Evaluation of Virulence of *Fusarium solani* Isolates on Pea

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Abstract

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The virulence of 166 *Fusarium solani* isolates collected in the Czech Republic from infected pea plants was evaluated. Based on the ability to produce phytotoxic naphtarazin (DHF – dihydrofusarubin), only two isolates from this collection (designated FS VG and FS We) corresponded with the characteristics of f.sp. *pisi*. Suitability and effectiveness of two methods of inoculation based on soaking of seed in the inoculum were evaluated. The possibility to use mixtures of inocula of different species (*F. solani* f.sp. *pisi* + *F. oxysporum* f.sp. *pisi* races 1 and 2) and of mixtures of two isolates with different virulences was explored. Employing an efficient testing method and the most virulent isolate FS VG, 184 semileafless types of field peas and garden peas were screened to find accessions with a higher level of resistance to *F. solani* f.sp. *pisi.*

Keywords: pea; germplasm, *Fusarium solani*; *Fusarium oxysporum*; resistance; laboratory and glasshouse screening; Czech Republic

The soil fungus *Fusarium solani* (Mart.) Sacc. (teleomorph: *Nectria haematococca* Berk. et Br.) is one of the most frequent causal agents of root rot on many host plants. It is a very variable species having both saprophytic and parasitic forms (Brayford 1993).

Nowadays, *F. solani* is defined as a complex of taxons having a wide morphological and biologically active spectrum, containing the different genetical mating groups I to VII (Brayford 1993). Snyder and Hansen (1941) had included 20 taxons, which were formerly separated, into one widely accepted species of *F. solani*; they had also determined, by host plant specificity, five parasitically specialised forms. Currently there are already 20 determined specialized forms (Brayford 1993). On pea (*Pisum sativum* L.) the specialised form *Fusarium solani* f.sp. *pisi* (Jones) Snyder et Hansen (syn. = *F. martii* app. et Wu var. *pisi* Jones) was determined. Van Etten (1978) has included the f.sp. *pisi* into the genetical and mating group MP VI.

The genetic background of virulence of *F. solani* f.sp. *pisi* is complicated. The following genes are responsible for virulence: CTT 1, CUT 2 and CUT 3. They control cutinase enzymes, while a range of other genes are responsible for CWDE (cell wall degrading enzymes) like cellulase, xylanase, pectinase, protease, PG1 (endopolygalactonase) and others (Köller *et al.* 1982; Roncero *et al.* 2000). The *F. solani* isolates from pea are virulent if they are able to detoxify the phytoalexin pisatin with help of the enzymes pisatin demethylase and

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cytochrome P-450. The pea isolates are divided into three groups based on their demethylation ability. The amount of pisatin-demethylase (PDA) was calculated by genetical analysis to be controlled by six genes. Nevertheless, other research has shown that PDA has only little influence on the level of virulence. Some isolates having no *PDA* (1–6) genes were even more virulent (Morrissey & Osbourn 1999). It is evident that there are also other genes whose functions and effects are not yet known.

Distinct physiological effects of the produced phytotoxins on hydrated seed germs and on the arising plants characterize the highly virulent isolates of *F. solani* f.sp. *pisi.* Kern *et al.* (1972) designated the naphtazarins as being responsible for the virulence of the *F. solani* f.sp. *pisi* isolates. From the produced naphtazarins (marticin, isomarticin and fusarubin), dihydrofusarubin (DHF) is responsible for the destruction of chlorophyll and for chlorosis, for deformities on the epicotyl basis bulge of germinating plants, for the production of multiple sprouts, for the growth inhibition of roots, lateral roots and epicotyls. The effects caused by DHF are comparable to those of the herbicide paraquat (Hollenstein & Défage 1983; Hartman *et al*. 1996a; Albrecht 1998).

The genetic resources of pea with resistance against *F. solani* f.sp. *pisi* are not known.

