Plant Pathology & Quarantine 10(1): 144–151 (2020) ISSN 2229-2217
www.ppqjournal.org
Article

Doi 10.5943/ppq/10/1/16

Performance of fungicides on the management of Glomerella leaf spot in southern Brazil

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Ogoshi C, Monteiro FP, Pinto FAMF, Perazzoli V, Cardoso DA 2020 – Performance of fungicides on the management of Glomerella leaf spot in southern Brazil. Plant Pathology & Quarantine 10(1), 144–151, Doi 10.5943/ppq/10/1/16

Abstract

Glomerella leaf spot (GLS) is an apple disease difficult to control, even with the use of chemical fungicides. In years of GLS high pressure, protective fungicides in the chemical group of dithiocarbamates, especially mancozeb, have been used frequently, and even so, the control has not been satisfactory. Because of this scenario, growers have been increasing the frequency of application and the dose recommended on the product label, a situation that will become unsustainable over the years, and resistance of some species belonging to *Colletotrichum acutatum* species complex to mancozeb has already been observed in Brazil. Our objective was to verify the efficiency of several chemicals' fungicides in the control of GLS in field conditions in three consecutive crop seasons. The experiments were conducted at the Experimental Station of Caçador in 2017/2018, 2018/2019 and 2019/2020 crop seasons. The tested fungicides were: mancozeb, mancozeb + trifloxystrobin, mancozeb + trifloxystrobin + tebuconazole, fluxapyroxad + pyraclostrobin, chlorothalonil, dithianon, captan, metiram + pyraclostrobin, thiophanate-methyl + fluazinam, fluazinam, metiram, dodine, folpet, and propineb. We assessed the GLS incidence over time, and with these data, the Area Under the Disease Incidence Progress Curve (AUDIPC) and the control efficiency of each treatment were calculated. The experimental design was in randomized blocks with four replications. The fungicides mancozeb, mancozeb + trifloxystrobin, mancozeb + trifloxystrobin + tebuconazole, chlorothalonil, dithianon, captan, thiophanate-methyl + fluazinam, metiram, fluazinam, folpet and propineb showed an efficient control of GLS. The fungicide metiram + pyraclostrobin showed an intermediate control and the fungicides dodine, and fluxapyroxad + pyraclostrobin were not efficient in GLS control. In general, systemic fungicides were not efficient in GLS control. Protectant fungicides were the most efficient in GSL control, being excellent options to be rotated with the fungicide mancozeb, which is used as a standard in GLS control in Brazil.

Key words – apple – *Colletotrichum* – non-systemic fungicides – protectant fungicides

Submitted 15 September 2020, Accepted 26 October 2020, Published 10 November 2020
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Introduction

Glomerella leaf spot (GLS) is one of the primary diseases of apple trees in Brazil (Velho et al. 2015, Denardi et al. 2019, Moreira et al. 2019a, b) and severe losses in fruit production can occur if adequate management strategies are not adopted (Valdebenito-Sanhueza et al. 2002). GLS was first described in 1970 in the United States (Taylor 1971) and 1983 in Brazil in the state of Paraná (Leite et al. 1988). Subsequently, it was disseminated to Santa Catarina state in the municipality of Fraiburgo, and today it is spread throughout the apple-producing region, including São Joaquim-SC and Vacaria-RS (Boneti et al. 2006).

GLS in Brazil is caused by several species of *Colletotrichum*. These species belong to *C. gloeosporioides*, *C. acutatum* and *C. boninense* species complexes (Velho et al. 2015, Moreira et al. 2019a). In Santa Catarina state, species of the group *C. gloeosporioides* are the most prevalent in apple orchards, with predominance of *C. fructicola* (Velho et al. 2015), while in Rio Grande do Sul and Paraná state there is a prevalent of *C. acutatum* species complex, with *C. nymphaeae* being the specie most predominant in the apple orchards (Moreira et al. 2019a).

GLS symptoms in the leaves are characterized by lesions that are initially reddish, without defined margins, randomly distributed in the leaf blade and whose size varies from 1 mm to 4 mm in diameter (Valdebenito-Sanhueza et al. 2002). The injured leaves usually turn yellow and fall off in a short period of time (Boneti et al. 2006).

