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Pathogenic, genetic and molecular characterisation of *Fusarium oxysporum* f.sp. *lilii*

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Abstract

Isolates of *Fusarium oxysporum* from lily were screened for pathogenicity, vegetative compatibility and DNA restriction fragment length polymorphisms, and compared to reference isolates of *F. oxysporum* f.sp. *gladioli* and *F. oxysporum* f.sp. *tulipae* to justify the distinction of *F. oxysporum* f.sp. *lilii*. Twenty-four isolates from different locations in The Netherlands (18 isolates), Italy (4 isolates), Poland and the United States (1 isolate each) shared unique RFLP patterns with probes D4 and pFOM7, while hybridization did not occur with a third probe (F9). Except for a self-incompatible isolate, these 24 isolates all belonged to a single vegetative compatibility group (VCG 0190). Isolates belonging to VCG 0190 were highly pathogenic to lily, but not to gladiolus or tulip, except for a single nonpathogenic isolate. Six saprophytic isolates of *F. oxysporum* from lily were nonpathogenic or only slightly aggressive to lily, gladiolus and tulip, belonged to unique VCGs and had distinct RFLP patterns. Three pathogenic isolates previously considered to belong to *F. oxysporum* f.sp. *lilii* were identified as *F. proliferatum* var. *minus*; all three belonged to the same VCG and shared unique RFLP patterns. These three isolates were moderately pathogenic to lily and nonpathogenic to gladiolus and tulip. The reference isolates of *F. oxysporum* f.sp. *tulipae* were pathogenic to tulip, but not to lily and gladiolus; they shared a distinct RFLP pattern, different from those encountered among pathogenic and saprophytic isolates from lily, and formed a separate new VCG (VCG 0230). Reference isolates of *F. oxysporum* f.sp. *gladioli* belonging to VCG 0340 proved pathogenic to both gladiolus and lily, but not to tulip. These isolates, as well as isolates belonging to VCGs 0341, 0342 and 0343 of *F. oxysporum* f.sp. *gladioli*, had RFLP patterns different from those encountered among the isolates from lily or tulip. These findings identify *F. oxysporum* f.sp. *lilii* as a single clonal lineage, distinct from *F. oxysporum* f.sp. *gladioli* and f.sp. *tulipae*.

Introduction

The cultivation of flower bulbs, a tradition of paramount economic importance to The Netherlands, is threatened by soil-borne diseases, among which bulb and corm rots caused by the fungus *Fusarium oxysporum* Schlecht.: Fr. take a prominent place (Baayen, 1992; Baayen and Förch, 1998). Specialized forms of this fungus exist for tulip (*F. oxysporum* f.sp. *tulipae* Apt), gladiolus (*F. oxysporum* f.sp. *gladioli* [Massey] Snyder & Hansen), crocus (*F. oxysporum* f.sp. *croci* Boerema & Hamers), hyacinthus (*F. oxysporum* f.sp. *hyacinthi* Muller), narcissus (*F. oxysporum* f.sp. *narcissi* Snyder & Hansen), lily (*F. oxysporum* f.sp. *lilii* Imle) and cyclamen (*F. oxysporum* f.sp. *cyclaminis* Gerlach) (Linderman, 1981; Boerema and Hamers, 1988, 1989; Roebroeck and Mes, 1992a; Woudt et al., 1995). The degree of specificity is questionable, however, since isolates of *F. oxysporum* f.sp. *gladioli* from gladiolus also affect other iridaceous species such as crocus, iris, ixia and freesia (McClellan, 1945). Moreover, isolates from the latter host species can also

infect gladiolus suggesting that this group of strains is pathogenic to the iris family, rather than to gladiolus alone (Linderman, 1981; Roebroeck and Mes, 1992a and unpublished data). Löffler and Mouris (1992) and Löffler et al. (1995) observed that isolates from gladiolus and iris were also pathogenic to lily, although their aggressiveness was low compared to isolates originating from lily itself (supposedly *F. oxysporum* f.sp. *lilii*). This was true for isolates belonging to VCG 0340 of *F. oxysporum* f.sp. *gladioli*; however, isolates from VCG 0341 and VCG 0342 were not pathogenic to lily (H.J.M. Löffler, unpublished data). On the contrary, an isolate considered to belong to *F. oxysporum* f.sp. *tulipae* also proved to be pathogenic to lily (Löffler et al., 1995). The distinction of *F. oxysporum* f.sp. *lilii* from *F. oxysporum* f.sp. *gladioli* or *F. oxysporum* f.sp. *tulipae* thus became questionable.

