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Fungicides in the control of septoriose in tomato plant

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Abstract

The disease septoriose causes severe defoliation in tomato plants that can reach 100% leaf fall. Consequently, the losses are significant due to the decrease in photoassimilate production and sun scald on tomato fruits. This work presents studies in vitro and in vivo of 15 active ingredients, alone or combined, at the recommended doses to control the septoriose in preventive or curative pulverization. The dose used must follow the fungicide label instructions to keep the resistance risk low and comply with current legislation. In addition, the efficiency of 24 active ingredients, alone or combined, recommended for other tomato diseases than septoriose in preventive pulverization was also explored to know its effect on *Septoria lycopersici*. In the preventive treatment fluxapyroxad + pyraclostrobin (58.5+116.6ppm of the active ingredient, respectively), mancozeb (4000ppm), difenoconazole (125ppm), chlorothalonil (1500ppm), propineb (2100ppm), fluazinam + thiophanate-methyl (375+375ppm) and metiram + pyraclostrobin (1100+100ppm) controlled the severity of the disease above 70%. In the curative treatment, applying the fungicides after seven days from spores pulverization, no fungicide control above 70% of the disease incidence and severity compared to the treatment without pulverization. Among the fungicides recommended for other tomato diseases than septoriose, those with mancozeb or chlorothalonil in doses higher than 1920 and 1200ppm, respectively, as part of the active ingredients and boscalide (75ppm) controlled above of 70% of the severity of the disease. The use of multi-site products (mancozeb, chlorothalonil, propineb or metiram) or fluazinam (protective fungicide) combined with efficient systemic fungicides (fluxapyroxad or difenoconazole) at the doses recommended in label for the control of *S. lycopersici* could control tomato septoriose efficiently. Those fungicides should be applied without a tank mixture. The fungicides metiram, fluazinam and fluxapiroxade are recommended to control septoriose in Brazil only when formulated with pyraclostrobin, thiophanate-methyl and pyraclostrobin, respectively.

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Introduction

The septoriose was reported for the first time in Argentina in 1882 (Sutton & Waterston 1966) and nowadays occur anywhere in tomato crops (Stevenson 1991). *S. lycopersici* infect tomato leaves by both stomata and direct penetration (Martin-Hernandez et al. 2000). This species is the unique etiologic agent of the septoriose in Brazil, and genetic diversity was not found for isolates from Brazil using Tub, Cal and EF1-α sequencing region (Costa 2019). Despite the lack of genetic diversity based on those sequencing regions, the isolates of *S. lycopersici* have some morphology variability and variation in aggressiveness to tomato plants (Costa 2019). Its importance depends on the favorable weather conditions, which occur when the relative humidity is above 85%; temperature is between 20 to 25ºC (Kurozawa & Pavan 2005) and wetting periods greater than 20h (Elmer & Ferrandino 1995).

Symptoms appear one week after inoculation, and after six weeks, defoliation is close to 100%, and losses are significant due to the sunscald on tomato fruits (Sohi & Sokhi 1974) when in humid conditions and no control measures are used (Parker et al. 1997). In each cultivation cycle, the disease begins in the leaves of the shallows due to the raindrops that fall on fragments of plants with Septoria spores and cause splashes spreading the spores to the surrounding tomato leaves (Douglas 2008).

Symptoms are circular-shaped spots with darkened edges and brown coloured centres, which initially appear at the bottom of the plant. After a few days, small black spots appear in the centre of the lesions, which correspond to the reproductive structures of the fungus (pycnids). Under favourable conditions, the lesions may coalesce, turn yellow, and then brown, which wither, dry and detach from the plant (Douglas 2008). Similar injuries can occur on the stem. The fruits are rarely affected. It can be confused with a bacterial spot when the lesions are at the beginning or with Alternaria leaf spot. A study to determine resistance to septoriosis with more than 500 plants, including strains, accessions and cultivars revealed that all tested cultivars demonstrated susceptibility to this disease (Poysa et al. 1993). Despite attempts to include septoriose resistance, no commercial cultivar has it. Since there is no genetic resistance, the recommended control tactics include crop rotation with a non-host species, removal of alternative hosts (*Datura stramonium*, *Physalis* sp., *Solanum carolinense*) and cultural remains (Seymour & Ridings 1980, Zitter 1987, Stevenson 1991, Malnati 1993, Bhardwaj et al. 1995), and the application of the recommended fungicides.

Fungicides, natural or synthetic, protect plants against invasion by fungi or eradicate established fungal infection to ensure the yield potential, measure as the quantity or quality of production (Oliver & Hewitt 2014). The effectiveness of some fungicides may vary from time to time because pathogens can develop resistance to it. The risk of fungicides resistance varies depending on the number of action sites of the active ingredients (Table 1) and pathogen propensity to develop resistance (Oliver & Hewitt 2014). Mobility is an important fungicide attribute, which occurs by interplant movement through vapour-phase activity or redistribution by rain, and intraplant movement through xylem or phloem transport and diffusion (Oliver & Hewitt 2014). Chlorothalonil and metalaxyl, for example, have some redistribution in the air due to the low vapour pressures they have. Fungal structures which are exposed to the air might be susceptible to fungicides active through the vapour phase (Oliver & Hewitt 2014). In addition, fungicides may reach places that were not sprayed via vapour phase (Oliver & Hewitt 2014). Fungicides can be systemic, non-systemic, protectant, curative or eradicant, and all those characteristics are important to outline a field management strategy. Systemic fungicide may also possess strong protectant characteristics (Oliver & Hewitt 2014).

Chemically distinct classes can have the same mode of action (Oliver & Hewitt 2014), which could be led to an emerging resistant lineage in a case without fungicide group rotation. The fungicide with multi-site action has a lower risk of developing resistant individuals, like dithiocarbamates and chlorothalonil, and because of this, it had been used in combination with

high-risk compounds to diminish the possibility of fungal resistance (Oliver & Hewitt 2014). The resistance risk for many groups of fungicides is discussed in Frac (2021).

Table 1 Fungicide mode of action used in tomato crop in Brazil and its modes of action

