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

Mejda Daami-Remadi1* • Hayfa Jabnoun-Khiareddine2 • Abir Sdiri2 • Mohamed El Mahjoub2

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.

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 2002), the assessment of Sclerotium tuber rot severity 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

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.
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 depositing 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:

\[ P (\text{mm}) = \frac{w/2 + (d-6)}{2} \]

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 (Wf) was determined, the rotten tissue was removed and tubers were weighed again (Wi). The percentage of rotten tissue was calculated as follows:

\[ \text{Rotten tissue (\%)} = \left( \frac{W_i - W_f}{W_i} \right) \times 100 \]

**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 \( P < 0.05 \)) 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 \( P < 0.05 \)) 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 \( P < 0.05 \)) with temperatures tested. Indeed, tubers incubated at 5, 10, 15, 20 and 40°C showed significantly similar...
Effect of temperature on Sclerotium tuber rot severity. Daami-Remadi et al.

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...
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 inoculated 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 Dashiel (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; Hildbrand 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 soil fungicides. The use of less susceptible of cultivars to Sclerotium rolfsii and will 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, Dashiel KE (1991) Detached shoot technique to evaluate the reaction of soybean cultivars to Sclerotium rolfsii. Crop Protection 10, 325-327
Djebali N, Tarbouni 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
Hildbrand S, Ninnemann H (1994) Kinetics of phytoalexin accumulation in potato tubers of different genotypes infected with Erwinia carotovora subsp. atroseptica. Physiological and Molecular Plant Pathology 44, 335-347
Lui L-H, 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
Sequences the potato genome: outline and first results. In: Benkeblia N, Tennant P (Eds) Potato IV, Food 3 (Special Issue 2), 23-29
Tivoli B (1981) Inventory, frequency and aggressivity of different species or varieties of Fusarium responsible of potato tuber dry rot. Agronomy 1, 787-794
Wokocha RC, Ebenwee AC, Erinle ID (1986) Biological control of the basal stem rot disease of tomato caused by Corticum rolfsii (Sacc) Curzi in Northern Nigeria. Tropical Pest Management 32, 35-39