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Effect of the Level of Seed Tuber Infection by *Colletotrichum coccodes* at Planting on Potato Growth, Black Dot Severity and Subsequent Yield Reduction

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ABSTRACT

A great deal of attention has been given to tuber infections of *Colletotrichum coccodes* as skin blemishes depreciate the tuber's commercial value and serve as means of pathogen spread into new productions areas. This study was carried out on cv. 'Spunta' potato plants to compare, quantitatively, the relative impact of different tuber-borne inoculum levels on black dot severity, plant growth and expected yield loss. Black dot severity recorded 60 (pot experiment) or 90 days post-planting (field experiment) on the below-ground plant parts varied significantly depending on inoculum levels tested. The severest symptoms were induced by the highest seed tubers infections corresponding to more than 50% of the seed tuber surface area covered with black dot lesions. The recorded disease severity was found to be highly and positively correlated with the level of seeds contamination. The aerial part fresh weight noted 60 DPP was reduced, compared to plants grown from the symptomless seed tubers, by 24 and 42% when heavily infested seeds were planted. This growth parameter was also negatively correlated with black dot severity on seed tubers and on the below-ground plant parts. In the pot experiment, tuber weight was reduced by about 14-21% on plants grown from extremely infected seeds as compared with the non infested control plants. Yield decrease, subsequent to the planting of diseased seeds, varied between 15 and 27% when *C. coccodes* inoculum levels ranged between 1 and 5. The field study revealed the existence of significant and negative correlations between the tuber yield, the level of seed-borne inoculum and disease severity on the below-ground stems, roots and stolons.

Keywords: correlations, degree, disease parameters, loss, *Solanum tuberosum* L., tuber-borne inoculum, tuber weight

INTRODUCTION

Several pathogens affecting potato tubers are tuber-borne and introduced into the non infested soils through planting diseased seeds as is the case of *Colletotrichum coccodes*. This pathogen once introduced into the soil, survives, affects the quality and yield of potatoes for seed and consumption, and serves for the buildup of a soil-borne inoculum for infection of future crops leading to an increasingly important black dot incidence (Read and Hide 1988; Read 1991; Barkdoll and Davis 1992; Read and Hide 1995a, 1995b; Read *et al*. 1995; Johnson *et al.* 1997). Of the disease-control measures available to growers, avoidance by planting healthy seed or avoiding contaminated fields is important (Lees *et al*. 2010). Lengthy crop rotations are suggested to significantly reduce the viable inoculum of *C. coccodes* and, therefore, attenuate inoculum pressure but they are limited by pathogen potential of survival in the soil for up to 8 years (Dillard and Cobb 1998; Cullen *et al.* 2002). Moreover, fungicides applied throughout the season for the control foliar diseases had no effect on alleviating the problem (Pennypacker and Sanogo 1995) and even chemicals generally applied to seed tubers failed to control the disease (Marais 1990; Read and Hide 1995b; Andrivon *et al*. 1997).

Tuber infections of *C. coccodes* were given more attention as the very important phase of the disease i.e. blemishing and lesions on the tuber periderm (Read and Hide 1995a; Lees and Hilton 2003) which not only depreciate tuber quality (Barkdoll and Davis 1992; Johnson 1994; Read and Hide 1995a; Tsror (Lahkim) *et al*. 1999b) but destroy the integrity of the natural barrier of the skin, forming an entry point for pathogenic microorganisms. Furthermore, blemishes may continue to develop during storage (Tsror and Johnson 2000; Lees and Hilton 2003; Glais and Andrivon 2004). The visual appearance of tuber skin is not only an important factor in attracting consumer but also it may seriously threaten plant development as a potential source of inoculum. Thus, the economical losses subsequent to tuber infection may concern all potato sectors, i.e. seed, consumption (including exportation), and processing (Hunger and McIntyre 1979; Ostrysko and Banville 1992; Secor 1994; Lees and Hilton 2003; Fiers *et al*. 2010).

Literature review focused on the relative contribution of the different inoculum sources on disease severity concluded to a general consensus that soil-borne inoculum is of greater importance than seed-borne inoculum (Read and Hide 1988, 1995; Denner *et al*. 1998; Nitzan *et al*. 2005, 2008; Lees *et al*. 2010). In fact, Denner *et al*. (1998) demonstrated that soil-borne inoculum doubled the incidence of disease on progeny tubers compared with seed-borne inoculum and Nitzan *et al*. (2008) described a non-linear relationship between soil-borne inoculum and disease severity. However, Read and Hide (1988) concluded that the relative importance of each inoculum source is dependent on disease severity or inoculum concentration.

