

Erwinia amylovora, a bacterial blight affecting fruit, is one of the most known and dangerous diseases affecting quince, pear and apple and other Rosaceae family plants. It was first discovered in America in 1780, and first appeared in Macedonian quinces in 1989. *Erwinia amylovora*, called bacterial blight of fruit trees and ornamental plants, is one of the most intensely pathogenic bacteria that have been studied. It is assumed that the disease was first recognised in the United States at the end of the 18th century in wild species of plants, from which the bacteria spread to sensitive varieties of pear (*Pyrus communis*), apple (*Malus silvestris*) and quince (*Cydonia oblongata*), introduced by early American settlers from Eastern Europe and Southwest Asia. Drying of fruit trees was first observed in mountainous areas on both sides of the Hudson River in New York. From this region, the bacteria spread at high speed to neighbouring countries. Symptoms of *E. amylovora* are generally seen in the flowers which dehydrate and then turn either brown or black. The most massive and intensive symptoms is spread from the flowers.



Sasa Mitrev
Emilija Arsov

Presence of fire blight in apple, pear and quince in Macedonia

Authors: Prof d-r Sasa Mitrev, full professor and Assist. prof. Emilija Arsov, PhD Plant Protection, Faculty of Agriculture, Goce Delcev University, Stip, Department for Plant and Environmental Protection. Scientific work based on Plant Protection from the pathogens such as bacteria, fungi, viruses and phytoplasmas, field and laboratory work.



978-613-9-86749-3

 **LAMBERT**
Academic Publishing

**Sasa Mitrev
Emilija Arsov**

Presence of fire blight in apple, pear and quince in Macedonia

**Sasa Mitrev
Emilija Arsov**

**Presence of fire blight in apple, pear
and quince in Macedonia**

LAP LAMBERT Academic Publishing

Imprint

Any brand names and product names mentioned in this book are subject to trademark, brand or patent protection and are trademarks or registered trademarks of their respective holders. The use of brand names, product names, common names, trade names, product descriptions etc. even without a particular marking in this work is in no way to be construed to mean that such names may be regarded as unrestricted in respect of trademark and brand protection legislation and could thus be used by anyone.

Cover image: www.ingimage.com

Publisher:

LAP LAMBERT Academic Publishing

is a trademark of

International Book Market Service Ltd., member of OmniScriptum Publishing Group

17 Meldrum Street, Beau Bassin 71504, Mauritius

Printed at: see last page

ISBN: 978-613-9-86749-3

Copyright © Sasa Mitrev, Emilija Arsov

Copyright © 2018 International Book Market Service Ltd., member of
OmniScriptum Publishing Group

All rights reserved. Beau Bassin 2018

***PRESENCE OF FIRE BLIGHT IN APPLE, PEAR AND
QUINCE IN THE REPUBLIC OF MACEDONIA***

Sasa Mitrev / Emilija Arsov

***“Goce Delcev” University
Faculty of Agriculture,
Department for Plant and Environmental Protection,
Republic of Macedonia***

**PRESENCE OF FIRE BLIGHT IN APPLE, PEAR AND QUINCE IN THE
REPUBLIC OF MACEDONIA**

Sasa Mitrev / Emilija Arsov

***Goce Delcev University
Faculty of Agriculture
Department for Plant and Environmental Protection
Republic of Macedonia***

Erwinia amylovora, a bacterial blight affecting fruit, is one of the most known and dangerous diseases affecting quince, pear and apple and other Rosaceae family plants. It was first discovered in America in 1780, and first appeared in Macedonian quinces in 1989.

Erwinia amylovora, called bacterial blight of fruit trees and ornamental plants, is one of the most intensely pathogenic bacteria that have been studied. It is assumed that the disease was first recognised in the United States at the end of the 18th century in wild species of plants, from which the bacteria spread to sensitive varieties of pear (*Pyrus communis*), apple (*Malus silvestris*) and quince (*Cydonia oblongata*), introduced by early American settlers from Eastern Europe and Southwest Asia. Drying of fruit trees was first observed in mountainous areas on both sides of the Hudson River in New York. From this region, the bacteria spread at high speed to neighbouring countries.

