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Phytophthora Root and Crown Rot on Apples in Bulgaria

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SUMMARY

Phytophthora is a genus of *Oomycota* responsible for some of the most serious diseases with great economic impact (Judelson and Blanco, 2005). While 54 species were found in the 20th century (Erwin and Ribeiro, 1996) another 51-54 new species have been identified (Brasier, 2008) since the year 2000. They are spread worldwide and have broad range of host plants – fruit trees, citrus, forest and park species. *Phytophthora* can cause serious damages in orchards and nurseries of apples, cherries, etc. In Bulgaria they have been found first on young apples and cherries (1998-1999) in Plovdiv region (Nakova, 2003).

Surveys have been done for discovering disease symptoms in Plovdiv and Kjustendil regions. Isolates have been obtained from infected plant material (roots and stem bases) applying baiting bioassay (green apples, variety Granny Smith) and/or PARP 10 selective media. *Phytophthora* strains were identified based on standard morphology methods – types of colonies on PDA, CMA, V 8, type and size of sporangia, oogonia and antheridia, and oospores. Cardial temperatures for their growth were tested on CMA and PDA.

For molecular studies, DNA was extracted from mycelium using the DNA extraction kit. DNA was amplified using universal primers ITS 6 and ITS 4. Amplification products concentrations were estimated by comparison with the standard DNA. Sequencing was done at the Scottish Crop Research Institute (SCRI, Dundee, Scotland).

Phytophthora root and crown rot symptoms first appear in early spring. Infected trees show bud break delay, have small chlorotic leaves, and branches die all of a sudden. Later symptoms are found in August-September. Leaves of the infected trees show reddish discoloration and drop down. Both symptoms are connected with lesions (wet, necrotic in appearance) at stem bases of the trees.

Disease spread was 2-3% in most gardens, only in an apple orchard in Bjaga (Plovdiv region) it was up to 8-10%.

Morphologically, the isolates acquired from the apple trees were identified as *Phytophthora cactorum, P. citrophthora* and *P. cryptogea*. Cardial temperatures for their growth were tested on CMA and PDA.

PCR tests with ITS primers 4 and 6 generated a band at about 800 bP. Consequent sequencing showed that 2 strains, Bg 1/1 and Bg ½, belong to *Phytophthora cryptogea*.

Keywords: *Phytophthora* root and crown rot; Apples; *Phytophthora* sp.

INTRODUCTION

Phytophthora is a genus of *Oomycota* responsible for some of the most serious diseases with great economic impact (Judelson and Blanco, 2005). While 54 species were found in the 20th century (Erwin and Ribeiro, 1996) another 51-54 new species have been identified after the year 2000 (Brasier, 2008). They are spread worldwide and have broad range of host plants – fruit trees, citrus, forest and park species. *Phytophthora* can cause serious damage to apples, cherries and other trees in orchards and nurseries (Newhook, 1959; Jeffers and Aldwinckle, 1987; Ellis, 1997; Growe, 1997; Teviotdale and Gubler, 1999; Wilcox, 1990, 1998). Pear and plum trees appear to be relatively resistant.

Diseases caused by *Phytophthora* species are known in literature as "*Phytophthora* root and crown rot". In Bulgaria, symptoms of *Phytophthora* root, crown and collar rots were first found on young apple and cherry trees in the region of Plovdiv during 1998-1999 (Nakova, 2003).

Trees declining and dying from *Phytophthora* root and crown rots are frequently misdiagnosed as suffering from "wet feet" (root asphyxiation) and sometimes confused with those suffering from winter injury (Ellis, 1997).

Symptom expression depends upon how much of the root or crown tissues are affected, and how quickly they are destroyed. Crown rots advance rapidly and trees collapse after the first warm weather in spring (Teviotdale and Gubler, 1999). Leaves wilt, dry and remain attached to the tree. *Phytophthora* infections typically kill young trees.

Ellis (1997) states that wet soils that remain saturated for extended periods are required for disease development. Above-ground symptoms vary between tree species but generally include: reduced tree vigor and growth, yellowing or chlorosis of leaves, and eventual collapse or death of the tree. Infected trees may decline slowly over one or more years, or they may collapse in spring. Trees may also appear healthy in spring but die suddenly in the latter part of the growing season. Below-ground symptoms include reddish-brown discoloration of the inner bark and wood. A sharp line demarcates the diseased and healthy portion of the crown. Similar symptoms can be found on the roots (Ellis, 1997; Hickey and Yoder, 2001).