For a proper selection of isolates to be used in breeding programmes for resistance and for studies on the etiology of the disease, the ambiguous status of *F. oxysporum* f.sp. *lilii* needs to be clarified. Besides data on the pathogenic ability of isolates, the overall genetic diversity present among isolates from lily needs to be investigated. Vegetative compatibility groups within *F. oxysporum* are commonly believed to represent single clonal lineages of the fungus, that are characterized by specific DNA fingerprints, isozyme profiles and karyotypes. While such studies have been carried out for many formae speciales of *F. oxysporum* (Aloi and Baayen, 1993; Baayen et al., 1997; Bosland and Williams, 1987; Correll, 1991; Elias et al., 1993; Kistler, 1997; Kistler et al., 1987; Manicom and Baayen, 1993; Migheli et al., 1995; Tantaoui et al., 1996), including f.sp. *gladioli* (Roebroeck and Mes, 1992a; Mes et al., 1994), only preliminary results have been published for *F. oxysporum* f.sp. *lilii* (Löffler and Rumine, 1991). Moreover, no data are currently available for *F. oxysporum* f.sp. *tulipae*. The present study was undertaken to compare the pathogenic ability of isolates of *F. oxysporum* from lily, gladiolus (VCG 0340 in particular) and tulip to these three hosts as well as to characterize the genetic diversity among these isolates by vegetative compatibility assays and RFLP analyses.

Materials and methods

Fungal isolates. Thirty isolates of *F. oxysporum* from lily from The Netherlands (21 isolates), Italy (5 isolates), Poland (1 isolate) and the U.S.A. (3 isolates) were obtained from various sources (Table 1). Two isolates from a previous study (Löffler et al., 1995) and an additional one received as *F. oxysporum* but all three now re-identified as *F. proliferatum* were included in this study. Seven reference isolates of *F. oxysporum* f.sp. *gladioli* were selected belonging to VCG 0340, VCG 0341, VCG 0342 and VCG 0343, as well as ten isolates of *F. oxysporum* f.sp. *tulipae*. Isolates were stored on potato dextrose agar (PDA), on 'Protect bacterial preserves' at $-80\,^{\circ}$C, or in liquid nitrogen. Morphological examination of isolates grown on potato dextrose agar or carnation leaf agar was performed according to Gerlach and Nirenberg (1982).

Pathogenicity tests. Pathogenicity for lily, gladiolus and tulip was tested in a greenhouse at $20\,^{\circ}$C using the cultivars Esther (*Lilium* sp., Asiatic hybrid group), Nymph (*Gladiolus* $\times$ *nanus* Hort.), Prominence and Rosario (*Tulipa gesneriana* L. hybrids). These cultivars are known to be highly susceptible to, respectively, *Fusarium oxysporum* f.sp. *lilii*, f.sp. *gladioli* (races 1 and 2) and f.sp. *tulipae* (Löffler et al., 1995; Roebroeck and Mes, 1992a and unpublished data; Straathof et al., 1993). Corms and bulbs appropriately pre-treated for cultivation were obtained from general stock at the institutes of the authors, and were surface-sterilized using 0.8% formaldehyde (gladiolus corms and tulip bulbs) or 1.5% sodium hypochlorite (lily scales). Inocula were prepared by culturing the isolates on potato dextrose broth on a reciprocal shaker at $22\,^{\circ}$C for nine days, filtering the cultures through cheese cloth and adjusting the conidial suspension to approximately 10^7 conidia/ml with a hemacytometer. Five gladiolus corms and ten tulip bulbs (five of cv. Prominence and five of cv. Rosario) per isolate were dipped into the conidial suspension and planted in soil (85% peat, 10% clay, 5% sand) in square pots (8 cm). Ten lily bulb scales per isolate were placed in heavily infested soil, prepared from steam-sterilized soil (enriched with 50 ml malt extract per liter soil) inoculated with 40 ml conidial suspension and incubated for one week at $20\,^{\circ}$C. Infested soil prepared in such a manner contains generally about 10^4 colony forming units per gram soil (R.P. Baayen, unpublished data). Symptom development of plants (bulbs/corms, roots, shoots) was assessed visually on an ordinal scale from 0 (unaffected) to 5 (rotten completely) after four weeks (tulip), six weeks (gladiolus) or eight weeks (lily). For lily scales, values up to 2 do not necessarily indicate rot development. Slight browning normally occurs at

