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Communication

Control of Bacterial Canker in Stone Fruit Trees by Chemical and Biological Products

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Abstract: Bacterial canker, caused by *Pseudomonas syringae* pv. *syringae* and pv. *morsprunorum*, is one of the most important diseases of stone fruit trees (*Prunus* spp.). The pathogen infects buds, flowers, fruitlets, leaves and shoots, from which the disease spreads to the branches, boughs and trunks, causing necrosis and cankers. The efficacy of different chemical and biological products for the control of bacterial canker on stone fruit trees was tested in 2018–2021. The experiments were conducted in sour cherry, plum and sweet cherry orchards in central Poland. Foliar application of the tested preparations was performed three times a season. The biological efficacy of the tested products in the control of bacterial canker was evaluated on sour cherry on the basis of infected leaves and fruits and on plum and sweet cherry on the basis of infected leaves. The highest efficacy was observed for products containing various forms of copper—copper oxide, copper oxychloride and copper hydroxide—as well as fertilizers with copper gluconate and the fungicide Luna Care 71.6 WG (fluopyram and fosetyl-Al). However, the biological preparations were significantly less effective. The conducted studies showed that preparations based on copper gluconate can be a valuable alternative to typical copper fungicides.

Keywords: bacterial canker; stone fruit trees; copper; copper gluconate; biological products

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1. Introduction

One of the most important factors influencing the quality and volume of stone fruit (*Prunus* spp.) production is tree diseases; for example, brown rot of stone fruits (*Monilinia* spp.), cherry leaf spot (*Blumeriella jaapii*) or leucostoma canker (*Leucostoma persoonii* and *Leucostoma cinctum*). Bacterial canker is one of the most important of these diseases, both in Poland and in many other parts of the world [1–4]. The causal agent of the disease—a bacteria belonging to *Pseudomonas syringae sensu lato*—is a polyphage; it infects all species of fruit trees as well as other species of plants, both annual (for example: *Cucumis melo*, *Cucumis sativus*, *Solanum lycopersicum*, *Triticum aestivum*, *Phaseolus vulgaris*, *Nicotiana tabacum*) and perennial (*Syringa vulgaris*, *Vaccinium* sp., *Acer* sp., *Salix* sp., *Ulmus* sp. and many other woody plants). In stone fruit trees such as cherries and plums, two pathovars can cause the disease: *Pseudomonas syringae* pv. *syringae* (Pss) van Hall and pv. *morsprunorum* (Psm) (Wormald) [5–9]. The pathogen infects buds, flowers, fruitlets, leaves and shoots, from which the disease spreads to the branches, boughs and trunks, causing necrosis and cankers. Moreover, pathogenic bacteria show synergism with frosts, resulting in increased damage at temperatures even slightly lower than 0 °C. Bacterial canker causes the greatest damage to orchards and stone fruit tree nurseries. Cankers on branches and trunks often lead to the death of entire trees, and the affected fruits are unfit for consumption [1,3,9–12].

Prevention plays an important role in protection against bacterial canker, with preventative actions including the use of healthy planting material; appropriate site selection (avoiding low-lying areas that are conducive to tree frost); cutting trees on dry, sunny days; accurate and systematic cutting and removal of all infected shoots and even whole trees; and quick protection of wounds after pruning. However, prophylactic measures may only limit, but not completely destroy, existing infections [1,3]. Currently, preparations based on various copper compounds, such as copper oxide, copper oxychloride and copper hydroxide, are the primary approach used to control bacterial canker on stone fruit trees [5,6]. However, due to the occurrence of resistance of some isolates or even populations of *P. syringae* pv. *syringae* to copper, its phytotoxicity and efforts to reduce soil contamination with copper, other measures are being sought [10,13]. To control bacterial diseases on various plants, resistance inducers, e.g., fosetyl-Al, potassium phosphite and acibenzolar-S-methyl, antagonistic microorganisms such as *Bacillus subtilis* and *B. amyloliquefaciens* [10] and synthetic essential oils [14], are used. Moreover, there are various products available on the market, often registered as foliar fertilizers or preparations for improving plant health, in which copper in the form of nanoparticles is complexed with organic molecules, but their exact formulation is a trade secret [15].

The aim of our research was to evaluate the effectiveness of typical copper preparations, foliar fertilizers and biological preparations against bacterial canker of stone fruit trees under field conditions.

