Dissipation of glyphosate from grapevine soils in Sonora, Mexico

Disipación de glifosato en suelos de viñedos en Sonora, México

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SUMMARY

Grapevine is one of the important crops in Sonora, due to revenue generation from its export to foreign countries. Among the most widely used herbicides for this crop is glyphosate, which is considered moderately toxic and persistent. The present research evaluates the dissipation of glyphosate in grapevine planted soil at three depths (5, 30 and 60 cm). Sampling was carried out before glyphosate application, and 5, 10, 18, 27, and 65 days after. Glyphosate was extracted from soil samples using ammonium hydroxide. The derivate extracts were partitioned with dichloromethane and analyzed using gas chromatography with pulsed flame photometric detector (PFPD). The results showed that average glyphosate residues are significantly greater at 5 cm (0.09 mg kg^{-1}) than the other depths (30 and 60 cm), having a difference of 0.078 mg kg^{-1} between them ($P < 0.03$). Glyphosate concentration time profiles were similar; it reached maximum soil concentration in a range of 10 to 18 days after application. The half-life of glyphosate in soil has an average of 39 days at all depths. Our data suggests that the release in soil of glyphosate applied to weeds delays its transference to soil by 14 days, and extends residue half life to 55 days after application. These results could be the basis for further research, including more environmental parameters that could affect the dissipation or degradation process in soil.

Index words: dissipation; glyphosate; grapevine; soil.

RESUMEN

La vid para producción de uva de mesa es uno de los cultivos de importancia en Sonora, ya que su exportación a varios mercados mundiales genera divisas. Entre los herbicidas más usados en este cultivo está el glifosato, el cual es considerado moderadamente tóxico y persistente. En la presente investigación se evalúa la disipación de glifosato a tres profundidades (5, 30 y 60 cm) en el suelo de plantaciones de viñedos. El muestreo se llevó a cabo antes de la aplicación de glifosato, y en 5, 10, 18, 27 y 65 días después. El glifosato se extrajo de las muestras de suelo utilizando hidróxido de amonio. Los extractos derivatizados se mezclaron con diclorometano y se analizaron mediante cromatografía de gases con detector fotométrico de flama pulsada (PFPD). Los resultados mostraron que el promedio de los residuos de glifosato fueron significativamente mayores a los 5 cm (0.09 mg kg^{-1}) que a los 30 y 60 cm de profundidad, con una diferencia entre ellos de 0.078 mg kg^{-1} ($P < 0.03$). Los tiempos de disipación en la concentración de glifosato fueron similares; se alcanzó la máxima concentración en el suelo en un rango de 10 a 18 días después de la aplicación. La vida media de glifosato en el suelo es en promedio de 39 días en todas las profundidades. Nuestros datos sugieren que la liberación en el suelo de glifosato aplicado para el control de la maleza retrasa su transferencia al suelo por 14 días, y la vida media de los residuos se extiende después de la aplicación hasta 55 días. Estos resultados podrían ser la base de una amplia investigación, incluyendo algunos parámetros del ambiente que pueden tener efecto en el proceso de disolución y degradación en el suelo.

Palabras clave: disipación; glifosato; vid; suelo.

INTRODUCTION

Grape production in Sonora was 223 600 Mg per year (75.3% of the country grape production) between 2007 and 2011, with an average yearly acreage of

19 105 ha, yielding 11.66 Mg ha^{-1} (INEGI, 2012). Within the state, Hermosillo Valley had an acreage of 10912 ha, and Caborca 7903 ha. In 2010 Mexico exported 171 325 tons of table grapes, ranking seventh in the list of world exporters (FAOSTAT, 2010; INEGI, 2012).

