NOTA PRÉVIA

APPLICATIONS OF COPPER-BASED FUNGICIDES AND INFESTATIONS OF *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) IN COFFEE PLANTS

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ABSTRACT: The present study aimed to evaluate the effects of applying fungicides with different sources of copper and of the number of applications on the occurrence of *Leucoptera coffeella* (Guérin-Menéville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) and on the wax content on leaves in a coffee plantation. Four applications of fungicides were carried out, and the effects on the number of leaves mined by the insect and on the wax content on the leaf surface were evaluated. The copper-based fungicides increased the number of leaves mined by the leaf-miner and reduced the wax content on the coffee leaf surfaces in both periods studied.

Index terms: *Coffea arabica*, pulverization, epicuticular wax, occurrence, leaf-miner.

APLICAÇÃO DE FUNGICIDAS CÚPRICOS E INFESTAÇÃO DE *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) NO CAFEEIRO

RESUMO: O presente trabalho teve como objetivo avaliar os efeitos de aplicações de diferentes fontes de fungicidas cúpricos e de números de aplicações, na ocorrência de *Leucoptera coffeella* (Guérin-Menéville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) e no teor de cera das folhas em lavoura cafeeira. Foram realizadas quatro aplicações de fungicidas e foi avaliado os efeitos no número de folhas minadas pelo inseto e no teor de cera da superfície foliar. Os fungicidas cúpricos aumentaram o número de folhas minadas pelo bicho-mineiro e reduziram o teor de cera das superfícies das folhas do cafeeiro nas duas épocas estudadas.

Termo de indexação: *Coffea arabica*, pulverização, cera epicuticular, ocorrência, bicho-mineiro.

The coffee leaf-miner, *Leucoptera coffeella* (Guérin-Mèneville, 1842) (Lepidoptera: Lyonetiidae), is the main pest of coffee plants (*Coffea arabica* L.) (BARRERA, 2008). The occurrence of this insect pest in plantations is associated with various factors, and notable among these is the application of copper-based fungicides, based on copper hydroxide and copper oxychloride. These compounds are applied in coffee plantations throughout the crop cycle in order to carry out preventive control of the fungus *Hemileia vastatrix* (CARVALHO et al., 2008) and also of bacterial infections. Copper fungicides may act on the wax layer of leaves, which is an important defense structure against attack by herbivorous insects (LICHSTON; GODOY, 2006), and changes to this layer can increase *L. coffeella* egg-laying and population size.

In planning management programs for this insect, it is very important to confirm if the application of copper fungicides is correlated with an increase in the population of the coffee leaf-miner. Therefore, the present study aimed to evaluate the effects of applying fungicides with different sources of copper and the effects of the number of applications on the occurrence of *L. coffeella* and on the wax layer of leaves in a coffee plantation.

The assays were carried out at Fazenda Vitória, a farm belonging to the José do Rosário Vellano University (UNIFENAS), in the county of Fama, MG, Brazil, between 21º 24’ 23” S and 45º 49’ 43” W, at 776 m altitude. The climate is Cwa according to the Köppen classification. Six-year-old coffee cv. Catuai plants, cultivated with spacing of 3.5 x 0.80 m, were used to conduct two assays in different periods of the year. The period from November 2016 to February 2017 was denominated period 1, the period from June 2017 to September 2017 was denominated period 2, and all assays were carried out on the same piece of land. Each assay received four applications of copper fungicides.

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The experimental design was randomized blocks in a 4x4 factorial scheme. It consisted of four applications of fungicide in a combination with three copper-based products plus the control treatment (water), with four replicates, totaling 64 experimental plots, each of which contained 10 plants. The useful area of each plot was composed of the six central plants, and between the blocks a line of coffee plants was left as a barrier. The fungicides and the doses used were: Cuprozeb® (copper oxychloride) – 3 kg of commercial product (c.p.).ha^{-1}, Supera® (copper hydroxide) – 3 L c.p. ha^{-1} and Supa cobre (copper hydroxide – product used for plant nutrition) – 2 L c.p. ha^{-1}.