Black dot development from tuber-borne inoculum may be managed by using disease-free certified seed tubers and chemical seed treatments prior to planting (Tsror (Lahkim) and Johnson 2000; Lees and Hilton 2003). However, limited infection is allowed even on certified seeds (Hunger and McIntyre 1979) and planting of these seeds does not guarantee freedom from disease or disease-causing agents (Secor *et al.* 1997). In fact, disease-free seed tubers produc-

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tion may be compromised by tuber infection during the last phases of their multiplication i.e in seed production areas (Dashwood *et al*. 1992; Johnson *et al*. 1997). Indeed, *C. coccodes* is well known to be carried on certified potato seed tubers (Komm and Stevenson 1978; Read and Hide 1988; Barkdoll and Davis 1992; Uribe and Loria 1994). In fact, In Idaho, *C. coccodes* was detected in 60% of seed tuber lots whereas in Indiana, incidence of *C. coccodes* in certified seed tubers ranged up to nearly 88% (Komm and Stevenson 1978). In Israel, black dot was observed in a considerable portion of the shipments from Holland where 34% of the lots were infected whereas tubers originating from France and Germany were infected at low levels (11-19%) (Tsror (Lahkim) *et al*. 1999a) even though *C. coccodes* was reported to be widespread in French potato cropping areas (Andrivon *et al*. 1997). In Tunisia, seed tubers infection as high as 80% (depending on cultivars) may be observed on self-produced or imported seeds and the 5% authorized infection per seed lot may be largely exceeded due to latent infections and to possibility of expansion of initial lesions during storage and transport. Thus, planting of seed tubers completely free of black dot symptoms is almost impossible and inevitable.

Infected seed tubers are an important means of pathogen widespread into new growing areas (Komm and Stevenson 1978; Barkdoll and Davis 1992). Furthermore, planting of heavily infected seed tubers might negate the effects of pre-plant soil treatments and long rotations between potato crops (Komm and Stevenson 1978) as young, underground organs are susceptible to *C. coccodes* infection from both soil and tuber-borne inocula (Read and Hide 1995a; Andrivon *et al*. 1998). Therefore, due to the increasing economic impact of the disease worldwide, and particularly in Tunisia, a better understanding of the importance of tuber-borne inoculum in black dot development and epidemiology is needed. Thus, this study was carried out to determine, quantitatively, the relative importance of the contribution of different tuber-inoculum levels in causing black dot under controlled-environment (pot experiment i.e. *in vivo*) and field conditions (field experiment i.e. *in situ*). Their impact on plant growth and expected yield loss were also elucidated.

MATERIALS AND METHODS

Plant material

Potato (*Solanum tuberosum* L. cv. 'Spunta') seed tubers were used. This cultivar is the most cultivated in Tunisia. If seed tubers were not well sprouted prior to planting, they were washed with tap water, air dried and placed under favorable environmental conditions to sprout (15-20°C, 60-80% relative humidity and natural room light) for approximately 14 days prior to use.

Pot experiment

To determine the effect of tuber-borne inoculum level in black dot development and severity, naturally infected potato seeds from the same lot and differing in incidence of initial inoculum were used. Seeds were classified visually into six disease categories (i.e. inoculum levels) based on the percentage of tuber surface area covered with black dot symptoms (lesions with sclerotia) as shown in **Fig. 1**. *C. coccodes* contamination i.e. sclerotia presence was also confirmed with a magnifying glass. Symptoms were assessed according to a 0-5 scale as follows: 0 = no lesion present, 1 = 1 to 10%, 2 = 10 to 30%, 3 = 30 to 50%, 4 = 50 to 75%, and 5 = 75 to 100% of the tuber surface area covered with black dot lesions.

Seed tubers from each disease category were individually planted in plastic pots (25 cm diameter, 1.6 l volume) containing a non-infested culture substrate composed of a mixture of peat and perlite (2: 1), previously sterilized at 110°C for 1 h. 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.

Fig. 1 Pictorial representation of the scale used for the assessment of black dot severity on potato seed tubers. 0 = no lesion present 1 = 1 to 10%, 2 = 10 to 30%, 3 = 30 to 50%, 4 = 50 to 75%, and 5 = 75 to 100% of the tuber surface area covered with black dot lesions.

