Bioactive Flavonoids from Endophyte-Infected Blue Grass (*Poa ampla*)

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Ethanol extract of leaves of *Poa ampla*, either infected or uninfected with the symbiotic fungus *Neotyphodium typhnium*, were tested for bioactivity against the mosquito larvae. Results of these tests indicated that only the extracts obtained from endophyte-infected grass are active against mosquito larvae. Relatively high activity was detected in stromata, seeds, spikelets, and leaves of plants, while only weak or no activity was found in extracts of the isolated fungus. The ethyl acetate fraction had approximately the same activity as that of the crude extract, while the activities from 1-butanol and hexane were intermediate and low, respectively. Bioassay-guided fractionation of the ethyl acetate fraction yielded tricin (**1**), 7-*O*-(β-D-glucopyranosyl)tricin (**2**), and isoorientin (**3**) as the bioactive constituents. A fourth compound, 7-*O*-[α-L-rhamnopyranosyl(1−6)−β-D-glucopyranosyl]tricin (**4**), was also isolated from the active fraction but showed little activity in this assay.

INTRODUCTION

Many grasses infected with symbiotic endophytes have been reported to be resistant to damage or infestation by certain insects. These endophytes, which live intercellularly in grass tissues, appear to bestow characteristics on the grass which act as defense mechanisms, thereby conferring resistance or tolerance to deleterious things such as exogenous pathogenic fungi, water stress, weed competition, and insect pests (White et al., 1992; Clay, 1988; Breen, 1994; Siegel, 1993).

In most research dealing with insect resistance conferred on grasses by endophyte infection, the effects on insects have been attributed to specific alkaloids which are believed to be produced by the endophytes, since they are not found in plant tissues (Clay, 1988; Eichenseer and Dahlman, 1993; Siegel, 1993; Rowan, 1993; Bush et al., 1993; Garner et al., 1993). In some instances, alkaloids associated with grass endophytes have been shown to act as feeding deterrents (Clay and Cheplick, 1989; Hardy et al., 1986; Patterson et al., 1991; Eichenseer and Dahlman, 1992).

A casual observation by the authors which indicated that spidermites selectively attacked greenhouse grown endophyte-free *Poa ampla* (−), but not endophyte-infected *Poa ampla* (+), led to the initiation of this investigation. The purpose of the present work was undertaken to determine whether the *Poa ampla* (+) plants contain compound/s which are toxic to insects and to identify such compounds, using a mosquito larvae bioassay.

EXPERIMENTAL PROCEDURES

Plant Material. Endophyte-infected (+) and endophyte-free (−) grasses (*Poa ampla*) were collected at Rutgers University Turf Farm in August 1996. Sheath leaves were checked microscopically for the absence or presence of the endophyte using rose bengal staining (Saha et al., 1988). A voucher specimen was deposited with the Plant Science Department, Rutgers University, New Brunswick, NJ.

Extraction and Isolation. Fresh aerial parts of both (+) and (−) *Poa ampla* (2.5 kg each) were extracted 4 times with 95% EtOH at room temperature. The combined extracts were concentrated under reduced pressure to provide a crude extract. Initial toxicity bioassays with mosquito larvae indicated that only the crude extract from endophyte-infected grass was lethal to mosquito larvae, whereas extracts of uninfected grass had little or no effect. Therefore, only the crude extract from endophyte-infected grass was used for the subsequent isolation. The entire crude extract (40 g) was mixed with H_2O (500 mL) and partitioned 3 times each with hexane, ethyl acetate, and 1-butanol, respectively, in the order shown. The relative volumes of water−organic solvent used in partitioning in each case were roughly 3:1. The ethyl acetate extract (3 g) was the major portion showing insecticidal activity. This portion was chromatographed over a Si gel column using a stepwise gradient of $CHCl_3$−MeOH (10:1, 8:1, 5:1, 3:1). The bioactive fractions were found only in the 10:1 and 5:1 eluents.

