RJH Vol. V, 2024: 99-106
10.51258/RJH.2024.12

Effectiveness of active ingredients in controlling fireblight (*Erwinia amylovora)* in modern apple orchards

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ABSTRACT

This study investigated the effects of different registered active ingredients aiming to control fireblight *Erwinia amylovora* Burill Winslow, in modern apple bearing orchards. Five type active ingredients and combinations were assessed as regard their protective effect against fire blight on the apples. *Copper oxiclorure* provided an average efficacy E% 78.74-80.81 Better results were obtained with *copper hydroxide* E% 80.70-81.66. The mixture *copper oxiclorure+hydroxide* displayed a superior field efficacy ranging between 81.45 - 83.91. *Aluminum fosetyl,* applied during the flowering period and fruit set provided an average efficacy E% of 90.70 -81.26. The other mixture tested *fluopyram+aluminum fosetyl* offered an average efficacy E% in fireblight control, within 91.20 - 93.18 especially on growing shoots and fruits. However, in order to increase the bactericides efficacy in apple crop protection, it will be useful and opportune additional researches, on new active ingredients complementarity, water quality monitoring, spray equipment calibration, and improvement of applications quality.

Keywords: *apple, orchards, fireblight, chemical control*.

INTRODUCTION

Fireblight - *Erwinia amylovora* Burrill 1882 Winslow *et al.* 1920 (Phylum *Pseudomonadota*, Class *Gammaproteobacteria*, Order *Enterobacterales,* Family *Erwiniaceae)* is a contagious bacterial disease affecting More than 200 species from 40 genera, worldwide, in 46 countries and among these, 75 members of *Rosaceae* Family are susceptible, which is a major concern for the pome fruits producers. (Momol and Aldwincklke 2000; Babadoost, 2005; Beckerman, 2007; Sundin, 2014). This destructive crop bacterium initially originated in North America. Today, *E. amylovora* can currently be found in all the provinces of Canada, as well as in some parts of the USA. Nowadays it is believed that the pathogen was first introduced into Northern Europe in the 1950s through fruit containers, contaminated with bacterial ooze, imported from the USA. During the following decades, *E. amylovora* spread through much of Northern Europe. In Romania was reported first time in 1991 and since then spread constantly especially among sensitive and highly sensitive commercial varieties. (Amzăr and Ivașcu, 2003). *Bacteria overwinter in cankers. In the spring, bacteria multiply fast at the canker margins and in adjacent bark tissues, giving rise to inoculum (*Van der Zweet and Beer 1995*,* Hartman and Hershman, 2002). *Schroth, 2010;* Cooley *et al.,* 2015; Ivey & Ellis, 2016; Nischwitz and Hubbel, 2018; DuPont, 2023; Peter, 2023*).* The bacteria infect first the trees flowers travels more quickly in one- and two-year-old wood, and within 2-3 years especially the young trees are killed. Honeybees and other insects are attracted to the colonies ooze and can spread bacteria to susceptible tissue, such as flower stigmata. Birds, rain and wind can also transmit the bacterium to susceptible tissue, the colonization of

which will be heavily decided by temperature (21-28-31 ^{O}C is most favourable) and moisture either from rain or heavy dew. A few minutes of heavy hail can spread the disease throughout an entire orchard, killing the trees. (*Thomson, 1986;* Steiner *et al*., 2000; Babadoost, 2005; Teviotdale, 2011, *Malnoy et al., 2012; Cooley et al., 2015;* Johnson, 2000; Johnson, 2015; *Koski and Jacobi, 2015;* Smith, 2017; Nischwitz and Hubbel, 2018; DuPont, 2023; *Martinez, and Pearce, 2023).*
Climate changes, strong winds and drought periods, also can contribute to spread the disease in the apple orchards. (Roșu-Mareș *et al*., 2024).
Most commercially successful apple cultivars (12.5% autochthonous and 44.0% foreign ones) grown in recent years which includes: 'Elstar' 'Fuji', 'Gala', 'Braeburn', 'Golden Delicious', 'Red Delicious', 'Granny Smith', 'Idared', 'Jonathan', 'Jonagold', 'Mutsu', 'Rome Beauty', (Braniste and Andries, 1990; Janick, 1996; Beckerman, 2007; Sobiczewski, 2014; Elis, 2016; DuPont, 2023), 'Topaz', 'Auriu de Bistrita' (Jakab-llyefalvi and Platon, 2012; Militaru *et al.,* 2012) and other valuable varieties, are more vulnerable to fireblight than many other cultivars. Due to the damages produced by this terrible disease, for its containment researches are carried out in many research units and entities from major apple producing countries (USA, 14; Canada, 1; Australia, 1 New Zealand 1; UK, 2; Germany, 2; Italy, 6; France, 2; Switzerland, 2, Poland, 1; Belarus, 2; Romania, 7).
Therefore, the purpose of this work was to assess the effectiveness of some registered active ingredients in control of fireblight under the harsh conditions of the latest years.

