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Review paper

The Status of *Erwinia amylovora* in the Former Yugoslav Republics over the Past Two Decades

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SUMMARY

Erwinia amylovora, the causal agent of fire blight (FB) on fruit trees and ornamental plants, rapidly spread across eastern Mediterranean countries in the early 1980s. This quarantine bacterium probably arrived in the southern parts of the former Yugoslavia (now FYR Macedonia) from Greece. Based on symptoms, and isolation and identification data, it was concluded that *Erwinia amylovora* was the causal agent of pear drying in Macedonia (1989). It was the first experimental confirmation of a presence of *E. amylovora* in the territory of the former Yugoslavia. The presence of *E. amylovora* was also proved in Serbia that same year. In Bosnia and Herzegovina, FB was detected during 1990. Based on an official report filed with the Federal Ministry of Agriculture in Belgrade, the presence of *E. amylovora* in Yugoslavia was confirmed (EPPO – Reporting Service, 1991). Therefore, the presence of the bacterium *E. amylovora* in the territory of Yugoslavia was officially confirmed in 1990. In Croatia, FB was first observed in villages near the border on Serbia in 1995. In Montenegro, FB was first detected in 1996. In Slovenia, FB appeared as late as in 2001. *E. amylovora* is now present on 10 hosts (pear, wild pear, apple, quince, medlar, mountainash, hawthorn, firethorn, cotoneaster and Japanese quince) in the territory of the former Yugoslav republics. Based on literature data, losses caused by FB in the former Yugoslav republics in the period 1989-1992 were estimated at about 12,000,000 DEM (mostly in Macedonia) and in the period 1992–1996 at 6,000,000 DEM. Total damage in a more recent epiphytotic year in Slovenia (2003) was estimated at about 474,200 EUR.

Conventional and up-to-date rapid methods (PCR, ELISA and IF, BIOLOG and API System, FAME and SDS-PAGE) have been used to identify *E. amylovora*. Mainly preventive measures

have been used to control *E. amylovora* in the former Yugoslav republics. Spraying with copper products has been practiced during the dormant period and in early spring. In rare cases, spraying has been done at the flowering stage (MARYBLYT), but the problem is that no synthetic bactericides are available on the market. When symptoms occur, only mechanical measures are being applied.

Keywords: *Erwinia amylovora*; Former Yugoslavia; Occurrence; Distribution; Control

INTRODUCTION

Erwinia amylovora (Burr.) Winslow et al., the causal agent of fire blight (FB) on fruit trees and ornamental plants, is one of the most intensively studied phytopathogenic bacteria. It is assumed that this disease first occurred in the USA at the end of the 18th century on wild plant species (crab apple, hawthorn and mountainash), from which the bacterium spread to susceptible cultivars of pear (*Pyrus communis*), apple (*Malus silvestris*) and quince (*Cydonia oblonga*) introduced by first American settlers from Eastern Europe and Southwestern Asia (Van der Zwet and Keil, 1979). The first drying of fruit trees was observed in mountain areas at both sides of the Hudson river in the State of New York. From this region, FB spread to neighboring countries.

At the beginning of the 19th century, FB was recognized in some US states as the biggest problem of fruit production. Epidemics of high intensity occurred on pear and apple in eastern parts of the USA. In about 1840, FB reached Ohio, Indiana and Illinois (Bonn and van der Van der Zwet, 2000). Catastrophic damage was recorded in California (1901-1909). Major epidemics were recorded in Oregon and Washington State as well. *E. amylovora* is currently a widespread phytopathogenic bacterium in all fruit-growing regions in the USA.

For more than 100 years the etiology of this threatening disease was unknown. Many researchers investigated this problem before professor T. Burill of the Illinois University in 1878 pointed to the bacterial nature of the disease. Several years later (1882) the bacterium was given a name, *Micrococcus amylovorus*, and became the first described phytopathogenic bacterium. The bacterium got its current name and was classified in 1920 as *Erwinia amylovora* (Burill: Winnslow et al).

Based on its economic significance and the level of threat to the production of pome fruits and ornamental plants, the bacterium was declared a quarantine organism in many countries. It crossed the Pacific and was introduced to Japan on planting material, and it was later found on apples (Uyeda, 1903). Harrison (1904) (cited by Bonn and Van der Zwet, 2000) speculated that this disease had occurred in Canada as early as 1840 and that it had spread to many areas of the Ontario province by 1870. It reached New Zealand in 1919. In Mexico it was first reported in 1921 and by 1943 it was observed on pear and apple.

After World War II, devastating damage occurred in all regions where the bacterium was reported. For this reason, FB was expected in Europe with anxiety. The first occurrence of *E. amylovora* in England was recorded in 1957 on pear trees (Crosse et al., 1958). In continental Europe, first reports of FB came from two distant locations – the Netherlands and Poland (1966). In Denmark it occurred in 1968, in Germany in 1971, and in France and Belgium in 1972.

E. amylovora rapidly spread over Europe in the second half of the 1980s and the first half of the 1990s. During that period it was found for the first time in Northern and Central European countries (Sweden and Norway, 1986; Czechoslovakia, 1987; Switzerland, 1989).

