Ribosomal DNA analyses challenge the status of *Fusarium* sections *Liseola* and *Elegans*

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Waalwijk, C., R. P. Baayen, J. R. A. de Koning & W. Gams (1996). Ribosomal DNA analyses challenge the status of *Fusarium* sections *Liseola* and *Elegans*. – Sydowia 48 (1): 90–104.

The internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) of species in *Fusarium* sections *Elegans*, *Liseola* and *Dlaminia* and species considered to be related to these sections was analyzed by amplification and subsequent digestion with several restriction enzymes. Two distinct RFLPs were encountered among the species studied that differed from one another in size and in restriction pattern with *Hha*I, *Hin*fI and *Pst*I. RFLP pattern I was shared by *F. oxysporum*, *F. anthophilum*, *F. napiforme*, *F. polyphialidicum*, *F. sacchari* var. *sacchari* and var. *elongatum*, *F. subglutinans*, *F. succisae* and *F. verticillioides*. RFLP pattern II was shared by *F. redolens*, *F. annulatum*, *F. beomiforme*, *F. dlaminii*, *F. fujikuroi*, *F. nygamai* and *F. proliferatum*. Neither RFLP was encountered in species belonging to sections *Fusarium*, *Sporotrichiella*, *Gibbosum* or *Martiella*. Sequence analysis for *F. oxysporum* and *F. redolens* revealed that diversity was mainly located in the ITS2 region. The separation of the sections *Elegans*, *Liseola* and *Dlaminia* becomes debatable.

Keywords: *Fusarium*, molecular taxonomy, ITS region, ribosomal DNA.

The basis for all *Fusarium* systematics is the monograph of Wollenweber & Reinking (1935), who recognized 65 species grouped together into 16 sections. Their classification has been followed and their system further extended by Gerlach & Nirenberg (1982) and Nirenberg (1989), who recognized more than 90 species. A different interpretation was presented by Snyder & Hansen (1940, 1941, 1945) who reduced the entire genus to nine species, each corresponding to one or more of the sections described by Wollenweber & Reinking. With the work of Nelson & al. (1983), who considered 30 well-documented and 16 questionable species grouped into 13 sections, a certain convergence between the two schools was observed. One of the sections, *Arachnites*, encompasses species now excluded from the

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genus (Gams & Nirenberg, 1989), with the exception of *F. larvarum* which is better placed in one of the other sections. A more detailed discussion of the history of *Fusarium* taxonomy is found in Nelson (1991).

Perfect states (teleomorphs) of species considered to belong to the deuteromycete genus *Fusarium* have been described mainly in the ascomycete genera *Gibberella* and *Nectria*. The teleomorphs of species in seven *Fusarium* sections [*Fusarium* (= *Discolor*), *Gibbosum*, *Roseum*, *Liseola*, *Lateritium*, *Sporotrichiella* and *Arthrosporiella*] have been classified in the genus *Gibberella* and four sections (*Eupionnotes*, *Macroconia*, *Martiella* and *Ventricosum*) have teleomorphs in *Nectria*. In contrast, no teleomorphs have been described for species in section *Elegans* (Nelson & al., 1983). Sequence analyses of the large subunit ribosomal RNA of species belonging to *Fusarium* and related genera have indicated that the core sections of *Fusarium*, and also section *Elegans*, are related to *Gibberella*. According to these analyses, section *Elegans* is closely related to section *Liseola* (Guadet & al., 1989; Bruns & al., 1991).

The distinction between the sections *Liseola*, without chlamydospores, and *Elegans*, with chlamydospores, has generally been accepted as valid. Species in section *Liseola* have slightly basally tapering and truncate microconidia produced from mono- or polyphialides in slimy heads or in chains and many of these species are toxinogenic. Species in section *Elegans*, *F. oxysporum* and *F. redolens*, have oval microconidia produced in heads from monophialides. Both species in section *Elegans* are known to be generally non-toxigenic (Thrane, 1989).

Several species have been described in recent years that combine characteristics of both sections. *F. nygamai*, *F. dlaminii*, *F. beomiforme* and *F. napiforme* cannot properly be accommodated in either section *Elegans* or *Liseola*. A new section, *Dlaminia*, was therefore proposed for these species by Kwasna & al. (1991). In this light, it is interesting that Bilai (1955, 1977) already chose to combine the sections *Liseola* and *Elegans* under the name *Elegans*. More remote species with possible affinity to sections *Liseola* and *Elegans* are *F. polyphialidicum* (Marasas & al., 1986) and *Fusarium* sp., isolate BBA 65467.

