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The Elegans fusaria causing wilt disease of carnation. II. Distinction of vegetative compatibility groups

R.P. BAAYEN and J. KLEIJN

Willie Commelin Scholten Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, the Netherlands

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Abstract

The vegetative compatibility patterns among isolates of *Elegans* fusaria causing wilt disease of carnation were investigated. Nitrate non-utilizing mutants were generated from 16 isolates labelled *F. redolens*, nine of which came from carnation, and from 33 isolates labelled *F. oxysporum*, 19 of which came from carnation. Pairings of the mutants revealed five vegetative compatibility groups among the isolates from carnation, corresponding with *F. oxysporum* f.sp. *dianthi* race 1 (VCG 1), race 2 (VCG 2) and race 4 (VCG 3), *F. redolens* f.sp. *dianthi* (VCG 4) and *F. redolens* isolates from foot rot-diseased carnations (VCG 5). Besides three isolates typical of *F. redolens*, VCG 4 comprised a now slightly deviating subculture of the type isolate of *F. redolens* f.sp. *dianthi* of which the cultural characteristics correspond to *F. oxysporum* instead of *F. redolens*. This observation may be taken to support previous conclusions that the distinction between both taxa is not justified. Otherwise, the compatibility patterns did not provide decisive evidence to accept or reject conspecificity of both taxa. Isolates from carnation did not form heterokaryons with other formae speciales of *F. oxysporum*.

Additional keywords: Fusarium oxysporum, *Fusarium redolens*, vegetative compatibility, taxonomy, *Dianthus caryophyllus*.

Introduction

The distinction of *Fusarium* (*oxysporum* var.) *redolens* from *F. oxysporum* (var. *oxysporum*) has recently been questioned (Baayen and Gams, 1988). On morphological grounds, *F.* (*oxysporum* var.) *redolens* and *F. oxysporum* were found by these authors to form one variable complex. On the basis of pathogenicity to carnation, *F. redolens* f.sp. *dianthi* appeared to be identical to *F. oxysporum* f.sp. *dianthi*, since isolates of both taxa caused indistinguishable diseases, and isolate DSM 62392 of *F. redolens* f.sp. *dianthi* corresponded in virulence (sensu Vanderplank, 1984) with the common race 2 of *F. oxysporum* f.sp. *dianthi*.

The present paper provides data on the genetic interrelationships among the *Elegans* fusaria causing wilt disease of carnation. We tested heterokaryon formation between nitrate non-utilizing (*nit*) mutants of isolates of *F. oxysporum* and *F. redolens*. These auxotrophic mutants grow as thin expansive colonies without any aerial mycelium on a minimal agar medium (MM) that contains sodium nitrate as the sole nitrogen source. Using selective media containing different nitrogen sources, *nit* mutants can be identified into three phenotypic classes: 1) mutations at a nitrate reductase structural locus

(*nit 1*), 2) mutations at a nitrate-assimilation pathway-specific regulatory locus (*nit 3*), and 3) mutations at loci that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (nit M). As the deficiency can be located on different sites in the genome, combinations of certain *nit* mutants on MM can complement each other in the heterokaryons which may be formed by hyphal fusion (anastomosis) between related mutants. In such cases dense aerial mycelium develops where the thin *nit* mutant colonies come in contact. This complementation is an indicator of vegetative compatibility; pairings between vegetatively incompatible isolates or isolates of identical defect remain thin. Complementation occurs most frequently when the pairing involves at least one nit M mutant. All isolates that form mutual heterokaryons are considered to belong to the same vegetative compatibility group or VCG (Puhalla, 1984a, 1984b, 1985; Correll et al., 1986a, 1987).

The vegetative compatibility patterns among the *Elegans* fusaria may provide additional information concerning the relation between *F. oxysporum* and *F. redolens*. If the distinction between these taxa is justified, vegetative compatibility should be restricted to isolates within either taxon. The distinction is not justified if isolates of *F. redolens* f.sp. *dianthi* and of *F. oxysporum* f.sp. *dianthi* are vegetatively compatible with one another, but not with isolates of other formae speciales of either species. *Nit* mutants of isolates of *F. oxysporum* and *F. redolens* from carnation and from several other host plants were therefore generated and paired. Largely the same isolates were used as in the previous, taxonomic study (Baayen and Gams, 1988).

