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Clavicipitaceous Anamorphic Endophytes in *Hordeum* germplasm

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Abstract: The incidence of clavicipitaceous anamorphic endophytes, non-choke inducing endosymbiotic fungi of the genus *Neotyphodium* that systemically infect grasses, in eighteen *Hordeum* species from the U.S. National Plant Germplasm System was examined using light and Scanning Electron Microscopy (SEM). Seventeen plant inventory accessions from only three *Hordeum* species, including *H. bogdanii*, *H. brevisubulatum* subsp. *violaceum* and *H. comosum*, were found to contain significant levels of seed and seedling infection ranging from 18-99%. *Neotyphodium*-endophytes were found in *Hordeum* germplasm from one country (Argentina) in South America and four countries (Afghanistan, China, Iran and Kazakhstan) in Asia. The viability of endophytic mycelium in seeds was confirmed by culturing these fungi on potato dextrose agar medium from aleurone tissue of seeds and by direct observations of hyphae in leaf sheaths of 3-4 week-old seedlings. Morphological characteristics of these fungi were further characterized using SEM to determine similarities and differences in conidiophores and conidia produced in culture. The importance, significance and potential benefits of *Neotyphodium*-endophytes from wild *Hordeum* as sources of insect resistance in cultivated barley, other cereal grasses and in wild grasses included in the Conservation Reserve Program are discussed. Suggested strategies for the proper maintenance of this valuable germplasm also are elucidated.

Key words: *Neotyphodium* species, wild barley, biological control, Clavicipitaceae, insect resistance

INTRODUCTION

Clavicipitaceous endophytes are an important group of fungi with world-wide distribution in graminaceous hosts of the Poaceae. These fungi form relationships with grasses ranging from mutualistic to parasitic (Carroll, 1988; Clay, 1988). Consequently, they may be divided into two major groups, Clavicipitaceous Teleomorphic (CT) endophytes and Clavicipitaceous Anamorphic (CA) endophytes, based on the relationships and trophic interactions formed with their grass hosts (Wilson *et al*., 1991a; Wilson, 1996). Clavicipitaceous teleomorphic endophytes (Clavicipitaceae: tribe Balansiae) are pathogenic and induce choke diseases of grasses that abort flowering and preclude seed production due to the formation of subterminal sclerotia on the culm. This effect is different from the ergot fungi caused by *Claviceps* species that do not prevent flowering, but cause ergotised grain. Clavicipitaceous anamorphic endophytes of the genus *Neotyphodium* Glenn, Bacon, Price and Hanlin gen. nov. (formerly *Acremonium* section *Albolanosa* Morgan-Jones and Gams) (Glenn *et al*., 1996), are a closely related group of fungi, probably derivatives of the teleomorphic forms (Clay, 1990; Schardl and Phillips, 1997), that have lost the ability to produce a sexual stage (teleomorph) and to cause diseases of grasses. This group of mutualistic endophytic fungi has received considerable attention since the late 1970s when it was discovered that they are responsible for causing serious toxicoses of livestock (primarily cattle, sheep and horses) that consume endophyte-infected forage grasses (Bacon *et al*., 1997), but also provide important sources of resistance in grasses to a wide variety of insects (Clement *et al*., 1994; Rowan and Latch, 1994; Anderson *et al*., 2006) and diseases (Schmidt, 1990). The biologically active compounds responsible for the toxic syndromes in livestock and insect resistance were later discovered to be ergot alkaloids (clavine, lysergic acid and ergopeptine) (Lyons *et al*., 1986; Yates and Powell, 1988), loline (pyrrolizidine) alkaloids (Yates *et al*., 1990; Siegel *et al*., 1990; Bush *et al*., 1993), lolitrems (Miles *et al*., 1992, 1993) and peramine (pyrrolopyrazine) alkaloids (Siegel *et al*., 1990, Rowan *et al*., 1986). Economic losses in animal production caused by endophyte toxicoses in cattle and sheep annually exceed $100s millions in U.S. dollars (Siegel *et al*., 1985). Endophyte-infected grass cultivars have been utilized for the biocontrol of insect and disease

pests primarily in the lawn or turf-grass industry (Gwinn and Gavin, 1992; Greulich *et al*., 1999; Clarke *et al*, 2006).

Clavicipitaceous anamorphic endophytes of the genus *Neotyphodium* are known only from the subfamily Pooideae that dominate temperate areas of the world and have the C3 photosynthetic mechanism (Clay, 1994, 1997). Neotyphodium-endophytes are very host specific (Clay, 1992) and have been found in many cool-season grasses such as *Achnatherum* (*Stipa*) (Bruehl *et al*., 1994; Jones *et al*., 2000), *Alopecurus* (Zabalgogeazcoa *et al*., 1999), *Bromus* (White *et al*., 2001; Brem and Leuchtmann, 2003; Mirlohi *et al*., 2006), *Dactylis* (Zabalgogeazcoa *et al*., 1999), *Echinopogon* (Miles *et al*., 1998), *Elymus* (Vinton *et al*., 2001; Nan and Li, 2001), *Festuca* (Hahn *et al*., 2003; Koh *et al*., 2006), *Holcus* (Zabalgogeazcoa *et al*., 1999), *Hordeum* (Wilson *et al*., 1991a, 1991b; 1991c), *Lolium* (Wilson *et al*., 1992; Lewis *et al*., 1997), *Poa* (Cabral *et al*., 1999) and diploid *Triticum* species (Marshall *et al*., 1999). The discoveries of *Neotyphodium*-endophytes in goatgrass species, *Aegilops mutica* (Boiss.) Eig and *A. uniaristata* Vis., represent the first diploid cereal grass species with endophytes that could be hybridized with cultivated wheat (*Triticum aestivum* L.) (Marshall, 1993). All *Neotyphodium*-endophytes form predominantly asymptomatic interactions with their hosts, systemically infect only aboveground parts within intercellular spaces, overwinter in meristematic tissues, are maternally transmitted via the seed and often impart superior performance to their grass hosts (Wilson, 1996; Clay, 1997; Malinowski and Belesky, 2000).

The National Plant Germplasm System (NPGS), administered by the United States Department of Agriculture, Agricultural Research Service (ARS), is the largest source of plant germplasm maintained in the United States. A large number of Plant Inventory (PI) accessions of numerous grass genera are available in the NPGS which potentially could be endophyte infected (Wilson, 1996). Surveys of this germplasm, especially in the cool-season grasses, have proven useful for discovering many new sources of endophyte-infected PI accessions in *Festuca* (Springer and Kindler, 1990), *Lolium* (Wilson *et al*., 1991d) and *Poa* species (Wilson, 1996). The objectives of this research were to conduct a microscopic survey of plant inventory accessions of a portion of the *Hordeum* species found in the NPGS to determine the incidence and rates of infection of seeds and seedlings by CA-endophytes, particularly endosymbiotic fungi of the genus *Neotyphodium* and to culture and characterize isolates from infected seeds and seedlings using scanning electron microscopy. Supporting information about *Hordeum* germplasm characteristics, collection data and some preliminary results of this research were reported previously (Wilson *et al*., 1991a-c).

MATERIALS AND METHODS

Seed source: Seed samples of 142 PI accession lines from a portion of the *Hordeum* sp. germplasm collection, previously held at the Western Regional Plant Introduction Station (WRPIS) prior to 22 November 1989, were obtained from the Small Grains Germplasm Research Facility in Aberdeen, Idaho that presently maintains the U.S. *Hordeum* collection in the NSGC. Seeds were previously stored at 4-5°C and 30-35% relative humidity at the WRPIS and subsequently at 6-7°C and 36-38% relative humidity since 22 November 1989 at the NSGC. Most accessions had been increased one or two times since original seeds were received into the collection. The oldest seeds of each accession were selected since original seeds often were unavailable.

