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Cross-pathogenicity of *Verticillium dahliae* between potato and sunflower

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Abstract This study examined cross-pathogenicity of the soilborne pathogen *Verticillium dahliae* between potato and sunflower. Four week-old potato and sunflower seedlings were inoculated with ten isolates from each of the two host species. Potato cultivars (Kennebec, susceptible, and Ranger Russet, moderately resistant) and sunflower hybrids (IS8048, susceptible, and 6946, moderately resistant) were assessed for disease severity and percent infection at 2 weeks, 3 weeks, 4 weeks, 5 weeks, and 6 weeks after inoculation (w.a.i), and for vascular discolouration at 6 w.a.i., using visual scales developed for each host species. The experiments were conducted in 2006 and repeated in 2007. Based on percent infection and disease severity, most *V. dahliae* isolates were highly aggressive on both host species. The tested isolates caused higher disease levels in the susceptible than in the moderately resistant phenotypes. They also caused more vascular discolouration in their original than in the alternative host. However, the isolates originating from sunflower caused less infection and disease severity on both hosts, compared to their potato counterparts. Cluster analysis based on all of the criteria used to assess pathogenicity led to three groups of isolates: (i) most *V. dahliae* potato isolates, which ranged with the highly aggressive control isolates, (ii) one *V. dahliae* sunflower isolate, which showed a similar pathogenicity level to the weakly-aggressive *V. albo-atrum* sub-group II control isolate, with no more symptoms than in the non-inoculated plants, and (iii) most *V. dahliae* sunflower isolates with mildly- to weakly-aggressive levels. Based on these results, *V. dahliae* cross-pathogenicity is very effective between potato and sunflower. Therefore, rotations involving these species should be avoided, especially where sunflower follows potato.

Keywords Alternate host · Cross-pathogenicity · Disease assessment · Host of origin · Potato · Rotation · Sunflower · Verticillium wilt

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Abbreviations

AUDPC	area under the disease progress curve
PDA	potato dextrose agar
PCR	polymerase chain reaction
SDW	sterile distilled water
w.a.i.	weeks after inoculation

Introduction

Potato and sunflower are important crops worldwide. Both are hosts to *Verticillium* spp. (*V. dahliae* and *V. albo-atrum*), which reduce yield and tuber quality in potato (Rowe and Powelson 2002) and both head size and oil content in sunflower (Hoes 1972). These fungal pathogens are soil-borne and cause wilts in several other crops worldwide (Pegg and Brady 2002). Rotation is one of the major components of Verticillium wilt management. However, many field crops and vegetables are susceptible to the pathogens causing this disease. In addition, *V. dahliae's* resting structures (microsclerotia) can survive in the soil for >10 years (Heale and Karapapa 1999). Its disease-cycle starts with the germination of microsclerotia after stimulation by root exudates (Mol 1995). The germinated hyphae penetrate the root tip, colonise the cortex tissues and move upwards through the vascular system to the above-ground part of the plant (Bowers et al. 1996). The ability of microsclerotia to germinate and produce secondary microsclerotia is a significant factor that can maintain a higher inoculum pressure in the soil for several years (Coley-Smith and Cooke 1971).

Although *V. dahliae* has a wide host range, it varies in its level of pathogenicity on different hosts (Bhat and Subbarao 1999). Isolates from one host are able to cause disease on other plant species but symptoms are often more severe on the host of origin. For instance, isolates from cocoa or strawberry were shown to be more aggressive on these host plants than on other tested crops (Resende et al. 1994; Gordon et al. 2006). Cross-pathogenicity was also reported in other pathogens, i.e., *Fusarium oxysporum* f. sp. *cucumerinum* on cucumber and melon (Cafri et al. 2005), *Gaeumannomyces graminis* var. *graminis* on bermudagrass, St. Augustine grass, and rice (Datnoff et al. 1997), and *Fusarium moniliforme* on corn and asparagus (Damicone et al. 1988). *Verticillium dahliae* cross-pathogenicity was also reported on artichoke, bell pepper, broccoli, cabbage, cauliflower, chili pepper, cotton, eggplant, mint, lettuce, potato, strawberry, tomato, and watermelon (Qin et al. 2006). However, it is not known whether sunflower isolates cause disease on potato, to what extent, and *vice-versa*. This information is important in locations where both crops are widely grown and could be considered for rotation such as in Manitoba, Canada.

The objectives of this study were to: (i) isolate *Verticillium* spp. from infected potato and sunflower tissues grown in commercial or experimental fields in Manitoba; (ii) determine the prevalent species of *Verticillium* spp. across the sampled potato and sunflower fields; (iii) develop an accurate rating scale for both potato and sunflower in order to assess both external and internal symptoms; and (iv) evaluate the cross-pathogenicity of *V. dahliae* isolates recovered from potato on sunflower and *vice-versa*.

Materials and methods

Plant material and growth conditions

Two potato cultivars and two sunflower hybrids were selected for this study, based on their level of susceptibility to *V. dahliae*. Cultivar Kennebec and hybrid IS8048 are susceptible while cv. Ranger Russet and hybrid 6946 are moderately resistant. Potato seed pieces and sunflower seeds were sown in 10 cm-diam plastic pots filled with a pasteurised mixture of sand and soil (1:1, v/v) containing NPK fertiliser granules (16:20:16). Pots were incubated for 4 weeks in a growth room set at 20/16±2°C day/night under 16 h-photoperiod with a light intensity of 350 μmol m^{-2} s^{-1}. After inoculation, seedlings were transplanted into 15 cm-diam pots filled with pasteurised soil mixture composed of soil, sand, peat and perlite (4:4:4:1, v/v/v/v) and received NPK treatment (20:20:20). During all experimentation, plants were watered regularly and kept clean from aphids, spider mites and other pests that may interfere with Verticillium wilt assessment.

Verticillium spp. characterisation

All *Verticillium* spp. used in this study were single-spore isolates recovered in 2004, 2005 and 2006, from potato and sunflower tissues collected from commercial and experimental fields across the province of Manitoba, Canada. Altogether, 23 *V. dahliae* isolates were used, 10 from each plant species (potato: 04–07, 04–09, 04–17, 04–21, 04–28, 04–25, 04–35, 04–38, 04–47; sunflower: 06–01, 06–02, 06–03, 06–06, 06–07, 06–09, 06–11, 06–13, 06–14, 06–20), two highly aggressive *V. dahliae* control isolates from potato (Vd1396-9 and Vd1398-21, Uppal et al. 2007), and

one weakly aggressive *V. albo-atrum* sub-group II control isolate from potato (V104b, P.E.I.-Canada).

For morphological identification of the isolates, mycelia originating from three week-old cultures on PDA were examined under the microscope for typical features such as the presence of microsclerotia and shape, and the verticillate branching of phialides on the conidiophores.