Active ingredient	Group	Mode of action of fungicides
Thiophanate-methyl	Benzimidazole	Mitosis: assembly of β-tubulin (Frac 2021)
Difenoconazole Metconazole Tebuconazole Trifloxistrobin Tetraconazole	Triazole	Demethylation of C-14 in sterol biosynthesis (Frac 2021)
Mancozeb Metiram Propineb	Dithiocarbamate	Multi-site contact activity (Frac 2021)
Azoxystrobin Pyraclostrobin	Strobilurins	Breathing complex 3: ubiquinol oxidase, local Qo (Frac 2021)
Chlorothalonil	Chloronitriles	Multi-site contact activity (Frac 2021)
Fluxapyroxad Boscalid	Carboxamide	Breathing complex 2: succinate dehydrogenase inhibitors (Frac 2021)
Benzalkonium chloride	Quaternary ammonium compound	Perturbation and disruption of the membrane bilayers (Wessels & Ingmer 2013)
Fluazinam	Phenylpyridinylamine	Mitochondrial oxidative phosphorylation inhibitors (Vitoratos 2014)
Cymoxanil	Acetamide	Might inhibits nucleic acid and protein biosynthesis induced via interaction with host metabolic processes (Joshi 2003)
Isopropyl benthiavalicarb	Valinamide carbamate	Inhibits processes involved in cell-wall biosynthesis and assembly (Gisi et al. 2019)
Pyrimethanil	Anilino-pyrimidine	Methionine aminoacid biosynthesis inhibition, which affects the protein synthesis (Frac 2021)
Iprodione Procimidone	Dicarboxamide	Involve the interference with kinase signalling (Frac 2021)
Captan	Dicarboxamide (phthalimides)	React with enzyme sulfhydryl groups but may also attack amino groups and inhibit enzymes that do not contain sulfhydryl groups (Luo & Lewis 1992)
Kasugamycin	Antibiotic	Binds to the ribosomal subunit 30S and inhibit the protein elongation (Okuyama et al. 1971)
Mandipropamid	Mandelic acid amides	Target the enzyme cellulose synthase that affect the cell wall biosynthesis (Frac 2021)
Propamocarb hydrochloride	Carbamate	The action is related to membrane function, causing an efflux of cell compounds (Kilian & Steiner 2003, Papavizas et al. 1978)
Fluopicolide	Pyridinylmethyl-benzamide	Affect spectrin-like proteins in the cytoskeleton of oomycetes (Toquin et al. 2019)
Metalaxyl-M	Acylalanines	Inhibiting ribosomal RNA synthesis via the RNA polymerase I-template complex, which disrupts the protein synthesis (Fisher & Hayes 1982)
Dimethomorph	Carboxylic acid amides	Affect the cellulose synthase, which interfere in the cell wall biosynthesis (Kuhn et al. 1991)
Famoxadone	Oxazolidinedione	Quinone outside inhibitors affecting the respiration process (Frac 2021)
Copper oxychloride Copper hydroxide Cuprous oxide	Inorganic	Inactivate enzymes, which possess sulfhydryl, hydroxyl, amino or carboxyl groups, leading to a general disruption of metabolism and breakdown of cell integrity (Frac 2021)

Scientific data on the efficiency of fungicides in controlling tomato septoriosis are scarce, highlighting the use of chlorothalonil (Poysa & Tu 1993) and mancozeb (Dillard et al. 1997). The objective of this work was to determine the efficiency of registered and non-registered fungicides for septoriose at the label dosage in Brazil to control this disease.

Materials & Methods

In vitro assays

Mycelial growth evaluation of *Septoria* when in contact with the registered fungicides at the label dosage

The fungicides were mixed with the culture medium malt-extract (20g malt extract/L and 20g agar/L) at the recommended dose for field application (Table 2) to determine the efficiency of the product in inhibiting mycelial growth. The experiment was conducted in Petri dishes of 9cm. After pouring the culture media mixed with the fungicide, a mycelial disc of 0.5cm was placed at the centre. The mycelial growth was measured after 14 days of incubation at 25ºC and a photoperiod of 12h. The data was transformed into a percentage of the control. The experiment was repeated twice.

Table 2 Concentration (part per million of the active ingredient-ppm) and Frac group code of the registered fungicides for *Septoria* control in Brazil

Registered fungicides	ppm of a.i[1] recommended	FRAC code
Thiophanate-methyl	490	B1
Azoxystrobin	80	C3
Benzalkonium chloride	250	-
Propineb	2100	M3
Tebuconazole	213	G1
Cuprous oxide	1344 (1200)[2]	M1
Metconazole	90	G1
Metiram + Pyraclostrobin	1100+100	M3+C3
Chlorothalonil	1500	M5
Fluazinam + Thiophanate-methyl	375+375	B1+C5
Difenoconazole	125	G1
Mancozeb	4000	M3
Fluxapyroxad + Pyraclostrobin	58.5+116.6	C2+C3
Pyraclostrobin	100	C3
Tetraconazole	75	G1

[1]Active ingredient

[2]The value between parentheses is the amount of metallic copper present

Germination of *Septoria* spores when in contact with the registered fungicides at the label dose – Method 1

The fungicides were mixed with the culture medium water-agar (20g agar/L) at the recommended dose for field application (Table 2) to determine the efficiency of the active ingredient in inhibiting spore germination. The experiment was conducted in Petri dishes of 9cm. After pouring the culture media mixed with the fungicide, 100µL of a spore suspension at 10^5 spores/mL was spread over the culture media surface. *S. lycopersici* spores were produced by the method proposed by Monteiro et al. (2019). The spore germination was measured after 2 days from incubation at 25ºC and a photoperiod of 12h. The data was transformed into a percentage of the control. The experiment was repeated twice.

Germination of *Septoria* spores when in contact with the registered fungicides at the label dose – Method 2

The efficiency of the fungicides in inhibiting spore germination was tested by another

method. The experiment was conducted in Petri dishes of 9cm. After pouring the culture media malt extract (malt extract 20 g/L and agar 20 g/L), 100µL of a spore suspension at 10^5 spores per ml was spread over the culture media surface using a Drigalski handle. In the centre of the Petri dish was placed 10 µL of the fungicides solution at the recommended dose. The inhibition halo was measured after 14 days of incubation at 25ºC and a photoperiod of 12h. The data was transformed into a percentage of the control. The experiment was repeated twice.

Mycelial growth influenced by a range of concentrations of some registered fungicide

The fungicides azoxystrobin, chlorothalonil, cuprous oxide, difenoconazole, mancozeb, metconazole, methyl-thiophanate, propineb, pyraclostrobin, tebuconazol and tetraconazole were mixed in malt culture medium for the final doses of 50, 100, 1000, 2000 and 4000 ppm of active ingredients to know the effect of growing doses on the mycelial growth. Five isolates with morphology differences were used to show how the efficiency of control might change with the *Septoria* isolate.

Comparison between pyraclostrobin and pyraclostrobin + fluxapyroxad in equivalent doses

The experiment was performed to know the effect of the pyraclostrobin in the control of the spore germination of *Septoria*. The dose for pyraclostrobin was 116.6 ppm and for fluxapyroxad + pyraclostrobin were 58.5 and 116.6 ppm, respectively. The experiment was conducted in Petri dishes of 9cm. After pouring the culture media malt extract (malt extract 20 g/L and agar 20 g/L), 100µL of a spore suspension at 10^5 spores/mL was spread over the culture media surface using a Drigalski handle. In the centre of the Petri dish was placed 10 µL of the fungicides solution at the recommended dose. The inhibition halo was measured after 14 days of incubation at 25ºC and a photoperiod of 12h.

***In vivo* assays**

Incidence and severity of septoriose under the effect of the registered fungicides at the label dose in the greenhouse

Tomato plants were cultivated in vessels with five-litre of capacity. When plants reach four leaflets completely developed, fungicides at the recommended doses (Table 2) were sprayed until the rain off point (1,000L/ha). The water used to prepare the fungicides solutions had pH 7. After two hours from the fungicides spraying, a spore suspension at 10^5 spores/mL was sprayed over plants. Plants were incubated in a greenhouse for 14 days. After this period, the incidence and severity of the disease were evaluated using a diagrammatic scale for *S. lycopersici* of the tomato (Monteiro et al. 2021) and via Measure Picture software. The experiment was replicated three times. The data were transformed to the percentage of the control. A fungicide was considered efficient when it was able to control above 70% of disease incidence and severity compared to the check (without fungicide pulverization).