Periods of 24 hrs 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). Disease is more often observed in low areas of the orchard on heavy, poorly drained soils (Wilcox, 1998; Hickey and Yoder, 2001).

Phytophthora root and crown rots are caused by several *Phytophthora* species. All of them require extremely wet soils in order to infect and cause significant damage but they differ in destructiveness depending on plant species (Ellis, 1997).

MATERIAL AND METHODS

Phytophthora fungi causing root and crown rot on apples in Bulgaria were first reported in the period 1998-1999 (Nakova, 2003). Further surveys for discovering disease spread in fruit-growing regions of Bulgaria were done over the period 1999-2009.

The spread of *Phytophthora* root and crown rot was calculated based on the percentage of infected trees. From the infected plant material (root, stem base), isolates were obtained applying the "baiting bioassay" method and using PARP 10 media [Corn Meal Agar amended after autoclaving (μg/ml) with pimaricin (10), ampicillin (250), rifampicin (10) and hymexazol (25)] (Erwin and Ribeiro, 1996).

Both procedures are standard methods for isolating *Phytophthora* fungi from infected wood and soils. Plant tissue showing symptoms (from root or crown zones) was thoroughly washed with running water, surface sterilized with alcohol (90%) and cut into small pieces. They were then plated into Petri dishes with PARP media.

Sterilized woody tissues were also used in "baiting bioassay". Green apples served as "trap culture". They were surface sterilized, and then sterile cone-shaped cuts were made. Diseased tissue was placed in the holes, then 4-5 ml of sterile water was added, and wax and parafilm as a cover. The infected fruits were incubated in a growth chamber (25°C, RH 75% and 12 hrs photoperiod) for 7-10 days until disease symptoms appeared. Fruit tissues from the edge of rotten zones were transferred onto PARP media.

Phytophthora strains were identified based on standard morphology methods: types of colonies on PDA, CMA, V 8, type and size of sporangia, oogonia and antheridia, and oospores. Effects of temperatures on their mycelia growth were tested on CMA, V-8 and PDA in a temperature range from 2 to 35-36°C.

For molecular studies, DNA was extracted from mycelium using a DNA extraction kit. DNA was amplified using the universal primers ITS 6 and ITS 4 (www.

phytophthoradb.org). Amplification products concentrations were estimated by comparison with standard DNA. Sequencing was done at the Scottish Crop Research Institute (M. Cooke, SCRI, Dundee, Scotland).

RESULTS

In Bulgaria, disease symptoms were found first on 2-3-year old apple trees in the region of Bjaga village (Plovdiv) during 1998-1999, and also on 2-year old cherry rootstocks (Katunitza, Plovdiv) and 2-year old cherries (Trilistnik, Plovdiv). *Phythophthora* root and crown rot was later registered in Kjustendil, Sliven, Jambol, Karnobat, Bourgas and Svichov regions.

Concerning apples, the disease has been proved to be caused by *Phythophthora sp.* in Plovdiv (Bjaga, Asenovgrad, Tzalapitza), Kjustendil, Pazardzik and Haskovo (Stalevo) regions.

Symptoms of *Phytophthora* root rot disease first appear in early spring. Infected trees show bud break delay, have small chlorotic leaves, and branches die all of a sudden. These symptoms, however, are not enough for a diagnose, and point out that root systems or crowns of the trees can be affected. Reddish brown lesions with wet, necrotic appearance were found on crowns and roots of the infected trees. There is a distinct margin between diseased and healthy wood. Infected wood becomes dark brown and lesions can spread as a ring and also upwards on the trunk of the tree (Figure 1, 2).

