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Physiological races and vegetative compatibility groups within Fusarium oxysporum f.sp. gladioli

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Abstract

The pathogenicity and vegetative compatibility of mainly Dutch isolates of *Fusarium oxysporum* collected from diseased gladioli and other Iridaceae were investigated. Based on their pathogenicity to two differential gladiolus cultivars, the isolates could tentatively be divided into two races. All self-compatible isolates of *Fusarium oxysporum* f.sp. *gladioli* belonged to one of three distinct vegetative compatibility groups, VCG 0340, 0341 or 0342, and were incompatible with isolates that were not pathogenic to gladiolus. Isolates of one of the two races were restricted to one VCG while isolates of the other race were present in all three VCGs.

Additional keywords: Gladiolus, Iridaceae.

Introduction

Fusarium yellows and corm rot are considered to be serious threats to gladiolus cultivation. The diseases are caused by *Fusarium oxysporum* Schlecht. : Fr. f.sp. *gladioli* (Mass.) Snyder & Hansen (Massey, 1926; Bald et al., 1971; Nelson et al., 1981; Boerema and Hamers, 1989). McClellan (1945) found that the same pathogen can also cause disease symptoms on other genera within the Iridaceae.

The host specificity of isolates of *F. oxysporum* led Snyder and Hansen (1940) to subdivide the species into formae, later called formae speciales, based on the host or group of hosts the pathogen is able to infect. Many formae speciales have been subdivided into races on the basis of their pathogenicity to differential host-plant genotypes (Armstrong and Armstrong, 1966; Garibaldi, 1977; Bosland and Williams, 1987; Jacobson and Gordon, 1988).

Knowledge about the genetic variation of the pathogen and especially the existence of different races is of great importance in order to breed effectively for resistance to *Fusarium*. Determination of host specificity of isolates and genetic relationships between isolates is a prerequisite for research on pathogenicity factors and can be the basis for the development of a specific detection system. Incorporation of isolates from other Iridaceae in this study provides insight in the host specificity of this pathogen and therefore in the importance of crop rotation.

Another way of grouping related isolates is based on the capability of auxotrophic mutants to complement each other by forming a heterokaryon after anastomosis. Buxton (1954; 1956), using isolates of *F. oxysporum* f.sp. *gladioli*, was the first to desc-

ribe heterokaryosis in *Fusarium* and the pairing of complementary auxotrophic mutants to force heterokaryon formation. Puhalla (1984) modified this technique using nitrate-non-utilizing (*nit*) mutants recovered from cultures growing on a chlorate-containing agar medium. If complementation occurs between two isolates, they must be vegetatively compatible and are placed in one and the same vegetative compatibility group (VCG) (Puhalla, 1984; Correll et al., 1987).

Some studies have proved the usefulness of VCG analysis in identifying races within a forma specialis (Correll et al., 1986; Baayen and Kleijn, 1989; Larkin et al., 1990). Others have found more complex relationships between VCGs and races, such as in *F. oxysporum* f.sp. *asparagi* (Elmer and Stephens, 1989), f.sp. *cubense* (Ploetz and Correll, 1988), f.sp. *melonis* (Jacobson and Gordon, 1990a) and f.sp. *lycopersici* (Elias and Schneider, 1991).

Preliminary experiments have shown that so-called 'large-flowered' gladiolus cultivars were more or less susceptible to a certain group of isolates, while 'small-flowered' gladiolus cultivars were susceptible to a wider range of isolates including the previous group. In this article we report on the subdivision of *F. oxysporum* f.sp. *gladioli* based on the results of screening mainly Dutch isolates obtained from gladioli and other Iridaceae. The screening consisted of a pathogenicity test on two differential cultivars and a vegetative compatibility test using different *nit* mutants in order to establish VCGs.

Materials and methods

Fungal isolates. Twenty-three of the isolates (G1-G19, G21-G24) were isolated from gladiolus corms showing characteristic disease symptoms (Table 1). Twelve came from 'large-flowered' and nine from 'small-flowered' gladiolus cultivars. The isolates G13 and G15 originate from European gladiolus species. One of these isolates (G13) was identified as *F. proliferatum* (Matsushima) Nirenberg.

The isolates CBS 151.27, CBS 253.52, CBS 160.57 (from gladiolus) and CBS 620.72 (from crocus) were obtained from the Centraal Bureau voor Schimmelcultures (CBS) in Baarn, the Netherlands.

