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Epidemiology of Fire Blight in Fruit Crops in Kazakhstan

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ABSTRACT

A survey on 13 regions of Kazakhstan was carried out to identify foci of fire blight, the extent of its spread, the range of affected crops, and the identification of infection reserves. The disease was mainly concentrated in the main fruit growing zone.at the south and southeast of Kazakhstan. Symptoms of fire blight were characteristic of two bacterial diseases: namely, necrosis, by *Pseudomonas syringae* Van. Hall. and fire blight by *Erwinia amylovora* (Burrill) Winslow et al. The authors performed bacteriological analyses to isolate and identify the causative agent of fire blight from various fruit and wild cultures of the Rosaceae family, using classical bacteriological and molecular genetic methods. Two types of bacteria were isolated from the samples affected by the disease, namely *E. amylovora*, the causative agent of fire blight, and *P. syringae*, the causative agent of bacterial necrosis. The results of the studies on the identification of bacterial species *E. amylovora* and *P. syringae* were confirmed by Swiss scientists from the Agroscope research center based on an immuno-chromatographic test and by Russian scientists at the All-Russian Research Institute of Plant Quarantine using enzyme-linked immunosorbent assay, FLASH polymerase chain reaction, and polymerase chain reaction in real-time.

INTRODUCTION

Fire blight is one of the most harmful fruit crop diseases caused by enterobacteria *Erwinia amylovora* (Burrill) Winslow et al. (Bonn & van der Zwet, 2000; Braun & Hildebrand, 2006). Currently, the disease has been reported in more than 50 countries (Zhao et al., 2019). Such a widespread occurrence of fire blight in fruit crops, despite the differences in the climatic conditions of the countries where this disease is registered, indicates the ecological plasticity of its pathogen. Consequently, the threat of expansion of the disease area will persist. The disease affects all the above ground parts of fruit crops - buds, ovary, leaves, shoots, bark, and stem. Its harmfulness lies in its rapid spread, in large crop losses, death of trees, high expenses for uprooting dead and affected trees, as well as for the restoration of new gardens (Karimova, Shneyder, Zaets, & Smirnova, 2013).

For Kazakhstan, this disease is quarantined. However, in 2008, signals began to come from phytosanitary services and individual farms about the damage to the apple and pear trees caused by a disease similar to fire blight. In 2010, a significant spread of this disease was observed in several areas of the Almaty fruit zone (Drenova, Isin, Dzhaimurzina, Zharmukhamedova, & Aitkulov, 2013). In appearance, fire blight is similar to another bacterial disease, bacterial necrosis, caused by the bacterium *Pseudomonas syringae* Van. Hall

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(Kennelly, Cazorla, de Vicente, Ramos, & Sundin, 2007). In the gardens of southeastern Kazakhstan, bacterial necrosis has been encountered since the 1980s and mainly affected pear trees and stone fruits. It was not widespread. Recently, however, this pathogen has often become isolated from diseased apple trees. Fruit necrosis, caused by the *P. syringae* bacteria, is also a highly harmful disease in fruit crops and affects all the aerial organs of plants. In addition to the symptoms, these two diseases are similar in the source of infection, the mode of distribution, and the influence of weather conditions. Fruit necrosis also affects a wide range of plants of the Rosaceae family (Hulin et al., 2018; Konavko, Moročko-Bičevska, & Bankina, 2014).

The most likely route of invasion of the fire blight pathogen is through the import of planting stock of fruit trees and ornamental trees of the Rosaceae family. According to Jock et al. (2013), the main way of spreading the fire blight pathogen to many countries is the commercial trade in planting stock of fruit and ornamental trees of the Rosaceae family. It was probably in this way that the disease entered Kazakhstan. Thus, from 2008 to 2015, over 10 million seedlings and stocks of apple, pear, and quince trees were imported to Kazakhstan from countries where the disease was widespread. The possibility of invasion by this pathogen is confirmed by molecular genetic analyzes of the strains of *E. amylovora* from the southern and southeastern regions. It was established that they originated from genotype A and corresponded to the southern European-Mediterranean genotype (Drenova, Matiashova, Belkin, & Kondratyev, 2016; Rezzonico et al., 2016).

