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Durability of resistance in lily to basal rot: evaluation of virulence and aggressiveness among isolates of *Fusarium oxysporum* f. sp. *lilii*

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Abstract

Prospects of durability of resistance in lily to basal rot have been evaluated by testing the virulence and aggressiveness of 31 isolates of *Fusarium oxysporum* f. sp. *lilii* towards a number of different resistance sources in *Lilium* spp. Isolates differed strongly in aggressiveness as did species and cultivars of *Lilium* spp. in resistance. Significant interactions were observed between isolates of the pathogen and genotypes of *Lilium* spp., but the magnitude was very small compared to the main effects. The interactions were mainly due to a small group of isolates with low aggressiveness. It is argued that the interactions might be based on minor genes. No major break down of the resistance was found. For practical purposes it will be sufficient to use highly aggressive isolates in screening tests.

Introduction

The soil-borne fungus *Fusarium oxysporum* is a pathogen which occurs worldwide and causes, in specialized form, serious diseases in many crops. In lily the forma specialis *lilii* causes bulb rot, which threatens the bulb production worldwide. The predominant symptom of the disease is a brownish rot at the base of the bulb scales. This rot may spread over the whole bulb in a later stage of the disease, resulting in the total loss of the bulb [Imle, 1942; Linderman, 1981; McRae, 1988].

The disease can be controlled by disinfecting bulbs before planting in combination with soil fumigation. In the Netherlands, however, the application of chemical control will be reduced strongly in the near future in order to reduce environmental pollution. Control of the disease by cultivation of Fusarium-resistant cultivars will be the first alternative.

High levels of partial resistance were found in some Asiatic hybrid lilies and some species. A low level of resistance was found in cultivars of *Lilium longiflorum* and almost none in the Oriental hybrid lilies [Straathof and Van Tuyl, 1994]. The sources of resistance can be used in (interspecific) breeding programs to develop new resistant lily cultivars.

It is not clear, however, what the durability of those resistances will be. Non-durable resistance evidently is of limited value in a breeding program. In other crops, resistance to *Fusarium oxysporum* sometimes was found to be overcome by the pathogen, resulting in the emergence of different races of the pathogenic forma specialis [Armstrong and Armstrong, 1981; Roebroeck and Mes, 1992]. The specificity of the interaction caused by races will be designated with the term 'virulence' throughout this paper, whereas the term 'aggressiveness' refers to the overall rate of disease development [Van der Plank, 1984]. Apart from a preliminary study in vegetative compatibility, aggressiveness and virulence in *F. oxysporum* f. sp. *lilii* [Löffler and Rumine, 1991],

data on the existence of races within the f. sp. *lilii* are lacking. The present study assesses the prospects of durability of the resistance in lily to basal rot.

Durability is governed by two distinct factors. In the first place, genetic variation for virulence may exist already within the population of the pathogen. Cultivation of resistant cultivars might thus lead to selection of more virulent genotypes of the fungus. In the second place, the pathogen might be able to adapt easily to the resistance of the host plant. In that case a breaking down of the resistance would be due to changes in the genome of the pathogen. The present study reports on the first aspect; the second aspect will be dealt with in a separate paper [Löffler *et al.*, in preparation]. We tested the virulence of a large number of isolates from diverse geographic origin to a series of lily cultivars differing in resistance to basal rot. For comparison a number of isolates of other rot-inducing formae speciales were included. Such isolates may cross-react with lilies [Löffler and Mouris, 1993].

Materials and methods

Fungi

Isolates of *F. oxysporum* f. sp. *lilii* (Fol), *F. oxysporum* f. sp. *gladioli* (Fog; Foi) and *F. oxysporum* f. sp. *tulipae* (Fot) were kindly provided by E. J. A. Roebroeck (LBO: Bulb Research Centre, Lisse, The Netherlands), G. Bollen (LUW: Wageningen Agricultural University, The Netherlands), J. van Tuyl (CPRO-DLO: DLO Centre for Plant Breeding and Reproduction Research, Wageningen, The Netherlands), P. Rumine (ISF: Istituto Sperimentale per la Floricoltura, Pescia, Italy), L. B. Orlikowski (RIPF: Research Institute of Pomology and Floriculture, Skierniwice, Poland) and P. E. Nelson (FRC: The Fusarium Research Centrum, State College (PA), USA). The origin of the isolates is summarized in Table 1. Single-spore isolates were made and screened for pathogenicity to a number of susceptible lily cultivars in a scale-assay [Smith and Maginnes, 1969; Maginnes and Smith, 1971; Löffler and Mouris, 1989]. Non-pathogenic isolates were discarded; pathogenic ones were stored on PROTECT bacterial preserves (Technical Service Consultants) at −80 °C. Isolates selected for virulence tests were revitalized on OXOID Czapek Dox agar plates (CDA).

Plant material

Lily genotypes known to be highly resistant to basal rot were used [Straathof and Van Tuyl, 1994; Straathof *et al.*, 1993] along with moderately resistant and susceptible ones (Table 2). Bulbs were not disinfected after harvest but were stored as such at −0.5 °C until use. For the screening, a bulb scale assay was used [Maginnes and Smith, 1971; Löffler and Mouris, 1989]. This assay is efficient in time and space and renders reliable data [Straathof and Löffler, 1994]. Therefore bulbs were brought at room temperature and scales were detached one day before planting. Apparently healthy scales were disinfected for 10 minutes in a 1% hypochlorite solution with a drop of Tween 20, rinsed with tap water, spread on filter paper and dried overnight at room temperature.