2. Materials and Methods

The efficacy of different chemical and biological products for the control of bacterial canker on stone fruit trees was tested in 2018–2021. Sour cherry trees in the experimental orchard in Dąbrowice near Skierniewice (φ 20.106525 E, λ 51.916996 N) and sweet cherry and plum trees in the orchard in Skierniewice (φ 20.170636 E, λ 51.962595 N) were used. Both orchards were located in central Poland in a warm, temperate, transitional climate, with an average annual temperature of 8–9 °C and annual total rainfall of approximately 600 mm [16]. Sour cherry cv. "Nefris" (susceptible to bacterial canker), planted in 1999 at a spacing of 1.5 m within rows and 4 m between rows, sweet cherry cv. "Van", planted in 2018 at a spacing of 2.8 × 4 m, and European plum (*Prunus domestica*) cv. "Jubileum", planted in 2018 at a spacing of 2 × 4 m, were used. Experimental plots were set up in randomized blocks. Each combination consisted of 4 plots/replications with 5–6 trees in each plot. Each year, three treatments were performed with the tested preparations: the first was performed before flowering (BBCH 54/59), the second was performed during flowering (BBCH 60/67) and the third was performed after flowering (BBCH 69/73) [17]. A "Stihl" motor knapsack sprayer was used to apply the products, using 600 l/ha of water. The average monthly air temperature during the experiment ranged from 6.5 to 21.6 °C, and the average monthly rainfall ranged from 7.8 to 113.6 mm (Figure 1). The list of products tested in each year and their doses and composition are presented in Table 1.

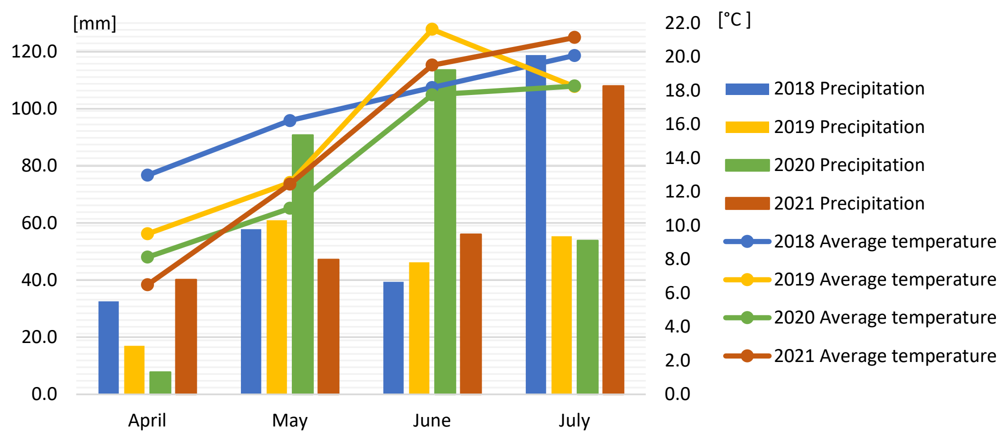

Figure 1. The average monthly air temperature and total precipitation during the experiment in 2018–2021 at experimental orchard in Dąbrowice near Skierniewice.

Table 1. Tested products and their doses and composition.

Product	Dose per 1 ha or Concentration	Composition
2018—sour cherry		
Nordox 75 WG	1.0 kg/ha	copper oxide—750 g/kg
Miedzian Extra 350 SC	3.0/1.5 L/ha *	copper oxychloride—350 g/L
Funguran Forte A-PLUS NEW 50 WP	2.0/1.5 kg/ha *	copper hydroxide—500 g/kg
2019—sour cherry, sweet cherry and plum		
Nordox 75 WG	1.0 kg	copper oxide—750 g/kg
Controlphyt Cu	0.4%	6.5% copper in the form of copper gluconate
Sergomil L-60	2.0 L	5.5% copper in the form of copper gluconate
Viflo CuB	2.0 L	4% copper in the form of copper gluconate, 2% potassium (K), 1.8% boron (B) and 0.5% manganese (Mn)
2020—sour cherry, sweet cherry and plum		
Nordox 75 WG	1.0 kg	copper oxide—750 g/kg
Serenade ASO	8.0 L	bacteria *Bacillus amyloliquifaciens* strain QST 713—13.96 g/L (minimum concentration 1.042 × 10^{12} CFU/L)
Luna Care 71.6 WG	3.0 L	fluopyram—50 g/kg and fosetyl-Al—666 g/kg
AM3/18	73.5 g/1 L of water	prototype formulation containing bacteria *Pantoea agglomerans*—107 CUF/mL
2021—sour cherry, sweet cherry and plum		
Nordox 75 WG	1.0 kg	copper oxide—750 g/kg
Serenade ASO	8.0 L	bacteria *Bacillus amyloliquifaciens* strain QST 713—13.96 g/L (minimum concentration 1042 × 10^{12} CFU/L)
Luna Care 71.6 WG	3.0 L	fluopyram—50 g/kg and fosetyl-Al—666 g/kg
IonBlue	1.5 L	copper—5.1% and sulfur—4.97% in the form of nanoparticles

