Dissipation of Spirotetramat and Spirotetramat-Enol in Leaf and Fruit of Grape Variety Red Globe (Vitis Vinifera L.)

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Abstract

The dissipation of spirotetramat (sp) and spirotetramat-enol (sp-enol) was determined in leaves and fruit of grapevine (Vitis vinífera L.) in a commercial vineyard. sp and sp-enol were extracted with acetonitrile:water, and quantification of residues was done on LC-DAD/MS. The LOQ of this method was found 0.002 mg kg^{-1}, LOD being 0.001 mg kg^{-1} for both. Recovery rates were in the range of 92 to 103% with coefficients of variation less than 9 and 6%, respectively at three levels (0.02, 0.2 and 0.6 mg kg^{-1}). sp degradation in leaves followed first-order kinetics with R^2=0.84 (p < 0.03). In fruit, only 35% of the original concentration of sp was detected at 5 days after application. These results suggest that residual levels of sp and sp-enol in grapes grown under the conditions of this study pose minimal risk to consumers, since residues did not exceed the limit established by the EPA.

Keywords: Spirotetramat; Spirotetramat-Enol; Grape; Dissipation; Grapevine.

1. Introduction

The application of agrochemicals to control crop pests may result in their presence in foods (Aldana et al., 2008a; Aldana et al., 2008b; Aldana et al., 2011) together with their physicochemical properties and environmental conditions under which they are applied (Babczinski and Hellpointner, 2008). Their residues may be of interest when products receive no processing before consumption, as in the case of table grapes (Oliva and Beard, 2002; Han et al., 2013).

Sp is an insecticide derived from the ambimobile insecticide tetramic acid aimed at the control of sucking pests in their juvenile stages (Nauen et al., 2008). It acts as an inhibitor of lipid biosynthesis, causing incomplete ecdysis, significant reduction in fecundity and fertility of insects and death (Fischer and Weiß, 2008; Kühnhold et al., 2008; Schöning, 2008). In experimental animals sp can cause eye irritation, skin sensitization, sperm abnormalities, hypospermia, tubular degeneration, and decreased testicular weight (FAO, 2008). Meanwhile, they may be the cause of contact dermatitis in humans (Funk, 2011). In toxicological studies in Cerioda phniadubia, it was reported that the mixture of an agricultural adjuvant (Destiny) and sp produced stronger toxicological effects (Chen and Stark, 2010). Even with its advantages and disadvantages, sp is a new alternative for the control of Planococcus ficus, which is a current problem in the cultivation of grapes on the coast of Sonora (Fu and del Real, 2009; Mansour et al., 2011). Accordingly, the objective of this study was to determine the dissipation of sp and sp-enol in leaves and fruit during cultivation of the grape variety (Vitis vinifera L.) Red Globe.

2. Materials and Methods

We used the standards of sp (cis-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro[4.5]dec-3-en-2-one) with 99.3% purity (Chem Service, West Chester, PA) and its enol metabolite (cis-3-(2,5-dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one) with 98.5% purity (Dr. Ehrenstorfer GmbH, Germany), HPLC grade acetonitrile (JT Baker, USA), reagent grade formic acid (88%) and SPE LC-18 cartridges (Supelco analytical No. 505471, USA). sp (Movento OD) at 15% was used in the field, where a dose of 600 cc per hectare was applied, equivalent to 90 g ha^{-1} of active ingredient. The insecticide was applied by spraying on the foliage in a volume of 1500 L of water per hectare. During application, the temperature was 23.4°C, wind speed 2.1 m s^{-1}, relative humidity 37.5% and solar radiation 0.331 kW m^{-2}. The samples were collected in San Luis Vineyard located at Km 19.5 of North 12th Street in Costa de Hermosillo, Sonora, Mexico at 29° 00' 53'' N, 111° 29' 16'' W (Datum WGS84) at an altitude of 78 m. The cultivation area consisted of 106 rows of plants separated by four meters, each row with 182 vines separated by 1.4 m. A drip irrigation system was used, set approximately 50 cm from the ground. Within the vineyard, five points were randomly selected, where plots were formed which in turn were made up of three plants located in the same row, for collection of samples of leaves and fruits at six time points; the day before the application of insecticide and on days 1, 5, 10, 15 and 48 after application. About 300 g of each sample were collected, placed in plastic bags and stored at -20°C on a laboratory freezer until analysis.

