CERCETĂRI PRIVIND AVERTIZAREA TIMPURIE ASUPRA RISCULUI INFECȚIILOR CU BACTERIA *ERWINIA AMYLOVORA* LA CÂTEVA SOIURI NOI DE MĂR

RESEARCHES ON THE EARLY WARNING OF INFECTIONS RISK WITH FIREBLIGHT *ERWINIA AMYLOVORA* AND BEHAVIOR OF SOME NEW APPLE CULTIVARS

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Abstract

The paper presents the results regarding the early warning of the infections risk and the behavior of some new released and introduced apple cultivars and their vulnerability on fire blight (*Erwinia amylovora*). 20 apple cultivars, grafted on 6 rootstocks were assessed about their vulnerability and susceptibility on the infection with *Erwinia amylovora* on shoots. During 2009-2011, using common and specific software it was noticed that daily mean temperatures in May ranged between 14.8-19.9 ºC, but in June and July oscillated between 19.2-19.5 and 20.9-21.5 ºC. Also, daily mean air relative humidity ranged between 70.4-75.8% in May, 69.9-75.7% in June and 69.6-77.1% in July. The conditions were favorable for the apple blooming and shoots development, but for the pathogen as well. Investigations on the infections risks using the plants cells electric conductivity revealed that apple leaves and green fruits could be tolerant to first infections with *Erwinia amylovora,* as long as their juice or sap electric conductivity range between 190-250 mV, and their pH oscillate between 2.5-4.5. Artificial infections with *Erwinia amylovora* on growing shoot, sampled from some new apple cultivar-rootstock combinations, evidenced that the shoots collected from the cultivars 'Initial /M9', 'Rustic /M9', 'Ariane /M9', 'Crimson Crisp /Pajam 1' and 'Golden Orange' were not damaged, the disease attack frequency and severity being null 0%. Opposite, the attack higher frequency F% was noticed on the artificially infected shoots sampled from the cultivars 'Dalinred/EMLA', 'Topaz /M9' and 'Topaz /T337', and was between 5.88-9.78%. Among the artificially infected shoots, the most severely damaged (s=100%) were the ones sampled from the cultivars 'Crimson Crisp /PI80', 'Dalinred /PI 80', 'Dalinred /T337', 'Dalinbel /EMLA', 'Goldrush /EMLA' and 'Topaz /M9'. All these aspects are very useful for schedule and precise execution of the preventive phytosanitary treatments against the fire blight, especially on the vulnerable cultivars.

Key words: apple cultivars, infection risk, artificial inoculation, shoots, fireblight
Cuvinte cheie: soiuri de măr, risc de infectie, inoculari artificiale, foc bacterian

1. Introduction

Fireblight is one of the oldest diseases of the *Rosaceous* species one of the most damaging for the apple culture allover the world. Under ideal conditions it can destroy an entire apple or pear orchard in a single growing season, and by consequence very devastating not only in the most favorable seasons but for the apple and pear growing industry and nurseries as well. (Babadoost M., 2006; Cazelles et Hasler T. O., 2004; Hartman J., Hershman D., 2002, Ritchie D.F., Sutton T.B., 2002, Steiner P.W., 1998, 2000; Steiner P.W., Van der Zwet T., Biggs A.R. 2000; Van der Zwet T., Keil L. H., 1979; Amzar V. and Braniste N., 2000, Amzar V. and Ivascu A., 2003; Severin et colab., 1985, 2007, Teodorescu G., 2000, Teodorescu G. et colab., 2004; Tomşa M., Tomşa Elena, 2003).

The causal agent (*Erwinia amylovora* Burr Winslow*, Bacteriophyta, Enterobacteriaceae*) was spotted for the first in England in the 18 Century spread allover the world, being noticed in Canada (1840), USA (1780-1888), New Zealand (1919), China (1926-1959), Mexico (1943), England (1957), Chile (1959), some ex-USSR countries (1960) Egypt (1962), Italy (1963), Nederland and Poland (1966), Guatemala (1967), Denmark (1968), Germany (1971), Belgium, France and Turkey (1972), Romania (1981), but nowadays is present in many apple and pear growing countries. This one can be found on 75 hosts plant species and genera, on many genera of fruit bearing and decorative species.

When cultivated on artificial growing media, the bacteria, a gram-negative one (0.9 -1.5 x 0.7-1.0µm), forms small, white, round-shape colonies, in (the non-virulent ones) or sunny-side up eggs like colonies (the virulent ones).

The pathogen over winters in diseased shoots and surrounding bark plagues (Biggs and Steiner, 2000), and, during the vegetation period the bacteria are spread on host plants by bees, insects, birds, winds, rains water and human interventions.

On the host plants the attack symptoms were observed on all aerial parts.

During the warm springs bacterial ooze drops appear first the on flowers stalks. The flowers which are hydrolyzed, became brown, are fading, and soon turn in black. The leaves are affected starting with the stalk and the mid vein, and then the infection spread between leaves and along the shoots which turns in brown on apples, or in black on pears. In the wet periods, on the diseased shoots of the sensible cultivars, bacterial ooze drops having white, brown-yellow, orange or even red colors suddenly appears.

On green fruits, the disease appears especially after hails and thunderstorms. The heavy diseased fruits became brown on apples and black on pears in no more than a couple of weeks.

During the vegetation period secondary infections occurs, the bacteria being spread by bees, insects, birds, winds, rains water and human interventions. The bacteria enter into the host using the gates like wounds, broken stalks or shoots tips, stomata, lenticels, etc., even the tolerant cultivars being menaced.

On the sensible varieties, the infection moves down through the phloem to the trees limbs and trunk producing plagues and exudates and within few years the death of the entire tree.

