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Host Specialization among Vegetative Compatibility Groups of *Verticillium dahliae* in Relation to *Verticillium longisporum*

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With 2 figures

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Abstract

A collection of 24 isolates of *Verticillium dahliae* and 10 isolates of *Verticillium longisporum* originating from nine different host plants and from several geographic regions was tested for host specificity on 11 economically important crops such as potato, tomato, strawberry, linseed, three legumes and four *Brassica* species. In order to reveal host specificity the potential of each isolate to induce disease and affect plant yield was recorded for all isolate–host combinations. The collected data were statistically processed by means of a cluster analysis. As a result, the host range of individual isolates was found to be more dependent on the vegetative compatibility group (VCG) of the isolate than on its original host plant provenance. Twenty-two out of 24 *V. dahliae* isolates belonged to either VCG 2B or 4B. VCG 2B isolates showed specificity for legumes, strawberry, potato and linseed, whereas VCG 4B was specifically virulent on potato, strawberry and linseed. Subgroups within VCG 2B and 4B almost lacking any host preference were designated 2B* and 4B*. Three isolates from VCG 2B*, however, severely attacked tomato which is a host outside the authentic host range of VCG 2B. The pathogenicity of *V. longisporum* isolates was restricted to cruciferous hosts. Conversely, cruciferous plants were not affected by isolates from VCGs 2B and 4B of *V. dahliae*. This lack of cross-infectivity of certain subpopulations of *V. dahliae* and of *V. longisporum* may be useful in the management of this soil-borne wilt disease.

Introduction

Verticillium dahliae Kleb. causes wilt diseases on a wide range of host plants, which, on a large scale may account for severe economic losses. Chemical control of this soil-borne pathogen is not possible. For agronomic reasons it is therefore of significant interest to know whether this pathogen develops host specificity and whether this could be utilized in crop rotation to minimize the accumulation of inoculum in soils.

Populations of *V. dahliae* have been successfully subdivided into vegetative compatibility groups (VCGs) as a method of physiological differentiation (Puhalla, 1979; Joaquim and Rowe, 1990, 1991). Members of individual VCGs form discrete subpopulations which are exposed to selection, mutation, migration, and drift (Leslie, 1993). As VCGs represent genetically divergent subpopulations, they are assumed to also reflect significant differences in physiological and pathogenic traits (Puhalla, 1979). Several attempts to correlate host preferences with the vegetative compatibility of isolates have been successful. However, most of the published studies dealt with only one or two potential host species of *Verticillium* and thus, their conclusions were restricted to single VCG–host plant interactions such as VCG 1 on cotton (Puhalla, 1979; Zhengjun et al., 1998; Korolev et al., 2000a, b) or VCG 4 on potato (Joaquim and Rowe, 1990, 1991; Strausbaugh, 1993). The present study attempted a more comprehensive approach to assign the VCGs previously established in a collection of European isolates of *V. dahliae* (Zeise and Tiedemann, 2001) with host specificity on a broad range of 11 potential host species and to compare their host ranges with those of *Verticillium longisporum* (formerly *V. dahliae* var. *longisporum*), which does not form VCGs. Evidence is presented for an existing host specialization among the VCGs of *V. dahliae* and a significant differentiation between the host range of *V. dahliae* and *V. longisporum*.

Materials and Methods

Fungal inoculum production and inoculation procedures

The 34 fungal isolates used in this study were characterized by their geographic origin, the original host plants, the place where they were isolated and their VCG (Table 1). Isolates were maintained as conidial

Table 1
Assignment to vegetative compatibility groups (VCG), original host provenances and geographic origin of fungal isolates under study

