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Occurrence of fungal endophytes in cultivars of *Triticum aestivum* in South Africa

Pedro W. Crous[1], Orlando Petrini[2], Gideon F. Marais[3], Zaccharia A. Pretorius[4] and Franziska Rehder[1]

[1] Department of Plant Pathology, University of Stellenbosch, Stellenbosch 7600, South Africa
[2] Mikrobiologisches Institut ETH, ETH-Zentrum, Zürich, Switzerland
[3] Department of Genetics, University of stellenbosch, Stellenbosch 7600, South Africa
[4] Department of Plant Pathology, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa

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Fungal endophytes were isolated from leaves, roots and stems of four wheat cultivars and a breeding line at three different sampling dates during the 1993 growing season. Of the 55 different fungal taxa encountered, 19 were present at relative importance values of more than 5%. No cultivar-related differences in the assembleges of endophytes were observed. *Phoma glomerata* was not restricted to only one tissue type, whereas *Alternaria alternata*, basidiomycete sp. 1, *Pleospora herbarum* and *Epicoccum nigrum* occurred primarily in the leaves, and *Fusarium avenaceum* was extremely frequent in roots. In general, colonization by endophytes increased with the age of the plants. Most endophytes were isolated from wheat leaves. Successional colonization of a given tissue type was quantitative rather than qualitative, with a given fungal taxon increasing or decreasing over the period sampled, rather than replacing the fungi initially encountered.

Key Words——fungal endophytes; South Africa; *Triticum aestivum*.

Introduction

Fungal endophytes occurring in foliage and other plant parts are known from numerous hosts (Carroll et al., 1977; Petrini and Carroll, 1981; Petrini et al., 1982, 1992), including graminicolous genera (Bannon, 1978; Riesen and Close, 1987; Sieber et al., 1988; Dewan and Sivasithamparam, 1988, 1989; Fernandez, 1991). In a study of endophytic fungi occurring in coniferous foliage, Carroll and Petrini (1983) reported them to be either symbionts, weak parasites or latent saprophytes. In a similar study of endophytes occurring in different wheat cultivars, the majority were found to be facultative symbionts (Sieber et al., 1988).

In different case studies endophytes have been demonstrated to protect their host against aphids, beetles, cattle or insects (Webber and Gibbs, 1984; Carroll, 1988; Clay, 1988; Petrini et al., 1989; Scott and Schardl, 1993), to stimulate seed germination (Luginbühl and Müller, 1980), and increase growth (Leuchtmann and Clay, 1988). Significant increases of wheat yield have been obtained after fungicide applications in the absence of disease symptoms, suggesting that the presence of endophytes may also influence the eventual yield. Furthermore, a pathogen such as *Stagonospora nodorum* (Berk.) Cast. & Germ. was shown to have an extended period of host colonization in the absence of obvious disease symptoms (Sieber et al., 1988).

Previous studies of endophytes in wheat (Bannon, 1978; Luginbühl et al., 1979; Sieber et al., 1988) have shown several pathogens such as *S. nodorum, Didymella exitialis* (Morini) Müller, *Fusarium* spp. and *Rhizoctonia solani* Kühn to be present in symptomless leaves. Sieber et al. (1988) reported that several other minor pathogens and species more specific to wheat also occurred, that the number of isolates generally increased with increasing plant age, and that several species could be correlated with specific plant organs. They also concluded that the frequency with which *S. nodorum* occurred among endophyte isolates from wheat tissue could not serve as a reliable basis for the selection of resistant genotypes, as no distinct relationship was found between the number of isolates and disease symptom expression.

A breeding line, Alpha, which is a semi solid stemmed wheat of which the basal 2–3 internodes are normally completely filled with pith, and four hollow stemmed wheat cultivars were selected for the present study. In field nurseries, leaves of solid stemmed genotypes appeared to remain longer healthy and green when compared to hollow stemmed selections. The aim of the present study was to determine which endophytes occurred in wheat in the western Cape Province of South Africa, and if their frequencies were affected by the wheat genotypes included in the trial. A further aim was to investigate the distribution of the different fungi within

*Present address: Téra d'Sott 5, CH-6949, Comano, Switzerland.

the different plant parts, and to determine whether there was a season-related succession during the growing period.

