

Comparative Reaction of Potato Cultivars to *Sclerotium rolfsii* Assessed by Stem Rot and Tuber Decay Severity

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ABSTRACT

Experiments were conducted to evaluate the relative susceptibility of 11 local potato cultivars to *Sclerotium rolfsii* by using several stem and tuber disease parameters. Based on stem rot severity, noted three weeks post-inoculation, pathogen penetration, and percentage of rotten tissue, recorded after 8 days of incubation at 30°C, none of the cultivars tested was resistant to *S. rolfsii*. However, there was a variable degree of susceptibility in which cultivar ‘Tango’ was found to be the most susceptible whilst ‘Daisy’ was the most tolerant to the disease. The most severe stem rot attributed to pathogen inoculation was observed, three weeks post-inoculation, on ‘Tango’ and ‘Spunta’ plants. For all cultivars combined, the percentage of rotten tuber tissue was related to the lesion diameter formed on the tuber surface and to the pathogen penetration.

Keywords: atypical soft rot, cultivar behaviour, disease severity, inoculation, Southern blight

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an economically important crop worldwide. It is classified as the third most important food crop after wheat and rice (Wang *et al.* 2008; Schieber and Aranda Saldaña 2009; Visser *et al.* 2009). In Tunisia, it is a strategic crop (Azzouz 1996) with an average yield of about 14 t/ha (Djébali and Tarhouni 2010). As potato is frequently grown in monoculture and rotated with other vegetable crops (Daami-Remadi *et al.* 2011), it is threatened by several soil-borne fungi. Amongst these fungal pathogens, agents causing wilt and root and tuber rots are responsible of yield losses (Priou and El Mahjoub 1999).

Sclerotium rolfsii is a soil-borne plant pathogen of worldwide occurrence that infects more than 500 plant species (Aycock 1966; Mordue 1974; Punja 1985; Wokocho 2001; Ozgonen *et al.* 2010). *Sclerotium* wilt, incited by *S. rolfsii* is an important field disease of potato particularly in tropical, subtropical and warm temperate areas (Aycock 1966; de Icochea 1981).

The large number of sclerotia produced by *S. rolfsii*, their survival ability for several years, and the abundant growth rate of the fungus are key factors making this pathogen of major importance (Punja 1988). The first confirmed report of losses due to the pathogen in the USA was made by Rolfs in 1892 on tomato (*Lycopersicon esculentum* Miller) in Florida (Aycock 1966). The disease was particularly severe, in Alessandria located in Northern Italy, on potato cv. ‘Monalisa’ causing 5 to 15% yield losses because of premature plant death and rotting of tubers (Garibaldi *et al.* 2006).

In Tunisia, the disease has been observed, since 2006, on potato plants and rotting tubers (Daami-Remadi *et al.* 2007) and was found to cause serious soft rots under the same thermal conditions of traditional improved storage or non-stored potatoes, where temperature ranges between 25 and 35°C (Daami-Remadi *et al.* 2010). Moreover, the reaction of potato cultivars to stem rot disease has not been well established, although cv. ‘Spunta’ usually showed pathogen

infection and exhibited typical symptoms (Daami-Remadi *et al.* 2007, 2010). To the best of our knowledge, there are limited published data concerning the relative susceptibility of commercial potato cultivars to *S. rolfsii*, in Tunisia and the world at large, with the exception of the work of Holm *et al.* (1987) and Garibaldi *et al.* (2006). Thus, as potato production areas are limited under Tunisian conditions and rotation is often difficult to implement, assessing the local cultivars for their reaction to *S. rolfsii* is needed. This would consequently identify the use of resistant or less susceptible cultivars which would obviously reduce economic loss and damage attributed to the disease.

Due to the occurrence of *S. rolfsii* in soil and plants and their effects on plants and tubers, the present work conducted to assess the susceptibility of local cultivars to stem and tuber infection and rots.

MATERIALS AND METHODS

Plant material

Relatively healthy and undamaged potato (*Solanum tuberosum* L.) tubers belonging to 11 cultivars mentioned in A class (cultivars distributed to farmers for growing potatoes for consumption and/or common seed production for late season crop) of the Tunisian varietal assortment, were tested. They were kindly provided by the Technical Potato Center, Essaïda, Tunisia. Just before use, tubers were washed to remove excess soil, superficially disinfected with a 10% sodium hypochlorite solution for 5 min, rinsed with sterile distilled water and air dried.

