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IMPACT OF BACTERIOPHAGES ON THE FIRE BLIGHT DISEASE PREVALENCE ON QIUNCE

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Summary. The effect of the phage therapy in the fire blight disease control was studied at the quince experimental plot and in the quince orchard. Quince plants at the experimental plot were treated with *Erwinia amylovora* bacteriophages over the last ten vegetation seasons while quince trees at the orchard were treated with preparations recommended for the fire blight control. It was found out that in the conditions, favorable for the fire blight causative agent development, the disease prevalence in the orchard where the phage therapy was not used was significantly higher than on the quince trees treated with bacteriophages.

Keywords: fire blight, *Erwinia amylovora*, bacteriophages, phage therapy, quince.

Introduction

Fire blight is a plant disease which affects the *Amygdaloideae* subfamily (previously *Spiroideae*) which contains such economically important pome fruits as apples, pears and quince as well as the *Rubus* genus [van der Zwet et al., 2012]. The disease causes serious economic damage, not only because it directly destroys pome fruit crops, but also because producers spend significant outlays for effective protection against the pathogen.

The most intensive development of the disease occurs in spring during flowering and shoot growth and in autumn during the secondary flowering. There are several forms of the disease: blossom blight, shoot or twig blight, leaf blight, fruit blight, limb or trunk blight, or collar or rootstock blight [van der Zwet & Keil, 1979].

The causal agent of fire blight - bacterium *Erwinia amylovora* (Burrill 1882) Winslow, Broadhust, Buchanan, Krumwiede, Roders and Smith 1920. Synonyms: *Micrococcus amylovorus* (Burrill, 1882), *Bacillus amylovorus* (Burrill, 1882) Trevisan, 1889, *Bacterium amylovorus* (Burrill, 1882) Chester, 1897, *Erwinia amylovora* f. sp. *Rubi* (Starr, 1951), Cardona & Falson [EPPO, 2022]. The bacterium is transmitted to healthy tissues mainly by insects, wind and rain splashes. The causative agents of fire blight can be found on the surface of plants in the epiphytic state. It is epiphytic bacterial populations on flowers that in most cases cause the spread of the fire blight.

The disease management, aimed to reducing the amount of the pathogen in orchards and preventing infection by the pathogen, includes pruning of infected twigs and branches, application of chemical and biological pesticides, antibiotics and the integration of tolerant cultivars [Gusberti et al., 2015].

Biological control agents, such as antagonistic bacteria, fungi, viruses as well as natural bactericides, which the mentioned organisms produce, are the most eco-friendly and sustainable means for protection against fire blight and are proposed for the fire blight control.

Bacteriophages, i.e. highly specific bacterial viruses that infect and lyse bacteria and are the natural components of ecosystems, are under intensive evaluation as the biocontrol agents against fire blight [Sabri et al., 2022, Kim et al., 2022].

The study of the interaction of the bacteriophages *E. amylovora* isolated and characterized at IGFPP with bacteria *E. amylovora* in quince tissues *in vitro* and *in vivo* revealed that phages effectiveness, although reduced, remains at the level provided by chemical means of protection against this bacteriosis [Samoilova, 2023].

Thus, the aim of the present research was to compare the effect of the long term quince plants treatment with *Erwinia amylovora* bacteriophages on the fire blight control and quince plants protection against fire blight without using bacteriophages.

Materials and methods

Quince tissues with fire blight infection symptoms were collected from the quince trees growing at the experimental plot of the IGFPP and in the quince orchard.

The virulent bacteria *Erwinia amylovora* were detected using immature pear slices inoculated with the collected plant tissues extracts. Inoculated immature pear slicers were incubated in a wet chamber at 28˚C and monitored for fire blight symptoms development in 24, 48 and 72 hours after inoculation. No less than five immature pear slices were inoculated per extract.

Bacteria were grown on plates with LB agar (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, 20 g/L agar) and on levan medium (2 g/L yeast extract, 5 g/L bactopeptone, 5 g/L NaCl , 50 g/L sucrose, agar 20 g/L, pH 7,2) at 28˚C. Liquid cultures were grown in LB medium (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl) at 28˚C.

The fire blight disease prevalence was calculated as follows:

$$P = n / N \times 100\%,$$

where

n – number of affected plants;

N – total number of plants sampled.

Results and discussions

Quince plants at the IGFPP experimental plot were treated with *E. amylovora* bacteriophages suspension in concentration 10^8 PFU/ml. Treatments were carried out over ten years when the high risks of the fire blight development occurred, i.e. during blossoming and when the high relative humidity was accompanied by the temperature from +24˚C till +28˚C. Quince trees at the orchard were treated with copper containing preparations.

Monitoring of the fire blight occurrence at the experimental plot and in the orchard was carried out in the late spring after several days with heavy rains and temperature from +24˚C till +28˚C (Figure 1).

A  B 

Figure 1. Quince plants at the IGFPP experimental plot (A) and in the quince orchard (B)

Dry leaves and dry shoots were found on all investigated trees in the quince orchard. Characteristic symptoms such as the typical “shepherd’s crook” of the twigs and the milky-yellowish bacterial exudate were not observed.

