

# Effect of the Level of Seed Tuber Infection by *Rhizoctonia solani* at Planting on Potato Growth and Disease Severity

Mejda Daami-Remadi<sup>1\*</sup> • Samia Zammouri<sup>2</sup> • Mohamed El Mahjoub<sup>2</sup>

<sup>1</sup> Centre Régional des Recherches en Horticulture et Agriculture Biologique, 4042, Chott-Mariem, Sousse, Tunisia

<sup>2</sup> Institut Supérieur Agronomique de Chott-Mariem, 4042, Chott-Mariem, Sousse, Tunisia

Corresponding author: \* daami\_rm@yahoo.fr

## ABSTRACT

The effect of different levels of seed tuber infection by *Rhizoctonia solani* sclerotia at planting was assessed on the severity of stem canker and black scurf and on the growth and yield of potato (*Solanum tuberosum*) plants cv. 'Spunta'. Collar necrosis and tuber infection by *R. solani*, observed four weeks after planting, increased with the level of seed tuber infection by the pathogen. In fact, plants that developed from tubers with a 4 to 5 severity index at planting showed the presence of large stem cankers and a high incidence of black scurf. The vigour of the affected plants, estimated by the fresh and dry weights of the aerial and the subterranean plant parts, was negatively proportional to the level of tuber infection. Furthermore, the most important losses in the mean fresh tuber weights, depending of the level of seed tuber infection, reached 60% for plants forming from tuber with the highest level of infection. This study showed the role of initial seed tuber infection on disease development, severity and expected impact on yield.

**Keywords:** black scurf, *Rhizoctonia solani*, seed tuber, *Solanum tuberosum*, stem canker severity

## INTRODUCTION

*Rhizoctonia solani* Kuhn is an important fungal pathogen that causes both stem canker and black scurf of potato, and which can lead to reduced tuber yield and losses in tuber quality due to the development of malformed tubers and the alteration in target size and number of tubers (Baker 1970; Hide *et al.* 1973; Frank 1978, 1981; Anderson 1982; Carling *et al.* 1989; Jager *et al.* 1991; Jeger *et al.* 1996). Thus, the marketable tuber yield is considerably reduced. Furthermore, stem lesions can occur and reduce the total tuber yield by limiting the transport of nutrients throughout the infected plant (Brewer and Larkin 2005; Roland 2006).

This disease affects potato development from emergence to harvest and typical symptoms include death of pre-emerging sprouts, cankers on underground stem parts and stolons, a diminished root system, and the formation of sclerotia on progeny tubers (El Bakali and Martín 2006). The major problem of this disease resides when infected tubers are used as seeds serving as major sources of inoculum for future potato crops. In fact, under cold and wet soil conditions, the germination of sclerotia present on these tubers was favoured. Consequently, the development of sprouts was slowed leading to the development of cankers characterised by brown and black sunken lesions on young and under-developed tissues (Tsror (Lahkim) *et al.* 2001).

In Tunisia, *R. solani* sporadically infects potato and it has not been previously studied because even the infected tubers are locally marketed. However, this disease has become a veritable threat to potato culture even in newly created production areas such as Gafsa where potato has never been cultivated. Furthermore, it is actually responsible for the serious refusal of tubers for export, a developing and a promising market, especially those of cv. 'Nicola' and it was recently observed even on tubers destined to the local market, mainly cv. 'Spunta'. As the inoculum level of seed tubers is so important for the establishment of this disease, especially in non-infested areas, and self-produced uncontrolled seed tubers are frequently used for late crop produc-

tion (autumn crop), the objective of this study was to assess the effect of different levels of seed tuber infection by *R. solani* sclerotia, at planting, on the severity of stem canker and black scurf and on the growth and yield of potato plants under controlled conditions.

## MATERIALS AND METHODS

### Plant material

Potato tubers (cv. 'Spunta') naturally infected by *R. solani* sclerotia, produced at the Regional Center of Research in Horticulture and Organic Agriculture of Chott-Mariem (Tunisia), were used in this study. They were stored for about three months at 5-6°C in the dark before use in February, 2007.

Tested tubers showed variable amounts of *R. solani* sclerotia on their surfaces and their level of infection was estimated and classified according to a 1 to 5 French scale used for tuber disease assessment (Anonymous 2007).

