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Effect of Temperature on Aggressivity of Tunisian *Fusarium* species Causing Potato (*Solanum tuberosum* L.) Tuber Dry Rot

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Abstract: Mycelial growth on PDA of *Fusarium graminearum*, *F. oxysporum* f. sp. *tuberosi*, *F. sambucinum* and *F. solani*, causal agents of potato tuber dry rot, depends upon temperatures ranging from 5 to 40°C. An interaction between pathogens tested and temperatures used for culture incubation was observed (at $p \leq 0.05$). These *Fusarium* species showed *in vivo* both thermal pics of aggressivity: a first one at low temperatures (10-15°C) and a second at higher temperatures (30-35°C). Nevertheless, at temperatures less than 25°C, *F. sambucinum* and *F. graminearum* were the most aggressive, while *F. solani* was the most aggressive at temperatures superior to 30°C.

Key words: *Solanum tuberosum*, mycelial growth, incubation, lesions, thermal optimum

INTRODUCTION

Potato tuber dry rot incidence varies upon *Fusarium* spp. involved in disease development, soil, environmental conditions and cultivars. *F. sambucinum*, *F. solani* var *coeruleum* and *F. oxysporum* are the most aggressive agents (Tivoli *et al.*, 1986; Theron and Holz, 1989, 1990; Desjardins *et al.*, 1992; Choiseul, 1996; Carnegie *et al.*, 2001). However, these pathogens are site specific and their aggressivity varies upon experimental conditions such as temperature used for tuber incubation (Jellis and Starling, 1983; Bjor, 1987; Theron and Holz, 1987).

Fusarium has a thermal spore formation and development optima at 20°C; but this temperature does not necessarily coincide with that required for tuber infection. *Fusarium* thermal infection optima are specific for every country showing pathogen adaptation aptitude (Tivoli and Jouan, 1981; Tivoli *et al.*, 1986; Seppanen, 1981).

Thermal requirements of tuber infection depend also on *Fusarium* species. They varied from 24 to 30°C for *F. avenaceum*, from 18 to 24°C for *F. coeruleum* and from 12 to 30°C for *F. sambucinum* (Seppanen, 1982).

Temperature affects also the installation phase of different *Fusarium* species, the number of tuber infections as well as the degree of virulence of *Fusarium* species and the cultivar's resistance (Henrikson, 1977; Tivoli and Jouan, 1981; Corsini and Pavek, 1982).

In Tunisia, dry rot incidence tends to increase from year to another due to the importance of the soil phase of the causal agents, non respect of sufficiently long rotation, application of monoculture and absence of resistant cultivars and registered fungicides (Daami-Remadi and El Mahjoub, 1996a,b; 2004, 2006; Priou and El Mahjoub 1999; Triki *et al.*, 2001). Furthermore, dry rot causal agents are frequently present in rotten tubers as mixed infections and these phenomena are observed in refrigerated and traditional storages. Losses due to dry rot development in store are reported to exceed 50% even in refrigerated stores (Chérif *et al.*, 2000). However, fungal isolations showed variation in *Fusarium* spp. frequency between both types of storages without a qualitative variation in pathogen population (Daami-Remadi, unpublished data). Thus, in order to determine the specific thermal requirements of the Tunisian *Fusarium* species, representing the major causal agents of tuber dry rot in local potato storage

conditions, a range of temperatures is tested *in vitro* and *in vivo* on the development of *F. solani*, *F. graminearum*, *F. sambucinum* and *F. oxysporum* f. sp. *tuberosi*. This study, which has never been investigated in Tunisia, will also explain the presence of mixed infections observed in refrigerated and/or traditional potato storages and the predominant species of *Fusarium* involved.

MATERIALS AND METHODS

Pathogens: *Fusarium* spp. were isolated from tubers showing typical symptoms of dry rot or potato plants partially or totally wilted. It is a fungi complex composed of *F. solani*, *F. graminearum* (isolated on 2003), *F. sambucinum* and *F. oxysporum* f. sp. *tuberosi* (isolated on 2004). These *Fusarium* species are cultured at 25°C on PDA (Potato Dextrose Agar) containing streptomycin-sulphate at 300 mg L⁻¹.