Identification of fire blight in the southeast of Kazakhstan, in the main industrial zone of fruit growing, can lead to its massive spread. The pathogen can be in a latent state for a long time and, when favorable conditions occur, can cause the epiphytotic development of the disease. According to phytosanitary risk analysis, the southeast of Kazakhstan, where the main fruit growing zone is located, is favorable for acclimatization and justification of this pathogen (Djaimurzina et al., 2014). Of particular concern is the invasion of the pathogen into the geographical center of the origin of pome fruits, which poses a potential threat to the wild fruit forests of southeastern Kazakhstan, and the Sievers apple tree.

According to scientists of the European Union, a long-term program to eliminate the pathogen is necessary for efficient control of fire blight (Shtienberg et al., 2015). Herewith, the program should consider important local factors determining the disease progression in each season, the inoculum potential, the number of susceptible crops, and favorable weather conditions. At the local level, it is important to know the symptoms of the disease manifestation, to determine the optimal timing for protective measures, and to choose more effective chemical and biological preventive measures. The goal of the program is to reduce the pathogen population and damage rate to an economically acceptable threshold. A local control strategy should be developed for each country.

In this regard, the purposes of the research were to identify foci of fire blight in the republic of Kazakhstan and to study its epidemiology in this case the dependence of the seasonal dynamics of the disease on the prevailing meteorological conditions, reveal the susceptibility of the apple tree development phase to the disease and the range of affected plants, as well as isolate and identify the causative agents of the disease. Based on the results obtained could be used to develop a control strategy concerning local conditions. This strategy will allow reducing the harmfulness of fire blight and preventing its further spread.

MATERIALS AND METHODS

Research Location

The studies were carried out in laboratory and field conditions. We examined gardens in fruit growing farms and private sectors of various regions of Kazakhstan (in the south, southeast, west, east, and north). Examinations were carried out annually from 2013 to 2018 during the period of flowering and regrowth of young shoots. Seasonal dynamics were studied in the southeast of the country, in the main fruit growing zone, during the entire growing season every 10 days.

The Examination Revealed Foci of Fire Blight, the Spread of the Disease, and Its Severity

When examining large industrial gardens and large farms, at least 10% of trees were examined. In the private sector, all available trees (100%) were examined. Tree planting scheme in the surveyed gardens: between trees in a row 3 m;

between rows 5 m. The examination was carried out along four sides and two diagonals of the garden. The spread of the disease and the extent of damage were based on susceptibility of fruit crops, the prevalence and the degree of development of disease.

The susceptibility of fruit crops (apple, pear) was carried out according to a 5-point scale i.e. 0 = healthy tree; 0.1 = barely noticeable signs of the disease; 1 = the initial degree of manifestation of the disease (single wilting and blackening of flowers and twisting and browning of shoots and leaves are noticeable); 2 = more than 10% of affected flowers, shoots, and leaves; 3 = damage to the bark of branches, trunks, and fruits (bacterial exudate is released in the affected areas); 4 = more than 75% of the crown is burned, the trees stand as after a fire; and 5 = a tree that died from the disease.

The prevalence (frequency of occurrence) of the disease is calculated using the formula:

$$P = \frac{H \text{ x } 100}{N}$$

Where: P = the prevalence or incidence of the disease (%); H = the number of diseased trees; N = the number of surveyed trees.

The degree of development of the disease is determined using the formula:

$$R = \frac{\sum(a \text{ x } b) \text{ x } 100}{NK}$$

Where: R = the degree of development of the disease (%); a = the number of trees with the same signs of disease development; b = the score of the damage corresponding to this sign; N = the number of surveyed trees; K = the highest score on the damage scale.

Based on the survey, a map of the south and southeast of the republic, the main zone of industrial horticulture, was created, where the largest number of foci was identified. The map shows the coordinates of the largest fire blight foci using GPS. During the examination for laboratory analyzes, samples were taken of affected ovaries, shoots, branches, parts of the bark, leaves, and fruits with well-pronounced symptoms of fire blight.