### Effect of level of seed tuber infection on severity of *Rhizoctonia* stem canker

Tubers were superficially disinfected in a sodium hypochlorite solution diluted to 5%, for 5 min then rinsed with water and air dried. Tubers were placed under environmental conditions favourable for pre-germination (15-20°C, 60 to 80% relative humidity and natural room light) for two weeks before planting. Germinating tubers were then individually planted in plastic pots (6 litres) containing a mixture of perlite and peat (25:75) previously sterilized for 60 min at 107°C. After emergence, plants were irrigated every 2 to 3 days.

Four weeks post-plantation, plants were uprooted and their roots were washed with tap water, then air dried. An index of collar necrosis and tuber infection was determined for all treatments according to the scale presented in Fig. 1. Other parameters such as tuber fresh weight and aerial and subterranean parts fresh and dry weights were also noted per plant.

The pathogen was isolated from diseased plants and from every treatment to confirm that *R. solani* was responsible for the



Fig. 1 Scale used for the assessment of *Rhizoctonia* stem canker severity. From Zammouri 2007.

observed symptoms.

The assay was arranged in a completely randomized experimental design where the sole fixed factor corresponded to the level of seed tuber infection. Six replicates were used per treatment. Data were analyzed using SPSS (Ver. 11) and subjected to analysis of variance and Fisher's least significant difference test, LSD at  $p \leq 0.05$ .

## RESULTS

### Effect of level of infection of seed tubers on severity of *Rhizoctonia* stem canker and Black scurf

The effect of the infection level of seed tubers on the severity *Rhizoctonia* stem canker was assessed on plants that developed from naturally infected tubers showing a variable amount of sclerotia at their surface at planting. In fact, two weeks after plantation, the first symptoms of *Rhizoctonia* stem canker were observed and they were accompanied by an irregular emergence of plants, mainly when derived from seed tubers showing the forth and the fifth levels of infection. However, three to four weeks post-plantation, necrosis and cankers appeared at the stem bases of plants. This phenomenon was followed by wilting and yellowing of leaves of plants that emerged from seed tubers with the fifth level of infection.

### Collar necrosis and tuber infection indexes

Figs. 2 and 3 show that the indexes of collar necrosis and tuber infection – noted by the presence of *R. solani* – four weeks after planting, increased with the initial level of seed tuber infection by the pathogen. In fact, the collar index was about 0.4 for the first level and reached 2.25 for the fifth one. However, on tubers, these indexes varied from 0 to 2.37 for the first and the sixth level, respectively. These values reflected the severity of stem canker and black scurf present on plants and progeny tubers, respectively. In fact, plants developing from seed tubers having the levels 1 and 2 showed small brown necrotic lesions whereas the tubers produced had no sclerotia on their surface and limited lenticel infection. However, plants derived from tubers with levels 4 to 5 at planting had large stem cankers and sclerotia covering the progeny tubers almost totally (Fig. 4).

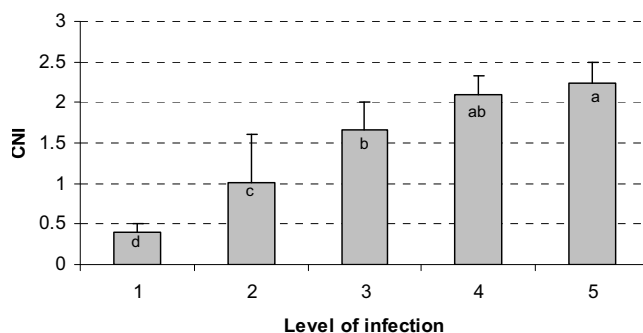


Fig. 2 Collar necrosis indexes (CNI) noted four weeks post-plantation on cv. 'Spunta' plants derived from seed tubers showing different levels of infection by *R. solani* sclerotia at planting.

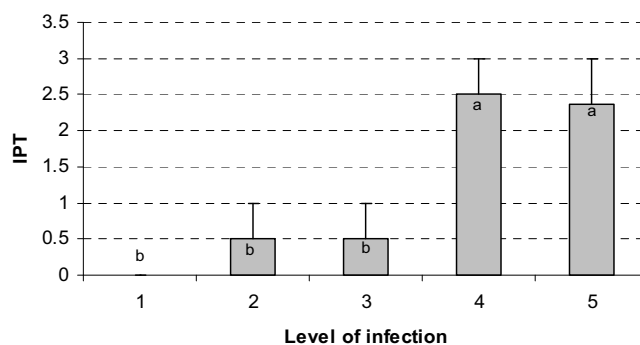


Fig. 3 Indexes of progeny tuber (IPT) infection by *R. solani* sclerotia noted four weeks post-plantation, on cv. 'Spunta' plants derived from seed tubers presenting different levels of infection at planting.

