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The fire blight prevalence on different varieties of apple trees in the main industrial zone of horticulture (south and south-east of Kazakhstan)

Abstract. *Apple is the most significant fruit in Kazakhstan, as well as in many other countries of the world. According to the Statistics Agency in 2020, the apple cultivation area in Kazakhstan is about 36 thousand hectares with a gross har-vest of 259.1 thousand tons. The fire blight of fruit crops, in particular apple trees, is one of the most harmful infectious diseases, rapidly spreading and cre-ating a constant threat to the apple plantations of Kazakhstan. In order to avoid the further spread of fire blight in the territory of Kazakhstan, it is very important to grow varieties of fruit trees resistant to fire blight. The article pre-sents the results on the spread of fire blight on zoned, introduced, and promis-ing apple varieties in plantations of the main industrial zone of horticulture (Turkestan, Zhambyl, Almaty regions). As a result of the bacteriological analy-sis of the selected samples, 8 bacterial isolates were extracted from the varieties such as Aport, Voskhod, Sinap Almaty, Maksat, Golden Delicious, Kon-fetnoye, Pinova, Pink Lady, which, and were like the causative agent of fire blight by morphological and cultural characteristics. Bacterial pathogenicity testing on immature apple fruit showed positive test results confirming the presence of the bacterium Erwinia amylovora, the causative agent of fire blight.*
Keywords: *apple tree, disease, fire blight, phytopathological monitoring, varieties, Erwinia amylovora, the resistance of varieties, the prevalence of the disease.*

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Introduction

Apple is the most popular fruit in Kazakhstan, as well as in many other countries of the world. Kazakhstan is a leader in increasing fruit production. Apple orchards cover 77% out of 45.0 thousand hectares of pome and stone fruit orchards. Southern and southeastern regions of Kazakhstan have the most favorable climatic conditions for growing fruit crops, in particular apple trees. According to the Statistics Agency in 2020, the apple cultivation area in Kazakhstan is about 36 thousand hectares with a gross harvest of 259.1 thousand tons. According to the varieties research of the plantation in the country, a big assortment of fruit crops is recommended for use, with about 69 apple varieties included in the State register in Kazakhstan.

One of the most harmful infectious diseases of fruit crops is fire blight (*Erwinia amylovora*). It is a quarantine object in Kazakhstan. The disease is caused by the bacteria *Erwinia amylovora (Burrill) Winslow et al* and affects apples (*Malus domestica*) and pear (*Pyrus communis*). The disease affects all apple tree organs: flowers, blossoming buds, fruits, leaves, shoots, boughs, bark stem.

Erwinia amylovora was the first bacterium described as a causal agent of plant disease by Burrill [1]. It was reported in North America and was later detected in New Zealand in 1920. In Europe, fire blight was reported in 1957 in the United Kingdom and has since been identified in most areas where susceptible hosts are cultivated. *Erwinia amylovora* is now present in more than 40 countries [2, 3], but it has not been recorded in South America, Asia, or sub-Saharan African countries. It has been recorded in some North African countries and only once in Australia [3, 4]. Bacterial fire blight in Kazakhstan was identified in 2010 for the first time [5]. It represents a threat to the pome fruit industry of all the countries. Details on geographical distribution can be found in the EPPO Plant Quarantine Data Retrieval system [6].

Field observations have already shown that Aport was one of the susceptible varieties to this disease. To localize and eliminate the disease, uprooting and burning of trees and all vegetation in this area within a radius of 30 kilometers will be required. This will result in multi-billion-dollar losses. Losses could amount to more than 50 percent of all fruit plantations in Kazakhstan, which could lead to an environmental disaster [7].

E. amylovora is highly virulent and capable of rapid systemic movement within plant hosts and of rapid dissemination among rosaceous species, including apple and pear trees, when environmental conditions are favorable. The internal movement of the pathogen through the vascular system of plants and the ability of the pathogen to infect flowers, actively growing shoots, and rootstocks makes the management of fire blight difficult [8, 9]. The first fire blight manifestation in Kazakhstan was in 2008, and by 2010 it began to cause significant damage to the apple and pear orchards of the republic. In some peasant farms of the Almaty region, the proportion of affected trees in apple orchards reached 50-60% or more with a high degree of symptom development. In terms of severity, fire blight has not equal among known diseases of fruit crops [10, 11].