For their long term preservation, monoconidial cultures are maintained at -20°C in a solution containing 20% of glycerol.

Fusarium spp. identification is realized via several morphological (phialides, microconidies, macroconidies, chlamydospores) and cultural criteria such as colony aspect, pigmentation, front growth following keys proposed by Messiaen and Cassini (1968) and Tivoli (1988).

These agents are inoculated to healthy tubers bimonthly and re-isolated and purified after dry rot development for maintaining their virulence.

Potato cultivars: Tubers used for pathogenicity tests of *Fusarium* spp. belong to cv. Spunta the most cultivated in Tunisia. They are obtained on 2004 from the Technical Potato Centre of Tunisia and maintained, in the dark, at 6°C for two months and moved to room temperature several hours before inoculation.

Isolation of *Fusarium* spp.: Potato tuber tissues showing dry rot symptoms were superficially disinfected with an hypochlorite-sodium solution diluted at 1% during 4 min, rinsed with sterile distilled water then air dried under a laminar flow. Disinfected fragments are placed on PDA (Potato Dextrose Agar) containing streptomycin-sulphate at 300 mg L⁻¹. Purification of isolates is realized via successive cultures from front growth of colonies, followed by a monoconidial culture. Pathogen cultures are incubated at 25°C for 7 days.

Determination of thermal optimum for *Fusarium* spp.

***in vitro* growth:** The technique used consists of aseptically depositing a fragment of mycelium (diameter 6 mm), removed from a seven days pathogen culture, at the centre of a Petri dish (90 mm in diameter) containing PDA. Fungal cultures were incubated, in the dark, in growth chamber under different thermal conditions: 5, 10, 15, 20, 25, 30, 35 and 40°C during 6 days.

One isolate per *Fusarium* specie is used. Every elementary treatment is repeated eight times. At the end of incubation period, mean colony diameter is noted.

Statistical analyses (ANOVA) are performed following a completely randomised factorial design where *Fusarium* isolates and temperatures are both fixed factors. Means are separated using Fisher's protected LSD test ($p \leq 0.05$).

Effect of temperature of incubation on *Fusarium* spp.

aggressivity on potato tubers: Tubers of cv. Spunta are individually inoculated by four *Fusarium* species. Inoculation technique consists of depositing an agar disc (6 mm diameter) colonized by pathogen at wounds (6 mm diameter and depth) occasioned on tubers by a corer bore. Tuber incubation is realized in growth chamber at 5, 10, 15, 20, 25, 30, 35 and 40°C for 21 days at high relative humidity. Every elementary treatment is repeated twenty times (ten tubers × two wounds).

After incubation period, tubers were cut longitudinally via sites of inoculation. Parameters of dry rot induced (maximal width (w) and depth (d)) are noted. The pathogen penetration within tubers, is calculated following formula of Lapwood *et al.* (1984) where:

$$\text{Penetration (mm)} = (w/2 + (d-6))/2$$

Statistical analyses (ANOVA) are performed following a completely randomised factorial design where fungal isolates and temperatures are both fixed factors. Means are separated using Fisher's protected LSD test ($p \leq 0.05$).

RESULTS

Thermal optimum for mycelial growth of *Fusarium*

spp.: Mean diameter of colonies formed after 6 days of culture on PDA depends on *Fusarium* species and temperatures of incubation. A significant interaction (at $p \leq 0.05$) was observed between both fixed factors.

