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Variability in Pathogenicity among Tunisian Isolates of *Phytophthora cactorum* as Measured by Their Ability to Cause Crown Rot on Four Apple Cultivars and MM106 Roostock

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Abstract: Studies on two isolates of *Phytophthora cactorum* recovered from apple plants identified the presence of diversity in pathogenicity. These isolates appeared pathogenic to tested apple trees. It revealed that Golden Delicious, Star Crimson and the rootstock MM106 were more susceptible than Richared and Red Delicious cultivars, but with variable levels of aggressiveness according to physiological stages of tested segments of apple plants. Necrosis caused by *Phytophthora cactorum* isolates was more important on shoot segments than on wood segments.

Key words: Crown rot, apple tree cultivars, *Phytophthora cactorum*, pathogenicity, physiological stages

INTRODUCTION

The decay of apple trees is a serious fungal disease in Tunisia these latest years. This problem is essentially caused by *Phytophthora* spp. (Personal surveys since 2003-2004). These fungi can infect a number of plants including citrus, stone fruit trees, peach and cherry (Erwin and Ribeiro, 1996). A number of *Phytophthora* species have been associated with the symptoms of crown rot of cherry trees in different parts of the world (Mircetich and Matheron, 1976; Wilcox and Mircetich, 1985). The first serious cases of attacks of *Phytophthora* spp. on the fruit trees appeared in the years 50 in apple trees in Germany (Braun, 1952; Braun and Krôber, 1953), England (Smith, 1953), Netherlands (Ten Houten, 1953; Buddenhagen, 1955), Belgium (Soenen and Busschots, 1954) and Italy (Pratella, 1959). It was especially about the type damages collar rot caused by *P. cactorum*. The bark and underlying cambium tissues, starting from the base of the tree and often extending several centimetres above soil level become damaged and gum exudation from the bark tissue is plentiful. The second type of apoplexy occurs in late winter or early spring and is due to *P. syringae* (Kouyeas, 1977). On young trees this type of apoplexy produces typical *Phytophthora* symptoms and the buds usually fail to open. Mature trees, in contrast, produce weak, stunted and chlorotic shoots. Finally, affected trees die in May or early June. Other species of *Phytophthora* have also been associated with crown rot of peach trees; these include *P. cinnamomi*, *P. crypogea* and *P. cambivora* (Wilcox and Ellis, 1989). Recently, *P. cactorum* and *P. syringae* isolates from almond trees and *P. citrophthora* isolate from citrus have been found to cause crown rot on different peach, plum and cherry rootstocks after artificial inoculations (Thomidis, 2001). Its symptoms are sudden death of the trees sometimes but not always, proceeded by mild leaf chlorosis as identified by Kouyeas (1977).

Phytophthora cactorum is responsible for the losses of production of several fruit trees in New York (Jeffers and Aldwinckle, 1986), Colombia and Canada (Utkhede, 1986) and Greece (Chitzanidis and Stylianides, 1987; Thomidis 2000a, b, 2001). In normal year, 1 to 3% of the apple trees die following the decay; these losses increase to 10% or more in humid year. This complex meets on the apple trees suffering asphyxia of the roots following an obstruction in water from soil (Kelmer, 1989; Erwin and Ribeiro, 1996).

Variation in virulence among isolates and geographical regions has long been recognized within *Phytophthora* species (Hamm and Hansen, 1981; Hantula *et al*., 1997; Hwang *et al*., 1996; Lebreton and Andrivon, 1998; Matheron and Mircetich, 1990). Thomas (2002) demonstrated the variation of the virulence of *P. citrophthora* isolated from different plant on peach rootstocks. This information is needed to determine the potential threat to commercial apple trees from *Phytopthora* spp. infecting plants.

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The goal of this study was to test the pathogenicity of *Phytophthora* spp. recovered from apple trees to present varieties and one rootstock at two physiological stages.

MATERIALS AND METHODS

Sampling and isolation of *Phytophthora* spp.: Isolation from root and crown of infected trees samples localized in Foussana and Oued Eddarb areas of Kasserine government in Tunisia. To isolate the pathogen(s) from the plant tissues, small pieces from the margin of the discolored area, including a healthy part of the tissue, were placed on malt medium amended with antibiotic. Isolates were maintained on Malt at 22°C in the culture collection of the Phytopathological laboratory of ESHE-CM (Tunisia). Fresh cultures were prepared by transferring an agar disc bearing actively growing mycelium of *Phytophthora* to plates containing fresh malt. Two isolates were considered in pathogenicity assays, Ph1 and Ph2 identified from Foussana and Oued Eddarb areas, respectively.

