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Article

Evaluating response variables to the reversion of the 'engurruñadera del cacao' through the control of *Aceria reyesi* (Nuzzaci) (Acari: Eriophyidae) with abamectin and pruning

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Abstract

The cacao bud mite, *Aceria reyesi* (Nuzzaci), has been identified as the causal agent of the 'engurruñadera del cacao', characterized by the apparent accumulation of stipules on the buds, followed by die-back, with premature fall of leaves, shortening of internodes and proliferation of lateral branches, or witches'-broom, resulting in production losses or even in the death of plants. Our objective was to evaluate four response variables (numbers of leaves/plant, stipules/bud, internodes/branch and branches/plant) that could be indicative of a possible reversion of the 'engurruñadera' after the application of the acaricide abamectin and/or pruning. Two experiments were carried out between November 2020 and April 2021 in a greenhouse at UESC, with cacao seedlings of the genetic group 'Forastero' (cacao 'Common') infested with *A. reyesi* and showing typical symptoms of the 'engurruñadera'. The first experiment was conducted with 4 treatments and 8 replicates, with the application of (1) Abamectin + pruning, (2) Abamectin without pruning, (3) Water + pruning and (4) Water without pruning (control). Sprays were carried out fortnightly with an Abamex® solution (18 g/L of abamectin, in a concentration of 2 mL/L of distilled water + neutral detergent). Pruning was performed only once, at the beginning of the experiment, by removing the apical 10 centimeters of all plant branches. Response variables were evaluated one day before the application of the treatments (Day 0), and afterward (~Days 75 and 150 after the first evaluation, respectively). The second experiment was conducted with 2 treatments and 12 replicates, with the application of (1) Abamectin (treated) and (2) Water (control). Pulverizations and evaluations were carried out as in the first experiment. The number of leaves/plant and internodes/branch were the only response variables that indicated some reversion of the 'engurruñadera' in both experiments among treated and control groups, although only 150 days after the application of abamectin and/or pruning. The number of branches/plant showed a significant increase from 75 days after the treatments with pruning, and no difference was found among treated and control groups in terms of numbers of stipules/bud in both experiments. We concluded that the numbers of leaves/plant and internodes/branch seem to be adequate monitoring tools to indicate the reversion of the 'engurruñadera' after the application of abamectin and/or pruning. Pruning was effective in the reversion of symptoms of the 'engurruñadera' on cacao seedlings as well as the abamectin application of a 2 mL dose of Abamex® per liter of water fortnightly.

Keywords: *Theobroma cacao*, cocoa, cacao bud mite, control

Introduction

The cacao tree, *Theobroma cacao* L. (Malvaceae) is a perennial and arboreal plant native to the Amazon basin, with great economic importance in humid tropical regions as a primary component of chocolate (Wood 2001). Brazil is the seventh producer of cocoa beans in the world, with

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approximately 250 thousand tons produced in 2019, with the states of Pará and Bahia leading the ranking, producing together about 242 thousand tons of beans in that year (MAPA 2021). Although Brazilian cacao cultivation occupies a prominent place in world production, the occurrence of diseases and pests, such as the cacao bud mite, *Aceria reyesi* (Nuzzaci 1973) (Acari: Eriophyidae), have been one of the obstacles to the increasing of Brazilian productivity (Carvalho *et al*. 2018; Nakayama 2019; MAPA 2021).

Aceria reyesi has been identified as the causal agent of the cacao 'engurruñadera', causing serious damage to the culture, and even death of plants under high incidence (Sánchez *et al.* 1978). Described from Venezuela in the 1970s as 'engurruñadera del cacao' (Doreste *et al.* 1975), the first records of this problem in Brazil occurred in 1979, in the municipalities of Itapebi and Itajú do Colônia, Bahia (Soria & Silva 1983). More recently, the 'engurruñadera' has been reported in the states of Amazonas, Bahia, Ceará, Espírito Santo, and Rondônia (Carvalho *et al.* 2018; Nakayama 2019).

The 'engurruñadera' is identified as a result of the feeding of *A. reyesi* in the buds, and has being characterized by the apparent accumulation of stipules and leaf primordia on them, followed by die-back, with premature fall of leaves, shortening of internodes by the inhibition of elongation of stems, and increase of the production of lateral branches, or witches'-broom (Carvalho *et al*. 2018; Nakayama 2019), as commonly caused by other eriophyid mites in several cultures (Vacante 2015). Due to the intense foliar reduction and the exhaustion of the plant's reserves, affecting the production or even conducting plants to death in a period of 2–5 years, the advance of the 'engurruñadera' in Brazil has caused great concern (Oliveira & Navia 2013; Nakayama 2019).