Soil infestation

Inoculum was prepared by infecting 100-ml glass jars containing sterilized oatmeal-soil mixture (20% w/w oatmeal) with five 5-mm agar plugs taken from CDA plates with the proper isolate and incubating the mixture for 2 weeks at 23 °C. Subsequently, the mixture was ground by pressing it through a sieve (experiment 1) or by using a food processor (experiment 2). Ten litres of soil in 20-litre open trays was mixed with the ground mixture of the appropriate isolate (1% v/v) and incubated for 2 weeks prior to use to allow the fungal population to stabilize [Löffler and Mouris, 1989].

Virulence screening

Twenty genotypes of lily were screened in two experiments with 31 fungal isolates in a randomized block design of two blocks. Both blocks contained trays of all isolates and a single tray with uninfested soil. Scales were planted in rows of six according to genotype (nine in experiment 1 and 13 in experiment 2). Rows within trays and trays within blocks were fully randomized. In this way each combination of genotype and isolate was tested in 12-fold. After 8 weeks incubation at 18 °C in the greenhouse, the scales were harvested and indexed for disease severity using the

Table 1. Origin of isolates of *F. oxysporum* f. sp. *lilii* (Fol), f. sp. *gladioli* (Fog; Foi) and f. sp. *tulipae* (Fot) used in this study

Isolate	Host	Cultivar	Source[1]	Country
Fol-3	Asiatic hybrid lily	Enchantment	LBO	The Netherlands
Fol-4	Asiatic hybrid lily	Pirate	LBO	The Netherlands
Fol-5	Asiatic hybrid lily	Pirate	LBO	The Netherlands
Fol-7	*Lilium formosum*	–	LBO	The Netherlands
Fol-9	Asiatic hybrid lily	Amigo	LBO	The Netherlands
Fol-10	Asiatic hybrid lily	Krista	LUW	The Netherlands
Fol-11	Asiatic hybrid lily	Esther	LUW	The Netherlands
Fol-15	*Lilium concolor*	–	CPRO-DLO	The Netherlands
Fol-18	Lily	–	LBO	The Netherlands
Fol-19	Lily	–	LBO	The Netherlands
Fol-21	Asiatic hybrid lily	Casa Blanca	LBO	The Netherlands
Fol-28	Lily	–	ISF	Italy
Fol-30	Lily	–	ISF	Italy
Fol-33	Asiatic hybrid lily	Aleida	LBO	The Netherlands
Fol-35	Asiatic hybrid lily	Avignon	LBO	The Netherlands
Fol-36	Asiatic hybrid lily	Montreux	LBO	The Netherlands
Fol-38	Asiatic hybrid lily	Snow Queen	LBO	The Netherlands
Fol-40	Asiatic hybrid lily	Moulin Rouge	LBO	The Netherlands
Fol-42	Asiatic hybrid lily	Yellow Present	LBO	The Netherlands
Fol-43	Asiatic hybrid lily	Connecticut King	LBO	The Netherlands
Fol-63	Lily	–	FRC	USA
Fol-69	Asiatic hybrid lily	Escapade	ISF	Italy
Fol-71	*Lilium longiflorum*	Snow Queen	ISF	Italy
Fol-73	Asiatic hybrid lily	Enchantment	ISF	Italy
Fol-78	Lily	–	ISF	Italy
Fol-79	Asiatic hybrid lily	Boston	RIPF	Poland
Fol-80	Asiatic hybrid lily	Nellie White	RIPF	Poland
Fog-15	Gladiolus	–	LBO	The Netherlands
Foi-2	Iris	–	LBO	The Netherlands
Foi-7	Iris	–	LBO	The Netherlands
Fot-8	Tulip	–	LBO	The Netherlands

[1] For abbreviations see Materials and methods.

following scale: 0 (healthy), 1 (slightly rotten), 2 (moderately rotten), 3 (heavily rotten), 4 (very heavily rotten) and 5 (completely decayed).

Statistical analysis

Average disease indices (DI) were calculated per row of six scales and subjected to analysis of variance (ANOVA) to estimate the block effect, the main effects (aggressiveness of isolates and resistance of *Lilium* genotypes) and the isolate-genotype interaction. Alternatively, the disease indices were transformed from the non-linear categorical scale to a linear disease severity score (DSS) applying a threshold model, which is a generalized linear model (GLM), according to Straathof *et al.* [1993]. This analysis was restricted to the main factors.

Non-additive variance was further analyzed using two approaches. In the first one, individual isolates with the largest effects were excluded from the model until the variance in the remaining model could be explained by additivity. In the second one, fungal isolates and lily genotypes were stepwise clustered according to Corsten and Denis [1990]. This procedure identifies simultaneously groups of unclustered rows and groups of unclustered columns in an orthogonal two-way table of uncorrelated normally distributed observations with common variance, such that the interaction between row and column factors is due to interactions between those groups. In the first approach, interaction effects are removed and the model is reduced to additivity; in the second one additive effects are removed and fungal and host

Table 2. Resistant level and taxonomical position of cultivars and accessions of lilies used in experiments 1 and 2

