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Chemical products for corn bacterial streak control¹

Alex Junior Pietrobon², José Barbosa Duarte Júnior², Odair José Kuhn²

ABSTRACT

Xanthomonas vasicola pv. *vasculorum* is one of the main bacterial diseases in corn, which causes damage to crops due to its fast dissemination and difficult control. This study aimed to evaluate the effects of chemical products on its control and corn grain yield. The experimental design was randomized blocks, in a 2 x 7 factorial arrangement, with three replications. The first factor consisted of the corn hybrids P30F53VYHR (more resistant to the disease) and P4285VYHR (more susceptible) and the second one of six active ingredients (kasugamycin, sulfur, cuprous oxide, copper oxychloride, quaternary ammonia and potassium phosphite) and one control (no application). The obtained data were used to estimate the area under the disease progress curve. The products based on quaternary ammonia, cuprous oxide, copper oxychloride and kasugamycin provided a greater disease control. P30F53VYHR presented a higher grain yield than that for P4285VYHR. Therefore, the use of a genetically resistant hybrid is an efficient alternative for the management of this bacteriosis.

KEYWORDS: *Xanthomonas vasicola* pv. *vasculorum*, *Zea mays*, genetic control.

RESUMO

Produtos químicos no controle de estria bacteriana do milho

Xanthomonas vasicola pv. *vasculorum* é uma das principais bacterioses na cultura do milho, a qual causa danos às lavouras, devido à sua rápida disseminação e difícil controle. Objetivou-se avaliar o efeito de produtos químicos no seu controle e na produtividade de grãos de milho. O delineamento experimental foi em blocos casualizados, em esquema fatorial 2 x 7, com três repetições. O primeiro fator foi constituído pelos híbridos de milho P30F53VYHR (mais resistente à doença) e P4285VYHR (mais suscetível) e o segundo por seis ingredientes ativos (casugamicina, enxofre, óxido cuproso, oxiclureto de cobre, amônia quaternária e fosfito de potássio) e uma testemunha (sem aplicação). Os dados obtidos foram utilizados para estimar a área abaixo da curva de progresso da doença. Os produtos à base de amônia quaternária, óxido cuproso, oxiclureto de cobre e casugamicina proporcionaram maior controle da doença. P30F53VYHR apresentou produtividade de grãos superior à de P4285VYHR. Portanto, o uso de híbrido geneticamente resistente representa uma alternativa eficiente ao manejo dessa bacteriose.

PALAVRAS-CHAVE: *Xanthomonas vasicola* pv. *vasculorum*, *Zea mays*, controle genético.

INTRODUCTION

Corn is the most important and most cultivated cereal in the world. Currently, Brazil is the second largest producer of this grain, with a record production of 102.1 million tons in the 2019/2020 harvest (Conab 2020). However, many areas cultivated with corn use direct sowing and monoculture, and no crop rotation systems. Thus, there is an increase in the incidence of diseases and, above all, in the intensity of infections by pathogens.

Among the limiting conditions of corn cultivation, diseases caused by bacteria are of great relevance, as they affect roots, stalk, leaf tissue or

grains (Medeiros et al. 2018). Among them, the corn bacterial streak, caused by *Xanthomonas vasicola* pv. *vasculorum* (Xvv), is responsible for significant damages, with symptoms that may occur at any development stage (Leite Junior et al. 2018).

It is considered one of the main bacteriosis in corn crops in the Paraná state, and may be found in several producing regions. This disease mainly affects the leaf tissue, causing elongated and narrow lesions, and, in more severe cases, it extends to the stem and bracts (Leite Junior et al. 2018). In epidemic dimensions, it presents incidence levels above 90 % and may compromise more than 50 % of the leaf area in susceptible hybrids (Broders 2017).

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Bacteria can survive in weeds and crop residues, which are thus a source of inoculum for the next crop. It can spread over long distances by wind (Broders 2017), rain and/or irrigation (Ortiz-Castro et al. 2020). These factors make the process of preventing the spread of this disease to uninfected crops even more difficult.

