Incidence of the pathogens *Erwinia amylovora* and *Venturia inaequalis* in apple orchards in northern Transylvania

SUMMARY OF THE DOCTORAL THESIS

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1. INTRODUCTION

The apple is one of the first fruits known to man and one of the most appreciated due to the versatility of its uses as well as the very favorable composition for human health. Obtaining economically profitable productions, however, requires the application of well-developed culture technologies, in which the control of pathogens and specific pests plays an extremely important role.

Human society as we know it today would not exist in the absence of intensive agriculture. The last 20 years, however, come with a particular specificity given by the general context of globalization, the intense circulation of goods and people at the planetary level, the concern for ecology as well as climate change.

All these aspects make up a very different picture from the one in the apple culture of the last century and the research within the thesis aims to clarify some aspects of controlling fire blight and apple scab, two of the most damaging apple diseases.

2. STRUCTURE OF THE DOCTORAL THESIS

Doctoral thesis: The incidence of the pathogens *Erwinia amylovora* and *Venturia inaequalis* in apple plantations in the northern area of Transylvania, comprises 108 of pages with a number of 7 chapters, 25 figures and 34 tables. The paper is structured into two parts. The first part includes the bibliographic documentation on the discussed issue. It consists of two chapters and comprises 34 pages (31% of the total paper) and 3 figures. The second part is represented by the personal contribution that extends over 74 pages, includes 5 chapters and contains the research from the experimental years 2019-2022. The bibliography includes both domestic and foreign scientific works, it lists 160 sources, of which 144 citations from publications and 16 web citations.

Current state of knowledge:

Chapter 1. Brief retrospective on apple cultivation in the world and in Romania - this chapter presents the evolution of culture systems and the influence of intensification on the phytopathological aspects of apple orchards.

Chapter 2. General characteristics of the monitored pathogens - this chapter refers to the biology of pathogens and methods of phytosanitary control or with the help of genetic resistance of apple cultivars.

Personal contribution:

Chapter 3. The main objectives - presents the main objectives proposed within the scientific research

Chapter 4. Characteristics of the experimental environment - in this chapter the factors of the environment in which the experiments were carried out are described

Chapter 5. Study 1. Control of the fire blight caused by the bacterium *Erwinia amylovora* - this chapter presents how the experiments were conducted and the results obtained in the study of the epidemiology and control of the pathogenic bacterium *Erwinia amylovora*

Chapter 6. Study 2 - Control of apple scab caused by the fungus *Venturia inaequalis* - this chapter presents the way the experiments were carried out and the results obtained in the study of the epidemiology and control of the pathogenic fungus *Venturia inaequalis*

Chapter 7. The originality and innovative contributions of the thesis - the innovative ideas of the personal contribution in the case of the debated topic are presented.

3. THE PURPOSE AND THE OBJECTIVES OF RESEARCH

The main purpose of the thesis was to study the manifestation of the diseases caused by the pathogens *Erwinia amylovora* and *Venturia inaequalis* in the current climatic context of the Bistrita area and to optimize their control solutions.

In order to achieve the goal, the following main objectives were established:

1. The behavior of cultivars showing resistance or tolerance to the infection with the pathogens *Erwinia amylovora* and *Venturia inaequalis* compared to cultivars sensitive to them;

2. Identification of possible changes in the pathogenesis and ecology of the pathogens *Erwinia amylovora* and *Venturia inaequalis* in the local climate context;

3. Testing the effectiveness of certified phytosanitary products and in vivo treatment schemes, in order to establish treatment schemes adapted to the experimental area/experimental fields at S.C.DP. Bistriţa, Bistriţa-Năsăud county;

4. Elaboration of treatment recipes in order to obtain the best possible results in the control of the most common bacterial and fungal diseases of the apple

4. CHARACTERISTICS OF THE EXPERIMENTAL ENVIRONMENT

The experiments took place at the Bistriţa Fruit Research and Development Station (FRDS Bistriţa) which is located in the northern part of Romania on the hills of Bistriţa, having the coordinates 47°09'57"N 24°29'47"E and is 358 m above sea level.