Depending on the experiment being conducted, tubers were either kept at 15-20°C, 60-80% relative humidity under natural room light for pre-sprouting (for pot experiment) or used directly for inoculation (tuber experiment). After sprout induction (occurrence), tubers were planted (one tuber per pot) in plastic pots (25 cm diameter) containing a mixture of peat and perlite (3/4: 1/4), previously sterilized at 110°C for 1 h. After emergence, potato plants were watered every 2-3 days, depending on environmental conditions and plant’s needs, until inoculation date.

Pathogen

S. rolfisii SS1 isolate, originally obtained from rotted potato tubers, was used for tuber and plant inoculation. Its pathogenicity as well as its aggressiveness was previously characterized on potato cv. 'Spunta' (Daami-Remadi *et al.* 2007, 2010). The pathogen was cultured for 6 to 10 days at 25°C on potato dextrose agar (PDA) medium amended with 300 mg/l of streptomycin sulphate (Pharmadrug Production GmbH-Hamburg, Germany) before use. For plant inoculations, a mixture of mycelium and sclerotia, prepared by adding 10 plates of the pathogen grown on media to one liter of sterile distilled water, was used.

Tuber inoculation and rot severity assessment

Tubers were wounded by a 6-mm diameter disinfected cork borer which was used to induce wounds in tubers. Tuber inoculation was accomplished by depositing a hyphal plug (6 mm diameter) removed from the edge of actively growing colonies of *S. rolfisii* on PDA. Inoculated tubers were placed in moistened trays and incubated in a growth chamber at 30°C for 72 h. Ten tubers were used per cultivar tested. Tubers that had been similarly wounded but not inoculated were used as controls. The experiment had eleven experimental treatments (tubers from 11 cultivars) which were completely randomized within a growth chamber.

The progress of the disease on tubers (i.e. external lesion diameter formed as a consequent of pathogen infection) was quantified by measuring surface lesion diameter on all inoculated tubers after 72 h of incubation. However, rot severity was quantified, after 8 days of incubation on a sample of tubers by cutting the tubers longitudinally at each wound site in half. Maximal width (w) and depth (d) of soft rot were recorded and pathogen penetration or lesion depth (P) was calculated according to the formula of Lapwood *et al.* (1984) as follows:

$$P(\text{mm}) = (w/2 + (d-6))/2.$$

Disease severity was also estimated via the percentage of rotten tissue as previously described (Daami-Remadi *et al.* 2010). Inoculated tubers were weighed before (Wi) and after (Wf) rotten tissue was removed and the percentage of rotten tissue was calculated as follows:

$$\text{Rotten tissue (\%)} = ((Wi-Wf)/Wi) \times 100$$

Plant inoculation and stem rot severity assessment

Potato plants from the cultivars were inoculated two weeks after their emergence. Inoculation was conducted by watering each potted plant, next to the collar region, with 100 ml of a mixture of mycelium and sclerotia (\approx 25-30 sclerotia per 100 ml). Non-inoculated control plants were watered only with 100 ml of sterile distilled water. Six plants were used per cultivar. Plants were placed under greenhouse conditions where temperatures ranged between 10 and 29°C (minimum and maximum, respectively). During all experimentation, plants were watered regularly and fertilized with a nutrient solution (20 N: 20 K₂O: 20 P₂O₅) (Manici and Cerato 1994).

Three weeks post-inoculation, plants were uprooted, washed with tap water, air dried, and scored for stem rot severity. In fact, each stem was examined for signs of stem rot disease based on a 0-5-point scale, according to Wokocho (1990), as follows:

- 0: No visible *S. rolfisii* infection;
- 1: 1-25% of stem circumference girdled;
- 2: 26-75% of stem circumference girdled;
- 3: 76-100% of stem circumference girdled;
- 4: Entire plant wilted;
- 5: Plant dead.

The disease severity index (DSI) of *S. rolfisii* attack (i.e. stem rot severity) per plant was determined as follows: DSI = Sum of all numerical ratings/total number of stems.