Data on the resistance of domestic and foreign apple varieties to fire blight in Kazakhstan is extremely segmentary, which prevents us to recommend a suitable assortment for industrial and private orchards in regions with the higher risk of disease development.

Materials and research methods

The research was carried out in the field and in laboratory conditions. Route inspections of apple plantations to identify the causative agent of fire blight on apple varieties and rootstocks were carried out in large industrial orchards and farms of Turkistan, Zhambyl, and Almaty regions and collection plantations of Kazakh Research Institute of Fruits and Vegetables LLP, located in the Talgar district of Almaty region.

In order to detect the pathogen, the apple trees without symptoms and with highly expressed symptoms of fire blight were selected. Branches, leaves, and ovaries were taken from trees with symptoms of fire blight for microbiological studies.

For the timely detection of fire blight, regular inspections of apple plantations in the south and southeast of Kazakhstan were carried out during the growing season according to the methods for detecting and identifying the fire blight agent of fruit trees [12, 13]. For bacteriological analysis, confirming the presence of *Erwinia amylovora* in the plant, fresh samples were taken from the bark taken at the border of the canker, as well as from bacterial exudate, and bent tips of young shoots. The pathogenicity of the strains on young immature apple fruits was tested according to the White method. This test is one of the main ones in determining the causative agent of fire blight.

The degree of study of the topic. In terms of its damage to horticulture during the years of epiphytosis, fire blight is more harmful than all fruit diseases combined. According to its economic importance, this disease is recognized as the most dangerous in the world and is included in the EPPO A2 list [14, 15, 16]. Gradually spreading, it reached almost all continents. Its penetration into Europe dates back to 1957. Currently, there is an expansion of the disease around the globe, penetrating into the territory of more and more new countries. Such a widespread prevalence of fire blight, despite the difference in natural and climatic conditions of the countries where the disease is registered, indicates the ecological plasticity of the pathogen. Consequently, the threat of expanding the range of the disease will continue in the future. Despite numerous studies conducted in different countries, several issues of fruit crop varieties' resistance to fire blight have not been sufficiently studied. Data on the resistance of domestic and foreign apple varieties to fire blight in Kazakhstan is extremely segmentary, which prevents us to recommend a suitable assortment for industrial and private orchards in regions with the higher risk of disease development.

Analysis. The fire blight causative agent diagnosis will allow for assessing the level of infection and identifying resistant apple varieties for breeding and further reproduction. The cultivation of such varieties will reduce the pesticide rate, and obtain environmentally friendly products for fresh consumption and food production, including those based on organic production.

Results

A monitoring survey of several farms to identify the fire blight causative agent and sampling for a subsequent molecular genetic analysis was carried out on 53 apple varieties, including 21 zoned, 5 promising, and 27 introduced in Turkestan, Zhambyl, and Almaty regions and collection plantations of "Kazakh Research Institute of fruits and vegetables" LLP. Sampling was carried out in double and more replicates at 2 and more sampling points. Thorough analysis of the disease symptoms was carried out during inspection and samples of the affected tree organs (leaves, fruits, shoots, branches, bark) were selected.

Apple plantations of Almaty regions were examined in the farms of Baiseit, Kyzylsharyk, Koram, Malovodnoe villages in Enbekshikazakh district, and collectable plantations of "Kazakh Research Institute of fruits and vegetables" LLP in Talgar district. The apple plantations in the Turkistan region were examined in farms and production orchards of Akzhar and Sharbulak villages of Kazygurt region and Shakpak village of Tulkibas district. In Zhambyl region, there were examined apple orchards of Merke village in the Merken district. The results of apple varieties evaluation to fire blight are given in table 1.