GLS occurs most severely during the summer in Brazil, causing severe defoliation of apple trees above 75% (Katsurayama & Boneti 2009). This severe and early defoliation damage particularly the good flowering bud's differentiation in the next season, which translates into low production, and poor quality of fruits (Kretzschmar et al. 2005, Anzanello et al. 2012).

The leaf wetting period of at least 10 hours with a temperature above 15ºC favours the GLS occurrence (Valdebenito-Sanhueza et al. 2002), and the higher is the temperature; the shorter is the leaf wetting period necessary for the disease occurs (Hamada 2005).

The GLS management has been complicated in all apple-producing regions in Brazil. The leading causes are: the disease progress in the leaves is fast (Moreira et al. 2019b); the incubation period is short, approximately 45 hours on leaves, and 96 hours on fruits (Valdebenito-Sanhueza et al. 2002, Moreira & May De Mio 2015); there are large variations in the number of species, and species complexes of *Colletotrichum* that cause the disease in the field (Moreira et al. 2019a), and "gala" clones, which are highly susceptible to GLS (Stadnik et al. 2009, Denardi et al. 2019), occupy approximately 60% of the planted area with the apple in Brazil (Petri & Hawerroth 2014).

Therefore, in order to control GLS efficiently, growers have been spraying a large amount of fungicides, reaching more than 20 applications of protectant fungicides from the chemical group of dithiocarbamates (propineb, metiram and mancozeb) per season (Katsurayama & Boneti 2012, Hamada & May De Mio 2017, Moreira et al. 2019b). Even with this large number of fungicide applications, growers have been reporting failures in GLS control, and to get around this problem, they are applying high doses of mancozeb. This situation concerns the longevity of this chemical molecule because it increases the selection pressure of resistant individuals of *Colletotrichum*, a scenario already been observed in *C. acutatum* species complex in apple trees in Paraná state of Brazil (Moreira et al. 2019b). Despite all concern with the disease, information about levels of control with recommended fungicides are scarce and must always be updated, since the complex fungi are in constant changing. Furthermore, there is a continuous threat of banning mancozeb use in the European Union, being essential to provide new tools to replace it.

Materials & Methods

The experiment was performed in the field at Caçador Experimental Station, belonging to the Agricultural Research and Rural Extension Enterprise of Santa Catarina (EPAGRI / EECd), in Caçador city, Santa Catarina State, Brazil.

The experimental area is at an average altitude that corresponds to 964 m above sea level, under the following geographical coordinates: 26º50'07"S latitude and 50º58'29"W longitude. The local climate is the humid subtropical climate (Cfa), according to the Köppen classification, with an average air temperature of 8.5 and 28.8ºC, in the coldest and hottest months, respectively. The area's soil is classified as typical Nitossolo Bruno dystrophic (Embrapa 2013).

The experiment was carried out in three crop seasons 2017/2018, 2018/2019 and 2019/2020. The Royal Gala apple cultivar grafted under the Marubakaido rootstock with an M-9 filter was used. The pollinating cultivars were Fuji and Granny Smith, and the orchard is 8 years old. The planting spacing is 4 m between lines and 2 m between plants. The orchard has 10 lines with 500 plants in total.

The plant conduction system is a central leader in an east-west direction, and the weed management is with brush hog between rows and chemical in the planting line. Others cultural management practices used in this experiment were carried out following the technical recommendations available in Production System for Apple in Santa Catarina State, Brazil (Epagri 2018).

The chemical fungicides used as a treatment in our experiment are described in detail in Table 1, with the trade name, respective dose, active ingredient name, and which crop season were used. The fungicides application was carried out with a turbo atomizer sprayer adapted with a manual spray lance, and the spray volume used was approximately 1000 L. ha^{-1} or 1.0 L per plant.

The first applications of treatments in each crop season were performed when the disease outset was observed on 10/24/2017 (2017/2018); 10/05/2018 (2018/2019) and 10/10/2019 (2019/2020). The other sprays were carried out weekly, and the last one was on 01/09/2018 (2017/2018); 01/16/2019 (2018/2019); 12/06/2019 (2019/2020).