After all of the organic solvent was removed by rotary evaporation under reduced pressure, the 10:1 fraction was rechromatographed three times on a silica column with 10:1 $CHCl_3$−MeOH as the eluent. The bioactive fractions, detected using Si TLC, were combined, and the solvent was removed under reduced pressure, to yield compound **1**. The 5:1 fraction was concentrated and was also rechromatographed on a silica column using a linear gradient of 5:1 to 3:1 $CHCl_3$−MeOH, taking portions of the eluate to determine fractions possessing activity in our bioassay. Identical or similar fractions were detected using TLC and combined. The combined fractions were then reduced in volume and concentrated, to give an impure mixture of compounds **2** and **3** from the 5:1 $CHCl_3$−MeOH eluate and compound **4** from the 3:1 $CHCl_3$−MeOH eluate. These impure compounds (**2**−**3** and **4**) which were obtained from the silica gel column were further purified on Sephadex LH-20 with MeOH as the eluant to remove any nonflavonoid compounds, and yielded pure compounds **2**−**4**.

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Mosquito Larval Bioassay Screening Protocol. Mosquito larvae, *Culex pipiens*, were obtained at weekly intervals from Carolina Biological Supply, Burlington, NC, and allowed to develop and grow in distilled water to which was added a small amount of powdered milk. Larvae were allowed to develop for 6 days before assay. Bioassays were conducted in wells of 24-well, flat-bottom "Multiwell" plastic tissue culture plates. Extracts were transferred to wells, and all traces of the organic solvent were evaporated. For the water control, in addition to pure distilled water, a small amount of the organic solvents used in extraction and purification procedures was also added to an additional well and evaporated prior to addition of water. Ten 6-day old larvae were then transferred to wells containing 0.5 mL of distilled water and test samples, i.e., crude extracts, fractions, or pure compounds at concentrations of 100 or 400 μg/mL. After 24 h at room temperature, the surviving larvae were counted. The same protocol was used for controls containing distilled water. All bioassays were conducted in triplicate.

Culture of *Neotyphodium typhinium*. The *Neotyphodium typhinium* (*N. typhinium*) endophte was cultured in vitro on PDA plates. To isolate the endophytic fungus, small pieces of leaf sheath tissue were surface-sterilized for 15 min in 1.25% sodium hypochlorite, rinsed in sterile water, and then placed on PDA plates. After 2−3 weeks at 24 °C, fungal mycelia began to emerge from the plant tissue. Lindstrom et al. (1993). The *N. typhinium* cultures growing on PDA plates were harvested and extracted using 95% EtOH.

Acid Hydrolysis on TLC and Identification of Resulting Aglycon and Monosaccharides. Flavonoid glycosides were hydrolyzed with HCl vapor on a Si gel TLC plate (80 °C water bath for 30−40 min), followed respectively by 10:1 CHCl$_3$−MeOH and CHCl$_3$−MeOH−H$_2$O (7:3:1, lower layer) as developing solvent, and compared with authentic samples using 5% H$_2$SO$_4$−EtOH as detector for aglycon and sugar.

General Experimental Procedures. Melting points were measured with a Kofler apparatus and are uncorrected. Infrared spectra were measured on a Mattson CYGNUS 100 Fourier transform infrared spectrometer. UV spectra were obtained using a Shimadzu UV-160 UV−visible recorder spectrometer and MeOH as solvent. ^{1}H (500 MHz), ^{13}C (125.7 MHz) NMR spectra were recorded using the Bruker AMX-500 spectrometer. Dimethyl-d_6 sulfoxide (DMSO-d_6) was used as solvent and tetramethylsilane (TMS) as internal standard. Mass spectra were obtained on a VG ZAB-HS spectrometer using fast-atom-bombment ionization and thio−glycerol as matrix. Silica gel (Selecto Scientific, 230−400 mesh) was used for CC, and precoated silica gel plates (Waterman, Al Si G) were used for TLC. A UV lamp (254 and 365 nm) and 5% H$_2$SO$_4$−EtOH was used for detection of areas of migration.

