Simazine Degradation Rates in Orchard and Vineyard Soils with Varying Simazine Use Histories

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Introduction

Due to its relatively low cost and long residual activity, simazine is a commonly used herbicide in California vineyards and orchards. Simazine is applied as a preemergent herbicide, controlling broadleaf and some grass weeds as they emerge. Weed seeds can germinate throughout a growing season, so extended herbicidal activity is desired in order to minimize the number of applications. The average simazine half-life in the field is 60 days (WSSA Herbicide Handbook, 2002). Microbial populations that are able to metabolize a chemical and use it as an energy or nutrient source can increase with repeated applications of a herbicide or related herbicides. The process of enhanced biodegradation can result in reduced residual weed control (Arbeli and Fuente, 2007). Several microorganisms, including both bacteria and fungi, with the ability to metabolize simazine have been identified (Santiago-Mora et al, 2005). It is important for Central Valley and Ventura County growers to know if simazine is subject to enhanced biodegradation as weed control strategies in several important perennial cropping systems may be affected.

Objective

To determine if enhanced biodegradation of simazine occurs in Central Valley and Ventura County soils due to repeated simazine applications.

Materials and Methods

Soil Collection

Vineyard and orchard soils were collected in fall of 2007 and stored in five gallon containers. The vineyard soils were collected from established (>15 yr) vineyards near Parlier, CA. One vineyard has been treated annually with simazine (adapted) and is located at the University of California Kearney Agricultural Center, and one had no recent simazine applications (non-adapted) and is located at the USDA ARS San Joaquin Valley Agricultural Sciences Center. The two sites are located within one mile of each other and both have Hanford sandy loam soil. The orchard soils were collected from nine citrus and avocado orchards in Tulare and Ventura counties. The simazine use history for each orchard soil is included in Table 1.

Simazine Degradation Experiment

Four lab experiments were conducted to determine simazine degradation rates, two for each set of soils. The experiments were set up in a completely randomized design with three replications. In each experiment, half of each soil was triple autoclaved to eliminate existing microbial populations. The other half was left unsterilized. Autoclaved (sterile) or non-autoclaved (live) soil (100 g) was added to Wheaton jars with Teflon-lined lids and mixed with either 15 mL water or 15 mL of 66.67 µg/mL simazine in water for a final simazine concentration of 10 µg per g soil (w/w). Additional water was added to some soils at the time of mixing to achieve more uniform moisture levels between the sterile and live soils. Simazine was extracted from soil using a water-based process described by Shaner et al (2007) and extraction efficiency was 68-71% in preliminary experiments. Samples were taken 0, 1, 3, 7, 14, 21, and 35 days after treatment (DAT) for the vineyard soils and 0, 1, 2, 3, 7, 14, 21, 28 and 35 DAT for the orchard soils. At each time point, five g moist soil and five g water were placed in a 50 mL centrifuge tube and mixed on a reciprocating shaker for one hour. The samples were then centrifuged at 2000 x g for 20 minutes. One-half to one mL aliquots of the supernatant were transferred to Microfree eppendorf tubes with 0.22 µM Teflon filter inserts and centrifuged at 10,000 x g for 10 minutes. The filtrate was transferred to 1.5 mL glass vials and analyzed using a high-performance liquid chromatography (HPLC) system equipped with a multiple wavelength detector and a C18 column. The mobile phase was 35% acetonitrile:65% water:0.05% phosphoric acid run isocratically at 40^{o}C at a flow rate of 1 mL/min. The injection volume was 100 µL. Simazine was detected at 223 nm and the retention time under these conditions was between 5.5 and 6.5 minutes. To determine the simazine concentration, the HPLC data were fitted to a linear equation developed from the analysis of known concentrations of simazine at each time point. The data were standardized to µg/g soil on a dry weight basis for statistical analyses.

Simazine Mineralization Experiment

Separate experiments were run to assess the mineralization rate of simazine in vineyard and orchard soils. In biometer flasks, 25 g soil (dry weight equivalent) was mixed with ring-labeled ^{14}C-simazine for an initial concentration of 4 µg/g and radioactivity of 190 Bq/g. Evolved $^{14}C\text{-}CO_2$ was collected in sodium hydroxide traps and quantified by liquid scintillation spectroscopy (LSS). The sodium hydroxide solution in the traps was replaced after each sampling. Samples were taken 0, 2, 7, 11, 14, 18, 22, 25, 28, 32, and 35 DAT. At the conclusion of the experiment, the soil was destructively sampled and the remaining $^{14}C\text{-}CO_2$ was obtained using a combustion method and added to the cumulative evolved $^{14}C\text{-}CO_2$ for mass balance determination.