The other spreading direction of *E. amylovora* in Europe was from the south. In the early 1980s, rainy weather during flowering contributed to an epiphytotic outbreak in Egypt (where it occurred for the first time in 1962-1964). In Cyprus, it occurred in 1984, and in Israel and Turkey in 1985. Once *E. amylovora* had established in the triangle Egypt-Cyprus-Israel, it was only a matter of time before it appeared in neighboring countries (Bonn and van der Van der Zwet, 2000). FB was very soon observed in Lebanon, Jordan and Iran. From Turkey, it reached Greece (1986) over Crete, and from Greece it arrived in the SFRY (Socialistic Federal Republic of Yugoslavia) (Bonn and van der Van der Zwet, 2000).

These data clearly show that *E. amylovora* reached the territory of the former Yugoslavia from the south, entering the FYR Macedonia from Greece, and it was experimentally proved in 1989. Injac and Dulić (1994) reported that *E. amylovora* had reached Serbia from two directions: into the South Morava region (Vranje, Vladičin Han, Leskovac and Vučje) from the south (Macedonia), and towards Nova Gradiška, Vukovar, Loznica, Šid and Belgrade from the west.

According to EPPO (2012), *E. amylovora* is currently present in more than 50 countries in the world.

OCCURRENCE, SPREADING, IDENTIFICATION AND CONTROL OF *Erwinia amylovora* IN THE FORMER YUGOSLAV REPUBLICS (1989-1992)

In the mid-1980s, *E. amylovora* was already present in several neighboring states and its occurrence was expected in the territory of the former Yugoslavia.

Occurrence and distribution

In 1988, information on sudden pear drying of unknown cause in Macedonia began to arrive from producers and extension services. Based on characteristic symptoms and conventional methods used for identification of the isolated bacteria it was proved that *E. amylovora* was the causal agent of sudden drying of pear during 1988 and 1989. It was the first experimentally proved finding of *E. amylovora* in the territory of the former Yugoslavia. Based on these results, an official report was filed with the Federal Ministry of Agriculture in Belgrade (23rd March, 1989) confirming that pathological changes found on pear trees on the plantation of ZIK Radoviško Pole in Macedonia were caused by the quarantine bacterium *E. amylovora* (Panić et al., 1989, cited by Panić and Arsenijević, 1996). In 1989, the presence of *E. amylovora* was soon experimentally proved in Serbia in the vicinity of Šabac (on quince, June 1989) (Arsenijević, 1990, cited by Panić and Arsenijević, 1996). Based on an official report of the Federal Ministry of Agriculture of January 1991, the presence of *E. amylovora* was confirmed at several localities in Yugoslavia (EPPO – Reporting Service, 1991; Arsenijević and Panić, 1992a). In 1990, the former Yugoslavia thus became the 24th country in the world with officially confirmed presence of the bacterium *E. amylovora* on its territory (Arsenijević and Panić, 1992b).

According to Pejchinovski (1996), the first symptoms characteristic of FB were observed in Macedonia in 1986 in commercial quince orchards near the Greek border and in commercial pear orchards (45 ha) near the Radoviš locality. In 1987, FB spread to a commercial pear orchard of 33 ha near Gevgelija and to areas near Negotin and Strumica. In 1988, FB appeared in two commercial pear orchards in the region of Vinica. Favorable weather conditions contributed to a serious outbreak of the disease in 1989. *E. amylovora* spread across the entire Macedonia by 1989 and 1990. Apart from pear and quince, FB was in 1991 also observed on certain varieties of apple and *Crategus* spp.

Mitrev (1993) also painted that the disease had already been present in some places (at low intensity) before 1987 because the disease was known to take at least two or three years after initial infection before developing such high intensity. The author pointed out that total damage caused by FB in Macedonia was estimated at about 10,000,000 DM.

By the end of 1990, *E. amylovora* was detected in 16 municipalities in Yugoslavia, mostly on pear and quince, and rarely on apple. In Macedonia, the bacterium was detected in 11 municipalities (Kriva Palanka, Probištip, Bitola, Kavadarci, Negotin, Štip, Vinica, Radoviš, Perovo, Strumica and Gevgelija) on a total of 252 ha of pear orchards, 44 ha of quince orchards and several individual apple trees. It was found in 4 municipalities in Serbia (Šabac, Vladimirci, Koceljeva and Đakovica in Kosovo). In all cases, individual pear and quince trees were infected, only in the Koceljeva municipality it was a single apple tree. In Bosnia and Hezegovina, FB was found in the municipality of Bosanska Gradiška on an area of about 178 ha and on individual apple trees (Panić and Arsenijević, 1991). In Southern Serbia (Leskovac, Bojnik, Vranje and Vladičin Han) a strong outbreak of FB was recorded in 1991.

In the former Yugoslavia, the presence of this bacterium in 1991 was confirmed on pear, quince, apple, medlar, hawthorn and wild pear trees (Gavrilović et al., 2001).

In the former Yugoslavia, *E. amylovora* was by 10th November 1992 registered: in Eastern Macedonia (14 municipalities) on about 500 ha of pear, 60 ha of quince and some individual apple trees; in Northern Bosnia (Bosanska Gradiška) on 288 ha of pear and individual apple trees; in Central Serbia (Barajevo, Sopot and Kragujevac) on 67 ha of quince and individual medlar trees; in Western Serbia (Koceljeva, Šabac and Vladimirovci) on 6 ha of quince and individual pear and apple trees; in Southern Serbia (Leskovac) on 40 ha of pear trees; in Kosovo (Đakovica) on individual pear trees; in Eastern Slavonia (Vukovar/Trpinja) on individual trees in pear plantations (Panić and Arsenijević, 1996).