MATERIALS AND METHODS

The researches were conducted between 2021 - 2024, at the Research Institute for Fruit Growing Pitesti Romania. The experimental device was located on a plain terrain placed on the second terrace of the Argeș River, on a low to medium fertile illuvial clay unit (more than 30% of clay; humus less than 1.7%; nitrogen index 0.33-1.43; PAL 1.3-2.5 mg/100g, but well supplied with potassium, up to 40 mg/100 g). Soil reaction is slightly acid (pH=5.8-6.8). The orchard floor was covered with grass between the tree rows and cleared with total herbicides on stripes of 1.0-1.2 m wide, along the tree rows. The fertilization was well balanced in nutrients by applying N200-P240-K280-Ca20 (1.0: 1.2: 1.4: 0.2 ratio) recipe and 4-5 foliar fertilizations with Haifa products. During the dry periods the experimental plots received 6-7 l water/ m2/day.
Biological material consisted in 'Jonathan' and 'Idared' varieties grafted on low vigour vegetative rootstocks grown under super-intensive system, with over 2222 trees /ha, trained as 'slender spindle' and supplied with water and nutrients by fertirrigation. Each trial variant included 4 replicates of 5 apple trees.
During the study period 5 type active ingredients and combinations were assessed as regard their protective effect against fire blight on the apples following EPPO and national and international guidelines. The variants experimented in detail are presented in Table 1.
The treatments were precise forecasted, based on the reserve of the pathogens which survived over winter, the apple trees phenology stages, and also related to the evolution of the climatic parameters, monitored and registered with automate iMetos weather station and were stored, processed and analyzed using its software Field Climate, with fireblight early warning module. Using this module, the risk of infections with fireblight was assessed using the Cougar scale (where '0'=no risk; '1'=low infection risk; '2'=medium infection risk and '3'=high infection risk). In the experimental plots the phytosanitary treatments with fungicides were applied with the atomizer STIHL, 400 series. The coverage quality was verified using Novartis sensitive paper and assessed using Snap Card mobile application, developed by the University of Western Australia and the Department

of Agriculture and Food. The rests of products, solution, and water from washing the spraying equipment were neutralized in installations type Phytobac, Heliosec or RemDry.
The damages caused in by fireblig t on apple shoots were estimated periodically, both as attack frequency/incidence **F%=[(n/N) x 100]**. The attack intensity/severity (I) was evaluated with notes upon a 0-4 scale damages degree was calculated using the formula **DD% = [(F% x I) x 100]**. The tested products biological main indicator, biological efficacy, the was calculated upon Abbott's formula, **E%=[(1-T/UT) x 100].**
The experimental data were stored, processed and analysed, using MS Office Excel, 2010, statistic and graphic facilities and verified once more with SPSS.14 statistic software.

