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Comparative Aggressiveness of *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus* on Potato as Measured by their Effects on Wilt Severity, Plant Growth and Subsequent Yield Loss

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ABSTRACT

Verticillium wilt is among the most serious diseases of potato in Tunisia. Three *Verticillium* species are involved in potato vascular wilt symptoms. The present study evaluated the pathogenic abilities of different *Verticillium* spp. isolates on the basis of their effects on wilt severity, plant growth and subsequent yield loss. Artificial inoculation of potato cv. 'Spunta' plants showed, 60 days post-inoculation (DPI), that all the *Verticillium* spp. isolates adversely affected the inoculated plants compared to the non-inoculated control. A high degree of pathogenic variability among *Verticillium* spp. isolates, and especially those of *V. dahliae* was detected, according to their differential ability to cause wilt and browning of the vascular system together with a reduction in plant height, aerial part weight and tuber yield. Although, *V. dahliae* isolates were the most aggressive on potato plants, some *V. albo-atrum* and *V. tricorpus* isolates were found to be as aggressive as some *V. dahliae* isolates, according to our experiment conditions. All three *Verticillium* spp. seem to contribute, to variable degrees, to the potato Verticillium wilt complex in Tunisia. To our knowledge this is the first report of *V. albo-atrum* and *V. tricorpus* with comparable aggressiveness as some *V. dahliae* isolates on potato in Tunisia.

Keywords: cross-pathogenicity, leaf damage, *Solanum tuberosum* L., tuber weight, Tunisia, vascular discoloration

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the third most important food crop in the world after wheat and rice (Wang *et al*. 2008; Schieber and Aranda Saldaña 2009; Visser *et al*. 2009). In Tunisia, it is a strategic crop; there are three major potato growing seasons i.e. early season, season and late season. All these potato crops are affected to a variable degree by vascular wilts depending on the cultivars used, seed origins, fields, and environmental conditions. Verticillium wilt (VW) is among the most serious potato diseases in Tunisia, after late blight, worldwide the most serious disease (Wolski *et al*. 2009). The main symptoms are resumed to early senescence, including yellowing of the foliage and, in some cases, stunting of the plants. A brownish discoloration of the vascular tissue in stems and roots was commonly observed in plants showing early dying (Rowe *et al*. 1987).

Worldwide, VW is one of the most important yield-limiting diseases in potato production (Powelson and Rowe 1993). Yield losses due to the premature senescence induced by the disease reaching up to 50% have been reported (Nachmias and Krikun 1985; Riedel and Rowe 1985; Rowe *et al.* 1987). These yield losses are adversely proportionally related with the degree of soil infestation. In fact, in North America, yields can be reduced 10 to 15% in moderately infested fields and 30 to 50% in heavily infested fields (Rowe and Powelson 2002). In addition to affecting yield and tuber size distribution, VW affects other important quality factors in potatoes i.e., diseased seed tubers occurring mainly in susceptible cultivars (Davis *et al.* 1995; Lynch *et al.* 1997; Goth and Haynes 2000; Robinson *et al.* 2007). Moreover, the vasculature of tubers issued from plants infected by *Verticillium* spp. are known to often develop darkened brown areas that ruin marketability (Lulai 2005).

The disease is caused by a complex of *Verticillium* spp., among which the most recognized causal agents are the root-infecting pathogens *V. albo-atrum* and *V. dahliae* (Mol *et al.* 1996; Heale and Karapapa 1999; Stevenson *et al.* 2001; Robinson *et al.* 2007; Alkher *et al.* 2009). Two other species associated with the VW complex are *V. tricorpus* and *V. albo-atrum* "group 2" (Robb *et al*. 1993; Mahuku *et al.* 1999; Robinson *et al.* 2007). However, in Tunisia, the most common potato pathogens involved in vascular wilt symptoms are *V. dahliae* and *Fusarium oxysporum* f. sp. *tuberosi* (Daami-Remadi and El Mahjoub 2004; Khiareddine 2004). However, during potato crop surveys conducted in a 2005-2006 agricultural campaign in several potato-growing areas, fields exhibiting early dying symptoms most commonly yielded isolates of *V. dahliae* (71%) and to a lesser degree, *V. albo-atrum* (12%) and *V. tricorpus* (17%), from collected samples (unpublished data). *V. albo-atrum* and *V. tricorpus*, both emergent pathogens on potato, and *V. dahliae*, the predominant species, were isolated singly or mixed with other soil-borne root-infecting fungi such as *F. solani*, *F. graminearum* and *Colletotrichum coccodes* (Daami-Remadi and El Mahjoub 2004; Ayed *et al.* 2006; Jabnoun-Khiareddine *et al.* 2009).

Potato is frequently grown in monoculture and rotated with other vegetable crops and cultivated, in some cases, between olive trees. However, no data are available for the contribution of *Verticillium* spp. isolates from potato or other hosts in the VW syndrome. Thus, comparative studies of their aggressiveness on potato need to be clarified in order to obtain an additional understanding of their role in the disease development and severity and to assess the pathogenic variability within inventoried species based on their effects on wilt severity, plant growth and subsequent yield loss.

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Original Research Paper

Table 1 *Verticillium* spp. isolates tested and their origins.

Verticillium species	Isolate	Original host	Geographic origin
V. dahliae	Vd14	Potato	Nabeul
	Vd18	Tomato	Sousse
	Vd20	Potato	Sousse
	Vd23	Potato	Sousse
	Vd28	Potato	Sousse
	Vd29	Potato	Sousse
	Vd30	Potato	Bizerte
	Vd31	Potato	Monastir
	Vd37	Olive	Sousse
	Vd57	Potato	Nabeul
	Vd69	Artichoke	Sousse
	Vd80	Melon	Sousse
	Vd82	Potato	Nabeul
	Vd89	Potato	Jendouba
V. albo-atrum	Vaa1	Tomato	Sousse
	Vaa4	Potato	Sousse
	Vaa5	Tomato	Sousse
V. tricorpus	Vt13	Potato	Monastir
	Vt15	Melon	Mahdia
	Vt17	Tomato	Sousse

MATERIALS AND METHODS

Plant material

Potato (*Solanum tuberosum* L.) cv. 'Spunta' seed tubers were used. This cultivar is the most cultivated in Tunisia and is known to be infected with *V. dahliae*. Tubers were superficially disinfected with a 10% sodium hypochlorite solution for 5 min, rinsed with tap water and air dried. They were placed under favorable environmental conditions for pre-germination (15-20°C, 60-80% relative humidity and natural room light).