During 1992, FB was also recorded at the following locations in Serbia: Arilje (Western Serbia), Ćuprija, Smederevo, Grocka (Central Serbia) (Gavrilović et al., 2001).

Identification

In Macedonia, the presence of FB was experimentally confirmed by Arsenijević and Panić (1992a). Further investigation of numerous strains obtained from pear and quince trees in Macedonia was continued by

Mitrev (1993). Different conventional and serological identification methods were used (Šutić and Panić, 1969; Schaad, 1980; Lelliott and Stead, 1987; Klement et al., 1990). Based on the studies of numerous isolates from pear (79) and quince (76), the author concluded that a homogenous population of the bacterium *E. amylovora* was present in Macedonia. Further detailed serological studies (cross agglutination using three antiserums) showed that the investigated isolates could be differentiated into three groups (a, b and c) based on the somatic composition of thermostable antigens.

Control

The health status of pear and quince orchards in Macedonia was placed under permanent monitoring by the State Phytosanitary Inspection. According to Pejchinovski (1996), rigorous action was taken after the appearance of the disease. For that reason, total eradication of commercial orchards and individual trees was conducted. As a result, over 400 ha of pear and 60 ha of quince orchards in Macedonia were eradicated in 1990. Besides, a number of individual trees in gardens and in wild environments were uprooted. In other pear and quince orchards, mechanical removal of infected tree parts was performed during vegetation and during winter pruning. Regarding chemical protection, spraying with copper products in autumn, copper hydroxide in combination with mineral oil in early spring (at bud burst) and streptomycin sulfate during flowering or later was recommended (Pejchinovski , 1996).

In this period, 1.3 ha of quince trees in Serbia and 58 ha of pear trees in Bosnia and Hezegovina were eradicated.

Losses caused by FB in former Yugoslav republics in 1989-1992 were estimated at about 12,000,000 DEM (Panić and Arsenijević, 1996).

OCCURRENCE, SPREADING, IDENTIFICATION AND CONTROL OF *Erwinia amylovora* IN THE FORMER YUGOSLAV REPUBLICS (1993-2012)

MACEDONIA

The presence of *E. amylovora* in the former Yugoslavia was first experimentally proved in Macedonia (1989) (see section 1). The intensity of infection rapidly increased over the years following the disease outbreak. For this reason, rigorous action was taken after the disease first occurred (Pejchinovski, 1996). Detailed data on the occurrence, spreading, identification and control of *E. amylovora* in Macedonia are given in section 1.

Recent studies conducted by Mitrev and Kostadinovska (2012) showed a renewed interest in FB in Macedonia. The authors found a new host of *E. amylovora* in Macedonia - the wild pear (*Pyrus pyraster*). The isolates were identified using conventional and up-to-date identification methods (PCR, BIOLOG). The authors pointed out in their paper that *E. amylovora* was still spreading in that area and that the highest damage was caused to pear trees.

SERBIA

Occurrence and distribution

After the first occurrence of FB in the vicinity of Šabac (1989) the disease continued to spread in Western, Southern and Central Serbia until the end of 1992 (see section 1).

In the territory of Serbia, new foci of FB were found in northern and eastern parts of Serbia in 1993.

In Northern Serbia (Vojvodina), *E. amylovora* was first recorded on a Branjevina pear plantation in Srem (near Šid) in 1993 (Balaž and Stamenov, 1996). Some 360 pear trees on that plantation were reported to be uprooted in 1993 and additional 1,500 pear trees in 1994. On the same plantation, young pear trees were eradicated on over 30 ha in 1995. During the same year, FB was also registered on a large apple plantation nearby (Morović) and on individual apple trees at a distant location (Bajmok) in Northern Bačka.

Besides these localities, FB also spread significantly across Vojvodina (around Sombor, Zrenjanin, Bačka Palanka, Horgoš, Kikinda, Bečej, Srpska Crnja, Tavankut, Bečej, Sremska Mitrovica) until the end of 1996 (Balaž et al., 1997). The most common host was apple, but it was also found on pear and quince trees. According to Panić and Arsenijević (1996), FB was also present on 10 ha of quince trees in Subotica and on 2 ha of apple trees in Banat (Alibunar).

In Eastern Serbia (Negotin), FB occurred in 1993 on pear, quince and medlar trees (Gavrilović et al., 2001).

Panić and Arsenijević (1996) estimated that total losses caused by FB in FR Yugoslavia in the 1992-1996 period amounted to 6,000,000 DEM.

In 1997, another host of *E. amylovora* was found in Serbia (Novi Sad), i.e. firethorn (*Pyracantha coccinea*) (Gavrilović and Arsenijević, 1998).

During a four-year study (1994-1997), Jovanović (1999) monitored FB on plantations and in small orchards, gardens and nurseries in Southern Serbia (Jablanički and

Pčinjski county). In this region *E. amylovora* was registered on pear, quince, apple, medlar, wild pear and hawthorn trees. According to Jovanović et al. (1997), *E. amylovora* was in 1995 and 1996 also found on parent trees of pear and on apple seedlings in nurseries. In 2000, *E. amylovora* was also observed on apple parent trees and quince seedlings in Southern Serbia (Gavrilović et al., 2001).

The year 2000 will be remembered for an epiphytotic outbreak of FB on apples in Serbia (Balaž, 2000a; Balaž, 2000b). Extremely favorable weather conditions during the flowering period of apple stimulated a strong FB infection that year. Strong disease intensity was registered as early as in the first half of May. The presence of larger amounts of inoculum on apples in 2000 contributed to the infection of two new hosts: *Cotoneaster horisontalis* (cotoneaster) and *Chaenomeles japonica* (Japanese quince) in Vojvodina Province (Balaž and Smiljanić, 2004; Balaž et al., 2004).