At the start of 19th century, in some US states, this bacteria was singled out as the biggest problem in the fruit production. The biggest epidemic occurred in pears and apples in the eastern parts of the US. In the period of 1840, the bacteria reach Ohio, Indiana and Illinois.

Symptoms of *E. amylovora* are generally seen in the flowers which dehydrate and then turn either brown or black. The most massive and intensive symptoms is spread from the flowers. The rate at which the disease spreads among fruits depends

mostly on the turgidity of the plant as well as air temperature and humidity. The disease spreads when humidity is greater than 70% and in temperatures exceeding 23°C.

TABLE OF CONTENTS

1. INTRODUCTION	6
1.1. <i>Erwinia amylovora</i> , bacterial blight on apple, pear and quince	7
2. REVIEW OF LITERATURE	10
2.1. Occurrence, distribution, identification and control of <i>E. amylovora</i> in the region	10
2.1.1. Macedonia	14
2.1.2. Bosnia and Herzegovina	15
2.1.3. Croatia	15
2.1.4. Montenegro	16
2.1.5. Slovenia	16
2.1.6. Albania	17
2.1.7. Bulgaria	17
3. MATERIAL AND METHODS	18
3.1. Field analysis	18
3.2. General laboratory techniques	18
3.2.1. Methods for isolating the bacteria	19
3.2.2. Determination of the pathogenic characteristics	20
3.2.3. Hypersensitive reaction in tobacco variety Samsun (<i>Nicotiana tabacum</i> L.cv. <i>Samsun</i>)	20
3.2.4. Check pathogenicity of leaves of pear	20
3.2.5. Check pathogenicity of young green fruits	20
3.2.6. Check pathogenicity of shoots from apple, quince and sour thorn	20
3.2.7. Breeding characteristics of isolates	21
3.2.8. Gram-staining reaction	22
3.2.9. Levan production	22

3.3. Molecular methods for diagnosis of <i>E. amylovora</i>	22
3.3.1. Extraction of DNA	22
3.3.2. Amplification	23
4. RESULTS	26
4.1. Symptoms caused by <i>E. amylovora</i> in apple, pear and quince orchards in Macedonia	26
4.2. Biology of the pathogen	30
4.3. Reproduction	31
4.4. Laboratory diagnostics	32
4.4.1. Isolation and general characteristics of the bacteria	32
5. DISCUSSION	40
5.1. Characteristic of fire blight in the Republic of Macedonia	40
5.2. Measurement for protection	41
7. CONCLUSION	42
8. LITERATURE	44

1. INTRODUCTION

Bacterial blight, caused by *Erwinia amylovora*, is an economically important bacterial disease of apples and pears which is present in North America, most countries in Western Europe, large areas around the Mediterranean Sea and New Zealand (Bonn & Van der Zwet, 2000).

This bacteria is responsible for serious economic losses in countries where it has appeared. In 1976, a loss of \$4.7 million was reported in California, mainly affecting pears. In 1991, a loss of about 3.8 million dollars was reported in Southwest Michigan (Smith & Lattimore, 1997). In Egypt in 1988, the bacteria destroyed 80 % of pear trees, with around 50 % of the crop uprooted (Bonn & Van der Zwet, 2000). It is estimated that the epidemic which occurred in southwest Michigan in 2000 caused damage that cost approximately \$42 million (Longstroth, 2000).

Even in areas where climatic conditions are generally not favourable for the development of bacterial blight epidemics, the disease may arise from time to time, such as in the UK in the early 1980's (Gwynne, 1984).

There are three factors that contribute to the high-economic importance of *Erwinia amylovora*.

The first is the ability of bacteria to multiply on the surface of plants without causing disease (Thomson, 2000). However, when a favourable climate for the disease occurs, the bacteria is suddenly able to infect all plants. Epidemics of bacterial blight during the fall can be as unpredictable as the weather in this period. The second factor is the ability of the disease to spread relatively quickly over short distances. The pathogen can be easily transported by insects, wind and rain (Thomson, 2000). The ability for rapid expansion creates the potential for the bacteria to be distributed over parts of Europe and North America and indicates why eradication of the disease is rarely successful. The third factor is concerning the inherent difficult control of bacterial diseases (Thomson, 2000).