Once pure single-spore cultures were obtained, DNA was extracted from each isolate following the protocol described by El Hadrami et al. (2007). Briefly, harvested mycelia (~50–100 mg) placed in a sterile 1.5 ml Eppendorf tube were mixed with 125 µl of 0.5 M NaOH and ground using a plastic micro-pestle. After 5 min of incubation at room temperature, 500 µl of 0.1 M Tris-HCl buffer containing 0.5 M EDTA (pH 8.0) were added to the extracts and the mixture was centrifuged for 6 min at 14000 r.p.m. Three hundred µl of the supernatant, representing the DNA solutions, were transferred to new tubes and stored at −20°C until further analysis by PCR. PCR identification of *Verticillium* spp. consisted of using a universal primer set (UVd-F: 5′-CTCATAACCCTTTGT GAACC-3′: UVd-R: 5′- CCGAGGTCAACCGTTGC CG-3′), *Verticillium* genus-specific that amplifies a 452 bp product, as well as a *V. dahliae*-specific primer set (Vd-F: 5′-CCGGTCCATCAGTCTCTCTG-3′: Vd-R: 5′-ACTCCGCATCAGTCTCTCCTG-3′) that yields a 334 bp (Nazar et al. 1991; Robb et al. 1994). One µl DNA was used as a template in 25 µl PCR reaction mixture containing 1X PCR buffer, 1 mM dNTPs mix, 1 µM of each primer, 5 mM $MgCl_2$, 0.1 U Taq DNA polymerase and H_2O. PCR amplification was carried out in a Flexigene thermocycler (Techne, Princeton, N.J.) with an initial denaturation at 94°C for 5 min followed by 35 reaction cycles consisting of 30 s denaturation at 94°C, 45 s annealing at 60°C and 1 min elongation at 72°C, and a final extension for 10 min at 72°C. PCR products were then analysed by electrophoresis using a 1.5% agarose gel containing 1% ethidium bromide at 105 V for 30 min. The gels were visualised using an AlphaImager HP version 6 (Alpha Ease FC software, Alpha Innotech, San Leandro, U.S.A).

Inoculation of *V. dahliae* isolates and evaluation of their pathogenicity

Verticillium dahliae cultures incubated at 25°C for 3 weeks on PDA were used to produce inoculum. Conidial suspensions were made in SDW and calibrated at 10^6 conidia·ml^{-1}. Four week-old seedlings grown in a pasteurised sandy soil mixture were gently removed and washed under running tap water. Following the rinsing, a few mm of the root tips were trimmed then dipped for approximately 75 s into the inoculum solutions prepared from each tested isolate as described by Daayf et al. (1998).

Development of disease rating scales

In a preliminary study, both sunflower and potato seedlings were inoculated as described above using a reference *V. dahliae* isolate Vd1396-9 in order to accurately develop rating scales for percent infection and disease severity using the image analysis software Assess 2.0 (Lamari 2002). For that purpose, ten symptomatic leaves with various degrees of chlorosis and necrosis were chosen from infected potato and sunflower seedlings at different stages after inoculation. The percentage of infected area (chlorosis and necrosis) was deduced from the ratio of infected area over the total leaf area. Based on these percentages, a 0–5 qualitative visual scale was developed for both potato and sunflower (Fig. 1). In these scales, 0 = no necrosis or chlorosis, 1: visible chlorosis with <1% necrosis, 2: up to 40% chlorosis and 1–20% necrosis, 3: up to 65% chlorosis and 20–35% necrosis, 4: 100% chlorosis and 35–70% necrosis, 5: 100% chlorosis and 70–100% necrosis.

Similarly, a scale was also developed to assess the degree of discolouration of the vascular system. Cross-sections taken from the lower, middle and upper parts of the stems of infected plants were chosen to represent various degrees of vascular discolouration at 6 w.a.i. Using the software Assess 2.0 (Lamari 2002), the vascular discolouration percentages were calculated and a visual scale of 0–5 was developed according to the following: 0 = no vascular discolouration, 1 = trace to <9% of the cross-section showing discolouration, 2= 10–24%, 3= 25–49%, 4= 50–74%, and 5= 75–100% discolouration (Fig. 1). Due to the hollow nature of the upper part of sunflower seedlings, the vascular discolouration was assessed using another scale that was developed for this particular part based on the same principle (Fig. 1).

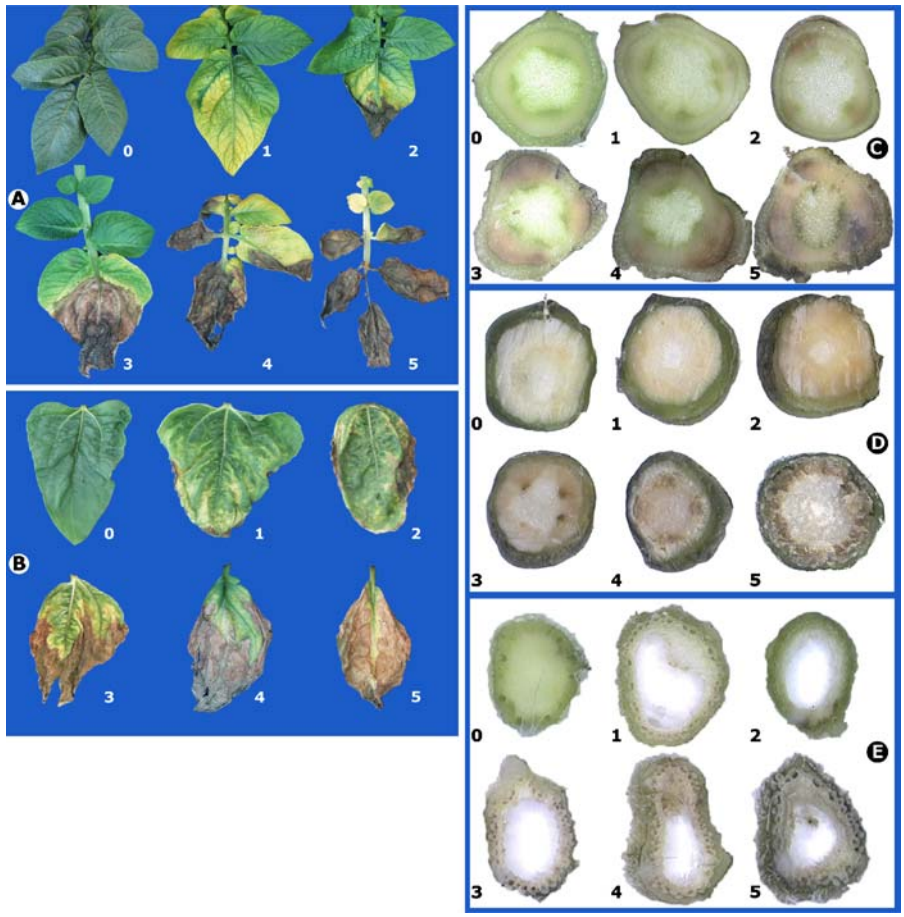

Fig. 1 Visual scales (0–5) used to assess the external (chlorosis and necrosis in **a** and **b**) and internal symptoms (vascular discolouration in **c**, **d** and **e**) of Verticillium wilt in potato (**a**, **c**) and sunflower (**b**, **d**, **e**). **d** and **e** represent the scales used to rate vascular discolouration of sunflower cross-sections from the lower and upper stem parts, respectively. In **a** and **b**, 0 = no necrosis or chlorosis, 1: visible chlorosis with <1% necrosis, 2: up to 40% chlorosis and 1–20% necrosis, 3: up to 65% chlorosis and 20–35% necrosis, 4: 100% chlorosis and 35–70% necrosis, 5: 100% chlorosis and 70–100% necrosis. In **c**, **d** and **e**, 0 = no vascular discolouration (VD); 1: traces to <9% VD; 2: 10–24%; 3: 25–49%; 4: 50–74%; and 5: 75–100%. These percentages were calculated using the image analysis software Assess (Lamari 2002)