The applications of the products were carried out every 30 days in each of periods 1 and 2, using a manual back-sprayer (Jacto®, model PJH 20, with a cone-shaped nozzle) and with application volume of 200 $L.ha^{1}$. The adjuvant Agral® was added at a concentration of 0.5% (v/v) to the chemical mixture and to the control water treatment. Crop treatment followed the agronomic practices recommended for coffee. No insecticides were applied in the experimental area.

For the evaluations of insect pest attack, four branches were marked in the middle third of the plant, by means of colored tape, with two on each side, making a total of 24 branches marked per plot. The applications of copper fungicides were carried out monthly. Twenty-four hours before the first application, the leaves that had already been mined were removed from the marked branches, in order not to count them during the first evaluation. The evaluations of the number of leaves with mines made by leaf-miner insects were carried out one day before each application, by counting the leaves with at least one intact mine; in other words, with no sign of predation by wasps. Immediately after each evaluation, the leaves with mines were removed from all the marked branches. In this way, it was possible to determine the infestation by leaf-miner throughout the evaluation periods.

In period 1 and in period 2, the wax content on leaf surfaces was analyzed at 10 days after the fourth fungicide application. This amount of time is sufficient for copper-based products to have been absorbed. Leaves from the third and fourth pair of branches in the middle third of the plants were collected, which were exactly those that had previously been marked with colored tape. Two leaves were taken from each side of the plant. These leaves were taken to the laboratory to quantify the wax content, following the methodology modified by Guimarães et al. (2009). For this, the leaves were separately placed on to a Petri dish with 100 mL of chloroform for 30 seconds, with gentle shaking. This procedure was carried out with great care to avoid rupturing the leaf tissues, thereby releasing cell compounds. The extracts obtained were filtered through filter paper and transferred to a beaker, in which they were evaporated over a steam bath until the volume was reduced to approximately 15 mL. This solution (chloroform and wax) was transferred to 25 mL test tubes of known weight. The chloroform was evaporated in a steam bath to obtain the solid residue (wax). The quantity of wax was expressed in mass per leaf area unit ($\mu g.\ cm^{-2}$).

The data on mined leaves and wax content were submitted to the Shapiro-Wilk test for analysis of normality. The data were transformed into $\sqrt{x} + 0.5$ and submitted to analysis of variance with the use of software R version 3.2.4 (R CORE TEAM, 2016). The means were grouped by the Scott-Knott test at 5% significance.

In relation to the percentage of leaves mined in the assay carried out in period 1, there was a significant effect on the interaction between the evaluated products and the number of applications (gl = 9; F = 2.53; $p < 0.05$) (Table 1). After the first two applications, no difference was observed between the evaluated products regarding the increase in leaf-miner infestation; however, from the third application onwards, the copper-based fungicides provoked an increase in the percentage of mines in relation to the control treatment. With three and four applications of the fungicides Supa cobre, Cuprozeb® and Supera®, an increase in the percentage of mined leaves was noted (Table 1).

There was a significant effect on the interaction between the evaluated fungicides and the number of applications with regard to the percentage of mined leaves in period 2 (gl = 9; F = 1.96; $p < 0.05$) (Table 1). There was no difference between the products in the first three applications; however, when the fourth application took place, the products Supa cobre and Cuprozeb® differed from Supera® and from the control treatment (Table 1). The number of applications of copper fungicides contributed to the increase in the percentage of mined leaves, and with four applications the number of mined leaves was higher.

The economic threshold of coffee leaf-miner in Brazil was previously determined to be 20% of mined leaves with no signs of predation, mainly in the third and fourth pair of leaves (REIS; SOUZA, 1996).

TABLE 1 – Percentage of leaves mined by the coffee leaf-miner (± standard error) after applications of copper fungicides in period 1 (November 2016 to February 2017) and period 2 (June 2017 to September 2017) in field conditions.