Field experiment

The effect of the six tuber-borne inoculum levels on black dot severity was also assessed under natural conditions on an autumn crop season. The field experiment was conducted at the domain of the Regional Center of Research in Horticulture and Organic Agriculture of Chott-Mariem, Tunisia. The chosen field was not cropped to potato for three years ago. Tubers were planted according to a completely randomized design with 32 replications (i.e. 32 tubers) per elementary treatment (i.e. per inoculum level).

The experiment was managed (fertilization, late blight management and cultural practices) as commonly done for potato farming in the region.

Disease assessments

Black dot severity was assessed based on several parameters at 60 or 90 days post-planting (DPP) for the pot and the field experiment, respectively. In fact, individual stem bases and their attached root systems were examined for the presence of microsclerotia (sclerotia) of *C. coccodes* and necrotic lesions. The sclerotial density and the black dot lesions progress on the below-ground organs (below-ground stems, roots and stolons) were estimated visually according to a 0-4 scale detailed in Daami-Remadi *et al*. (2010) where 0 = no microsclerotia and 4 = 76 to 100% of plant tissue colonized by microsclerotia. The assessments of the disease severity index (DSI) were done for each stem individually and the mean for each plant was recorded.

The total weight of harvested tubers per plant was noted for both experiments. The fresh weight of the aerial plant parts was noted only in the pot experiment.

Fungal isolations on PDA were also done per elementary treatment for confirmation of the involvement of *C. coccodes* in the symptoms observed and scored.

Statistical analyses

Statistical analyses were performed, for all parameters measured, following a completely randomized design where the different inoculum levels were the only fixed factor. Five and 32 replicates (plants) were used per elementary treatment for the pot and the field experiment, respectively.

Data were statistically analyzed by SPSS Software version 11 and subjected to one-way analysis of variance. Means were differentiated using Fisher's protected least significant difference (LSD) test ($P \leq 0.05$). Correlations between tuber-borne inoculum levels, disease severity indexes and horticultural parameters were calculated using the Pearson correlation coefficient.

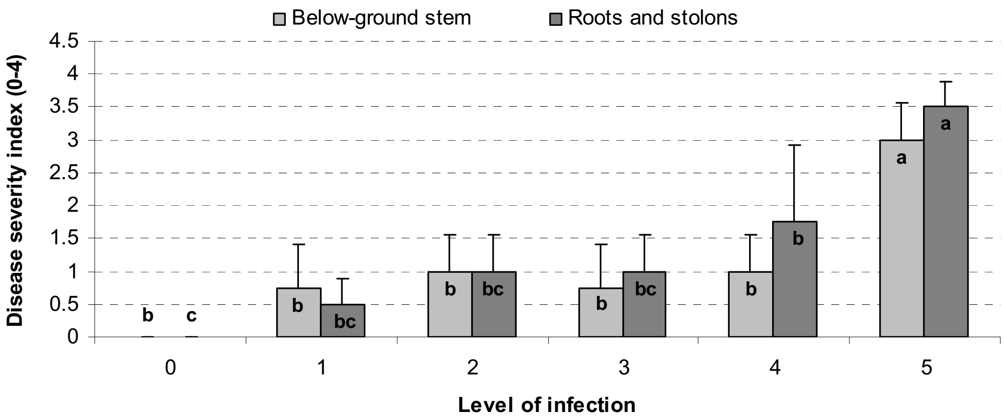

Fig. 2 Effect of tuber-borne *Colletotrichum coccodes* inoculum level on black dot severity recorded 60 DPP on the below-ground parts of potato cv. 'Spunta' plants (pot experiment). Bars with the same colour and with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$). The scale of the level of infection on the X-axis varied from 0 to 5 where 0 = no lesion present, 1 = 1 to 10%, 2 = 10 to 30%, 3 = 30 to 50%, 4 = 50 to 75%, and 5 = 75 to 100% of the tuber surface area covered with black dot lesions.

