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Monitoring of *Phytophthora* species on fruit trees in Bulgaria

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Abstract Disease on fruit trees in Bulgaria caused by *Phytopthora cactorum* and *P. citrophthora* was found in the period 1998–1999. Leaves of some trees become reddish during July, and later in the season fall off. Infected trees die during the same season, or the next season. Observations on symptom development and spread of *Phytophthora* root and crown rot of fruit trees was undertaken from 1999 to 2009. Disease incidence is between 2% and 14% in some gardens and nurseries. The disease was registered in the regions of Plovdiv, Kjustendil, Sliven, Yambol, Karnobat, Bourgas and Svishtov. Samples from infected plant tissues were taken and isolations were done on selective PARP media, or by applying a baiting bioassay. Based on morphological and cultural characteristics and temperature requirements the following *Phytophthora* species have been identified: *Phytophthora cactorum*, *P. citrophthora, P. drechsleri*, *P. cryptogea,* hybrid and *Pythium*. Pathogenicity of the isolates was tested on green apple fruits or one-year-old apple rootstocks. Laboratory studies of the effect of temperature on mycelia growth showed that most isolates can grow from 5° up to 30°C, with an optimum from 18° to 25°C. Only three strains grew at 35–36°C, two developed slowly, one grew well. The optimal pH for mycelia development was tested. Aiming at control of disease, in vivo pot trials have been carried out for studying resistance of rootstocks to *P. cactorum*. At the end of the growing season a good level of resistance has been shown in the rootstocks M29C, Gizela 6, and MAXMA 14.

Keywords *Phytophthora* root and crown rot · Spread · Rootstocks susceptibility

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Introduction

Fruit production is a traditional feature of Bulgarian agriculture. The climate and soils in most regions of the country are suitable for growing fruit tree species—apples, pears, plums, cherries, almonds and peaches. Renovation of old fruit gardens has taken place in Bulgaria since 1990 and new orchards, based on modern market-orientated varieties, have been established. Fruit gardens have been planted with imported and locally produced planting material, in regions where ecological conditions are favourable for the biological requirements of fruit species.

Studies of climatic conditions were done during 1999. The data indicated that during the months of May, June to mid July, there was significant rainfall

which can flood the lower sites of orchards. That is a problem especially when soils are heavy. Rain periods are usually followed by temperatures up to 35–38°C, and dry conditions in July–August. That combination of climate factors leads to water and temperature stress for the plants. Such conditions have recurred in 2001, 2003, 2004, 2005, 2007, and 2009. In some gardens, where soils are soaked for extended periods, the following symptoms were observed: yellowing or chlorosis of leaves, followed by wilting and eventually collapse of some trees. Some specialists misdiagnose those symptoms as "wet feet" (root asphyxiation) or winter injury, caused by water and temperature stress. The roots of the trees, killed by excessive water, are usually completely black (have no line of demarcation to healthy tissue) and often have an unpleasant smell. Discolouration from winter injury is usually confined to the above ground part of the trunk, while the below-ground portion of the tree may still appear healthy (Ellis 2008; Browne and Mircetich 1988). In the literature there are reports that pathogens from genus *Phytophthora* cause symptoms such as root, crown and collar rots (Ellis 2008; Teviotdale and Gubler 2009; Wilcox 1990, 1998). Above-ground symptoms vary among the tree species, but generally they include reduced tree vigour and growth, yellowing or chlorosis of the leaves and eventually collapse or death of the tree. Below-ground symptoms include reddish-brown discolouration of the inner bark and wood. A sharp line shows the contrast between the infected and the healthy portions of the crown (Ellis 2008). Literature reviews show that *Phytophthora* fungi are widely spread on fruit trees, citrus trees, forest trees, park trees and other species. Since 1980 they have affected the following fruit trees: apples, cherries, apricots, peaches and almonds (Braun and Kröber 1958; Jeffers and Aldwinckle 1987; Ellis 2008; Grove 1997; Teviotdale and Gubler 2009; Wilcox 1990, 1998; Kang et al. 2008; Mbaga et al. 2008; Brasier 2008). The following *Phytophthora* species have been reported on apples: *P. cactorum*, *P. cambivora*, *P. cryptogea*, *P. drechsleri*, *P. megasperma*, *P. citricola*, *P. citrophthora*; and on cherries: *P. citrophthora*, *P. cactorum*, *P. cambivora*, *P. citricola* (Erwin and Ribeiro 1996).

