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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 *et al*. 2008), South Africa (Denner *et al*. 1997) and southern Australia (Harding and Wicks 2005). It is signalled 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 senescing 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 reported on susceptible cultivars in addition to reduction of tuber quality. Moreover, the reported percentages of yield reductions 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 population 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 (Thirumalachar 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*.

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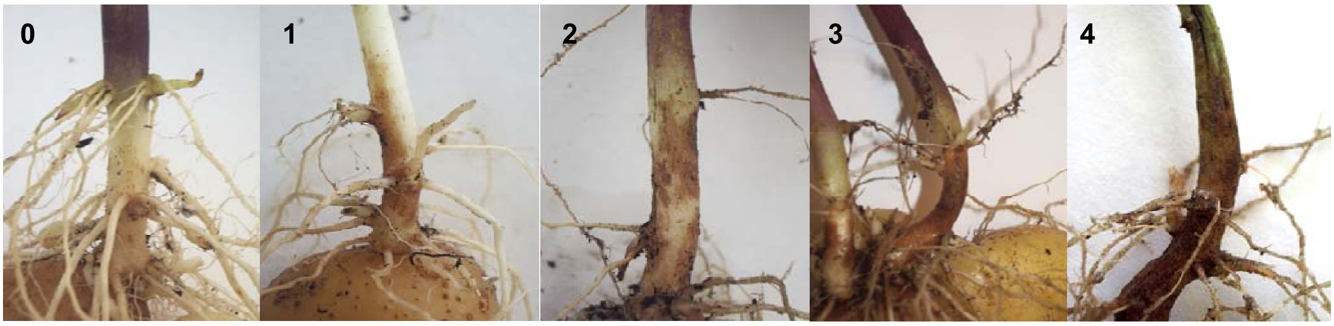

Fig. 1 Pictorial representation of the scale used for the assessment of black dot severity on the below-ground organs of potato plants.

1978; Barkdoll and Davis 1992; Johnson and Miliczky 1993; Johnson 1994; Read and Hide 1988 1995; Johnson *et al*. 1997; Andrivon *et al*. 1997 1998; Denner *et al*. 1997 1998; Tsror (Lahkim) *et al*. 1999a; Tsror (Lahkim) and Johnson 2000; Tsror (Lahkim) and Hazanovsky 2001).

In Tunisia, black dot was often regarded as a secondary disease because infections of the potato periderm are superficial and the contribution of *C. coccodes* in plant wilting was not quantified due to the lack of specific disease symptoms on potato foliage (Nitzan *et al*. 2006a). In wilted plants, the pathogen was often isolated in association with *Fusarium oxysporum* f. sp. *tuberosi*, *F. solani* and *V. dahliae* (Priou and El Mahjoub 1999; Daami-Remadi and El Mahjoub 2004; Daami-Remadi *et al*. 2009). Furthermore, mixed infections of *C. coccodes* with other soil-borne fungi such as *Rhizoctonia solani*, *R. bataticola*, *F. graminearum*, were also observed (Daami-Remadi, unpublished data). Black dot agent has not been previously studied given that the infected tubers are locally marketed and planted as self-produced seeds. However, this disease has become a veritable threat to potato crop even in areas where potato is newly introduced, such as Gafsa. Furthermore, it is actually responsible for the serious rejection of exported tubers, a growing and a promising market for Tunisian economy.

Given the soil- and seed-borne nature of the pathogen and its extensive host range, accurate assessment of potato 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 (Pharmadrug 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 10^6 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 (10^6 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 K_2O: 20 P_2O_5) (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

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

Regions	Roots	Tubers	Stems
Bizerte (4)[C]	0-18[a] (4.5)	0-10[a] (5.5)	6-12[a] (9)
Gafsa (4)	20-100 (60)	18-80 (53.25)	30-100 (57.5)
Sidi Bouzid (4)	0-30 (12)	0-100 (29.25)	0-10 (5.75)
Jendouba (4)	0-75 (43.25)	18-45 (30)	0-45 (26.25)
Cap-Bon (4)	0-85 (23.25)	0-0 (0)	0-22 (7.5)
Sousse (4)	0-18 (10.5)	0-45 (15.74)	8-40 (18.75)
Béja (2)	0-65 (32.5)	0-10 (5)	6-9 (7.5)
Mahdia (6)	4-22 (15.16)	0-15 (5)	0-10 (1.66)

[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

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. cocoodes* 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-

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.