Grape (*Vitis vinífera* L.) is a perennial crop that could last up to 20 years in good standing. In Hermosillo Valley the most common cultivars are Perlette, Flame, Superior (Sugraone) and Red Globe (INIFAP, 2010). Vineyards are planted mostly in yermosol soil. This soil type is salt-free, sandy, with medium texture, low organic matter, and a depth that ranges between 20 and 100 cm (INEGI, 2012). Most vineyards utilize a "Y" trellis system with drip irrigation systems positioned 50 cm above the soil surface (INIFAP, 2010). Irrigation water comes from two aquifers (superior and inferior) proceeding from the Bacoachi and Sonora river basins (Quevedo[1], 2007).

Among other factors, grape production is susceptible to weeds; thus, cultivation practices include herbicide applications to control weeds and reduce their negative effects on plant development and grape quality. Glyphosate is one of the herbicides recommended to control knotgrass (*Convolvulus arvensis* L.), Johnson grass (*Sorghum halepense* L.), and Bermuda grass (*Cynodon dactylon* L.) in Sonora's vineyards (INIFAP, 2010). Glyphosate [N-(phosphonomethyl) glycine, CAS No. 1071-83-6] is an isopropyl amine salt with a molecular weight of 228.18 g mol^{-1}. Classified as an organophosphorus broad-spectrum herbicide, glyphosate has non-selective and systemic foliar action (Prata *et al*., 2003; Gimsing *et al.,* 2007; Sanchís *et al*., 2012).

Several studies focus on the mechanisms of glyphosate leakage, runoff, translocation, or interaction with soil particles, minerals and microorganisms. In general, they agree that glyphosate is fairly stable in the soil, and its degradation depends on the type of soil, where soil structure, pH, organic matter, microorganisms, metals and fertilization are the factors involved. Soil pH is an important factor for glyphosate sorption, correlating negatively with low pH (Helander *et al*., 2012). The high solubility of glyphosate in water (12 000 mg L^{-1}) aids its transportation by drip irrigation water from the planted soil surface to deeper soil layers. Such molecules can be transported to surface and/or ground water, either in solution or in suspension when bound to sediments (Sanchís *et al*., 2012). Leaching and subsurface runoff are important processes with respect to contamination risk in the aquatic environment (ground or drainage water) (Kjaer *et al*., 2005, 2011; Borggaard and Gimsing 2008). Torstensson *et al*. (2005), reported glyphosate residues above 0.1 µg L^{-1} in ground water samples.

To date, there are no reported studies on the impact of glyphosate on Sonora's vineyards soils; therefore, the aim of this research is to determine the behavior of glyphosate residues in grapevine planted soils at three different depths, before application and until grape maturity. The results of this research will aid in evaluating the potential environmental risks involved with the use of glyphosate.

MATERIALS AND METHODS

Experiment Description

The experiment was conducted in a commercial vineyard situated in Costa de Hermosillo District of Sonora (Mexico) located at 29° 00' 53'' N, 111° 29' 16'' W (Datum WGS84) coordinates, and 78 m above sea level. The cultivar used was Red Globe, which was planted in 2006.

The vineyard consisted of 106 plant rows (4 m between rows), each row with 182 plants 1.4 m apart. The vines were grown on a "Y" trellis system.

As stated before, the soils are mostly yermosol with alkaline pH, low organic matter content, and a predominance of sandy loam structure. Rainfall is scarce in this region, occurring mainly in the summer. Average yearly precipitation is 200 mm. Temperatures during the growing season ranged from -3 to 46 °C (INEGI, 2012). All the crops in the district, including vineyards, are irrigated. A drip irrigation system was located at the vineyard. Drip irrigation involves dripping water onto the soil at very low rates from a system of small diameter plastic pipes fitted with outlets called emitters or drippers that are 50 cm above the soil surface. This irrigation system aims to reduce the amount of water used to reach the grapevine roots. Although the amount of water is reduced, it is incessant, and at level surfaces the sandy soil texture favors downward water filtration (Poling, 2007).

