

Fungal pathogens associated with crown and collar rot of apple trees in southern Syria

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ABSTRACT

Crown and collar rot of apple trees is a destructive and widespread disease in most areas of the world. Surveys have been done to describe disease symptoms, estimate the disease incidence, and identify the pathogens associated with this disease in southern Syria. Disease incidence was 0.08 – 10 % in most studied sites, only in Alroom location it was up to 14.7 % in 2014 and 17.8 % in 2015, with average of 11.8 %. Symptoms included small pale green leaves, sparse foliage, and a reddish-brown discoloration of inner bark of the infected area at the base of infected tree. The isolated fungi belonged to genera and form genera *Phytophthora*, *Rosellinia*, *Rhizoctonia*, *Phialophora*, *Acremonium*, *Pestalotiopsis*, *Cylindrocarpon* and *Verticillium*. *Phytophthora* was isolated from all infected trees, and was the most frequent pathogen (53.7 % of total isolates). *Phytophthora* isolates recovered from crown cankers of apple were identified as *P. cactorum* (91.5 %) and *P. cambivora* (8.5 %). The results of this study are the first report of crown and collar root rot of apple in Syria.

Key words: crown and collar rot; apple tree; associated fungi, *Phytophthora*; Syria

IZVLEČEK

GLIVNI PATOGENI, POVEZANI Z ODMIRANJEM KROŠNJE IN GNILOBO KORENINSKEGA VRATU JABLANE V JUŽNI SIRIJI

Odmiranje krošnje in gniloba koreninskega vratu jablane je uničujoča in široko razširjena bolezen na večini njenih pridelovalnih območij. Na območju južne Sirije so bile opravljene raziskave za opis bolezenskih znakov, pogostosti pojavljanja boleznih in prepoznavanja njenih povzročiteljev. Na večini raziskanih mest se je bolezen pojavljala z 0,08 do 10 %, samo na lokaciji Alroom pa do 14,7 % v letu 2014 in do 17,8 % v letu 2015, povprečno 11,8 %. Bolezenski znaki so bili majhni blede zeleni listi, redka olistanost in rdeče-rjavo obarvano ličje okuženih delov na bazi debel drevces. Izolati gliv so pripadali naslednjim rodovom gliv: *Phytophthora*, *Rosellinia*, *Rhizoctonia*, *Phialophora*, *Acremonium*, *Pestalotiopsis*, *Cylindrocarpon* in *Verticillium*. Glive iz rodu *Phytophthora* so bile najpogostejši patogen in izolirane iz vseh okuženih drevces, 53,7 % vseh izolatov. Izolati vrst iz rodu *Phytophthora*, ki so se razvili iz rakov v krošnji jablan, so bili določeni kot vrsti *P. cactorum* (91,5 %) in *P. cambivora* (8,5 %). Izsledki te raziskave so prvi o pojavljanju boleznih odmiranja krošnje in gnilobe koreninskega vratu jablane v Siriji.

Ključne besede: odmiranje krošnje; gniloba koreninskega vratu; jablana; z boleznijo povezane glive; *Phytophthora*; Syria

1 INTRODUCTION

Crown rot is an important soil-borne disease of apple *Malus domestica* Borkh. and pear trees *Pyrus communis* L. in most production regions of the world (Jeffers and Wilcox, 1990; Thomidis et al., 2002). Symptoms generally include: reduced tree vigor and growth, yellowing or chlorosis of leaves

and eventual collapse or death of the tree (Nakova, 2010). Crown rots and trees declining and dying are frequently misdiagnosed as suffering from root asphyxiation, and sometimes confused with those suffering from winter injury (Ellis, 2008). Crown rot advances rapidly and trees collapse after the

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first warm weather in spring (Teviotdale and Gubler, 1999).