Analysis

Simazine degradation data (% of applied) were fitted to a logistic, 4-parameter model [$y=y_0+(a/(1+(x/x_0)b))$] to estimate the simazine half-life (x_0) and the slope (b) at the half-life. The max and min values of the model were constrained to 100 and <1, respectively (data not shown). Mineralization data were fitted to a Gompertz, 3-parameter model [$y=a*\exp(-\exp(-x-x_0/b))$] to estimate the maximum % CO_2 evolved (a) and the lag phase (x_0). Graphs and parameter estimates were generated using SigmaPlot 11.0 (2008).

References

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Results and Discussion

Simazine Degradation Rates

In the vineyard soil experiment, reduction in simazine concentration was clearly due primarily to biotic degradation (Figure 1). Simazine concentration was reduced by only 20-30% in the sterile soils while nearly 100% of the simazine in the live soils degraded over the course of the experiment. In addition, the vineyard experiment indicated that simazine degradation can be strongly affected by simazine use history. Simazine had a half-life of 5.3 days in the adapted soil compared to 26.6 days in the non-adapted soil, a five-fold difference. The vineyard soils represent two extremes in simazine use history, while the orchard soils represent a more comprehensive range. The simazine half-life data for all of the orchard soils are included in Table 1. The simazine half-life in the soil with no recent simazine use (soil A) was 30.3 days, 2.2-fold greater than the next closest half-life of 13.6 days (soil D). However, in soils B-I, simazine degradation was only loosely correlated to simazine use history (Figure 2), suggesting that the microbial population responsible for enhanced degradation can persist even when simazine applications are discontinued. Microbial population dynamics and differences in soil characteristics, such as pH or moisture, can influence microbial activity and may also contribute to the relatively poor correlation between degradation rate and simazine exposure.

Simazine Mineralization

The results from the simazine mineralization experiments indicate that reduction of simazine concentration over time in these assays was due to microbial activity. In the vineyard soils, simazine mineralization was strongly affected by simazine use history (Figure 3). The lag phase for simazine mineralization in the adapted soil was 3.8 days compared to 16.1 days in the non-adapted soil, a 4.2-fold difference. This suggests that high populations of the simazine degrading microbes were present in the adapted soil. In the orchard soils, the longest lag phase was 35.1 days in soil A (no recent simazine use). This was 4.1 times greater than the next longest lag phase of 8.5 days (soil D). The shortest lag phase was 3.6 days in soil F. The soils with the shortest and longest lag phase correspond to the soils with the shortest and longest half-life in the simazine degradation experiment, indicating the soils with the most active (soil F) and least active (soil A) microbial simazine degradation. The lag phase and maximum % CO_2 evolved data for all orchard soils are included in Table 2 and graphically represented in Figure 4.

Soil	Simazine Use History	Half-life±SE* (d)	Slope±SE	r^2
A	no use for at least 15 yrs	30.3±19.9	17.8	0.8749
B	annual for 42 yrs, break for 7 yrs	10.7±0.6	4.8±0.8	0.9547
C	annual for 15 yrs, break for 4 yrs	9.6±1.5	9.0±4.3	0.9090
D	annual for 35 yrs, break for 14 yrs, 1 yr use	13.6±10.3	17.3	0.8930
E	annual for 42 yrs, break for 7 yrs, 1 yr use	6.5±0.3	4.9±1.8	0.9658
F	annual for 42 yrs, break for 7 yrs, 2 yr use	3.1	42.3	0.9590
G	annual for 5 yrs	5.1±0.1	3.5±0.2	0.9910
H	annual for 13 yrs	10.4±0.5	2.6±0.3	0.9743
I	annual for 15 yrs	13.2	35.9	0.9374

*Standard error is not included in the table when greater than the estimate by at least one order of magnitude. High standard errors were due to rapid simazine degradation between sampling time points.

Table 1. Soils were collected in Tulare and Ventura Counties in 2007 from nine citrus and avocado orchards. Simazine degradation rates were assessed for each soil and the half-life and slope at the half-life determined using a logistic, 4-parameter model.