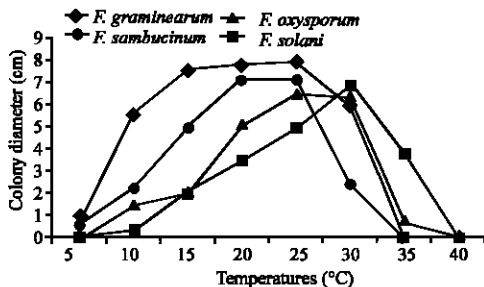


Fig. 1: Effect of temperatures of incubation on mycelial growth of *Fusarium* spp. causing potato tuber dry rot (PDA, after 6 days) LSD (*Fusarium* spp.×Temperature) = 0.222 cm (at $p \leq 0.05$).

At higher temperatures such as 40°C, none mycelial development was observed for all *Fusarium* species tested, whereas at lower temperatures such as 5°C, there is no mycelial growth for *F. solani* and *F. oxysporum* f. sp. *tuberosi* but a minimum growth is noted for *F. sambucinum* and at a lesser degree, for *F. graminearum*.

Thermal optima for mycelial growth of *Fusarium* spp. are specific for every specie. In fact, for *F. sambucinum* and *F. graminearum*, the optimum is comprised between 20 and 25°C and this last specie also grows at 15°C.

F. oxysporum f. sp. *tuberosi* seems to have its thermal optimum of *in vitro* growth between 25 and 30°C whereas that of *F. solani* is at 30°C. Figure 1 also shows that at temperatures inferior to 25°C, *F. sambucinum* and *F. graminearum* grow faster but at temperatures superior to 25-30°C, *F. solani* and *F. oxysporum* f. sp. *tuberosi* are most favoured.

Effect of temperature of incubation on dry rot development on tubers inoculated by *Fusarium* spp.:

Development of tuber dry rot is assessed on cv. Spunta depending on *Fusarium* species and temperatures of incubation. Variance analyses of mean pathogen penetration into tubers revealed existence of a significant interaction (at $p = 0.05$) between both fixed factors. Figure 2 showed that aggressivity of different *Fusarium* species varies upon temperatures of incubation and their thermal optimum for dry rot induction seems to be specific. In fact, that of *F. graminearum* is comprised between 25 and 30°C, but this pathogen occasioned a lesser rot at lower temperatures such as 5 and 10°C. However, at 10-15°C, *F. sambucinum* seems to be more aggressive but at these temperatures, rots observed tend to develop in large then in depth (Fig. 3).

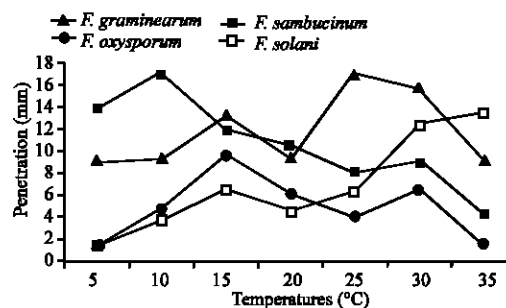


Fig. 2: Effect of temperatures of incubation on dry rot development on potato tubers cv. Spunta inoculated by *Fusarium* spp. (after 21 days) LSD (*Fusarium* spp.×Temperatures) = 2.779 mm (at $p \leq 0.05$).

It is important to note that majority of *Fusarium* species showed both thermal pics one at lower temperatures and a second at higher temperatures. In fact, *F. sambucinum* showed a pic of aggressivity at 10°C and a second but less important at 30°C. *F. graminearum* has a first pic at 15°C and a second at 25-30°C.

At temperatures inferior to 25°C, *F. sambucinum* and *F. graminearum* seem to be more aggressive than *F. solani* and *F. oxysporum* f. sp. *tuberosi*. Furthermore, for these both agents, none dry rot development was observed at 5°C, contrary to that noted for *F. sambucinum* and *F. graminearum*. For *F. oxysporum* f. sp. *tuberosi*, maximum of aggressivity was reached at 15°C whereas at higher temperatures such as 30 and 35°C, it was *F. solani* that occasioned the most important dry rot (Fig. 4).

When aggressivity of *Fusarium* spp. was observed depending on incubation temperatures, two groups can be distinguished, one containing *F. sambucinum* and *F. graminearum* that are more aggressive at temperatures inferior or equal to 20°C and the second group includes *F. oxysporum* f. sp. *tuberosi* and *F. solani*.

