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Assessing Simazine Degradation Patterns in California Citrus Orchards with Different Simazine Use Histories

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Abstract: Simazine is commonly used to control broadleaf weeds and annual grasses in perennial tree and vine crops because of its relatively low cost and long residual activity. Simazine may be subject to enhanced biodegradation in some areas which can result in decreased herbicide persistence and reduced residual weed control. Laboratory studies were conducted to determine if rapid simazine degradation occurs in California citrus orchards and if degradation rates are correlated with simazine use history. In the Central Valley, simazine degradation curves indicate that simazine degradation rate is more rapid in soils with a simazine use history (adapted) compared to soils with no recent use (non-adapted). In these soils, simazine dissipation was two- to three-fold faster in adapted compared with the non-adapted soils. However, in southern California, simazine dissipation and mineralization were not substantially different among soils with different simazine use histories. Repeated simazine use in California orchards can lead to the development of enhanced microbial degradation of the herbicide. However, soil type and long-term cropping factors can affect persistence and distribution of herbicide-degrading microbial populations in California orchards.

Keywords: enhanced degradation, simazine, *s*-triazine herbicides, herbicide use history

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Introduction

Simazine is commonly applied as a preemergence herbicide during fall and winter in California orchards and vineyards. Simazine controls broadleaf weeds and annual grasses including several problem species such as horseweed [*Conyza canadensis* (L.) Cronq.], hairy fleabane [*Conyza bonariensis* (L.) Cronq.], and annual bluegrass (*Poa annua* L.) in citrus orchards. In 2009, growers applied an average rate of 2.5 kg ai ha^{-1} of simazine on 25% of citrus orchards in California.[1,2] Simazine has moderate residual activity with an average field half-life of 60 days, but persistence increases with low organic matter content and high pH soils.[3–5] Persistence of simazine in soil is particularly important because it determines the duration of weed control. However, growers and extension agents have periodically reported unsatisfactory residual weed control in orchards with an extensive simazine use history.

Enhanced degradation is a phenomenon whereby a soil-applied pesticide is rapidly degraded by a population of microorganisms that has developed the ability to use the pesticide as a carbon, energy, or nutrient source because of previous exposure to the pesticide or an analog. This enhanced biodegradation can greatly decrease the half-life of the herbicide and result in reduced residual weed control efficacy. Repeated applications of simazine under laboratory and field conditions have been associated with increased rates of microbial degradation in soil. In 2000, Rouchaud et al[6] reported an accelerated (by a factor of 1.3) rate of simazine degradation in plots treated with consecutive annual applications compared to plots treated for the first time. Krutz et al[7] reported that soils exhibiting enhanced atrazine degradation also rapidly degraded simazine, a phenomenon referred to as cross-adaptation. Further, simazine degradation potential acquired by repeated herbicide treatment may also confer to bacterial communities a higher resilience to the impact of the herbicide.[8]

Reports indicate that enhanced *s*-triazine degradation is positively correlated with *s*-triazine exposure history.[9–11] Repeated use of simazine in California citrus orchards, therefore, could select for microbial populations with the ability to rapidly degrade the herbicide and contribute to the reported weed control failures. This project was initiated to determine if (1) rapid simazine degradation occurs in California orchards and (2) degradation rates are correlated with simazine use history.

Materials and Methods

Soil collection

Twenty-seven orchards in the Central Valley (Tulare or Fresno Counties) and Ventura County with different simazine use histories were identified and soil samples were collected. The targeted simazine use histories included: (1) annual simazine use, (2) recent simazine use only, (3) a short break since the last simazine application, and (4) no recent simazine use. In this context, annual use was defined as at least 15 consecutive years of at least one simazine application; recent use only means less than 7 years of annual use; short break since last means 2–5 years since last simazine use, and no recent use means at least 15 years since the last simazine application. Soil was collected in October 2006 from the top 15 cm within the tree row in each orchard, placed in a plastic bag, and stored in a sealed 20-L plastic container until further analysis. Based on herbicide use information from the growers nine of the 27 sites were classified as annual use, five as short recent use only, seven as short break since last use, and six as no recent use (Table 1).