At the multi-germ stage, tubers were planted in plastic pots (25 cm diameter) containing a peat and perlite mixture (2: 1), previously sterilized at 110°C for 1 h. After emergence, plants were watered every 2-3 days, depending on the environmental conditions and the plant's needs, until inoculation date.

Pathogen

All *Verticillium* spp. isolates (**Table 1**) used in the present study (14 of *V. dahliae*, 3 of *V. albo-atrum* and 3 of *V. tricorpus*) were single-spore isolates recovered from potato, mainly, and from other hosts (tomato, melon, artichoke, olive trees). They were isolated from diseased plants showing wilt symptoms and vascular discoloration.

They were cultured at 20°C on potato dextrose agar (PDA) medium amended with 300 mg/l of streptomycin sulphate (Pharmadrug Production Gmbh-Hambourg, Germany). Liquid cultures used for substrate inoculation were prepared on potato dextrose broth (PDB) and incubated at 20°C under continuous agitation at 150 rpm during 4 to 5 days. The conidial suspensions were adjusted to 10^7 conidia/ml by a Malassez cytometer. For their long term preservation, pathogen isolates were stored up to 12 months at -20°C in a 40% glycerol solution.

Potato inoculation and culture conditions

Ten days post-emergence of potato plants, inoculation was conducted by watering each potted plant with 100 ml of a conidial suspension (10^7 conidia/ml). Non-inoculated control (NIC) plants were watered with only 100 ml of sterile distilled water.

During all experimentation, plants were watered regularly, fertilized with a nutrient solution (20 N: 20 K_2O: 20 P_2O_5) (Manici and Cerato 1994) and kept clean from aphids and other pests that may interfere with VW assessment.

VW severity was assessed, 60 DPI, based on both external and internal symptoms. In fact, the leaf damage index (LDI) was noted according to a 0-4 scale depending on symptom severity on leaves as previously in Jabnoun-Khiareddine *et al.* (2006). Based on LDI recorded, isolates were classified into different aggressiveness levels as follows: LDI: 0-0.9: weakly aggressive, LDI: 1-1.9: moderately aggressive, LDI: 2-2.9: aggressive LDI ≥ 3: highly aggressive.

In addition, presence of vascular discoloration was verified visually by longitudinally cutting the crown and main roots and its extent was measured from the crown to the top of each stem. The relative vascular discoloration, which is percentage of stem height exhibiting vascular discoloration, is calculated as follows:

(Vascular discoloration extent/ plant height) *100.

These assessments were done for each stem individually and the mean for each plant was recorded.

The effects of inoculations were also evaluated via plant growth and production parameters. In fact, the length of all stems from the ground level was measured and the average per plant was used to calculate the mean height. However, for the stems, roots and tubers, the total weight for each plant was recorded.

Statistical analyses

Statistical analyses were performed, for all parameters measured, following a completely randomized design where treatments (inoculated or non-inoculated control) were the only fixed factor. Five replicates were used per elementary treatment and means were separated using Fisher's protected LSD test (at $P \leq 0.05$).

The relationships between LDI and plant height, stem weight and tuber weight were compared using Pearson's correlation analysis.

RESULTS

Analysis of variance of pathogenicity data indicated that all the variables were affected significantly ($P \leq 0.05$) by *Verticillium* spp. isolates compared to the non-inoculated control.

Comparative effects of *Verticillium* spp. on wilt severity

All inoculated 'Spunta' plants exhibited typical VW symptoms in response to inoculation with the tested *Verticillium* spp. isolates. First disease symptoms were visible at 2 to 3 weeks post-inoculation and developed over time from chlorosis to necrosis, concerning mainly the lower leaves, and total wilting and drying (**Fig. 1**). Thus, all *Verticillium* spp.

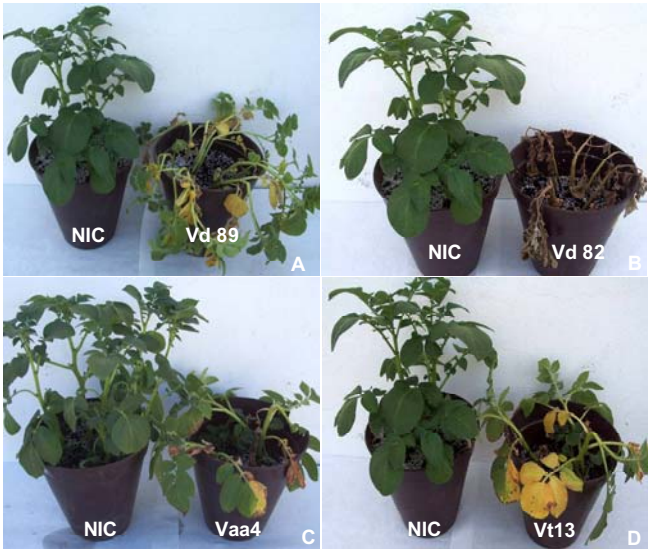

Fig. 1 Early dying symptoms observed 60 days post-inoculation on potato 'Spunta' plants inoculated with two *V. dahliae* isolates (A and B), *V. albo-atrum* (C) and *V. tricorpus* compared with non-inoculated control (NIC) plants. (**A**) Chlorosis and wilting caused by *V. dahliae* (Vd89); (**B**) Total drying of leaves and stems caused by *V. dahliae* (Vd82); (**C**) and (**D**) Yellowing of lower leaves caused by *V. albo-atrum* (Vaa4) and *V. tricorpus* (Vt13), respectively; 5 < T < 28°C.

