Comparative Aggressiveness of Tunisian 

Colletotrichum coccodes Isolates on Potato Assessed via 
Black Dot Severity, Plant Growth and Yield Loss

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ABSTRACT

Black dot of potato has become a serious disease in Tunisia. Colletotrichum coccodes, the causal agent, was found to be widely dispersed throughout the major potato-growing areas. Disease incidence varied depending on regions surveyed and potato organs sampled. The aggressiveness of local C. coccodes isolates was tested on potato cv. ‘Spunta’ plants based on disease severity records on the below-ground plant parts, plant growth and expected yield. Black dot severity, noted 60 days post-planting, was influenced by C. coccodes isolates used for inoculation and a variation in pathogen aggressiveness was recorded. The sclerotial density was higher on roots and stolons than on the below-ground stems. C. coccodes aggressiveness was directly associated with reduced growth, i.e. fresh and dry weights, of the below- and above-ground plant parts. For all fungal treatments or isolates combined, no significant correlation was found between black dot severity and tuber yield although 47% of yield loss was recorded subsequent to inoculation with some Tunisian C. coccodes isolates. Therefore, as shown in the present study, this pathogen may by itself influence potato growth and disease intensity and should be considered as a primary-disease-causing organism.

Keywords: anthracnose, early dying, inoculation, sclerotial density, Solanum tuberosum L., stunting, tuber weight

INTRODUCTION

Black dot (or anthracnose) of potato (Solanum tuberosum L.) is caused by the polyphagous and cosmopolitan soil-borne fungus Colletotrichum coccodes (Wallr.) Hughes (synonyms C. atramentarium (Berk. & Broome) Taubenh. and C. phomoides (Sacc.) Chester). The disease name refers to the abundant, small, black sclerotia produced on infected tubers, stolons, roots and stems (Dillard 1992; Ingram and Johnson 2010). It is most important in areas with dry and hot conditions, such as the Mediterranean regions (Daami-Remadi and El Mahjoub 2004), USA (Barkdoll and Davis 1992; Aqeel and Cobb 1997), South Africa (Denner et al. 1997) and southern Australia (Harding and Wicks 2005). It is sig- nalled in more temperate areas, such as UK, France, the Netherlands and Germany (Scholte et al. 1985; Reid and Hide 1988; Carnegie et al. 2003; Glais-Varlet et al. 2004; Tsror (Lahkim) et al. 2007).

Symptoms caused by C. coccodes include sloughing of the root cortex, brown lesions on roots and blemishes on tubers (Dickson 1926; Stevenson et al. 2001). The disease is characterized by the development of sclerotia on senecing and dead potato roots, stolons, and stems, and on potato tubers (Read and Hide 1988, 1995; Johnson and Miliczky 1993; Tsror (Lahkim) and Johnson 2000; Nitzan et al. 2002; Lees and Hilton 2003; Nitzan et al. 2005). The blemishes usually develop prior to harvest, become more evident during storage and reduce tuber quality (Tsror (Lahkim) and Johnson 2000). Blemishes are usually superficial, but if the infection is severe it may cause tubers to shrivel (Read 1991) and may progress to more deep and sunken lesions (Glais and Andrivon 2004; Griffiths et al. 2010). C. coccodes can cause premature foliage death and yield losses at harvest as well as weight loss in storage (Hunger and McIntyre 1979). In fact, yield losses as much as 30% were repor- ted on susceptible cultivars in addition to reduction of tuber quality. Moreover, the reported percentages of yield reduc- tions refer to the total tuber yield, but real losses of marketable product are higher (Tsror (Lahkim) et al. 1999a; Tsror (Lahkim) and Johnson 2000; Lees and Hilton 2003; Nitzan et al. 2006b).

