Modeling pesticide translocation injected by endotherapy into the stem of coconut tree (Cocos nucifera L.)

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Abstract

Aim of study: To present a mathematical model to simulate the translocation of pesticides injected into coconut trees. Pesticide residues in water and coconut pulp were also evaluated.

Area of study: The data were obtained in coconut plants of the Itaporanga Experimental Field, located in the Municipality of Itaporanga d’Ajuda, Sergipe State, Brazil.

Material and methods: To estimate the effect of pesticide site-sap coefficients and retardation factors on translocation and its phytosanitary behavior and risk of contamination of coconut fruits, the stipe was modeled by a classic dispersion-advection equation. The pesticides cyproconazole, azoxyostrobin, dimethoate, imidacloprid, metalaxyl, and thiamethoxam were injected into the coconut palm stipe. The method used to extract pesticide residues from pulp, water and coconut sap samples was based on the QuEChERS methodology with modifications.

Main results: Simulations showed that (i) the pesticides dimethoate, metalaxyl, and thiamethoxan were the active ingredients showing the greatest potential for translocation in the sap of the coconut tree stem; (ii) the pesticides imidacloprid and metalaxyl translocated upward in the stipe, and more rapidly than pesticides abamectin and cyproconazole, which moved slower to the aerial part of coconut plants. In chromatography analysis, no pesticide residues were quantified in water and coconut fruit pulp samples of coconut trees injected with pesticides, after the evaluated intervals.

Research highlights: The proposed model allowed us to observe that the translocation is inversely proportional to the sorption of the pesticide in the stem of coconut trees.

Additional key words: pesticide application technique; dispersion-advection equation; stipe-sap partition coefficient; mass spectrometry.

Parameters: A (cross sectional area of stipe); Cclean_sap (concentration of the pesticide in the sample clean sap); C0 (initial concentration); Cgeneral_sap (concentration of the pesticide in the powdered stipe); Cstipe (concentration of the pesticide in the stipe); D (hydrodynamic dispersion coefficient); Dl (diffusivity of oxygen); Dp (diffusion of the pesticide in the sap); Kstipe-sap (stipe-sap partition coefficient); L (empirical parameter); LogKow (logarithm of pesticide octanol-water partition coefficient); LogSol (logarithm of pesticide water solubility); m (mass of the pesticide); n0 (molar mass of the pesticide); R (pesticide retardation factor); t (time after the pesticide injection); Vclean_sap (volume of clean sap); Vpowdered_stipe (volume of powdered stipe); s (height of the stipe); a (Dirac delta distribution pulse function); εstipe (porosity of the stipe); Vwater (sap water velocity); qstipe (water sap volumetric content); r (density of the stipe).

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<table>
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<th>Funding agencies/institutions</th>
<th>Project / Grant</th>
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</table>

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Introduction

Approximately 96% of the world's coconuts production comes from properties ranging from 0.5 to 4 hectares, which shows its relative importance for farmers who have this crop as their main source of income (Persley, 1992). In Brazil, coconut trees (Cocos nucifera L.) are grown in areas of approximately 280,000 hectares, mostly by small farmers, distributed in almost all states of the national territory. With a production equivalent to 2 billion fruits, it is an important generator of income and revenue.

Pesticides are used for controlling the incidence of coconut pests and diseases, a limiting factor for production (Ferreira et al., 2016). In Brazil, only a small number of pesticides are registered for use on coconut trees (MAPA, 2020). When applied to the plant, pesticides can remain on its surface after deposition on the leaves or fruits, and can also be absorbed by the plant and translocate to the water and to the coconut pulp, which are the two most important commercial components of this crop. Translocation is the movement of the pesticide within the plant's body to tissues distant from the application or deposition site. Pesticide residues in the environment can cause a number of imbalances in the agroecosystem, such as pest outbreaks, the development of resistance to pesticides by insects and pathogens, contamination of humans and animals due to their persistence in soils, water, and food, and it can also impact production costs (Geiger et al., 2011; Ferreira et al., 2018).

The environmental behavior of a pesticide is estimated by its physico-chemical characteristics, its metabolites, and degradation products. Systemic pesticides can be absorbed by roots or leaves and later be translocate inside the plant via xylem, by the transpiration flow. The translocation of the pesticide via xylem or acropetal is defined as the movement of the pesticide that occurs from the lower to the upper parts of the plants through the transpiration flow (Souza, 2006). Modeling the dynamics of pesticides applied to plants helps in choosing pesticides that control pests or diseases. For example, the decision to use a particular fungicide to control a pathogen can be evaluated using mathematical models (Pfender, 2006; Pfender & Eynard, 2009; Caffi & Rossi, 2018).

The technique of injecting pesticides into the stems of coconut or palm trees to control diseases and pests is not new and has been previously addressed by several authors (Nadarajan et al., 1980; Nadarajan & Channabasavanna, 1981; Moore & Alexander, 1987; Ferry & Gomez, 2014). The success of this phytosanitary technique for application on coconut palms is based on the absorption and transpiration of pesticides in palm trees. A pesticide, when injected into the stem (endotherapy), is diluted in the sap and, through the innumerable xylem bundles of the stem, transported to the leaves (acropetal translocation) as a direct consequence of the plant's transpiration. Regardless of the type of pest or disease, the injection of pesticides in the stem of palm trees tends to give good results because the palm trees do not heal in the region of the interior of the injection in the stem, which allows the transport of pesticides diluted in the sap to the site of action in the plant (Ferry & Gomez, 2014).