Under such conditions, many breeding programs were developed on in order to release resistant or tolerant cultivars and many researches are carried on to prevent and control the disease.

These work goals were to assess risks and early warning possibilities for the fire blight (*Erwinia amylovora*) infections, under our climatic conditions, using modern computer software and to assess the behavior of some new released and introduced apple cultivars and their susceptibility to artificial infections carried on active growing shoots.

2. Material and method

The researches were conducted during 2009-2011, at Research Institute for Fruit Growing Pitesti Romania [44.51.30 N, 24.52.00 E; 240 m altitude]. The weather data were collected using the WatchDog Spectrum Technologies Inc. automate weather station and were stored, processed and analyzed using the facilities of the MS Office Excel 97-2003 and with SpecWare 7.0 Professional software facilities.

Biological material was represented by 20 apple cultivars recently released or introduced in our country, grafted on six different low vigor vegetative rootstocks, grown in high density intensive orchard plots (over 3000 trees/ha, trained as slender spindle and supplied with water and nutrients by fertirrigation). The experimental devices, are located on a plane ground situated on the second terrace of the Argeş River, on a low to medium fertile illuvial-clay soil unit (over 30% clay; humus less than 3%; nitrogen index 0.33-1.43; PAL 1.3-2.5 mg/100g), but well supplied with potassium up to 40 mg /100g). Soil reaction is slightly acid (pH 5.8-6.8) up to very acid (pH 4.8-4.9) with the depth and therefore the soil need amendments. Orchard floor was covered with grass between the trees rows and cleared with herbicides on stripes of 1.0-1.2 m wide, along the trees rows.

From the 20 apple cultivars samples of at least 10 green fruits were collected for the assessed of the pH value and electric conductivity of the fruits juice and samples of at least 6 shoots of 20 cm long for artificial inoculations with *Erwinia amylovora* ooze droplets.

All the samples were collected using a disinfected pruning tool, packed and labeled.

The leaves surface pH and green fruits juice pH and electric conductivity were investigated using an ISFET portable pH-meter.

The inoculum source was the infected fruits sampled from the 'James Grieve' variety which was spotted in an abandoned apple orchard. The diseased fruits were incubated in humid chambers at 21 ºC.

Then *Erwinia amylovora* bacteria were inoculated first on sterile nutrient agar [NA] artificial media (agar-agar 23 g/L) and incubated 3 days at 25 ºC. In the next step, the bacteria colonies were suspended in physiological serum (NaCl conc. 0.85%) and passed on the NYDA nutritive media (yeast 5 g/L; dextrose 10 g/L agar-agar 23 g/L) and grown 5 days at 27 ºC, to obtain the bacteria colonization and enough inoculum quantity. The bacteria cultures were grown in a BMT Incucell Comfort incubator.

Shoots samples were disinfected with sodium hypochlorite conc. 0.5% for 10 minutes and then well rinsed with distilled water. Each sampled shoot was then double-infected with the inoculum in the stalk of the sixth leaf, counting from the shoot top, using a hypodermic needle (Crosse-Schaffer method, 1969).

The infected shoots were then put in the lab, in glass vials with water, at 21 º C and covered with humid cheese cloth, and incubated one week for pathogen damages assessment.

The data were collected, stored, processed and analyzed using the MS Office Excel 97-2003 facilities.

3. Results and discussions

From the climatic point of view, assessment of the figure 1 reveal that location belong to the second climatic area of Romania. In this warm and sub-humid area, the multi-annual climatic data (1969-2009) reveals annual rainfall plus of 109 mm, from October to February and annual rainfall deficits of 151 mm, from March to September.

Under these conditions the analyses of the figure 2 shows that in the last three years (2009-2011) daily average temperature in May ranged between 14.8-19.9 ºC. In June and July daily average temperatures oscillated between 19.2-19.5 and 20.9-21.5 ºC favorable for the apple blooming and shoots development and optimum for their contamination with bacteria.

Examination of the figure 3 reveal that in the last three years (2009-2011), the most rainy spring and half of summer was in 2010, when daily average rainfall was 4.2 mm in May and 5.2 mm in June.

Assessment of the figure 4 reveal that in the years of the study, average air relative humidity was ranging daily between 70.4-75.8% in May, 69.9-75.7% in June and 69.6-77.1% in July.

Till this point of the analysis the meteorological data assessment offer only monthly general trends for the infection risk with *Erwinia amylovora.* Far more accurate information were obtained with the SpecWare 7.0 Professional software facilities. Using this software the risk of the infections with *Erwinia amylovora* was estimated daily according the Cougar scale 0-4, where '0'=very low infection risk; 4=very high infection risk.

Assessment of the figure 5 shows that in 2009, the most critical period for possible infections with *Erwinia amylovora* was the moth of May and the first half of June.
In this period, the infection risk was high between 8-22, 27-29 May and first half of June and scored with the notes 2.5-3.0.

Analysis of the figure 6 evidenced that in 2010 the most critical period for the possible infections with fire blight *Erwinia amylovora* was also the moths of May and June.

In May, the infection risk was high between 6-7 May and 13-15 May (noted with 2.5-3.0), as well as in 26-29 May, when was scored with the notes 2.0-3.0. In June, the infection risk was high between 7-8, 10-13 and 15-23 June, as well as in 27-29 and 30 June, when it was scored with the notes 2.0-3.0.

Results on the infection risks assessment for the fire blight (*Erwinia amylovora*) infections using some organs pH and electric conductivity changes

Early, in 1968, Goodman indicated that on plants the infections with pathogens can modify the host cells wall permeability. Some other researchers (Fisher, 1959; Parker, 1961; Van der Zwet and Keil, 1979) explains the host cells wall permeability changes, by their insufficient provisions in some mineral elements, present into soil but partially immobilized on the soil colloids.