* Correlation is significant at the 0.05 level.

For the sour cherry trees, the biological efficacy of the tested products in the control of bacterial canker was evaluated on the basis of infected leaves (characterized by visible small, round spots that began light brown then turned necrotic) and fruits (characterized by visible first spots that began hydrated and dark green before turning brown to black). The first assessment was performed after the occurrence of disease symptoms on untreated trees, and the second assessment was performed just before harvest. In each of the 4 replications, 10 leaves on 20 young shoots (200 leaves total), located in different places of the tree crown, and 100 fruits were evaluated. For the sweet cherry and plum trees, only the percentage of infected leaves was evaluated because the experiments were conducted on young trees that have not yet produced fruit.

The results were evaluated by single factor analysis of variance. Due to the step character of the percentage variable, the values were transformed according to the Bliss transformation. The differences between means were estimated by the Student Newman–Keuls test at a 5% significance level. The efficacy was calculated according to Abbott's formula (e = (1 – percentage of infected leaves/fruits in treated plots/percentage of infected leaves/fruits in untreated plots) × 100%).

3. Results

On sour cherry cv. "Nefris", in all seasons (2018–2021), moderately severe symptoms of bacterial canker were observed on both leaves and fruit, and the level of infected leaves between seasons ranged from 22.2 to 64.6%, while that of infected fruit ranged from 12.7 to 48%.

In the 2018 season, the tested fungicides limited disease on the leaves and on the fruits. During the first assessment, the efficacy of the tested fungicides (Table 1) in the protection of leaves was lower than 41.4%, and that in the protection of fruits was lower than 48.9%. However, statistical analyses did not show any significant differences between untreated plots and plots treated with the tested fungicides. During the second assessment, the efficacy of the tested fungicides in the protection of leaves was similar and ranged from 35.7–39.8%. In the protection of fruits, the highest efficacy (85.4%) was achieved with the application of Funguran Forte A-Plus NEW 50 WP (Figure 2A).