Endophyte seed and seedling assays: Seeds of 96 PI accessions of seventeen *Hordeum* species including *H. agriocrithon* Aberg, *H. bogdanii* Wilensky, *H. brachyantherum* Nevski, *H. brevisubulatum* (Trin.) Link, *H. bulbosum* L., *H. californicum* Covas and Stebbins (= *H. brachyantherum* subsp. *californicum* (Covas and Stebbins) Bothmer *et al*.), *H. capense* Thunb., *H. chilense* Roem. and Schult., *H. comosum* J. Presl, *H. jubatum* L., *H. lechleri* (Steud.) Schenck, *H. marinum* Huds., *H. murinum* L., *H. procerum* Nevski, *H. secalinum* Schreb., *H. stenostachys* Godr. and *H. vulgare* L. were examined microscopically for clavicipitaceous anamorphic endophyte, specific endosymbiotic fungi of grasses as defined by Wilson (1996). This survey represented 67.6% of available accessions that were examined. At least 40% of available PI accessions of *H. brevisubulatum* subsp. *violaceum* (Boiss. and Hohen.) Tzvelev, *H. marinum* subsp. *gussoneanum* (Parl.) Thell., *H. murinum* subsp. *glaucum* (Steud.) Tzvelev and *H. murinum* subsp. *leporinum* (Link) Arcang. also were examined. The *Hordeum* germplasm examined here originated from many countries within five continents of the world, including North America (NA), South America (SA), Europe (EU), Africa (AF) and Asia (AS).

Seed samples, 0.5-1.3 g depending on seed size, were soaked overnight in 5% sodium hydroxide at 22°C, rinsed with tap water and stained for several days at 22°C in 0.07% analine blue as described previously (Wilson *et al*., 1991d). Seeds were then rinsed, squashed and mounted on slides in 1:1 v/v glycerol-distilled water and examined with a Zeiss compound microscope at 100-400x

magnification within tissues of the aleurone layer and outer integument. Seed infection rates were based on examinations of 50 seeds in endophyte-free accessions and 100 seeds in endophyte-infected accessions. Seedlings of some infected accessions were grown in a greenhouse under natural light in 15 cm pots containing 55% peatmoss, 35% pumice and 10% sand. Leaf sheaths from 50-100 7-9 week old seedlings were examined microscopically at 400× to determine endophyte viability.

Isolation and culture: Clavicipitaceous endosymbiotic fungi from endophyte-infected accessions were isolated on Potato Dextrose Agar (PDA) from aleurone tissue of surface-sterilized seeds and from infected leaf sheaths of seedlings. For seed isolations, seed were soaked in 10% Clorox for 3-5 min, rinsed several times in sterile distilled water and plated on 3.5% PDA with or without 0.2% streptomycin sulfate to reduce bacterial contamination. For leaf sheath isolations, seedlings were generated from seed taken from NSGC storage by placing seeds on moist filter papers under 12 h fluorescent lights until germination. Germinated seeds were then transferred to a 55% peat moss 35% pumice and 10% sand soil mixture and grown in the greenhouse under conditions described previously (Wilson *et al*., 1991d). Seedlings were allowed to grow for several weeks to allow the endophytic fungi to grow out of the seed into leaf sheath tissue. Leaf sheaths were then collected from the base of stems, surface sterilized in 5% Clorox for 1-5 min. and plated onto 3.5% PDA using aseptic techniques. Colonies generated on plant tissues were further purified by transferring small amounts of clean inoculum to new plates.

Scanning electron microscopy: Mycelial plugs taken from cultures in exponential growth were fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1M Pipes buffer, rinsed in 0.1M potassium phosphate buffer, postfixed in 1.0% osmium tetroxide and rinsed again in phosphate buffer. The mycelial plugs were dehydrated in an ethanol series, critical point dried and sputter coated with gold. Morphological characteristics of mycelia, conidiophores and conidia from each isolate were examined and compared at 5-15,000x magnification using a Hitachi 5571 scanning electron microscope. Data from SEM observations of CA-endophytes from each *Hordeum* species were taken including conidiophore grouping, conidiophore shape and taper, presence or absence of basal septum, conidia shape, conidial orientation on conidiophores and spore dimensions. A minimum of fifty conidial measurements were taken from SEM photographs for determining the range of dimensions for endophytes of each *Hordeum* species.

RESULTS

Endophyte seed and seedling assays: Endophytic hyphae in seeds of infected *Hordeum* species were concentrated primarily in the aleurone tissue and to a lesser extent, in the outer integument tissue of the seed. A microscopic survey of CA-endophytes in aleurone seed tissue of 96 PI accession lines, representing seventeen species of *Hordeum* germplasm from the NSGC, indicated that the majority of accessions of most *Hordeum* species examined were CA-endophyte free (Table 1). Only 17 (18%) of the 96 accessions examined contained hyphae of *Neotyphodium* species within aleurone and outer integument seed tissues. These *Neotyphodium* endophyte-infected accessions were found in only three *Hordeum* species (17.6%) of the species examined in this survey. Most of the endophyte-infected accessions were found in *H. bogdanii* (70.6%) and *H. brevisubulatum* subsp. *violaceum* (11.8%) germplasm that originated from Asia. The remainder of *Neotyphodium* endophyte-infected accessions was found in *H. comosum* seeds originating from South America. None of the endophyte-infected *Hordeum* germplasm originated from North America, Europe, Africa, or Australia. All source countries of infected germplasm were in temperate regions either north or south of the equator (Table 2). *Neotyphodium*-infected accessions of *H. bogdanii* originated primarily from Kazakhstan and China, although one infected accession was found from Afghanistan. The *H. brevisubulatum* subsp. *violaceum* infected accessions originated from Kazakhstan and Iran whereas all *Neotyphodium*-infected accessions of *H. comosum* originated from Argentina.

Endophyte infection rates in *Hordeum* seeds and seedlings germplasm varied widely among individual accessions (Table 2). Seed infection rates within individual accessions ranged from 18-99% infection in *H. bogdanii*, 68-98% infection in *H. brevisubulatum* subsp. *violaceum* and 74-92% infection in *H. comosum*. Infection rates determined from growth of endophytes up into leaf sheafs of infected seedlings were usually similar or higher than seed infection rates from the same accession, indicating that most inoculum in aleurone seed tissue was viable (Table 2). Higher rates of infection in seedlings relative to seeds indicated that small amounts of inoculum not detected in seeds were detectable in seedlings. Seedling infection rates lower than apparent seed infection rates in the same accession generally indicated that inoculum in some individual seeds was not viable. Plant inventory accessions collected from wet habitats on uncultivated lands tended to have higher seed-infection rates, whereas accessions from dry habitats on cultivated or grazed lands were often endophyte-free.

Table 1: Incidence of clavicipitaceous anamorphic endophytes in seeds of the NSGC *Hordeum* germplasm collection[1]

	Number of accessions[2]			Continent of origin[3]				
Species	Available	Examined	Infected	NA	SA	EU	AF	AS
H. agriocrithon	1	1	0	0	0	1	0	0
H. bogdanii	16	16	12	0	0	0	0	16
H. brachyantherum	5	5	0	5	0	0	0	0
H. brevisubulatum	2	2	0	0	0	0	0	2
H. brevisubulatum subsp. *violaceum*	25	15	2	0	0	0	0	15
H. bulbosum	54	22	0	0	0	4	2	16
H. californicum	2	2	0	2	0	0	0	0
H. capense	1	1	0	0	0	0	1	0
H. chilense	3	3	0	0	3	0	0	0
H. comosum	4	4	3	0	4	0	0	0
H. jubatum	3	3	0	2	0	0	0	1
H. lechleri	2	2	0	0	2	0	0	0
H. marinum	2	2	0	0	0	0	0	2
H. marinum subsp. *gussoneanum*	2	2	0	0	0	0	0	2
H. murinum	1	1	0	0	1	0	0	0
H. murinum subsp. *glaucum*	3	3	0	0	0	0	0	3
H. murinum subsp. *leporinum*	6	6	0	0	0	0	0	6
H. procerum	3	1	0	0	1	0	0	0
H. secalinum	4	2	0	0	0	2	0	0
H. stenostachys	1	1	0	0	0	0	1	0
H. vulgare	2	2	0	0	0	2	0	0
Total	142	96	17	9	11	9	4	63

[1]Incidence is based on examinations of 100 seeds in endophyte-infected accessions and 50 seeds in endophyte-free accessions of the National Small Grains Collection (NSGC) of *Hordeum* species. [2]Accessions from the National Small Grains Collection (NSGC) in Aberdeen, Idaho. [3]Abbreviations for continents of origin of all accessions examined: North America (NA), South America (SA), Europe (EU), Africa (AF), Asia (AS). Information on specific endophyte-free accessions is available from the Germplasm Resources Information Network (GRIN) database, USDA-ARS