Rapid laboratory dissipation assay

One hundred g subsamples of each soil were weighed into wide-mouth 250-mL Wheaton jars with Teflon-lined lids. Soil was treated with 15 mL of 66.67 μg mL^{-1} formulated simazine (Princep 4L, Syngenta Crop Protection, Greensboro, NC) in water and manually homogenized to achieve a nominal simazine concentration of 10 μg g^{-1} soil, a level that approximates initial simazine residues following applications in California orchards. Samples were taken at 0, 1, 2, 3, 4, 6, 9, 14, 21, 28, and 35 d after treatment (DAT), and simazine was extracted using a water-based procedure described by Shaner et al.[12] At each sampling time, soil samples were physically stirred and a 5 g subsample of moist soil was weighed into a 50 mL centrifuge tube. An equivalent amount (w/w) of distilled water was added to each tube and samples were mixed on a reciprocating shaker for 1 hour then centrifuged at 2000 × g for 20 min at 20 °C. One-half to 1-mL aliquots of the supernatant were transferred to microfuge tubes (Millipore Corporation, Bedford, MA) with 0.22-μM

Table 1. Location, simazine use history, and soil classification of 27 California citrus orchard soils collected in 2006.

Soil sample	County	City	Simazine use history	Simazine use classification	Soil series
1	Fresno	Orange Cove	Annual use until 1990, none since	No recent use	Cometa loam
2	Fresno	Orange Cove	Annual use until 1990, none since	No recent use	Cometa loam
3	Tulare	Dinuba	Annual use since 1963	Annual use	San Joaquin loam
4	Tulare	Dinuba	Annual use since 1963, trees were pulled out in 2004	Annual use	San Joaquin loam
5	Tulare	Ivanhoe	Annual use from 1955 to 1990; no use from 1990 to 2004; simazine use in 2005	Short break since last use	San Joaquin loam
6	Tulare	Ivanhoe	No simazine use in the past 15 years	No recent use	San Joaquin loam
7	Tulare	Ivanhoe	No simazine use in the past 15 years	No recent use	San Joaquin loam
8	Tulare	Ivanhoe	No simazine use in the past 15 years	No recent use	San Joaquin loam
9	Tulare	Lindsay	No simazine use since 2002	Recent use only	Exeter loam
10	Tulare	Lindsay	Annual use for at least 15 years	Annual use	Exeter loam
11	Tulare	Exeter	No simazine use since 2002; annual use for at least 15 years prior; simazine in 2005	Short break since last use	Exeter loam
12	Tulare	Exeter	No simazine use since 2002; annual use for at least 15 years prior; simazine in 2005	Short break since last use	Exeter loam
13	Tulare	Exeter	No simazine use since 2002; annual use for at least 15 years prior; simazine in 2005	Short break since last use	Flamen loam
14	Tulare	Dinuba	Annual use for at least 20 years	Annual use	San Joaquin loam
15	Tulare	Dinuba	Annual use for at least 20 years	Annual use	San Joaquin loam
16	Ventura	Santa Paula	Annual use for the last 6 years	Recent use only	Garretson loam
17	Ventura	Santa Paula	Annual use since 2005; no simazine use prior	Recent use only	Garretson loam
18	Ventura	Santa Paula	Annual use for the last 2 years; no simazine use prior	Recent use only	Sorrento loam
19	Ventura	Fillmore	Annual use from 1956 to 1998; no use from 1998 to 2004; simazine use in 2005	Short break since last use	Mocho loam
20	Ventura	Fillmore	Annual use from 1956 to 1990; none since 1991	No recent use	Mocho loam
21	Ventura	Fillmore	Annual use from 1956 to 1998; no use from 1998 to 2005; simazine use in 2006	Short break since last use	Mocho loam
22	Ventura	Somis	Annual use since 1960's; last application 2005	Annual use	Mocho loam
23	Ventura	Somis	Annual use since 1960's; last application 2003	Annual use	Mocho loam
24	Ventura	Somis	Annual use since 2001; none before 2001; last application 2005	Recent use only	Mocho loam
25	Ventura	Saticoy	Annual use for at least 20 years	Annual use	Pico loam
26	Ventura	Ojai	Annual use for at least 13 years	Annual use	Sorrento loam
27	Ventura	Moorpark	Annual use for the last 2 years; no use prior	Recent use only	Sorrento loam