Table 1

Evaluation of apple varieties to fire blight in the farms of the Turkestan, Zhambyl, and Almaty regions, 2021

Varieties	Almaty region	Turkistan region	Zhambyl region	Trees with symptoms
Zoned varieties				
Idared	+	+	+	+
Ainur	+	+	–	–
Aport	+	+	+	+
Bayterek	+	–	–	–
Voskhod	+	–	–	+
Gala	+	+	+	
Golden Delicious	+	+	+	+
Granny Smith	+	+	+	+
Danalyk	+	–	–	–
Zarya Alatau	+	–	–	–
Maksat	+	–	–	+
Mantet	+	–	–	–
Melba	+	+	–	–
Red Delicious	+	+	–	–
Renet Simirenko	–	+	–	–
Renet Burkhardta	+	–	–	–
Saltanat	–	+	–	–
Starkrimson	+	+	+	+

Talgarskoe	+	–	–	–
Fuji	+	+	+	+
Pestrushka	+	–	–	–
Promising varieties of domestic breeding				
Damira	+	–	–	–
Zharkyn	+	–	–	–
Yessen	+	–	–	–
Sarkyt	+	–	–	–
Rakhat	+	–	–	–
Introduced varieties				
Braeburn	+	–	–	–
Jeromine	+	+	+	–
Jonagold	+	+	–	–
Kandil Sinap	–	+	–	–
Quinte	+		+	–
Pink Lady	+	+	–	+
Pinova	+	+	–	+
Prima	+	–	–	–
Scarlet Spur		+	+	–
Stark's Earliest	+	–	–	–
Rashida	+	–	–	+
Sinap Almatinski	+	–	–	+
Konfetnoe	–	+	–	+
Diana	+	–	–	–
Red Chief	–	+	–	–
Elstar	+	–	–	–
Monroe	+	–	–	–
Red free	+	–	–	–
Lady Williams	+	–	–	–
Diana	+	–	–	–
Gala Mitch	+	–	–	–
Honeycrisp	+	–	–	–
Golden Spur	+	–	–	–
Korea	+	–	–	–
Red Topaz	+	–	–	+
Diligence	+	–	–	–
Santana	+	–	–	–

The presence of fire blight symptoms characteristic of apple tree was noted during a visual examination of the samples: drying of the tops of young shoots, the tips of which are bent in a hook-like manner, leaf necrosis, brown spots on immature fruits, which are gradually mummified, "marbling" on the bark cut, wedge-shaped ulcers on the bark, exudate on the affected organs (Figure 1).

(а) (б)

Figure 1. Symptoms of a fire blight (a) drying out of the tops of young shoots, shepherd's crook; (b) release of bacterial exudate

According to the results of the surveys, a focal spread of the fire blight of fruit crops in the surveyed farms of Almaty, Turkestan, and Zhambyl regions was established. Due to the dry weather conditions observed during the 2021 growing season, there was noted a sharp decrease in the disease harmfulness compared to previous years. In order to control and exterminate the infection on farms, pruning and removal of trees, as well as treatments with copper-containing preparations, are used.

Surveys of apple plantations in the main horticulture production areas (south and southeast of the republic) showed that fire blight was quite widespread. In three surveyed farms of the Turkistan region, 6 released varieties out of 12 were with symptoms of fire blight damage, and out of 8 introduced varieties of foreign breeding, 3 were with symptoms of disease damage. In the Almaty region, in five surveyed farms out of 18 released varieties, 8 apple trees were damaged by fire blight, and out of 5 promising and 22 varieties of foreign selection, 5 were with symptoms of disease damage. In the Merke district of the Zhambyl region, out of 7 released and 3 foreign varieties, 6 varieties were found with a fire blight effect on an apple tree. In all surveyed farms of Almaty, Tukristan, and Zhambyl regions, apple varieties Idored, Aport, Voskhod, Maksat, Golden Delicious, Granny Smith, Pinova are most susceptible to fire blight, where the prevalence of the disease was 27-50%, with a degree of development – 9.0-16.2%, respectively.

Thus, in the surveyed production orchards and farms of Turkestan, Zhambyl, Almaty regions and collection plantations of "Kazakh Research Institute of fruits and vegetables" LLP, among 21 released varieties, apple trees damaged by fire blight was found on most varieties: Idored, Aport, Golden Delicious, Granny Smith, Maksat, Sunrise, Starkrimson, and Fuji. Of the 27 introduced varieties of foreign breeding, symptoms of the disease were noted on the apple varieties Pinova, Pink Lady, Rashida, Konfetnoye, Sinap Almaty, and Red Topaz. The promising domestic apple varieties like Damira, Zharkyn, Yesen, Sarkyt, Rakhat were free of a fire blight symptom, which brings a significant interest in apple production.

