The African Journal of Plant Science and Biotechnology ©2010 Global Science Books

Effect of Temperature on *Sclerotium rolfsii* Mycelial Growth and Rot Severity on Potato Tubers

Mejda Daami-Remadi[1*] • Hayfa Jabnoun-Khiareddine[2] • Abir Sdiri[2] • Mohamed El Mahjoub[2]

[1] Centre Régional des Recherches en Horticulture et Agriculture Biologique, 4042, Chott-Mariem, Sousse, Tunisia
[2] Institut Supérieur Agronomique de Chott-Mariem, 4042, Chott-Mariem, Sousse, Tunisia

Corresponding author: * **daami_rm@yahoo.fr**

ABSTRACT

Sclerotium tuber rot incited by *Sclerotium rolfsii* is an emergent potato disease in Tunisia. Due to the known effects of temperature on several post-harvest pathogens of potato, the present study focused on the assessment of pathogen development *in vitro* and *in vivo* under different thermal conditions. The present study showed significant differences in the mycelial growth rate of *S. rolfsii*, as measured by mean colony diameter recorded after 24, 48 and 72 h at various temperatures (5-40°C) where the optimum was found to be 30-35°C on PDA. Significant differences in pathogen external and internal development were also noted on inoculated potato cv. 'Spunta' tubers. In fact, the maximum lesion diameter noted at tuber surface was observed at 30°C. However, the most severe soft (atypical) rot and the highest percentage of rotten tissue were recorded after 8 days of incubation at 35°C. Statistically significant positive correlations were noted between the tuber lesion diameter, pathogen penetration and the percentage of rotten tissue.

Keywords: atypical soft rot, disease severity, inoculation, optimum temperature, rotten tissue, storage

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) and it is a strategic crop in Tunisia (Azzouz 1996). It occupies about 16% of the areas cultivated for vegetable crops and it is mainly grown in two seasons i.e. spring and autumn and an additional crop may be grown in winter in coastal areas (Fahem and Haverkort 1988). Despite this strategic importance, the average potato yield does not exceed 14 t/ha due to several factors, including fungal diseases (Djébali and Tarhouni 2010).

Sclerotium rolfsii (teleomorph *Athelia rolfsii*) is a soil-borne fungal pathogen that causes Southern blight disease on a wide range of agricultural and horticultural crops, weeds and forest trees. The fungus is distributed around the world in tropical and subtropical regions (Aycock 1966). Although no statistical data are available, disease caused by this pathogen lead to heavy yield losses in vegetable crops especially during the wet season when weather conditions are favorable for both crop production and growth and dissemination of pathogen sclerotia (Wokocha *et al*. 1986). In Tunisia, it has been observed on potato on plants and rotting tubers on 2006 (Daami-Remadi *et al*. 2007). Due to its soil- and tuber-borne development, this fungus may lead to serious losses in plants, as wilting agent, and in stored tubers, as rot agent.

Temperature has been recognized as an important factor in the development of post-harvest diseases of potato. In fact, in hot conditions and when immature potatoes are harvested, significant losses can occur in storage. Thus, the monitoring of storage environment could be efficient in controlling post-harvest diseases (Tivoli and Jouan 1981; Bartz and Kelman 1984; Barr *et al*. 1996; Triki *et al*. 2001; Daami-Remadi *et al*. 2006). The health of seed potatoes plays a significant role in tuber rot incidence in stores as well as in the soil before plant emergence. As tubers are stored at different thermal conditions in Tunisia i.e. refrigerated and unrefrigerated traditional storage under open-air (under trees) or controlled conditions (Khamassy *et al*. 2002), the assessment of Sclerotium tuber rot severity under different temperatures is crucial. In fact, it may give additional information concerning the disease rate progress and consequently the threat of this pathogen emergence and may help future decision-making about suitable storage conditions with minimal rot development risk.

MATERIALS AND METHODS

Plant material

Apparently healthy and undamaged potato tubers cv. 'Spunta' were used. This cultivar is the most cultivated in Tunisia and known to be infected with *S. rolfsii* (Daami-Remadi *et al*. 2007). Tubers, kindly provided by the Technical Center of Potato, Essaïda (Tunisia), were stored at 6°C for two months before use. Just before inoculation, tubers were washed to remove excess soil, superficially sterilized in 10% sodium hypochlorite solution during 5 min, rinsed in distilled water and air dried.