Table 2: *Neotyphodium* endophyte-infected accessions of the NSGC *Hordeum* germplasm collection[1]

Species	Accession (PI)	Country of origin	Year received[2]	Mean % endophyte[3]
H. bogdanii	269406	Afghanistan	1960	47 (53)
	314696	Kazakhstan	1966	62 (47)
	440413	Kazakhstan	1978	88 (95)
	440414	Kazakhstan	1978	80 (80)
	499499	China	1984	18
	499500	China	1984	52
	499501	China	1984	77
	499643	China	1985	47
	499644	China	1985	99
	499645	China	1985	94
	499646	China	1985	97
	531761	China	1988	93
H. brevisubulatum ssp. *violaceum*	401386	Iran	1975	68 (63)
	440420	Kazakhstan	1978	98 (100)
H. comosum	264404	Argentina	1960	86
	264405	Argentina	1960	92
	269648	Argentina	1960	74

[1]Plant Inventory (PI) accessions of the National Small Grains Collection (NSGC) in Aberdeen, ID, were held at the Western Regional Plant Introduction Station (WRPIS) in Pullman, WA prior to 22 Nov 1989. [2]Date the accession was received into the WRPIS *Hordeum* germ plasm collection. [3]Infection rates were based on examinations of 100 seeds. Values in parentheses indicate viable endophyte as determined from examinations of 50-100 leaf sheaths of 7-9 week-old seedlings

Microscopic examinations of *Neotyphodium*-endophytic hyphae in aleurone seed tissues and leaf sheafs of 7- to 9-week old seedlings revealed a diversity of morphological types in accessions of endophyte-infected *Hordeum* species. *Neotyphodium* hyphae in *H. bogdanii* PI accession 440413 was highly torulose with little branching in aleurone seed tissue (Fig. 1A), but relatively straight between leaf sheaf cells of seedlings (Fig. 1B). Somatic hyphae of the *Neotyphodium* endophyte in *H. brevisubulatum* subsp. *violaceum* aleurone tissue of PI accession 440420 was sinuous, of finer diameter and contained some hyphal segments that were straighter and less curvilinear (Fig. 2A). By contrast, endophytic hyphae in leaf sheafs of PI accession 440420 were much thicker, straight and with occasional branching between cells across the long axis of the leaf sheaf (Fig. 2B). Hyphae of the *Neotyphodium* endophyte in aleurone tissue of *H. comosum* PI accession 264405 were much less sinuous, but straighter, with abundant branching (Fig. 3A). Hyphae within the outer integument of the seed tissue in PI accession 264405 also exhibited frequent branching perpendicular to the long axis of the cells (Fig. 3B). All *Neotyphodium* hyphae within aleurone and outer integument seed tissues and in leaf sheaf tissues of seedlings grew only intercellularly and never penetrated cell walls, typical of true endophytes.

Cultural characteristics of endophytes: The CA-endophytes isolated from infected aleurone seed tissue were identified as *Neotyphodium* species in all cases based on cultural characteristics, somatic hyphae and

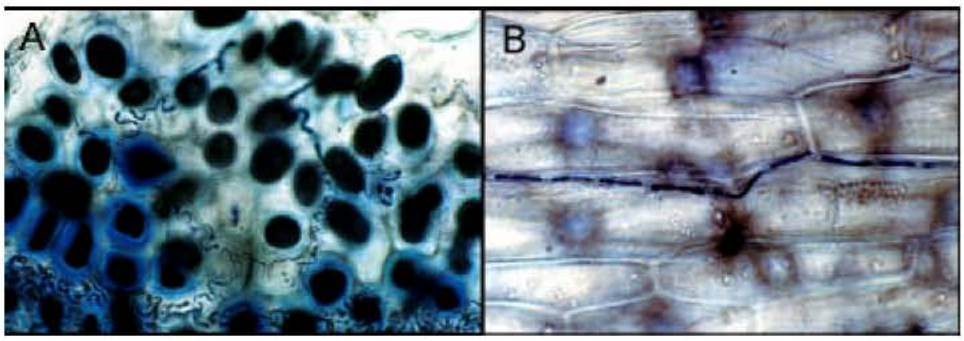

Fig. 1: *Neotyphodium*-endophyte in *H. bogdanii* tissue. (A) Torulose hyphae within the aleurone layer of seed, PI accession 499499. (B) Straight hyphae within leaf sheath at the base of stem, PI accession 440413

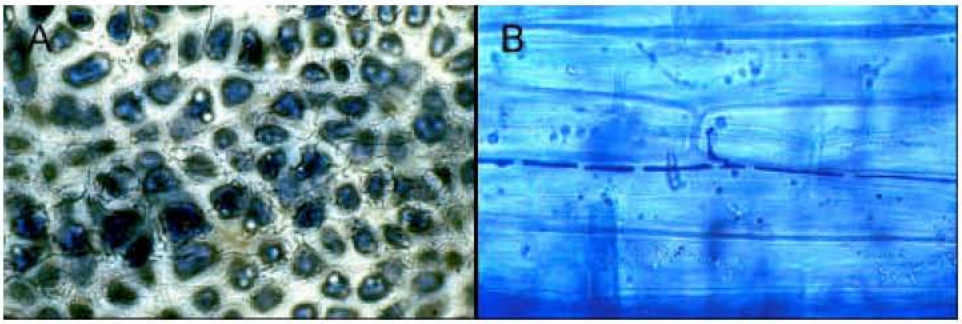

Fig. 2: *Neotyphodium*-endophyte in *H. brevisubulatum* subsp. *violaceum* tissue of PI accession 440420. (A) Convoluted hyphae within the aleurone layer of seed. (B) Straight hyphae with a branch between two cells in the leaf sheath, parallel to the long-axis of the stem

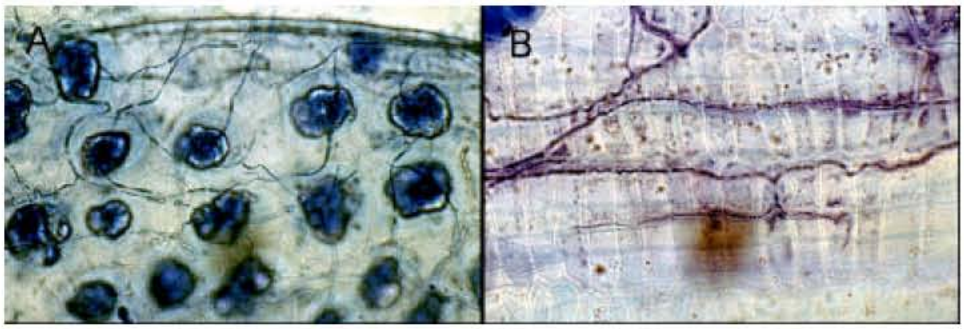

Fig. 3: *Neotyphodium*-endophyte in *H. comosum* tissue of PI accession 264405. (A) Sinuous hyphae within the aleurone layer of seed. (B) Straight and branched hyphae in the outer integument of seed

conidiophore morphology. These identifications were confirmed by subsequent observations of endophytic hyphae in the leaf sheafs of infected *Hordeum* seedlings and in SEM observations. Cultures of *Neotyphodium*-endophytes from *Hordeum* germplasm were usually slow-growing (< 20 mm/week), white to tan in color, with oppressed or raised growth, waxy to cottony, often puckering the agar as moisture was removed and producing seed colonies from germinating conidia as they were formed and released from conidiophores. Somatic hyphae of *Neotyphodium*-endophytes in *Hordeum* germplasm were typical of CA-endophytes in that they tended to be fine (<5 μm diameter), convoluted, torulose, or sinuous, highly vacuolated cells, rarely or infrequently branching, occasionally constricted at the septa and having septa that stained poorly or not at all. Sporulation was abundant with most strains. Conidiophores were produced abundantly, usually perpendicular to hyphal

strands and singly or grouped into synnemata. Cresent-shaped and elliptical or allantoid conidia formed singly at the ends of determinant conidiophores via holoblastic conidiogenesis.