Experimental design and data analysis

All trials were conducted following a randomised complete block design. Each experiment consisted of four individual replicates per treatment isolate x cultivar/hybrid and each trial was conducted in 2006 and repeated in 2007. Both non-inoculated control plants and plants inoculated with highly-aggressive *V. dahliae* isolates Vd1396-9 and Vd1398-21 or weakly-aggressive *V. albo-atrum* isolate V104b were included in all trials. In each experiment, plants were evaluated for disease development and progress at 2 w.a.i., 3 w.a.i., 4 w.a.i., 5 w.a.i., and 6 w.a.i. Percent infection and disease severity were calculated as follows: Percent Infection $= \left({}^{IL}/_{TL}\right) \times 100$; where I_L is the number of leaves exhibiting external Verticillium wilt symptoms (i.e. chlorosis, necrosis, wilting), and T_L is the total number of leaves of the rated plant; Disease Severity $= \left[\sum_{i=0}^{n}(n \times b)\right] \times 100/T \times (N-1)$ (Gauhl et al. 1993), where b is the chlorosis/necrosis grade (0–5 referring to the pre-developed scale) and n is the number of leaves with necrosis grade b, N is the total number of chlorosis/necrosis grades used on the scale and T is the total number of leaves. Based on the over-time percent infection and disease severity, the area under the disease progress curve (AUDPC) was calculated using the following formula (Campbell and Madden 1990): $\text{AUDPC} = \sum_{i=1}^{n-1}[((yi+yi+1)/2)(ti+1-ti)]$, where n is the total number of assessments in weeks, y_i is the percent infection or disease severity at the i^{th} assessment week, and the term t_{i+1}-t_i is the time duration between two assessments.

Internal Verticillium wilt symptoms were also determined by assessing the degree of vascular discolouration in the plant stems. Since this is a destructive method, cross-sections from the lower, middle, and upper stem parts were assessed for vascular discolouration only at the end of each experiment (6 w.a.i.).

All collected data was statistically analysed using the General Linear Model (GLM) in the S.A.S. Package (Statistical Analysis Systems Institute Inc., Cary, N.C, and U.S.A.). Differences among *V. dahliae* isolates in terms of total AUDPC for percent infection and disease severity were determined by analysis of variance followed by means comparison using Newman-Keul's test ($P<0.05$). Comparing the data gathered in 2006 and 2007 showed significant differences ($P<0.05$) between the 2 years with higher disease ratings in 2007. Although the overall trend remained similar between the 2 years, data are presented in separate graphics.

Cluster analysis of Statistica v. 8 (statSoft, 1999) was also used to classify the tested isolates and determine similarities among them. In these analyses, all criteria used to assess the disease (percent infection, disease severity and vascular discolouration) in both 2006 and 2007 were used to generate a horizontal hierarchical tree plot representing similarity among the tested isolates. Linkage distances were calculated based on the Euclidean distance ($\text{Distance}(x,y) = \left\{\sum_i (xi + yi)^2\right\}^{1/2}$), which is the geometric distance in the multidimensional space. Isolate clusters were generated using unweighted pair-group averages, in which the distance between two clusters was calculated as the average distance between all pairs of isolates in different clusters. This method was chosen because it performs equally well with elongated 'chain' type clusters such as variation of pathogenicity among isolates.

Results

Morphological and PCR identification of *V. dahliae*

All tested isolates were morphologically identified *in vitro* as *V. dahliae*. Their septated mycelia harboured 3–6 phialides in verticillate arrangement on the conidiophores (Fig. 2a$_1$). Microsclerotia were also observed on all tested cultures as globose to oblongate dark melanised structures throughout the colonies with no presence of dark mycelia (Fig. 2a$_{2,3}$). PCR analysis confirmed all tested isolates belonged to the genus *Verticillium*, since a 452 bp specific fragment was positively amplified from their DNA using *Verticillium* universal primers. When this first PCR product was used as a template for a nested PCR with *V. dahliae*-specific primers, a 334 bp product was amplified, confirming the tested isolates belonged to the *V. dahliae* species (Fig. 2b).

Plant responses to infection with *V. dahliae* isolates

Potato cultivars and sunflower hybrids all exhibited typical Verticillium wilt symptoms in response to inoculation with the tested *V. dahliae* isolates (Fig. 3a, b). The disease was much more pronounced, and progressed faster, on the susceptible cultivars/hybrids than on the moderately resistant ones. In potato, disease symptoms were visible 2 w.a.i. and developed over time from chlorosis to necrosis and wilting. At late stages (≥6 w.a.i.) disease severity was high and stunting was apparent on the rated plants, especially the susceptible cv. Kennebec. When lower, middle and upper stem sections of this cultivar were dissected, they all showed vascular discolouration and most of them had microsclerotia (Fig. 3d). Cultivar Ranger Russet, on the other hand, exhibited chlorosis only on the lower leaves 3 w.a.i. Six w.a.i., plants of this cultivar dropped their mostly affected leaves and produced new branches. At 8 w.a.i., only a few plants displayed vascular discolouration in response to highly aggressive isolates.

Verticillium wilt symptoms observed on sunflower hybrids were different in shape and appearance from those seen on potato cultivars (Fig. 3a), and were generally visible before the second w.a.i. Generally, wilting and stunting accompanied chlorosis and necrosis and all analysed cross-sections displayed vascular discolouration (Fig. 3c). Significant differences ($P<0.05$) were recorded among data sets collected in 2006 and 2007, although a similar trend was observed between the 2 years (Fig. 4).