FB was in 2000 registered on three large apple plantations in Vojvodina's Southern Bačka region (Odžaci, Kisač and Bačka Palanka) on an area of about 400 ha. On the „Jedinstvo" apple plantation in Odžaci, about 2,000 young apple trees were uprooted. On the Irmovo plantation at Kisač, many individual trees of susceptible summer cultivars were uprooted. Bede (2000) recorded that FB had caused an economic loss of about 1,000,000 DEM in the Subotica municipality alone.

A pear plantation of about 100 ha in Bela Crkva (Southern Banat) was eradicated in 2001 (Arsenijević and Gavrilović, 2007). The epiphytotic outbreak of FB on quince trees in Serbia in 2003 was reported by Obradović et al. (2003).

In the summer of 2005, symptoms of FB were observed on a new host, *Sorbus* spp. (mountainash) in south-eastern parts of Serbia (Gavrilović et al., 2007). *Sorbus* spp. thus became the 10th registered host of *E. amylovora* in Serbia.

According to Vojinović (2008), FB was in 1997 observed for the first time in the Nišava County and it was on pear and quince. In the period from 2004 to 2007, *E. amylovora* was experimentally confirmed in that county on quince, pear, mountainash, medlar and apple.

In Southern Bačka, *E. amylovora* was monitored during 2008 within a project titled „Monitoring of *Erwinia amylovora* in the territory of Southern Bačka County as a basis for implementing an eradication program", which was funded by the Serbian Ministry of Agriculture, Forestry and Water Management. Eradication was conducted in six orchards. Apple trees (83,474) were uprooted on over 40 ha of two old apple plantations (Irmovo of Kisač, and Budućnost of Bačka Palanka), in which *E. amylovora* has been endemically present since 1995. Other objects included small quince, pear and apple orchards. Among them, 648 quince, 152 pear and 67 apple trees were uprooted (Balaž, unpublished).

Identification

A number of researchers have studied the bacterium *E. amylovora* in Serbia. Conventional laboratory methods were mostly employed for identification (Schaad, 1980; Lelliott and Stead, 1987; Klement et al., 1990) but up-to-date rapid identification methods were also included. Most commonly used were the PCR analyses using primers A and B (Bereswill et al., 1992), Nested-PCR (Llop et al., 2000), ELISA serological method and commercial automated techniques – BIOLOG test.

The first bacterium identification data in the former Yugoslavia were presented by Arsenijević et al. (1991), Arsenijević and Panić (1992a; 1992b), Panić and Arsenijević (1993), Arsenijević et al. (1994), Panić and Arsenijević (1996). The authors studied bacterial isolates obtained primarily from pear, quince and apple trees in different regions of the former Yugoslavia (mostly from Western, Southern and Central Serbia and Macedonia). The isolates were identified by conventional identification methods.

Jovanović (1999) investigated a number of isolates (about 150) of this bacterium obtained from pear, quince, apple, medlar, wild pear and hawthorn trees. The identification was conducted using conventional and serological methods (agglutination).

Besides conventional methods, BIOLOG System GN (Hayward, California, USA) and ATB Expert System (Bio Merieux, France) were used for identification of the isolates obtained from the region of Vojvodina (Balaž, 1998; Balaž, 1999; Balaž et al., 2000). Dulić et al. (1999) used indirect ELISA test to confirm the presence of *E. amylovora* in plant samples.

Gavrilović (1998), and Gavrilović and Arsenijević (1999) studied the pathogenic, cultural and biochemical-physiological characteristics of *E. amylovora* isolates derived from different hosts and from different parts of the world. The results showed a uniformity of investigated bacterial populations. ELISA and PCR assays, besides conventional identification methods, were also employed to identify isolates obtained from apple crown (Gavrilović et al., 2008).

Vojinović (2008) identified *E. amylovora* isolates from the Nišava County by testing several basic bacteriological characteristics by conventional methods and ELISA assays.

Experimental studies conducted as part of the project „Monitoring of *Erwinia amylovora* in the territory of

Southern Bačka County as a basis for implementing an eradication program" included a survey of the health status of about 606 ha of plantations, small orchards and 3,150 individual trees. The most frequent were apple trees (about 85%) and the remaining were pear, quince and medlar trees. Suspected samples (218) were submitted to and analyzed at the Laboratory for Phytobacteriology, Faculty of Agriculture, University of Novi Sad. The isolates were identified on the basis of specific bacteriological characteristics using conventional methods. Some of these isolates and numerous samples (asymptomatic) from nurseries (9), were analyzed by molecular methods (OEPP/EPPO, 1992; OEPP/EPPO, 2004). Out of the total number of samples from plantations, small orchards and individual trees, 31.6% were positive and 68.4% negative (low disease intensity was expected because the year 2008 was not favorable for FB infection in Serbia). All of the samples from nurseries were negative (Balaž, unpublished).

Obradović et al. (2007) developed a new molecular method for identification of *E. amylovora* based on detection of chromosomal DNA sequence, using specific primers (FER1-FER1-R), which enables a detection of *E. amylovora* isolates lacking the plasmid pEA29.