Incidence and severity of septoriose under the effect of fungicides used to control other diseases (without register for septoriose control) in tomato crops at the label dosage in the greenhouse

To know the effect of the other fungicides not registered for septoriose control, but that is applied in the tomato crop for the control of other pathogens such as *Colletotrichum*, *Fulvia*, *Stemphylium*, *Phytophthora*, *Alternaria*, *Sclerotinia*, *Erwinia*, *Clavibacter* or *Xanthomonas*, we performed an experiment using the major doses in commercial product labels against those pathogens (Table 3). The experiment was performed in the same way as for the recommended fungicides experiment. The experiment was repeated twice. A fungicide was considered efficient when it controlled above 70% of incidence and severity of the disease compared to the check (without fungicide pulverization).

Table 3 Non-registered fungicides for *Septoria* control used in the tomato crop to control other diseases and Frac group codes

Fungicides	ppm of a.i[2] recommended	FRAC code
Cymoxanil + Famoxadone	240+180	Unknown+C3
Isopropyl benthiavalicarb + Chlorothalonil	93.75+937.50	H5+M5
Pyrimethanil	900	D1
Iprodione	750	E3
Kasugamycin	60	D3
Procymidone	750	E3
Mandipropamid	150	H5
Propamocarb hydrochloride	2166	F4
Propamocarb hydrochloride + Fluopicolide	937.5+93.75	F4+B5
Chlorothalonil + Metalaxyl-M	1200+120	M5+A1
Captan	1200	M4
Dimethomorph	750	H5
Trifloxystrobin + Tebuconazole	75+150	C3+G1
Boscalid	75	C2
Azoxystrobin + Diphenoconazole	80+50	C3+G1
Fluazinam	500	C5
Metiram	2100	M3
Copper oxychloride	1680(1000)[1]	M1
Copper hydroxide	2073(1350)[1]	M1
Metalaxyl-M + Mancozeb	120+1920	A1+M3
Cymoxanil + Mancozeb	240+1920	Unknown+M3
Isopropyl bentiavalicarb + Fluazinam	70+175	H5+C5
Copper oxychloride + Mancozeb	600(340)[1]+880	M1+M3
Famoxadone + Mancozeb	100+1000	C3+M3
Azoxystrobin + Mancozeb	166.65+2333.10	C3+M3

[1]The value between parentheses is the amount of metallic copper present
[2]Active ingredient

Curative effect of the fungicides applied after seven days from spore pulverization

Tomato plants were cultivated in vessels with five-litre of capacity. When plants reach four leaflets completely developed, the spore suspension at 10^5 spores/mL was sprayed over plants. The fungicides were applied after the appearance of the first symptom (seven days after spores pulverization) at the doses in commercial product labels (Table 2). The water used to prepare the fungicides solutions had pH 7. Plants were incubated in a greenhouse for 14 days. After this period, the incidence and severity of the disease were evaluated using a diagrammatic scale for *S. lycopersici* of the tomato (Monteiro et al. 2021) and via Measure picture software. The experiment was replicated twice. The data were transformed to percentage of the control.

Control of *S. lycopersici* in the field comparing a fungicide with seven days of withholding period and another with one day of withholding period

We performed a field experiment with pyraclostrobin + fluxapiroxad and pyraclostrobin at the doses in commercial product labels (Table 2) to study whether using a fungicide with a lower withholding period, since the harvest of tomatoes can be made three times per week. In addition, this experiment was performed to know the contribution of the pyraclostrobin (1 day of withholding period), when it is used together with fluxapiroxad (7 days of withholding period). Tomato plants were cultivated in a field. The space was 45cm and 1m between plants and lines, respectively, and each line had 20 tomato plants. When plants reach four leaflets completely developed, the fungicides at the recommended doses were sprayed until the rain off point (1,000L/ha). The water used to prepare the fungicides solutions had pH 7. After two hours from the fungicides spraying, a spore suspension at 10^5 spores/mL was sprayed over plants. After 14 days

from the pulverization, the incidence and the severity of the disease was evaluated by using a diagrammatic scale for *S. lycopersici* of the tomato (Monteiro et al. 2021) and by via Measure picture software. The data was transformed to the percentage of the control. A fungicide was considered efficient when it was able to control above 70% of the incidence and severity of the disease compared to the check (without fungicide pulverization).

Phytotoxicity effect of fungicides applied on tomato plants

Some fungicides had a phytotoxic effect while conducting the previous experiments. Because of that, we experimented to confirm this deleterious effect at the recommended dose. Nine plants and three replicates composed each treatment. This experiment was performed with metconazole (90ppm), pyraclostrobin (100ppm), pyrimethanil (900ppm), tebuconazole (213ppm), tetraconazole (75ppm) and one treatment without fungicide pulverization.

Statistical analyses

The results were submitted to analysis of variance, when significant by the F test, the means were compared by the Scott-Knott statistical test at 5% of probability ($P \leq 0.05$).

Results

Excepted for the fungicides thiophanate-methyl and azoxystrobin, all fungicides control over 80% of the *S. lycopersici* mycelial growth. By method 1, thiophanate-methyl and azoxystrobin were the only fungicides that allowed some spore germination. By method 2, the fungicides that prevent the germination of the spores were fluxapyroxad + pyraclostrobin, mancozeb, difenoconazole, fluazinam + thiophanate-methyl, benzalkonium chloride, chlorothalonil and metiram + pyraclostrobin (Table 4).

Table 4 *In vitro* assays for fungicides registered to control *S. lycopersici* in tomato crop

Registered fungicides	Mycelial growth		Spore germination			
	Control (%)	Control (%)[1]	Method 1		Method 2	
			Control (%)	Control (%)[1]	Control (%)	Control (%)[1]
Check	0 c[3]	0 e	0 d	0 d	0 g	0 f
Thiophanate-methyl	50.82± 28.81[2] b	33.25± 12.26 d	95.84± 1.32 b	98.61± 0.60 b	0 g	0 f
Azoxystrobin	53.86± 5.27 b	47.80± 4.23 c	92.41± 1.02 c	97.91± 1.04 c	0 g	0 f
Benzalkonium chloride	94.34± 6.27	85.54± 1.55 b	100 a	100 a	12.04± 1.79 e	13.89± 1.81 d
Propineb	100 a	100 a	100 a	100 a	0 g	0 f
Tebuconazole	100 a	100 a	100 a	100 a	0 g	0 f
Cuprous oxide	100 a	100 a	100 a	100 a	0 g	0 f
Metconazole	100 a	100 a	100 a	100 a	0 g	0 f
Metiram + Pyraclostrobin	100 a	100 a	100 a	100 a	1.85± 1.60 g	8.04± 3.72 e
Chlorothalonil	100 a	100 a	100 a	100 a	7.59± 5.01 f	27.47± 2.04 b
Fluazinam + Thiophanate-methyl	100 a	100 a	100 a	100 a	19.82± 2.25 d	16.08± 3.17 d
Difenoconazole	100 a	100 a	100 a	100 a	28.52± 5.04 c	19.70± 4.07 c
Mancozeb	100 a	100 a	100 a	100 a	48.52± 5.16 b	47.38± 1.98 a
Fluxapyroxad + Pyraclostrobin	100 a	100 a	100 a	100 a	61.48± 2.25 a	46.27± 1.18 a
CV (%)	7.09	3.48	0.24	0.17	11.99	11.44

