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Major Diseases of Apple Trees in Kyustendil Region of Bulgaria

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Abstract

Surveys were done in different apple orchards at the Institute of Agriculture –Kyustendil and private orchards and nurseries in Kyustendil region of Bulgaria between 2004 and 2013. Generally accepted methods in plant pathology were used. Fungal diseases such as scab (*Venturia inaequalis)* and powdery mildew (*Podosphaera leucotricha*) are the most important diseases of apple in Kyustendil region. Crop losses mainly depend on the frequency of infection periods of *V. inaequalis* and cultivar susceptibility. Damages by black rot *(Botryosphaeria obtusa)* of trunks and branches are problem at biological growing system and apple orchards with cut-wounded, cold, hail or insect–injured apple trees. Brown rot (*Monilinia fructigena)* and blue mold (*Penicillium* spp) radically infect apple fruits damaged by *Cydia pomonella.* Bacterial disease fire blight *(Erwinia amylovora*) occurs in some years, depending on certain abiotic and biotic factors in region and caused damages. The most common virus infected apples in Kyustendil region is *Apple chlorotic leaf spot virus* (ACLSV), followed by *Apple stem grooving virus* (ASGV). *Apple mosaic virus* (ApMV) has not been detected up to now in the species *Malus domestica* in the region. The phytoplasma *'Candidatus* Phytoplasma mali' the causal agent of Apple proliferation disease (AP) was identified, too.

Keywords: apple, diseases, fungi, bacteria, viruses, Bulgaria

Introduction

Malus domestica which is more commonly known as the commercial apple is a high value crop. The climatic and topographical conditions in the valleys and the hilly regions of Bulgaria are favorable for apple growing and production of high quality fruits. In 2010 apple plantations in farms are 5239 ha and their region distribution is as follows: South Central - 37%, followed by the Southwest - 22% with Kyustendil location inside, South East - 18%, North and Northwest - 8% and North Central - 7%. The total apple production is approximately 43 235 t (Radomirska et al., 2012).

Apple plants are associated internationally with a number of important plant pests and diseases, including fungi, bacteria, viruses, phytoplasma, viroids and diseases of unknown aetiology. The information about the phytosanitary status of apple orchards relating virus, fungi and bacterial diseases and their distribution in the specific region is a warranty for the implementation of strict quarantine and sanitary measures and adequate plant protection technologies to restrict the spread and development of pathogens causing diseases.

This article presents the results obtained over a long period of study on the agents of viral, bacterial and fungal diseases of apple trees growing in Kyustendil region of Bulgaria.

The investigation was carried out during the period 2004 - 2013 at apple orchards of the Institute of Agriculture - Kyustendil and private orchards and nurseries in the municipalities of Kyustendil, Nevestino, Dupnitza, Bobov Dol.

Generally accepted methods for pathogens identification were used.

Organisms and Diseases Recorded on *Malus domestica* in KyustendiL Region

More than forty virus and virus-like diseases known under different names have been described in pome fruit trees (Nemeth, 1986). Among the virus diseases affecting apple, those caused by *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV) and *Tomato ringspot virus* (ToRSV) are common and the most important economically (Mink, 1989; Desvignes, 1999; Sutic *et al*., 1999).

The general prevalence viruses infected apples in Kyustendil region are in the genus *Trichovirus and Capillovirus.* The most common among them are viruses ACLSV (genus *Trichovirus*) and ASGV (genus *Capillovirus*) (Borisova, 2005; Borisova, 2009; Borisova, 2012).

ApMV of the genus *Ilarvirus* has not been detected in the species *Malus domestica* in the region of Kyustendil, Bulgaria (Borisova, 2009). Still, no information is available on the virus status of apple trees in respect to ASPV and ToRSV and their distribution in Kyustendil region.

In a survey as part of the official fruit trees monitoring program of Bulgarian Food Safety Agency '*Candidatus* Phytoplasma mali' the agent of apple proliferation (AP) was found in samples from five different regions of Bulgarian, including Kyustendil. Samples were collected from fruit tree nurseries and mother trees (Etropolska and Laginova, 2012).