Thus, the aims of the study were:

(1) to find a suitable, highly virulent isolate of *F. solani* that corresponds to the characteristics of f.sp. *pisi,* and use it for testing the reaction of field and garden pea genotypes to *F. solani*;

(2) to verify the suitability and the effectivity of the method of inoculating seeds by soaking them in the inoculum, and explore the possible use of mixtures of inocula with different species (*F. solani* f.sp. *pisi* + *F. oxysporum* f.sp. *pisi* race 1 and 2) and of mixtures with isolates of different virulence;

(3) to find genotypes of pea which have a higher degree of resistance (or tolerance) to *F. solani* f.sp. *pisi.*

MATERIAL AND METHODS

Plant material

Seed samples of both field (round seeds) and garden (wrinkled seeds) pea were obtained from the gene banks of AGRITEC Šumperk and SEMO Smržice (Table 1). The accessions were used in the various tests on method of inoculation and reaction to isolates of *F. solani* and *F. oxysporum.*

Detection of highly virulent isolates

In 2003-2004, pea plants infected with *Fusarium solani* were collected at different locations in the Czech Republic (Lužany, Chlumec nad Cidlinou, Chrudim, Bernartice, Temenice, Vikýřovice and Smržice). From the collections, 166 pure monospore isolates were established and maintained on the agar substrates Czapek-Dox (CzD) or PDA.

The *F. oxysporum* f.sp. *pisi* isolates were obtained from abroad (race 1 from Cambridge, UK; race 1 and 2 from Wageningen, NL).

Glasshouse and laboratory screening tests

The most effective, original method to test accessions of pea for their reaction to the fungus has been described as follows. Pea seeds are soaked overnight in a conidia suspension adjusted to 1×10^6 ml. Inoculated seeds are then planted in coarse Perlite in plastic flats and incubated in a growth chamber at 24°C with a 16h photoperiod at 500 ft – c (6480 lux). The Perlite is kept moist throughout the 2-week inoculation period by watering with millipore filtered water. The plants are then scored for disease severity on a 0–5 scale (Kraft & Kaiser 1993; Kraft *et al.* 1994):

Degree of damage:
0 – healthy (without any damage)
1 – weak damage (1–10%)
2 – medium damage (10–25%)
3 – medium strong damage (26–50%)
4 – strong damage (51–75%)
5 – very strong damage to total destruction (76 to 100%)

The inoculation method by Kraft and Kaiser (1993) was modified by first soaking the seeds in water for 24 h. The presoaked = primed seed is then soaked for 24 h in the propagule suspension (Agritec), or in a suspension of microconidia (Semo).

Sowing inoculated seeds

(a) To test for phytotoxicity and to detect virulent f.sp. *pisi* isolates: seeds were placed on wet filter

Table 1. Sources of resistant germplasm and commercial cultivars

Name	Accession	Status	Resistance	Origin	Seed surface
LPKE 36		germplasm	–	GER	round
Herold		cultivar	–	CR	round
Kamelot		cultivar	–	CR	round
Gotik		cultivar	*Fw*	CR	round
Mozart		cultivar	*er*-1	CAN	round
Sponzor		cultivar	–	CR	round
Hardy		cultivar	–	FR	round
Zekon		cultivar	–	CR	round
Garde		cultivar	–	Holland	round
Melfort		cultivar	*er*-1	CAN	round
Franklin	PI 628275	cultivar	*Fw, Frr, er-1, En, sbm*-1	US	round
Novella II	PI 600875	cultivar	*Fw, er-1, En*	US	wrinkled
Freezer	PI 596702	cultivar	–	US	wrinkled
Sommerwood		cultivar	–	US	wrinkled
Almoto	W6 175 35	cultivar	–	US	wrinkled
Dakota		cultivar	-	US	wrinkled
Melton		germplasm	*er*-1	CR	wrinkled
B 99/118		germplasm	*Fw, Fnw, er-1, En, sbm*-1	US	wrinkled
Midget	PI 600965	cultivar		US	wrinkled
G 9174	PI 257593	germplasm	*Frr*	Ethiopia	round
Hohenheimer Pink Flowered	PI 180693	germplasm		GER	round
Line 260	PI 600942	germplasm		US	round
Joel	PI 619080	cultivar	*Fw, Frr, er-1, En, sbm*-1	US	round
WR-1167	PI 618635	germplasm	*Fw*	US	round
Roi des Conserves	PI 244222	germplasm		Holland	round
Lincoln	PI 250447	germplasm		CR	wrinkled
WSU 23	CSR 200	germplasm	*Fw, Fnw, Fwf*	US	wrinkled
B 99/103		germplasm	*Fw, Fnw, er-1, En, sbm*-1	US	wrinkled
Gypsy	PI 595575	cultivar	*Fw*	US	wrinkled
Duke	PI 600765	cultivar	*Fw*	US	wrinkled
OSU 667	W6 175 23	germplasm		US	wrinkled