[1]Experiment replication
[2]Values presented after the symbol ± are standard deviations
[3]The same means in columns were not different compared by the Scott-Knott statistical test at 5% of probability ($P \leq 0.05$)

Mancozeb, difenoconazole, metconazole, tetraconazole, chlorothalonil and propineb at the doses of 50, 100, 1000, 2000 and 4000ppm active ingredients controlled 100% of the mycelial growth of five *S. lycopersici* isolates. Tebuconazole allows the growth of two isolates at the 50ppm active ingredient. Pyraclostrobin, methyl-thiophanate, cuprous oxide and azoxystrobin control depend on the concentration and *Septoria* isolate (Fig. 1). Azoxystrobin, methyl-thiophanate and pyraclostrobin at the recommended dose seem not sufficient to control 100% of the mycelial growth (Fig. 1).

For incidence, considering the average value of the percentage of control of the three replicates, the fungicides that had the percentage of control above 70% were fluxapyroxad+pyraclostrobin, mancozeb, difenoconazole, chlorothalonil, propineb and fluazinam+thiophanate-methyl (95.64; 89,36; 89.14; 75.69; 70.66 and 70.42% of control, respectively). For severity, the fungicides that had the percentage of control above 70%, on average of the percentage of control of the three replicates were fluxapyroxad + pyraclostrobin, mancozeb, difenoconazole, chlorothalonil, propineb, fluazinam + thiophanate-methyl and metiram+pyraclostrobin (97.77; 89.89; 92.26; 86.89; 78.97; 85.09 and 75.48% of control, respectively) (Table 5).

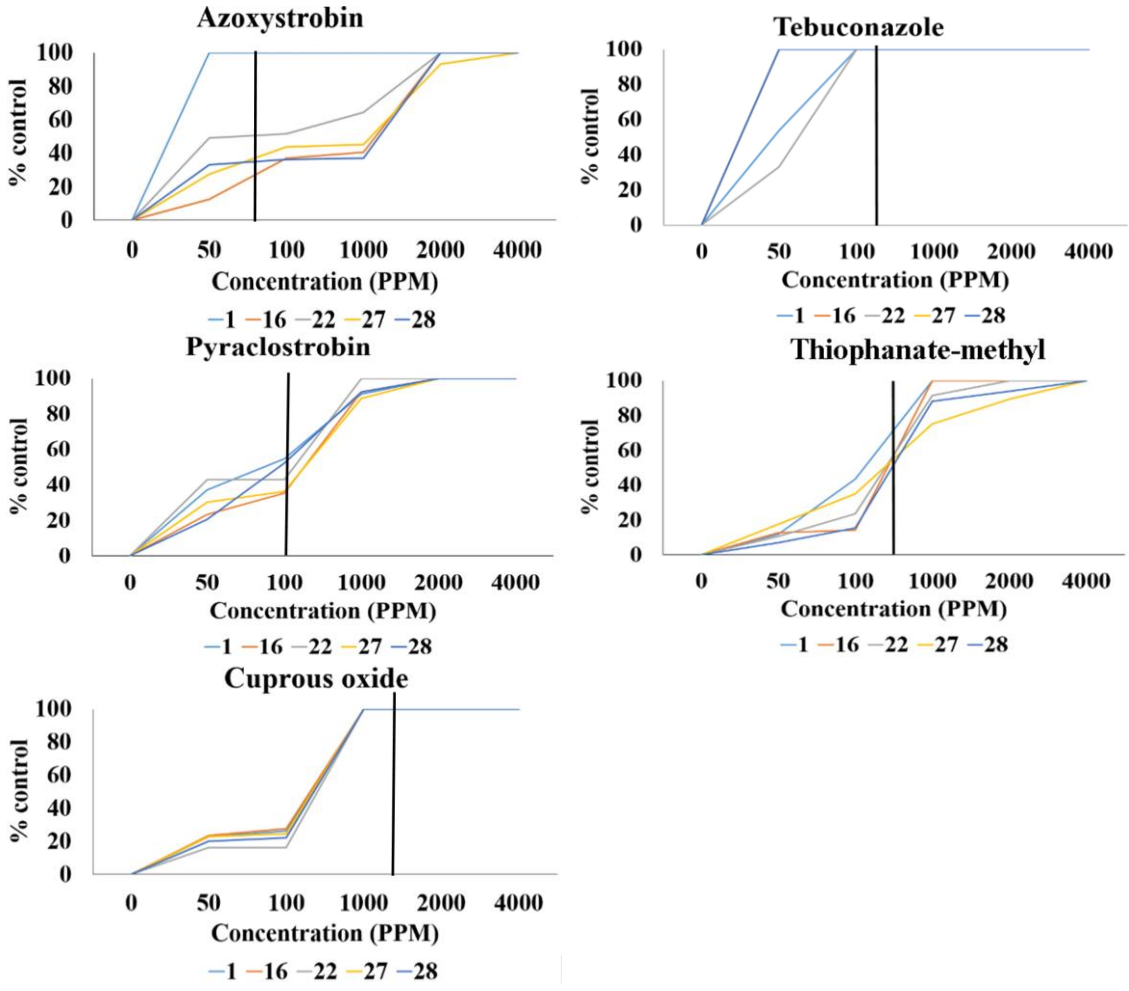

Fig. 1 – Percentage of control of the mycelial growth of *S. lycopersici* under five doses of the registered fungicides. Black vertical bars indicate the recommended doses of the active ingredient and colored lines indicate the *S. lycopersici* isolates.

After the appearance of the first symptoms, seven days from the spores pulverization, no fungicides was considered efficient, because they did not control above 70% of the incidence and severity compared to the treatment without fungicides (Table 6).