Isolation of the pathogen from diseased tubers was performed after disease evaluation to confirm the results and ascertain that the symptoms observed were indeed induced by *S. rolfisii*. Simi-

larly, stem segments of diseased tissues were surface disinfected in 0.5% sodium hypochlorite solution for 5 min and rinsed three times with sterile distilled water. Dried segments were then plated (3 pieces per plate) on PDA supplemented with streptomycin sulphate (300 mg/l), and incubated for 7 days at 25°C to isolate the pathogen. Some disinfected stem sections were also placed in a humid chamber at 25°C for visual observation of the fan-like mycelium development, which is characteristic of morphological growth of *S. rolfisii* pathogen.

Statistical analyses

As all non-inoculated plants and tubers were symptomless; therefore, only the data of inoculated plants and tubers were considered in the statistical analyses. The disease severity parameters were analyzed using Analysis of variance (ANOVA) to assess treatment effects (cultivars) on lesion diameter and stem rot in the various experiments. The effect of treatment on lesion diameter (tuber rot) and stem rot were also compared by computing treatment means using Statistical Analysis System (SPSS). Means were separated using Fisher's protected LSD test (at $P \leq 0.05$).

The relationships between the surface lesion diameter on tubers, penetration and the percentage of rotten tissue were compared using Pearson's correlation analysis (SPSS Ver. 11) where $P < 0.05$ was considered statistically significant.

RESULTS

Analysis of variance of cultivar's data indicated that all the variables were affected significantly ($P \leq 0.01$) by the plant material tested (Table 1).

Effect of potato cultivars on tuber surface lesion diameter incited by *S. rolfisii*

Inoculated tubers of all cultivars, showed fan-like mycelial growth, forming symmetrical circles around the site of inoculation, typical of *S. rolfisii* infection. However, all non-inoculated tubers were symptomless. Significant ($P \leq 0.05$) differences were found in the mycelial growth, as measured by mean colony diameter noted after 72 h of incubation at 30°C. Data shown in Fig. 1 indicates that tuber surface lesions in excess of 4 cm in diameter were recorded on tubers of all cultivars. The cv. 'Bellini', showed the highest external lesion growth on tubers (6 cm) while the cvs. 'Elodie' and 'Daisy' exhibited the lowest lesion growth (approximately 4 cm). The remaining cultivars showed intermediate reaction to *S. rolfisii* infection based on lesion diameter. Thus, potato cultivars tested varied in their susceptibility to *S. rolfisii* but none of these was completely resistant. Furthermore, lesions expanded rapidly and completely girdled the inoculated tubers within 6-8 days.

Table 1 Analysis of variance of Sclerotium disease ratings on potato cultivars inoculated with *Sclerotium rolfisii*.

Disease ratings/Source	df	Mean square	F value ^a
Lesion diameter			
Cultivars	10	3.920	5.899**
Error	99	0.665	
Pathogen penetration			
Cultivars	10	22.205	3.565**
Error	99	6.228	
Rotten tissue			
Cultivars	10	710.250	7.282**
Error	99	97.542	
Stem rot			
Cultivars	10	10.145	11.601**
Error	55	0.874	

^a Values followed by ** are statistically significant at $P \leq 0.01$. df: degree of freedom.

