ACIBENZOLAR-S-METHYL IN RUST AND BLOTCH CONTROL IN FIELD CONDITIONS

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ABSTRACT: The objective of this study was to evaluate the effect of acibenzolar-S-methyl (ASM) resistance inductor, associated or not with the fungicide azoxystrobin + cyproconazole base, the control of Cercospora leaf rust and coffee under field conditions. The study was conducted during two harvest years (2007/2008 and 2008/2009), in commercial planting of the Mundo Novo cultivar. Used treatments were: ASM (25 g ha^{-1}) applied omnthly five times (from December to April of each harvest year); fungicide (500 mL ha^{-1}) and the mixture fungicide (500 mL ha^{-1}) + ASM applied three times (December, February and April of each harvest year); the mixture fungicide (500 mL ha^{-1}) applied three times (December, February and April of each harvest year) + ASM applied five times (December to April of each harvest year); the mixture fungicide (750 mL ha^{-1}) applied two times (December and March of each harvest year) + ASM applied five times (December to April of each harvest year) and witness. Rust control (84%) given by ASM was statistically equal to the treatment with fungicide (94%) in the low harvest year (2008/2009), when diseases intensity was not as high as in the high harvest year (2007/2008). In the high harvest year, ASM did not control blotch, while in the low harvest year this product controlled blotch (77%) at an inferior rate compared to fungicide (92%) and mixtures (89 to 94%). In low harvest years, ASM can be used in rust and blotch control for coffee trees and showed compatibility with the fungicide ciproconazole + azoxystrobin.

Index terms: *Coffea arabica* L., *Hemileia vastatrix*, *Cercospora coffeicola*, induction of resistance.

ACIBENZOLAR-S-METIL NO CONTROLE DA FERRUGEM E DA CERCOSPORIOSE DO CAFEEIRO EM CONDIÇÕES DE CAMPO

***RESUMO:** Objetivou-se, neste trabalho, avaliar o efeito do indutor de resistência acibenzolar-S-metil (ASM), associado ou não com fungicida à base de ciproconazol + azoxystrobin, no controle da ferrugem e cercosporiose do cafeeiro em condições de campo. O ensaio foi realizado em dois anos safra (2007/2008 e 2008/2009), em plantio comercial com a cultivar Mundo Novo. Os tratamentos utilizados foram: ASM (25 g ha^{-1}) aplicado mensalmente 5 vezes (dezembro a abril de cada ano safra); fungicida (500 mL ha^{-1}) e a mistura fungicida (500 mL ha^{-1}) + ASM aplicados 3 vezes (dezembro, fevereiro e abril de cada ano safra); a mistura fungicida (500 mL ha^{-1}) aplicado 3 vezes (dezembro, fevereiro e abril de cada ano safra) + ASM aplicado 5 vezes (dezembro a abril de cada ano safra); a mistura fungicida (750 mL ha^{-1}) aplicados 2 vezes (dezembro e março de cada ano safra) + ASM aplicado 5 vezes (dezembro a abril de cada ano safra) e testemunha. O controle da ferrugem (84%) proporcionado pelo ASM foi estatisticamente igual ao tratamento com o fungicida (94%) no ano de safra baixa (2008/2009), quando a intensidade das doenças não foi tão elevada quanto no ano de safra alta (2007/2008). No ano de safra alta, o ASM não controlou a cercosporiose, enquanto que no ano de safra baixa esse produto controlou a cercosporiose (77%) de forma inferior ao fungicida (92%) e suas misturas (89 a 94%). Em ano de safra baixa, o ASM pode ser usado no controle da ferrugem e cercosporiose do cafeeiro e apresentou compatibidade com o fungicida ciproconazol + azoxystrobin.*

