

NOTA PRÉVIA

NITROGEN FERTILIZERS AND OCCURRENCE OF *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) IN TRANSPLANTED COFFEE SEEDLINGS

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ABSTRACT: The coffee leaf-miner (CLM) *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) is one of the main pests of coffee plants in Brazil. Its occurrence in the crop is directly related to the physiological state and growth characteristics of coffee plants, in turn related to plant nutrition. The present work, therefore, aimed to evaluate the effect of nitrogen sources on the occurrence of CLM in coffee seedlings. The fertilizers used were ammonium sulfate, urea, and organomineral. The number of leaves mined by CLM and the relative contents of chlorophyll and crude protein in the leaves were recorded. Ammonium sulfate and urea favored higher occurrence of leaves mined and organomineral fertilizer provided the lowest incidence of leaves mined by CLM. The three sources of nitrogen increased the chlorophyll content, but only ammonium sulfate caused an increased percentage of crude protein in the leaves of coffee seedlings. For the improvement of management strategies for *L. coffeella* it is fundamental to understand favorable conditions, nutritional management it is one of the pest control mechanisms, thus making it possible to control the insect and prevent the population of CLM from reaching an economic damage threshold.

Index terms: *Coffea arabica*, nitrogen, coffee leaf-miner, infestation, fertilization.

FERTILIZANTES NITROGENADOS E OCORRÊNCIA DE *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) EM MUDAS DE CAFEEIRO TRANSPLANTADAS

RESUMO: O bicho-mineiro-do-cafeiro (BMC) *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) é uma das principais pragas dos cafeeiros no Brasil. Sua ocorrência na cultura está diretamente relacionada ao estado fisiológico e as características de crescimento dos cafeeiros por sua vez relacionados à nutrição das plantas. O presente trabalho, portanto, objetivou avaliar o efeito de fontes de nitrogênio na ocorrência de BMC em mudas de café. Os fertilizantes utilizados foram sulfato de amônio, uréia e organomineral. O número de folhas minadas pelo BMC e o teor relativo de clorofila e de proteína bruta nas folhas foram registrados. Sulfato de amônio e uréia favoreceram o aumento da ocorrência de folhas minadas e o fertilizante organomineral promoveu menor incidência de folhas minadas pelo BMC. As três fontes de nitrogênio aumentaram o teor de clorofila, no entanto apenas sulfato de amônio provocou incremento da porcentagem de proteína bruta nas folhas das mudas do cafeeiro. Para o aprimoramento das estratégias de manejo de *L. coffeella* é fundamental o conhecimento das condições favoráveis, o manejo nutricional é um dos mecanismos de controle de pragas, possibilitando o controle do inseto e impedindo que a população do BMC atinja o nível de dano econômico.

Termos para indexação: *Coffea arabica*, nitrogênio, bicho-mineiro-do-cafeeiro, infestação, adubação.

The coffee leaf-miner (CLM) *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) is considered to be the main pest of coffee plants (REIS; SOUZA, 1998). This microlepidopteran is monophagous, i.e. only the coffee plant constitutes a suitable host for CLM development. The moths lay eggs on the leaves, and after hatching, the larvae penetrate in the leaves and consume the parenchyma, causing leaf fall and reducing plant's photosynthetic capacity. Occurrence of the CLM is highest in dry periods of the year, when conditions are favorable for its reproduction and development (REIS; SOUZA, 1998).

Fertilization interferes with the physiological state and growth characteristics of coffee plants, and may be related to higher occurrence of CLM (NESTEL; DICHSCHEIN; ALTIERI, 1994). Availability of mineral nutrients usually influences host-plant selection by herbivorous insects as nutrients alter plants' chemical composition, morphology, anatomy and phenology (HAN et al., 2014; OLIVEIRA et al., 2014; RASHID; UESUGI, 2015; JAHAN; ISLAM, 2016).

Nitrogen is important for synthesis of amino acids and proteins, which are limiting nutrients for insect survival (RASHID; JAHAN; ISLAM, 2017). High nitrogen availability in soil increases

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leaf content of amino acids and proteins, as well as improving vegetative growth, delaying maturity and lignification of plant tissues, and making the plant more attractive to insect herbivores (COQUERET et al., 2017). Thus, it is important to investigate the influence of sources of nitrogen fertilizers used in coffee production on CLM occurrence.

The present work reported the effect of nitrogen fertilization on the occurrence of CLM in coffee seedlings. We expect that the preliminary results of this study can provide useful information on appropriate use of nitrogen fertilizers in coffee-producing regions where CLM is the major insect pest.

The experiment was carried out in an experimental area of the University José do Rosário Vellano, UNIFENAS, in Alfenas, Minas Gerais state, Brazil, between 21° 25' 45" S and 45° 56' 50" W, and 881 m altitude. The climate is Cwa according to the Köppen classification. The sub-superficial layer (40 to 80 cm) of the soil (red oxisol) present in the region of Alfenas, Minas Gerais state, was used.