Table 1. Isolates of *Fusarium oxysporum* and *F. proliferatum* from lily, gladiolus and tulip, listed according to pathogenicity to these three host species, vegetative compatibility group (VCG) and RFLP pattern with probes D4, F9 and pFOM7

Isolate[a]	From	Pathogenicity[b]			VCG[c]	RFLP[d]			Origin	Source[e]
		Lily	Gladiolus	Tulip		D4	F9	pFOM7		
					Fusarium oxysporum f.sp. *lilii*					
Fol-71#	Lily	**4.8** a	0.0 a	0.9 abc	0190	a	—	*a*	Italy	ISF
Fol-36	Lily	**4.7** ab	0.0 a	1.0 abcd	0190	a	—	*a*	The Netherlands	LBO
Fol-73#	Lily	**4.7** ab	0.0 ab	1.0 abcd	0190	a	—	*a*	Italy	ISF
Fol-4	Lily	**4.6** abc	0.0 a	0.7 abc	0190	a	—	*a*	The Netherlands	LBO
Fol-33	Lily	**4.6** abc	0.2 ab	1.3 abcd	0190	a	—	*a*	The Netherlands	LBO
Fol-18	Lily	**4.5** abcd	0.8 abcd	1.2 abcd	0190	a	—	*a*	The Netherlands	LBO
Fol-38#	Lily	**4.5** abcd	0.2 ab	1.1 abcd	0190	a	—	*a*	The Netherlands	LBO
Fol-43	Lily	**4.5** abcd	1.4 abcd	**2.4** def	0190	a	—	*a*	The Netherlands	LBO
Fol-80#	Lily	**4.5** abcd	0.2 ab	1.1 abcd	0190	a	—	*a*	Poland	RIPF
Fol-9	Lily	**4.4** abcde	0.0 a	1.3 abcd	0190	a	—	*a*	The Netherlands	LBO
Fol-10#	Lily	**4.4** abcde	0.0 a	1.2 abcd	0190	a	—	*a*	The Netherlands	WAU
Fol-35	Lily	**4.4** abcde	0.0 a	0.9 abc	0190	a	—	*a*	The Netherlands	LBO
Fol-40	Lily	**4.4** abcde	0.0 a	0.8 abc	0190	a	—	*a*	The Netherlands	LBO
Fol-11	Lily	**4.3** abcde	0.0 a	1.1 abcd	0190	a	—	*a*	The Netherlands	WAU
Fol-78#	Lily	**4.3** abcde	1.0 abc	0.9 abc	0190	a	—	*a*	Italy	ISF
Fol-19	Lily	**4.2** abcdef	0.0 a	0.4 ab	0190	a	—	*a*	The Netherlands	LBO
Fol-42	Lily	**4.2** abcdef	0.6 abc	1.3 abcd	0190	a	—	*a*	The Netherlands	LBO
Fol-63	Lily	**4.2** abcdef	0.0 a	1.1 abcd	019–	a	—	*a*	U.S.A.	FRC
Fol-28#	Lily	**4.1** bcdef	0.0 a	0.6 ab	0190	a	—	*a*	Italy	ISF
Fol-3#	Lily	**4.0** bcdef	0.0 a	1.3 abcd	0190	a	—	*a*	The Netherlands	LBO
Fol-27#	Lily	**4.0** def	0.0 a	1.0 abcd	0190	a	—	*a*	The Netherlands	LBO
Fol-15	Lily	**3.9** def	0.0 a	0.8 abc	0190	a	—	*a*	The Netherlands	CPRO-DLO
Fol-5#	Lily	**3.9** abcdef	0.0 a	0.7 abc	0190	a	—	*a*	The Netherlands	LBO
Fol-7	Lily	1.9 hi	0.4 ab	1.1 abcd	0190	a	—	*a*	The Netherlands	LBO
					Saprophytic *Fusarium oxysporum* from lily					