The multiannual temperature average has risen in recent years in the Bistriţa area as well, in line with the global climate warming trends, and in the 2014-2022 period the annual average was over 10.45°C. The growing seasons in the experimental period generally included months warmer than the multiannual monthly averages, with only 16.(6) % of the months being thermally normal.

From a pluviometric point of view, the year 2019 stood out as dry with an amount of precipitation of 538.8 mm, while the years 2020-2022 were normal from this point of view. The distribution of precipitation in the summer months was variable from year to year, with May 2019 being the rainiest and June 2022 being the driest during the experimental period.

5. Study 1. Control of the fire blight caused by the bacterium *Erwinia amylovora*

The purpose of the study

The aim of the study is to identify the changes in the way the fire blight manifests itself under climate change conditions and to find ways to reduce the impact of this bacterial disease. To achieve this goal, we conducted three experiments that address different aspects related to the manifestation of *Erwinia amylovora* infections in apple orchards.

The first experience focuses on the epidemiology of fire blight, respectively on the identification of the new patterns in which the *Erwinia amylovora* epidemics fall and the critical moments when it is necessary to intervene with bactericidal treatments.

The second experiment aimed to evaluate the effectiveness of different products to control bacterial diseases.

The third experience aims to identify cultivars that show resistance or tolerance to natural infections with *Erwinia amylovora*, in the specific climatic conditions of the Bistriţa area.

Materials and methods used in Experiment Ea1 – Epidemiology of fire blight in the conditions of climate change in Bistrita

The epidemiological study was carried out on six apple cultivars: Auriu de Bistrita, Salva, Jonathan, Idared, Goldprim and Generos. Some of these cultivars are known to be susceptible to Erwinia amylovora infection, such as Idared or Auriu de Bistrita, while others behave better, such as the cultivar Golprim.

Visual observations were made weekly during the summer from 2019 to 2022. The meteorological data collected were used to calculate active temperature degrees and then summed to compare with the thresholds indicated in the MARYBLYTTM model (STEINER, 1990a, 1990b) for favorable conditions for *Erwinia amylovora* infections.

The difference between the frequency of symptoms observed on the six cultivars used in this experience, in each of the years and in the period 2019-2022 was statistically analyzed using the Duncan test.

Materials and methods used in Experiment Ea2 - Testing the effectiveness of some bactericides to control the pathogen *Erwinia amylovora*

The effectiveness of the tested products was done on the cultivar Jonathan, due to the known sensitivity of this cultivar.

As a biological bactericide, a product based on *Bacillus subtilis* strain QWT713 was tested, which is a bacterium with an antagonistic action against *Erwinia amylovora.*

The conventional bactericides tested were based on copper hydroxide, copper oxychloride, a combination of fluopyram with fosetyl aluminum and prohexadione-calcium.

The method of execution of the field experiments was the application of the treatments with the help of the atomizer and the recommendations of the producers

regarding the favorable moments of the application and the restrictions related to certain phenological phases were respected.

The observations carried out aimed at detecting the specific symptoms of the fire blight described in the specialized literature, the frequency of the damage was calculated and the differences between the variants were tested using the Duncan test.

Materials and methods used in Experiment Ea3 – The behavior of some apple cultivars against the attack of the bacterium *Erwinia amylovora*

Fifteen apple cultivars were studied, ten of them are of Romanian origin: Auriu de Bistriţa, Generos, Salva, Bistriţean, Starkprim, Jonaprim, Goldprim, Alex, Dany, Doina and five of them are well-known foreign cultivars: Jonathan, Florina, Golden Delicious, Idared and Starkrimson.

All specific symptoms known to occur on infected apple plants were evaluated: wilting of flowers, characteristic crutch-like wilting or necrosis of shoots, presence of bacterial exudate on organs, tissue staining, bark necrosis. The main symptom observed was wilting and necrosis of young shoots, and we used the frequency of this symptom to evaluate cultivar susceptibility. Differences between cultivars were subsequently tested using Duncan's test.

Results and discussions regarding the epidemiology of fire blight in the climatic conditions of Bistrita (Ea1)

The first experimental year, 2019, was characterized by the absence of fire blight symptoms on the trees in the experimental plot.