Corn bacterial streak control is difficult and still unknown (Broders 2017). Furthermore, the use of fungicides showed to be ineffective in controlling the pathogen (Leite Junior et al. 2018). Due to the great difficulty in controlling bacterial streak, the use of chemical products for the management of *Xvv* is an important tool for phytosanitary measures.

Despite the dimension reached by the corn bacterial streak in Brazil, little information is available, and, at a national level, there are no studies capable of measuring the effects of agrochemicals for the control of this disease. Therefore, it is necessary to study their potential for this purpose.

Thus, this study aimed to evaluate the effects of chemical products on the *Xanthomonas vasicola* pv. *vasculorum* control and corn grain yield.

MATERIAL AND METHODS

The experiment was carried out during the second harvest of 2020, in Toledo, Paraná state,

Brazil (24°67'30"S, 53°75'34"W and altitude of 540 m), in an area with no history of the disease. The soil in this area is an Eutroferic Red Latossol (Santos et al. 2018), or Oxisol (USDA 1999), whose chemical properties are summarized in Table 1.

Information regarding rainfall and average maximum and minimum weekly temperatures observed during the experiment were obtained from a meteorological station located 100 m away from the experimental area (Figure 1).

During the conduction of the experiment, there were periods with low rainfall, mainly from the beginning of the first week of March to the second week of May. To offset the water deficit and create a microclimate favorable to the pathogen's development, a sprinkler irrigation system was used, which allowed for a total daily water depth of approximately 10 mm.

The sowing was carried out on February 1, 2020, using a Semeato experimental seeder. Two corn hybrids were used: P30F53VYHR (resistant to the disease) and P4285VYHR (susceptible to the disease). The base fertilization was 300 kg ha⁻¹, formula 13-24-12, from YaraMila. The seedling emergence occurred at five days after sowing. The N application was carried out at the V₄ phenological stage, at a dose of 400 kg ha⁻¹ and formulation 19-04-19.

Table 1. Soil chemical properties in the experimental area, according to laboratory analysis¹.

pH (CaCl ₂)	Ca	Mg	K	P	Al	H + Al	T	V (%)	OM (g dm ⁻³)
5.6	10.07	3.01	0.94	38.5	0	3.41	14.02	80.3	33.1
Sand	Silt	Clay	Fe	Mn	Cu	Zn	Ca/Mg	Ca/K	Mg/K
17.5	17.38	65	22.9	32.3	2.05	4.85	3.36	10.74	3.21

¹ Micronutrient and P contents are expressed in mg dm⁻³; other nutrients in cmol dm⁻³.

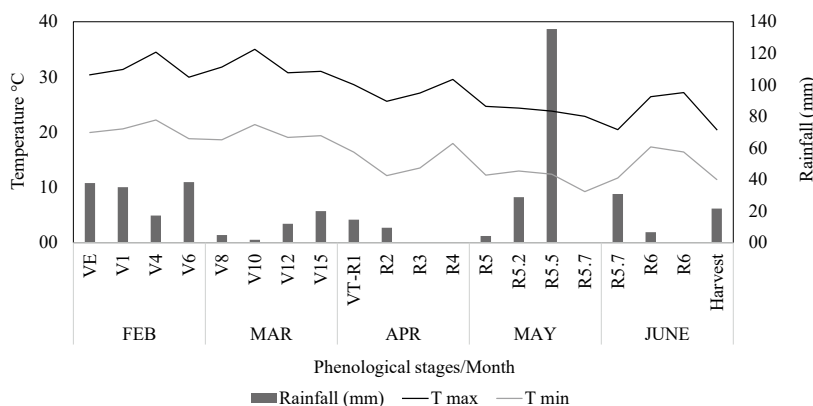


Figure 1. Rainfall, weekly average temperature and phenological stages of the corn plants during the experiment (Toledo, Paraná state, Brazil, 2020).

For pest control, the insecticide Galil (Imidacloprid + Bifentrin) was used, at a dose of 400 mL ha⁻¹, at the V₁ phenological stage. Atrazine herbicides were used at a dose of 2,250 g ha⁻¹ and tembotrione at 100 g ha⁻¹, when the crop was at the V₃ phenological stage.