Teflon filter inserts and centrifuged at 10,000 × g for 10 min. The filtrates were analyzed using a high-performance liquid chromatograph (HPLC) (Agilent Technologies, Wilmington DE) equipped with a multiple wavelength detector and a 4.6- by 250-mm C18 column (Agilent Technologies, Wilmington DE). The mobile phase was acetonitrile:water:phosphoric acid (35:65:0.05 vol/vol/vol) and was run isocratically at 1 mL min^{-1} at 40 °C. The injection volume was 100 µL, and simazine was detected at 223 nm. A series of simazine standards were included with each sample run to determine herbicide concentration and retention. Simazine retention time under these conditions was between 5.5 and 6.5 min and extraction efficiency in these soils was 69.5% ± 1.5% in preliminary experiments.

Microbial activity determination

From the initial 27 orchards, eight soils (one of each simazine use class from the two regions) were selected for more detailed simazine dissipation analyses. Soil samples 10 and 26 (annual simazine use), 5 and 24 (recent simazine use only), 12 and 20 (short break since last), and 6 and 21 (no simazine use) were selected for this study (Tables 1 and 2). Approximately 20 kg soil (dry weight equivalent) from each sample was divided into two; half was triple autoclaved to eliminate existing microbial populations and the other half was left unsterilized. Autoclaved (sterile) or non-autoclaved (live) soil was passed through a 4-mm sieve and soil moisture was measured. Three 100 g replicate subsamples of each soil were weighed into wide-mouth 250-mL Wheaton jars with Teflon-lined lids. Soil was treated with either 15 mL water or 15 mL of 66.67 µg mL^{-1} simazine in water and manually homogenized resulting in a nominal final simazine concentration of 10 µg g^{-1} soil (w/w). Samples were taken at 0, 1, 2, 3, 7, 14, 21, 28, and 35 d after treatment (DAT), and simazine was extracted using the previously described water-based extraction procedure. Data were analyzed as a completely randomized experimental design with three replications and the experiment was repeated.

Mineralization assay

Mineralization of ^{14}C-ring-labed simazine in eight representative soils (Table 2) was evaluated in biometer flasks as described by Krutz et al.[13] Thirty g soil (dry weight equivalent) was mixed with ring-labeled ^{14}C-simazine (≥95% radiological purity with specific activity of 9.9 mCi $mmol^{-1}$) and analytical grade simazine (99% purity) for an initial concentration of 1 µg simazine g^{-1} soil and radioactivity of 58.6 Bq g^{-1} soil. The experiment was arranged as a completely randomized design with three replications and was repeated. Final soil moisture content in each flask was adjusted to 30% (w/w) by addition of deionized water, and biometers were incubated in the dark at 25 °C ± 2 °C. Evolved $^{14}C\text{-}CO_2$ was collected in sodium hydroxide traps and quantified by liquid scintillation spectroscopy (LSS) using Hionic-Fluor (Perkin Elmer, Shelton, CT). The sodium hydroxide solution in the traps was replaced after each sampling. Samples were collected at 0, 1, 7, 11, 14, 18, 21, 25, 28, 32, and 35 DAT. Soil was destructively sampled 35 d after herbicide application. Air-dried soil was manually crushed into uniform particle size, and duplicate samples (0.30 g) were weighed onto Whatman 1 qualitative filter paper (Whatman Inc., Florham Park, NJ). Samples were combusted in a Packard model 306 oxidizer (Packard

Table 2. Location, simazine use history, and physical properties of California citrus orchard soils used for microbial activity and mineralization assays in 2006.