Genotype	Abbreviation	Group	Resistance	Experiment
Aristo	AR	A	S	1, 2
Gelria	GE	L	S	1
Nellie White	NW	L	S	2
Star Gazer	SG	O	S	1
Banga	BA	A	MR	2
CPRO-02 (*L.hansonii*)	Han	S	MR	2
CPRO-10 (*L.tigrinum*)	Tig	S	MR	2
CPRO-12 (*L.henryi*)	Han	S	MR	2
CPRO-22 (*L.davidii*)	Dav	S	MR	2
CPRO-26	Hyb	*	MR	2
Napoli	NA	A	MR	1
White Europe	WE	L	MR	2
Yellow Star	YS	A	MR	2
Connecticut King	CK	A	R	1, 2
CPRO-01 (*L.dauricum*)	Dau	S	R	2
Mont Blanc	MB	A	R	1
Orlito	OR	A	R	1
Prominence	PR	A	R	1
Vonnie	VO	A	R	2
Yellow Blaze	YB	A	R	1

A: Asiatic hybrid lily; L: *Lilium longiflorum*; O: Oriental hybrid lily; S: botanical species; *: hybrid originating from Asiatic hybrid × (Asiatic hybrid × *L. henryi*); R: resistant; MR: moderately resistant; S: susceptible [Straathof and Van Tuyl, 1994].

genotypes are stepwize clustered so that only variance due to interaction effects is being analyzed.

Results

Isolates differed considerably in aggressiveness to *Lilium* spp, as did the lily genotypes in resistance level to *Fusarium* (non-transformed data: Table 3 and 4). This was found both without transformation of the data and after transformation into DSS values using a threshold model. Transformed and non-transformed data correlate highly for the main effect aggressiveness (Fig. 1a, b) and for the main effect resistance (data not shown). Due to computational limitations, the DSS values of each isolate-cultivar interaction could not be calculated separately. Therefore the transformed data can not be used for analyzing interactions between the two main effects; further analyses are based on non-transformed data. ANOVA showed that in both experiments the variance was mainly due to the main effects (aggressiveness of isolates and resistance of *Lilium* genotypes); block effects were not significant. The isolate-by-lily genotype effects were significant ($P < 0.001$) in both experiments, but their contribution to the total variance was relatively small (Table 5). In both experiments, regardless of the different host genotypes used, the interaction was found to be mainly due to the seven least aggressive isolates Fol-7, Fot-8, Fol-79, Fol-69, Fol-30, Foi-7 and Fog-15. Subsequent ANOVA showed that excluding those isolates precluded almost all interaction (Table 6), demonstrating that indeed those isolates are responsible for the interaction. Some inversions were found. In experiment 1, the overall susceptible cultivar 'Gelria' is less affected by the isolates Fog-15, Fol-30 and Fol-69 than most overall resistant cultivars. The same holds for the isolates Fog-15, Fol-30, Fol-69 and Foi-7 on 'Nellie White' and 'White Europe' and for Fol-79 on 'Nellie White' in experiment 2. All these isolates belong to the seven least aggressive ones.

In the Corsten-analysis, isolates and lily genotypes were clustered according to a minimal contribution to non-additive variance. This approach identifies the same seven isolates as first-responsible for the interaction in experiment 2 (Fig. 3). Clustering of these isolates is less distinct in experiment 1 (Fig. 2).

Table 3. Average disease indices of nine lily genotypes inoculated with 31 isolates of *F. oxysporum* in a scale assay (n = 12), sorted towards increasing aggressiveness of isolates (vertically) and decreasing resistance of the genotypes (horizontally). Data are from experiment 1. Combinations with a large interaction effect are underlined. The abbreviations of the genotypes are explained in Table 1

Isolate	Genotype									
	PR	YB	NA	MB	CK	OR	SG	GE	AR	AVG[1]
Fol-79	0.3	0.9	0.9	1.3	1.0	1.1	1.2	2.5	2.5	1.3
Fog-15	0.4	1.0	1.5	0.9	2.1	0.9	2.6	0.3	2.8	1.4
Fol-7	0.5	1.6	1.7	0.4	1.8	1.9	2.5	2.3	2.9	1.7
Fol-30	1.3	1.8	2.3	1.3	2.1	2.1	1.7	1.0	2.4	1.8
Fol-69	1.0	1.7	2.0	1.4	1.9	1.6	1.9	1.5	3.2	1.8
Foi-7	0.7	1.6	1.7	2.3	2.3	2.1	2.6	2.3	3.0	2.0
Fot-8	1.7	1.5	2.3	2.1	1.8	2.3	1.8	3.1	3.2	2.2
Foi-2	0.9	1.1	1.6	1.5	2.5	2.2	3.8	4.6	3.3	2.4
Fol-63	1.0	2.0	2.2	1.6	2.4	2.3	2.6	3.8	4.1	2.4
Fol-3	1.9	2.3	2.4	2.3	2.5	2.6	3.3	3.8	4.0	2.8
Fol-28	2.0	2.3	2.3	2.1	2.2	3.1	3.3	3.7	4.2	2.8
Fol-33	2.4	2.7	2.3	2.4	2.3	2.8	3.6	4.1	4.3	3.0
Fol-4	2.1	2.3	2.6	2.5	2.5	2.6	4.1	4.1	4.2	3.0
Fol-10	2.4	2.8	2.6	2.6	2.3	2.7	3.3	4.3	4.4	3.0
Fol-15	2.6	2.6	3.1	2.8	2.8	2.8	3.5	3.6	4.3	3.1
Fol-71	2.7	2.6	2.8	2.8	2.6	2.7	3.7	4.3	4.3	3.1
Fol-5	2.1	2.6	3.0	2.9	2.6	3.0	3.8	4.3	4.1	3.2
Fol-19	2.7	2.9	2.5	2.8	2.8	2.9	3.5	4.1	4.5	3.2
Fol-36	2.6	2.6	2.7	3.0	2.5	2.8	3.8	4.4	4.3	3.2
Fol-73	2.1	2.6	2.6	2.8	2.8	3.0	4.3	4.7	4.2	3.2
Fol-78	2.5	2.8	2.4	2.9	3.0	2.9	3.8	4.8	4.3	3.3
Fol-9	2.8	2.7	2.8	3.0	3.0	3.1	4.1	4.1	4.2	3.3
Fol-18	2.8	2.8	2.6	3.0	2.5	3.1	4.4	4.7	4.3	3.4
Fol-43	2.8	3.0	3.0	3.2	2.8	3.3	4.0	4.6	4.3	3.4
Fol-42	3.1	2.9	2.6	3.0	2.8	3.3	4.1	5.0	4.3	3.4
Fol-21	2.8	3.3	3.2	3.0	2.6	3.3	4.3	4.9	4.4	3.5
Fol-80	2.9	3.0	2.8	3.3	3.0	3.6	4.5	4.5	4.5	3.6
Fol-40	2.4	3.0	2.9	3.3	3.0	3.7	4.7	4.8	4.4	3.6
Fol-38	3.3	3.5	2.8	3.4	2.8	3.2	3.9	4.8	4.5	3.6
Fol-35	3.0	3.6	2.8	3.5	3.4	3.2	4.2	4.8	4.3	3.6
Fol-11	2.9	3.5	3.3	3.5	3.1	3.8	4.9	4.9	4.7	3.8
AVG[1]	2.1	2.4	2.4	2.5	2.5	2.7	3.5	3.8	3.9	2.9