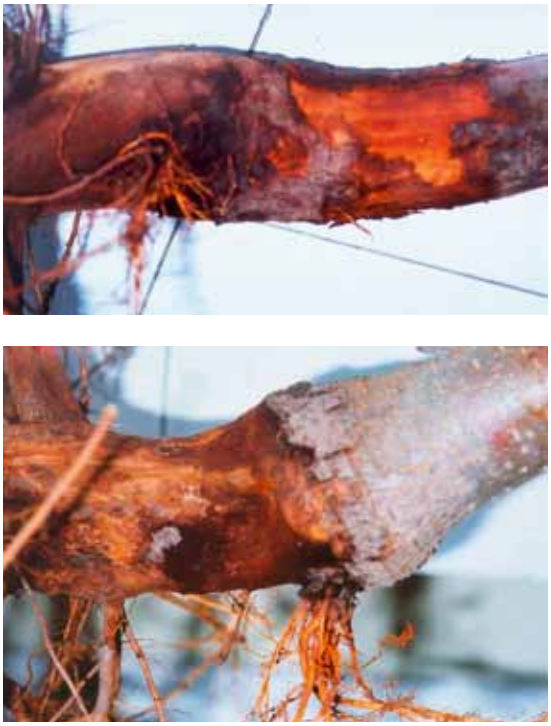

Figure 1. 3-year old apples showing symptoms of *Phytophthora* collar rot – discoloration and wet appearances of inner bark

Diagnostic symptoms are found also bellow the grafting zone as large dark spots clearly defined from healthy tissues. When bark is removed orange to reddish discoloration shows and a dark line marking the border with healthy tissue. Roots show similar symptoms when infected.

Trees with healthy appearance in the spring can all of a sudden wilt and dry out later in the season (mid-August to September). Leaves on the infected trees drop down early and have reddish discoloration by the end of August.

Studies have pointed out that disease spread is favored by wet, heavy and poorly-drained soils. Heavy rains also provoke disease symptoms.

During the year 1999, a complicated disease syndrome was observed after heavy rainfalls of over 100 l/m^2 and flooding of young apple gardens aged 2-3 years. In August, when temperatures have reached 30-35°C at midday, leaves of some trees became reddish or yellowish, started to wilt and entire trees collapsed in a short time. Fruit-growing experts have attributed those symptoms to water and temperature stress. Similar reports, though not always correct, are found also in some publications (Newhook, 1959; Browne and Krober, 1958). Monitoring of some typical fruit growing regions has shown that the disease syndrome has spread some 2-10% and more in some orchards (Table 1).

Spread of the disease varies from 0.63-1% to 8-10%. In an apple garden (Plovdiv, Bjaga) where *Phytophthora* root and collar rot was first recorded, there was 8-10% of infected trees. Up to 5% was the disease incidence in Kjustendil – the second infection focus. Later, the percentage of diseased trees was between 0.63 and 5%, with one exception of the apple garden in Tzalapitza (close to Plovdiv) – 7%.

The isolates obtained in our Laboratory on PARP media or as a result of baiting technique belong mainly to the genus *Phytophthora* (Table 1). Their pathogenicity was proved by inoculation of young apple fruits and 1-year old apple rootstocks.

Identification of the first 12 isolates obtained from 3-year old apples with symptoms of collar rot (region Plovdiv, village Bjaga) was done on PDA based on fungal morphology and cultural characteristics.

Table 1. Disease spread on apple trees in some regions of Bulgaria

Region – Year	No. of trees		Infected trees %	Fungi isolates from genus
	Total	Diseased		
Plovdiv (Bjaga) – 1999	1000	100	10	*Phythophthora* sp.
Kjustendil – 2002	4000	200	5	*Phythophthora* sp.
Pazardzik – 2007	1000	10	1	*Phythophthora* sp.
Plovdiv (Asenovgrad) – 2007	1000	18	1.8	*Phythophthora* sp.
Plovdiv (Tzalapitza) – 2006	3000	210	7	*Phythophthora* sp.
Jambol – 2007	18000	150	0.83	*Phythophthora* sp.
Plovdiv (Stalevo) – 2009	5500	138	2.51	*Phythophthora* sp.
Svichov – 2009	1200	56	4.7	*Phythophthora* sp.

Table 2. Influence of temperatures on mycelia growth (*in vitro* tests) on apple isolate, region Bjaga (Plovdiv), mm