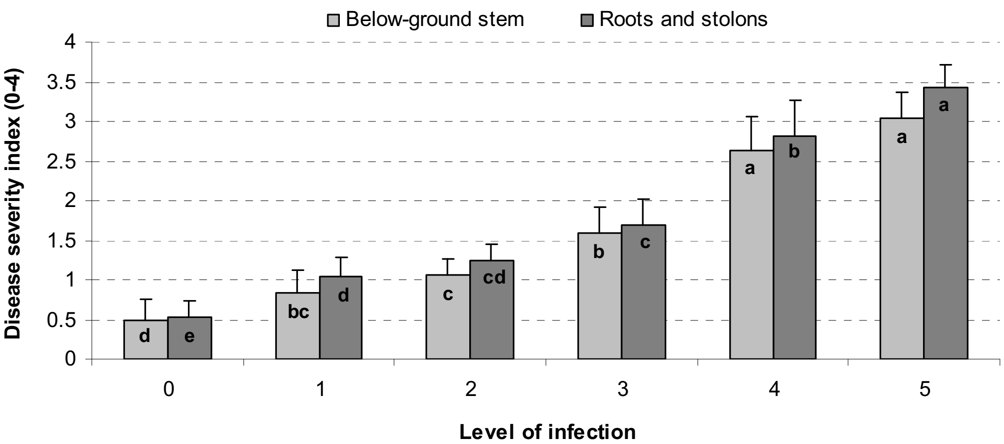

Fig. 3 Effect of tuber-borne *Colletotrichum coccodes* inoculum level on black dot severity recorded 90 DPP on the below-ground parts of potato cv. 'Spunta' plants (field experiment). Bars with the same colour and with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$). The scale of the level of infection on the X-axis varied from 0 to 5 where 0 = no lesion present, 1 = 1 to 10%, 2 = 10 to 30%, 3 = 30 to 50%, 4 = 50 to 75%, and 5 = 75 to 100% of the tuber surface area covered with black dot lesions.

RESULTS

Effect of the tuber-borne inoculum level on black severity

Chlorotic foliage resembling early dying symptoms was noted on all potato cv. 'Spunta' plants grown from infected seed tubers whereas non infected plants seemed to be apparently healthy. When diseased plants were harvested and their below-ground organs washed with tap water, light brown lesions were observed, with variable degrees, on their below-ground stems, roots and stolons but progeny tubers seemed to be none infested.

Black dot severity recorded 60 DPP on the below-ground plant parts varied significantly ($P \leq 0.05$) depending on tuber-borne inoculum levels tested. In fact, as shown in **Fig. 2**, the most severe black dot symptoms on the below-ground stems as well as on roots and stolons (DSI of 3 and 3.5, respectively) were induced by the greatest inoculum level (i.e. 5). The sclerotial density and lesions formed were 4 and 7 times higher on the below-ground stems and roots and stolons, respectively, when the tuber's infestation level varied from 1 to 5. Thus, black dot severity significantly raised as the level of *C. coccodes* inoculum increased. However, black dot severity was significantly similar when seed tubers showing *C. coccodes* inoculum levels ranging from 1 to 4 were used. It should be also mentioned that no black dot symptoms developed on plants issued from the symptomless seed tubers (i.e. level 0) which may reflect the rigor in tuber sorting before planting and the absence of latent infection in the seed lot.

Correlation analysis revealed that the levels of tuber infection tested were highly and positively associated with DSI records on the below-ground stems, roots and stolons ($r = 0.682$, $p = 0.00003$, n = 30; $r = 0.784$, $p = 0.0000003$, n = 30, respectively) showing their impact on disease development and intensity. Moreover, a highly significant and positive correlation was found between both disease parameters records (i.e. DSI) ($r = 0.713$, $p = 0.00001$, n = 30).

Field experiment data also revealed a significant ($P \leq 0.05$) variation in black dot severity on the below ground plant parts when seed tubers presenting different levels of infection by *C. coccodes* were used (**Fig. 3**). In fact, the highest disease records on the below-ground stems, which were significantly similar, were noted with the severely infected seed tubers i.e. levels 4 and 5 (DSI = 2.5 and 3, respectively). Moreover, these DSI were about three times greater than that recorded with seed tubers having 1 as inoculum level. The severest black dot symptoms on roots and stolons were obtained with the heavily infected tubers where the DSI ranged from 1 to 3.5 when the seed inoculum level varied from 1 to 5, respectively.

Fig. 3 also revealed a slight disease development on potato plants grown from the apparently symptomless seed tubers; the DSI recorded did not exceed 0.5 and was significantly lower than those of the other inoculum levels tested.

C. coccodes was successfully isolated on PDA from all diseased plants which confirmed its involvement in the symptoms observed and assessed.