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Experimental Design

Five rows from the vineyard were selected randomly, and a plot of three consecutive plants within each row was selected as the experimental unit. A composite soil sample of 1 kg was extracted from 3 holes near each plant at three different soil depths (5, 30 and 60 cm). Soil samples were taken 0 (before glyphosate application), 5, 10, 18, 27 and 65 days after application (from March to June of 2011). The experimental design was completely random with three soil depths as treatments, six repeated measures in time, and five replicates (plots with three grapevine plants each). Soil samples were collected in polyethylene bags, transported to the lab and stored at -20 °C until soil and glyphosate residues analyses.

The soil was analyzed for pH, organic matter and texture. The pH determination was carried out by the AS-02 method of NOM-021-SEMARNAT-2000, which is based on the activity of the H^+ ion in a mixture of soil and water (1:2), measured by a potentiometer (Orio Star 3, Thermo Electron Co, USA). Organic matter was measured by the AS-07 method of NOM-021-SEMARNAT-2000, which is based on measurement of soil organic carbon oxidation by a heated potassium dichromate solution. Texture of the soil was determined by the AS-09 method of the same Mexican Official Norm.

Herbicide Application

Glyphosate commercial grade FAENA FORTE (Bayer Crop Science, Germany) was diluted with water by field workers following the instructions on the label. The solution was sprayed over the weeds with a hand operated backpack sprayer. Application rate was 1.3 L ha^{-1} (equivalent to 702 g active ingredient per ha). During application, the temperature was 23.5 °C, wind velocity 1.7 m s^{-1}, relative humidity 32.4%, foliar humidity was zero percent and solar radiation was 0.362 kW m^2.

Quantification of Glyphosate Residues in Soil

Chemicals. The analytical standard of glyphosate (99.5%) was obtained from Chem Service Inc. (USA). Methanol (99.97%) HPLC grade was from JT Baker (USA). Hydrochloric acid (37.5%),

ammonium hydroxide (30%), phosphoric acid (85%) and methylene chloride (99.9%) were from Fermont (Mexico). Trifluoroacetic anhydride (TFAA) and trifluoroethanol (TFE, 99%) were from Fluka (USA). Anhydrous sodium sulfate (99%) was from Merck (USA) and ethyl acetate (99.9%) was from Burdick & Jackson (USA).

Extraction, cleanup and derivatization. Samples were crushed, dried at room temperature and sieved to 2 mm maximum particle size. The extraction of glyphosate was based on a method by Hu *et al.* (2008). A homogenized soil sample (5 g) was extracted (2X) with 16 mL of 2 *M* NH$_4$OH using a shaker for 40 min (Burrell, USA, Model 71). The samples were centrifuged for 20 min at 3500 rpm and the supernatant was poured into a 50 mL conical vessel. Subsequently, the supernatant was evaporated to dryness at 75 °C with a gentle stream of air using an N-EVAP 112 (model OA-SYS, Organomation Associates Inc., USA). The extract was re-suspended with 5 mL of water:methanol:HCl (160:40:2.7, v/v/v), 20 µL of concentrated phosphoric acid was added and the extract was kept at room temperature for 40 min. The extract was centrifuge for 20 min at 3500 rpm; the supernatant was transferred to a derivatization tube and evaporated to dryness at 80 °C with a gentle stream of air. The tube was cooled to room temperature and, after adding 1 mL of TFAA and 0.5 mL of TFE, it was kept in an ice-bath. The tube was then put on a hot plate (Thermolyne type 16 500 Dry-Bath, USA) at 100 °C for one hour. In this method of derivatization, the functional groups of carboxylic and phosphoric acid are derivatized to form the corresponding trifluoroethyl ester, while the amino functional group is derivatized to form the corresponding trifluoroacetyl (Hu *et al.*, 2008). The excess reagents were removed by a gentle stream of air at 40 °C. The extract was re-suspended with 6 mL of distilled water and transferred to another vessel. This process was repeated twice; 20 mL of methylene chloride was added to a tube and shaken for 2 min, the methylene chloride layer was collected into a 50 mL tube. The aqueous layer was extracted (2X) with 20 mL of methylene chloride. The extract was dried over a bed of anhydrous sodium sulfate (2.5 g) and evaporated to dryness with air at 40 °C. The final extract was dissolved with 1 mL of ethyl acetate, filtered using a 0.2 µm, 13 mm syringe filter unit (Pall, USA) and analyzed by gas chromatography.