Sands and McIntyre in 1975, Van der Zwet and Keil in 1979, associated the cell pH lower than 4.0 with the leaves tolerance to the infections with fire blight (*Erwinia amylovora*).

By consequence, we expect from the biological material to reveal pretty similar levels of the pH and cell electric conductivity. The results with our material are presented in figures 8 and 11.

Examination of the figure 8 and 9 shows that on the studied material the green fruits juice electric conductivity was variable, depending on the genetic background of the cultivars, the cultivar/rootstock combination, physiological and nutritional status, organs water content, etc.

In the first set of measurements, this indicator was oscillating between 207.0mV (at 'Dalinbel /EMLA') and 240.0 mV (at 'Ariwa /T337'). The exception was 'Rustic /M9' with fruits juice electric conductivity of 189 mV. In the second set of measurements, this indicator was ranging between 127.7 mV (at 'Fuji Kiku Clone 8') and 248 mV (at 'Kalther Bohmer'). The exception was 'Red Jonaprince /M9' with fruits juice electric conductivity of 93,4 mV.

Assessment of the data presented in figure 10 reveal that on the studied cultivars the pH ranged between 5.2 and 5.9 on leaves and 2.8 and 4.0 on green fruits, the two indicators being medium correlated [R^2=0.2285; r=0.4780; n=77], and depending on the genetic background of the cultivars, the cultivar/rootstock combination, physiological and nutritional status, water content of the organs, etc.

Examination of the data presented in figure 11 evidenced strong correlation [R^2=0.676; r=0.8222; n=32] between the green fruits juice pH and its electric conductivity. It was observed that when the fruit juice pH is moving up from 2.5 to 5.5, its electric conductivity is dropping from 190-250 mV to 90-180 mV.Based on these data we estimated that both juice pH and its electric conductivity of the shoots might follow the same trends, and can be explained by starch and other organic matters accumulation during the assimilation process in spring and summer. After these measurements we estimate that lower levels of the electric conductivity registered in the juice of fruits sampled from 'Red Jonaprince/M9', 'Fuji Kiku Clone 8 /M9', 'Dalinbel /EMLA', 'Dalinred /T337', 'Dalinred /PI 80', 'Topaz /M9' and 'Topaz/ T337' (207-215 mV) or the ones registered in the juice of fruits sampled from 'Red Jonaprince/M9', 'Fuji Kiku Clone 8 /M9', 'Braeburn /M9', 'Idared /M9', 'Golden Delicious Clone B /M9', 'Jonathan/M106' 'Nured Jonathan /M106', (93.4-188 mV) offer additional information on the possible vulnerability on the fire blight attack on the shoots.In the light of Sands and McIntyre hypothesis, the apple leaves and immature fruits are tolerant to the first infections with *Erwinia amylovora* as long as their juice or sap electric conductivity ranges between 180-250 mV and the pH is oscillating between 2.5-4.5, for *Erwinia amylovora* the literature indicating an optimum pH domain between 6.0 and 7.2.

Results on the artificial infections with *Erwinia amylovora* on growing shoots to establish the susceptibility of some apple cultivar-rootstock combinations

Evaluation of the cultivars susceptibility on *Erwinia amylovora* using the artificial infections on active growing shoots is very important, and a reliable method as well.

To establish the susceptibility of the new apple cultivar-rootstock combinations, active growing shoots were infected in the lab using bacterial ooze. Then the attack frequency F [%] and the attack severity [% of the shoots length] were assessed.

The results of our experiments are presented in the figures 12 to 14.

Assessment of the figure 12 reveal that the attack frequency F% on the shoots artificially infected with fire blight *Erwinia amylovora* was very different among the cultivar-rootstock combinations. It can be seen that the shoots sampled from the cultivars 'Initial /M9', 'Rustic /M9', 'Ariane /M9', 'Crimson Crisp /Pajam 1' and 'Golden Orange' were not damaged, the attack frequency F% being null 0% and were judged as very tolerant. On the shoots sampled from the cultivars 'Rebra /M9', 'Goldrush /Pajam2', 'Goldrush /PI 80', 'Golden Lassa /T337', 'Dalinred /T337', 'Dalinred /PI 80', and 'Goldrush /EMLA', the attack frequency oscillated between 0.87% and 1.81%. The shoots sampled from the cultivars 'Ariwa/T337', 'Dalinbel /EMLA' and 'Goldrush /Pajam 1' were attacked with a frequency oscillating between 2.80% and 4.6%. However, the attack higher frequency was noticed on the shoots sampled from the cultivars 'Dalinred/EMLA', 'Topaz /M9' and 'Topaz /T337', and was ranging between 5.88-9.78%.

Examination of the damaged shoots indicate that the ones sampled from 'Golden Lassa /T337', 'Goldrush /Pajam1', 'Goldrush /Pajam2', 'Goldrush /Pajam1', were quite severely damaged, between 67-75% from the shoots total length (13.4-15.0 / 20.0 cm). Other shoots, sampled from 'Rebra /M9', Ariwa /T337 and Topaz /T337 were severely damaged, between 87.5-93.8% from the shoots total length (17.5-18.75 cm / 20.0 cm). The most severely damaged (100%) were the shoots sampled and infected belonging to the cultivars 'Crimson Crisp /PI80','Dalinred /PI 80', 'Dalinred /T337', 'Dalinbel /EMLA', 'Goldrush /EMLA' and 'Topaz /M9', and for these reasons, these cultivars might be judged as vulnerable on the pathogen attack, if all the condition for pathogenesis are met.
However, the analysis of the figure 14 indicate a weak correlation [R^2=0.0597; r=0.2443; n=102 between the attack frequency F% and the attack severity on artificially infected shoots.