Pathogenicity of potato isolates on potato cultivars (P/P)

In 2006, no significant differences ($P<0.05$) were observed among the tested potato isolates, based on the percent infection and disease severity they caused on either potato cultivar (Figs. 5 and 6). In 2007, however, significant differences ($P<0.05$) were detected among the same isolates, especially on the susceptible cv. Kennebec (Figs. 5 and 6). With the exception of isolates 04–09, 04–47 and 04–25, all other tested potato isolates displayed high disease levels on cv. Kennebec, similar to the highly-

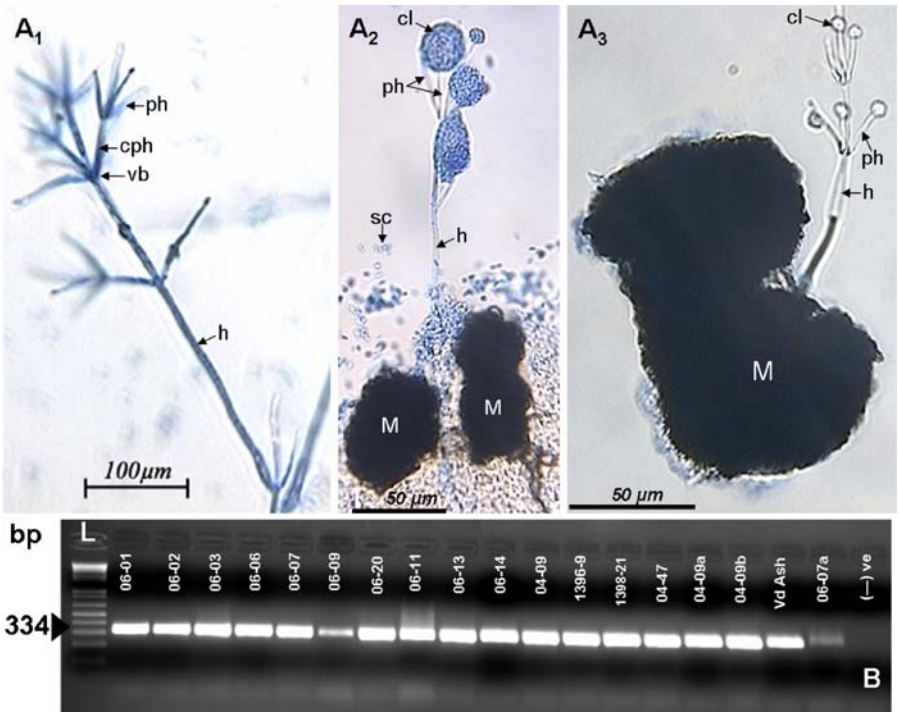

Fig. 2 Morphological characteristics of a potato *V. dahliae* isolate showing the number of phialides (ph) per conidiophore (cph) and their arrangement in verticillate (whorled) branching (vb) on the conidiophores ($\mathbf{a}_1$) (magnification ×125) and the presence of microsclerotia (M) when grown on PDA ($\mathbf{a}_{2,3}$) (magnification ×400). $A_{2,3}$ show also branches of phialides carrying apical clusters of conidia (cl). The size of these phialides depends on the number of carried conidia. Single conidia (sc) are also observed. h: septated hyphae. B represents an agarose gel showing the amplification of a 334 bp fragment using *V. dahliae*-specific primers set Vd-F and Vd-R. 06-0x isolates were recovered from sunflower while 04-0x were recovered from potato. Vd Ash: *V. dahliae* control isolate from an ash tree. L: 1Kb+ ladder (Invitrogen Inc.). (-)ve: negative control

aggressive control isolates Vd1396-9 and Vd1398-21 (Figs. 5 and 6). Based on vascular discolouration ratings, most potato isolates ranked 4.5–5 (55–100% discolouration) and were comparable to the highly-aggressive control isolates Vd1396-9 and Vd1398-21 on cv. Kennebec in both 2006 and 2007 trials (Fig. 7). Isolates Vs04-09, Vs04-25 and Vs04-47, on the other hand, induced ($P<0.05$) significantly lower vascular discolouration ratings (level 2–3: 10–49% discolouration) compared to the remaining isolates in both years. Significant differences were also recorded among potato isolates on cv. Ranger Russet, which displayed lower levels of vascular discolouration in comparison to Kennebec (Fig. 7).

Pathogenicity of potato isolates on sunflower hybrids (P/S)

Overall, potato isolates induced higher percent infection and disease severity on sunflower hybrids than on potato cultivars, especially on the susceptible hybrid IS8048 (Figs. 5 and 6). No significant differences ($P<0.05$) were detectable among isolates for percent infection, disease severity, or vascular discolouration in either sunflower hybrid (Fig. 7).

Pathogenicity of sunflower isolates on sunflower hybrids (S/S)

Significant differences ($P<0.05$) were observed among the tested sunflower isolates depending on the hybrid used for inoculations (Figs. 5 and 6). Lower percent infection and disease severity scores were recorded on the moderately tolerant hybrid 6946, compared to the susceptible IS8048, especially in 2007. Differences among isolates were more prominent in terms of the vascular discolouration levels they caused on the two sunflower hybrids (Fig. 7). The majority of the tested sunflower isolates led to vascular discolouration levels ranging from

Fig. 3 Verticillium wilt symptoms on sunflowers (**a**, **c**) and potatoes (**b**, **d**). **a** and **b** show chlorosis, necrosis, and wilting of the foliage; **c** = vascular discolouration in cross- and longitudinal-sections of sunflower hybrid IS8048 stems; **d** shows the presence of microsclerotia in potato stem sections of cv. Kennebec

40% to 100% in the susceptible hybrid, whereas these levels ranged from traces to 24% discolouration in the moderately resistant hybrid (Fig. 7).

Pathogenicity of sunflower isolates on potato cultivars (S/P)

Percent infection and disease severity scores recorded with sunflower isolates inoculated on either susceptible or moderately resistant potato cultivars were comparable (Figs. 5 and 6) in both 2006 and 2007. Low levels of vascular discolouration were observed in cv. Ranger Russet in response to all tested sunflower isolates in both years. Kennebec's response to the isolates was significantly different between 2006 and 2007 (Fig. 7). However, no significant differences were recorded among isolates in terms of the amount of vascular discolouration they induced.

Relative aggressiveness similarity among *V. dahliae* isolates

Hierarchical analysis was used to compare all of the tested isolates based on the percent infection, disease severity, and vascular discolouration caused in both susceptible and moderately resistant cultivars/hybrids from both species. Using the Euclidean distance analysis, the tested isolates were ranged into three pathogenicity groups (Fig. 8). Group 1 included *V. albo-atrum* V104b, which was used as a weakly-aggressive isolate control on potato, and isolate 06–09, which is a sunflower isolate that induced limited symptoms on both potato and sunflower, similar to non-inoculated wounded and non-wounded controls. Group 2 had most of the tested potato isolates, including the two highly-aggressive control isolates on potato Vd1398-21 and Vd1396-9. Group 3 encompassed all sunflower isolates except the one