***Termos para indexação:** Coffea arabica, Hemileia vastatrix, Cercospora coffeicola, indução de resistência.*

1 INTRODUCTION

Coffee is one of the main commodities produced in Brazil, and stands out as the world's biggest producer and exporter. According to the National Supplying Company (Companhia Nacional de Abastecimento - CONAB (2012)), coffe arabica production for the 2012/2013 harvest is estimated 37,9 million sacs. Although coffee culture shows great growth potential, some diseases have limited plantation productivity, such as coffee leaf rust, caused by *Hemileia vastatrix* Berk. & Br. And blotch, caused by *Cercospora coffeicola* Berk. & Cooke (ZAMBOLIM; VALE; ZAMBOLIM, 2005). The losses due to rust can reach up to 50%, since the production damage extends to the following years (POZZA, 2008). This disease causes precocious leaf drop and withering of productive branches and therefore do not produce during the following year,

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lowering productivity and beverage quality. This constant branch withering reduces the coffee trees' lifespan, making the plantation gradually antieconomic. Other disease of great importance in coffee culture is blotch, capable of intense plant defoliation, production reducing damage to beans and lowering beverage quality. In greenhouse conditions, blotch causes severe defoliation of seedlings, causing development delay and possibly making them rickety (ZAMBOLIM; VALE; ZAMBOLIM, 2005). Blotch setbacks achieved economic importance in Minas Gerais state after the setting of plantations at the cerrado, which show low natural soil fertility, or in badly nutrition handled plantations (POZZA et al., 2000).

Resistance induction in plants against phytopathogens representsan alternative method in disease control, which triggers latent defense mechanisms in the plants. Such activation could be achieved through biotic treatment (CAVALCANTI et al., 2006) or by chemical activators such as acibenzolar-S-metil (ASM) (LOON; BAKKER; PIETERSE, 1998). Among the defense mechanisms could be quoted the activation of protein genes related to the pathogen (PR proteins) (LOON; REP; PIETERSE, 2006), activation of genes that code for enzimes, which regulate secondary metabolic routes of substances of the phytoalexin types od structural defense compounds such as lignin (GLAZEBROOK, 2005).

The only commercial product registered at the Agriculture Ministery as a plant activator in Brazil is 500 WG® (Syngenta Proteção de Cultivos), whose active is acibenzolar-S-metil (ASM). This product is derived from salicylic acid molecule and was developed to promote activation of the metabolic aparatus described above. Many studies proved the protective effect of ASM in coffee plants against a large spectrum of pathogens. Guzzo et al. (2001) observed that ASM provided local protection of 97% and 94% systemic protection in coffee seedlings against coffee leaf rust. According to the author, the fact highlights the products equitable diffusion capacity starting from applying point and/or activation of resistance machanisms in plants (systemic aquired resistance - RSA), which stops or hampers the pathogens' stablishment and/or development, even though no pre-penetration (germination or apresorium formation) phase effect has been observed. This inductive effect of ASM against coffee leaf rust was confirmed by Pereira et al. (2009), reporting harshness reduction of this disease by 70% when compared to witness. In another study, Guzzo et al. (2004) observed increase in chitinase and β-1,3-glucanase PR proteins activities, one day after foliar application of ASM, which maintained high up to 35 days after application, showing reduction if rust harshness from 60% to 80%, in controlled conditions. Nardi et al. (2006) verified in coffee leaves, 16 hours after ASM application, the super expression of genes responsible for PR proteins such as chitinase and genes responsible for celular wall fortification, such as peroxidase.

The objective of this study was to evaluate the action of acibenzolar-S-metil, associated or not to standard fungicide (ciproconazol 80 g L^{-1} + azoxystrobin 200 g L^{-1}), in coffee leaf rust and blotch control in the field.

2 MATERIALS AND METHODS

The experiment was conducted in Cascavel Farm, Carmo da Cachoeira municipality, southern Minas Gerais, in the harvest years of 2007/2008 and 2008/2009. It was used coffee plantation (Coffee arabica cv. Mundo Novo), sensitive to coffee leaf rust and blotch, spaced by 3,5 m x 0,8 m, six years after re-strain, taken over conventional cultivation system. During the time of the experiment the plantation was fertilized according to ground analyses and growth recommendations for Minas Gerais state (RIBEIRO; GUIMARÃES; ALVAREZ, 1999). As control pattern to compare the action of acibenzolar-S-metil a fungicide product composed by ciproconazole 80 g L^{-1} + azoxystrobin 200 g L^{-1} was used. Treatments used in the experiment are described in Chart 1, as well as the dosages and times of used applications. Experimental delimitation used was of randomised blocks, with six treatments and four repetitions. Parcels were made out of three planting lines with ten plants each, Only the six center plants from the central parcel line were used as useful parcels.