RESULTS AND DISCUSSIONS

Romania holding the 8th place on regard the apple production (593.700 t /year) and planted surfaces (53.820 ha) (FAOSTAT, 2021).
The diversity of host tissues susceptible to infection, combined with the limited number of management tools available to control the disease, has made it difficult to stop or slow the progress of fire blight epidemics (Norelli et all, 2007; Fellows 2024).
a) Results regarding the orchard microclimate infections and infection risks.
Assessment of the Table 2 revealed that during the study period (2021-2023) the monthly average temperature was favorable for the infection's occurrence from 15.1-16.4 C at the beginning of May and 22.4-23.3 C in August. The average number of wet hours ranged between 110-255 h in June, during the active growth of the shoots and 62.3-189.8 h in September. Assessment of the figures 1-3 highlight the infection risks with the *Erwinia amylovora* registered with the aid of the early warning module of iMetos software.
In this sense, can be observed that in 2021, first light infections (100 DIV) occurred in April-May, with peaks (DIV 300-400) min 21 May, and second and third decade of June. Then during the entire vegetation period, the infection risk was maximum until 21 September (DIV 400). Another period of maximum infection risk (DIV 400) was in the mid of September and in 2021 October, a medium one (DIV 200). Last infections (DIV 100) were in mid of November. In 2022, first light infections (100 DIV) occurred in 22 April, the medium ones (DIV 200) in 19-20 May, then the infection risk was medium or higher (DIV 200-400), from the end of May till 22 September, maximum (DIV 400) at the end of September and debut of October. Since 22 of October till 22 November, the infection risk was medium (DIV 200). In 2023 first light infections (100 DIV) occurred in April-May, with a small peak in 23 May, and a medium one (DIV 200) in first decade of June. The infection risk was maximum (DIV 300-400) from the last decade of June till the end of September. Another maximum risk of infections was registered between 20-23 September, and a medium one (DIV 100-200) between 20-25 September and 20 November.
We can conclude, that according the early warning module of iMetos software, in our area, the risk of infections with fireblight, stretched from late April to late November.
Under the most favorable conditions coupled with varietal sensitivity and the lack of treatments, the fireblight infections progresses fast on every apple organs especially in the growing shoots (see Fig. 4 and Fig. 5) which lead to the necessity of preventive, quality treatments with various registered active ingredients, multisite or with a specific action chain.
b) Results with the active ingredients tested against fireblight.
Assessment of Fig. 6 reveal that among the studied and accepted active ingredients tested many of them were polyvalent, at reasonable rate of application controlling well and very well fireblight and more than two major pathogens of the apple crop, which explain their use in practice for a long period. Some active substances, copper compounds, aluminum fosetyl and the ones acting on phosphonates metabolism, seem to trigger the defense mechanisms of the plants. A particular attention must be allocated during the growing season to the use of the

copper hydroxide not to overpass the accepted rate (6.0 kg metallic copper/ha/season). To increase the active efficacy and avoid any mistakes, it can be displayed better at the beginning and the end of the vegetation period (Fig. 6). Examination of the figure 7 it can be seen that the number of applications per season ranged between 2 and 3 and the interval between treatments ranged according to the active ingredients tested between 10 and 14 days, with the exception of the mixture copper hydroxide + copper oxychloride, on which the interval between treatments was in average 7 days. Assessment of figure 8 reveal the variation of the field efficacy of the active ingredients tested, against fireblight attack on apple shoots. In this sense, copper oxychloride provided an average efficacy E% ranging between 78.74-80.81 (stdev=0.8703; var=1.0917). Better results were obtained with copper hydroxide which provided an average efficacy E% between 80.70-81.66 (stdev=0.5404; var=0.6669). The mixture hydroxide + copper oxychloride displayed a superior field efficacy ranging between 81.45 - 83.91 (stdev=1.3539; var=1.6440). All these coppers based active ingredients showed a good contact (multisite) effect against the infections with Erwinia amylovora. Aluminium fosetyl, applied during the flowering period and fruit set provided an average efficacy E% ranging between 90.70-81.26 (stdev=0.2802; var=0.3080). Belonging to the ethyl-phosphonates group of fungicides, acts by translaminar effect, against fireblight progression in young shoots and fruitlets. The mixture fluopyram + aluminum fosetyl offered an average efficacy E% in fireblight control, within 91.20-93.18 (stdev=0.9954; var=1.0791), especially on growing shoots and fruits. The mixture insured the fireblight control during the maximum risk infection periods and provided the simultaneously control of some others key pathogens of the apples such as, apple scab, powdery mildew and dry cancer due the fluopyram component which acts on inhibition of sterol biosynthesis DMI of mentioned pathogens.
Our researches results confirm the newest ones that active ingredients effectiveness are very strong influenced by the yearly orchard conditions and to many changes. (See Table 2).
However, to increase the bactericides efficacy in apple crop protection, it will be useful and opportune additional researches, on new active ingredients complementarity and products agreed in biological and integrated production systems, water quality monitoring, spray equipment calibration, and improvement of applications quality.
In USA, various copper liquid formulation Bacillus subtilis QST 713 strain (Blossom Protect), essential oils or peracetic acid-peroxide products (e.g. Thyme Guard, Cinnerate, Oxidate 5.0, Jet Ag) were applied in apple orchards in several trials at petal fall have also had control similar good effects in control fireblight (DuPont *et. al* 2023). In Germany, complexed sulfuric clays (Myco-Sin) showed good results to prevent infection after artificial hail simulation. (Bantleon, G. *et al.*, 2021).