Table 5 *In vivo* assays measuring incidence and severity of *S. lycopersici* affected by the preventive pulverization of the registered fungicides

Registered fungicides	Exp. 1		Exp. 2		Exp. 3		Exp. 1		Exp. 2		Exp. 3	
	Incidence	Control (%)	Incidence	Control (%)	Incidence	Control (%)	Severity	Control (%)	Severity	Control (%)	Severity	Control (%)
Fluxapyroxad + Pyraclostrobin	3.25± 2.17 a[23]	95.24± 3.17	0.00± 0.00 a	100.00± 0.00	3.00± 1.47 a	91.67± 4.09	0.20± 0.15 a	99.80± 0.15	0.00± 0.00 a	100.00± 0.00	6.44± 2.95 a	93.52± 2.97
Mancozeb	5.75± 1.79 a	91.58± 2.62	5.75± 3.11 b	91.32± 4.70	5.33± 2.63 a	85.19± 7.29	8.76± 4.43 a	91.25± 4.43	0.76± 0.31 a	91.82± 3.38	13.33± 5.14 a	86.59± 5.17
Difenoconazole	7.25± 4.66 a	89.38± 6.82	3.50± 2.29 b	94.72± 3.46	6.00± 2.16 a	83.33± 6.00	0.51± 0.35 a	99.49± 0.35	0.37± 0.35 a	96.00± 3.78	18.58± 9.30 a	81.30± 9.36
Chlorothalonil	22.50± 6.18 b	67.03± 9.06	11.75± 2.95 c	82.26± 4.45	8.00± 1.41 b	77.78± 3.93	9.86± 4.25 b	90.14± 4.25	0.79± 0.34 a	91.48± 3.64	20.83± 12.18 b	79.04± 12.25
Propineb	24.00± 8.06 b	64.84± 11.81	10.50± 2.60 c	84.15± 3.92	13.33± 4.50 c	62.99± 12.49	27.24± 20.00 c	72.77± 20.00	0.83± 0.32 a	91.06± 3.44	26.75± 21.79 b	73.08± 21.93
Fluazinam + Thiophanate-methyl	34.00± 6.44 c	50.18± 9.44	10.75± 2.95 c	83.77± 4.45	8.17± 3.17 b	77.31± 8.81	10.85± 3.28 b	89.15± 3.28	1.04± 0.50 a	88.77± 5.44	22.50± 9.19 b	77.36± 9.24
Metiram + Pyraclostrobin	43.50± 2.60 d	36.26± 3.81	17.75± 9.26 c	73.21± 13.98	6.33± 3.30 a	82.41± 9.17	27.61± 19.63 c	72.39± 19.63	1.48± 0.80 a	84.07± 8.69	29.83± 16.28 b	69.98± 16.38
Benzalkonium chloride	45.00± 3.67 d	34.07± 5.38	20.00± 11.51 c	69.81± 17.38	23.00± 5.89 c	36.11± 16.36	25.87± 11.13 c	74.13± 11.13	3.33± 1.55 a	64.05± 16.71	41.25± 13.62 b	58.49± 13.70
Cuprous oxide	58.00± 20.32 e	15.02± 29.78	41.00± 14.73 d	38.11± 22.24	20.83± 7.53 c	42.13± 20.92	33.54± 2.63 c	66.46± 2.63	16.86± 10.80 c	0.00± 0.00	63.13± 38.71 c	36.48± 38.95
Thiophanate-methyl	64.00± 28.10 e	6.23± 41.17	52.00± 23.88 d	21.51± 36.04	17.67± 6.36 c	50.92± 17.65	95.20± 7.25 d	4.80± 7.25	22.46± 11.99 c	0.00± 0.00	36.56± 40.74 b	63.21± 40.99
Azoxystrobin	64.50± 11.97 e	5.49± 17.54	52.50± 14.53 d	20.75± 21.94	33.00± 5.66 d	8.33± 15.71	92.22± 10.41 d	7.78± 10.41	20.22± 14.46 c	0.00± 0.00	99.17± 1.18 d	0.21± 1.19
Check	68.20± 7.98 e	0.00± 0.00	66.25± 7.79 e	0.00± 0.00	36.00± 7.75 d	0.00± 0.00	100.00± 0.00 d	0.00± 0.00	9.26± 5.46 b	0.00± 0.00	98.38± 1.25 d	0.00± 0.00
Metconazole[1]	-	-	-	-	-	-	-	-	-	-	-	-
Tebuconazole[1]	-	-	-	-	-	-	-	-	-	-	-	-
CV (%)	**14.68**		**21.14**		**17.10**		**17.17**		**36.97**		**25.06**	

[1]Those fungicides had a phytotoxic effect

[2]Values presented after the symbol ± are standard deviations

[3]Means followed by the same letters in columns were not different when compared by the Scott-Knott statistical test at 5% of probability ($P \leq 0.05$)

Table 6 Fungicides applied after seven days from spores pulverization as curative treatment

Registered fungicides	Exp. 1		Exp. 2		Exp. 1		Exp. 2	
	Incidence	Control (%)	Incidence	Control (%)	Severity	Control (%)	Severity	Control (%)
Fluxapyroxad + Pyraclostrobin	9.50± 1.91 a[23]	51.28± 9.82	16.50± 5.00 a	53.52± 14.09	26.88± 8.75 [ns]	63.56± 11.86	54.38± 35.14 a	45.63± 35.14
Metiram + Pyraclostrobin	11.00± 3.74 a	43.59± 19.19	27.75± 9.57 b	21.83± 26.96	39.38± 17.49	46.61± 23.71	100.00± 0.00 c	0.00± 0.00
Difenoconazole	10.00± 2.45 a	48.72± 12.72	18.25± 4.19 a	48.59± 11.81	56.88± 30.23	22.88± 40.99	73.75± 20.56 b	26.25± 20.56
Tetraconazole	11.50± 4.12 a	41.03± 21.14	19.25± 3.30 a	45.77± 9.31	40.00± 29.58	45.76± 40.11	31.25± 22.78 a	68.75± 22.78
Propineb	14.67± 2.05 b	24.78± 10.54	29.00± 3.56 b	18.31± 10.03	56.88± 49.81	22.88± 67.53	100.00± 0.00 c	0.00± 0.00
Mancozeb	14.00± 4.32 b	28.21± 22.16	27.33± 1.89 b	23.00± 5.31	43.75± 26.97	40.68± 36.56	81.25± 37.50 c	18.75± 37.50
Tebuconazole[1]	12.75± 2.22 a	34.62± 11.37	20.75± 4.92 a	41.55± 13.87	36.88± 10.08	50.00± 13.67	68.13± 31.45 b	31.88± 31.45
Fluazinam + Thiophanate-methyl	13.50± 3.87 a	30.77± 19.86	21.25± 4.27 a	40.14± 12.03	65.00± 25.58	11.86± 34.68	100.00± 0.00 c	0.00± 0.00
Benzalkonium chloride	14.00± 1.83 b	28.21± 9.36	30.25± 4.03 b	14.79± 11.36	65.00± 37.64	11.86± 51.04	100.00± 0.00 c	0.00± 0.00
Pyraclostrobin	15.50± 1.73 b	20.51± 8.88	28.00± 3.74 b	21.13± 10.54	60.63± 17.37	17.80± 23.55	100.00± 0.00 c	0.00± 0.00
Thiophanate-methyl	15.75± 2.06 b	19.23± 10.57	26.25± 8.81 b	26.06± 24.81	73.75± 20.87	0.00± 28.29	100.00± 0.00 c	0.00± 0.00
Metconazole[1]	15.75± 4.79 b	19.23± 24.55	24.50± 4.51 b	30.99± 12.70	68.75± 30.03	6.78± 40.73	46.25± 21.26 a	53.75± 21.26
Azoxystrobin	16.25± 7.63 b	16.67± 39.14	26.25± 4.79 b	26.06± 13.48	72.50± 34.03	1.69± 46.15	100.00± 0.00 c	0.00± 0.00
Cuprous oxide	17.00± 4.69 b	12.82± 24.05	28.25± 5.74 b	20.42± 16.16	36.88± 5.15	50.00± 6.99	100.00± 0.00 c	0.00± 0.00
Chlorothalonil	19.50± 5.80 b	0.00± 29.75	23.75± 2.87 b	33.10± 8.09	60.63± 29.47	17.80± 39.95	100.00± 0.00 c	0.00± 0.00
Check	19.50± 3.11 b	0.00± 0.00	35.50± 5.69 b	0.00± 0.00	73.75± 24.71	0.00± 0.00	100.00± 0.00 c	0.00± 0.00
CV (%)	12.64	-	9.76	-	24.85	-	20.33	-