All of the pulverizations were performed using motorized backpack sprayers, applying syrup volumes equivalent to 400 L ha^{-1}. The rust and blotch assesments took place every 30 days, using diagram scales of Cunha et al. (2001) and Custodio et al. (2011), respectively. The third and fourth pairs of leaves from six plagiotropic branches from the mi third of each plant were assessed (three branches from each side of the planting line). Then, for all the treatments, the areas below the incidence progress (AACPI) and harshness (AACPS) curves were calculated, using Shaner and Finney's (1977) formula.

CHART 1 - Treatments assessed in Mundo Novo cultivar coffee plantations during the harvest years of 2007/2008 and 2008/2009, their respective dosages and periods of application. Lavras, Minas Gerais, Brasil.

Treatments		Doses ha^{-1} (mL ou g)	Periods of application (harvest years)									
			2007/2008					2008/2009				
			dec	jan	feb	mar	apr	dec	jan	feb	mar	apr
1.	Fungicide	500	x	-	x	-	x	x	-	x	-	x
2.	Fungicide	500	x	-	x	-	x	x	-	x	-	x
	ASM	25	x	-	x	-	x	x	-	x	-	x
3.	Fungicide	500	x	-	x	-	x	x	-	x	-	x
	ASM	25	x	x	x	x	x	x	x	x	x	x
4.	Fungicide	750	x	-	-	x	-	x	-	-	x	-
	ASM	25	x	x	x	x	x	x	x	x	x	x
5.	ASM	25	x	x	x	x	x	x	x	x	x	x
6.	Witness	-	-	-	-	-	-	-	-	-	-	-

* ASM: acibenzolar-S-metil; Fungicide: ciproconazole + azoxystrobin; In every treatment paraffinic mineral oil at 0,5% concentration syrup volume was used.

Coffee tree productivity was also assessed during the harvest years of 2007/2008 and 2008/2009. In order to do só, beans from the six plants from the center of the parcel were machanically picked, using tractor attached harvester. Those were wieghted, and the numbers collected were turned into the equivalent of sacs per hectare. The assessments of coffee tree leafing began just before harvesting, and took place according to Boldini's (2001) grade scale. Grades from 1 to 5 represent from 0% to 20%, 21% to 40%, 41% to 60%, 61% to 80% and 81% to 100% leafing, respectively. Statistic analysis were performed using Sisvar v. 5.1 (FERREIRA, 2008) statistic software and average comparison were performed by Turkey test (p<0,05).

3 RESULTS AND DISCUSSION

During the experiment, no signs of toxicity were verified in coffee trees due to treatment applications. Generally, higher and harsher coffee leaf rust incidences during the harvest year of 2007/2008 took place between the months of March and October, peaking in June and July. As for the harvest year of 2008/2009, the disease's peak happened from the month of March (data not shown). As shown in Chart 2, all of the treatments significantly reduced the areas below the incidence progress (AACPI) and harshness (AACPS) curves of coffee leaf rust during the first disease assessment year (2007/2008).

The commercial mixture fo fungicides ciproconazole + azoxystrobin applied alone of associated to ASM, regardless of the application periods, reduced in 97% the AACPIF and in 98% the AACPSF, followed by ASM ASM, which differed from the witness and reduced in 18% and 23% AACPIF and AACPSF, respectively.

During the second year of application (2008/2009), under less disease pressure, treatments constituted by the fungicides ciproconazole + azoxystrobin or by the association of these with ASM, regardless of the period of application, reduced AACPIF and AACPSF in a similar way, with decreases from 89% to 96% in relation to witness (Chart 2). The ASM treatment did not differ and reduced in 84% and 85% AACPIF and AACPSF, respectively, being as efficient as evaluated fungicides and associations.