RESULTS AND DISCUSSION

Initial toxicity tests with mosquito larvae using crude extracts indicated that crude extracts of aerial parts of endophyte-infected *Poa ampla* were generally lethal to mosquito larvae within a 24 h period, while exposure to the endophyte-free grass extract had no detectable effect (data not shown). Therefore, only tissue from endophyte-infected grass was used for the subsequent tests. The crude ethanol extract of the endophyte-infected *Poa ampla* was partitioned between hexane, ethyl acetate, and 1-butanol and water. The greatest proportion of insecticidal activity was found in the ethyl acetate fraction (Table 1). A series of chromatographic separations of the bioactive fractions provided purified compounds **1**−**4**, and of these four compounds, bioassay indicated that only compounds **1**−**3** were significantly active (Table 2). To our knowledge, this is the first time these compounds have been reported to be isolated from this species (Tulloch, 1977).

Compound 1 (15 mg). This purified compound showed a pink color in Mg−HCl test. The mass

Table 1. Mortality of Mosquito Larvae Exposed to Two Concentrations of Extracts of Endophyte-Infected *Poa ampla* Using Various Extraction Solvents[a]

	100 μL/mL	400 μL/mL
distilled H$_2$O	0a	0a
crude extract	100c	100b
ethyl acetate	100c	100b
hexane	11a	33a
1-butanol	56b	75b

[a] Means within columns followed by the same letter are not significantly different, using χ^2 test. $P < 0.05$.

Table 2. Mortality of Mosquito Larvae Exposed to Two Concentrations of Purified Compounds Isolated from Endophyte-Infected *Poa ampla*[a,b]

	100 μg/mL	400 μg/mL
distilled H$_2$O	0a	0a
compound 1	90b	100b
compound 2	80b	80b
compound 3	80b	100b
compound 4	0a	0a

[a] The amounts of purified compounds **1**−**3** (15, 30, and 50 mg, respectively) are obtained from 2.5 kg of tissue. [b] Means within columns followed by the same letter are not significantly different, using χ^2 test. $P < 0.05$.

spectrum of compound **1** gave M$^+$ at m/e 330 and major fragment ions at m/e 178, 163, 158, and 153, which suggested it to be a flavonoid containing two hydroxyls in the A-ring and one hydroxyl and two methoxyl in the B-ring (Markham, 1982). ^{1}H NMR revealed that compound **1** was 5,7-dihydroxyl in its A-ring and a symmetric structure in the B-ring. Compound **1** was identified to be tricin by comparison of spectral data and TLC with the literature and with an authentic sample data (Miles et al., 1994; Bhattacharyya et al., 1978).

Compound 2 (30 mg). The Mg−HCl color and Molisch tests indicated **2** to be a flavonoid glycoside. The FAB mass spectrum gave the quasimolecular ion at m/e 493 ([M + H]$^+$), which suggested the Mr to be 492, and fragments at m/e 330 ([M − 162]$^+$, 100%). On acid hydrolysis, **2** afforded tricin as an aglycon with glucose as the sugar component by comparison with an authentic sample on TLC. In the ^{13}C NMR spectrum, **2** displayed one anomeric carbon signal at δ 104.8 and other sugar moiety signals due to glucopyranoside, indicating that there was one glucose unit. The anomeric proton signal at δ 5.12 (d, $J = 6.5$ Hz) in the ^{1}H NMR spectrum led to the assignment of the anomeric configuration of glucose as β. The δ 11.5 signal in the ^{1}H NMR spectrum indicated there was a free hydroxyl in the 5-position of the A-ring. By comparison of the ^{1}H NMR spectrum with that of **1** (tricin), the chemical shifts of H8 and H6 of **2** were downshifted 0.31 and 0.36 ppm, respectively. The significant shifts clearly showed that the glucopyranosyl was linked to the 7-position hydroxyl group (Soe et al., 1978; Markham, 1982). This conclusion was also verified by its ^{13}C NMR data (Agrawal, 1989). From the above evidence, **2** was established as 7-*O*-β-D-glucopyranosyltricin. The spectral data were identical with the literature (Kurlin et al., 1982).