Soil	Simazine Use History	Max % Mineralization±SE	Lag Phase±SE (d)
A	no use for at least 15 yrs	84.7±10.5	35.1±1.9
B	annual for 42 yrs, break for 7 yrs	76.4±0.4	8.5±0.1
C	annual use for 15 yrs, break for 4 yrs	74.1±1.0	5.0±0.3
D	annual for 35 yrs, break for 14 yrs, 1 yr use	76.7±0.6	6.7±0.2
E	annual for 42 yrs, break for 7 yrs, 1 yr use	71.5±0.8	6.4±0.2
F	annual for 42 yrs, break for 7 yrs, 2 yr use	79.3±0.5	3.6±0.1
G	annual for 5 yrs	67.1±0.7	4.5±0.2
H	annual for 13 yrs	60.1±0.5	7.2±0.1
I	annual for 15 yrs	79.6±0.5	6.9±0.1

Table 2. Soils were collected in Tulare and Ventura Counties in 2007 from nine citrus and avocado orchards. Each soil was treated with ^{14}C-simazine and sampled at regular time points to determine cumulative % $^{14}C\text{-}CO_2$ evolved. Data were fitted to a Gompertz, 3-parameter curve to estimate the maximum % CO_2 evolved and the lag phase.

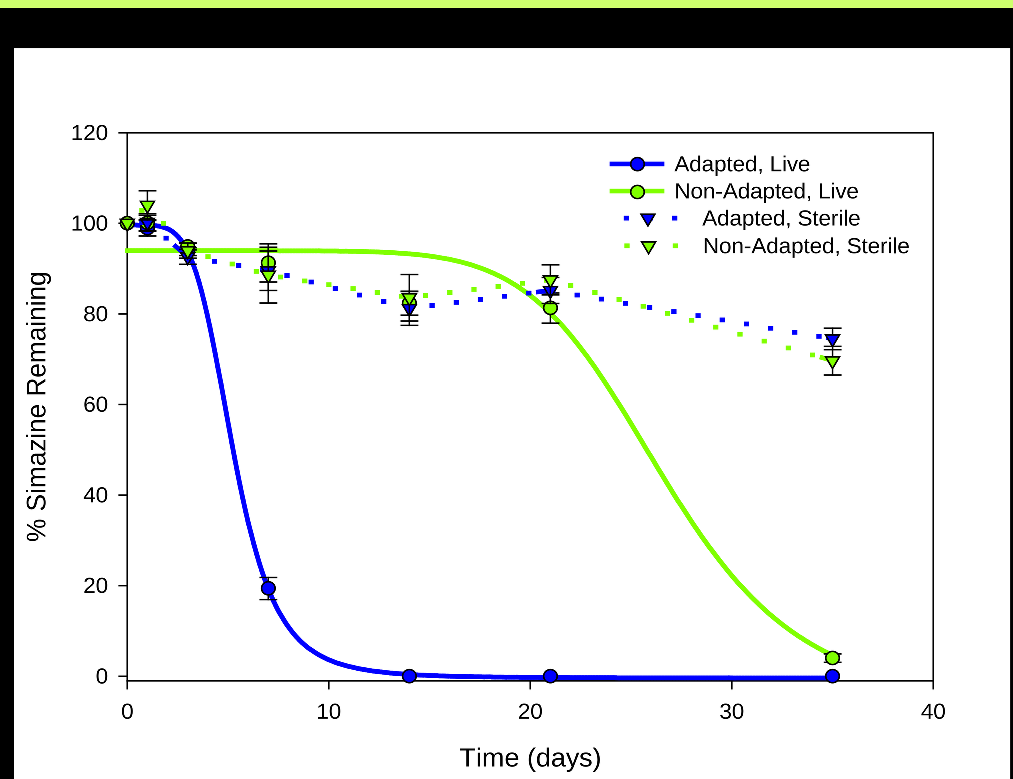

Figure 1. Simazine degradation in two vineyard soils with annual simazine use (adapted) and no simazine use (non-adapted) histories. Data were fitted to a logistic, 4-parameter model. The half-life and r^2 are 5.3 d and 0.9965, respectively, for the adapted and 26.6 d and 0.9388 for the non-adapted, live soils. Due to limited degradation during the experiment, a regression curve was not fitted to data for the sterile treatments. Error bars around the mean values indicate +/- one standard error.

