

# Endophytic fungi in a *Hordeum* germplasm collection

A.D. Wilson<sup>1</sup>, S.L. Clement<sup>1</sup> and W.J. Kaiser<sup>1</sup>

## Summary

The incidence of clavicipitaceous anamorphic endophytes in a *Hordeum* spp. germplasm collection is reported. The potential application of endophytes as biocontrol agents against pests of cereal crops is recognized. Suggestions are proposed to modify existing germplasm maintenance procedures to ensure that both seed viability and endophyte viability and diversity are maintained in grass germplasm collections.

## Introduction

A group of seed-borne fungi known as clavicipitaceous endophytes have been known to occur in grasses for almost a century (see Vogl, 1898), but their economic importance has been recognized only within the last 10-12 years. Clavicipitaceous endophytes were first associated with toxicoses of cattle in the United States and sheep in New Zealand that consumed endophyte-infected tall fescue and perennial ryegrass. Further research showed that endophytes produce poisonous ergot and related alkaloids within their hosts that are toxic to a wide range of insects and mammalian herbivores (Clay, 1990). The toxic effects of endophyte-infected forage grasses on domestic livestock have resulted in economic losses exceeding \$US 100 million annually in the eastern United States and in New Zealand (Siegel *et al.*, 1987).

Clavicipitaceous endophytes can significantly affect host physiology, reproductive biology and ecology. They are considered obligate biotrophs that complete their entire life cycle within their host, growing intercellularly and systemically from aleurone tissues of seeds into culm piths, leaf sheaths, and eventually into tissues of the developing inflorescence by which they enter progeny seeds. The fungi are confined to the apical meristem region when plants are dormant. Resistance to herbivory is enhanced by the release of the alkaloids from mycelium into all infected parts of the plant. In addition to the protection provided by alkaloids against pests of infected hosts, plants also may benefit from endophyte infection through increased growth and vigour, tolerance to drought and water stress, competitive ability and enhanced photosynthetic efficiency (Siegel *et al.*, 1987). The mechanisms of growth promotion in endophyte-infected

plants are unknown, although clavicipitaceous endophytes have been shown to produce the growth-promoting phytohormone IAA (auxin) in culture (De Battista *et al.*, 1990).

The endophytic fungi considered here can be divided into two natural groups: 1) clavicipitaceous teleomorphic endophytes, and 2) clavicipitaceous anamorphic endophytes (Wilson *et al.*, 1991a). Teleomorphic endophytes are ascomycetes (Clavicipitaceae: tribe Balansieae) that infect members of the Poaceae (grasses), Cyperaceae (sedges) and Juncaceae (rushes) (Clay, 1989). They produce a sexual stage (teleomorph) on stromata and induce choke diseases which may disrupt reproduction of their hosts (Diehl, 1950). Consequently, infected plants are often sterile due to inhibition of flowering or abortion of inflorescences. Anamorphic endophytes appear to be asexual derivatives of teleomorphic endophytes, but they lack a sexual stage and form mutualistic associations with their hosts. They are classified as imperfect fungi (Deuteromycetes) in the genus *Acremonium* sect. *Albolanosa* and in related anamorphic genera (e.g. *Gliocladium* and *Phialophora*) (Morgan-Jones and Gams, 1982; Latch *et al.*, 1984). Anamorphic endophytes infect primarily cool-season grasses of the tribe Pooideae (Clay, 1990).

Anamorphic endophytes are more applicable and offer greater potential benefits as biocontrol agents against pests of grasses than teleomorphic endophytes because the anamorphic forms do not cause disease symptoms and they are self-perpetuated (maternally inherited) through seed infection since flowering and seed development are not disrupted. Because the interactions between anamorphic endophytes and their hosts are mutualistic (asymptomatic) and these fungi rarely sporulate on their hosts, infected plants can usually be detected only by microscopic examination or chemical analyses. Most surveys of anamorphic endophytes have concentrated on turf and forage grass collections primarily from Europe, North America and New Zealand (Latch *et al.*, 1987; Saha *et al.*, 1987; White, 1987). Few surveys have included

<sup>1</sup> Plant Germplasm Introduction and Testing Research Unit, US Department of Agriculture, Agricultural Research Service, 59 Johnson Hall, Washington State University, Pullman, Washington, 99164-6402. Present address of first author: Southern Forest Experiment Station, Southern Hardwoods Laboratory, USDA Forest Service, PO Box 227, Stoneville, MS 38776 USA

grass germplasm from South America, Africa and Asia. Wilson et al. (1991a) first reported on the incidence of endophytes in a US grass germplasm repository. This survey indicated the occurrence of endophytes in *Lolium* germplasm principally from Europe and Asia. Wilson et al. (1991b) provided a preliminary report documenting the incidence of anamorphic endophytes in a *Hordeum* germplasm collection.

The economic losses to the livestock industry and the potential benefits of endophytes as additional sources of pest resistance in grasses increase the need to determine the incidence of these mutualistic fungi in world germplasm collections of grasses. The largest collection of grass germplasm in the United States is maintained in the National Small Grains Collections (NSGC) at Aberdeen, Idaho. The NSGC contains almost 27 000 accessions of *Hordeum* spp., the majority of which are *H. vulgare* L., *H. spontaneum* C. Koch and unidentified *Hordeum* species. This paper reports on the incidence of anamorphic endophytes in a portion of this collection.