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.

sented in **Fig. 4** shows that 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 ($P \leq 0.05$) 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 ($P \leq 0.05$)

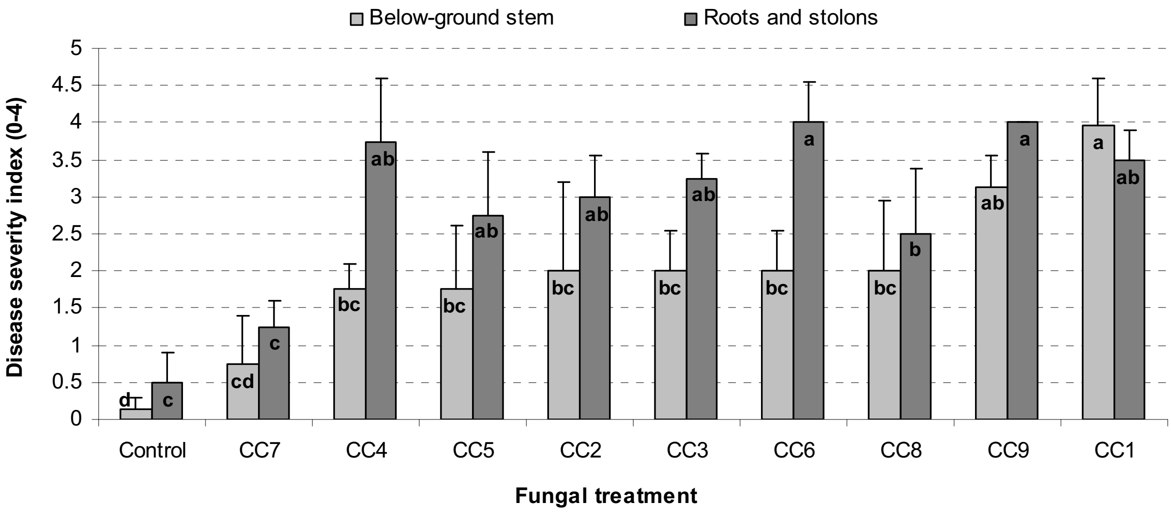

Fig. 4 Black dot severity recorded 60 DPP on the below-ground organs of potato cv. 'Spunta' plants inoculated with different *C. coccodes* isolates compared with the non-inoculated control. Bars with the same colour and with the same letters are non-significantly different according to the LSD test ($P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