Nutritive media	Temperature °C	Mycelia growth, mm/day							
		1st	2nd	3rd	6th	7th	8th	9th	10th
CMA		–	–	–	–	–	–	–	–
PDA	1-2	–	–	–	–	–	–	–	–
V–8		–	–	–	–	–	–	–	–
CMA		–	–	–	–	–	–	–	–
PDA	5	–	–	–	–	–	–	–	–
V–8		–	–	–	9.15	13.0	15.5	16.5	16.5
CMA		–	9.0	13.0	23.0	26.0	27.0	30.0	33.0
PDA	9-–10	–	–	6.0	22.0	25.0	28.5	31.0	34.0
V–8		–	12.0	15.0	35.0	43.0	48.0	50.0	55.0
CMA		–	24.0	27.0	35.0	37.5	41.0	43.5	46.5
PDA	15	–	16.0	19.0	28.0	31.5	35.6	39.0	43.5
V–8		–	20.0	24.0	36.0	41.5	45.0	50.0	54.0
CMA		15.0	23.5	35.0	60.0	65.0	69.0	71.0	76.8
PDA	20	14.0	20.5	29.0	57.5	66.0	73.5	81.5	83.0
V–8		16.0	27.0	42.0	75.0	78.0	79.0	80.0	83.0
CMA		12.5	21.3	29.5	41.3	44.5	48.6	53.8	53.8
PDA	25	13.0	16.0	20.3	44.3	54.3	61.3	71.0	71.0
V–8		13.0	15.0	24.0	38.3	75.0	84.0	85.0	85.0
CMA		–	15.0	22.0	30.0	34.5	35.0	35.0	35.0
PDA	30	–	12.3	22.0	48.7	59.0	65.0	71.0	74.0
V–8		–	15.5	25.0	55.0	65.0	73.8	80.0	80.0
CMA		–	–	–	–	–	–	–	–
PDA	35-36	–	–	–	–	–	–	–	–
V-8		–	–	–	–	–	–	–	-

Figure 2. 1-year old apple showing symptoms of *Phytophthora* collar rot – distinct line between healthy and infected tissues

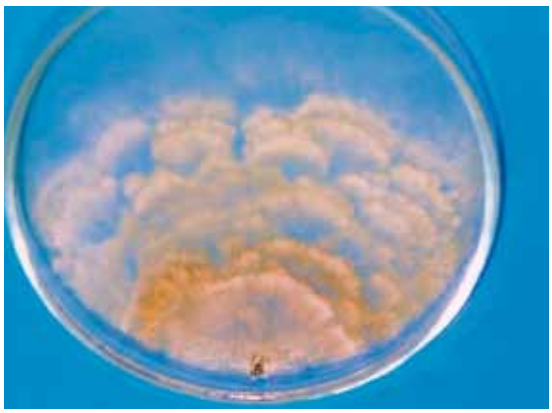

Figure 3. Colony type of the first group isolates from 3-year old apples (Plovdiv region, Bjaga village) (*P. cryptogea* type)

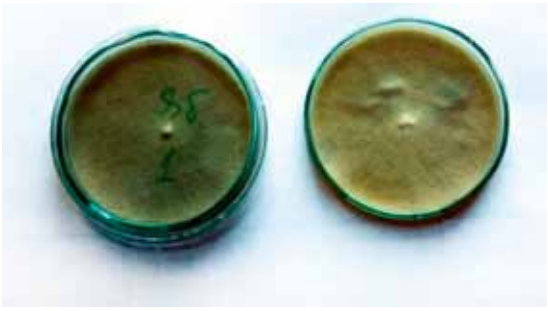

Figure 4. Colony type of the second group isolates from 3-year old apples (Plovdiv region, Bjaga village) (*P. cactorum* type)

Figure 5. Colony appearance of *P. citrophthora* type of isolates

Strains isolated from apple trees differ in appearance. The first group on PDA developed whitish, slightly smoky, featherlike colonies with stellate growth (starlike). Sporangiophores are simple or branched, zoosporangia are oval, fussiform or irregular in shape (38-40 x 31-33 μm). Terminal chlamidospores are rarely formed, especially when NH_4NO_3 was added to the media. Sparse oospores were found (*P. cryptogea* type).

The second group had whitish, smoky, fluffy mycelia. Mycelia hyphae were long, branched, normal or slightly swollen at the point of branching. Conidiophores were simple or sympodially branched. Zoosporangia were oval to elongated (lemon shape), mostly with papillae, average size 33-34 x 26-28 μm on the media. After a culture period of more than 7 days, terminal or intercalary chlamydospores were found. Antheridia and oogonia, as well oospores, formed in large numbers (*P. cactorum* type).

The morphology of isolates obtained later from different regions of the country was studied on PDA, V8 and CMA. They showed growth that is typical for *Phythophthora*:

Colonies were stellate or rosette, or patternless on V8 agar. Sporangia were extremely variable in shape – ellipsoid, ovoid, limoniform or irregural. Conidiophores were irregulary branched or in loose sympodium (*P. citrophthora* type).

