Evaluation of the efficacy of Metalosate copper on infections by *Lasiodiplodia theobromae*, causal agent of arm dead vine under controlled conditions

1. METHODOLOGY

The experiment was conducted in a House mesh of the facilities of the Department of health vegetable of the Universidad Nacional San Luis Gonzaga. The temperature conditions were between 20 - 24 ° C and 65-75% relative humidity.

Vines were used in poly bag of five-month-old cultivar Red Globe grafted on pattern MGT-101-14. The products listed in table 1 were used in trials.

The isolated Las 1 was used as inoculum, of *l. theobromae* from vine, contained in Petri dishes.

Table 1. Treatments used for the control of *l. theobromae* in artificial inoculations in vine.

Treatments	Kind of Application
T0: Witness (no application)	
T1: Metalosate copper:	0.3 L/200 L leaf
T2: Metalosate copper:	0.5 L/200 L leaf
T3: Metalosate copper:	0.7 L/200 L leaf
T4: Metalosate copper: + Metalosate boron: T5: Prochloraz (Bucaner 450 EC): Drench	0.5 L / 200 L 0.2 L/200 L leaf 0.3 L/200 L
T6: Thiabendazol (Mertect 500 SC):	0.4 L/200 L Drench

Products applied following two different application modes: by foliar and drench (table 1). Previously the calculations of the cost of water for each type of application were performed per plant.

a. By foliar spray applied an average volume of 60 ml of broth fungicide for plant, a backpack using hydraulic pressure spray prior.

b. Applied via drench was made directly to the substrate from each pot. He was previously

calculated the volume of water of the substrate

in the pot, and used spending was 150 ml of broth fungicide by plant.

Evaluation of the efficacy of products strategies

A. Preventive applications:

Two consecutive applications of Metalosate copper were performed at intervals of 7 days to plants of different shaped treatments. The plant group witness was applied in the same intervals with water alone.

Five days after the last treatment were inoculated plants of each treatment with the isolated of *l. thebromae*. Plants were inoculated at the center of the stem in the area of the pattern, and the Woody part of the outbreak in the area of the variety. Groups of ten plants each treatment and dose of the product to be evaluated were applied only on the leaf, and similar groups of plants via drench. The objective of this type of application was to assess the effect of the evaluated products in applications prior to infection, and also as drench or foliar applications in the area of injury affected by the pathogen.

B. Curative application:

Groups of plants of each treatment (dose of fungicide products) were previously inoculated with isolated from *l. thebromae*. Five days after inoculation were two consecutive applications at 7 days intervals between applications of different treatments to each group of plants. The objective of such applications was to assess the efficacy of products and dose used in stop infection by the pathogen. Similar to that described above, plants were inoculated in the area of the pattern and the variety, and were independent of plants applications drench or foliar application.

Process of inoculation of plants

The bark of the stem in the area of the pattern and the Woody part of the area of the variety, were superficially disinfected with ethyl alcohol 96 °. Of this area was extracted a disc of cortex using a sterile 5 mm diameter punch. In the wound was placed in direct contact with the exposed cambium, a disc of PDA of similar diameter colonized with mycelium of the isolated of *l. thebromae*, and the wound covered with bark disk extracted. The area of inoculation is wetted with a few drops of sterile water, wrapped with parafilm tape to protect the inoculated area of desiccation (**Figure 1**).

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Figure 1. Inoculation process of greenhouse vine plants with L. theobromae

Evaluation of results

Four weeks after inoculation was carefully removed the crust of each inoculated plant around the area of the wound. The resulting lesion area delineated on a transparent plastic sheet, was transferred to a sheet of white paper, and are scanned **(Figure 2).** Through the program Assess (American Phytopathological Society, St. Paul MN), was quantified the area of the lesion of each treatment by image analysis. The area of inoculation wound size is seized of the obtained total area to calculate the actual size of the lesion. To confirm that the injuries were caused by *l. theobromae,* were sowing of tissues affected in medium PDA with at least two floors of each treatment.

Figure 2. Evaluation of the area of the lesion to. Area of vines injury**. b.** Area of lesions on paper, **c.** Digital image processing program

Statistical analysis

Results were expressed as lesion area in cm^2 by treatment. It placed special emphasis on the areas of injury both in the area of the variety as well as the pattern in both developed assessment strategies. Injury factors in study areas were subjected to analysis of variance (ANOVA), fixing the level of significance at $P < 0,05$. The factors that were significant in the analysis ANOVA was performed a separation test using analysis of Fisher's least significant difference (LSD). The statistical software used for the analysis was SAS 9.1.