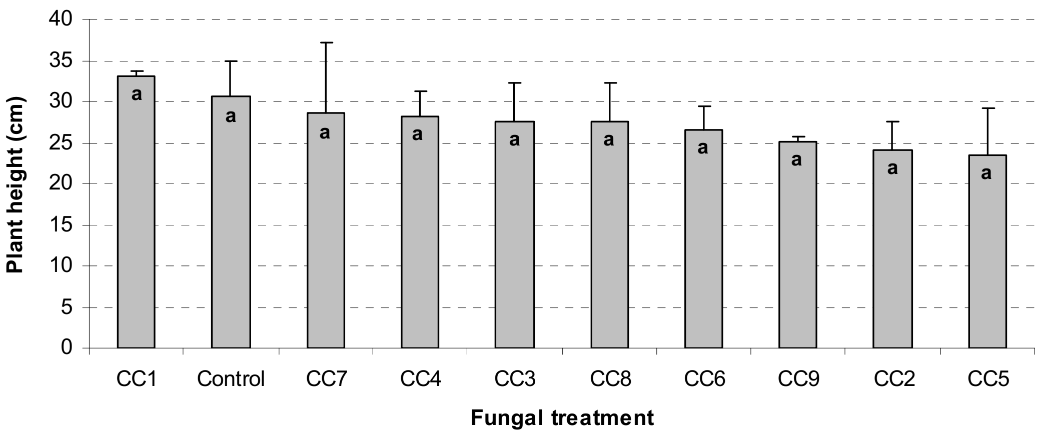

Fig. 5 Plant height recorded 60 DPP on potato cv. 'Spunta' plants inoculated with different *C. coccodes* isolates compared with the non-inoculated control. Bars with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$).

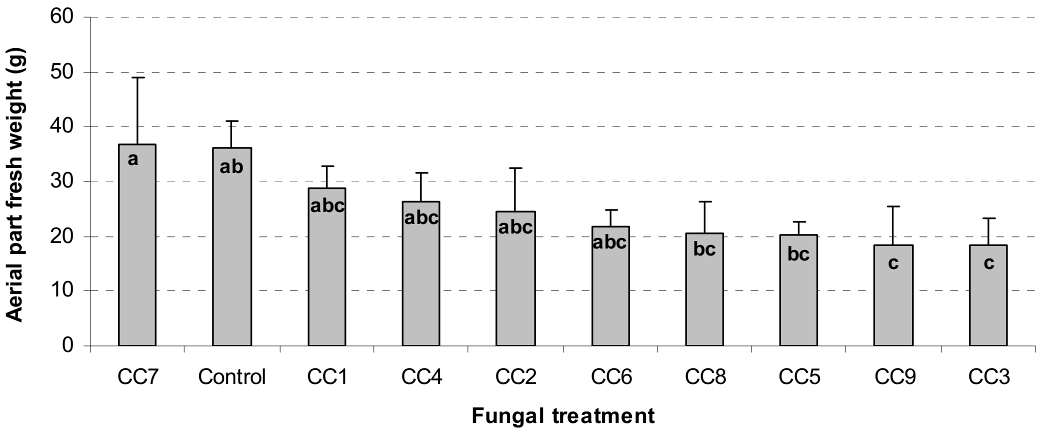

Fig. 6 Aerial part fresh weight recorded 60 DPP on potato cv. 'Spunta' plants inoculated with different *C. coccodes* isolates compared with the non-inoculated control. Bars with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$).

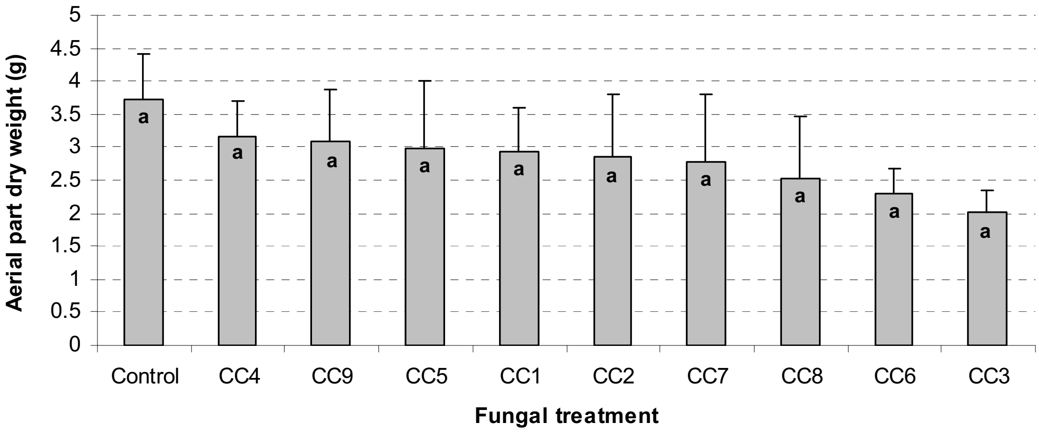

Fig. 7 Aerial part dry weight recorded 60 DPP on potato cv. 'Spunta' plants inoculated with different *C. coccodes* isolates compared with the non-inoculated control. Bars with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$).