Table 1. Work variants RIFG Pitesti Romania, Lat. N 44.513; Long. E 24.52; Alt 287m, 2021-2023.

Variant	Active ingredient	BBCH	Rate [kg/ha/appl.]	Applications [n]
V1	Untreated	-	-	-
V2	copper oxiclorure (50% metallic copper)	54;72; 75	2.0	3
V3	copper hydroxide (50% metallic copper)	54;72	3.0	2
V4	copper oxiclorure+hydroxide (280 g copper/kg)	56;72	2.0	2
V5	aluminum fosetyl (800 g/kg)	59;73	3.75	2
V6	fluopyram+aluminum fosetyl (50+666 g/kg)	72;75	3.0	2

Table 2. Orchard microclimate conditions during the study period
RIFG Pitesti Romania, Lat. N 44.513; Long. E 24.52; Alt 287m, 2021-2023

Month/ Year	Temperature									Degree Days			Chill Hours			Rain Fall [mm]			Wet Hours			Wet Days			Rainy Days		
	High			Low			Mean																				
	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023
January	13,3	17,8	20,3	-14,1	-12,0	-4,2	0,5	0,8	4,0	0,8	4,4	8,7	438,5	396,3	540,5	73,6	6,4	101,7	0,0	0,0	3,8	25,0	13,0	28,0	21,0	6,0	15,0
Feburuary	23,3	17,8	19,6	-10,3	-7,6	-11,4	3,0	3,1	2,6	13,9	7,8	10,7	365,3	385,5	348,0	12,4	11,0	7,9	2,0	0,3	1,3	18,0	12,0	13,0	7,0	9,0	7,0
March	18,8	22,1	21,7	-6,2	-9,3	-5,7	4,1	3,6	7,1	16,7	29,1	36,7	456,8	363,8	467,3	66,8	19,4	18,8	0,0	2,5	9,5	17,0	14,0	20,0	15,0	10,0	8,0
April	25,3	24,5	21,3	-3,3	-3,8	-1,0	8,6	10,1	9,7	47,4	75,2	52,8	432,3	333,5	384,5	38,4	88,0	80,4	8,3	38,5	45,5	22,0	20,0	28,0	12,0	13,0	20,0
May	28,4	31,5	27,1	2,5	2,9	3,9	15,6	16,4	15,1	184,0	211,3	165,6	102,3	117,0	107,3	65,4	72,6	77,4	132,0	96,5	150,3	25,0	23,0	28,0	16,0	13,0	16,0
June	34,0	36,8	32,6	7,0	11,0	9,7	19,3	21,1	19,4	278,2	329,3	281,8	24,8	0,0	2,3	104,0	25,6	78,7	225,0	154,3	154,5	29,0	25,0	26,0	18,0	100,0	18,0
July	36,8	38,3	35,2	12,5	8,7	8,9	23,5	22,8	23,2	408,6	383,7	402,8	0,0	3,3	2,5	33,5	25,3	77,7	66,0	92,8	110,5	18,0	17,0	27,0	9,0	12,0	12,0