Additionally, included values averages for the percentage of control of each treatment. This figure was calculated by the following formula:

Control = 100 - [(Area of injury x 100) / injury to the witness Area].

2. RESULTS

Figure 3a and 3b. Area of injury in the inoculations in area of the variety of (arrows) in the control treatment. Note the progression of lesion from the inoculation point.

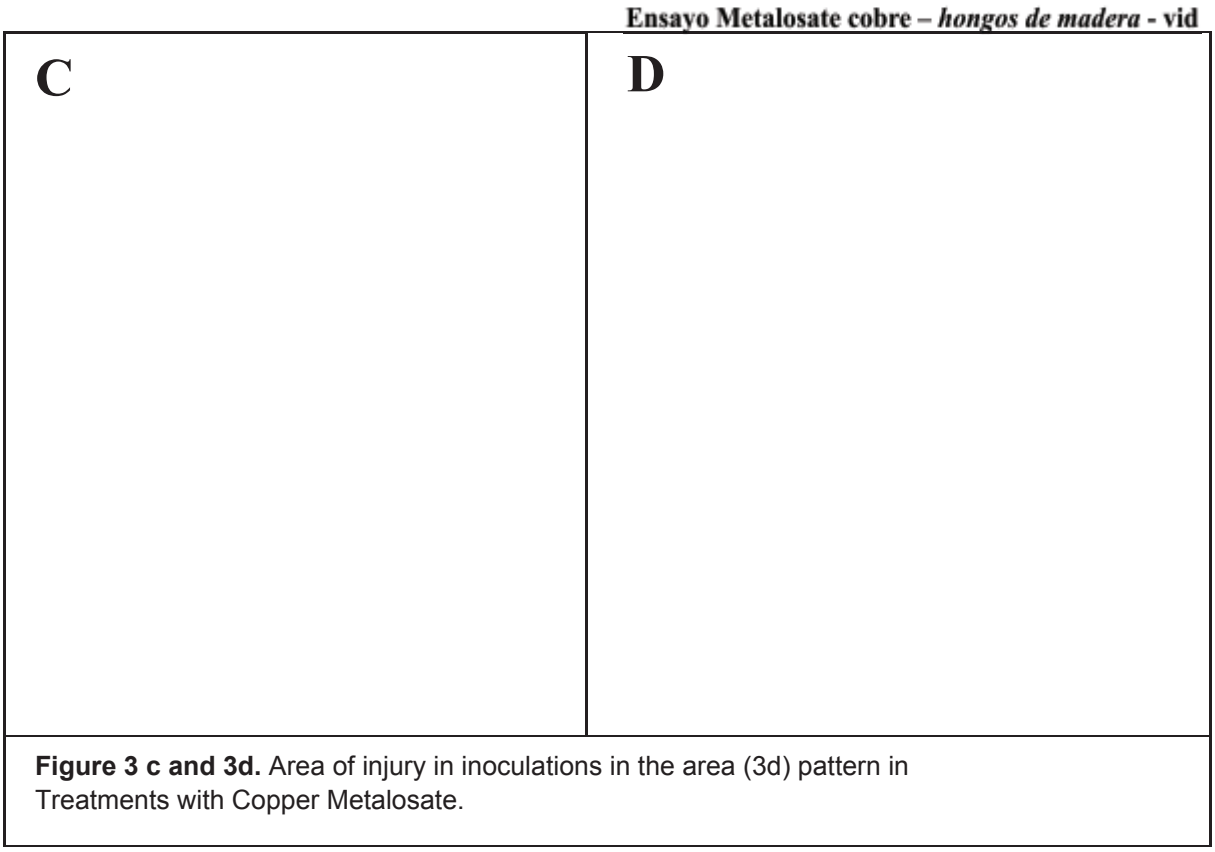

Figure 3 c and 3d. Area of injury in inoculations in the area (3d) pattern in Treatments with Copper Metalosate.

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Table 2: Metalosate copper effect on the efficacy of infection control strategies by *Lasiodiplodia theobromae* in artificial inoculations of young plants of vine. Ica, April - June of 2015.