Pathogen

S. rolfsii isolate used for tuber inoculation was previously cultured for 6 days at 25°C on potato dextrose agar (PDA) medium amended with 300 mg/l of streptomycin sulphate (Pharmadrug Production Gmbh, Hamburg, Germany). Its pathogenicity was already confirmed on potato cv. 'Spunta' (Daami-Remadi *et al.* 2007).

In vitro experiment

The effect of temperature on mycelial growth was evaluated on PDA supplemented with streptomycin sulphate (300 mg/l). Plates (Petri dishes, 85 mm in diameter) were inoculated with agar discs (diameter 6 mm) colonized by the pathogen and incubated at 5, 10, 15, 20, 25, 30, 35 or 40°C in the dark. In each incubator (at each temperature), plates (five replicates per temperature tested) were arranged in a completely randomized design. The colony diameter

Received: 21 August, 2010. Accepted: 28 September, 2010.

Original Research Paper

in each plate was measured along two axes perpendicular to one another at 24, 48 and 72 h after inoculation, and the two measurements for each incubation period were averaged.

Tuber inoculation and rot severity assessment

Tubers were wounded by a 6 mm diameter disinfected cork borer occasioning wounds of 6 mm in diameter and depth, which serve as sites of infection. Tuber inoculation was made by deposing a mycelial agar disc (6 mm diameter) colonized by the pathogen removed from a 7-day-old culture at 25°C. Inoculated tubers were incubated in a growth chamber at 5, 10, 15, 20, 25, 30, 35 or 40°C for 48 and 72 h at high relative humidity. Five tubers were used per elementary treatment (i.e. per temperature tested). Tubers that had been similarly wounded but non-inoculated were used as controls.

Pathogen external progress (i.e. external lesion diameter formed at the tuber surface) was estimated (as done for in the *in vitro* experiment) on all inoculated tubers at both incubation periods (48 and 72 h).

After 8 days of incubation, inoculated tubers were cut longitudinally at each wound site in half and the cut surfaces were checked for rot severity. Maximal width (w) and depth (d) were noted and the pathogen penetration (P) was calculated according to the formula of Lapwood *et al*. (1984) as follows:

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

Disease severity was also estimated via the percentage of rotten tissue according to Bourne *et al.* (1981) and Hildenbrand and Ninnemann (1994) methods used for soft rot susceptibility assessment. In fact, just prior to rot removal, the weight of each tuber (Wi) was determined, the rotten tissue was removed and tubers were weighed again (Wf). The percentage of rotten tissue was calculated as follows:

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

Statistical analyses

The mean radial growth (colony diameter) and the disease severity parameters were analyzed using completely randomized design. 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 the external lesion diameter, penetration and the percentage of rotten tissue were compared using Pearson's correlation analysis where $P < 0.05$ was considered statistically significant. Data were analyzed using SPSS ver. 11.

RESULTS

Effect of temperature on *S. rolfsii* mycelial growth

The mean radial growth of *S. rolfsii* recorded on PDA after 24, 48 and 72 h of incubation (**Fig. 1**) depended significantly (at $P \leq 0.05$) on temperatures tested. In fact, no mycelial growth was noted at 5, 10 and 40°C for all incubation periods. However, at 15 and 20°C, the mean colony diameter recorded after 72 h of incubation was approximately 2 and 4 cm, respectively, compared to more than 7.5 cm noted at 30 and 35°C. *S. rolfsii* mycelial growth was optimum at 30-35°C.

Effect of temperature on pathogen external progress at tuber surface

Inoculated tubers developed fan-like mycelial growth as external symptom whereas the non-inoculated control tubers were symptomless. The lesion diameter occasioned by *S. rolfsii* on potato cv. 'Spunta' tuber surface and recorded after 48 and 72 h of incubation (**Fig. 2**) varied significantly (at $P \leq 0.05$) with the different temperatures tested. In fact, no external disease progress i.e. no mycelium development was noted at 5, 10 and 40°C for both incubation periods. At 15 and 20°C, very little lesion diameter, of about 0.4 cm which was significantly similar to that noted at 5-15°C, was recorded after 48 h. However, temperatures comprised between 25 and 35°C had a significantly similar effect on disease external progress. However, after 72 h, the highest lesion diameter of about 3.5 cm was noted at 30°C compared to 2.5 cm recorded at 25 and 35°C. The external tuber colonization by *S. rolfsii* mycelium was optimum at 30°C.