Fol-67#	Lily	2.5 hi	0.0 a	1.6 bcde	I	b	A	*b*	U.S.A.	FRC
Fol-17#	Lily	2.3 hij	0.2 ab	2.0 cde	II	c	B	*c*	The Netherlands	CPRO-DLO
Fol-65#	Lily	2.3 hij	0.1 ab	1.0 abcd	III	d	C	*d*	U.S.A.	FRC
Fol-77#	Lily	2.0 hij	0.6 abc	0.7 abc	IV	e	D	*e*	Italy	ISF
Fol-14#	Lily	2.0 hij	0.0 a	1,0 abc	V	f	E	*f*	The Netherlands	CPRO-DLO
Fol-6#	Lily	1.6 hij	0.0 a	0.7 abc	VI	g	—	*g*	The Netherlands	WAU
					Fusarium oxysporum f.sp. *gladioli*					
G15#	Gladiolus	**4.5** abcd	**4.4** d	0.7 abc	0340	h	F	*h*	The Netherlands	LBO
Ir7#	Iris	**4.3** abcde	**4.2** d	1.4 abcde	0340	h	F	*h*	The Netherlands	LBO
Ir2#	Iris	**4.3** abcde	**2.6** cd	0.7 abc	0340	h	F	*h*	The Netherlands	LBO
X1#	Ixia	2.4 hi	**4.0** d	0.5 ab	0340	h	F	*h*	The Netherlands	LBO
G23#	Gladiolus				0341	i		*i*	The Netherlands	LBO
Cr1#	Crocus				0342	j		*j*	The Netherlands	LBO
G82#	Gladiolus				0343	k		*k*	Italy	LBO
					Fusarium oxysporum f.sp. *tulipae*					
Tu4#	Tulip	2.4 hi	0.0 a	**3.7** fg	0230	l	G	*l*	The Netherlands	LBO
Tu3	Tulip	2.3 hij	0.4 abc	**2.8** ef	0230	l	G	*l*	The Netherlands	LBO
Tu8	Tulip	2.1 hij	0.0 a	**3.8** fg	0230	l	G	*l*	The Netherlands	LBO
Tu10	Tulip	2.0 hij	0.2 ab	**4.2** g	0230	l	G	*l*	The Netherlands	LBO
Tu13	Tulip	2.0 hij	0.0 a	**3.9** fg	0230	l	G	*l*	The Netherlands	LBO
Tu5	Tulip	1.8 hij	0.4 abc	**2.8** ef	0230	l	G	*l*	The Netherlands	LBO
Tu9	Tulip	1.8 hij	0.2 ab	**3.6** fg	0230	l	G	*l*	The Netherlands	LBO
Tu1	Tulip	1.6 ij	0.4 abc	**2.6** ef	0230	l	G	*l*	The Netherlands	LBO
Tu14	Tulip	1.5 ij	0.0 a	**3.7** fg	0230	l	G	*l*	The Netherlands	LBO
Tu11	Tulip	1.5 ij	0.0 a	**3.6** fg	0230				The Netherlands	LBO

Table 1. Continued

Isolate[a]	From	Pathogenicity[b]			VCG[c]	RFLP[d]			Origin	Source[e]
		Lily	Gladiolus	Tulip		D4	F9	pFOM7		
Fusarium proliferatum from lily										
Fol-69#	Lily	**3.7** efg	0.0 a	0.9 abc	VII	m	—	*m*	Italy	ISF
Fol-75#	Lily	**3.5** fg	0.2 ab	1.1 abcd	VII	m	—	*m*	Italy	ISF
Fol-30#	Lily	**2.6** ghi	0.0 a	0.7 abc	VII	m	—	*m*	Italy	ISF
Control		1.5 j	1.3 bc	0.0 a						

[a] Isolates of which the classification was checked by means of ITS-RFLP have been marked (#). Isolates Fol-28 and Fol-30 have been referred to in a previous study (Löffler and Rumine, 1991) as FolA and FolC, respectively.