In all other years, the infection appeared only on the shoots, manifesting itself in the characteristic crutch shape. During the flowering period, as well as during the growth and ripening period of the fruits, no symptoms of fire blight were observed on the flowers and quite sporadically on the fruits. Infection that first appears on shoots is often found in areas with milder winters. Recent climate changes have led to milder winters and in the Bistrita area, the average monthly temperatures in the winter months are higher by up to 6.36°C (November 2019) compared to the monthly averages of the last century.

Auriu de Bistrita was affected in 2021 and 2022, Salva in 2020 and 2021 and Jonathan in 2020 and 2022, while the others showed symptoms only in 2022. In the three years in which fire blight manifested itself, there were significant differences between symptom frequencies on shoots. In the cultivars where it occurred, the frequency varied between 11.4% and 0.3%.

Goldprim and Generos, followed by Idared, were the least affected cultivars during the experimental period, by *Erwinia amylovora*, while Auriu de Bistrita recorded the highest frequencies.

Observing the average daily temperatures in the months of May and June and calculating the sum of the temperature degrees (∑GT) according to the MARYBLYT™ system, we found that in all four years, the thresholds for the initiation of infections, respectively for the appearance of symptoms in the orchard, were exceeded in the

month June. For this reason, the fire blight did not appear on the flowers but only on the shoots. This is the first epidemiological change to appear in the Bistrita area where previous sources mention the occurrence of infections on flowers.

In 2022, a new wave of infections appeared in the cultivars Auriu de Bistrita, Jonathan and Idared, in September with a lower frequency of symptoms than in June. This is the second change we report in the epidemiology of fire blight in the Bistrita area.

Results and discussions regarding the effectiveness of the tested bactericides for the control of the pathogen *Erwinia amylovora* (Ea2)

The calculation of the effectiveness of the bactericides was made based on the frequency of the fire blight symptoms appearing in the treated variants compared to the untreated control variant V6. Although there were symptoms in all variants in the treated ones, the frequency was significantly lower, a fact highlighted with the help of the limit difference test. In terms of effectiveness, the highest was recorded at V2 (Copper oxychloride 380g/l) and the lowest at V4 (Prohexadione calcium 100g/kg). Cultivars V1 and V2 treated with copper-based substances obtained the best control of fire blight with the least symptoms on shoots. The two copper-based products had similar efficiencies: 95.33% (V1 – Cu hydroxide) and 96.96% (V2 – Cu oxychloride), slightly higher in the case of copper oxychloride, between these two variants the differences were insignificant.

In the case of the combination of the substances fluopiram+fosetil aluminum (V3) the effectiveness was over 90%, significantly lower than in the case of cupric substances and significantly higher than the biological product (V4) respectively than prohexadione-calcium (V5). The tested biological product based on *Bacillus subtilis* race QST 713 (V5) achieved a satisfactory effectiveness of 74.59%, while Prohexadione-calcium, a compound with the role of stimulating plant defense mechanisms, recorded an effectiveness of 67, 71%, the difference between them not being statistically ensured.

Results and discussions regarding the behavior of the studied apple cultivarss against the attack of the bacterium *Erwinia amylovora* (Ea3)

The Ea3 experience in Study 1 took place in 2021-2022.

In 2021, fourteen of the cultivars were included in a single significance group when the data were statistically analyzed. In the field, this translated into the sporadic appearance or absence of the attack on the shoots of these cultivars, with the exception of the Auriu de Bistrita cultivar, the only one affected by fire blight in 2021, in the untreated lot where the observations were made. There were symptoms both on the cultivar Idared, a sensitive cultivar, and on the cultivar Florina, considered resistant, but the values were very low, the damage being sporadic. In the same situation we find the cultivars Salva and Bistriţean which also showed sporadic symptoms. In 2021, the damage was absent from the other ten cultivars studied.

In 2022, the cultivars can be grouped into five groups, depending on the frequency of fire blight symptoms. 'Auriu de Bistrita' suffered the most damage caused

by fire blight (10%), confirming the existing data in the specialized literature. Damage to the cultivar Florina in 2022 was much higher (5%) than in 2021 (0.03%).

The cultivars Dany and Doina were affected with a frequency of 3% and 2% respectively and formed distinct significance groups. The other eleven cultivars showed sporadic symptoms or were not affected at all (Jonaprim).