PERIOD 1				
Number of applications of copper fungicides				
Treatments	1	2	3	4
Supa cobre	2.27 ± 0.66 a B*	2.49 ± 0.7 a B	4.09 ± 1.56 a A	4.19 ± 0.58 a A
Cuprozeb	2.13 ± 0.6 a B	2.59 ± 0.68 a B	4.66 ± 1.3 a A	5.12 ± 0.77 a A
Supera	1.83 ± 0.69 a B	1.99 ± 0.8 a B	3.98 ± 1.11 a A	4.32 ± 0.57 a A
Control	1.83 ± 0.58 a A	1.83 ± 0.8 a A	1.92 ± 0.63 b A	2.11 ± 0.51 b A
PERIOD 2				
Number of applications of copper fungicides				
Treatments	1	2	3	4
Supa cobre	4.20 ± 1.64 a C*	8.40 ± 3.54 a B	10.75 ± 3.04 a B	22.47 ± 5.34 a A
Cuprozeb	2.31 ± 1.37 a C	11.52 ± 3.15 a B	13.41 ± 3.27 a B	22.72 ± 4.99 a A
Supera	5.47 ± 2.43 a C	10.21 ± 2.13 a B	11.09 ± 1.95 a B	16.83 ± 3.02 b A
Control	2.47 ± 1.21 a C	5.97 ± 3.59 a C	12.13 ± 3.14 a B	17.06 ± 3.6 b A

*Lower-case letters in the column and upper-case letters in the line do not differ among themselves by Scott-Knott test at a 5% level of significance.

In our study, the percentage of leaves mined in September was higher than 20% in plants treated with supa cobre (22.47%) and cuprozeb (22.72%) in older leaves. This information is important for improving management strategies for the coffee leaf-miner.

In period 1 (November 2016 to February 2017) the infestation of the coffee leaf-miner did not reach the economic threshold. These results were also observed by Custódio et al. (2009), in a study that identified low infestation of the leaf-miner during the same period in the south of Minas Gerais state. The South of Minas region presents high levels of rainfall, which leads to unsuitable conditions for the reproduction of the coffee leaf-miner (ASSIS et al., 2012).

The wax content in coffee leaves differed between the treatments from periods 1 and 2 (gl = 3; F = 4.91; $p < 0.05$ and gl = 3; F = 4.94; $p < 0.05$) (Table 2). The three copper-based fungicides reduced the wax content on leaves in both periods 1 and 2.

Studies have been carried out on the effects of copper fungicide application on the wax content of coffee leaves; however, little is known about its influence on infestation by *L. coffeella*. Linchoston and Godoy (2006) performed four cumulative applications of fungicides based on copper oxychloride on coffee plants and affirmed that these applications diminished the wax content on the leaf surface. Therefore, they concluded that substances in contact with the leaf surface may damage the leaf morphology and wax content, jeopardizing the plant by reducing its capacity to retain water by means of the wax. This may cause hydric stress, which is favorable to leaf-miner attack, because the moth prefers to lay eggs on leaves with a drier surface (CUSTÓDIO et al., 2009).

According to Cunha, Mendes and Chalfoun (2004), applications of copper fungicides make the coffee leaves greener and shinier. The greener coloration of leaves with less wax may be considered an attractive characteristic for the leaf-miner moth, when it makes an egg-laying choice under field conditions. Another hypothesis that could explain the increase in mined leaves is that the fungicides may interact with the plant, affecting its metabolic routes, with the release of volatiles that attract the leaf-miner (COQUERET et al., 2017; VEROMANN et al., 2013). However, the real cause is not yet clear, and more research is needed to examine this event more deeply.

TABLE 2 - Mass of epicuticular wax (µg. cm^{-2}) extracted from coffee leaves (± standard error) after four applications of different copper fungicides in period 1 (November 2016 to February 2017) and in period 2 (June 2017 to September 2017) in field conditions.

Treatments	Period 1	Period 2
Cuprozeb	22.31 ± 2.48 b*	51.53 ± 5.57 b
Supera	32.24 ± 3.89 b	57.06 ± 3.26 b
Supa cobre	33.94 ± 7.37 b	41.45 ± 2.74 b
Control	46.50 ± 1.71 a	76.01 ± 4.69 a

*Means followed by the same lowercase letter in the column belong to the same cluster by Scott-Knott test at a 5% level of significance.

In coffee crops of the Minas Gerais savanna the occurrence of *L. coffeella* is higher than in the south of the same state, due to environmental conditions (REIS; SOUZA, 1996). It is therefore also important to carry out the present study in the savanna region, in order to see the effects of copper fungicides on pest occurrence. It was concluded that the application of fungicides based on copper hydroxide and copper oxychloride in coffee plantations increases the infestation of *L. coffeella* and reduces the wax layer on coffee leaves.

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