5th IMPB INTERNATIONAL MEETING ON PLANT BREEDING

INTEGRATED DATA ANALYSIS AS A TOOL TO REVOLUTIONIZE PLANT BREEDING

In vitro SENSITIVITY OF *Colletotrichum* spp. ASSOCIATED WITH *Citrus sinensis* TO TRIAZOIS FUNGICIDES (DMI) AND THEIR COMBINATIONS WITH STROBILURINS (QoI)

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Introduction

Brazil stands out as the largest producer and exporter of orange juice in the world, the 2020 harvest was 268.63 million boxes. Among the main fungal diseases, the prostbloom fruit drop (PFD) stands out. In Brazil, this disease is caused by *Colletotrichum gloeosporioides* and *Colletotrichum abscissum*. Also there, was found the presence of *C. karstii* in citrus plants, with confirmation of the pathogenic expression of symptoms typical of those of PFD. The fungicides recommended for the management of PFD belong to the groups of triazoles (sterol demethylation inhibitors - DMI) and strobilurins (external quinone inhibitors - QoI). The commercially formulated mixture of fungicides of these chemical groups are the most efficient under field conditions, being the most used in the management of the disease.

Objective

The present work aims to evaluate the in vitro sensitivity *of C. abscissum, C. gloeosporioides* and *C. karstii,* all from sweet orange trees, to the two fungicides, of the triazole group (DMI), registered for the crop, as well as to commercial formulations most used in disease management, derived from the combination of DMI fungicides and those of the strobilurin group (QoIs).

Conclusion

The species *C. gloeosporiodes, C. abscissim* and *C. karstii* are highly sensitive to the fungicides tebuconazole, difenoconazole and trifloxystrobin + tebuconazole in an in vitro test. The mixture trifloxystrobin + tebuconazole is more efficient compared to the fungicides tebuconazole and difenoconazole.

Materials and methods

Isolates of *Colletotrichum* spp. were obtained from leaves, flowers and fruits of asymptomatic plants and fruits of symptomatic plants from five cities located in the state of São Paulo. Isolation of *Colletotrichum* spp. was performed according to protocols. The taxonomy of the isolates used in this work was previously determined, being characterized by *C. gloeosporiodes, C. abscissum* and *C. karstii*. Nine *Colletotrichum* isolates were evaluated. The fungicides used were: tebuconozole, difenoconazole, trifloxystrobin + tebuconazole and azoxystrobin + difenoconazole. The experimental design adopted was completely randomized blocks, in a 9x5x3 factorial scheme, corresponding to nine isolates, with five concentrations of fungicides (0.01, 0.10, 1, 10 and 100 µg mL^{-1}) and a control. From the colony size data, the percentage of growth inhibition was determined. Statistical analysis was performed using the AgroEstat program, proceeding with analysis of variance. Subsequently, the means of the treatments were separated by the Scott-Knott method at a 5% probability level. The mycelial growth data were previously transformed into x+k, where k = 0.5 and when significant, the regression analysis. The mycelial growth index was determined by the formula described by Oliveira (1991). EC50 was determined by average of mycelial growth inhibition and the logarithm of the fungicide concentration. To classify the level of sensitivity of the isolates, was addopted a scale similar to the one used by Joshi et al. (2013).

Results and discussion

Table 1. Percentage inhibition of mycelial growth of isolates of *Colletotrichum gloeosporioides, Colletotrichum abscissum* and *Colletotrichum karstii* at different concentrations (µg mL^{-1}) of the fungicide tebuconazole *in vitro*.