The attack frequency offers information on the disease spread in the trees canopy, but the attack severity is linked to the pathogen virulence and the cultivar defense capacity against the pathogen invasion.

All these aspects are very useful for the new orchards management and for planning and precise execution of the preventive phytosanitary treatments against the fire blight, especially on the vulnerable cultivars.

4. Conclusions

Fire blight (*Erwinia amylovora*) is one of the oldest diseases of the *Rosaceous* species one of the most damaging for the apple culture allover the world, and nowadays modern apple orchards can not be extended in favorable areas, without knowledge on the disease occurrence risks and the new released or introduced cultivars reaction on the possible infections with the pathogen.

During 2009-2011, daily mean temperatures in May ranged between 14.8-19.9 ºC, but in June and July oscillated between 19.2-19.5 and 20.9-21.5 ºC; lso, daily mean air relative humidity ranged between 70.4-75.8% in May, 69.9-75.7% in June and 69.6-77.1% in July. The conditions were favorable for the apple blooming and shoots development, but for the pathogen as well.

Meteorological data analysis to forecast the risk of infections with *Erwinia amylovora,* offered only general trends when MS Office Excel 97-2003 facilities were used but with the SpecWare 7.0 Professional software facilities far more accurate information were obtained. During the study period the most critical periods for possible infections with *Erwinia amylovora* were the moths of May and June.
Infection risks assessment some organs pH and electric conductivity changes offered additional information on the possible vulnerability on the fire blight attack on the shoots.

The apple leaves and immature fruits are tolerant to the first infections with *Erwinia amylovora* as long as their juice or sap electric conductivity ranges between 180-250 mV and the pH is oscillating between 2.5-4.5.

Evaluation of the cultivars susceptibility on *Erwinia amylovora* using the artificial infections on active growing shoots is very important, and a reliable method as well.

The shoots sampled from the cultivars 'Initial /M9', 'Rustic /M9', 'Ariane /M9', 'Crimson Crisp /Pajam 1' and 'Golden Orange' were not damaged, the attack frequency F% being null, and were judged as very tolerant.

After the artificial infections, the attack higher frequency (5.88-9.78%) was noticed on the shoots sampled from the cultivars 'Dalinred', and 'Topaz', but, the maximum attack severity occurred on the shoots sampled from the cultivars, 'Dalinred', 'Dalinbel' and 'Topaz /M9'.

All these aspects are very useful for the new orchards management and for planning and precise execution of the preventive phytosanitary treatments against the fire blight, especially on these potential vulnerable cultivars.

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Tables and figures

1. Results on the risks assessment and early warning possibilities for the fire blight (*Erwinia amylovora*) infections using the meteo data

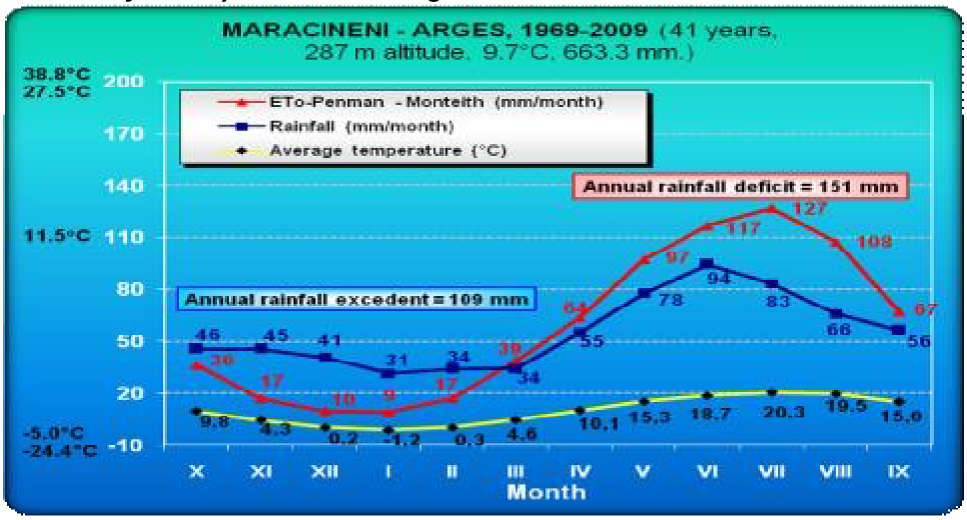
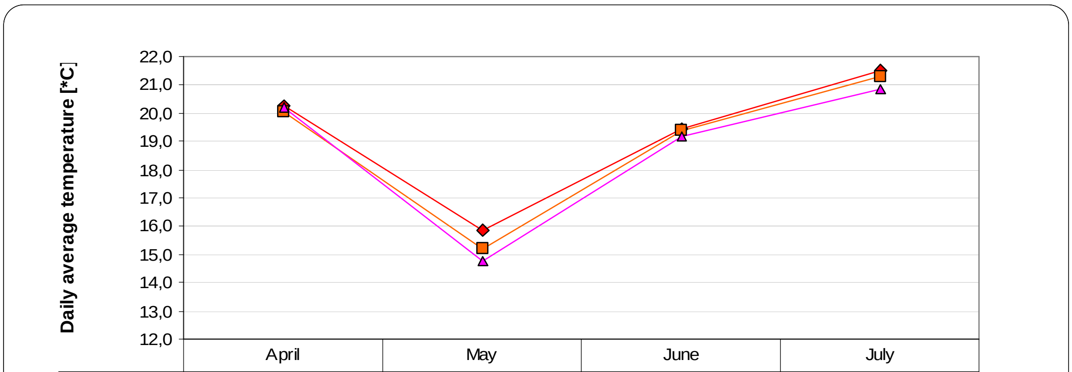


