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Trunk injection as an alternative approach to insecticide spraying: an experience with cashew trees

Injeção no tronco como tática alternativa as pulverizações de inseticidas: uma experiência com cajueiros

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Endotherapy is a tactic for phytosanitary products application to trees and palm. The goal of this study was to verify the efficiency of trunk injections in cashew trees to control of whitefly (*Aleurodicus cocois*) (Curtis, 1846) (Hemiptera: Aleyrodidae). To test this technology were used 17 cashew trees with one trunk or more. The injections were applied in just one trunk in plants with two or more trunks. Two doses of acephate were tested. For each centimeter of trunk diameter were applied 5.4 g or 0.25 g of active ingredient. Immediately before the application of the insecticide and at 1, 6, 9 and 16 months later, 12 mature leaves per plant were collected in the median part of the cashew canopy to count the number of adult whiteflies. There was no significant difference in the number of *A. cocois* over the months after the insecticide trunk injection in plants with a single trunk. Injections with acephate in cashew tree trunk are effective on whitefly control.
Keywords: *Aleurodicus cocois*, *Anacardium occidentale*, endotherapy.

A endoterapia é uma tática de aplicação de produtos fitossanitários em árvores e palmeiras. Objetivou-se verificar a eficiência de injeções em cajueiros no controle da mosca-branca (*Aleurodicus cocois*) (Curtis, 1846) (Hemiptera: Aleyrodidae). Para testar esta tecnologia foram utilizados 17 cajueiros com um tronco ou mais. As injeções foram aplicadas em apenas um tronco nas plantas com dois troncos ou mais. Testaram-se duas doses de acefato. Para cada centímetro do diâmetro do tronco foram aplicados 5,4 g ou 0,25 g do ingrediente ativo. Imediatamente antes da aplicação do inseticida e aos 1, 6, 9 e 16 meses após, 12 folhas maduras por planta foram coletadas da na parte mediana da copa dos cajueiros para a contagem do número desses aleirodídeos adultos. Não houve diferença significativa no número de *A. cocois* ao longo dos meses após a injeção do inseticida nas plantas com tronco único. Injeções com acefato em tronco de cajueiros são eficientes no controle da mosca-branca.
Palavras-chave: *Aleurodicus cocois*, *Anacardium occidentale*, endoterapia.

1. INTRODUCTION

Injections on trees date back to the 15th century and over the centuries some experiments were conducted. However, its understanding became difficult due to the lack of knowledge of basic sciences such as botany, plant physiology, agriculture, and forestry. With new information and the emergence of the theory of cohesion-tension in the movement of water, endotherapy has become better understood [1]. Later, it was complemented when the healing processes of trees, called compartmentalization, were studied in detail by Shigo and Marx (1977) [2].

Endotherapy as a tactic in phytosanitary management probably started after the spread of the Dutch elm disease, caused by the fungus *Ophiostoma ulmi* Biusman, on elm trees (*Ulmus americana* L.) in the urban landscape of the United States of America (USA). Because it is a pathogen that attacks vascular tissue, topical spraying is not satisfactory in addition to being prohibitive in public areas. Thus, tests with trunk injection of systemic fungicides were conducted and proved to be efficient in the preventive and therapeutic action of the disease for more than two years with a single injection [3].

Foliar spraying and applications via soil are the most common chemical methods in the control of pests and diseases of urban, fruit and forest trees. However, motorized spraying becomes impractical on tall trees. In turn, even when the canopy can be reached by the phytosanitary product, the losses of these products are significant [4].

Endotherapy is a phytopharmaceutical tactic that consists of the phytosanitary product trunk injection or infusion in a tree or palm, which is translocated through the vascular tissues to the higher parts of the canopy where conventional spraying cannot reach. It is considered an environmental-friendly method, due to the non-exposure to non-target organisms, soil, water, air, wildlife, in addition it is not negatively affected by the weather such as rain and solar radiation [5]. Furthermore, biological control agents such as fungi, bacteria and resistance inducers can be used in endotherapy [6, 7].