Because of their structural and cultural diversity, plasticity and variability, the *Phytophthora* species are highly adaptable to new host species (Erwin and Ribeiro 1996). Before 1996, 54 species were found (Erwin and Ribeiro 1996). By year 2000 the published species had already become 60, and in the period 2000–2008 a total of 51 (really 54, because for *P. alni* three subspecies could be counted as one species or as a multiple taxa) new species were discovered (Brasier 2008; Coffey 2008). Scientists assume that only 10% of the "fungi" are known (Hawkswarth 2001). If we accept that figure for *Phytophthora*, it means that there are about 540 new unknown species existing in nature. The question is how many of them can become invasive and dangerous for ecosystems (Brasier 2008).

The biological requirements of *Phytophthora* concerning temperature are very important for their development and spread. In the literature there are data about specific temperature needs of different species, and values that vary from 4–5°C to 35–36°C, and up to 37.5–40°C for some *Phytophthora* species. The optimum is within the limits of 24–27°C (Tucker 1931; Frezzi 1950; Erwin 1950; Jiang and Erwin 1993; Erwin and Ribeiro 1996; Ho 1992; Wilkinson et al. 1982; Legenkaja 1971). Most authors consider the mycelial growth temperature interval as a major identification tool (Erwin and Ribeiro 1996; Coffey 2008). *P. cryptogea* and *P. drechsleri* can be distinguished based on temperature requirements (*P. drechsleri* grows up to 36°C). For example, temperatures higher than 30°C normally restrict epidemic development (Wilkinson et al. 1982). Optimal pH values for most *Phytophthora* species are about 6.5–7 (Sneh et al. 1981; Jiang 1991; Jiang and Erwin 1993).

Their biological characteristics makes *Phytophthora* difficult to control. Because of that, a combination of quarantine and sanitary practices is recommended to protect soil from *Phytophthora* infestation (Pegg 1978; Hansen et al. 1979; Newhook 1988). These can be combined with fungicide treatments (Zentmyer 1984; Coffey and Wilson 1983; Wilhelm and Paulus 1980) and biological control practices (Gabriel and Cook 1990; Baker 1978; Mlagczuk 1983). Growing resistant varieties is an especially promising tool in integrated and biological fruit production. Rootstocks vary in susceptibility to different *Phytophthora* species. Among dwarfing apple rootstocks M-9, M-2 and M-4 are relatively resistant. The Canadian rootstock Ottawa-3 has an M-9 type of resistance. M-7 and

Table 1 Soil substrate composition, before planting in 2007

pH	$CaCo_3$,%	Active $CaCo_3$,%	Humus,%	Total N
7,18	2,70	0,30	6,70	0,39
Content of the major nutritive elements/ppm/mg/kg				
NH_4	NO_3	N	P_2O_5	K_2O
11,20	103,60	114,80	2162,5	1570

MM-111 are moderately susceptible, M-26 and MM-106 are susceptible and MM-104 is highly susceptible (Ellis 2008; Wilcox 1998). Teviotdale and Gubler (2009) report that MM-104 and MM-106 are more susceptible than M-9 and M-26. Among stone fruits, plums are relatively resistant (Ellis 2008; Gubler et al. 2009). Mahaleb is the most susceptible cherry rootstock whereas Mazzard, Morrelo and Colt are more resistant and recommended on heavier soils (Ellis 2008). Breeding of resistant rootstocks and varieties is of extreme importance and is a major component in the strategy for *Phytophthora* root and crown rot control. There is a discussion about the nature of plant resistance to *Phytophthora* species, and most authors consider resistance to be species-specific (Keen and Yoshikawa 1983; Coffey and Wilson 1983; Ebel and Oxon 1986).

In Bulgaria *Phytophthora* symptoms were first found on apple and cherry trees during 1998–1999 (Nakova 2003, 2004).

This paper studies the spread of *Phytophthora* root and crown rot in Bulgaria. It also examines the causal agents' morphological characteristics, including the effect of temperature on mycelial growth. Rootstocks susceptibility to *P. cactorum*, has also been tested for further control of the disease.