Consensus on the position of species and varieties in section *Liseola* was greatly stimulated by laboratory crosses between isolates as performed by Leslie and coworkers (Leslie, 1991). At least six mating populations exist (Leslie, 1991) that partly coincide with species or varieties in the classification of Nirenberg (1976) and Nelson & al. (1983). However, not all species in section *Liseola* and none in sections *Elegans* and *Dlaminia* have known teleomorphs, which restricts the use of crosses in the taxonomy of these sections.

In addition to morphological criteria or crossing experiments, molecular characteristics such as DNA homology studied by hybridization or analysis of the ribosomal DNA (rDNA) now provide taxonomic insight. The 18S, 5.8S and 28S RNA genes are highly conserved; sequence variations in these genes reflect major evolutionary events and can therefore be used to reconstruct higher order phylogenies. The three RNA-encoding regions are separated by internal transcribed spacer (ITS) regions that are less conserved and are useful to study lower order phylogeny (Bruns & al., 1991; Samuels & Seifert, 1995). In the present study this approach is applied to the sections *Elegans*, *Liseola* and *Dlaminia*.

In the course of our studies on the *Elegans* fusaria causing wilt disease of carnation, we were puzzled by the difficult distinction between *F. oxysporum* and *F. redolens*. The latter was distinguished at species level by Wollenweber & Reinking (1935) and Gerlach & Nirenberg (1982), but only as a variety by Booth (1971). Isolates typical of *F. redolens* have much blunter macroconidia and paler colonies, but they tend to lose these features during prolonged culture, after which they strongly resemble *F. oxysporum*. Many isolates have a morphology intermediate to the description of both species. The pathogenicity patterns in *F. redolens* also resemble those of *F. oxysporum*. Baayen & Gams (1988) therefore considered *F. redolens* as synonymous with *F. oxysporum*. Genetic diversity was further investigated by Baayen & Kleijn (1989) and Aloi & Baayen (1993) who described distinct vegetative compatibility groups (VCGs) both in *F. oxysporum* f.sp. *dianthi* and *F. redolens* f.sp. *dianthi*. VCGs common to both species were not detected. Different VCGs proved to have distinct restriction fragment length polymorphisms (RFLPs) of total DNA with probe D4 (Manicom & Baayen, 1993; Waalwijk & Baayen, 1995).

In an initial study, RFLP patterns of the ITS regions of isolates of *F. oxysporum* f.sp. *dianthi* and *F. redolens* f.sp. *dianthi* were found to be markedly distinct. These patterns were also found in other formae speciales and non-pathogenic isolates of both species, thus apparently confirming the separation of *F. redolens* from *F. oxysporum* at the species level. However, of two species from section *Liseola* included as outgroup, one (*F. sacchari* var. *elongatum*) had the same RFLP pattern as *F. oxysporum* while the other (*F. proliferatum*) matched the pattern of *F. redolens*. The possibility that ITS restriction patterns distinguish at a higher level than that of species was investigated by extending the study to other species in section *Liseola*.

The present study describes the diversity in ITS restriction sites in sections *Elegans*, *Liseola* and *Dlaminia* and compares these patterns with those in four other sections of the genus, three of which have teleomorphs in *Gibberella* and one with teleomorphs in *Nectria*.

Materials and methods

The isolates used in this study (Tab. 1) were collected from various sources and comprise the major species in sections *Elegans* and *Liseola*. Monospore cultures were prepared from all isolates before DNA was extracted.

Tab. 1. – List of isolates used and their origin.
CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; IPO, DLO Research Institute for Plant Protection (IPO–DLO), Wageningen, The Netherlands; PD, Plantenziektenkundige Dienst, Wageningen, The Netherlands. Isolates not preceded by one of these acronyms originate from various private collections (see footnotes).