### Effect of the level of infection of seed tubers on potato aerial part weight

Fresh and dry weights of the aerial part of potato plants (Figs. 5 and 6), noted one month post-planting, varied significantly depending on the level of seed tuber infection by *R. solani* sclerotia at planting. Generally, when the level of infection is higher, the aerial fresh weight is less, showing that vigour of the affected plants is negatively proportional to the tuber infection level.

Plants derived from seed tubers at the first level of in-



Fig. 4 Severity of stem canker and black scurf on potato cv. ‘Spunta’ plants derived from seed tubers presenting level 5 of infection by *R. solani* sclerotia at planting.

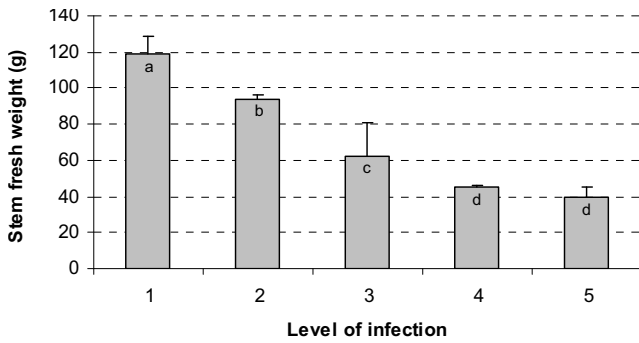


Fig. 5 Fresh weight of the aerial part noted, four weeks post-planting, on potato cv. ‘Spunta’ plants derived from seed tubers presenting different levels of infection by *R. solani* sclerotia at planting.

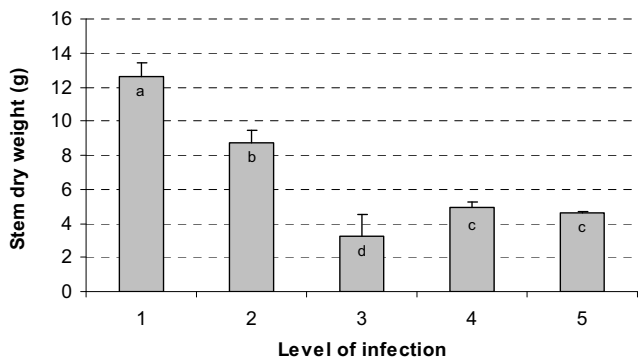


Fig. 6 Dry weight of the aerial part noted, four weeks post-planting, on potato cv. ‘Spunta’ plants derived from seed tubers presenting different levels of infection by *R. solani* sclerotia at planting.

fection showed no reduction in fresh and dry weights whereas for those derived from the second one, both parameters were reduced by about 15% relative to the first level of infection. However, in the case of fourth and fifth levels of infection, plants showed yellowing and wilting symptoms. Furthermore, losses in fresh and dry weights, under the environmental conditions of the culture, reached 78% when tubers with level 5 were planted, when compared to level 1.

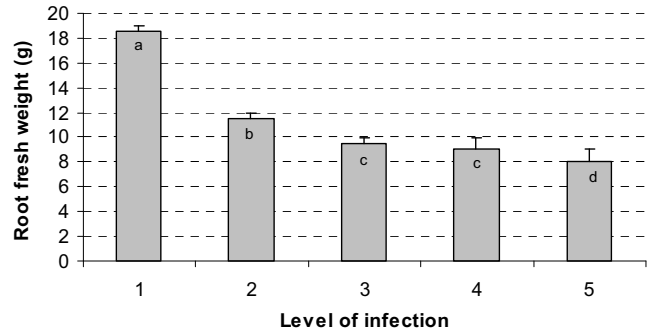


Fig. 7 Fresh weight of the underground parts noted four weeks post-planting, on potato cv. ‘Spunta’ plants derived from seed tubers showing different levels of infection by *R. solani* sclerotia at planting.

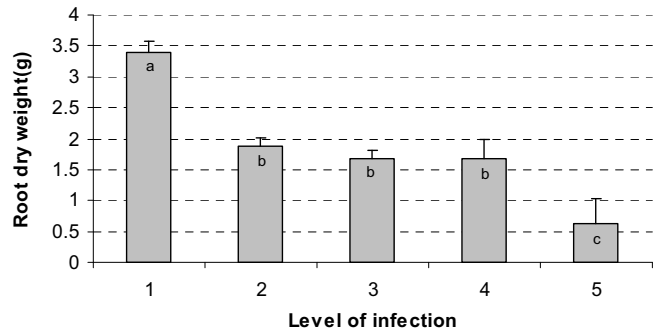


Fig. 8 Dry weight of the underground parts noted four weeks post-planting on potato cv. ‘Spunta’ plants derived from seed tubers showing different levels of infection by *R. solani* sclerotia at planting.