Effect of temperature on Sclerotium tuber rot severity

The pathogen internal progress i.e. penetration (**Fig. 3**), noted after 8 days of incubation, depended significantly (at $P \leq 0.05$) on temperatures tested. In fact, no soft rot developed on tubers inoculated with *S. rolfsii* and incubated at 5, 10 and 40°C; the penetration recorded was of about 1.5 mm (calculated with wound dimensions) which was also significantly similar to that noted at 20°C (2.5 mm). However, the highest penetration (15.4 mm) was recorded at 35°C (**Fig. 4**) compared to 12 and 9.6 mm noted at 30 and 25°C, respectively. Thus, the pathogen was found to be most aggressive at 35°C.

The percentage of rotten tissue recorded after 8 days of incubation (**Fig. 5**) on inoculated tubers varied significantly (at $P \leq 0.05$) with temperatures tested. Indeed, tubers incubated at 5, 10, 15, 20 and 40°C showed significantly similar

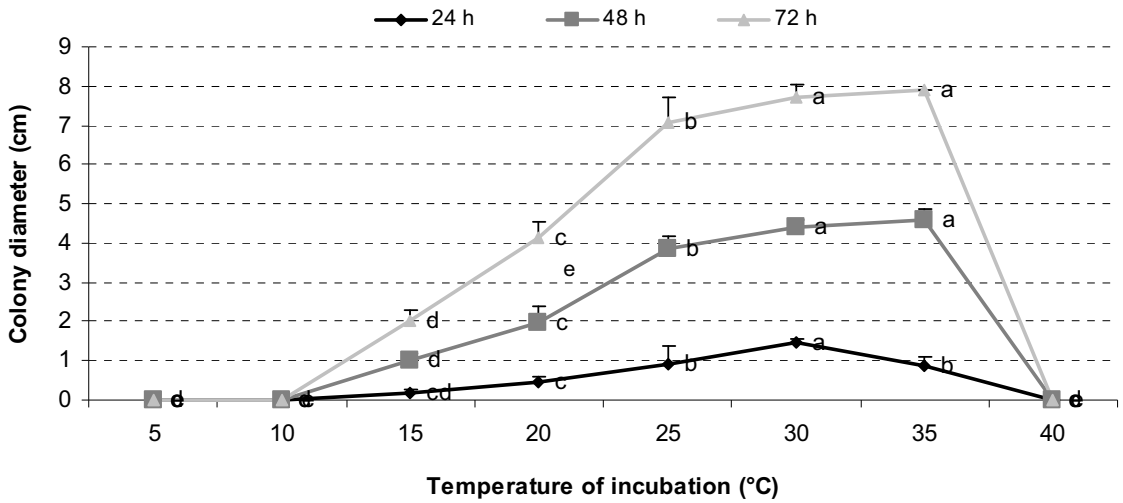

Fig. 1 Mean radial growth of *S. rolfsii* recorded on PDA after 24, 48 and 72 h of incubation at different temperatures. For each incubation period, means followed by different letters were significantly different according to the LSD test ($P \leq 0.05$).