Isolate	VCG[a]	Original host	Geographic origin	Year[b]	Source[c]
2	4B	*Fragaria x ananassa*	Münsterland/Germany	1996	1
3	2B	*Fragaria x ananassa*	Münsterland/Germany	1997	1
4	2B	*Fragaria x ananassa*	Münsterland/Germany	1997	1
5	4B	*Fragaria x ananassa*	Münsterland/Germany	1996	1
8	4B	*Solanum tuberosum*	Münsterland/Germany	1997	1
9	4B	*Solanum tuberosum*	Brandenburg/Germany	1995	1
13	HSI	*Gossypium hirsutum*	Cordoba/Spain	1987	2
15	2B	*Linum usitatissimum*	Mecklenburg/Germany	1988	1
16	2B	*Solanum tuberosum*	Mecklenburg/Germany	1988	1
18	Lsp	*Brassica napus*	Mecklenburg/Germany	1989	1
19	Lsp	*Brassica napus*	Mecklenburg/Germany	1989	1
32	Lsp	*Brassica napus*	Mecklenburg/Germany	1988	1
38	2B	*Helianthus annuus*	Hessen/Germany	1996	3 (22/5)
39	4B	*Helianthus annuus*	Hessen/Germany	1997	1
40	Lsp	*Brassica napus*	Mecklenburg/Germany	1990	1
41	2B	*Brassica rapa*	Mecklenburg/Germany		3 (1/1)
42	2B	*Brassica rapa*	Krasnodar/Russia	1994	4 (K1-4)
43	Lsp	*Brassica napus.*	Mecklenburg/Germany	1990	1
49	2B	*Capsicum annuum*	Burgenland/Austria		5 (MD 1Vd.)
52	2B	*Capsicum annuum*	Burgenland/Austria		5 (MD49V.d)
54	2B	*Capsicum annuum*	Burgenland/Austria		5 (MD 6Vd.)
57	2B	*Fragaria x ananassa*	Mecklenburg/Germany	1995	1
59	Lsp	*B. oleracea* var. *botrytis*	California/USA	1997	6 (90–03)
60	Lsp	*B. oleracea* var. *botrytis*	California/USA	1997	6 (90–10)
73	2B	*Linum usitatissimum*	Mecklenburg/Germany	1993	1
74	4B	*Helianthus annuus*	Mecklenburg/Germany	1994	1
82	Lsp	*Brassica napus*	Mecklenburg/Germany	1997	1
83	Lsp	*Brassica napus*	Mecklenburg/Germany	1997	1
830	4 A	*Solanum tuberosum*	Ohio/USA		7 (83)
84	Lsp	*Brassica napus*	Mecklenburg/Germany	1997	1
85	2B	*Solanum tuberosum*	Mecklenburg/Germany	1997	1
87	2B	*Solanum tuberosum*	Mecklenburg/Germany	1997	1
88	4B	*Solanum tuberosum*	Mecklenburg/Germany	1997	1
89	2B	*Lupinum luteus*	Mecklenburg/Germany	1997	1

[a] VCG, vegetative compatibility group; VCG 2B, 4A and 4B according to Joaquim and Rowe (1990); HSI, heterokaryon self incompatible isolate; lsp, *V. longisporum*;
[b] Missing entry means that information was not available;
[c] Source of the tested isolates: 1, K. Zeise, Germany; 2, R. Jimenez-Diaz, Spain; 3, P. Lüth, Germany; 4, L. Portenko, Russia; 5, H.-J. Prillinger, Austria; 6, S. Koike, California/USA; 7, R. Rowe, Ohio/USA; denomination in brackets refers to the original isolate code.

suspensions (about 10^8 conidia/ml) in Czapek-Dox medium supplemented with 25% glycerol at –84°C. The inoculum was produced from 1 ml conidial suspensions taken from the freezer and added to 50 ml liquid Czapek-Dox medium. After 3 days of incubation at 23°C on a rotary shaker the conidial suspensions were ready for use.

Inoculations were carried out in two different ways. For direct root dip inoculation, the plant roots were submerged in conidial suspensions adjusted to 10^6 conidia/ml. Soil inoculum was prepared in 500 ml tissue culture flasks containing sterilised soil substrate (200 g sand, 18 g cornmeal, 30 ml water) which was inoculated with 5 ml of conidial suspension (10^6 conidia/ml) and incubated for 4 weeks at 23°C in the greenhouse under natural light. The protocols of virulence tests, involving both root dipping and soil inoculation techniques, are described in more detail in Table 2.

Plant material

In total, 11 potential host plant species were tested: oilseed rape (*Brassica napus* L), Pak Choi (*Brassica rapa* L. spp. *chinensis* (L) Hanelt), cauliflower (*Brassica oleracea* L. var. *botrytis* L), broccoli (*Brassica oleracea* var. *italica* Plenck), pea (*Pisum sativum* L), faba bean (*Vicia faba* L), lupin (*Lupinum luteus* L), linseed (*Linum usitatissimum* L), tomato (*Lycopersicon esculentum* L), potato (*Solanum tuberosum* L), and strawberry (*Fragaria* × *ananassa* Duchense).

Assessment of virulence

A detailed description of experimental design is given in Table 2. In the virulence tests with system (a) the first symptoms were visible about 3 weeks after inoculation. At weekly intervals each plant was visually scored for disease symptoms using a 1–9 assessment key (Table 3; see also Zeise, 1992). In general, the tests were stopped after four scores. At this time, about half of the test plants of compatible host–isolate combinations were dead or showed severe wilting. From the disease indices given for each replication the area under the disease progress curve (AUDPC) was calculated. Additionally, the plant fresh weight per replicate was recorded, except for tomato, in which the degree of stunting was assessed.