Fusarium acuminatum Ellis & Kellerm. – CBS 618.87, ex soil, Denmark.
Fusarium annulatum Bugnic. – CBS 258.54, ex *Oryza sativa*, Vietnam, type isolate of *F. annulatum*.
Fusarium anthophilum (A. Braun) Wollenw. – CBS 136.95, ex *Hippeastrum* sp., The Netherlands.
Fusarium beomiforme Nelson & al. – CBS 482.94 (= FRC M–1090), ex soil debris, Papua New Guinea.
Fusarium culmorum (W. G. Smith) Sacc. – PD 90/283, ex *Hordeum vulgare*, Finland.
Fusarium dlaminii Marasas & al. – CBS 481.94 (= FRC M–1581) ex plant debris, CBS 671.94 (= MRC 3023 = BBA 69046) and CBS 672.94 (= MRC 3024 = BBA 69047), ex soil, South Africa.
Fusarium equiseti (Corda) Sacc. – CBS 394.93 (= BBA 64265), ex *Disphyma crassifolium*, Germany.
Fusarium fujikuroi Nirenb. – CBS 221.76 (= BBA 12428), ex *Oryza sativa*, Taiwan, type isolate of *F. fujikuroi*.
Fusarium graminearum Schwabe. – PD 88/790, ex *Dianthus caryophyllus*, The Netherlands.
Fusarium longipes Wollenw. & Reinking. – CBS 739.79, ex soil, Malaysia.
Fusarium napiforme Marasas & al. – CBS 673.94 (= BBA 67629 = Nelson M 3560) and CBS 674.94 (= BBA 67630 = Nelson M 3566).
Fusarium nygamai Burgess & Trimboli. – CBS 572.94 (= BBA 64375), ex *Cajanus indicus*, Germany; CBS 675.94 (= BBA 65862), ex *Striga hermonthica*, Sudan.
Fusarium oxysporum Schlecht. : Fr. f.sp. *chrysanthemi* Littrell & al. – CBS 127.81, ex *Chrysanthemum* sp., U.S.A.
Fusarium oxysporum f.sp. *conglutinans* (Wollenw.) Snyder & Hansen. – IPO DPS–10186, ex *Brassica* sp.
Fusarium oxysporum f.sp. *cyclaminis* Gerlach. – IPO F92–13, ex *Cyclamen persicum*, The Netherlands.
Fusarium oxysporum f.sp. *dianthi* (Prill. & Del.) Snyder & Hansen. – FOD–E114 (source: T. K.), Israel; F1, F79, F100, F101, F107, F165, F256, F276, F310, F639, F1168 and IPO F94– 19 (source: A. G.), Italy; IPO WCS–816, IPO WCS–842, IPO F95–1 (formerly referred to as NAKS 3) and PD 90/291, The Netherlands; O–4 (source: T. K.), U.S.A.; all ex *Dianthus caryophyllus*.
Fusarium oxysporum f.sp. *gladioli* (Massey) Snyder & Hansen. – G24 (Source: E. R.), ex *Gladiolus* sp., The Netherlands.
Fusarium oxysporum f.sp. *lilii* Imle. – Fol–4 (source: H. L.), ex *Lilium* sp., The Netherlands.
Fusarium oxysporum f.sp. *lycopersici* (Sacc.) Snyder & Hansen – IPO DPS–1530, ex *Lycopersicon esculentum*.

Fusarium oxysporum f.sp. *narcissi* (Cooke & Massee) Snyder & Hansen. – CBS 169.65, ex *Narcissus* sp.

Fusarium oxysporum, non-pathogenic isolates. – FO–47 (source: C. A.), ex soil, France; F62 (source: K. E.), Greece; X40 and X48 (source: B. M.), Israel; CBS 366.87 (formerly misidentified as *F. redolens*; belongs to the same VCG as CBS 840.88), CBS 840.88, IPO F95–3, PD 90/440, PD 90/1580.1 and PD 90/1580.2, all ex *Dianthus caryophyllus*, The Netherlands; PD 90/1445, ex *Dianthus barbatus*, The Netherlands.

Fusarium poae (Peck) Wollenw. – PD 93/1780, ex *Dianthus caryophyllus*, The Netherlands.

Fusarium polyphialidicum Marasas & al. – CBS 676.94 (= MRC 2405), ex soil, South Africa; CBS 677.94 (= Nelson M 2435 = BBA 67742).

Fusarium proliferatum (Matsushima) Nirenb. – CBS 133.95, ex *Dianthus caryophyllus*, Italy; CBS 134.95, CBS 135.95, IPO F95–4, IPO F95–5, IPO F95–6 (formerly referred to as NAKS 9, 11 and 16, respectively) and PD 89/1863a, ex *Dianthus caryophyllus*, The Netherlands; CBS 217.76, ex *Cattleya* sp., Germany.

Fusarium sp. Nirenb. & Tiedemann ined. – CBS 573.94 (= BBA 65467), ex soil, Germany.

Fusarium redolens Wollenw. f.sp. *dianthi* Gerlach. – IPO F94–14, ex *Dianthus caryophyllus*; DSM 62390 (= CBS 360.87), type isolate of *F. redolens* f.sp. *dianthi*, DSM 62391 and DSM 62392 (= CBS 362.87), ex *Dianthus caryophyllus*, Germany; DSM 62393 (= CBS 363.87), ex *Dianthus barbatus*, Germany; PD 89/1825 and PD 90/1882, ex *Dianthus barbatus*, The Netherlands.