More than 57 species of fungi can attack apple and about 21 of them are found out on apple in Bulgaria (Christoff, 1972; Anonymous, 2007; Bobev, 2009).

Apple scab, caused by *Venturia inaequalis* is the most economically important disease in countries with favourable weather conditions like Bulgaria. All commercially apple cultivars (with the exception of cultivars with genes of resistance) are susceptible to scab and the disease is controlled by 10-18 fungicide applications (Holb, 2000; Turechek and Wilcox, 2005; Holb, 2006; Borovinova, 2006). The powdery mildew *(Podosphaera leucotricha)* is second important disease on susceptible to scab apple cultivars but its control could be integrated with scab (Sestraş, 2003; Borovinova, 2006). The most economically important disease of resistant to scab apple cultivars as Florina, Freedom, Liberty, Miora is powdery mildew (Borovinova, 1994; Cline et al., 1998; Borovinova and Ivanova, 2004).

Bacterial disease fire blight caused by *Erwinia amylovora* was observed in 1990 in Bulgaria and it is problem for susceptible apple cultivars during some years (Bobev, 2007; Borovinova et al., 2011)

During the period of investigation 2004–2013 it was established that some fungal diseases such as black rot (*Botryosphaeria obtusa),* Leucostoma cancer (*Leucostoma* spp), *Schizophyllum alneum*), brown rot (*Monilinia fructigena)* and blue mold (*Penicillium* spp) caused also damages on apple in Kyustendil region.

Sanitary Status of Apple Trees in Kyustendil Region

Viruses and Phytoplasma

In total 581 trees of them 461 apples from 61 cultivars and 8 hybrids and 120 rootstocks MM 106 were tested by DAS-ELISA method (Clark and Adams, 1977) for infection with ApMV in the period 2004-2013. DAS- ELISA for ACLSV and ASGV detection was performed according to the modification suggested by Flegg and Clark (1979). Diagnostic kits (Loewe Phytodiagnostica GmbH) were used.

The percentage of virus infection in tested apple trees was 55.3 % and in rootstocks MM 106 - 3.3 % (Fig.1).

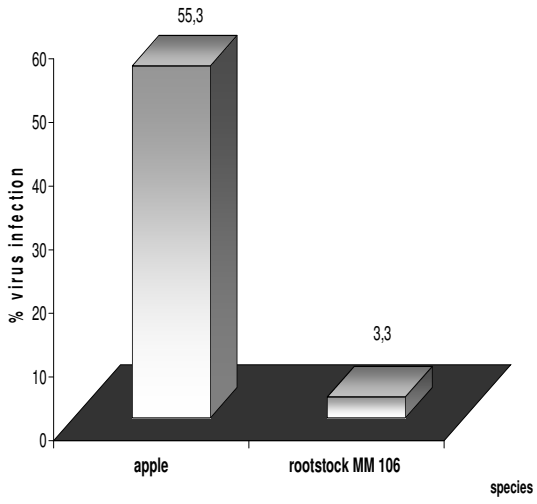

Figure 1. Incidence of virus infection in apple trees and rootstocks in Kyustendil region of Bulgaria (%)

The frequency of single and mixed viral infection in apple is represented in fig. 2. ACLSV infection was found at the highest rate (36.4 %), followed by mixed virus infection in combination ACLSV + ASGV with 16.7 %. ASGV was found in only 2.2 % of tested apple trees in single combination.

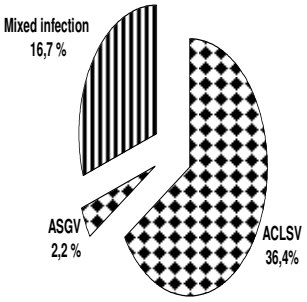

Figure 2. Viruses detected in apple collection orchards at the Institute of Agriculture -Kyustendil and private orchards by ELISA.

In 2.5 % of tested rootstock MM 106 ACLSV was identified, while ASGV was detected only in mixed infection with ACLSV in 0.8 % of the analyzed samples- fig.3.