Table 2
Inoculation systems used for testing the virulence of *V. dahliae* and *V. longisporum* on various host plant species

System	Root dip inoculation (a)	Root dip inoculation (b)	Soil inoculation (c)
Hosts	Oilseed rape, broccoli, cauliflower, Pak Choi, faba bean, lupine, pea, linseed, tomato	Strawberry	Potato
Target plant material	Seedlings at the first true leaf stage	Frigo plants	Tubers
Substrate	Sterilized poor substrate (compost, field soil, peat; 1 : 2 : 1/v, v, v)	Sterilized poor substrate, gravel; 4 : 1/v, v	Sterilized poor substrate, gravel; 4 : 1/v, v
Pot volume	Small pots (80 mm × 80 mm × 70 mm)	5 l Mitscherlich pots	5 l Mitscherlich pots
Incubation	Controlled greenhouse conditions	Outdoor conditions	Greenhouse without climate control

(a) The seedling roots were washed, injured by cutting the tips, and placed in the prepared spore suspensions for 30 min. Control plants remained in sterilized tap water for the same time. During the incubation period at 20 ± 0.5°C with a 12 h photoperiod per day no fertilization was carried out. Each host–isolate combination was represented by three replicates consisting of four plants each (= 12 plants in total per host and isolate).
(b) After the root dip inoculation at the beginning of May the strawberry plants were grown solitary in 5 l Mitscherlich pots. For each pot 100 ml of 0.2% Hakaphos solution (fertilizer with 15% N, 10% P, 15% K, and 2% Mg) were applied 3 and 5 weeks after planting. Ten plants were inoculated with each isolate. Each solitary plant was regarded as one replicate.
(c) For soil inoculation one part of the prepared cornmeal/sand-inoculum was mixed with 30 parts of soil substrate. One potato tuber (less than 30 mm in diameter) was planted per pot. Nutrients were added 3 and 5 weeks after planting with 100 ml of 0.2% Hakaphos solution. *Phytophthora* control started with the onset of flowering and was repeated weekly with alternating applications of Zineb and Acrobat Plus (dimethomorph + mancozeb). Ten plants per isolate were inoculated. Each plant was regarded as one replicate.

Table 3
Assessment key for scoring the disease severity in virulence tests with root dip inoculation of tomato, *Brassica* species, legume species and linseed

Index	Tomato, *Brassica* species	Legumes, Linseed
1	No symptoms	No symptoms
2	Some veins on the oldest leaf become black	Less than 25% of the plant is defoliated
3	Black veins also on the next younger leaves	Less than 33% of the plant is defoliated
4	The oldest leaf with black veins is lost	Less than 50% of the plant is defoliated
5	About 50% of leaves show black veins	Less than 67% of the plant is defoliated
6	Up to 50% of the leaves are lost	Less than 75% of the plant is defoliated
7	More than 50% of the leaves are lost	More than 75% of the plant is defoliated
8	Only the terminal bud is still alive	Only the terminal bud is still alive
9	The plant is dead	The plant is dead

Table 4
Indices for disease severity assessment in virulence tests with strawberry and potato

Index		Strawberry	Potato
1	No symptoms		
2	Weak symptoms	Oldest leaves appear leathery	Oldest leaves with chlorosis and necrosis
3	Marked symptoms	Oldest leaves are dead, stunting of the inflorescence	About 50% of the leaves are dead
4	Severe symptoms	Nearly all leaves with symptoms; stunting of the whole plant	More than 75% of the leaves are dead; stunting may occur
5	Plant is dead		

The first symptoms on strawberry (system b) occurred at the beginning of fruit setting, and on potato (system c) at the beginning of flowering (Table 4). The symptoms were scored visually until harvest at weekly intervals. For each plant the AUDPC and the yield were recorded.

Statistical analysis of the data

An analysis of variance (one-way ANOVA with 95 or 99% least significant difference, LSD) was carried out for each host plant, in order to identify (i) differences between the individual isolates, and (ii) differences between the VCGs of *V. dahliae* and *V. longisporum*.