Establishing the etiology of the disease in laboratory conditions was required for bacteriological analysis of selected samples to be carried out for isolating and identifying the causative agent of the disease. Isolation of bacteria was performed on King B nutrient medium. Initially, the surface was thoroughly sterilized. Fruits, stems, and bark were dipped in alcohol, then rinsed three times in sterile water; leaves and flowers were initially washed in running water, then washed three times in sterile water. After that, they were ground in a sterile mortar (maceration) with the addition of a small amount of sterile water and sown on a nutrient medium after a series of dilutions. Petri dishes were placed in a thermostat at a temperature of 26-27°C. After 2-3 days, colonies of bacteria appeared on the nutrient medium, then their growth was monitored. The final analysis of the grown colonies was performed in 10-12 days. During this period, morphological signs of bacteria were formed, such as size, color, shape, profile, edge consistency. Based on these morphological features, colonies were selected, which, according to the description in the literature, corresponded to the *E. amylovora* and *P. syringae* phytopathogenic bacteria.

Selected colonies were inoculated into test tubes with a solid nutrient medium, then inoculated into plates, and, with the growth of similar colonies, inoculated into schools. Along with phytopathogenic bacteria, saprophytic microorganisms grew on a nutrient medium in petri dishes, often in the form of a colony similar to phytopathogenic one, and thus saprophytic forms could also be isolated into a pure culture. Therefore, before proceeding with a detailed study of bacteria isolated into a pure culture, their pathogenicity had to be checked and only pathogenic bacteria were to be selected for further study.

The pathogenic properties of isolated bacteria were checked by the infectious-infiltration method by A. Klement according to the hypersensitivity reaction on an indicator plant of room geranium (*Pelargonium zonala L.*). For this purpose, the daily culture of isolated bacteria was used, the inoculum concentration was 109 cells/ml according to the turbidity standard. The bacterial suspension was injected with a sterile syringe into the extracellular space of the geranium leaf. In the control, sterile water was used on the same indicator plant. If the suspension contained phytopathogenic bacteria, necrotic spots (in the form of parchment) formed at the infiltration site after 12-24 hours. This was

a reaction of the indicator plant's hypersensitivity to pathogenic bacterial species. Nonpathogenic bacteria (saprophytes) did not cause such a reaction (necrosis), even when high concentrations of inoculum were administered.

Simultaneously with the hypersensitivity reaction, the pathogenicity of bacteria was checked on young unripe pear fruits according to White's method. This method was one of the main methods used for determining the causative agent of the fire blight. Unripe pears were harvested and refrigerated for 5 months. Pear fruits were superficially sterilized with alcohol, then rinsed three times with sterile water. The bacterial suspension was applied to the surface of the pear, then the skin of the fruit was pricked three times with a sterile entomological pin to different depths. The pricking was carried out several times, then the bacterial suspension was again applied to these places. Inoculated pear fruits were placed in humid chambers, where high humidity was maintained, and kept at 25-27°C; sterile water was used for the control. The test was positive, if dark necrotic spots and milky-white exudate appeared on the surface in 2-5 days, then the isolated bacterium was the bacterium *E. amylovora*. In the control variant, where similar inoculation was also performed with water, the fruits of unripe pears remained without change.

Further FLASH (Fluorescent Amplification-based Specific Hybridization) polymerase chain reaction (PCR) analysis (AgroDiagnostica, 2008), was used to confirm the correct identification of bacteria *E. amylovora* and *P. syringae,* 96 isolates were transferred to the Agroscope Research Center in Zurich, Switzerland. The identity of isolates as *E. amylovora* was verified using side-stream immunochromatographic analysis (AgriStrip, Bioreba AG), which responded positively to the fire blight pathogen and several very similar species (Braun-Kiewnick et al., 2011). All samples giving a positive reaction in AgriStrip tests were confirmed as *E. amylovora* in loop-mediated isothermal amplification (LAMP) PCR (Buhmann et al., 2013). Concomitant microflora was identified using matrix-assisted laser desorption/ionization (MALDI), time-of-flight mass spectrometry (ToF MS), and Mabritec AgPuen (Switzerland) (Djaimurzina et al., 2014).