Table 2 Influence of temperatures on mycelial growth ("in vitro" tests) of apple isolate, region of Plovdiv (village of Bjaga)

Nutritive media	Temperature,°C	Mycelial growth, in mm, on day							
		1st	2nd	3rd	6th	7th	8th	9th	10th
CMA	1–2°C	–	–	–	–	–	–	–	–
PDA		–	–	–	–	–	–	–	–
V-8		–	–	–	–	–	–	–	–
CMA	5°C	–	–	–	–	–	–	–	–
PDA		–	–	–	–	–	–	–	–
V-8		–	–	–	9.15	13.0	15.5	16.5	16.5
CMA	9–10°C	–	9.0	13.0	23.0	26.0	27.0	30.0	33.0
PDA		–	–	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	15°C	–	24.0	27.0	35.0	37.5	41.0	43.5	46.5
PDA		–	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	20°C	15.0	23.5	35.0	60.0	65.0	69.0	71.0	76.8
PDA		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	25°C	12.5	21.3	29.5	41.3	44.5	48.6	53.8	53.8
PDA		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	30°C	–	15.0	22.0	30.0	34.5	35.0	35.0	35.0
PDA		–	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	35–36°C	–	–	–	–	–	–	–	–
PDA		–	–	–	–	–	–	–	–
V-8		–	–	–	–	–	–	–	–

Table 3 Influence of temperatures on mycelial growth ("in vitro" tests) of almond isolate, region of Sliven

Nutritive media	Temperature,°C	Mycelial growth, in mm, on day							
		1st	2nd	3rd	6th	7th	8th	9th	10th
CMA	1–2°C	–	–	–	–	–	–	–	–
PDA		–	–	–	–	–	–	–	–
V-8		–	–	–	–	–	–	–	–
CMA	5°C	–	–	–	–	3.5	3.5	3.5	3.5
PDA		–	–	–	–	–	–	–	–
V-8		–	–	–	–	–	–	–	–
CMA	9–10°C	–	–	6.0	9.0	10.0	12.0	14.0	17.0
PDA		–	–	–	8.0	10.0	12.0	14.0	16.0
V-8		–	–	–	6.0	7.5	8.5	9.0	11.0
CMA	15°C	–	11.5	12.0	18.0	20.0	22.0	25.0	30.0
PDA		–	12.0	14.0	20.0	23.0	26.0	30.0	35.0
V-8		–	12.5	15.0	23.0	26.0	29.0	34.0	37.0
CMA	20°C	9.0	14.5	22.0	43.0	46.0	55.0	60.0	65.0
PDA		8.5	15.0	25.0	60.0	66.0	75.0	80.0	83.0
V-8		9.5	17.0	27.0	52.0	57.0	64.0	70.0	75.0
CMA	25°C	7.0	13.5	23.5	45.25	53.8	58.8	67.5	75.0
PDA		7.0	16.8	31.8	66.0	77.0	85.0	85.0	85.0
V-8		11.5	22.0	34.25	71.0	83.0	85.0	85.0	85.0
CMA	30°C	13.0	18.5	23.5	44.3	56.8	64.8	80.0	80.0
PDA		11.5	19.5	27.25	60.5	70.0	74.5	82.5	82.5
V-8		15.3	23.5	31.5	73.5	85.0	85.0	85.0	85.0
CMA	35–36°C	8.0	9.5	11.5	23.0	45.0	49.0	56.5	59.5
PDA		8.0	9.0	12.0	25.0	48.5	55.0	69.0	72.5
V-8		8.0	10.0	13.5	20.0	29.5	66.5	75.0	75.0

Materials and methods

During the period 1999–2009 observations on symptom development and spread of *Phytophthora* root and crown rot of fruit trees were undertaken. Disease incidence was calculated according to the formula (Nakov et al. 2007):

$$P = a/A \times 100,$$

where

P Incidence %
a Number of diseased plants
A Total number of plants

Isolates were received from infected plant materials (root, stem base) by using selective PARP media (corn meal agar amended after autoclaving with pimaricin (10 μm), ampicillin (250 μm), rifampicin (10 μm), and hymexazol (25 μm)). The other method applied was a baiting bioassay (Erwin and Ribeiro 1996). Small green apple fruits (variety Granny Smith) were used as trap cultures to isolate *Phytophthora* from surface-sterilized infected woody tissues (Erwin and Ribeiro 1996). Fruit tissues from the edge of the rotten zone were transferred on to PARP media, suitable for isolating *Phytophthora*. PARP media suppresses the growth of bacteria and some *Pythium* species. Pathogenicity of the isolates is usually proved by inoculation of the small green apple fruits, and/or one-year-apple rootstocks.

Phytophthora strains were identified based on standard morphology methods—type of colonies on PDA, CMA, V 8, type and size of sporangia, oogonia and antheridia, and oospores (Erwin and Ribeiro 1996; Abad and Coffey 2008).