For cluster analysis, the absolute AUDPC values and yield data were transformed for each host plant–isolate combination into a simple matrix of four virulence groups as follows:
Group 1, non-virulent isolates: disease parameters (AUDPC, plant fresh weight, stunting, yield) were not statistically different from uninoculated control plants;
Group 2, weakly virulent isolates: disease parameters were significantly different from control plants on the one hand and from the reference isolates for high virulence on the respective host plant on the other hand. Reference isolates for high host-specific virulence were isolate 73 (VCG 2B) on linseed, strawberry and the three legumes, isolate 9 (VCG 4B) on potato, isolate 49 (VCG 2B*) on tomato, and isolate 59 (*V. longisporum*; lsp) on *Brassica* spp.;

Group 3, highly virulent isolates: disease parameters were significantly different from control plants but not significantly different from the reference isolate for high virulence;
Group 4, a fourth group included extremely virulent isolates yet significantly (95% LSD) exceeding the virulence level of the reference isolates.

Results

Grouping of isolates according to their virulence

The host-specific virulence of 34 isolates was assessed on 11 host plants based on the disease progress and one yield parameter. The disease and yield data were processed in a cluster analysis in order to group the isolates according to their host specificity traits. The dendrogram deriving from cluster analysis (Fig. 1) showed that the *V. longisporum* isolates were very different from those of *V. dahliae*. The latter were separated into three subclusters that reflect to a large extent the VCG differentiation. One subcluster included nearly all isolates of VCG 4A and 4B, except for isolate 39, which belonged to a second subcluster together with eight isolates of VCG 2B. Seven of them have recently been proved to also differ morphologically and physiologically from the authentic VCG 2B (Zeise and Tiedemann 2001), and therefore, were especially labelled and regarded as a distinct subunit VCG 2B* throughout this study. Most isolates of the authentic VCG 2B and the single heterokaryon self incompatible isolate (HSI) made up the third subcluster.

In a second step the pathotypes revealed were connected by strong virulence characteristics on each tested host plant.

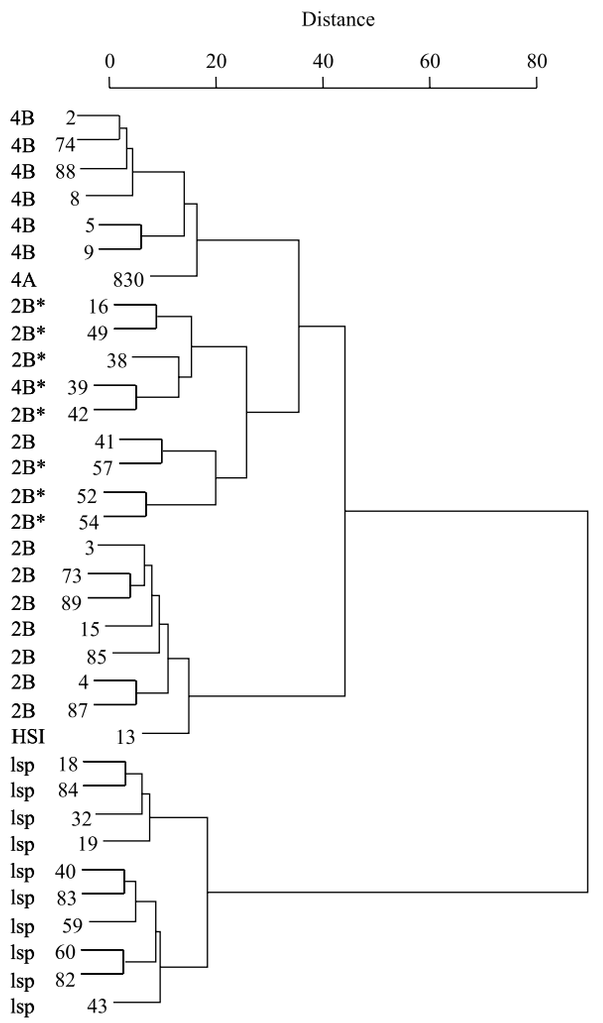

Fig. 1 Tree diagram derived from cluster analysis (Ward minimum variance method) from the rated virulence characteristics showing relationships among the tested isolates. Scale derives from Manhattan distance as distance metric

Virulence on *Brassica* species

Among the four tested *Brassica* species only oilseed rape (cv. Ceres) showed typical wilt symptoms (Table 5). Comparing the VCGs among each other and with the untreated control, the *V. longisporum* isolates were significantly the most virulent, causing losses in plant fresh weight of 49% (Table 6). The AUDPC values of VCG 2B, 2B*, 4A and 4B were not significantly higher than the control; however, VCG 2B* significantly reduced the plant fresh weight by 21%.