In addition to their direct effects on tuber quality, blemish presence can increase inoculum potential for subsequent planting seasons. In fact, C. coccodes is diverse from a pathological point of view: it infects a range of plant species within and outside the Solanaceae, and shows some specific interactions with individual potato cultivars (Dillard and Cobb 1997; Tsror (Lahkim) et al. 2007). Furthermore, it was recently reported that C. coccodes sclerotia survive in soil for at least 8 years (Dillard and Cobb 1998). Data obtained to date suggest that the number of potato crops planted in a field directly influences the soil popula- tion of C. coccodes and may lead to substantial yield losses even in the absence of pests or pathogens well known to reduce tuber yield (Gudmestad et al. 2007). Thus, once established in a field, the recommended 3- to 4-year crop rotation is not sufficient to result in a significant decrease in C. coccodes viable inoculum.

In some countries the fungus causes wilting (Thiru- malchar 1967), but it is generally regarded as a weak pathogen capable only of colonizing the vascular system of plants weakened by some other biotic and/or abiotic stresses (Stevenson et al. 1976; Otazu et al. 1978; Reid and Hide 1988; Johnson and Miliczky 1993; Olanya et al. 2010). Moreover, it was reported that the early dying complex in the USA is exacerbated by the presence of C. coccodes. The fungus can cause reductions in yield and quality by itself (Johnson 1994; Tsror (Lahkim) et al. 1999a), but appears to be particularly important with co-infections of Verticillium dahliae and other soil-borne fungi and parasites (Otazu et al.
Plant material

MATERIALS AND METHODS

The purpose of this study was to quantify the intensity of black dot severity and appropriate control measures are necessary to avoid high disease incidence. Therefore, the purpose of this study was to quantify the intensity of pathogen attack and the subsequent effect on plant growth and the expected yield based on several parameters.

MATERIALS AND METHODS

Plant material

Potato (Solanum tuberosum L.) cv. ‘Spunta’ seed tubers were used to test the aggressiveness of the selected C. coccodes isolates. This cultivar is the most cultivated in Tunisia and is known to be infected with C. coccodes (Daami-Remadi and El Mahjoub 2004). Visually healthy tubers were superficially disinfected with a 10% sodium hypochlorite solution for 5 min, rinsed with tap water, air dried and placed under favorable environmental conditions to sprout (15-20°C, 60-80% relative humidity and natural room light).

At the multi-germ stage, tubers were individually planted in plastic pots (25 cm diameter, 1.6 l volume) containing a mixture of peat and perlite (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 need until inoculation date.

Pathogen

C. coccodes isolates were collected from either self-produced seed tubers or tubers for consumption, as well from infected stems and roots collected from different locations in the major potato-growing regions in Tunisia.

They were cultured on potato dextrose agar (PDA) medium amended with 300 mg/l of streptomycin sulphate (Pharmadru Production GmbH, Hamburg, Germany). Plates were incubated at 25°C in the dark for 7 days.

Liquid cultures used for inoculation were prepared on potato dextrose broth (PDB) and incubated at 25°C under continuous agitation at 150 rpm during 4 to 5 days. The obtained suspension was filtered through four layers of cheesecloth. The conidial suspension was adjusted with sterile distilled water to a final concentration of 10⁶ conidia/ml with a Malassez cytometer.

For their long term preservation, pathogen isolates were stored up to 12 months at -20°C in a 30% glycerol solution.

Assessment of black dot incidence

Field surveys were made on 2008 in the major potato-growing areas in Tunisia (32 fields belonging to 8 growing regions) to determine the incidence of black dot disease. Crop surveys were done before total plant senescence. Twenty potato cv. ‘Spunta’ plants showing chlorotic foliage and early dying symptoms were diagonally sampled per field. In the laboratory, the below-ground parts (stems bases, roots, stolons, tubers) were washed with tap water and examined for C. coccodes infection based on the presence of microsclerotia and cortical symptoms (tuber blemishing or cortical rot).

Ten segments of the below-ground organs (each 1 cm in length) taken from each plant were surface-sterilized with 10% sodium hypochlorite for 4 min and then placed in PDA with streptomycin to confirm their infection. After one week of incubation at 25°C, plated segments were observed for growth of C. coccodes and disease incidence was estimated based on the percentage of infected segments per organ type and per region.