The experimental design was randomized blocks, in a 2 x 7 factorial design, with three replications. The first factor was the corn hybrids P30F53VYHR and P4285VYHR and the second one six products and one control (without application) (Table 2). The combination of factors resulted in 14 treatments and 42 experimental units.

Sowing was carried out in blocks, where hybrids and products remained randomized. Each experimental unit consisted of 12 lines spaced 0.50 m apart. Only the four central lines were analyzed to reduce the border effect. The total length of each row was 4.20 m, with a total population estimated at 67,500 plants ha⁻¹. In addition, 1.1 m from each end were disregarded to reduce the drift effects from the adjacent treatment; thus, each plot had a useful area of 4 m².

For plant inoculation, the isolate of *Xv* (strain SAM 119), belonging to the phytopathology laboratory of the Universidade Estadual do Oeste do Paraná, was used. The bacterial suspension was prepared as described by Mafia et al. (2016), adjusted in a spectrophotometer UV-Vis, for Abs₅₈₀ = 0.300, corresponding to approximately 2.7 x 10⁹ CFU mL⁻¹, according to the bacterial concentration curve described by Hendges et al. (2020). The total production of inoculum for spraying in the field was 11 L.

The inoculation was performed at the end of the same day of inoculum preparation. This procedure was carried out in all experimental units by spraying approximately 1 mL of bacterial suspension in the cartridge of each plant vessel at the V₆ development stage.

The monitoring of symptoms and disease severity levels was performed according to a diagrammatic scale previously developed and validated by the authors. There were 12 levels of severity ranging from 1 to 22 %, as during the period of the experiment there was no defined diagrammatic scale in the literature to quantify corn bacterial streak.

The disease incidence evaluations were carried out in the useful portion of all treatments, after counting the number of plants with visible symptoms at the following phenological stages: V₆, V₈, V₁₀, V₁₂, V_T, R₁, R₂, R₃, R₄, R₅ and R₆. The disease progress was also monitored by evaluating the percentage of bacterial streak severity over time in each treatment.

To evaluate the disease severity levels, ten plants were identified in the useful area of each plot, by marking two leaves per plot, totaling 20 leaves evaluated per experimental unit. The evaluations were carried out every seven days over six weeks, totaling six evaluations, during the phenological stages (V₁₂ to R₃).

From the scores obtained in severity assessments, the disease evolution was determined by calculating the area under the disease progress curve (AUDPC), following the standardization of Shaner & Finney (1977): $AUDPC = \sum [(y_i + y_{i+1})/2] \times (t_{i+1} - t_i)$, where y_i is the initial disease severity; y_{i+1} the final disease severity; and $t_{i+1} - t_i$ the time interval between the initial and final readings.

The applications of the chemical products were carried out at the doses recommended by the manufacturers. For this, a back sprayer pressurized with CO₂ was used. It has a bar with six nozzles, spray volume of 250 L ha⁻¹, pressure of 50 psi and a fan nozzle 110-0.2. For this activity, individual application protection equipment was used.

After the onset of the disease symptoms, three applications were performed in the morning: the first at the V₁₃ phenological stage or 21 days

Table 2. Active ingredient, concentration and dose of chemical products used to control the corn bacterial streak.

Active ingredient	Concentration (g a.i. L ⁻¹ or kg)	Dose (mL ha ⁻¹)
Kasugamycin	20	3,000
Sulfur + nitrogen	250 + 110	1,500
Cuprous oxide	500	100
Copper oxychloride + nitrogen	499 + 68	500
Calcium + nitrogen + (quaternary ammonia + salicylic acid)	20 + 10	1,500
Potassium phosphite + magnesium + sulfur + copper + nickel	450 + 250 + 23 + 29 + 10 + 5	500
Control (no application)	0	0

after the bacterial inoculation; the second at the R_1 reproductive stage or 13 days after the first application; and the last at the R_2 stage or 12 days after the previous application. At each exchange of bactericidal product, the spraying equipment was cleaned with clean water.