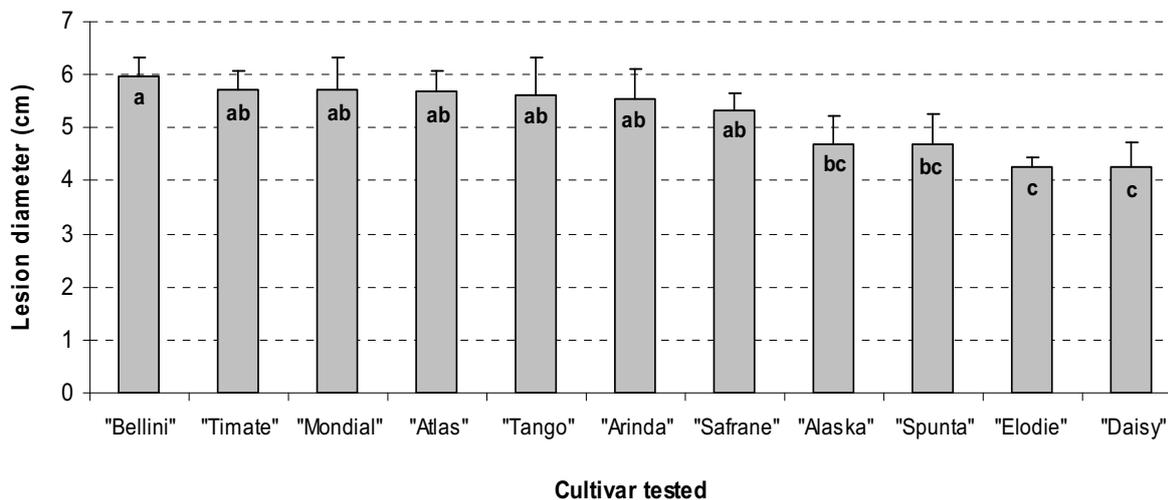


Fig. 1 Lesion diameter incited by *Sclerotium rolfsii* on potato cultivars after 72 h of incubation at 30°C. Different letters indicate significant differences in lesion diameter among cultivars. The error bars represent a 95% confidence interval.

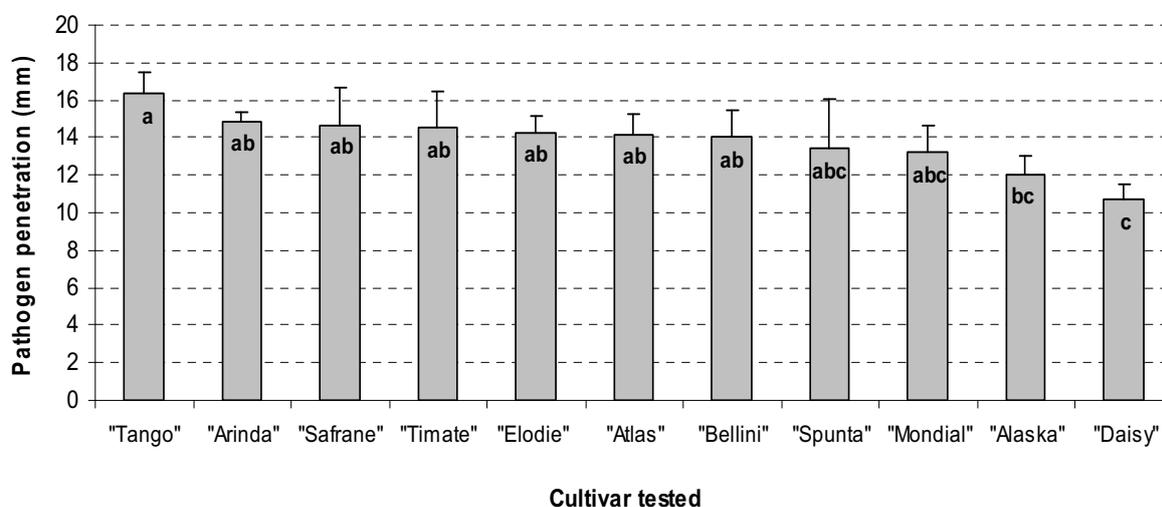


Fig. 2 Effect of potato cultivar on tuber rot severity incited by *Sclerotium rolfsii* and incubation for 8 days (30°C). The means with different values indicate significant differences among cultivars. The error bars represent a 95% confidence interval.

Effect of potato cultivar on tuber rot severity induced by *S. rolfsii*

In the inoculated tubers, disintegration of tuber tissue and subsequent rotting were observed, whereas no rot deve-

loped on the non-inoculated tubers. Tuber rot severity varied significantly ($P \leq 0.05$) among the cultivars tested. The lesion depth recorded after 8 days of incubation at 30°C ranged from between 10 to 16 mm (Fig. 2). The majority of cultivars tested showed significantly similar rot seve-