Application of kind preventive Area injury cm2			applications such healing Lesion area cm2		
Treatments	**Variety**	**Pattern**	**Treatments**	**Variety**	**Pattern**
T 0	8.9	5.8	T 0	5.2	4.7
T 0	6.4	4.7	T 0	6.3	4.3
T 0	7.6	3.4	T 0	6.5	5.5
T 0	5.1	5.6	T 0	7.1	3.8
T 0	7.8	6.1	T 0	7.4	4.4
T 0	5.7	5.7	T 0	8.2	4.4
T 0	8.5	3.4	T 0	4.9	5.1
T 0	4.9	4.1	T 0	6.1	3.2
T 0	7.3	5.2	T 0	4.6	4.6
T 0	8.1	4.9	T 0	7.3	3.1
Average	**7.0**	**4.9**	**Average**	**6.4**	**4.3**
T1	3.6	3.1	T1	4.9	4.1
T1	6.2	4.3	T1	5.6	4.7
T1	4.1	2.1	T1	6.7	5.6
T1	5.7	3.6	T1	5.1	4.6
T1	3.4	3.7	T1	7.4	5.7
T1	4.9	2.3	T1	5.5	3.8
T1	5.3	3.9	T1	7.0	5.1
T1	3.4	2.6	T1	4.9	4.1
T1	4.2	3.6	T1	6.3	3.9
T1	5.4	4.1	T1	6.6	5.3
Average	**4.6**	**3.3**	**Average**	**6.0**	**4.7**
T2	2.8	2.8	T2	5.2	5.0
T2	3.1	1.4	T2	4.2	3.2
T2	2.4	1.8	T2	6.1	4.7
T2	1.8	1.3	T2	4.2	5.2
T2	3.5	2.4	T2	5.5	4.0
T2	4.4	1.8	T2	4.9	3.3
T2	2.2	1.9	T2	4.3	3.9
T2	2.8	2.5	T2	5.9	4.4
T2	1.6	1.6	T2	4.1	4.3
T2	3.7	2.2	T2	4.7	4.5
Average	**2.8**	**2.0**	**Average**	**4.9**	**4.2**
T3	3.2	1.6	T3	3.6	3.9

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T3	1.9	1.7	T3	4.4	3.1
T3	2.6	2.2	T3	5.9	4.7

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T3	1.8	1.2	T3	4.3	3.8
T3	2.6	2.5	T3	5.9	4.2
T3	2.1	2.4	T3	5.1	4.9
T3	2.7	1.2	T3	5.2	3.8
T3	3.2	1.7	T3	4.2	3.2
T3	1.9	1.4	T3	3.4	3.1
T3	3.5	2.2	T3	5.1	4.7
Average	**2.6**	**1.8**	**Average**	**4.7**	**3.9**
T4	1.7	1.1	T4	4.1	3.8
T4	0.5	1.5	T4	5.1	3.7
T4	2.2	0.4	T4	4.2	4.4
T4	1.5	1.3	T4	3.9	3.7
T4	0.7	0.8	T4	4.1	4.2
T4	1.8	1.1	T4	5.1	3.5
T4	1.3	0.8	T4	4.7	4.2
T4	0.7	1.4	T4	4.3	4.5
T4	2.1	1.3	T4	4.1	3.9
T4	0.8	0.9	T4	5.1	3.9
Average	**1.3**	**1.1**	**Average**	**4.5**	**4.0**
T5	7.1	2.9	T5	4.5	2.2
T5	3.9	4.6	T5	4.8	1.4
T5	5.5	3.8	T5	3.6	3.2
T5	3.3	2.3	T5	3.0	1.4
T5	6.4	3.9	T5	5.8	2.8
T5	4.7	4.7	T5	4.3	3.6
T5	5.8	2.4	T5	5.3	1.5
T5	6.1	3.1	T5	3.5	2.0
T5	5.3	2.4	T5	3.9	3.5
T5	6.2	4.3	T5	4.6	2.8
Average	**5.4**	**3.5**	**Average**	**4.3**	**2.4**
T6	1.1	0.8	T6	1.8	2.0
T6	0	0.5	T6	2.7	1.5
T6	1.5	0.3	T6	2.2	1.7
T6	0.3	0.2	T6	1.4	1.9
T6	0.4	0.8	T6	1.5	2.2
T6	1.7	0.7	T6	2.7	1.7
T6	0.8	0.5	T6	1.8	1.6
T6	0.5	0.9	T6	2.4	1.9
T6	0.3	0.2	T6	1.6	1.6
T6	1.2	0.3	T6	2.5	1.4
Average	**0.8**	**0.5**	**Average**	**2.1**	**1.7**

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A. Preventive strategies

Table 3. Efficacy of metalosate copper and other products on the area of injury by *l. theobromae* vine in the area of the variety. Evaluation of preventive application strategies.