RESULTS AND DISCUSSION

Distribution and Seasonal Dynamics of Fire Blight

To establish the extent of the spread of fire blight in Kazakhstan, we examined 1,250 ha of gardens in 135 peasant farms. The results showed, the largest number of foci of fire blight was detected in the south and southeast of Kazakhstan, in the main fruit growing zone. In the South Kazakhstan region, fire blight caused damage on apple and pear tress on 9 of 17 examined peasant farms. In the Zhambyl region, 20 households were examined, where 15 outbreaks of fire blight were found. Most of the foci of fire blight were detected in the Almaty region. Of the 49 surveyed farms, fire blight injury was noted in 35 farms. Based on the survey, a map of the south and southeast of Kazakhstan was compiled using the ArcGIS Pro software. Using GPS, the coordinates of the largest foci of fire blight were marked on the map where the degree of development of the disease averaged 3 points with the prevalence from 30 to 100%. The largest number of disease developments was found in the foothill zone of the Almaty region (Fig. 1). Individual foci of fire blight were identified in the western, central, and eastern regions of Kazakhstan in the private sector. No fire blight was detected in the northern regions of Kazakhstan.

The examination showed the presence of foci of fire blight in all examined regions of Kazakhstan, except the northern ones. They were most numerous in the south and southeast, in the main zone of industrial fruit growing. The dependence of disease development on weather conditions during the growing season by years of research was established. There was a tendency for the epiphytotinous development of the disease. This is especially dangerous in years with wet and warm spring during the flowering and regrowth of young shoots. The identification of numerous foci of fire blight in the south and southeast of Kazakhstan in the main fruit growing zone can lead to its massive spread and large economic losses. In the countries of Europe and America, as well as in many other countries where this disease had an epiphytotic development, hundreds of thousands of fruit and ornamental trees were uprooted. In the United States, for example, between $42 million and $100 million was spent annually to fight this disease (Norelli, Jones, & Aldwinckle, 2003).

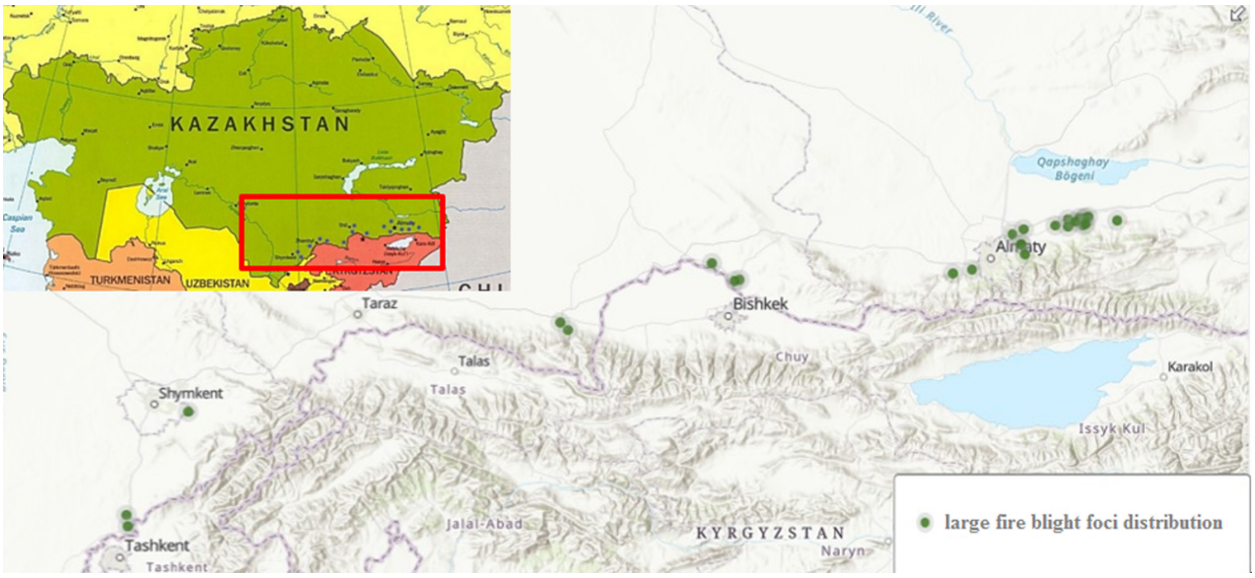

Fig. 1. Map of large fire blight foci distribution in the south and southeast of Kazakhstan

Epidemiology of Fire Blight in Fruit Crops

According to published data, the epidemiological features of fire blight are associated with factors such as temperature, rainfall, and humidity (Shtienberg et al., 2015). In this regard, from 2013 to 2018, analyses were carried out of the dependence of fire blight dynamics in fruit crops on the development phase of the apple tree and the weather conditions during the growing season divided by years of research.