Potato inoculation and culture conditions

C. coccodes inoculum was added to the culture substrate 15 days post-planting (DPP). Inoculation was conducted by watering each potted plant with 100 ml of a conidial suspension (10⁶ conidia/ml) next to the collar region. Non-inoculated control plants were watered with 100 ml of sterile distilled water. During all experimentation, plants were watered regularly and fertilized with a nutrient solution (20 N: 20 K₂O: 20 P₂O₅) (Manici and Cerato 1994), as needed, to control plant stresses and to promote normal growth.

Black dot severity was assessed, 60 DPP, based on several horticultural and disease severity parameters. In fact, the below-ground organs were carefully removed from the pots and gently washed with water to remove the remaining culture substrate. Individual stem bases and their attached root systems were examined for the presence of microsclerotia of C. coccodes and necrotic lesions. The sclerotial density on the below-ground stems, roots and stolons was estimated visually according to the scale (disease severity index i.e. DSI) adopted by Nitzan et al. (2006c) and which was modified (Fig. 1) based on necrotic lesions progress and percentage of area covered by black dot sclerotia where 0 = no microsclerotia, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100% of plant tissue colonized by microsclerotia. 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 aerial and the below-ground parts (below-ground stems, roots and stolons) and tubers, the total weight (fresh and dry 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 (5 potato plants) were used per elementary treatment.

Data were statistically analyzed by SPSS Software version 11 and subjected to analysis of variance and Fisher’s least significant difference test, LSD at $P \leq 0.05$.

The relationship between disease severity and horticultural parameters was compared using Pearson’s correlation analysis.

RESULTS

Black dot incidence

Data presented in Table 1 reveals that black dot presence was confirmed in all regions inspected. *C. coccodes* was detected on several potato organs i.e. stems, roots, stolons and tubers either solely or as disease complex with other root-infecting fungi such as *V. dahliae*, *F. solani*, *F. graminearum*, *R. solani*, *R. bataticola*, *Pythium* spp., and *Phytophthora* spp. However, disease incidence, i.e. frequency of pathogen isolation, varied depending on fields prospected. In fact, for all regions combined, the percentage of *C. coccodes* from potato cv. ‘Spunta’ roots ranged between 0 and 100 whereas the maximum average was recorded at Gafsa and Jendouba (60 and 43.25%, respectively). Similarly, isolations made from tubers confirmed the involvement of pathogen in diseased plants and the percentage of segments exhibiting typical *C. coccodes* colonies varied from 0 to 100%. The highest average of the percentage of pathogen isolation was recorded in samples belonging to Gafsa and Jendouba (53.25 and 30%, respectively). *C. coccodes* presence was confirmed in 0 to 100% in stems of all diseased plants collected with an average ranging between 1.66 and 57.5%. The highest percentage of pathogen isolation was recorded at Gafsa and at a lesser degree at Jendouba (26.25%).

Effect of *C. coccodes* isolates on black dot severity

All inoculated plants showed, at the end of the assay (i.e. 60 DPP), chlorotic foliage, resembling early senescence symptoms, compared with the non-inoculated control plants. However, the above-ground symptoms appeared as cupping and pinching of the leaves (Fig. 2) rather than the wilt characteristic induced by vascular pathogens. When harvested and washed with tap water, light brown lesions were observed on the below-ground stems, roots and stolons. The most severe below-ground symptoms were expressed as large brown to grey lesions covered with small black sclerotia (Fig. 3) and cortical sloughing on severely infected fine roots.

Black dot severity recorded on the below-ground parts of potato plants 60 DPP varied significantly ($P \leq 0.05$) with fungal treatments realized and organs. In fact, data pre-