Identification of *Phytophthora* spp.: *Phytophthora* spp. is an Oomycete, belonging to the order of Pythiales, family of Pythiaceae, characterized by a siphoned spawn. *Phytophthora cactorum* is characterized by its compact colonies without clean margin, that push a radial way and that are downy and present short aerial hyphes. The sexual reproduction of *Phytophthora* depends on species, either in the cloths of diseased plants (*P. cactorum* and *P. cambivora*) either only in the cultures (*P. infestans*). For *Phytophthora cactorum*, the antheridia applies against the oogona and send to its inside a tube copulate with a reproduction homothallic. Morphologically, the antheridia are spherical to globular. The oogona is uncolored and presents a smooth partition. The oospores germinate producing a sporangia or mycelium. The sporanges presents a characteristic papilla. They are terminal and rarely inset. They are deciduous and carry a short pedicel.

Pathogenicity test: Wood samples of diseased trees were collected in January 2005 from orchard planted between, 1987 and 1992. The rootstock used was MM106. Shoot samples were collected in April 2005.

The excised twig assay, developed by Jeffers *et al.* (1981), used by Matheron and Matejka (1988) and also Thomidis (2003), was applied in these experiments. These tests occurred for wood and shoot samples of 4 cultivars Golden delicious, Richared, Red Delicious, Star Crimson and a rootstock MM 106.

This method is based on the choice of segments having uniform length and diameter. Two types of segments were used to test the influence of physiological stage on pathogenicity tests. For this reason wood and shoot segments were considered in these tests. Segments, 10 cm long and 20 mm in diameter, were disinfected in 10% domestic chloride for 3 min and washed three times in distilled water. The basal end of each twig segment was the pared by tangential cuts 6 mm long and 1-2 mm deep on opposite sides, exposing the cambium region in the center of every segment. Thereafter, we applied a strip of agar covered directly by the fungal colony on the exposed cambium. The twig segments were sealed with parafilm and incubated at 22-24°C in the dark for four days. Strips of agar without mycelium were used for control segments. Then twig segments were then removed and stripped of their epidermis with scalpel. The length of necrosis on each twig segment and for each variety and *Phytophthora* isolate were measured.

Data analysis: The experimental design used throughout the laboratory experiments was completely randomized. All experiments were conducted twice. To combine experiments, the Bartlett's test of homogeneity of variance was used. Data were analyzed by one-way analyses of variance (ANOVA) by SPSS Software program (SPSS Inc. Headquarters, Chicago, Illinois). Means values and standard errors were also calculated to compare the average length of cankers obtained with each isolates of *Phytophthora* spp.

RESULTS

Isolation of *Phytophthora* spp. identification: Based on morphological features, isolation showed the presence of *Phytophthora cactorum* identified for all samples collected the two surveyed areas Foussana and Oued Eddarb.

Pathogenicity tests: In apple trees inoculated with isolates, the first disease appeared as bark discoloration at the point of inoculation and around it, with different level of severity depending on the degree of cultivars susceptibility (Fig. 1). It is to note that isolate Ph1 is more virulent than isolate Ph2.

Essay on wood segments: The length of necrosis developed on Golden Delicious varied from 9.40±0.80 to 11.78±3.87 and Star Crimson cultivar between 8.06±4.65 and 9.75±5.87 mm. For Red Delicious and Richared, the cankers ranged from 1.88±0.89 to 2.29±1.47 and 2.89±1.06 to 3.25±1.67 mm (Table 1).