paper in a Petri dish (10 cm diameter, 3 sectors and 30 seeds per dish).

(b) To determine the degree of resistance of an accession: placed seed under the wet Agroperlite surface (app. 3 cm)in plastic boxes, with 40–50 seeds/box (Agritec), or into a multipot Modiform at 5 seeds/cell and 20–50 seeds of each genotype (Semo). After sowing, the boxes were placed in a greenhouse on sill boards under a non-regulated regime; night/day temperature varied in the range from 10°C to 35°C (Agritec). Multipots were placed in a growth room with strip lighting 12 h day/12 h night, at a temperature of 20–25°C (Semo).

Inoculation with *F. solani* isolate FS VG

Variant (1), simple and expeditious (Agritec). The contents of one CzD agar plate with a pure culture of *F. solani* isolate VG, 15-30 days old, were mixed

in a blender with 250 ml water. The obtained suspension of propagules (macroconidia, microconidia and mycelial segments) was diluted 1:1 with water, which usually resulted in a propagule concentration of about 10^6/ml. The suspension was further diluted and adjusted to 10^5 propagule/1 ml. This suspension was poured over primed pea seeds in plastic dishes; the seeds stayed immersed for 24 hours.

Variant (2), more demanding (SEMO). A disc of 8 mm diameter from a pure culture of *F. solani* isolate VG cultures on CzD agar was placed into 50 ml Kerr medium in a 250 ml Erlenmeyer flask. Flasks were put on a rotation shaker (80 cycles/min) and the fungus cultured for 4 days at 28°C and with permanent lighting. The suspension cultures were then strained through sieves (mesh size 3 and 1 mm) and the concentrations of microspores in 1 ml filtrate were determined. The filtrate was diluted with water to a concentration of 10^6 microspores/ml. Primed seeds, in plastic pots, were immersed in this inoculum for 24 hours.

Evaluation

The germination capacity of seeds was determined. The inhibitory effect of phytotoxins was gauged by measuring the length of lateral roots, root sprouts and epicotyl. In the tests done on Agroperlite, the level of field germination and the differences in retardation of field germination were determined (the more resistant or tolerant genotypes emerge sooner). After emergence, the frequency of morphosis (chlorotic deformed plants) was recorded. During growth of the plants their regeneration ability, i.e. to overcome the initial phytotoxic growth depression, was consid-

Table 2. Influence of the length of time of seed soaking in inocula (titer of spores 10^5/ml) with different virulence of *Fusarium solani* f.sp. *pisi* (isolates VG, Te, Be, Ra, 61 and We) and *F. oxysporum* f.sp. *pisi* (races 1 and 2) – laboratory test (*in vitro*)

Isolate	Germination (%)	Sprout length (cm)	Lateral root (pcs)	Lateral root length (mm)	Epicotyl frequency (%)	Epicotyl length (mm)
Soaking seeds in the inoculum for 24 hours						
Control	98.3	11.8	20.7	17.2	100	18
F. solani Te	100	9.8	20.2	17	100	16.5
F. solani Be	100	9.6	19.6	17.5	100	14.4
F. solani Ra	96.6	7.5	19	18	100	14
F. solani 61	91.6	7	15.3	10.3	100	13.6
F. solani We	75	5.1	4.5	6.4	55.4	6.3
F. solani VG	58.3	3.6	0	0	16.6	1.4
F. oxysporum 1	95	11.2	20.6	17.3	100	18
F. oxysporum 2	33.3	5.4	0	0	11.5	4.2
Soaking seeds in the inoculum for 48 hours						
Control	100	11.6	19.5	18.3	100	18.2
F. solani Te	96.6	6.8	10.3	11.4	100	14.4
F. solani Be	95	8	11	13.6	100	13.5
F. solani Ra	88.3	6.4	9.8	11	100	12.6
F. solani 61	75	5.5	7	6.3	100	11.4
F. solani We	60	4	0	0	28.5	3.3
F. solani VG	28.3	2.2	0	0	0	0
F. oxysporum 1	100	9.5	20	14.6	100	15.6
F. oxysporum 2	16.6	3.9	0	0	0	0