Materials and Methods

Four wheat cultivars (SST66, Dias, Nantes and Palmiet) and a breeding line, Alpha, were grown in a randomised complete block trial at the Welgevallen Experiment Farm, Stellenbosch in 1993. The trial was planted at the beginning of May. Each genotype was replicated five times. A plot consisted of two 3-m rows planted 37 cm apart. The sowing density was 54 kg/ha. Standard fertilizer applications were made but no chemical control of insects or diseases was done. Five plants were collected randomly from each replicate of each cultivar at three different growth stages. The plants were sampled after 12 weeks (5 Aug., Zadoks growth stage 22-24), 14 weeks (19 Aug., Zadoks growth stage 39-45) and 17 weeks (9 Sept., Zadoks growth stage 60-62), respectively (Zadoks et al., 1974). The presence of endophytes in the leaves, culms, and roots was detected by plating out surface sterilized plant segments on malt extract agar (MEA) (10 g Biolab malt extract, 15 g agar, 1000 ml H_2O). Plants were initially washed under running tap water for 5 min, divided into ten segments (10–15 cm in length) as described by Sieber et al. (1988): 1, leaf tip; 2, leaf mid-section; 3, leaf base; 4, leaf sheath; 5, root tip; 6, root mid-section; 7, root crown; 8, first basal node; 9, first basal internode; 10, second basal node, and then surface sterilized. Segments were dipped successively into 96% ethanol for 1 min, sodium-hypochlorite (2% available chlorine) for 3 min, and 96% ethanol again for 30 sec. (Luginbühl et al., 1979). Surface-sterilized segments were then transferred aseptically to marked positions in 90-mm Petri dishes containing MEA, supplemented with 10 mg/l Streptomycin to suppress bacterial growth. Dishes were incubated on the laboratory bench at 25°C, and emerging mycelia transferred to MEA slants. The latter were incubated at 25°C under continuous near-ultraviolet light. Cultures were identified directly from the slants, or subcultured onto two plates divided in the middle, each containing 1.5% tap water agar with autoclaved pieces of wheat leaf tissue (10 cm in length) on the one side, and potato dextrose agar (PDA) on the other (Nelson et al., 1983). To induce sporulation one plate was incubated at 25°C, and the other at 15°C under near-ultraviolet light. Single-spored *Fusarium* species were placed on divided plates containing carnation-leaf agar (Fisher et al., 1982; Crous et al., 1992) and PDA, and treated according to the methods explained in Nelson et al. (1983).

The relative importance values (RI) of endophyte species isolated from the different plant parts were computed according to Ludwig and Reynolds (1988). After standardization of the RI values within each sample by assigning the most frequent species the value of 100% and computing the other RI values as a percentage of it, a graphical display was prepared including all fungal species with standardized RI values of at least 10% in one of the three samples. For correspondence analysis, only those fungi with a standardized RI of at least 5% in either roots, culms, or leaves were used for the ordination analysis. Simple correspondence analysis was performed with the package SimCA 2.1 (Greenacre, 1986) on the raw data, since for each organ the same sample size had