[b] Per column, average disease indices followed by the same letter reflect frequency distributions that do not differ significantly from one another ($P < 0.01$). Values in bold are markedly higher than those of control plants.

[c] VCG 019-, self-incompatible isolate of *F. oxysporum* f.sp. *lilii* of which the mutants could not be placed in VCG 0190.

[d] Per column, isolates with the same letter have the same RFLP pattern. -, no hybridisation.

[e] Abbreviations: CPRO-DLO, DLO Centre for Plant Breeding and Reproduction Research, Wageningen, The Netherlands; FRC, Fusarium Research Centre, University Park, Pennsylvania, U.S.A.; ISF, Istituto Sperimentale per la Floricoltura, Pescia, Italy; LBO, Bulb Research Centre, Lisse, The Netherlands; WAU, Wageningen Agricultural University, Wageningen, The Netherlands.

the wound surface upon detachment of scales from the mother bulb, and intense brown defense responses may develop over several mm from the wound in incompatible combinations. The frequency distributions of numbers of plants (or scales) in the six symptom classes were compared non-parametrically using Wilcoxon's test corrected for ties.

Vegetative compatibility tests. Nitrate nonutilizing (nit) mutants were generated and characterized as *nit*1, *nit*3 or NitM mutants as described previously (Correll et al., 1987; Aloi and Baayen, 1993). Enhanced chlorate concentrations (30 g $KClO_3$ per l) proved necessary for obtaining mutants from isolates of *F. oxysporum* f.sp. *tulipae*. Pairings were made on minimal medium (Puhalla, 1985) and incubated at room temperature for at least two weeks. Complementary mutants producing abundant aerial mycelium at the interface of colonies were placed in the same vegetative compatibility group (VCG). Tester mutants (Fol-42 *nit*1, Fol-43 *nit*1, Fol-3 NitM, Fol-42 NitM) of the major VCG found among isolates from lily were selected for further complementation tests. Mutants of isolates from lily that could not be placed in the main VCG were tested against all other such mutants and were either placed in the main VCG by linkage to other mutants than the testers, or were classified as distinct. Representative isolates of all VCGs were subsequently tested for complementation with *nit*1 and NitM tester mutants of VCGs 0340, 0341, 0342 and 0343 of *F. oxysporum* f.sp. *gladioli* and with complementary mutants representing the single VCG found in this study for *F. oxysporum* f.sp. *tulipae*.

Ribosomal DNA analysis. Classification of the various vegetative compatibility groups as *F. oxysporum*, *F. redolens* or *F. proliferatum* was performed by restriction fragment analysis of the internal transcribed spacer (ITS) region of the ribosomal DNA (Waalwijk et al., 1996a, 1996b). Amplification was performed directly from mycelium (Blakemore et al., 1994) in a PCR reaction using the primers ITS-1 and ITS-4 as previously described (Waalwijk et al., 1996a). 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 denaturing 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. Amplified products (circa 550 bp) were digested individually with *Hinf*I and analyzed on 1.5% agarose gels. Isolates with fragments of 90, 180 and 265 base pairs were considered to have been properly classified as *F. oxysporum*. Isolates with fragments of 265 and 290 base

pairs (apparently not belonging to *F. oxysporum*) were further analysed with *Alu*I, *Msp*I, *Rsa*I and *Sma*I for distinction between *F. redolens* (no cleavage sites with *Alu*I, *Rsa*I or *Sma*I, and two with *Msp*I) and *F. proliferatum* var. *proliferatum* (a single cleavage site with *Alu*I, *Rsa*I and *Sma*I, and three with *Msp*I). Variant populations of *F. proliferatum* may lack the *Sma*I site and thus also lack one of the three *Msp*I sites (Waalwijk and Baayen, 1995; Waalwijk et al., 1996a and unpublished data; Elmer and Vossbrinck, 1997).