Soil sample	County	City	History classification	Soil pH	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
5	Tulare	Ivanhoe	Recent simazine use	7.2	1.24	58	29	13
6	Tulare	Ivanhoe	No simazine use	6.9	1.85	63	25	12
10	Tulare	Lindsay	Annual simazine use	6.6	0.87	50	35	15
12	Tulare	Exeter	Short break since last	7.4	0.77	57	30	13
20	Ventura	Fillmore	Short break since last	7.5	0.89	66	23	11
21	Ventura	Fillmore	No simazine use	7.6	0.83	67	21	12
24	Ventura	Somis	Recent simazine use	6.5	2.63	26	41	33
26	Ventura	Ojai	Annual simazine use	6.0	1.54	72	21	7

Instruments, Chicago, IL), and evolved $^{14}CO_2$ was trapped in scintillation vials containing Carbo-Sorb and Permafluor (20 mL; 1 + 1 by volume) (Perkin Elmer, Meridian, CT). Radioactivity was determined by LSS. The amount of ^{14}C-CO_2 recovered from the combusted samples was added to the cumulative ^{14}C-CO_2 evolved to determine the ^{14}C mass balance.

Statistical Analyses

Rapid dissipation assay

Dissipation data were converted to percent simazine extracted 1 hr after equilibration, ie, time zero, and were grouped together on the basis of simazine history. Percent simazine extracted data were described using a first order kinetics model using Sigma Plot 11:

$$Y = Ae^{-kt} \qquad (1)$$

where Y is the response variable, A is the concentration of simazine in soil at time zero (%), t is the time variable, and k is the rate constant.

Microbial activity determination

Data on simazine dissipation was analyzed using mixed procedure in Statistical Analysis System (SAS version 9.1; SAS Institute Inc., Cary, NC) with sampling time as fixed effects and replications and experimental runs as random effects. Sampling time means for simazine dissipation were then separated using LSMEANS at the 5% level of significance. The relationship between dissipation data to sampling time for sterile soils was described using a first order kinetics model. The live soils dissipation data were described using the three-parameter, sigmoidal logistic function:

$$Y = a/[1 + (t/t_0)^b] \qquad (2)$$

where Y represents the remaining simazine (%), a represents the maximum value of Y, t represents the number of days, t_0 is the number of days required to 50% simazine dissipation, and b is the slope of curve around t_0.

Mineralization assay

Analysis of variance and mean separation of mineralization assay data were performed using Proc Mixed at $P \leq 0.05$. When interactions with the sampling time main effect were noted, the relationship between time and simazine mineralization was described by the Gompertz growth model in Sigma Plot 11. The general form of the Gompertz model utilized is as follows:

$$y = a^*\exp(-\exp(-(t - t_0)/k) \qquad (3)$$

where a is the plateau representing the maximum mineralization (%); t_0, is the abscissa of the inflection point representing the lag phase (d); k is the inverse of the Gompertz mineralization rate constant (d); and t is time (d).

Results and Discussion

Rapid dissipation assay

When the 27 citrus orchard soils were grouped according to broad simazine use classes, simazine dissipation was more rapid in soils with a history of simazine use compared to soils with no recent use (Fig. 1). In soils with simazine use history, 90% or greater was dissipated by 14 days in the 35 day laboratory assay. In contrast, in soils with no use history (for at least 15 years), simazine was at least 40% higher at 35 days after treatment. Soil from orchards treated recently with simazine dissipated the herbicide the fastest which suggests that enhanced degradation can develop after relatively few years of simazine treatment. Others have also reported that enhanced atrazine degradation can occur following a single atrazine application.[14] Moreover, a break of a few years between simazine treatments appears to slow the degradation rate but does not fully reset degradation to the level in non-adapted fields.