In the other *Brassica* species only faint wilt symptoms were visible. Even *V. longisporum* isolates rarely caused black veins or necrosis of the leaf tissue. However, the leaf loss was accelerated in the compatible host–isolate combinations and therefore was used to score the virulence and to discriminate between the VCGs. In this way, *V. longisporum* isolates induced the highest AUDPC levels in the *Brassica* species tested, whereas VCG 4B was weakly virulent. The HSI isolate caused wilt symptoms in broccoli and Pak Choi only, and isolates from all other VCGs of *V. dahliae* were non-virulent. Plant fresh weight was only affected by *V. longisporum* isolates, which induced losses of 38, 22 and 14% in Pak Choi, cauliflower and broccoli, respectively.

Because of the weak symptoms on cauliflower cv. Herbstriese, the virulence testing was repeated on cv. Karfiol Alpha 6. Although the absolute data for AUDPC were higher in the second test, the ranking of the VCGs on cauliflower was the same (data not shown). Table 7 shows the disease indices induced by lsp isolates at 42 days post-inoculation on cauliflower (cv. Karfiol Alpha 6) and oilseed rape. Whereas the highly virulent isolates 40, 43, 59 and 83 killed about half of the oilseed rape plants and isolates 18, 60, 82 and 84 induced significant disease severity, in cauliflower only isolate 40 induced severe symptoms that were significantly different from the untreated control.

Virulence on legumes

The three tested species were shown to be suitable hosts to differentiate between the VCGs and between the two *Verticillium* species. The most severe symptoms were induced by VCG 2B, whereas the subgroup VCG 2B* was less virulent. Losses in plant fresh weight due to VCG 2B were 33% in pea, 53% in lupin and 76% in faba bean. The VCGs 4A and 4B were only nearly as virulent as VCG 2B on lupin, reducing the plant fresh

Table 5
Disease severity induced on various host plants (expressed as mean AUDPC) by *V. dahliae* isolates from different VCGs and their subgroups, and by *V. longisporum* (lsp)

	Control	VCG 2B	VCG 2B*	VCG 4A	VCG 4B	VCG 4B*	lsp	HIS
Oilseed rape	64 ab	68 a	76 ab	78 ab	77 b		114 c	72 ab
Cauliflower	43 ab	41 a	40 a	46 ab	48 b		57 c	36 a
Broccoli	41 a	50 b	49 b	53 bc	56 c		66 d	60 c
Pak Choi	50 a	59 ab	55 a	58 ab	62 b		78 d	76 d
Pea	35 a	86 c	48 b	38 ab	35 a		30 a	84 c
Lupine	38 a	90 d	74 b	60 b	87 cd		38 a	72 bc
Faba bean	33 a	107 d	86 c	46 ab	55 b		42 a	64 b
Linseed	34 a	87 b	50 a	33 a	89 b		40 a	104 b
Tomato	14 a	19 a	36 b	24 ab	20 a		14 a	75 c
Potato	47 a	112 d	92 c	110 d	125 e	74 b	64 b	102 cd
Strawberry I	28 a	93 d	44 b	65 c	62 c		28 a	–
Strawberry II	35 a	104 b	39 a	–	104 b	42 a	36 a	102 b

Mean AUDPC values in each line followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$);
Control, uninoculated plants;
AUDPC values represent the means of all isolates of individual VCGs.

Table 6
Effects of individual VCGs of *V. dahliae* and their subgroups, and *V. longisporum* (lsp) on plant yields [fresh weight (g) of plants, fruits, or tubers] of various potential host plants

	Control	VCG 2B	VCG 2B*	VCG 4A	VCG 4B	VCG 4B*	lsp	HIS
Oilseed rape[a]	29 d	26 cd	23 c	21 bc	26 cd		15 a	28 bcd
Cauliflower[a]	44 b	42 b	41 b	38 ab	43 b		34 a	42 b
Broccoli[a]	25 b	23 b	23 b	24 b	23 b		21 a	22 ab
Pak Choi[a]	33 bc	33 bc	34 c	28 bc	33 bc		21 a	27 ab
Pea[a]	34 ab	26 a	31 ab	35 ab	31 b		31 ab	23 ab
Lupine[a]	32 d	15 a	34 c	18 abc	23 bc		29 d	17 ab
Faba bean[a]	72 c	17 a	36 b	67 c	64 c		69 c	59 c
Linseed[a]	12 cd	9 b	11 c	13 d	7 a		12 d	7 a
Potato[b]	485 d	383 b	436 cd	442 bcd	334 a	411 bcd	469 d	374 abc
Strawberry I[c]	117 d	18 a	74 c	44 b	52 b		100 d	
Strawberry II[c]	120 b	69 a	105 b		43 a	125 b	112 b	46 a

Mean values in each line followed by the same letter are not significantly different according to LSD analysis ($P \leq 0.05$);
Control, uninoculated plants;
Values for plant yields represent the means of all isolates of individual VCGs;
[a] Mean plant fresh weight per replication (g);
[b] Mean tuber yield per plant (g);
[c] Mean fruit yield per plant (g).