In several countries, many experiments with trunk injections have been carried out aiming to control pests and diseases in dicots and monocots plants. In the USA different types of tests with injections of imidacloprid, emamectin benzoate and potassium phosphate were conducted with apple trees (*Malus domestica* Borkh) to determinate the efficiency in the control of pests and diseases. Most of the experimental results were highly promising in addition to showed low residues in the fruits below the limits established by official organs of government and, when analyzed in nectar and pollen they were undetectable [4-6, 8, 9].

In California, USA, the Huanglongbing disease (HLB, yellow dragon disease) also known as citrus greening, is one of the most destructive diseases of citrus. The causal agent is the bacterium *Candidatus* Liberibacter asiaticus (Las) which is transmitted by the psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). Shin et al. (2016) [10] and Hu et al. (2017) [11] found that sprays with the antibiotics streptomycin and oxytetracycline do not reach the biological target (bacteria) in the phloem, in addition to the losses by spray drift and degradation by ultraviolet rays. However, when the antibiotics were applied via soil drench the microbial degradation occurs; requires a greater amount of antibiotics and causes environmental problems. On the other hand, trunk injection with these antibiotics accurately reaches the biological target, has preventive and curative action, and does not contaminate the environment, although it has a higher cost than conventional spraying.

In Portugal and others European countries, pine wilt is the most important disease of *Pinus pinaster* Aiton. The causal agent of this disease is the nematode *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle, and *Monochamus galloprovincialis* (Olivier) (Coleoptera: Cerambycidae) is the vector. To determine the efficiency of emamectin benzoate in protecting pines against nematodes, Souza et al. (2013) [12] conducted tests with injections of emamectin benzoate in concentrations of 0.032; 0.064 and 0.128 g of a.i./cm of tree diameter. According to these authors, after 26 months, no pine tree died in contrast to control plants with 33% mortality.

The acephate is a systemic organophosphate with insecticidal and acaricidal action. It has high solubility in water (790 g L^{-1}) and low adsorption coefficient (K_{oc} = 0.48) [13]. These characteristics are important to rapid systemic mobility and due to this fact, its use to trunk injection is commercialized in the USA [13].

Between 2015 and 2016 a strong drought occurred in the municipalities of Ilhéus (14º47′50″ S latitude, 39º2′8″ W longitude); Una (15º17'36" S latitude, 39º04'31" W longitude) and Canavieiras (15º39′1″ S latitude, 38º57′42″ W longitude) in the state of Bahia, Brazil. After this phenomenon, numerous cashew trees (*Anacardium occidentale* L.) were observed intensely injured by the whitefly *Aleurodicus cocois* (Curtis, 1846) (Hemiptera: Aleyrodidae). Aleirodids continuously suck sap and nutrients causing plants weakening, affecting their development and cause death of cashew tree, depending on the whitefly population density. Like the other species of *Aleurodicus*, females lay their eggs in a spiral on the abaxial leaf surfaces, covering the entire attacked leaf with a white powdery wax. Both adults and nymphs suck sap from the leaves and, thus, their sugary excreta (honeydew) promote the proliferation of fumagine (*Capnodium* spp.). This fungus develops on the adaxial leaf surfaces, inhibiting photosynthesis, respiration and, consequently, reducing crop productivity [14, 15].

Due to few studies on endotherapy in Brazil, this work aims to determine the efficiency of trunk injections in cashew trees in the control of the whitefly (*A. cocois*).

2. MATERIAL AND METHODS

The cashew trees are of the common type and spontaneously vegetate in restinga forests; they are planted in residential backyards or in small farms. In general, they are old and tall cashew trees, with more than five meters with a wide canopy. However, they have economic and social importance complementing family income.

Due to the lack of commercial crops of cashew trees for carrying out experimental tests with phytosanitary product injections, the plants used in this study were cashew trees growing spontaneously and attacked by whitefly in areas among the municipalities of Ilhéus, Una and Canavieiras.