August	36,4	35,2	36,0	9,0	12,9	9,8	22,4	22,6	23,3	372,4	384,3	398,6	2,8	0,0	0,5	74,0	142,1	17,0	82,3	128,3	63,8	16,0	25,0	17,0	9,0	15,0	7,0
September	30,4	29,4	32,1	2,7	2,3	8,2	15,6	15,6	19,4	178,4	184,5	280,8	129,0	111,8	15,5	14,3	49,6	26,1	62,3	189,8	111,3	27,0	29,0	18,0	6,0	11,0	9,0
October	22,7	27,1	29,5	-2,0	1,2	-0,8	8,6	12,0	13,5	27,5	104,5	140,5	360,0	304,5	240,0	36,1	4,3	4,9	29,5	46,0	55,0	23,0	30,0	26,0	9,0	3,0	6,0
November	26,3	26,7	20,3	-2,2	-3,7	-5,7	6,7	7,6	6,6	25,4	31,9	25,9	514,0	531,3	436,8	25,6	40,8	53,8	8,8	7,5	47,0	26,0	27,0	25,0	11,0	13,0	16,0
December	13,1	16,9	19,6	-11,6	-7,1	-4,8	1,6	2,8	3,1	0,6	5,8	10,2	500,3	500,0	487,0	91,9	40,4	18,4	0,5	8,8	0,0	25,0	28,0	26,0	16,0	16,0	10,0
AVG	***25,7***	***27,0***	***26,3***	***-1,3***	***-0,4***	***0,6***	***10,8***	***11,5***	***12,3***	***129,5***	***146,0***	***151,3***	***277,2***	***253,9***	***252,7***	***53,0***	***43,8***	***46,9***	***51,4***	***63,8***	***62,7***	***22,6***	***21,9***	***23,5***	***12,4***	***18,4***	***12,0***
STDEV	*8,04*	*7,50*	*6,50*	*8,40*	*8,22*	*7,29*	*8,19*	*8,12*	*7,77*	*151,24*	*149,20*	*152,88*	*206,97*	*196,97*	*215,45*	*30,30*	*40,23*	*34,74*	*69,01*	*66,84*	*56,69*	*4,34*	*6,52*	*5,13*	*4,72*	*25,95*	*4,82*
VAR	*31,25*	*27,77*	*24,76*	*-629,97*	*-2192,13*	*1268,37*	*75,86*	*70,31*	*63,41*	*116,79*	*102,20*	*101,07*	*74,67*	*77,57*	*85,26*	*57,17*	*91,87*	*74,08*	*134,28*	*104,81*	*90,40*	*19,20*	*29,73*	*21,81*	*38,00*	*140,92*	*40,20*

Figure 1. *Erwinia amylovora* attack risk
RIFG Pitesti Romania, Lat. N 44.513; Long. E 24.52; Alt 287m, 2021.

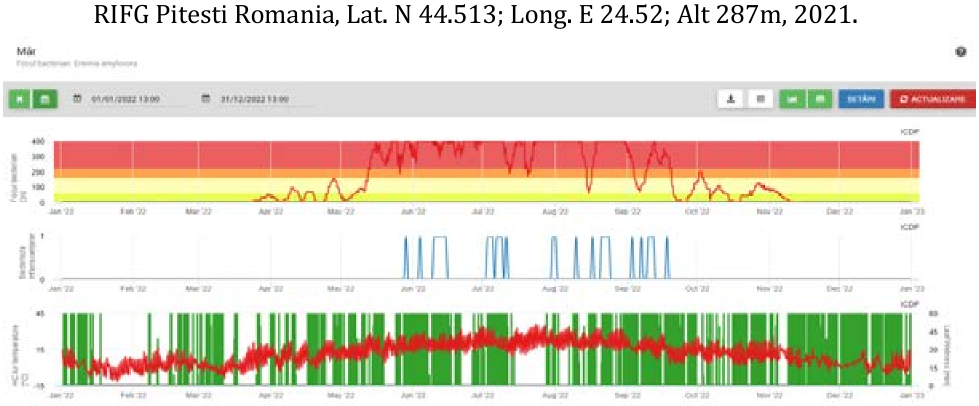

Figure 2. *Erwinia amylovora attack risk*

RIFG Pitesti Romania, Lat. N 44.513; Long. E 24.52; Alt 287m, 2022

Figure 3. *Erwinia amylovora* attack risk
RIFG Pitesti Romania Lat. N 44.513; Long. E 24.52; Alt 287m, 2023.