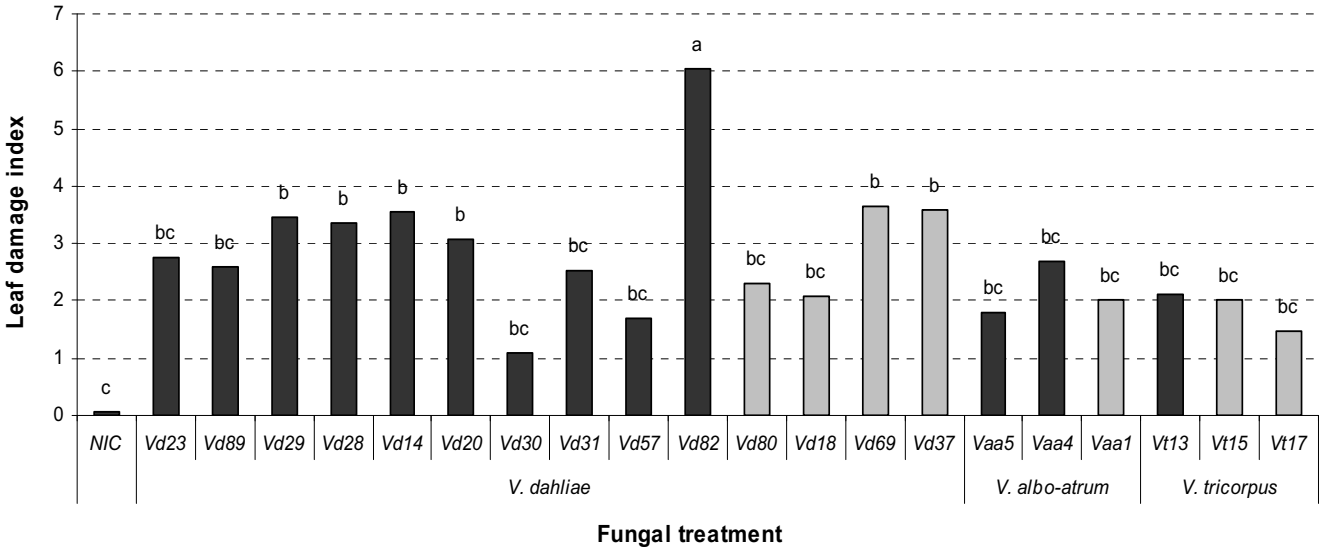

Fig. 2 Leaf damage index noted 60 days post-inoculation on potato 'Spunta' plants inoculated with some *V. dahliae*, *V. albo-atrum* and *V. tricorpus* isolates compared to the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$); (NIC) non-inoculated control; 5 < T <28°C; (Vd23-Vd82) *V. dahliae* isolates from potato; (Vd80, Vd18, Vd69 and Vd37) *V. dahliae* isolates from melon, tomato, artichoke and olive, respectively; (Vaa5 and Vaa4) *V. albo-atrum* from potato; (Vaa1) *V. albo-atrum* from tomato; (Vt13) *V. tricorpus* from potato; (Vt15 and Vt17) *V. tricorpus* from melon and tomato, respectively.

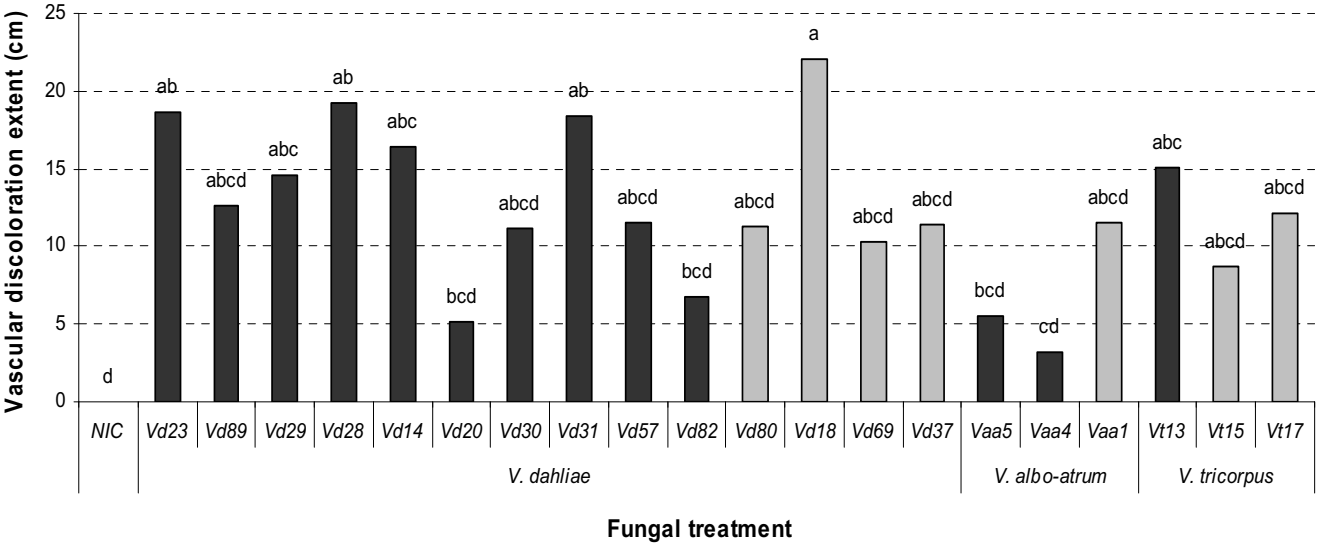

Fig. 3 Vascular discoloration extent noted 60 days post-inoculation on potato 'Spunta' plants inoculated with some *V. dahliae*, *V. albo-atrum* and *V. tricorpus* isolates compared to the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$); (NIC) non-inoculated control; 5 < T <28°C; (Vd23-Vd82) *V. dahliae* isolates from potato; (Vd80, Vd18, Vd69 and Vd37) *V. dahliae* isolates from melon, tomato, artichoke and olive, respectively; (Vaa5 and Vaa4) *V. albo-atrum* from potato; (Vaa1) *V. albo-atrum* from tomato; Vt13: *V. tricorpus* from potato; (Vt15 and Vt17) *V. tricorpus* from melon and tomato, respectively.

