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Effects of Long-Term Use on Simazine Dissipation in Central California Vineyards

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Simazine is an important management tool for weed control in vineyards because of its relatively low price, reliable control of several problem weeds, and long residual activity. After repeated and extensive use of simazine, several growers in the Central Valley of California expressed concerns about reduced, residual weed control with this herbicide. Experiments were conducted to evaluate the rate of simazine dissipation in soils with differing simazine-use histories and to determine whether residual weed control differed among sites. Two raisin vineyards were used in all studies, one with extensive simazine-use history (adapted) and one with no recent simazine-use history (nonadapted). Results indicated that simazine dissipation from biotic processes was fourfold greater in soil with a long simazine-use history relative to soil with no recent simazine applications. In the field, simazine persisted longer at the nonadapted site, and weed-control duration was affected by dissipation rate. Central Valley vineyard soils that have had repeated simazine applications can develop enhanced, microbial degradation, and reduced, residual weed control is possible; however, weed control is also affected by environmental conditions and other crop management practices.

Nomenclature: Simazine, 6-chloro-*N*,*N*′-diethyl-1,3,5-triazine-2,4-diamine; raisin grapes, *Vitis vinifera* L.

Key words: Enhanced degradation, atrazine, *s*-triazine herbicides, herbicide use history.

Simazine is a selective *s*-triazine herbicide used to control broad-leaved weeds and annual grasses in field crops, orchards, and vineyards. In California vineyards, simazine commonly is applied at 2.25 to 4.5 kg ai ha^{-1} in the vine row during December through February after vine pruning but before winter precipitation ends (UC-ANR 2009). Simazine can be mixed with oryzalin or norflurazon to improve PRE weed control and is often combined with POST herbicides, such as paraquat, glyphosate, or glufosinate, if emerged weeds are present. Simazine is valued for its relatively low price, reliable control of several problem weeds, including horseweed [*Conyza canadensis* (L.) Cronq.] and hairy fleabane [*Conyza bonariensis* (L.) Cronq.], and long residual activity (Senseman 2007; Hembree and Shrestha 2005).

Residual weed control depends on how persistent the herbicide is in the soil environment. Like other *s*-triazine herbicides, simazine is considered to be moderately persistent (average half-life of 60 d) in soil (Senseman 2007); however, persistence is affected by a number of biotic and abiotic factors, and half-life estimates range widely (16 to 186 d)(Best and Weber 1974; Nearpass et al. 1978; Walker 1976; Wauchope 1992). Degradation of *s*-triazines in soil occurs via chemical and biological pathways; however, chemical hydrolysis was thought for many years to be the primary mechanism because metabolites from biological degradation retained some phytotoxic properties (Khan and Marriage 1977; Sirons et al. 1973; Wackett et al. 2002).

The incomplete biological degradation of these herbicides has been attributed to the halogen, methyl thioether, and *N*-alkyl substitutions, which impede the ability of soil microorganisms to metabolize the *s*-triazine ring (Cooke 1987; Mandelbaum et al. 2008). Although Cook (1987) isolated bacteria that grew on cyanuric acid and related *s*-triazines, these microorganisms were not able to survive on *s*-triazine herbicides. Between 1993 and 1995, however, independent laboratories isolated bacteria with the ability to rapidly mineralize *s*-triazine herbicides (Mandelbaum et al. 1993, 1995; Radosevich et al. 1995). In subsequent decades, the genes responsible for rapid *s*-triazine mineralization were identified, isolated, and confirmed to be widespread in agricultural soils (Krutz et al. 2010).

Rapid or ''enhanced'' microbial degradation of a soil-applied pesticide can have a significant effect on agricultural pest management. Reduced, residual activity in adapted soil has been attributed to enhanced degradation of several soil-applied insecticides and herbicides (Anderson and Lafuerza 1992; Arbeli and Fuentes 2007; Krutz et al. 2007, 2009; Roeth 1986; Suett et al. 1993). There is evidence that simazine may be subject to enhanced degradation. Krutz et al. (2008) reported that soils exhibiting enhanced atrazine degradation also rapidly degraded simazine, i.e., a phenomenon referred to as cross-adaptation. Rouchaud et al. (2000) reported an accelerated (by a factor of 1.3) rate of simazine degradation in plots treated with consecutive annual applications compared with plots treated for the first time.

Following repeated and extensive annual use of simazine, several California vineyard and orchard growers expressed concern about reduced, residual weed control. Because several weed species were affected, poor simazine performance was suspected to be related to enhanced degradation rather than herbicide resistance. To address local grower concerns, laboratory and field experiments were conducted to determine whether enhanced simazine degradation occurs in Central Valley, California, vineyards, and whether residual weed control is affected.

Materials and Methods

Site Description. Two raisin vineyards located within 1 km of each other near Parlier, CA, were used in the experiments. One vineyard was located at the University of California Kearney Agriculture Center (KAC) (36°36′3.90″N, 119°30′41.01″W) and the other at the U.S. Department of Agriculture (USDA)–Agricultural Research Service, San

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Joaquin Valley Agricultural Sciences Center (SJVASC) (36°35′44.67″N, 119°30′41.75″W). Both vineyards had well-established (> 20 yr) Thompson Seedless raisin grapes. The under-vine soil areas of the KAC site had been treated annually with simazine for at least 15 yr (adapted). The adapted soil was a Hanford sandy loam with pH 7.6, 9.2 mEq 100 g^{-1} cation exchange capacity (CEC), 0.78% organic matter, with 68% sand, 26% silt, and 6% clay. The soil at the SJVASC site had no simazine use for at least 15 yr (nonadapted). The nonadapted soil was Hanford sandy loam with pH 7.8, 9.4 mEq 100 g^{-1} CEC, 0.77% organic matter, with 64% sand, 30% silt, and 6% clay. The KAC vineyard was furrow-irrigated, whereas the SJVASC vineyard was drip irrigated.