ered. The plants were taken out of the Agroperlite (30–40 days after the test was set up) and the degree of damage was determined on a scale of 0–5 (see above). The data were used to calculate the disease index DI (DI = 1 × *a* + 2 × *b* + 3 × *c* + 4 × *d* + 5 × *e*)/*n*.

Statistical evaluation

Greenhouse and laboratory *in vitro* trials were evaluated by analysis of variance and multiple comparisons by ANOVA statistical program UNISTAT 4.53 g (UNISTAT Ltd., London, UK) at the 0.05 significance level.

RESULTS AND DISCUSSION

A large group of 166 *Fusarium solani* isolates, which originated from infected field pea plants, were investigated by Agritec Šumperk and Semo Smržice experts. Of these isolates only two, isolates FS VG and FS We, corresponded with the f.sp. *pisi* characteristics, based on the ability to produce the phytotoxin naphtazarin (DHF – dihydrofusarubin) (Kern *et al*. 1972; Holenstein & Défago 1983; Hartman *et al*. 1996b; Albrecht *et al*. 1998).

In a laboratory test *in vitro* we compared the phytotoxic activity of six selected *F. solani* isolates with that of the *F. oxysporum* f.sp. *pisi* isolates, races 1 and 2 (Table 2). In this context it was also determined whether the length of time (24 or 48 h) the pea seeds were soaked in the inoculum (titer 10^5) had an influence. Only the isolates FS VG, FS We and *F. oxysporum* race 2 showed a high phytotoxic activity. The longer time of soaking (48 h) increased the phytotoxic effect (reduction of germination capacity, growth dynamics, root germ, side roots and epicotyl length). The phyto-

Table 3. Influence of titer concentration (spores per ml) of *Fusarium solani* f.sp. *pisi* isolate VG on the severity of symptoms on two pea accessions in a greenhouse test

Titer	Plant survival (%)		Frequency of morphosis (%)		Length of stem (cm)	
	Gotik	B99/118	Gotik	B99/118	Gotik	B99/118
Control	97.5	100	0	0	23.5	22.4
10^8	2.5	0	62.5	–	7.3	–
10^7	10	0	27.5	–	9.8	–
10^6	22.5	5	7.5	100	12.4	5
10^5	47.5	17.5	2.5	67.5	19.6	106
10^4	87.5	37.5	0	47.5	22.8	17

Table 4. Evaluation of the susceptibility of four pea accessions after greenhouse inoculation to differently virulent isolates of *Fusarium solani* f.sp. *pisi* and *F. oxysporum* f.sp. *pisi*

Isolate	Plant survival (%)				Disease index (0–5)			
	Gotik	Zekon	Almoto	B99/118	Gotik	Zekon	Almoto	B99/118
Control	97.50	93.70	92.50	93.70	1.39	1.40	1.60	1.74
F. solani Te	98.10	87.50	91.10	73.90	1.51	1.58	1.90	1.90
F. solani Be	99.70	75.60	79.50	71.20	1.57	1.80	1.88	1.83
F. solani Ra	98.10	88.70	82.50	66.20	1.66	1.88	2.00	2.20
F. solani 61	95.50	82.20	73.00	60.70	2.00	2.15	2.25	2.77
F. solani We	72.50	69.30	38.00	21.80	2.36	2.50	2.60	3.54
F. solani VG	60.00	50.00	12.70	5.10	2.40	2.75	3.75	4.00
F. oxysporum 1	93.70	88.10	90.60	94.30	1.60	1.85	1.88	1.78
F. oxysporum 2	21.20	20.60	41.80	65.00	4.02	3.75	2.66	2.40

toxic effect from the other isolates FS Te, FS Be, Fs Ra, FS 61 and F. *oxysporum* race 1 was very low even with the 48 h soaking time.