Factor			Value de *P* [x] Area of injury cm²	
Main effects				
Treatment			<0,0001 (**)	
Treatments	**Application Methods**	**Place of inoculation**	**Area injury**	**Tukey**
T0: Witness		Variety	7,0[y]	a[z]
T1: Metalosate copper: 0,3 L/200 L	Leaf	Variety	4,6	b
T2: Metalosate copper: 0,5 L/200 L	leaf	Variety	2,8	c
T3: Metalosate copper: 0,7 L/200 L	Leaf	Variety	2,6	cd
T4: Met. copper: 0,5 L/200 L + Met. Boron: 0,2 L/200 L	Leaf	Variety	1,3	de
T5: Prochloraz: 0,3 L/200 L	Drench	Variety	5,4	b
T6: Thiabendazol: 0,4 L/200 L	Drench	Variety	0,8	e
Average			3,5	
Coefficient of variability			26,9 %	

Values of *P*> 0.05 are not significantly different according to Tukey's test. (**) High significance, (NS) there is no significance. Each data is average of 10 values (plants), and refers to the area of injury in cm2 developed from the area of inoculation in infections by *Lasiodiplodia theobromae*; [z] Numbers in column followed by the same letter are not significantly different according to Tukey's test.

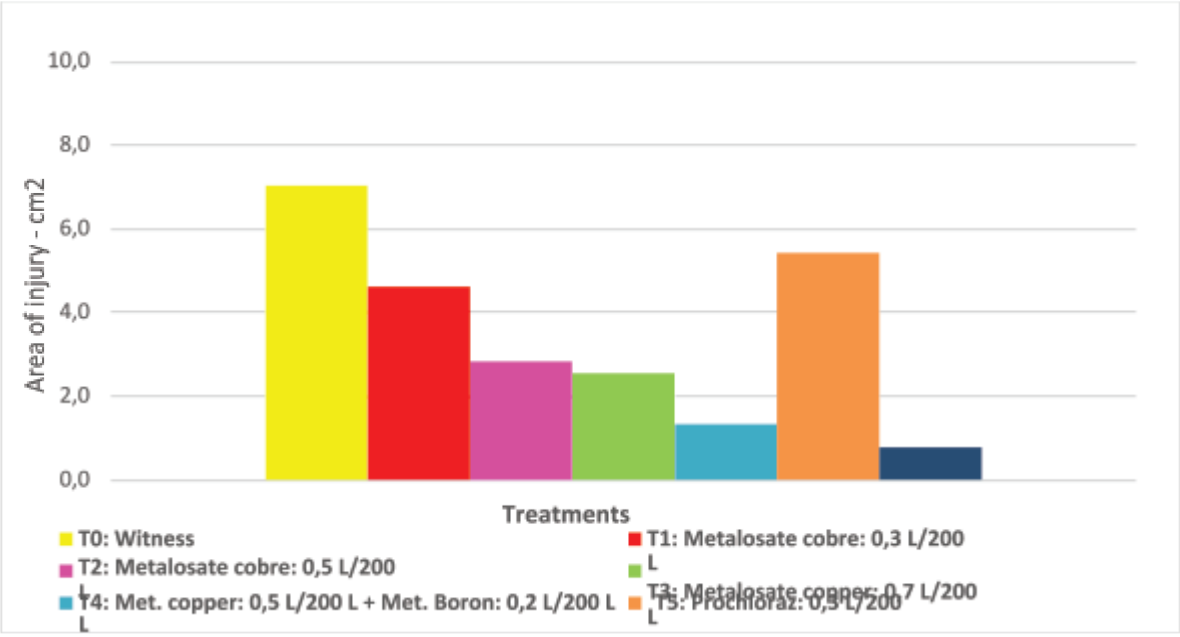

Figure 4. Influence of metalosate copper on the severity of the lesions (area of injury in cm2)

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caused by *Lasiodiplodia* in vines in the area of the variety in preventive applications

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Table 4. Efficacy of metalosate copper and other products on the area of injury by *l. theobromae* vine in the area of the pattern Evaluation of preventive application strategies.

Factor Main effects			Value de *P* [x] Area of injury cm^2	
Treatments			<0,0001 (**)	
Treatments	**Method de application**	**Lugar inoculation**	**Area injury**	**Tukey**
T0: Witness		Pattern	4,9[y]	a[z]
T1: Metalosate copper: 0,3 L/200 L	Leaf	Pattern	3,3	b
T2: Metalosate copper: 0,5 L/200 L	Leaf	Pattern	2,0	c
T3: Metalosate copper: 0,7 L/200 L	Leaf	Pattern	1,8	cd
T4: Met. Copper: 0,5 L/200 L + Met. boron: 0,2 L/200 L	Leaf	Pattern	1,1	de
T5: Prochloraz: 0,3 L/200 L	Drench	Pattern	3,5	b
T6: Thiabendazol: 0,4 L/200 L	Drench	Pattern	0,5	e
Average			2,4	
Coefficient of variability			27,3 %	

Values of *P*> 0.05 are not significantly different according to Tukey's test. (**) High significance, (NS) there is no significance. Each data is average of 10 values (plants), and refers to the area of injury in cm2 developed from the area of inoculation in infections by *Lasiodiplodia theobromae*; [z] Numbers in column followed by the same letter are not significantly different according to Tukey's test.