Seedlings of coffee cv. Catuaí Vermelho IAC 144 with four pairs of leaves and without the presence of mines made by CLM were used in the experiment. The seedlings were transplanted in November 2016 into 7-L pots with circular holes (1 cm diameter) on the bottom to allow roots aeration and excess water to drain off. The need for chemical correction with fertilizers was based on soil analysis and recommendations of Novais et al. (1991) for experiments with potted plants. After being transplanted to pots, the seedlings received appropriate irrigation, aiming to keep soil humidity close to field capacity, maximum water capacity that the soil is able to retain. During the experiment, weeds and disease among plants were controlled when necessary, and no insecticides were applied. The products and respective doses used were: Comet® (pyraclostrobin) at 600 ml of commercial product (c.p.) ha⁻¹ and Niphokam 10-08-08 at 250 ml c.p. 100⁻¹ L of water. The products were applied three times during the experiment.

The nitrogen fertilizers and respective concentrations of N were as follows: ammonium sulfate (21% N and 23% S), urea (45% N), and organomineral fertilizer (20% N). The design used in the experiment was randomized blocks in 4 x 3 factorial scheme, with three nitrogen fertilizers (urea, ammonium sulfate, organomineral fertilizer, and control), and three CLM evaluation periods that were performed in May, June, and July 2017, with four blocks as replicates, totaling 48

experimental plots. Each plot was composed of four plants, making a total of 192 evaluated plants.

All treatments, except the control, received the same amount of N (2 g plant⁻¹) that was defined according to Novais et al. (1991), which corresponded in function of the N concentration to 4.4 g of urea, 9.5 g of ammonium sulfate, and 10 g of organomineral per plant. The doses of applied N were divided into equal parts, the first taking place upon transplanting, and the second and third applications at 45 and 90 days after transplanting. At planting-stage fertilization, the fertilizers were mixed into the soil volume in each pot, and for topdressing fertilizations (45 and 90 days) the N sources were applied on soil surface in the pots, followed by irrigation, aiming to incorporate them into the soil and avoid N loss by volatilization.

The experiment was carried out for 240 days after transplanting, and the first evaluation took place in May 2017, when CLM infestations initiated. The second and third evaluations were performed in June and July 2017. All coffee plants (192 potted plants) were inspected for CLM presence in each evaluation period. The evaluations were done by counting the leaves with at least one intact mine of CLM; in other words, without any sign of predation by wasps. Immediately after each evaluation, the leaves with mines were detached from the plants; it was thus possible to determine the CLM infestation, with data expressed as percentage of mined leaves.

At 240 days after transplanting, indirect determination of chlorophyll content was carried out on recently developed leaves using a portable measure device (SPAD-502, Konica Minolta Sensing Americas, Inc). The leaves used for evaluation of chlorophyll content were collected from the plants, stored in bags and taken to the laboratory, where they were washed and dried in an oven with forced air circulation. Next, crude protein analysis was conducted following methodology of Silva and Queiroz (2002).

Data on CLM infestation were submitted to the Shapiro-Wilk test to check for normality of residuals. The data presented normal distribution ($p > 0.05$) and were submitted to analysis of variance. The interactive effects of treatments with N fertilizers and evaluation periods were analyzed. Means of chlorophyll and crude protein contents were compared. The means of treatments were grouped by the Scott-Knott test at 5% significance using the software R, version 3.2.4 (R CORE TEAM, 2016).

There was a significant interactive effect between N fertilization and period of evaluation on the percentage of leaves mined by CLM ($df = 6$; $dfR = 33$; $F = 1.85$, $p < 0.05$) (Table 1). In the evaluation of May, no influence was observed for the sources of N on pest infestation. In June and July, ammonium sulfate and urea significantly differed from the organomineral fertilizer and control. These results indicated that the ammonium sulfate and urea sources of N increased CLM infestation.

There was a significant effect on the three evaluation periods from the different sources of nitrogen used, and an increase in the percentage of mined leaves was seen from May onwards (Table 1). These results are similar to those found by Theodoro, Guimarães and Mendes (2014), in a study that observed an increase in the infestation by CLM in those same periods and noticed that these periods are characterized by cold and dry conditions in the south of Minas Gerais state, making it a propitious period for the development of the leaf-miner (REIS; SOUZA, 1998).

In commercial coffee plantations, the percentage of mined leaves is used to determine the economic damage threshold of CLM, which is the recommended timing for insecticide application to prevent the pest from causing economic yield loss. The economic threshold of CLM in Brazil is established at 20% of mined leaves with no signs of predation, mainly in the third and fourth pair of leaves (REIS; SOUZA, 1998). In our study, the percentage of leaves mined in July was higher than 20% in plants treated with urea (22.52%) and ammonium sulfate (23.67%) in older leaves. This information is important for improvement of management strategies for CLM.