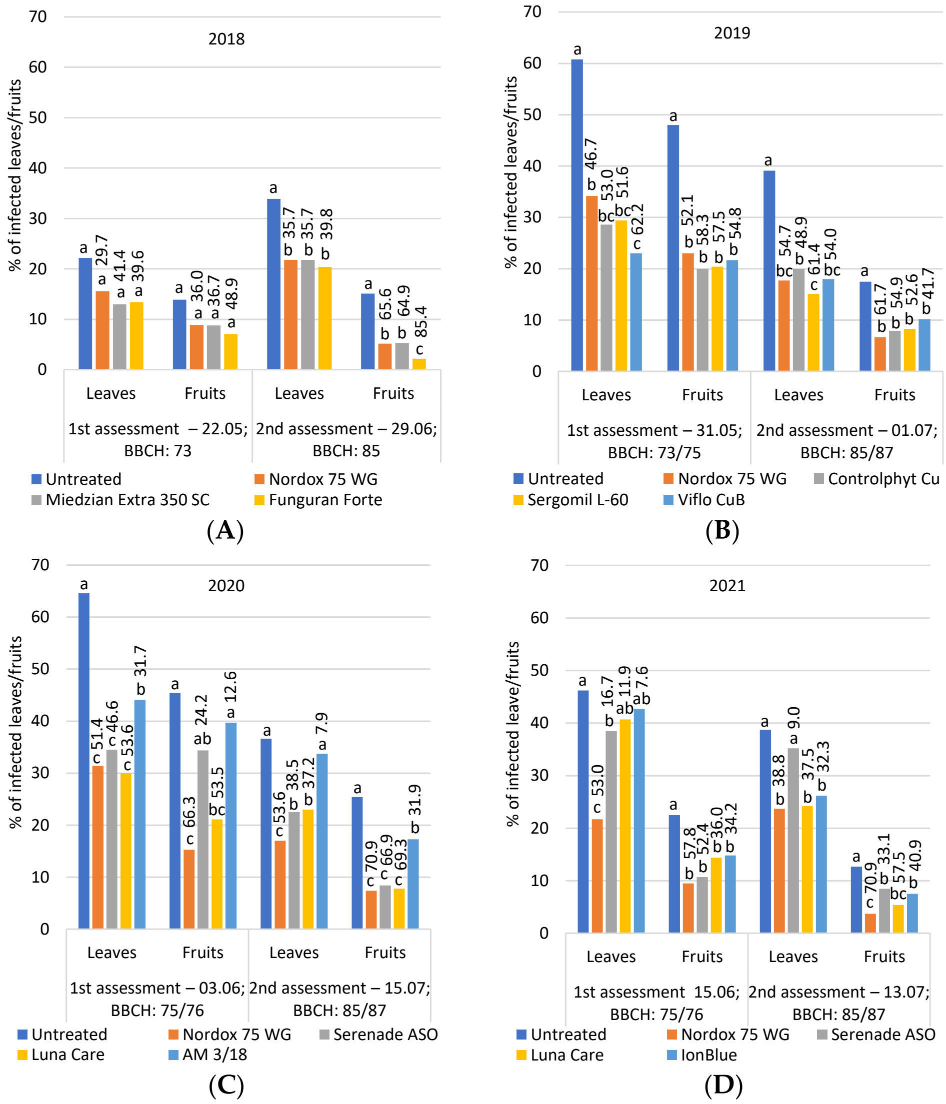

Figure 2. (**A**–**D**). Incidence of infection by bacterial canker (caused by *P. syringae*) on leaves and fruits of sour cherry after foliar applications of different products during the flowering period in 2018–2021. Efficacy values are shown above each bar. The means marked by the same letter do not differ according to the Newman–Keuls test at the 5% significance level.

In the 2019 season, the tested products (Table 1) limited disease on the leaves and on the fruits. During the first assessment on leaves, the highest efficacy was achieved by the application of Viflo CuB with a value of 62.2%. Similar levels of efficacy were observed for Controlphyt Cu and Sergomil L-60, but lower levels were observed for Nordox 75 WG. In the protection of fruits, the efficacy of the tested products was similar and ranged from 52.1 to 58.3%. During the second assessment, the highest efficacy in leaf protection was observed for Sergomil L-60; the efficacy of this product was similar to that of Nordox 75 WG and Viflo CuB but higher than that of Controlphyt Cu. The efficacy of the tested products in the protection of fruits was similar to that in the protection of leaves, but statistical analyses did not show any significant differences between the tested products (Figure 2B).

In the 2020 season, during all the assessments of leaves and fruits, out of all the tested products, Nordox 75 WG and Luna Care 71.6 WG showed the highest efficacy, while the lowest efficacy was observed for *P. agglomerans* AM3/18. In the case of Serenade ASO, the efficacy was variable depending on the date of assessment and plant organ (Figure 2C).

In the 2021 season, the effectiveness of the same products as in 2020 was tested with the exception of AM3/18, which was replaced by Ion Blue (Table 1). In the 2021 season, all the tested products resulted in limited disease occurrence on the leaves of the trees and on the fruits and leaves of the sour cherry trees. During the first and second assessments, the highest efficacy both on leaves and fruits was observed for Nordox 75 WG. During the first assessment on leaves and fruits, the lowest efficacy was observed for Ion Blue, while during the second assessment, the lowest efficacy was observed for Serenade ASO (Figure 2D).

For the plum and sweet cherry trees, in the first year (2019) of the study, the symptoms of bacterial canker occurred at very low severity, and it was not possible to assess the efficacies of the tested products (Table 1). The statistical analyses did not show any significant differences between the untreated plots and plots treated with the tested products (Figures 3A and 4A).