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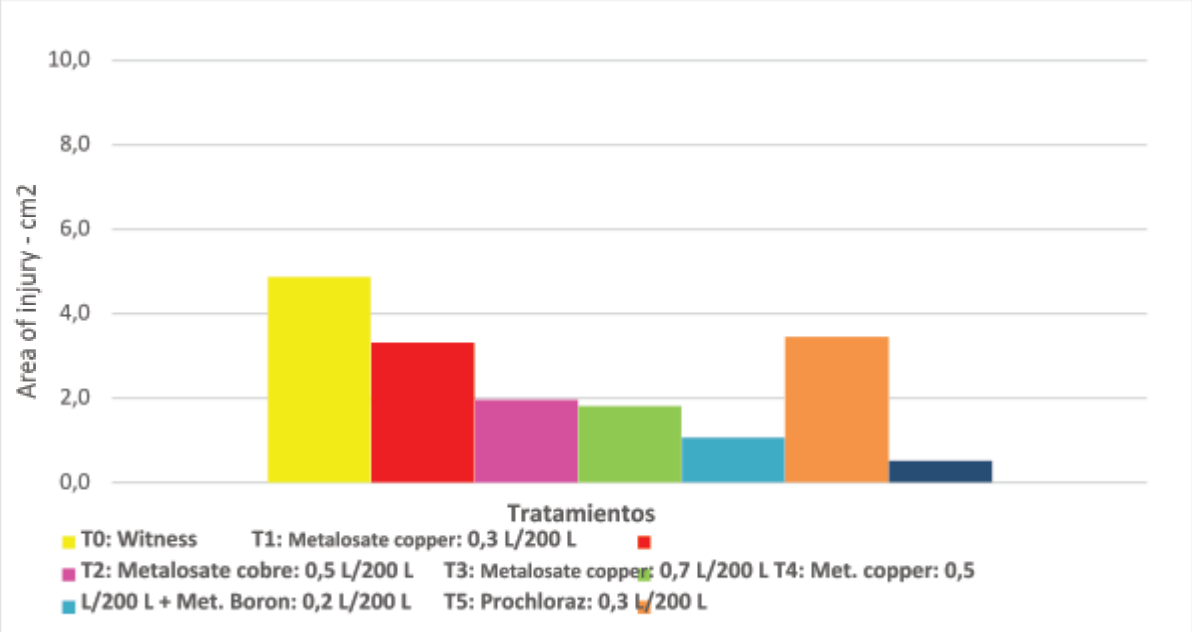

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Figure 5. Influence of metalosate copper on the severity of the lesions (area of injury in cm^2) caused by *Lasiodiplodia* in vine pattern on preventive applications area.

Curative strategies

Table 5. Efficacy of Metalosate copper and other products on the area of injury by *l. theobromae* vine in the area of the variety. Evaluation of healing application strategies.

Factor Main effects			**Value de *P* [x] Area of injury - cm²**	
Treatments			<0,0001 (**)	
Treatments	**Application Method**	**Place of inoculation**	**Area injury**	**Tukey**
T0: Witness		Variety	6,4[y]	a[z]
T1: Metalosate copper: 0,3 L/200 L	Foliar	Variety	6,0	ab
T2: Metalosate copper: 0,5 L/200 L	Foliar	Variety	4,9	bc
T3: Metalosate cobre: 0,7 L/200 L	Foliar	Variety	4,7	c
T4: Met. copper: 0,5 L/200 L + Met. Boron: 0,2 L/200 L	Foliar	Variety	4,5	c
T5: Prochloraz: 0,3 L/200 L	Drench	Variety	4,3	c
T6: Thiabendazol: 0,4 L/200 L	Drench	Variety	2,1	d
Average			4,7	
Coefficient of variability			17,5 %	

Values of *P*> 0.05 are not significantly different according to Tukey's test. (**) High significance, (NS) there is no significance. Each data is average of 10 values (plants), and refers to the area of injury in cm2 developed from the area of inoculation in infections by *Lasiodiplodia theobromae*; [z] Numbers in column followed by the same letter are not significantly different according to Tukey's test.