In another trial we tested whether length of seed soaking and propagule concentration influenced the level of phytotoxicity and degree of damage on two pea accessions. The primed seed of cv. Gotik (field peas) and line B 99/118 (garden peas) were soaked in inocula with graded propagule concentrations, at titers 10^8, 10^7, 10^6, 10^5, 10^4 and H_2O as control; they were then sown in Agroperlite in greenhouse conditions (Table 3). Seed soaking in inoculum concentrations 10^8, 10^7 and 10^6 showed a strong phytotoxic effect (the germination capacity was very low, the germinating seeds were susceptible to rot, the surviving plants were chlorotic and deformed). The 10^5 and 10^4 concentrations were less phytotoxic, but allowed better differentiation of the reaction of the two tested accessions.

Next, the field pea cvs Gotik and Zekon, and the garden pea accessions cv. Almoto and line B99/118 were inoculated with a group of selected either avirulent or virulent isolates of *F. solani* f.sp. *pisi* and *F. oxysporum* f.sp. *pisi*. The tests were planted in Agroperlite. Sensitivity to the isolates of F. *solani* and to *F. oxysporum* f.sp. *pisi* race 1 was not much different. Larger differences were found only with the virulent isolates F. *solani* VG and We and the *F. oxysporum* f.sp. *pisi* race 2 (Table 4). The two accessions of garden pea were highly susceptible to *F. solani* isolate VG, while they were relatively more resistant to F. *oxysporum* f.sp. *pisi* race 2. With the two accessions of field pea the reverse was observed, they were more resistant to *F. solani* isolate VG.

The suitability of using isolates of *F. solani* with different virulence in a mixed inoculum, or of a mixture of *F. solani* + *F. oxysporum* f.sp. *pisi* was tested (Table 5). Virulence was decreased if the inoculum was a mixture of a virulent isolate of *F. solani* with an avirulent isolate of that species; a mixture of the races 1 and 2 of *F. oxysporum* f.sp. *pisi* gave a similar effect. However, there was no decrease in virulence if the inoculum was a mixture of the two virulent strains F. *solani* isolate VG + *F. oxysporum* f.sp. *pisi* race 2.

A test in Agroperlite was performed to determine whether virulent *F. solani* isolate VG can be used to distinguish between susceptible and resistant reactions among 19 selected pea accessions (11 field types and 8 garden types). The results clearly demonstrated the differences in reaction of the accessions to that isolate (Table 6). A higher level of resistance (survival rate over 70%,

Table 5. The suitability of using a mixture of virulent isolates of *Fusarium solani* f.sp. *pisi* (VG) and *F. oxysporum* f.sp. *pisi* race 2 with an avirulent isolate of *F. solani* (Te, 61) and *F. oxysporum* race 1 for inoculation tests

Mixture of isolates	Plant survival of accession (%)					Mean
	LPKE 36	Gotik	Zekon	Garde	Sommerwood	
Control	97.5	97.5	93.3	92.5	98.3	95.82
F. oxysporum 1	90.8	92.5	96.6	93.3	90	92.64
F. oxysporum 2	30	25	7.5	6.6	46.6	23.14
F. oxysporum 1 + 2	73.3	74.1	75	67.5	82.5	74.48
F. solani VG	60.8	44.1	12.5	9.1	5.8	26.46
F. solani Te	99.1	98.3	83.3	80	85	89.14
F. solani 61	90	85	67.5	68.3	70.8	76.32
F. solani VG + 61	90.8	91.6	77.5	40	12.5	62.48
F. solani VG + Te	80	72.5	61.6	35.8	9.1	51.8
F. solani VG + FOX 1	83.3	63.3	56.6	67.5	42.5	62.64
F. solani 61 + FOX 1	98.3	96.6	95	61.2	95.3	89.28
F. solani VG + FOX 2	56.6	43.3	10	5.8	4.1	23.96
F. solani 61 + FOX 2	69.1	70	44.1	38.3	75	59.3
Mean	78.4	73.4	60	51.2	55.2	