Colonies may be slightly radiate, but less defined, compact, fluffy; sporangia – distinctively papillate, broadly ellipsoidal, obpyriform, or ovoid; produced oospores in media and in diseased plant tissues (*P. catorum* type).

Analysing the data from our studies of the morphology of *Phytophthora* fungi and comparing them with results published by other authors (Smith and Smith, 1906; Smith, 1953, 1955; Novotelnova, 1974; Ellis, 1997; Hickey and Yoder, 2001; Erwin and Ribeiro, 1996) we concluded that strains isolated from apples belonged to the species:

Table 3. Influence of temperatures on mycelia growth (*in vitro* tests) on apple isolate, region Kjustendil, mm

Temperature, °C	Nutritive media	Mycelia growth, mm/day						
		1st	2nd	3rd	4th	7th	8th	9th
2	PDA	0	0	0	0	4.0	5.0	6.0
	CMA	0	0	0	0	0	0	0
5	PDA	0	0	0	0	29.0	30.0	45.0
	CMA	0	0	0	0	15.0	16.0	23.0
10-12	PDA	12.0	15.0	43.0	53,5	69.0	85.0	85.0
	CMA	0	17.0	20.0	25.0	27,5	30.0	33,5
20	PDA	25.0	41.0	58.0	70,5	85.0	85.0	85.0
	CMA	9.0	20.0	30.0	37,5	65.0	71.0	80.0
25	PDA	24.0	51.0	76.0	85.0	85.0	85.0	85.0
	CMA	7.0	15.0	20.0	25.0	46.0	54.0	55.0
30	PDA	20.0	36.0	50.0	60.0	67.0	71.0	80.0
35-36	PDA	0	0	0	0	6.0	8.0	14.0
	CMA	0	0	0	0	0	0	0