Materials and methods

Fungal strains. Sixteen isolates labelled *F. redolens* Wollenw. were tested, of which nine originated from carnation while seven of these were recorded as f.sp. *dianthi* Gerlach. Of the 33 isolates labelled *F. oxysporum* Schlecht. : Fr. which were tested, 19 originated from carnation and 18 of them belonged to f.sp. *dianthi* (Prill. & Del.) Snyder & Hansen. The isolates are listed in Tables 1 and 2. All stock cultures were kept on oatmeal agar (OA; Gams et al., 1987).

Recovery and pairing of nit mutants. Nitrate non-utilizing mutants were selected by the method of Puhalla (1985). The fungus was transferred from OA cultures to minimal medium agar (MM; Puhalla, 1985) amended with $KClO_3$ and L-asparagine. Growth on this chlorate medium was restricted until normally growing, chlorate-tolerant mutant sectors arose. These mutants were screened for auxotrophy for organic nitrogen sources (which frequently accompanies tolerance to chlorate) by transferring them to MM. Mutants lacking aerial mycelium on MM were thereafter identified at *nit 1*, *nit 3* or nit M by their growth on MM in which nitrate had been replaced by nitrite, hypoxanthine or ammonium (Correll et al., 1987). At least one mutant was generated from each of the isolates. Three different *nit* mutants were generated from 8 isolates and two different ones from 13 isolates, while only one *nit* mutant was generated from the remaining 26 isolates in spite of repeated attempts (Tables 1 and 2).

The mutants were paired by placing them several centimetres apart in a 9 cm diam Petri dish containing MM. The pairings were incubated at room temperature and scored for complementation 7-14 days later. Development of dense aerial mycelium where the two thin mutant colonies came in contact (Fig. 1) indicated complementation. Initial-

Table 1. List of isolates of *Elegans* fusaria from carnation, and of the mutants generated from these isolates. CBS = culture collection of the Centraalbureau voor Schimmelcultures, Baarn, the Netherlands; DSM = Deutsche Sammlung von Mikroorganismen, Göttingen / Braunschweig, German Federal Republic (1974 catalogue); A.G. = culture collection of Prof. Dr A. Garibaldi, Turin, Italy; WCS = culture collection of the Willie Commelin Scholten Phytopathological Laboratory, Baarn, the Netherlands.

Isolate	Taxon	Race[a]	Origin	Other codes	Nit mutants
WCS 828	*F. oxysporum* f.sp. *dianthi*	race 1	Italy	A.G. 1	1, 3, M
WCS 828	*F. oxysporum* f.sp. *dianthi*	race 1	Italy	A.G. F100	1, 3, M
WCS 829	*F. oxysporum* f.sp. *dianthi*	race 1	Italy	A.G. F101	1
WCS 816	*F. oxysporum* f.sp. *dianthi*	race 2	Netherlands		3, M
WCS 830	*F. oxysporum* f.sp. *dianthi*	race 2	Netherlands		3
WCS 834	*F. oxysporum* f.sp. *dianthi*	race 2	Italy	A.G. 75	1, M
WCS 835	*F. oxysporum* f.sp. *dianthi*	race 2	Italy	A.G. 259	3
WCS 836	*F. oxysporum* f.sp. *dianthi*	race 2	Italy	A.G. F107	3
WCS 843	*F. oxysporum* f.sp. *dianthi*	race 2	Netherlands		1, M
WCS 847	*F. oxysporum* f.sp. *dianthi*	race 2	Netherlands		1
WCS 848	*F. oxysporum* f.sp. *dianthi*	race 2	Netherlands		M
WCS 849	*F. oxysporum* f.sp. *dianthi*	race 2	Netherlands		1
WCS 850	*F. oxysporum* f.sp. *dianthi*	race 2	Netherlands		1, 3, M
WCS 851	*F. oxysporum* f.sp. *dianthi*	race 2	France		1
WCS 837	*F. oxysporum* f.sp. *dianthi*	race 4	Italy	A.G. 261	1, 3
WCS 838	*F. oxysporum* f.sp. *dianthi*	race 4	Italy	A.G. 310	1, 3, M
WCS 839	*F. oxysporum* f.sp. *dianthi*	race 4	Italy	A.G. F79	1
WCS 840	*F. oxysporum* f.sp. *dianthi*	race 8	Italy	A.G. s.n.	1, 3, M
WCS 832	*F. oxysporum* (non-pathogenic)		Netherlands	CBS 742.88	1, M
CBS 248.61	*F. redolens* f.sp. *dianthi*		Germany	DSM 62390[b]	1, 3
CBS 366.87	*F. redolens* f.sp. *dianthi*		Netherlands		1
DSM 62390[c]	*F. redolens* f.sp. *dianthi*		Germany	CBS 360.87	M
DSM 62391	*F. redolens* f.sp. *dianthi*		Germany	CBS 361.87	3
DSM 62392	*F. redolens* f.sp. *dianthi*		Germany	CBS 362.87	1
DSM 62393[d]	*F. redolens* f.sp. *dianthi*		Germany	CBS 363.87	3
WCS 842	*F. redolens* f.sp. *dianthi*		Netherlands		1, 3
DSM 62378	*F. redolens* (non-pathogenic)		Germany	CBS 364.87	1
DSM 62379	*F. redolens* (non-pathogenic)		Germany	CBS 365.87	1, M