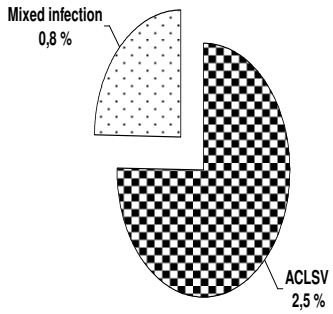

Figure 3. Viruses detected in rootstocks MM 106 at the Institute of Agriculture -Kyustendil by ELISA.

No ApMV was serologically identified in any of the tested apple trees of studied cultivars and hybrids and in tested rootstocks MM 106 in Kyustendil region.

Our study demonstrated the high rate of virus infection of apple cultivars from collection orchards at the Institute of Agriculture-Kyustendil and private orchards and nurseries in Kyustendil region. A similar situation was reported from nearby countries. The study of apple cultivars collection in Albania showed a very high level of virus contamination: ASPV (98.6%), ACLSV (97.8%), ASGV (91.4%) (Myrta et al., 2004). In Romania ACLSV was found in 37. 2% from the tested accessions, ASGV and ASPV in 7. 8% (Popescu et al., 2004). Detection of pome fruit viruses in germplasm collection in the Czech Republic showed that 28 out of 29 tested cultivars were infected with viruses and ACLSV was the most prevalent in apple trees (96.5%), followed by ASPV (89.7%) and ASGV (34.5%)(Hassan et al., 2008). In Turkey, 126 out of 174 tested samples of apple in orchards and commercial gardens were infected with at least one virus (Caglayan et al., 2006). Another study conducted in the Mediterranean Region of Eastern Turkey also demonstrates a high rate of virus infections for apples ACLSV (46.8%), ASGV (60.8%) and ASPV found present in 54.5% of the tested samples (Birişik et al., 2008).

Results of our investigation on the lack of ApMV infection in apple trees growing in Kysutendil region of Bulgaria are similar to those obtained from studies in neighboring countries. In Romania ApMV was found only in cv. 'Granny Smith', and serological also in the rootstock selection G_{21}, together with ACLSV (Popescu et al., 2004), in Czech Republic only one apple tree, cv. Angold, was infected with the four viruses including ApMV (Hassan et al., 2008), in Kosovo 1% of 219 tested apples was infected with ApMV (Krasniqi et al., 2013).

In the autumn of 2013 a survey for identification of causal agent of AP was performed. Leaf midribs and phloem of branches were taken from 8 symptomatic apple plants with 'witches' broom', enlarged stipules of leaves and colouration in the early autumn. Total DNA extraction was done according Dellaporta et al., 1983 and Doyle and Doyle, 1990. PCR was performed using the PCR-set for AP group phytoplasma of Loewe Phytodiagnostica GmbH. PCR analyses detected phytoplasmas in four of the eight samples tested (Kamenova and Borisova, unpublished data).

Fungi and Bacteria

Weather conditions are favourable for development of *Venturia inaequalis* in the region of Kyustendil. For the period of the investigation it was registered minimum 14 Mills infection periods in 2012 and 2013 and maximum 30 Mills infection periods in 2005 (Tab.1).

Table 1. Infectious periods and treatments (No) of *V. inaequalis, P. leucotricha* and *E. amylovora,* rate of attack on leaves and fruits during 2004-2013 r.

Year	Apple scab *Venturia inaequalis*				Powdery mildew *Podosphaera leucotricha*		Fire blight *Erwinia amylovora*	
	Infec. periods	Treat-ments, No	**P*** on Granny Smith,% leaves	fruits	Treat-ments, No	**P*** on Florina,% leaves	Treat-ments, No	Infected shoots and twigs of Erwin Bauer / mean of 1 tree, %
2004	22	10	2.24	3.12	3	2.9	0	0
2005	30	9	2.88	2.24	5	1.5	4	5.86
2006	26	12	6.56	1.84	6	1.6	0	1
2007	19	11	3.52	0.8	6	10.8	2	3.41
2008	16	13	20.32	7.68	5	11.6	2	0.59
2009	20	9	16.64	7.76	7	5.6	0	0
2010	19	12	38.08	18.67	6	6.8	0	0
2011	15	13	22.72	17.52	5	23.5	0	0.045
2012	14	10	1,08	7.2	4	21.36	0	0.18
2013	14	7	32,24	22.4	3	42.5	0	0.015