Fusarium redolens Wollenw., unspecified isolates. – DSM 62378 (= CBS 364.87) and DSM 62379 (= CBS 365.87), ex *Dianthus caryophyllus*, Germany; PD 90/1377, ex *Dianthus caryophyllus*, The Netherlands; DSM 62380, ex *Asparagus* sp.; DSM 62383, ex *Helleborus* sp.; DSM 62384, ex *Fragaria* sp.; DSM 62385, ex *Convallaria majalis*; DSM 62386, ex *Fritillaria* sp.; DSM 64524, ex *Solanum tuberosum*; DSM 64613, ex *Pisum* sp.

Fusarium sacchari (Butler) W. Gams var. *sacchari*. – CBS 223.76 (= BBA 63340), ex *Saccharum officinarum*, India.

Fusarium sacchari var. *elongatum* Nirenb. – PD 88/317, ex *Aechmea* sp., The Netherlands.

Fusarium subglutinans Wollenw. & Reinking. – CBS 215.76, ex *Zea mays*, Germany.

Fusarium sambucinum Fuckel. – PD 90/250, ex soil, The Netherlands.

Fusarium scirpi Lambotte & Fautrey. – CBS 448.84, ex pasture soil, Australia.

Fusarium solani (Mart.) Sacc. – Fol 44 (source: H. L.), ex *Lilium* sp., The Netherlands; IPO F94–23 and IPO F94–24 (source: P. O.), ex *Pisum sativum*; PD 87/712, ex *Gerbera* sp., The Netherlands.

Fusarium sporotrichioides Sherb. – PD 82/776, ex *Daucus carota*, The Netherlands.

Fusarium succisae (Schröter) Sacc. – CBS 219.76, ex *Succisa pratensis*, Germany.

Fusarium tricinctum (Corda) Sacc. – CBS 393.93, ex *Triticum aestivum*, Germany.

Fusarium verticillioides (Sacc.) Nirenb. – CBS 218.76, ex *Zea mays*, Germany.

Source acronyms: A. G., A. Garibaldi, Turin, Italy; B. M., B. Q. Manicom, Nelspruit, South Africa; C. A., C. Alabouvette, Dijon, France; E. R., E. J. A. Roebroeck, Lisse, The Netherlands; H. L., H. J. M. Löffler, Wageningen, The Netherlands; K. E., K. Elena, Athens, Greece; P. O., P. Oyarzun, Wageningen, The Netherlands; T. K., T. Katan, Bet Dagan, Israel.

Fungal isolates were grown at 27 C in potato-dextrose broth at 150 rpm. Mycelium was harvested, lyophilized, ground under liquid N_2 and suspended in extraction buffer (0.15 M NaCl, 50 mM Tris-HCl,

pH 8.0, 10 mM EDTA, 1% SDS). Following two phenol/chloroform extractions, RNA was degraded by treatment with RNase A (50 µg/ml) for 30 min at 37 C. After an additional phenol/chloroform extraction, the DNA was precipitated and resuspended in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

The internal transcribed spacer region was amplified in a PCR reaction using the primers ITS-1 and ITS-4 (White & al., 1990). Amplifications were performed in a 100 µl reaction volume containing 60 µM of both primers, 2 units Taq DNA polymerase (Gibco-BRL) and 1.5 mM $MgCl_2$. PCR conditions consisted of a denaturation step of 1 min at 94 C, 35 amplification cycles and an extension step of 10 min at 72 C. Amplification cycles were 1 min at 94 C, 1 min at 56 C and 1.5 min at 72 C. Some DNAs that did not allow amplification of the ITS region with these primers were amplified with ITS-4 and a modified ITS-1 primer, ITS-1* (TCCGTTGGTGAACCAGCGG), based on the sequences published by O'Donnell (1992). The ITS region from *F. fujikuroi* could only be amplified by lowering the annealing temperature from 56 C to 50 C. Amplified products were digested individually with 12 restriction enzymes and analyzed on 1.5% agarose gels.

Amplified DNA fragments from two independent PCR reactions were cloned into the PCR cloning vectors pCRII (Invitrogen) or pGEM-T (Promega) and plasmid DNAs were subjected to automatic DNA sequencing using ampliTaq DNA polymerase and the –21M13 forward primer and M13 reverse primer. Alternatively, the SP6- and T7-sequencing primers were used. Pairwise alignments were done using the GCG program Best fit and dendrograms were constructed with GCG program Pileup (Genetics Computer Group, Inc., Madison, Wisconsin).