[a] Numbering of races according to Garibaldi (1983).
[b] Originally = DSM 62390, type isolate of *F. redolens* f.sp. *dianthi*; morphology now different.
[c] Type isolate of *F. redolens* f.sp. *dianthi*, newly received from the Institut für Mikrobiologie, Berlin-Dahlem.
[d] Isolate from a sweet william (*Dianthus barbatus*) instead of a carnation (*D. caryophyllus*).

ly, 51 mutants were generated from the isolates, each of which was paired with all other mutants. All pairings between isolates from carnation, and all other pairings that scored positive for complementation, were repeated at least once. This resulted in the distinction of several VCGs among the isolates from carnation. However, subsequent mutant phenotype identification revealed that some VCGs lacked nit M mutants. Since only nit M × *nit 1* or nit M × *nit 3* pairings ensure an optimal complementation (Correll et al., 1987), 19 additional mutants from carnation and tomato isolates were generated and paired with all mutants of isolates from the same host. All finally distinguished VCGs contained *nit 1*, *nit 3* and nit M mutants, except for VCG 5 which lacked a *nit 3* mutant. Finally, nit M × *nit 1* or nit M × *nit 3* pairings were established between

Table 2. List of isolates of *Elegans* fusaria from other plants than carnation, and of the mutants generated from these isolates. Abbreviations as in Table 1.

Isolate	Taxon	Isolated from	Nit mutants
CBS 742.79	*F. oxysporum* f.sp. *cattleyae*	*Phalaenopsis* sp.	3
CBS 127.81	*F. oxysporum* f.sp. *chrysanthemi*	*Chrysanthemum* sp.	1
WCS 845	*F. oxysporum* f.sp. *cyclaminis*	*Cyclamen* sp.	1, 3
WCS 801	*F. oxysporum* f.sp. *lycopersici* race 1	*Lycopersicon esculentum*	1, 3, M
WCS 861	*F. oxysporum* f.sp. *lycopersici* race 1	*L. esculentum*	1, 3, M
WCS E30	*F. oxysporum* f.sp. *lycopersici* race 1	*L. esculentum*	1, M
WCS E31	*F. oxysporum* f.sp. *lycopersici* race 1	*L. esculentum*	1
WCS E79	*F. oxysporum* f.sp. *lycopersici* race 1	*L. esculentum*	3, M
WCS E182	*F. oxysporum* f.sp. *lycopersici* race 1	*L. esculentum*	1, M
WCS E183	*F. oxysporum* f.sp. *lycopersici* race 1	*L. esculentum*	1
WCS 862	*F. oxysporum* f.sp. *lycopersici* race 2	*L. esculentum*	3, M
CBS 196.65	*F. oxysporum* f.sp. *narcissi*	*Narcissus* sp.	3
CBS 743.79	*F. oxysporum* f.sp. *opuntiarum*	*Zygocactus* sp.	1
WCS 863	*F. oxysporum* f.sp. *radicis-lycopersici*	*L. esculentum*	1, M
CBS 128.73	*F. oxysporum* var. *redolens*, unspecified	*L. esculentum*	1, 3, M
DSM 62384	*F. redolens*, unspecified	*Fragaria* sp.	M
DSM 62385	*F. redolens*, unspecified	*Convallaria majalis*	1
DSM 62386	*F. redolens*, unspecified	*Fritillaria* sp.	1
DSM 64524	*F. redolens*, unspecified	*Solanum tuberosum*	1
DSM 64613	*F. redolens*, unspecified	*Pisum* sp.	3
WCS 846	*F. redolens*, unspecified		1

the mutants of isolates apparently not belonging to any of the VCGs and single representative mutants from each of the VCGs.