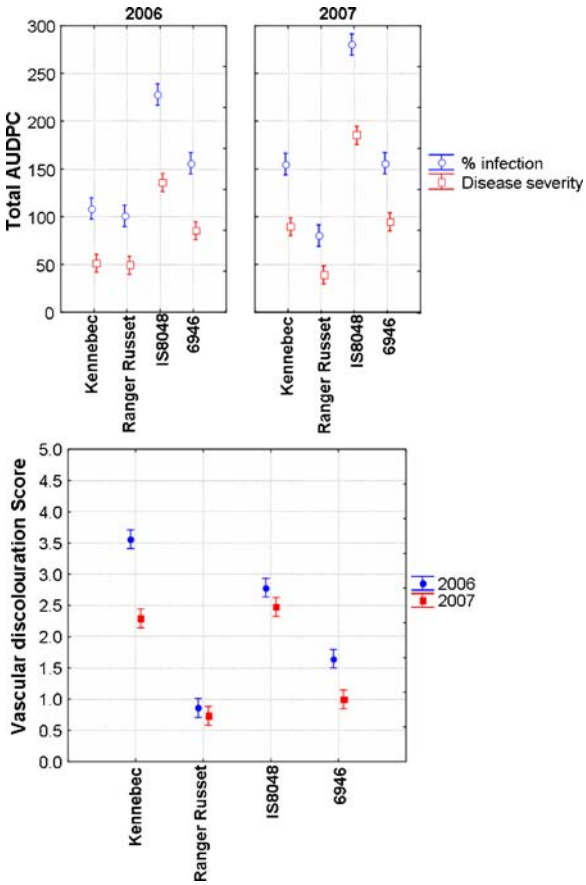

Fig. 4 Percent infection, disease severity, and vascular discolouration recorded in 2006 and 2007 on potato cvs Kennebec (susceptible) and Ranger Russet (moderately resistant), and two sunflower hybrids IS8048 (susceptible) and 6946 (moderately resistant) infected with 22 *V. dahliae* isolates (12 originating from potato and 10 from sunflower). Each data point represents the mean across isolates × replicates. A significant year effect was observed at $P<0.05$ even if the trend remains similar

found with group 1. It also included three potato isolates (Vs04-09, Vs04-25 and Vs04-47) similar to the sunflower isolates in terms of their aggressiveness on both potato and sunflower.

Discussion

In this study, we report on *V. dahliae* cross-pathogenicity between potato and sunflower. Higher disease severity was recorded when the isolates were inoculated on their original host, as opposed to the alternate one, which concurs with previous reports in other host species (Bhat and Subbarao 1999; Resende et al. 1994). Regardless of the isolate host of origin, variability was observed in their pathogenicity on each host plant, as well as between the two hosts, as shown in other pathosystems involving this pathogen (Daayf et al. 1995; Katan 2000; Korolev et al. 2001; Sebastjan et al. 2006). In *V. dahliae*, pathogenic variability is related to several factors, including the ability to produce pathogenicity factors such as toxins, i.e., glycoproteins and protein-lipopolysaccharide complexes, which induce necrosis and wilting in host plants (Mansoori et al. 1995; Meyer et al. 1994; Palmer et al. 2005). Pathogenicity was also reported to be dependent on the host of origin and vegetative compatibility groups (VCGs). For instance, most *V. dahliae* isolates from potato were grouped in VCG_4 (Rowe 1995) while cotton-defoliating strains were ranged into $VCG_{1,}$ and cotton non-defoliating strains in either VCG_2 or VCG_4 (Daayf et al. 1995).

In the present study, trials in both 2006 and 2007 have shown that most *V. dahliae* isolates obtained from potato were highly aggressive on potato cv. Kennebec (susceptible) and less aggressive on Ranger Russet (moderately resistant). These isolates were also very aggressive on sunflower hybrids, particularly on the susceptible phenotype. Verticillium wilt symptoms appeared on sunflower hybrids about 10 days after inoculation, whereas a full 2 w.a.i were necessary to see the earliest symptoms on potato. In some cases, symptoms on the leaves did not reflect the true potential of an isolate to cause disease, because they could be induced by other stress factors that often occur in growth rooms. On the other hand, vascular discolouration in stem cross-sections seemed to be a good criterion to discriminate highly- from weakly-aggressive isolates, probably because vascular discolouration is permanent and can extend beyond infected tissues (Mace 1989). Even when plants recover quickly from the disease, as noticed on cv. Ranger Russet, by forming new vascular tissues, vascular discolouration remains visible in the old xylem tissues. For instance, both sunflower hybrids developed strong vascular discolouration at the upper part of their stems in response to most *V. dahliae* isolates whereas in potato, such a response was apparent only in the susceptible cultivar infected with highly-aggressive potato isolates. This may be partly due to the anatomical differences between the two hosts. Sunflower has broader leaves and larger stems