Several *Phytophthora* spp. are known to cause crown, collar and root rots of apple trees in most areas of the world where this crop is grown (Erwin and Ribeiro, 1996; Jeffers and Wilcox, 1990; Judelson and Blanco, 2005; Brasier, 2008). *Ph. cactorum* (Lebert & Cohn) J. Schröt. has been the species most frequently associated with crown and root rot of apple throughout much of the world, but other species of *Phytophthora* have been associated with crown rot of apple or pears trees such as *Ph. cambivora* (Petri) Buisman., *P. citricola* Sawada, *P. cryptogea* Pethybr. & Laff., *P. drechsleri* Tucker, *P. megasperma* Drechsler., *P. parasitica* Dastur, *Pseudomonas syringae* Van Hall, 1904, and several unidentified *Phytophthora* spp. (Jeffers et al., 1981; Jeffers and Aldwinckle,

1986, 1988; Browne et al., 1995; Thomidis et al., 2002). Crown rot incidence was highly variable, depending on rootstock, pathogen, and flooding duration (Wilcox, 1993). Ellis (2008) states that wet soils that remain saturated for extended periods are required for disease development. Periods of 24 h or more of saturated soil favor *Phytophthora* infections. Conversely, good soil drainage and more frequent but shorter irrigation reduce the risk of root and crown rot (Teviotdale and Gubler, 1999).

In Syria, no previous studies have been conducted on decline and death of apple trees. The purpose of this study was to discover disease symptoms, to evaluate the incidence of this disease, and to identify the fungi associated with crown rot of apple trees in southern Syria where apples and pears are grown.

2 MATERIALS AND METHODS

2.1 Surveying and sampling

The survey was conducted from January 2014 to May 2015 in apple orchards distributed in eight different locations in Daher Aljabal region where apples are grown in the province of Swaida in southern Syria. Percentage of infected orchards at each site, and the percentage of infected trees in each orchard was estimated, then the disease incidence in each site was calculated.

A hundred and forty nine samples of infected plant materials (stem base) were collected from 35 different orchards of approximately 408 acres.

2.2 Fungal Isolation

Necrotic crown tissues were removed from diseased apple trees, thoroughly washed with

running water, surface sterilized with 70 % ethanol for 30 seconds, washed with sterile distilled water, dipped in 6 % sodium hypochlorite for 5 minutes, washed twice with sterile distilled water, dried on a sterilized filter paper, and cut into small pieces (2 x 5 mm). They were then plated into Petri dishes with two media: potato dextrose agar PDA (39 g/l) and cornmeal agar CMA (30 g/l cornmeal and 20 g/l agar in 1 l of sterile water) both supplemented with antibiotic (Amoxicillin 100 ppm). Petri dishes were incubated in darkness at 21°C ± 2 for 7 days. Pure cultures were made by additional transfers (several sub-culture). Fungal isolates were identified based on their morphology according to International Mycological Institute (I.M.I.) Descriptions of Pathogenic Fungi and Bacteria (Minter and Cannon 2015).

3 RESULTS AND DISCUSSION

3.1 Incidence and distribution of apple crown rot

Incidence of the disease varied significantly depending on the location and on the orchards within the same site. The percentage of orchards

where the disease was observed ranged from 0 % to 100 %. The disease incidence was 0.08 - 17.8 %, and the average of the disease incidence was 11.8 % in Daher Aljabal region (Table 1).

Table 1: Incidence of crown and collar rot disease of apple trees in southern Syria

Site	Date	Number of Orchards	Area of studied orchards (hectares)	Percentage (%) of infected orchards	Disease incidence (%)
Ayon Alsoufer	30/01/2014	5	6.3	80	2.5
Albassaa	09/02/2014	7	9.6	71.42	9.6
Alshoaf Algharbi	05/06/2014	5	11.5	20	0.08
Almazlaghat	11/08/2014	10	6.8	10	0.1
Almouaker	11/08/2014	2	2.0	100	10
Alroom	11/08/2014	4	3.0	100	14.7
	03/5/2015	4	3.0	100	17.8
Aljeemah	08/09/2014	1	1.2	100	0.8
Kanawat	09/09/2014	1	0.4	0	0
	2014	35	43.8	47.52	11.8

Disease incidence ranged from 0.08 % to 10 % in most studied sites, only in Alroom location it was 14.7 % in 2014 and 17.8 % in 2015. This high percentage of infected trees was more often observed in lowland areas of the orchards, especially when soils are clay, heavy and poorly drained. In fact, similar results have been reported in some previous studies (Wilcox, 1998; Hickey and Yoder, 2001). Nakova (2010) showed that crown rot spread was 2-3 % in most gardens, and only in an apple orchard in Bjaga (Plovdiv region) it was up to 8-10 % and also pointed out that disease spread is favored by wet, heavy and poorly-drained soils, and heavy rains also provoke disease symptoms.