[1]Those fungicides had a phytotoxic effect
[2]Values presented after the symbol ± are standard deviations
[3]Means followed by the same letters in columns were not different when compared by the Scott-Knott statistical test at 5% of probability ($P \leq 0.05$)

Fungicides used in the tomato crop to control other diseases were not able to control above 70% of the incidence of *S. lycopersici* on average. For severity, the fungicides which have a percentage of control above 70% were boscalide, cymoxanil + mancozeb, azoxystrobin + mancozeb, metalaxyl-M + mancozeb and metalaxyl-M + chlorothalonil (88.73; 81.91; 80.35; 79.38 and 77.03%, on average), when applied preventively (Table 7).

Table 7 *In vivo* assays measuring incidence and severity of septoriose affected by preventive treatment used in the tomato crop to control other diseases

Non-registered fungicides	Exp. 1		Exp. 2		Exp. 1		Exp. 2	
	Incidence	Control (%)	Incidence	Control (%)	Severity	Control (%)	Severity	Control (%)
Azoxystrobin + Mancozeb	14.00± 5.72 a[23]	49.09± 20.78	22.50± 7.14 b	56.94± 6.54	20.38± 13.72 a	76.63± 13.72	15.94± 10.96 b	84.06± 10.96
Cymoxanil + Mancozeb	14.75± 2.36 a	46.36± 8.59	13.25± 6.80 a	74.64± 13.02	23.13± 4.84 a	76.88± 4.84	13.06± 10.31 b	86.94± 10.31
Metalaxyl-M + Mancozeb	16.75± 7.89 a	39.09± 28.69	25.75± 4.99 b	50.72± 9.55	25.00± 11.73 a	75.00± 11.73	16.25± 3.06 b	83.75± 3.06
Boscalid	18.00± 5.83 a	34.55± 21.20	7.50± 3.11 a	85.65± 5.95	18.50± 9.95 a	81.50± 9.95	4.06± 1.53 a	95.94± 1.53
Metalaxyl-M + Chlorothalonil	20.00± 5.10 a	27.27± 18.54	24.50± 5.80 b	53.11± 11.11	21.25± 7.14 a	78.75± 7.14	24.69± 17.51 c	75.31± 17.51
Copper oxychloride	23.00± 4.08 b	16.36± 14.85	46.00± 6.06 d	11.96± 11.59	100.00± 0.00 d	0.00± 0.00	100.00± 0.00 e	0.00± 0.00
Propamocarb hydrochloride + Fluopicolide	23.75± 2.63 b	13.64± 9.56	39.25± 3.59 c	24.88± 6.88	100.00± 0.00 d	0.00± 0.00	100.00± 0.00 e	0.00± 0.00
Propamocarb hydrochloride	24.00± 2.45 b	12.73± 8.91	40.50± 6.03 c	22.49± 11.54	100.00± 0.00 d	0.00± 0.00	100.00± 0.00 e	0.00± 0.00
Procymidone	24.00± 4.97 b	12.73± 18.06	39.75± 12.20 c	23.92± 23.36	100.00± 0.00 d	0.00± 0.00	100.00± 0.00 e	0.00± 0.00
Famoxadone + Mancozeb	24.50± 5.07 b	10.91± 18.42	24.25± 4.50 b	53.59± 8.61	48.13± 19.08 b	51.88± 19.08	16.56± 8.50 b	83.44± 8.50
Metiram	24.75± 1.26 b	10.00± 4.58	41.00± 5.83 c	21.53± 11.16	76.88± 2.39 c	23.13± 2.39	28.75± 10.85 c	71.25± 10.85
Copper hydroxide	25.25± 2.87 b	8.18± 10.44	46.25± 3.30 d	11.48± 6.32	100.00± 0.00 d	0.00± 0.00	100.00± 0.00 e	0.00± 0.00

Table 7 Continued.

Non-registered fungicides	Exp. 1		Exp. 2		Exp. 1		Exp. 2	
	Incidence	**Control (%)**	**Incidence**	**Control (%)**	**Severity**	**Control (%)**	**Severity**	**Control (%)**
Azoxystrobin + Diphenoconazole	26.00± 2.94 b	5.45± 10.71	40.50± 3.32 c	22.49± 6.35	37.19± 7.10 b	62.84± 7.10	50.00± 17.56 d	50.00± 17.56
Trifloxystrobin + Tebuconazole	26.75± 4.11 b	2.73± 14.96	43.50± 7.14 d	16.75± 13.67	85.00± 22.45 c	15.00± 22.45	80.63± 13.75 e	19.38± 13.75
Captan	27.00± 2.94 b	1.82± 10.71	37.50± 5.57 c	28.23± 10.66	72.50± 13.69 c	27.50± 13.69	39.69± 21.32 d	60.31± 21.32
Isopropyl bentiavalicarb + Fluazinam	28.25± 4.99 b	0.00± 0.00	39.75± 3.95 c	23.92± 7.56	100.00± 0.00 d	0.00± 0.00	36.56± 22.04 d	63.44± 22.04
Iprodione	28.25± 4.35 b	0.00± 0.00	54.75± 8.38 d	0.00± 0.00	100.00± 0.00 d	0.00± 0.00	100.00± 0.00 e	0.00± 0.00
Dimethomorph	28.75± 3.50 b	0.00± 0.00	37.00± 6.22 c	29.19± 11.90	100.00± 0.00 d	0.00± 0.00	100.00± 0.00 e	0.00± 0.00
Isopropyl bentiavalicarb + Chlorothalonil	28.75± 7.27 b	0.00± 0.00	36.00± 6.88 c	31.10± 13.17	47.50± 14.72 b	52.50± 14.72	24.69± 6.32 c	75.31± 6.32
Mandipropamid	29.00± 4.32 b	0.00± 0.00	46.00± 5.16 d	11.96± 9.88	100.00± 0.00 d	0.00± 0.00	100.00± 0.00 e	0.00± 0.00
Cymoxanil + Famoxadone	29.25± 4.03 b	0.00± 0.00	39.50± 11.93 c	24.40± 22.83	100.00± 0.00 d	0.00± 0.00	100.00± 0.00 e	0.00± 0.00
Copper oxychloride + Mancozeb	29.75± 10.44 b	0.00± 0.00	36.75± 2.87 c	29.67± 5.50	94.38± 6.57 d	5.63± 6.57	40.31± 6.16 d	59.69± 6.16
Kasugamycin	31.25± 2.75 b	0.00± 0.00	45.75± 7.93 d	12.44± 15.18	100.00± 0.00 d	0.00± 0.00	100.00± 0.00 e	0.00± 0.00
Fluazinam	31.50± 4.12 b	0.00± 0.00	25.50± 9.98 b	51.20± 19.11	61.88± 11.06 c	38.13± 11.06	13.19± 6.69 b	86.81± 6.69
Check	27.50± 5.51 b	-	52.25± 7.14 d	-	100.00± 0.00 d	-	100.00± 0.00 e	-
Pyrimethanil[1]	-	-	-	-	-	-	-	-
CV (%)	9.22		9.68		8.74		12.08	

