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Identification of Cauliflower Cultivars that Differ in Susceptibility to *Verticillium longisporum* using Different Inoculation Methods

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Abstract

The response of 13 European cauliflower cultivars to *Verticillium longisporum* was evaluated using two greenhouse tests and one *in vitro* inoculation test. The greenhouse tests involved dipping roots of 3-week-old seedlings in a conidial suspension or inoculating the soil of 3-week-old seedlings with *Verticillium* microsclerotia. The *in vitro* test involved the inoculation of 9-day-old seedlings with *Verticillium* conidia. Useful disease parameters were the area under disease progress curve and plant growth reduction for the greenhouse tests and fresh weight reduction for the *in vitro* test. Significant correlations were found among the three inoculation methods. Irrespective of the inoculation method used, cultivar 'Sernio' was most resistant to *V. longisporum*, while 'Minaret' was the most susceptible cultivar. The pathogen could be re-isolated from the hypocotyls and from the stem of 'Minaret' 4 and 49 days after inoculation respectively, whereas *V. longisporum* could never be re-isolated from 'Sernio'. These results suggest that the more resistant cauliflower cultivar 'Sernio' can suppress the ascent and the proliferation of *V. longisporum* into the plant.

Introduction

Cauliflower (*Brassica oleraceae* var. *botrytis*) is an important vegetable crop commercially grown in Europe. Important cauliflower-producing areas are 'Pfalz' in Germany, 'Bretange' and 'Pas de Calais' in France, 'West-Friesland' in the Netherlands and 'Vlaanderen' in Belgium. Verticillium wilt of cauliflower was first recorded in 1990 in coastal California (USA; Koike et al., 1994), but more recently the problem also emerged in Europe (Debode et al., 2005). Symptoms consist of chlorosis, defoliation, stunting, wilting and vascular discoloration.

Verticillium isolates from crucifers used to be classified as *Verticilllium dahliae* var. *longisporum* (Stark, 1961). On the basis of work by Karapapa et al. (1997), who stated that all crucifer isolates are host-specific and long-spored, a new species, *V. longisporum*, was recognized. However, there is currently controversy regarding the recognition of *V. longisporum* as a separate species (Fahleson et al., 2004) because also short-spored *Verticillium* isolates can infect crucifers (Collins et al., 2003), while *V. longisporum* can also infect plant species outside the Brassicaceae family (Fahleson et al., 2004). Collins et al. (2003) divided *Verticillium* isolates that infect crucifers in three distinct molecular groups by amplified fragment length polymorphism (AFLP); a short-spored group and two groups with long-spored isolates (AFLP-α and AFLP-β). Debode et al. (2005) isolated and characterized seven European *Verticillium* isolates from cauliflower. All isolates belonged to AFLP group-α (Debode et al., 2005), which also comprises the Californian cauliflower isolates, and the majority of European oilseed rape isolates (Collins et al., 2003). The Californian cauliflower isolates were also included in the AFLP study conducted by Fahleson et al. (2003), where the Californian isolates clustered together with European oilseed rape isolates in AFLP group A. Fahleson et al. (2003) stated that isolates belonging to this group should be regarded as members of *V. longisporum*.

All crucifer isolates of *Verticillium* produce microsclerotia (Collins et al., 2003) and the control of microsclerotia is especially difficult because they are thick wall melanized aggregates, which can survive for more than a decade in soil (Schnathorst, 1981). Chemical fumigants (e.g. methyl bromide) usually have been used to reduce the amount of microsclerotia in soil. Because of the environmental impact and the non-selective mode of action, such treatments are now restricted by many national governments. Therefore, research on environmental friendly alternatives is needed. Various forms of organic amendments have shown to contribute to the suppression of the viability of *Verticillium* microsclerotia in soil (reviewed by Bailey and Lazarovits, 2003). Incorporation of broccoli residue in soil can reduce the number of *Verticillium* microsclerotia from

cauliflower (Subbarao and Hubbard, 1996; Subbarao et al., 1999). Recently, we have shown that lignin is involved in crop residue-mediated reduction of *Verticillium* inoculum in soil (Debode et al., 2005).