Detection and Quantification of Sp and Sp-Enol by LC-MS

The instrumentation used was an Agilent Technologies 1100 Series LC-MS chromatograph coupled to a simple quadrupole mass spectrometer model VL and DAD, equipped with a quaternary pump (1310a) and G1313A autosampler. Separation was carried out with an XBridge column, 3.5 µm and 3.0 x 150 mm, and XBridge C18 precolumn (Waters), 3.5 µm and 3.0 x 20 mm. Data were collected using Rev B.03.02 Chemstation software. The analysis of sp and sp-enol was according to Schöning (2008). The precursor ions and quantification of sp and sp-enol were 374→216, 302 m/z and 302→216, 270 m/z, respectively. The retention times for sp and sp-enol were 9.17 and 7.6 minutes, respectively. Satisfactory linarites were observed for sp and sp-enol ($R^2 \geq 0.9926$) for both compounds.

Statistical Analysis

We used a completely randomized design, with six repeated observations on days 0, 1, 5, 10, 15 and 48, after application of sp, and five replicates. Response variables were the amounts of sp and sp-enol on fruits and leaves. Descriptive statistics were carried out for each time in leaves and fruit. Analysis of variance with repeated measures was performed to determine: a) persistence [difference between initial time (before) and time with maximum concentration detected] and b) assessment of dissipation profiles of the insecticide and its metabolite in the plant.

3. Results and Discussion

Spirotetramat recovery percentages obtained for levels of 0.02, 0.2 and 0.6 mg kg^{-1} are show on **Table 1**. The results were similar to those reported by Singh et al. (2013) in okra, eggplant and peppers (82-97%) and Schöning (2008) in tomato, avocado, citrus, potato and hops (84-104%), and higher than those reported by Mohapatra et al. (2012) in mango and cabbage (sp and sp-enol respectively 73-93 and 84-116% in mango; sp and sp-enol respectively 74-91 and 87-109% in cabbage).

Table 1: Spirotetramat Recovery Percentages Obtained for Levels of Compounds				
Compound	Compound level (mg kg^{-1})	Leaves (%)	Immature fruit (%)	Ripe fruit (%)
Spirotetramat	0.02 0.2 0.6	99 ± 5 98 ± 3 99 ± 1	101 ± 8 96 ± 1 100 ± 2	98 ± 3 95 ± 4 93 ± 4
Spirotetramat-enol	0.02 0.2 0.6	99 ± 6 103 ± 2 99 ± 2	99 ± 2 98 ± 1 103 ± 9	92 ± 3 97 ± 4 93 ± 5
n=5				

The dissipation of sp in leaves was fitted to a first-order kinetics model with R^2 = 0.84 (p < 0.03), the initial concentration found a day after the application was 0.14 mg kg-1; after 5 days, there was a dissipation of 13%, and after 48 days, this increased up to 79%, resulting in a concentration of 0.03 mg kg^{-1}. The half-life of the insecticide in leaves was 9 days with a 95% confidence interval of (7-11). Dissipation data were similar to those previously reported by Maclachlan and Hamilton (2010) for the degradation of sp in apple. sp degradation in fruit was 65% during the first 5 days after application, giving a concentration of 0.08 mg kg^{-1}; at 48 days after the application, a residual concentration of 0.02 mg kg^{-1} was detected. Vemuri et al. (2014) reported a dissipation rate less than that observed in this study, possibly because of the combined application of imidacloprid and sp on grapevines. However, in both studies, concentrations below the maximum residue limit (MRL) established by the EPA (2011) (1.3 mg kg^{-1}) are reported. In this study, the half-life of sp in fruit was 5 ± 2 days. These results differed from those reported by Chahil et al. (2015), who obtained a half-life of 1.91 days for sp in the fruit of chili pepper; the shorter half-life could be due to the high relative humidity of the crop (48-100%). Residual levels of sp were higher in leaves compared to fruit (Fig. 1A). This behavior was previously reported by Sur (2008), who evaluated the concentration of sp in the fruit and leaves of apple, as well as in the tuber and leaves of potato, finding a higher concentration in the leaves in both cases. This was possibly due to the greater density of leaves than fruit of the plant, and thus leaves may be exposed to a larger amount of insecticide. The results for sp-enol in leaves and fruit at 5 days after application showed 70 and 80% dissipation, respectively, of the residue in relation to the concentration at day 1 (0.03 and 0.04 mg kg^{-1}). After day 5, residual levels slowly decreased to 0.005 and 0.008 mg kg^{-1} in leaves and fruit, respectively. It was clear that this metabolite showed different degradation kinetics as sp, but it was not possible to determine the dissipation kinetics of sp-enol in leaves and fruit, since the data showed no significant differences after day 5 (Fig. 1B). It would be interesting in future studies to take samples daily, to elucidate its degradation kinetics.

The sp dissipation model (first-order kinetics) coincides with that reported for the ketoenols spirodiclofen and spiromesifen (Sharma et al., 2006), and likewise, the sp-enol metabolite was detected at 12 hours after application of the pesticide. In the present investigation, sp-enol metabolite was first detected at 24 hours, since no samples were taken on the same day the insecticide was applied.