Restriction fragment length polymorphisms of total DNA. Isolates were inoculated onto PDA (Oxoid) plates covered by sterile cellophane and cultured for seven days at 20 °C. Mycelium was harvested by scraping off the cellophane, and was lyophilized and processed according to the methods described by Raeder and Broda (1985). Alternatively, DNA was extracted from liquid cultures grown for five days in potato dextrose broth at 27 °C. DNA preparations digested with *Hin*dIII were electrophoretically resolved on agarose gels, blotted to nylon membranes and hybridized with one of the following probes: D4, a probe from *F. oxysporum* f.sp. *dianthi* (Manicom et al., 1987); probe F9, a 0.5 kb PCR product from *F. oxysporum* f.sp. *dianthi* (Baayen et al., 1997), and pFOM7, a mitochondrial probe from *F. oxysporum* f.sp. *melonis* (Jacobson and Gordon, 1990). Probes were either labelled radioactively (^{32}P), or with digoxinin using a Boehringer DIG DNA labelling and detection kit.

Results

Uninoculated lily scales developed a narrow brown barrier zone at the wound surface, resulting in indices classified between 1 and 2 as usual for healthy propagative material of lily (Table 1). However, control corms of gladiolus developed rot symptoms due to latent infections, which are extremely difficult to avoid in propagative material of gladioli (Roebroeck and Mes, 1992a). Control bulbs of tulip remained wholly unaffected.

Twenty-three isolates of *F. oxysporum* from lily could be placed in a single VCG, to which we assign the four-digit code 0190 according to the system of Puhalla (1985). Classification of these isolates as *F. oxysporum* was confirmed by ITS-RFLP for ten random members of VCG 0190. Two isolates (Fol-5 and Fol-28) strongly complemented each other but could only be linked to the main group by weak reactions with the mutants of isolate Fol-80. The isolates in VCG 0190 shared unique RFLP patterns with probes D4 and pFOM7 while no hybridization occurred with probe F9 (Table 1; Figure 1). For some isolates minor deviations from the basic RFLP patterns were observed (an impression is given in Figure 1). Self-incompatible isolate Fol-63 shared the RFLP pattern of the previous isolates but could not be placed in VCG 0190. With the exception of isolate Fol-7, all isolates sharing the characteristic RFLP pattern of VCG 0190 were highly pathogenic to lily cv. Esther (Table 1). None of these isolates was distinctly pathogenic to gladiolus, but most were slightly aggressive to tulip.

Six saprophytic isolates of *F. oxysporum* from lily each belonged to single-member VCGs, and all had unique RFLP patterns (Table 1). All six isolates were identified as *F. oxysporum* by ITS-RFLP. None of these was distinctly pathogenic to lily or gladiolus, while they were slightly aggressive to tulip.

Ten reference isolates of *F. oxysporum* f.sp. *tulipae* could be placed in a single VCG, to which we assign the four-digit code 0230. However, *nit* mutants could be obtained only with difficulty for these isolates

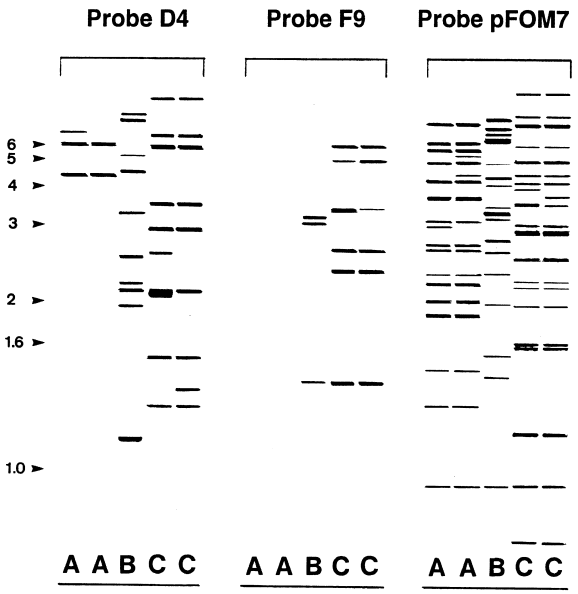

Figure 1. Schematic diagram of the characteristic RFLP profiles with probes D4, F9 and pFOM7 for isolates of A, *Fusarium oxysporum* f.sp. *lilii* (VCG 0190); B, *F. oxysporum* f.sp. *gladioli* (VCG 0340), and C, *F. oxysporum* f.sp. *tulipae* (VCG 0230). Arrowheads indicate markers (in kb). RFLP profiles for other VCGs in *F. oxysporum* f.sp. *gladioli* have been published elsewhere (Mes et al., 1994).