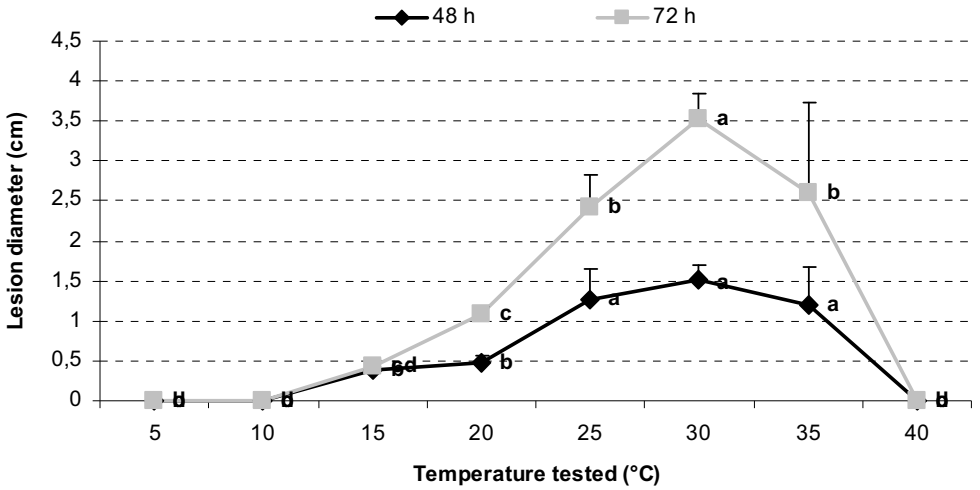

Fig. 2 Lesion diameter occasioned by *S. rolfsii* on potato cv. 'Spunta' tuber surface after 48 and 72 h of incubation at different temperatures. For each incubation period, means followed by different letters were significantly different according to the LSD test ($P \leq 0.05$).

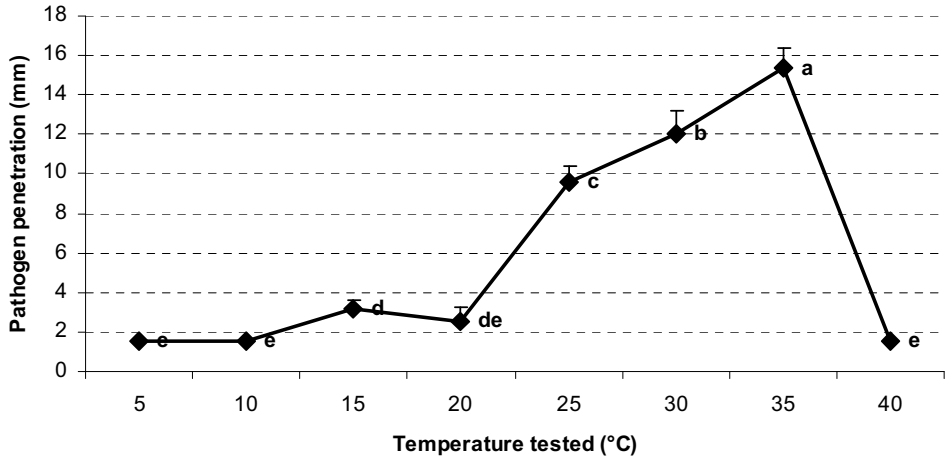

Fig. 3 Effect of temperature on Sclerotium tuber rot severity noted on cv. 'Spunta' after 8 days of incubation. Means followed by different letters were significantly different according to the LSD test ($P \leq 0.05$).

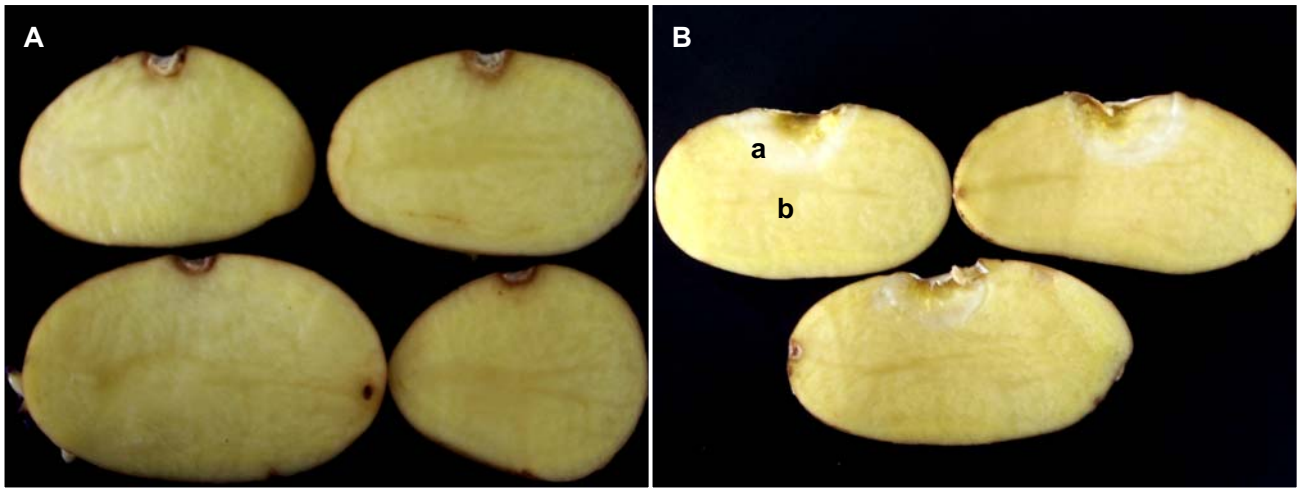

Fig. 4 Soft rot severity noted at 20°C (A) and 35°C (B) on potato tubers cv. 'Spunta' 8 days post-inoculation with *S. rolfsii.* a: rotten tissue; b: healthy tissue.