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<tr>
<th>Regions</th>
<th>Roots</th>
<th>Tubers</th>
<th>Stems</th>
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<tr>
<td>Bizerte (4)$^a$</td>
<td>0-18$^a$(4.5)</td>
<td>0-10$^a$(5.5)</td>
<td>6-12$^a$(9)</td>
</tr>
<tr>
<td>Gafsa (4)</td>
<td>20-100 (60)</td>
<td>18-80 (53.25)</td>
<td>30-100 (57.5)</td>
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<td>Sidi Bouzid (4)</td>
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<td>0-100 (29.25)</td>
<td>0-10 (5.75)</td>
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<tr>
<td>Jendouba (4)</td>
<td>0-75 (43.25)</td>
<td>18-45 (30)</td>
<td>0-45 (26.25)</td>
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<tr>
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<td>0-85 (23.25)</td>
<td>0-0 (0)</td>
<td>0-22 (7.5)</td>
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<td>0-45 (15.74)</td>
<td>8-40 (18.75)</td>
</tr>
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<td>0-65 (32.5)</td>
<td>0-10 (5)</td>
<td>6-9 (7.5)</td>
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<tr>
<td>Mahdia (6)</td>
<td>4-22 (15.16)</td>
<td>0-15 (5)</td>
<td>0-10 (1.66)</td>
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$^a$ Values represent the minimum and the maximum incidence of black dot per region
$^b$ Values in brackets represent the mean incidence of black dot
$^c$ Values in brackets represent the number of inspected fields per region

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**Table 1** Percentage of *C. coccodes* isolation per inspected region and per potato organ.

**Fig. 2** Premature senescence symptoms including chlorotic foliage, cupping and pinching of the leaves observed on potato plants cv. ‘Spunta’ inoculated with *C. coccodes* compared with the non-inoculated control.
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Fig. 3 Severe black dot infection on potato cv. ‘Spunta’ plants inoculated with C. coccodes showing on the below-ground parts large brown to grey lesions covered with small black sclerotia.

Fig. 4 Black dot severity recorded 60 DPP on the below-ground organs of potato cv. ‘Spunta’ plants inoculated with different C. coccodes isolates compared with the non-inoculated control. Bars with the same colour and with the same letters are non-significantly different according to the LSD test (P ≤ 0.05).

Fig. 5 Plant height recorded 60 DPP on potato cv. ‘Spunta’ plants inoculated with different C. coccodes isolates compared with the non-inoculated control. Bars with the same letters are non-significantly different according to the LSD test (P ≤ 0.05).

The sclerotial density was higher on roots and stolons than on the below-ground stems. Moreover, for each organ examined, disease severity was affected by C. coccodes isolate used for inoculation which reveals a variation in pathogen aggressiveness. Thus, the most severe below-ground stem colonization was induced by isolates CC1 and CC9 with disease scores of about 3.957 and 3.125, respectively, whereas the lowest infection on inoculated plants was caused by isolate CC7 (DSI = 0.75). The remaining isolates exhibited comparable aggressiveness (DSI ≈ 2) based on below-ground stem degree of infection. It is also to note than even the non-inoculated control plants showed black dot symptoms with a very weak disease score of about 0.125. However, roots and stolons were found to be severely infected by the majority of C. coccodes isolates. In fact, 7 out of the 9 isolates tested showed DSI exceeding 2.5 among them 4 had a DSI comprised between 3.5 and 4 (based on a 0-4 scale). This disease severity reflects that roots and stolons were covered at 76 to 100% with C. coccodes sclerotia in addition to the associated root sloughing and to the cortical lesions induced on the below-ground stems which may affect plant growth and production.

Effect of C. coccodes isolates on potato growth

The plant height noted 60 DPP on potato cv. ‘Spunta’ plants inoculated or not with C. coccodes did not depend significantly (P ≥ 0.05) on the fungal treatments tested (Fig. 5). In fact, the height of all inoculated plants was significantly similar to that of the non-inoculated control. However, the overall trend is that the inoculated plants were slightly shorter compared to the control except the case of the isolate CC1. Indeed, the percentage of height reduction compared to the control varied from 6% (isolate CC7) to 23% (isolate CC5).

Data presented in Fig. 6 reveals a significant (P ≤ 0.05)
variation in isolate effect on plant growth estimated via the aerial part fresh weight. In fact, the majority of isolates showed a significantly comparable aerial part fresh weight as the non-inoculated control except CC9 and CC3. Moreover, the reduction of this parameter compared to the non-inoculated control ranged between 0 (isolate CC7) and 49% (isolate CC3); for 4 (CC8, CC5, CC9, CC3) out of the 9 isolates tested, the aerial part fresh weight was reduced by more than 40% compared to the control. These results revealed the variable impact of this pathogen on potato vegetative growth depending on isolate (different geographical origin) used for inoculation.