[1] Average disease indices per isolate and cultivar.

Discussion

In this study the existence of races of *Fusarium oxysporum* f. sp. *lilii* was investigated by studying the interaction between a number of fungal isolates with Fusarium susceptible and resistant lily genotypes. The level of interaction between isolates and lily genotypes was determined using a disease index (DI). Since DI data are qualitative and ordinal rather than quantitative and linear, care must be taken when applying statistics. Average disease indices may not be appropriate since lilies indexed DI = 4 are not necessarily twice as diseased as lilies indexed DI = 2. Straathof *et al.* [1993] showed that such categorical data can be analyzed efficiently using a threshold model. With this model, disease severity scores (DSS) can be calculated by transforming the categorical data to an underlying linear scale. Unfortunately it is not possible to analyze large data sets such as gathered in this study due to computational limitations. Therefore the DSS values

Table 4. Average disease indices of 13 lily genotypes inoculated with 31 isolates of *F. oxysporum* in a scale assay (n = 12), and sorted towards increasing aggressiveness of the isolates (vertically) and decreasing resistance of the genotypes (horizontally). Data are from experiment 2. Combinations with a large interaction effect are underlined. The abbreviations of the genotypes are explained in Table 1

Isolate	Genotype													
	CK	VO	Dau	Tig	Han	BA	Dav	Hen	Hyb	YS	WE	AR	NW	AVG[1]
Fol-79	0.6	1.0	0.6	1.0	0.4	0.8	1.0	1.0	1.0	1.7	1.1	0.7	0.2	0.8
Fol-69	1.0	0.9	1.0	1.2	0.8	1.2	1.1	1.0	0.7	1.0	0.3	1.5	0.4	0.9
Fol-30	1.0	1.1	1.2	1.0	1.0	1.2	1.4	1.3	1.0	1.1	0.3	2.3	0.4	1.1
Fot-8	1.0	0.8	1.3	1.0	0.8	1.3	1.3	1.3	1.3	1.2	0.9	1.3	2.3	1.2
Fol-7	1.0	1.2	1.2	1.3	1.0	1.1	2.2	1.2	1.3	1.5	1.5	1.3	1.3	1.3
Fog-15	1.2	0.5	1.3	1.6	1.3	1.1	1.7	2.1	1.8	3.2	0.4	2.1	0.1	1.4
Foi-7	1.0	0.8	1.1	0.9	1.0	0.9	1.1	2.7	2.7	2.8	0.3	3.2	0.5	1.4
Fol-63	1.1	1.2	1.5	1.8	1.4	1.3	1.8	2.3	2.3	2.2	3.8	3.6	3.7	2.1
Fol-5	1.1	1.3	1.3	1.6	2.3	1.5	2.8	3.5	2.6	3.3	3.1	3.2	4.3	2.4
Foi-2	1.3	1.4	2.0	2.0	1.4	1.7	2.4	3.3	1.9	3.3	3.7	3.3	4.3	2.5
Fol-3	1.2	1.8	1.6	2.3	1.2	1.9	1.9	2.9	2.8	3.8	3.8	4.4	4.8	2.7
Fol-28	1.2	1.4	1.5	1.6	1.5	3.2	2.5	2.9	2.2	3.7	4.3	4.3	4.7	2.7
Fol-11	1.4	1.8	1.8	1.8	1.5	2.4	2.5	2.4	2.5	3.8	4.1	4.2	4.8	2.7
Fol-15	1.3	1.9	1.9	2.6	1.7	3.1	2.1	2.5	2.9	3.6	3.8	3.6	4.0	2.7
Fol-4	1.2	1.8	1.3	2.4	1.6	2.2	2.9	3.2	3.7	3.3	3.5	3.6	4.4	2.7
Fol-73	1.2	1.4	2.4	1.5	2.4	2.0	3.0	2.8	3.7	2.8	4.0	3.6	4.7	2.7
Fol-71	1.1	1.7	1.7	2.3	2.7	3.1	3.1	3.0	2.9	3.4	3.6	3.5	4.1	2.8
Fol-36	1.3	1.5	1.5	2.1	2.1	3.2	2.5	2.3	3.6	3.8	3.9	4.2	4.6	2.8
Fol-33	1.2	1.8	1.9	2.8	3.4	3.3	2.9	3.5	3.2	4.0	3.8	4.5	4.8	3.2
Fol-35	1.3	2.3	2.0	2.4	3.3	3.1	3.0	3.3	3.3	4.2	4.7	4.8	4.8	3.3
Fol-40	1.3	2.3	3.5	3.3	3.6	2.7	3.0	3.2	3.4	3.6	4.1	4.7	4.9	3.3
Fol-80	1.3	2.3	2.6	2.5	3.6	3.1	3.3	3.3	4.2	4.0	4.7	4.6	4.8	3.4
Fol-21	1.3	2.7	2.3	2.8	3.9	3.2	3.7	3.3	3.6	4.3	4.0	5.0	4.7	3.4
Fol-42	1.3	2.3	2.6	1.9	4.3	3.4	3.9	3.3	3.6	3.7	4.8	4.8	4.8	3.4
Fol-19	1.3	2.0	2.0	3.8	4.0	4.3	3.4	3.3	3.7	4.1	4.6	4.9	4.8	3.5
Fol-43	1.7	2.0	3.0	3.8	3.8	3.8	4.0	3.8	3.1	3.8	4.6	4.6	4.8	3.6
Fol-18	1.6	2.2	2.2	3.9	4.0	3.8	3.8	4.0	4.2	4.0	4.2	4.5	4.6	3.6
Fol-38	1.2	2.2	3.3	3.4	3.4	3.8	3.7	3.4	3.8	4.5	4.4	4.7	5.0	3.6
Fol-10	1.5	1.8	3.1	3.1	4.3	4.0	3.7	3.5	4.0	4.4	4.3	4.8	4.9	3.6
Fol-9	2.0	2.8	3.0	3.8	3.4	3.3	4.1	4.0	3.3	3.9	4.8	4.4	4.9	3.7
Fol-78	1.5	2.9	3.7	3.2	4.1	3.8	4.3	3.0	3.9	4.6	4.3	4.8	5.1	3.8
AVG[1]	1.2	1.7	2.0	2.3	2.4	2.5	2.7	2.8	2.8	3.3	3.3	3.7	3.7	2.7

[1] Average disease indices per isolate and cultivar.

were only calculated for the main effects. These values were found to be highly correlated to the average disease indices (Fig. 1). Although the application of statistics to the average DI data in general may not be adequate, it therefore seems to be justified in this occasion. Apparently classes of the DI categories were chosen in such a way that they are more or less quantitative. This is in line with the observations of Straathof *et al.* [1993] that the width of the DI classes for this screening assay on the underlying linear scale is almost constant.

No major break-down of resistance was detected in this study. With all isolates, resistant genotypes were less affected than the susceptible controls. If the interaction would follow a gene-for-gene relationship in which major resistance genes and (a)virulence genes are involved, virulent isolates would presumably incite disease levels in compatible 'resistant' genotypes to the same extent as in susceptible genotypes, which is clearly not the case in the present study. A gene-for-gene relationship, however, might also exist at minor gene level, as argued by Parlevliet and Zadoks