Compound 3 (50 mg). Acid hydrolysis yielded two products, one of which was found to be compound **3** by TLC analysis (Wessely−Moser isomerization) but not glucose, which suggested compound **3** to be a flavonoid *C*-glucoside, and the ^{13}C NMR spectrum showed that the pattern of sugar carbon signals identified the sugar as glucose (Mabry et al., 1970; Markham, 1982). The

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FAB mass spectrum indicated a pseudomolecular ion: 471 ([M + Na]$^+$) and 449 ([M+H]$^+$), and the characteristic fragment ions of luteolin C-glucose showed compound **3** was a luteolin *C*-glucoside. In the ^{1}H NMR spectrum, the signal δ 4.56 (d, J = 9.5 Hz) was assigned to the anomeric proton of the glucose confirming the linkage between the sugar residue and the aglycon as C-glucosidic in character. Comparison of the ^{13}C NMR spectral data with the literature indicates the presence of a C-linked substituent at C6, and it is evident from the shift of the C6 signal to δ 108.8 ppm from its normal position at δ 98.0 ppm in the 5,7-dihydroxyl flavonoids that compound **3** was luteolin-6-*C*-glucoside (isoorientin) (Agrawal, 1989; Minh-Duc-luong et al., 1976).

Compound 4 responded positively as a flavonoid glucoside to Mg−HCl color and Molisch tests. On acid hydrolysis, **4** yielded tricin as the aglycon and glucose and rhamnose as the sugar moiety by comparison with an authentic sample on TLC. The FAB mass spectrum gave the quasimolecular ions at *m/e* 663 ([M + Na]$^+$), 640 ([M$^+$]), which suggested the M_r is 640, and fragment ions at *m/e* 493 ([M − 146 + H]$^+$), 330 ([M − 146 − 162 + H]$^+$, 100%), indicating **4** was a biglycoside with glucosyl as the inner unit and rhamnosyl as the terminal unit. The ^{1}H NMR and ^{13}C NMR signal patterns of the aglycon of **4** were superimposable with that of **2**, which suggested the sugar chain was also linked to the 7-position hydroxyl of tricin. The anomeric proton signal of glucose of **4** at δ 5.21 (d, J = 6.5 Hz) showed that the glucosyl band of **4** had the β-configuration. However, on the basis of the coupling constant of the anomeric proton of rhamnose in the ^{1}H NMR spectrum, the α,β nature could not be deduced, but α-rhamnose could be identified from its ^{13}C NMR chemical shifts, with C5 of α- and β-rhamnose appearing at δ 69.8 and 73.5, respectively (Soe et al., 1978). Since the C5 signal of rhamnose in compound **4** was at δ 69.8, it was concluded that the rhamnose had the α-configuration. Finally, **4** was determined as 7-*O*-[α-L-rhamnopyranosyl(1−6)−β-D-glucopyranosyl]tricin, and the spectral data were identical to the literature (Chari et al., 1977, Kurlin et al., 1982). The structures of these four compounds are shown in Figure 1.

To determine whether the active component came from the grass or from the fungus, the leaves, spikelets, stromata, and seeds were collected. *Acremonium typhnium* was cultured and extracted as described above. Extracts from each of these portions were tested for activity, and the results indicated that only the extracts from, or including, the plant tissue possessed the toxic properties of the crude extract and that extracts of the isolated fungus displayed little or no toxicity (Table 3). We conclude that the bioactive components appear to be produced by the fungal-infected grass tissues and not by the isolated endophyte itself. In a literature search, no reports could be found by the authors which indicate that flavonoids are synthesized by fungi.