3.2 Symptoms of apple crown rot

The trees infected by crown and collar rots showed symptoms including bud break delay in early spring, the presence of small pale green leaves,

sparse foliage and the absence of vigorous growth of terminal shoots. In late spring, leaves of infected trees show reddish discoloration and drop down. Twigs and branches dieback. Infected trees may decline slowly, or they may die suddenly in the latter part of the growing season.

A reddish-brown discoloration of inner bark can be seen by removing the outer bark layer of the infected area at the base of infected tree several centimeters above the ground surface (Fig.1). These symptoms were reported in many previous studies of *Phytophthora* root and crown rot (Ellis, 2008; Nakova, 2003, 2010). We also noted that trees showing symptoms of crown rot disease were more sensitive to be infected by bacterial canker and papery bark disease caused by *Pseudomonas syringae* pv. *syringae*. Approximately 30 % of the trees infected by crown rot disease were also infected later by bacterial canker.

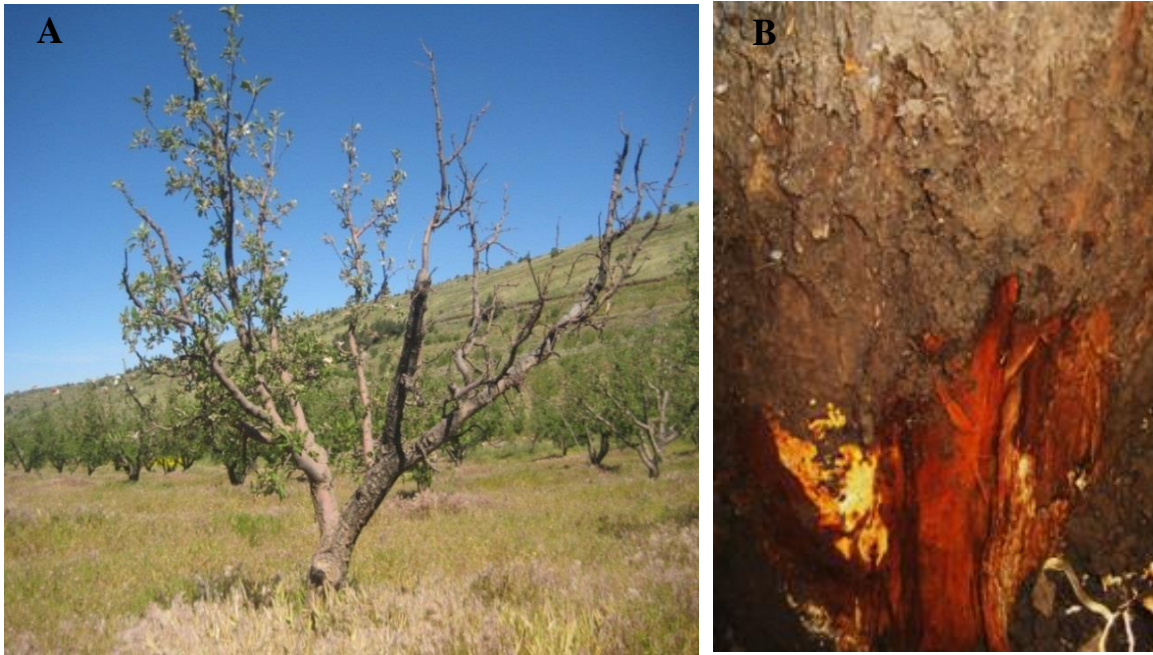


Figure 1: (A) Infected tree showing sparse small chlorotic leaves, die back of branches. The tree was later infected by bacterial canker. (B) A reddish-brown discoloration of inner bark of the infected area at the base of infected tree.