GSL incidence was the quantified variable. For this, 100 leaves were evaluated at random on each side of the plant, with a total of 200 leaves evaluated per plan. The experimental plot consisted of an apple plant. With GLS incidence data evaluated at different times, the Area Under the Disease Incidence Progress Curve was calculated (AUDIPC) (Campbell & Madden 1990).

In total, eight disease assessments were performed in 2017/2018, which occurred on: 10/24/2017 (to standardize treatments); 10/30/2017; 11/14/2017; 11/22/2017; 12/06/2017; 12/20/2017; 1/8/2018, and 1/14/2018. In 2018/2019, nine disease assessments were made, which occurred on: dates: 10/19/2018; 10/25/2018; 11/08/2018; 11/20/2018; 12/05/2018; 12/18/2018; 01/04/2019; 01/15/2019, and 01/23/2019. Finally, in 2018/2019, only four disease assessments were carried out, which occurred on 11/11/2019; 11/19/2019; 12/03/2019, and 12/19/2019.

AUDIPC from treatment and control were used to calculate the efficacy of fungicide control using Abbott's formula: Efc (%) = (T – t)*100/T, wherein Efc (%) = control efficacy of Fungicides in the function of control treatment; T = AUDIPC observed in the control treatment and, t: AUDIPC observed in fungicide treatment (Abbott 1925).

The experimental design used was randomized blocks with four replications. The data were subjected to analysis of variance by F test with 5% probability of experimental error. Then, the treatments means were compared by Scott-Knott test, using the same level of significance mentioned above. All analyzes were performed using the SISVAR program (Ferreira 2011).

Table 1 Description of the fungicides tested in our experiments with trade name, dose, active ingredients and which season crops were sprayed.

Trade name	Dose (g or mL/100L)	Active ingredients	17/18	18/19	19/20
Dithane NT™	300	mancozeb (80%)	X	X	X
Dithane NT™ + Flint™	300 + 10	mancozeb (80%) + trifloxystrobin (50%)	X		
Dithane NT™ + Nativo™	300 + 40	mancozeb + trifloxystrobin (10%) + tebuconazole (20%)	X		
Orkestra SC™	40	fluxapyroxad (16,7%) + pyraclostrobin (33,3%)	X	X	
Bravonil Ultrex™	150	chlorothalonil (82,5%)	X	X	X
Delan™	125	dithianon (75%)	X	X	X
Captan SC™	250	captan (48%)	X	X	X

Table 1 Continued.

Trade name	Dose (g or mL/100L)	Active ingredients	17/18	18/19	19/20
Cabrio Top™	250	metiram (55%) + pyraclostrobin (5%)	X	X	X
Approve™	100	thiophanate-methyl (37,5%) + fluazinam (37,5%)	X	X	X
Polyram™ DF	300	metiram (70%)		X	X
Frowncide 500 SC™	100	fluazinam (50%)		X	X
Dodex 450 SC™	100	dodine (45%)		X	X
Folpan Agricur 500 WP™	210	folpet (50%)		X	X
Antracol 700 WP™	300	propineb (70%)		X	X
Control	--------	--------	X	X	X

Results

The fungicides mancozeb, mancozeb + trifloxystrobin, mancozeb + trifloxystrobin + tebuconazole, dithianon, captan and chlorotalonil showed the lowest AUDIPC in 2017/2018 ($p < 0.05$), being statistically equal to each other ($p > 0.05$). The fungicides metiram + pyraclostrobin, thiophanate-methyl + fluazinam and fluxapyroxad + pyraclostrobin showed the same AUDIPC in relation to control treatment ($p > 0.05$). The control efficiency of all fungicides was low, not exceeding 25% (Table 2).

In relation to 2018/2019, the fungicides mancozeb, dithianon, captan, chlorothalonil, metiram + pyraclostrobin, thiophanate-methyl + fluazinam, metiram, fluazinam, folpet and propineb presented the lowest AUDIPC in relation to the other treatments ($p<0.05$). The control efficiency of these fungicides varied from 87.33% to 95.72%. The fluxapyroxad + pyraclostrobin and dodine fungicides showed lower AUDIPC only in relation to control, however, both showed low control efficiency between 17.33% to 18.64% (Table 2).