Brito et al. (2002) proposed a chromatographic method for detecting pesticide residues in coconut water analyzing 15 real coconut water samples (TetraPak, plastic bottles and fresh fruit) collected from six commercial markets located in Araraquara and Campinas cities, São Paulo State, Southeast, Brazil. None of the target analytes were detected in these samples under the experimental conditions described.

Deme et al. (2013) developed a method for determining residues of organophosphorus pesticides in coconut water extracted by solid phase extraction (SPE) and analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) technique. The values of pesticide residues in coconut water of the developed method were below the maximum residue level (MRL) proposed by the European Union (EC, 2008; Villaverde et al., 2016). However, there is an urgent need to set limits for fresh/packaged coconut water to prevent consumers from contamination with pesticide residues, as the country needs to have its own standards.

Dos Anjos & De Andrade (2014) determined residues of nineteen pesticides (organophosphates, organochlorine, pyrethroids, carbamate, thiocarbamate and strobilurin) in coconut water by single-drop microextraction followed by gas chromatography-mass spectrometry (SDME/GC–MS). The developed method was successfully applied to real samples of coconut water. The method enabled the identification and quantification of pesticides from different chemical classes in the analyzed samples, and presence of pesticides sulfotep, demeton-O, dimethoate, disulfoton, fenitrothion, and malathion was verified. It was found that the highest concentrations of pesticides were observed for samples of industrialized coconut water.

Ferreira et al. (2016) developed a method for analyzing pesticide residues in coconut fruits (water and pulp) using modified Quick, Easy, Cheap, Effective, Ruged and Safe (QuEChERS) multiresidue extraction method, and LC-MS/MS. The proposed method was applied to samples collected from different regions of Brazil. In order to demonstrate the applicability of the validated analytical method for the purpose of routine pesticide residue analysis in coconut crops, 36 samples were monitored, of which 18 samples were from coconut water and 18 samples were from the pulp, all from different regions of Brazil. The results obtained show that all samples (both matrices) were contaminated with carbofuran. Coconut water samples from Goianésia-GO and Campinas-SP showed only difenoconazole. Most of the samples analyzed from Neópolis-SE had some pesticides detected, such as:
carbendazim, thiacarbazone, carbosulfan, cyproconazole and difenoconazole. However, all samples showed levels below the limit of quantification (10 µg/kg) and maximum residue limits established by the European Union regulations (EC, 2008; Villaverde et al., 2016).

Silva et al. (2008) developed a chromatographic method for simultaneous analysis of residues of eight pesticides in coconut in India. Their investigation for OCs residue levels indicated residues of organochlorine (OCs) pesticides in coconuts in the Indian environment. Their investigation for OCs residue levels indicated presence of residue level of hexachlorocyclohexane isomers (HCHs) (0.099 mg/kg), dichlorodiphenyltrichloroethanes (DDTs) (0.047 mg/kg) and endosulfan (0.017 mg/kg). In Brito et al. (2002), Pandey et al. (2010), Silva et al. (2008), Deme et al. (2013), Dos Anjos & De Andrade (2014) and Ferreira et al. (2016) the phytosanitary techniques used in the applications of pesticides in the coconut trees of the analyzed fruits were not informed.

The assumptions made in model formulation allow for consideration of a dispersion-advection equation of the concentration of the pesticide in the plant stem to describe the translocation of the pesticide over time and the vertical height of the stem, towards the crown of the coconut tree, and starting from the injection point, which occurs simultaneously with the translocation of water and nutrients during the plant's transpiration process. Therefore, the equation

\[ D \frac{\partial^2 C_{\text{sap}}}{\partial z^2} - v_{\text{sap}} \frac{\partial C_{\text{sap}}}{\partial z} - R_f \frac{\partial C_{\text{sap}}}{\partial t} = 0 \]  

(1)

describes the dynamics of the concentration of the diluted pesticide in the sap after being injected, by means of packets, at a spot in the stipe at a certain height from the soil surface (Freijer et al., 1998).

In Eq. (1), \( D \) (m²/day) is the hydrodynamic dispersion coefficient of the pesticide in the stipe, \( C_{\text{sap}} \) (ug/L) is the concentration of the pesticide in the sap, \( V_{\text{stripes}} \) (m²/day) is the sap water velocity, \( t \) (days) is the time after the pesticide injection in the stipe, and \( z \) (m) is the height of the stipe between the pesticide injection spot in the stipe and the height of the coconut tree canopy.

The retardation factor, \( R_f \), is a numerical value which expresses the retardation of the upward movement of the concentration of the pesticide in the sap of the stipe in relation to the upward movement of the sap without the pesticide. The retardation factor in Eq. (1) is defined by

\[ R_f = 1 + \rho_{\text{stipe}} \frac{K_{\text{stipe-sap}}}{\theta_{\text{stipe}}} \]  

(2)

where \( \rho_{\text{stipe}} \) (kg/L) is the density of the stipe, \( K_{\text{stipe-sap}} \) (L/kg) is the pesticide partition coefficients between the stipe and the sap (pesticide stipe-sap partition coefficients) and \( \theta_{\text{stipe}} \) (m³/m³) is the stipe volumetric sap content at plant field capacity. The coefficient of stipe-sap partition of a pesticide, which depends on the physicochemical characteristics of the pesticide and the constituent material of the stipe, can be determined experimentally (Trapp et al., 2001). As it will be noted later, this coefficient is the numerical value that determines the dynamics of translocation.
location of the concentration of pesticides injected into the coconut tree.