Table 4 Influence of temperatures on mycelial growth ("in vitro" tests) of cherry isolate No 1, region of Sliven

Nutritive media	Temperature,°C	Mycelial growth, in mm, on day							
		1st	2nd	3rd	6th	7th	8th	9th	10th
CMA	1–2°C	–	–	–	–	–	–	–	–
PDA		–	–	–	–	–	–	–	–
V-8		–	–	–	–	–	–	–	–
CMA	5°C	–	–	–	–	–	–	–	–
PDA		–	–	–	–	–	–	–	–
V-8		–	–	–	–	–	–	–	–
CMA	9–10°C	–	–	6.0	10.0	14.0	16.0	17.0	20.0
PDA		–	6.0	8.5	14.0	17.0	19.5	20.5	23.0
V-8		–	7.0	11.0	18.0	23.0	25.0	27.0	30.0
CMA	15°C	–	–	6.0	19.0	31.0	44.0	46.0	46.0
PDA		–	–	8.0	15.0	27.0	35.5	37.5	37.5
V-8		–	–	7.0	18.0	37.0	43.0	47.3	55.0
CMA	20°C	9.0	14.5	25.0	50.0	55.3	60.0	66.5	70.0
PDA		9.0	14.0	23.5	47.0	53.0	62.0	70.0	75.0
V-8		10.5	17.0	24.0	50.0	55.0	58.5	60.0	65.0
CMA	25°C	–	3.5	13.5	37.0	46.3	54.3	63.5	63.5
PDA		–	3.5	13.5	36.5	45.8	53.5	60.5	60.5
V-8		–	4.5	16.0	35.5	44.5	51.0	56.5	56.5
CMA	30°C	–	–	–	18.0	25.3	30.8	39.0	39.0
PDA		–	–	3.8	17.3	25.0	29.0	38.3	38.3
V-8		–	–	–	–	7.5	8.3	15.0	15.0
CMA	35–36°C	–	–	–	–	–	–	–	–
PDA		–	–	–	–	–	–	–	–
V-8		–	–	–	–	–	–	–	–

The effect of temperature on mycelial growth of isolates from apples (region of Plovdiv), cherries (isolates 1, 2, 3 from the region of Sliven) and almond (region of Sliven) were also studied. In vitro tests were carried out on PDA, CMA and V-8 media, within a temperature range of 1–2°C to 35–36°C in growth chambers (Vindon Scientific, United Kingdom).

The response of rootstocks to *P. cactorum* was investigated in 2007. In the trials the following rootstocks used for production of the planting material, were evaluated: M 9, MM 106, M 26, M29C, SP 80, OHF, Gizela 6, MAXMA 14, CXN, PHLC, GF 677, Mahaleb, Wild peach, Quince Provance, Quince Anger, Quince BA 29, Quince B 12. Tests were carried out on sterile soil substrate, rich in humus as organic material, nitrogen, phosphorus and potassium (Table 1). The soil substrate is optimal for the plants growth, and free from pathogenic microflora.

Each variant, including untreated controls, had 15 plants. Inoculation of rootstocks was done with a culture of *P. cactorum*, 10 to 12 days old, grown on PDA. The following treatments were applied (Erwin and Ribeiro 1996):

I. Rootstocks were dipped in mycelia and spore suspension, and then planted in plastic containers (0.35×0.75 cm). Plants were inoculated for a second time after 15 days by watering with mycelia and spore suspension, and for a third time 12 days later.
II. Rootstocks were watered with mycelia and spore suspension three times: at planting, 15 days after planting, and 12 days later.
III. Rootstocks were dipped in/or watered with mycelia and spore suspension at planting, followed by inoculation with a mycelia plug 15 days later. The mycelia plug was placed on a sterile cut, 0.8 to 1 cm

Table 5 Rootstock susceptibility (reaction) to *Phytophthora cactorum* variant three, 120 days (01.08.2007) after 1st inoculation