Populations of *E. amylovora* in Serbia were studied in detail by Ivanović (2010) and Ivanović et al. (2012). Conventional and up-to-date methods were employed in identification (BIOLOG System, fatty acid methyl ester - FAME and molecular techniques). The results of that investigation of bacteriological characteristics showed that the studied strains (40 from Serbia and one from Montenegro) expressed characteristics typical of *E. amylovora*. Identification by FAME analysis using MIDI technical instructions (MIDI Technical Note # 101, 2001) provided a Fatty Acid Profile (FAP) of the studied strains. According to the obtained results all strains were clustered into three groups (α, β and γ). The restriction analysis of the genomic DNA using *Xab*I and pulsed-field gel electrophoresis (PFGE) resulted in six different patterns and differentiated the strains into six groups. Most of the investigated strains clustered in one group, having the pattern type similar to the Pt2 group, described earlier as dominant in East Europe and the Mediterranean region.

Control

Control of *E. amylovora* is difficult because implementation of the available control measures is limited. Positive results in control of this disease can be expected only from implementation of integrated disease management, which should focus on three main goals: inoculum reduction, reduced susceptibility of host plants and prevention of infection establishment (Aldwinckle and Beer, 1978).

Until the end of 1992, FB was managed in the former Yugoslavia by uprooting highly infected orchards and individual trees (see section 1). After 1992, control of *E. amylovora* has not been systemically organised by the authorities in Serbia, yet all regulations and directives for quarantine parasites on the A_2 list have been implemented.

Inoculum reduction is achieved by administrative and mechanical protection measures. Administrative measures are implemented in compliance with the Plant Protection Act and their aim is to prevent the bacterium from entering new areas. They are primarily focused on examining the health status of planting material. Mechanical removal of infected branches is usually done during winter and summer pruning. For winter pruning disinfection of pruning shears is not needed, but disinfection with ethanol or sodium hypochlorite is required when symptoms occur during the vegetation period. Infected plant material should be removed from an orchard immediately and burned (Panić and Arsenijević, 1996; Balaž et al., 1997; Dulić and Mićić, 1997; Arsenijević and Gavrilović, 2007; Balaž, 2008; Balaž et al., 2009).

Crop susceptibility can be reduced by planting less susceptible cultivars. However, this measure is generally not implemented in Serbia because susceptible cultivars had been planted before *E. amylovora* was first detected in Serbia and when knowledge of this pathogen was poor (Babović et al., 1995).

Many researchers have noted that most pome fruit cultivars in Serbia are susceptible. The most susceptible apple cultivars are Idared, Jonathan and Gloster. Regarding pear susceptibility, Williams, Passe Crassenne and Santa Maria are the most susceptible. In Serbia, quince (cultivars Leskovačka and Vranjska) is the most threatened fruit species (Arsenijević and Panić, 1992b; Panić and Arsenijević, 1993; Balaž and Stamenov, 1996; Balaž, 2000b; Jovanović, 1999; Arsenijević and Gavrilović, 2007).

Infection is mostly prevented by treatments with copper products at concentrations of 0.2 - 0.5% (depending on product applied) in autumn and immediately after bud burst. This measure is regularly applied and has a positive effect. The problem is that there are no synthetic bactericides available on the market to be applied during the flowering stage, when the risk of infection is high. For this reason, treatments in the flowering stage are not usually applied. At some locations in Serbia there are automatic meteorological stations with programs for *E. amylovora* forecast (METOS System). When weather conditions are extremely favorable for infection (high temperature) at the flowering stage, growers can apply a product based on

copper-hydroxide, FUNGURAN-OH. This product has been registered in Serbia for use at this stage but only at low concentrations (0.04-0.06%) to avoid phyxtotoxicity.

Balaž et al. (2002) evaluated the efficacy of some bactericides on artificially inoculated pear blossoms in field trials. The products were applied preventively. In 1999, infection intensity in control plots ranged from 91-95%. In plots treated with Starner 20 WP it ranged from 17-23%, and in those treated with the other tested products it was 40-50%. In 2000, infection intensity in control plots was 80-98%, in plots treated with Starner 20 WP it was 5-8%, while it was 16-24% in those treated with Streptomycin 20 WP. In plots treated with Blauvit it was >40%.

Many researchers in Serbia have worked on *E. amylovora* control (Panić and Arsenijević, 1996; Balaž et al., 1997; Dulić and Mićić, 1997; Arsenijević and Gavrilović, 2007; Balaž, 2008; Balaž et al., 2009).

BOSNIA AND HERZEGOVINA

There is relatively little data on the present status of *E. amylovora* in the territory of Bosnia and Herzegovina, although that area was among the first reported foci of FB in the former Yugoslavia (Arsenijević, et al., 1991; Panić and Arsenijević, 1991; Panić and Arsenijević, 1993). According to Panić and Arsenijević (1996), in the territory of the Republic of Srpska, *E. amylovora* was detected for the first time on pear trees near Bosanska Gradiška in 1990. Data on the spreading and eradication of *E. amylovora* on pear in the period from 1990 to 1992 are already given (see section 1). The fact that the first occurrence of *E. amylovora* was registered on an unusually large area (178 ha) indicates that FB was probably present in that area (in smaller foci) before 1990.

Trkulja and Stojčić (2001) reported that FB had an epiphytotic character in the Republic of Srpska in 2000. The authors suggested that strong disease outbreak was stimulated by weather conditions in 2000, which enabled propagation of epiphytic microflora and massive infection at the flowering stage. The presence of *E. amylovora* was experimentally confirmed on apple, pear and quince samples collected near Banja Luka, as well as on a stretch from Novi Grad to Bijeljina. The importance of implementation of integrated protection measures in control of this quarantine bacterium was also addressed in the paper.