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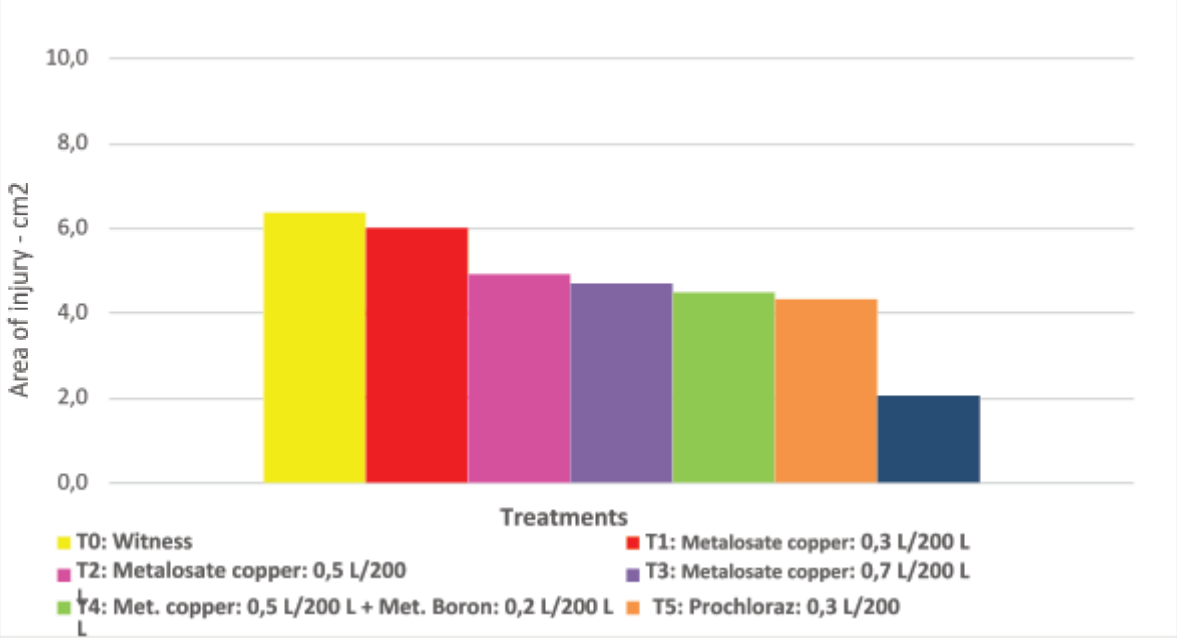

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Figure 6. Influence of metalosate copper on the severity of the lesions (area of injury in cm^2) caused by *Lasiodiplodia* in vines in the area of the variety of cures.

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Table 6. Efficacy of metalosate copper and other products on the area of injury by *l. theobromae* vine in the area of the pattern. Evaluation of healing application strategies.

Factor	Value de *P* [x] Área of injury - cm²			
Main effects				
Treatments	<0,0001 (**)			
Treatments	**Method of application**	**Lugar de inoculation**	**Area injury**	**Tukey**
T0: Witness		Pattern	4.3[y]	a[z]
T1: Metalosate copper: 0,3 L/200 L	Leaf	Pattern	4.7	a
T2: Metalosate copper: 0,5 L/200 L	Leaf	Pattern	4.2	a
T3: Metalosate copper: 0,7 L/200 L	Leaf	Pattern	3.9	a
T4: Met. Copper: 0,5 L/200 L + Met. Boron: 0,2 L/200 L	Leaf	Pattern	4.0	a
T5: Prochloraz: 0,3 L/200 L	Drench	Pattern	2.4	b
T6: Thiabendazol: 0,4 L/200 L	Drench	Pattern	1.7	b
Average			3,6	
Coefficient of variability			17,7 %	

Values of *P*> 0.05 are not significantly different according to Tukey's test. (**) High significance, (NS) there is no significance. Each data is average of 10 values (plants), and refers to the area of injury in cm2 developed from the area of inoculation in infections by *Lasiodiplodia theobromae*; [z] Numbers in column followed by the same letter are not significantly different according to Tukey's test.