percentage of rotten tissue (≈ 0%). The highest disease severity was recorded at 35°C with a rotten tissue representing ≈ 10% of the total tuber weight compared to 7 and 2% noted at 30 and 25°C, respectively.

Thus, pathogen penetration and the percentage of rotten tissue were found to be optimum at 35°C.

S. rolfsii was consistently reisolated from all the inoculated tubers, while no symptoms or signs were observed on the non-inoculated control tubers.

Correlation analyses

Pearson's correlation analysis revealed, for all temperatures combined, statistically significant positive correlations between tuber lesion diameter, noted at 48 (r = 0.85, P = 0.000000000004; n = 40) and 72 h (r = 0.86, P = 0.000000000001; n = 40), and penetration.

Correlations analysis, for all temperatures pooled, showed a significant positive correlation between the tuber

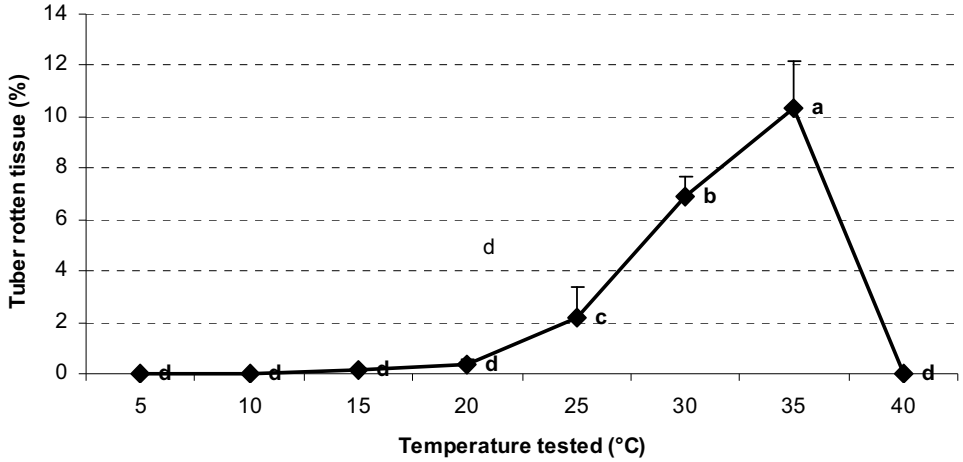

Fig. 5 Effect of temperature on the percentage of rotten tissue noted, after 8 days of incubation, on potato cv. 'Spunta' tubers inoculated with *S. rolfsii*. Means followed by different letters were significantly different according to the LSD test ($P \leq 0.05$).

lesion diameter, noted at 48 h ($r = 0.684$, $P = 0.000001$; n = 40) and 72 h ($r = 0.695$, $P = 0.000001$; n = 40), and the percentage of rotten tissue.

The percentage of rotten tissue was found to be significantly and positively correlated with pathogen penetration ($r = 0.898$, $P = 0.000000000000004$; n = 40).

DISCUSSION

Stem rot, Southern blight, Sclerotium rot or Sclerotium tuber rot is a soil-borne disease of economic importance on various legumes and numerous other cultivated plants including tomatoes, potatoes and ornamentals (Domsch *et al*. 1980). For potato crops, Lopes *et al*. (2008), cited by Ghini *et al.* (2008), considered that if the predictions about a rise in global temperature actually take place, subsequent to climate change, rainy season potato crops will be more stricken with diseases, mainly those of broad host range such as *S. rolfsii*, than winter crops. In fact, climate change was reported to invoke changes in epidemiology of pests and pathogens (Termorshuizen 2008).