Assuming that the injection of pesticides into the stipe by means of pickets is described occasionally by a pulse equation (Veling, 1993), the boundary condition of Eq. (1) is given by

\[ C_{\text{stipe}}(0,t) = \frac{D}{\nu_{\text{stipe}}} \frac{\partial C_{\text{stipe}}}{\partial z}(0,t) = m \frac{\nu_{\text{stipe}}}{\nu_{\text{stipe}} A} \delta(t), \quad t \geq 0 \quad (3) \]

where \( m \) (ug) is the mass of the pesticide at the dose injected into the stipe, \( A \) (m²) is the cross-sectional area of the stipe at the injection spot parallel to the soil surface and \( \delta(t) \) is the Dirac delta distribution pulse function that describes the instant injection of the pesticide (pulse equation).

The analytical solution of Eq. (1), with the boundary condition given by Eq. (3), is given by Freijer et al. (1998):

\[ C_{\text{stipe}}(\zeta, \tau) = C_0 \left[ 1 + \frac{1}{\sqrt{\pi \tau}} \exp \left( -\frac{(\zeta - r)^2}{4\tau} \right) + \frac{1}{2} \exp(\zeta) \text{erfc} \left( \frac{\zeta + \tau}{2\sqrt{\tau}} \right) \right] \quad (4) \]

where \( C_0 = \frac{m_{\text{powdered_stipe}}}{\theta_{\text{powdered_stipe}} \nu_{\text{stipe}} A C_{\text{clean_sap}}} \) is the initial concentration of the pesticide in the stipe at the height of 0.50 m from the soil surface and immediately after the injection of the pesticide in the stipe. The values \( \nu_{\text{stipe}} \) and \( \theta_{\text{powdered_stipe}} \) are changes in variables to facilitate calculations and obtain the analytical solution (Freijer et al., 1998).

Equations \( D = D_0 \frac{\theta_{\text{powdered_stipe}}}{\omega_{\text{powdered_stipe}}} \omega_{\text{stipe}} \nu_{\text{stipe}} \nu_{\text{powdered_stipe}} \) and \( D_t = D_0 \frac{\theta_{\text{powdered_stipe}}}{\omega_{\text{powdered_stipe}}} \omega_{\text{stipe}} \nu_{\text{stipe}} \nu_{\text{powdered_stipe}} \) were applied to simulate the translocation dynamics of the pesticide in the stipe, with \( m_{\text{powdered_stipe}} \) denoting the molar mass of the pesticide, \( D_0 \) is the diffusion of the pesticide in the sap and \( D_t \) is the diffusivity of oxygen in the sap, \( L_{\text{disp}} \) is an empirical parameter, normally agreed to be between 0.005 and 0.05 in porous media of nature such as soil and wood, and \( \theta_{\text{stipe}} \) is the porosity of the stipe. All of the above definitions and equations can be found in Freijer et al. (1998).

The stipe volumetric sap content at field capacity (\( \theta_{\text{stipe}} \)), the stipe density (\( \rho_{\text{stipe}} \)) and the stipe porosity (\( \theta_{\text{stipe}} \)) were determined to be 0.38, 0.44 kg/L, and 0.65, respectively. These values were obtained experimentally at the Soil Laboratory of Embrapa Meio Ambiente (Jaguaruana, São Paulo, Brazil), using a 1000 cm³ sample of the interior of the stipe taken from a healthy coconut tree. The mean transverse area (\( A \), m²) of the stipe was estimated at 95 cm² using an average stipe of 15 cm radius. Diffusivity was estimated at 180×10⁻⁷ cm²/sec in coconut stipe and is compatible, in order of magnitude, with values determined by Baduru et al. (2008) in their study of diffusivity of several low molecular weight organic compounds in different types of wood. The molar weights of the pesticides abamectin, azoxyxystrobin, dimethoate, imidacloprid and thiamethoxam are 873, 403, 229, 255 and 291 g/mol, respectively. The molar weights of these pesticides are compatible with the values of the compounds studied by Baduru et al. (2008). The physicochemical characteristics of the pesticides abamectin, azoxyxystrobin, cyproconazole, dimethoate, imidacloprid, metalaxyl, thiamethoxan are shown in Table 1.

### Pesticide stipe-sap partition coefficient

A number of works have previously determined experimentally several wood-sap partition coefficients of organic substances for different tree woods and functional relationships between physicochemical characteristics of the analyzed substances and their respective wood-sap partition coefficients were also studied and determined experimentally (Trapp et al., 2001; Baduru et al., 2009; Rodriguez-Cruz et al., 2009). These functional relationships are useful for simulation studies where the aim is to study the behavior of substances injected or absorbed by plants (Trapp et al., 2001).