Attempts were made to assay the mutants for cross-feeding by physically separating the paired *nit* mutants with a strip of sterilized cellophane (Enka Glanzstoff Cuprophan Dialysierfolie 250 PM) as described by Puhalla (1984a) and Correll et al. (1986a). According to these authors, cellophane prevents hyphal contact but not the passage of small molecules. Therefore, dense growth of aerial mycelium even when the nitrate non-utilizing mutants are separated by cellophane would indicate cross-feeding. The method was found to be unreliable, however, because the fungi repeatedly penetrated the cellophane already after 4 to 5 days growth. The fungus was also able to grow through sterilized cellulose nitrate membrane filters (Sartorius, pore diam 0.2 μm). Further assays for cross-feeding were therefore not carried out.

Results

Twenty-three out of the 28 isolates from carnation could be assigned to vegetative compatibility groups (Table 3):

– VCG 1. All three isolates (WCS 827, WCS 828 and WCS 829) of *F. oxysporum* f.sp. *dianthi* race 1. A weak positive reaction was obtained between WCS 840 nit M (*F. oxysporum* f.sp. *dianthi* race 8) and WCS 829 *nit 1*, whereas ten other nit M × *nit 1* or

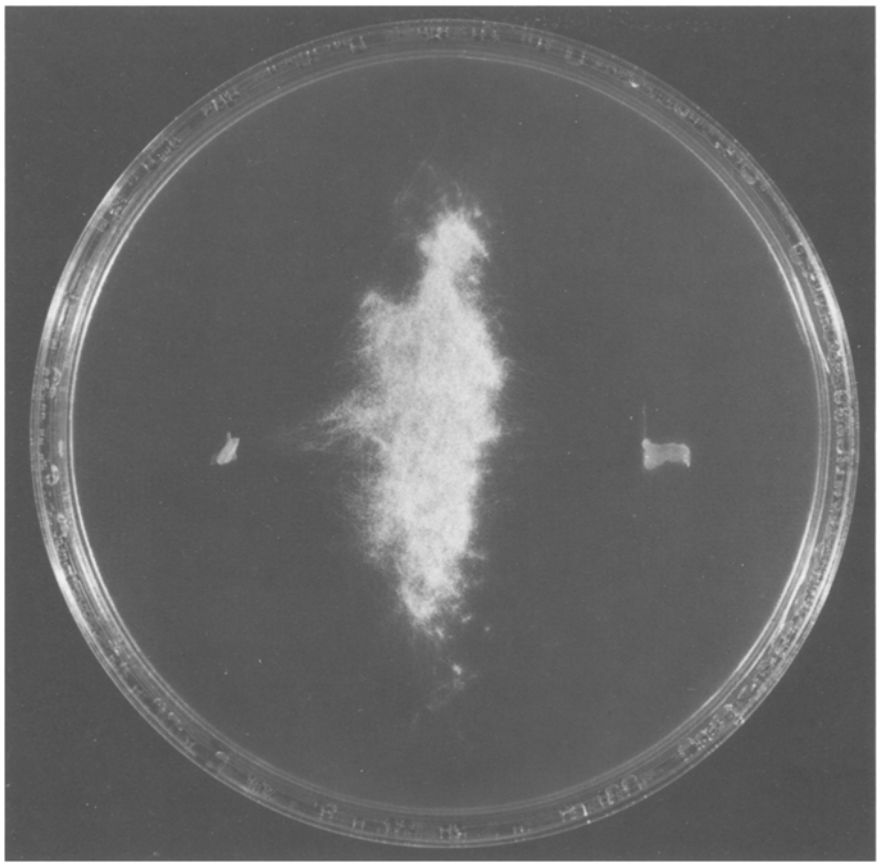

Fig. 1. Growth of aerial mycelium at the contact line between two compatible complementary *nit* mutants of *Fusarium oxysporum* f.sp. *dianthi* grown on MM.

nit M × *nit 3* pairings between races 1 and 8 did not react positively. The studied isolates of races 1 and 8 are of Italian origin.