The harvest was carried out manually by collecting all the ears of the useful portion of each experimental unit. The yield of the treatments was adjusted to 13 % of moisture and converted into kg ha^{-1} . The data were subjected to analysis of variance (Anova) and the effects analyzed by the Tukey test at 5 % of probability, using the Sisvar statistical software (Ferreira 2011).

RESULTS AND DISCUSSION

The temperature conditions were adequate for the development of the crop throughout the cycle. There was a water deficit, especially in March and April. However, the irrigation system complemented the low rainfall volume and provided the necessary conditions for the development of the pathogen. This fact may be associated with the pathogen's adaptation to warm climates, where periodic irrigations facilitate its spread throughout the crop (Broders 2017).

The initial disease symptoms for the hybrid P4285VYHR appeared during the V_8 phenological stage. However, the disease incidence reached all plants in the plot only from the VT phenological stage. The chemical treatments were not effective in reducing the disease incidence for the hybrid P4285VYHR, as there were symptoms in 100 % of the plants until their physiological maturation (Figure 2).

As for the hybrid P30F53VYHR, there was no characteristic symptom of bacterial streak throughout its cycle. This fact shows a genetic resistance of the material to the bacteria.

In this context, Silva et al. (2020) pointed out genetic resistance as an efficient strategy to control leaf diseases in corn, since, in addition to not increasing production costs, it also provides opportunities to reduce the use of agrochemicals.

The disease progress curves for the hybrid P4285VYHR showed a lower AUDPC for the active ingredients cuprous oxide and quaternary ammonia, and later copper oxychloride and kasugamycin, by which the progress curves of bacterial streak were similar to each other. The highest AUDPC occurred in the control (32.37). For sulfur and potassium phosphite, there was a disease progress close to that shown by the control, that is, the products were less efficient in controlling the disease (Figure 3).

Conversely, Junqueira et al. (2011) worked with *Xanthomonas axonopodis* pv. *passiflorae* and found that fortnightly potassium phosphite sprays are efficient in controlling bacteriosis. Furthermore, the authors found that this type of spraying contributes to an increased yield.

Núñez et al. (2018) studied the black rot of crucifers and found that sulfur-based products have no bactericidal effect against *Xanthomonas campestris* pv. *campestris*. Leite Junior et al. (2018) had already reported that, commonly, as in other diseases, fungicidal products do not have a control action on plant bacteriosis.

For the corn bacterial streak's AUDPC, quaternary ammonia, cupric and kasugamycin-based

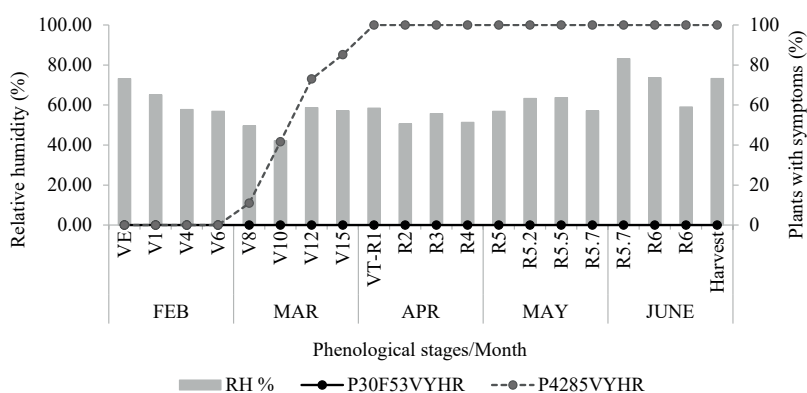


Figure 2. Incidence of bacterial streak caused by *Xanthomonas vasicola* pv. *vasculorum* over time at different development stages of two corn hybrids (Toledo, Paraná state, Brazil, 2020).