The cashew trees used were taller than five meters height, leafy and showed a high population density of whiteflies. Trees in the fruiting period were not included.

In this study, 17 cashew trees with one trunk or more were used. However, in those with more than one trunk, injections were applied in only one. Cashew trees with more than one trunk were used as source of infestation. The crowns intertwined and it was possible to estimate how long the leaves of the treated trunks were protected from the attack of whiteflies from untreated trunks.

Twelve mature leaves per cashew tree treated with insecticide were collected in the median part of the crown. Immediately before the application of the insecticide and at 1, 6, 9 and 16 months the number of adult whiteflies was counted on the leaf samples. To prevent the aleirodids from taking flight, the leaves of each plant were quickly placed in plastic bags and taken to Ceplac's Lemos Maia Station laboratory in Una, Bahia, Brazil to count adult whiteflies (*A. cocois*). This procedure was carried out on the day of the trunk injection application (time zero - control) and 1, 6, 9 and 16 months later.

First, we tried to use trunk injections with imidacloprid but its emulsifiable concentrate did not allow it to pass through the syringe nozzle due to viscosity. Even with several dilutions in water it was not possible. Thus, it was used the acephate.

To calculate the diameter (D), the circumference (C) was divided by 3.14 ($D = C/\pi$). Cashew trees that had a single trunk, the circumference length was measured a few centimeters above the ground (never more than 20 cm). As for trees that had more than one trunk, the trunk with the largest diameter was chosen. The distance between each injection point around the trunk was 10 cm. To obtain the required number of points, the circumference length was divided by 10 and consequently, the number of syringes needed, and insecticide dose diluted in deionized water were estimated. Two doses of acephate (5.4 g and 0.25 g ai cm^{-1} of trunk diameter) were tested. The concentrations of acephate used in the present study were based on the studies by Byrne et al. (2014) [16] and Gous and Richardson (2008) [17] with injections. The amount of water was according to the number of syringes.

The injection syringe used in this study was the Australian-made ChemJet®, with 20 ml capacity and screw-shaped tip. To adapt the syringes in the trunk of the cashew trees a port with 4.4 mm in diameter and 2.5 cm depth were drilled with a battery-powered drill. The amount of insecticide calculated was aspirated into the syringe and the plunger is pulled and rotated counterclockwise to be locked. Then, the syringe was screwed into the port and adjusted. The plunger was unlocked to infuse the phytosanitary product via pressure by a spring pushing the plunger slowly.

The experiment was carried out in a 2x2x4 + 1 factorial scheme (two types of trees with one or more trunks, two doses of insecticide, four evaluation periods and a control with plants evaluated before the application of the insecticide. The experiment was conducted in a completely randomized design. It was used plots subdivided with the factor trunk type in the plot, doses in the subplot and evaluation periods in the sub-subplot. The data were transformed by $\sqrt{(x + 0.5)}$.

3. RESULTS AND DISCUSSION

The number of whitefly adults showed a significant effect to type of trunk and evaluation period factors, as well as the interaction between these two factors. There was also a significant effect on the factorial versus additional treatment. There were no significant effects for the other sources of variance.

There was no significant variation in the number of adult *A. cocois* over the months after applying the insecticide to plants with a single trunk (Figure 1).