Control strategies against the 'engurruñadera' include soil correction, fertilization, pruning, replacement of old trees, and shade management (Nakayama 2019). Although the effectiveness of some acaricides in the control of *A. reyesi* has already been demonstrated, the published works are old and bring formulations and products that are no longer commercialized (Sánchez *et al*. 1978; Soria *et al*. 1984, 1991), with no commercial product nowadays registered to be used against this mite in the cacao cultivation in Brazil (Nakayama 2019; Agrofit/Mapa 2022). The abamectin, for example, is an acaricide with translaminar activity (Hoy 2011), with potential to reach mites protected inside the buds, effective against some *Aceria* species, as *A. guerreronis* Keifer, *A. litchii* (Keifer) and *A. sheldoni* (Ewing) (Lacasa *et al.* 1990; Azevedo *et al.* 2013; Silva *et al.* 2017), but is still untested against *A. reyesi*.

Considering that studies on the relative resistance and susceptibility of different cacao genotypes to 'engurruñadera' are still starting (De Jesus 2022), and the lack of information on the effectiveness of the control strategies that have been adopted against that problem, the objective of this work was to evaluate four response variables (numbers of leaves/plant, stipules/bud, internodes/branch and branches/plant) that could be indicative of possible reversion of the 'engurruñadera' through the application of abamectin and/or pruning.

Materials and methods

Two experiments were carried out in a greenhouse (~25º C and 80% RH) at the Santa Cruz State University (UESC), 14º45'35" S, 39º13'49" W, Ilhéus, BA, Brazil, between November 2020 and April 2021. Cacao seedlings of the genetic group 'Forastero' (cacao 'Common') were used, produced through fruits collected from areas of the 'Comissão Executiva do Plano da Lavoura Cacaueira' (CEPLAC), Ilhéus, BA, Brazil, and showing abnormalities typical of the 'engurruñadera', were evaluated in both experiments. Four response variables respectively related to the abortion of leaf primordia, the accumulation of stipules on the buds, the shortening of internodes and the increase of the production of lateral branches were measured: the numbers of (1) leaves/plant, (2) stipules/bud, (3) internodes/branch and (4) branches/plant. Only leaves longer than 5 cm in length and the apical 10 cm of each branch were considered in the counts of the number of leaves/plant and of internodes/branch, respectively. The plants were sprayed fortnightly with a solution of Abamex®, containing 18 g/L of abamectin, in the concentration of 2 mL/L of distilled water, with the addition of 2 mL of neutral detergent. Manual plants watering (0.5 L of water/plant) and fertilization with N-P-K (10 mL/plant of PG MIX Yara 14-16-18 in a concentration of 5g/L) were performed every two days and quarterly, respectively. Plants were cleaned weekly with the aid of a sponge moistened with water and a fine brush against any initial colonization by mealybugs, aphids, ants, spider mites or other pests.

The 32 cacao plants evaluated in the first experiment were 30 months old, originated from seeds that were removed from the fruits, washed in running water, and then cleaned with paper towels. Seeds were mixed with sawdust with sand 1:1 and moistened for five days for germination. Each germinated seed was sown in 5L pots, filled with Carolina Soil® substrate. Watering was carried out to maintain the substrate moisture at 70% of the maximum water retention capacity. To keep the plants properly nourished, slow-release fertilizers containing macros and micronutrients were added fortnightly to the pots according to Sodré *et al*. (2017), and artificially infested with *A. reyesi*, through continuous superposition of infested buds from the field on healthy buds during 12 months before the experiment. The experiment was conducted with 4 treatments and 8 replicates, by the application of (1) Abamectin + pruning, (2) Abamectin without pruning, (3) water + pruning and (4) water without pruning (control). About 200 mL of the previously described solution of Abamex® or distilled water were pulverized per plant from November 5th, 2020. Plants pruning was performed only once, on November 5th, 2020, by removing the apical 10 centimeters of all plant branches. The 24 cacao plants evaluated in the second experiment were 18 months old, planted in soil substrate, naturally infested by *A. reyesi*, and taken to the greenhouse in October 2020. The experiment was conducted with 2 treatments and 12 replicates, by the application of (1) water (control) and (2) Abamectin (treated), with the spray of 100 mL of the previously described solution of Abamex® or distilled water per plant from November 5th, 2020.