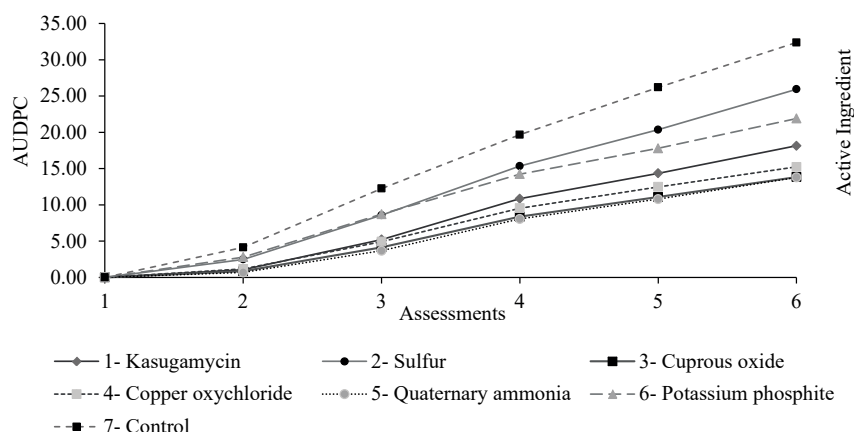


Figure 3. Area under the bacterial streak progress curve caused by *Xanthomonas vasicola* pv. *vasculorum*, hybrid P4285VYHR, during the six weekly assessments.

products showed a greater efficiency in disease control in the susceptible hybrid. The average AUDPC was 55 % lower than that of the control (Table 3).

When studying the chemical control over the *Pantoea ananatis* severity in corn, Burtet (2018) found a low sensitivity of the bacteria to kasugamycin under laboratory conditions. In addition, this same product showed, in the field, a lower disease control and yield, when compared to the other treatments. On the other hand, Garcia et al. (2019) evaluated the effects of kasugamycin on growth and endogenous infection by *Leifsonia xyli* subsp. *xyli* and found a reduction in the bacterial load. This, therefore, suggests an efficient alternative to control the bacteria.

Patil et al. (2017) tested chemical products for the management of *Xanthomonas oryzae* pv. *oryzae* in rice and observed a 59.78 % reduction in

the disease intensity, when they used the antibiotic streptomycin combined with copper oxychloride. However, the treatment with kasugamycin showed a control of 44.62 %.

Other important data regarding quaternary ammonia are mentioned by Pontes et al. (2012). They identified benzalkonium chloride and dodecyl dimethyl ammonium chloride with a high and low efficiency, respectively, in controlling the severity of bacterial spot caused by *Xanthomonas perforans* in tomato plants.

Cuprous oxide, copper oxychloride and quaternary ammonia differed statistically from the other active ingredients at 5 % of probability. These were the treatments that presented the lowest disease severity rates for the hybrid P4285VYHR (Table 4).

The maximum severity of corn bacterial streak obtained in the last evaluation during the reproductive stage (R_3) demonstrated relatively low severity levels.

Table 3. Area under the bacterial streak progress curve for two corn hybrids and control efficacy, in relation to the control.

Active principle	P30F53VYHR	CE (%)	P4285VYHR	CE (%)
Kasugamycin	0 ^{ns} A ¹	0	49.61 abB	47.56
Sulfur	0 A	0	72.68 cdB	23.17
Cuprous oxide	0 A	0	38.26 aB	59.56
Copper oxychloride	0 A	0	43.34 aB	54.19
Quaternary ammonia	0 A	0	36.95 aB	60.94
Potassium phosphite	0 A	0	65.45 bcB	30.81
Control	0 A	0	94.60 dB	0
Mean	0		57.27	
CV (%)	0		19.55	

¹ Means followed by the same lowercase letter in the rows and uppercase letter in the lines do not differ by the Tukey test ($p \leq 0.05$). Original AUDPC data. For analysis purposes, the AUDPC points data were transformed into $\sqrt{+5}$; CE (%): means for control efficacy. ^{ns} non-significant at 0.05.

Table 4. Maximum severity of bacterial streak in two corn hybrids, as a function of each active ingredient.