Gas chromatography conditions. A Varian CP 3800 gas chromatograph (Varian, USA) equipped with a capillary column DB-5 (30 m × 0.25 mm ID, 0.25 μm-film thickness, J & W Scientific, USA) and pulsed flame photometric detector (PFPD, phosphorus mode) was used. The chromatographic conditions used for the analysis of glyphosate residues were as follows: detector temperature 310 °C; injector temperature 220 °C; initial oven temperature was 85 °C (1 min hold), increased 20 °C min^{-1} to 150 °C, ramped at 20 °C min^{-1} to 200 °C (2 min hold), followed by a final ramp of 20 °C min^{-1} to 250 °C (4 min hold). The total run time was 18.5 minutes, and injection volume was 2 μL. Nitrogen (N$_2$) was used as the carrier gas, maintained at a constant flow rate of 1.1 mL min^{-1}, which resulted in a retention time of 6.3 min for glyphosate. Data acquisition and integration was controlled by Galaxy Chromatography Data System (Varian, USA).

In order to establish method linearity the following standards were used: 20, 50, 100, 200, 300, 500 and 1000 μg L^{-1}. Concentration *vs* response plots were obtained and their correlation coefficient was calculated. All injections were done in triplicate.

Percentage recovery of glyphosate in soil samples. The effectiveness of the analytical process was measured with percent recovery (% Rec) using spiked control samples at three different fortification levels (0.02, 0.2 and 0.6 mg kg^{-1}) with three replicates. Soil samples (5 g) were fortified and subsequently analyzed according to the previously described extraction method. The acceptable recovery percentage was stated in the range of 70-120%, with a variation coefficient lower than 15% (FDA, 2012).

Statistical Analysis

Data for soil properties were analyzed by ANOVA with three depths (5, 30 and 60 cm) and five replications. Depth means were compared by orthogonal contrasts ($P < 0.05$).

Glyphosate residues were analyzed as a repeated measures model with the following model: $Y = \mu + Depth_i + Rep\ (Depth)_{k(i)} + Day_j + Depth{\times}Day_{ij} + \varepsilon_{ijk}$. Mean comparisons and confidence intervals were calculated at $P < 0.05$.

Since dissipation must be modeled starting from the highest concentration, simple regression models to fit glyphosate dissipation were analyzed for each depth. From these model results, glyphosate half-life and its 95% confidence limit were estimated.

RESULTS AND DISCUSSION

The peak associated with glyphosate was identified at the retention time of 6.3 min, showing good resolution with no interference related to the soil matrix (Figure 1). Linearity was achieved over the range of 20 to 1000 μg L^{-1} with a coefficient of determination (R^2) of 0.99 and a coefficient of variation of 2.71%. The detection limit of the method was 5.45 μg L^{-1} and the limit of quantitation was 20 μg L^{-1}. The percent recovery obtained from fortified soil samples at 0.02, 0.2 and 0.6 mg kg^{-1} were 86 ± 9, 103 ± 6 and 93 ± 3, respectively. The average pH of the soil samples was 8.8 ± 0.8, and there were no significant differences among the three soil depths or sampling times ($P < 0.05$). Mean content of organic matter was slightly higher at 5 cm depth (1.16 ± 0.35) than at 30 and 60 cm (0.78 ± 0.26 and 0.69 ± 0.19), respectively. Soil texture was determined as type I at the three depths, with sandy soil (75%), low silt (14%) and low clay (11%).
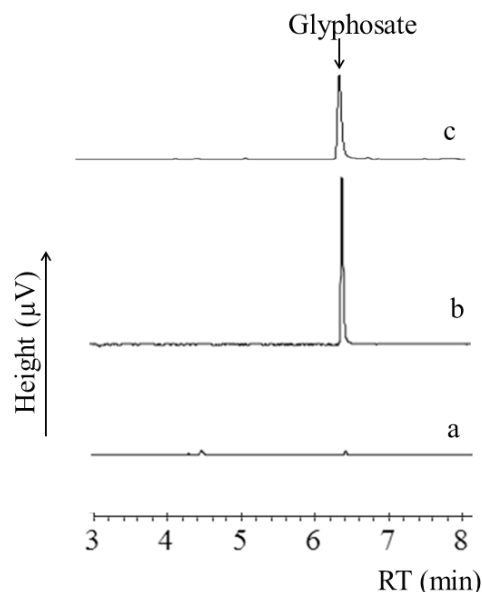
Figure 1. GC-PFPD chromatogram of glyphosate: (a) Control soil, (b) Fortified soil at 0.2 mg kg^{-1}, (c) Treated soil sample after 27 days with 0.12 mg kg^{-1} of glyphosate.