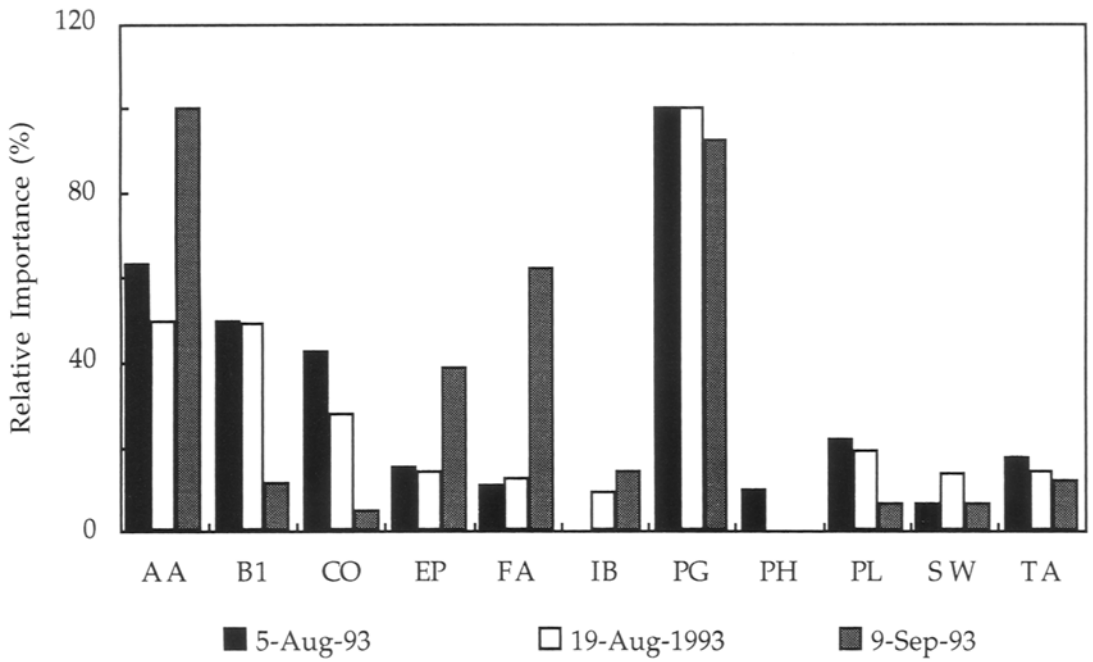

Fig. 1. Bar chart displaying the overall relative importance of selected wheat endophytes at the three sampling dates, irrespective of the tissue type. Only those fungi that account for at least 10% relative importance at any of the sampling dates are shown. For additional details see text. Fungal codes are explained in Table 1.