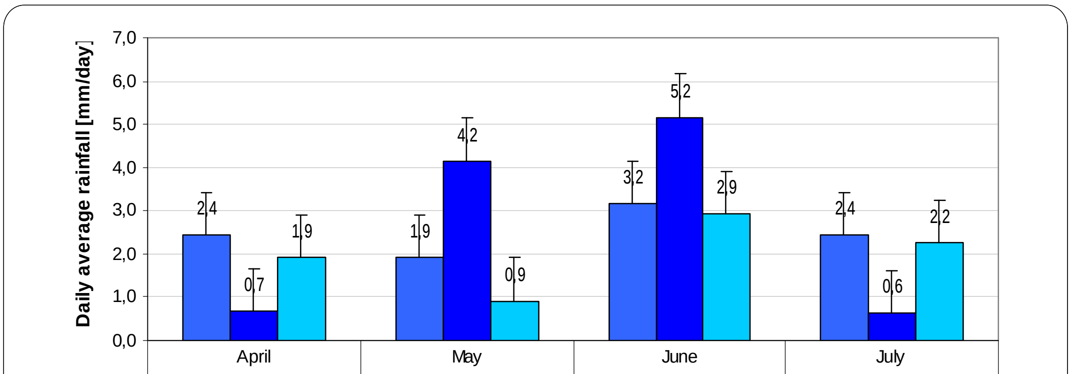
Fig. 1. The multi-annual clima-diagram RIFG Pitesti, Romania

The Eco-Physiology Lab, 1969-2009



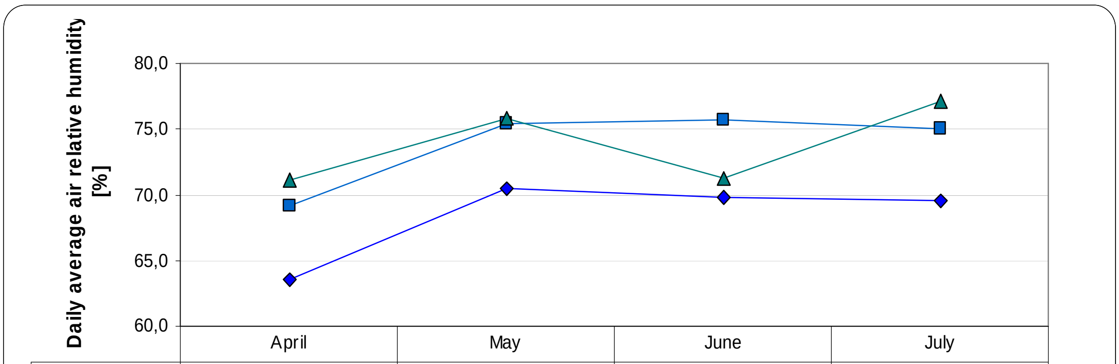
	April	May	June	July
MEDT-09	20,3	15,9	19,5	21,5
MEDT-10	20,1	15,2	19,4	21,3
MEDT-11	20,2	14,8	19,2	20,9

Fig. 2. Daily average temperatures dynamic in spring and first half of the summer RIFG Pitesti, 2009-2011



	April	May	June	July
RAIN-09	2,4	1,9	3,2	2,4
RAIN-10	0,7	4,2	5,2	0,6
RAIN-11	1,9	0,9	2,9	2,2

Fig. 3. Daily average rainfall dynamic in spring and first half of the summer RIFG Pitesti, 2009-2011



	April	May	June	July
AIRHUM-09	63,6	70,4	69,9	69,6
AIRHUM-10	69,2	75,4	75,7	75,1
AIRHUM-11	71,1	75,8	71,2	77,1

Fig. 4. Daily average air relative humidity dynamic in spring and first half of the summer RIFG Pitesti, 2009-2011

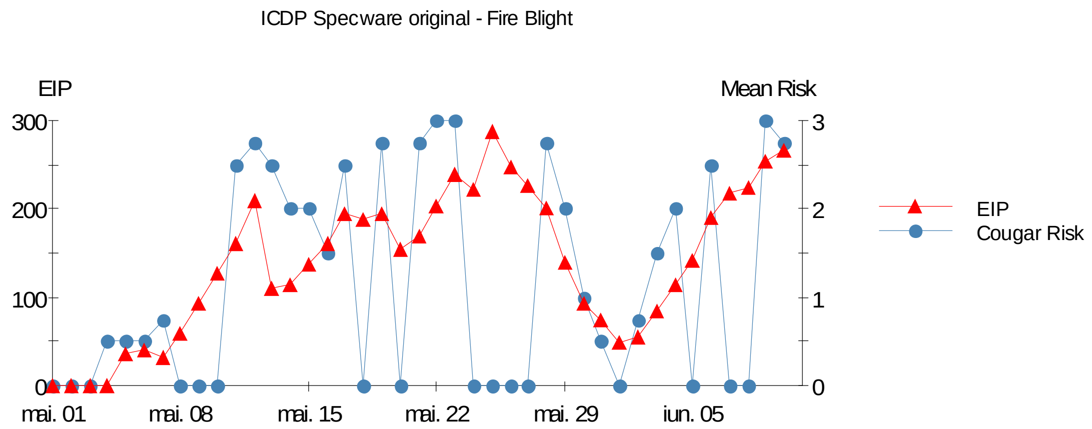


Fig. 5. The risk of the infections with *Erwinia amylovora* RIFG Pitesti, May 2009

