheath. Four of the seven amino acids present in sheaths in amounts greater than 1.0 μmol/g fresh weight (Figs. 1 and 2) were significantly affected by the endophyte. Asparagine and glutamine, particularly at high rates of N, were the most obviously affected. In addition to those shown in Figures 1 and 2, several amino acids present in sheaths at concentrations below 1.0 μmol/g fresh weight were also affected by the endophyte. Concentrations of lysine and arginine were in excess of 0.1 μmol/g fresh weight in all treatments but were more than doubled in sheaths of endophyte-infected plants at high rates of N. Under these same conditions, histidine levels, also present in excess of 0.1 μmol/g fresh weight, were less than half those in uninfected plants.

In the blade, the level of one amino acid, asparagine, was significantly affected by the endophyte. The concentration of asparagine in the blade of infected plants was more than four times that in uninfected plants at the high rate of KNO_3 fertilization and nearly twice as great as the high rate of $(NH_4)_2SO_4$.

Glutamine Synthetase and Nitrate Reductase

In the leaf blade, the form of N fertilization had no significant effect on GS activity, nor were there any significant interactions among the main effects in this experiment. There-

Table II. *Effects of Fungal Endophyte, A. coenophialum, on Total Amino Acid Concentration in KY 31 Tall Fescue Leaves*

Values are the mean concentrations (±SE) in leaf blades and leaf sheaths of endophyte-infected or uninfected tall fescue fertilized at low (0.05 mM) or high (10 mM) rate with KNO_3 or NH_4SO_4. Means were calculated by summing the concentrations of the individual amino acids in Figures 1 and 2. Endophyte infection significantly affected total amino acid concentrations in leaf blade and sheath (P = 0.05).

Leaf Part	Infection Status	Total Free Amino Acid			
		Nitrate fertilized		Ammonia fertilized	
		Low N	High N	Low N	High N
		μmol/g dry wt			
Blade	+	20.2 ± 2.2	44.6 ± 3.6	16.8 ± 1.7	38.9 ± 4.7
	−	19.0 ± 0.7	39.3 ± 1.4	14.6 ± 0.8	30.9 ± 1.0
Sheath	+	14.1 ± 1.4	81.4 ± 12.1	13.5 ± 2.2	122.2 ± 13.7
	−	13.5 ± 2.4	40.8 ± 4.5	12.8 ± 2.3	92.6 ± 23.0

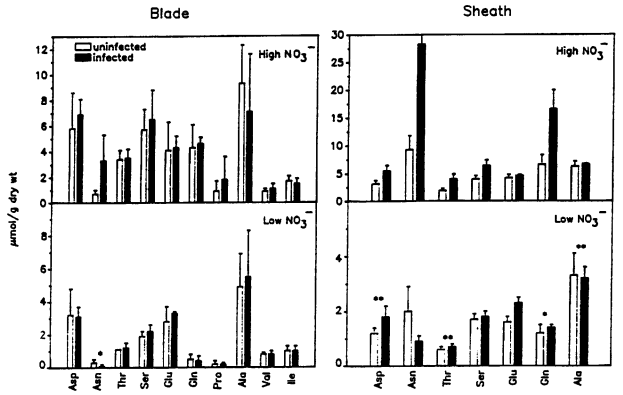

Figure 1. Amino acid concentrations in leaf blades and sheaths of endophyte-infected and uninfected KY 31 tall fescue fertilized at low (0.50 mM) and high (10 mM) rates of KNO_3. Bars indicate standard error. Asterisks indicate those amino acids that were significantly affected by endophyte infection as determined by ANOVA (**, P = 0.01; *, P = 0.05).

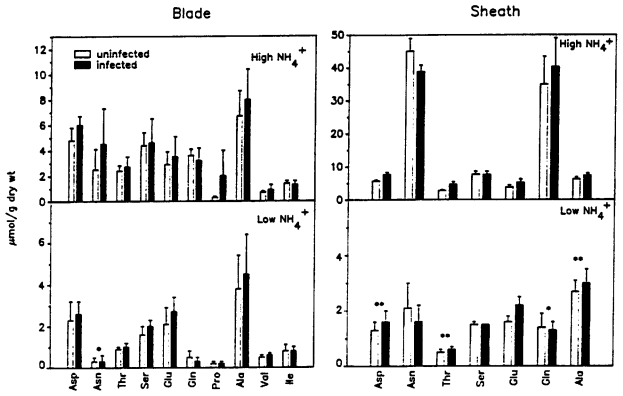

Figure 2. Amino acid concentrations in leaf blades and sheaths of endophyte-infected and uninfected KY 31 tall fescue fertilized at low (0.50 mM) and high (10 mM) rates of $(NH_4)SO_4$. Bars indicate standard error. Asterisks indicate those amino acids that were significantly affected by endophyte infection as determined by ANOVA (**, P = 0.01; *, P = 0.05).

fore, data from the two N forms of treatments were pooled, and the results are presented for the four remaining treatment combinations involving the endophyte and the rate of N fertilization (Table III). Both infection and N rate significantly affected GS activity. Infection resulted in a mean increase in GS activity of 32% when data were averaged over the two infected and two uninfected treatment combinations at the two rates of fertilization. By comparison, the mean increase in GS activity for treatments receiving the high rate of N fertilization *versus* the low rate was 19% when data were averaged over the infected and uninfected treatments.