Table 7
Disease indices in oilseed rape and cauliflower 42 days after inoculation with 10 isolates of *V. longisporum*

		Isolates of *V. longisporum*									
Host	Control	18	19	32	40	43	59	60	82	83	84
Oilseed rape	4.9	6.8*	5.4	6.1	7.8*	7.4*	7.9*	6.8*	6.9*	8.4*	6.4*
Cauliflower	4.3	4.8	4.6	4.3	5.3*	5.0	5.0	5.0	4.9	5.0	4.8

*Significantly different from untreated control (99% Tukey test);
Control: uninoculated plants; cauliflower cv. was Karfiol Alpha 6.

weight by 43 and 28%, respectively. VCG 4B produced weak symptoms on faba bean, and no disease on pea. The HSI isolate was highly virulent on pea and lupin and weakly virulent on faba bean. The lsp isolates caused no symptoms on legumes. The HSI isolate induced severe symptoms and losses in plant weight on lupin, faba bean and pea.

Virulence on linseed

The symptoms on linseed (cv. True Blue) were most visible between 21 and 28 days post-inoculation. Strong growth starting in the fourth week mitigated further symptom development. Nevertheless, differentiation between the VCGs was possible. The HSI isolate and VCGs 4B and 2B were the most virulent,

causing losses in plant fresh weight of 42, 42 and 25%, respectively. The AUDPC values of VCG 2B*, VCG 4A and the lsp isolates were not significantly different from each other or from the untreated control (Tables 5 and 6).

Virulence on tomato

On tomato (cv. Moneymaker) only the HSI isolate from cotton (13) and three isolates from sweet pepper (49, 52 and 54) from VCG 2B* induced severe wilt symptoms accompanied by stunting. With respect to this pathogenic differentiation within VCG 2B the subgroup 'tomato strains' was established and the AUDPC was calculated separately for both subgroups, in addition to the VCG 2B calculation as a whole (Table 5). The 'tomato strains' accounted for an AUDPC value of 36, whereas the remaining VCG 2B isolates (16, 38, 42 and 57) did not induce any significant disease.

Virulence on strawberry

Due to its high demand for space, the experiment with strawberry (cv. Elsanta) was split up in two separate tests. Each included an untreated control and isolate 73 as reference isolate for high virulence. In both tests, the strawberry plants were inoculated and potted at the beginning of May, scored and harvested in June/July (Tables 5 and 6), then covered with leaves and straw for the winter and harvested again in the subsequent summer (data not shown).

The most severe wilt symptoms and almost complete yield losses were induced by isolates of VCG 2B and the HSI isolate. None of these inoculated plants survived the winter period. Isolates of VCG 4B and VCG 4A also induced disease, but were less virulent than VCG 2B. Nevertheless, most of them also killed nearly all of the inoculated plants during the winter period. Only one VCG 4B isolate (39) did not induce either wilt or yield loss. It also caused no decay of inoculated plants during the winter period. Therefore, isolate 39 was assigned to a separate subgroup VCG 4B*. Most plants that had been inoculated with VCG 2B* showed only slight symptoms and produced yield also in the second year. No disease symptoms or yield reduction were induced by any of the *V. longisporum* isolates.

Virulence on potato

Isolates from VCG 4B were the most virulent on potato (cv. Likaria). Yield losses due to these isolates ranged from 25% (isolate 5) to 42% (isolate 9). Isolates from VCGs 2B and 4A and the HSI isolate were also virulent but to a significantly lesser degree than 4B. Yield losses varied from 16% (isolate 73) to 31% (isolate 15), whereas losses due to isolate 830 (VCG 4A) were only 9%. Similar to strawberry, the isolate 39 (VCG 4B) induced only weak wilt symptoms also on potato and significantly less yield loss, thus supporting its differentiation as 4B*. The *V. longisporum* isolates were weakly virulent, and caused no yield losses at all.