No cauliflower cultivars with complete resistance to *Verticillium* have been reported (Koike et al., 1994). However, also cauliflower cultivars with partial resistance to *Verticillium* have the potential to reduce crop losses in an environmental safe, cost-effective manner. Moreover, cultivars with partial resistance can be combined with other control measurements (such as the incorporation of crop residues) to achieve optimal levels of disease management.

The first objective of this work was to screen cauliflower cultivars, frequently grown in Europe, for *V. longisporum* susceptibility, so that cauliflower cultivars with partial resistance can be identified and used in future breeding programmes. The second objective was to evaluate and compare three different inoculation methods for screening the effect of *Verticillium* on cauliflower cultivars: root dip inoculation, the most common method to inoculate plants with *Verticillium*; microsclerotia inoculation, a method that is less aggressive; and a more rapid *in vitro* inoculation method.

Materials and Methods

Cauliflower cultivars and *Verticillium* isolate

The 13 European cauliflower cultivars used in this study are listed in Table 1. The cauliflower isolate K1 of *V. longisporum* was used throughout this study. This isolate was chosen after a preliminary virulence evaluation of seven European *Verticillium* strains from cauliflower previously described (Debode et al., 2005). The ITS sequences of the isolate K1 has been deposited in GenBank (*Verticillium* isolate K1 from cauliflower: ITS1, 5.8S ribosomal RNA gene, and ITS2; complete sequence: AY566600; functional rRNA intergenic region V-region: AY566594). The isolate was routinely grown on potato dextrose agar (PDA) plates at 24°C and conserved in a Microbank TM (Pro-lab Diagnostics, Toronto, Canada) at −80°C.

Greenhouse conidia root dip method

Conidial suspensions were prepared by adding 10 ml of sterile distilled water to a 4-week-old PDA culture of *V. longisporum*. The culture was scraped with a rubber spatula and the inoculum concentration was adjusted to 10^7 conidia/ml. Cauliflower seeds of the 13 cultivars were planted in small pots containing 1 cm of non-sterile potting soil [Substrate 4, Klasmann Benelux, B. V. EC = 360 μS/cm, pH = 5.5–6.5, NPK fertilizer = 1.5 kg/m^3, dry matter (DM) = 25%, organic matter (OM) = 20%]. After 2 days, the germinating seeds were transferred to trays (22 × 15 × 6 cm) filled with 4 cm potting soil. About 1 cm non-sterile potting soil was put on top of the seeds. The trays were placed in a greenhouse with a 16 h photoperiod at 20 ± 2°C. After the development of the first three true leaves (approximately 21 days after sowing), 12 seedlings of each cultivar were inoculated according to the root dip procedure of Koike et al. (1994). Ten plants of each cultivar were treated with sterile water and served as control treatments. All plants were planted individually in 12 cm^2 pots filled with non-sterile potting soil and placed randomly in a greenhouse with a 16 h photoperiod at 20 ± 2°C. Starting 21 days after inoculation (DAI), following disease parameters were assessed weekly for 49 days: plant height (above the soil line), number of fully expanded leaves and yellowing of the leaves. The yellowing of the leaves was recorded using a 0–5 disease scale with 0 = no symptoms; 1 = ≤25% yellowing of the leaf; 2 = 26–50% yellowing of the leaf; 3 = 51–75% yellowing of the leaf; 4 = 76–100% yellowing of the leaf and 5 = dead leaf. A disease index (DI) per plant was calculated using following formula: $DI = [\sum(i \times x_i)]/(5 \times \text{total amount of leaves})$ with $i = 0 - 5$ (and x_i is the number of leaves with rating i). Using the DI, the area under the disease progress curve (AUDPC) was calculated (Campbell and Madden, 1990; López-Escudero et al., 2004). Plant height, plant growth reduction (PGR), number of leaves and leaf formation reduction (LFR) were analysed with ANOVA univariate repeated measures analysis.