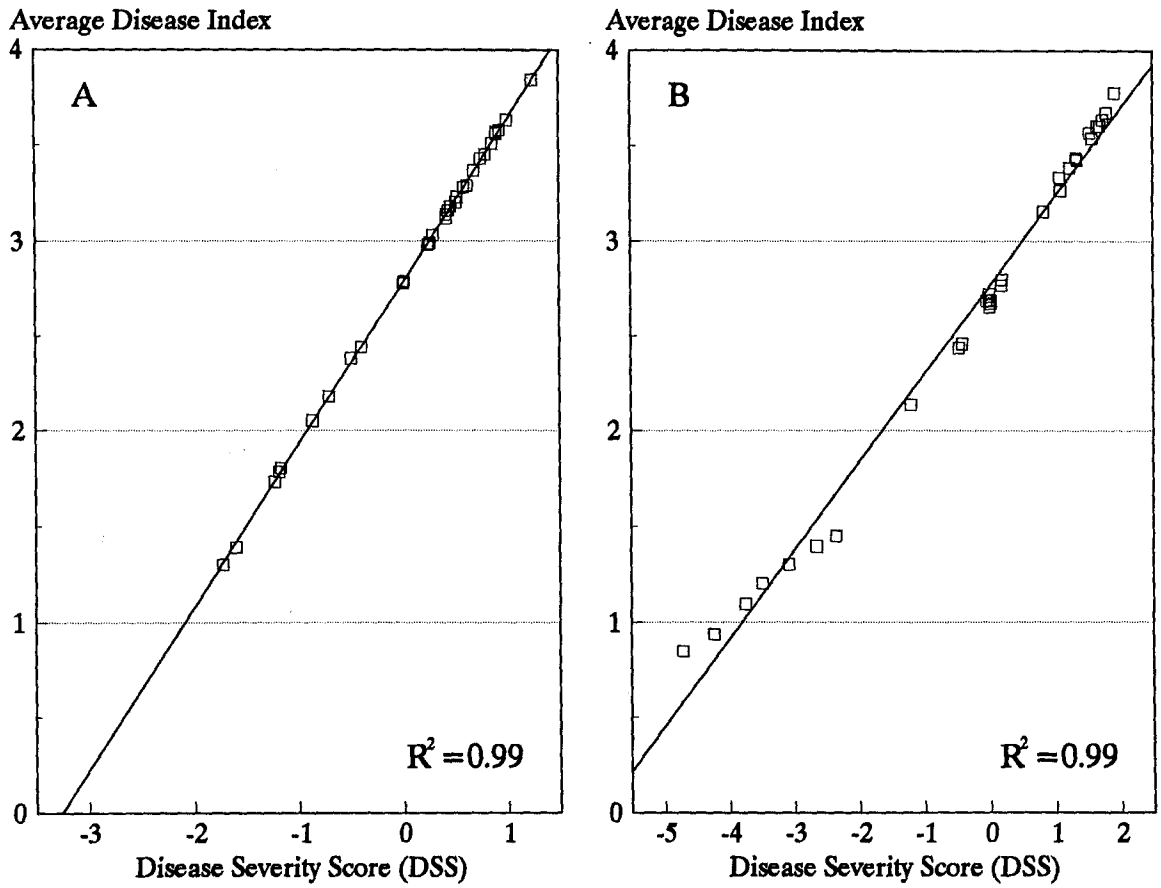

Fig. 1. Correlation between the aggressiveness of isolates, calculated as average disease indices over all genotypes, with the average disease severity score (DSS) calculated by applying a threshold model. A: data from experiment 1; B: data from experiment 2.

Table 5. Analysis of variance of average disease indices from experiment 1 and 2

Exp	Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio	Significance
1	block	1	0.562	0.562	1.69	NS
1	isolate	30	272.677	9.089	27.29	*
1	genotype	8	230.880	28.860	232.66	*
1	isolate × genotype	240	74.500	0.310	2.50	*
1	residual	248	30.763	0.124		
1	total	557	619.375			
2	block	1	0.290	0.290	0.88	NS
2	isolate	30	663.565	22.119	67.16	*
2	genotype	12	422.463	35.205	156.16	*
2	isolate × genotype	360	251.759	0.701	3.11	*
2	residual	372	82.962	0.225		
2	total	805	1415.843			

NS: not significant; *: significant at $P < 0.001$.

Table 6. Analysis of variance of average disease indices from experiment 1 and 2, excluding the seven least aggressive isolates

Exp	Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio	Significance
1	block	1	0.016	0.016	0.06	NS
1	isolate	23	53.938	2.345	8.99	*
1	genotype	8	220.306	27.538	239.87	*
1	isolate × genotype	184	28.266	0.154	1.34	NS
1	residual	192	22.042	0.115		
1	total	431				
2	block	1	1.165	1.165	4.13	NS
2	isolate	23	139.881	6.082	21.56	*
2	genotype	12	516.544	43.045	168.72	*
2	isolate × genotype	276	101.708	0.369	1.44	NS
2	residual	288	73.222	0.255		
2	total	623	834.256			

NS: not significant; *: significant at $P < 0.001$.