Species of *Neotyphodium* endophytes isolated from *Hordeum* germplasm were not determined because of the common overlap in spore and conidiophore measurements between *Neotyphodium* species and the limited number of morphological and cultural characteristics that are available to differentiate species. Consequently, reliable identifications of individual *Neotyphodium* endophytes to species generally require the use of DNA-sequencing data. Such identifications were beyond the scope of this study.

Hyphal growth of *Neotyphodium* endophytes on the agar surface generally resulted in highly wrinkled colonies as growth continued and moisture was removed from the surface layers of the agar (Fig. 4A). Mycelium observed on the edge of colonies grew radially in dense, parallel masses of convoluted hyphae that tended to form appressed growth, particularly in the endophyte of *H. brevisubulatum* subsp. *violaceum* from PI accession 440420 (Fig. 4 B). Convoluted hyphae that grew radially, relative to the center of the colony, maintained a parallel growth pattern and rarely crossed over adjacent hyphae. By contrast, endophyte cultures that tended to form synnemata, such as those from certain *H. bogdanii* accessions, produced ropelike strands of synnemata that frequently crossed adjacent strands and produced raised aerial cottony growth (Fig. 4 C). The torulose somatic growth form, characteristic of appressed colonies in exponential growth, continued until the colony was sufficiently old to initiate sporulation. Sporulation from determinate conidiophores was initiated in 2- to 4-week old cultures. Seed colonies often formed later from germinating conidia released from conidiophores.

Scanning electron microscopy: Analysis of *Neotyphodium*-endophyte conidiophores and conidia morphology using SEM revealed differences in groupings, shape and taper and occurrence of basal septa with conidiophores, as well as variations in shape, orientation and dimensions of conidia of endophytes from different *Hordeum* species and accessions. Conidiophores of the *H. bogdanii* endophyte in PI accession 440413 were densely compacted to form synnemata composed of long, thin-tapering conidiophores that contained a basal septum (Table 3). By contrast, conidiophores of the *H. bogdanii* endophyte isolated from PI accession 314696 occurred singly and

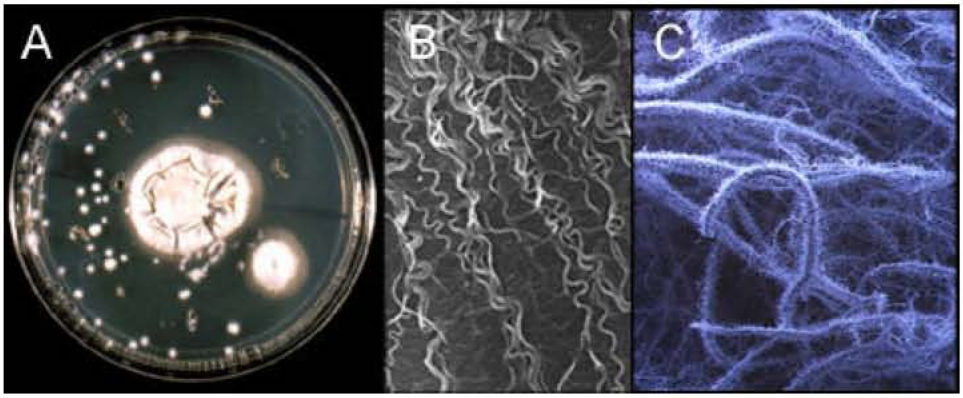

Fig. 4: Growth and culture of *Neotyphodium*-endophytes on 3.5% PDA medium. (A) Culture of endophyte isolated from *H. brevisubulatum* subsp. *violaceum* (PI accession 440420) with seed colonies arising from the mother colony at the center of the Petri dish. (B) SEM of convoluted hyphae of *H. bogdanii* endophyte from PI accession 314696 on the agar surface. (C) SEM of raised synnematal strands of *H. bogdanii* endophyte from PI accession 269406

Table 3: Morphological characteristics *in vitro* of conidiophores and conidia of *Neotyphodium* strains isolated from accessions of *Hordeum* germplasm

	Accessions	Conidiophores[1]			Conidia[2]		
Species	(PI)	Grouping	Shape and taper	Basal septum	Shape	Orientation	Dimensions
H. bogdanii	440413	Synnemata	Long, thin-tapering	Present	Ellipsoid	Perpendicular	3.7-5.1×1.4-2.2
	314696	Single	Short, thick-tapering	Absent	Ellipsoid	Perpendicular	2.6-4.4×1.5-2.4
	269406	Single or clusters	Long, slight-tapering	Present	Allantoid	Vertical	4.1-6.9×1.1-3.9
H. brevisubulatum	440420	Single	Short, thick-tapering	Absent	Ellipsoid	Vertical	3.3-5.2×1.4-1.8
ssp. *violaceum*	401386	Single	Short, thick-tapering	Absent	Ellipsoid	Vertical	2.8-4.3×1.0-1.6

[1]Conidiophores occurring singly (ungrouped), loosely clustered, or organized into synnemata (bundles). A single septum was either absent or present at the base of conidiophores. [2] Conidia shape indicates the predominant form for the majority of spores. Conidial orientation is relative to the central axis of conidiophores in scanning electron micrographs. Dimensions (length×width, in μm) are mean values from 50 spores determined using measurements from scanning electron micrographs

were short and stout, thick-tapering and lacked a basal septum, very similar to the conidiophores of the endophyte from two accessions of *H. brevisubulatum* subsp. *violaceum* .

Conidia shape was ellipsoid for most *Neotyphodium*-endophytes from *Hordeum* species, but conidia of the *H. bogdanii* endophyte (PI accession 269406) were distinctly allantoid. Orientation of conidia on conidiophores was often vertical in early stages of conidiogenesis, but mature detached spores were usually oriented perpendicular to the apex of the conidiophores. Thus, orientation of conidia on conidiophores may be more a function of conidial age rather than morphological specificity that can be associated with specific endophytes. Conidiogenesis was holoblastic for all strains of *Neotyphodium*-endophytes isolated here from *Hordeum* species. Conidia dimensions varied with endophyte strains isolated from different *Hordeum* species and accessions, but there were considerable overlaps in measurements that may be affected by growth