Table 2 Area Under the Disease Incidence Progress Curve was calculated (AUDIPC), and control efficacy of Fungicides (Efc) in 2017/2018, 2018/2019 e 2019/2020 crop season.

Treatments	AUDIPC 17/18	Efc (%)	AUDIPC 18/19	Efc (%)	AUDIPC 19/20	Efc (%)
mancozeb	3522 B*	24,19	502,19 C*	92,18	68,38 E*	100
mancozeb + trifloxystrobin	3899 B	16,08				
mancozeb + trifloxystrobin + tebuconazole	3877 B	16,55				
dithianon	3809 B	18,01	547,94 C	91,47	0,00 E	100
captan	3785 B	18,53	562,63 C	91,24	0,00 E	100
chlorothalonil	3674 B	20,92	274,69 C	95,72	0,00 E	100
metiram + pyraclostrobin	4155 A	10,57	813,94 C	87,33	334,25 D	86,58
thiophanate-methyl + fluazinam	4492 A	3,31	625,56 C	90,26	0,00 E	100
fluxapyroxad + pyraclostrobin	4670 A	0	5309,00 B	17,33		
metiram			656,69 C	89,77	590,38 C	76,29
fluazinam			423,94 C	93,40	32,87 E	98,68
dodine			5225,19 B	18,64	903,63 B	63,71
folpet			395,00 C	93,85	0,00 E	100
propineb			551,94 C	91,41	0,00 E	100
control	4646 A	0	6422,06 A	0	2490,13 A	0

* Means followed by the same letter in columns belong to the same group by the Scott-Knott test ($p < 0.05$).

For 2018/2019 crop season, the fungicides mancozeb, dithianon, captan, chlorotalonil, thiophanate-methyl + fluazinam, fluazinam, folpet and propineb demonstrated the lowest AUDIPC in relation to the other treatments (p <0.05), with high control efficiency (100%) (Table 2). The fungicide metiram + pyraclostrobin showed a higher AUDIPC in relation to previous fungicides, but demonstrated a lower AUDIPC in regard to control and the fungicides metiram and dodine, and presented a high control efficiency of 86.58% (Table 2). The fungicide metiram showed lower AUDIPC only in relation to the fungicide dodine and the control without fungicide application with control efficiency of 76.29%. Finally, the fungicide dodine demonstrated AUDIPC inferior only to the control, with a control efficiency of 63.71% (Table 2).

The average air temperatures and rainfall that occurred at EECd / EPAGRI region during the period of experiment conduction are shown in Fig. 1.

The average air temperature for 2017/2018 crop season ranged from 16.76°C in October to 20.47°C in January (Fig. 1). While total rainfall in October / 2017 was 351.8mm, 171mm in November / 2017, 146.2mm in December / 2017 and 193.4mm in January / 2018. For 2018/2019, the average air temperature between the months of October and January was 17.05ºC to 22.65ºC respectively the total precipitation for October / 2018 was 280.4mm, 143.2mm in November / 2018, 146.0mm in December / 2018 and 226.8mm in January / 2019 (Fig. 1). In relation to 2019/2020 crop season, the average air temperature between the months of October and January was 19.30ºC to 21.16ºC, respectively. While the total rainfall in October/2018 was 142.4mm, 153.4mm in November/2018,101.8 mm in December/2018 and 117.0mm in January 2020 (Fig. 1).