According to Trkulja et al. (2004), monitoring of apple, pear and quince trees was carried out in the northern part of the Republic of Srpska over a ten-year period (1995-2004). In some years, infection intensity varied, ranging from sporadic to strong outbreaks (epiphytotic in 2000). During 2003 and 2004, an intensive work on *E. amylovora* was initiated in the Federation of Bosnia and Herzegovina. Field samples were collected and laboratory bacteriological analyses were performed. On the basis of those analyses, *E. amylovora* presence on pear, apple, quince and medlar samples from the Una-Sana Canton was confirmed. Numić et al. (2005) reported that all necessary legal measures for successful control of this bacterium were taken after the isolation of *E. amylovora* (quarantine parasite on the A_2 list).

CROATIA

Occurrence and distribution

In Croatia, *E. amylovora* has been continuously studied from its first occurrence to present day. Cvjetković et al. (1999) reported that *E. amylovora* had been first observed in 1995 in Croatian villages bordering on Serbia, from where it gradually spread across Eastern Croatia. It was detected on apple, pear, quince and medlar. *Cotoneaster* spp. was also confirmed as a host of *E. amylovora*. After the first occurrence of this quarantine bacterium in Croatia, all available quarantine protection measures were applied. Until 1998, a total of 199,432 apple trees, 142,479 pear trees, 148 quince trees and 152 medlar trees were uprooted.

According to Šubić and Cvjetković (2005), after the first occurrence of FB around Vinkovci and Osjek, the disease spread westards. An epiphytotic outbreak of FB was recorded in Međumurje in 2004. In Nedelišće, 3 ha of apple trees, the Gloster cultivar, were eradicated during that summer. Symptoms of FB were observed on apple, pear, quince and firethorn. In the Međumurje County, FB was detected in the localities Gornji Hrašćan, Strahoninec, Kuršanec, Mihovljan, Šenkovec and Čakovec. In total, 9 foci of the disease were found (four plantations and five cases of individual plants in gardens).

Križanac et al. (2008) detected FB on *Cotoneaster dammeri* cv. Skogholm in a commercial nursery in the Vukovarsko-Srijemska County.

Identification

Cvjetković et al. (1999) reported the first results on identification of *E. amylovora* in Croatia. The tested isolates were obtained from pear, apple, quince and medlar trees in several localities in Eastern Croatia. Conventional laboratory methods were used for identification of isolates (Calzolari et al., 1992) and characteristic colonies were formed on D_3 medium (Kado, 1970).

Halupecki et al. (2006) studied the population of *E. amylovora* in Croatia. The isolates were obtained from different hosts in the period from 1998 to 2001. Conventional and up-to-date (serological and molecular) methods were used for identification. The methods described by Schroth and Hildebrand (1988) were employed for conventional identification of the isolates. Serological characteristics of isolates were tested by ELISA-DASI enrichment method using monoclonal antiserum. According to the same authors, European population of the bacterium *E. amylovora* can be divided into several subtypes based on differences in restriction fragments length polymorphism of the *Xba*1 genomic DNA digest analysed with pulsed-field gel electrophoresis. This technique was also used to determine the genetic relatedness of six Croatian isolates. The obtained results showed that the tested isolates from Croatia were homogenous and belonged to the Pt2 pattern type that is characteristic of the Eastern Mediterranean basin. All tested isolates gave essentially the same total cell protein pattern in SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Certain differences between the isolates were expressed in microbiological assays, such as amylovoran synthesis, levan formation, siderophore production and color on coliform medium.

For identification of the isolates derived from *Cotoneaster dammeri* cv. Skogholm, a conventional bacteriological procedure, immunofluorescence assay (IFA) and PCR were used (Križanac et al., 2008).

Control

According to Cvjetković et al. (1999), eradication was conducted after *E. amylovora* appeared in eastern parts of Croatia (1995). Šubić and Cvjetković (2005) reported of a range of administrative and technical measures that had been applied, so that disease spreading was stopped at the so-called sanitary coridor Virovitica-Požega-Slavonski Brod. According to Cvjetković et al. (1999), the efficacy of some bactericides was evaluated experimentally on pear in the flowering stage. The most efficient was the product Firestop, followed by Champ F-2 and streptomycin sulphate. Aliette provided the weakest protection of all tested products.

For *E. amylovora* forecast, MARYBLYT (Steiner et al., 1996) and BIS Systems (Berrie and Billing, 1997) were used (Cvjetković and Halupecki 2002). In the period from 1997 to 2001, both forecast systems were simultaneously used and their adequacy was evaluated. According to these forecast systems, the years 1998 and 2001 were not favorable either for infection of the flower or for disease development. Flower infections were observed in 1997, 1999 and 2001, which was in accordance with predictions given by both models. In 2001, infection occurred after flowering in the form of "shoot blight". In that year, MARYBLYT model was more useful because it correctly predicted "shoot blight".

According to Cvjetković et al. (1999), the most susceptible pear cultivars in Croatia were Passe Crassane and Santa Maria, and apple cultivars Gloster and Idared.