As noted for the *in vivo* experiment (i.e. pot experiment), field assessments of disease severity in relation to tuber-borne infection level also revealed, via correlation analyses, that the inoculum levels were highly and significantly related to disease severity recorded on the below-ground stems, roots and stolons (r = 0.701, p = 1.12e-029, n = 192; r = 0.739, p = 2.202e-037, n = 192, respectively). Thus, black dot severity was highly dependent on the concentration of tuber-borne inoculum. Furthermore, a high correlation level among both disease severity scores on the below-ground plant parts was noted (r = 0.816, p = 3.59e-047, n = 192). These results also highlighted the adverse effects of contaminated seeds on black dot severity.

Effect of the tuber-borne inoculum level on the aerial part fresh weight

The aerial part fresh weight noted 60 DPP did not vary significantly (P = 0.061) when seed tubers with different black dot severities were used. However, even statistically insignificant, this parameter was found to decrease, compared to plants issued from the symptomless seed tubers, by 24 and 42% with the heavily infected ones i.e. levels 4 and 5, respectively (**Fig. 4**). Thus, the highest infestation level used resulted in significant adverse effects on plant normal growth.

This parameter was not considered in the field experiment due to early senescence and drying of some severely infected plants.

Correlation analysis also showed that the aerial part fresh weight of potato plants was influenced by inoculum levels at seed tuber surfaces. In fact, a significant and negative correlation occurred between these both parameters (r = -0.483, p = 0.007, n = 30). This growth parameter was also related to both disease severity scores on the below-ground stems, roots and stolons; a negative and significant correlation was recorded (r = -0.458, p = 0.011, n = 30; r = -0.742, p = 0.000003, n = 30).

Effect of the tuber-borne inoculum level on tuber weight

The pot experiment of the effect of the tuber-borne level of infection with *C. coccodes* on disease severity demonstrated that the tuber weight recorded 60 DPP was significantly similar (P = 0. 583) for all inoculum levels tested (**Fig. 5**). However, even statistically insignificant, tuber yield reduction on plants grown from seed tubers having the highest levels of infection was about 14-21%, compared to the lowest inoculum level (i.e. 0). This data revealed the negative impact of *C. coccodes* on tuber yield when naturally contaminated seeds were used.

It should be mentioned that no correlation was found between the tuber weight, the inoculum levels tested and black dot severity on the below-ground stems, roots and stolons (r = -0.319, p = 0.086, n = 30; r = -0.085, p = 0.654, n = 30; r = -0.358, p = 0.052, n = 30, respectively).

Under field conditions, tuber weight noted 90 DPP varied significantly (P = 0.006) depending on treatments tested (**Fig. 6**); it decreased with the increase of the tuber-borne inoculum level. In fact, the highest tuber yield was recorded on potato plants issued from seed tubers with no apparent black dot symptoms whereas the lowest records were noted with the heavily infected ones. Yield decrease, subsequent to the planting of diseased seeds, varied between 15 and 27% when their inoculum levels ranged from 1 to 5.

Contrarily to the pot experiment, the field study revealed the existence of significant and negative correlations between the tuber yield and the degree of seed-borne inoculum as well as both disease severity parameters (i.e. DSI) on the below-ground stems, roots and stolons (r = -0.257, p = 0.0003, n = 192; r = -0.169, p = 0.019, n = 192; r = -0.163, p = 0.024, n = 192, respectively).

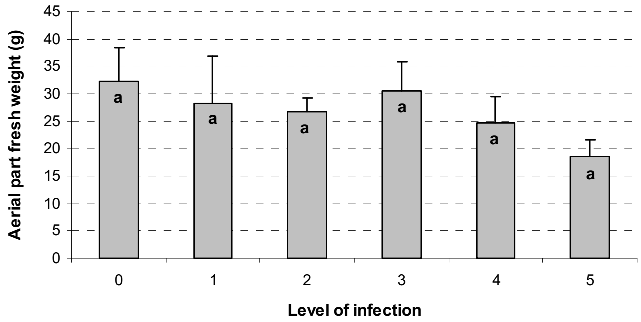

Fig. 4 Effect of tuber-borne *Colletotrichum coccodes* inoculum level on the aerial part fresh weight recorded 60 DPP on potato cv. 'Spunta' plants (pot experiment). Bars with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$). The scale of the level of infection on the X-axis varied from 0 to 5 where 0 = no lesion present, 1 = 1 to 10%, 2 = 10 to 30%, 3 = 30 to 50%, 4 = 50 to 75%, and 5 = 75 to 100% of the tuber surface area covered with black dot lesions.