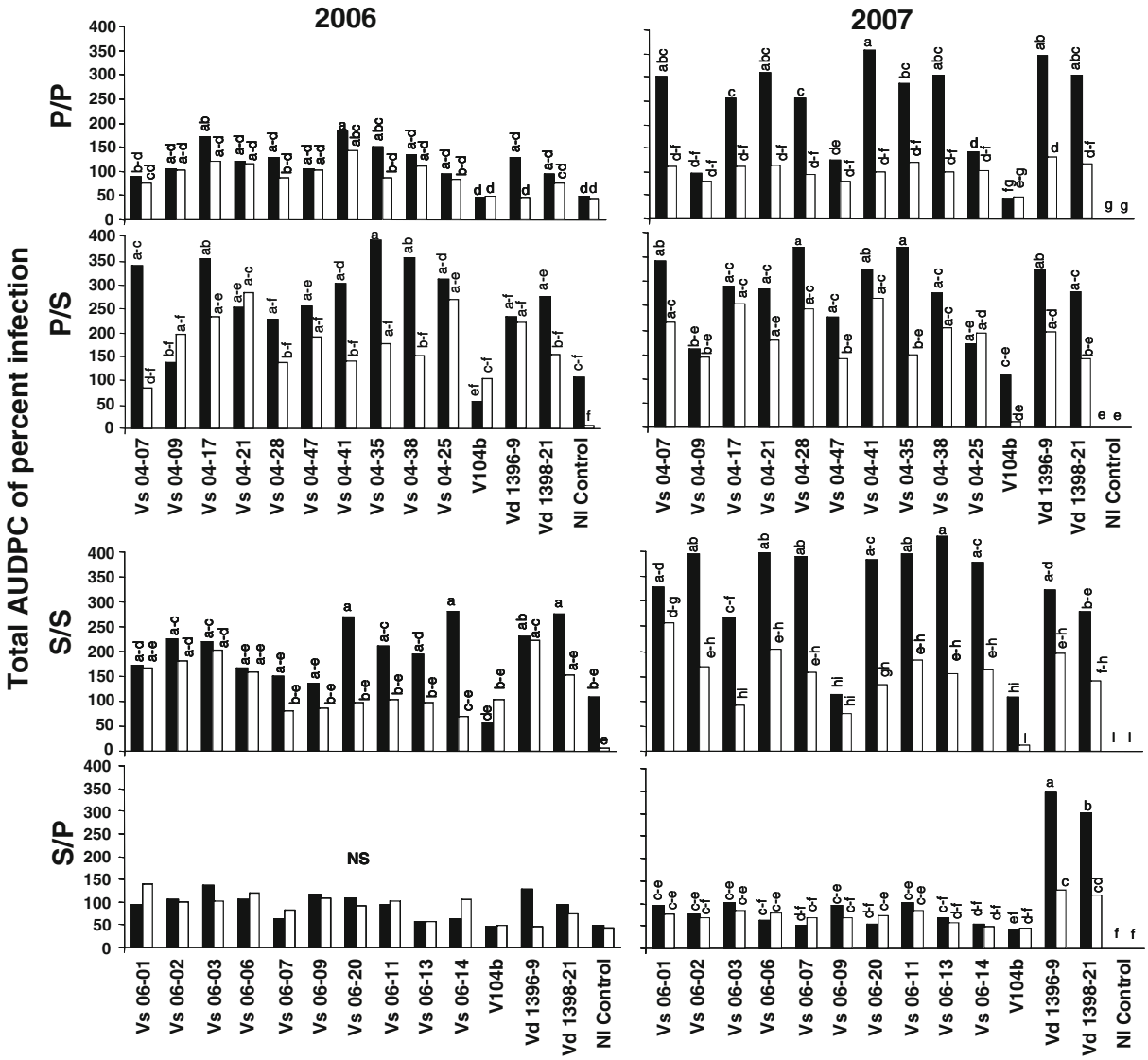

Fig. 5 Total AUDPC based on the percent infection of potato cultivars and sunflower hybrids inoculated with *V. dahliae* isolates recovered from potato (Vs04s) or from sunflower (Vs06s) in 2006 and 2007. NI Control = non-inoculated control. P/P = potato isolates on susceptible Kennebec (■) and moderately resistant Ranger Russet (□) potato cultivars; P/S = potato isolates on susceptible IS8048 (■) and moderately resistant 6946 (□) sunflower hybrids; S/S shows sunflower isolates on susceptible IS8048 (■) and moderately resistant 6946 (□) sunflower hybrids; S/P = sunflower isolates on susceptible Kennebec (■) and moderately resistant Ranger Russet (□) potato cultivars. Columns with the same letter are not significantly different according to Newman Keul's test at $P<0.05$; NS=not significant

with wider vessels, denoting a stronger sap flow and suggesting a greater upward movement of the conidia to the top part of the plant (Beckman et al. 1976). Potato, on the other hand, displays a greater ability for ramification, which can possibly prevent these spores from quickly reaching the top part of the plant.

Based on percent infection and disease severity, the higher level of aggressiveness on potato cultivars of *V. dahliae* isolates originating from potato, compared to those from sunflower, suggests that selection or adaptation of the pathogen to the host may have occurred over time (Okoli et al. 1994; Resende et al. 1994). This seems also to be true for sunflower isolates inoculated onto sunflower. However, cross-infection of sunflower with potato isolates has shown that these maintained a higher level of aggressiveness,

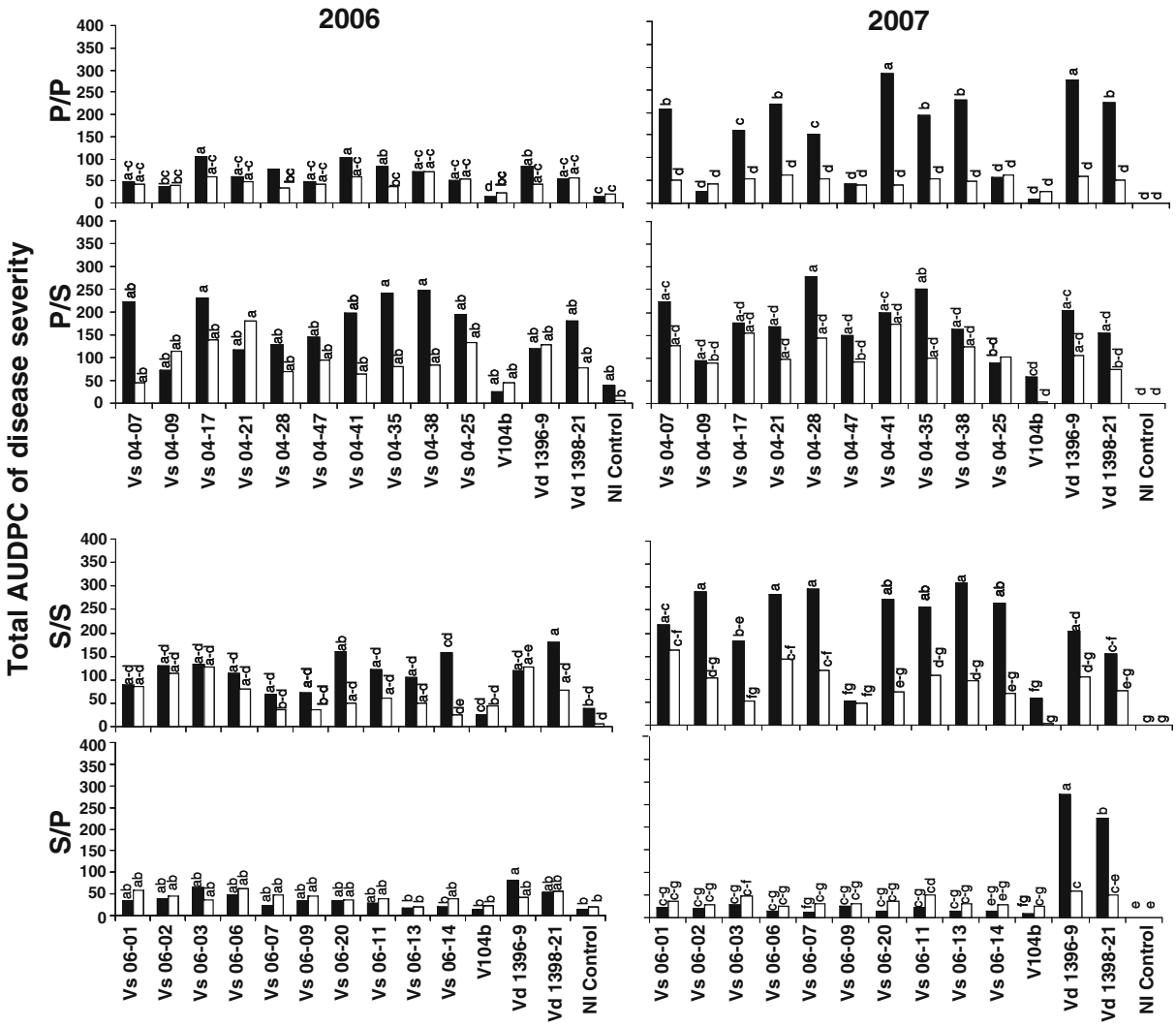

Fig. 6 Total AUDPC based on disease severity of potato cultivars and sunflower hybrids inoculated with *V. dahliae* isolates recovered from potato (Vs04s) or from sunflower (Vs06s) in 2006 and 2007. NI Control = the non-inoculated control. P/P = potato isolates on susceptible Kennebec (■) and moderately resistant Ranger Russet (□) potato cultivars; P/S = potato isolates on susceptible IS8048 (■) and moderately resistant 6946 (□) sunflower hybrids; S/S shows sunflower isolates on susceptible IS8048 (■) and moderately resistant 6946 (□) sunflower hybrids; S/P = sunflower isolates on susceptible Kennebec (■) and moderately resistant Ranger Russet (□) potato cultivars. Columns with the same letter are not significantly different according to Newman Keul's test at $P<0.05$