In the leaf sheath, GS activities averaged over the KNO_3 and $(NH_4)_2SO_4$ treatments were 3.7 and 6.6 µmol γ-glutamyl hydroxamate $min^{-1}g^{-1}$ fresh weight, respectively, for plants receiving low and high N fertilization. These activities were 5 to 10% of those measured in the leaf blade and were not significantly affected by the endophyte.

Table III. *Effects of Fungal Endophyte, A. coenophialum, on GS Activities in Blades of KY 31 Tall Fescue*

Values are mean activities (±SE) fresh wt at low (0.5 mM) and high (10 mM) rates of fertilization. The values represent data that were combined from the KNO_3 and NH_4SO_4 treatments which ANOVA indicated to have no significant effect on enzyme activity. Endophyte infection significantly affected GS activities in lead blades (P = 0.01).

Infection Status	N Rate	
	Low N	High N
	µmol γ-glutamyl hydroxamate min^{-1} g^{-1}	
+	63.7 ± 1.5	72.9 ± 1.9
−	46.8 ± 0.8	56.7 ± 1.7

Table IV. *Assimilation of ^{14}C into Leaf Blade Fractions following Administration of $^{14}CO_2$ to Leaf Blades of KY31 Tall Fescue Plants*

Values are the means of four separate paired experiments in which leaf blades of endophyte-infected and uninfected tall fescue plants were separated into amino acid, sugar, organic acid and residue fractions for determination of the amount of ^{14}C incorporated following a 1-h exposure to $^{14}CO_2$. Statistical analysis of the paired differences for each fraction indicated that ^{14}C incorporation into amino acids and organic acids of the leaf blade was significantly affected (P = 0.01) by infection.

Fraction	Blade		Sheath	
	Infected	Uninfected	Infected	Uninfected
	dpm × 10^3/g fresh wt		dpm/g fresh wt	
Amino acid	885	584	11,525	8,700
Sugar	10,556	9,762	158,096	191,906
Organic acid	506	625	7,316	5,747
Residue	1,682	1,755	7,413	10,378
Totals	13,819	12,821	183,897	216,923

Infection had no significant effect on NR activities in the leaf sheath or leaf blade under any treatment combination receiving the high rate of KNO_3 fertilization. Activities of this enzyme were typically low or variable under conditions other than the high rate of KNO_3 fertilization. Therefore, results under these conditions were not considered conclusive.

$^{14}CO_2$ Assimilation

Infection significantly affected the incorporation of $^{14}CO_2$ into the free amino acid and organic acid fractions of leaf blades. Leaf blades of infected plants accumulated an average of 52% more ^{14}C in the free amino acid fraction than did leaf blades of uninfected plants (Table IV). Correspondingly, there was a 19% decrease of ^{14}C in the organic acid fraction. No significant differences were found in the sugar-containing fraction or in the cell residue. No significant differences occurred between infected and uninfected plants in any fraction from leaf sheaths (Table IV).

Autoradiography of thin-layer chromatograms of the amino acid fractions from leaf blades of infected and uninfected plants revealed no qualitative differences in those amino acids which were labeled. In both treatments, alanine was the most heavily labeled amino acid, but significant radioactivity was

also detected in serine, glycine, glutamic acid, aspartic acid, valine, and γ-aminobutyric acid.

DISCUSSION

This study has shown that infection of tall fescue by *A. coenophialum* can substantially alter N metabolism in both infected and uninfected parts of the grass leaf. These changes appeared to involve both assimilatory and basic N metabolism, because the concentrations of NO_3^-, NH_4^+, and amino acids, the level of GS activity, and the assimilation of carbon into amino acids were all affected. Since the fungus does not invade the leaf blade, this study demonstrated unequivocally that it can significantly affect N metabolism in parts of the grass which remain entirely free of fungal mycelia as well as in parts of the plant which are actually colonized. Previous reports have indicated that N-containing secondary metabolites produced by the endophyte, *i.e.* ergot alkaloids (3, 16) and other N-containing secondary metabolites produced only in the infected plant, *i.e.* pyrollizidine alkaloids (12,13), may be important to at least some of the economic characteristic changes associated with infection. The results of this study also provides evidence of the significance of N metabolism to this symbiotic relationship.

Some of the aspects affected by the endophyte were dependent upon other factors, the most obvious interactive effect being the rate of N fertilization. This implies that the extent to which the endophyte might affect N metabolism under field conditions could also depend considerably upon other interacting factors.

As with all of the effects in the blade, which is not colonized by the endophyte, this increase in soluble compounds might represent a change in host metabolism exclusive of any direct fungal contribution or differential rates of translocation. Perhaps the most notable effect of the endophyte on tall fescue observed in this study was the increase in GS activity in the leaf blade. In contrast, an increase in NR activity in the leaves, which would be essential for increased primary NO_3^- assimilation in the leaves, did not occur. This suggests that the change in GS activity in the blade was probably associated with reassimilation rather than primary NH_4^+ assimilation. Since NH_4^+ reassimilation is thought to be associated primarily with the cytoplasmic rather than the chloroplastic form of GS (24), a study of the effects on these two isozymes could be informative as to the actual significance of this effect.