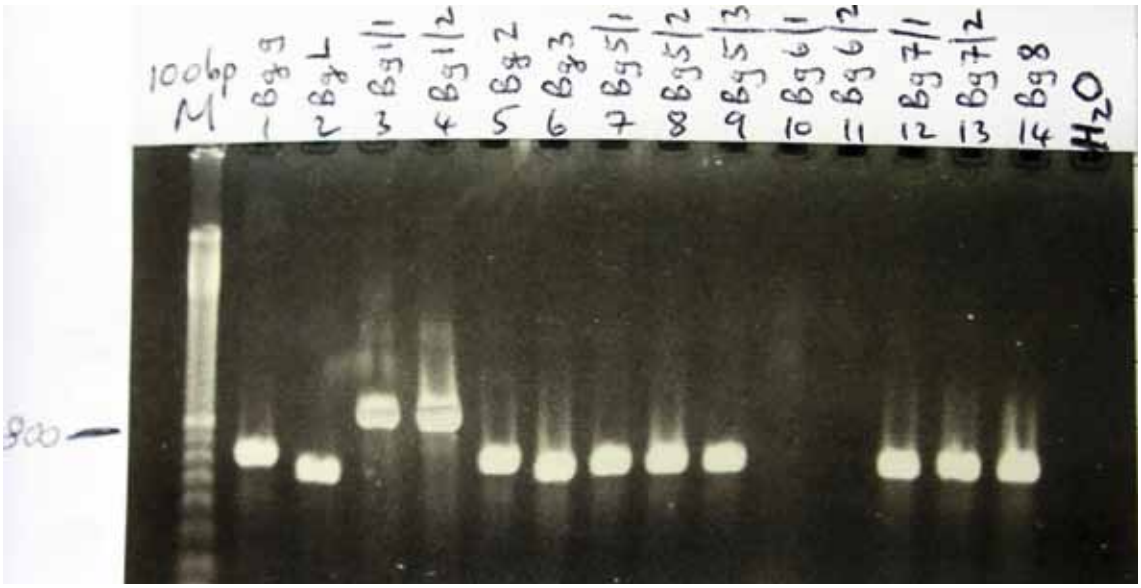

Figure 6. PCR with ITS 4 and ITS 6 primers, isolates Bg1-Bg9

bg1/1
ATTGAGATGCGCGCCGAAGCGCACACAACGTTTCCCAAATGGATCGACCC
TCGTCCACCCCAGCTTGCGCCGGAACAGACACCCTACTTCGCACCACACC
GCCGGTTCAAACGCCAAGCCTAGCACAGCTACGGTTCACCAGTCCATCCA
GCCACAGCAGGAAAAGCACCCAATAAGCGTCTGTTCAGCCGAAGCCAATC
ATACCGCGAATCGAACACTCCTCCATTAAACGCCGCAGCAGACAAACCGG
TCGCCGACATGCCACACAGGCAGCCTCCACAACCAGCAACGTCACGCTTT
TCGAGCAAAGAGAAGTACAGTGTAGTACATTTCAAAGGACTCGCAGCCGG
ACCGAAGACCAGCCGCAAGACACTTCACATCTGCGCGTCCCCATCCACCG
ACTACACGGAAGGAAGGGAGCCAAGTTTAGTGTACGGACACTGATACAGG
CATACTCCCAGGACTAACCCGGAAGTGCAATATGCGTTCAAAATTTCGAT
GACTCACTGAATCCTGCAATTCGCATTACGTATCGCAGTTCGCAGCGTTC
TTCATCGATGTGCGAGCCTAGACATCCACTGCTGAAAGTTGCTATCTAGT
TAAAAGCAGAGACTTTCGTCCCCACAGTATATTCAGTAATTAGGAATGGG
TTTAAAAAATACGCTACTAGCCCAGGCCGAGGCCCAAGCGGTCGCCATGA
TAGAGCCGCCACCCAGCCAGCCGAAAACCGGCCGGCCAGACGGGAGCCCC
CAATTTAAAAAGGTTGATACGGTTCACGTGGAAAGTTTTTAGGTGTGGTA
AT
Matches *P. cryptogea*

Figure 7. Sequence of ITS region of strain Bg1/1

bg1/2
GGTATTTTGCTGATATCAGTCCATTGAGATGCGCGCCGAAGCGCACACAA
CGTTTCCCAAATGGATCGACCCTCGTCCACCCCAGCTTGCGCCGGAACAG
ACACCCTACTTCGCACCACACCGCCGGTTCAAACGCCAAGCCTAGCACAG
CTACGGTTCACCAGTCCATCCAGCCACAGCAGGAAAAGCACCCAATAAGC
GTCTGTTCAGCCGAAGCCAATCATACCGCGAATCGAACACTCCTCCATTA
AACGCCGCAGCAGACAAACCGGTCGCCGACATGCCACACAGGCAGCCTCC
ACAACCAGCAACGTCACGCTTTTCGAGCAAAGAGAAGTACAGTGTAGTAC
ATTTCAAAGGACTCGCAGCCGGACCGAAGACCAGCCGCAAGACACTTCAC
ATCTGCGCGTCCCCATCCACCGACTACACGGAAGGAAGGGAGCCAAGTTT
AGTGTACGGACACTGATACAGGCATACTCCCAGGACTAACCCGGAAGTGC
AATATGCGTTCAAAATTTCGATGACTCACTGAATCCTGCAATTCGCATTA
CGTATCGCAGTTCGCAGCGTTCTTCATCGATGTGCGAGCCTAGACATCCA
CTGCTGAAAGTTGCTATCTAGTTAAAAGCAGAGACTTTCGTCCCCACAGT
ATATTCAGTAATTAGGAATGGGTTTAAAAAATACGCTACTAGCCCAGGCC
GAGGCCCAAGCGGTCGCCATGATAGAGCCGCCACCCAGCCAGCCGAAAAC
CGGCCGGCCAGACGGGAGCCCCCAATTTAAAAAGGTTGATACGGTTCACG
TGGAAAGTTTTTAGGTGTGGTATGATCTTCCGCAGGTT
BLAST matches *P. cryptogea*

Figure 8. Sequence of ITS region of strain Bg 1/2

Phytophthora cactorum [Leb. and Cohn (Schröeter)] *Phytophthora citrophthora* [R.E. Smith and E.H. Smith (Leonian)] and *Phytophthora cryptogea* (Pethybridge and Lafferty).

Data concerning the effect of temperature on mycelia growth showed that most of *Phytophthora*'s isolates from diseased apple trees developed in the interval of 5-30°C, the optimum being 20-25°C. One of the strains started to grow at 2°C and at 35-36°C, but slowly on the 7th day. At high temperatures mycelia were depressed (Tables 2, 3).