isolates tested were pathogenic on 'Spunta' plants. However, their pathogenicity in terms of disease severity was highly variable depending on the *Verticillium* species and isolates tested. In fact, data shown in **Fig. 2** indicates that the highest LDI (i.e. 6) was recorded on plants inoculated with *V. dahliae* isolate Vd82 originally isolated from potato. Furthermore, 7 out of the 14 *V. dahliae* tested showed an LDI exceeding 3 and classified, consequently, as highly aggressive. Five out of the 14 *V. dahliae* isolates showed an LDI comprised between 2 and 2.9 (qualified as aggressive) which was significantly comparable to that noted on plants inoculated with 2 *V. albo-atrum* and 2 *V. tricorpus* isolates. The remaining *Verticillium* spp. isolates were moderately aggressive and none of the tested isolates was qualified as weak pathogen (i.e. weakly aggressive). Moreover, based on LDI records, no intraspecific variability within the three *Verticillium* species, independent of their host origin, was detected as all isolates, excepting the most aggressive Vd82 (*V. dahliae*), induced significantly comparable effects on potato plants.

When lower, middle and upper stem sections of inoculated 'Spunta' plants were dissected, they all showed vascular discoloration reflecting the endophytic Verticillium disease progress (second disease severity parameter). Nevertheless, 12 out of the 14 *V. dahliae* isolates colonized potato stems, in a statistically comparable way as the most aggressive (based on vascular discoloration extent) isolate Vd18 although the extent recorded varied between 10 and 23 cm (**Fig. 3**). *V. dahliae* isolates collected from the other hosts (tomato, artichoke and olive) were as aggressive as isolates issued from potato. Moreover, *V. albo-atrum* and *V. tricorpus* isolates induced significantly similar disease severity as some *V. dahliae* isolates and the vascular discoloration extent ranged between 4 and 15 cm.

However, the relative vascular discoloration (**Fig. 4**) revealed the important colonization of more than 50% of stem's height by 8 out of 14 *V. dahliae* and 2 out of 3 *V. tricorpus* isolates tested. This colonization exceeded 70% of the total plant height for 4 *V. dahliae* isolates (Vd23, Vd28 and Vd14), originally isolated from potato, and Vd18 col-

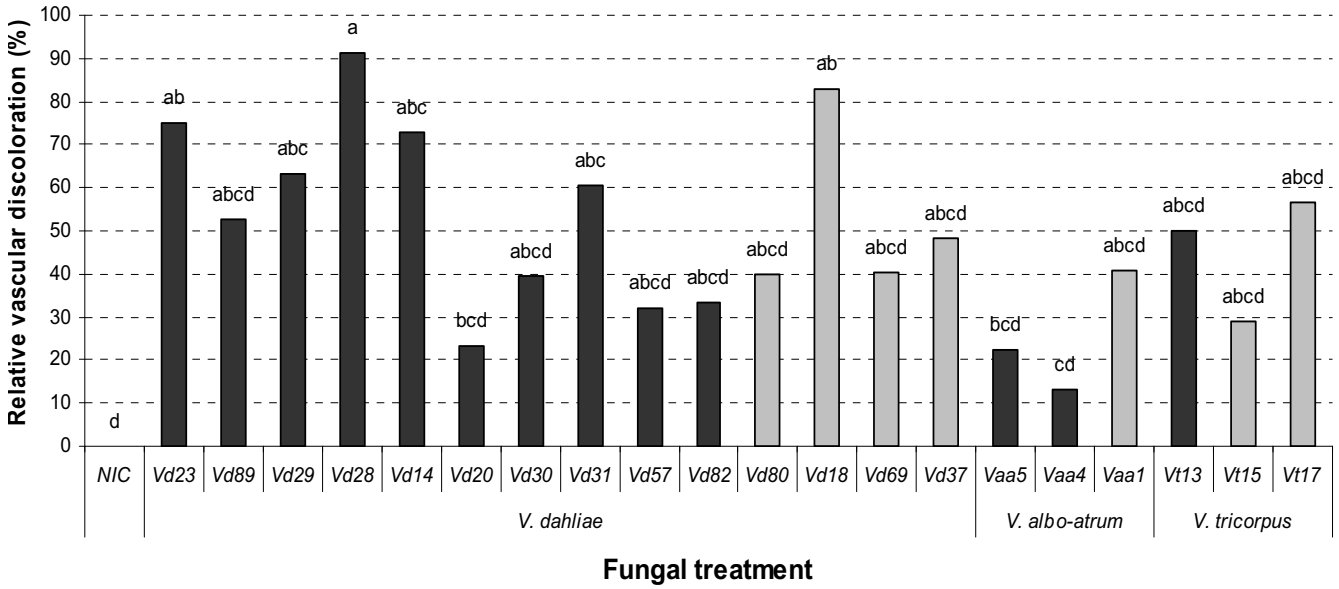

Fig. 4 Relative vascular discoloration noted 60 days post-inoculation on potato 'Spunta' plants inoculated with some *V. dahliae*, *V. albo-atrum* and *V. tricorpus* isolates compared to the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$); (NIC) non-inoculated control; 5 < T < 28°C; (Vd23-Vd82) *V. dahliae* isolates from potato; (Vd80, Vd18, Vd69 and Vd37) *V. dahliae* isolates from melon, tomato, artichoke and olive, respectively; (Vaa5 and Vaa4) *V. albo-atrum* from potato; (Vaa1) *V. albo-atrum* from tomato; Vt13: *V. tricorpus* from potato; (Vt15 and Vt17) *V. tricorpus* from melon and tomato, respectively.