Greenhouse microsclerotia method

An experiment identical to the above described was set up, only the inoculum preparation and inoculation procedure were different. For the inoculum preparation, suspensions of microsclerotia were prepared according to the procedure of Hawke and Lazarovits

Table 1
Description of the European cauliflower cultivars used in this study

Cultivar	Harvest season	Variety	Seed company
Alverda	Autumn	Green	Rijk Zwaan Breeding
Brabant	Summer–autumn	White	Rijk Zwaan Breeding
Casper	Summer	White	Rijk Zwaan Breeding
Céveline	Autumn	White	Rijk Zwaan Breeding
Cornell	Summer	White	Royal Sluis-Seminis
Fremont	Summer–autumn	White	Royal Sluis-Seminis
Gregor	Autumn	White	Rijk Zwaan Breeding
Hermon	Summer	White	Royal Sluis-Seminis
Limburg	Summer–autumn	White	Rijk Zwaan Breeding
Marine	Summer	White	Novartis-Syngenta Seeds
Minaret	Autumn–winter	Green (romanesco)	Rijk Zwaan Breeding
Sernio	Summer–autumn	White	Royal Sluis-Seminis
Tomba	Summer–autumn	White	Rijk Zwaan Breeding

(1994). Two plates of 4-week-old semisolid Modified Czapek-Dox (MCDX) cultures of *V. longisporum* were blended with 1 l distilled water. The resulting suspension was passed through a 75 μm sieve, collected, rinsed with running tap water and resuspended in 1 l of distilled water. The concentration of the suspension was estimated by counting the number of microsclerotia in subsamples under the microscope. Inoculations were made on 21-days-old seedlings in 12 cm^2 pots containing non-sterile potting soil. About 10 ml of the microsclerotia suspension (1500 microsclerotia/ml) was injected with a pipette into the soil next to the roots.

In vitro inoculation method

Six cauliflower cultivars with high, moderate and low susceptibility to *V. longisporum* were selected of the 13 cauliflower cultivars tested in the greenhouse tests mentioned above. About 10 ml of Murashige and Skoog (MS) medium (Murashige and Skoog, 1962; Duchefa, Haarlem, the Netherlands) augmented with 1% sucrose (MS-1) and solidified with 10 g of plant agar (Duchefa) per litre, were added to glass tubes ($\varnothing = 2.5$ cm, $L = 15$ cm). Cauliflower seeds were surface-sterilized with 0.5% NaOCl for 1 h and 70% ethanol for 1 min, followed by three washes in sterile distilled water. Per glass tube, one seed was placed on the sterile MS-1 medium. The glass tubes were placed in a growth chamber with a 16 h photoperiod at 18 (dark) to 22°C (light). Conidial suspensions were prepared as mentioned above. After 9 days, 150 μl of the conidial suspension was added to the roots of 12 plants per cultivar. Ten control treatments per cultivar were prepared using sterile water. After inoculation, the glass tubes were placed in a growth chamber with a 16 h photoperiod at 18 (dark) to 22°C (light). Disease progression was assessed by recording visual symptoms such as anthocyanin production (Steventon et al., 2001) and yellowing of the leaves and by measuring the total fresh weight (roots included) of each plant 14 DAI.

Presence of the pathogen in inoculated plants

Attempts were made to re-isolate the pathogen from the hypocotyl and from the stem (at 0, 5 and 10 cm height) of inoculated plants, 3–8 and 49 DAI, respectively. Plant material of four root dip inoculated plants per cultivar was soaked for 2 min in 0.5% NaOCl, rinsed in two washes of sterile distilled water, blotted dry and aseptically pressed on PDA amended with 100 μg/ml streptomycin (Melouk, 1992). The plates were incubated for 2–3 weeks at 24°C at which time *V. longisporum* could be detected by the presence of microsclerotia.

Statistical analysis

Data was statistically analysed with the software package SPSS 11.0 (SPSS Inc., Chicago, IL, USA). Mean values were compared using an ANOVA Tukey test ($P = 0.05$) and correlations were calculated using Pearson correlation analysis ($P = 0.01$).