From the bioactive fractions, two flavonoid glucosides of tricin were isolated, compound **2**, a monosaccharide glycoside which was quite toxic to mosquito larvae, while compound **4**, the disaccharide, possessed little toxicity. The lack of toxicity afforded tricin by addition of a single sugar group to a flavanoid may give a significant clue in studying the structure−activity relationship of these secondary metabolites of endophyte-infected grasses (Figure 1).
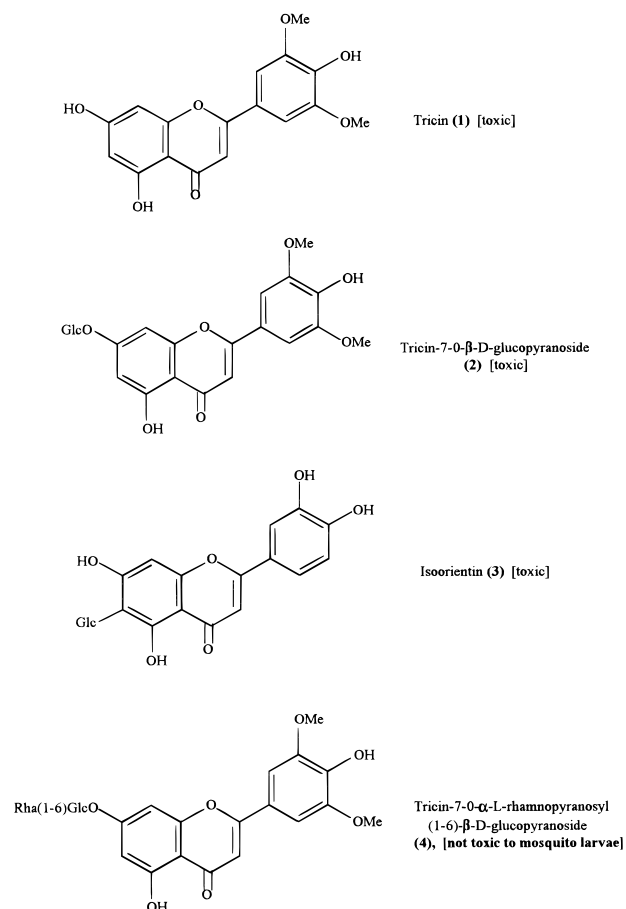

Figure 1. Structures of biologically active and inactive compounds isolated from endophyte-infected grass (*Poa ampla*).

Table 3. Mortality of Mosquito Larvae Exposed to Two Concentrations of Extracts of Various Portions of Endophyte-Infected *Poa ampla* (from 2.5 kg of Plant Tissue) and from Isolated Endophyte[a]

	100 μL/mL	400 μL/mL
distilled H$_2$O	0a	0a
isolated fungus	19b	23b
leaves	61c	90c
seeds	52c	97c
spikelets	71c	100c
stromata	70c	93c

[a] Means within columns followed by the same letter are not significantly different, using χ^2 test. P < 0.05.

In summary, three of the four compounds isolated from endophyte-infected grass were found to be active in a mosquito larvae bioassay. Although these compounds have been previously isolated, this is the first report of the investigation of their bioactivity against mosquito larvae. The plant tissues and not the isolated endophyte were found to possess activity in this bioassay.

Tricin (1). Yellow powder; mp 285−289 °C. IR (ν^{KBr}, cm^{-1}): 3330 (OH), 2947, 1654 (C=O), 1611, 1506 (Ar−H). UV (MeOH; λ_{max}, nm): 215, 259, 269, 348. ^{1}H NMR (DMSO-d_6): δ 11.47.17 (1H, s, 12-OH), 7.30 (2H, s, H2′, H6′), 7.00 (1H, s, H3), 6.56 (1H, d, J = 1.8 Hz, H8), 6.21 (1H, d, J = 1.8 Hz, H6), 3.88 (6H, s, 2× OMe). EI-MS (*m/e*): 330 (M$^+$, 100%), 302, 287, 213, 178, 153, 135.

7-*O*-β-D-Glucopyranosyltricin (2). Yellow crystal; mp 300 °C (decomp.). IR (ν^{KBr}, cm^{-1}): 3400 (OH), 2919, 1629 (C=O), 1615, 1491 (Ar−H). UV (MeOH; λ_{max},

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nm): 212, 250, 278, 242. FAB-MS (*m/e*): 493 ([M + H]$^+$), 492 (M$^+$), 330 ([M − 162]$^+$, 100%), 178, 163, 148, 153. ^{1}H NMR: δ 11.50 (1H, s, 12-OH), 9.24 (2H, br, OH), 7.31 (2H, s, H2′, H6′), 6.87 (1H, s, H3), 6.87 (1H, d, *J* = 1.9 Hz, H8), 6.57 (1H, d, *J* = 1.9 Hz, H6), 5.12 (1H, d, *J* = 6.5 Hz, anomeric proton of Glu-H‴), 3.90 (6H, s, 2 OMe). ^{13}C NMR (aglycon): δ 164.7, 105.7, 184.0, 163.0, 101.0, 164.7, 99.3, 158.9, 104.9, 122.4, 149.7, 141.0, 149.6, 105.1, 57.0. ^{13}C NMR (Glu): δ 104.7, 74.1, 78.6, 71.3, 77.3, 62.6. Acid hydrolysis of **2** afforded tricin and glucose by comparison the TLC with authentic samples.