even on an alternate host, and suggests that their acquired pathogenicity factors are effective at the multi-host level. Conversely, cross-infection of potato cultivars with sunflower isolates has shown that these were weakly-aggressive. This may be due to a loss of certain essential pathogenicity factors by these isolates while adapting to sunflower, or to the initial transmission of these isolates to sunflower from another alternate host, which had attenuated their aggressiveness. Another possible explanation of these findings is that potato defences are much more sophisticated for non-adapted isolates coming from sunflower to potato, while potato isolates probably have already acquired counter-defences against these mechanisms. It is important to keep in mind that if isolates are collected from diseased plants, they are possibly the most adapted among those that initiated the infection.

Verticillium dahliae isolates from sunflower were, in general, mildly- to weakly-aggressive on potato cultivars, where they induced symptoms about

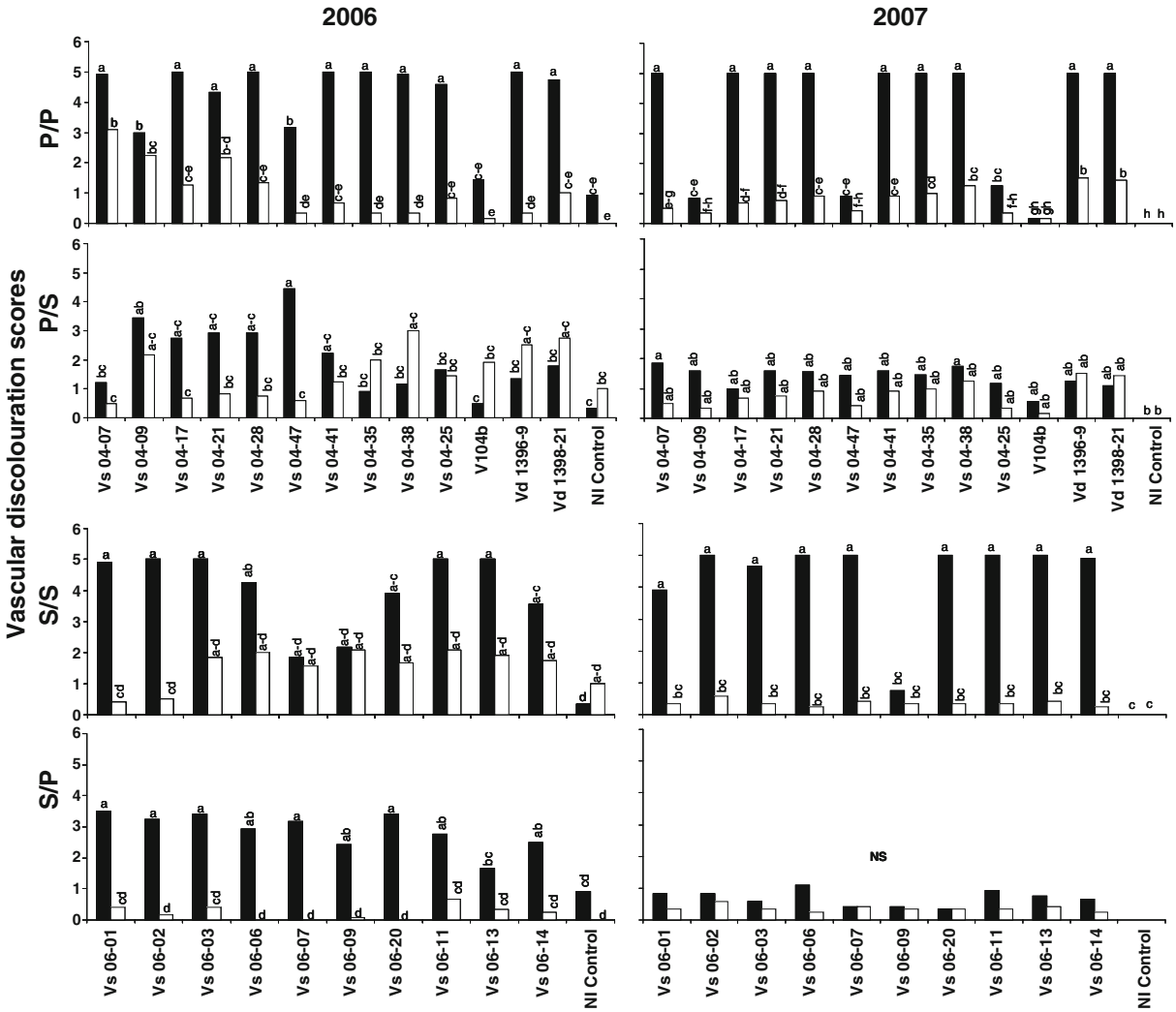

Fig. 7 Vascular discolouration average scores based on the lower, middle and upper cross-sections of the stems of two potato cultivars and two sunflower hybrids 6 w.a.i. with *V. dahliae* isolates recovered from potato (Vs04s) or sunflower (Vs06s) in 2006 and 2007. NI Control = the non-inoculated control. P/P = potato isolates on susceptible Kennebec (■) and moderately resistant Ranger Russet (□) potato cultivars; P/S = potato isolates on susceptible IS8048 (■) and moderately resistant 6946 (□) sunflower hybrids; S/S shows sunflower isolates on susceptible IS8048 (■) and moderately resistant 6946 (□) sunflower hybrids; S/P = sunflower isolates on susceptible Kennebec (■) and moderately resistant Ranger Russet (□) potato cultivars. Columns with the same letter are not significantly different according to Newman Keul's test at $P<0.05$; NS=not significant

2–3 w.a.i. These symptoms were mainly in the form of chlorosis, rather than necrosis, in comparison with the ones caused by potato isolates. This suggests that infections by these isolates are restricted rather early by the various mechanisms that potato puts in place to reduce the impact of diseases in general (Wang et al. 2004, 2005, 2008). The presence of chlorosis implies that, if toxins are involved (Pu et al. 2007), they possibly reach the top of the plant through the vascular system even though the progress of the pathogen is blocked early on by the host. The ability to induce more or less of these symptoms may depend on the differential expression of genes controlling pathogenicity factors in *V. dahliae* isolates (El-Bebany et al. 2008).