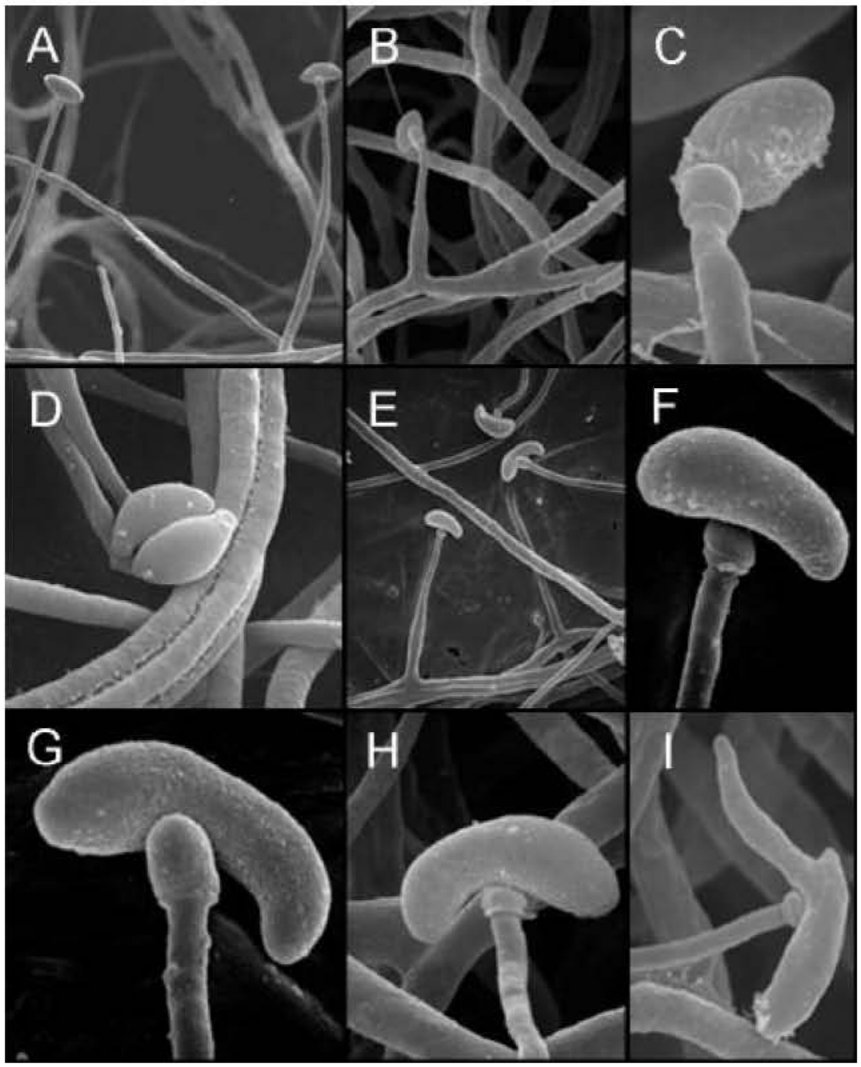

Fig. 5: Scanning electron micrographs of conidiophores and crescent-shaped conidia of *Neotyphodium*-endophyte from different *H. bogdanii* PI accessions. (A) Long thin-tapering conidiophores (PI accession 440413), (B) Short thick-tapering conidiophores (PI accession 314696), (C) Immature conidium forming by holoblastic conidiogenesis (PI accession 314696) (D) Two detached ellipsoid conidia with conidiophore-attachment scars (PI accession 440413) (E) Conidiophores with swollen apices and perpendicular conidial orientation (PI accession 269406) (F) Closeup of swollen conidiophore apex and perpendicular conidial orientation (PI accession 269406) (G) Elongated swelling of conidiophore apex with perpendicular conidial orientation (PI accession 269406) (H) Bulbous swelling of conidiophore apex with perpendicular orientation (PI accession 269406) (I) Germinating conidium on a swollen conidiophore apex (PI accession 269406)

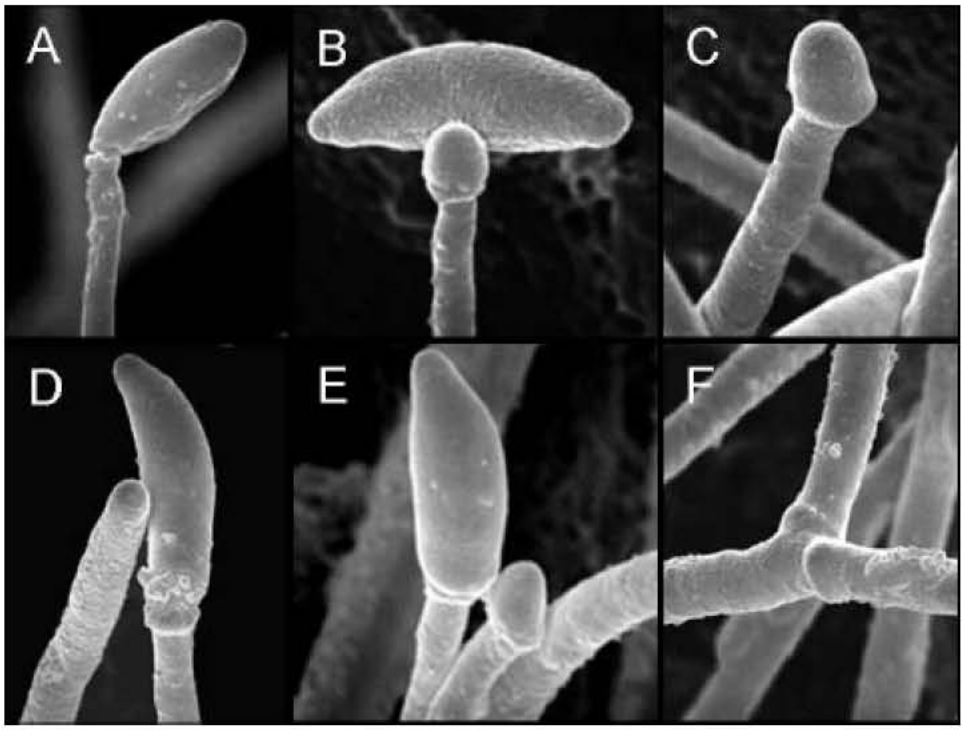

Fig. 6: Scanning electron micrographs of conidiophores and conidia of *Neotyphodium*-endophyte from different *H. brevisubulatum* subsp. *violaceum* PI accessions. (A) Conidium separating from conidiophore following holoblastic conidiogenesis (PI accession 440420), (B) Swollen conidiophore apex with straight elliptical conidium in perpendicular orientation (PI accession 401396) (C) Closeup of swollen conidiophore apex (PI accession 401386) (D) Immature conidiophore apices prior to swelling and with young conidium in vertical orientation (PI accession 401386) (E) Swollen conidiophore apex and adjacent developing conidium (PI accession 401386) (F) Closeup of septum at the base of conidiophore (PI accession 401386)

conditions and genetic differences in individual strains. Consequently, conidia dimensions were not useful for distinguishing species of *Neotyphodium*-endophytes from wild *Hordeum* species.

The diversity of conidiophore and conidial morphologies found among endophytes isolated from *H. bogdanii* accessions were revealed by SEM analyses of conidiogenesis. The long, thin-tapering condiophores of the endophyte from PI accession 440413 with perpendicular conidial configuration and unswollen conidiophore apices (Fig. 5A) were distinguished from the short thick-tapering conidiophores of the endophyte from PI accession 314696 (Fig. 5B) having conidia formed from swollen conidiophore apices (Fig. 5C). Conidia from the endophyte from PI accession 440413 were broadly ellipsoid (Fig. 5D), whereas the conidia of the endophyte isolated from PI accession 269406 were allantoid to broadly lunate (crescent shaped) and formed on swollen conidiophore apices, either elongated or bulbous (Fig. 5E-H). Conidia occasionally germinated while still on the apex of the conidiophore prior to release (Fig. 5I).

The morphologies of conidiophores and conidia of *Neotyphodium*-endophytes isolated from *H. brevisubulatum* subsp. *violaceum* also showed differences among strains isolated from different PI accessions. The endophyte from PI accession 440420 formed elliptical conidia on conidiophore apices that were not swollen (Fig. 6A), but the endophyte isolated from PI accession 401386 contained swollen conidiophore apices with slightly curved conidia (Fig. 6B, C and E) and conidiophores usually formed a basal septum (Fig. 6F). The surfaces of most conidia were glabrous (Fig. 6A, D and E), but some conidia had roughened surface architecture (Fig. 6B). Detachment scars usually remained on conidiophores after conidia were released (Fig. 6A and D). Conidia of all endophytes from *H. brevisubulatum* also were formed by holoblastic conidiogenesis.

DISCUSSION

The *Hordeum* species surveyed here are representative of germplasm from many different climatic regions, habitat types and land-use areas within

predominantly temperate regions from five continents of the world. The three endophyte-infected cool-season perennial species (*H. bogdanii*, *H. brevisubulatum* and *H. comosum*) are all in the tertiary gene pool (von Bothmer *et al*., 1991). The habitat types and land-use areas from which these endophyte-infected *Hordeum* species originated were reported previously (Wilson *et al*., 1991c). Some *Neotyphodium*-infected *Hordeum* PI accessions have been maintained in NPGR seed storage for up to 34 years, but others for six or fewer years. Seeds of infected accessions maintained in storage for many years often had reduced endophyte viability compared with accessions stored for much shorter duration (Wilson *et al*., 1991d). The apparent absence of *Neotyphodium*-endophytes from tropical regions suggests that there is a requirement for temperate hosts or that environmental conditions are not favorable for growth of endophytic fungi in the warmer regions of the tropics. This might be explained by the sensitivity of *Neotyphodium*-endophytes within seeds to high temperatures and high moisture content (Welty *et al*., 1987). Thus, the variability in environmental conditions within areas where endophyte-infected grasses are endemic may also determine the range and variability of endophyte incidence and infection rates in individual grass species and accessions.