Microbial activity determination

More detailed degradation assays conducted with a subset of the orchard soils indicated that microbial activity is the primary contributor to simazine degradation. In autoclaved soil, simazine dissipation was reduced four-fold compared to non-sterilized soil (Fig. 2A and B, dashed line) indicating that simazine persistence in these orchard soils is principally governed by biotic activity.[11,14]

In soils from Tulare County, simazine was quickly degraded below analytical detection limits if the orchard had a history of simazine use (Fig. 2A). The fitted logistic curve revealed that the simazine half-life in

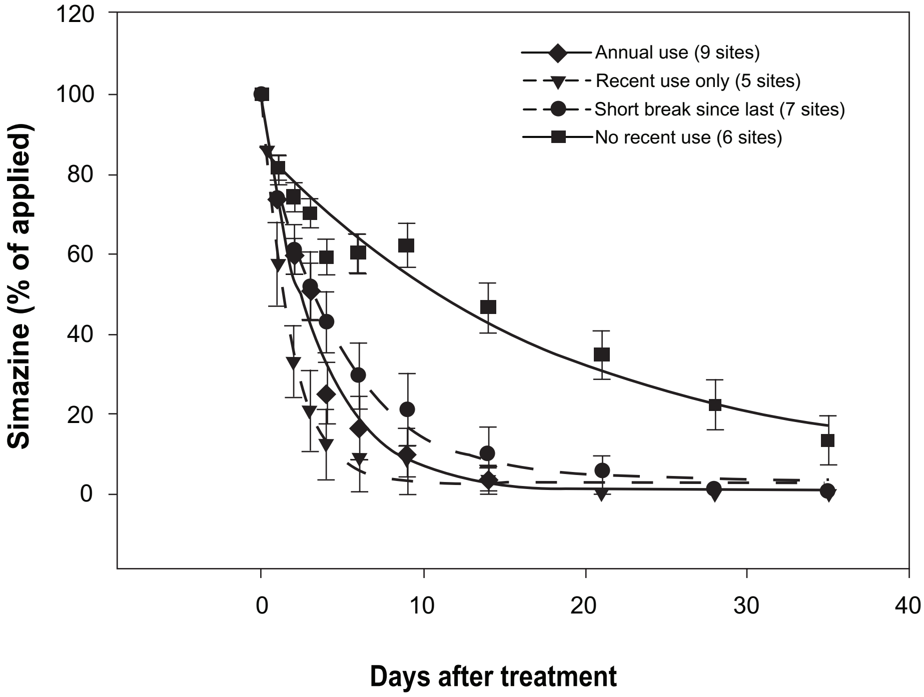

Figure 1. Simazine soil dissipation in a laboratory assay using soil from citrus orchards with various simazine use histories.

soils with simazine use histories were 2-fold (recent use only) and 3-fold (annual use and short break after last use) faster compared with soil with no recent simazine use history. Similar degradation rates between adapted and non-adapted soils were reported in a related vineyard experiment.[15] In this experiment, reduced simazine persistence in soils with long use history and short break after last use were expected. However, it is interesting to note that soils with only recent simazine use also exhibited enhanced degradation of the herbicide which suggests that the microbial population was already present and can build up quickly after one or two applications of simazine. In the Ventura County soils, fitted logistic curves indicated that the half-life of all soils regardless of simazine use history was 11 d or shorter (Fig. 2B). These half-life values are similar to soils with long simazine use and short break after last use in the Tulare County soils which suggests that the microbial population that can quickly degrade simazine is widely spread in these orchards regardless of recent simazine use. A similar phenomenon has been reported in western and southern U.S. corn production regions.[16]

Mineralization

Cumulative mineralization of ^{14}C ring-labeled *s*-triazine herbicides can be used as a diagnostic test to confirm enhanced degradation although the critical threshold may vary among specific pesticides and environments. Krutz et al[16] suggested that soils that mineralize available *s*-triazine herbicide more than 50% within 30 d could be considered adapted. In Tulare County, soils with a short break after use, annual use, and recent use only had cumulative simazine mineralization that exceeded 50% in less than 15 days indicating that these soils are adapted (Fig. 3A). Simazine mineralization in soils with long annual use and short break after long use peaked at 15 days then leveled off. Simazine mineralization in soils recently treated with simazine plateaued after 19 days of incubation in the laboratory. Conversely, cumulative mineralization in soil with no simazine history did not exceed 30% after 30 days of incubation, which is typical of non-adapted soils. In Ventura County, all soils had cumulative mineralization of 14C-simazine that exceeded 50% before 30 d of incubation indicating that these soils are adapted to simazine regardless of simazine use history in the last 15 years (Fig. 3B).