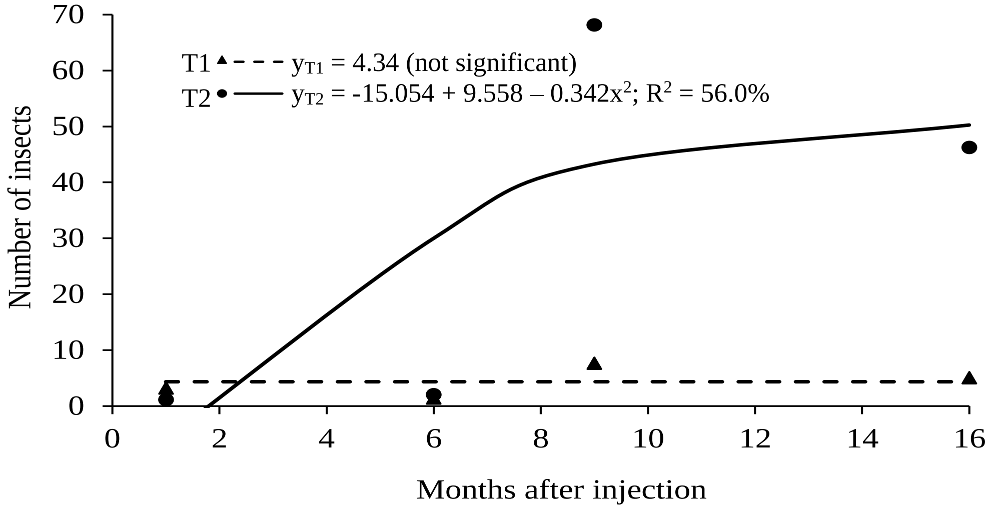

Figure 1. Number of adult Aleurodicus cocois in cashews trees with a single trunk type (T1) and more than one trunk (T2), treated with acephate and evaluated at different times after the injections were applied.

On the other hand, in plants with more than one trunk a significant increase was observed on number of whiteflies over the different evaluation periods, especially after nine months after the injections. This result showed that is necessary to apply the insecticide in all the plant trunks to be successful in controlling *A. cocois* in cashew trees with acephate via trunk injection. Probably the trunk of the plant that did not receive treatment was a source of infestation.

There was no significant difference in the number of *A. cocois* between the two types of trunk when the plants were evaluated at 1 and 6 months after the application of the insecticide injections. However, in subsequent evaluations, 9 and 16 months after the application of the insecticide, plants with only one trunk showed number of whiteflies significantly less when compared to plants with two or more trunks (Figure 2).

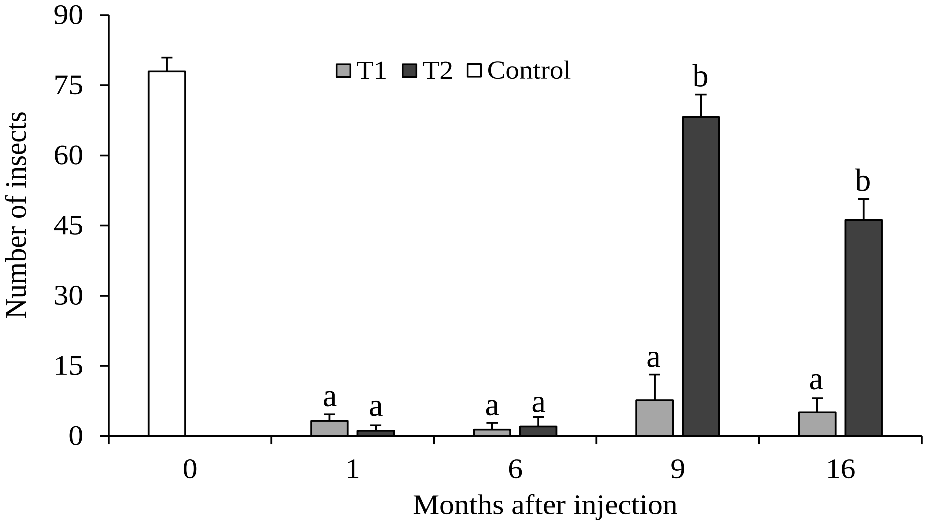

Figure 2. Number of adult Aleurodicus cocois in cashew trees with a single trunk type (T1) and more than one trunk (T2), treated with acephate trunk injections and evaluated at different times after application. Means followed by the same letter do not differ significantly by the F test, in each evaluation period, at 1% probability. Bars at the tops of the columns indicate the standard error of the mean.

