# Results of using bactericides in the control of phytoplasmosis Bois Noir (pathogen *Candidatus* Ph. solani) to preserve grape yield

Ya.E. Radionovskaya<sup>1,\*</sup>, N.V. Aleinikova<sup>1</sup>, E.S. Galkina<sup>1</sup>, O.I. Tikhomirova<sup>2</sup>, and I.P. Borisova<sup>2</sup>

<sup>1</sup>All-Russian National Research Institute of Viticulture and Winemaking Magarach of the RAS, 31 Kirova str., 298600, Yalta, Republic of Crimea, Russia

<sup>2</sup>SEO LLC Scientific and Biological Center Pharmbiomed, 14 Solntsevsky Prospect, 119620, Moscow, Russia

**Abstract.** For finding effective means of controlling phytoplasmosis Bois Noir (BN) in 2020-2022, the effect of bactericides on the intensity of *Candidatus* Ph. Solani infection and quantitative yield indicators were studied in the vineyards of 2 soil-climatic Crimean regions. Behind the moderate and epiphytotic phytoplasmosis development (15-31% and 56-76% symptomatic plants, respectively), when using Pharmayod, GS (0.06-0.1%), Phytolavin, SC (0.2%), Phytoplasmin, SC (0.2-0.3%) preparations, a tendency to a decrease in the intensity of symptoms to 24% and 39% in the 2nd and 3d research years was established, followed by a decrease in the number of plants, completely affected by BN, by 5.3-11 times. A steady tendency to an increase in the "yield per bush" indicator was shown: on symptomatic plants within 11-39%, on asymptomatic - within 12-25%; and to a larger bunch weight: within 12-28% for plants with BN symptoms. The obtained results indicate good prospects for using the studied bactericides.

# **1** Introduction

In the vineyards of the main winemaking regions of the world, in conditions of wide spreading and significant harmfulness of phytoplasmosis Bois Noir grapevine trunk disease (BN) associated with the bacterium *Candidatus* Phytoplasma solani from the Stolbur group, subgroup 16SrXII-A, the problem of controlling this disease remains relevant [1, 2]. The difficulties in developing effective strategies for BN prevention and treatment are due to its complex ecology, based on the large genetic diversity and adaptability of the pathogen [3-5], a wide list of infection vectors, host plants, etc. [6, 7]. BN management strategies adopted to date consist in the use of phytoplasma–free material for grape propagation, various agrotechnical techniques in vineyards (pruning of varying intensity, in extreme cases, destruction of affected plants), vector control directly by insecticidal treatments, as well as herbicidal treatments to destroy wild insect host plants and phytoplasmic infection reserve plants [2, 8-11].

<sup>\*</sup> Corresponding author: vovkayalta@mail.ru

<sup>©</sup> The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

These areas continue to develop. For example, protocols for the improvement of grape plants based on tissue culture in combination with heat treatment and subsequent micropropagation of healthy plants are being developed [12]; good results have been obtained for plant restoration and yield increase using such agronomic techniques as grafting materials from restored vines onto symptomatic plants, processing BN-affected vineyards with resistance inducers [1, 13] and biostimulants [14]. In addition, promising areas are the use of grape varieties that are poorly susceptible to BN, the selection of new grape varieties by horizontal gene transfer, the use of non-pathogenic endophytic bacteria of grapes, the use of entomopathogenic fungi and nematodes as biological protection agents by vectors, etc. [2, 15].

The purpose of this research was to study the effect of grape protection system based on the use of preparations with bactericidal properties Pharmayod, GS, Phytolavin, SC, and Phytoplasmin, SC on the intensity of damage to grape plants with BN and quantitative yield indicators in two soil-climatic regions of Crimea in the search for effective means of controlling phytoplasmosis.

# 2 Materials and Methods

The research was carried out in 2020-2021 on the territory of Piedmont (Kirovsky district) and Mountain-Valley-Coastal (Alushta city district) soil and climatic regions of Crimea, in the plots of 'Chardonnay' grape variety as mostly affected by phytoplasmosis. In previous years, the intensity of visual signs of plant damage with BN in these vineyards ranged from weak to epiphytotic levels; the presence of *Candidatus* Ph. solani infection was confirmed by molecular genetic methods in laboratory conditions. Experimental plot in the Piedmont area (PA): the year of planting is 2009, the planting scheme is 3x3x0.3 m, bush training is a one-armed cordon on the middle trunk, the rootstock is 'Berlandieri x Riparia Kober 5BB', the supplier country of seedlings is Serbia; without irrigation. Experimental plot in Mountain-Valley-Coastal area (MVCA): year of planting – 2016, planting scheme – 3x1.25 m; training – AZOS-1, rootstock – 'Berlandieri x Riparia Kober 5BB', seedling supplier country – Serbia; without irrigation.