At a temperature such as 30°C, the difference between *Fusarium* spp. aggressivity is well distinguished. In fact, all *Fusarium* species occasioned dry rot which importance was determined by the aggressivity of the pathogens. *F. graminearum* and *F. sambucinum* are more aggressive than the other species. However at 35°C, *F. graminearum* showed decrease of their development *in vivo* and *F. solani* becomes more aggressive.

It is important to note that at 40°C, latent infections due to *Erwinia* spp. and interaction with other tuber rot agents inhibits assessment of dry rot development at this temperature.

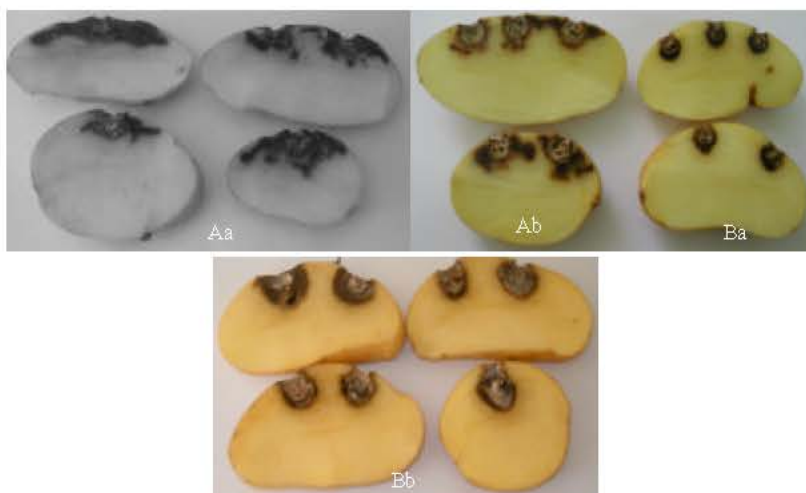


Fig. 3: Dry rot occasioned by *F. sambucinum* on potato tubers cv. Spunta incubated for 21 days at 10°C (Aa) and 5°C (Ab) and by *F. graminearum* at 5°C (Ba) and 15°C (Bb)

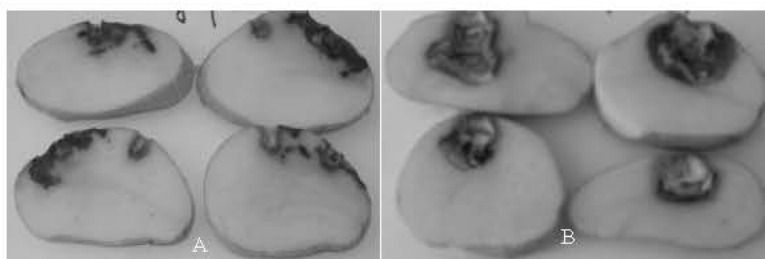


Fig. 4: Dry rot occasioned by *F. oxysporum* f. sp. *tuberosi* on potato tubers cv. Spunta incubated for 21 days at 15°C (A) and by *F. solani* at 35°C (B)

DISCUSSION

As temperature has a significant role on pathogen development and tuber susceptibility to dry rot, a range of temperatures of incubation was tested on mycelial growth and aggressivity of *Fusarium* complex involved in this disease. Obtained results showed that thermal optima for *in vitro* and *in vivo* growth are specific for each *Fusarium* specie as observed by Tivoli and Jouan (1981) who found that necrosis future is not the same for all *Fusarium* species.

Furthermore, this is the first study in Tunisia revealing interaction of the *Fusarium* complex with temperatures of incubation showing, consequently, variable mycelial growth and aggressivity. This result joins on the whole that of Theron and Holz (1991) reporting that *Fusarium* spp. incidence depends considerably upon temperatures of incubation of inoculated tubers.