Organomineral fertilization led to the lowest incidence of mined leaves, and the percentage of infestation remained below 20%, suggesting a potential benefit this fertilizer can

bring to pest management programs in coffee plantations. Theodoro, Guimarães and Mendes (2013) evaluated the use of different sources of organic matter and the interaction between CLM behavior and the production of total soluble sugars (TSS). The authors stated that organic fertilization affected TSS production, which may possibly have competed for an increase in plant resistance to CLM.

High N concentrations reduce the production of lignin, reducing plant resistance to insect pests. Nitrogen also increases concentrations of amino acids in cell apoplast, which favor the incidence of pests. Release of N from the organomineral fertilizer is more gradual than in the other sources. Therefore, gradual release of N from this fertilizer may have provided lower synthesis of amino acids and sugars in the apoplast, and this may explain the lower incidence of CLM in our study (CARDOSO; LUZ; LANA, 2015; CORRÊA et al., 2016).

Regarding the relative chlorophyll content in coffee leaves, there was significant difference among N fertilizers ($df = 3$; $dfR = 9$; $F = 5.51$; $p < 0.05$) (Table 2). Ammonium sulfate (68.01), urea (75.66) and organomineral (78.30) fertilizers caused an increase in the chlorophyll content relative to control (54.08) (Table 2). Oliveira et al. (2014) reported that leaf color is important to insect-pests when selecting the host plant, and those insects prefer to lay eggs on greener leaves rather than yellowish ones, presenting a relationship with the better nutritional status of plants and the suitable development of the insect.

There was significant difference among treatments for crude protein content in coffee leaves, with ammonium sulfate (1.87%) causing a higher percentage compared to the other nitrogen fertilizers ($df = 3$; $dfR = 9$; $F = 7.43$; $p < 0.05$) (Table 2).

TABLE 1 - Percentage of leaves mined by coffee leaf-miner (mean \pm standard error) after fertilization with nitrogen fertilizers.

Fertilizers	May	June	July
Ammonium sulfate	4.64 \pm 0.27 aC*	13.97 \pm 0.38 aB	23.67 \pm 0.52 aA
Urea	1.70 \pm 0.48 aC	13.23 \pm 0.46 aB	22.52 \pm 0.54 aA
Organomineral	2.20 \pm 0.25 aC	8.55 \pm 0.78 bB	17.88 \pm 0.44 bA
Control	2.67 \pm 0.30 aC	8.08 \pm 0.42 bB	13.70 \pm 0.55 bA

*Means followed by the same uppercase letter in rows and same lowercase letter in columns belong to the same cluster by Scott-Knott test at 5% level of significance.

TABLE 2 - Percentage of crude protein and relative chlorophyll content in leaves of coffee plants (mean \pm standard error) fertilized with sources of nitrogen.

Fertilizers	Crude protein (%)	Relative chlorophyll content
Ammonium sulfate	1.87 \pm 0.04 a*	68.01 \pm 5.43 a
Urea	1.49 \pm 0.03 b	75.66 \pm 5.65 a
Organomineral	1.58 \pm 0.12 b	78.30 \pm 1.02 a
Control	1.37 \pm 0.08 b	54.08 \pm 2.79 b

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

Ammonium sulfate was the only tested fertilizer with sulfur in the formulation, and according to Prado (2008), sulfur acts in protein metabolism in plant tissues, contributing to conversion of non-protein N into protein. Sulfur can also be associated with various enzymatic reactions and synthesis of three essential amino acids, namely cystine, cysteine, and methionine. This may explain the higher crude protein content found in coffee leaves treated with ammonium sulfate.

According to Nestel, Dickschen and Altieri (1994), the population density of CLM during the year is directly related to the physiological state of the plants, considering that egg-laying increases during the period in which plant material is most digestible by the larvae. Thus, the CLM moths probably select plants that are more nutritious for development of their offspring (RASHID; JAHAN; ISLAM, 2017; SANTOS et al., 2017). Another hypothesis that helps explain the increase in leaves mined by CLM is that the nitrogen sources may interact with the plant by affecting metabolic pathways, with release of volatile organic compounds that are attractive to CLM adults (COQUERET et al., 2017; VEROMANN et al., 2013). However, this hypothesis deserves further evaluation.

We conclude that fertilization with ammonium sulfate and urea favors higher CLM infestation, hence higher percentage of mined leaves in coffee plants. All sources of nitrogen herein evaluated increased chlorophyll content, and ammonium sulfate also increased the percentage of crude protein in the leaves.

The preliminary findings of the present study show the importance of correct use of nitrogen fertilization in integrated management of CLM in coffee plantations, given that gradually released fertilizers were shown to slightly reduce the pest infestation. New studies related to the

influence of nitrogen fertilization on coffee plant-CLM interaction should be performed, among them the evaluation of plants' release of volatile organic compounds that are attractive to CLM adults.

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