Results

Initial experiments were directed to the analysis of possible variation in the ITS regions of the rDNA repeat between *F. oxysporum* f.sp. *dianthi* and *F. redolens* f.sp. *dianthi*. Amplification reactions were performed on 12 isolates representing the VCGs 0020 to 0027 and a non-pathogenic isolate. The PCR products of these isolates appeared to fall within one of two different size classes of either 545 bp or 560 bp. All isolates identified as *F. oxysporum* gave the 545 bp fragment, whereas the three isolates that gave the 560 bp amplification product had originally been identified as *F. redolens*. The amplified DNA fragments were digested with twelve restriction enzymes (Tab. 2). Apart from the difference in overall size, eight

Tab. 2. – Restriction fragment length sizes of amplified DNAs from the ITS region of *F. oxysporum* and *F. redolens*.

Enzyme	*F. oxysporum*	*F. redolens*
*Alu*I	60, 135, 350	560
*Cla*I	215, 330	215, 345
*Dde*I	155, 390	155, 405
*Eco*RI	260, 285	260, 300
*Hae*III	90, 120, 340	80, 90, 120, 275
*Hha*I	250, 295	90, 175, 295
*Hinc*II	55, 490	55, 505
*Hinf*I	90, 180, 265	265, 290
*Msp*I	100, 445	100, 170, 290
*Mbo*I	25, 50, 60, 110, 300	25, 50, 60, 110, 135, 175
*Pst*I	545	135, 425
*Taq*YI	50, 60, 85, 135, 215	50, 60, 215, 230

enzymes were found to produce RFLP patterns that further discriminated between the two size class groups, that are subsequently designated RFLP pattern I (*F. oxysporum*) and RFLP pattern II (*F. redolens*).

Three enzymes, *Eco*RI, *Hha*I and *Hinf*I, were selected to screen a collection of isolates consisting of 36 isolates of *F. oxysporum* and 17 isolates of *F. redolens*, encompassing several formae speciales as well as non-pathogenic isolates of both species. All isolates originally classified as *F. redolens* had RFLP pattern II while all of the *F. oxysporum* isolates had RFLP pattern I. A restriction map of the ITS regions of *F. oxysporum* and *F. redolens* is given in Fig. 1. Three isolates of *F. proliferatum*, one of *F. sacchari* var. *elongatum* and one of *F. sporotrichioides* were included as possible outgroup isolates. While DNA from *F. sporotrichioides* produced a unique RFLP pattern, DNA from *F. sacchari* var. *elongatum* and *F. proliferatum* gave RFLP patterns I and II, respectively.

RFLP analyses of ITS regions were extended to 15 species and varieties in section *Liseola* (and *Dlaminia*), represented by one to eight isolates (Tab. 1). Isolates A.G. F101 and A.G. F639 were included as references for *F. oxysporum* and DSM 62390 and DSM 62393 as references for *F. redolens*, respectively. Amplified DNA of all isolates was restricted with *Hha*I, *Hinf*I and *Pst*I. Six species and varieties in section *Liseola* along with *F. napiforme* (section *Dlaminia*), *F. polyphialidicum* and *Fusarium* sp. isolate BBA 65467 produced the RFLP pattern I, while the remaining species in sections *Liseola* and *Dlaminia* produced RFLP pattern II (Tab. 3). RFLP patterns of the ITS region in sections *Elegans* and *Liseola* were compared with those in 11 species in four other sections of the genus using the same restriction enzymes (Tab. 3). Six additional RFLP patterns were

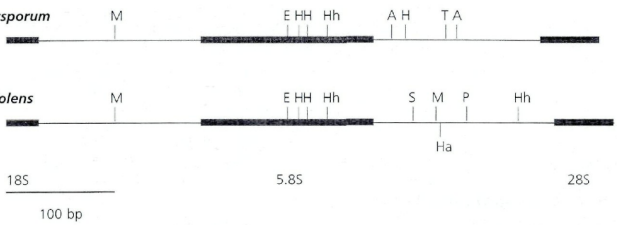

Fig. 1. – Schematic representation of the ITS regions and the 5.8S rRNA of the rDNA repeat in *F. oxysporum* and *F. redolens*. Some of the restriction sites that discriminate between the two species are indicated: *Alu*I (A), *Eco*RI (E), *Hae*III (Ha), *Hha*I (Hh), *Hinf*I (H), *Msp*I (M), *Pst*I (P), *Sau*3AI (S) and *Taq*I (T).

detected among these species, some of which were confined within a single section while others traversed section boundaries. None of these species had RFLP patterns I or II.