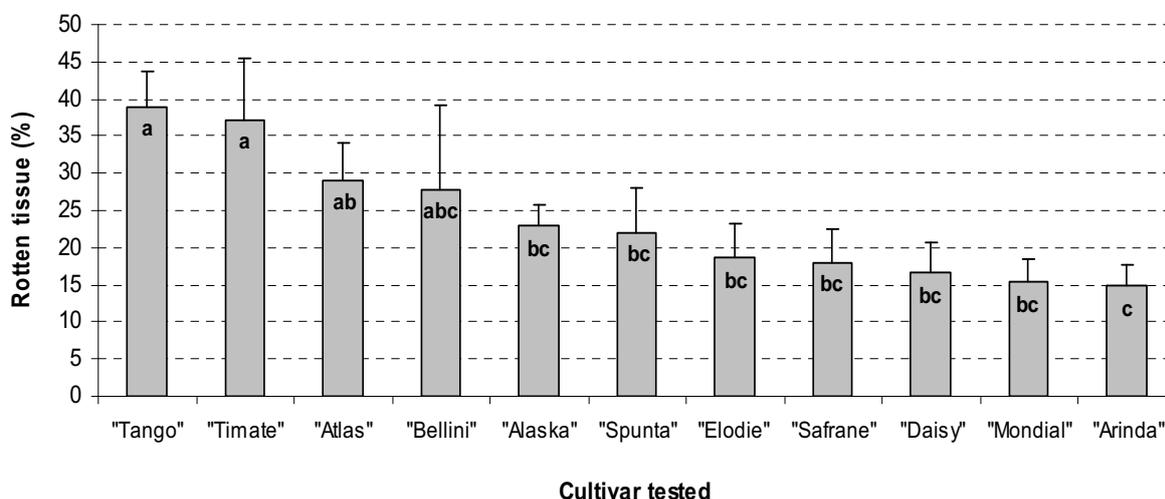


Fig. 3 The susceptibility of potato tubers of different cultivars to *Sclerotium rolfsii* following artificial inoculation. Tubers were assessed after 8 days of incubation at 30°C. Means with different letters indicate significant differences in tuber decay among cultivars. The error bars represent a 95% confidence interval.

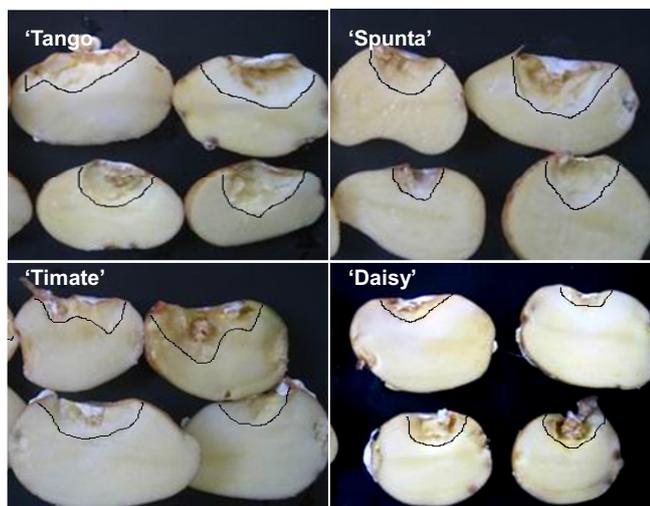


Fig. 4 Tuber rot severity induced by *Sclerotium rolfsii* following artificial inoculation and incubation at 30°C for 8 days. The cultivars 'Tango', 'Spunta' and 'Timate' were susceptible while 'Daisy' was tolerant to the pathogen. The markings with black line indicate the extent of tuber decay.

riety with the exception of 'Tango' and 'Daisy', which showed the highest and the lowest pathogen penetration (lesion depth), respectively.

Similarly, the percentage of rotten tissue recorded after 8 days of incubation at 30°C (Fig. 3) on inoculated tubers varied significantly ($P \leq 0.05$) with cultivars tested. The highest percentage (40%) was recorded on tubers belonging to cvs. 'Tango' and 'Timate' (Fig. 4) and to a lesser degree, to cvs. 'Atlas' and 'Bellini'. However, the other cultivars showed a significantly similar (15-23%) percentage of rotten tissue.

Pearson's correlation analysis revealed, a statistically significant positive correlation between the percentage of rotten tubers and the lesion diameter ($r = 0.224$, $P = 0.0181$; $n = 110$) when data was combined for cultivars. Nevertheless, analysis made on each cultivar showed that these parameters were significantly correlated only in the case of cv. 'Atlas' ($r = 0.689$, $P = 0.028$; $n = 10$)

Correlations analysis, for all cultivars pooled, showed a significant positive correlation between lesion depth (pathogen penetration) and the percentage of rotten tissue ($r = 0.383$, $P = 0.00004$; $n = 110$). However, when correlation analysis was done for each cultivar separately, the parameters were found to be significantly correlated only in the case of cvs. 'Spunta' and 'Timate' ($r = 0.755$, $P = 0.012$; $n =$

10 and $r = 0.765$, $P = 0.010$; $n = 10$, respectively).