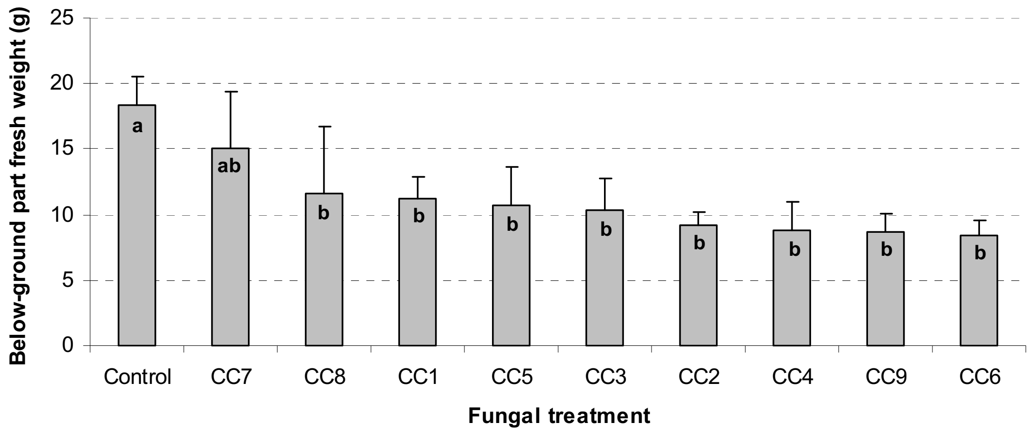

Fig. 8 Below-ground parts fresh weight recorded 60 DPP on potato cv. 'Spunta' plants inoculated with different *C. coccodes* isolates compared with the non-inoculated control. Bars with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$).

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 *C. coccodes* 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) 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

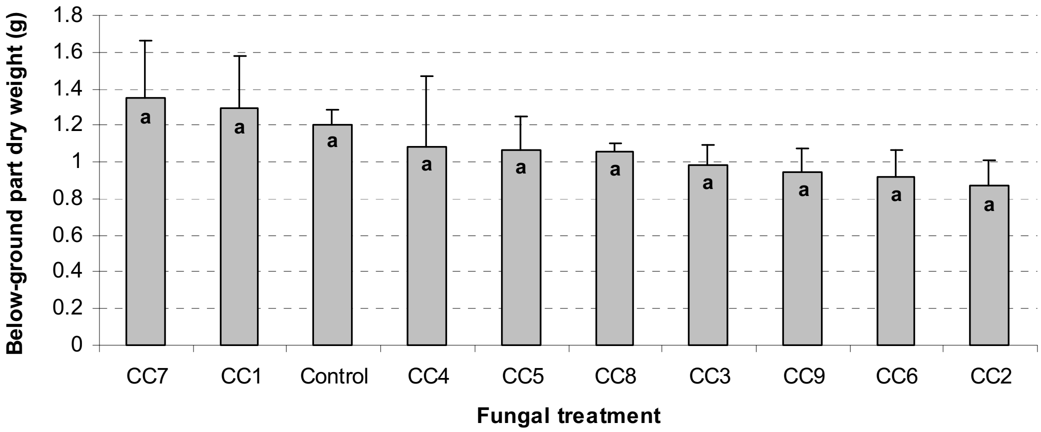

Fig. 9 Below-ground parts dry weight recorded 60 DPP on potato cv. 'Spunta' plants inoculated with different *C. coccodes* isolates compared with the non-inoculated control. Bars with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$)