S. rolfsii is an emergent pathogen in Tunisia (Daami-Remadi *et al*. 2007) and no data was available concerning its aggressiveness under different climatic conditions and especially temperature. This abiotic factor affects significantly the development of post-harvest potato pathogens especially in unrefrigerated traditional stores (Triki *et al*. 2001; Daami-Remadi *et al*. 2006).

The present study showed significant differences in the mycelial growth rate, as measured by mean colony diameter recorded after 24, 48 and 72 h at various temperatures. In fact, the optimum temperature required for the *in vitro* mycelial growth was found to be 30-35°C on PDA compared to 30°C on tuber surface, whilst the most severe soft rot and the highest percentage of rotten tissue were recorded at 35°C. Under Tunisian weather conditions, temperatures occurring in open-air traditional stores may even exceed 40°C whereas in improved traditional stores, temperatures are comprised between 25 and 30°C (Khamassy *et al.* 2002). These thermal conditions were known to favor *S. rolfsii* development (de Icochea 1981). Browne *et al*. (2002) reported that, in California, this pathogen is most active at relatively warm temperatures (27 to 32°C). Nevertheless, reports of Vannacci *et al*. (1988) indicated that sclerotia may degrade rapidly at temperatures exceeding 35°C. The local isolate of *S. rolfsii* used for tuber inoculation seems to have a slightly higher optimum temperature than the other reports and it seems to be more tolerant. Similarly, under natural conditions of a commercial field near Alessandria (Northern Italy), Garibaldi *et al*. (2006) observed potato plants showing severe basal rot symptoms during early July associated with a strong increase of air temperature (as much as 38°C) and relative humidity. These symptoms were artificially reproduced when inoculated plants were kept at temperatures ranging between 25 and 32°C. Moreover, according to Pane *et al*. (2007), the optimum growth temperature of *S. rolfsii* was recorded at 30 ± 2°C. Similarly, an invasive, white, cottony mycelium with a fan-like pattern and numerous, small, brown spherical sclerotia (0.5 to 4.0 mm in diameter) developed on infected tissues of ornamental citrus plants inoculated with *S. rolfsii* and incubated at 28 to 30°C. However, Raabe (1988) incubated Kiwi plants inoculated with *S. rolfsii* at 32.2 ± 2°C and 26.6 ± 2°C. Ambient temperature (24°C) was used in the pathogenicity tests of *S. rolfsii* on common chickweed and typical disease symptoms were reproduced on inoculated plants (Hollowell and Shew 2004).

This literature review, concerning temperatures used for *S. rolfsii* incubation, reveals the specificity of local isolates and supports the importance of the assessment of pathogen aggressiveness under Tunisian weather conditions characterizing potato stores.

The absence of pathogen growth and soft rot development at 40°C noted in the present study may be attributed to the loss of sclerotia viability beyond 30°C (Vannacci *et al*. 1988). The slight mycelium development on tuber surface recorded at 15 and 20°C may have an epidemiological impact when these tubers will be used as seeds. Indeed, severe plant wilting may occur in the field and mainly under late season conditions which are suitable for stem rot development. Many potato diseases are initiated as inoculum in seed tubers. Thus, as *S. rolfsii* mycelium may spread over and into the soil, sudden wilting may occur as the first symptom, followed by the appearance of a collar of fan-like, white fungal mycelium and seed tubers may decay before plant emergence (Alexander and Stewart 1994).

Sclerotium rot is known to be initiated at the stolon end of the tuber but can also occur at lenticels and wounds (de Icochea 1981). The current study reveals the rot severity that may occur on wounded and inoculated tubers. Moreover, even though the incubation period used did not exceed 8 days, for all the data presented, significant rots were observed. More tuber losses may occur under natural conditions where storage duration may exceed two months.

For the assessment of pathogen aggressiveness and consequently Sclerotium rot severity, several parameters were used i.e. external progress, penetration and importance of rotten tissue. These parameters were found to be positively and significantly correlated to each other and may increase the precision of the results obtained. In fact, the rate of lesion expansion, sclerotia production and viability are used by Akem and Dashiell (1991) to rank soybean cultivars for reaction to *S. rolfsii* under controlled conditions. However, Browne *et al.* (2002) graded on each tuber according to a visual estimate of the percentage of the tuber surface covered by pathogen mycelium. However, in the

present study, disease severity was not only estimated based on lesion diameter but also rot parameters i.e. penetration and percentage of rotten tissue as done for typical soft rot caused by *Pectobacterium* spp. (syn. *Erwinia* spp.) and other post-harvest fungal pathogens such as *Pythium* spp. and *Fusarium* spp. (Bourne *et al*. 1981; Hildenbrand and Ninnemann 1994; Triki *et al*. 2001; Lui and Kushalappa 2003; Daami-Remadi *et al*. 2006).