		Tebuconazole (µg mL^{-1})				
Species[1]	Isolateds	0,01	0,10	1,0	10	100
Glo	OO1.11	5,109 aC	4,024 aD	7,779 aB	8,941 aA	9,673 aA
Abs	OO2.9	2,481 bC	1,769 cC	7,832 aB	9,078 aA	9,078 aA
Glo	OO3.20	2,943 bD	4,838 aC	6,939 bB	8,994 aA	9,488 aA
Glo	SO2.6	2,439 bC	3,211 bC	7,062 bB	8,720 aA	9,648 aA
Glo	MON1.5	1,985 cC	1,796 cC	7,486 aB	9,006 aA	9,630 aA
Glo	TO1.3.6	2,790 bC	3,572 bC	7,698 aB	9,410 aA	9,668 aA
K	TO2.3.10	1,434 cC	2,943 bB	8,453 aA	9,164 aA	9,518 aA
Glo	TO2.3.11	1,521 cD	4,103 aC	7,037 bB	8,531 aA	9,056 aA
K	BO1.5	1,485 cD	3,499 bC	7,781 aB	8,964 aA	9,500 aA

*Averages followed by the same letter do not differ statistically from each other, uppercase in the column and lowercase in the row, by the Scott-Knott test at 5% probability.[1] Species of *Colletotrichum* (Glo: *C. gloeosporioides*; Abs: *C. abscissum* and K: *C. karstii*).

Table 2. Percentage inhibition of mycelial growth of *Colletotrichum gloeosporioides, Colletotrichum abscissum* and *Colletotrichum karstii* isolates at different concentrations (µg mL^{-1}) of the fungicide difenoconazole *in vitro*.

		Difenoconazole (µg mL^{-1})				
Species[1]	Isolateds	0,01	0,10	1,0	10	100
Glo	OO1.11	3,170 cE	6,499 cD	7,949 bC	9,004 aB	9,727 aA
Abs	OO2.9	5,649 aC	8,277 aB	9,306 aA	9,515 aA	9,533 aA
Glo	OO3.20	3,136 cE	6,014 dD	7,612 bC	8,466 bA	9,543 aA
Glo	SO2.6	2,863 cE	6,266 cD	7,811 bC	8,721 bB	9,220 aA
Glo	MON1.5	3,294 cD	6,284 cC	7,679 bB	9,032 aA	9,252 aA
Glo	TO1.3.6	3,400 cD	5,590 dC	8,177 bB	9,225 aA	9,680 aA
K	TO2.3.10	4,284 bD	6,040 dC	7,951 bB	9,205 aA	9,560 aA
Glo	TO2.3.11	4,199 bD	7,097 bC	7,540 bC	8,811 bB	9,669 aA
K	BO1.5	4,118 bE	6,617 cD	7,563 bC	8,827 bB	9,557 aA

*Averages followed by the same letter do not differ statistically from each other, uppercase in the column and lowercase in the row, by the Scott-Knott test at 5% probability.[1] Species of *Colletotrichum* (Glo: *C. gloeosporioides*; Abs: *C. abscissum* e K: *C. karstii*).

Table 3. Percentage inhibition of mycelial growth of *Colletotrichum gloeosporioides, Colletotrichum abscissum* and *Colletotrichum karstii* isolates at different concentrations (µg mL-1) of the fungicide trifloxystrobin + tebuconazole *in vitro*.

		Trifloxistrobin + Tebuconazole (µg mL^{-1})				
Species[1]	Isolateds	0,01	0,10	1,0	10	100
Glo	OO1.11	6,489 aD	7,908 aC	8,888 bB	8,784 bB	9,369 aA
Abs	OO2.9	6,305 aC	8,021 aB	9,078 aA	9,078 bA	9,078 aA
Glo	OO3.20	6,433 aD	7,375 bC	8,510 bB	8,588 bB	9,298 aA
Glo	SO2.6	4,582 cD	7,183 bC	8,505 bB	8,388 bB	9,203 aA
Glo	MON1.5	5,565 bC	8,116 aB	9,427 aA	9,630 aA	9,630 aA
Glo	TO1.3.6	6,601 aC	8,157 aB	9,310 aA	9,372 aA	9,668 aA
K	TO2.3.10	5,672 bC	8,530 aB	9,518 aA	9,518 aA	9,518 aA
Glo	TO2.3.11	3,715 dC	7,013 bB	8,587 bA	8,643 bA	9,056 aA
K	BO1.5	5,703 bC	7,826 aB	9,002 aA	8,983 bA	9,500 aA

*Averages followed by the same letter do not differ statistically from each other, uppercase in the column and lowercase in the row, by the Scott-Knott test at 5% probability. [1] Species of *Colletotrichum* (Glo: *C. gloeosporioides*; Abs: *C. abscissum* e K: *C. karstii*).