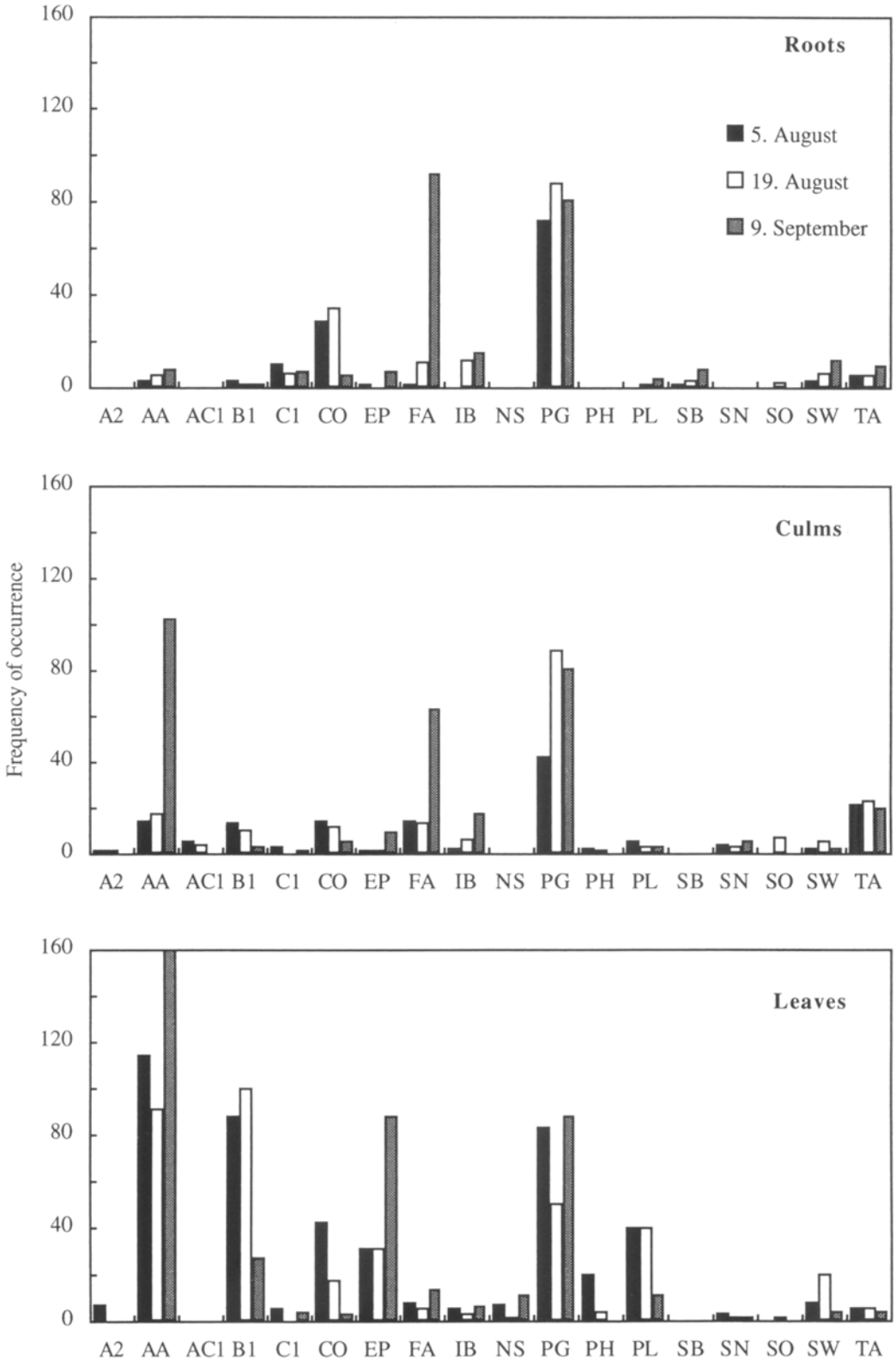

Fig. 2. Bar chart displaying the frequency of occurrence of selected wheat endophytes at the three sampling dates, in correlation with the tissue type. Only those fungi that have been used for the statistical analysis or account for at least 5% RI at any of the sampling dates are shown. For additional details see text. Fungal codes are explained in Table 1.

been used. The fungal codes used in the graphical display are given in Table 1, and the numbers of the various plant part (1-10) are explained above.

Results and Discussion

Of the 55 different fungal taxa isolated, including four distinctive sterile mycelia, only 19 were present at RI values of more than 5% (Table 1), and only 11 taxa were recorded at standardized RI larger than 10% (Fig. 1).

Correspondence analysis on the data gathered from the different samplings showed that in a specific growth stage the different plant parts of each cultivar were similarly colonized by the primary fungi, i.e., there is no evidence of a host genotypic effect. No cultivar-related differences relating to fungal assembleges could be detected at any sampling time (results of the analysis not shown). In the first two samplings, the percentage of total inertia explained by the first four factors was approximately 50%, indicating only a moderate fit of the model to the data. In the third sampling, on the other hand, the percentage increased to 68%. This indicates that in the first two sampling dates the fungal assemblages of the organs are still not well defined and no clear grouping can be detected, whereas later in the growing season the fungal assemblages of the different tissues investigated become more tissue-specific. However, even at the first sampling date the fungal assemblages of the leaves appear to be distinct from those of other tissues and are mainly composed of *Alternaria alternata* (Fr.) Keissler, *Epicoccum nigrum* Link, basidiomycete sp. 1, *Nigrospora sphaerica* (Sacc.) Mason, and *Pleospora herbarum* (Fr.) Rabenh. Root and culm tissues, on the other hand, form a rather compact group, although there is a tendency for the root tissues to separate from those of the culms, the roots being colonized more heavily by a *Coniothyrium* sp. and having virtually no other dominant endophyte, with the exception of the ubiquitous *Phoma glomerata*. At the second sampling time the same pattern can be seen, the leaf segments once again hosting an endophyte assemblage composed mainly of the same fungi reported for sampling date 1 and distinct from those of the other tissues. In the last sampling the endophytic assemblages of leaves are well defined, and the leaf segments form a compact cluster in all cultivars. In addition, the root collar region is distinct from all other parts by being rather heavily colonized by *F. avenaceum*. *Stagonospora nodorum*, a pathogen reported by Sieber et al. (1988) as a common wheat endophyte, is particularly frequent only in the cv. Nantes and missing in all other cultivars. The latter is the only real cultivar difference, and may be because 1993 was a year with relatively low rainfall, and subsequently few leaf diseases were observed in wheat growing in the area.

Several of the fungi listed in Table 1 show an appreciable degree of tissue specificity (Figs. 2, 3). Data of all cultivars have been pooled, since no cultivar-related differences were observed. *Phoma glomerata* (Cda) Wollenw. & Hochapf., a common endophyte of wheat (Sieber, 1985) is apparently not restricted to only one tis-