Most of the cultivars tested responded well to the natural infection with *Erwinia amylovora* in the specific conditions of 2022, which was very favorable to fire blight in both apple and quince species.

Conclusions and recommendations following Experiment Ea1

The obtained results justify us to recommend the inclusion of the periods from the beginning of the rapid growth of the shoots in June as well as the end of summer as critical moments for the warning of preventive treatments for the control of fire blight. The warning of a treatment in this last period is required when the cooling of the weather in the second half of August caused by the occurrence of frequent rains ensures optimal conditions of temperature and humidity for the appearance of new infections with *Erwinia amylovora*.

Conclusions and recommendations following Experiment Ea2

The copper-based products as well as the fosetyl aluminum + fluopyram product and *Bacillus subtilis* race QST 713 all achieved good control of fire blight in the experimental plot. Also, the product based on prohexadione-calcium demonstrated a good ability to reduce the risks of *Erwinia amylovora* infection.

Conclusions and recommendations following Experiment Ea3

The behavior of the Romanian cultivars: Jonaprim, Goldprim, Generos, Salva, Bistrițean, Starkprim and Alex, as well as the foreign cultivars Golden Delicious and Starkrimson, from the experience, recommend them for cultivation in areas with a temperate climate similar to those of Transylvania, as well as for breeding programs to improve apple resistance to *Erwinia amylovora*. When choosing the other cultivars for cultivation, it is recommended to pay more attention to preventive treatments against fire blight.

6. Study 2. Control of apple scab caused by the fungus *Venturia inaequalis*

Study 2 focusing on apple scab was inspired by the new challenge that has recently emerged in apple scab control, namely the overcoming of V*f* genetic resistance by *Venturia inaequalis* (Cooke) Winter.

The purpose of the study

The purpose of the study was to identify the most appropriate way to control *Venturia inaequalis* infections, both in terms of costs and environmental impact, in the current context. To this end, we pursued the achievement of the following objectives:

The first objective pursued consisted in verifying the practical effectiveness of the V*f*-type genetic resistance, based on the Rvi6 gene, against the population of *Venturia inaequalis* in the Bistrita area under field conditions with conventional treatments.

The second objective was to investigate the type of opportune antifungal treatment schemes in the case of sensitive and resistant cultivars respectively.

The two objectives were achieved within two experiences, Vi1 and Vi2, carried out in the experimental period 2019-2021 and 2021-2022 respectively.

Materials and methods used in Experiment Vi1 - The behavior of some apple cultivars against the attack of the fungus *Venturia inaequalis*

Seven apple cultivars were used in the Vi1 experience, five of which have genetic resistance to scab (V*f*) and two known to be very sensitive: Idared and Golden Delicious. The genotypes with the gene Rvi6 used were: Florina, Prima, Aura, Salva and Bistriţean.

The cultivars were grafted onto M9 rootstocks and planted at 3.8m/1m resulting in a density of 2631 trees/ha. The trees were treated every year, with conventional fungicides performing 10-12 treatments per year.

Apple scab sensitivity was observed for three consecutive years 2019, 2020, 2021. The degree of damage on leaves and the frequency of attack on fruits were evaluated, and the data were interpreted statistically.

Materials and methods used in Experiment Vi2 - Efficacy of treatment schemes tested for the control of apple rot caused by the fungus *Venturia inaequalis*

The biological material consisted of 21-year-old trees of the cultivars Golden Delicious, Starkrimson, Idared, Aura, Florina and Generos.

The testing was carried out in two plots in which an extended scheme of treatments (V1) and a reduced scheme (V2) with a minimum of fungicide treatments were applied. The untreated control variant (V3) was treated only with insecticides. The results obtained regarding the frequency of scab symptoms on leaves and the effectiveness of the treatment schemes were statistically analyzed.

Results and discussions regarding the behavior of some apple cultivars against the attack of the fungus *Venturia inaequalis* (Vi1)

Cultivars with type V*f* resistance showed their resistance in 2019, with four out of five having no symptoms on the leaves, except for the Bistriţean cultivar where a frequency of 36% was recorded. Throughout 2019, the frequency of leaf symptoms was high in the cultivars Idared and Golden Delicious, being above 80% in both sensitive cultivars.