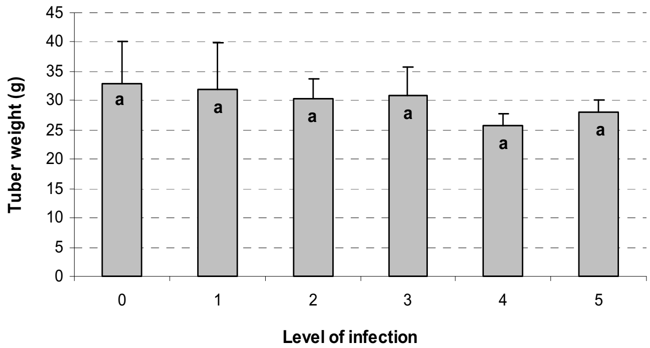

Fig. 5 Effect of tuber-borne *Colletotrichum coccodes* inoculum level on tuber weight recorded 60 DPP on potato cv. 'Spunta' plants (pot experiment). Bars with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$). The scale of the level of infection on the X-axis varied from 0 to 5 where 0 = no lesion present, 1 = 1 to 10%, 2 = 10 to 30%, 3 = 30 to 50%, 4 = 50 to 75%, and 5 = 75 to 100% of the tuber surface area covered with black dot lesions.

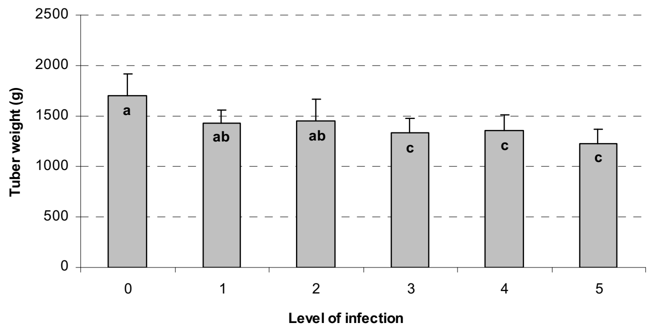

Fig. 6 Effect of tuber-borne *Colletotrichum coccodes* inoculum level on tuber weight recorded 90 DPP on potato cv. 'Spunta' plants (field experiment). Bars with the same letters are non-significantly different according to the LSD test ($P \leq 0.05$). The scale of the level of infection on the X-axis varied from 0 to 5 where 0 = no lesion present, 1 = 1 to 10%, 2 = 10 to 30%, 3 = 30 to 50%, 4 = 50 to 75%, and 5 = 75 to 100% of the tuber surface area covered with black dot lesions.

DISCUSSION

The literature review of black dot severity in relation to the effect of inoculum sources on black dot severity concluded in general to a more severe effect of soil inoculum compared to folair and tuber-borne infections (Read and Hide 1988, 1995a; Denner *et al.* 1998; Nitzan *et al.* 2005, 2008; Lees *et al.* 2010) and that disease severity remained constant above a threshold of soil-borne inoculum (Nitzan *et al.* 2008). In all of the above mentioned studies, experiments were mainly based on artificial soil infections or tuber inoculations for the assessment of inoculum levels influence on disease development and incidence (Dashwood *et al.* 1992; Reid and Hide 1995; Nitzan *et al*. 2008; Pasche *et al*. 2010). However, very little or no experiments were conducted based on natural infection, except the work of Dashwood *et al.* (1992) and Nitzan *et al.* (2005), as done in the present study with seed tubers naturally infected with different *C. coccodes* inoculum levels. Thus, the current study is the first in Tunisia and probably in the world that compares, quantitatively, the incidence of seed tuber inoculum levels on black dot development based on disease, growth and yield measurements.