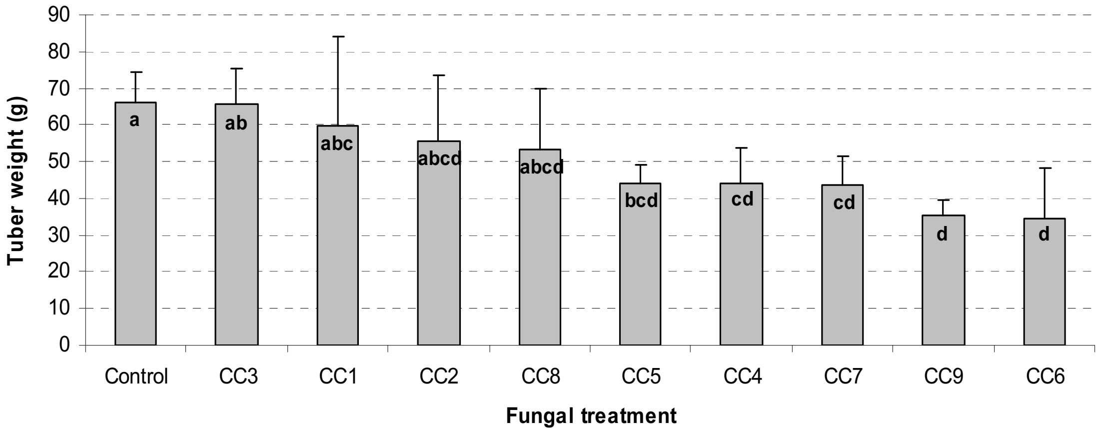

Fig. 10 Tuber weight recorded 60 DPP on potato cv. 'Spunta' plants inoculated with different *C. coccodes* isolates compared with the non-inoculated control. Bars with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$).

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.

Fig. 9 showed that the below-ground parts dry weight noted on potato plants cv. 'Spunta' 60 DPP was significantly ($P \leq 0.05$) similar for all fungal treatments tested. However, the overall trend, even statistically insignificant, is that excepting 2 out of the 9 *C. coccodes* isolates tested, all the remaining isolates adversely affected below-ground organs growth. In fact, this parameter was reduced by more than 18% by 4 out of the total isolates tested (CC3, CC9, CC6, CC2).

Effect of *C. coccodes* isolates on potato production

The weight of potato cv. 'Spunta' tubers harvested 60 DPP varied significantly ($P \leq 0.05$) 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 stem 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 a 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 symptom ambiguity prior to sclerotia development can make field detection of the pathogen difficult as demonstrated when *C. coc-*

codes 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 the present study, *C. coccodes* was detected on 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-infecting 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; Barkdoll and Davis 1992; Johnson and Miliczky 1993; Johnson 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 Barkdoll and Davis (1992) who assessed nine isolates of *C. coccodes* for their ability to cause disease symptoms on the foliage of potato cultivar ‘Russett Burbank’ utilizing root inoculations. These differences in aggressiveness were also identified among vegetative compatibility groups or VCGs (Nitzan *et al*. 2002, 2006c; Aqeel *et al*. 2008) based on stem colonization level and sclerotial density on roots and crowns (Shcolnick *et al*. 2007). The present study was 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. In fact, both scoring systems used for isolates characterization focused on black dot severity on the below-ground plant parts (below-ground stem, roots and stolons). The results pointed to a variation in aggressiveness to potato plants and to more severe disease on roots and stolons than on the below-ground stem. These records are in agreement with previous findings showing that all underground parts of a potato plant are susceptible to infection by *C. coccodes* and that roots and stolons were infected more rapidly and to a greater extent than stems (Johnson 1994; Andrivon *et al.* 1998; Aqeel *et al*. 2008; Pasche *et al*. 2010). This phenomenon could be explained by the greater affinity of the fungal growth towards the roots than the plant apex (Nitzan *et al*. 2006a). Furthermore, similarly to our essay duration, symptoms were reported to develop on stems and stolons 6 and 8 weeks after planting, respectively (Lee and Hilton 2003). Similarly, this colonization of roots and stolons was restricted in the early stages of plant growth, similar to what was previously reported for potato stems (Nitzan *et al*. 2006a). Roots susceptibility to infection by the fungus is favored by their spread through 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.