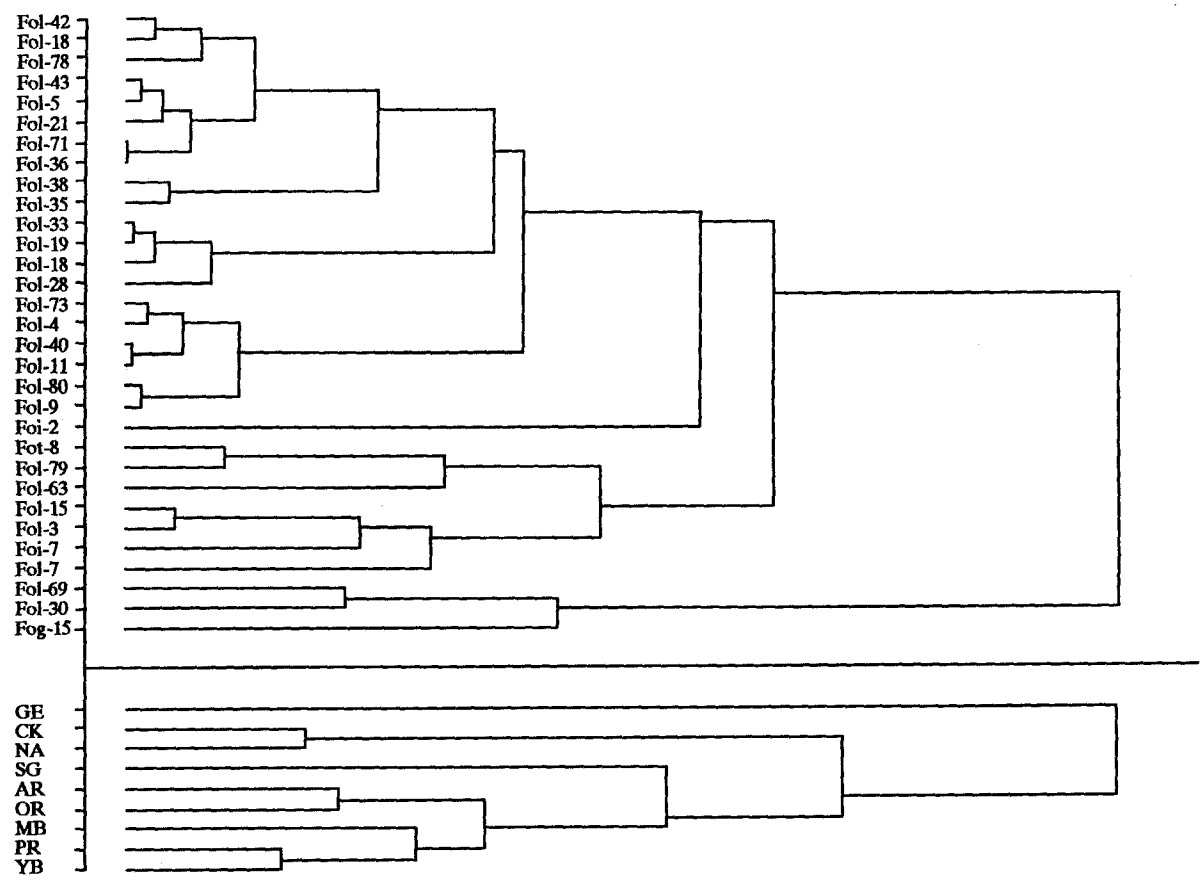

Fig. 2. Dendogram produced by cluster analysis of fungal isolates and host cultivars according to Corsten and Denis [1990]. The analysis was performed on average disease indices from experiment 1. Isolates and cultivars are clustered succesively according to similarity exclusively in terms of minimal contribution to variance for interaction.