3.3 Fungi associated with crown rot of apple trees

Two hundred sixty isolates were obtained from crown cankers of apple trees. A hundred seventy five were identified according to their morphology. The isolated fungi belonged to genera and form genera such as *Phytophthora*, *Rosellinia*, *Rhizoctonia*, *Phialophora*, *Acremonium*, *Pestalotiopsis*, *Cylindrocarpon* and *Verticillium*. However, the relative dominance of individual species in the fungal community isolated from apple varied among the orchards, and some fungi of this complex were absent in specific orchards. *Phytophthora* was isolated from all infected trees, and dominated the fungal population (53.7 % of

total isolates) recovered from crown cankers of apple. According to their morphology, 91.5 % of *Phytophthora* isolates were identified as *P. cactorum*, and 8.5 % as *P. cambivora*.

Colonies of *P. cactorum* were whitish, fluffy or smooth on PDA. Zoosporangia were oval to elongated (lemon shape) with papillae. Terminal or intercalary chlamydospores were found. Antheridia and oogonia, as well oospores, were formed in large numbers (Fig. 2). Colonies of *P. cambivora* were whitish, fluffy with dense aerial hypha. Zoosporangia were nonpapillate, ellipsoid or ovoid. Chlamydospores were absent.

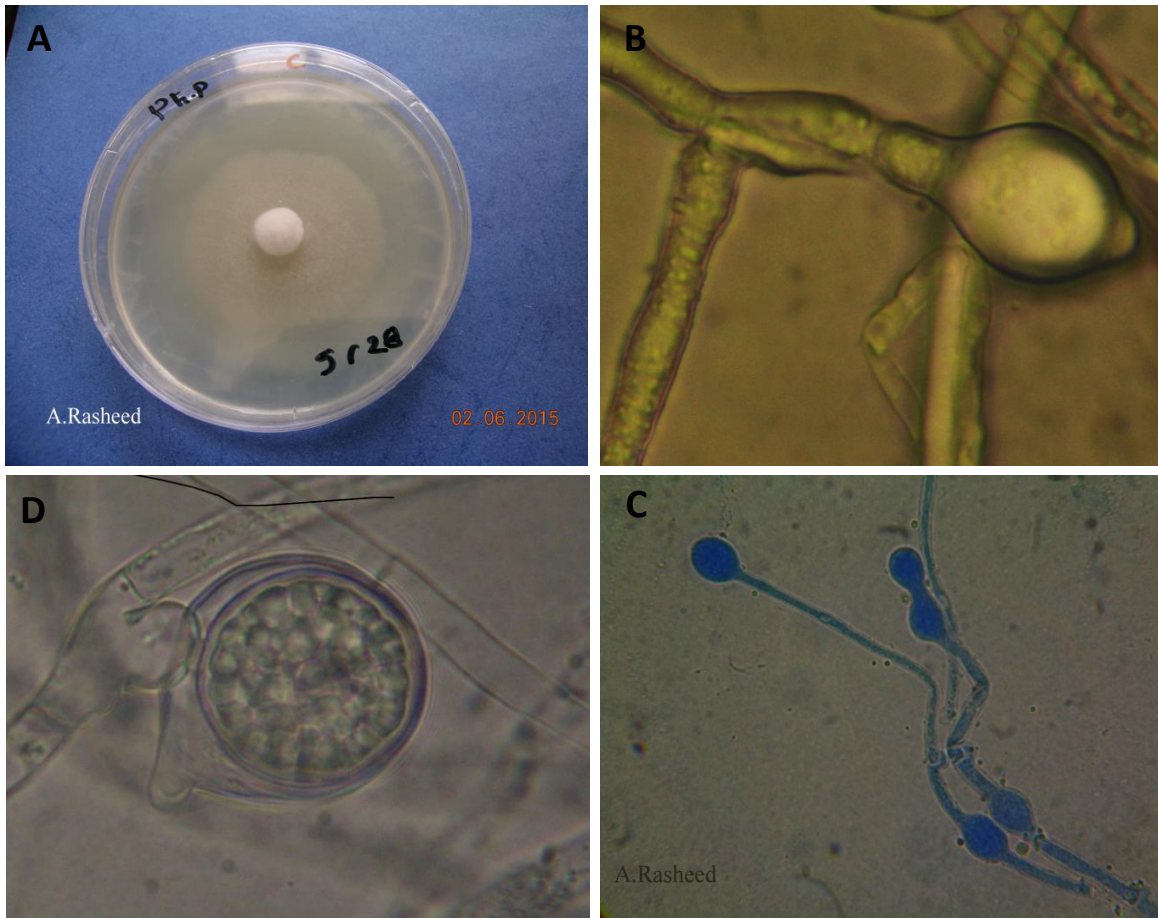


Figure 2: (A) colony of *P. cactorum* on PDA. (B) Limon shape sporangium with papillae. (C) Terminal and intercalary chlamydospores. (D) Sexual organs (antheridium and oogonium).

The morphology of sporangia, and general morphology of isolates of the two identified *Phytophthora* species were similar to those described in many studies (Waterhouse, 1963, 1970; Nakova, 2010; Welsh, 2011). Matheron et al. (1988) showed that *P. cactorum* and *P. cambivora* were highly virulent and caused rapid decline and death of apple seedlings, and these pathogens also were the most frequently isolated *Phytophthora* spp. in commercial apple orchards in Arizona.

The frequency of other fungi associated with crown and collar rot of apple trees varied from 1.7 % for *Verticillium* sp. to 18.3 for *Rhizoctonia solani* J.G. Kühn (Table 2).