The aerial part dry weight recorded on potato plants 60 DPP was not significantly different depending on fungal treatments tested (Fig. 7). However, the overall trend is that all inoculated plants tend to have lowest records compared to the non-inoculated control. Moreover, this parameter was found to be reduced by 15% (isolate CC4) to 46% (isolate CC3) compared to the non-infected control; the reduction recorded exceeded 20% for 6 out of the 9 isolates tested which reflect the negative effect of inoculation with *C. coccodes* isolates on the aerial part dry weight.

As presented in Fig. 8, the below-ground parts (tubers not considered) fresh weight recorded 60 DPP was significantly variable depending on fungal treatments tested. Indeed, all *C. coccodes* isolates tested induced significantly similar effect on this parameter. However, except the isolate CC7, all the remaining isolates showed significantly lesser root fresh weight compared to the non-inoculated control where this parameter was reduced by more...
than 36%; the reduction exceeded 50% for 4 (CC2, CC4, CC9, CC6) out of the 9 isolates tested. These observations reveal the adverse effect of black dot disease induced by Tunisian isolates on these below-ground organs growth.

**Effect of** *C. coccodes* **isolates on potato production**

The weight of potato cv. ‘Spunta’ tubers harvested 60 DPP varied significantly (*P* ≤ 0.05) depending on fungal treatments tested. In fact, inoculation with *C. coccodes* isolates reduced this parameter compared to the non-inoculated control except the case of CC3 isolate (Fig. 10). Indeed, yield reduction subsequent to black dot development ranged between 0 (isolate CC3) and 47% (isolates CC9 and CC6). The expected tuber yield, due to inoculation with local isolates, seems to be severely threatened.

**Correlation analyses**

Both disease severity indexes noted on the below-ground stems, roots and stolons (all fungal treatments combined) were found to be significantly and positively correlated according to Pearson’s correlation analysis (*r* = 0.67, *P* = 0.0000001; *n* = 50).

The disease index of the below-ground stems was significantly and negatively related to the below-ground parts fresh weight (*r* = -0.435, *P* = 0.002; *n* = 50) and to the aerial part fresh (*r* = -0.343, *P* = 0.015; *n* = 50) and dry weight (*r* = -0.297, *P* = 0.036; *n* = 50).

Significant but negative correlation was recorded between black dot severity on roots and stolons and the fresh and dry weight of the below-ground (*r* = -0.626, *P* = 0.000001; *n* = 50; *r* = -0.381, *P* = 0.006; *n* = 50) and aerial parts (*r* = -0.478, *P* = 0.0004; *n* = 50; *r* = -0.345, *P* = 0.014; *n* = 50), respectively.

For all fungal treatments or isolates combined, both disease severity parameters were not correlated to tuber yield. However, when correlation analysis was done on an isolate to isolate basis, black dot severity on the below-ground stems was found to be significantly correlated only in the case of CC6 isolate (*r* = 0.941, *P* = 0.017; *n* = 5).

**DISCUSSION**

Black dot has become a serious disease that causes early senescence and plant wilting which impacts tuber quality due to tuber skin blemishing resulting in lots rejection for export. The present study emphasized on the geographical distribution of the disease and is the first to investigate the effect of *C. coccodes* isolates on plant growth and production.

Prospecting done by midseason led to successful pathogen isolation from the majority of fields surveyed. In fact, *C. coccodes* was frequently and successfully isolated relatively early in the growing season from below- and above-ground potato stems and from a high proportion of plants by midseason (Johnson and Miliczky 1993; Johnson et al. 1997). It was reported that early season symptoms ambiguity prior to sclerotia development can make field detection of the pathogen difficult as demonstrated when *C. coc-
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**C. coccodes** was isolated from asymptomatic plants in commercial potato fields (Otazu et al. 1978). Indeed, underground infection on potato occurs soon after emergence and develops in the stems as early as 7-11 weeks (Johnson and Miliczky 1993b; Read and Hide 1995; Andrivon et al. 1998). Thus, the choice of crop stage and susceptible organs are the main factors involved in the successful of pathogen isolation for a more precise estimation of its prevalence in Tunisia.