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 (Mohan *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 tuber-borne as well as soil inoculum (Read and Hide 1995; Andrivon *et al*. 1998).

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 Mclntyre 1979) and Tsror (Lahkim) *et al*. (1999b) suggested that soil and tuber inoculations with *C. coccodes* result in greater yield reduction than foliar inoculations. Findings from our study 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 scoring systems i.e. disease index on the below-ground stems, roots and stolons. In potato, Nitzan *et al*. (2002, 2006c) used 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 roots was not correlated with tuber weight reductions. 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 (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 measure of aggressiveness (Shcolnick *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) *et al*. 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 the potato 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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REFERENCES

Andrivon D, Ramage K, Guerin C, Lucas JM, Jouan B (1997) Distribution and fungicide sensitivity of *Colletotrichum coccodes* in French potato-producing areas. *Plant Pathology* **46**, 722-728

Andrivon D, Lucas JM, Guérin C, Jouan B (1998) Colonization of roots, stolons, tubers and stems of various potato (*Solanum tuberosum*) cultivars by the black-dot fungus *Colletotrichum coccodes*. *Plant Pathology* **47**, 440-445

Aqeel AM, Pasche JS, Gudmestad NC (2008) Variability in morphology and aggressiveness among vegetative compatibility groups of *Colletotrichum coccodes*. *Phytopathology* **98**, 901-909

Barkdoll AW, Davis JR (1992) Distribution of *Colletotrichum coccodes* in Idaho and variation in pathogenicity on potato. *Plant Disease* **76**, 131-135

Carnegie SF, Choiseul JW, Roberts AMI (2003) Detection of *Colletotrichum coccodes* and *Helminthosporium solani* in soils by bioassay. *Plant Pathology* **52**, 13-21

Cummings TF, Johnson DA (2008) Effectiveness of early-season, single applications of azoxystrobin for the control of potato black dot as evaluated by three assessment methods. *American Journal of Potato Research* **85**, 422-431

Daami-Remadi M, El Mahjoub M (2004) Emergence en Tunisie de *Fusarium oxysporum* f. sp. *tuberosi* agent de flétrissure vasculaire des plants et de pourriture sèche des tubercules de pomme de terre. *Bulletin EOPP/EPPO* **34**, 407-411.

Daami-Remadi M, Sayes S, Horrigue-Raouani N, Hlaoua-Ben Hassine W (2009) Effects of *Verticillium dahliae* Kleb., *Fusarium oxysporum* Schlecht. f. sp. *tuberosi* Snyder and Hansen and *Meloidogyne javanica* (Treub.) Chitwood inoculated individually or in combination on potato growth, wilt severity and nematode development. *African Journal of Microbiology Research* **3**, 595-604

Denner FDN, Millard C, Geldenhuys A, Wehner FC (1997) Treatment of seed potatoes with prochloraz for simultaneous control of silver scurf and black dot on progeny tubers. *Potato Research* **40**, 221-227

Denner FDN, Millard C, Wehner FC (1998) The effect of seed- and soilborne inoculum of *Colletotrichum coccodes* on the incidence of black dot on potatoes. *Potato Research* **41**, 51-56

Dickson BT (1926) The black dot disease of potato. *Phytopathology* **16**, 23-40

Dillard HR (1992) *Colletotrichum coccodes*: the pathogen and its hosts. In: Bailey JA, Jeger MJ (Eds) *Colletotrichum*: *Biology, Pathology and Control*, CAB International, Wallingford, UK, pp 225-236

Dillard HR, Cobb AC (1997) Disease progress of black dot on tomato roots and reduction in incidence with foliar applied fungicides. *Plant Disease* **81**, 1439-1442

Dillard HR, Cobb AC (1998) Survival of *Colletotrichum coccodes* in infected tomato tissue and in soil. *Plant Disease* **82**, 235-238

Glais I, Andrivon D (2004) Deep sunken lesions – an atypical symptom on potato tubers caused by *Colletotrichum coccodes* during storage. *Plant Pathology* **53**, 254