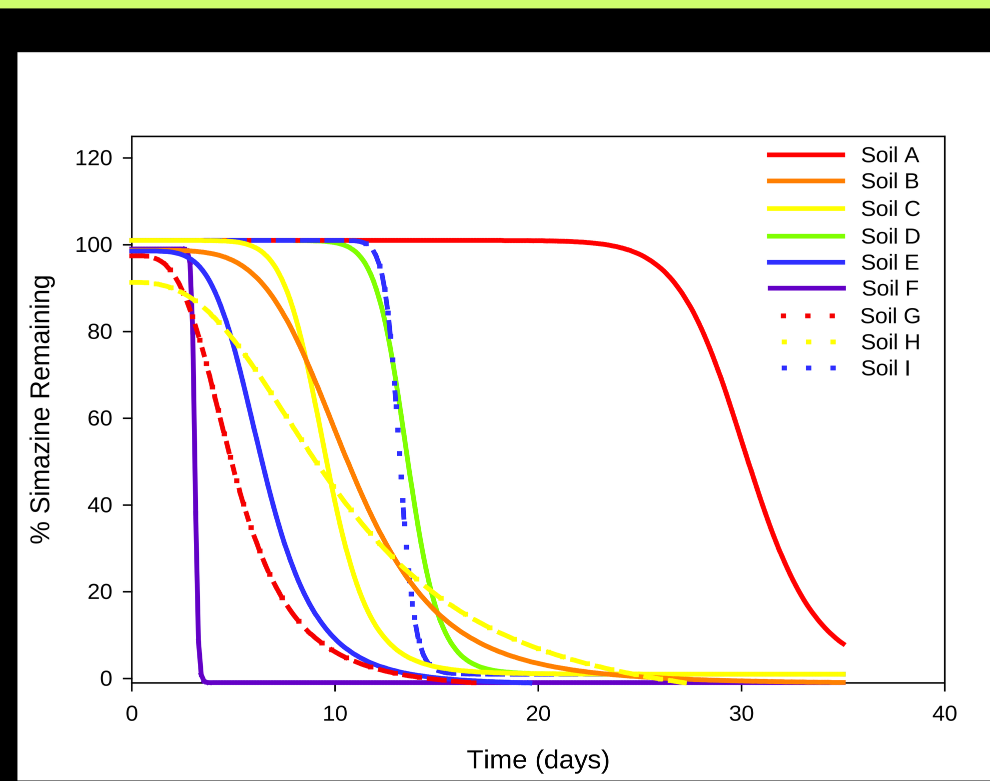

Figure 2. Simazine degradation was evaluated in nine citrus orchard soils with varying simazine use histories. Data were fitted to a logistic, 4-parameter model. The half-life, slope and r^2 for each soil are included in Table 1.