Table 6. Evaluation of the reaction of pea accessions to *Fusarium solani* f.sp. *pisi* isolate VG. Suspension of propagules, titer 10^5 (greenhouse test, Agritec Šumperk)

Accession	Plant survival (%)	$P > 0.05$	Frequency of morphosis (14 days after germination in %)	$P > 0.05$	Disease index (0–5)
LPKE 36	90.00	A	6.00	A	2.02
Herold	87.60	A	5.40	A	2.11
Kamelot	88.20	A	6.20	A	2.20
Gotik	78.40	AB	3.70	A	2.15
Mozart	68.30	AB	12.60	AB	2.58
Sponzor	63.30	BC	16.70	BC	2.54
Hardy	60.60	C	20.00	BC	2.48
Zekon	56.30	CD	21.50	CD	2.50
Garde	55.00	DE	22.20	CD	2.48
Melfort	50.70	EF	37.20	DE	2.80
Franklin	43.30	EF	42.50	E	3.12
Novella II	38.00	FG	27.60	F	3.00
Freezer	30.20	FG	58.00	FG	3.22
Sommerwood	25.80	FG	60.10	GH	3.40
Almoto	16.60	GH	60.50	H	3.63
Dakota	15.80	HI	63.40	HI	3.46
Melton	12.50	I	72.70	IJ	3.95
B 99/118	11.00	I	75.40	J	3.89
Midget	8.60	I	78.00	J	4.02

*morphosis = destruction of chlorophyll, plant deformities, production of multiple sprouts, growth inhibition of roots and epicotyl basis, bulge of epicotyl

and lower frequency of chlorotic and deformed plants, about < 6%) was shown by the accessions LPKE 36, Herold, Kamelot and Gotik. The garden pea accessions generally appeared to be highly predisposed to the inoculum (survival rate only up to 30%, and high frequency of chlorotic and deformed plants of > 50%).

At Semo Smržice an extensive screening test evaluated the reaction of 184 accessions to the virulent *F. solani* isolate VG (the testing method and evaluation were changed). Five of the accessions had high tolerance, with a survival rate of over 70%; a survival rate ranging from 45% to 60% means that 34 accessions were on the tolerance level of the control cv. Gotik; 73 accessions were in the group with a survival rate of 30–45%; and 72 accessions had low survival (10–29%) and thus low tolerance. The reactions of 19 accessions selected from this collection are shown in Table 7. The results show that *F. solani* isolate VG is suitable to define the level of susceptibility or resistance of individual accessions. The two lines PI 257593 and WR–1167 were included in the test; they are considered to be the most resistant to *F. solani* isolate VG among 24 pea accessions (Kraft 1984, 1986; Kraft *et al.* 1994).

The effectivity and reliability of the inoculation tests with *F. solani* f.sp. *pisi* depend on the selection of suitable virulent isolates. The *F. solani* f.sp. *pisi* isolates are characterized by different virulence. These differences were outlined by Reiking (1950). Based on a comparison of the virulence of pea isolates he has distinguished the two different types:

(1) the strongly virulent type. The infection expands from the seed to the neck, roots and epicotyl.

Table 7. Evaluation of the reaction of pea accessions to *Fusarium solani* f.sp. *pisi* isolate VG. Suspension of microconidia, titer 10^6 (greenhouse test Semo Smržice). Selection from screening tests of 184 accessions

Accession	Plant survival after inoculation with FS VG (%)
G 9174 (PI 257593)	100.00
Hohenheimer Pink Flowered	94.50
260 (PI 600942)	73.70
Joel (PI 619080)	59.10
WR-1167 (PI 618635)	57.40
Roi des Conserves	50.10
Gotik	47.50
Lincoln (PI 250447)	41.80
Franklin (PI 628275)	41.20
WSU 23 (W6 175 19)	37.60
Novella II (PI 600875)	35.50
Dakota	34.20
Zekon	31.80
Lifter (PI 628276)	26.40
Midget (PI 600965)	24.20
B 99/103	23.30
Gypsy	18.60
Duke (PI 600 765)	16.70
OSU 667 (W6 175 23)	14.50

This type inhibits the germinative capacity, growth and formation of secondary roots;

(2) the less virulent type. The infection expands from the soil through the root tips or the injured roots and step by step kills the root system. The formation of side roots is not influenced.