In fact, **C. coccodes** was detected in several potato organs i.e. stems, roots, stolons and tubers either solely or as mixed infections in different combinations with vascular wilt agents *V. dahliae* and *F. oxysporum* f. sp. *tuberosi*, and other root-infesting fungi such as *F. solani*, *F. graminearum*, *R. solani*, *R. bataticola*, *Pythium* spp., and *Phytophthora* spp. These findings joined our previous observations (Daami-Remadi and El Mahjoub 2004; Daami-Remadi et al. 2009). Similar disease complexes including, in addition to **C. coccodes**, other soil-borne fungi were reported elsewhere as biotic factors responsible of the early dying syndrome (Kotcon et al. 1985; Read and Hide 1988; Barkdol and Davis 1992; Johnson and Miliczky 1993; Johnon 1994; Read and Hide 1995; Johnson et al. 1997; Andrivon et al. 1997; Denner et al. 1997; Andrivon et al. 1998; Denner et al. 1998; Tsror (Lahkim) et al. 1999a; Tsror (Lahkim) and Johnson 2000; Tsror (Lahkim) and Hazanovsky 2001).

The maximum average of **C. coccodes** isolation from major potato-growing areas in Tunisia was recorded at Gafsa and Jendouba regions representing new and old zones of production, respectively. If presence of pathogen at Jendouba was justified by the frequent potato cultivation (mainly late season crop) and the short rotation, the highest incidence of black dot at Gafsa may be explained by an important soil infestation occurring via infected seed tubers. In fact, some **C. coccodes** isolates were collected from local seed tubers as well as from certified potato seed tuber lots imported to Tunisia from Holland, Belgium or France. Both seed and soil-borne inoculum were reported to be important in the epidemiology of black dot disease (Read and Hide 1988). Thus, over time, infected roots issued from infected seed tubers could significantly increase the inoculum density of **C. coccodes** in field soils and result in increased disease incidence and severity.

Differences in black dot severity were recorded on all inoculated potato plants implying variation in pathogen aggressiveness depending on isolates used for inoculation. Variation in pathogenicity was examined by Barkdol and Davis (1992) who assessed nine isolates of **C. coccodes** for their ability to cause disease symptoms on the foliage of potato cv. ‘Spunta’ based on growth, yield and disease parameters. In fact, isolates CC6 and CC9 exhibited the highest aggressiveness.

In the present study, the first study in Tunisia in which **C. coccodes** isolates were tested for aggressiveness to potato cv. ‘Spunta’ based on growth, yield and disease parameters, **C. coccodes** isolates were collected from local potato fields and result in increased disease incidence and severity. The infection of control plants may be attributed to the seed tubers which were checked only visually before planting and may have been latently infected with the pathogen. However, since we used tubers from the same lot in the inoculated and control pots, any significant difference in the results can only be attributed to inoculation and **C. coccodes** aggressiveness. In fact, latent infections by this blemishing agent on tubers were reported in several studies (Mohamed et al. 1992; Johnson and Miliczky 1993; Johnson et al. 1997; Tsror (Lahkim) et al. 1999b; Glais-Varlet et al. 2004). This slight disease severity recorded on control plants may lead to more pathogen development on roots and stolons when exposure duration to colonization between planting and harvest increased. This hypothesis was supported by the fact that young, underground organs are susceptible to **C. coccodes**-infection from the inoculum in the culture substrate leading to greater contact with inoculum added (Carnegie et al. 2003). However, roots considered as so susceptible and insufficient to detect differences in isolate aggressiveness (Aqeel et al. 2008) presented in our study sufficient sclerotial density to classify local **C. coccodes** isolates.