In the experimental protection systems, three preparations were used: SEO LLC Pharmbiomed (city of Moscow, Russia): 1) Pharmayod, GS (iodine, 100 g/l) – a preparation with high antimicrobial activity against various fungi, viruses, and bacteria; 2) Phytolavin, SC (BA-120,000 EA/ml, 32 g/l) – a systemic biopesticide of immunizing action, the active ingredient is phytobacteriomycin – combination of substances with antibacterial action produced by strain 696 *Streptomyces griseus*; 3) Phytoplasmin, SC (macrolide tylosin complex, 200 g/l, the producer *Streptomyces fradiae*), a broad-spectrum systemic biopesticide to protect plants from bacteriosis and phytoplasmosis, with a pronounced effect against stolbur.

Annually, grape protection system included 1-4 sprayings with Pharmayod, GS at a rate of 0.06% (from May to August) for vegetating plants, and 0.1% (during leaf fall, November) for washing vines (Table 1). The preparation Phytoplasmin, SC was used 2-4 times at a rate of 0.2% in the first half of the growing season, and 0.25-0.3% mainly in the second half of the growing season, during periods of grape growing and ripening. The frequency of treatments with Phytolavin, SC (0.2%) was 1-3 times during the growing season. In the vineyards under study, experimental preparations were used in tank mixtures with other pesticides on the plots of 2 hectares using tractor spraying of plants at a working fluid consumption rate of 250-800\%.

Variant, preparation	Concentrati	r lienological stage of grape development according	
	on of	to the BBCH scale	
	working		at the moment of treatment
	solution, %	No.	Title
I Control (without the use of bactericides), 2020-2022			
II Experiment (application of bactericides) 2020			
1. Pharmayod, GS	0.06	55	"Inflorescences swelling"
2. Phytoplasmin, SC	0.2	61	"Beginning of flowering"
3. Pharmayod, GS	0.06	69	"End of flowering"
4. Phytoplasmin, SC	0.3	75	"Berries pea-sized"
5. Phytolavin, SC	0.2	77	"Berries beginning to touch"
6. Pharmayod, GS	0.06	81	"Beginning of berry ripening"
7. Pharmayod, GS	0.1	97	"End of leaf fall"
II Experiment (application of bactericides) 2021			
1. Pharmayod, GS	0.06	11; 13	"1st leaf unfolded"; "3d leaves unfolded"
2. Phytolavin, SC	0.2	55	"Inflorescences swelling"
3. Phytoplasmin, SC	0.2	61	"Beginning of flowering"
4. Phytolavin, SC	0.2	69	"End of flowering"
5. Phytoplasmin, SC	0.3	77	"Berries beginning to touch"
6. Phytoplasmin, SC	0.2	79	"Majority of berries touching"
7. Pharmayod, GS	0.06	81	"Beginning of berry ripening"
8. Pharmayod, GS	0.1	97	"End of leaf fall"
II Experiment (application of bactericides) 2022			
1. Phytolavin, SC	0.2	55	"Inflorescences swelling"
2. Phytoplasmin, SC	0.2	61	"Beginning of flowering"
3. Phytolavin, SC	0.2	69	"End of flowering"
4. Phytoplasmin, SC	0.2	77	"Berries beginning to touch"
5. Phytoplasmin, SC	0.3	79	"Majority of berries touching"
6. Phytolavin, SC	0.2	81	"Beginning of berry ripening"
7. Phytoplasmin, SC	0.25	85	"Softening of berries"
8. Pharmayod, GS	0.1	97	"End of leaf fall"

 Table 1. The scheme of three years of experiment on the study of effectiveness of bactericides of SEO LLC Pharmbiomed in the control of BN in the vineyards of Crimea

Discuster in the set of succession in the second in the

Source: compiled by the authors.

The level of BN development in the experimental plots was assessed by the indicator of the number of symptomatic plants, expressed as a percentage of the total number of bushes examined. The records were carried out three times during the growing season, the last one - during harvesting. The degree of BN damage to grape plants was determined according to the developed scale: weak – 1-2 shoots per bush with symptoms of BN; medium – 3-5 shoots per bush with symptoms of BN, strong – more than 5 shoots per bush with symptoms of BN. The level (intensity) of phytoplasmosis development was characterized using the author's gradation: weak – up to 10 % of plants with signs of BN to a weak degree or up to 5 % of plants with signs of BN to varying degrees; epiphytotic – more than 40 % of plants with signs of BN to varying degrees.