[1]Those fungicides had a phytotoxic effect

[2]Values presented after the symbol ± are standard deviations

[3]Means followed by the same letters in columns were not different when compared by the Scott-Knott statistical test at 5% of probability ($P \leq 0.05$)

In the field experiment, for the check treatment the disease incidence and severity was 89.28 ± 6.40 and 100 ± 0.00, respectively. The pyraclostrobin alone was ineffective against septoriose since the disease incidence and severity was 87.56 ± 3.19 (1.94% ± 3.57 of control) and 100 ±

0.00 (0% of control), respectively. While for the pyraclostrobin + fluxapiroxad the disease incidence and severity was 4.08 ± 3.58 (95.42 ± 4.01 of control) and 0.62 ± 0.15 (99.31 ± 0.17 of control), respectively. The coefficient of variation for incidence and severity was 7.83% and 12.75%, respectively.

Comparing the results *in vitro* between pyraclostrobin and pyraclostrobin + fluxapyroxad in equivalent doses of pyraclostrobin, we observed that pyraclostrobin did not control the *S. lycopersici*, because there was no halo inhibition formed, while pyraclostrobin + fluxapyroxad induced an inhibition halo of 19.33 ± 2.63 mm.

Pyraclostrobin pulverization had no harmful effect on the tomato leaves compared to the plants without fungicides application (Fig. 2b). Pyrimethanil pulverization caused white-brown spots on the tomato leaves (Fig. 2d). Metconazole and tebuconazole deformed and delayed leaves development (Fig. 2c, e). Tetraconazole promoted the shriveling of the leaves (Fig. 2f). The harmful effect of some fungicides also affected the growth of the plant, since the height (mm) of the check, pyraclostrobin, pyrimethanil, metconazole, tebuconazole and tetraconazole was 176.72mm ± 7.08, 174.19mm ± 10.07, 174.06mm ± 6.99, 138.87mm ± 15.50, 124.05mm ± 10.33 and 91.05mm ± 14.04, respectively.

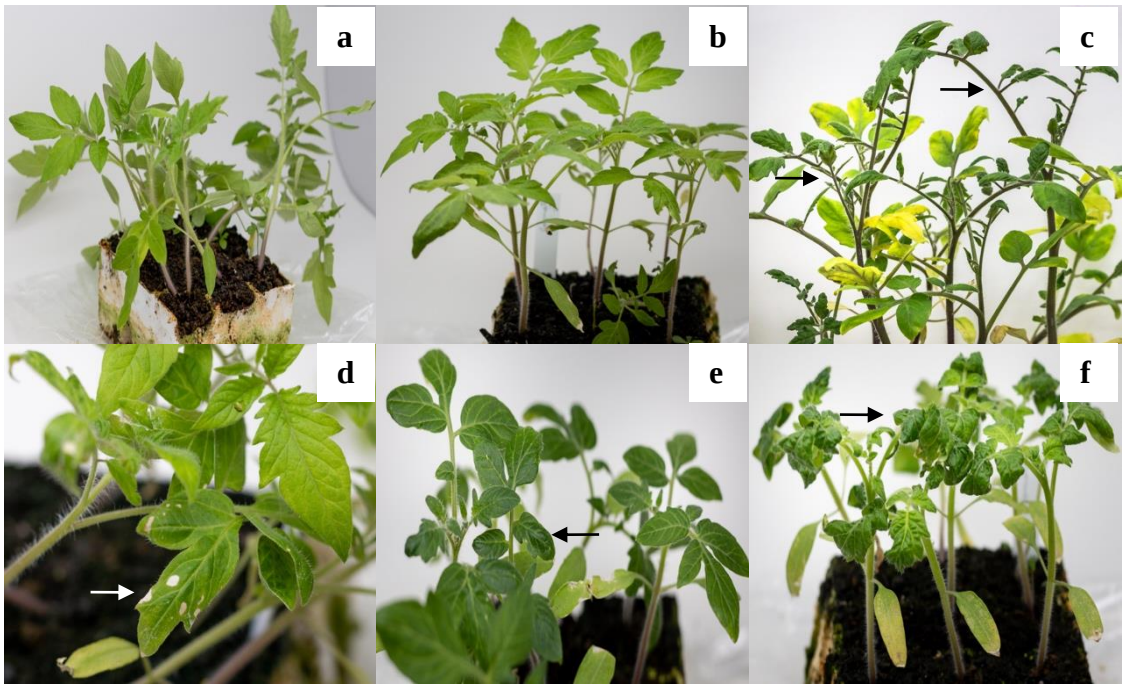

Fig. 2 – Phytotoxicity effect of fungicides applied on tomato leaves. a Tomato plants without the application of fungicides. b with the application of pyraclostrobin-100ppm. c metconazole-90ppm. d pyrimethanil-900ppm. e tebuconazole-213ppm. f tetraconazole-75ppm. Arrows indicate the specific symptom of the phytotoxicity triggered by the fungicide. Photos by André Sezerino.

Discussion

One of the first experiments carried out to determine which active ingredients and doses are efficient in controlling plant pathogens was *in vitro*. In our work, considering the *in vitro* experiments for mycelial growth control and spore germination-method 1 (Table 4), there seems to be no adequate separation between fungicides that have high efficiency from those that do not have, when applied to plants. In fact, *in vitro* test tends to generate many false-positive results, which occur when a compound that inhibits growth in the plate assay fails to inhibit growth in the plant (Oliver & Hewitt 2014). Sometimes there is no relationship between the IC_{50} determined in the laboratory and the recommended dose for use in crops (Reis et al. 2016).

On the other hand, method 2 of inhibiting spore germination seems to demonstrate the reality of what happens *in vivo*, when the fungicide is applied to tomato plants, aiming to control

S. lycopersici, and with the advantage of not needing a microscope for the spore count. In general, the greater the halo of inhibition caused by the fungicide in method 2, the more efficient the fungicide was in controlling the incidence and severity of *S. lycopersici* in tomato plants (Tables 4, 5). In this context, we believe that the proposed method can be a fast and effective method for determining fungicide efficiency, aiming at field control. The disadvantage of *in vitro* methods is that it is impossible to know any deleterious effects to plants caused by applying some fungicides in some doses. *In vivo* screening is the most time-consuming and expensive and the most predictive of final success (Oliver & Hewitt 2014).