MONTENEGRO

In the territory of Montenegro, FB symptoms were recorded for the first time on a pear tree near Bijelo Polje in 1996 (Arsenijević and Gavrilović, 2007). According to these authors, the presence of this bacterium was experimentally confirmed on apple trees (on an area of about 2 ha) in the vicinity of Nikšić (Župa Nikšićka). Epiphytotic occurrence of FB on quince in Montenegro was registered in 2003 (Bijelo Polje, Berane, Mojkovac and Župa Nikšićka) (Obradović et al., 2003). Recent literature data (Balaž et al., 2012) suggest that *E. amylovora* is currently widespread in Montenegro. In a health survey of pome fruit plantations and smaller orchards and back yards (June and July 2012), low or high intensity infections with this bacterium were experimentally proved in northern, western and central parts of Montenegro. Besides its territorial spreading, the host range of *E. amylovora* has also gradually expanded. Previous literature data (Panić and Arsenijević, 1996; Obradović et al., 2003; Arsenijević and Gavrilović, 2007) had shown the presence of the bacterium on pear, quince and apple. The latest data (Balaž et al., 2012) show that *E. amylovora* besides mentioned hosts is also present on medlar (*Mespilus germanica*) and hawthorn (*Crategus* spp). The survey of all mentioned fruit trees growing in various regions in Montenegro revealed that quince was the species most threatened by *E. amylovora*. Isolates collected from apple, pear, quince, medlar and hawthron were identified by testing their basic bacteriological characteristics using conventional bacteriological methods.

Organized measures to control *E. amylovora* have not been applied in Montenegro.

SLOVENIA

Occurrence and distribution

Slovenia was the last country of the former Yugoslavia to detect *E. amylovora* on its territory (OEPP/EPPO – 2001). According to Dreo et al. (2006), it is one

of the European Union's countries in which FB last occurred. Well organized protection measures by the state authorities contributed to a delayed occurrence of *E. amylovora* in that country. One of these measures included the monitoring of *E. amylovora*, which was initiated in 1998, i.e. before the bacterium appeared in Slovenia.

The first infection with *E. amylovora* in Slovenia was detected on an old pear tree in the vicinity of Kranj (Naklo) in the Gorenjska region (north-western part) in the summer of 2001. It was considered the only infected "quarantine area" among 791 locations placed under organized monitoring of *E. amylovora* in Slovenia. The bacterium was identified at the National Institute of Biology, Ljubljana. Identification was confirmed at the Plant Protection Service in Wageningen (Netherlands). In 2002, *E. amylovora* was found again at the same site (Naklo) and within a 1 km radius during a systematic survey. Therefore, two locations were found positive in 2002, while the remaining 875 monitored locations were fireblight-free (Knapic et al., 2004; Demšar et al., 2004).

In 2003, FB infection had an epiphytotic character. FB symptoms were observed on some old pear trees on 20th May as a result of canker infection. A focus was found in a garden in the town of Škofja Loka some 13 km southward of the first sighting location at Naklo. In 2003, FB spread throughout the Gorenjska region with a tendency to spread further into eastern and southern parts of Slovenia. At the end of the season of 2003, a systematic survey was conducted by the National Plant Protection Organisation (NPPO) of Slovenia. A total of 184 foci were registered in the Gorenjska region, and 23 in other regions. During the survey, 195 infected plants were confirmed by testing: *Malus* (50%), *Pyrus communis* (21%), *Cydonia* 15%, *Cotoneaster* 10%, *Pyrocantha* (2%), *Crategus* (1%) and *Chaenomeles* (1%) (Knapic et al., 2004).

In the region of Gorenjska in the year of epyphitotic (2003), 9 nurseries with a production of about 120,000 seedlings and an area of 35 ha of intensively grown apple plantations were endangered. All seedlings from the nurseries were eradicated (although no visible symptoms were observed at the time of destruction), as well as the infected species and cultivars in orchards and gardens (only plants with symptoms and plants in their vicinity within a radius of 10 m) (Knapic et al., 2004).

Detail data on the economic loss caused by FB in Slovenia in 2003 were presented by Knapic et al. (2004). Damage was estimated at 161,300 EUR in the nurseries, at 140,400 EUR in intensive orchards, and 174,500 EUR in extensive orchards and gardens. After eradication, the total damage was estimated at 476,200 EUR.

Identification

In Slovenia, all suspected samples (beginning from 1998) were officially delivered to the Laboratory for Fireblight Diagnostics at the National Institute of Biology in Ljubljana. The collecting, packing and sending of samples for analysis was conducted in accordance with the CSL (1998) and OEPP/EPPO (1992) (Štebih et al., 2003; Demšar et al., 2004).

Different methods were employed for detection. The isolation was done on nutrient, selective and non-selective media (SNA, King B, CCT) and pathogenicity was tested on immature pear fruits. Serological methods were also used (indirect immunofluorescence and agglutination test), as well as molecular and automatized methods (PCR with different primers and API 20E biochemical test).

According to Dreo et al. (2006), identification of the bacteria obtained from suspected samples in the year of epiphytotic (2003) was carried out according to a SMT protocol developed as part of the project SMT-4-CT98-2252 and EPPO quarantine procedure No. 40 (EPPO, 1992). A total of 441 samples was delivered to the Laboratory by November 2003. The samples having more or less typical FB symptoms were tested, and 44.2% were found to be positive for *E. amylovora*. The largest proportion of positive samples was found on *Cydonia oblonga* and *Cotoneaster*.

Efforts to improve *E. amylovora* diagnostics in Slovenia were continuous. Demšar et al. (2001) introduced a molecular method (PCR) which both confirms the presence of *E. amylovora* in a bacterial culture and enables to distinguish this bacterium from other bacteria such as *Pseudomonas syringae* pv. *syringae.* Pirc et al. (2007) and Pirc et al. (2009) used Real-time PCR to improve *E. amylovora* diagnostic.