Discussion

Until recently host specificity has been assumed not to exist in *V. dahliae* (Subbarao et al., 1995). Nevertheless, several reports addressing host-specific strains have been published in the past. Races of the pathogen that are specific on peppermint and Brussels sprout have been already mentioned in 1954 and 1957, respectively (Horner, 1954; Isaac, 1957). On tomato, two races are known, which differ in their virulence on cultivars with and without the *Ve* resistance gene (Hall and Kimble, 1972; Gold et al., 1996). On cotton, two pathotypes of *V. dahliae* have been distinguished, one defoliating and the other non-defoliating (Schnathorst and Mathre, 1966). In principle, the term host specificity lacks a clear definition. Within the present study host-specific isolates were considered to be those not simply occurring on a certain host plant but also having the potential to infect and specifically damage it.

Several recent references demonstrate a relationship of host preference with the vegetative compatibility of individual isolates. Among the four main vegetative compatibility groups (VCG 1–4), originally established in order to differentiate the world-wide *V. dahliae* population, VCG 1 was found to consist of cotton-defoliating strains. Likewise, isolates of VCG 1 predominantly occur in cotton-growing areas of the United States, Mexico, Peru, Spain, Middle Asia and China (Puhalla, 1979; Joaquim and Rowe, 1990; Zhengjun et al., 1998; Korolev et al., 2000a, b). Isolates of VCG 4 are known to damage especially potato. According to Rowe et al. (2000) all 270 isolates collected from potato tubers in several regions of the USA and Canada belonged to VCG 4, with subgroup 4A predominating. Isolates from subgroup 4A were collectively more virulent on potato (cvs. Russet Burbank and Superior) than subgroups 4A/B and 4B (Joaquim and Rowe, 1991; Strausbaugh, 1993).

Among the 34 isolates tested in the present study only one belonged to VCG 4A, and this isolate originated from a potato-growing region in Ohio. However, in the present study it caused only slight yield losses on potato (cv. Likaria). Although the seven European isolates of VCG 4B originated from various plant species, all were collected from fields with frequent potato cultivation. This is reflected by their high virulence on potato in the assessments made during this study. None of them caused wilt symptoms on tomato, even though it belongs to the same plant family. The occurrence of VCG 4B on tomato was reported several times, but without showing virulence data from inoculation experiments. Thus these isolates could be attributed to the previous growing of potato in the same field (Daafy et al., 1995; Harrington and Dobinson, 2000; Korolev et al., 2000b). In addition to potato, the host range of VCG 4B also included lupin, linseed and strawberry, but the latter was significantly more severely damaged by isolates from VCG 2B. Although isolate 39 belongs to VCG 4B it showed a completely divergent host

specialization. In a previous study (Zeise and Tiedemann, 2001) this same isolate showed differing morphological and physiological properties. Instead of white to greyish colonies with reduced formation of globular microsclerotia, which is typical for VCG 4B, it produced black colonies with elongated microsclerotia.

The isolates assigned to VCG 2 complemented with the tester strains of the subgroup B only. Regarding their host provenance and geographical origin, this seems to be a very heterogeneous group. VCG 2B is known to occur world-wide on a relatively wide range of hosts (Bhat and Subbarao, 1999; Korolev et al., 2000b). The isolates of VCG 2B in this study had the widest host range. With the exception of the *Brassica* hosts, all other plant species tested were susceptible hosts. However, at least three subgroups within VCG 2B that differed in their host preferences were detectable. One subgroup consisted of isolates of the authentic type of VCG 2B. These isolates were highly virulent on all tested non-cruciferous host plants, except tomato. Secondly, a morphologically distinct group (Zeise and von Tiedemann, 2001), characterized as VCG 2B* within this study, was significantly less virulent on all plant species. With the exception of three isolates, this subgroup did not show any clear preference for any of the tested plants. However, those three isolates from sweet pepper specifically induced severe wilt on tomato.

The isolate from cotton was HSI and therefore could not be assigned to any of the VCGs. It showed the broadest host range of any isolate, still exceeding that of authentic isolates of VCG 2B, with high virulence on broccoli, Pak Choi and tomato.

It was not possible to obtain nitrate non-utilizing mutants from 10 isolates in the authors' collection and so the VCG could not be determined. On the basis of their morphological traits these isolates were assigned to *V. longisporum* (Zeise and Tiedemann, 2001). This morphotype was first defined by Stark (1961) as var. *longisporum* and included isolates from horse radish with extremely long conidia. Jackson and Heale (1985) assigned two Swedish isolates from sugar beet and oilseed rape with long conidia to the same variety, and assumed them to be stable diploid. Isolates of var. *longisporum* exhibited a limited host range and were host-specific on oilseed rape and other cruciferous plants (Horiuchi et al., 1990; Zeise, 1995; Karapapa et al., 1997; Bhat and Subbarao, 1999). Because they were distinguished from *V. dahliae* using molecular markers (Okoli et al., 1994; Subbarao et al., 1995; Koike et al., 1996; Karapapa et al., 1997), Karapapa et al. (1997) considered var. *longisporum* to be a distinct species, *V. longisporum*.