Three isolates were obtained from diseased bulbs or corms of other genera within the Iridaceae: iris (Ir7), freesia (Fr1) and crocus (Cr1). An isolate of *F. proliferatum* (Matsushima) Nirenberg (M-685) obtained from gladiolus and formerly called *F. moniliforme* (Woltz et al., 1978) was also included.

All cultures were single-spored and kept at 4 °C on malt-agar slants.

Pathogenicity tests. Inoculation experiments were carried out using corms of two gladiolus cultivars: the 'large-flowered' gladiolus cultivar Peter Pears and the 'small-flowered' gladiolus cultivar Nymph. Corms of these cultivars were harvested, dried, cleaned and stored at 5 °C until used.

Inoculum consisted of a conidial suspension prepared from 14-day-old cultures on potato dextrose agar (PDA), grown at 20-23 °C in the dark. Husks of corms were removed, corms were surface-disinfected in 0.8% formaldehyde for 30 min, rinsed in running tap water, dipped in a conidial suspension of 10^5 conidia/ml and planted directly in potting soil. Corms dipped in sterile water were used as controls. Five replicates with one corm per pot were used. The plants were maintained in the greenhouse

at 23-30 °C. After a period of 6 weeks, disease symptoms in the corms and the length of the shoots were recorded.

Pathogenicity tests were carried out in several experiments throughout the year. The effect of inoculation on shoot length was calculated for each isolate separately by performing analysis of variance on shootlengths of inoculated and control plants. Afterwards, results were expressed as relative shoot lengths. Therefore, relative shoot length of different isolates cannot be compared mutually. Severity of corm symptoms was assessed visually after cutting the corms in half and scored using a disease index: 0 = no symptoms; 1 = slight superficial browning; 2 = slightly infected (< 10% of corm tissue rotted); 3 = moderately infected (10-50% of corm tissue rotted); 4 = heavily infected (50-100% of corm tissue rotted).

Pathogenicity of isolates from iris, freesia and crocus to their respective hosts was confirmed using the same inoculation technique as described for gladiolus.

Vegetative compatibility tests. We used the method developed by Puhalla (1985) and Correll et al. (1987), and modified by Löffler and Rumine (1991). Nit mutants were obtained by placing mycelium of each isolate on Czapek Dox agar (CDA) (Oxoid; Hampshire, UK) containing 5% (w/v) $KClO_3$. Rapidly growing, chlorate-resistant sectors were characterized as *nit*1, *nit*3 or NitM mutants. At least two phenotypic classes were obtained from each isolate.

Pairings were made on CDA, incubated at room temperature and scored for complementation. Pairings showing no complementation were left for four weeks before definitively scored as negative. All complementation tests were performed at least twice.

Results

Pathogenicity tests. Results of pathogenicity tests are given in Table 1. In all pathogenicity tests, corms of control plants either did not develop any symptoms (disease index 0), or developed some superficial browning (disease index 1). Corms of inoculated plants with disease index 1 could therefore not be considered diseased.

Some isolates (G10, G13, G22, CBS 253.52, CBS 160.57 and M685) were not pathogenic to 'Peter Pears' or 'Nymph'. Other isolates could be divided into two groups on the basis of their pathogenicity to the differential cultivars. A first group of 13 isolates, obtained from 'large-flowered' cultivars, *G. italicus* or iris, was pathogenic to both cultivars (Table 1). Plants of 'Peter Pears' and 'Nymph' inoculated with these isolates showed heavy corm-rot symptoms and the length of the shoots was strongly reduced. A second group of 12 isolates, from 'small-flowered' gladiolus cultivars, freesia or crocus, was pathogenic to 'Nymph' but not to 'Peter Pears'. Corms of 'Peter Pears' inoculated with these isolates showed some slight superficial browning (disease index 1) or no symptoms at all (disease index 0). Shoot length did not differ significantly from those of control plants. On 'Nymph' these isolates caused symptoms comparable to those caused by isolates of the first group or more moderate symptoms. Shoot length of 'Nymph' was significantly reduced by most of these isolates.