The partition of a pesticide between the stipe and the sap, is the stipe-sap partition coefficient of a pesticide or pesticide partition coefficient, which is given by

\[ K_{\text{stipe-sap}} = \frac{C_{\text{powdered_stipe}}}{C_{\text{clean_sap}}} \quad (5) \]

where \( C_{\text{powdered_stipe}} \) (mg pesticide/kg powdered_stipe) is the concentration of the pesticide in the powdered stipe, and \( C_{\text{clean_sap}} \) (mg pesticide/L clean_sap) is the concentration of the pesticide in the clean sap sample. In partition experiments, the concentration of the pesticide in the stipe is estimated by the relationship

\[ C_{\text{stipe}} = \frac{V_{\text{clean_sap}}}{V_{\text{powdered_stipe}}} (C_i - C_{\text{clean_sap}}) \quad (6) \]

where \( V_{\text{clean_sap}} \) (L) is the volume of sap, \( V_{\text{powdered_stipe}} \) (mg) is the volume of stipe, and \( C_i \) (mg/L) is the initial concentration of the pesticide in the stipe-sap-pesticide solution mixture of the procedure used for experimental determination of the pesticide stipe-sap partition coefficients (Trapp et al., 2001; De Wilde et al., 2009; Cerdeira et al., 2015). Linear regressions analyses were used to determine the partition isotherms and partition coefficients of pesticides between the stipe and sap (Fig. 1).

### Table 1. Physico-chemical characteristics of the pesticides injected into the coconut tree used in the modeling of the translocation in the tree and observed residues in coconut water and coconut pulp (Sol is the pesticide water solubility; LogKow is the logarithm of octanol-water partition coefficient).

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Sol (g/L)</th>
<th>LogKow (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abamectin</td>
<td>10.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Azoxyxystrobin</td>
<td>0.006</td>
<td>2.5</td>
</tr>
<tr>
<td>Cyproconazole</td>
<td>0.14</td>
<td>2.9</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>23</td>
<td>0.7</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>0.61</td>
<td>0.57</td>
</tr>
<tr>
<td>Metalaxyl</td>
<td>8.4</td>
<td>1.75</td>
</tr>
<tr>
<td>Thiamethoxan</td>
<td>4.1</td>
<td>-0.13</td>
</tr>
</tbody>
</table>
Laboratory determination of pesticide stipe-sap partition coefficient

Laboratory and field tests were performed using pesticides based on commercial formulations of abamectin (Abamex), cyproconazole (Alto100), azoxystrobin+cyproconazole (Priori xtra), dimethoate (Dimetoato 500EC Nortox), metalaxyl+mancozebe (Ridomil 350SC), and thiamethoxam (Actara 250WG). All the commercial formulations were obtained from local suppliers. Abamectin, cyproconazole, and thiamethoxam analytical standards, of purity above >98%, were purchased from Sigma Aldrich (Germany). Azoxystrobin, dimethoate, and metalaxyl analytical standards, of purity above 98%, were purchased from Chem Service (USA). Metalaxyl (98.7% purity) analytical standard were purchased from Dr. Ehrenstorfer (Germany).

Pesticide partition tests were performed using mixtures of pesticide formulations based on commercial formulations of abamectin, cyproconazole, azoxystrobin, dimethoate, imidacloprid, metalaxyl and thiamethoxam. A sample of approximately 2500 g of stipe from a healthy coconut plant was ground, washed with distilled water, and dried in an oven with forced air circulation for 72 h at 60 °C. To obtain the isotherms of stipe-sap partition and their respective partition coefficients ($K_{stipe-sap}$), a ratio of 1 g of powdered stipe and 10 mL of each pesticide solution was used. Six different pesticide concentrations between 0.1 and 8 µg/mL were used in duplicate. The samples were kept under agitation (150 rpm), at 24 °C for 24 h. Then, they were centrifuged at 5000 rpm for 15 min to separate the supernatant and quantify the pesticides.

Abamectin was quantified by a liquid chromatography system coupled to a mass spectrometer (Ion trap analyser, Amazon, Brucker). The chromatographic separation was carried out in a 5 µm (150 mm × 210 mm) Zorbax Eclipse – XD8 – C18 column at 40 °C using a mobile phase consisting of Milli-Q® water with 0.1% formic acid and 2% methanol and B-phase methanol with 0.1% formic acid. The flow rate was 0.4 mL/min in gradient elution mode. The other studied pesticides were quantified by a liquid chromatography system and diode array detector (UFLC-DAD, Prominence 20 A, Shimadzu) at specific conditions (Table 2) using a LiChrosorb RP-18 100 A column, 5 µ (250 mm × 4.60 mm), flow of 0.6 mL/min and gradient elution mode. Linear analytical curves were constructed in the mobile phase at seven concentration levels of pesticide mixtures in saps (clean saps) extracted from healthy coconut trees and without pesticide applications and (0.1; 0.2; 0.5; 0.8; 1.0; 2.0; 5.0 and 8.0 µg pesticide mixture/mL clean-sap) for quantification of each pesticide.

Experimental field design

Field experiments consisted of nine treatments (seven pesticides tested alone; a “mix” solution of all pesticides; and the control treatment in which 15 mL of distilled water were injected), three coconut tree plants for each treatment, totaling 27 plants. For pesticides residues, a number of six fruits/treatment (two fruits/tree) were collected at 48 h, 15 days, 30 days, 50 days, 70 days, 90 days, 110 days, 130 days, 150 days, 180 days, 210 days, and 240 days after the pesticide’s application, totaling 12 harvests and 54 fruits/harvest or a total of 648 fruits at the end of experiment.