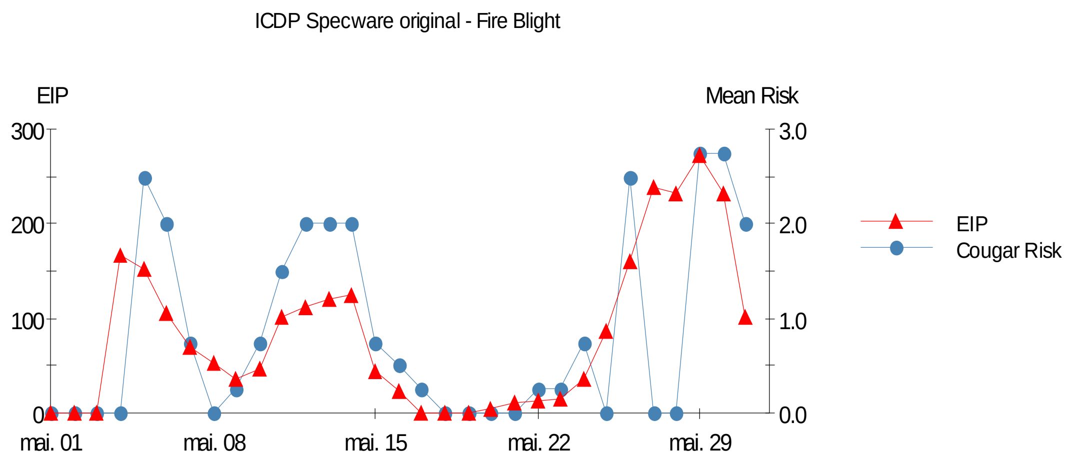


Fig. 6. The risk of the infections with *Erwinia amylovora* RIFG Pite□ti, May 2010

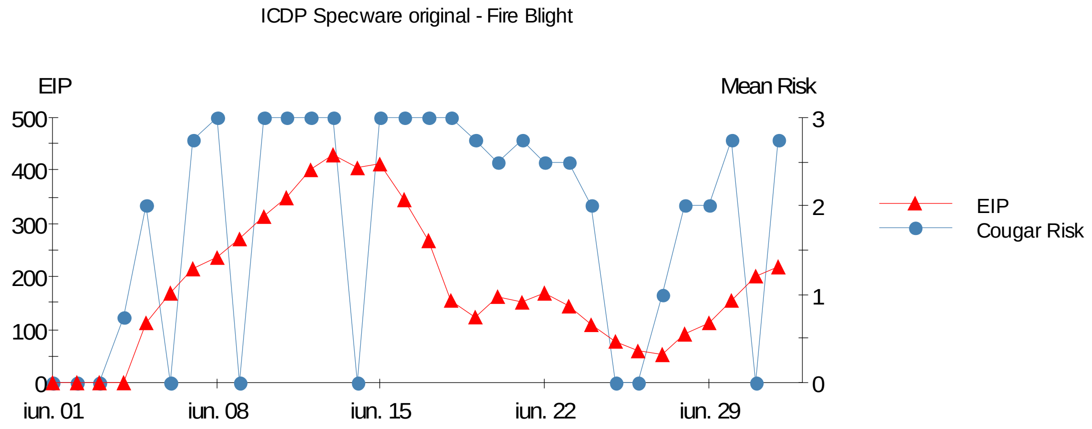


Fig. 7. The risk of the infections with *Erwinia amylovora* RIFG Pite□ti, June 2010

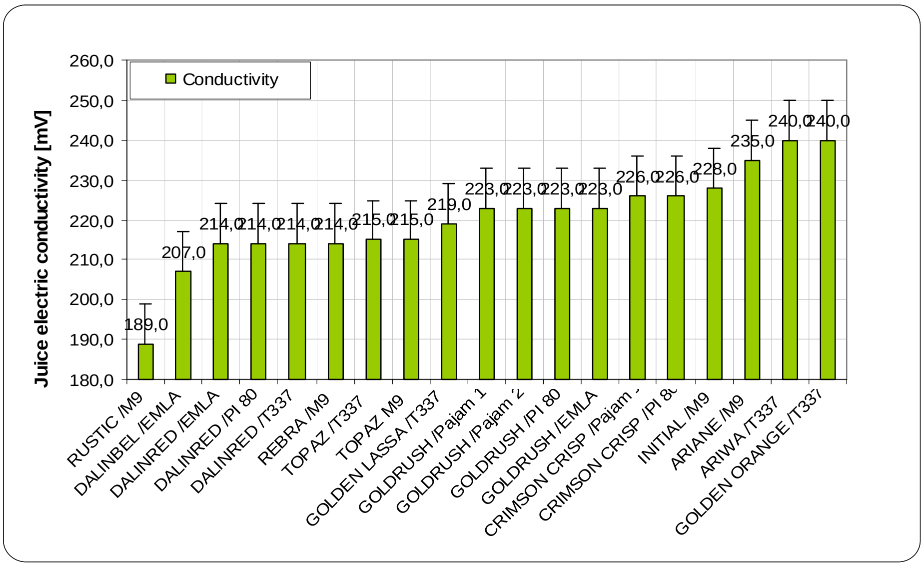


Fig. 8. Electric conductivity of green fruits juice on several apple cultivars (1) RIFG Pitesti-Romania, 2011