When it came to blotch, disease incidences and harshness during the harvest year of 2007/2008 were observed starting in March, with peaks in August and September. As for the harvest year of 2008/2009, higher disease incidences and harshness were observed between January and June (data not shown). As observed in Chart 3, all of the treatments significantly reduced the areas below the incidence progress (AACPI) and harshness (AACPS) curves of blotch in the first assessment year (2007/2008). Mixture of fungicides ciproconazole + azoxystrobin, associated or not to the inductor ASM, regardless of the assessment period, reduced AACPIC AACPSC in similar ways, with decreases from 73% to 79% from 74% to 79% respectively.

CHART 2 – Effect of treatments in reducing (%) and areas below the incidence progress (AACPI) and harshness (AACPS) curves of coffee leaf rust in Mundo Novo cultivar coffee trees, during the harvest years of 2007/2008 and 2008/2009.

Treatments*	Doses ha^{-1} (mL ou g)	2007/2008 AACPIF	2007/2008 Reduction (%)	2007/2008 AACPSF	2007/2008 Reduction (%)	2008/2009 AACPIF	2008/2009 Reduction (%)	2008/2009 AACPSF	2008/2009 Reduction (%)
1. Fungicide[a]	500	126,5 a	99	10,3 a	98,2	416,0 a	94	12,8 a	94
2. Fungicide[a] ASM[a]	500 25	252,9 a	98	16,5 a	97,1	554,7 a	92	17,0 a	92
3. Fungicide[a] ASM[b]	500 25	379,4 a	97	16,5 a	97,1	762,7 a	89	23,4 a	89
4. Fungicide[c] ASM[b]	750 25	252,9 a	98	18,5 a	96,8	277,3 a	96	8,5 a	96
5. ASM[b]	25	10369,0 b	18	442,6 b	23,2	1109,3 a	84	32,0 a	85
6. Witness	---	12645,2 c	---	576,5 c	---	6933,3 b		213,0 b	---

* ASM: acibenzolar-S-metil; Fungicide: ciproconazole+azoxystrobin; Averages with the same letter on the column do not differ by the Tukey test ($p \leq 0,05$).

[a] Applications: December (2007), February and April (2008), December (2008), February and April (2009);

[b] Applications: December (2007), January, February, March, April and December (2008), January, February, March and April (2009);

[c] Applications: December (2007), March and December (2008), March (2009).

CHART 3 - Effect of treatments in reducing (%) and in the areas below the incidence progress (AACPI) and harshness (AACPS) curves of blotch in Mundo Novo cultivar coffee trees, in the years of 2007/3008 and 2008/2009.

Treatments*	Doses ha^{-1} (mL ou g)	2007/2008				2008/2009			
		AACPIC	Reduction (%)	AACPSC	Reduction (%)	AACPIC	Reduction (%)	AACPSC	Reduction (%)
1. Fungicide[a]	500	2442,9 a	73	78,9 a	74	501,5 a	92	13,8 a	93
2. Fungicide[a] ASM[a]	500 25	1900,0 a	79	63,7 a	79	689,6 a	89	21,7 a	89
3. Fungicide[a] ASM[b]	500 25	2171,4 a	76	72,8 a	76	564,2 a	91	15,8 a	92
4. Fungicide[c] ASM[b]	750 25	2171,4 a	76	72,0 a	76	376,1 a	94	9,8 a	95
5. ASM[b]	25	8233,3 b	9	300,0 b	1	1441,8 b	77	45,3 b	77
6. Witness	---	9047,6 b	---	303,3 b	---	6268,7 c	---	196,9 c	---

* ASM: acibenzolar-S-metil; Fungicide: ciproconazole+azoxystrobin; Averages with the same letter in column do not differ by the Tukey test ($p \leq 0,05$).

[a] Applications: December (2007), February and April (2008), December (2008), February and April (2009);

[b] Applications: December (2007), January, February, March, April and December (2008), January, February, March and April (2009);

[c] Applications: December (2007), March and December (2008), March (2009).

During this first assessment year, ASM inductor did not control blotch in a satisfactory way, as it did no differ from the witness.