Glais-Varlet I, Bouchek-Mechiche K, Andrivon D (2004) Growth *in vitro* and infectivity of *Colletotrichum coccodes* on potato tubers at different temperatures. *Plant Pathology* **53**, 398-404

Griffiths HM , Zitter TA , Loeffler K, De Jong WS, Menasha S (2010) First report in North America of atypical symptoms caused by *Colletotrichum coccodes* on field-grown tubers during storage. *Plant Health Progress* July 2010 (Abstract). Available online: http://www.plantmanagementnetwork.org/php/elements/sum.aspx?id=8749&photo=4992

Gudmestad NC, Taylor RJ, Pasche JS (2007) Management of soilborne diseases of potato. *Australasian Plant Pathology* **36**, 109-115

Harding RB, Wicks TJ (2005) Black dot... Alternative hosts and cultivar susceptibility. Newsletters of the South Australian Research and Development Institute. Available online: http://www.sardi.sa.gov.au/_data/assets/pdf_file/0009/46593/black_dot.pdf

Hunger RM, Mclntyre GA (1979) Occurrence, development and losses associated with silver scurf and black dot on Colorado potatoes. *American Potato Journal* **56**, 289-306

Ingram J, Johnson DA (2010) Colonization of potato roots and stolons by *Colletotrichum coccodes* from tuberborne inoculum. *American Journal of Potato Research* **87**, 382-389

Johnson DA (1994) Effect of foliar infection caused by *Colletotrichum coccodes* on yield of Russet Burbank potato. *Plant Disease* **78**, 1075-1078

Johnson DA, Miliczky ER (1993) Effects of wounding and wetting duration on infection of potato foliage by *Colletotrichum coccodes*. *Plant Disease* **77**, 13-17

Johnson DA, Rowe RC, Cummings TF (1997) Incidence of *Colletotrichum coccodes* in certified potato seed tubers planted in Washington State. *Plant Disease* **81**, 1199-1202

Komm DA, Stevenson WR (1978) Tuber-borne infection of *Solanum tuberosum* ‘Superior’ by *Colletotrichum coccodes*. *Plant Disease Reporter* **62**, 682-687

Kotcon JB, Rouse DI, Mitchell JE (1985) Interactions of *Verticillium dahliae*, *Colletotrichum coccodes*, *Rhizoctonia solani*, and *Pratylenchus penetrans* in the early dying syndrome of Russet Burbank potatoes. *Phytopathology* **75**, 68-74

Lees AK, Hilton AJ (2003) Black dot (*Colletotrichum coccodes*): an increasingly important disease of potato. *Plant Pathology* **52**, 3-12

Manici LM, Cerato C (1994) Pathogenicity of *Fusarium oxysporum* f. sp. *tuberosi* isolates from tubers and potato plants. *Potato Research* **37**, 129-134

Mohan SK, Davis JR, Sorensen LH, Schneider AT (1992) Infection of aerial parts of potato plants by *Colletotrichum coccodes* and its effects on premature death and yield. *American Potato Journal* **69**, 547-559

Nitzan N, Cummings TF, Johnson DA (2005) Effect of seedtuber generation, soilborne inoculum, and azoxystrobin application on development of potato black dot caused by *Colletotrichum coccodes*. *Plant Disease* **89**, 1181-1185

Nitzan N, Evans M, Johnson DA (2006a). Colonization of potato plants after aerial infection by *Colletotrichum coccodes*, the causal agent of potato black dot. *Plant Disease* **90**, 999-1003

Nitzan N, Hazanovsky M, Tal M, Tsror (Lahkim) L (2002) Vegetative compatibility groups in *Colletotrichum coccodes*, the causal agent of black dot on potato. *Phytopathology* **92**, 827-832

Nitzan N, Lucas BS, Christ BJ (2006b) Colonization of rotation crops and weeds by the potato black dot pathogen *Colletotrichum coccodes. American Journal of Potato Research* **83**, 503-507