The relatively low incidence of CA-endophytes in *Hordeum* germplasm suggests that the occurrence these endosymbiotic fungi of the genus *Neotyphodium* are relatively rare in natural populations of wild *Hordeum* species from which most NPGS collections are made. This relatively low incidence of *Neotyphodium*-endophytes in wild barley provides evidence that endophyte-infected *Hordeum* germplasm is potentially very valuable. However, the diversity of *Neotyphodium*-morphological types in wild *Hordeum* species suggest that several different endophyte species may be represented in this germplasm and that wild barley may be a good source of endophyte diversity among temperate grasses. The high potential importance of this relatively rare germplasm strongly suggests the need to provide special treatment and care for this germplasm within germplasm banks to assure that it is handled properly to maintain endophyte as well as seed viability. Such special handling might include more frequent seed increases, maintaining low humidity or liquid nitrogen storage conditions and checking for endophyte viability after each seed increase prior to storage. The abundance of world germplasm banks containing CA-endophytes of grasses suggests that a large diversity of these fungi may be available for many beneficial uses in plant-protection applications.

All *Neotyphodium* endophytes described here produced only intercellular hyphae that did not penetrate through plant cell walls into the tonoplast. This growth habit is typical of true endosymbiotic fungi that do not appear to cause any adverse pathogenic effects on host morphology within the vicinity of endophytic hyphae. *Neotyphodium* species function as mild parasites which utilize nutrients that leach through cell walls into intercellular spaces of their host. Infection was symptomless with no evidence of disease, hyperplasia or hypertrophy. No adverse effects on the middle lamella were produced causing any type of separation or maceration between plant cells. *Neotyphodium* hyphae in all cases grew systemically through the tillers and into the floral parts by which new seed infections arose. The absence of pathogenesis associated with these *Hordeum* grass-endophyte associations indicates that the relationship is more mutualistic than commensalistic or parasitic because both plant and fungus derive benefit from the interaction. The fungal endophyte derives nutrients, an effective means of passive dispersal via infected *Hordeum* seeds and can remain in the vegetative (somatic) state indefinitely without the need to sporulate or to engage in metabolically-expensive activities such as sporulation or forming a sexual stage. However, some *Hordeum* endophytes do occasionally sporulate on their host. The *H. brevisubulatum* subsp. *violaceum* endophyte from PI accession 440420 was found to sporulate within various leaf tissues of its host (Youssef and Dugan, 2000). Hyphae can continue to grow in the somatic phase as long as it is associated with a grass host. The fungus overwinters in the meristem of the plant and remains viable whether or not tillers and leaves are removed by herbivores.

The first discovery of *Neotyphodium*-endophytes in wild *Hordeum* (Wilson *et al*., 1991b) and *Lolium* (Wilson *et al*., 1991d) NPGS germplasm stimulated a considerable amount of new endophyte research because of the great potential possibility and importance of utilizing these mutualistic endosymbiontic fungi as biocontrol agents of insect and disease pests to enhance production yields of cereal crops (Wilson *et al*., 1991; Wilson, 1996; Clay, 1989, 1990; Clement *et al*., 2001). Additional surveys of NPGS and other germplasm collections led to discoveries of *Neotyphodium*-endophytes in other grass species, including some that are wild relatives of crop grasses with potential for hybridizations or genetic crosses with cereal species. Subsequent research has focused on the distribution and sporulation of *Neotyphodium*-endophytes on their *Hordeum* hosts (Youssef and Dugan, 2000; Dugan *et al*., 2002), identifying the biologically active alkaloids

produced by *Hordeum*-endophytes (TePaske *et al*., 1993) and determining their effects on insects pests (Clement *et al*., 1991, 1992, 1993, 1996, 1997, 2004a, 2004b, 2005).

The discovery of *Neotyphodium*-endophytes in *Hordeum* species is significant for several reasons. *Neotyphodium*-endophytes of *Hordeum* species produce secondary metabolites that are biologically active against plant pests. *Neotyphodium*-infected accessions of *H. bogdanii* and *H. brevisubulatum* subsp. *violaceum* have been shown to exhibit significant resistance to several insects including the Russian wheat aphid (*Diuraphis noxia* Mordvilko) (Clement *et al*., 1997), Rose-grass aphid (*Metopolophium dirhodum* Walker), Bird cherry-oat aphid (*Rhopalosiphum padi* L.) and Hessian fly (*Mayetiola destructor* Say) (Clement *et al*., 2005). Ergoclavine ergot alkaloids (ergovaline, ergosine and ergotamine) and N-formylloline (loline alkaloid) have been detected in *Neotyphodium*-infected seeds and forage from *H. bogdanii* (PI accession 314696) and *H. brevisubulatum* subsp. *violaceum* (PI accession 440420) (TePaske *et al*., 1993). Tests with insect bioassays have shown that *Neotyphodium*-endophytes (microbial germplasm within plants) are extra-genomic sources of resistance against numerous insects, providing fortuitous means for controlling major insect and disease pests in grasses that lack genetic resistance (Clay, 1988; Wilson, 1996; Clement *et al*., 1994). The high level of resistance of *H. bogdanii* accessions to *R. padi*, previously attributed to genetic resistance (Weibull, 1988), was more likely due to *Neotyphodium*-infected plants because endophyte-free accessions have little resistance to this aphid (Clement *et al*., 2005). Seed from these PI accessions eventually may be used in breeding programs, in genetically-modified plants through biotechnological approaches, or by artificial inoculations with CA-endophytes to produce new cultivars of commercial barley or other crop grasses with resistance to a number of insect and disease pests.

The systemic nature of CA-endophyte infections is a characteristic of great potential usefulness in breeding programs because these specialized fungi are seed-borne. Once the endophyte is introduced by inoculation into the female parent, the symbiotic association becomes self-replicating thereafter and is passed on to the progeny of genetic crosses (Clay, 1989). Unfortunately, interspecific crosses of *H. bogdanii* (section *Stenostachys*) and *H. comosum* (section *Critesion*), both diploid species, with *H. vulgare* L. (cultivated barley, section *Hordeum*) have not produced viable plants beyond the seedling stage, but crosses of *H. brevisubulatum* (a diploid, tetraploid, or hexaploid species of section *Stenostachys*) with *H. vulgare* have produced viable adult plants (von Bothmer *et al*., 1983). Crosses were more successful when *H. vulgare* was used as a pollen donor rather than as the female parent. Another potential benefit of *Neotyphodium*-endophytes in infected wild barley, fescue and ryegrass species is their potential for use in the Conservation Reserve Program (CRP) to significantly reduce the buildup of insect and disease pests in endophyte-infected reservoir plants, particularly wild alternate hosts of agronomic grass pests that are planted on CRP lands (fallow crop fields) between years of crop cultivations. Endophyte-infected CRP grasses growing adjacent to or surrounding grass crops also should significantly reduce the influx of insect pests into these crop fields much like barrier crops.

Endophyte-infected grasses receive many potential benefits from the fungus including increased water use efficiency, improved growth and vigor in associated with endophyte-associated auxin production, tolerance to drought and water stress, increased competitive advantage, resistance or tolerance to herbivory and protection against certain pathogenic fungi, such as *Rhizoctonia cerealis* Van der Hoeven and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, and Van Kesteren) (White and Cole, 1985), nematodes *Heterodera glycines* Ichinohe and *Meloidogyne incognita* (Kofoid and White) Chit. (Petersen *et al*., 1988) and viruses (Barley yellow dwarf virus) (Latch *et al*., 1985). New applications of CA-endophytes for the biocontrol of insects and diseases of turf grasses and other non-pasture situations are being discovered continuously (Clement *et al*., 1994; Rowan and Latch, 1994; Clay, 1989; Anderson *et al*. 2006; Clarke *et al*., 2006).

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REFERENCES

Anderson, W.G., T.M. Heng-Moss and F.P. Baxendale, 2006. Evaluation of cool- and warm-season grasses for resistance to multiple Chinch bug (Hemiptera: Blissidae) species. J. Econ. Entomol., 99: 203-211.

Bacon, C.W., J.K. Porter, J.D. Robbins and E.S. Luttrell, 1997. *Epichloë typhina* from toxic tall fescue grasses. Applied Environ. Microbiol., 34: 576-581.

Brem, D. and A. Leuchtmann, 2003. Molecular evidence for host-adapted races of the fungal endophyte *Epichloë bromicola* after presumed host shifts. Evolution, 57: 37-51.