The control of this pathogen is difficult due to production of sclerotia which overwinter in the soil, emerge as inoculum and cause disease in the following season (Cilliers *et al.* 2003) and also due to the un-availability of soil fungicides (Maurya *et al*. 2007). Moreover, as potato areas are limited, and rotation is often difficult to apply in Tunisia, the use of less susceptible of cultivars to Sclerotium rot and wilt would obviously improve the control of this emergent disease.

ACKNOWLEDGEMENTS

Authors thank the Technical Potato Center of Tunisia (CTPT) for financial contribution. Many thanks to Aymen Youssef for technical assistance.

REFERENCES

Akem CN, Dashiell KE (1991) Detached shoot technique to evaluate the reaction of soybean cultivars to *Sclerotium rolfsii. Crop Protection* **10**, 325-327

Alexander BJR, Stewart A (1994) Survival of sclerotia of *Sclerotinia* and *Sclerotium* spp. in New Zealand horticultural soil. *Soil Biology and Biochemistry* **26**, 1323-1329

Aycock R (1966) Stem rot and other disease caused by *Sclerotium rolfsii*. North Carolina Agricultural Experiment Station Technical Bulletin No. 174

Azzouz M (1996) La pomme de terre en Tunisie. In: Rousselle P, Robert Y, Crosnier JC (Eds) *La Pomme de Terre*, INRA Editions, Paris, France, pp 552-577

Barr DJS, Warwick SI, Desaulnierx NL (1996) Isozyme variation, morphology, and growth response to temperature in *Pythium ultimum*. *Canadian Journal of Botany* **74**, 753-761

Bartz JA, Kelman A (1984) Bacterial soft rot potential in washed potato tubers in relation to temperature of tubers and water during simulated commercial handling practices. *American Potato Journal* **61**, 485-493

Bourne WF, McCalmont DC, Wastie RL (1981) Assessing potato tubers for susceptibility to bacterial soft rot (*Erwinia carotovora* subsp. *atroseptica*). *Potato Research* **24**, 409-415

Browne GT, DeTar WR, Sanden BL, Phene CJ (2002) Comparison of drip and sprinkler irrigation systems for applying metam sodium and managing stem rot on potato. *Plant Disease* **86**, 1211-1218

Cilliers AJ, Pretorius ZA, van Wyk PS (2003) Integrated control of *Sclerotium rolfsii* on groundnut in South Africa. *Journal of Phytopathology* **151**, 249-258

Daami-Remadi M, Jabnoun-Khiareddine H, Ayed F, El Mahjoub M (2006) Effect of temperature on aggressivity of Tunisian *Fusarium* species causing potato (*Solanum tuberosum* L.) tuber dry rot. *Journal of Agronomy* **5**, 350-355

Daami-Remadi M, Jabnoun-Khiareddine H, Ayed F, Hibar K, El Mahjoub M (2007) First report of *Sclerotium rolfsii* causing atypical soft rot on potato tubers in Tunisia. *Tunisian Journal of Plant Protection* **2**, 59-62

de Icochea TA (1981) Stem rot. In: Hooker WJ (Ed) *Compendium of Potato Diseases*, American Phytopathological Society, St. Paul., MN, USA, pp 50-51

Djébali N, Tarhouni B (2010) Field study of the relative susceptibility of eleven potato (*Solanum tuberosum* L.) varieties and the efficacy of two fungicides against *Rhizoctonia solani* attack. *Crop Protection* **29**, 998-1002

Domsch KH, Gams W, Anderson Z (1980) *Compendium of Soil Fungi*, Academic Press, London, UK, 859 pp

Fahem M, Haverkort AJ (1988) Comparison of the growth of potato crops grown in autumn and spring in North Africa. *Potato Research* **31**, 557-568

Garibaldi A, Gilardi G, Gullino ML (2006) First report of Southern blight incited by *Sclerotium rolfsii* on potato (*Solanum tuberosum*) in Northern Italy. *Plant Disease* **90**, 1114