At the same time quince plants at the IGFPP experimental plot treated with *Erwinia amylovora* bacteriophages revealed no symptoms of the fire blight damage.

Plant material from the quince orchard and experimental plot was collected for detection of the bacteria *E. amylovora* by infection of the immature pear slices and selective media inoculation (Figure 2). These methods are not sensitive enough for detection of the low concentrations of bacteria but they provide information concerning the presence of virulent *E. amylovora* bacteria at potentially dangerous levels.

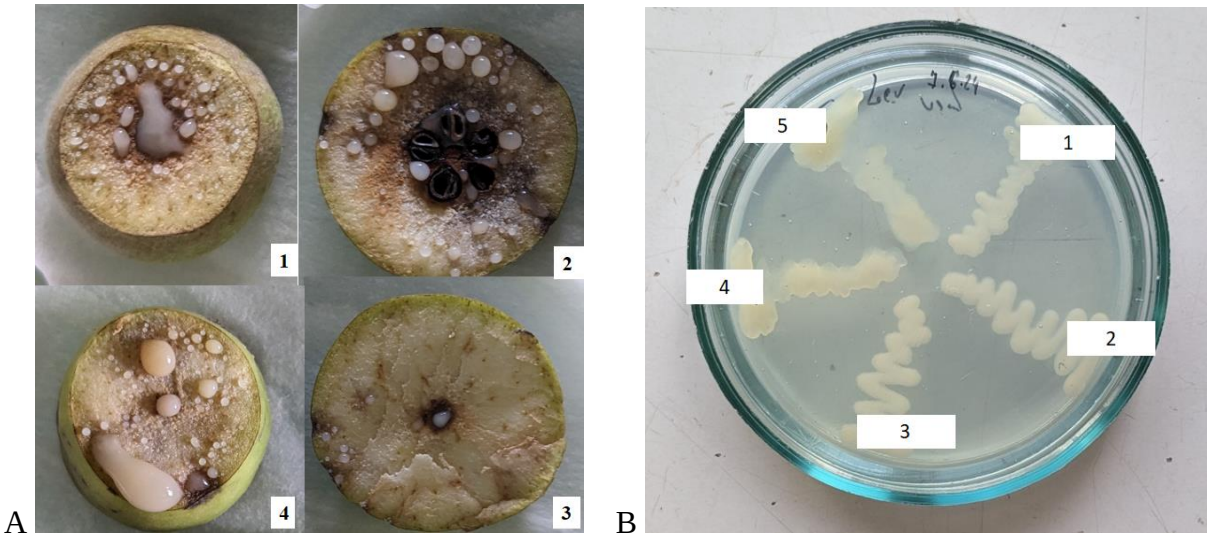

Figure 2. Immature pear slices inoculated with quince tissues extracts collected in the quince orchard (A) and levan medium inoculated with ooze from the immature pear slices (B):
1- dry orange quince bark, 2, 3 - dry quince leaves, 4 - dry shoots,
5- *E. amylovora* strain from the laboratory collection

Milky white ooze on the surface of the immature pear slices produced after 24 – 48 hours incubation at 28˚C and 100% humidity as well as milky, domed, smooth, mucoid colonies on the levan medium inoculated with the ooze, showed presence of the virulent bacteria *E. amylovora* in quince dry leaves, shoots and bark at the quince orchard. Plant tissue extracts collected at the experimental plot didn't cause on the immature pear slices producing of the typical for virulent *E. amylovora* strains ooze.

Thus, it has been found out that the fire blight disease prevalence in the quince orchard in the weather conditions favorable for pathogen growth consisted 100%, while at the experimental plot there were found several dry leaves in which the virulent *E. amylovora* bacteria were not detected by immature pear test.

It is reasonably considered that biopesticides effectiveness for fire blight control is generally low due to their highly influenced by environmental conditions and disease severities in each orchard [Ngugi et al., 2011]. The conducted experiments confirmed the advantage of bacteriophages as widely spread natural components of ecosystems which infect only bacteria sensitive to them. Furthermore, bacteriophages can regulate themselves in the sites of infection, depending on the bacterial population density. This study demonstrated that beneficial microorganisms such as *E. amylovora* bacteriophages, applied in the conditions favorable for the pathogen development, could be one of the possible solutions for reducing the economic losses without damaging the environment and at the same time maintaining high crop quality.

Conclusions

It is shown that long term application of *Erwinia amylovora* bacteriophages, non-toxic to plants, animals and humans biopecticides, in the periods of the fire blight disease high risk development significantly reduce virulent bacteria *Erwinia amylovora* amount in plants tissues. Consiquently, phage therapy provides protection of the quince trees against the fire blight disease development and prevents the spread of the pathogen among the other host plants. The obtained

results demonstrate that bacteriophages, isolated from the fruit orchards, where the disease causative agent, bacteria *Erwinia amylovora*, presents, could be involved in the fire blight control systems.

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