Effect of potato cultivar on stem rot severity

Inoculated plants showed typical symptoms of stem rot with variable severity depending on the cultivar when assessed after three weeks of inoculation. Initially, infected stem tissues were soft, depressed, and brownish. The Southern blight fungus caused sudden wilting as the first symptom, on the severely affected plants, followed by the appearance of a fan-like, white fungal mycelia at the collar region and even the subsequent formation of sclerotia which first appeared as white nodules, but later turned brown.

Data presented in Fig. 5 showed that the most severe stem rot (of about 3-4) was observed on 'Tango' and 'Spunta' plants whereas 'Arinda' and 'Bellini' resulted in a disease score of 2 (DSI). However, all the other cultivars showed significantly similar stem rot severity and infection score did not exceed 1.

The isolation on PDA of the pathogen from diseased plants confirmed the involvement of *S. rolfsii* in the symptoms observed and assessed.

DISCUSSION

The present study reports, for the first time in Tunisia, the evaluation of the relative susceptibility of local potato cultivars to stem and tuber rots caused by *S. rolfsii*. In general potato cultivar susceptibility or resistance to this pathogen has not been quantified in many parts of the world, compared to the abundant literature on *S. rolfsii* involvement in other plants such as peanut (Branch and Csinos 1987; Smith *et al.* 1989; Breneman *et al.* 1990; Besler *et al.* 1997). In a previous research, Holm *et al.* (1987) described the resistance of an oblong russet 'Ute Russet' potato cultivar to leaf-roll net necrosis and *S. rolfsii*. The cultivar 'Patronis' was also reported to be highly susceptible to potato wilt caused by *S. rolfsii* (Bakr and Khan 1981) in another study. Similarly, Garibaldi *et al.* (2006) mentioned the susceptibility of cultivars 'Hermes' and 'Monalisa' based on premature plant death and rotting of tubers. Potato cultivars have also been reported to vary in their degree of susceptibility and reaction to *S. rolfsii*, but the researchers noted that many current cultivars have not been well characterized with respect to their reaction (Browne *et al.* 2002; Davis *et al.* 2007). Thus, more comparative studies for both stem and tuber rot intensity would yield much needed data and information in this pathosystem on potato.

Cultivar resistance or lower susceptibility to the disease could constitute a promising alternative method of controlling stem and tuber rots caused by *S. rolfsii*. This could be

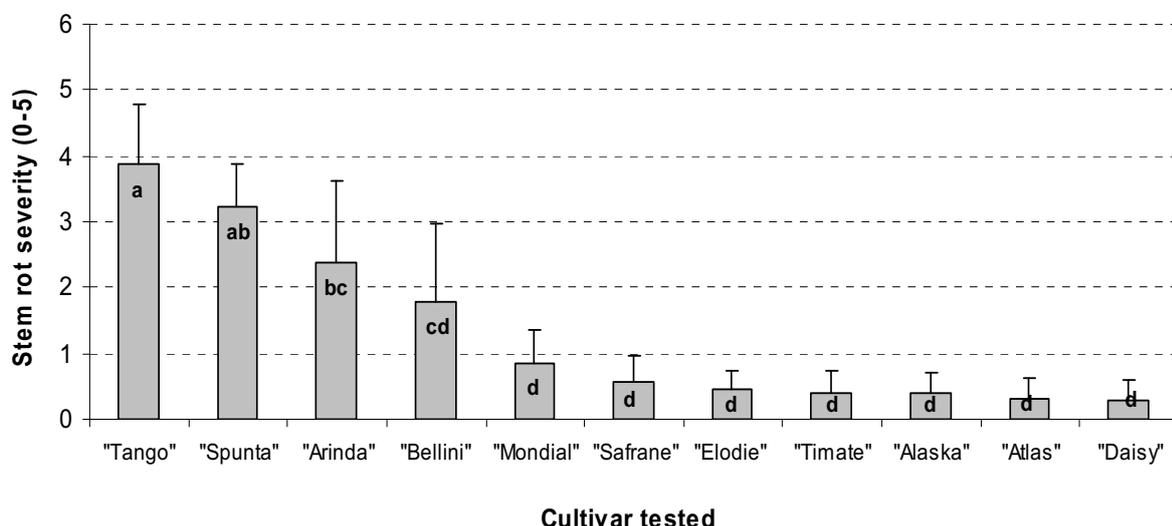


Fig. 5 Relative stem rot severity on potato cultivars incited by *Sclerotium rolfsii* after 3 weeks of incubation. The values with different letters indicate significant differences in stem rot severity among cultivars. The error bars represent a 95% confidence interval.