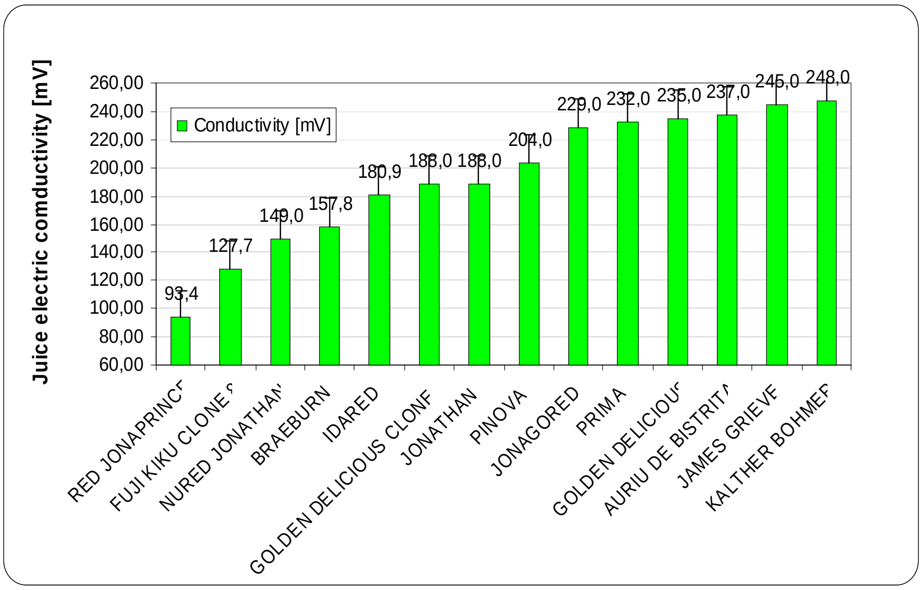


Fig. 9. Electric conductivity of green fruits juice on several apple cultivars (2) RIFG Pitesti-Romania, 2011

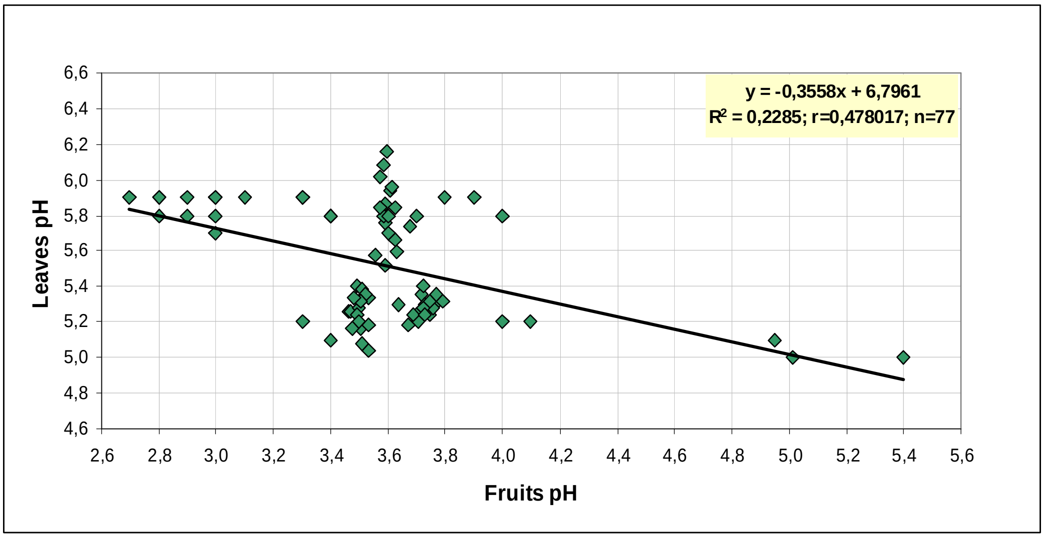


Fig 10. Correlation between fruits pH and leaves pH on some apple cultivars, RIFG Pitesti 2011

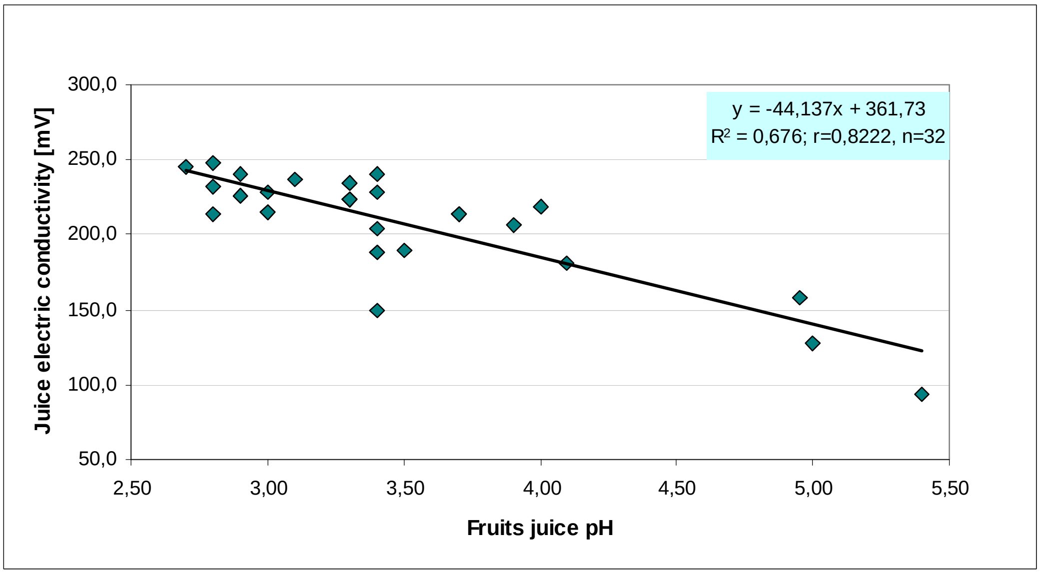


Fig 11. Correlation between fruits juice electric conductivity and some apple cultivars, RIFG Pitesti 2011