This study showed that *P. cactorum* was the most important fungal species causing crown and collar rot of apple in Swaida where the apple trees are grown in southern Syria. In fact, *P. cactorum* has been the species most frequently associated with crown and root rot of apple throughout much of the world (Jeffers et al. 1981; Jeffers and Aldwinckle, 1986, 1988; Browne et al. 1995; Thomidis et al., 2002). Isolation of *P. cactorum* has been possible only from the margins of active lesions. There is evidence that the activity of *P. cactorum* is inhibited in rotted tissues by the antagonistic effect of one or more secondary organisms. These results are in accordance to those of Welsh (2011).

Table 2: Percentage of fungi associated with crown and collar rot of apple

Fungi	Ro	Rh	Ph	Phi	Ac	P	C	V
Number of isolates	11	32	94	13	10	5	7	3
Percentage %	6.3	18.3	53.7	7.4	5.7	2.9	4	1.7

Ro: *Rosellinia*, Rh: *Rhizoctonia* sp., Ph: *Phytophthora* sp., Phi: *Phialophora* sp., Ac: *Acremonium* sp., P: *Pestalotiopsis* sp., C: *Cylindrocarpon* sp., V: *Verticillium* sp.

In addition to *Phytophthora* spp., a fungal complex consisting of *Rosellinia* sp. (6.3 %), *Rhizoctonia solani* (18.3 %), *Phialophora* sp. (7.4 %), *Acremonium* sp. (5.7 %), *Pestalotiopsis* sp. (2.9 %), *Cylindrocarpon* sp. (4 %) and *Verticillium* sp. (1.7 %), was associated with crown and collar rot of apple. Many studies conducted in Washington State indicated that a fungal pathogen complex, consisting of *Nectria radicola* Gerlach & L. Nilsson (*Cylindrocarpon destructans* (Zinssm.) Scholten), *Phytophthora cactorum*, *Pythium* spp., and *Rhizoctonia solani* was the predominant cause of replant disease of apple trees, and were

consistently isolated from symptomatic trees in all orchards, and these fungi were pathogenic to apple in greenhouse tests (Mazzola, 1998; Mazzola et al., 2001; Braun, 1995). *Pestalotiopsis* spp. have been reported to cause root and crown rot of strawberry in Spain (Chamorro et al., 2016), canker and twig dieback of blueberry in Chile (Espinoza et al., 2008), black foot of grapevine in Portugal (Oliveira et al., 1998). In fact, the soilborne fungus *Rosellinia necatrix* Berl. ex Prill., is the causal agent of white root rot disease on numerous plant species, including apple (Pasini et al., 2016).

4 CONCLUSIONS

Crown rot of apple in Swaida in the south of Syria is associated with many fungi, but the main cause of this disease, and the most frequently isolated

pathogen is *P. cactorum*. The result of this study is the first report of crown rot of apple trees in Syria.

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