Bruehl, G.W., W.J. Kaiser and R.E. Klein, 1994. An endophyte of *Achnatherum inebrians*, an intoxicating grass of northwest China. Mycologia, 86: 773-776.

Bush, L.P., F.F. Fannin, M.R. Siegel, D.L. Dahlman and H.R. Burton, 1993. Chemistry of Compounds Associated with Endophyte-Grass Interactions: Saturated Pyrrolizidines Alkaloids. Vol. 44. *Acremonium/Grass Interactions*. Joost, R. and S. Quisenberry (Eds.), pp: 81-82. Agricultural, Ecosystems and Environment, Elsevier, Amsterdam.

Cabral, D., M.J. Cafaro, B. Saidman, M. Lugo, P. V. Reddy and J.F. White, Jr., 1999. Evidence supporting the occurrence of a new species of endophyte in some South American grasses. Mycologia, 91: 315-325.

Carroll, G., 1988. Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. Ecology, 69: 2-9.

Clarke, B.B., J.F. White, Jr. and R.H. Hurley, 2006. Endophyte-mediated suppression of dollar spot disease in fine fescues. Plant Dis., 90: 994-998.

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

Clay, K., 1989. Clavicipitaceous endophytes of grasses: Their potential as biocontrol agents. Mycol. Res., 92: 1-12.

Clay, K., 1990. Fungal endophytes of grasses. Ann. Rev. Ecol. Syst., 21: 275-297.

Clay, K., 1992. Fungal endophytes of plants: biological and chemical diversity. Nat Toxins, 1: 147-149.

Clay, K., 1994. The Potential Role of Endophytes in Ecosystems. Chapter 6. Biotechnology of Endophytic Fungi of Grasses. Bacon, C.W. and J.F. White, Jr. (Eds.), CRC Press, Boca Raton, FL., pp: 73-86.

Clay, K., 1997. Fungal endophytes, herbivores and the structure of grassland communities. Multi-trophic interactions in terrestrial systems. Gange, A.C. and V.K. Brown (Eds.), Blackwell Science, London. pp: 151-170.

Clement, S.L., K.S. Pike, W.J. Kaiser and A.D. Wilson. 1991. Resistance of endophyte-infected plants of tall fescue and perennial ryegrass to the Russian wheat aphid (Homoptera: Aphididae). J. Kans. Ento. Soc., 63: 646-648.

Clement, S.L., D.G. Lester, A.D. Wilson and K.S. Pike, 1992. Behavior and performance of *Diuraphis noxia* (Homoptera: Aphididae) on fungal endophyte-infected and uninfected perennial ryegrass. J. Econ. Ento., 85: 583-588.

Clement, S.L., D.G. Lester, A.D. Wilson, R.C. Johnson and J.H. Bouton. 1993. Effect of Fungal Endophyte in Tall Fescue and Wild Barley on Aphid Performance. Proceedings of the 2nd International Symposium on Acremonium/ Grass Interactions. Hume, D.E., G.C.M. Latch and H.S. Easton (Eds.), AgResearch, Grassland Research Centre, Palmerston North, New Zealand. pp: 155-157.

Clement, S.L., W.J. Kaiser and H. Eichenseer, 1994. *Acremonium* Endophytes in Germplasms of Major Grasses and Their Utilization for Insect Resistance. Chapter 13. Biotechnology of Endophytic Fungi of Grasses. Bacon, C.W. and J.F. White, Jr. (Eds.), CRC Press, Boca Raton, FL. pp: 185-199.

Clement, S.L., D.G. Lester, A.D. Wilson, R.C. Johnson and J.H. Bouton, 1996. Expression of Russian wheat aphid (Homoptera: Aphididae) resistance in genotypes of tall fescue harboring different isolates of *Acremonium* endophyte. J. Econ. Ento., 89: 766-770.

Clement, S.L., A.D. Wilson, D.G. Lester and C.M. Davitt. 1997. Fungal endophytes of wild barley and their effects on *Diuraphis noxia* population development Entomol. Exp. et Applic., 82: 275-281.

Clement, S.L., L.R. Elberson, N.N. Youssef, C.M. Davitt and R.P. Doss, 2001. Incidence and diversity of *Neotyphodium* fungal endophytes in tall fescue from Morocco, Tunisia and Sardinia. Crop Sci., 41: 570-576.

Clement, S.L., L.R. Elberson, B.L. Waldron and T.J. Kisha, 2004a. Variable Effects of Wild fescue-*Neotyphodium* Associations on Bird Cherry-oat Aphid Survival. Proceedings of the 5th International Symposium on *Neotyphodium*/grass Interactions. Kallenbach, R., C. Rosenkuans, Jr. and T.R. Lock (Eds.), paper 308. University of Arkansas, Fayetteville, AR.

Clement, S.L., N. Youssef, G.W. Bruehl, W.J. Kaiser, L.R. Elberson and V. Bradley, 2004b. Effects of Different Storage Temperatures on Grass Seed Germination and Neotyphodium Survival. Proceedings 5th International Symposium on Neotyphodium/Grass Interactions. Kallenbach, R., C. Rosenkuans, Jr. and T.R. Lock (Eds.), Paper 511. University of Arkansas, Fayetteville, AR.

Clement, S.L., L.R. Elberson, N.A. Bosque-Pérez and D.J. Schotzko, 2005. Detrimental and neutral effects of wild barley-*Neotyphodium* fungal endophyte associations on insect survival. Entomol. Exp. Applicata, 114: 119-125.

Dugan, F.M., J.W. Sitton, R.F. Sullivan and J.F. White Jr. 2002. The *Neotyphodium* endophyte of wild barley, (*Hordeum brevisubulatem* subsp. *violaceum*) grows and sporulates on leaf surfaces of the host. Symbiosis, 32: 147-159.

Glenn, A.E, C.W. Bacon, R. Price and R.T. Hanlin, 1996. Molecular phylogeny of *Acremonium* and its taxonomic implications. Mycologia, 88: 369-383.

Greulich, F., E. Horio, T. Schimanuki and T. Yoshihara, 1999. Field results confirm natural plant protection by the endophyte fungus *Epichloë typhina* against the pathogenic fungus *Cladosporium phlei* on timothy leaves. Ann. Phytopathol. Soc. Jpn., 65: 454-459.

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

Hahn, H., W. Huth, W. Schöberlein, W. Diepenbrock, 2003. Detection of endophytic fungi in *Festuca* sp. by means of tissue print immunoassay. Plant Breed., 122: 217-222.

Jones, T.A., M.H. Ralphs, D.R. Gardner and N.J. Chatterton, 2000. Cattle prefer endophyte-free robust needlegrass. J. Range Manage., 53: 427-431.

Koh, S., M. Vicari, J.P. Ball, T. Rakocevic, S. Zaheer, D.S. Hik and D.R. Bazely, 2006. Rapid detection of fungal endophytes in grasses for large-scale studies. Funct. Ecol., 20: 736-742.

Latch, G.C.M., M.J. Christensen and D.L. Gaynor, 1985. Aphid detection of endophyte infection in tall fescue. N.Z. J. Agric. Res., 28: 129-132.

Lewis, G.C., C. Ravel, W. Naffaa, C. Astier and G.W. Charmet, 1997. Occurrence of *Acremonium*-endophytes of wild populations of *Lolium* sp. in European countries and a relationship between level of infection and climate in France. Ann. Applied Biol., 130: 227-238.

Lyons, P.C., R.D. Plattner and C.W. Bacon, 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue. Science, 232: 487-489.

Malinowski, D.P. and D.P. Belesky, 2000. Adaptation of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. Crop Sci., 40: 923-940.

Marshall, D., L.R. Nelson and B. Tunali, 1993. The Occurrence of *Acremonium* and Other Endophytic Fungi in the Indigenous Wild Cereals of Turkey. Proceedings of the 2nd. International Symposium on Acremonium/Grass Interactions. Hume, D.E., G.C.M. Latch and H.S. Easton (Eds.), AgResearch, Grasslands Research Centre, Palmerston North, New Zealand, pp: 8-10.

Marshall, D., B. Tunali and L.R. Nelson, 1999. Occurrence of fungal endophytes in species of wild *Triticum*. Crop Sci., 39: 1507-1512.