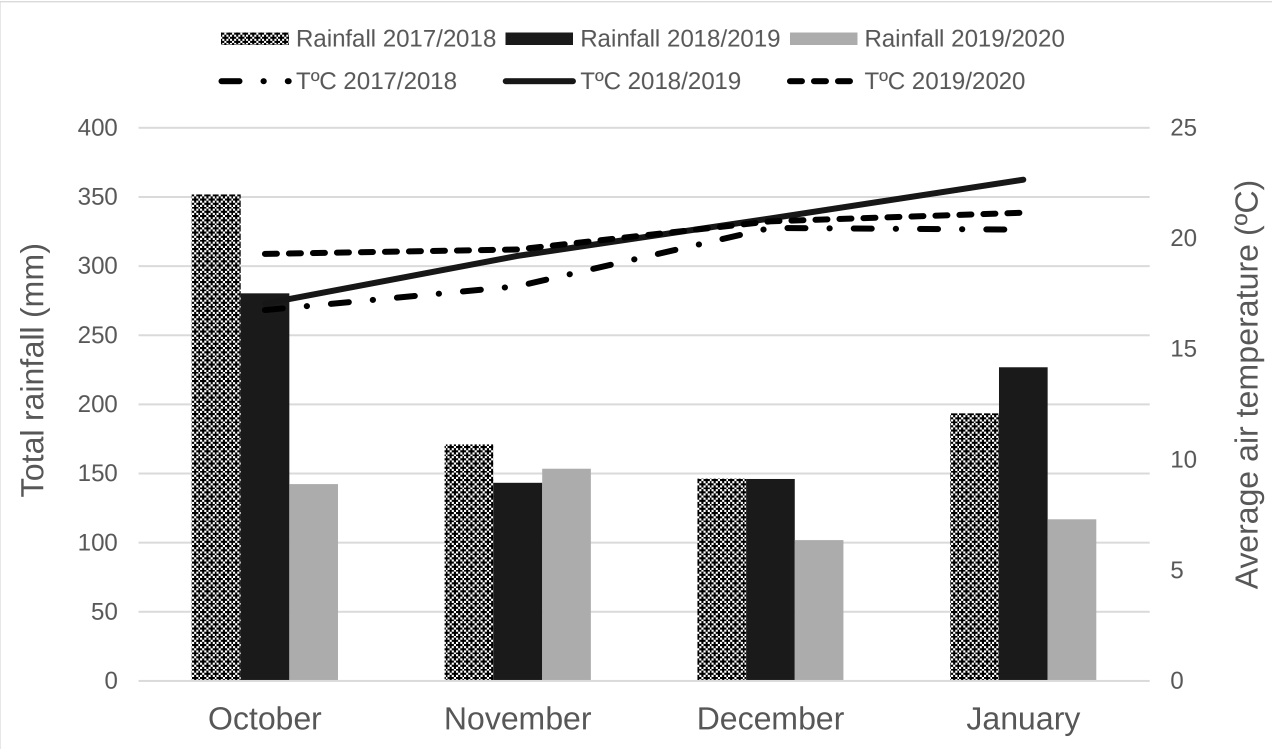

Fig. 1 – Average air temperature (ºC) and total rainfall (mm) occurred from October to January in 2017/2018, 2018/2019 and 2019/2020 crop seasons at EECd / EPAGRI. (Data obtained from the EECd / EPAGRI automated weather station), available at: http://www.ciram.sc.gov.br/agroconnect

Discussion

The first GLS symptoms appearance in 2017/2018 possibly started between the 7th to 9th of October 2017, therefore, at the beginning of treatments spraying (10/24/2017), there was already approximately 50% of disease incidence in all treatments (data not shown). Unlike 2018/2019 and 2019/2020, when the start of treatments spraying coincided with the appearance of the first disease symptoms. Therefore, we believe that the low efficiency of fungicide control in 2017/2018 is related to the timing of fungicides application, once preventive application is ideal, that is, before the disease appearance onset.

Boneti et al. (2006) mention that GLS control with fungicides should be performed preventively or at most when the disease first symptoms are found and should be reapplied at intervals of 7 to 10 days or whenever there is a rain accumulation of 25mm or more. Becker et al. (2004) reported that the monitoring of climatic conditions, especially the possibility of a cold front occurring, is fundamental to GLS control with protectant fungicides.

However, studies carried out in Paraná State, Brazil have shown that in regions where the pressure of GLS is higher, monitoring of climatic conditions is not sufficient for effective control with fungicides, being essential to monitor *Colletotrichum* spores in the air (Hamada et al. 2019). Some authors believe that this occurs due to the disease short incubation period, and the rapid development of symptoms in Gala group cultivars (Moreira et al. 2019a).