At each location, the cumulative mineralization and degradation data were in agreement. In Tulare county, adapted soils had shorter half-life and exceeded the critical cumulative mineralization threshold level faster than the non-adapted soil.

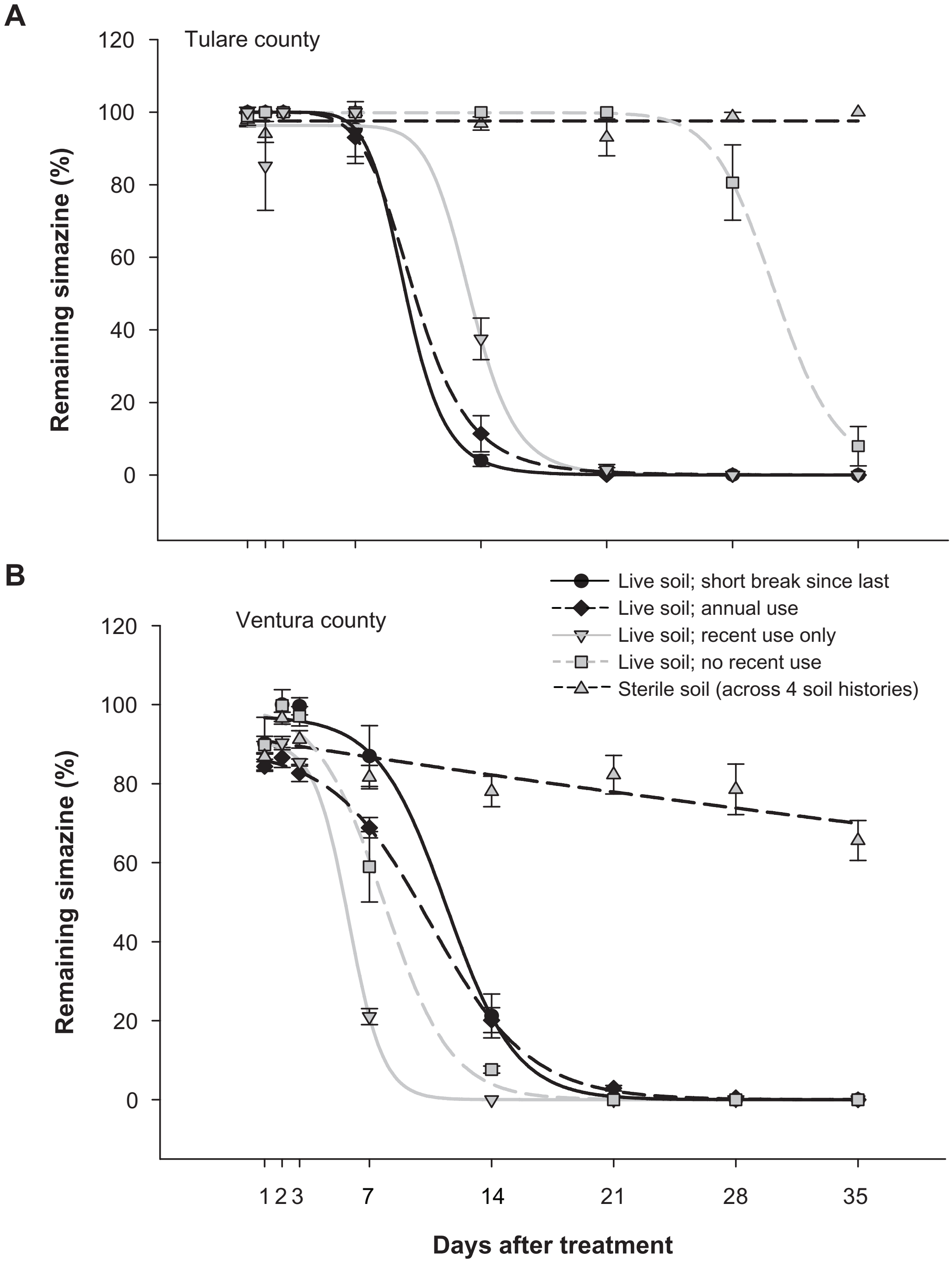

Figure 2. Effects of soil sterilization and simazine use history on simazine dissipation in California citrus orchard soils.