Figure 1. Mycelial growth index of species of *C. gloeosporioides, C. karstii* and *C. abscissum*.

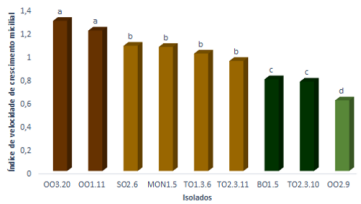

Table 4. Regression equations, R^2, CE50 and sensitivity of *Colletotrichum gloeosporioides, Colletotrichum abscissum* and *Colletotrichum karstii* isolates associated with symptoms of postbloom fruit drop, exposed to different concentrations (µg mL^{-1}) of the fungicides tebuconazole, difenoconazole and trifloxystrobin + tebuconazole.

	Equations, R^2 values, CE50 and Sensitivity response											
Isolateds	Tebuconazole				Difenoconazole				Trifloxystrobin + Tebuconazole			
	Regression Equation	R^2	CE_{50}*	Sensib.**	Regression Equation	R^2	CE_{50}*	Sensib.**	Regression Equation	R^2	CE_{50}*	Sensib.**
OO1.11	y = -1,4217x + 3,2433	0,887	1,483	AS	y = -1,7867x + 3,81	0,9696	0,7	AS	y = -0,7483x + 2,2033	0,8807	1,522	AS
OO2.9	y = -0,8383x + 1,4733	0,852	1,607	AS	y = -0,4583x + 1,0233	0,7192	0,35	AS	y = -0,2833x + 0,8333	0,7605	1	AS
OO3.20	y = -1,6467x + 3,79	0,975	1,578	AS	y = -1,8833x + 4,0033	0,9879	1,23	AS	y = -0,9183x + 2,6033	0,9519	3,038	AS
SO2.6	y = -1,5617x + 3,5597	0,969	1,736	AS	y = -1,4133x + 3,46	0,9515	1,06	AS	y = -0,9833x + 2,73	0,8688	1,137	AS
MON1.5	y = -1,8367x + 3,4067	0,921	1,521	AS	y = -1,3467x + 3,18	0,9542	1,05	AS	y = -0,97x + 1,6933	0,7965	0,272	AS
TO1.3.6	y = -1,7433x + 3,4067	0,924	1,203	AS	y = -1,6033x + 3,1467	0,959	0,84	AS	y = -0,8817x + 1,77	0,8628	0,505	AS
TO2.3.10	y = -1,2733x + 2,48	0,877	0,993	AS	y = -1,0533x + 2,2933	0,9601	1,08	AS	y = -0,68x + 1,27	0,7062	0,214	AS
TO2.3.11	y = -1,1217x + 2,9133	0,971	2,072	AS	y = -0,955x + 3,2733	0,819	2,14	AS	y = -0,84x + 2,18	0,8395	0,825	AS
BO1.5	y = -1,1017x + 2,5067	0,938	1,228	AS	y = -0,9083x + 2,0933	0,9801	2,08	AS	y = -0,8583x + 1,53	0,8571	0,638	AS

*CE_{50}= Minimum concentration required for 50% inhibition of colony growth.
** Classification according to CE50, where: CE50 < 100 µg/mL- highly sensitive (AS); 101 ≥ 200 µg/mL – sensitive (S); 201 ≥ 300 µg/mL - moderately insensitive (MI); 301 ≥ 400 µg/mL – insensitive (I) and > 400 µg/mL – highly insensitive (AI).