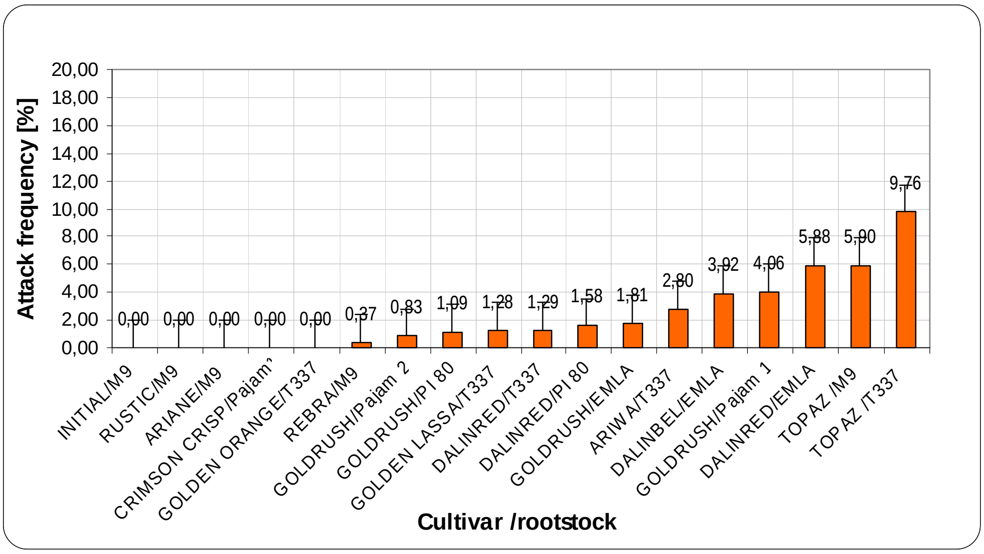


Fig. 12. Attack frequency F% on shoots artificially infected with fireblight *Erwinia amylovora* on some apple cultivar-rootstock combinations, RIFG Pitesti-ROMANIA, 2011 [average 4.43%; STDEV 7,9826]

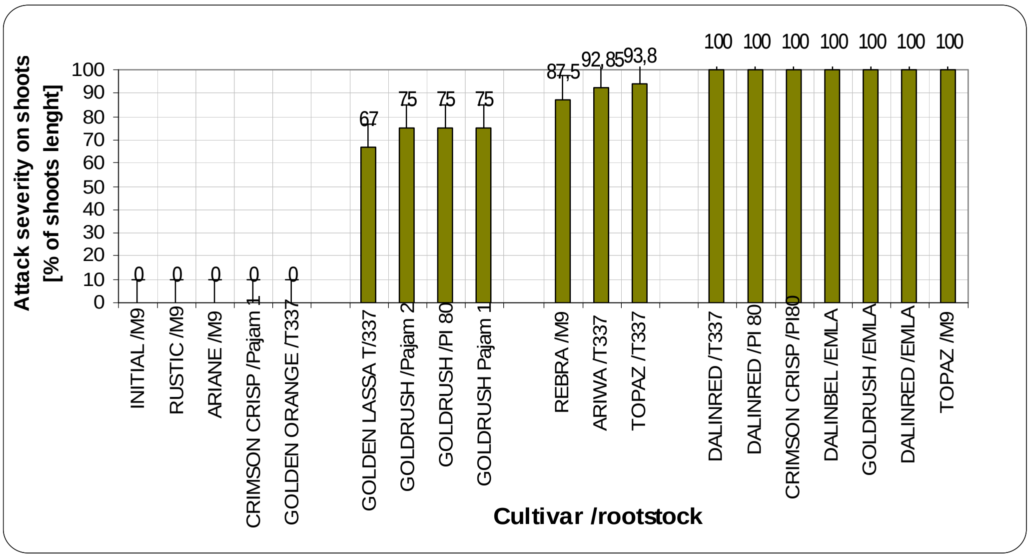


Fig. 13 Attack severity on shoots artificially infected with fireblight *Erwinia amylovora* on some apple cultivar-rootstock combinations, RIFG Pitesti-ROMANIA, 2011 [average 91.08; STDEV 11.9983]

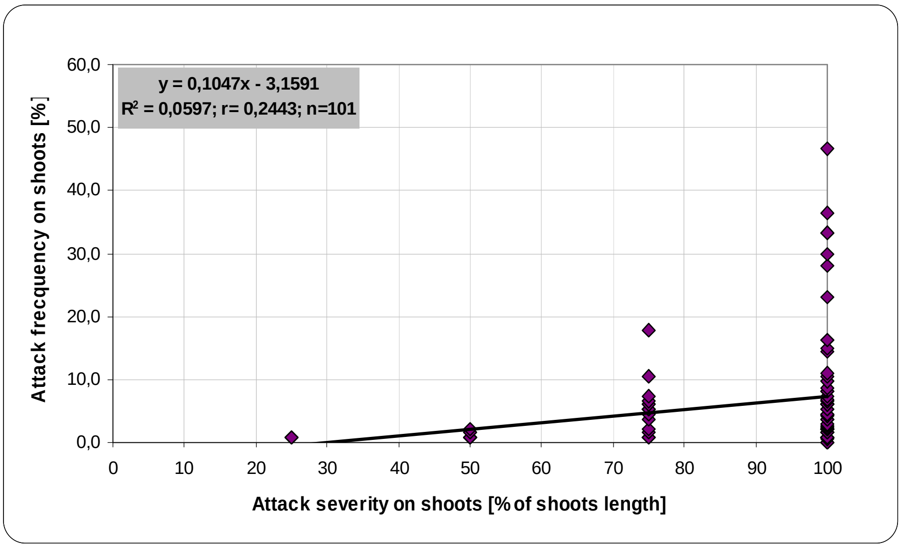


Fig. 14 Correlation between the attack severity and attack frequency on the shoots of some apple cultivar-rootstock combinations, artificially infected with fireblight *Erwinia amylovora*, RIFG Pitesti-ROMANIA, 2011