Despite the low efficiency of GLS control with fungicides in 2017/2018, fungicides non-systemic showed lowest AUDIPC in relation to control without fungicides and with the systemic fungicides. The systemic fungicides trifloxystrobin and trifloxystrobin + tebuconazole, did not increase the GLS control in mixture with the fungicide mancozeb, which is used as a standard for disease control by apple growers in Brazil.

In all crops season, fungicides with exclusively systemic action, such as fluxapyroxade + pyraclostrobin and dodine, always presented higher AUDIPC in relation to fungicides with protective action, this situation that directly reflected in low efficiency of disease control in the field. Becker et al. (2004) mention that in years that GLS pressure is high, the disease control by growers is deficient mainly by the lack of efficient systemic fungicides.

The GLS in 2019/2020 was not as severe as in previous years, possibly due to low rainfall during the entire period of the experiment (Fig. 1). Because we did not observe a considerable variation in air temperature average between the crop years of conducting the experiments (Fig. 1). The GLS aggressiveness is directly involved with the leaf wetting duration at least 10 hours with a temperature above 15ºC (Valdebenito-Sanhueza et al. 2002), and the higher the temperature, the shorter leaf wetting period is necessary for disease development (Hamada 2005).

The 2017/2018 crop season had the highest rainfall in virtually all months of the experiments, and consequently, had the highest probability of GLS occurrence. This situation also contributes to explaining the low efficiency of the fungicides tested in 2017/2018, since in years with increased frequency of rains and high disease pressure, chemical control has not been satisfactory (Becker et al. 2004, Hamada et al. 2012, Moreira et al. 2019b).

Non-systemic fungicides were the most efficient in GLS control in the 2018/2019 and 2019/2020. The GLS control in Brazil, under conditions of high disease pressure, has been done by application of the fungicides mancozeb and dithianon (Boneti et al. 2006), however, due to the continuous application of mancozeb by apple growers in Brazil, GLS control failures have been observed.

This situation has been causing an increase in the number of applications and in the number of mancozeb doses to improve the control efficiency in the field; however, over the years, this scenario will become unsustainable, as it harms the environment, favours the selection pressure of *Colletotrichum* resistant individuals, and increases the food risk for society.

Despite the mancozeb fungicide being a multi-site activity, and considered as a low risk of resistance development (Frac 2020), there are already reports of several pathogens resistant to it, including for apple x Colletotrichum pathosystem (Moreira et al. 2019b).

Moreira et al. (2019b) verified the sensitivity of different species of *Colletotrichum* from the *C. acutatum* species complex obtained in Paraná state, and observed that 25% of the isolates were resistant to mancozeb. In addition, several isolates resistant to thiophanate-methyl and azoxystrobin were found, a situation that concerns the GLS chemical control longevity in Brazil.

Johnson (2018) in North Carolina, USA applying a non-rotational fungicide field experiment found pyraclostrobin plus fluxapyroxad, captan plus phosphorous acid, and captan at the 5.6 kg/ha rate as most effectively to control GLS, although Mancozeb was not utilized as standard control since there is only allowed spray Mancozeb until 77 days before harvest.

Boneti et al. (2006) reported that fungicides chlorothalonil, fluazinam, captan, and folpet are not efficient in controlling GLS in years with high disease pressure, that is, with increased precipitation and average air temperature between 22 to 27ºC. However, data from our field experiments in three consecutive crop seasons demonstrate that these fungicides were useful in the disease control since it was applied at the right time even in years of high disease pressure. In addition, Hamada et al. (2012) found that chlorothalonil fungicide have an GLS control efficiency higher than mancozeb which is used as a standard GLS control in Brazil.

As a result, although systemic fungicides are not effective in GSL control in Brazil, others non-systemic fungicides have shown efficiency in the disease control, being excellent rotation options with the mancozeb fungicide.

Acknowledgements

We are thankful to EPAGRI and to Research and Innovation Foundation of Santa Catarina State (FAPESC).

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