Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 2458-9403
Vol. 3 Issue 3, March - 2016

Distribution Of Fire Blight Erwinia Amylovora And Its Control At The District Of Tirana, Albania

Dhurata Shehu
Plant Protection Department,
Agricultural University of Tirana
Plant Protection Laboratory, Durres
Albania
dhuratashehu@info.al

Harallamb Paçe
Plant Protection Department,
Agricultural University of Tirana
Plant Protection Laboratory, Durres
Albania
ha.pace@yahoo.com

Aleksandra Aleksoska
State Phitosanitary Laboratory – Skopje, Macedonia
Ministry of Agriculture, Forestry and water Economy
REPUBLIC OF MACEDONIA
aleksoska05@yahoo.com

Abstract—Fire blight is a bacterial disease caused by the bacterium *Erwinia amylovora* (Burrill) Winsolow et al and is considered as one of the most dangerous diseases of pome fruit worldwide. It is listed as a quarantined disease Plant Protection Directive of the European Union and is one of the main pests in the list A2 European Organization Plant Protection (EPPO). It destroys apple, pear, quince crops and other species of Rosaceae family. Seeing it's devastating effects, huge economic damages and higher control costs, it was attained a monitoring of this disease in the district of Tirana city for 2015 year. During vegetative period of pome fruits, apple, pear and quince observations have been conducted in orchard production, the orchard in production and the production of planting nurseries. From these observations have resulted to suspicious symptoms of the disease, considering it as the first step of the pathogen diagnosis. During this period, plant samples were collected with suspicious signs of the disease, which were brought to the laboratory and underwent laboratory tests to determining the final identification of the pathogen *Erwinia amylovora* on Laknas area and Zhurje village. Then were conducted control measures with Cu fungicides reducting bacterial infection till 30 %.

Keywords: Fire blight, observation, Erwinia amylovora

I. INTRODUCTION

The name "bacterial fire" (referred to as "Fire Blight" in the international literature and in the traditional literature in Albania as "bacterial scorch of fruit trees" is attributable to the quick and destructive effects of the pathogen on the vegetative mass, on certain branches or on the whole tree, which wilts and blackens rapidly in few days upon the onset of the disease [11]. *Erwinia amylovora* is most destructive to pears, apples, quinces and crab-apples, as well as several other species of Rosaceae family [12]. The fire blight affects the host trees by causing the flowers, leaves and young shoots to become limp and wilt, by creating cankers (cancerous wounds) in the bark of tree trunks, causing the tree to ultimately dry all over. Pathogen infects all aerial parts of the plant host [1, 7]. While the following plants have been considered to be the most important host plants from the economic and epidemiologic perspective: Amelanchier, A.alnifolia, Canadensis, Malus, Chaenomeles spp., Cotoneaster spp., Crataegus spp., Cydonia spp, Eryobotrya, Mespilus, Pyrus, P. amygdalifformis, Pyracantha spp., Sorbus spp. This disease has caused major economic losses in many countries of Europe and the Mediterranean, where commercial varieties of apple and pear are often susceptible to fire blight [3, 9]. Fire blight is big threat for the region EPPO and *Erwinia amylovora* is one from the most important diseases in the list A2 to European Organization for Plant Protection (EPPO) [8]. This disease is on the list of quarantine facilities A2 of the European Union where currently being strengthened quarantine regulations against this pathogen. Every year decreased the level of infection in the apple and pear orchard because of to quarantine measures [5]. All plant organs except the seeds are considered as possible sources for the spread of the pathogen. In many cases, is difficult to determine the origin of the fire blight in the protected areas, non-epidemic [4,6].

II. MATERIAL AND METHODS

Sampling in the districts of Tirana was done between May and September primarily in trees indicating an unnatural development and growth in which one could suspect symptoms of disease onset. The samples comprised in the main leaves, green growths, parts of twigs and branches, bits of bark, blossoms and ripe fruits. They were put into plastic bags and taken straight to a laboratory where they were preserved in refrigerators at temperatures within the 4 - 5° C range until the point when they were inspected and analyzed. The study also made use of other laboratory analysis for final identification of the disease.

For purposes of pathogen isolation was used the method as applied according to E. Billing et al., [2] and R. A. Lelliott [10]. To this end tissues from the areas between discoloration and the healthy parts were obtained from the leaves and shoots which indicated suspicious symptoms of

disease. These tissues were pounded into a sterile mortar combined with 1 - 2 droplets of sterile distilled water until a homogeneous concoction was formed. This mass of concoction (suspension) was planted through a sterile loop into petri dish fitted with a NSA feeding setting, by sliding the hook over the setting in a free manner as if drawing. The plates planted in this way were incubated in thermostats at temperatures 25 - 27°C. After a period of 2 - 3 days, once the bacterial colonies emerged in the planted plates they were transferred into other plates with the same NSA feeding setting in place. Transferals occurred twice in a row in order to obtain cleaned colonies. The clean colonies were transferred into other Petri-type plates with a NSA subtract and with the other King B subtract.