In 2020, symptoms also appeared on the cultivar Aura V*f* (F=9%) and on the cultivar Bistriţean, the frequency was 14%. The sensitive cultivars were again the most affected with slightly lower values but still around 80%.

In 2021, scab symptoms appeared on all seven cultivars in the experimental plot, thus confirming the defeat of V*f*-type resistance in the Bistrita area. The frequency values revealed statistically guaranteed differences between cultivars, less so between

Florina and Salva, the least affected. The results of the least significant difference test applied to the average degree of damage from the period 2019-2021 compared to sensitive control cultivars (Golden Delicious, Idared) and resistant control (Florina, Prima), respectively, revealed the existence of very significant differences between sensitive and resistant cultivars in this period .

Infections on the fruit typically manifested as rough irregular gray-brown spots, even cracking the fruit. It is noted the low frequency with which the symptoms were manifested on the fruits of the cultivars: Salva, Aura, Bistrițean and Florina which were significantly less affected than the other cultivars according to the results of the Duncan test carried out for the annual data respectively for the average of the 3 years. The highest frequency was registered with the Idared cultivar in 2019.

In the first two of the Vi1 experience there was a statistically significant difference between the two susceptible cultivars as well as between them and all genetically resistant cultivars. In 2021, however, the differences between the resistant cultivars increased as follows: Aura and Bistrițean showed a good reaction to the fruit rot infection and the Salva cultivar was more affected, recording a frequency of 11%, on the opposite side, the fruits of the cultivar Florina were not infected with apple scab. The difference between the cultivar Prima (V*f*) and the cultivar Golden Delicious (susceptible) became statistically insignificant in the year 2021 unlike the same difference in previous years.

The phenotypic response in the field to infections with *Venturia inaequalis* of the cultivars Aura and Bistrițean, from Bistrita, refutes the data obtained by other authors and underlines the recent decrease in the effectiveness of the V*f*-type genetic resistance, based on the Rvi6.

Results and discussions regarding the effectiveness of the treatment schemes tested for the control of apple scab caused by the fungus *Venturia inaequalis* (Vi2)

The data obtained show that the application of a reduced treatment plan can be used for the cultivar Generos in which no symptoms of scab have appeared. In the case of the cultivars Florina and Aura, the values of the fully registered damage degree in the V2 variant were low in both years, while for the sensitive cultivars the damage degree values were high, 14.43% for Idared and 32.25% for Golden Delicious, in the year 2022.

Conclusions and recommendations of experiment Vi1

We found the defeat of V*f*-type resistance in the Bistrita area and therefore we recommend greater attention compared to previous years in preventing infections with *Venturia inaequalis*. In the case of cultivars possessing the V*f*-type resistance, we recommend the application of fungicides in the critical moments of scab infection and reducing the number of treatments only in periods with less favorable conditions for the development of the pathogen from July to August.

Conclusions and recommendations of the Vi2 experiment

During the Vi2 experience, we noted the superiority of the treatment variant with the extended treatment scheme for most of the cultivars in the experience. Applying a reduced treatment plan can still be used for cultivars Generos, Florina and Aura. In the case of sensitive cultivars, the effectiveness of the reduced scheme was oscillating and its application did not have satisfactory results, therefore we recommend the use of extended treatment schemes.

7. ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The presentation of the current knowledge about the two pathogens brings together the latest data on their biology and control and integrates them in an original way constituting a solid basis for the experiments that were carried out in the framework of the thesis.

In study 1, the element of originality consisted in the detailed approach to the epidemiology of fire blight in the Bistrita area in the context of climate change, thus identifying two new critical moments for the evolution of this disease. Consequently, we performed complementary efficacy experiments of some bactericidal substances and evaluated fifteen cultivars from the perspective of their behavior in natural infection with *Erwinia amylovora*. So that at the end of the experience we can recommend a unified approach to the problem of fire blight both from the point of view of the farmers in production and the breeders involved in research.

The novelty element of study 2 that targeted the apple scab was the finding of the defeat of the resistance to *Venturia inaequalis* V*f*-type in the Bistrita area and the identification of the most suitable phytosanitary measures in the new context created. The thesis can have an international character because the recommended measures can be applied in all areas with a climate similar to the Bistrita region.