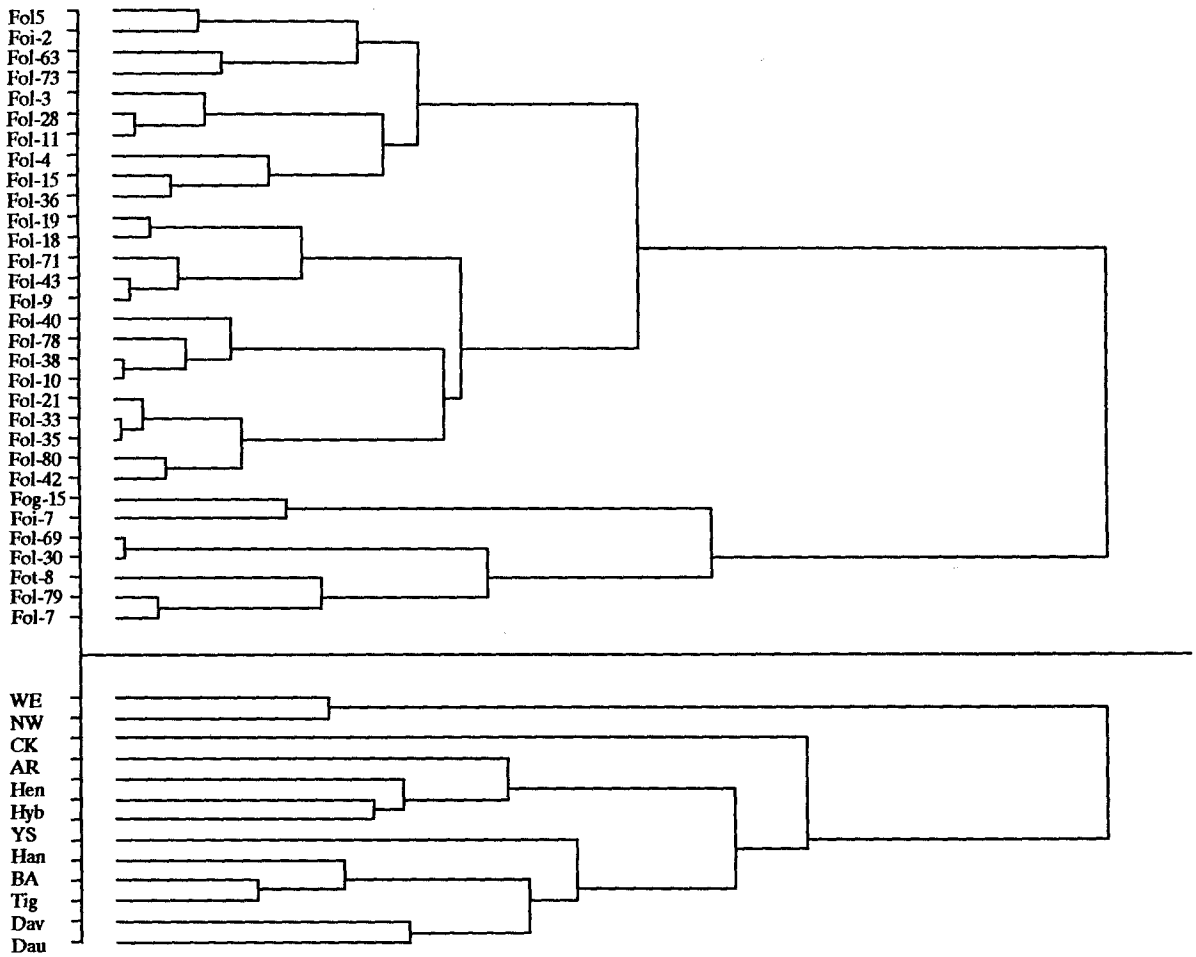

Fig. 3. Dendogram produced by cluster analysis of fungal isolates and host cultivars according to Corsten and Denis [1990]. The analysis was performed on average disease indices from experiment 2. Isolates and cultivars are clustered succesively according to similarity exclusively in terms of minimal contribution to variance for interaction.