NSA substracts [1]

Bacto nutrient broth	8.0 gr
Sucrose	50.0 gr
Bacto agar	15.0 gr
Distillate water	1000.0 ml

The ingredients are dissolved in Mary wash for 15 min, pH 7.0 is properly regulated and is distributed in bottles (elermayer) of 150 ml each and later sterilized in autoclaves as in the case of the NSA setting.

Description of colonies

The colonies which are obtained, acting on the basis of knowledge and experience gained, underwent a macroscopic description by taking fully into account these elements:
a-The shape of colony (round, erratic).
b-The size of the colony.
c–The color of colony.
d - Position on the setting (flat, elevated, convex, hemispherical, etc.).
- Nature of the colony (mucoid, dry, etc.).
f - Opacity (transparent, opaque)
g - Structure (homogeneous or not)
k - Area (wrinkled, smooth, etc.)

There might be some other characteristic element which may have been mentioned in the description.

Coloring of bacteria

For the purpose of coloring bacteria fixed bacterial microscopic preparations were created at an early stage. To this end a droplet of sterile distilled water was placed on a clean microscopic slide. Then, a little bit of material obtained from the 24 hour old bacterial culture to be examined was placed in this droplet of water which was mixed with water. The mixture was evenly distributed on the slide and was left to dry in the laboratory temperature. After drying the slide was fixed over the flame of the lamp which was later subjected to coloring using the Gram method based on the following procedure:

a. Coloring with Gentian violet for 2 minutes.
b. Rinsing with water.
c. Lugoli solution 1-1.5 minutes.
d. Rinsing
e. Rinsing with alcohol for 1 minute.
f. Coloring with Fuchsine for 2 minutes.
g. Rinsing with water and drying by means of blotting paper which is later examined in a microscope.

Examination in a microscope was conducted with the object immersed, where the cells of the bacterial cells were detected along with their shapes, form, etc. Then treatment with fungicide for to limit fire blight is performed in a parcel, which is defined in Zhurje area. The land, which is located experiment is part of the orchard, which is pure good, has uniform fertility, etc. Treatments are performed in the apple tree cv. "Gala", the pear tree cv. "Williams" and quince (quince country).

III. RESULTS AND DISCUSSION

As seen from the data presented above, some pear and apple samples failed to prove the presence of bacteria *E. amylovora* despite displaying suspicious symptoms the trees from which the samples were collected. From these samples were isolated bacterial colonies that even though they had some similarities with those of the pathogen in word, again showed some different qualities and gave not pathogenicity during artificial inoculation in pear fruit. They can be considered saprophytic bacteria. Following are the results of monitoring.

The observations performed in pears, apples and quinces in nurseries, orchards under mature trees and those already under production have revealed the presence of symptoms similar to those of the fire blight (table 1).

TABLE 1. THE PRESENCE OF FIRE BLIGHT - *E. AMYLOVORA* IN SAMPLES COLLECTED IN 2015 YEAR

Sample	*The origin of the sample*	*Culture*	*Cultivar*	*The result achieved Positive + Negative -*
P_1	Zhurje – Tirana	Pear	Williams	+
P_2	Laknas – Tirana	Pear	Gentile Bianca	+
P_3	Laprakë – Tirana	Pear	Gentile Bianca	-
P_4	Ndroq - Tirana	Pear	Koshia prekoçe	-
P_5	Sauk – Tirana	Pear	Koshia prekoçe	-
P_6	Kurorë – Tirana	Pear	Gentile Bianca	-
P_7	Bathore – Tirana	Pear	Koshia prekoçe	-
P_8	Tapizë – Tirana	Pear	Abate fetel	-
A	Zhurje – Tirana	Apple	Gala	+
Q	Zhurje – Tirana	Quince	Q. country	+

Monitoring results in the district of Tirana for the 2015 year are presented in the figure 1.

Fig. 1. (Pezë Helmës, Ndroq, Qinam, Sauk, Tapizë, Bathore, Laprakeë (No infection); Zhurie, Laknas (With infection). Monitoring results in the district of Tirana for the 2015 year.

From the totality of symptoms present in test samples (taken for analyze), the results of tests carried out for testing the morphological peculiarity, biochemical and pathogenicity; result that to the samples (P1, P2, A, Q) is present bacterium *Erwinia amylovora* (Burill) Winslow et al. While in other samples of different bacteria were isolated that do not possess the qualities of the pathogen E. amylovora. From the analyzes performed result that the pathogen *E. amylovora* (Burrill) Winslow et al, cause of the fire blight was isolated from samples of pear, apple and quince with origin: Zhurje - Tirana and Laknas – Tirana Chemical treatments are used during sensitive periods to infection, which should be the main concern of cultivators after has important the effectiveness of treatment and the result of interventions, because this saves the number of treatments and prevent problems.

Treatments before flowering and after flowering with copper preparations, as Neoram WG the dose 200-250 gr/100 l water. In cases where is raining used dose 250 gr. After connecting the fruit the elimination of injuries with cutting and treatment every 10 days until the end of June. In this period, the amount of rainfall has been substantial. Self-climatic conditions have significantly affected to the progress of development to fire blight as and self-plant host, influencing on its sensitivity at the disease.

