

Research Article

Dissipation and Effects of Thiamethoxam on Aphid Control and Non-Targets in Apple Tree Canopy by Trunk Injection Application

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Trunk injection is an effective alternative to spraying or soil applied pesticides for tree pest control to induce their side function on environment. Here, we investigated the dissipation behavior of thiamethoxam applied by trunk injection in canopy of apple tree and evaluated the control effect on the aphid pests. The results showed that thiamethoxam was quickly taken up by the vascular system and distributed throughout the tree. The cultivars, sample location of canopy and tree age affected the absorption of thiamethoxam, resulting in the differences in their dissipation. Under the same amount of thiamethoxam applications, the initial (2h) concentration was 20% higher in cultivar 'Guoguang' than in 'Fuji'. Thiamethoxam had a similar trend in both cultivars, which declined within the first 1-3 days and reach to less than 10% after 21 days of application. The outer canopy had higher thiamethoxam than inner canopy. The final control effect was up to about 90%. The results showed that the injection application method had a good lasting validity and high final control effect on *Aphis citricola*. This knowledge will contribute to effective control of aphids by using trunk injection.

Keywords: Dissipation; Thiamethoxam; *Aphis citricola*; Non-targets; Injection application method**Introduction**

With greater emphasis on protecting the environment and the improvement of pesticide use regulations, it is important to manage treatments to minimise insecticide spray drift. Alternative methods of application, including trunk injection, are considered as effective for tree pest control. It is highly efficient for liquid drugs, can be used with a broad spectrum of insecticides, and is relatively pollution free, safe, simple to apply, and is less affected by weather. Previous studies had shown that chemicals such as systemic pesticides, minerals, and plant growth regulators could be injected into the tree by self-flow or high pressure [1]. These pesticides would be transported and then distributed throughout the plant with the transpiration stream [2]. Extensive research has shown that trunk injection can play an important role in disease or insect pest control [3-6].

Some researchers have tested a variety of insecticide options in the application of trunk injection technology [7,8]. Trunk injections of neonictinoids insecticides, such as imidacloprid and thiamethoxam were shown to be effective in pest control [9]. Therefore, evaluations of pest control using trunk injections of these chemicals provide a guidance in pest control.

In China, the most common aphid species in apple orchards is the *Aphis citricola* Van der Goot (Homoptera) [10]. Severe infestation of aphid may curl leaves, reduce tree growth and non-structural carbohydrate concentration in young apple trees. Thiamethoxam is common to be sprayed in apple orchard when controlling this aphid [11]. However, little has been reported on the success of insecticide treatments using trunk injection techniques to control aphid on apple

trees.

In order to evaluate the environmental pressure of thiamethoxam and scientifically conduct post-pesticide risk assessment during production, an analytical method for thiamethoxam residue in apple leaves by high-performance liquid chromatography was set up, its dissipation in different parts of the apple tree canopy was studied, and the effects of thiamethoxam on *Aphis citricola* and non-targets with injection application method were studied, at the same time.

Materials and Methods**Laboratory work**

Thiamethoxam standard stock solution of 100mg·L⁻¹ were prepared in acetonitrile, and then was used to prepare working standard solutions and standard calibration. All standard solutions were stored at 4°C before use.

Thiamethoxam stock solution was diluted to a standard solution of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 mg·L⁻¹. 5μL were injected under stable chromatographic conditions to obtain the injection concentration and corresponding peak area of thiamethoxam, then standard curve was established.

Extraction and purification of thiamethoxam from apple leaf samples were conducted by using modified QuEChERS method [12]. A sample (1.00g) was placed into 15mL polypropylene tubes with 10mL acetonitrile : water = 80:20(v/v). 2.0g NaCl were added and vortexed for 5min. The tube was centrifuged at 10,000r/min for 5 min. 7mL of the upper acetonitrile extract was collected in a new tube and cleaned up by dispersive solid-phase extraction with 100

mg PSA and 100mg GCB. The sample was again vortexed for 5 min, and then centrifuged at 10,000 $\text{r}\cdot\text{min}^{-1}$ for 5 min. 6mL of the upper acetonitrile extract was collected and concentrated to dryness with a rotary vacuum evaporator at 35°C. Then, the supernatant was taken, filtered through a 0.45 μm PTFE filter and transferred into a glass vial for HPLC-DAD analysis.