No distinct differences in cumulative mineralization and simazine degradation were observed among soils with different simazine use histories in the Ventura County soils. All soils appear to have shorter half-lives and exceeded the critical cumulative mineralization threshold level before 30 days. Although available records indicated that no simazine use had been used for at least 15 years in the orchard classified as no recent simazine use, the similarity of cumulative mineralization and simazine degradation among the soils in Ventura County suggest that all four orchards have some level of enhanced degradation. Because of the long history of orchard crops and simazine availability in this area, it is fairly likely that the soil we classified as non-adapted had actually been treated with an *s*-triazine herbicide at some time in the past.

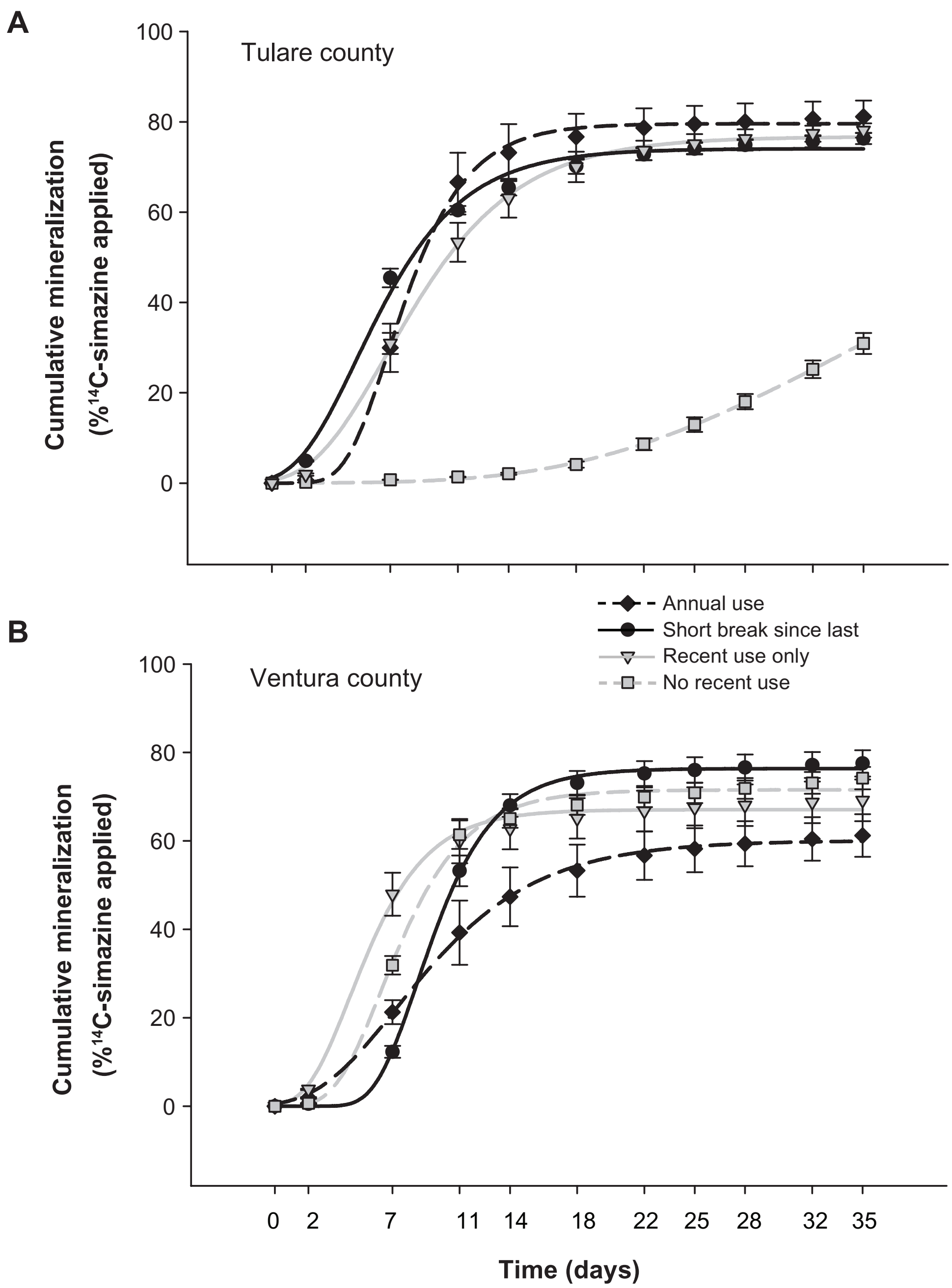

Figure 3. Cumulative mineralization of ^{14}C-simazine in citrus orchard soils with different simazine use histories.

The triazine-degrading microbial population may have been previously selected but had decreased to a low level following at least 15 years of no *s*-triazine treatments. However, the re-application of simazine to these soils may have re-activated the remaining degraders in the soil thus field dissipation kinetics was generally similar on all soils. Krutz et al[13] reported that once a soil is adapted to *s*-triazine herbicides, the *s*-triazine degrader population will persist at some level in the soil. In the absence of continued *s*-triazine use, the population declines to a low but static level but can quickly rebound following a subsequent application of an *s*-triazine herbicide. It should be noted, however, that not all soils that received *s*-triazine treatments mineralized the herbicides despite a history of application.

The biological degradation of *s*-triazine in the soil depends on the presence and activity of indigenous microorganisms that possess enzymatic mechanism to transform and degrade the *s*-triazine molecule.[8,17,18] Several factors are thought to influence these microorganisms in soils including microbial composition (diversity and biomass) or soil conditions (nutrient limitations and specificity, moisture requirement, pH, temperature, soil texture and porosity, and organic matter content).[4,19–22] Depending on the microbial population and the factor(s) affecting these microorganisms, simazine half-life may vary from few weeks to months.