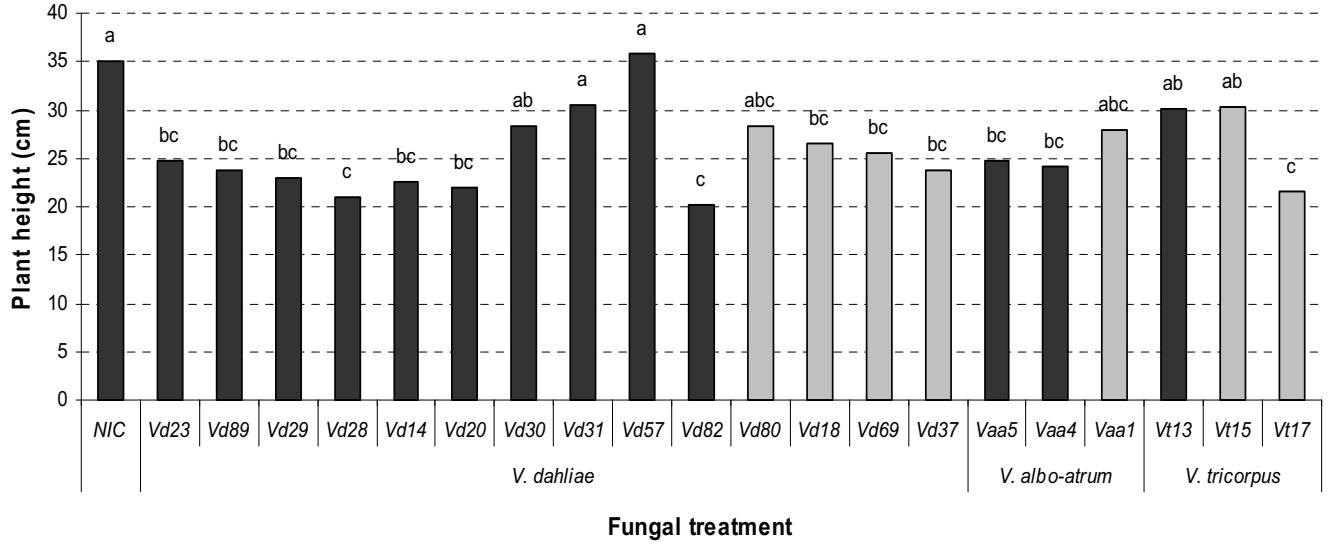

Fig. 5 Plant height noted 60 days post-inoculation on potato 'Spunta' plants inoculated with some *V. dahliae*, *V. albo-atrum* and *V. tricorpus* isolates compared to the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$); (NIC) non-inoculated control; 5 < T < 28°C; (Vd23-Vd82) *V. dahliae* isolates from potato; (Vd80, Vd18, Vd69 and Vd37) *V. dahliae* isolates from melon, tomato, artichoke and olive, respectively; (Vaa5 and Vaa4) *V. albo-atrum* from potato; Vaa1: *V. albo-atrum* from tomato; (Vt13) *V. tricorpus* from potato; (Vt15 and Vt17) *V. tricorpus* from melon and tomato, respectively.

lected from tomato.

All the *Verticillium* spp. isolates were successfully re-isolated on PDA from the inoculated potato plants, especially from basal stems.

Comparative effects of *Verticillium* spp. on growth parameters

The mean plant height, noted at 60 DPI, was significantly reduced compared to the non-inoculated control plants, excepting plants inoculated with 4 *V. dahliae*, 1 *V. albo-atrum* and 2 *V. tricorpus* isolates (**Fig. 5**). In fact, depending on isolates used for inoculation, the height decrease ranged between 0 and 42% for *V. dahliae* isolates issued from potato, and between 19 and 32% for *V. dahliae* collected from other hosts. Potato plants inoculated with *V. albo-atrum* and *V. tricorpus* showed reduction in height of about 20-31% and 14-38%, respectively, compared to the non-inoculated control plants.

Intraspecific variability within *V. dahliae* and *V. tricorpus* was recorded based on stunting effect on potato plants subsequent to inoculation.

Data shown in **Fig. 6** indicates that, except isolates Vd30 and Vd57 (*V. dahliae*) and Vt15 (*V. tricorpus*), all the *Verticillium* spp. isolates had significantly reduced the weight of the aerial part compared with the non-inoculated control plants. In fact, the range of decrease was 20 to 64% for *V. dahliae* collected from potato, and 34 to 62% for those originated from the other hosts. However, for *V. albo-atrum* and *V. tricorpus*, the decrease in the aerial part weight varied from 34 to 54% and from 21 to 54%, respectively, compared with the non-inoculated control.

Intraspecific variability was only recorded within *V. dahliae* based on effects on plant weight caused by inoculation.

Significant correlations between these growth parameters and the LDI recorded on potato plants inoculated with *Verticillium* species were found. In fact, for *V. dahliae*,