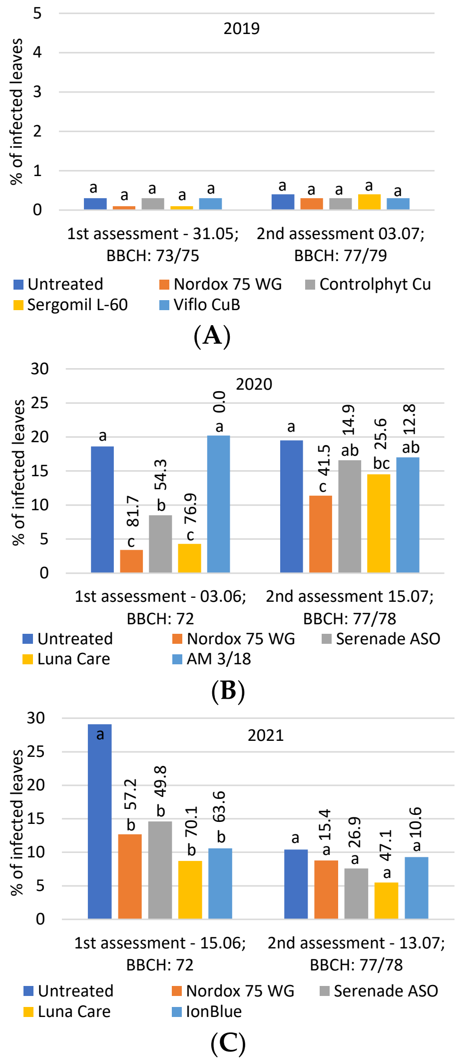

Figure 3. (**A**–**C**) Incidence Figure 4A–C. Incidence of infection by bacterial canker (caused by *P. syringae*) on leaves of plum after foliar applications of different products during the flowering period in 2019–2021. Efficacy values are shown above each bar. The means marked by the same letter do not differ according to the Newman-Keuls test at the 5% significance level.

In both the 2020 and 2021 seasons, the symptoms of bacterial canker on the leaves of plum and sweet cherry trees occurred at a moderate severity. In the 2020 season, the highest efficacy in the control of disease was achieved by Nordox 75 WG and Luna Care 71.6 WG during both assessments. Strain *P. agglomerans* AM3/18 did not control the disease at all (Figures 3B and 4B).