Essay on shoot segments: The results expressed in length of necrosis (mm) revealed the high susceptibility of Golden Delicious (28.00±11.04 to 29.75±15.57 mm) and Star Krimson (38.06±14.00 to 40.71±13.34 mm) and the

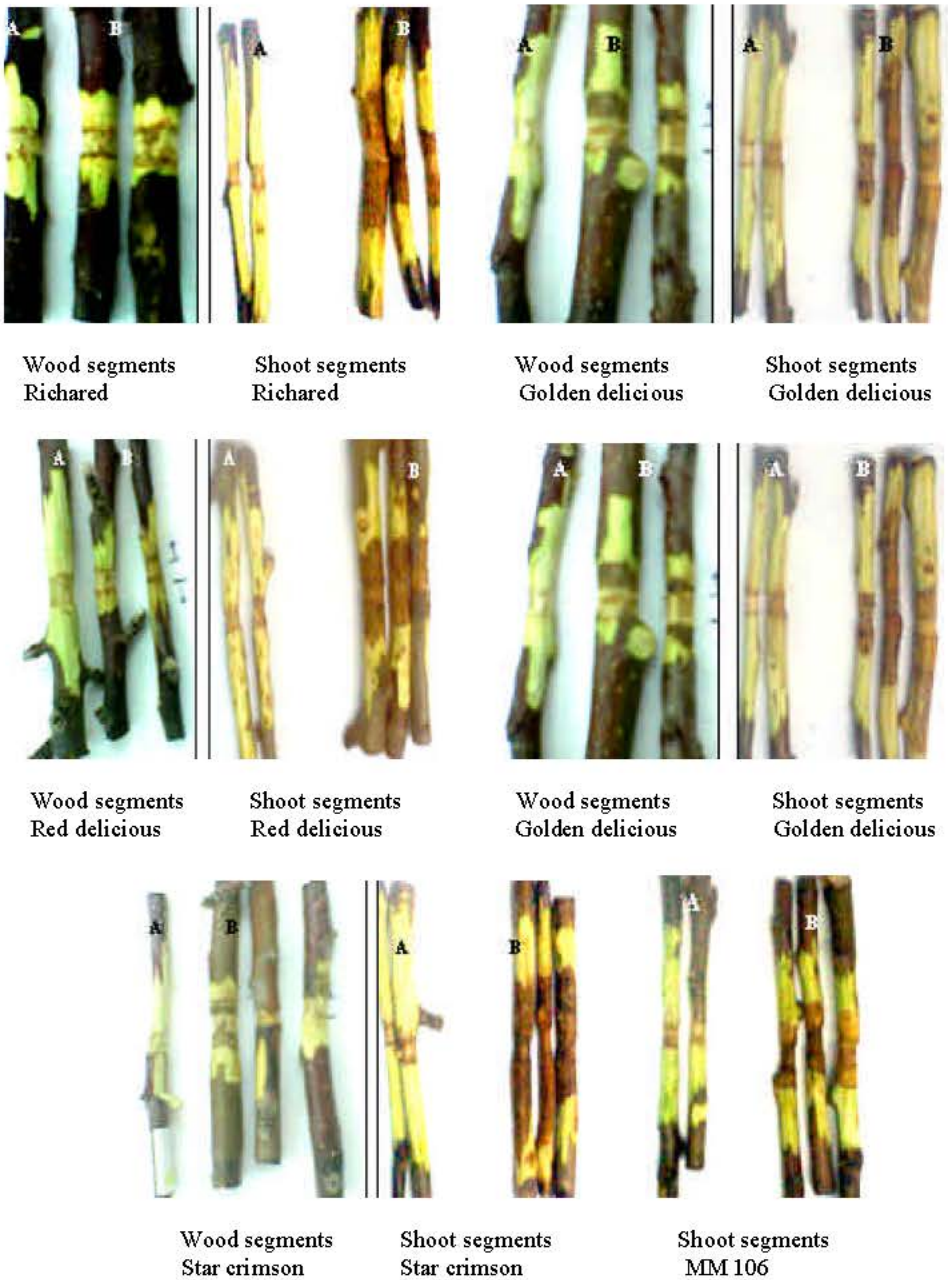

Fig. 1: Importance of canker necrosis developed on four wood and shoot segments of apple cultivars and shoot segments of the rootstock MM 106 inoculated with isolate Ph1 of *Phytophthora cactorum* compared to the controls (A: Control, B: inoculated segments)

Table 1: Pathogenicity (as measured by lesion length (mm)) of two isolates recovered from apple tree plants on four apple cultivars and one apple rootstock at two physiological stages of plant