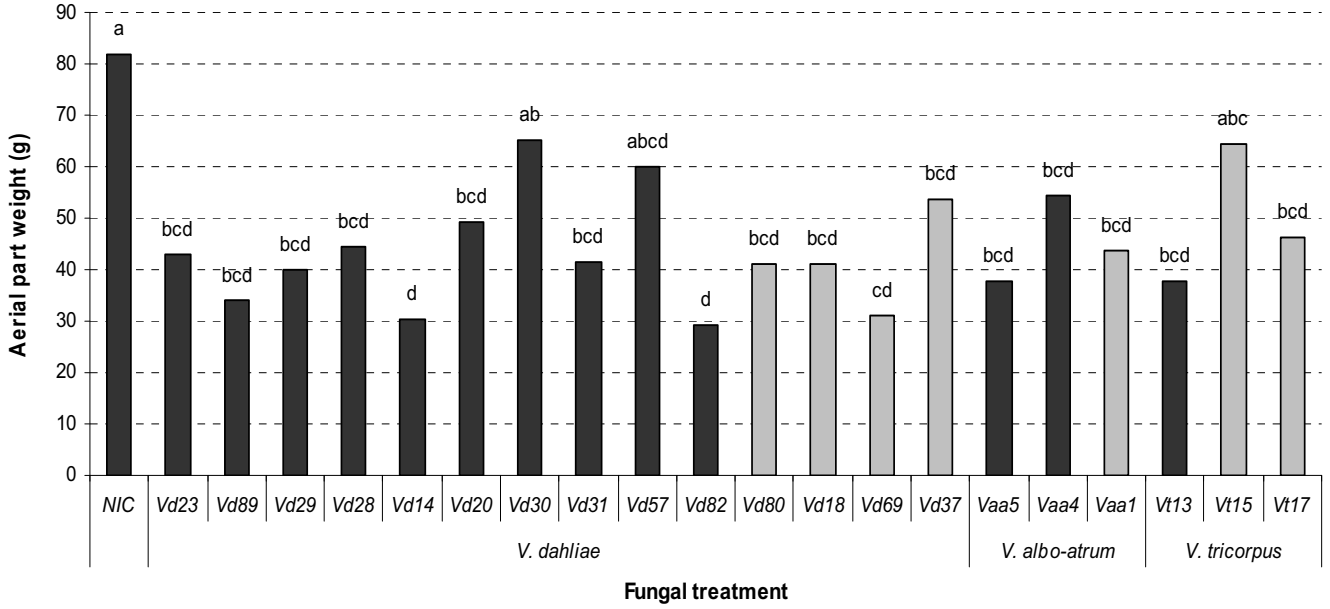

Fig. 6 Aerial part weight noted 60 days post-inoculation on potato 'Spunta' plants inoculated with some *V. dahliae*, *V. albo-atrum* and *V. tricorpus* isolates compared to the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$); (NIC) non-inoculated control; 5 < T < 28°C; (Vd23-Vd82) *V. dahliae* isolates from potato; (Vd80, Vd18, Vd69 and Vd37) *V. dahliae* isolates from melon, tomato, artichoke and olive, respectively; (Vaa5 and Vaa4) *V. albo-atrum* from potato; (Vaa1) *V. albo-atrum* from tomato; (Vt13) *V. tricorpus* from potato; (Vt15 and Vt17) *V. tricorpus* from melon and tomato, respectively.

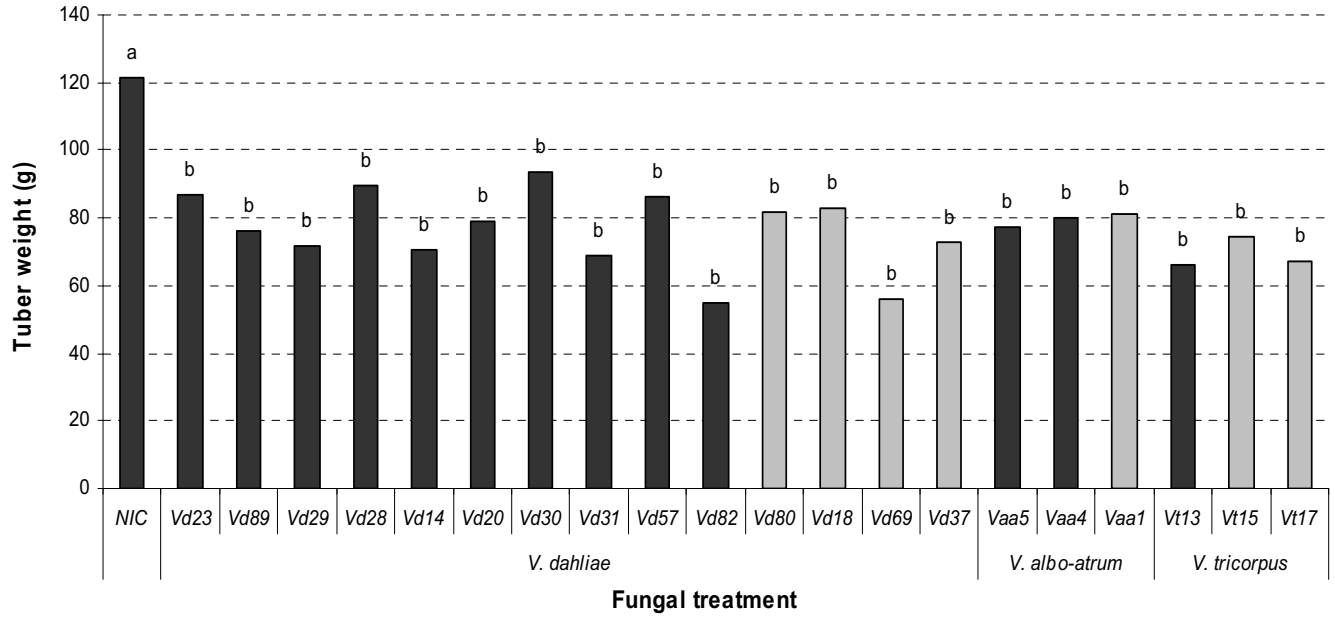

Fig. 7 Tuber weight noted 60 days post-inoculation on potato 'Spunta' plants inoculated with some *V. dahliae*, *V. albo-atrum* and *V. tricorpus* isolates compared to the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$); (NIC) non-inoculated control; 5 < T < 28°C; (Vd23-Vd82) *V. dahliae* isolates from potato; (Vd80, Vd18, Vd69 and Vd37) *V. dahliae* isolates from melon, tomato, artichoke and olive, respectively; (Vaa5 and Vaa4) *V. albo-atrum* from potato; (Vaa1) *V. albo-atrum* from tomato; (Vt13) *V. tricorpus* from potato; (Vt15 and Vt17) *V. tricorpus* from melon and tomato, respectively.

significant negative correlations were found between LDI and plant height (Pearson R = -0.300) and weight of the aerial part (Pearson R = -0.460). For *V. albo-atrum*, similar negative correlations were noted between LDI and plant height (Pearson R= -0.444) and weight of the aerial part (Pearson R = -0.555). Plants inoculated with isolates of *V. dahliae* and *V. albo-atrum* showing the highest LDI records, exhibited the lowest plant heights and aerial part weights. For *V. tricorpus*, although no significant correlation was found between LDI and plant height, a significant negative correlation was found between LDI and the aerial part weight of potato plants (Pearson R = -0.501).