Treatment/Rootstocks	Symptoms development				% Diseased plants	% Healthy plants
	Chlorosis wilting leave fall,%	Injury on roots and collar,%	Necrotic lesions in wood			
			up to 2 cm,%	more than 2 cm,%		
MM 106						
untreated	0.0	0.0	0.0	0.0	0.0	100.0
inoculated	10.0	15.0	15.0	40.0	80.0	20.0
M 26						
untreated	0.0	0.0	0.0	0.0	0.0	100.0
inoculated	5.0	10.0	25.0	35.0	75.0	25.0
M 29 C						
untreated	0.0	0.0	0.0	0.0	0.0	100.0
inoculated	0.0	5.0	10.0	20.0	35.0	65.0
SP 80						
untreated	0.0	0.0	0.0	0.0	0.0	100.0
inoculated	20.0	14.0	30.0	15.0	79.0	21.0
Gizela 6						
untreated	0.0	0.0	0.0	0.0	0.0	100.0
inoculated	10.0	15.0	5.0	15.0	45.0	55.0
M 9						
untreated	0.0	0.0	0.0	0.0	0.0	100.0
inoculated	17.0	8.0	20.0	25.0	70.0	30.0
Quince B12-inoculated	10.0	15.0	15.0	30.0	70.0	30.0
Quince BA 29-inoculated	15.0	20.0	10.0	30.0	75.0	25.0
QuinceAnger- inoculated	15.0	25.0	10.0	30.0	80.0	20.0
Quince Provance -inoculated	25.0	20.0	0.0	55.0	100.0	0.0
CXN -inoculated	100.0	40.0	15.0	40.0	100.0	0.0
OHF -inoculated	100.0	35.0	0.0	55.0	100.0	0.0
PHLC -inoculated	10.0	25.0	10.0	10.0	55.0	45.0
MAXMA 14-inoculated	0.0	0.0	0.0	0.0	0.0	100.0
Gizela MD-inoculated	10.0	0.0	10.0	45.0	65.0	35.0
Mahaleb -inoculated	100.0	60.0	0.0	20.0	100.0	0.0
Wild peach-inoculated	Clear necrotic symptoms on crown, roots and stems. Plants collapse.				100.0	0.0
GF 677-inoculated	chlorosis upper leaves	20.0	20.0	10.0	50.0	50.0

long, covered with cotton, dipped in sterile water and parafilm. Plants were watered with mycelia and spore suspension 12 days after.

IV. A sterile cut was made on the control plants and was covered with cotton, dipped in sterile water and parafilm.

Different inoculation techniques were compared to obtain maximum infection in field conditions. Rainfall has a positive effect on disease development (Browne and Mircetich 1988; Teviotdale and Gubler 2009; Wilcox 1998).

The plants in the plastic containers were grown in open air conditions from the end of March to mid October 2007. During the growing period of 2007, between 20–25

June, the rootstocks were under water stress caused by rainfall, with the water layer in the plastic containers 5–6 cm deep. In this way the need of water stress, when rootstock resistance to *Phytoph-*

thora is tested, was fulfilled (Aldwinckle, personal communication).

Observations for symptom appearance were done on a daily basis. Above-ground symptoms were recorded: chlorosis, yellowing of the leaves and wilting at the top of the branches, followed by drying of branches or whole plants. Infections on the roots and at the collar of the plants were also studied at the end of the growing season. When cankers were found, their size (length, width, depth) was measured.

Results and discussion

In the period 1998–9 a new disease on fruit trees in Bulgaria caused by *Phytophthora cactorum* and *P. citrophthora* was found (Nakova 2003, 2004). Monitoring of *Phytophthora* root and crown rot spread from 1999 to 2009, points out disease incidence between 2% and 14% and sometimes more in single gardens. First symptoms were discovered on 2–3-year-old apple trees in the village of Bjaga, near Plovdiv and also on 2-year-old cherry rootstocks in the village of Katunitza, near Plovdiv, and 2-year-old cherry trees in the village of Trilistnik, near Plovdiv (Nakova 2003). Later, *Phythophthora* root and crown rot was often recorded in young apple and cherry orchards or nurseries, in the regions of Plovdiv, Kjustendil, Sliven, Yambol, Karnobat, Bourgas and Svishtov. Typical *Phytophthora* root and crown rot symptoms were also found on almonds and peaches.

Infected plant tissues were taken from orchards in the different parts of southern Bulgaria and more than 100 isolations were made on selective PARP media or by applying the baiting bioassay. Morphological analysis and microscope studies on cultures grown on PDA, CMA and V-8 agar media, proved that a major part of isolates belonged to genus *Phytophthora*. Data from microscope observations on the oospores suggest that hybrid forms also exist in the regions of Sliven and Kjustendil. *Pythium* sp. was isolated in the region of Kjustendil from infected apples.

The results from the studies on the effect of temperature on mycelial growth of some isolates, from apples, cherries and almond are presented in Tables 2, 3, 4.