P* **-** rate of attack

Cultivars as Granny Smith, Fuji, Golden delicious, Mutsu, Melrose, Cortland are very susceptible to *V. inaequalis.* For control of apple scab during the period of investigation it was applied 2 fungicide programs – with only protective and combination of protective and postinfections treatments. Mixed program including 3 protective treatments - 2 before flowering, 1 - during flowering and postinfections sprayings after flowering which number depends on Mills infections periods. Apple scab was effectively controlled with fungicides, such as copper hydroxide, captan (only for pre bloom) and flusilazole, myclobutanil, difenconazole, tebuconazole, trifloxystrobin, kresoxym-methyl and cyprodinil. During period of investigation it was applied minimum 7 in 2013 and maximum 13 in 2008 and 2011 treatments. The leaves and fruits of cultivars were protected from scab very good. Rate of attack of leaves and fruits of Granny Smith by *V. inaequalis* was very low from 1.08 in 2012 to 6.56 in 2006, exception of years with long rainfalls. During such years it was impossible to apply treatments in time and rate of attack of leaves and fruits reach to 50% (2004).

The applied fungicide programs were effective also for control of powdery mildew. The rate of attack of leaves by *P. leucotricha* was low from 0 to 8. 8 in 2006 at Granny Smith.

Powdery mildew is important disease of resistant to scab cultivars as Florina, Freedom, Liberty, COOP 10, Moira, Prima, Britegold. Cultivars Liberty and Moira are very susceptible to powdery mildew in region of Kyustendil and rate of attack of leaves reach to 76 % in 2011 of control (untreated trees). Powdery mildew was controlled by removal of infected twigs and buds by dormant-season pruning and applications of min 3 in 2004 and 2013 and maximum 7 (2009) fungicide treatments with triadimenol, flusilazole, myclobutanil, difenoconazole, tebuconazole, trifloxystrobin, kresoxym-methyl and sulphur (Table1).

Fungi *Monilinia fructigena* and *Penicillium* spp. is not important and radically infect apple fruits damaged by *Cydia pomonella.*

Bacterial disease fire blight *(Erwinia amylovora*) occurs only in some years, depending on certain abiotic and biotic factors in region and caused damages. During period of investigation were found out symptoms of fire blight only on individually single annual growth and flower clusters. Fire blight is controlled by removing infected spurs and with 2 or 4 preventive treatments with fungicides, such as copper hydroxide (Table1).

Botryosphaeria obtusa, Schizophyllum alneum and *Leucostoma* spp caused cankers on trunk and branches, but only *B. obtusa* is dangerous for cut-wounded, cold, hail or insect–injured apple trees. *B. obtusa* is not a problem for conventional and integrated growing systems. The damages by *B. obtusa* of trunks and branches were problem at biological growing system. Reduced vitality of apple trees growing with out pesticides and mineral fertilizers at biological growing system was the reason for strong infection of *B. obtusa* and attack of *Synanthedon myopaeformis* and *Scolytus rugulosus*.

Conclusions

The overall average of virus infection rate in apple was 55.3% and in rootstocks MM106 – 3.3%. The most frequently detected virus was ACLSV in 36.4 % of all apple samples tested and 2.5 % in rootstocks, followed by ASGV 2.2 % in apple. The frequency of mixed infection was 16.7 % in combination ACLSV+ASGV in tested apple trees and in rootstocks – 0.8 %.

No infection of ApMV in tested apple trees of studied cultivars and hybrids and in tested rootstocks MM 106 in Kyustendil region was detected.

The identification of '*Candidatus* Phytoplasma mali' in Kyustendil region requires further research in this field to be continued.

The fungal diseases apple scab (*V. inaequalis)* and powdery mildew (*P. leucotricha*) are the most important fungal diseases of apple in Kyustendil region and crop losses depending on the frequency of infection periods of *V. inaequalis* and cultivar susceptibility.

The damages by *B. obtusa* of trunks and branches were problem at biological growing system and apple orchards with cut-wounded, cold, hail or insect–injured apple trees.

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