Grape harvest in the conditions of MVCA was carried out on 24.08.2020, 08.09.2021, and 7.09.2022; in the conditions of PA - 03.09.2020, 07.09.2021, and 8.09.2022. The

3

quantitative indicators of the harvest (the number of bunches per bush, the average bunch weight, the harvest per bush) were evaluated separately on asymptomatic and symptomatic grape bushes in the experimental variants. The experimental data obtained were processed using generally accepted statistical methods using the Excel spreadsheet data analysis package.

# 3 Results

#### 3.1 The effect of the use of bactericides on the level of BN development

During the research period, epiphytotic development of BN was observed in the conditions of 2021 and 2022: the number of symptomatic grape plants in the experiment and control reached 56-76 %, whereas in 2020 the values of this indicator varied within a moderate level -15-17 % in PA, and 30-31 % in MVCA areas of Crimea.

Since the second year of research, there was a decrease in the number of symptomatic plants on plots treated with bactericides by 7-14 % (PA) and 24-39 % (MVCA), relative to the control plots (Fig. 1).



**Fig. 1.** The extension of BN in the conditions of three-year use of bactericides: A – PA, 'Chardonnay' vineyard; B - MVCA, 'Chardonnay' vineyard. *Source:* compiled by the authors.

The level of BN damage to grape plants in the first year of observation was characterized by a weak degree (experiment, control in MVCA and experiment in PA) and an average degree (control in PA). In the next two years, in the conditions of MVCA, the average degree of phytoplasmosis damage was recorded on the experimental and control variants: within 3-5 shoots per bush with symptoms of BN; in the vineyards of PA, a strong degree of damage was noted in the experiment (2021) and control (2021-2022). In general, over three years of research, the decrease in the intensity of damage to BN grape bushes against the background of bactericide treatments did not exceed 1-2 shoots/bush and amounted to 13-33 % relative to the control. In addition, a decrease of 5.3-11 times in the number of plants completely infected by BN was recorded.

# 3.2 The effect of the use of bactericides on the quantitative indicators of the harvest

In the experimental variant of PA vineyard, in case of symptomatic plants, all three years at the time of harvest, a significantly higher (by 24-37 %) number of bunches was registered compared to the control variant, whereas in the asymptomatic plants of experimental variant, a significantly higher (31 %) number of bunches per bush was recorded only in the third year of observations (Fig. 2).



Fig. 2. The effect of experimental bactericides on the indicator of the number of bunches in the conditions of PA: A – grape plants without symptoms of BN; B – grape plants with symptoms of BN. *Source:* compiled by the authors.

Accordingly, in the conditions of PA, against the background of the use of the studied system, a significantly higher yield per bush was recorded: from asymptomatic plants – by 12 % only in the third year of the experiment; from symptomatic plants – all three years by 27-39 % (Fig. 3), with an increase in the average bunch weight by 12 % in 2020.



Fig. 3. The effect of experimental bactericides on the yield per bush in the following conditions: A - grape plants without symptoms of BN; B - grape plants with symptoms of BN. *Source:* compiled by the authors.

In the conditions of another soil and climatic region (MVCA), the number of bunches per grape plant of the experimental variant was significantly higher than in the control variant for all three years: on asymptomatic plants - by 19-28 % (Fig. 4, A), on symptomatic plants - by 8-28 % (Fig. 4, B).





Fig. 4. The effect of experimental bactericides on the indicator of the number of bunches in the conditions of MVCA: A – grape plants without symptoms of BN; B – grape plants with symptoms of BN. *Source:* compiled by the authors.

Against the background of the use of experimental system in the MVCA vineyard, a significantly higher yield per bush was also recorded: by 19 % and 25 % from asymptomatic plants, respectively, in the second and third years of bactericide use (Fig. 5, A); by 11-33 % from symptomatic plants all three years (Fig. 5, B) with an increase in the average bunch weight - 24-28 %.



Fig. 5. The effect of experimental bactericides on the yield per bush in the conditions of MVCA: A – grape plants without symptoms of BN; B – grape plants with symptoms of BN. *Source:* compiled by the authors.