Soil Glyphosate Quantification

The recoveries of glyphosate obtained in fortified soil samples are similar to those reported by Hu *et al.* (2008), with recoveries of 84-94%. The average pH of the soil samples were not significantly different among soil depths and was between alkaline to strong alkaline pH range for a soil, according to the NOM-021-SEMARNAT-2000 classification. Organic matter content was low according to the classification of the same official norm, this result agrees with the values reported by Padilla *et al.* (2006) who reported low organic matter content, in the range of 0.6 to 1.5%, in the soil of Costa de Hermosillo. The predominant soil texture was type I at the three depths.

Significant differences ($P < 0.03$) in average residual glyphosate concentration were found between 5 cm (0.092 mg kg^{-1}) and the deeper soil layers (30 and 60) 0.021 and 0.009 mg kg^{-1}, respectively. Eighteen days after herbicide application, residues increased from 0.017 mg kg^{-1} to 0.195 mg kg^{-1} (5 cm depth), representing a difference of 11.5 times the initial concentration (before application). A similar time concentration pattern was observed at 30 and 60 cm; however, at 30 cm the peak (0.058 mg kg^{-1}) was reached 10 days after application, while at 60 cm the peak occurred 18 days after application and reached only 0.020 mg kg^{-1} (Figure 2).

From the peak concentration at 18 days, the glyphosate dissipation in the soil surface (5 cm) follows a linear relationship, which was non-significant ($P > 0.05$) with R^2 of 0.82 and an estimated half-life of 45 days. At 30 cm the half-life was 34 days, starting from 10 days; at 60 cm depth the peak concentration was at 18 days and the half-life was 38 days. Summarizing, average half-life from application was 55 days.

The results on the soil residual glyphosate levels are similar to those observed by Locke *et al.* (2008) and Doublet *et al.* (2009). They mentioned that once glyphosate is adsorbed by the weeds, it is released again into the soil depending on the speed of decomposition of the plant tissue. Moreover, glyphosate residues remain partly in weed tissue until the weed dies and breaks down. The release of glyphosate causes an increase of the residual concentration in soil. According to Devlin *et al.* (1986), weeds treated with glyphosate die at least 10 days after the herbicide was applied. On the other hand, Laitinen *et al.* (2007) demonstrated that a part of the herbicide applied on the weeds is driven toward their roots and is able to reach the soil later.