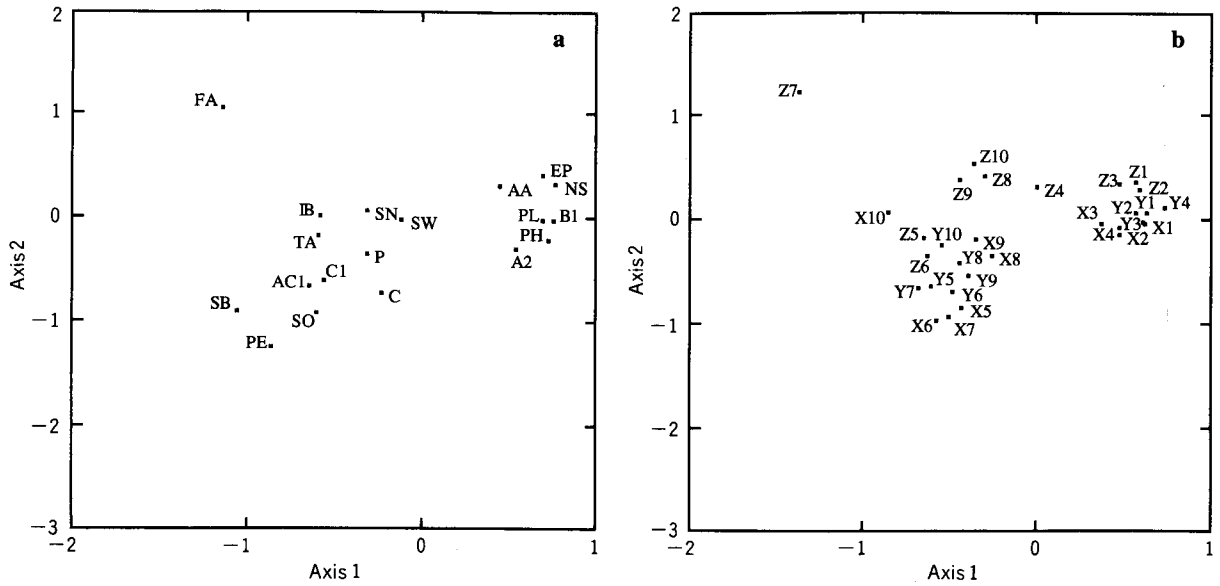

Fig. 3. Results of the ordination by simple correspondence analysis for all sampling dates. Data from all cultivars were pooled, since no significant differences could be seen among cultivars: (a, graphical display of the co-ordinates of the fungal taxa; b, coordinates of the plant parts). Only the fungi that were present at standardized RI higher than 5% have been used in the analysis. Symbols of plant parts that lie close in the graphical display tend to be colonized by similar endophyte assemblages. Correspondingly, fungi close on the display are frequent in the same tissues. To detect which fungi are present in a given tissue, the two displays can be superimposed and the relative position of fungal and plant symbols compared in the fashion described. Abbreviations: see Table 1 for fungal codes, and methods for plant parts. X, Y and Z refer to the three sampling dates, respectively. Total inertia explained by the first four co-ordinates: 72.5%.

Table 1. Most frequent endophytic fungi isolated from wheat plants at three different dates. The raw frequencies are given. The figures are the total number of isolations from a given plant organ of five different cultivars. The code indicates the symbols used in the display of the statistical analysis. Only those fungi which have been included in the statistical analysis have been considered*.

Taxon	Code	Roots			Culms			Leaves		
		5.8.	19.8.	9.9.	5.8.	19.8.	9.9.	5.8.	19.8.	9.9.
Ascochyta sp. 2	A2	0	0	0	1	1	0	7	0	0
Alternaria alternata	AA	3	5	8	14	17	102	114	91	160
Acremonium sp. 1	AC1	0	0	0	5	4	0	0	0	0
Basidiomycete sp. 1	B1	3	1	1	13	10	3	88	100	27
Chaetomium sp. 1	C1	10	6	7	3	0	1	5	0	4
Coniothyrium sp.	CO	29	34	5	14	12	5	42	17	3
Epicoccum nigrum	EP	1	0	7	1	1	9	31	31	88
Fusarium avenaceum	FA	1	11	92	14	13	63	8	5	13
Microdochium bolleyi	IB	0	12	15	2	6	17	5	3	6
Nigrospora sphaerica	NS	0	0	0	0	0	0	7	1	11
Periconia sp.	PE	1	2	0	0	2	0	0	0	0
Phoma glomerata	PG	72	88	81	42	89	81	83	50	88
Phomopsis sp.	PH	0	0	0	2	1	0	20	4	0
Pleospora herbarum	PL	0	1	4	5	3	3	40	40	11
Black sterile mycelium	SB	1	3	8	0	0	0	0	0	0
Stagonospora nodorum	SN	0	0	0	4	3	5	3	1	1
Orange sterile mycelium	SO	0	2	0	0	7	0	0	1	0
White sterile mycelium	SW	3	6	12	2	5	2	8	20	4
Truncatella angustata	TA	5	5	9	21	23	20	5	5	4