In order to diagnose apple fire blight by isolating the causative agent of the disease in pure cultures, bacteriological analyzes of samples taken as a result of the examination were carried out in laboratory conditions. Samples were taken on 14 varieties with symptoms of the disease: Idored, Aport, Golden Delicious, Granny Smith, Maksat, Voskhod, Starkrimson, Fuji, Pinova, Pink Lady, Rashida, Candy, Sinap Almaty, Red Topaz.

The pathogenicity of the bacteria *E. amylovora* was isolated, identified, and characterized. The records of the bacteria grown on a nutrient medium result and description were carried out within 3-10 days. Colonies of *E. amylovora* on King B medium are off-white, rounded, smooth, flat to slightly convex. Good results were obtained by isolation on a Levan medium. Colonies on Levan medium are white, rounded, and smooth, the profile is from convex to drop-shaped with smooth edges, shiny, translucent, and size from 2 mm to 5 mm. Colonies on potato agar are off-white or yellow, round, smooth, flat to slightly convex, and punctate to 5 mm in size. The selected colonies were sifted into Petri dishes with the nutrient medium of King B and Levan. With the growth of the same type of colonies, they were re-seeded for further research.

As a result of the bacteriological analysis of the selected samples, 8 bacterial isolates were isolated from the varieties: Aport, Voskhod, Sinap Almaty, Maksat, Golden Delicious, Konfetnoye, Pinova, Pink Lady, which were similar in morphological and cultural characteristics to *Erwinia amylovora*, the causative agent of fire blight.

To comply with Koch's postulates and confirm the pathogenicity of bacteria, the test was carried out by the White method on immature apple fruits. For inoculation, the Pinova apple variety susceptible to *E. amylovora* was used. A suspension of bacteria (concentration 10 cells/ml) was applied to fresh incisions and pricks made with an entomological pin on the surface of a disinfected apple fruit under the skin, at different depths. Then the inoculated apple fruits were placed in a humidity chamber using closed desiccators with a sterile cotton pad. The control was individual apple fruits with sterile water applied to the incisions and injections. Fruits were incubated in a humidity chamber at 25ºC for 3-7 days. Observations of the inoculated fruits showed that after 3 days necrotic spots appeared around the injection site and milky-white exudate was released at the injection sites. In the control, there were no ulcers at the site of inoculation or only a small necrotic ulcer was observed (Figure 2).

(а)

(б)

Figure 2. Testing the pathogenicity of *E. amylovora* on immature apple fruits: (a) inoculated fruits; (B) control without inoculation

Discussion

An increased level of resistance to diseases is one of the most important requirements for a modern variety of agricultural plants, including fruit ones. The problem at present is the search for fire-blight-resistant domestic and introduced varieties of apple trees, which does not allow us to recommend a suitable assortment for industrial and private gardens. Despite numerous studies conducted in different countries, a number of issues of fruit crop varieties' resistance to fire blight have not been sufficiently studied. The cultivation of such varieties will reduce the pesticide load and obtain environmentally friendly products for fresh consumption and food production, including those based on organic production. As a result of this work, the area of spread of fire blight in the orchards of Turkestan, Zhambyl, and Almaty regions was estimated and zoned and introduced apple tree varieties are identified as the most susceptible to disease. The disease-resistant promising apple varieties of domestic breeding such as Damira, Zharkyn, Yessen, Sarkyt, Rakhat were selected, which should be of a production considerable interest. The pathogenicity of the bacterium *E. amylovora* has been isolated, identified, and characterized. The fire blight causative agent was identified on the examined apple varieties on the basis of microbiological analysis. In the future, it is planned to identify genetically resistant apple varieties to the blight pathogen using modern molecular genetic methods based on DNA markers and develop recommendations for improving the breeding process and increasing the productivity of plantations.

Conclusion

As a result of a survey of apple plantations in the main industrial zone of horticulture (Turkestan, Zhambyl, Almaty regions), it was found that fire blight has become quite widespread. The most susceptible to bacterial blight among 53 zoned, promising, and introduced apple varieties were identified, which should be of considerable interest for apple production.