An Agilent 1200 (Agilent Technology, USA) was used for residue analysis of thiamethoxam with quaternary pump, autosampler injector, thermostat compartment for the column and photodiode array detector. The chromatographic column was Zorbax XDB-C18 (250mm $\times$ 4.6mm, 5 μm). The column was kept at 30°C. Flow rate of mobile phase was 1.0mL/min, and injection volume was 5 μL . The mobile phase ratios of thiamethoxam was acetonitrile:water=15:85 (v/v). Detection wavelength was set at 252nm.

Field experiment design

Field studies of thiamethoxam were conducted in 2018 and 2019 at north suburbs of Beijing which referred to the method of Kou [13]. The apple trees tested were 10-year-old of the cultivar Fuji and 30-year-old varieties Guoguang. The experimental field soil was of loam type. Drip irrigation technology was used in irrigation. We used a drill with a diameter of 5 mm to tilt the drill down 45° at a distance of 10cm from the ground, then drilled a hole with a depth of 5cm, and finally insert the needle of the infusion tube into the trunk hole as long as the liquid did not leak out of the tree. Prior to application of thiamethoxam, fresh apple leaves were plucked to detect the presence of residues and no residue was detected in the collected apple leaves, indicating that the experimental plots had no previous history of using thiamethoxam. To ensure the reliability of the experimental results, orchard management was carried out in accordance with local management practices.

Apple trees were injected with the commercial formulation once in the experiment plots. The dosage of thiamethoxam was 31.5g a.i. ha^{-1} (the recommended dosage from the label use). There were two treatments (injection treatment and blank control treatment) with total 240 m^2 and two experimental plots for each treatment with “Guoguang”. Similarly, there were two treatments (injection treatment and blank control treatment) with 240 m^2 and two experimental plots for each treatment with the cultivar “Fuji”. Blank control (water with injection treatment) of the same size but with no thiamethoxam application was compared simultaneously. A buffer zone with two trees was used to separate these plots with different treatments. Samples (apple leaves) were drawn at eight collecting points (four directions of the north, east, south, west and two positions of the inside and outside of the canopy) at 0 (2 h post treatment), 1, 3, 5, 7, 14, 21 and 28 d interval from the treated and blank control plots, respectively. Each time two trees in each plot were randomly selected as sampling objects. Samples were collected into clean polyethylene bags, transported to the laboratory, stored at -20°C until analysis.

Control effect on aphids

The experiment design referred to the method of Kou [13]. Two trees in each plot each time were randomly selected as the objects of investigation. *Aphis citricola* was observed and counted at ten branches of each observing points (four directions of the north, east, south and west of the canopy) at 0 (before application), 1, 3, 7 and 14

d interval from the treated and blank control plots separately. And in order to further develop IPM efficiency, it's important to evaluate the side effects of pesticides on non-targets [14]. So, yellow sticky traps were hanged with four directions of the north, east, south and west of the canopy, which were used to monitor the population dynamics of non-targets.

Results

Method validation

There was a high correlation between different injection concentrations (x) and corresponding peak areas (y), $y = 27.162x - 1.4631$, correlation coefficient $r = 0.9992$ ($n = 8$), within the range of 0.1-20 $\text{mg}\cdot\text{L}^{-1}$. Respectively, the LOD and the LOQ were 0.1ng and 0.02 $\text{mg}\cdot\text{L}^{-1}$. The recoveries of thiamethoxam at the three levels were 97.64-105.85% with the RSDs 5.90-9.56%, which complied with pesticide residue analysis standards [15,16].