PCR tests with *Phytophthora* isolates from apples were run with ITS primers 4 and 6. All had bands above 700 bP (Figure 6). Two isolates generated a band at about 800 bP. Consequent sequencing of the isolate strains Bg 1/1 and Bg ½ showed that the two strains belonged to *Phytophthora cryptogea (*Pethybridge and Lafferty) (Figures 7 and 8).

DISCUSSION

In Bulgaria *Phytophthora*-like type of symptoms were detected first on 2-3-year old apple trees in the region of Bjaga village (Plovdiv) during 1998-1999, and also on 2-year old cherry rootstocks (Katunitza, Plovdiv) and 2-year old cherries (Trilistnik, Plovdiv) (Nakova, 2003). Disease had caused serious damage in apple, cherry and other fruit tree orchards and nurseries. Disease spread-

ing varied from 0.63-1.0% to 8-10.0%, calculated based on the percentage of infected trees. Symptoms differ depending on a fruit tree species but generally include: suppressed tree vigor and growth, yellowing or chlorosis of leaves, reddish-brown discoloration of inner bark and wood of the trunk, and finally the collapse or death of the trees (Ellis, 1997; Hickey and Yoder, 2001).

Isolates were obtained from infected plant tissues applying "baiting bioassay" and using PARP 10 media (Erwin and Ribeiro, 1996). *Phytophthora* strains were identified based on morphology characteristics, cardial temperatures for mycelia growth and molecular tools. Mycelia of most *Phytophthora* isolates grew in the temperature interval from 5 to 30°C, with the exception of one isolate that slowly developed at 2°C and 35-36°C. High temperatures depressed mycelia growth. PCR tests done on *Phytophthora* isolates with ITS primers 4 and 6 showed bands above 700 bP. Two isolates generated a band at about 800 bP. Consequent sequencing of those isolates showed that they belonged to *Phytophthora cryptogea* (Pethybridge and Lafferty).

Comparing our data with those published by other authors, we concluded that the strains isolated from apples belonged to *Phytophthora cactorum* [Leb. and Cohn (Schröeter)], *Phytophthora citrophthora* [R.E. Smith and E.H. Smith (Leonian)] and *Phytophthora cryptogea* (Pethybridge and Lafferty) (Smith, 1906, 1937, 1953, 1956; Novotelnova, 1974; Erwin and Ribeiro, 1996).

LITERATURE

Brown, H. and Krober, H.: Untersuchungen uber die durch *Phytophthora cactorum* /Leb. et Cohn/ Schroet hervorgerufene kragenfaule des Apfels. Phytopathologische Zeitschrift, 32: 34-94, 1958.

Brasier, C.: *Phytophthora* biodiversity: How many *Phytophthora* species are there? Proceedings 4th IUFRO Workshop on *Phytophthoras* in Forest and Natural Ecosystems, Working party S07.02.09, Monterey, California, USA, 2008, pp. 101-115.

Ellis, M.A.: *Phytophthora* root and crown rot of fruit trees. Ohio State University, Extension Fact Sheet HYG-3029-95, 1997.

Erwin, D.C. and Ribeiro, O.K.: *Phytophthora* disease worldwide. APS Press, St. Paul, Minnesota, 1996, pp. 1-556.

Grove, G.G.: Collar Rot of Apple. Washington State University, Tree Fruit Research and Extension Center, 1997.

Hickey, K.D. and Yoder, K.S.: Crown or Collar Rot, *Phytophthora cactorum*, Kearneyscille. Fruit Research and Education Center, West Virginia University, 2001.

Jeffers, S.N. and Aldwinckle, H.S.: Enhancing detection of *Phytophthora cactorum* in naturally infested soils. Phytopathology, 77(10): 1475-1482, 1987.

Judelson, H.S. and Blanco, F.A.: The spores of *Phytophthora*: weapons of the plants destroyer. Nature Reviews Microbiology, 3: 47-48, 2005.

Nakova, M.B.: *Phytophthora* root and crown rot of fruit trees in Bulgaria. Proceedings 3rd International Plant Protection Symposium: From Ideas to Implementation, Debrecen, Hungary, 2003, pp. 196-203.

Newhook, F.J.: The association of *Phytophthora spp*. with mortality of *Pinus radiate* and other conifers. I. Symptoms and epidemiology in shelterbelts. New Zealand Journal of Academic Research, 2(4): 808-893, 1959.