Lutelin-6-*C*-β-D-glucopyranoside (isoorientin, 3). Mp 270 °C (decomp.). IR (ν^{KBr}, cm^{-1}): 3400, 2920, 1653 (C=O), 1622, 1482 (Ar−H). UV (MeOH; λ_{max}, nm): 214, 257, 279, 347, 361. FAB-MS (*m/e*): 471 ([M + Na]$^+$), 449 ([M + H]$^+$), 353, 329, 315, 167, 155, 135, 119. ^{1}H NMR (DMSO-d_6): δ 12.55 (1H, s, 12-OH), 10.57 (1H, br, OH), 9.90 (1H, br. OH), 9.41 (1H, OH), 7.4 (2H, m, H2′, H6′), 6.87 (1H, d, *J* = 8.0 Hz, H5′), 6.66 (1H, s, H8), 6.46 (1H, s, H3), 4.56 (1H, d, *J* = 9.5 Hz). ^{13}C NMR: δ 163.9, 102.8, 181.8, 160.6, 108.8, 163.2, 93.5, 156.2, 103.4, 121.4, 113.3, 145.7, 149.7, 116.0, 118.9. ^{13}C NMR (*C*-Glu): δ 73.0, 70.6, 78.9, 70.2, 81.5, 61.5.

7-*O*-[α-L-Rhamnopyranosyl(1−6)−β-D-glucopyranosyl]tricin(4). Yellow powder; mp 310 °C. IR (ν^{KBr}, cm^{-1}: 3400 (OH), 2923, 1655 (C=O),1616, 1495 (Ar−H). UV (MeOH; λ_{max}, nm): 213, 248, 276, 348. FAB-MS (*m/e*): 678 ([M + K]$^+$, 662 ([M+Na]$^+$), 640 (M$^+$), 493 ([M − 146 + H]$^+$), 331 ([M − 146 − 162 + H]$^+$). ^{1}H NMR: δ 12.50 (1H, s, 12-OH), 7.38 (2H, s, H2′, H6′), 7.06 (1H, s, H3), 6.86 (1H, d, *J* = 1.9 Hz, H8), 6.50 (1H, d, *J* = 1.9 Hz, H6), 5.21 (1H, d, *J* = 6.5 Hz, anomeric proton of Glu-H‴), 4.70 (1H, s, anemmeric proton of Rha), 3.90 (6H, s, 2× OMe), 1.09 (3H, d, *J* = 5.6 Hz, Rha−CH$_3$). ^{13}C NMR (aglycon): δ 166.5, 105.7, 184.0, 163.0, 102.0, 164.7, 96.4, 158.9, 104.9, 122.5, 104.9, 149.7, 141.0, 149.7, 105.7, 57.2. ^{13}C NMR (inner glu): δ 101.4, 74.1, 77.8, 71.2, 77.1, 67.3. ^{13}C NMR (terminal Rha): δ 101.0, 72.3, 72.1, 74.8, 69.8, 17.9. Acid hydrolysis of **2** afforded tricin, rhamnose, and glucose by comparison the TLC with authentic sample.

LITERATURE CITED

Agrawal, P. K. *Carbon-13 NMR of flavonoids*; Elsevier: Amsterdam, 1989; 330−331.

Bhattacharyya, J.; Stagg, D.; Mody N. Y.; Miles, D. H., Constituents of *Spartin cynuroides*: Isolation and ^{13}C NMR analysis. *J. Pharm. Sci.* **1978**, *67*, 1325−1236.

Breen, J. P. Acremonium endophyte interactions with enhanced plant resistance to insects. *Annu. Rev. Entomol.* **1994**, *39*, 401−423.

Bush, L. P.; Fannin, F. F.; Siegel, M. R.; Dahlman, D. L.; Burton, H. R. Chemistry, occurrence and biological effects of saturated pyrrolizidine alkaloids associated with endophyte-grass interactions. *Agric. Ecosystems Environ.* **1993**, *44*, 81−102.