Considering the rotation of different modes of action recommended by FRAC (2021), fungicides considered inefficient in this work, such as thiophanate-methyl, azoxystrobin and cuprous oxide, could have their label dosage revised by the manufacturers, seeking to improve the efficiency of the product against *S. lycopersici*, making the management of the disease more efficient. The low sensibility of some *S. lycopersici* isolates to azoxystrobin was reported at the dose of 40ppm (Costa 2019). However, higher doses of azoxystrobin may improve the performance of the fungicide (Anand et al. 2014). Despite the salicyl hydroxamic acid (SHAM) was not being used to inhibit the fungal alternative oxidase *in vitro*, the *in vivo* assays results corroborate the inefficiency of azoxystrobin or pyraclostrobin, since this via is presumed to be inhibited by secondary metabolites of plants (Liang et al. 2019). This inefficiency may is related to the emergence of the resistant *S. lycopersici* population. For this reason, the use of a single fungicide to control this disease is not recommended herein, as it has a temporary effect and increases the selection pressure for the fungicide used, increasing the chances of resistant spores to the fungicide arise due to adaptability and mutations.

In this work, thiophanate-methyl was not considered efficient. However, Awuah (1997) reported that the dose of 413ppm delayed the septoriose progress. The adaptability of *S. lycopersici* isolates to some fungicides became clearer when increasing doses of azoxystrobin, pyraclostrobin and thiophanate-methyl were used, suggesting that the label dosage may not be efficient as it once was in controlling the pathogen. This fact is explained by the adaptation of the isolates, which could trigger the development of resistance against fungicides with just one mode of action. Despite the efficiency of cuprous oxide demonstrated *in vitro*, *in vivo* experiments did not confirm its efficiency in controlling *S. lycopersici*.

At the doses tested, two of the most efficient fungicides against *S. lycopersici* seem to be succinate dehydrogenase inhibitors, which interfere in the fungus's energy generation (Keon et al. 1991), herein represented by fluxapyroxad and boscalid. Silva (2018) also showed an efficient control of septoriose by using fluxapyroxad + pyraclostrobin at the dose of 50ppm + 99ppm of the active ingredient, respectively.

The efficiency of mancozeb, a multi-site fungicide, is related to the recommended dose since, in the copper oxychloride+mancozeb formulation, which was considered ineffective in controlling septoriose, the dose of mancozeb is 218.18% lower than other formulations containing the active ingredient (azoxystrobin + mancozeb; cymoxanil + mancozeb), which obtains a severity control around 80%. It seems to happen with the chlorothalonil as well. Based on the results, fungicides with mancozeb or chlorothalonil should be present at a minimum of 1900 or 1200ppm, respectively, to achieve at least 70% of septoriose control. Mancozeb is reported to be efficient against *S. lycopersici* at the dose of 2500ppm in combination with carbendazim (2000ppm of active ingredient) (Lal et al. 2015) and at the dose of 1260ppm in combination with carbendazim (240ppm) (Naik et al. 2010), while chlorothalonil at the dose of 2969ppm was reported to reduce the final foliar disease development of septoriose (Poysa et al. 1993). In addition, chlorothalonil and azoxystrobin as preventive treatment, and difenoconazole and metconazole as curative treatments were reported to be efficient in controlling septorioses (Baldicera et al. 2020).

The control of septoriose should be exclusively preventive, as all the recommended fungicides have an efficiency below 70% when used curatively (Table 6). The effect of dose on the emergence of resistance has been the subject of intense debate (Shaw & Pijls 1994, Zziwa & Burnett 1994). It is now established for the great majority of cases that the lower the dose the lower

the risk of resistance because the selection pressure is higher at higher doses (Van den Bosch et al. 2011). However, this lower dose must be sufficient to control the disease in the field, even in an excellent condition to pathogen development and disease progress. Rotate fungicides based on the mode of action is recommended to avoid or delay the emergence of the resistance, but the subsequent fungicide must be effective and recommended in an effective dose. Fungicides that act via one specific site such IDMs (triazoles), IQes (strobilurins) and ISDHs (fluxapyroxad and boscalid), only one mutation in the target of the fungicide action may result in a resistant strain, and therefore, they should be used in a system of rotation modes of action. To mitigate the outcome of resistant *S. lycopersici* strains, we recommended herein the use of one-site fungicides only combined with multisite fungicides as such mancozeb or chlorothalonil, or combined with efficient protective fungicides, always rotating the mode of action as recommended by FRAC (2021).

The fungicidal action (fungitoxicity) is a function of the concentration in the leaf tissue. Sun, rain and metabolization through the plant will reduce the fungicide effective concentration. In crops like tomato, the spraying of protective fungicides is weekly in an open field, and in case where there is a rainfall of 13mm it removes the deposit of protective fungicides in tomato (Reis et al. 2016), requiring a reapplication. In fact, the half-lives of the fungicides applied on plants rarely extend to more than one week. Azoxystrobin, boscalid, captan, chlorothalonil, difenoconazole, famoxadone, fluazinam, iprodione, mancozeb, mandipropamide, metalaxyl-M, procymidone, pyraclostrobin, tebuconazole and tetraconazole have 3.53; 6.63; 4.45; 5.02; 5.02; 5.63; 3.77; 6.92; 4.69; 3.46; 3.74; 9.89; 3.90; 7.67 and 5.27 half-lives in days, respectively, measure by dissipation (Fantke et al. 2014). In addition, the deposition does not always cover or protect all infection sites (Reis et al. 2016). The higher the dose, the longer the protection period (Reis et al. 2016), but the dose used must follow the fungicide label instructions to keep the risk of resistance low and to comply with current legislation.

IDM have plant growth-regulating activity by likely inhibiting the production of gibberellins and sterol biosynthesis (phytosterol), which slows or reduces growth in some plants, and the increase in the green colour is the result of the increase in the chlorophyll content and the activity of the enzyme nitrate reductase (Reis et al. 2016). The overexpression of those characteristics might lead to the occurrence of plant phytotoxicity as observed for metconazole, tebuconazole and tetraconazole at the doses used. It is important to note that the phytotoxicity is dependent on the type of fungicide, dose and specific environmental conditions, and might vary according to the plant cultivar.

Based on the results of the *in vivo* experiments, a crucial factor for the study of fungicide efficiency is the existence of an optimum favourable environment for the pathogen and the progress of the disease. When the environment is unfavourable, even though the disease occurs, it does not occur with great intensity, leading to wrong conclusions about the fungicides efficiency since it also depends on the environmental conditions. Similar to what exists for soybeans (Godoy et al. 2020) we suggest a joint action program to determine the efficiency of the active ingredients at their recommended doses in several regions with different edaphoclimatic conditions.

Therefore, growers are faced with numerous decisions on how best to use fungicides, choosing which type of fungicide to use and when applying them. Fungicide chosen must be sufficient to control the disease efficiently in the field to ensure higher yields, do not cause any harmful effect on plants and be economically acceptable with good cost/benefits. To manage *S. lycopersici* in tomato plants, the pulverization of multi-sites fungicides (mancozeb, chlorothalonil, propineb or metiram) or fluazinam (protective fungicide) combined with systemic fungicides (fluxapyroxad or difenoconazole) at the doses recommended without a tank mixture and performing the action mode rotation should provide an efficient level of control. The fungicides metiram, fluazinam and fluxapiroxade are recommended to control septoriose in Brazil only when formulated with pyraclostrobin, thiophanate-methyl and pyraclostrobin, respectively.

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