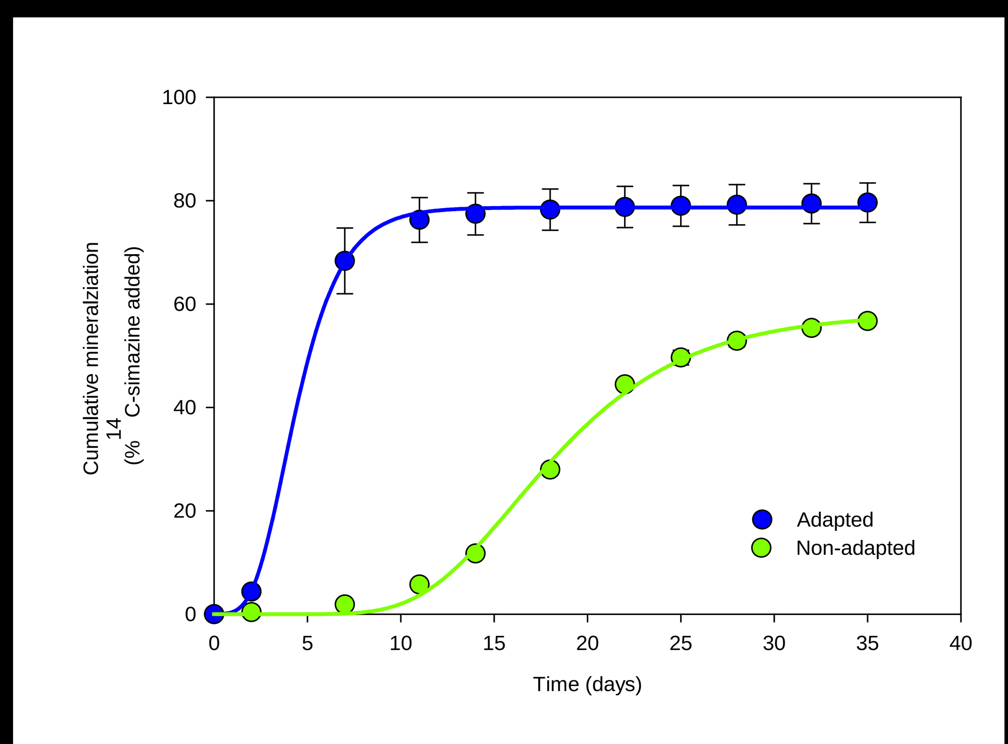

Figure 3. Cumulative mineralization (%) of ^{14}C-simazine was determined for two vineyard soils with annual simazine use (adapted) and no simazine use (non-adapted) histories. Mean data were fitted to a Gompertz, 3-parameter model. ^{14}C-simazine was mineralized more rapidly in the adapted soil. The lag phase and maximum % mineralization were 3.8 d and 78.7%, respectively, for the adapted soil and 16.1 d and 58.3% for the non-adapted soil.

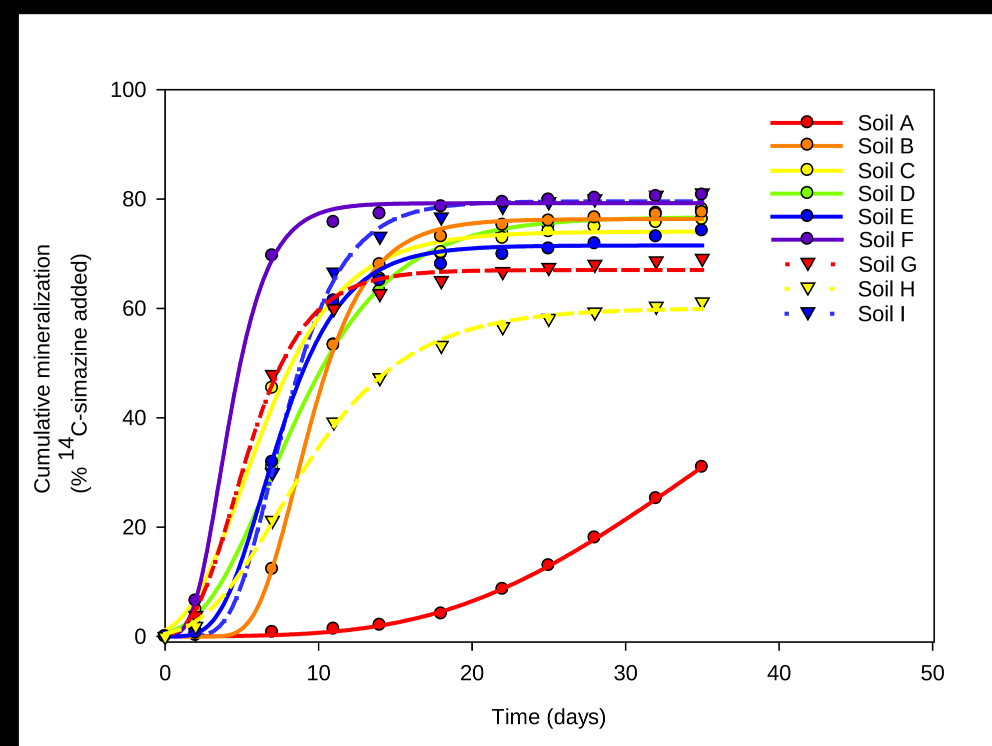

Figure 4. Cumulative mineralization (%) of ^{14}C-simazine was determined for nine orchard soils with varying simazine use histories. Mean data were fitted to a Gompertz, 3-parameter model. The lag phase and maximum % mineralization are included in Table 2.

Conclusion

Data from these experiments indicate that enhanced biodegradation of simazine does occur in California vineyards and citrus orchards and that microbial populations with the ability to degrade simazine are still present in soil after breaks in simazine treatments. Additional research is ongoing to evaluate the effect of enhanced biodegradation on simazine efficacy in field and greenhouse trials.

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