As a result of the bacteriological analysis of the selected samples, 8 bacterial isolates were extracted from the varieties: Aport, Voskhod, Sinap Almatinsky, Maksat, Golden Delicious, Candy, Pinova, Pink Lady, which, in morphological and cultural characteristics, were similar to *Erwinia amylovora*, the pathogen fire blight.

Testing the pathogenicity of bacteria on immature apple fruits showed positive test results confirming the presence of the bacteria *Erwinia amylovora*, the causative agent of fire blight.

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Бағбандықтың негізгі өнеркәсіптік аймағында (Қазақстанның оңтүстік және оңтүстік-шығысы) алма ағашының әртүрлі сорттарында бактериялық күйіктің таралуы

Аңдатпа. Қазақстанда, әлемнің көптеген елдеріндегідей, жеміс дақылдарының ішіндегі ең маңыздысы алма ағашы болып табылады. 45,0 мың га аймақта егілетін жеміс дақылдары бақтарының 77%-ы алма бақтары. Жеміс-жидек дақылдарының, атап айтқанда, алма ағаштарының бактериялық күйігі Республика бақтарында тұрақты қауіп төндіретін және тез таралатын ең зиянды жұқпалы аурулардың бірі болып табылады. Қазақстан аумағында бактериялық күйіктің одан әрі таралуына жол бермеу үшін ауруға төзімді жеміс ағаштарының сорттарын өсіру өте маңызды. Мақалада негізгі өнеркәсіптік бағбандық аймақтың (Түркістан, Жамбыл, Алматы облыстары) плантацияларындағы аудандастырылған, интродукцияланған және перспективалы алма сорттарына бактериалды өрттің таралуы бойынша нәтижелер берілген. Таңдалған сынамаларды бактериологиялық талдау нәтижесінде морфологиялық және культуралдық белгілері бойынша Апорт, Восход, Синап Алматинский, Мақсат, Голден Делишес, Конфетное, Пинова, Пинк Леди сорттарынан бактериялық күйік қоздырғышына ұқсас 8 изоляты бөлініп алынды. Піспеген алма жемістеріне жүргізілген бактериалды патогендік сынама, бактериялық күйік қоздырғышы *Erwinia amylovora* бактериясының болуын растайтын оң сынақ нәтижелерін көрсетті.

Түйін сөздер: алма ағашы, ауру, бактериалық күйік, фитопатологиялық мониторинг, сорттар, *Erwinia amilovora*, сорттардың төзімділігі, аурудың таралуы.

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Распространенность бактериального ожога на различных сортах яблони в основной промышленной зоне садоводства (юг и юго-восток Казахстана)

Аннотация. В Казахстане, как и в большинстве стран мира, наиболее значимой из плодовых культур является яблоня. Из 45,0 тысячи га садов, занятых семечковыми и косточковыми культурами, 77% составляют яблоневые сады. Бактериальный ожог плодовых культур, в частности яблони, является одним из наиболее вредоносных инфекционных заболеваний, стремительно распространяющихся и создающих постоянную угрозу насаждениям республики. Во избежание дальнейшего распространения бактериального ожога на территории Казахстана очень важно выращивать устойчивые к данному заболеванию сорта плодовых деревьев. В статье изложены результаты по распространению бактериального ожога на районированных, интродуцированных и перспективных сортах яблони в насаждениях основной промышленной зоны садоводства (Туркестанской, Жамбылской, Алматинской областей). В результате бактериологического анализа отобранных образцов было выделено 8 изолятов бактерий с сортов Апорт, Восход, Синап Алматинский, Максат, Голден Делишес, Конфетное, Пинова, Пинк Леди, которые по морфологическим и культуральным признакам были схожи с возбудителем бактериального ожога. Проверка патогенности бактерий на незрелых плодах яблони показала положительные результаты теста, подтверждающие наличие бактерии *Erwinia amylovora*, возбудителя бактериального ожога.

Ключевые слова: яблоня, болезнь, бактериальный ожог, фитопатологический мониторинг, сорта, *Erwinia amylovora*, устойчивость сортов, распространенность болезни.

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