In the harvest year of 2008/2009, treatments constituted of ciproconazole + azoxystrobin or the association of these and ASM, regardles of the application period, showed similar behavior in the harvest year of 2007/2008, decreasing AACPIC from 89% to 94% and AACPSC from 89% to 95% (Chart 3). As for the ASM treatment, it showed a different behavior, for it differed from the other treatments, as well as from the witness, reducing in 77% AACPIC and AACPSC.

In relation to the leafing of the coffee tree during 2008/2009, it was observed that plants sprayed with ciproconazole + azoxystrobin, associated or not with ASM, showed leafings from 89,2% to 94,2%, while plants sprayed with ASM and witness plants did not differ between themselves and showed leafings of 44,8% and 48,3%, respectively (Chart 4). During the second year of assessment (2008/2009) a significative leafing difference was not observed in plants sprayed with fungicide and/or ASM and witness plants, which showed leafings of 89,2% and 94,1%.

No significative difference was observed in plants' productivity between the treatments during the first year of assessment, which produced from 71,1 to 75,1 sacs ha^{-1} (Chart 4).

On the counterpart, during the year of 2008/2009, considered a low production year, plants sprayed with fungicide, except ASM (25 g ha^{-1}), showed productivities higher than witness, which varied from 19,8 to 23,9 sacs ha^{-1}. Witness plants and plants sprayed only with ASM produced the equivalent of 10,2 and 10,3 sacs ha^{-1}, respectively.

As the field studies went by, it was observed that the ASM activator showed satisfactory results, mostly in the year of low pending load (2008/2009). However, other studies with higher doses and different application periods must be performed, mainly for the years of high pending load.

During the first assessment year (2007/2008) it was observed that ASM applied alone, even though it showed a certain rust control, was not as satisfactory as the other treatments which contained the fungicide ciproconazole + azoxystrobin. It is known, however, that this was an year of high pending load, which contributed to the unsatisfactory effect of the activator. Similar results were found for blotch and, in this case, ASM did not differ statistically from the witness.

In the second year of assessment (2008/2009), for blotch, ASM showed itself effective in decrasing areas below the incidence progress (AACPI) and harshness (AACPS) curves, with 77% reduction.

CHART 4 - Effect of treatments of leafing percentage and productivity (sacs ha^{-1}) of Mundo Novo cultivar coffee tree during the harvest years of 2007/2008 and 2008/2009.

Treatments	Doses ha^{-1} (mL ou g)	Leafing (%)		Productivity (sacs ha^{-1})	
		2007/2008	2008/2009	2007/2008	2008/2009
1. Fungicide[a]	500	89,2 a	92,3 a	72,8 a	19,8 a
2. Fungicide[a] ASM[a]	500 25	91,7 a	93,6 a	71,4 a	19,8 a
3. Fungicide[a] ASM[b]	500 25	94,2 a	92,7 a	75,1 a	23,9 a
4. Fungicide[c] ASM[b]	750 25	94,2 a	94,1 a	71,5 a	21,5 a
5. ASM[b]	25	44,8 b	91,3 a	74,7 a	10,3 b
6. Witness	---	48,3 b	89,2 a	71,1 a	10,2 b

* ASM: acibenzolar-S-metil; Fungicide: ciproconazole+azoxystrobin; Averages with the same letter in column do not differ by the Tukey test (p≤0,05).

[a] Applications: December (2007), February and April (2008), December (2008), February and April (2009);

[b] Applications: December (2007), January, February, March, April and December (2008), January, February, March and April (2009);

[c] Applications: December (2007), March and December (2008), March (2009).

Such result was below the achieved by the fungicide, but above the control treatment (witness). For coffee leaf rust, ASM showed itself as efficient as the fungicide mixture, reaching disease reduction levels up to 85%, statistically not differing from the treatments in which that mixture was used. Data like these reinforce that Aquired systemic resistance is more effective for biotrophic fungi, such as coffee leaf rust, than for necrotrophic fungi, such as blotch (PIETERSE; TON; LOON, 2001).