## 4 Conclusion

In the vineyards of 'Chardonnay' wine grape variety, as the most sensitive to phytoplasmosis BN, in the conditions of two soil and climatic regions of Crimea, during 2020-2022, the possibility of controlling this disease using grape protection system based on the use of three bactericides by SEO LLC Pharmbiomed was studied.

Against the background of moderate and epiphytotic levels of phytoplasmosis (15-31 % and 56-76 % of symptomatic plants, respectively), there was a tendency to decrease the disease intensity with repeated use of bactericides Pharmayod, GS (0.06-0.1 %), Phytolavin, SC (0.2 %), and Phytoplasmin, SC (0.2-0.3 %): the maximum decrease in the number of symptomatic plants reached 24 % and 39 % in the MVCA vineyards in the second and third years of research; at the time of harvest, the decrease in the intensity of bush infection varied between 13-33 % with a decrease of 5.3-11 times in the number of plants completely affected by BN. In general, a higher effect on reducing plant infestation was observed in the conditions of MVCA in the vineyard of the 2016 planting year, relative to the vineyard in the PA of the 2009 planting year, which suggests an impact on the effectiveness of BN control, including soil and climatic conditions of the growing area, the intensity of pathogen damage and the age of grape plants.

The results of three-year observations demonstrate a steady increase in the "harvest per bush" indicator, to a greater extent on plants with symptoms of BN disease (within 11-39 %), than on asymptomatic plants (within 12-25 %), due to the positive effect of the used bactericides on preservation of grapes (at the level of 8-37 % for symptomatic plants and 19-37 % for asymptomatic plants), as well as for the formation of a larger bunch weight for plants with the symptoms of phytoplasmosis (within 12-28 %). The results obtained indicate the expediency of conducting further research to study a wider range of issues of the use of bactericides in the vineyards in order to search for effective means of reducing negative impact of phytoplasmosis on the productivity and service life of vineyards.

### References

- 1. A. Bertaccini, Antibiotics 10, 1398 (2021)
- 2. CABI International, Candidatus Phytoplasma solani (Stolbur phytoplasma), (2022)
- 3. S. Murolo, M. Garbarino, V. Mancini, G. Romanazzi, Sci Rep 10, 9801 (2020)
- 4. W. Wei, Y. Zhao, Biology **11**(8), 1119 (2022)
- A. Bertaccini, Y. Arocha-Rosete, N. Contaldo, B. Duduk, N. Fiore, H. Montano, M. Kube, C.-H. Kuo, M. Martini, K. Oshima, F. Quaglino, B. Schneider, W. Wie, A. Zamorano, Int J Syst Evol Microbiol 72. 005353 (2022)
- A. Alma, F. Lessio, H. Nickel, *Insects as Phytoplasma Vectors: Ecological and Epidemiological Aspects*, In: Bertaccini, A., Weintraub, P., Rao, G., Mori, N. (eds) Phytoplasmas: Plant Pathogenic Bacteria II. Springer, Singapore (2019)
- F. Quaglino, F. Sanna, A. Moussa, M. Faccincani, A. Passera, P. Casati, P. A. Bianco, N. Mori, Sci Rep 9, 19522 (2019)
- R. Pierro, A. Moussa, N. Mori, C. Marcone, F. Quaglino, G. Romanazzi, Front. Plant Sci. 15, 1364241 (2024)
- 9. F. Pavan, D. Frizzera, M. Martini, C. Lujan, E. Cargnus, Agronomy, 14(4), 643 (2024)
- 10. Y. Radionovskaya, N. Aleinikova, G. Bondarenko, B. Khamaeva, E. Bolotianskaia,

S. Belash, J Magarach. Viticulture and Winemaking, 25(4), 356–362 (2023)

- 11. M. Riedle-Bauer, G. Brader, J Plant Dis Prot 130, 1057–1074 (2023)
- V. Klimenko, I. Pavlova, V. Volodin, S. Gorislavets, Scientific Works of NCFSCHVW, 30, 112–117 (2020)
- 13. G. Romanazzi, S. Murolo, E. Feliziani, Phytopathology 103, 785–791 (2013)
- A. Moussa, C. Barbieri, S. Torcoli, A. Passera, E. Guerrieri, F. Serina, N. Mori, F. Quaglino, *Evaluation of biostimulant effectiveness in "Bois noir" control in vineyards*, Intervento presentato al 6. convegno European Bois noir workshop. 1st International Pro-AECOGY conference tenutosi a Bordeaux (2024)
- L. Stojanović, O. Popov, J. Stepanovic, B. Duduk, A. Kosovac, Pestic Phytomed 39(1), 1–12 (2024)