On the basis of its economic importance and the level of threat to fruit and ornamental plants, the bacteria have been designated as a quarantine organism in many countries. The bacteria crossed the Pacific and appeared in Japan, first affected seedlings and later it was affected all apples (Uyeda, 1903).

Harrison (1904) speculated that this disease had occurred in Canada since 1840 and that it has spread to many areas in the province of Ontario in 1870 (cited by Bonn & Van der Zwet, 2000). It reached New Zealand in 1919 (Bonn & Van der Zwet, 2000).

In Mexico, the disease was first reported in 1921, and in 1943 was observed in pear and apple trees (Bonn & Van der Zwet, 2000).

After World War II, devastating damage occurred in all regions where the presence of bacteria was reported. Therefore, its rapid appearance was expected in Europe. England was the first country in Europe where the disease was observed; this was in 1957 (Crosse et al., 1958).

***Erwinia amylovora* - bacterial blight of quince, pears and apples**

Quince (*Cydonia oblonga*) belongs to the genus *Cydonia*, subfamily *Pomoidae* and family *Rosaceae*. It has been used for at least 4000 years. The fruit has always been appreciated. It was first introduced as a culture in the Caucasus (Potter et al., 2007). America imported the fruit in the late 18th century. Varieties of quince are different in their morphological and physiological characteristics. They are distinguished according to the shape of the fruit, the shape of the crown and the colour of the leaf, and can be divided into five groups: varieties with pear form (variety *Pyriformis*), varieties with apple form (variety *Maliformis*), varieties with elongated ribbed fruits and coarse sheets (*Lusitanica*), varieties with pyramidal growth crowns (*Pyramidalis*) and varieties with variegated leaves and whitish colour (*Mormorata*) (Lauffenburger, 2006.).

Quince development requires fairly hot summers. Quince blossoms in May and June and it has white and light pink flower. It ripens from late October to December and contains several useful substances. It can withstand temperatures of between -30 °C in the winter and up to 52 °C in the summer. Most of this fruit is grown in areas with a continental climate and an average annual temperature of 10 to 15°C. Unlike apple and pear, quince is less tolerant of winter temperatures (Carlton, 2013).

Quince is represented in Central Asia, around the Black Sea, Asia Minor, in Mediterranean countries and countries bordering the Atlantic, in Scandinavia and America.

In the coastal locations, the fruit can be found at up to 1000 meters altitude, and in high continental areas up to 600 meters. Today, the biggest producers of quince are Portugal, Canada, France and Turkey. In Macedonia quince is represented in a small area, and the reason for this is no processing facilities because this culture is intended for fresh consumption.

In the pharmaceutical industry, quince fruit is used for making syrups to treat bronchitis, influenza and bleeding. Quince has a much simpler growing technology than apple, pear and peach, and also the measurement for protection is easier. It makes production much cheaper, but this fruit also can be attacked by many diseases and insect pests.



Figure 1. Production of quince in the Valandovo region

Diseases that attack quince are canker, rot, brown rot, soft rot, rust, brown spots and blight, the most important and most harmful is bacterial blight (Balaz et al., 2013).

Insects that attack the quince are shaggy beetle, cherry leaf wasp and, causing the greatest damage, apple worm.

The bacterial blight disease is the most harmful for pears, apples, quince and other plants of the family *Rosaceae*.

Fire blight belongs to the family *Enterobacteriaceae*, the genus *Erwinia*, type *E. amylovora*, which are polyphagous (Balaz et al., 2013) (Table 1).

Erwinia amylovora attack and parasitise a large number of plants from different family, but mostly of the family *Rosaceae*. About 129 species from 37 genera of the family *Rosaceae* are sensitive. The most important genera are *Malus*, *Pyrus* and *Cynodonia*.

In Macedonia fire blight is present in pears, apples, quinces and medlar-tree, cultivation.