– VCG 2. All isolates of *F. oxysporum* f.sp. *dianthi* race 2, except WCS 834. The lack of complementation of WCS 834 with other race 2 isolates is apparently due to complete inability of this isolate to form heterokaryons ('self-incompatibility'; Correll et al., 1987): WCS 834 nit M did not complement any of three different *nit 1* mutants of WCS 834 itself either. VCG 2 comprised isolates from the Netherlands, France and Italy.

– VCG 3. All three isolates (WCS 837, WCS 838 and WCS 839) of *F. oxysporum* f.sp. *dianthi* race 4. These isolates are of Italian origin.

– VCG 4. Isolates CBS 248.61, DSM 62390, DSM 62391 and DSM 62392 of *F. redolens* f.sp. *dianthi*. These isolates inflict wilt disease on carnation (Hantschke, 1961; Baayen and Gams, 1988) from which they were isolated: DSM 62390 and its subculture CBS 248.61 came from a nursery in Hamburg, and DSM 62391 and DSM 62392 came from nurseries in Berlin (Hantschke, 1961).

Table 3. Heterokaryon formation between isolates of *F. oxysporum* and *F. redolens* from carnation. NT, not tested; ?, ambiguous results. A, *F. oxysporum* f.sp. *dianthi* race 1; B, race 2; C, race 4; D, race 8; E, *F. oxysporum* but not f.sp. *dianthi*; F, *F. redolens* f.sp. *dianthi*; G, *F. redolens* but not f.sp. *dianthi*.

		WCS 827	WCS 828	WCS 829	WCS 816	WCS 830	WCS 834	WCS 835	WCS 836	WCS 843	WCS 847	WCS 848	WCS 849	WCS 850	WCS 851	WCS 837	WCS 838	WCS 839	WCS 840	WCS 832	CBS 248.61	CBS 366.87	DSM 62390	DSM 62391	DSM 62392	DSM 62393	WCS 842	DSM 62378	DSM 62379
A	WCS 827	+																											
	WCS 828	+	+																										
	WCS 829	+	+	NT																									
B	WCS 816	–	–	–	+																								
	WCS 830	–	–	–	+	NT																							
	WCS 834	–	–	–	–	–	–																						
	WCS 835	–	–	–	–	–	–	NT																					
	WCS 836	–	–	–	–	–	–	–	NT																				
	WCS 843	–	–	–	+	+	–	+	+	+																			
	WCS 847	–	–	–	+	–	–	–	–	+	NT																		
	WCS 848	–	–	–	+	+	–	–	+	+	+	NT																	
	WCS 849	–	–	–	+	–	–	–	–	+	–	+	NT																
	WCS 850	–	–	–	+	+	–	+	–	+	+	–	+	+															
	WCS 851	–	–	–	+	+	–	+	–	+	–	+	–	+	NT														
C	WCS 837	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–													
	WCS 838	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+												
	WCS 839	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	NT											
D	WCS 840	–	–	?	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+										
E	WCS 832	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+									
F	CBS 248.61	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+								
	CBS 366.87	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NT							
	DSM 62390	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	NT						
	DSM 62391	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	NT					
	DSM 62392	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	NT				
	DSM 62393	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NT			
	WCS 842	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+		
G	DSM 62378	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NT	
	DSM 62379	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+

– VCG 5. Isolates DSM 62378 and 62379 from foot rot-diseased carnations from nurseries in Waiblingen (Württemberg, Germany). The isolates belong to *F. redolens* but not to f.sp. *dianthi* as they do not inflict wilt disease on carnation (Gerlach and Pag, 1961; R.P. Baayen, unpublished data).

Five isolates from carnation (WCS 832, WCS 834, WCS 842, CBS 366.87 and DSM 62393) could not be assigned to any of the VCGs.

Pairings of the various mutants revealed compatibility patterns only among isolates from the same host. Except for two mutually compatible isolates from tomato, the isolates from host plants other than carnation appeared incompatible with all other isolates studied.