Results from our study, including disease severity, growth and yield data, highlighted the potential threat of pathogen development in potato crops if contaminated seeds were used. In fact, black dot symptom's intensity on all the below-ground organs (below-ground stems, roots and stolons) increased proportionally with the rise of inoculum levels on seed tubers and all disease parameters were found to be highly correlated. These findings were recorded both in the pot and the field experiment where important DSI scores as high as 3 and 3.5 (based on 0-4 scale) were noted with heavily infested tubers. Similarly, Komm and Stevenson (1978) observed a linear association between the level of tuber infection on the surface and the level of disease. In the same way, Read and Hide (1995a) observed that black dot on tubers was significantly more severe from severely affected than from disease-free seeds, and was most severe where inoculum, especially large amounts, had been added at planting. However, Nitzan *et al*. (2008) described a non-linear relationship between soil-borne inoculum and disease severity. Read (1993), cited by Denner *et al*. (1998), specified that seed infection, unless severe, is only important early in the season in when soil-borne inoculum level is low. Moreover, the impact seed-borne inoculum seems to vary depending on experiment duration. In fact, Ingram and Johnson (2010) reported that the extent of infection of the root system and progeny tubers grown from an infected seed piece as the sole source of infection, as is the case in our pot experiment, is a function of time, the longer the time the more extensive is infection. Infected seed tubers may result in early-season plant infection (Komm and Stevenson 1978; Barkdoll and Davis 1992) as observed in the present study, where severe symptoms developed within 60 days. Read and Hide (1988) also indicated that the relative importance of each inoculum source (soil or tuber) depends on disease severity or inoculum concentration. In fact, the serious below ground infections subsequent to the planting heavily infected seed tubers, as shown in our study, were reported to be more economically damaging than above ground infections (Aqeel *et al*. 2008) because they directly impacted plant nutrition. Furthermore, even though tuber-borne inoculum is generally restricted to a small area around seeds, compared with soil-borne inoculum causing multiple sites of infection (Ingram and Johnson 2010; Lees *et al.* 2010), it may cause severe symptoms when heavily infested seeds were used. Thus, the present work presented some relevant knowledge elements concerning the quantitative contribution of each tuber-borne inoculum level on black dot severity and, consequently, their epidemiological impact under Tunisian conditions.

The absence of disease symptoms on plants developed from the non infected seed tubers (level 0) confirmed the absence of latent infection in the seed lot used even though the sorting and assessment were done based on visual observations only. In fact, tuber's classification was made based on the presence and the frequency of typical black lesions and sclerotia (Read and Hide 1988, 1995a; Denner *et al*. 1998) which were also confirmed microscopically as suggested by several authors working on black dot (Dashwood *et al.* 1992; Andrivon *et al*. 1997; Lees *et al*. 2010; Olanya *et al*. 2010) because symptoms may be frequently confused with other tuber blemishing diseases such as silver scurf (Hunger and Mclntyre 1979; Denner *et al*. 1997; Olanya *et al*. 2010). Moreover, even under natural conditions (i.e. in the field experiment), the non infected seed tubers resulted in slight disease development which confirmed the previous conclusions made from the pot experiment. This small disease development may be attributed to the latent soil-borne inoculum because the field was not cropped to potato for three years ago but the pre-existing inoculum may be developed on weeds or on the other host plants grown. In fact, *C. coccodes* is a polyphagous pathogen which may infect several cultivated Solanaceae and Cucurbitaceae plants as well as weeds (Jellis and Taylor 1977; Raid and Pennypacker 1987; Barkdoll and Davis 1992; Dillard and Cobb 1997, 1998; Denner *et al*. 1998; Nitzan *et al*. 2006b; Ben-Daniel *et al*. 2009) and can survive in soil for more than 8 years (Dillard and Cobb 1998; Cullen *et al*. 2002). However, the proportional rise of disease severity with the increase of seed-borne inoculum level, and the successful *C. coccodes* isolation from diseased plants, are more solid reasons for more supporting the involvement of tuber infections in black dot development and intensity than soil-borne inoculum.

It should also be mentioned that the pot experiment reflected the individual contribution of each tuber-borne inoculum level in disease development as the culture substrate was non infested with the pathogen as reflected in symptomless plants grown from the apparently disease-free tubers. The relative effect of inoculum levels was also confirmed *in situ* i.e. field experiment under similar soil and environmental conditions. Moreover, data from both experiments converged on a more severe disease on roots and stolons than on the below-ground stems. These findings are in agreement with our previous records when the aggressiveness of several local *C. coccodes* isolated was assessed on potato cv. 'Spunta' plants (Daami-Remadi *et al*. 2010). Similarly, Andrivon *et al*. (1998) observed that symptoms resulting from seed-borne inoculum developed first on roots, then on stolons and finally on stems and daughter tubers. However, Read and Hide (1988) and Denner *et al*. (1998) demonstrated that there was no significant difference in disease severity on progeny tubers when seeds carrying different levels of inoculum were planted. Nevertheless, Lees *et al*. (2010) stated that in trials where both seed- and soil-borne inocula were present, the presence of seed inoculum increased the level of black dot on progeny tubers. Thus, tuber infection with *C. coccodes* should be considered as seriously as soil-borne inoculum. Indeed, diseases tubers constitute the main means of pathogen spread to new areas (Komm and Stevenson 1978; Barkdoll and Davis 1992; Johnson *et al*. 1997); the subsequent root and stolon colonisation (Read and Hide 1988; Johnson and Miliczky 1993; Read and Hide 1995a; Tsror (Lahkim) and Johnson 2000; Nitzan *et al*. 2002; Lees and Hilton 2003; Nitzan *et al.* 2005) may led to increase in the soil inoculum level and contamination of progeny tubers during harvesting or in store (Hide and Adams 1980; Dashwood *et al*. 1993). However, Read *et al*. (1995) indicated that there was no relationship between the amount of black dot on seed tubers and that developing on the daughter tubers. Due to these contradictory conclusions, effect of tuber-borne inoculum levels on progeny tubers degree of infection needs to be also deeply studied.