To compare the two greenhouse experiments, a $2 \times 13 \times 5$ mixed factorial design was analysed with 'inoculation method' (condia root dip vs. microsclerotia) and 'cultivar' as between-subject factors, 'time' as a within-subject factor and DI as dependent variable.

Results

Greenhouse conidia root dip method

Symptoms observed were typical asymmetric yellowing of the leaves (measured as AUDPC) and stunted growth (measured as PGR). The control plants were without symptoms during the whole experiment. Table 2 shows the AUDPC, the plant height, the PGR, the number of fully expanded leaves and the LFR of 13 cauliflower cultivars after conidia root dip inoculation. 'Minaret' was most susceptible, with an AUDPC at least twice as high compared with the AUDPC of the other 12 cultivars. Within these 12 cultivars, only 'Casper' and 'Marine' were significantly different from the cultivar 'Sernio'. PGR was highest for 'Marine', 'Casper' and 'Minaret'. LFR was highest

Table 2
Area under disease progress curve (AUDPC), plant height, plant growth reduction (PGR), number of leaves and leaf formation reduction (LFR) of 13 cauliflower cultivars after conidia root dip inoculation with *Verticillium longisporum*

		Plant height (cm)			Number of leaves		
Cultivar	AUDPC[a]	Control ± SE	Inoculated ± SE	PGR[a] (%)	Control ± SE	Inoculated ± SE	LFR[a] (%)
Sernio	247.9 a	7.8 ± 0.5	6.5 ± 0.4	16.7 ab	7.5 ± 0.2	7.5 ± 0.2	0.0 ab
Tomba	376.4 ab	9.7 ± 0.5	7.5 ± 0.4	22.7 ab	7.7 ± 0.2	7.3 ± 0.2	5.2 ab
Brabant	379.4 ab	8.4 ± 0.5	7.8 ± 0.5	7.1 a	6.5 ± 0.2	6.9 ± 0.2	−5.8 a
Gregor	492.9 ab	10.1 ± 0.5	7.3 ± 0.5	27.7 ab	8.0 ± 0.2	7.9 ± 0.2	1.3 ab
Fremont	529.8 ab	10.9 ± 0.5	7.7 ± 0.5	29.4 ab	7.4 ± 0.2	7.3 ± 0.2	1.4 ab
Alverda	579.3 ab	13.3 ± 0.5	10.7 ± 0.5	19.5 ab	8.2 ± 0.2	7.3 ± 0.2	11.0 b
Céveline	603.3 ab	7.9 ± 0.5	7.6 ± 0.5	3.8 a	7.3 ± 0.2	7.8 ± 0.2	−6.4 a
Hermon	617.3 ab	9.5 ± 0.5	7.6 ± 0.5	20.0 ab	8.4 ± 0.2	8.4 ± 0.2	0.0 ab
Cornell	635.5 ab	9.4 ± 0.6	7.3 ± 0.5	22.3 ab	8.5 ± 0.3	8.6 ± 0.2	−1.2 ab
Limburg	646.9 ab	7.6 ± 0.5	6.0 ± 0.5	21.1 ab	7.4 ± 0.2	7.7 ± 0.2	−3.9 ab
Marine	869.9 b	11.8 ± 0.5	7.3 ± 0.5	38.1 bc	7.8 ± 0.2	7.1 ± 0.2	9.0 b
Casper	914.3 b	9.0 ± 0.5	5.2 ± 0.5	42.2 c	8.3 ± 0.2	8.1 ± 0.2	2.4 ab
Minaret	1928.9 c	11.0 ± 0.5	4.1 ± 0.5	62.7 c	7.4 ± 0.2	7.3 ± 0.2	1.4 ab

[a]Within columns, values followed by the same letter are not significantly different for $P = 0.05$.