Three evaluations of the response variables were carried out in order to determine the possibility of reversion of the 'engurruñadera' through the application of abamectin and/or pruning: on November 4th, 2020, one day before the application of the treatments (Day 0), on January 18th, 2021 and on April 5th, 2021 (~Days 75 and 150 after the first evaluation, respectively). A completely randomized design (CRD) was adopted in both experiments, with the averages obtained from the counts of response variables in each treatment/day being submitted to analysis of variance, transformed into square roots, and compared by the Scott-Knott test at 5% significance with the Sisvar software version 5.6 (Ferreira 2019).

Results

No differences in the numbers of stipules/bud, leaves/plant, internodes/branch and branches/plant between treatments in both experiments were found on Day 0 ($P > 0.05$) (Figs 1, 2), reflecting an acceptable uniformity for the use of the statistical design adopted and comparison of the results obtained after the treatments applications.

In the first experiment, although no differences were found in the numbers of stipules/bud between treatments on Days 75 or 150 ($P = 0.2291$) (Fig. 1A), the numbers of leaves/plant, internodes/branch and branches/plant differed between treatments in those days ($P < 0.05$) (Figs 1B-D). There was a general increase in the numbers of leaves/plant in all treatments from Day 0 to 150

(Fig. 1B), with the numbers of leaves/plant in Day 75 in the treatments Abamectin without pruning and Water without pruning (averages of 16 and 10 leaves/plant, respectively) significantly lower if compared to the treatments Abamectin + pruning and water + pruning (averages of 25.0 and 22.5 leaves/plant, respectively). In Day 150, however, the numbers of leaves/plant were significantly lower in the treatment Water without pruning (average of 23.9 leaves/plant) if compared with the other three treatments, with abamectin and/or pruning (averages ranging from 38.0 to 43.5 leaves/plant).

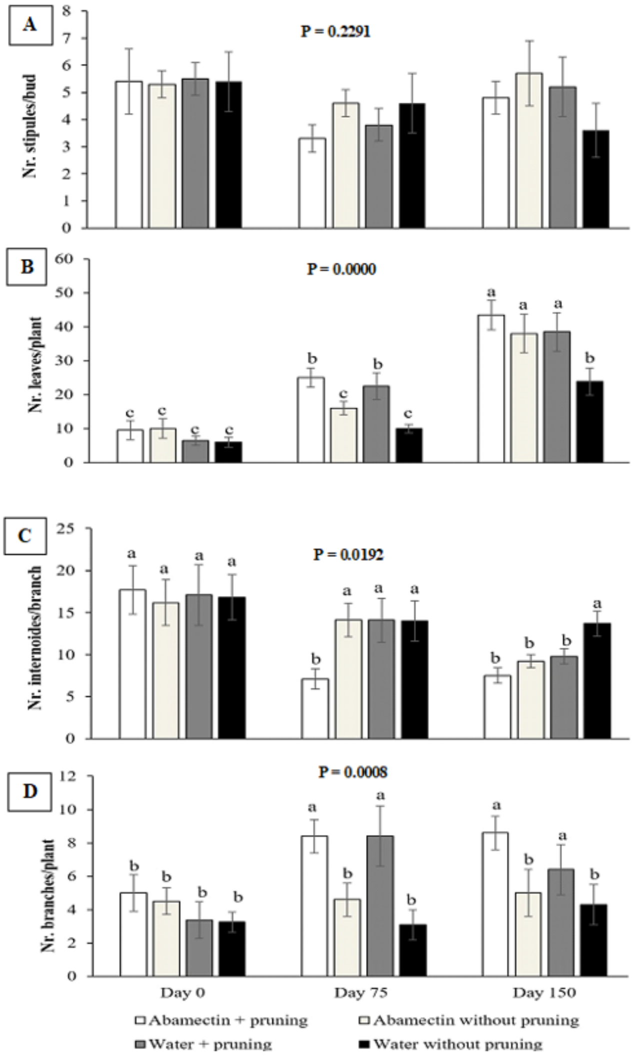

FIGURE 1. Averages for the response variables analyzed to evaluate the possibility of reversion of the 'engurruñadera' in potted cacao trees treated with Abamectin and/or pruning and control (Water without pruning). Number of stipules/bud (A), leaves/plant (B), internodes/branch (C) and branches/plant (D). Evaluations carried out one day before (Day 0) and after the application (~Days 75 and 150) of the treatments in a greenhouse at UESC, Ilhéus, Brazil. Means followed by the same letter do not differ statistically from each other by the Scott Knott test at 5% significance. Error bars represent standard errors.