The adverse effects of seed tubers infections were assessed on black dot severity as well as on plant growth and production. In fact, the aerial part fresh weight was negatively affected by the highest inoculum levels tested. This growth parameter was correlated with disease severity recorded on seed tubers, below-ground stems, roots and stolons and may be, thus, considered as indicator of disease severity as the other parameters reported in previous studies (Barkdoll and Davis 1992; Johnson 1994; Reid and Hide 1995; Tsror (Lahkim) *et al*. 1999b; Nitzan *et al*. 2008 2009; Ingram and Johnson 2010; Pasche *et al*. 2010). Similarly, Shcolnick *et al*. (2007), when comparing variation of *C. coccodes* aggressiveness depending on isolates, reported the existence of a significant negative correlation between crown symptoms (stem base) and shoot fresh weight whereas they noted that root symptoms were not significantly correlated with these parameters. Our study revealed, based on natural tuber infection, that this growth parameter is highly related to roots and stolons degree of infection (correlation coefficient of about -0.742) rather than to that of the below-ground stem (correlation coefficient of about -0.458).

Data recorded in the current study also revealed the direct effects of the degree of infection of seed tubers, black dot development and the subsequent yield reduction. Indeed, although no significant correlations were observed in the pot experiment between all disease parameters (on seed tubers and below-ground parts) and tuber weight, this parameter was significantly related to disease intensity in the field experiment. The essay duration which was 60 days for the pot experiment compared with 90 days for the field study may be responsible of these variations in correlation analyses results. However, the overall trend in both experiments in that yield loss increased with the rise of the inoculum levels at the seed tuber surfaces. This reduction in tuber weight subsequent to *C. coccodes* infection is in accordance with the findings of Read and Hide (1995a) where severely affected seed tubers yielded significantly less than healthy seeds. In the same way, Dashwood *et al.* (1993) indicated that infection of potato roots by fungal pathogens may result in poor plant emergence and growth, early senescence and reduced tuber yield. Similarly, Nitzan *et al.* (2008) mentioned that plants grown from infected tubers had reduced yields even though they produced similar numbers of tubers as the non-inoculated plants in all trials. Also, Tsror (Lahkim) *et al.* (1999b) suggested that tuber infections with *C. coccodes* result in greater yield reduction compared to foliar inoculations.

Furthermore, it should be signaled that seed tubers inspection and classification in different disease categories were made based on external symptoms only and no quantifications of vascular or stem end colonization were done. In fact, tuber-borne *C. coccodes* inoculum was known to be external i.e. sclerotia on the epidermis or internal as hyphae situated in the vascular bundles associated with the stolon end (Tsror (Lahkim) *et al.* 1999a; Lees and Hilton 2003; Nitzan *et al.* 2008). However, this method of tuber inspection was reported to be valid and rapid for regular assessments (Nitzan *et al.* 2005; Lees *et al.* 2010) and may be adopted by farmers during tuber's visual selection before planting as done for the other tuber diseases or infection such as dry rots and late blight. However, more accurate methods were developed for the detection of latent tuber infections which were based on pathogen isolation frequency from the stem end or tuber periderm (Johnson *et al.* 1997; Nitzan *et al.* 2006a), PCR (Cullen *et al.* 2002) and recently, Fourier Transform Infrared Microscopy (FT-IR microscopy) (Erukhimovitch *et al.* 2007). These techniques may be of great use in research studies and quarantine laboratories during seed lots inspections of this increasingly important worldwide pathogen.

CONCLUSIONS

The present study elucidated the relative contributions of different inoculum levels in causing black dot disease and impacting plant growth and yield. Thus, disease-risk and expected yield loss, depending on degree of seed tubers infection, can be estimated in the light of our records on the most grown cultivar in Tunisia. However, additional information is needed concerning the influence of local potato cultivars on black dot severity and impact.

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