Table 3
Area under disease progress curve (AUDPC), plant height, plant growth reduction (PGR), number of leaves and leaf formation reduction (LFR) of 13 cauliflower cultivars after microsclerotia inoculation with *Verticillium longisporum*

Cultivar	AUDPC[a]	Plant height (cm)		PGR[a] (%)	Number of leaves		LFR[a] (%)
		Control ± SE	Inoculated ± SE		Control ± SE	Inoculated ± SE	
Sernio	193.0 a	7.8 ± 0.5	8.8 ± 0.5	−11.4 b	7.5 ± 0.2	8.0 ± 0.2	−6.3 a
Limburg	307.5 ab	7.6 ± 0.5	8.2 ± 0.5	−7.3 bc	7.4 ± 0.2	7.5 ± 0.2	−1.3 ab
Brabant	320.3 ab	7.7 ± 0.6	8.6 ± 0.5	−10.5 bc	6.3 ± 0.2	6.5 ± 0.2	−3.1 ab
Tomba	369.5 ab	9.7 ± 0.5	12.9 ± 0.8	−24.8 a	7.7 ± 0.2	8.5 ± 0.3	−9.4 a
Casper	398.1 ab	9.0 ± 0.5	8.6 ± 0.5	4.4 bc	8.3 ± 0.2	8.0 ± 0.2	3.6 ab
Cornell	418.7 ab	9.4 ± 0.6	9.9 ± 0.5	−5.1 bc	8.5 ± 0.3	9.4 ± 0.2	−9.6 a
Marine	468.9 ab	11.8 ± 0.5	11.3 ± 0.5	4.2 bc	7.8 ± 0.2	7.9 ± 0.2	−1.3 ab
Hermon	474.4 ab	9.5 ± 0.5	8.8 ± 0.5	7.4 bc	8.4 ± 0.2	8.4 ± 0.2	0.0 ab
Gregor	477.7 ab	10.1 ± 0.6	10.3 ± 0.5	−1.9 bc	8.0 ± 0.2	8.7 ± 0.2	−8.0 a
Alverda	608.0 b	13.3 ± 0.5	12.0 ± 0.5	9.8 bc	8.2 ± 0.2	7.4 ± 0.2	9.8 b
Céveline	645.4 b	7.9 ± 0.5	8.9 ± 0.5	−11.2 bc	7.3 ± 0.2	8.2 ± 0.2	−11.0 a
Fremont	713.8 b	10.9 ± 0.5	10.3 ± 0.5	5.5 bc	7.4 ± 0.2	7.8 ± 0.2	−5.1 ab
Minaret	1420.4 c	11.1 ± 0.5	7.5 ± 0.9	32.4 c	7.5 ± 0.2	6.8 ± 0.4	9.3 b

[a]Within columns, values followed by the same letter are not significantly different for P = 0.05.

for 'Alverda' and 'Marine', whereas 'Brabant' and 'Céveline' showed an increase in number of leaves after inoculation. The disease parameters AUDPC and PGR were highly correlated ($r = 0.86$), whereas AUDPC and LFR were not significantly correlated ($r = 0.13$).

Greenhouse microsclerotia method

Similar symptoms were observed as mentioned in the above described experiment. As shown in Table 3, 'Minaret' was most susceptible, with an AUDPC at least twice as high compared with the AUDPC of the other 12 cultivars. Within these 12 cultivars, only 'Alverda', 'Céveline' and 'Fremont' were significantly different from the cultivar 'Sernio'. PGR after inoculation was highest for 'Minaret', whereas 'Tomba' showed a significant stimulation of plant growth after inoculation. 'Minaret' and 'Alverda' showed the highest LFR, whereas the number of leaves was increased for 'Sernio', 'Tomba', 'Cornell', 'Gregor' and 'Céveline' after inoculation. The disease parameters AUDPC and PGR were significantly correlated ($r = 0.75$), whereas AUDPC and LFR were not significantly correlated ($r = 0.49$).

***In vitro* inoculation method**

Symptoms included stunted growth, measured as fresh weight reduction (FWR), and anthocyanin accumulation. However, anthocyanin accumulation was also observed in some non-inoculated cauliflower cultivars. Therefore, anthocyanin accumulation could not be used as disease parameter. FWR was significant higher for 'Minaret' compared with 'Sernio' (Table 4). No asymmetric yellowing of the leaves could be observed.