Assessment of infection

The severity of disease progress has been expressed as a percentage of the affected area in the bloom crown of the plant on a scale from nine the lowest to one the highest. The degree of affection for treatments with Neoram WG is based on the scale system as encountered in figure 2.

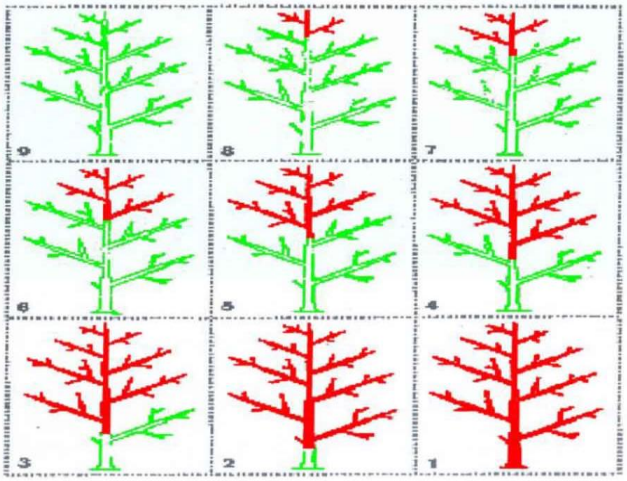

Fig 2: Severity scale of disease affection: 1. 100% of crown affected; 2. 90% of crown affected; 3. 80% of crown affected; 4. 50% of crown affected; 5. 40% of crown affected; 6. 30% of crown affected; 7. 20% of crown affected, 8. 10% of crown affected and 9. There is no infection.

For calculation of intensity of touch formula Mekinejit was used:

The severity of affection from disease in that variant is calculated through the formula:

$$I = \frac{\sum (n \times v)}{N \times 9} \times 100$$

Where:

I = Intensity (severity) in %

$\sum$ = Sum

n = number of trees evaluated on each scale

v = 0, 1, ... 9 number of evaluation scales

N = Total number of trees subjected to observation

(indicates number of scale)

The preparation of copper for to limit the infection has affected on the parameters considerably, to 30%.

View to fire blight in the village Zhurje and Laknas are presented in the figures 3 and 4.

Fig 3: Fire blight on Zhurje

Fig 4: Fire blight on Laknas

IV. CONCLUSIONS

From the monitoring process of the disease administered in area under the focus of the study it is indicated that there are new outbreaks of disease in Albania city posing a risk which could easily spread further to seed-bearing fruit trees.

The chemical measures adopted to control the disease will have to be integrated with the alternative methods and should be accompanied with the agro-technical and mechanical practice of removing and burning the infected parts so as to minimize and inhibit the disease

We should aim at a minimum damage of the disease as being taught in some cohabitation with it.

V. REFERENCES

[1] C.M.E. Garrett, C.G. Panagopoulos, J.E. Crosse, "Comparison of plant pathogenic pseudomonads from fruit trees", Journal of Applied Bacteriology 1966, vol. 29: pp. 342-356.

[2] E. Billing, "An association between capsulation and phage sensitivity in Erwinia amylovona", Nature 1960, 186, pp. 819-820.

[3] H. Paçe, "Menaxhimi i integruar i sëmundjeve në pemët frutore. Zjarri bakterial – *Erwinia amylovora*. Njohuri mbi sëmundjen dhe luftimin e saj", 2005, 5 – 19, pp. 91 – 101.

[4] I. Llorente, E. Badosa, P. Vilardell, E. Montesinos, "Epidemiological studies of fire blight (Erwinia amylovora) in Spain", Acta Hortic 2002, Vol. 596: pp. 535-538.

[5] K. Vrasken, M. Holtappels, H. Schoofs, T. Deckers and R. Valcke, "Pathognicity and infection strategies of the fire blight pathogen Erwinia amylovoa in Rosaceae", State of the art, 2013.

[6] M. Hasani, "Patologjia e pemëve frutore", 2003, pp. 49 – 51.

[7] M. Van Teylingen, "Ornamental hosts of Erwinia amylovora and the effect of the fire blight control policy in the Netherlands", Acta Hortic, 2002, Vol. 590: pp. 81-87.

[8] OEPP/EPPO. EPPO Standards PM 7/20. Diagnostic Protocol for regulated Erwinia amylovora. 2004. Bulletin EPPO Bulletin 34: pp. 159-171.

[9] R. Lídia, C. Moragrega, E. Montesinos, "Evaluation of four whole-plant inoculation methods to analyze the pathogenicity of Erwinia amylovora under quarantine conditions", International Microbiology, 2008, Vol. 11: pp. 111-119.

[10] R. A. Lelliott and D. E. Stead, "Methods for the Diagnosis of Bacterial Diseases of Plants", Blackwell, Oxford (GB), 1987.

[11] T. Kaltani, B. Çelo, "Fitopatologjia bujqësore (Pjesa e veçantë)" Botim I ILB, 1982, pp. 375 – 379.

[12] T. van der Zwet, and H. l. Keil "Fire Blight: A Bacterial Disease of Rosaceous Plants, Handbook 510, US Department of Agriculture, 1979, pp. 200.