To substantiate the polymorphisms observed between *F. oxysporum* and *F. redolens*, the DNA sequences of ITS1, ITS2 and the 5.8S rDNA gene were determined for two clones each of *F. oxysporum* isolates A.G. F101 and A.G. F639 and *F. redolens* isolates DSM 62390 and DSM 62393. The sequences obtained for both isolates of *F. oxysporum* were completely identical as were the sequences of the two isolates of *F. redolens*. Between the species considerable sequence divergence was found (Fig. 2). The sequences of the rDNA genes are identical for both species. Only minor differences between both species occur in the ITS1 region, whereas considerable sequence differences are observed in ITS2. All the observed polymorphisms could be attributed to sequence divergences within ITS2. The ITS sequence of *F. oxysporum* was compared with ITS sequences of *F. oxysporum* f.sp. *vasinfectum* submitted to the EMBL database (accession X78258, X78259 and X78260) (Moricca & al., 1995) and this revealed sequence divergence (1 or 2 additional T-residues in *F. oxysporum* f.sp. *vasinfectum*) at nucleotide 68 in Fig. 2, the only position where *F. oxysporum* and *F. redolens* show divergence in ITS1. A comparison of the sequences of both species with published data on ITS sequences in the *F. sambucinum* complex (O'Donnell, 1992) is shown in Fig. 3. Three distinct species have recently been described in this complex (Nirenberg, 1995): *F. sambucinum sensu stricto*, *F. torulosum* and *F. venenatum*, which coincide with respectively ITS types B, A and C sensu O'Donnell (1992) (H. I. Nirenberg, pers. comm. to W. Gams; we have confirmed this by size comparison of ITS1 and ITS2 for three isolates from Nirenberg's collection of each of these

Tab. 3. – Restriction fragment length sizes of amplified DNA from the ITS region restricted with *Hha*I, *Hin*fI and *Pst*I and the inferred RFLP grouping for 28 species and varieties in seven sections of the genus *Fusarium*.

	*Hha*I	*Hin*fI	*Pst*I	RFLP
ELEGANS				
F. oxysporum	250, 295	90, 180, 265	545	I
F. redolens	90, 175, 295	265, 290	135, 425	II
LISEOLA, DLAMINIA (#) and related species (##)				
F. anthophilum	250, 295	90, 180, 265	545	I
F. napiforme#	250, 295	90, 180, 265	545	I
F. polyphialidicum##	250, 295	90, 180, 265	545	I
Fusarium sp., BBA65467	250, 295	90, 180, 265	545	I
F. sacchari var. *sacchari*	250, 295	90, 180, 265	545	I
F. sacchari var. *elongatum*	250, 295	90, 180, 265	545	I
F. subglutinans	250, 295	90, 180, 265	545	I
F. succisae	250, 295	90, 180, 265	545	I
F. verticillioides	250, 295	90, 180, 265	545	I
F. annulatum	90, 175, 295	265, 290	135, 425	II
F. beomiforme#	90, 90, 175, 200	265, 290	135, 425	II*
F. dlaminii#	90, 175, 295	265, 290	135, 425	II
F. fujikuroi	90, 175, 295	265, 290	135, 425	II
F. nygamai#	90, 175, 295	265, 290	135, 425	II
F. proliferatum	90, 175, 295	265, 290	135, 425	II
FUSARIUM				
F. culmorum	90, 220, 250	265, 290	160, 400	III
F. graminearum	90, 220, 250	265, 290	160, 400	III
F. sambucinum	260, 285	265, 270	545	IV
SPOROTRICHIELLA				
F. poae	260, 285	265, 270	545	IV
F. sporotrichioides	90, 220, 250	265, 290	560	V
F. tricinctum	100, 200, 220	265, 290	135, 425	VI
GIBBOSUM				
F. acuminatum	100, 200, 220	265, 290	135, 425	VI
F. longipes	100, 200, 220	265, 290	135, 425	VI
F. equiseti	90, 200, 250	90, 180, 265	545	VII
F. scirpi	90, 200, 250	90, 180, 265	545	VII
MARTIELLA				
F. solani	90, 175, 295	60, 230, 265	560	VIII

* *F. beomiforme* has an additional restriction site for *Hha*I.

species). *F. sambucinum sensu stricto* forms a distinct mating population, while no fertile crosses could be obtained with *F. torulosum* or *F. venenatum* (Desjardins & Nelson, 1995). The similarity dendrogram suggests that *F. oxysporum* would be more