Analysis of the seasonal dynamics of fire blight in 2013-2015 showed that the development of the disease was intensive in the first half of the growing season, especially in May and June when the temperature reached 16-20°C and air humidity was 60% and higher. The first signs of the disease were found in the first decade of May. The blossoming flowers suddenly faded, turned brown, and dried out, but did not fall throughout the season. The spread of the disease equaled 2%. The spread of the disease in the third decade of May was 18%. In the first decade of June, 28% of trees were affected. By the end of June, the spread of the disease had reached 47%. By this period, the infection had accumulated and a favorable microclimate had formed for the development of fire blight, which was ensured by the presence of drip-liquid moisture on the surface of tree organs, which is one of the necessary conditions for infection. The presence of drip-liquid moisture was due to the compaction of the leaves, which contributed to its retention on the surface of tree organs. Differences in night and day temperatures contributed to the formation of condensate (water droplets), which were on the leaves for a long time. All this contributed to the intensive infection process. The most susceptible phase for the development of the disease was the period of flowering and regrowth of young shoots, which are more susceptible to the disease.

In the second half of the growing season (July and August), the development of the disease stopped due to a rise in temperature to 30°C and higher; relative humidity was in the range of 30-40% and precipitation had stopped. The results of the studies showed that fire blight affected all the aerial organs of the apple tree, namely inflorescences, buds, leaves, fruits, young shoots, branches of various orders, skeletal branches, tree stem, and trunk. Along with the characteristic signs of fire blight (Zhao et al., 2019), there was a frequent manifestation of the disease on the trunk in the form of vague dark spots at the base and cracking of the cortex in the form of a crack along the trunk with the expiration of the exudate (Fig. 2). This manifestation of the disease can be explained by longer preservation of moisture on the bark of the tree and the effect of a lower temperature, due to shadowing at high temperatures in the summer.

Fig. 2. Symptoms of fire blight on the apple tree trunk: a) and b) cracking of the bark along the trunk with the outflow of exudate on Zolotoye prevoskhodnoye variety; c) vague dark wet spots at the base of the trunk on the Aport variety

Observations of the dynamics of fire blight in 2016-2018 showed that during the flowering period in April and May, due to the prevailing weather conditions (lingering cool spring), the manifestation of fire blight was not observed; the temperature was in the range of 7-13°C and air humidity was 60% or higher. For intensive propagation of the pathogen, the temperature needs to be in the range of 18-28°C (Burse & Ullrich, 2002). However, the temperature parameters in these years did not correspond to these indicators. The first signs of fire blight appeared only at the end of the third decade of May. The prevalence of the disease during this period did not exceed 2%.

In June and the first decade of July, during the growth of young shoots, unstable weather persisted with a predominance of elevated temperature; the average daily temperature was in the range of 23-25°C and humidity was 54-60%. During this period, the development of the disease increased slightly and the spread of the disease reached 16%. In the second half of July and in August, steady dry and

hot weather (temperature above 30°C and humidity within 30-40%) restrained the further spread and development of fire blights.

Thus, an analysis of the seasonal dynamics of fire blight showed that the periods of flowering and regrowth of young shoots were dangerous phases for apple trees to the diseases. The epiphytotic development of fire blight during this period was associated with such factors as temperature and humidity. According to the literature, it is during this period that the pathogen intensifies and the disease spreads rapidly (Yelin et al., 2016). It is important to prevent infection during the flowering period since nectar is a favorable environment for the growth of bacteria (Shtienberg, 2016).