*R. solani* was isolated from stem cankers developed on all diseased plants and this confirmed its involvement in these symptoms.

### Effect of degree of seed tuber infection by *R. solani* sclerotia at planting on weight of underground parts

Root fresh and dry weights (Figs. 7 and 8) significantly varied depending on the level of seed tuber infection by *R. solani* sclerotia at planting. Plants derived from tubers having level 1 infection were characterized by a vigorous root system compared to roots of plants originating from tubers with 3, 4 and 5 levels of infection. The percentages of fresh and dry weight loss varied from 9 to 14% compared to the first level of infection by *R. solani* sclerotia.

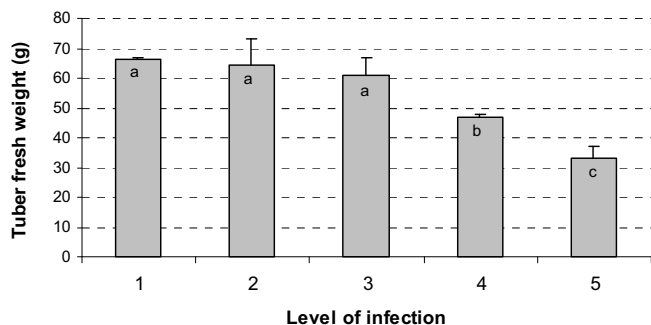
For lower levels of infection such as 1 or 2, the fungus present at the tuber surface as sclerotia had no significant effect on the root system development and damage was limited to some necrosis formed on stolons and roots. However, for levels superior or equal to 3, the root system was affected and finally rotted in potato plants derived from seed tubers with the level 5 of infection by *R. solani* at planting.

*R. solani* was isolated from diseased underground parts and this confirmed its involvement in these symptoms.

### Effect of level of infection of seed tubers by *R. solani* before planting on the weight of progeny tubers

Fig. 9 shows that tuber fresh weight is negatively proportional to the level of seed tuber infection by *R. solani* sclerotia. In fact, for plants derived from tubers having infection levels 1, 2 and 3, this parameter was statistically comparable whereas for those showing the fourth and the fifth levels of infection, tuber fresh weight was significantly reduced by about 20% compared to the other levels of infection.

Furthermore, the most important losses in mean tuber



**Fig. 9** Tubers fresh weight noted, four weeks post-planting on potato cv. 'Spunta' plants derived from seed tubers having different levels of infection by *R. solani* sclerotia at planting.

weight, depending on the level of initial seed tuber infection, reached 60% for plants derived from the highest tuber infection level. These plants also showed weak vigour, estimated via the relative fresh and dry weights of the aerial and the subterranean plant parts (Figs. 5, 6, 7 and 8), wilting and reduced tuber size. The tubers that formed were small, deformed and had sclerotia at their surface (Fig. 4).

*R. solani* was isolated from tubers showing black scurf and this confirmed its involvement in the infection of tubers.

## DISCUSSION

The present study showed the profound negative effect of the level of initial seed tuber infection by *R. solani* sclerotia at planting on plant growth and yield under controlled conditions. The present pot experiment has the advantage of following disease severity on a sterile substrate (peat and perlite) where only tuber-borne *R. solani* sclerotia are normally responsible for the induced symptoms. The negative effect on plant growth and yield is expected to be more severe under field conditions because both soil-borne and tuber-borne inoculum could interact in disease development and severity. The initial tuber infection led to the development of necrosis and cankers at the stem bases of plants in culture followed by the wilting and yellowing of leaves mainly when seed tubers were heavily infected. Similar symptoms were reported by Betencourt *et al.* (1971) in France under field conditions on cultivars 'Arran', 'Banner', 'B.F.15', 'Bintje', 'Farfa-dette', 'Ke Pondy', 'Saskia' and 'Sirt' and by Roland (2006) in the United Kingdom. This initial tuber infection by sclerotia served for pathogen dissemination into new areas within or even outside countries during plant material transfer and inhibited potato development when heavily infected seed tubers were planted as reported by Wharton *et al.* (2007) in Michigan, USA.