Novotelnova, N.C.: *Phytophthora* fungi – Family *Phytophthoraceae*. Nauka, Leningrad, Russia, 1974. (in Russian)

Smith, C.O.: Inoculation of some economic plants with *Phytophthora cactorum* and *P. citrophthora*. Phytopathology, 27:1106-1109, 1937

Smith, H.C.: Collar rot and crown rot of apple trees in Essex. Plant Pathology, 2: 85-86, 1953.

Smith, H.C.: Collar rot of apricots, peaches and cherries. Orchard New Zeland, 29:22-23, 1956.

Smith, E.R. and Smith, E.H.: A new fungus of economic importance. Botanical Gazette, 42: 215-221, 1906.

Teviotdate, B.L. and Grubler, W.D.: UC Pest Management Guidelines, Apple – *Phytophthora* root and crown rot. UC DANR Publication 3339, 1999.

Wilcox, W.F.: *Phytophthora* spp. In: Compendium of Apple and Pear Diseases (Jones A.L. and Aldwinckle H.S., eds.), APS Press, St. Paul, MN, USA, 1990.

Wilcox, W.F.: *Phytophthora* Root, Crown and Collar Rots *Phytophthora spp.* Fruit Focus USA, 1998. www.phytophthoradb.org

Phytophthora, prouzrokovač plamenjače korena i krošnje jabuke u Bugarskoj

REZIME

Phytophthora je rod *Oomycota* odgovoran za neke od najozbiljnijih bolesti sa velikim ekonomskim posledicama (Judelson i Blanco, 2005). Tokom 20. veka identifikovane su 54 vrste (Erwin i Ribeiro, 1996), a posle 2000. godine još 51-54 (Brasier, 2008). Ove vrste su raširene po celom svetu na velikom broju biljaka domaćina – stablima različitih voćaka, citrusnih, šumskih i parkovskih vrsta. *Phytophthora* može da prouzrokuje ozbiljnu štetu u voćnjacima i rasadnicima jabuke, trešnje i drugih voćaka. U Bugarskoj je bolest najpre pronađena na mladicama jabuke i trešnje (1998-1999) u regionu Plovdiva (Nakova, 2003).

Sprovedena su posmatranja kako bi se otkrili simptomi bolesti u području Plovdiva i Kjustendila. Izolati su dobijeni sa zaraženog biljnog materijala (korena i korenovog vrata) „metodom zamke" u biotestu (zelena jabuka, sorta greni smit) i/ili na selektivnom medijumu PARP 10. Sojevi *Phytophthora* su identifikovani standardnim morfološkim metodama na osnovu: vrsta kolonija na PDA, CMA i V 8 medijumima, tipa i veličine sporangija, oogonija, anteridija, kao i oospora. Kardijalna temperatura rasta ispitivana je na CMA i PDA medijumima.

U molekularnim ispitivanjima DNK je ekstrahovana iz micelija pomoću DNK ekstrakcionog kita. DNK je umnožena pomoću univerzalnih prajmera ITS 6 i ITS 4. Koncentracije produkata amplifikacije ocenjivane su poređenjem sa standardnom DNK. Sekvencioniranje je urađeno u Scottish Crop Research Institute (SCRI, Dundee, ŠkotskaPrvi simptomi plamenjače korena i krošnje uzrokovani rodom *Phytophthora* pojavljuju se u rano proleće. Kod zaraženih stabala javlja se okasnelo pucanje pupoljaka i mali hlorotični listovi, a grane iznenada odumiru. Kasniji simptomi javljaju se u avgustu i septembru. Listovi zaraženog drveća imaju crvenkastu boju i opadaju. Oba simptoma su u vezi sa lezijama (vlažnog, nekrotičnog izgleda) u vratu korena drveća.

U većini voćnjaka, rasprostranjenost bolesti je bila 2-3%, osim u jednom voćnjaku u Bjagi (Plovdiv) gde je raširenost bila 8-10%.

Izolati dobijeni sa stabala jabuke morfološki su identifikovani kao *Phytophthora cactorum, P. citrophthora* i *P. cryptogea*. Kardijalne temperature za njihov rast ispitivane su na CMA i PDA medijumima.

PCR testovi sa ITS prajmerima 4 i 6 dali su traku od oko 800 bP. Sekvencioniranje je pokazalo da dva soja, Bg 1/1 i Bg 1/2, pripadaju vrsti *Phytophthora cryptogea*.

Ključne reči: Plamenjača korena i krošnje prouzrokovana rodom *Phytophthora*; jabuka; *Phytophthora* sp.