Comparisons between inoculation methods

Analysis of variance showed that there was no 'inoculation method' × 'cultivar' × 'time' interaction, indicating that, irrespective the greenhouse inoculation method used, a similar AUDPC was measured for all 13 cultivars. However, the conidia inoculation method caused more rapid and more severe symptoms compared with the microsclerotia inoculation (Table 1, Fig. 1). For example, the cultivar 'Minaret', showed a DI of almost 15% 3 weeks after root dip inoculation, while its DI was only 4% 3 weeks after inoculation with the microsclerotia method (Fig. 1). The disease parameters AUDPC and PGR of both greenhouse tests were significantly correlated ($r = 0.85$ for AUDPC and $r = 0.72$ for PGR), whereas the disease parameter LFR of both greenhouse tests was not significantly correlated ($r = 0.33$).

Table 4
Fresh weight and fresh weight reduction (FWR) of six cauliflower cultivars after *in vitro* inoculation with *Verticillium longisporum*

Cultivar	Fresh weight (mg)		FWR[a] (%)
	Control ± SE	Inoculated ± SE	
Sernio	77 ± 0.9	63 ± 0.3	18.8 a
Marine	98 ± 0.9	72 ± 0.6	26.5 ab
Brabant	133 ± 2.2	98 ± 1.1	26.5 ab
Hermon	183 ± 2.2	131 ± 0.8	36.6 ab
Fremont	185 ± 2.1	108 ± 1.1	41.6 ab
Minaret	265 ± 3.2	127 ± 1.1	52.1 b

[a]Values followed by the same letter are not significantly different for P = 0.05.

The correlation coefficients between the *in vitro* and the conidia test ($r = 0.78$ for AUDPC and $r = 0.74$ for PGR), and the *in vitro* and the microsclerotia test ($r = 0.93$ for AUDPC and $r = 0.92$ for PGR) were high, although not significant for the conidia test. Partial resistance could be detected more rapidly with the *in vitro* test compared with the greenhouse tests. The first symptoms could be evaluated at 84 days after sowing for the greenhouse tests and at 23 days after sowing for the *in vitro* test.

Presence of the pathogen in inoculated plants

Verticillium longisporum could already be re-isolated from the hypocotyls of 'Minaret' and 'Fremont' 4 and 5 DAI respectively, whereas the pathogen could not be

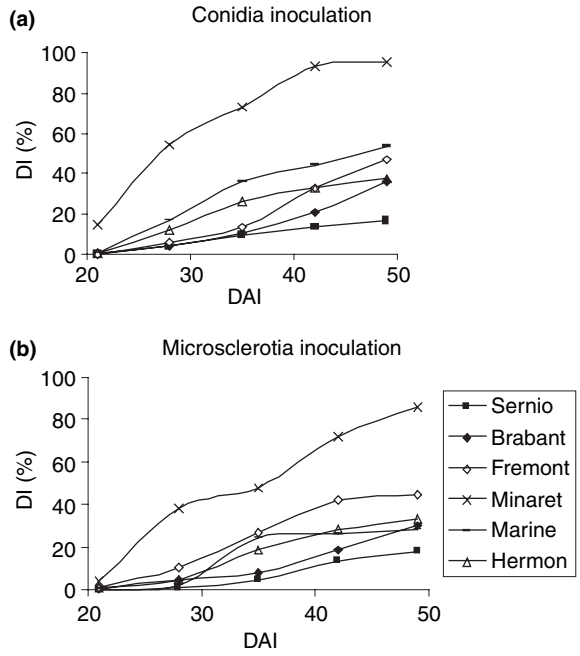

Fig. 1 Progress of the disease index (DI) of selected cauliflower cultivars inoculated with conidia (a) and microsclerotia (b) of *Verticillium longisporum*. The DI was assessed weekly for 49 days, starting 21 days after inoculation (DAI)

re-isolated from the hypocotyls of 'Brabant' and 'Sernio' 3–8 DAI (Table 5).