Regarding the numbers of internodes/branch (Fig. 1C), a significant decrease in this response variable was observed, first on Day 75 for the treatment Abamectin + pruning, and mainly on Day

150 in the three groups treated with abamectin and/or pruning (averages ranging from 7.5 to 9.8 internodes/branch), what was different from the number of internodes/branch observed in the treatment water without pruning (average of 13.7 internodes/branch). In relation to the number of branches/plant (Fig. 1D), it was observed a significant increase on them on both Days 75 and 150 for the treatments Abamectin + pruning and Water + pruning (averages ranging from 6.4 to 8.6 branches/plant) in relation to the treatments Abamectin without pruning and water without pruning (averages ranging from 3.1 to 5.5 branches/plant).

In the second experiment, no differences were observed between treatments Abamectin and water on Days 75 or 150 for the numbers of stipules/bud (P = 0.1774) and branches/plant (P = 0.5310) (Figs 2A, B), while the numbers of leaves/plant and internodes/branch differed between treatments in those days ($P < 0.05$) (Figs 2C, D). There was a general increase in the numbers of leaves/plant in the two treatments from Day 0 to 150 (Fig 2C), with the number of leaves/plant on Day 150 in the treatment Abamectin significantly higher if compared to treatment water (averages of 21.6 and 14.0 leaves/plant, respectively). Regarding the numbers of internodes/branch (Fig. 2D), a significant decrease in this response variable was observed in the treatment Abamectin in relation to the treatment water on Day 150 (averages of 9.4 and 27.8 internodes/branch, respectively).

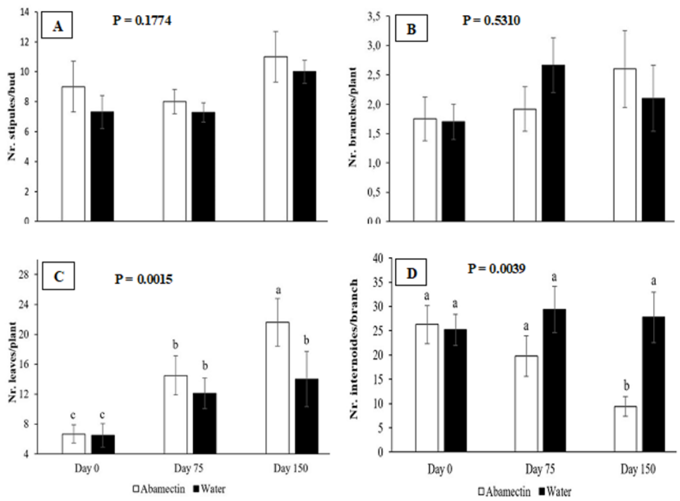

FIGURE 2. Averages for the response variables analyzed to evaluate the possibility of reversion of the ‘engurruñadera’ in potted cacao trees treated with Abamectin and control (Water). Number of stipules/bud (A), branches/plant (B), leaves/plant (C) and internodes/branch (D). Evaluations carried out one day before (Day 0) and after the application (~Days 75 and 150) of the treatments in a greenhouse at UESC, Ilhéus, Brazil. Means followed by the same letter do not differ statistically from each other by the Scott Knott test at 5% significance. Error bars represent standard errors.

Discussion

Although little is known about the mechanisms by which eriophyid mites feeding cause plant abnormalities (Keifer *et al*. 1982; de Lillo *et al.* 2018), it is probably that *A. reyesi*, when feeding on the leaf primordia inside the buds, somehow induces the plant to cause the abscission of the young leaves and stipules, leading it to try new foliar releases (Nakayama 2019). As a result of the constant leaf abortion, however, and without the consolidation of new leaf shoots, there is an accumulation of scars of the aborted parts on the branch, leading to shortening of the internodes (Nakayama &

Encarnação 2012; Sodré *et al*. 2017; Nakayama 2019), with a consequent increase of the number of internodes on a shoot length unit, a common abnormality caused by eriophyid mites (Keifer *et al*. 1982; Khederi *et al.* 2014, 2018). Our results showed that the typical symptoms of the 'engurruñadera', represented by the response variables evaluated, may be reverted with the use of abamectin and/or pruning to control *A. reyesi*, what corroborate results found for some other *Aceria* pests controlled with those strategies (Mariau & Tchibozo 1973; Muthiah *et al*. 2001; Moreira & Nascimento 2002; Lacasa *et al.* 1990; Monteiro *et al.* 2012; Azevedo *et al*. 2013).