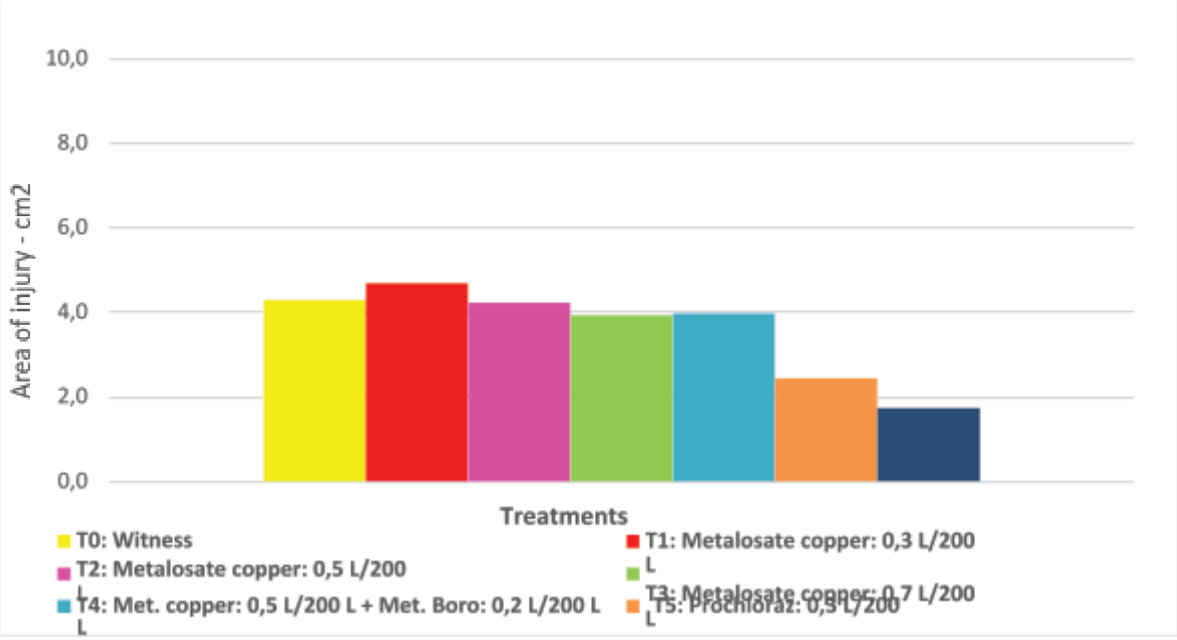

Figure 7. Influence of metalosate copper on the severity of the lesions (area of injury in cm^2) caused by *Lasiodiplodia* in vines in the area of the pattern in cures

Table 7. Calculation of the percentage of control of each treatment for the trial of the efficacy of metalosate copper and other products on *Lasiodiplodia theobromae* on vine.

	Aplicacionais de índole			
	preventive		curative	
Treatments	Variety	Pattern	Variety	Pattern
	de índole			
T1: Metalosate copper: 0,3 L/200 L	34,3[z]	31,9	5,7	0,0
T2: Metalosate copper: 0,5 L/200 L	59,7	59,8	22,8	1,6
T3: Metalosate copper: 0,7 L/200 L	63,7	62,9	26,2	8,5
T4: Met. Copper: 0,5 L/200 L + Met. Boron: 0,2 L/200 L	63,1	65,2	8,4	3,6
T5: Prochloraz: 0,3 L/200 L	22,8	29,1	32,0	43,2
T6: Thiabendazol: 0,4 L/200 L	88,9	89,5	67,8	59,6

[z] Values calculated by the following formula: % control = 100-(area of lesion in the treatment treated by 100/area of injury in the control treatment witness)

3. CONCLUSIONS

1. Were evaluated two strategies for the implementation of products, preventative and curative applications. The results obtained indicate that best results were obtained by preventive applications, because generated lesion area averages by *l. theobromae*, in general, were minor compared to the registered areas of injury to healing applications (table 2).
2. All doses evaluated Metalosate copper areas of injury decreased by *l. thebromae*, particularly when the products were applied preventively (tables 3 and 4).
3. When applications were preemptively, to the areas of injury reported in the area of the variety, the best treatments were T6 (Thiabendazol: 0.4 L / 200 L) and T4 (Metalosate copper: 0.5 L / 200 L + Metalosate boron: 0.2 L / 200 L), without significant statistical differences among treatments. Then stood in the T3 treatment (Metalosate copper: 0.7 L / 200 L); treatment T2 (Metalosate copper: 0.5 L / 200 L) did not differ significantly (table

3) T3 treatment. The same trend of efficacy of treatment was recorded when areas of injury in the area of the pattern were evaluated (table 4).