* Rare isolates (less than 5% standardized relative importance) include:
Acremonium spp., *Ascochyta* sp. 1, basidiomycete sp. 1 and sp. 2, *Bipolaris australis, Brachysporiella setosa, Chaetomium* sp. 2, *Cladorrhinum* sp., *Cladosporium* sp., *Cochliobolus sativus, Colletotrichum gloeosporioides, Cylindrocarpon destructans, Didymella* sp., *Fusarium acuminatum, F. culmorum, F. equiseti, F. oxysporum, F. scirpi, Geotrichum candidum, Gliocladium roseum, Gliocladium* sp., *Gnomonia* sp., *Hyalodendron* sp., *Leptosphaeria* sp., *Ophiobolus* sp., *Periconia* sp., *Phialophora* sp., *Pyrenophora tritici-repentis, Pyrenophora* sp., *Phaeoseptoria* sp., *Pyrenochaeta* sp., *Pythium* sp., *Robillarda sessilis, Septoria tritici, Sporormiella australis, Verticillium* sp.

sue type. In contrast, *A. alternata*, basidiomycete sp. 1, *P. herbarum* and *E. nigrum* colonize preferentially the wheat leaves. *A. alternata* to a lesser extent also colonizes the culms, while *Fusarium avenaceum* (Fr.) Sacc. is extremely frequent in roots and culms. The latter is comparatively rare in leaves. There was an overall tendency for the frequency of a given endophyte species to increase over the sampling period. Exceptions in this regard were *A. alternata* (all tissues) and *P. glomerata* (leaves), which were already present in large numbers in the first two sampling dates, and basidiomycete sp. 1, which showed a sharp decrease in the third sampling date. *P. glomerata* did not show any appreciable variation over the sampling time (Fig. 1). For both *A. alternata* and *E. nigrum* the increase could be due to the rapid growth and probably also to the epiphytic spread of these organisms, as reported by Sieber (1985) and O'Donnell and Dickinson (1980).

Several of the taxa identified as endophytes (Table 1) have pathological implications for wheat cultivation. Wiese (1987) and Zillinsky (1983) listed, among others, *Alternaria, Aschochyta* and *Epicoccum* sp., *Phoma glomerata*, and *Pleospora herbarum* as saprohytic or weakly pathogenic on wheat. These fungi are commonly associated with wheat throughout its life span in the field but are most frequent when humid conditions coincide with maturation (Wiese, 1987). *F. avenaceum* causes root and crown rot, and has also been associated with head scab of wheat (Wiese, 1987). In South Africa, this species was commonly isolated from the crowns of wheat plants displaying white heads, as well as from symptomless plants (Klaasen et al., 1992). Zillinsky (1983) regarded *P. glomerata* and other *Phoma* spp., which are usually encountered as secondary invaders of host tissues, to have limited pathogenic potential on cereal crops and grasses. *P. herbarum* also invades lesions caused by other pathogens, or can produce leaf spots (Zillinsky, 1983). *Stemphylium botryosum* Wallr., the anamorph of *P. herbarum*, is known to contribute to black mold of wheat heads (Wiese, 1987). *S. nodorum* is a well-known and globally damaging disease of wheat (Eyal et al., 1987). It frequently occurs in all wheat

production areas of the Cape Province, but only sporadically in Natal and the Orange Free State (Scott, 1990).

To test the hypothesis of a seasonal succession within endophyte assemblages as postulated by Widler and Mueller (1984) and Sieber (1985), a correspondence analysis was carried out on the pooled data of all cultivars, since the previous analysis failed to detect cultivar-specific patterns. The results presented in Fig. 3 (only axes 1 and 2 represented) confirm some tissue-specificity but only partly support the hypothesis of seasonal succession in the endophyte assemblages. The percentage of total inertia explained by the first four factors is 72%, and by the first two 53%, indicating a good fit of the model to the data. The first factor (axis 1) discriminates between leaf segments (1-4) and the other (axis 2) root (5-7) and culm (8-10) segments. The second factor separates the root collar from the other root and culm segments. On this factor a gradient can be seen that gradually, but not significantly, separates the samplings, with the sampling units of the first sampling on the negative part of the axis and those of the last on the positive one. We conclude that a succession corresponding to its classical definition cannot be detected in wheat. Changes in the assemblages are not qualitative, with given endophyte species gradually replaced by others, but rather quantitative, with colonization by a given fungal taxon increasing or decreasing according to the vegetation period. While succession in endophytic assemblages can be expected and has already been shown in few cases in woody perennials (Chapela and Boddy, 1988), season-related succession of endophyte assemblages in annual grasses and other plants apparently does not occur.

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