Pesticide injections into coconut stem

Field tests were performed using pesticides based on commercial formulations of abamectin (Abamex), cyproconazole (Alto100), azoxystrobin+cyproconazole (Priori Xtra), dimethoate (Dimetoato 500EC Nortox), imidacloprid (Rotaprid 350SC), metalaxyl+mancozebe (Ridomil 350SC), and thiamethoxam (Actara 250WG). For the pesticides Abamex, Alto 100, Priori Xtra, Dimetoato, Rotaprid, and Ridomil a volume of 15 mL of the pure commercial formulation were tested per tree. For thiamethoxam treatment, 15 g of the commercial product were dissolved in 45 mL of water to produce a liquid solution with a concentration of 3 g/mL of thiamethoxam. For this concentration, 15 mL of thiamethoxam were injected per tree. In the treatment with the “mix” solution, 15 mL of each commercial product was mixed, and 15 mL of
that mixture was injected into the plants. All commercial formulations were obtained from local market suppliers and were tested alone or in mixture at the concentration of 1.5% of a.i. per tree.

The commercial products and the “mix” solution were injected into the stem of coconut plants, in a single dose, through the picket from the company SOSPalm (https://www.sospalm.com) using a 20 mL plastic syringe. The SOSPalm picket was previously installed into the stem of each coconut tree inside an orifice of 45 cm depth, at a height of 50 cm from the soil surface and a downward slope angle of 25 degrees in relation to the vertical of plant stem. The injection of pesticides follows the procedures defined in SOSPalm protocols (https://www.sospalm.com/).

The coconut cultivar used in the experiment was the Brazil green dwarf (BGD) planted at the Itaporanga Experimental Field, located in the Municipality of Itaporanga d’Ajuda, Sergipe State, Brazil (11°07’S and 37°11’W), 28 km from Aracaju city, the capital of Sergipe State. This coconut cultivar was 13 years old, in full state of coconuts production and had an excellent phytosanitary appearance. The experiments were carried out between January 1, 2017 and January 11, 2018.

Stem sap, water, and coconut pulp sampling for pesticide residue analysis

A total of 12 samples of coconut water and pulp were collected during the experiment at 20-30 days intervals. Coconut water samples were collected from six fruits per treatment per harvest and mixed together in composed sample, as well as, the coconut pulps. From this mixture, a single sample of 250 mL for coconut water and of 100 g to 300 g for coconut pulp were carefully packed in sterile plastic bottles of 300 mL and 500 mL, respectively, totaling 108 for coconut water and 108 for coconut pulp. The samples were identified with the date of collection and the respective treatment, then frozen and sent for pesticide residues analysis at the Embrapa Meio Ambiente Analytical Residue and Contaminant Center in Jaguariúna, São Paulo State, Brazil. All samples were collected from bunches monthly harvested for coconut water – green coconuts with six to seven months of maturity – intended for coconut water market.

The first three samples were collected obeying the regulation for the registration of pesticides, and the other samples were collected obeying the coconut tree's biological cycle dictated by seasonal climatic variations that regulate the fruit's maturation point. The fruits harvested in August 2018 (last sample) were the one protected inside the spathe no 9 (spathe still closed), at the time of pesticide injection.

In the same experimental field, sap samples were collected from a coconut tree without pesticide injection, but with the same phytosanitary characteristics of those where pesticide were injected. Five pickets were installed around the stipe of the plant with an angle of approximately 210 degrees between them. A volume of sap of approx. 100 to 300 mL was collected every sixty days. All sap samples collected were used to prepare the chromatographic method for the analysis of residues and to estimate the coefficients of stipe-sap partition of each of the pesticides injected in other coconut trees.

Chromatography determination of pesticide residues in water, pulp, and coconut sap

The method used to extract pesticides from pulp, water and coconut sap samples was based on the QuEChERS methodology (AOAC, 2007). In a tube containing 10 g of sample, 10 mL of acidified acetonitrile (1% acetic acid) were added and subjected to mechanical stirring for 2 min.

### Table 2. Composition of the mobile phase, elution mode, and the wavelength used to determine the isotherms for pesticide partition between coconut sap.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Mobile phase</th>
<th>Elution mode</th>
<th>Wave-length (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoxystrobin</td>
<td>(A) 0.1% formic acid (B) methanol</td>
<td>Isocratic 60% (B)</td>
<td>254</td>
</tr>
<tr>
<td>Cyproconazole</td>
<td>(A) water (B) acetonitrile (60:40)</td>
<td>Gradient 0-25 min 40% (B); 30-35 min 80% (B); 35-40 min 40% (B)</td>
<td>210</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>(A) water (B) acetonitrile (60:40)</td>
<td>Gradient 0-20 min 35% (B); 20-25 min 80% (B); 30-40 min 35% (B)</td>
<td>210</td>
</tr>
<tr>
<td>Imidacloroprid</td>
<td>(A) 0.1% formic acid (B) methanol</td>
<td>Gradient 0-30 min 80% (B); 32-40 min 20% (B)</td>
<td>254</td>
</tr>
<tr>
<td>Metalaxy</td>
<td>(A) water (B) acetonitrile (60:40)</td>
<td>Isocratic 60% (B)</td>
<td>254</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>(A) 0.1% formic acid (B) methanol</td>
<td>Gradient 0-30 min 80% (B); 32-40 min 20% (B)</td>
<td>255</td>
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</tbody>
</table>
at 1600 rpm. To induce the separation of the phases, the salting out step performed by adding 4.0 g of MgSO4 and 1.7 g of anhydrous sodium acetate, subjected again to mechanical stirring and centrifugation (6500 rpm, 20°C, 5 min) to separate the phases. An 8 mL aliquot was removed from the supernatant and stored in a freezer at a temperature of -80°C for 15 min.