Nitzan N, Tsror (Lahkim) L, Johnson DA (2006c) Vegetative compatibility groups and aggressiveness of North American isolates of *Colletotrichum coccodes*, the causal agent of potato black dot. *Plant Disease* **90**, 1287-1292

Olanya OM, Porter GA, Lambert DH (2010) Supplemental irrigation and cultivar effects on potato tuber diseases. *Australian Journal of Crop Science*

4, 29-36

Otazu V, Gudmestad NC, Zink RT (1978) The role of *Colletotrichum atramentarium* in the potato wilt complex in North Dakota. *Plant Disease Reporter* **62**, 847-851

Pasche JS, Taylor RJ, Gudmestad NC (2010) Colonization of potato by *Colletotrichum coccodes*: Effect of soil infestation and seed tuber and foliar inoculation. *Plant Disease* **94**, 905-914

Priou S, El Mahjoub M (1999) Bacterial and fungal diseases in the major potato-growing areas of Tunisia. *EPPO Bulletin* **29**, 167-171

Read PJ (1991) The susceptibility of potato cultivars to black dot (*Colletotrichum coccodes*) Wallr. Hughes. *Annals of Applied Biology* **119**, 475-482

Read PJ, Hide GA (1988) Effects of inoculum source and irrigation on black dot disease of potatoes (*Colletotrichum coccodes* (Wallr.) Hughes) and its development during storage. *Potato Research* **31**, 493-500

Read PJ, Storey RMJ, Hudson DR (1995) A survey of black dot and other fungal tuber blemishing diseases in British potato crops at harvest. *Annals of Applied Biology* **126**, 249-258

Scholte K, Veenbaas-Rijks JW, Labruyere RE (1985) Potato growing in short rotations and the effect of *Streptomyces* spp., *Colletotrichum coccodes, Fusarium sambucinum,* and *Verticillium dahliae* on plant growth and tuber yield. *Potato Research* **28**, 331-348

Shcolnick S, Dinoor A, Tsror (Lahkim) L (2007) Additional vegetative compatibility groups in *Colletotrichum coccodes* subpopulations from Europe and Israel. *Plant Disease* **91**, 805-808

Stevenson WR, Loria R, Franc GD, Weingartner DP (2001) Diseases caused by fungi. In: Stevenson WR, Loria R, Franc GD, Weingartner DP (Eds) *Compendium of Potato Diseases*, APS Press, St. Paul, MN, USA, pp 216-218

Stevenson WR, Green RJ, Bergeson GB (1976) Occurrence and control of potato black dot root rot in Indiana. *Plant Disease Reporter* **60**, 248-251

Thirumalachar MS (1967) Pathogenicity of *Colletotrichum atramentarium* on some potato varieties. *American Potato Journal* **44**, 241-244

Tsror (Lahkim) L, Aharon M, Erlich O (1999a) Survey of bacterial and fungal seedborne diseases in imported and domestic potato seed tubers *Phytoparasitica* **27**, 1-12

Tsror (Lahkim) L, Erlich O, Hazanovsky M (1999b) Effect of *Colletotrichum coccodes* on potato yield, tuber quality, and stem colonization during spring and autumn. *Plant Disease* **83**, 561-565

Tsror (Lahkim) L, Ben-Daniel BH, Erlich O, Hazanovsky M, Lebiush S, Aharon M, Zig U (2007) Why have *Colletotrichum* and *Helminthosporium* become such a problem for fresh and ware potato quality - pathogen change or climate change. In: *Proceedings of the EAPR Pathology Section seminar on New and Old Pathogens of Potato in Changing Climate*, 2-6 July, 2007, Hattula, Finland, p 37 (Abstract)

Tsror (Lahkim) L, Johnson DA (2000) *Colletotrichum coccodes* on potato. In: Prusky D, Freeman S, Dickman MB (Eds) *Colletotrichum – Host Specificity, Pathology and Host – Pathogen Interaction*, APS Press, St. Paul, MN, USA, pp 362-373