Dreo et al. (2011) used newly developed and rapid methods (Real-time PCR, serological test – Ea AgriStrip, Bioreb and LAMP) for early detection of the bacterium during the fowering stage and for determination of its concentration. Dreo et al. (2012) pointed at the advantages of a Real-time PCR assay based on the *ams*C gene performed in a SmartCycler instrument.

Control

Administrative control measures mostly contributed to prevention and eradication of *E. amylovora* in Slovenia (ISPM 5, 1996; Knapič, 2004). Phytosanitary measures were conducted in accordance with international standards applied to prevent entrance and spreading of pest organisms. According to Knapič et al. (2004), FB

was present in low prevalence in the Gorenjska region and Northern Slovenia, but not yet established. The authors pointed out the significance of properly conducted quarantine measures, which prevented FB from spreading into the major fruit producing regions in Slovenia.

Epiphytotic occurrence of FB in the region of Gorenjska in 2003 was enabled by warm weather during the flowering stage of apple. Weather conditions were monitored by the MARYBLYT System. As the flowering stage is one of the most important stages in *E. amylovora* epidemiology, great efforts were made in Slovenia to improve methods for fast and reliable detection of latent blossom infections, i.e. for detection of epiphytic microflora (Dreo et al., 2011).

According to Dreo et al. (2006), Slovenia is a region with low intensity of FB infection in which protection measures have been conducted in an organized manner. Cvelbar et al. (2009) underlined the significance of survey and quarantine measures over the 2003-2008 period and the formation of a Protected Zone and Buffer Zones in Slovenia in compliance with relevant EU directives.

Miklavc et al. (2011) evaluated the efficacy of different products for control of *E. amylovora* at the flowering stage. Trials were conducted on the cultivar Gloster under conditions of spontaneous infection. According to their results, the efficacy of the product Blossom Protect was 85%, while Serenade, Kupro and Coptrel had 75% efficacy, Cuprablau Z 50%, Aliette 35%, and Regalis 0%. Lešnik et al. (2011) evaluated the efficacy of conventional copper products and copper products in combination with some organic substances, and compared them with the efficacy of products based on *Aureobasidium pullulans* and *Bacillus subtilis*. The best result (blossom/twigs) was achieved by the systemic activity of the products based on Cu-gluconate (57.7%-68.8%).

All available protection measures have been applied to control *E. amylovora* in Slovenia (Cvelbar, 2004; Seljak, 2004; Lešnik, 2004; Knapič, 2004).

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Status *Erwinia amylovora* na teritoriji bivše Jugoslavije tokom dve poslednje decenije

REZIME

Početkom 1980-ih *Erwinia amylovora* se brzo širila na teritoriji zemalja istočnog Mediterana. Iz Grčke (1986) ova karantinska bakterija je dospela u Makedoniju (južni deo Jugoslavije). Na osnovu simptoma, izvršenih izolacija i identifikacije dobijenih izolata, zaključeno je da je *E. amylovora* prouzrokovač sušenja kruške u Makedoniji (1989). To je bio prvi eksperimentalni dokaz o prisustvu *E. amylovora* na teritoriji bivše Jugoslavije. *E. amylovora* je iste godine dokazana i u Srbiji. U Bosni i Hercegovini je zapažena tokom 1990. Na osnovu zvaničnog dopisa Saveznom ministarstvu poljoprivrede u Beogradu, zvanično je potvrđeno prisustvo *E. amylovora* na teritoriji bivše Jugoslavije (EPPO – Reporting Service, 1991). Tako je prisustvo *E. amylovora* na teritoriji bivše Jugoslavije zvanično potvrđeno 1990. U Hrvatskoj je bakteriozna plamenjača prvi put zapažena 1995. u selima koja se nalaze u graničnom području prema Srbiji. U Crnoj Gori bakteriozna plamenjača je prvi put zapažena 1996. U Sloveniji se bakteriozna plamenjača pojavila tek 2001. Prisustvo *E. amylovora* je do sada utvrđeno na 10 domaćina (kruška, divlja kruška, jabuka, dunja, mušmula, oskoruša, glog, vatreni trn, kotonester i japanska dunja) na teritoriji bivše Jugoslavije. Na osnovu literaturnih podataka (1996) gubici prouzrokovani bakterioznom plamenjačom na teritoriji bivše Jugoslavije su procenjeni na oko 12,000,000 DM. Ukupni gubici u godini epifitocije u Sloveniji (2003) su procenjeni na oko 474,200 EUR.

Za identifikaciju *E. amylovora* korišćene su klasične i savremene metode identifikacije (PCR, ELISA, IF, BIOLOG i API sistem, FAME i SDS-PAGE). Za suzbijanje *E. amylovora* na teritoriji bivše Jugoslavije su uglavnom korišćene preventivne mere zaštite. Prskanje u periodu mirovanja i u rano proleće se izvodi bakarnim preparatima. U retkim slučajevima prskanje se primenjuje i u fazi cvetanja (MARYBLYT), ali je problem što na tržištu nemamo sintetičkih baktericida. Ukoliko se simptomi ipak pojave, primenjuju se mehaničke mere zaštite.

Ključne reči: *Erwinia amylovora*; bivša Jugoslavija; pojava; rasprostranjenost; suzbijanje