Isolation and Identification of the Causative Agent of Fire Blight

To establish the etiology of fire blight, we performed primary diagnosis during the examination of fruit crops. We studied carefully the symptoms of the manifestation of the disease on the apple tree and pear tree. It was found that they were, according to the main features, characteristic of two bacterial diseases, namely fire blight and necrosis. It is difficult to perform an accurate diagnosis of fire blight based on symptoms alone. For a more accurate diagnosis, during examinations, samples were taken with signs characteristic of fire blight. Bacteriological analyzes were carried out in laboratory conditions to isolate pathogens in pure cultures. All organs of fruit crops affected by the disease were analyzed (ovary, leaves, fruits, young shoots, bark). In total, over the years of research, we analyzed 492 samples of pome trees (apple, pear, quince), stone fruit trees (apricot, plum, sour cherry, cherry), and berries (raspberries, currants, gooseberries) from different regions (south, southeast, southwest, north) and the neighboring territory of Kyrgyzstan (Chu valley), as well as wild fruit (mountain ash, hawthorn, viburnum, cherry plum, and wild Sievers apple tree from Turgensky, Aksaysky, Talgar, and Medeu forestries, and Ile-Alatau State National Natural Park).

To eliminate saprophytic species, the pathogenicity of isolated bacteria was initially checked by the hypersensitivity reaction using the Klement infectious and infiltration method on indicator plants, tobacco, and room geranium (*Pelargonium zonala* L.), as it is a houseplant, convenient to use all year round. Studies showed that most of the isolates we isolated were similar to *P. syringae* and *E. amylovora* and after 12-24 hours caused leaf necrosis together with inoculum administration, while saprophytic species did not cause that reaction. The initial identification of bacteria isolated into a pure culture was carried out on the immature fruits of a pear tree according to the White test. This method is considered one of the reliable methods in the diagnosis of fire blight and is widely used by many researchers (Ashmawy, Zaghlou, & El-Sabagh, 2015; Doolotkeldieva & Bobusheva, 2016). It is very simple and allows one to quickly and accurately identify the bacterium *E. amylovora.* The only drawback of this method is the absence of unripe pear fruits throughout the year. However, unripe pear fruits can be stored in the refrigerator for six months. The unsuitability of mature pears is associated with physiological changes in ripened fruits. When the immature fruits of the pear were inoculated, the bacterium *E. amylovora* at the injection site caused a milky-white exudate (Fig. 3).

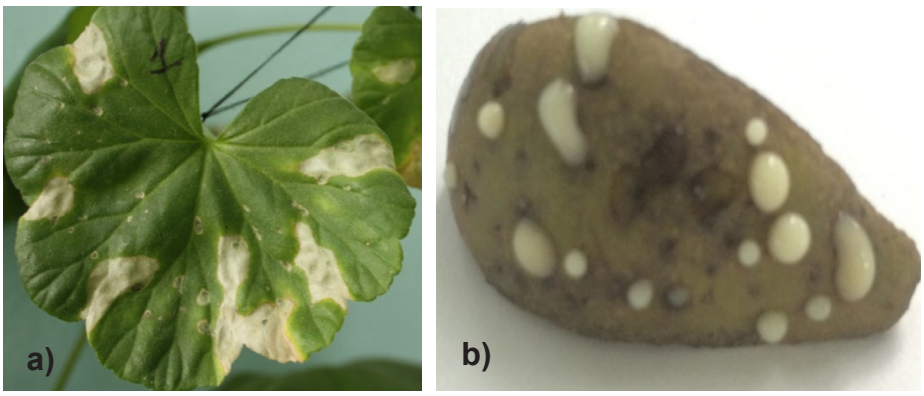

Fig. 3. Verification of pathogenicity on geranium (a) and unripe pear fruits (b)

In laboratory conditions, we analyzed 233 samples with signs of fire blight obtained during the examination of fruit crops on farms in the south and southeast of Kazakhstan. We obtained 1,143 isolates. The results of their testing on immature pear fruits by the White method confirmed the presence of bacteria *E. amylovora,* the causative agent of fruit crop fire blight (Table 1). Consequently, foci of this dangerous quarantine disease were found in all the examined regions of the south and southeast of Kazakhstan, as well as in the forestry of the SRPE (state research and production enterprise) on wild plant species. As can be seen on the data in Table 1, the largest number of isolates was obtained from samples of the Almaty region, which confirms the presence of a large number of foci in this region. A significant number of *E. amylovora* isolates were obtained from samples selected by us from the neighboring territory of Kyrgyzstan (Chuy valley) where grafting and planting materials were imported to Kazakhstan from this territory. Foci of fire blight in forest areas of the SRPE are considered as great danger. They can be perennial reserves of infection. They are especially dangerous for the Sievers apple tree, which grows in the Zaiyliysky and Zhongarsky Alatau.