In the present study, symptoms caused by *R. solani*, visualized by large stem cankers and sclerotia covering all progeny tubers, were shown to be more severe in plants derived from tubers having initial levels of infection of 4 to 5 at planting. These findings confirm results obtained by Tsror (Lahkim) and Peretz-Alon (2005) who found that both major sources of inoculum, seed tubers and soil, are important in disease development and that the subterranean symptoms were significantly higher in plots where both seed tubers and soil were contaminated. Furthermore, under these conditions, the incidence of black scurf and severity on daughter tubers was very high. In the same way, Simons and Gilligan (1997a) reported that when seed inoculum densities were of 10 and 40%, disease incidence varied between 6 and 57% and the percentage of lesions was about 7 to 72% for lowest and highest inoculum density, respectively. Simons and Gilligan (1997b) found that the differences in the incidence of stem canker at stem emergence were dominated by inoculum density with 7% and  $70 \pm 2.4\%$  at the lower and higher densities, respectively.

Inoculum initially present at seed tuber surface at planting is likely to infect more rapidly the subterranean parts of

potato plants. This was substantiated by Bailey *et al.* (2000), who reported that the lateral spread of pathogens such as *R. solani* depends on the endogenous supply and translocation of nutrients within the fungal colony, the growth-habit of the colony and the distances between susceptible host roots or other organs.

The present results showed that the vigour of the affected plants is negatively proportional to the tuber infection level by *R. solani* sclerotia and that plants derived from tubers having the level 1 were characterized by a vigorous root system compared to the other higher levels. Furthermore, losses in fresh and dry weights of aerial and subterranean parts of potato plants reached 78% when seed tubers having an initial level of 5 were used. Zammouri (2007) found that this reduction in plant vigour also varied depending on the potato cultivar used and that plant growth was reduced by 10% in tolerant cultivars such as 'Atlas', 'Arinda' and 'Safrane' compared to 30-70% in susceptible ones such as 'Tango' and 'Spunta'. However, preliminary field studies in Catalonia, Spain indicate that disease severity (*R. solani* AG-3) is not dependent on cultivar or related to yield reduction (10 to 65%), but is related to soil and seed contamination with sclerotia and mycelium (El Bakali *et al.* 2000).

This negative effect on plant growth is related in part, according to Simons and Gilligan (1997b), to the number of stolons and stems formed which seems to also be affected by seed tuber initial infection density. Aoki *et al.* (2005) reported that this negative effect of *R. solani* on plant growth is also due to some phenolic and glucosidic phytotoxic substances produced by the pathogen and involved in the inhibition of potato root growth.

Our results indicate that in plants derived from tubers having the fourth and the fifth levels of infection by *R. solani* sclerotia at planting, the fresh weight of progeny tubers was significantly reduced by about 20% compared to the other, lower levels of initial infection.

Most reports of significant yield reductions have been associated with low temperatures and moist conditions. In Tunisia, as in other areas with relatively high temperatures during the growing season, the major damage is to quality of the tubers and rarely to the total yield (Tsror (Lahkim) *et al.* 1996; Zammouri 2007). In the same way, Simons and Gilligan (1997b) also reported that the density of inoculum had no effect on total yield but it did affect the size distribution. In fact, even in our present work, tubers produced by plants issued from heavily infested seeds by *R. solani* sclerotia at planting, are small and deformed as observed by Betencourt *et al.* (1971) and have sclerotia at their surface as also reported by Simons and Gilligan (1997a, 1997b). Many others studies reported downgraded tuber quality by tuber-borne sclerotia (Jager *et al.* 1991), development of malformed (misshapen) tubers, alteration in target size and number of tubers and a marketable tuber yield reduction (Hide *et al.* 1973; Frank 1978, 1981; Anderson 1982; Carling *et al.* 1989; Read *et al.* 1989; Jeger *et al.* 1996). This damage seems to be directly related to the appearance of stem cankers on stolons and stems and due to problems in starch translocation and nutrient movement from leaves to tubers leading to these reduced yields (Brewer and Larkin 2005; Roland 2006).

In Tunisia, Rhizoctonia disease is mainly managed by cultural practices and methods that minimize prolonged contact of the plant or tubers with the pathogen, such as planting in warmer, drier conditions to promote rapid sprout emergence and early harvesting before sclerotia formation on tubers. Furthermore, the present study focused on the use of seed tubers free of *R. solani* infection for minimizing disease incidence and avoiding its spread to non-infected areas. However, as potato areas are limited, and rotation is often difficult to apply, and auto-produced uncontrolled seeds are frequently used by farmers, other studies focusing the study of the impact of *R. solani* inoculum on potato growth and yield under field conditions and depending on cultivars used are required. Testing of several seed tuber preplanting

treatments, even in the presence of *R. solani* sclerotia, are also needed for reducing emergence problems due to early infections by this pathogen.

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