A similar situation is known also from *F. oxysporum* f.sp. *pisi*, where the strongly virulent race 2 proceeds from the seeds to other plant parts, whereas race 1 advances only through the injured roots (Rogers *et al.* 1994; McPhee *et al.* 1999; Kraft 1996, 2000).

The selected highly virulent isolate *F. solani* VG corresponds (in laboratory tests *in vitro;* Table 2) to the f.sp. *pisi* characteristics. Its virulence is based on the production of phytotoxins of the group of naphtazarins, which are the cause of the chlorosis, inhibition of root growth and deformities, i.e. the morphoses of germinated plants (Reiking 1950; Kern *et al.* 1972; Hartman *et al.* 1996a, b; Dorn 1974; Holenstein & Défago 1983; Albrecht *et al.* 1998).

The *F. solani* f.sp. *pisi* isolate VG is suitable for fast routine tests, applying the method of seed soaking in the suspension of conidia or propagules for inoculation (Kraft & Kaiser 1993). The results of our tests do not contrast with the published experiences and results from other authors (King *et al.* 1960; Kraft 1975, 1984, 1986, 1996, 2000; Kraft & Kaiser 1993; Kraft *et al.* 1994).

The different methods of preparing the inoculum (either a propagule suspension obtained by macerating fungal cultures on agar, or a suspension of microconidia obtained by growing the fungus in agitated liquid medium) and different conditions of incubation (non-controlled regime in the greenhouse with a temperature range of 10–35°C, or a controlled regime in a growth room with a temperature range of 20°C to 25°C) did not influence the process of infection and subsequent symptoms of phytotoxicity.

However, it was necessary to modify the recommended method of inoculation (Kraft & Kaiser 1993). The short soaking (overnight) of non-primed pea seed in the inoculum did not result in sufficient infection. Non-primed or partly primed seed did not react to the inoculation. Successful infection was only initiated on fully primed seed (after 24 h soaking in water). Even after these 24 h, with some samples of not only fodder peas but also of field and garden pea, about 10 to 50% of the seeds had swollen only partially or not at all. For tests on the reaction of such accessions it was necessary to select only swollen, i.e. properly primed seeds.

For tests of the reactions of accessions to *F. solani* f.sp. *pisi* isolates which do not produce phytotoxins (type 2, Reiking 1950) it is necessary to choose another method of inoculation (through the injured roots). The soaking of seeds in a propagule suspension of these isolates is quite unsuccessful. Even the combination of such an isolate with the virulent isolate FS VG or *F. oxysporum* f.sp. *pisi* race 1 did not increase the pathogenic symptoms. Rather the reverse happened since these isolates decreased the effectivity of the virulent FS VG isolate. Possible reasons for this could be an antagonistic reaction between the isolates, or dilution or lower production of the phytotoxins in the mixture. No decrease of phytotoxicity and other symptoms of virulence (Table 5) was observed only with the mixed inocu-

lum that combined the two virulent isolates FS VG and *F. oxysporum* f.sp. *pisi* race 2.

The screening tests with virulent isolate FS VG (Tables 6 and 7) have proved differences in susceptibility/resistance amongst the pea accessions. Generally, the field semi-leafless pea types belong to the relatively more resistant strains, in contrast to the garden peas, which are more susceptible. In breeding programmes that emphasize improved resistance to diseases, resistance against *F. solani* f.sp. pisi has high priority. High resistance against the *F. solani* isolate VG was detected in less widely grown cultivars and lines which are similar to fodder peas (G 9174, Hohenheimer Pink Flower, Joel). Unfortunately, these resistant accessions have a wide range of undesirable characteristics that precludes them from being grown commercially for field or garden pea production. They can, however, be used as resistant controls in screening tests and as sources of resistance in hybridisation programmes. The only exception is line 260-PI 600942 (Green Giant Company MN) whose characteristics are suitable for cultivation as a garden pea.

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