At 49 DAI, *V. longisporum* could not be re-isolated from any cultivar at 0 cm plant height, but could be re-isolated at 5 and 10 cm plant height from 'Minaret' and at 10 cm plant height from 'Fremont' and 'Brabant'. The pathogen could not be re-isolated from the cultivar 'Sernio' (Table 6).

Table 5
Number of hypocotyls (of four) from which *Verticillium longisporum* could be re-isolated 3–8 days after inoculation (DAI)

	Cultivar			
DAI	Minaret	Fremont	Brabant	Sernio
3	0	0	0	0
4	2	0	0	0
5	2	2	0	0
6	4	2	0	0
7	3	3	0	0
8	4	4	0	0

Table 6
Number of stems (of four) from which *Verticillium longisporum* could be re-isolated at different heights 49 days after inoculation

	Cultivar			
Plant height (cm)	Minaret	Fremont	Brabant	Sernio
0	0	0	0	0
5	2	0	0	0
10	4	2	1	0

Discussion

In this study, clear differences in susceptibility to *V. longisporum* could be detected among European cauliflower cultivars. Irrespective of the inoculation method used, 'Sernio' was most resistant to *V. longisporum*, while 'Minaret' was the most susceptible cultivar. We decided to use various methods to infect the cauliflowers plants, because the root dip inoculation method, which is most frequently used, relies upon severe root damage and high spore inoculum (Heale, 2000). This method may not be suitable to study resistance mechanisms at the level of penetration, because the pathogen is brought in direct contact with the xylem. The microsclerotia method is less aggressive and roots are not damaged. Because both greenhouse inoculation methods take a long time (rating of disease symptoms can only be started 6–7 weeks after initiation of the experiment), we decided to include a more rapid *in vitro* method in our trials; an adaptation of the *in vitro* technique developed by Steventon et al. (2002) for the evaluation of the response of *B. napus* to Verticillium wilt.

The three inoculation methods correlated well with each other and all were effective for testing the response of cauliflower cultivars to *V. longisporum*. In all cultivars, however, conidia root dip inoculation led to PGR, while microsclerotia inoculation often resulted in plant growth stimulation, especially in the more resistant cultivars. Koike et al. (1994) observed that the number of leaves significantly increased after *Verticillium* root dip inoculation of cauliflower. We observed however, that the effect of *Verticillium* inoculation on leaf number was not consistent; in some cultivars leaf numbers increased, while other cultivars even had fewer leaves after *Verticillium* inoculation. It should be noted that Koike et al. (1994) tested only one cultivar (White Rock). Differences in susceptibility could be demonstrated at least 2.5 times more rapid with the *in vitro* test compared with the greenhouse tests.

Control plants of the more resistant cultivar 'Sernio' had a lower plant height and a lower fresh weight compared with control plants of 'Minaret' (Tables 2–4). Given this observation, one could assume that there is a positive correlation between plant growth and susceptibility to *V. longisporum*. However, for the greenhouse tests, the correlation between plant height of control plants and AUDPC was low and not significant ($r = 0.26$ for the conidia test and $r = 0.48$ for the microsclerotia test), indicating that this hypothesis was not supported. But for the *in vitro* inoculation method, the correlation between fresh weight of control plants and FWR was high and significant ($r = 0.98$), meaning that plants with a high fresh weight were more susceptible to *V. longisporum*. The extremely susceptible cultivar 'Minaret' is a green cauliflower variety of the romanesco type. Romanesco is a generic term for chartreuse-coloured varieties notable for their minaret- or turret-like structure. More romanesco type cauliflowers need to be tested to determine if there is a relation

between the romanesco type and high sensitivity to *V. longisporum*.