The data analyses (Tables 2, 3, 4) indicate some differences in the temperature requirements of the isolates:

- mycelia of apple isolates grew between 5°C and 30°C, with an optimum between 20°C to 25°C;
- mycelia of cherry isolate N°1 developed from 9°C–10°C to 30°C, optimal temperatures were also about 20–25°C. Mycelia of the cherry isolates N°2 and N°3, from the same region, grew slowly at 35°C;
- mycelial growth of an almond isolate occurred within the range of 5 to 35–36°C, with an optimum between 20 and 30°C. At 35–36°C the isolate also developed well. This can lead to the conclusion that *P. drechsleri* was also present in the pathogen complex (Jiang and Erwin 1993).

The temperature requirements of the isolates from apples, cherries and almonds are between 5 and 35–36°C (Jiang and Erwin 1993). In most strains, mycelial growth occurs up to 30°C, only those isolated from almond develop up to 35–36°C. Morphologically *P. drechsleri* and *P. cryptogea* are very close, and their response to temperature is the major identification characteristic (Tucker 1931; Erwin 1983; Coffey 2008). Both pathogens can co-parasitize (Coffey 2008). Differences in temperature requirements of the species are probably due to the adaptation process going on in *Phytophthora* sp. under changes in climate.

Mycelial growth of some isolates was also studied on culture media with pH varying from 4 to 10. *P. citrophthora* develops with a pH from 5 to 10, optimum 6–8. *P. cactorum* grows at pH 4 to 10, optimum 6–7. The results correspond to the data published by the authors mentioned below that *Phytophthora* species survive in different soils with pH varying from 3.5 to 8.5, and optimal pH values for most of them are about 6.5–7 (Braun and Kröber 1958; Jiang 1991; Jiang and Erwin 1993; Sneh et al. 1981).

Based on morphological and cultural characteristics of the isolated fungi (types of colonies, type and size of sporangia, oogonia, antheridia, and oospores), and studies done on the effect of cardial temperatures and pH values on mycelial growth, the following pathogens causing root and crown rot on fruit trees in Bulgaria were identified: *P. cactorum* (Leb. and Cohn (Schröeter)); *P. citrophthora* (R.E. Smith and E.H. Smith (Leonian)); and *P. cryptogea* (Pethybridge and

Lafferty) sometimes in complex with *P. drechsleri* (Tucker 1931), as was the case in almonds. The dominant species was *P. cactorum*, isolated from all seven regions. *Pythium* sp. and a hybrid were also present in the regions of Kjustendil and Sliven.

Considering that the *P.cryptogea-P.dreschelri* complex is difficult to differentiate morphologically, the two species were identified based on sequencing at the Scottish Crop Research Institute (SCRI, Invergowrie, Dundee, Scotland). The presence of *P. cryptogea* was confirmed in apple orchards in the region of Plovdiv (Bjaga village).

Rootstock susceptibility to *P. cactorum* was studied in in vivo pot trials. Differences between the rootstocks were more distinct in treatment III: plants dipped in spore and mycelia suspension at planting, and then inoculated with mycelia plug (Table 5).

The analysis of the results (treatment III) shows that most of the inoculated rootstocks have a high level of susceptibility. M29C, Gizela 6, and MAXMA 14 show comparatively good resistances. The results from treatments I and II show similar trends, but the symptoms are better expressed in treatment III.

Conclusions

Monitoring of *Phytophthora* root and crown rot spread done in Bulgaria from 1999 to 2009, showed a disease incidence between 2% and 14%, and sometimes more, in single gardens, orchards and nurseries. *Phythophthora* root and crown rot was often recorded in young apple and cherry orchards or nurseries, in the regions of Plovdiv, Kjustendil, Sliven, Yambol, Karnobat, Bourgas and Svishtov. Typical *Phytophthora* root and crown rot symptoms were also found on almonds and peaches.

The following species have been identified based on morphological and cultural characteristics and studies on the effect of the cardial temperatures on mycelial growth: *Phytophthora cactorum, P. cryptogea, P. citrophthora, P. drechsleri, Pythium sp.* and a hybrid *Phytophthora*.

The data received from the resistance tests confirm the susceptibility of MM-106, M-26 and Mahaleb (Ellis 2008; Teviotdale and Gubler 2009; Wilcox 1998). At the end of the growing season a good level of resistance was shown by rootstocks M29C, Gizela 6, and MAXMA 14. The other rootstocks included in the test were susceptible. Susceptible rootstocks require a combination of sanitary practices, biological control measures or fungicide treatments to avoid *Phytophthora* spread. M29C, Gizela 6, and MAXMA 14 should be widely used in production of planting material as a prevention of *Phytophthora* root and crown rot.

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