The respective isolates used in this study did not induce wilt symptoms on any of the non-cruciferous host plants. Conversely, all of them were highly virulent on the four *Brassica* hosts tested, although the severity of wilt symptoms varied among the host species. Oilseed rape was the most clearly preferred host plant of *V. longisporum* among the cruciferous hosts. About 50% of the plants were killed by the most virulent isolates within 42 days. Pak Choi, broccoli, and cauliflower only responded with an earlier leaf loss and a reduction in plant fresh weight, similar to results from a previous study (Subbarao et al., 1995). In the present study, cauliflower was not more susceptible than broccoli as reported by Koike et al. (1994), Subbarao et al. (1995), and Shetty et al. (2000). Both host plants, which belong to two different varieties of *B. oleracea,* proved to be comparatively tolerant to *V. longisporum* isolates, regardless of whether the fungal isolates originated from Germany or California (USA). Zeise and Buchmüller (1997) already realized the elevated tolerance of *B. oleracea* to the *V. longisporum* isolate 43, whereas *B. rapa* and *B. napus* were both susceptible. According to the present data, Pak Choi (*B. rapa* spp. *chinensis*) was notably less susceptible than oilseed rape. Despite the great variability of susceptibility among *Brassica* species, they represent a separate group of hosts that were preferred only by *V. longisporum* isolates. Indeed, broccoli and Pak Choi were also weakly affected by VCG 4B, but this may have been due to the extremely conducive, artificial conditions of infection, and would probably not occur under field conditions.

There are several important conclusions to be drawn from these data, with respect to the management of *Verticillium* wilt in fields under specific crop rotations. First, estimating the threat deriving from *Verticillium* soil infestation is not possible on the basis of a quantitative assessment of soil inoculum density alone. As already assumed by Joaquim and Rowe (1991), it is necessary to determine the predominant virulence type, or VCG, which in general is reflecting the cropping history of a certain field. Second, crucifers can be crop-rotated with any non-cruciferous crop plant at low risk, since the isolates from these two groups of hosts are not cross-infective. Third, when VCG 4B predominates in fields with intensive potato production, crops such as

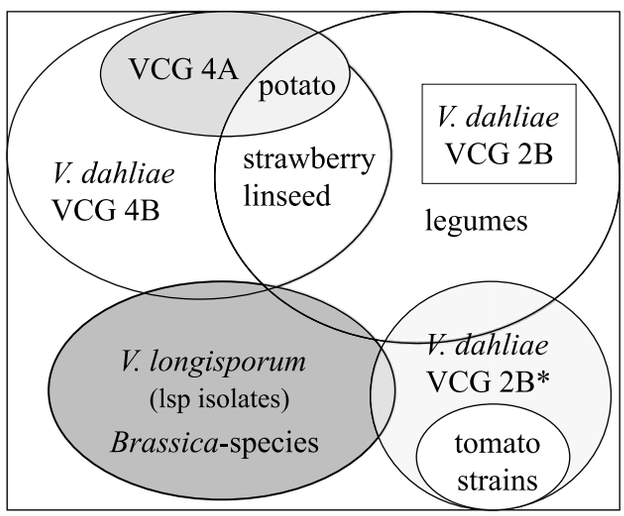

Fig. 2 Host ranges differentiated for VCGs of *V. dahliae* and for *V. longisporum*, based on disease severity (AUDPC) and yield losses induced by representative isolates

peas, crucifers, and tomato could be introduced at low risk, as they are only slightly affected by these isolates. In fields that are mainly infested with the authentic type of VCG 2B only crucifers and probably tomato can be expected to remain unaffected (Fig. 2). Subgroups of VCG 2B, such as VCG 2B*, are expected to rarely predominate in commercial fields due to their restricted host range. The tomato isolates used in the present study, for example, originated from an area in Austria that is known for intense sweet pepper production. The tested tomato isolates were non-virulent or very weakly virulent on *Brassica* species, pea, lupin, and strawberry, whereas they attacked potato, linseed, and faba bean rather more strongly.

The *Verticillium*–host relations investigated in this study may have to be extended on further, as yet unrecognized potential hosts, that are also basically susceptible to *Verticillium* wilt diseases.

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