#### Materials and Methods

Seed samples of 77 *Hordeum* PI lines, previously held at the Western Regional Plant Introduction Station (WRPIS) in Pullman, Washington prior to 22 November 1989, were obtained from the NSGC. Seeds were stored at 4-5°C and 30-35% relative humidity at the WRPIS, and subsequently at 6-7°C and 36-38% relative humidity at the NSGC. Seeds from the most recent seed increase of each accession were sampled for assay. The *Hordeum* species included in this survey are summarized in Table 1.

Seed samples 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 (Wilson et al., 1991a). Seeds were then rinsed, squashed and

mounted on slides in 1:1 v/v glycerol-distilled water, and examined microscopically at 100-400 × magnification. Seed infection rates were based on examinations of 50 seeds in endophyte-free accessions and 100 seeds in endophyte-infected accessions.

#### Results

Endophytes were found in three of ten species examined: *H. bogdani*, *H. brevisubulatum* ssp. *violaceum* and *H. comosum* (Table 1). Overall, 21% of examined accessions were infected with endophytic fungi. Most of the accessions examined originated from Asia (77%) and South America (10%). The endophytes in *H. bogdani* and *H. brevisubulatum* ssp. *violaceum* that originated from Asia were identified as *Acremonium* species. Accessions of *H. bogdani* had the highest incidence of endophyte infection.

Endophyte infection rates in seeds ranged from 18 to 99% for the three infected *Hordeum* species (Table 2). Accessions collected from wet habitats on uncultivated land tended to have higher seed infection rates. Accessions from dry habitats on cultivated or grazed land were often endophyte-free.

Endophyte-infected accessions of each species have been maintained for up to 30 years in storage. Seeds of some infected accessions that were maintained for many years have low viability, especially accessions of *H. comosum* (unpublished data). By contrast, the endophyte-free accession of *H. comosum* (PI 283379) had good seed viability. Accessions of *H. bogdani* and *H. brevisubulatum* ssp. *violaceum*, maintained for less than 12 years, tended to have higher levels of endophyte infection (Table 2) and seed viability than accessions maintained much longer.

#### Conclusions

This report is among the first to document the occurrence of anamorphic endophytes in grass germ-

Table 1. Occurrence of anamorphic endophytes in seeds of the NSGC *Hordeum* germplasm collection<sup>a</sup>

Species	Accessions <sup>b</sup>		Endophyte-free accessions <sup>c</sup>		Endophyte-infected accessions
	Available	Examined	Examined	Origin	
<i>H. bogdani</i>	16	12	1	AS	11
<i>H. brachyantherum</i>	6	1	1	NA	0
<i>H. brevisubulatum</i>	41	17	15	AS	2
<i>H. bulbosum</i>	218	22	22	AF, AS, EU	0
<i>H. chilense</i>	10	3	3	SA	0
<i>H. comosum</i>	4	4	1	SA	3
<i>H. jubatum</i>	20	3	3	NA, AS	0
<i>H. marinum</i>	20	4	4	AS	0
<i>H. murinum</i>	67	10	10	SA, AS	0
<i>H. stenostachys</i>	14	1	1	AF	0
Totals	424	77	61		16

<sup>a</sup> Occurrence 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.

<sup>b</sup> Accessions available as of 25 April 1990.

<sup>c</sup> Abbreviations for continents of origin: NA, North America; SA, South America; EU, Europe; AF, Africa; AS, Asia. Information on specific endophyte-free accessions is available from the Germplasm Resources Information Network (GRIN) data base, USDA-ARS.

Table 2 Endophyte-infected and endophyte-free accessions from the NSGC *Hordeum* germplasm collection

Barley species	Accession (PI) <sup>a</sup>	Collecting reporters <sup>b</sup>	Years stored <sup>c</sup>	Country of origin	Habitat type <sup>d</sup>	Land use <sup>e</sup>	Infection rates (%)
<i>H. bogdani</i>	269406	1	30	Afghanistan	—	—	47
	314696	2	24	USSR	—	—	62
	440413'	3,4	12	USSR	wet	wild	66
	440414	3,4	12	USSR	wet	wild	80
	499496	3	6	China	dry	cultivated	0
	499499	3	6	China	wet	wild	18
	499503	3	6	China	wet	wild	52
	499501	3	6	China	wet	wild	77
	499643	3,5	5	China	—	wild	47
	499644	3,5	5	China	—	wild	99
	499645	3,5	5	China	wet	wild	94
	499646	3,5	5	China	—	wild	97
	<i>H. brevisubulatum</i> ssp. <i>violaceum</i>	401386*	3	15	Iran	wet	wild
440420		3,4	12	USSR	wet	wild	98
229753		6	34	Iran	dry	grazed	0
461379		3	15	Iran	dry	wild	0
446419		3,4	12	USSR	dry	cultivated	0
<i>H. comosum</i>	264404*	—	30	Argentina	—	—	66
	264405'	—	30	Argentina	—	—	92
	269646	7	30	Argentina	—	—	74
	263379	—	30	Argentina	—	—	0

<sup>a</sup> Plant inventory (PI) accessions from the National Small Grains Collection (NSGC). Accessions marked by an (\*) were held at the Western Regional Plant Introduction Station (WRPIS) prior to 22 November 1969.