The identification results of pathogens of fire blight and fruit necrosis isolated from various cultures of the Rosaceae family are presented in Table 2. The results showed that the bacterium *E. amylovora* was isolated from nine cultures of the Rosaceae family. The largest number of isolates of *E. amylovora* was obtained from the apple tree since this crop occupies a larger specific weight (more than 90%) among all fruit crops, followed by the pear tree. In the analyses, we paid great attention to samples of the wild Sievers apple tree growing in the forest areas of the Zailiysky Alatau. The trees infected by fire blight were mainly detected in the Aksai forestry. In almost all forest districts (Turgensky, Aksaysky, Talgar, Medeus), we identified individual hawthorn bushes with fire blight symptoms and in the Medeusky and Turgensky forest districts, we found rowan with those symptoms.

Table 1. Identification results of bacteria, fire blight and necrosis pathogens isolated from fruit crops in the south and southeast of Kazakhstan, as well as from the neighboring territory of Kyrgyzstan (laboratory experiment, 2013)

Regions	Analyzed samples	Obtained isolates	Identified as *E. amylovora*	Identified as *P. syringae*	Related microorganisms
Almaty region	85	372	185	142	45
South Kazakhstan region	42	190	58	63	69
Zhambyl	66	284	71	137	76
Forestries	28	238	97	90	51
Kyrgyzstan	12	59	34	17	8
Total	233	1,143	445	432	241

Table 2. Results of the identification of pathogens for fire blight and necrosis isolated from plants of the Rosaceae family (laboratory experiment, 2013)

Culture	Analyzed samples	Obtained isolates	Identified as *E. amylovora*	Identified as *P. syringae*	Related microorganisms
Apple tree	102	657	254	240	175
Pear	68	166	85	69	22
Quince	12	25	12	10	7
Plum	11	16	4	7	4
Apricot	9	18	5	7	6
Cherry	3	11	2	4	5
Wild Sievers apple tree	11	98	47	41	15
Hawthorn	11	91	27	31	23
Rowan	6	48	9	18	21
Total	233	1,143	445	432	278

This is confirmed by the data on the wide specialization of the bacterium *E. amylovora* among the Rosaceae family (Ashmawy, Zaghlou, & El-Sabagh, 2015; van der Zwet, Orolaza-Halbrendt, & Zeller, 2016; Vrancken et al., 2013). Pome trees (apple, pear, and quince) are the main hosts of the bacteria. According to literature data, these trees are mainly marked by the strong harmfulness of the disease (Biggs & Turechek, 2010; Geider et al., 2009). In our conditions, more intensive development of the disease was noted on the pear tree, on which the ovary, leaves, branches, and fruits quickly blacken and have a charred appearance. Currently, in many farms, due to fire blight, pear gardens are being uprooted. According to Moltmann & Viehrig (2008), a pear tree may be the primary source of fire blight infection for other fruit crops, including apple trees. Therefore, it is necessary to observe the spatial isolation of apple gardens from pear gardens.

The damage to such stone fruit trees as plums, cherries, and apricots caused by fire blight was rare and manifested in the form of separate dry branches. The analyses showed that in many samples of fruit crops, mixed infection of *E. amylovora* and *P. syringae* was present (Fig. 4). Other researchers have also noted the joint isolation from the same samples of these two species of bacteria (Dreo, et al., 2006; Iakovleva et al., 2014). According to Iakovleva et al. (2014), in the case of joint inoculation of *E. amylovora* and *P. syringae*, the pathological process slowed down. Copper-containing fungicides are widely used against fire blight and necrosis of fruit crops (Mager et al., 2011; Norelli, Jones, & Aldwinckle, 2003). In laboratory conditions, *P. syringae* was found to be more sensitive to these fungicides (Djaimurzina et al., 2014). In the literature, there is an evidence that, in addition to temperature and humidity, epiphytic microflora is the limiting factor for the reproduction of the bacterium *E. amylovora* (Arafat, Hanan, & Rabab, 2015). In the concomitant microflora isolated by us, we identified the bacteria *Pseudomonas fluorescens*, *Panteoa aglomerans*, *Bacillus sp.*, and others, which, according to the literature, are antagonists of the bacterium *E. amylovora* (Arafat, Hanan, & Rabab, 2015; Sundin et al., 2009).