and complementation reactions between mutants were rather weak. The isolates studied shared distinct RFLP patterns with the probes used (Figure 1). None of them was distinctly pathogenic to lily or gladiolus, while all were highly pathogenic on tulip.

None of the VCGs detected among isolates of *F. oxysporum* from lily coincided with VCGs 0340, 0341, 0342 or 0343 of *F. oxysporum* f.sp. *gladioli* or VCG 0230 of *F. oxysporum* f.sp. *tulipae*. The RFLP patterns of the VCGs detected among isolates of *F. oxysporum* from lily also differed from those of *F. oxysporum* f.sp. *tulipae* and VCGs 0340, 0341, 0342 and 0343 of *F. oxysporum* f.sp. *gladioli* (Table 1). Out of four isolates belonging to VCG 0340 of *F. oxysporum* f.sp. *gladioli* tested, three were highly pathogenic to lily cv. Esther while not or only slightly aggressive to tulip. The isolates of *F. oxysporum* f.sp. *tulipae* were pathogenic only to tulip (Table 1).

Disease development on gladiolus corms inoculated with isolates belonging to *F. oxysporum* f.sp. *lilii* or *F. oxysporum* f.sp. *tulipae* was slightly lower than in uninoculated controls, suggestive of induced resistance responses as observed previously in gladiolus (Roebroeck and Mes, 1992b).

Three Italian isolates (Fol-30, Fol-69 and Fol-75), two of which were previously considered to belong to *F. oxysporum* f.sp. *lilii* (Löffler et al., 1995), were identified as *F. proliferatum* by ITS-RFLP. These isolates had a single cleavage site with *Alu*I and *Rsa*I, and two with *Msp*I, but lacked a *Sma*I site. On carnation leaf agar, these three isolates formed polyphialides with mostly two conidiogenous loci, and produced clavate but never pyriform or globose microconidia adhering in short chains (mostly <15 conidia), indicating that they probably belong to *F. proliferatum* var. *minus*. These isolates formed a distinct VCG, and shared unique RFLP patterns with probes D4 and pFOM7 that differed from all previous ones (Table 1). All three isolates were moderately pathogenic on lily cv. Esther, while they were nonpathogenic on gladiolus and tulip.

Discussion

A single group was detected by RFLP fingerprinting among pathogenic *F. oxysporum* isolates from lily from The Netherlands, Italy, Poland and the U.S.A. Given their distinctive pathogenic specialization and wide geographic distribution, the members of this group are considered to represent *F. oxysporum* f.sp. *lilii* proper. Virtually all members of this group belonged to VCG 0190, although some aberrant behaviour was observed for two isolates (Fol-5 and Fol-28) which were only weakly compatible with one of the mutants in VCG 0190. A third isolate (Fol-63) sharing the main RFLP fingerprint was incompatible with the isolates of VCG 0190. With the exception of isolate Fol-7, all members of this group were pathogenic to lily cv. Esther. In previous studies isolate Fol-7 also was at best weakly pathogenic to lily cultivars (Löffler and Rumine, 1991; Löffler et al., 1995). The aggressiveness levels of the pathogenic isolates within VCG 0190 found here correspond well with those reported by Löffler et al. (1995). A certain degree of evolutionary radiation within this group, originally a single clonal lineage, thus seems apparent at the genetic (VCG) and pathogenic level (aggressiveness). Other formae speciales have also been proposed to consist of one clonal lineage (Elmer et al., 1994; Tantaoui et al., 1996), and evolutionary radiation within clonal lineages of *F. oxysporum* is not unknown either (Baayen et al., 1997; Fernandez et al., 1997; Gordon and Martyn, 1997; Leslie, 1993; Mes et al., 1994). The remaining isolates of *F. oxysporum* from lily were nonpathogenic or only slightly aggressive on lily, gladiolus and tulip and probably can be considered random saprophytic strains of the fungus. In a number of preliminary trials, these isolates had also been found nonpathogenic to lily (H.J.M. Löffler, unpublished data).