Given the observation that 'Peter Pears' was resistant to the second group of isolates but fully susceptible to the first group, we propose to assign the two groups to different physiological races of *F. oxysporum* f.sp. *gladioli*. However, it cannot be ruled out that these differences are due to extreme quantitative differences in aggressiveness. We

Table 1. Origin, pathogenicity, race classification and vegetative compatibility groups (VCGs) of *Fusarium* isolates from gladiolus and other Iridaceae. PP, cultivar Peter Pears; N, cultivar Nymph; si, self-incompatible; lf, large-flowered; sf, small-flowered. Significance levels: $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

Code	Origin			Shoot length[1]	
	host	cultivar	group	'PP'	'N'
G1	*Gladiolus* sp.	unknown	lf	47***	0***
G2	*Gladiolus* sp.	unknown	lf	11***	0***
G3	*Gladiolus* sp.	Franz Liszt	lf	33***	1***
G4	*Gladiolus* sp.	Spotlight	lf	69**	2***
G7	*Gladiolus* sp.	Hunting Song	lf	32***	6**
G9	*Gladiolus* sp.	Tom Thumb	lf	42***	0***
G11	*Gladiolus* sp.	Hunting Song	lf	29***	38***
G12	*Gladiolus* sp.	Ben Trovato	lf	11***	16***
G15	*G. italicus*	–	–	48***	0***
G16	*Gladiolus* sp.	Superstar	lf	22***	0***
G19	*Gladiolus* sp.	Hunting Song	lf	13***	0***
G21	*Gladiolus* sp.	Peter Pears	lf	11***	0***
Ir7	*Iris* sp.	Prof. Blauw	–	3***	10***
G6	*Gladiolus* sp.	Nymph	sf	97	3***
G18	*Gladiolus* sp.	Nymph	sf	89	0***
G24	*Gladiolus* sp.	Nymph	sf	99	0***
G5	*Gladiolus* sp.	Robinetta	sf	101	13***
G8	*Gladiolus* sp.	Alba	sf	105	31*
G14	*Gladiolus* sp.	Alba	sf	92	74
G17	*Gladiolus* sp.	Robinetta	sf	103	36**
G23	*Gladiolus* sp.	Alba	sf	100	55
Fr1	*Freesia* sp.	unknown	–	102	2***
CBS620.72	*Crocus* sp.	unknown	–	93	1***
Cr1	*Crocus* sp.	unknown	–	96	2***
CBS151.27	*Gladiolus* sp.	unknown	–	107	4***
G10	*Gladiolus* sp.	Nymph	sf	97	95
CBS253.52	*Gladiolus* sp.	unknown	–	109*	95
CBS160.57	*Gladiolus* sp.	unknown	–	99	85*
G22	*Gladiolus* sp.	Peter Pears	lf	96	107
G13[2]	*G. illyricus*	–	–	100	98
M685[2]	*Gladiolus* sp.	unknown	–	101	93

[1] Shoot length relative to the control, in percentages.
[2] *F. proliferatum* instead of *F. oxysporum*.

tentatively designate the first group of isolates (pathogenic to both cultivars) race 1 and the second group (pathogenic to 'Nymph' only) race 2.

Corm-rot disease index		Race	VCG
'PP'	'N'		
4,4,4,4,4	4,4,4,4,4	1	0340
4,4,4,4,4	4,4,4,4,4	1	0340
3,4,4,4,4	4,4,4,4,4	1	0340
2,2,3,3,4	4,4,4,4,4	1	0340
3,4,4,4,4	4,4,4,4,4	1	0340
4,4,4,4,4	4,4,4,4,4	1	0340
3,3,3,4,4	4,4,4,4,4	1	0340
4,4,4,4,4	3,4,4,4,4	1	0340
4,4,4,4,4	4,4,4,4,4	1	0340
4,4,4,4,4	4,4,4,4,4	1	0340
4,4,4,4,4	4,4,4,4,4	1	0340
4,4,4,4,4	4,4,4,4,4	1	0340
4,4,4,4,4	4,4,4,4,4	1	0340
0,0,0,0,0	4,4,4,4,4	2	0340
0,0,0,1,2	4,4,4,4,4	2	0340
0,0,1,1,1	4,4,4,4,4	2	0340
0,0,0,0,1	3,3,3,3,3	2	0341
0,0,0,0,0	1,2,2,3,4	2	0341
0,0,0,0,0	0,1,2,2,3	2	0341
0,0,0,0,0	2,2,2,2,3	2	0341
0,0,0,0,0	1,2,3,3,4	2	0341
0,0,1,1,2	3,3,4,4,4	2	0341
0,1,1,2,2	4,4,4,4,4	2	0342
0,0,1,1,2	4,4,4,4,4	2	0342
0,0,0,1,1	3,4,4,4,4	2	034 – ^{si}
0,0,0,0,1	0,0,0,1,1	–	– ^{si}
0,0,0,0,1	0,0,0,0,1	–	– ^{si}
0,0,0,0,0	0,0,0,0,1	–	– ^{si}
0,0,0,0,0	0,0,0,0,0	–	–
0,0,0,0,1	0,0,0,1,1	–	–
0,0,0,0,0	0,0,0,0,0	–	–

Vegetative compatibility tests. The *nit* mutants of all self-compatible isolates were paired in all combinations, revealing three distinct VCGs. Following the numbering system proposed by Puhalla (1985), we designate these VCG 0340, 0341 and 0342. The number 034 refers to the number of the forma specialis *gladioli* in the list of Armstrong and Armstrong (1981).