Wood segments of cultivars					
Isolates	Golden delicious P1	Golden delicious P2	Richared	Red delicious	Star crimson
Ph1	10.88[a]±3.87[b]	10.19±1.00	3.25±1.67	2.29±1.47	9.75±5.87
Ph2	9.78±2.03	9.40±0.80	2.89±1.06	1.88±0.89	8.06±4.65
Control	1.00±0.61	0.89±0.60	0.87±0.67	0.70±0.57	0.81±0.52
Shoot segments of cultivars					
Isolates	Golden delicious P1	Richared	Red delicious	Star crimson	MM106 rootstock
Ph1	29.75±15.57	19.25±12.84	12.13±4.97	40.71±13.34	20.5±3.96
Ph2	28.00±11.04	16.25±11.24	11.64±3.00	38.06±14.00	18.86±2.88
Control	1.40±0.89	1.00±1.00	1.10±1.00	3.00±0.71	1.00±1.00

Ph1 and Ph2: Isolates of *Phytophthora cactorum* identified from Foussana and Oued Eddarb areas, respectively. a: Values are the means of two experiments, each with 10 repliccates b: Standard error

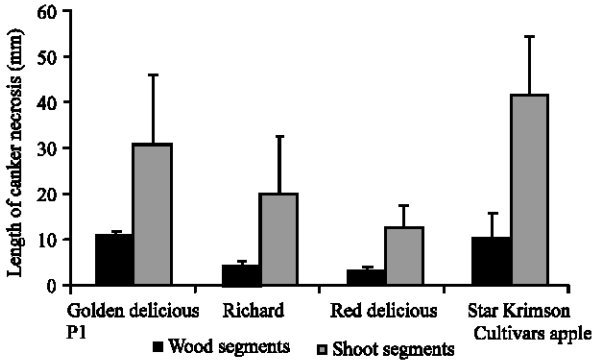

Fig. 2: Effect of physiological stage on the development of canker necrosis on different cultivars of apple inoculated with isolate Ph1 of *P. cactorum*

rootstock MM106 (18.86±2.88 to 20.50±3.96 mm) then the Richared cultivar (14.25±11.24 to, 19.25±12.84 mm) and finally Red Delicious (11.64±3.00 to 12.13±4.97 mm) (Table 1).

Effect of physiological stage of tested segments: Results analysis of effect of physiological stage of apple segments of different cultivars tested in this study showed that the necrosis developed on different segment cultivars inoculated with *Phytophthora cactorum* proved a significative difference of reaction of apple tree plants (Fig. 2). However, in the two cases, Golden Delicious and Star Krimson were the most susceptible, then Richared, Delicious golden, the rootstock MM106 and Red Delicious cultivar.

DISCUSSION

The most common cultivars of apple in Tunisia were tested for pathogenicity against *Phytophthora cactorum* isolated from crown and root of apple trees. In Tunisia, this is the first report of this fungus on apple trees. In the world, Thomidis (2001) reported *P. cactorum* originating from almond trees, as pathogen of cherry trees in Greece. The author showed that *P. cactorum*, *P. ctrophthora* and *P. syringae* to be the cause of the crown rot disease on peach, plum and cherry trees. Tested *Phytophthora* isolates exhibit differential virulence on stone and apple rootstocks, as opposed to strict host specificity. Isolates of *P. cactorum* caused the longest lesions on Golden Delicious and Star Crimson for the two types of segments (wood and shoot).

The excised-twig assay can be used to determine the pathogenicity of different species of Pythiaceous fungi and to compare relative resistance of different apple cultivars and rootstocks. It was also applied by Jeffers *et al.* (1981). Matheron and Mateijka (1988) used the excised-stem inoculation method to evaluate the resistance of apple rootstocks to *Phytophthora parasitica*. Both are reliable and quick methods that allow using ample replications.

The pathogenicity and the aggressiveness of individual isolates differed markedly depending on the cultivars and their levels of resistance. There were significant variations in the extent of tissues colonised. Present results showed that *P. cactorum* exhibit differential virulence on cultivars and one rootstock of apple, varying according to physiological stage. Similarly, Matheron and Mircetich (1985), reported seasonal variation in the susceptibility of *Juglans hindsii* and *Paradoy* rootstocks f. English walnut to *Phytophthora citricola*.

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