Inoculation with local **C. coccodes** isolates caused, in addition to black dot development, a reduced plant growth (plant height, fresh and dry weight of aerial and below-ground parts) and tuber yield compared with the non-inoculated control plants. Similar adverse effects on height and weight of plants were reported in other studies (Tsror (Lahkim) and Hazanovsky 2001). However, tuber yield and quality are both parameters which were considered in several works as indicators of pathogen aggressiveness but reports are sometimes controversial. In fact, the weight loss of seed tubers during sprouting was reported to increase with increasing amounts of black dot, but the disease had little effect on plant size through the season. Nevertheless, at harvest, the yield of ware tubers (> 50 mm) decreased with severe disease, the total tuber yields were not significantly affected and the total tuber number per plant increased (Read and Hide 1995). Johnson (1994) recorded a reduction in the total yield ranging between 19 and 32% and a reduction in the mean tuber weight of 29 to 43%.

This weight loss induced by black dot may occur at harvest as well as in storage (Hunger and McIntyre 1979) and Tsror (Lahkim) et al. (1999b) suggested that soil and tuber inoculations with **C. coccodes** result in greater yield reductions than foliar infections. Findings from some studies revealed reduced tuber weight, subsequent to inoculation, by more than 46% which may provide important information regarding aggressiveness of local **C. coccodes** isolates and their serious impact on expected yield.

Correlation analyses between parameters used in the present study for the assessment of pathogen aggressiveness revealed the significant relationship between both disease severity and disease symptoms on the below-ground organs, stems, roots and stolons. In potato, Nitzan et al. (2002, 2006c) found a microsclerotial density on roots as a method to determine isolate aggressiveness. Furthermore, in our work, the disease indexes were found to be correlated with the weight of the below-ground and the above-ground (aerial) plant parts but no with tuber weight. Similarly, Aqeel et al. (2008) found that microsclerotial density on below-ground organs was not correlated with tuber weight. Moreover, in the case of our study, some isolates (such as CC7) caused an important reduction in tuber weight of about 34% but had lower microsclerotial density compared with CC9 and CC6 isolates. However, CC1 isolate causing the highest disease severity on all below-ground parts caused a yield reduction of only 10%. When pooled data of all isolates or fungal treatments was considered, the absence of correlation between the disease scores and yield reduction may be due to the relatively short experiment duration.

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tion (two months). Moreover, correlation between these two parameters was found to be dependant on isolates tested which could be explained by variability in aggressiveness based on adverse effects on both plant health and growth. In other studies, root symptoms were not significantly correlated with shoot and crown symptoms but a significant negative correlation occurred between crown symptoms and shoot fresh weight suggesting that crown symptoms are the best indicator of aggressiveness (Sheehan et al. 2007). On the contrary, Carnegie et al. (2003) considered that root infection severity was the most sensitive and the reliable method for detecting soil infestations by *C. coccodes* but the visual sclerotia assessments would seem to have an advantage in determining relative disease responses at the end of the growing season (Cummings and Johnson 2008). Moreover, it will be also important to evaluate aggressiveness among *C. coccodes* population using more than one inoculation method and considering these reliable parameters.

The present investigation revealed that *C. coccodes* tested alone induced severe black dot symptoms on roots and the other underground parts, adversely affected plant growth and tuber yield in addition to the relatively accelerated leaf senescence i.e., early dying symptom. This pathogenic effect of *C. coccodes* when inoculated solely to potato plants was signalled in several other studies (Scholte et al. 1985; Johnson 1994; Tsror (Lahkim) 1999b) but the important percentages of reduction of plant (aerial and below-ground parts) and tuber weights may give additional information concerning variation in aggressiveness between isolates. Moreover, this determination of potential aggressiveness on potatoes in local *C. coccodes* populations is important for accurate selection of isolates for the screening/tuber cultivars behaviour against this increasingly important pathogen.

The health of seed potatoes has also a significant influence on the incidence of potato diseases in stores as well as in the soil before plant emergence or during plant development (Priou and El Mahjoub 1999). Moreover, crop rotation and other measures designed to reduce soil-borne inoculum of *C. coccodes* may be of limited value because of risk of reintroduction of the pathogen via potato seed stocks (Komm and Stevenson 1978). Thus, the assessment of black dot severity in response to the level of seed tuber infection by *C. coccodes* may elucidate the real impact of this disease and permit to assess its threat to potato crop.

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