Ghini R, Hamada E, Bettiol W (2008) Climate change and plant diseases. *Scientia Agricola* **65 (Special Issue)**, 98-107

Hildenbrand S, Ninnemann H (1994) Kinetics of phytoalexin accumulation in potato tubers of different genotypes infected with *Erwinia carotovora* ssp. *atroseptica*. *Physiological and Molecular Plant Pathology* **44**, 335-347

Hollowell JE, Shew BB (2004) First report of *Sclerotium rolfsii* on common chickweed in North Carolina. *Plant Disease* **88**, 426

Khamassy N, Ben Temim A, Arfaoui I (2002) Improved potato storage system. In: *15th Triennial Conference of the European Association for Potato Research*, 14-19 July, 2002, Hambourg Germany, p 162 (Abstract)

Lapwood DH, Read PJ, Spokes J (1984) Methods for assessing the susceptibility of potato tubers of different cultivars to rotting by *Erwinia carotovora* subsp. *atroseptica* and *carotovora. Plant Pathology* **33**, 13-20

Lui LH, Kushalappa AC (2003) Models to predict potato tuber infection by *Pythium ultimum* from duration of wetness and temperature, and leak-lesion expansion from storage duration and temperature. *Postharvest Biology and Technology* **27**, 313-322

Maurya S, Singh R, Singh DP, Singh HB, Srivastava JS, Singh UP (2007) Phenolic compounds of *Sorghum vulgare* in response to *Sclerotium rolfsii* infection. *Journal of Plant Interactions* **2**, 25-29

Pane A, Raudino F, Adornetto S, Proietto Russo G, Cacciola SO (2007) Blight of English ivy (*Hedera helix*) caused by *Sclerotium rolfsii* in Sicily. *Plant Disease* **91**, 635

Raabe RD (1988) *Sclerotium rolfsii* crown and root rot of kiwi (*Actinidia chinensis*). *Plant Disease* **72**, 1077

Schieber A, Aranda Saldaña MD (2009) Potato peels: a source of nutritionally and pharmacologically interesting compounds – A review. In: Yee N, Bussell WT (Eds) *Potato IV. Food* **3 (Special Issue 2)**, 23-29

Termorshuizen AJ (2008) Climate change and bioinvasiveness of plant pathogens: comparing pathogens from wild and cultivated hosts in the past and the present. In: *KNVP Symposium Pests and Climate Change*, 3 December, 2008, Wageningen, The Netherlands. Available online: http://www.knpv.org/db/upload/documents/Pests%20and%20climate%20change/ABSTRACTS_Pests_and_Climate_Change.pdf (Abstract)

Tivoli B Jouan B (1981) Inventory, frequency and aggressivity of different species or varieties of *Fusarium* responsible of potato tuber dry rot. *Agronomy* **1**, 787-794

Triki MA, Priou S, El Mahjoub M, Baudry A (2001) Leak syndrome of potato in Tunisia caused by *Pythium aphanidermatum* and *Pythium ultimum*. *Potato Research* **44**, 221-231

Vannacci G, Triolo E, Materazzi A (1988) Survival of *Sclerotinia minor* Jagger sclerotia in solarized soil. *Plant and Soil* **109**, 49-55

Visser RGF, Bachem CWB, de Boer JM, Bryan GJ, Chakrabati SK, Feingold S, Gromadka R, van Ham RCHJ, Huang S, Jacobs JME, Kuznetsov B, de Melo PE, Milbourne D, Orjeda G, Sagredo B, Tang X (2009) Sequencing the potato genome: outline and first results to come from the elucidation of the sequence of the world's third most important food crop. *American Journal of Potato Research* **86**, 417-429

Wang B, Yin Z, Feng C, Shi X, Li Y, Wang Q (2008) Cryopreservation of potato shoot tips. In: Benkeblia N, Tennant P (Eds) *Potato I. Fruit, Vegetable and Cereal Science and Biotechnology* **2 (Special Issue 1)**, 46-53

Wokocha RC, Ebenebe AC, Erinle ID (1986) Biological control of the basal stem rot disease of tomato caused by *Corticum rolsii* (Sacc) Curzi in Northern Nigeria. *Tropical Pest Management* **32**, 35-39