Table 1.Scientific classification of the bacterium *Erwinia amylovora*

Kingdom	<i>Eubacteria</i>
Stem	<i>Proteobacteria</i>
Class	<i>GammaProteobacteria</i>
Row	<i>Enterobacteriales</i>
Family	<i>Enterobacteriaceae</i>
Race	<i>Erwinia</i>
Kind	<i>Erwiniaamylovora</i>

In former Yugoslavia, the first occurrence of *Erwinia* was reported in 1986 in Bosnia and Serbia (Bonn & Van der Zwet, 2000), and in Macedonia, the first appearance of this bacteria was reported on quince in Gevgelija and pear in the Radovis region (Mitrev, 1993).

In the following years, the bacteria caused great damage, destroying over 400 ha of planted pears and over 60 ha of quince plantations.

It is especially harmful in plantations of pear crops, practically causing extinction of fruit trees. Younger trees of certain varieties and pear seedlings in nurseries can be destroyed in one year (Mitrev, 1993).

According to the latest data from Macedonia, the bacteria are harmful to certain varieties of apples (Mitrev & Kostadinovska, 2016). In addition, it causes great damage to some ornamental plants of the family *Rosaceae*, and certain forest species such as hawthorn (Balaz et al., 2013).

Moreover, it affects some bony fruit species. The bacteria attack virtually all parts of the plant, causing burning of flowers, leaves, young shoots and perennials, and cancer sores on the stem.

In apples and pears, the first symptoms appear in early spring during wet and hot weather (Van der Zwet & Keil, 1979).

The name of the disease is a description of its main features: the flowers and leaves of the fruit trees dry and change colour from brown to black, with the appearance of fire damage. Young fruits turn brown, dry and then change to a black colour with possible bacterial exudate (Van der Zwet & Keil, 1979; Van der Zwet & Beer, 1995).

2. LITERATURE REVIEW

Erwinia amylovora (Burrill, 1882), the causal agent of bacterial fire blight of fruit trees and ornamental plants, is the first bacterium that was discovered as a plant pathogen. The disease was first observed on wild plants of the *Rosaceae* family in the eastern part of North America. With the introduction to North America of domesticated varieties of apple and pear by the old Europeans, the disease quickly spread to these crops. The first written report of *Erwinia amylovora* was in 1780 when it was observed in apple and pear in mountainous areas on both sides of the Hudson River in New York (Johnson, 2000). More than 100 years, the specificity of the etiology of this dangerous disease was not known. Many researchers have investigated this problem until 1878, when a scientist named Burrill, together with his associates at the University of Illinois, proved the bacterial nature of the disease.

Two years later in 1880, Burrill described the bacterial blight. In 1883, the bacteria was named *Micrococcus amylovorus* (Burrill, 1883a). However, its name was incorrectly based on the concept that the bacterium destroyed starch (*amylo* + *vorus* = eat starch).

In 1889, the name of the bacterium was changed to *Bacillus amylovorus* (Burr.) Trevisan, and, in 1897, renamed as *Bacterium amylovorum* (Burr.) Serbinoff. In 1920, the bacterium was classified as *Erwinia amylovora*, its current name, and it became the first described phytopathogenic bacterium. This bacterium is a unique and interesting pathogen. Almost 150 years after its discovery as a cause of bacterial blight, there remain many questions about how and in what manner it causes the disease. The nature of its pathogenicity is not yet fully understood, nor is why it is the only type of bacteria that causes bacterial blight in certain species of the family *Rosaceae* (Vanneste, 2000).

2.1. Occurrence, distribution, identification and control of *E. amylovora* in the region

Once the pathogen was detected in North America in the late 18th century to early 19th century, it was identified in some states in the US as the biggest problem for fruit production. Epidemics of high intensity affected pears and apples in the eastern parts of the US. In the years around 1840, the disease expanded into Ohio, Indiana and Illinois (Bonn & Van der Zwet, 2000). Catastrophic damage was observed in California during the period 1901-1909. Large outbreaks have been seen in Oregon and Washington (Van der Zwet, 2002).