The isolate CBS 151.27, which is pathogenic to gladiolus but self-incompatible, was designated VCG 034-, a code suggested by Jacobson and Gordon (1988) for this type of isolates.

The remaining isolates were non-pathogenic and therefore considered not to be *F. oxysporum* f.sp. *gladioli*. Some of these isolates were self-incompatible, others were self-compatible but not vegetative compatible with any other isolate tested. So non of these non-pathogenic isolates could be assigned to a VCG.

Correlation between races and VCGs. The isolates pathogenic to both gladiolus cultivars were named race 1 and were all members of the same VCG (VCG 0340). Three out of twelve isolates pathogenic to the cultivar Nymph only (race 2) were compatible with the race 1 isolates assigned to VCG 0340, six isolates were assigned to VCG 0341 and two race 2 isolates, originating from crocus, belonged to a third distinct group (VCG 0342).

Discussion

The results of this study reveal the existence of at least two races and three VCGs within *F. oxysporum* f.sp. *gladioli*. As in other formae speciales (Bosland and Williams, 1987; Ploetz, 1990; Jacobson and Gordon, 1990a), no consistent correlation was found between VCGs and races.

The races are defined on the basis of pathogenicity to two differential gladiolus cultivars. 'Peter Pears', like other 'large-flowered' cultivars (unpublished results), was resistant to race 2. This resistance might be a feature of 'large-flowered' cultivars explained by their common ancestry (Lewis et al., 1972; Ohri and Khoshoo, 1983). Crosses between 'large-flowered' cultivars (resistant to race 2) and 'small-flowered' cultivars (susceptible to race 2) and backcrosses to universally susceptible cultivars are needed to confirm this hypothesis. As long as no genotypes are found that are susceptible to race 2 but not to race 1, it will be sufficient to use isolates of race 1 to screen for resistance to *Fusarium* in gladiolus.

It is noticeable that until now race 1 isolates, although able to affect 'small-flowered' cultivars, have never been isolated from corms of naturally diseased 'small-flowered' cultivars (Table 1).

Isolates G6, G18 and G24 have our special interest, because they have a high degree of genetic homology with the race 1 isolates of the same VCG (Puhalla and Spieth, 1985; Jacobson and Gordon, 1990b). This suggests that isolates of both races within VCG 0340 differ in a relatively small number of loci including those determining (a)virulence. These isolates therefore provide a good basis to study (a)virulence factors.

It is clear that race 2 does not represent a homogeneous group of isolates. These isolates are unified solely by their common pathogenicity to 'Nymph'. If additional cultivars are tested from gladiolus or from other genera of the Iridaceae, differences in virulence may become apparent.

The discovery that isolates from iris and freesia are vegetatively compatible with isolates from gladiolus, and the fact that isolates from crocus were pathogenic to 'Nymph', support the idea that the host range of *F. oxysporum* f.sp. *gladioli* includes more genera of the Iridaceae than mentioned here. This confirms McClellan's (1945),

finding that isolates from gladiolus can be pathogenic to other iridaceous crops. Consequently, successive plantings of these crops should be avoided.

Non-pathogenic *Fusarium* isolates were not vegetatively compatible to any pathogenic isolate. However, non-pathogenic isolates can only be excluded by the VCG technique when all existing VCGs of *F. oxysporum* f.sp. *gladioli* are known and all isolates are capable of forming heterokaryons. Four of the isolates tested could not be assigned to a VCG, because of the failure of *nit* mutants to form heterokaryons with complementary *nit* mutants generated from the same or other isolates. Since three of these isolates came from the culturel collection of the CBS self-incompatibility might be a result of prolonged storage in culture, as suggested by Jacobson and Gordon (1990a). Also the lack of pathogenicity of two of the isolates from the culturel collection might be due to prolonged storage.

In future studies we will try to determine genetic relationships within and between VCGs with the help of RFLP patterns (Jacobson and Gordon, 1990b; Manicom, 1990). In addition self-incompatible isolates might then be further identified.

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