Guzzo et al. (2001), using ASM on Mundo Novo cultivar coffee seedlings in greenhouses, verified reduction of up to 97% of number of rust inflicted lesions. They also observed that the protective effect of 90% persisted for up to ten weeks. Such result reinforces the fact that ASM inductor behaved in such a satisfactory way in disease control in the filed, mainly in the year of 2009, year of lower disease pressure.

Patrício et al. (2008) observed in a greenhouse experiment that strobilurin based fungicides were more effective in blotch control in coffee seedlings than others. The same was observed by Anesiadis, Karaoglanidis and Tzavella-Klonari (2003) and Karadimos, Karaoglanidis and Tzavella-Klonari (2005) in the pathosystem *Cercospora beticola* Sacc - beet (*Beta vulgaris* L.).

From the results shown, as for rust as for blotch, it became evident that the association of ASM activator with fungicides ciproconazole + azoxystrobin did not promote coffee leaf rust and blotch control increase in the field. During the first year of assessment (2007/2008), year of high pending load, the ASM treatment promoted a slight rust control and practically did not influentiate on blotch. On the other hand, treatment with fungicides ciproconazole + azoxystrobin, applied with no association with the inducer, was highly efficient in disease control. When ASM was associated with the fungicide, no potentiating effect of this inducer was verified in rust and blotch control. This fact occurred due to the high disease control index, mainly for rust which was above 92% during the two years of assessment. Similar results were obtained by Patrício et al. (2008), working with the pathosystem *C. coffeicola* – coffee tree, in which the authors did not verify aditional effect of ASM to copper oxychloride, for disease control.

It was observed that two applications of the fungicide mixture, at 750 mL ha^{-1} dosage, showed themselves as effective as three applications of the same, at 500 mL ha^{-1} dosage (Tabela 3). The first situation is more interesting, for it reduces the number of operations to be done at the plantation, decreasing soil compression, fuel and labor expenses, besides a lower exposure of the worker to toxic molecules.

During the first year of assessment, there was no difference in productivity of plants between the treatments, for that reflects the culture handlings delivered in the previous year, before the works on the area were initiated. When the first application of treatments was performed (December 2007), plantation production was already defined, for, in that time, coffee was already begining to grain (bean expansion phase– F2) and it would certainly not reflect difference between treatments in that year's production (2007/2008). however, for the harvest year of 2008/2009, the differences between treatments get evident, since these were the reflexes of two consecutive application years, so the second year of production was a reflex of the first sprayings done in the year of 2007/2008, when the study was already installed in the area.

As for coffee tree leafing, in the harvest year of 2007/2008 was observed that, when the pending load was high, the plant's draining power to the beans, which, allied to the high innoculation pressure in the area, made sure that there was a higher level of defoliation in the treatments more injured by rust and blotch, in this case, the ASM treatment and witness. This increased defoliation of these treatments resulted in lower production in the followeing year (2008/2009) (Chart 4). The production is decreased under high disease intensity, coffee leaf rust for example (KUSHALAPPA; ESKES, 1989), affecting since the formation of buds, consequence of the intense leaf dropping of the disease's previous cycle. Blotch, besides promoting defoliation, leads to premature bean drop and income loss, damaging the harvest (POZZA, 2008).

Witnesses and only ASM sprayed plants, which suffered from increased defoliation, needed higher metabolic and energetic expense to restablish themselves for the next year, in the emission of new plagiotropic branches, in branch growth and in the formation of leaves and roots. Thus, the plants that were highly damaged by defoliation during the first year (2007/2008), consequently produced less in the second year (2008/2009).

4 CONCLUSIONS

The plant activator acibenzolar-S-metil in the dosage of 25 g ha^{-1} has controled coffee leaf rust and blotch in a satisfactory way in low pending load year, but in the high pending load year it was not effective.

The commercial mixture of fungicides ciproconazole + azoxystrobin, used in the dosage of 750 mL ha^{-1} in two applications (January and March) controlled the coffee leaf rust and blotch effectively.

None of the products evaluated, in the dosages and condition used, caused phytotoxicity to the coffee tree.

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