4. When applications were healing way, to the areas of injury reported in the area of the variety, the best treatment was T6 (Thiabendazol: 0.4 L / 200 L). Then were the treatments T3, T4 and T5, no significant statistical differences among treatments (table 5). For the areas of injury reported in the area of the pattern, the best treatments were T6 and T5, no significant statistical differences among treatments. Then other treatments no significant differences among them were (table 6).
5. Control percentages obtained (table 7) are consistent with results from the separation of stockings, and sample that T4, T3 and T2 treatments obtained the highest percentages of control.
6. Between chemicals evaluated statistical significant differences between treatments prochloraz and thiabendazol were recorded. The lower efficiency was obtained with the product prochloraz, who won the largest areas of injury pattern and variety. These results may be due to differences in mobility of these products in soil and the interior of the plant.
7. The results indicate that Metalosate copper preventive applications have an effect directly above *Lasiodiplodia theobromae*, pathogen associated with "dead arm" of the vine, one of the most important diseases of the "fungi of timber" vine. *Lasiodiplodia* complex affects the internal tissues of the organs that affect and interfere directly with the free passage of water and nutrients in the plant.

4. RECOMMENDATIONS

Use metalosate copper at doses higher than 0.5 L / 200 L. A synergistic effect was observed when this product is used jointly with metalosate boron

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Evaluation of the efficacy of Metalosate copper on infections by *Lasiodiplodia theobromae* under controlled conditions. ICA, April - June of 2015.

Preventive applications (assessment of the area of the variety)

Dependent variable: Y

ANOVA procedure

source

	DF	Sum of squares	The average square	F-Valor	PR > F
Model	6	308.9820000	51.4970000	57.36	<.0001
Error	63	56.5610000	0.8977937		
Corrected total	69	365.5430000			

R-square	Coef, Var	Root MSE	Y Average
0.845269	26.99486	0.947520	3.510000

Source	DF	Anova SS	The average square	F-Valor	PR > F
TRAT	6	308.9820000	51.4970000	57.36	<.0001

Tukey grouping	Average	N	TRAT
A	7.0300	10	0
B	5.4300	10	5
B	4.6200	10	1
C	2.8300	10	2
D C	2.5500	10	3
D E	1.3300	10	4
E	0.7800	10	6

Preventive applications (assessment of the area of the pattern)

Dependent variable: Y

ANOVA procedure

source

	DF	Sum of squares	The average square	F-Valor	PR > F
Model	6	140.0268571	23.3378095	52.92	<.0001
Error	63	27.7840000	0.4410159		
Corrected total	69	167.8108571			

R-square	Coef, Var	Root MSE	Y Average
0.834433	27.31276	0.664090	2.431429

Source	DF	Anova SS	The average square	F-Valor	PR > F
TRAT	6	140.0268571	23.3378095	52.92	<.0001

Tukey grouping	Average	N	TRAT
A	4.8900	10	0
B	3.4400	10	5
B	3.3300	10	1

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	C	1.9700	10	2
D	C	1.8100	10	3
D	E	1.0600	10	4
	E	0.5200	10	6

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Application healing (evaluation of the area of the variety)

Dependent variable: Y

Procedure ANOVA

source

	DF	Sum of squares	The average square	F-Valor	PR > F
Model	6	116.4868571	19.4144762	28.59	<.0001
Error	63	42.7880000	0.6791746		
Corrected total	69	159.2748571			

R-Square	Coef, Var	Root MSE	Y Average
0.731357	17.56651	0.824121	4.691429

Source	Mexico City	Anova SS	The average square	F-Valor	PR > F
TRAT	6	116.4868571	19.4144762	28.59	<.0001

Tukey grouping	Average	N	TRAT
A	6.3600	10	0
B A	6.0000	10	1
B C	4.9100	10	2
C	4.7100	10	3
C	4.4700	10	4
C	4.3300	10	5
D	2.0600	10	6

Application healing (evaluation of the area of the pattern)

Dependent variable: Y

Procedure ANOVA

source

	DF	Sum of squares	The average square	F-Valor	PR > F
Model	6	71.39142857	11.89857143	28.86	<.0001
Error	63	25.97200000	0.41225397		
Corrected total	69	97.36342857			

R-Square	Coef, Var	Root MSE	Y Average
0.733247	17.72275	0.642070	3.622857

Source	Mexico City	Anova SS	The average square	F-Valor	PR > F
TRAT	6	71.39142857	11.89857143	28.86	<.0001

Tukey grouping	Average	N	TRAT
A	4.6900	10	1
A	4.3100	10	0
A	4.2500	10	2

Ensayo Metalosate cobre – *hongos de madera* - vid

A	3.9800	10	4
A	3.9400	10	3
B	2.4400	10	5
B	1.7500	10	6