Then cleanup was performed in a 5 mL aliquot by adding 600 mg of MgSO4, 500 mg of Octadecyl C18 and 100 mg of primary/secondary amine and mechanical agitation. After centrifugation (6500 rpm, 20°C, 5 min), a 2 mL aliquot was removed from the supernatant, filtered through a syringe filter (Millex-PVDF, 0.22 μm) directly into the injection vial. The quantification of pesticides was performed using an ultra-high-efficiency liquid chromatography system coupled to a mass spectrometer, triple-quadrupole type and electron-mist interface (UPLC-ESI-MS/MS), model Four Premier XE, Waters (Milford, MA, USA).

The chromatographic separation was performed using a Kinetex® C18 column (1.7 μm, 2.1 mm ID, 100 mm) (Phenomenex), temperature of 35°C, injection volume of 20 μL, with a flow of 0.225 mL/min, and mobile phase consisting of (A) 0.1% formic acid: methanol (98:2, v/v) and (B) methanol: 0.1% formic acid (98:2, v/v). Chromatographic elution was performed in gradient mode, with 60% B over 1.50 min, increasing linearly to 100% B in 4.5 min, and returning to the initial composition, totaling 8.0 min. The operating conditions of the mass spectrometer were, source temperature 120 °C, capillary voltage 3.0 KV, temperature and flow of the desolvation gas (N2) 400 °C and 500 L/H respectively. Argon was used as a collision gas at 0.20 mL/min. The acquisition was performed in MRM mode (Multiple Reaction Monitoring) in positive mode. Table 3 presents the precursor ion [M+H+] in MRM mode (Multiple Reaction Monitoring) in positive ion MRM 1 (m/z) 256.1 > 209.2, Cone energy 26 V, Retention time 1.21 min, Pesticide Imidacloprid, MRM 2 (m/z) 230.0 > 198.9, Cone energy 18 V, Retention time 1.40 min, Pesticide Dimethoate, MRM 1 (m/z) 292.2 > 70.2, Cone energy 30 V, Retention time 4.95 min, Pesticide Cyproconazole, MRM 2 (m/z) 280.1 > 125.0, Cone energy 22 V, Retention time 2.60 min, Pesticide Metalaxyl, MRM 1 (m/z) 292.1 > 211.2, Cone energy 24 V, Retention time 1.21 min, Pesticide Thiamethoxam.

Linear analytical curves (R² ≥ 0.99) were prepared in matrix matched using a mixture of pesticides at six different concentrations levels, from 0.005 to 0.05 μg/mL. The validated method presented quantification limit of 15 μg/kg for all pesticides, accuracy of 70 to 120% for both levels studied (15 and 30 μg/kg), and repeatability < 20% (n=5 for each level studied), as recommended by AOAC (1993).

### Results and discussion

In order to simulate the translocation of pesticides injected into coconut tree using Eq. (4), it was necessary to determine the pesticide stipe-sap partition coefficients and calculate the pesticide retardation factor. Pesticide stipe-sap partition coefficients, Kstipe-sap, were determined experimentally for five of the seven pesticides studied (abamectin, azoxystrobin, dimethoate, imidacloprid, and thiamethoxam) (Table 4). Pesticide partition of two of the seven pesticides (cyproconazole and metalaxyl) were estimated using multiple linear regression analysis from the physical-chemical data of the other five pesticides (abamectin, azoxystrobin, dimethoate, imidacloprid, and thiamethoxam) and their respective values of pesticide stipe-sap partition coefficients determined experimentally.

After the chromatographic analyses of the pesticides, simple linear regression analyses were used to estimate the values of pesticide stipe-sap partition coefficients (Fig. 1). The results are shown in Table 4, where coefficient of determination values R² indicate a good fit of the linear regression models. Table 4 also shows that the results of the partition experiments were not sufficient to estimate of pesticide stipe-sap partition coefficients of cyproconazole and metalaxyl. This was due to the fact that pesticide cyproconazole is a nonpolar molecule with low solubility in water (0.14 g/L) and high affinity to organic matter, which is verified by its octanol-water partition coefficient (LogKow = 2.9 or Kow = 795 L/kg). Concentrations of cyproconazole in the clean sap extracted of coconut were below the limit of quantification of the analytical method used, a phenomenon most likely due to the high affinity.