Dissipation of thiamethoxam in apple canopy

The distribution of thiamethoxam in the canopy leaves was detected after injection finished. The results showed that thiamethoxam could be detected after 2h from injection, and remained in apple tree leaves for about 14d, the variety and age of apple trees affected the absorption and transmission of thiamethoxam in trees. Compared with the Fuji cultivar with 10 years old, the Guoguang cultivar with 30-year-old absorbed and accumulated more thiamethoxam and transmit more quickly. The distribution of thiamethoxam in the outer leaves of the canopy was generally higher than that of the inner leaves, but there was no significant difference among the four directions (north, east, west and south).

The dissipation patterns of thiamethoxam showed a rapid decline within the first 3 days after application, and decreased slowly to less than 10% after 21 days of application. The only difference was in the initial days, the cultivar Fuji had a growth within the first 1d after application. The results showed that thiamethoxam was easy to degrade, and the dissipation curves followed the pharmacokinetic with the half-life ranging from 0.90 to 6.54 days.

Control effect of thiamethoxam on *Aphis citricola*

The control effect of thiamethoxam on *Aphis citricola* was near 50% in the day 3, and up to about 90% in the day 14. No significant difference in the four directions was found. The control effect in these two cultivars was similar.

Effect of thiamethoxam on non-targets

The population dynamics of non-targets, such as ladybugs, parasitoid wasps and predatory bugs, with thiamethoxam injection treatment group were consistent with its corresponding control group, and there was no significant difference within 14d after application. Take parasitoid wasps as an example, the effect of thiamethoxam on parasitoid wasps was shown as follows. The results indicated that the injection application method also had no obvious virulence effects and had a good ecological protection on non-targets in Guoguang and Fuji.

Discussion

Although the characteristics of injection application and the complex conduction process of pesticides in plants, there has been

some researches on the mechanism of insecticide conduction in plants so far [17-32]. A large number of research results showed that, whether it was stem injection or root application, the cumulative amount of the chemicals in the leaves was the highest [33-35,27]. The study showed that the distribution of thiamethoxam in the outer leaves of the canopy was higher than that in the inner leaves of the canopy with injection application method. This conclusion was similar to the conclusions obtained by Wu et al. [30] and Tang [36] using the single hole injection method of trunk. The outer branches and leaves of the canopy had strong growth vigor and transpiration, and the demand for nutrients and water was large, which was a strong "library" [30]. It showed that thiamethoxam entered the xylem of the tree and transported it up and down along with the transpiration fluid flow, at the same time, there was lateral transport, that was, it spread to both two sides when the xylem conducted upward, so that the canopy could be distributed in all parts of the canopy. Therefore, the amount of pesticides absorbed and accumulated by the water and nutrient flow was increased. It had been reported that the distribution of pesticides in the leaves after trunk injection was higher than other parts of the tree [36,27,37], which was conducive to the prevention and control of leaf-feeding pests. In addition, the difference of thiamethoxam dissipation in canopy between Guoguang and Fuji was probably due to the difference of tree age, which lead to the difference of thiamethoxam uptake rate in canopy. At the same time, less operation time and satisfactory control of Aphis citricol, Therefore, the injection application method can be used to prevent and control the Aphis citricol in the actual scene.

To decrease the side function of the pesticide, pesticide application technologies are more and more critical to the success of pest control. According to the results, the half-life was clarified, which could provide guidance for the efficient, safe and reasonable use of pesticides in the future, and also establish the foundation for the formulation of standards for the application of commonly used pesticides in apple orchards. The results could provide a basis for industry or policy decisions from the perspective of pesticide management.

Conclusions

In this paper, thiamethoxam had good conductivity and was easy to degrade in apple trees, and the residues in the outer leaves of the canopy were generally higher than that in the inner leaves. With regard the effects to Aphis citricol and non-target insects, the injection application method had a good lasting validity, high final control effect and good ecological protection.

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