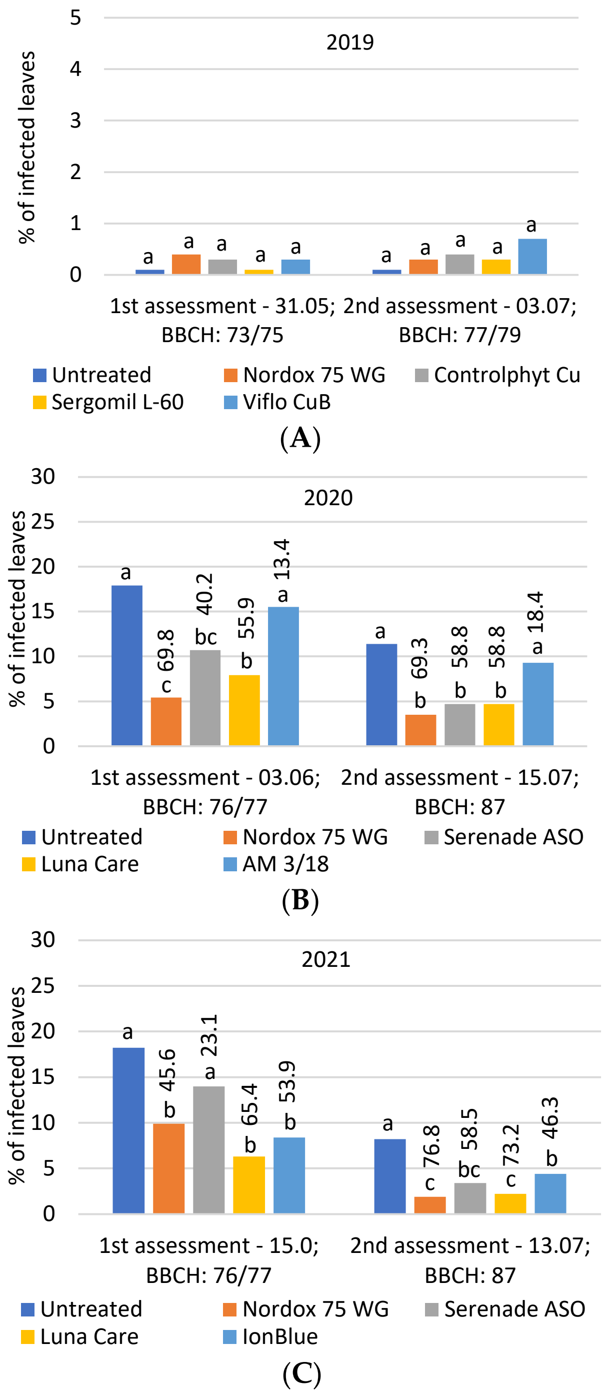

Figure 4. (**A**–**C**) Incidence of infection by bacterial canker (caused by *P. syringae*) on leaves of sweet cherry after foliar applications of different products during the flowering period in 2019–2021. Efficacy values are shown above each bar. The means marked by the same letter do not differ according to the Newman-Keuls test at the 5% significance level.

On plum leaves in the 2021 season, during the first and second assessments, the efficacies of the tested products were generally similar; the highest efficacy was observed for Luna Care 71.6 WG, while the lowest efficacy, depending on the date of assessment, was observed for Serenade ASO or Ion Blue (Figure 3C). For the sweet cherry leaves, during the 1st assessment, the highest efficacies were observed for Nordox 75 WG, Luna Care 71.6 WG and IonBlue, and their efficacies were higher than that of Serenade ASO. During the second assessment, Ion Blue had the lowest efficacy out of all tested products. The highest efficacies were observed for Nordox 75 WG and Luna Care 71.6 WG, and their efficacies were similar to that of Serenade ASO but significantly higher than that of Ion Blue (Figure 4C).

4. Discussion

The control of bacterial diseases of plants is generally more difficult than that of fungal diseases. The general approach for managing bacterial canker is to keep trees as tolerant as possible to the disease; however, in practice, this is not always an easy task. The control of bacterial canker on stone fruit trees is also challenging due to the chronic character of the disease. Chemical treatments are very limited because of the very small assortment of bactericides available and registered for use in plant production. The use of the most effective antibiotics is not allowed in the EU; thus, control is based mainly on treatments with copper preparations performed preventively during bud swelling, flowering and immediately after flowering, as well as at the beginning and at the end of leaf fall [10–12,14]. Typical copper preparations are surface agents, so they do not penetrate the plant tissues and do not move, for example, into infected buds. Copper ions, after entering the pathogen's structures, disrupt its energy processes by forming chelates of the Cu^{2+} cation with groups of respiratory chain enzymes. They damage the plasma membrane, which loses its semipermeable properties as a result [1,13,18].

In the present experiments, the effectiveness of different copper salts—copper oxide, copper oxychloride and copper hydroxide—in reducing the symptoms of bacterial canker on sour cherry leaves was similar, while copper hydroxide showed significantly higher efficacy on fruit (Figure 2A). Studies by other authors on the effectiveness of individual forms of copper in inhibiting the growth of pathogenic bacteria show mixed results [3]. For example, Eğerci [19] showed that copper sulfate was the most effective, followed by copper oxide, copper oxychloride and copper hydroxide. On the other hand, Martins et al. [20] indicated that the order of effectiveness was as follows: copper sulfate, copper hydroxide and copper oxide. Research by McLaren et al. [21] on nectarines showed that none of the tested forms of copper (copper oxide, copper oxychloride and copper hydroxide) limited the share of fruit without symptoms of bacterial canker compared to that observed in the control (untreated) combination.

In our studies carried out in 2019, the effectiveness of the preparations based on copper gluconate (Controlphyt Cu, Sergomil L-60 and Viflo CuB) in combating bacterial canker on sour cherry leaves ranged from 48.9 to 62.2%, while the effectiveness on fruit ranged from 41.7 to 58.3%, depending on the form of the preparation and the evaluation date. The effectiveness of these preparations did not differ significantly from that of the Nordox 75 WG (copper oxide) preparation (Figure 2B). On the other hand, studies by other authors [22] carried out in greenhouse conditions on kiwi plants showed that the effectiveness of traditional forms of copper (copper oxychloride and copper hydroxide) in combating bacterial canker was approximately 70%, while the effectiveness of preparations containing copper gluconate was lower.