### Table 3. Precursor ion [M+1], the energy of the cone, the retention time, and the transitions considered for quantification and confirmation.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Retention time (min)</th>
<th>[M+H+] m/z</th>
<th>MRM 1 quantification (m/z)</th>
<th>MRM 2 confirmation (m/z)</th>
<th>Cone energy (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abamectin</td>
<td>5.58</td>
<td>895.5</td>
<td>895.5 &gt; 327.3</td>
<td>895.5 &gt; 449.1</td>
<td>42</td>
</tr>
<tr>
<td>Afoxystrobin</td>
<td>3.45</td>
<td>404.1</td>
<td>404.1 &gt; 372.1</td>
<td>404.1 &gt; 329.0</td>
<td>26</td>
</tr>
<tr>
<td>Cyproconazole</td>
<td>4.95</td>
<td>292.2</td>
<td>292.2 &gt; 70.2</td>
<td>292.2 &gt; 125.0</td>
<td>30</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>1.40</td>
<td>230.0</td>
<td>230.0 &gt; 198.9</td>
<td>230.0 &gt; 124.9</td>
<td>18</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>1.21</td>
<td>256.1</td>
<td>256.1 &gt; 209.2</td>
<td>256.1 &gt; 175.1</td>
<td>30</td>
</tr>
<tr>
<td>Metalaxyl</td>
<td>2.60</td>
<td>280.1</td>
<td>280.1 &gt; 220.1</td>
<td>280.1 &gt; 192.1</td>
<td>22</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>1.21</td>
<td>292.1</td>
<td>292.1 &gt; 211.2</td>
<td>292.1 &gt; 132.0</td>
<td>24</td>
</tr>
</tbody>
</table>
of cyproconazole to the material of the stipe (organic matter). Therefore, the $K_{\text{stipe-sap}}$ partition coefficient of cyproconazole was not determined experimentally.

Since all metalaxyl remained diluted in the sap and it was not possible to determine the partition between the phases, the metalaxyl stipe-sap partition coefficient was not determined experimentally. This occurs when the pesticide has a low affinity for organic matter, as shown by the value of the octanol-water partition coefficient of metalaxyl ($\log K_{ow} = 1.75$; $K_{ow} = 56.23$ L/kg) and the high-water solubility of metalaxyl (8.4 g/L). Thus, the value of the $K_{\text{stipe-sap}}$ partition coefficient of metalaxyl was not also determined experimentally (Table 4).

In experiments aimed to determine substance partition coefficients between two media and situations such as those occurring with cyproconazole and metalaxyl, it is common to estimate the partition coefficient of these substances using values of the partition coefficients of other substances determined experimentally and physical-properties of substances by fitting a multiple linear regression model that functionally relates the physico-chemical characteristics of pesticides and the stipe-sap partition coefficients given by a the following model $\log K_{\text{stipe-sap}} = a + b \log K_{ow} + c \log Sol$, where $\log Sol$ is the logarithm of water solubility of the substance, and parameters $a$, $b$, and $c$ are unknown numerical values estimated by multiple linear regression. Values of the octanol-water partition coefficients ($\log K_{ow}$) and water solubility values ($\log Sol$) of the pesticides abamectin, azoxystrobin, dime- thoate, imidacloprid, and thiamethoxam (Table 1), as well as their respective values of the stipe-sap partition coefficients ($K_{\text{stipe-sap}}$) (Table 4) were used to estimate the values of parameters $a$, $b$, and $c$. The estimated values were $a = 1.14$, $b = 0.3$ and $c = -0.22$. Therefore, the multiple linear regression estimates of the coefficients of cyproconazole and metalaxyl stipe-sap partition were 157.35 L/kg and 29.15 L/kg, respectively. The estimated linear regression equation shows that the stipe-sap partition coefficient is directly proportional to the octanol-water partition coefficient ($b = 0.3 > 0$) and inversely proportional to water solubility ($c = -0.22 < 0$). In Table 4, it is observed that the translocation of abamectin proceeds slowly in the stipe and the pesticide remains for a longer time in the region of the stipe where it was injected into the plant due to the value of the stipe-sap partition coefficient ($K_{\text{stipe-sap}} = 208.46$ L/kg). From the linear regression analysis presented in Table 4, it can be observed that the translocation of abamectin is slowly processed in the stipe and the pesticide remains for a longer time in the region of the stipe where it was injected into the plant. This is due the value of the stipe-sap partition coefficient ($K_{\text{stipe-sap}} = 208.46$ L/kg). Pesticides dimethoate, thiamethoxam, and metalaxyl were the active ingredients displaying the greatest potential for translocation in the sap of coconut palms with low retain in the plant stem, as described by their values of the coefficient of stipe-sap partition of 6.43, 10.16, and 29.15, respectively (Table 4).

Another possibility presented by assuming the coconut tree stem as a porous medium and a hydrodynamic dispersion was to use the retardation factor ($R_f$) to speculate on the dynamics of pesticides in the stem column. Therefore, it was necessary to estimate the retardation factor that expresses the delay in the mobility of the pesticide in reaching the aerial part of the plant, i.e., in its diluted displacement in the sap in relation to the upward movement it makes after injected into the coconut stipe. The retardation factor in the coconut palm of the pesticides abamectin, cyproconazole, azoxystrobin, dimethoate, imidacloprid, metalaxyl, and thiamethoxam were estimated at 242.37, 206.50, 183.20, 8.45, 49.26, 34.75, and 12.77, respectively. From these values and the values of the coconut stipe-sap partition coefficient, it can be deduced that the pesticide imidacloprid and metalaxyl are transported upward faster in the stipe than the pesticides abamectin and cyproconazole (Figs. 2 and 3). Eq. (4) allowed the simulation of the movement of concentrations of these pesticides over time and along the height of the stipe.