A general estimation of the degree of compatibility among *nit 1*, *nit 3* and nit M mutants was made for the isolates from carnation belonging to the five VCGs. For each of the VCGs the actual number of complementary mutant combinations was compared with the potential number of complementary combinations as deduced from the number of *nit 1*, *nit 3* and nit M mutants that were tested against each other. VCG 2, for instance, comprised five *nit 1*, five *nit 3* and four nit M mutants. Nineteen out of the 20 *nit 1* × nit M pairings in VCG 2 reacted positively, 13 out of the 20 *nit 3* × nit M pairings, 10 out of the 25 *nit 1* × *nit 3* pairings, 4 out of the 6 nit M × nit M pairings, 1 out of the 10 *nit 1* × *nit 1* pairings and 0 out of the 10 *nit 3* × *nit 3* pairings. The actual and potential numbers of positively reacting mutant combinations for the combined VCGs 1 to 5 were calculated by summation of the results for the five VCGs separately. Because of its uncertain position, the single race 8 isolate was not included in the calculations. The results (Table 4) confirm previous findings that, concerning *nit 1* and *nit 3* mutants, complementation between mutants with different phenotypes occurs more frequently than among mutants with the same phenotype. However, complementation between *nit 1* and *nit 3* mutants occurred far more frequently than reported by Correll et al. (1987). On the other hand, the large differences in complementation frequency between *nit 1* × *nit 1* and *nit 3* × *nit 3* pairings compared with nit M × nit M pairings support Correll's hypothesis that *nit 1* and *nit 3* mutations affect single genes only, while nit M mutations may affect several genes.

Discussion

The vegetative compatibility patterns among the *Elegans* fusaria studied revealed a fine genetic differentiation among the isolates pathogenic to carnation. The detected VCGs

Table 4. Actual (n_{act}) and potential (n_{pot}) numbers of complementary mutant pairings for isolates of *Fusarium* VCGs 1 to 5 from carnation.

	n_{act}	n_{pot}	$n_{act} : n_{pot}$
nit 1 × *nit 1*	2	18	0.11
nit 1 × *nit 3*	18	41	0.44
nit 1 × nit M	34	36	0.94
nit 3 × *nit 3*	0	13	0.00
nit 3 × nit M	22	30	0.73
nit M × nit M	7	9	0.78

reflected differences in pathogenic potential. VCG 1, 2 and 3 coincided with the known physiologic races 1, 2 and 4 of *F. oxysporum* f.sp. *dianthi*; VCG 4 contained four isolates received as *F. redolens* f.sp. *dianthi*, and VCG 5 contained two non-pathogenic isolates of *F. redolens* from foot-rot diseased carnations. It is not clear whether VCG 1 includes *F. oxysporum* f.sp. *dianthi* race 8 in addition to race 1. Only a single isolate of race 8 was available that yielded ambiguous results.

Five isolates from carnation could not be assigned to any of the VCGs. Of these WCS 834 is self-incompatible. WCS 832, a non-pathogenic isolate of *F. oxysporum*, was isolated from an apparently healthy carnation plant and is probably a saprophytic soil inhabitant, accidentally found in carnation. Non-pathogenic *F. oxysporum* isolates are normally recovered now and then from healthy plants, and vegetative compatibility tests have proven useful to distinguish between non-pathogenic and pathogenic isolates in the cases of cotton (Katan and Katan, 1988), celery (Correll et al., 1986b) and even carnation (information from Dr T. Katan, Dept. of Plant Pathology, Volcani Center, Bet Dagan, Israel). *F. redolens* isolate DSM 62393 was received as belonging to f.sp. *dianthi*, but had been isolated from sweet william (*Dianthus barbatus*) and was not pathogenic to carnation in our experiments (unpublished data). Already Snyder (1941) framed a separate f.sp. *barbati* (within *F. oxysporum*) based on an isolate that was pathogenic to sweet william but not to carnation. However, Armstrong and Armstrong (1954) and Gerlach and Pag (1961) proposed that f.sp. *barbati* is merely a race of f.sp. *dianthi*, because the latter can be pathogenic to carnation as well as sweet william. DSM 62393 may indeed belong to a distinct race and a separate VCG, since it apparently differs in pathogenicity from the isolates in VCG 4 which are pathogenic to both carnation and sweet william (see Hantschke, 1961). *F. redolens* isolates CBS 366.87 and WCS 842 were incompatible with any isolate from carnation. WCS 842 is pathogenic to carnation (Baayen and Gams, 1988) and not self-incompatible, since its mutants (*nit 1* and *nit 3*) complemented each other.