Carbon assimilation into amino acids was also increased in leaf blades of infected plants. An increased rate of amino acid synthesis would be consistent with increased NH_4^+ assimilation or reassimilation since, with the exception of serine, in vascular plants, all amino acids are derived directly through transamination reactions involving glutamic acid, itself the final product of ammonia assimilation via GS/GOGAT (17). Although the ^{14}C-labeling experiments indicated that infection may have increased the concentration of some amino acids in the leaf blade, only a minor effect was actually observed on amino acid accumulation in the blade. An increase in amino acid synthesis could be accompanied by a compensating incorporation or conversion of the amino acids to other products or by translocation from the leaf blade. There was no evidence from the labeling experiments that the translocation of amino acids or other compounds to the sheath, the site of greatest fungal density, was increased by infection. This suggests that the endophyte may not represent an important sink for metabolites synthesized in the blade. Increased amino acid synthesis also did not appear to be related to the level of protein synthesis since protein concentrations were not affected. However, qualitative changes in soluble proteins or changes in the rate of protein turnover would not necessarily be reflected in total protein concentrations.

The small, but significant, increase in the total amino acid concentration in leaf blades at high rates of N fertilization was mainly reflected in the concentration of one amino acid, asparagine. Asparagine is an important N transport compound in plants (17, 18). Hence, its increased level in the blade could be the result of transport from the other sites of synthesis, such as the roots or the sheath. Failure to detect [^{14}C]asparagine in leaf blade amino acid fractions from the CO_2 assimilation experiments is consistent with this possibility. Ammonium released from asparagine which had been transported to the blade would probably be reassimilated by GS (18). An accumulation of NO_3^- in leaves has been interpreted as an indication that the amount of NO_3^- taken up by the roots exceeds the capacity of the root to assimilate it (18). Thus, the low NO_3^- levels in leaves of infected plants may mean that NO_3^- uptake is decreased, or that NO_3^- assimilation is increased, in the roots. An increase in NO_3^- assimilation in the roots could function to provide additional N to the sheath where fungal growth is most extensive. An increased demand for N in the sheath would decrease the N available to the blade, resulting in the necessity for the more efficient N utilization that would be provided by an increase in NH_4^+ reassimilation in the blade. An examination of the rate of N uptake and measurements of NO_3^- levels in xylem exudates could be very informative as to the extent to which the endophyte affects assimilatory processes in tall fescue.

Effects of the endophyte on N metabolism in the sheath mainly involved changes in the concentration of inorganic and organic N compounds. The substantial increases in concentrations of several amino acids suggest that amino acid synthesis in the sheath was increased by infection. Although there was no increase in the ^{14}C content in the sheath amino acid fraction following administration of $^{14}CO_2$, this does not represent evidence against increased amino acid synthesis since only blades were exposed directly to $^{14}CO_2$. In contrast to the leaf blade, the sheath is extensively invaded by the endophyte, and the extent to which host and fungus are responsible for the increased amino acid concentrations in this part of the leaf cannot be distinguished. That the endophyte is at least partly responsible for the increased concentration of asparagine and glutamine under certain conditions is suggested by the fact that most plants (17), including uninfected tall fescue, accumulate amide amino acids only under conditions of high NH_4^+ fertilization, whereas in the infected plant high concentration of these two amide amino acids occurred in plants grown at the high level of NO_3^- fertilization as well.

Although large increases in amino acid concentrations in the sheath might be attributed strictly to fungal metabolism,

it is unlikely that these compounds accumulated solely within fungal mycelium. *In vitro* studies showed that the total amino acid concentration in the mycelium grown in high-N medium was only 2.5 μmol/g dry weight (PC Lyons, unpublished data). In contrast, the concentration of asparagine in the sheath reached nearly 30 μmol/g dry weight under conditions of high KNO_3 fertilization. Furthermore, although fungal growth in the sheath can be quite dense, its mass has been estimated to represent less than 1% of the plant's dry weight (20).

From this discussion, then, it would appear that some of the changes in amino acid concentrations, particularly those of asparagine and glutamine, could be the result of fungal metabolism but that once synthesized by the fungus they may be made available for utilization by either member of this symbiosis. Infected plants exhibited obvious and substantial increases in the activity of GS in leaf blades at both rates of N fertilization. As suggested, this effect could represent a means by which the blade is able to compensate for an increased demand on N caused by the endophyte in the sheath. However, this effect on GS at the low rate of N could also have important implications for the overall N economy, hence competitive ability, of the plant particularly under conditions of low soil N. One of the infected tall fescue source plants used in this study showed an increase in growth rate and higher dry matter under drought conditions when compared to its cloned and endophyte-free ramet (2). Increased N economy would be consistent with observations that the endophyte decreases the effects of stress on growth and survival, which provides more evidence to the interpretation that endophyte-infected tall fescue is a mutualistic symbiosis (2, 5, 21).

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