<sup>b</sup> Reporters providing collection data on accessions are: 1, J.R. Harlan; 2, Q. Jones and W. Keller; 3, D.R. Dewey; 4, A.P. Plummer; 5, W. Tai; 6, H.S. Gentry; 7, R. Hernando.

<sup>c</sup> Years stored and maintained since accessions were received into the NSGC or WRPIS *Hordeum* germplasm collections, as of 25 April 1936.

<sup>d</sup> Soil habitats: wet = grassland in low-lying areas along irrigation or roadside ditches or near aquatic habitats; dry = upland grasslands in areas with good drainage.

<sup>e</sup> The habitats of the collection sites were either wild, cultivated, or grazed.

<sup>f</sup> Infection rates are based on examinations of 100 seeds.

plasm from Asia and South America. The discovery of endophytes in *H. comosum* from Argentina also represents one of the first reports of an endophyte in a perennial grass from a tropical zone. Tropical grasses hitherto have not been recognized as hosts of anamorphic endophytes (Clay, 1990). This indicates that endophytes may not be restricted to temperate grass hosts and that additional surveys for endophytic fungi in grasses from the tropics would be beneficial.

The occurrence of endophytic fungi in US and other world grass repositories necessitates that the beneficial and hazardous consequences of their presence be recognized. Since endophytes are maternally inherited through seeds and the resistance they provide is independent of genetic resistance, plant breeders may be able to use compatible endophyte-infected wild accessions as alternative sources of resistance in grass-crop breeding programmes. Similarly, forage and range managers should be warned that some grass germplasm could have detrimental effects on grazing livestock. The economic consequences of endophyte infection suggest that more extensive surveys of grass collections are needed to identify infected accessions so that they may be maintained using procedures that also preserve endophyte viability. Endophyte preservation is stressed here since disinfection procedures

are readily available to eliminate endophytes from seeds when desired (Siegel et al., 1987).

Most surveys of grass collections have indicated that endophyte-infected accessions comprise a relatively small portion of the total available accessions in any one collection. The rarity of infected accessions suggests that endophytes should be considered in germplasm maintenance programmes to preserve current levels of endophyte infection, viability and diversity. It is important to consider preservation of endophyte diversity since it is possible for several distinct strains and even separate species of endophytes to occur within an accession or a given host species (see Latch et al., 1984). Having a greater diversity of endophytes as sources of resistance to draw from would provide the same advantages as a diversity of genetic resistance. Endophyte diversity within accessions probably occurs due to the variability of plant genotypes that often occurs within an accession. Few accessions are genetically uniform.

Existing germplasm maintenance procedures could be modified to enhance endophyte viability and diversity. Once extensive surveys to identify infected accessions in grass collections are completed, infected accessions should be planted out and increased in sufficient numbers to yield adequate supplies of in-

fect seed to maintain the genetic diversity not only of the host, but also of the anamorphic endophyte(s) within each accession. Seed increases could be done more frequently, if necessary, to refresh endophyte inoculum in seeds and to improve seed viability. Alternatively, the seed storage conditions optimum for endophyte survival could be determined to reduce the frequency of seed increases needed to maintain viability. Finally, endophytes could be isolated from their hosts and the cultures maintained indefinitely through the American Type Culture Collection (ATCC), Beltsville, Maryland. Endophytes could then be reintroduced into their hosts through artificial inoculation techniques if the endophytes are lost during seed storage (see Latch and Christensen, 1985). Cultures of endophytes might also be used to produce novel endophyte-grass combinations for increased host vigour and pest resistance.

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#### Résumé

##### **Champignons endophytes dans une collection de matériel génétique de *Hordeum***

La présence de clavicipitacées endophytes anamorphes a été signalée dans une collection de matériel génétique de *Hordeum* spp. Il est reconnu que les champignons endophytes pourraient être utilisés pour la lutte biologique contre les parasites des céréales. Il est proposé de modifier les procédures actuelles de conservation du matériel génétique pour permettre de maintenir tout ensemble la faculté germinative des semences et la viabilité et la diversité des champignons endophytes dans les collections de matériel génétique de graminées.

#### Resumen

##### **Hongos endofíticos en una colección de germoplasma de *Hordeum***

En el artículo se informa de la presencia de hongos endofíticos anamórficos clavicipitáceos en una colección de germoplasma de *Hordeum* spp. Se estudia la posible aplicación de endofitos como agentes de lucha biológica contra las plagas de los cultivos de cereales. Se hacen propuestas de modificación de los procedimientos actuales de mantenimiento de germoplasma para asegurar la conservación de la viabilidad de las semillas y la viabilidad y la diversidad de los endofitos en las colecciones de germoplasma de gramíneas.