Comparative effects of *Verticillium* spp. on tuber yield

The mean tuber yield per plant recorded 60 DPI was adversely affected by inoculation with all the *Verticillium* spp. isolates tested (**Fig. 7**). This decrease varied from 23 to 55% for *V. dahliae* obtained from potato compared with 32 to 54% for those collected from other hosts. However, for *V. albo-atrum* and *V. tricorpus*, the reduction in tuber weight varied between 33 and 36% and between 38 and 45%, respectively, depending on the isolates used. It is important to note that all *Verticillium* spp. isolates caused statistically similar effect, i.e. decrease compared to the control, on tuber weight when inoculated to potato 'Spunta' plants.

It is also to note that no significant correlations between tuber yield and the extent of vascular discoloration were found when all the data are considered, except for *V. albo-atrum* in which a significant negative correlation was noted (Pearson R = -0.517). However, significant correlations between LDI and tuber yield were observed for isolates of the three *Verticillium* species. In fact, significant negative correlations were recorded for *V. dahliae* (Pearson R = -0.479), *V. albo-atrum* (Pearson R = -0.592) and *V. tricorpus* (Pearson R = -0.453). Potato plants inoculated with the *Verticillium* spp. isolates showing the highest LDI records had the lowest tuber yield.

DISCUSSION

VW is a chronic disease of potato and a leading chronic yield-limiting factor, in irrigated production regions worldwide, caused by a complex of *Verticillium* species (Rowe and Powelson 2002). As pathogen populations, plant material (cultivars), soil parameters and culture systems vary between countries, site specific studies on VW incidence and severity need to be done under Tunisian conditions.

The findings from our study give additional understanding of the role of each of the three *Verticillium* species, collected in Tunisia, in disease severity and subsequent adverse effects on plant growth and production. In fact, all the *Verticillium* spp. isolates, tested in the same experiment conditions, were found to be pathogenic on 'Spunta' plants. However, their aggressiveness based on main disease severity scoring systems, LDI and vascular discoloration extent, was highly variable depending on the *Verticillium* species and isolates tested. In fact, most *V. dahliae* isolates were highly aggressive while *V. albo-atrum* and *V. tricorpus* isolates showed significantly comparable LDI as the moderately aggressive or aggressive *V. dahliae* isolates. The higher aggressiveness of *V. dahliae* isolates is in agreement with previous findings (Robinson *et al.* 2006, 2007). However, *V. tricorpus*, reported to be a saprobe, unable to colonize healthy potato stems and proliferating in the soil or colonizing only plants severely infected by other pathogens (Isaac 1953; Mahuku *et al.* 1999), was found in the present study as aggressive or at least moderately aggressive as 50% of the *V. dahliae* isolates tested. Thus, Tunisian *V. tricorpus* isolates showed greater aggressiveness than isolates tested by Robinson *et al.* (2006) for which the percentage of symptomatic plants i.e. showing typical VW symptoms, was substantially lower (7%) although the pathogen was detected in roots and stems as well as in tubers. Other previous researches have also recognized *V. tricorpus* as a weak pathogen capable of causing VW in potato (Davis and McDole 1979; Heinz and Platt 2000).

V. albo-atrum isolates, tested in the present work, were moderately aggressive or aggressive as some *V. dahliae* isolates. This result is in agreement with other findings where the percentage of plants with foliar wilting ranged between 35 and 80% for *V. dahliae* (Schnathorst 1981; Easton *et al.* 1992; Davis *et al.* 1994; Mahuku *et al.* 1999; Lazarovits *et al.* 2001) and between 59 and 93% for *V. albo-atrum* group 1 (Sampson 1980; Mahuku *et al.* 1999). Moreover, no intraspecific variability was recorded within *V. albo-atrum* tested in the present study as estimated by LDI and vascular discoloration extent. Furthermore, contrary to previous findings (Okoli *et al.* 1994) reporting the existence of both groups within *V. albo-atrum* based on RFLPs which were pathogenic to their respective hosts only, Tunisian isolates originating from different hosts (potato and tomato in our case) showed significantly similar aggressiveness.

Nevertheless, *V. dahliae* isolates collected from the other hosts such as tomato, artichoke and olive tree were found to be significantly as aggressive as isolates issued from potato. This result indicated that Tunisian *V. dahliae* isolates were more adapted to this host due to the quasi absence of rotation and to the culture of vegetable crops between olive trees. *V. dahliae* cross-pathogenicity was also reported by Qin *et al.* (2006) on artichoke, bell pepper, broccoli, cabbage, cauliflower, chili pepper, cotton, eggplant, mint, lettuce, potato, strawberry, tomato, and watermelon. However, the level of pathogenicity varied on different hosts (Bhat and Subbarao 1999) and isolates from one host are able to cause disease on other plant species but symptoms are often more severe on the host of origin (Resende *et al.* 1994; Daayf *et al.* 1995; Bhat and Subbarao 1999; Katan 2000; Korolev *et al.* 2001; Qin *et al.* 2006; Sebastjan *et al.* 2006). This observation is evident in our study only in the case of the isolate Vd82 based on LDI records (**Fig. 2**). Higher level of aggressiveness on potato of *V. dahliae* isolates originating from potato may be due to the selection or adaptation, over time, of the pathogen to the host (Okoli *et al.* 1994; Resende *et al.* 1994). In the same way, according to Ispahani *et al.* (2008), enhanced pathogenicity of the *V. dahliae* isolates resulted from selection imposed by the continuous cultivation of a single host. In nature, a strong selection pressure is exerted on the field isolates, and only those isolates that are aggressive on a particular host can cause severe vascular discoloration and wilting (Bhat and Subbarao 1999). In fact, as shown in **Fig. 4**, the relative vascular discoloration exceeded 70% for four *V. dahliae* isolates of which three are issued from potato.