Copper-based compounds have many disadvantages for the environment, resulting in their addition to the list of active substances for which alternatives are needed according to European Commission regulations. First, these compounds may cause a phytotoxic effect on different plant species, mostly in the case of application at doses that are too high; however, application at too low a concentration does not allow effective protection against diseases. During rainfall, the copper is washed off the plants and into the soil, where it is retained for a long time. In some territories, orchard soils are already so contaminated with this metal that it is necessary to exclude them from cultivation [23]. In addition, copper causes pollen deactivation [24], which can cause lower yields, is lethal to honeybees and changes the properties of nectar, making it unattractive to bees [25]. Copper based fungicides were classified by the EU as candidate for substitution; however, it will be taken out from the list in 2023. There is no more effective alternative to control bacterial diseases. In 2020 and 2021, in addition to Nordox 75 WG, the fungicide Luna Care 71.6 WG (the active substances of which are flupyram and fosetyl-Al) and the biological preparation Serenade ASO, containing the bacterium *Bacillus amyloliquifaciens* QST713, were tested in our studies. Luna Care 71.6 WG and Serenade ASO are registered in Poland to combat bacterial canker of fruit on pear trees, and Serenade ASO is also registered for application on sour cherries, plums, sweet cherries, apricots and peaches. In the conducted studies, the effectiveness of Nordox 75 WG in combating bacterial canker on sour cherry, plum and sweet cherry leaves ranged from 15.4 to 81.7%, depending on the species, the evaluation period and the year of the study, and on sour cherry fruit, it ranged from 57.8 to 70.9%. The effectiveness of the fungicide Luna Care 71.6 WG ranged from 11.9 to 76.9% on sour cherry, plum and sweet cherry leaves and from 36 to 69.3% on sour cherry fruit. The effectiveness of the fungicide Luna Care 71.6 WG was similar, and in some evaluations, lower than that of Nordox 75 WG. Serenade ASO was generally less effective than the other preparations. Previous studies [21,22] showed relatively high efficacy of both Serenade ASO and fosetyl-Al. However, these studies were conducted in controlled greenhouse conditions or in vitro. In the present study, the experiments were carried out under field conditions, where high temperatures, large amounts of rainfall and strong sunlight may limit the effectiveness of fungicides and inhibit the development of the antagonistic bacteria constituting the active ingredient of Serenade ASO [11,12]. The least effective preparation was strain *P. agglomerans* AM3/18. This strain was isolated in our laboratory and found to be highly effective in the control of fire blight, a bacterial disease of apples and pears caused by *Erwinia amylovora* (data unpublished); however, in the case of bacterial canker, it showed almost no efficacy. The problem of the lower efficacy of biological products can also be related to the nature of the causal agent of bacterial canker. Generally, bacteria of the species *P. syringae* cause bacterial canker of fruit trees. However, the term *P. syringae sensu lato* should be used because *P. syringae* is a very heterogeneous species, actually consisting of several phylogenomic species that should be described and levelled up to the status of species [26]. Phylodiversity also results in the diversity of other features, such as host range and susceptibility to different compounds encoded in the genome of bacteria. Thus, in the case of bacterial canker, finding a universal biocontrol agent can be difficult.

5. Conclusions

The levels of effectiveness of the tested plant protection products containing various forms of copper—including copper oxide, copper oxychloride, copper hydroxide and fertilizers based on copper gluconate—in reducing bacterial canker leaf and fruit symptoms were similar, and high efficacy was observed. The fungicide Luna Care 71.6 WG (fluopyram and fosetyl-Al) was effective at levels similar to or lower than those of Nordox 75 WG, but the biological preparations Serenade ASO (*Bacillus subtilis* QST713) and *P. agglomerans* AM3/18 were significantly less effective. Taking into account limitations related to the usage of copper in agriculture and difficulties in finding proper biocontrol agents due to the diverse nature of the causal agents of bacterial canker, the control of this disease will encounter many difficulties in the future, not only for fruit trees but also for other plant species attacked by *P. syringae*.

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