In this regard, the use of copper-containing preparations during the growing season of fruit crops may adversely affect the epiphytic microflora in the tree phyllosphere since it may contain antagonists and competitors of the bacterium *E. amylovora*, which are more sensitive to these preparations. According to the proposed strategy, treatment with copper-containing preparations should be carried out in late autumn against the infection accumulated during the growing season as well as in early spring before bud break against the overwintered infection. In the period of flowering and regrowth of young shoots, it is necessary to spray them with biological products to saturate the phylosphere of the tree with antagonists and competitors as well as immunomodulators to increase resistance to the disease.

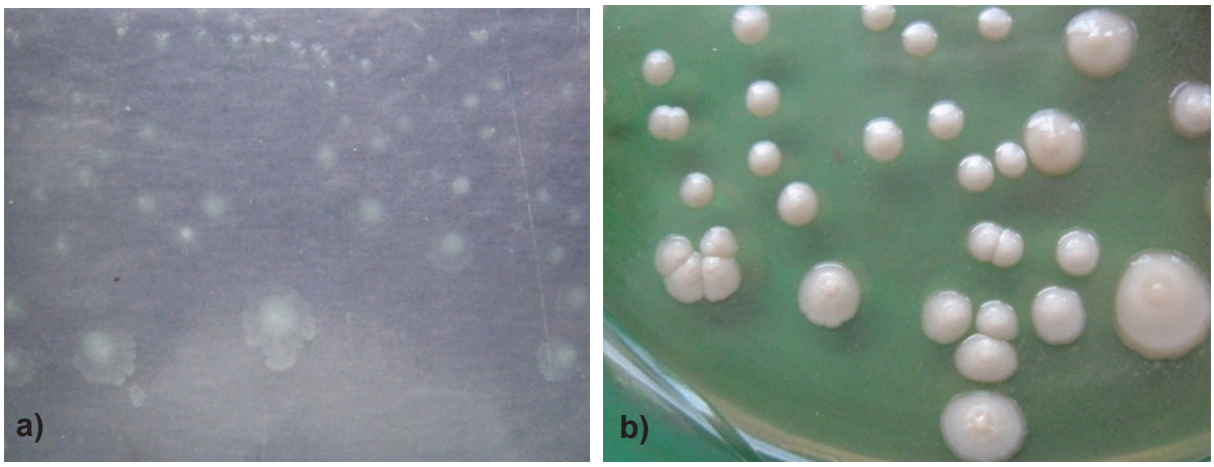

Fig. 4. Colonies of *P. syringae* (a) and *E. amylovora* bacteria (b) isolated from the same samples

The results of our studies on the identification of bacterial species *E. amylovora* and *P. syringae* were confirmed by Swiss scientists from the Agroscope research center based on an immuno-chromatographic test (Agri Strip Bioreba AG) and Russian scientists at the All-Russian Research Institute of Plant Quarantine using enzyme-linked immunosorbent assay (ELIA), FLASH PCR, and PCR in real-time (PCR-RT). Thus, we isolated and identified two types of phytopathogenic bacteria (*E. amylovora*), the causative agent of the dangerous quarantine fire blight disease, and *P. syringae*, the causative agent of fruit crop necrosis. The research results confirmed the presence of fire blight foci in the fruit zone of the south and southeast of Kazakhstan.

CONCLUSION

As a result of the study, foci of fire blight in the republic of Kazakhstan were revealed. The dependence of the development of the disease on meteorological conditions was established. The phase of the development of apple trees susceptible to the disease was revealed, and the causative agents of the disease were isolated and identified. Based on the research and analysis of literary sources, a strategy for combating fire blight in considered conditions was developed. It is aimed at preventing the accumulation of infection from season to season, reducing the intensity of reproduction of the pathogen, and increasing the plant's resistance to disease.

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