Differences in virulence towards specific lily cultivars have not been found within VCG 0190, even when testing 24 isolates (of which 23 also were included in this study) on 20 different lily accessions, representing widely different groups of hybrids as well as botanical species and species hybrids (Löffler et al., 1995). Besides differences in aggressiveness levels and a single isolate (Fol-7) having lost its pathogenicity, pathogenic diversity among isolates of *F. oxysporum* f.sp. *lilii* apparently is small, compared to the race diversity observed in other formae speciales (Baayen et al., 1997; Bosland and Williams, 1987; Elias et al., 1993; Roebroeck and Mes, 1992a). The present study thus confirms the previous conclusion of Löffler et al. (1995) that the use of a single highly aggressive isolate in screening tests should suffice in breeding programmes for resistance in lily to basal rot.

Three isolates of VCG 0340 of *F. oxysporum* f.sp. *gladioli* proved pathogenic to lily cv. Esther, implying that isolates pathogenic to lily need not necessarily belong to *F. oxysporum* f.sp. *lilii*. For unknown reasons, all three isolates were considerably more aggressive to lily than in previous trials (Löffler et al., 1995).

Lily can potentially be a secondary host for isolates belonging to other formae speciales of *F. oxysporum*, as reported for other monocots such as *Asparagus*, the secondary host for *F. oxysporum* f.sp. *apii* which normally infects celery (Armstrong and Armstrong, 1975; Elmer and Stephens, 1989). However, isolates belonging to *F. oxysporum* f.sp. *gladioli* have not been recovered from commercially grown lilies thus far (E.J.A. Roebroeck, unpublished data).

The isolates of *F. oxysporum* f.sp. *tulipae* tested were pathogenic to tulip cultivars, but not to lily or gladiolus. They shared unique RFLP fingerprints, and belonged to a single VCG that is presently assigned the four-digit code 0230. The isolates of *F. oxysporum* f.sp. *tulipae* that were included in the present study can therefore also be classified as a single clonal lineage. A single isolate (Fot-8) that had been pathogenic on lily in previous studies (Löffler et al., 1995) was reclassified in this study as a member of VCG 0190 and is considered a contaminant of the original isolate from tulip.

Three mildly pathogenic isolates (Fol-30, Fol-69 and Fol-75) received as *F. oxysporum* f.sp. *lilii* were reclassified in this study as *F. proliferatum* var. *minus*, a pathogen of thick-leaved monocots like *Sansevieria* spp., *Dracaena* spp. and *Gasteria* spp. on which it causes severe leaf lesions (Gerlach and Nirenberg, 1982) and of garlic bulbs (Simay, 1990). In lily, the bulb scales (thickened but subsoil leaves) are infected. The isolates involved were mildly pathogenic to lily while nonpathogenic to gladiolus and tulip, and belonged to a single VCG, suggestive of pathogenic specialization on lily in *F. proliferatum* var. *minus* like in *F. oxysporum*. However, with only three isolates from the same source at hand it cannot be judged whether *F. proliferatum* is truly associated with lily as primary host. The isolates involved are known to have a slightly different virulence towards lily cultivars from the *Lilium longiflorum* group, but their pathogenic potential otherwise closely resembles that of *F. oxysporum* f.sp. *lilii* (Löffler et al., 1995). Morphological characterization of the three isolates as *F. proliferatum* var. *minus* rather than *F. proliferatum* var. *proliferatum* was supported by an altered *Sma*I/*Msp*I site in the ITS region of the ribosomal DNA. A similar condition occurs in some populations of *F. proliferatum* from asparagus (Elmer and Vossbrinck, 1997) and carnation (Waalwijk and Baayen, unpublished data), which may similarly belong to *F. proliferatum* var. *minus*.

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