The low concentration detected at 60 cm depth, relative to the higher concentration at the soil surface, suggests that glyphosate is relatively immobile due to the phenomenon of adsorption. However, its mobility is not discarded since glyphosate is a hydrophilic compound, and can be transported by the irrigation
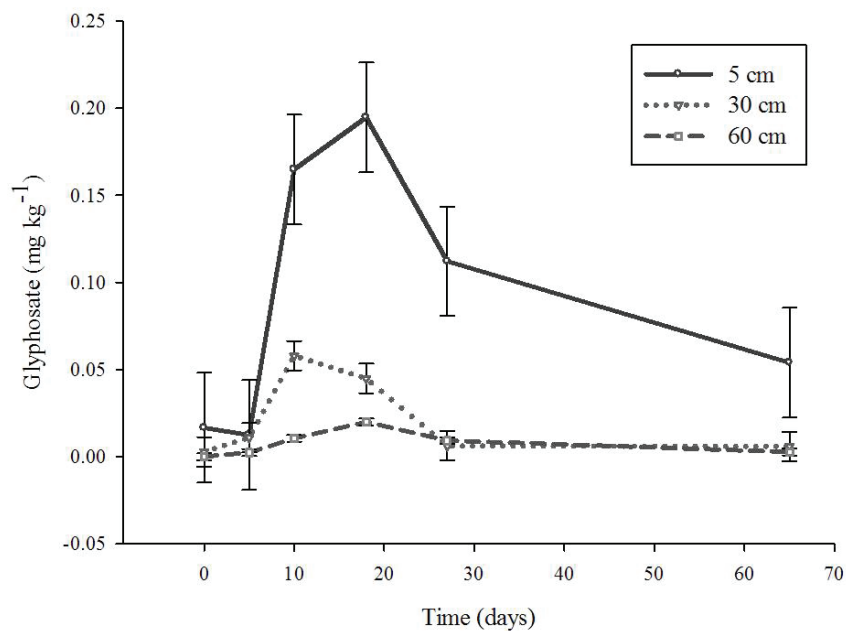
Figure 2. Dissipation rate of glyphosate in soil at different depths (5, 30 and 60 cm).
Points represent the average and lines the standard error.

water to deeper soil layers (Sanchís *et al.*, 2012). Following the application of glyphosate, the dissipation process happens in similar patterns at the three soil depths (Figure 2).

Glyphosate dissipation in the soil surface had a half-life of 55 days after application. This data agrees with Accinelli *et al.* (2005) and Sanchís *et al.* (2012), who found half-lives of 7 to 60 days and 49 days, respectively. However, assuming the herbicide application was similar to that applied in our study, the persistence of the herbicide after one year of the last application was 0.017 mg kg^{-1} on the surface, suggesting that a very low amount of glyphosate remains in the soil.

At depths of 30 and 60 cm, the data showed a rapid reduction in the concentration at the beginning, remaining at very low levels for the remainder of the experiment. This behavior can be attributed to several processes: the herbicide is being captured by the grapevine roots, or it has formed complexes with other minerals such as Al^{+3} and Fe^{+3}, making glyphosate less susceptible to removal (Schuette, 1998; Borggaard *et al.*, 2008).

At present, there is no soil maximum residue level (MRL) for glyphosate; however, all the residue levels found in the present study are below the MRL for grapevines and table grapes according to the European Union Pesticide Database (0.2 mg kg^{-1}, and 0.5 mg kg^{-1}, respectively).

According to Gomes *et al.* (2014), once in the soil, glyphosate may be adsorbed onto soil particles, degraded by microbes, or transferred to deeper soil horizons, migrating via soil pores or root canals. However, some agricultural practices, such as phosphorous amendment, may re-solubilize glyphosate in soils, making it available for leaching and transport to the rhizosphere of non-target plants. Glyphosate is quickly degraded to aminomethylphosphonic acid (AMPA) in soils by microorganisms; a similar mechanism of glyphosate degradation has been proposed in plants. AMPA is a phytotoxin, which amplifies the indirect effects of glyphosate on physiological processes. Because of its chemical similarity, AMPA can compete with glycine in biological sites and pathways, affecting chlorophyll biosynthesis and, consequently, the photosynthetic process.

CONCLUSIONS

This is the first study that was designed to evaluate the dissipation of glyphosate in soil from a commercial vineyard in Sonora. Our data suggests that glyphosate applied to weeds is subsequently released in the soil. This could delay its concentration and extend its half-life in soil. Once there, in sandy soils, irrigation water transports glyphosate to deeper soil layers, although its mobility seemed low during the final stages of the study. There are no clear explanations of the undergoing dissipation processes. The present research provides a basis for further studies, which should include more detailed measurements on dissipation and degradation processes in soil.

ACKNOWLEDGMENTS

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