integrated with the other cultural practices such as management of crop residue and crop rotation (Backman *et al.* 1984; Franke *et al.* 1998). Previous research has shown that significant yield losses due to the disease can occur when potatoes are grown continuously or in short rotations with other crops susceptible to the disease, such as tomato (Aycock 1966; de Icochea 1981). Furthermore, Tunisian climate conditions prevailing mainly in late spring and autumn crops are both favourable to stem rot development, and serious tuber decay may occur in traditional stores where ambient temperatures range between 25-35°C (Khamassy *et al.* 2002; Daami-Remadi *et al.* 2007, 2010). Therefore, utilization of cultivars with resistance to the disease or non-host, alternative crops in the rotation crops compatible with Tunisian growing conditions would be useful for disease control.

The present screening of potato cultivar to *S. rolfsii* was based on several parameters i.e. external pathogen progress, penetration, importance of rotten tissue and stem rot severity. These disease parameters were positively and significantly correlated to each other implying that an increase in disease level in one variable may invariably infer an increase in the other variable. This may also imply that the presence of stem rot incidence or severity could also contribute to development of tuber rot severity in this pathosystem. In this research, it has been shown that none of the cultivars tested was resistant to *S. rolfsii* suggesting that variable degree of resistance exists. Therefore, precautions should be exercised in deciding which cultivars can be designated resistant and used in disease control. The high susceptibility of the cultivar 'Tango' to stem rot severity suggests that this cultivar cannot be used for *S. rolfsii* management, while the cultivar 'Daisy' was the most tolerant to the disease, implying that it can readily be used for disease control. Furthermore, the similarity of the susceptibility of cultivars 'Bellini' and 'Timate' to 'Tango' suggest that they are of little importance for disease control.

A higher incidence of Sclerotium disease on tubers was recorded based on lesion diameter, penetration and percentage of rotten tissue compared to stem rot severity. Although inoculated tubers were incubated under controlled conditions (high humidity and at 30°C), pot trials were incubated at temperature ranges between 10 and 29°C. This could explain the variability in cultivars response (stem rot severity) to *S. rolfsii* as opposed to tuber rot severity. Stem rot severity was highest on cultivars 'Tango' and 'Spunta', and to a lesser degree on 'Arinda' and 'Bellini'. These results may be supported by the research reports of de Icochea (1981), which showed that germination of sclerotia and mycelial growth of *S. rolfsii* may be favoured by aerobic conditions, high temperatures (28-30°C), and high relative humidity. Similar reports have also indicated that sclerotia degrade rapidly at temperatures exceeding 35°C (Vannacci *et al.* 1988). Other reports have shown that the disease tends to occur near the end of the potato cropping season in spring because the pathogen is most active at relatively warm temperatures ranging between 27 and 32°C (Browne *et al.* 2002).

The susceptibility of 'Spunta', the most cultivated potato cultivar in Tunisia, to stem rot was in accordance with other previously published reports (Daami-Remadi *et al.* 2007). The fungus is best known as a stem parasite of a range of economically important fruit and vegetable crops (Aycock 1966; Alexander and Stewart 1994), because moisture, oxygen and perhaps nutritional needs of the pathogen are more easily satisfied and conditions are often ideal for infection and disease development. Pathogen penetration on the cultivar 'Spunta' was comparable to the susceptible cultivar 'Tango' as approximately 20% of rotten tissue was recorded in this experiment. Therefore, precautions should be taken in the management of this disease in storage facilities since thermal conditions for disease establishment and pathogen penetration are favourable (Daami-Remadi *et al.* 2010), leading to contamination of healthy tubers with decaying ones.

The findings from our research are important as stem

rot or Sclerotium blight continues to be a threat to potato production in Tunisia by affecting the most important crops (during spring and autumn). Our research has identified cultivar resistance / susceptibility to *S. rolfsii* under potted and storage incubation conditions. Various stem rot and tuber rot parameters were utilized to assess cultivar resistance or susceptibility to this disease. This research also indicates the urgent need of testing other disease management measures, such as biocontrol with indigenous antagonists, other than host-resistance alone, since this is a serious pathogen on potatoes and other crops.

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