Vegetative compatibility patterns may differ in significance among the various formae speciales of *F. oxysporum*. According to Puhalla (1984b), races 2 and 3 of *F. oxysporum* f.sp. *apii* belong to one, but race 1 to another VCG. The three vegetative compatibility groups among the *F. oxysporum* isolates from crucifers would correspond with the three 'formae speciales' (or races) *conglutinans*, *matthiolae* and *raphani*, two of which are known to contain several races (Bosland and Williams, 1987). On the other hand VCGs and races do not at all correspond among isolates of *F. oxysporum* f.sp. *melonis* (Jacobson and Gordon, 1988). The correlation observed in the present study between vegetative compatibility and pathogenicity among isolates from carnation is therefore not self-evident. If this correlation is confirmed with a larger number of isolates, *Elegans* fusaria from carnation may be identified at race level by means of vegetative compatibility tests without recourse to the usual pathogenicity tests which require much more time.

Vegetative compatibility between isolates of *F. redolens* and *F. oxysporum* was not encountered. However, *F. redolens* f.sp. *dianthi* isolates DSM 62390, DSM 62391 and DSM 62392 proved to be compatible with CBS 248.61. The latter isolate is a deviating subculture of the same isolate as DSM 62390 (the type isolate of *F. redolens* f.sp. *dianthi*), of which the cultural characteristics now correspond with *F. oxysporum* instead of *F. redolens* (Baayen and Gams, 1988). Thus *F. redolens* f.sp. *dianthi* may indeed be compatible with isolates from carnation corresponding to *F. oxysporum* in cultural

characteristics. This observation gives some support to Baayen and Gams' (1988) conclusion that the distinction of *F. redolens* from *F. oxysporum* is not justified. Moreover, the technique used here leads to such a fine subdivision, that a negative result in the remaining combinations between *F. oxysporum* f.sp. *dianthi* and *F. redolens* f.sp. *dianthi* cannot be taken as an argument against conspecificity: given the observed compatibility patterns, it cannot be decided whether the isolates belonging to VCG 4 ('*F. redolens* f.sp. *dianthi*') really classify as a separate taxon, or merely as a distinct race of *F. oxysporum* f.sp. *dianthi*. In a test with seven carnation cultivars used to differentiate among the more common races 1, 2 and 4 of *F. oxysporum* f.sp. *dianthi*, *F. redolens* f.sp. *dianthi* DSM 62392 correspond in virulence with race 2 (Baayen and Gams, 1988). However, the cultivars used in this test do not discriminate the remaining five races of *F. oxysporum* f.sp. *dianthi*. The possible existence of even more races of *F. oxysporum* f.sp. *dianthi* cannot be ruled out either. The observed compatibility patterns among the isolates from carnation do therefore not provide decisive evidence favouring or disfavouring conspecificity of *F. redolens* and *F. oxysporum*.

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Samenvatting

De Elegans-fusaria die verwelkingsziekte veroorzaken bij anjer. II. Compatibiliteitspatronen

De vegetatieve compatibiliteitspatronen bij isolaten van *Elegans*-fusaria die verwelkingsziekte bij anjer veroorzaken werden onderzocht. Van 16 isolaten van *F. redolens*, waarvan negen afkomstig van anjers, en van 33 isolaten van *F. oxysporum*, waarvan 19 afkomstig van anjers, werden mutanten gegenereerd die zonder een organische stikstofbron geen luchtmycelium meer konden vormen. Paringen tussen mutanten van isolaten afkomstig van anjers brachten een vijftal vegetatieve compatibiliteitsgroepen aan het licht, die overeenkwamen met *F. oxysporum* f.sp. *dianthi* fysio 1 (VCG 1), fysio 2 (VCG 2) en fysio 4 (VCG 3), *F. redolens* f.sp. *dianthi* (VCG 4) en *F. redolens* isolaten afkomstig van aan voetrot lijdende anjers (VCG 5). Naast drie voor *F. redolens* karakteristieke isolaten omvatte VCG 4 ook een afwijkende subculture van het type-isolaat van *F. redolens* f.sp. *dianthi*, die in cultuureigenschappen overeen kwam met *F. oxysporum* in plaats van *F. redolens*. Deze waarneming geeft enige steun aan eerdere conclusies dat het onderscheid tussen beide taxa niet gerechtvaardigd is. Daarbuiten gaven de compatibiliteitspatronen geen uitsluitsel over de mogelijke conspecificiteit van beide taxa. Isolaten afkomstig van anjers vormden geen heterokaryons met andere formae speciales van *F. oxysporum*.

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