There was a significantly higher number of *A. cocois* in the control, without insecticide, when compared to the other treatments, on average, indicating the efficiency of the application of acephate via trunk injections. The results allow us to affirm that the two concentrations protected the cashew trees against whiteflies attack for more than 6 months (Figure 2).

Leaves examined in stereomicroscope one month after trunk injections showed many dead nymphs attached to the leaf. In some, chrysopid larvae (Neuroptera: Chrysopidae) were observed feeding on dead nymphs. After six months, the intense green of the leaves from the cashew trees treated with injections of acephate, contrasted with the dark gray color of the leaves injured by the whiteflies from untreated cashew trees.

Acephate is toxic and degrades to methamidophos in the environment but when applied via trunk injection, it has the advantage of rapid systemic mobility, low adsorption coefficient (K_{oc}) and short residual power in the plant [13]. However, injection with acephate or any other pesticide to control whitefly in cashew trees is not economically viable for large areas. It is assumed that for small family farming it would be possible because its simplicity and, of course, the ecological and economic gains. Ecological because they do not contaminate the environment because there is no spray drift. Economical, as it does not require sophisticated equipment such as motorized sprayers.

However, in Brazil there are no pesticides formulated for phytopharmaceutical purposes and no syringes for trunk injections. Thus, it would be necessary to create mechanisms for commercialization in Brazil and/or to encourage technological innovations in endotherapy. This would bring a great economic, social, and ecological contribution because it is a sustainable chemical treatment.

The importance of cashew cultivation in some states in northeastern Brazil, is not restricted only to the production of chestnuts, pseudo fruits and derivatives, it is also in the creation of bees and honey production. *Apis mellifera* L. is strongly associated with the time of flowering of the cashew crop, essentially in migratory beekeeping [18]. However, when injuries caused by pest insects cause economic damage, spraying with phytosanitary products may be necessary and, deltamethrin is one of the pesticides authorized by the Ministry of Agriculture, Livestock and Supply (MAPA) for cashew trees [19].

In the studies by Byrne et al. (2012) [20] aiming the control of thrips *Scirtothrips perseae* (Thysanoptera: Thripidae) in avocado were used imidacloprid and dinotefuran (via trunk injection and soil application), avermectin via injection and acephate via injection in concentrations of 1.8 and 5.4 g a.i./tree. According to these authors, trunk injections compared to application via soil showed residues of imidacloprid and dinotefuran in the leaves 10 times greater compared application via soil. The residues in fruits were undetectable when trunk injections were used. Avermectin was not efficient in thrips mortality. Regarding acephate and its metabolite, methamidophos, its efficiency in the mortality of thrips was 50% to 80% and after 4-weeks the residues were no detectable in the fruits.

Subsequently, Byrne et al. (2014) [16], concluded that injections with acephate and imidacloprid can be used in the control of avocado thrips. Acephate showed rapid systemic mobility and lethality on thrips during the spring, i.e., its residues were lethal to thrips both on new shoots and on mature (green) leaves. According to these authors, this characteristic is strategic in case of sudden outbreaks of thrips. However, trunk injection with acephate was active against thrips for only four weeks. Imidacloprid showed no action in the beginning of spring but when the leaves began to expand, imidacloprid residues acted and were lethal to thrips for up to 12 weeks. The residues of acephate and imidacloprid were not detected in the avocado fruits.

The chrysopid larvae feeding on whitefly nymphs in cashew trees treated with acephate via trunk injection showed how much injection is a selective approach because circulates through the vascular system reaching different parts of the tree without exposure to the environment.

Although acephate is not registered in the MAPA for use in cashew trees, it can be more efficient against whitefly than spraying with vegetable oils by control of nymphs and adults of these aleirodids. In the trophic context, in the specific case of southern Bahia, cashew trees when killed by the whitefly attack, the loss is not only economic-social, but also biodiversity.

4. CONCLUSION

Acephate trunk injections in cashew trees are efficient in the control of the whitefly *Aleurodicus cocois*, being necessary the application in all the plant trunks for an effective pest control.

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