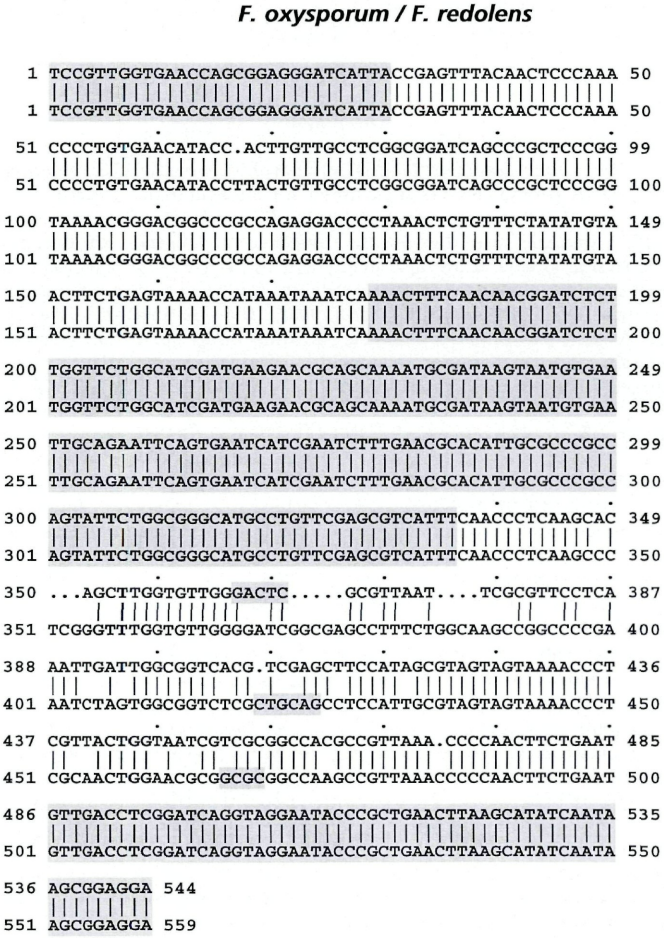

Fig. 2. – DNA sequence of the amplified fragment of the rDNA in *F. oxysporum* and *F. redolens*. The hatched boxes represent the 3' end of the 18S RNA gene, the 5.8S rRNA gene and the 5' end of the 28S rRNA gene, respectively. Small hatched boxes within the sequence indicate the polymorphic restriction sites for the enzymes *Hinf*I (position 363 in *F. oxysporum*) and *Pst*I and *Hha*I (positions 419 and 466, respectively) in the *F. redolens* sequence.

closely related to *F. sambucinum* and *F. venenatum* than to *F. redolens*, while *F. redolens* would instead be related to *F. torulosum*.

Discussion

Restriction fragment length polymorphisms in the ITS region of the rDNA provided a reliable, albeit not morphological, distinction between *F. oxysporum sensu stricto* and *F. redolens*. Differences in RFLP patterns are due to the presence or absence of specific restriction sites and are also evident in the DNA sequences for two different isolates of each of the species. Contrary to the conclusion drawn by Baayen & Gams (1988), the distinction between *F. oxysporum* and *F. redolens* is thus fully justified. Current classification systems for races and vegetative compatibility groups among isolates of *F. oxysporum* f.sp. *dianthi sensu lato* (Aloi & Baayen, 1993) must therefore be reconsidered. Of the eight races of *F. oxysporum* f.sp. *dianthi* described by Garibaldi (1983), one (race 3) had RFLP pattern II and should be classified as *F. redolens* f.sp. *dianthi* Gerlach. Originally race 3 had been identified as *F. redolens* (Garibaldi, 1979). Similarly, two of the VCGs (0023 and 0024) of *F. oxysporum* f.sp. *dianthi* described by Aloi & Baayen (1993) should be placed in *F. redolens* f.sp. *dianthi*. The sharp distinction of the two *Fusarium* species on the basis of ITS data conflicts with the observation by Rataj-Guranowska & Walkowiak-Cagara (1994) that some strains identified as *F. oxysporum* or *F. redolens* can form heterokaryons. This discrepancy needs to be resolved.

Analysis of the ITS region with several restriction enzymes revealed a close relationship between *F. oxysporum* in section *Elegans* and some taxa of the sections *Liseola* and *Dlaminia* on the one hand, and between *F. redolens* and the remaining species in these two sections on the other. Preliminary data on DNA sequences of the ITS region for these species (not shown) support the RFLP grouping. Our data indicate that the hitherto distinguished sections *Elegans* and *Liseola* (and *Dlaminia*) form unnatural groups of species. In recent years it has become obvious that the conventionally distinguished sections are unnatural entities that become progressively eroded by molecular data (O'Donnell, 1993).