The simulations of translocation of the pesticides in the stipe were obtained by means of the analytical solution of Eq. (1) given by Eq. (4), which describes the dynamics of

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>$K_{\text{stipe-sap}}$</th>
<th>Standard error</th>
<th>$t$ value</th>
<th>$p$-value</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abamectin</td>
<td>208.46</td>
<td>8.51</td>
<td>24.48</td>
<td>2.12E-06</td>
<td>0.992</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>177.47</td>
<td>11.23</td>
<td>15.80</td>
<td>3.98E-03</td>
<td>0.992</td>
</tr>
<tr>
<td>Cyproconazole</td>
<td>157.35*</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>6.43</td>
<td>0.51</td>
<td>12.70</td>
<td>4.35E-06</td>
<td>0.958</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>41.68</td>
<td>1.72</td>
<td>24.21</td>
<td>6.82E-11</td>
<td>0.982</td>
</tr>
<tr>
<td>Metalaxyl</td>
<td>29.15*</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>10.16</td>
<td>0.46</td>
<td>22.15</td>
<td>9.65E-08</td>
<td>0.986</td>
</tr>
</tbody>
</table>

*Values estimated by $\log K_{\text{stipe-sap}} = 1.144 + 0.299 \log K_{ow} - 0.219 \log Sol$
the concentrations of pesticides applied by injection into the stump of the coconut tree at a height of 50 cm of the soil surface (Figs. 2 and 3). Fig. 2 shows concentrations of pesticides in the sap as a function of the height of the stipe and the respective times required to reach the maximum heights simulated at each height. Fig. 3 presents the levels of pesticide concentrations in the sap as a function of the time required for the pesticide to reach certain simulated heights at each time. In both figures, it can be observed that concentrations of abamectin in the sap of the stripe are significantly lower than the concentrations of dimethoate at the same height of the stripe. This is given by the fact that the partition coefficient of the stipe-sap of abamectin is also significantly higher than the partition coefficient stipe-sap of dimethoate (Table 4). This demonstrates that part of abamectin is initially retained in the coconut tree's stem, unlike dimethoate, whose translocation occurred faster in the stem. The influence of the retardation factor on concentrations and translocations of these pesticides also suggests that abamectin needs much more time to reach the same height as that achieved by dimethoate. In Table 4, it is possible to observe the importance of the values of Rf and Kstipe-sap in the concentrations in the sap of the stipe for each of the active principles studied.

In general, pesticides classified agronomically as having systemic action on plants are also relatively more soluble in water than pesticides that are not classified as having systemic action. A pesticide is said to have a systemic action if when applied by aerial spray it penetrates the plant and has the ability of translocating and reaching its action point inside the plant. These pesticides, when injected into the coconut palm, also translocate inside the plant because they have low affinity to the solid matter of the stem, high water solubility, and low partition coe-

![Figure 2. Simulations of pesticide concentrations in the sap along the height of the stipe estimated by the model given by Eq. (4).](image-url)
fficient, which facilitates their translocation in the plant. It may happen that non-systemic pesticides translocate in the coconut tree when injected into the tree. The systemic or non-systemic characteristic of a pesticide injected into the palm stem will depend on the relative partition of the pesticide between the stem material and the palm sap described by the stipe-sap partition coefficient (Table 4).

In the coconut tree stump, it is likely that the concentration of pesticides in the plant’s transpiration flow depends on this coefficient in its translocation within the stump, which can lead to a pesticide even though it is not systemic in aerial applications to move upward in the stump even if slowly.

The simulations of translocation of the pesticides in the stipe were obtained by means of the analytical solution of Eq. (1) given by Eq. (4), which describes the dynamics of the concentrations of pesticides applied by injection into the stump of the coconut tree at a height of 50 cm of the soil surface (Figs. 2 and 3). Fig. 2 shows concentrations of pesticides in the sap as a function of the height of the stipe and the respective times required to reach the maximum heights simulated at each height. Fig. 3 presents the levels of pesticide concentrations in the sap as a function of the time required for the pesticide to reach certain simulated heights at each time. In both figures, it can be observed that concentrations of abamectin in the sap of the stipe are significantly lower than the concentrations of dimethoate at the same height of the stipe. This is given by the fact that the partition coefficient of the stipe-sap of abamectin is also significantly higher than the partition coefficient stipe-sap of dimethoate (Table 4). This demonstrates that part of abamectin is initially retained in the coconut tree’s stem, unlike dimethoate, whose translocation occurred faster in the stem. The influence of the retardation factor on concentrations and translocations of these pesticides also suggests that abamectin needs much more time to
reach the same height as that achieved by dimethoate. In Table 4, it is possible to observe the importance of the values of $R_f$ and $K_{stipe-sap}$ in the concentrations in the sap of the stipe for each of the active principles studied.

In general, pesticides classified agronomically as having systemic action on plants are also relatively more soluble in water than pesticides that are not classified as having systemic action. A pesticide is said to have a systemic action if when applied by aerial spray it penetrates the plant and has the ability of translocating and reaching its action point inside the plant. These pesticides, when injected into the coconut palm, also translocate inside the plant because they have low affinity to the solid matter of the stem, high water solubility, and low partition coefficient, which facilitates their translocation in the plant. It may happen that non-systemic pesticides translocate in the coconut stem when injected into the tree. The systemic or non-systemic characteristic of a pesticide injected into the palm stem will depend on the relative partition of the pesticide between the stem material and the palm sap described by the stipe-sap partition coefficient (Table 4). In the coconut tree stump, it is likely that the concentration of pesticides in the plant’s transpiration flow depends on this coefficient in its translocation within the stump, which can lead to a pesticide even though it is not systemic in aerial applications to move upward in the stump even if slowly.

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