Trials in both 2006 and 2007 showed high levels of vascular discolouration induced by each group of isolates in their original host. Significant differences occurred between the susceptible and moderately resistant potato cultivars and sunflower hybrids,

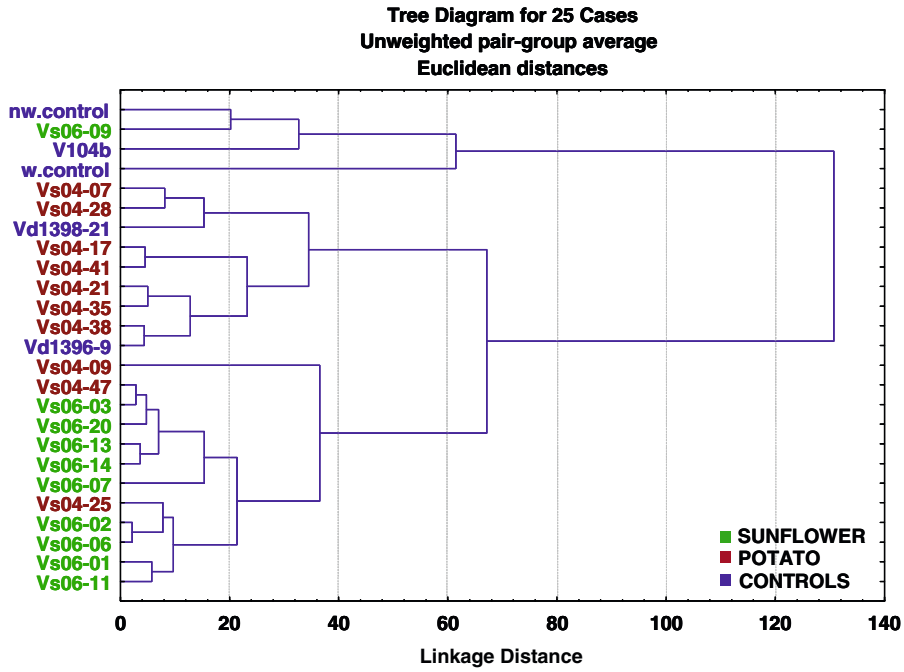

Fig. 8 Dendrogram representing disease assessment on control plants and those inoculated with *V. dahliae* isolates. The dendrogram was generated from cluster analysis based on the unweighted pair-group average method and the Euclidean distances were calculated for percent infection, disease severity and vascular discolouration scores on two potato cultivars and two sunflower hybrids, either susceptible or moderately resistant to *V. dahliae*. NW control = the unwounded healthy control plants; W control = healthy control wounded by trimming the root tips but non-inoculated; Vd1396-9 and Vd1398-21 are two highly aggressive *V. dahliae* used in this study as highly aggressive control isolates; V104b = *V. albo-atrum* sub-group II weakly aggressive control isolate. Vs04 isolates were recovered from potato. Vs06 isolates are from infected sunflowers

respectively. Most *V. dahliae* potato isolates had the ability to cause high levels of vascular discolouration (level 5: 75–100% discolouration) on the susceptible potato cv. Kennebec. However, these levels were significantly lower in the moderately resistant cv. Ranger Russet. Similar results were observed in sunflower hybrids when infected with sunflower isolates, which supports previous studies on host adaptability in *V. dahliae* and *V. albo-atrum* (Horiuchi et al. 1990; Okoli et al. 1994; Resende et al. 1994; Bhat and Subbarao 1999). This suggests that vascular discolouration is perhaps the best marker to assess Verticillium wilt (Pegg and Brady 2002). External symptoms are sometimes induced by factors other than the soilborne pathogen and may be overestimated. *Verticillium dahliae* has a wide host range and is able to cause both external and internal symptoms, sometimes similar to those induced by other biotic and abiotic stresses. In an attempt to accurately assess Verticillium wilt on both tested plant species, we have developed assessment scales for each of the two hosts. These scales were based on both external (chlorosis and necrosis) and internal symptoms (vascular discolouration). Even though both symptoms have been previously described (Church and McCartney 1995; Rowe and Powelson 2002; Fradin and Thomma 2006), we have observed differences, between the two plant species, that were not reported in previous studies. For instance, necrosis in potato leaves appears gradually from the edge after chlorosis then increase to produce a typical V-shaped lesion (Fig. 1). On the contrary, in sunflower leaves, chlorosis appears as flecks that increase in size with time. The necrosis appears as tiny dark brown spots in the chlorotic areas which are located in inter-veinal tissues. Later, these spots become larger and unite together to form larger necrotic areas. Scales developed for external symptoms were based on the symptomatic area (chlorosis and necrosis) on the leaves in each plant species. Although we have developed this assessment system under growth room conditions, we cannot rule out that such symptoms could be slightly different under field

conditions. Given the many factors prevailing in the field, our assessment system may be used as a tool to both quantitatively and qualitatively evaluate symptoms due to the pathogen and with a minimal influence from other external factors, especially when using vascular discolouration. Vascular discolouration scales were developed for each plant species, and measurements made on cross-sections of the lower, middle and upper parts of the stems. The appearance of vascular discolouration in the upper part of the sunflower hybrids infected with *V. dahliae* that was not noticed in potato cultivars, led to the development of another vascular discolouration scale for this particular section of sunflower plants having a hollow stem.

Cluster analysis used in the present study allowed for the placing of the tested isolates into pathogenicity groups with comparable aggressiveness levels. This analysis showed that isolates originating from sunflower were distinctly separated from those initially isolated from potato and closely related to weakly-aggressive control isolates of *V. albo-atrum*. These isolates like *V. albo-atrum* were weakly aggressive on both potato and sunflower and caused a low level of vascular discolouration. This result concurs with the findings of Bhat and Subbarao (1999), who showed that *V. albo-atrum* from alfalfa was able to cause vascular discolouration in potato but the plants never showed wilting. Isolates originating from potato were, on the other hand, clustered with highly-aggressive control isolates Vd1396-9 and Vd1398-21 from potato. The high level of aggressiveness of these isolates on their original host may be due to short rotations with alternate hosts. This may have resulted in increased selection pressure in favour of the isolates that colonise better, and effectively reproduce on the original host. Vigouroux (1971) suggested that the introduction of a preferential host in a rotation sequence could lead to an increase in the amount of inoculum with similar pathogenicity. He also reported that isolates from regions with permanent monoculture are similar, and are all highly aggressive on the particular monoculture crop but weakly aggressive on other host crops. The question is: when should an isolate be considered to originate from a given host and how to define 'the host of origin'? This refers to whether it spent several or only the last generation on the same host.

Even if each group of isolates was more aggressive on its original host, it caused various levels of infection on the alternate host. This suggests that the tested hosts are potential reservoirs for both of these isolates and in any case should not be considered in a rotation sequence, especially where sunflower follows potato, and knowing that most confection sunflower hybrids lack genetic resistance to Verticillium wilt (Rashid and Platford 1994). In this case, other biological control methods including the use of PGPRs (Ongena et al. 1999; El Hassni et al. 2007), Rhizobium (Arfaoui et al. 2007), and plant extracts (Uppal et al. 2008), or cross-protection using hypo-aggressive isolates (Daayf et al. 2003; El Hassni et al. 2004), may be more efficient in controlling this disease.

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