Miles, C.O., A.L. Wilkins, R.T. Gallagher, A.D. Hawkes, S.C. Munday and N. Towers, 1992. Synthesis and tremorgenicity of paxitrols and lolitriol: Possible biosynthetic precursors of lolitrem B. J. Agric. Food Chem., 40: 234-238.

Miles, C.O., S.C. Munday, A.L. Wilkins, R.M. Ede, A.D. Hawkes, P.P. Embling and N.R. Towers, 1993. Large Scale Isolation of Lolitrem B, Structure Determination of Some Minor Lolitrems and Tremorgenic Activities of Lolitrem B and Paxilline in Sheep. Proceedings of the 2nd. International Symposium on Acremonium/Grass Interactions. Hume, D.E., G.C.M. Latch and H.S. Easton (Eds.), AgResearch, Grasslands Research Centre, Palmerston North, New Zealand, pp: 85-86.

Miles, C.O., M.E. DiMenna, S.W.L. Jacobs, I. Garthwaite, G.A. Lane, R.A. Prestidge, S.L. Marshall, H.H. Wilkinson, C.L. Schardl, O.J.P. Ball and G.C.M. Latch, 1998. Endophytic fungi in indigenous Australasian grasses associate with toxicity to livestock. Applied Environ. Microbiol., 64: 601-606.

Mirlohi, A., M.R. Sabzalian, B. Sharifnabi and M.K. Nekoui, 2006. Widespread occurrence of *Neotyphodium*-like endophyte in populations of *Bromus tomentellus* Boiss. in Iran. FEMS Microbiol. Lett., 256: 126-131.

Nan, Z.B. and C.J. Li, 2001. *Neotyphodium* in Native Grasses in China and Observations on Endophyte/host Interactions. *Neotyphodium*/Grass Interactions Symposium. Paul, V.H. and P.D. Dapprich (Eds.), Proc. 4th Int. Fachbereich Agrarwirtschaft, Soest, Germany, pp: 41-50.

Petersen, J.F., R. Rodriguez-Kabana and R.A. Shelby, 1988. Ryegrass cultivars and endophyte in tall fescue affect nematode in grass and succeeding soybean. Agron. J., 80: 811-814.

Rowan, D.D., M.B. Hunt and D.L. Gaynor, 1986. Peramine, a novel insect feeding deterrent from ryegrass infected with the endophyte *Acremonium* loliae. J. Chem. Soc. Chem. Commun., 142: 935-936.

Rowan, D.D. and G.C.M. Latch, 1994. Utilization of Endophyte-infected Perennial Ryegrass for Increased Insect Resistance. Chapter 12. Biotechnology of Endophytic Fungi of Grasses. Bacon C.W. and J.F. White, Jr. (Eds.) CRC Press, Boca Raton, FL., pp: 169-183.

Schardl, C.L. and T.D. Phillips, 1997. Protective grass endophytes: Where are they from and where are they going? Plant Dis., 81: 430-438.

Schmidt, D., 1990. Antagonistic Effect of *Acremonium* sp. Against Potential Pathogens. Proceedings of the International Symposium on Acremonium/Grass Interactions. Quisenberry, S.S. and R.E. Joost (Eds.), Lousiana Agric. Exp. Stn., Baton Rouge, pp: 52-57.

Siegel, M.R., G.C.M. Latch and M.C. Johnson, 1985. *Acremonium* fungal endophytes of tall fescue and perennial ryegrass: Significance and control. Plant Dis., 69: 179-183.

Siegel, M.R., G.C.M. Latch, L.P. Bush, F.F. Fannin, D.D. Rowan, B.A. Tapper, C.W. Bacon and M.C. Johnson, 1990. Fungal endophyte-infected grasses: Alkaloid accumulation and aphid response. J. Chem. Ecol., 16: 3301-3316.

Springer, T.L. and S.D. Kindler, 1990. Endophyte-Infected Resistance to the Russian Wheat Aphid and the Incidence of Endophytes in Fescue Species. Proceedings of the International Symposium on Acremonium/Grass Interactions. Quisenberry, S.S. and R.E. Joost (Eds.), Lousiana Agric. Exp. Stn., Baton Rouge, LA, pp: 144.

TePaske, M.R., R.G. Powell and S.L. Clement, 1993. Analyses of selected endophyte-infected grasses for the presence of loline-type and ergot-type alkaloids. J. Agric. Food Chem., 41: 2299-2303.

Vinton, M.A., E.S. Kathol, K.P. Vogel and A.A. Hopkins, 2001. Endophytic fungi in Canada wild rye in natural grasslands. J. Range Manage., 54: 390-395.

von Bothmer, R., J. Flink, N. Jacobsen, M. Kotimäki and T. Landström, 1983. Interspecific hybridization with cultivated barley (*Hordeum vulgare* L.). Hereditas, 99: 219-244.

von Bothmer, R., N. Jacobsen, C. Baden, R.B. Jorgensen and I. Linde-Laursen, 1991. An ecogeographical study of the genus *Hordeum*, International Board for Plant Genetic Resources, Rome, Italy, pp: 127.

Weibull, J., 1988. Resistance in the wild crop relatives *Avena macrostachya* and *Hordeum bogdani* to the aphid *Rhopalosiphum padi*. Entomol. Exp. et Applic., 48: 225-232.

Welty, R.E., M.D. Azevedo and T.M. Cooper, 1987. Influence of moisture content, temperature and length of storage on seed germination and survival of endophytic fungi in seeds of tall fescue and perennial ryegrass. Phytopathol., 77: 893-900.

White, J.F., Jr. and G.T. Cole, 1985. Endophyte-host associations in forage grasses. III. *In vitro* inhibition of fungi by *Acremonium coenophialum*. Mycologia, 77: 487-489.

White, J.F., R.F. Sullivan, G.A. Balady, T.J. Gianfagna, Q. Yue, W.A. Meyer and D. Cabral, 2001. A Fungal endosymbiont of the grass *Bromus setifolius*: Distribution in some Andean populations, identification and examination of beneficial properties. Symbiosis (Rehovot), 31: 241-257.

Wilson, A.D., W.J. Kaiser and S.L. Clement, 1991a. Clavicipitaceous endophytes in wild *Hordeum* germ plasm. Phytopathology, 81: 1151.

Wilson, A.D., S.L. Clement, W.J. Kaiser and D.G. Lester, 1991b. First report of clavicipitaceous anamorphic endophytes in *Hordeum* species. Plant Dis., 75: 215.

Wilson, A.D., S.L. Clement and W.J. Kaiser, 1991c. Endophytic fungi in a wild *Hordeum* germplasm collection. Plant Gen. Res. Newslett., 87: 1-4.

Wilson, A.D., S.L. Clement and W.J. Kaiser, 1991d. Survey and detection of endophytic fungi in *Lolium* germplasm by direct staining and aphid assays. Plant Dis., 75: 169-173.

Wilson, A.D., C.C. Gay and S.C. Fransen, 1992. An *Acremonium* endophyte of *Lolium perenne* associated with hyperthermia of cattle in Pacific County, Washington. Plant Dis., 76: 212.

Wilson, A.D., 1996. Resources and testing of endophyte-infected germplasm in national grass repository collections. Chapter 9. Systematics, ecology and evolution of endophytic fungi in grasses and woody plants. Redlin, S.C. and L.M. Carris (Es.), pp: 79-196. APS Press, The American Phytopathological Society, St. Paul, Minnesota, pp: 223.

Yates, S.G. and R.G. Powell, 1988. Analysis of ergopeptine alkaloids in endophyte-infected tall fescue. J. Agric. Food Chem., 36: 337-340.

Yates, S.G., R.J. Petroski and R.G. Powell, 1990. Analysis of loline alkaloids in endophyte-infected tall fescue by capillary gas chromatography. J. Agric. Food Chem., 38: 182-185.

Youssef, N.N. and F.M. Dugan, 2000. Location of an endophytic *Neotyphodium* sp. within various leaf tissues of wild barley (*Hordeum brevisubulatum* subsp. *violaceum*). Plant Gen. Res. Newslett., 124: 17-19.

Zabalgogeazcoa, I., B.R. Vázquez de Aldana, A.G. Ciudad and B.G. Criado, 1999. A survey of grass species infected by fungal endophytes in dehesa pastures. Options Méditer., 39: 247-250.