We have not found any morphological criteria that support a subdivision of the present sections *Elegans*, *Liseola* and *Dlaminia* into two new sections according to the two RFLP patterns of the ITS regions of the rDNA. A detailed analysis of the secondary metabolites, including toxins, produced by species in both RFLP groups, may yield distinctive criteria. In any case, the distinction of a third section,

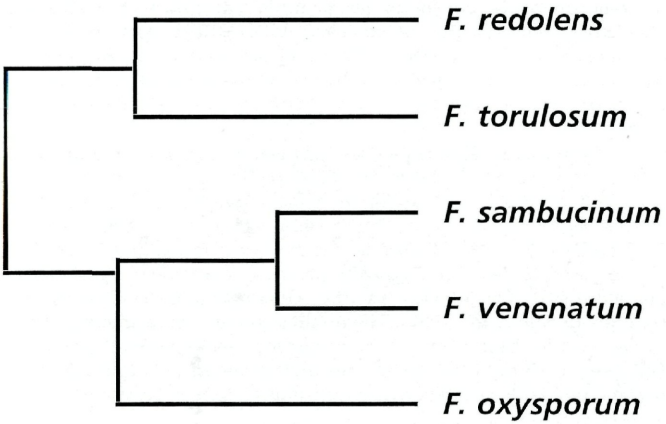

Fig. 3. – UPGMA dendrogram of the ITS sequences of *F. oxysporum* and *F. redolens* and published ITS sequences of *F. sambucinum sensu stricto* (ITS type B of O'Donnell, 1992; see Results), *F. torulosum* (ITS type A) and *F. venenatum* (ITS type C).

Dlaminia, is not supported by our data. Obviously, additional ITS sequence data are needed to substantiate these findings and to place the taxa into a phylogenetic context. Sequence analyses may also resolve the position of *F. polyphialidicum* and *Fusarium* sp., isolate BBA 65467, which both seem to be closely related to species giving RFLP pattern I such as *F. oxysporum*.

Analysis of RFLP groups among species in other sections of *Fusarium* revealed six patterns that were distinct from those in *Elegans* and *Liseola*. RFLP grouping followed existing sections (*F. culmorum* and *F. graminearum*) but occasionally traversed section boundaries (*F. sambucinum* and *F. poae*; *F. tricinctum*, *F. acuminatum* and *F. longipes*). RFLP analyses again suggest that the traditional sectional classification is unnatural. However, reclassification of the genus should not rely on sequence data from ITS regions alone. Additional DNA sequences from other regions of the fungal genome should be included, along with characteristics at biochemical and morphological levels.

No species of section *Elegans* has thus far been shown to have a teleomorph. However, analysis of VCGs and mtDNA in populations of *F. oxysporum* from agricultural soils led Appel & Gordon (1994) to the conclusion that sexual reproduction may have occurred fairly recently in these populations. The teleomorph of *F. oxysporum*, if it exists, is likely to be a *Gibberella* species close to *G. moniliformis* Winel.

(teleomorph of *F. verticillioides* [syn. *F. moniliforme*]) and other *Gibberella* teleomorphs of species with RFLP pattern I. The teleomorph of *F. redolens* might be closer to that of *F. fujikuroi* (*Gibberella fujikuroi* (Saw.) Wollenw.). Crosses between isolates of *F. redolens* may eventually reveal that this species has a teleomorph of its own.

Differences in ITS sequences between *F. oxysporum* and *F. redolens* were mainly found in the ITS2 region. This is in contrast to other fungal species such as *Rhizoctonia solani* (Liu & Sinclair, 1992) or *Gaeumannomyces* (Bryan & al., 1995) where divergence is mainly restricted to ITS1. The ITS sequences of *F. oxysporum* and *F. redolens* may be used to develop diagnostic primers as was recently shown to be successful for species in the *Gaeumannomyces-Phialophora* complex (Bryan & al., 1995). The application of such primers is likely to enable the discrimination between both pathogens of carnation. Until sequence data for the species in sections *Liseola* and *Dlaminia* become available, however, such an application is restricted to the afore-mentioned species in section *Elegans*.

Acknowledgments

The authors wish to acknowledge Dr. K. O'Donnell for critical reading and helpful suggestions to improve the manuscript.

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(Manuscript accepted 26th December 1995)