In the present study, all *Verticillium* spp. isolates caused vascular browning exceeding the basal parts of the stem and were, consequently, classified as highly aggressive or mildly aggressive depending on the importance of internal symptom progress recorded. In fact, weakly aggressive isolates were reported by Uppal *et al.* (2007) to colonize only the roots and were rarely detected in samples taken from the stems. Vascular discoloration in stem cross-sections was considered to be a good criterion to discriminate highly- from weakly-aggressive isolates (Mace 1989). Quantification of *Verticillium* species in the vascular tissue has also revealed differences that were not detected by visual disease assessments (Hoyos *et al.* 1991). Moreover, when using vascular discoloration i.e. endophytic disease progress, symptoms may be assessed both quantitatively and qualitatively with a minimal influence from other external factors such as abiotic stresses providing a better appreciation of plant susceptibility and evaluation of wilt agent pathogenicity (Uppal *et al.* 2007; Alkher *et al.* 2009).

These results corroborate other findings reporting pathogenic variability within *Verticillium* populations, and especially *V. dahliae*, on several plant species (Strausbaugh 1993; Resende *et al.* 1994; Daayf *et al.* 1995; Dobinson *et al.* 2000). This variability may result from the ability of isolates to produce pathogenicity factors such as toxins, phytotoxic peptides, glycopeptides, and protein–lipopolysaccharide (Gour and Dube 1985; Buchner *et al.* 1989; Meyer *et al.* 1994; Mansoori *et al.* 1995). These toxins are known for their local effect in inducing necrosis leading to plant wilting, as well as for their abilities to trigger host defence mechanisms and consequently, increased plant susceptibility to pathogen invasion (Zhen and Li 2004).

The findings from our study also reported decrease in plant height with the majority of *Verticillium* spp. isolates where the maximum reduction recorded was 42, 31 and 38% for *V. dahliae*, *V. albo-atrum* and *V. tricorpus* isolates, respectively. This effect of *V. dahliae* isolates on plant height is in accordance with other reports. In fact, in potato and at late stages i.e. more than six weeks after inoculation, disease severity was high and stunting was apparent on inoculated plants resulting in significant differences, depending on the isolates used, in tuber and plant growth (Bhat and Subbarao 1999). Additional information was obtained in our results concerning the variable adverse effects of *V. albo-atrum* and *V. tricorpus* isolates on 'Spunta' potato plants.

The decrease in the weight of the aerial part, noted in the current study, varied from 20-64%, 34-54% and 21-54% for *V. dahliae*, *V. albo-atrum* and *V. tricorpus* isolates used for inoculation, respectively. This growth reduction may be mainly due to the colonization of plant stem tissues by *Verticillium* spp. which results in a variety of responses in-

cluding plugging of the vessels with gum and pectin, cell death, lignification and accumulation of material thought to be suberin (Beckman 2000; Rowe and Powelson 2002). Differences in response to early dying, i.e. adverse effects on plant growth, were associated with the speed of colonization and the establishment of a higher population density by *V. dahliae* in the plant (Bae *et al.* 2007). Moreover, based on adverse effects on the weight of the aerial part, an intraspecific variability was recorded, in the present study, within *V. dahliae* species only; this may due to the relatively reduced number of isolates for *V. albo-atrum* and *V. tricorpus* (3) compared to *V. dahliae* (14).

Inoculation with *Verticillium* spp. isolates resulted in significantly similar tuber weight decrease compared to the non-inoculated control plants. In addition to their significant involvement in wilt severity, this study revealed, for the first time in Tunisia, the significant adverse effect of Tunisian *V. albo-atrum* and *V. tricorpus* isolates on potato yield subsequent to plant growth inhibition. Similarly, Goth and Haynes (2000) noted that greater reductions in yield were associated with a higher degree of wilting due to Verticillium infection. Yield reductions reported in previous studies were variable depending on the *Verticillium* species involved and countries. However, a yield reduction of 79% was reported in plants inoculated with *V. dahliae* alone, or in combination with *C. coccodes* (Sedegui *et al.* 2000). Yield losses, which are mainly due to a decrease in the photosynthesis rate caused by the premature death of the foliage, are estimated to 30-50% in infested fields (Uppal *et al.* 2007). Although Botseas and Rowe (1994) found no significant difference in total yield between the *V. dahliae* infested and non-infested soil, they concluded that size differences may reduce marketable yields. In fact, Uppal *et al.* (2007) recorded important losses with regard to the quality of harvested tubers due to VW. Similarly, Rowe and Powelson (2002) found that both *V. dahliae* and *V. albo-atrum* reduce yield and tuber quality in potato. The invasion and blockage of xylem vessels in plant vascular systems by *Verticillium* spp. are responsible of these serious yield and tuber quality problems (Beckman 1987; Tjamos *et al.* 2000; Pegg and Brady 2002; Rowe and Powelson 2002). Thus, comparative studies of variation in pathogenicity and/or aggressiveness within and among *Verticillium* species involved in potato early dying based on adverse effect on yield were considered as important as symptom development and both scoring methods were extensively used for evaluation of plant material resistance (Susnoschi *et al.* 1975, 1976; Davis *et al.* 1983; Platt and Sanderson 1987).

In the light of the results presented here, *V. albo-atrum* and *V. tricorpus* seem to play a significant role in the potato VW complex involving the traditionally recognised 'strong' pathogen *V. dahliae*, as the tested isolates were found to be as aggressive as some *V. dahliae* isolates. Moreover a high pathogenic variability was detected among *V. dahliae* isolates when inoculated to potato plants. In addition, 'Spunta', which is the most grown cultivar in Tunisia, have shown to be susceptible to all the tested *Verticillium* spp isolates. Thus, the development of VW resistant cultivars has an important part to play in managing this disease in an environmentally sound way, in order to minimize the negative impact of infection by *Verticillium* spp. on economically important traits such as yield and specific gravity.

An understanding of the genetic variation among *V. dahliae* strains from different hosts would contribute to the improvement of management schemes for this important disease.

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