F. sambucinum and *F. graminearum*, tested in the current study, are aggressive at lower temperatures; this result explains presence of mixed infections occasioned by *F. sambucinum* and *F. graminearum* in refrigerated storage in Tunisia and consequently their ability to cause dry rot at temperatures 5 and 10°C. These both *Fusarium* species are also able to induce disease even at higher temperatures, joining findings of Seppanen (1981) showing that *F. sambucinum* and *F. culmorum* have a double thermal optimum one at 10-12°C and a second at 24-27°C; this phenomenon was explained by optimised tuber defence reactions between 15 and 25°C.

Present study showed that optimum temperature for *F. sambucinum* *in vivo* development is reported to be 10°C however this pathogen induced rot of lesser importance under other thermal conditions whereas it was reported to be, respectively 10-15°C, 17°C, 15-20°C and even 25°C according to Seppanen (1982), Boyd (1972), Tivoli and Jouan (1981) and Theron and Holz (1990); this fact demonstrated that this pathogen has a large

thermal spectrum suggesting presence of an important ability of adaptation. Furthermore, *F. sambucinum* isolated in France induces symptoms between 5 and 30°C (Tivoli and Jouan, 1981).

Aptitude of *in vivo* development of *F. sambucinum* at lower temperatures was also observed by several authors such as Corsini and Pavek (1986) who found that 10°C, is more favourable for this pathogen growth than for that of *F. coeruleum* and according to Liu and Kushalappa (2002), when infection occurs, it is difficult to slow down lesion expansion even at lower temperatures mainly for *F. sambucinum*. This is also the case for *F. graminearum* as Ali *et al.* (2005) recently reported that, in North Dakota (USA), this pathogen was detected on cultivars Shepody and Russet Burbank stored at lower temperatures and Secor (personal communication) detailed that induced rots occurred on tuber lots stored at 10°C. However, *F. graminearum* occasioning, in the current study, a dry rot significantly important at 15°C, comparatively to other species, seems to have its thermal optimum of aggressivity comprised between 25 and 30°C. This result joins findings of Tivoli and Jouan (1981) who classified French isolates of *F. graminearum*, with *F. culmorum*, within a group of higher thermal optimum situated between 25 and 30°C.

F. solani seems to have its thermal optimum of *in vivo* development situated between 30 and 35°C contrary to 17, 15-20, 15 and 35°C reported, respectively by Boyd (1972), Tivoli and Jouan (1981), Seppanen (1982) and Theron and Holz (1990). In the same way, Carnegie *et al.* (2001) found that incubation of tubers for several weeks at lower temperatures, followed by a higher temperature, is more favourable for dry rot development by *F. solani* var. *coeruleum* than their incubation at a constant temperature of 15°C. However, at 5°C, our data showed none dry rot induction on tubers inoculated by *F. solani* and *F. oxysporum* f. sp. *tuberosi*. This result joins that obtained by Theron and Holz (1990) showing that *F. oxysporum*, *F. solani*, *F. equiseti* and *F. scripi* are the lesser active pathogens at 5°C but at lower temperatures, they found that minor species such *F. acuminatum* and *F. crookwellense* become more pathogenic than *F. oxysporum* and *F. solani* (Theron and Holz, 1991). Hide and Cayley (1985) concluded, via lower temperature effect on *in vivo Fusarium* spp. development, that a chemical treatment is more efficient when tubers are stored at 5°C than at 15°C, suggesting that inoculum introduced via wounds infected tubers slowly, at lower temperatures, being more accessible to fungicide.

Following our present results, Tunisian *Fusarium* species causing potato tuber dry rot have their specific thermal optimum of aggressivity different from previous

findings. Furthermore, the presence of a double thermal pics for major tested *Fusarium* species clarifies presence of mixed infections on rotten tubers and occurrence of *F. graminearum* and *F. sambucinum* in refrigerated stores. Finally, for the further studies and specially differential susceptibility of local potato cultivars and clones to tunisian *Fusarium* species, temperature effect should be implicated for the screening of tuber resistance to dry rot.

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