Chari. V. M.; Jordan, M.; Wagner; H.; Thies, P. W. A ^{13}C NMR study of the structure of an acl-linarin from *Valenriana wallichi. Phytochemistry* **1977**, *16*, 1110−1112.

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

Clay, K.; Cheplick, G. P. Effect of ergot alkaloids from fungal endophyte-infected grasses on fall armyworm (*Spodoptera frugiperda*). *J. Chem. Ecol.* **1989**, *15*, 169−182.

Eichenseer, H.; Dahlman, D. L. Antibiotic and Deterrent Qualities of Endophyte-Infected Tall Fescue to Two Aphid Species (*Homoptera: Aphididae*). *Environ. Entomol.* **1992**, 146−1051.

Eichenseer, H.; Dahlman, D. L. Survival and development of the true armyworm *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae), on endophyte-infected and endophyte-free tall fescue. *J. Entomol, Sci.* **1993**, *28*, 462−467.

Garner, G. B.; Rottinghaus, G. E.; Cornell, C. N.; Testereci, H. Chemistry of compounds associated with endophyte/grass interaction: Ergovaline- and ergopeptine-related alkaloids. *Agric. Ecosystems Environ.* **1993**, *44*, 65−80.

Hardy, T. N.; Clay, K.; Hammond, A. M. Leaf age and related factors affecting endophyte-mediated resistance to fall armyworm (Lepidoptera: Noctuidae) in tall fescue. *Environ. Entomol.* **1986**, *15*, 1083−1089.

Kurlin, V. A.; Zapesochnaya, G. C.; Klyaznika, V. G. Flavonoids from the rhizomes of *Rhodiola rosea*. I. Tricin glucosides. *Chem. Nat. Compd.* **1982**, *18* (5), 550−552.

Lindstrom, J. T.; Sun, S.; Belanger, F. C. A novel fungal protease expressed in endophytic infection of *Poa* species. *Plant Physiol.* **1993**, *102*, 645−650.

Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The systematic identification of flavonoids*; Springer: Berlin, 1970.

Markham, K. R. *Techniques of flavonoid identification*; Academic Press: London, 1982.

Miles, D. H.; Tunsuwan. K.; Chiiwong, V. Boll weevil antifeedants from *Eleocharis dulcis* Trin. *J. Agric. Food. Chem.* **1994**, *42*, 1561−1562.

Minh-Duc-luong; Hostettmann, K.; Jacot-Guilarmond, A. Phytochemistry of Genus Gentiana. XIV. Identification of new derivatives of isoorientin in the leaves of *Gentiana burseri* Lapeyr. *Helv. Chim. Acta* **1976**, *59* (4), 1294−1296.

Patterson, C. G.; Potter, D. A.; Fannin, F. F. Feeding deterrency of alkaloids from endophyte-infected grasses to Japanese beetle grubs. *Entomol. Exp. Appl.* **1991**, *61*, 285−289.

Rowan, D. D. Lolitrems, peramine and paxilline: Mycotoxins of the ryegrass/endophyte interaction. *Agric. Ecosystems Environ.* **1993**, *44*, 103−121.

Saha, D. C.; Jackson, M. A.; Johnson-Cicalese, J. M. A rapid staining method for detection of endophytic fungi in turf and forage grasses. *Phytopathology* **1988**, *78*, 237−239.

Siegel, M. R. *Acremonium* endophytes: Our current state of knowledge and future directions for research. *Agric., Ecosystems Environ.* **1993**, *44*, 301−321.

Soe, S.; Tomita, Y.; Tori, K.; Yoshimuri, Y. Determination of the absolute configuratioin of a secondary hydroxyl group in a chiral secondary alcohol using glycosidation shift in carbon-13 nuclear magnetic resonance spectroscopy. *J. Am. Chem. Soc.* **1978**, *100*, 3331−3338.

Tulloch, A. P. Epicuticular wax of *Poa ampla* leaves. *Phytochemistry* **1977**, *17*, 1613−1615.

White, R. H.; Engelke, M. C.; Morton, S. J.; Johnson-Cicalese, J. M.; Ruemmele, B. A. Acremonium endophyte effects on tall fescue drought resistance. *Crop Sci.* **1992**, *32*, 1392−1396.