Moreover, applying simazine in orchards before weeds emerge reduces its efficacy owing increased herbicide dissipation due to microbial or chemical degradation, photolysis, or leaching through the target zone.[23–26] To reduce early winter losses, citrus producers could delay simazine applications until late winter, although a tank mix partner would likely be required to control emerged weeds. This practice may also minimize the potential for off-site transport of the herbicide because fewer and smaller precipitation events would occur following the applications.

Conclusions

Results showed that repeated simazine use in California orchards can lead to more rapid degradation of the herbicide. In Tulare County soils, the dissipation of simazine in a laboratory assay was two- to three-fold greater in soils with history of simazine use than in soils with no simazine application. The mineralization assays verify that the enhanced simazine degradation is due to microbial activity. However, in contrast to the Tulare County soils, simazine dissipation and mineralization in the Ventura County soils were not substantially different among orchards with different simazine use histories. These results indicate that long-term cropping factors can affect herbicide degrader population persistence and distribution in California orchards. California growers and pest control consultants should be aware that simazine can perform differently under seemingly similar orchard conditions and monitor and adjust weed control strategies accordingly.

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Author Contributions

Conceived and designed the experiments: BDH, DLS, LJK. Analysed the data: MJMA, CMR, LJK. Wrote the first draft of the manuscript: MJMA, CMR, BDH. Contributed to the writing of the manuscript: MJMA, DLS, LJK, NVO, BAF, BDH. Agree with manuscript results and conclusions: MJMA, DLS, LJK, CMR, NVO, BAF, BDH. Jointly developed the structure and arguments for the paper: MJMA, DLS, LJK, BDH. Made critical revisions and approved final version: DLS, LJK, BDH. All authors reviewed and approved of the final manuscript.

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Competing Interests

Authors declare no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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