Among the response variables, just the number of leaves/plant and internodes/branch showed significant reversion in both experiments, although only 150 days after the application of abamectin and/or pruning. The number of branches/plant, on the other hand, showed a significant increase from 75 days after the treatments with pruning, and no difference was found between plants treated and untreated in relation to the number of stipules/bud. These results suggest that the numbers of leaves/plant and internodes/branch seem to be adequate response variables to indicate the reversion of the 'engurruñadera' after the application of the abamectin and/or pruning.

Abamectin is effective for several eriophyid mite species, including multiple gall/rust species such as the tomato russet mite, *Aculops lycopersici* (Masse), and important *Aceria* pests, as *A. guerreronis*, *A. litchii* and *A. sheldoni* (Lacasa *et al.* 1990; Azevedo *et al*. 2013; Silva *et al*. 2017; Vervaet *et al*. 2021), and this acaricide alone or associated with pruning seemed to have controlled *A. reyesi* to a point where positive effect were detected on the numbers of leaves/plant and internodes/branch. Although the abamectin is still not registered to be used against this mite in the cacao cultivation in Brazil (Nakayama 2019; Agrofit/Mapa 2022), the results of the present study suggest that this acaricide may be effective in the reversion of the 'engurruñadera'.

Also, the pruning, that has been recommended for the control *A. lichtii* and *A. reyesi* (Azevedo *et al*. 2013; Castro *et al*. 2018; Nakayama 2019), seemed to be effective in the reversion of some symptoms of the 'engurruñadera' in the first experiment, with an observed increase in the number of leaves/plant and a decrease in the number of internodes/branch in plants pruned. On the other hand, it is also possible that those positive responses may be related simply to mechanical and/or biochemical effects of pruning, once the counts of the number of internodes/branch could be affected by the removal of old branches, and the counts of the numbers of leaves/plant and internodes/branch could be affected by an increase in the frequency of emission of new lateral branches and leaves in plants pruned (Sodré *et al.* 2017). Once pruning apparently induced the production of normal, healthy branches, what was evidenced by both the increase of leaves/plant and decrease of internodes/branch, the significant increase in the number of branches/plant could not be related to the witches'-broom symptom of the 'engurruñadera', being a positive response in this case. Pruning is economically viable and is already part of the routine management of the cacao crop, helping to form the canopy architecture, reducing the height of the trees, increasing aeration and light, reducing self-shading of branches and the incidence of pathogen populations (Lik & Hussein 2001; Balasimha 2007, 2009).

The numbers of stipules/bud (in both the experiments) and branches/plant (in the second experiment) were not different between plants treated and untreated with abamectin and/or pruning, showing the ineffectiveness of those variables to indicate the reversion of the 'engurruñadera' or even of the treatments applied. A possible explanation for the ineffectiveness of the number of stipules/bud as a response variable for the reversion of the 'engurruñadera' is that the stipules can remain attached to the bud for some time before abscission (Nakayama 2019), affecting the response. Comparing the effectiveness of the response variable branches/plant between the first and second experiments, it can be concluded that it was affected by pruning but not by the application of abamectin, what suggests that this variable is also not adequate to indicate the reversion of the 'engurruñadera'.

Based on our results, we concluded that (1) the numbers of leaves/plant and internodes/branch seem to be adequate monitoring parameters and that (2) pruning was effective in the reversion of symptoms of the ‘engurruñadera’ on cacao seedlings, as well as the abamectin application of a 2 mL dose of Abamex® per liter of water fortnightly. It is important to observe, anyway, that the present study was conducted under controlled conditions in a greenhouse, on potted young trees, and in the maximum concentration recommended for the use of Abamex® in other crops for which the use of this product is already registered (Agrofit/Mapa 2022), what are factors quite adverse from those that should be observed for the application of the abamectin and/or pruning in field conditions, where temperature, solar radiation, air humidity, wind and water availability interfere in the physiological mechanisms of the plant (Muller & Valle 2012; Santos & Sodré 2017). Other factors, for example, that could affect the control of the ‘engurruñadera’ in field conditions are the severity of symptoms, that has been associated with low levels of shading of cacao trees (Navia *et al*. 2013; Araújo *et al*. 2016), and prolonged dry seasons (Reyes & Reyes 1972; Reyes *et al.* 1977; Sanchez & Caprilles De Reyes 1979; Soria & Silva 1983; Soria *et al*. 1984, 1991; Nakayama & Encarnação 2012; Trevisan *et al.* 2012; Oliveira & Navia 2013).

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