Figure 4. Severe infections with fireblight on growing shoots and fruitlets (untreated).

Figure 5. Severe infections with fireblight on growing shoots and fruits (untreated).

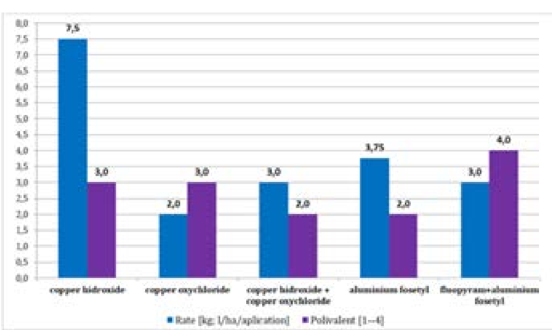

Figure 6. Active ingredients tested against fireblight type and the used rates limits.

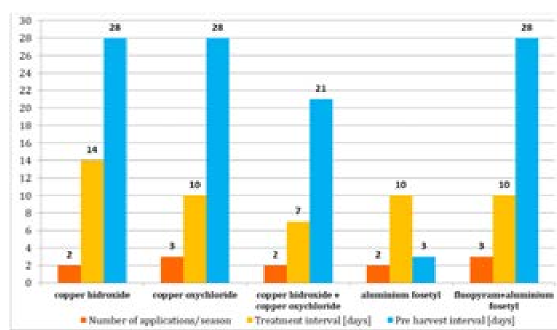

Figure 7. Active ingredients tested against multipurpose and some Good Agriculture Practice features.

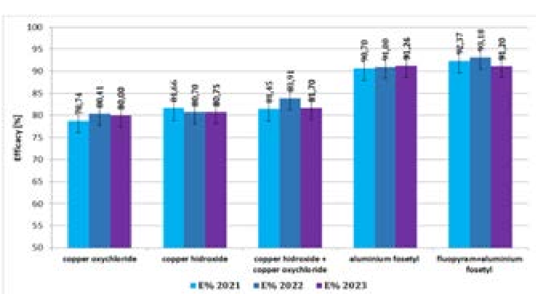

Figure 8. Efficacy of the tested active ingredients against fireblight growing shoots.

Figure 9. 'Idared' apples protected against fireblight attack.

Table 3. ANOVA analysis actives effectiveness correlated with the study years, ingredients dosage and number of their applications per season.

		Sum of Squares	df	Mean Square	F	Sig.
Year	Between Groups	,000	5	,000	,000	1,000
	Within Groups	12,000	12	1,000		
	Total	12,000	17			
Rate	Between Groups	28,000	5	5,600	.	.
	Within Groups	,000	12	,000		
	Total	28,000	17			
Applications	Between Groups	14,500	5	2,900	.	.
	Within Groups	,000	12	,000		
	Total	14,500	17			
Efficacy	Between Groups	18722,000	5	3744,400	5616,600	,000
	Within Groups	8,000	12	,667		
	Total	18730,000	17			

CONCLUSIONS

During 2021-2023, the meteorological conditions were very favorable to infections produced by *Erwinia amylovora* on apples.

The apple varieties behave different on the on the fireblight infections according their genitors, the affected organ (flowers, bark, shoots, fruits) or the entire tree/rootstock combination, meteorological conditions and local microclimate, the growing system and applied technology, production level, etc.

To increase the bactericides efficacy in apple crop protection, it will be useful and opportune additional researches, on new active ingredients complementarity, water quality monitoring, spray equipment calibration, and improvement of applications quality.

Technological interventions as fertigation and sanitation pruning and growing resistant or tolerant varieties, should be used as parts of an integrated fire blight management program, which includes using a risk assessment model, as well as cultural control when necessary.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support received from the Romanian Ministry of Agriculture and Rural Development for the Project ADER 25.2.1, within the framework of which this research was conducted.

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