Initiating of flowering is a very critical period for disease progression in the *Verticillium* pathosystem (Steventon et al., 2001; Veronese et al., 2003). In the field, Verticillium wilt symptoms on cauliflower are never observed until cauliflower plants develop curds. In a field study conducted by Rijk Zwaan in the Netherlands with 10 cauliflower cultivars, 'Minaret' appeared to be the most susceptible cultivar to *Verticillium* (Van den Berg, 2002; Declercq, 2004). 'Sernio' was not included in this trial. 'Sernio' has a poor performance in the field and is therefore not preferred by growers (Royal Sluis-Seminis, personel communication). Field studies in Belgium in 1999 with 20 cauliflower cultivars showed that cultivars with a superior field performance (in terms of temperature sensitivity, uniformity, leaf mass, colour, etc.) were most susceptible to *V. longisporum* (Callens et al., 2000). It is a challenge for breeders to obtain cauliflower cultivars with (partial) resistance to *V. longisporum* that also have good growth characteristics. 'Sernio' could be a good tool for breeders to include in breeding programmes.

Koike and Subbarao (1994) used field trials to evaluate the response of cauliflower cultivars frequently grown in the USA, to *Verticillium*. Similar to our research, significant differences in disease severity among cultivars were observed. 'White Rock' and 'Broccoflower' were most diseased, while 'Floriade' showed significantly less disease. Happstadius et al. (2003) screened 53 accessions of the *B. oleraceae* var. *botrytis* group and found variation in susceptibility to *V. longisporum*. *Brassica oleraceae* var. *botryris* accessions with low susceptibility to *Verticilllium* were used in breeding programmes to improve the level of resistance to Verticillium wilt in *B. napus*. It should be noted that in both studies and in our study, the majority of the tested cauliflower cultivars were partially resistant to *V. longisporum*. Resistance of Chinese cabbage (*B. oleraceae* L. var. *capitata*) to *V. longisporum* is controlled by dominant inheritance with multiple genes (Kemmochi et al., 2000). Resistance to *Verticillium* is polygenic in strawberry, potato and alfalfa, while in cotton, sunflower and tomato, resistance linked to a single dominant gene has been described (Lynch et al., 1997). The only characterized resistance gene is the tomato *Ve* gene that has been identified as a cell surface-like receptor (Kawchuk et al., 2001). Resistance genes in *B. oleraceae* var. *botrytis* genotypes to *V. longisporum* have not yet been characterized.

The presence of *V. longisporum* in the cauliflower plant was related to differences in susceptibility. The pathogen could be re-isolated very shortly after inoculation from susceptible cauliflower cultivars such as 'Minaret' and 'Fremont', but not from the more resistant cultivars 'Brabant' and 'Sernio', indicating that in these cauliflower plants the ascent of *V. longisporum* into the plant is limited. In 'Sernio' also the proliferation of *V. longisporum* appears to be limited, because the pathogen could not be re-isolated from inoculated 'Sernio' stems 49 DAI. This is different from the situation in mint, where the proliferation, but not the ascent of *Verticillium* is suppressed in the more resistant species compared with the more susceptible species (Brandt et al., 1984). Garber and Houston (1966) studied the development of *Verticillium* in infested cotton plants. The quantity of vessel elements invaded at primary invasion sites was not related to differences in levels of wilt tolerance between the varieties. However, after the first step of infection, the colonization was more intensive in susceptible than in resistant cultivars.

Our results indicate that 'Sernio' possesses certain resistance mechanisms that prevent the ascent and the proliferation of *V. longisporum* into the plant. Resistance mechanisms against *Verticillium* are assumed to be based on the exclusion or expulsion of the pathogen from the host or its restriction within the vascular system, using physical or chemical barriers (Pegg and Brady, 2002). Until now, no information exists on the possible underlying resistance mechanisms of cauliflower to *V. longisporum*.

Forty-nine days after inoculation, *V. longisporum* was present in the top of the plant (10 cm plant height) of the susceptible cultivars 'Minaret' and 'Fremont', but could not be detected anymore in the hypocotyls (0 cm plant height). This means that *V. longisporum* is transported with the sap stream and moves up with the growth of the plant.

In conclusion, this study has identified 'Sernio' as a cauliflower cultivar with a low susceptibility to *V. longisporum*. Studies are ongoing to determine the underlying resistance mechanisms. In addition, 'Sernio' can be included in breeding programmes for *V. longisporum* resistance.

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