Active principle	Severity (%) [*]	
	P30F53VYHR	P4285VYHR
Kasugamycin	0 ^{ns} A ¹	6.03 bcdB
Sulfur	0 A	7.04 cdB
Cuprous oxide	0 A	4.81 abB
Copper oxychloride	0 A	5.21 abcB
Quaternary ammonia	0 A	4.25 aB
Potassium phosphite	0 A	6.69 cdB
Control	0 A	7.48 dB
General mean	0	5.93
CV (%)	0	16.53

¹ Means followed by the same lowercase letter in the rows and uppercase letter in the lines do not differ by the Tukey test ($p \leq 0.05$). * Original data. For severity analysis purposes, the AUDPC points data were transformed into $\sqrt{+5}$. ^{ns} non-significant at 0.05.

Table 5. Mean grain yield for two corn hybrids with inoculation of *Xanthomonas vasculorum* pv. *vasculorum* and sprayed with different chemicals.

Active principle	Yield ¹	
	P30F53VYHR	P4285VYHR
Kasugamycin	13,279.0 ^{ns} A ¹	10,983.0 ^{ns} B
Sulfur	13,175.0 A	10,679.0 B
Cuprous oxide	13,295.0 A	10,816.0 B
Copper oxychloride	13,337.0 A	10,650.0 B
Quaternary ammonia	13,162.0 A	10,729.0 B
Potassium phosphite	13,133.0 A	10,929.0 B
Control	13,300.0 A	10,483.0 B
General mean	13,240.0	10,752.0
CV (%)	0.91	1.99

¹ and ^{ns}: no difference for variation (products) and, for means with the same capital letter in the rows (hybrids), they do not differ by the Tukey test ($p \leq 0.05$). Data presented in kg ha⁻¹.

The highest mean observed was for the control treatment, with levels of 7.48 % of severity.

Costa et al. (2017) evaluated the effects of chemical products on the severity of bacterial leaf spot in passion fruit and showed that treatments with copper oxychloride limit the number of colony-forming units of *Xanthomonas axonopodis* pv. *Passiflorae*. This control is related to the multi-site contact activity of copper products (Agrofit 2021).

A similar behavior as that of the present study was obtained by Strayer-Scherer et al. (2018), who carried out a research on copper and its action against *Xanthomonas perforans*, an agent of the main tomato bacteriosis. In this research, it was observed that copper compounds significantly reduce the disease severity, if compared to the control. A recent research on bacterial spot has confirmed the bactericidal action of copper-based products against *Xanthomonas euvesicatoria* and its influence in reducing the disease severity in hot peppers, in a greenhouse (Fan et al. 2021).

Based on these discussions, it is possible to observe an influence of copper-based products on bacteria of the *Xanthomonas* genus. This statement is also consistent with the results obtained in the present study. The copper products were able to reduce the corn bacterial streak severity under field conditions.

The variable grain yield presented a significant variation between the two hybrids ($p < 0.01$). There was no effect of the bactericidal products used (Table 5).

The hybrid P30F53VYHR presented a higher grain yield than the P4285VYHR in all treatments. The mean was 13,240 kg ha⁻¹, when compared to the previous 10,752 kg ha⁻¹, respectively. There was

a significant difference of 2,488 kg ha⁻¹ between the means of the treatments, which represents an increase of 19 % in yield.

In this context, Batista et al. (2018) evaluated the morphological characteristics and yield components of different corn hybrids cultivated off-season, in a dystrophic Red Latossolo, and found that the hybrid P30F53VYHR stood out among others, with a higher grain yield.

Thus, the use of corn hybrids adapted to the region and genetically resistant to the disease may be a good alternative for maintaining grain yield. In addition, it represents a way of reducing costs with agrochemical applications.

CONCLUSIONS

1. Products based on quaternary ammonia, cuprous oxide, copper oxychloride and kasugamycin reduce the bacterial streak severity in susceptible corn hybrids. The occurrence of the disease from the V₈ stage does not affect the corn grain yield;
2. The hybrid P30F53VYHR shows genetic resistance to the pathogen and is recommended for the disease management.

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