[1977]. Adaptation of the fungus to one or more minor resistance genes would result in strains differing slightly from one another in their virulence. The minor interactions found in both experiments point in this direction.

In both experiments the interaction can mainly be ascribed to the seven least aggressive isolates. If these are not included in the analysis, the interaction disappears. As argued by Straathof *et al.* [1993] the interaction might be partly due to a bias in the results, caused by the limitations of the ordinal observation scale. Weakly aggressive isolates can only affect any lily genotype slightly and thus will necessarily show less contrast between resistant and susceptible lily genotypes than highly aggressive isolates. This bias does not explain all interaction, however, since some inversions were found. The isolates Fog-15, Fol-30, Fol-69 and to some extent Foi-7 and Fol-79 affected the generally susceptible cultivars 'Gelria', 'White Europe' and 'Nellie White' less than expected. Apparently these cultivars possess some specific resistance to these isolates. Similar results were obtained by Löffler and Rumine [1991] for the combination of 'Gelria' and Fol-30 (in earlier work designated as Fol-C). Since 'Gelria', 'Nellie White' and 'White Europe' are the only *L. longiflorum* cultivars included in the experiments, it is likely that *L. longiflorum*, which is quite distinct from the Oriental and Asiatic

hybrids, differs in specific resistance to these particular isolates. The observed interactions, however, were relatively small and only occurred in isolates with rather low aggressiveness. Therefore the biological significance of the phenomenon seems to be limited.

Cluster analyses as proposed by Corsten [Corsten and Denis, 1990] separated the isolates and the lily genotypes into different groups (Figs. 2, 3). In experiment 1, the most divergent isolate group comprised Fol-69, Fol-30 and Fog-15, and the lily cultivar 'Gelria' deviated from the other lily genotypes. The interactions between the deviating groups are precisely those which showed inversions (Table 3).

In experiment 2 isolates Fol-69, Fol-30, Fog-15, Foi-7, Fol-79, Fol-7 and Fot-8 were clustered separately whereas lily genotypes 'Nellie White' and 'White Europe' differed from the remaining lily genotypes. Apart from Fol-7 and Fot-8, all these isolates interact specifically with both lily cultivars (Table 4). In general, therefore, isolates exerting specific interactions with *L. longiflorum* cultivars tended to cluster. At present it can not be judged, however, whether groups of isolates, differentiated based on their interaction patterns, are genetically distinct. Fog-15 and Foi-7 are related since they both belong to the same race in the formae specialis *'gladioli'* and share common RFLP-patterns [Roebroeck and Mes, 1992; Mes *et al.*, 1994]. Fol-30 and Fol-69 may be related to each other since they both originate from Italy. To elucidate whether these isolates might be related to those of f. sp. *gladioli*, further characterization of the isolates is necessary. Determination of vegetative compatibility among isolates [Aloi and Baayen, 1993; Roebroeck and Mes, 1992] and evaluation of DNA restriction fragment length polymorphisms [Manicom and Baayen, 1993; Mes *et al.*, 1994] are suitable techniques for this purpose and will be carried out in the future.

None of the deviating isolates led to a higher disease incidence than observed with highly aggressive isolates in the same cultivar. Therefore lily genotypes, selected for resistance to highly aggressive isolates, will not be affected by any of the deviating isolates. Thus for practical purposes it will be sufficient to screen genotypes against one well-characterized aggressive isolate.

As could be expected a large genotype effect was found. This apparently is due to the fact that in both experiments susceptible controls were included. Even the most resistant cultivars, however, were affected by the fungus in this study. This is partly due to the use of bulb scales in the screening test. The wounded base of detached scales forms an easy entrance for the fungus and thus scales will be affected more than bulbs [Straathof and Löffler, 1994]. Moreover, it confirms the partial character of the resistance. Still the level of resistance found in both experiments correlates well with the levels originally found and reported elsewhere [Straathof and Van Tuyl, 1994; Straathof *et al.*, 1993].

A large variation in aggressiveness of the isolates was also found. Since all isolates were used in both experiments, their relative aggressiveness can be compared. In general the two data sets correlate fairly well ($R^2 = 0.80$). Some discrepancies between the two data sets may be due to differences in the infection pressure [Straathof and Inggamer, 1992].

Isolates belonging to other formae speciales were able to infect lily as shown before [Löffler and Mouris, 1993]. Some cross-infection thus does occur as previously reported by Valásková [1976] for freesia. Those findings indicate that the concept of formae speciales may have to be re-evaluated for the rot-producing forms. Phylogenetic analyses, for example based on DNA patterns, using appropriately conserved areas of the genome may be necessary to answer the question raised above.

In conclusion, specific interactions were found between lily genotypes and isolates of *F. oxysporum*, suggesting the existence of physiological races. However, differences in virulence among the isolates are relatively small and seem to be of limited biological significance. The use of a single highly aggressive isolate in screening tests should suffice in breeding for resistance in lily to basal rot.

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