Sharma et al. (2007a) in their investigation of the dissipation kinetics of spiromesifen in tea (Camellia sinensis) grown under tropical conditions, showed that 99% of the compound was degraded between 33 and 57 days after application. Also, in chili pepper and cotton, 99% degradation of spiromesifen occurred between 14.5 and 16.8 days by first-order kinetics (Sharma et al., 2007b).

The results obtained in this study suggest that under the agricultural practices used in growing Red Globe table grapes in the state of Sonora, Mexico, residual levels of sp and its enol metabolite pose minimal risk to consumers, since they are below the MRLs established by the EPA. However, it would be interesting to conduct future studies with daily sampling after application of the insecticide and to determine the dissipation of sp and the formation and dissipation of its degradation products such as enol-glucoside, ketohydroxy and monohydroxy metabolites.
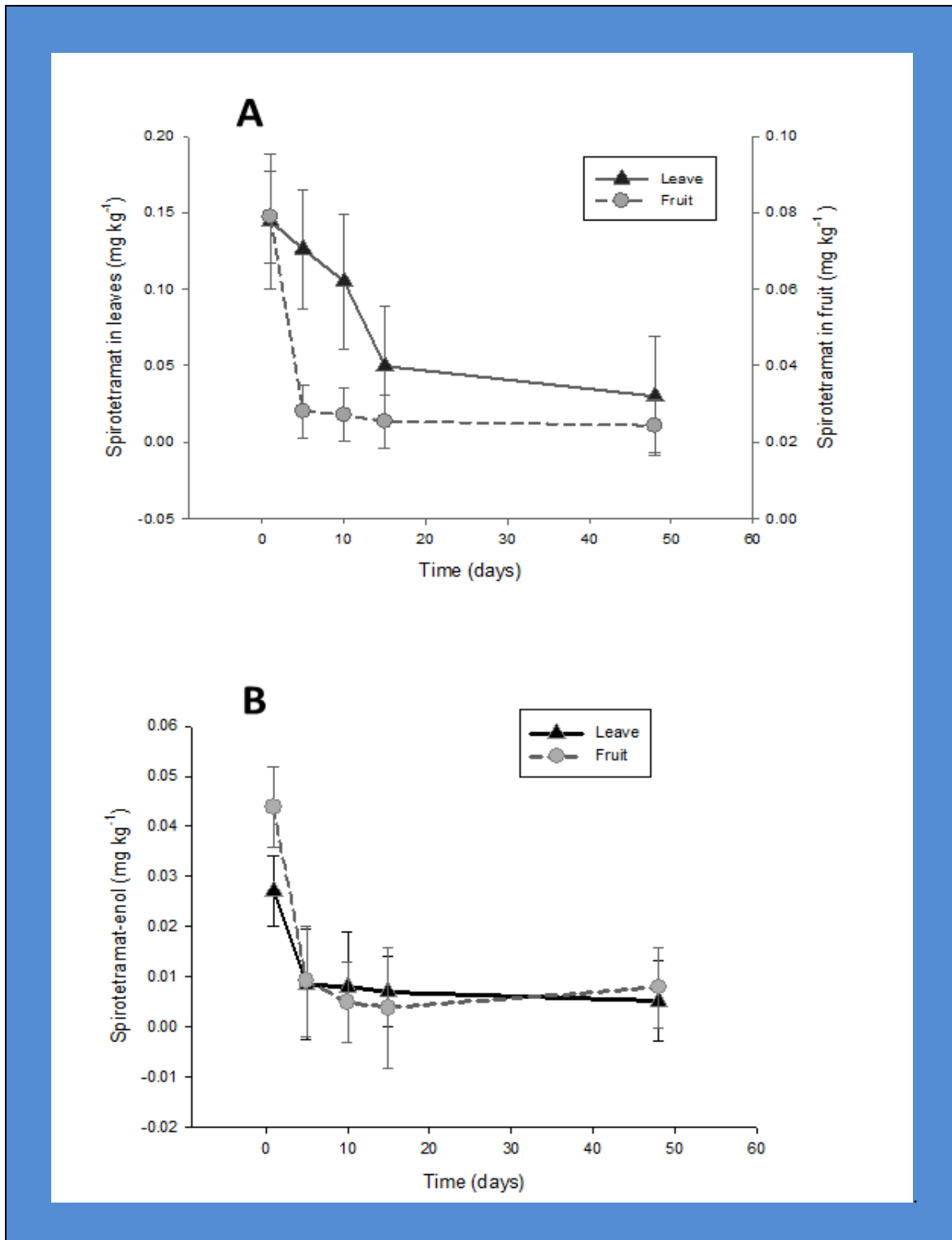
Fig 1: Dissipation of sp (A) and sp-enol (B) in leaves and fruit of grapevine with respect to time

Acknowledgements

4. References

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