Laboratory Experiments. Soil for the laboratory experiments was collected in the summer of 2007 from the surface 10 cm in vine rows adjacent to the field experimental plots in each vineyard. Moist soil was stored in a sealed, 20-L, plastic container at room temperature until the experiments were conducted. All laboratory experiments were arranged in a completely randomized design with three replicates, and the experiments were repeated.

Dissipation Assay. Approximately 20 kg of soil (dry wt equivalent) from each site was divided into two: half was triple-autoclaved to eliminate existing microbial populations, and the other half was left unsterilized. Autoclaved (sterile) or nonautoclaved (live) soil was passed through a 4-mm sieve (Fisher Scientific, Fair Lawn, NJ), 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 (T. C. Wheaton Glass Company, Millville, NJ). Soil was treated with either 15 ml water or 15 ml of 66.67 μg ml^{-1} formulated simazine (Princep 4L, Syngenta Crop Protection, Greensboro, NC) diluted in water, and the soil was manually homogenized resulting in a final, nominal, simazine concentration of 10 μg g^{-1} soil (wt/wt). Samples were taken at 0, 1, 3, 7, 14, 21, and 35 d after treatment (DAT), and simazine was extracted using a water-based procedure (Shaner et al. 2007). At each time point, soil samples were physically stirred, and a 5-g subsample of moist soil was weighed into a 50-ml centrifuge tube (Thermo Fisher Scientific Inc., Austin TX). An equivalent amount (wt/wt) of distilled water was added to each tube, and samples were mixed on a reciprocating shaker for 1 h, then centrifuged at 2,000 × *g* for 20 min at 20 C. One-half to 1-ml aliquots of the supernatant were transferred to microfuge tubes with 0.22 μM Teflon filter inserts (Millipore Corp., Bedford, MA) and centrifuged at 10,000 × *g* for 10 min. The filtrates were analyzed using high-performance liquid chromatography (HPLC)(Agilent Technologies, Wilmington, DE), which was equipped with a multiple wavelength detector (Agilent Technologies) and a 4.6- by 250-mm C18 column (ZORBAX, Agilent Technologies) and a 4.6- by 12.5-mm guard column. The mobile phase was HPLC grade acetonitrile : water : phosphoric acid (35 : 65 : 0.05 v/v/v)(Fisher Scientific) 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.

Mineralization Assay. Mineralization of ^{14}C-ring-labed simazine was evaluated in biometer flasks (Krutz et al. 2007). Briefly, soil (30 g dry wt equivalent) was mixed with ring-labeled ^{14}C-simazine (≥ 95% radiological purity with specific activity of 9.9 mCi $mmol^{-1}$)(Syngenta Crop Protection) and analytical grade simazine (99% purity)(Syngenta Crop Protection) for an initial concentration of 1 μg simazine per gram of soil and a radioactivity of 58.6 Bq per gram of soil. Final soil-moisture content was adjusted to 30% (wt/wt) by addition of deionized water, and biometers were incubated in the dark at 25 ± 2 C. Evolved $^{14}C–CO_2$ was collected in sodium hydroxide traps (Sigma-Aldrich, St. Louis, MO) 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, 2, 7, 11, 14, 18, 22, 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 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). 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 (Weaver et al. 2007).

Field Experiment. Field experiments were conducted during the 2008 and 2009 growing seasons to determine the practical implications of simazine degradation on weed control in vineyards. Simazine was applied at 4.5 kg ha^{-1} using a CO_2 backpack sprayer calibrated to deliver 187 L ha^{-1}. In 2008, simazine was applied on February 6 and 27 at the nonadapted and adapted sites, respectively. In 2009, the nonadapted site was treated on March 3 and the adapted site on March 10. A nontreated control was included for comparison at both locations. The experiments were arranged as randomized complete blocks with 8.5- by 1.5-m plots (included five vines) in the nonadapted site and 4.25- by 1.5-m plots (included three vines) in the adapted site. Treatments were replicated four times. Soil samples were taken 0, 14, 28, 56, 112, 176, and 224 DAT at 0 to 5 cm depth to assess the rate of simazine degradation in the field. Simazine was extracted from soil as described in the laboratory dissipation assay experiment. Weed density was determined 56, 112, and 176 DAT in a 0.5- by 2-m area between two grapevines in each plot. Following each weed count, plots were treated with paraquat (Gramoxone Inteon, Syngenta Crop Protection, Greensboro, NC) at 0.56 kg ai ha^{-1} plus glufosinate (Rely 200, Bayer CropScience, Greensboro, NC) at 0.93 kg ai ha^{-1}.

Statistical Analysis. *Dissipation Assay*. Simazine data were standardized to micrograms per gram of soil on a dry-wt basis. Data were converted to the percentage of simazine extracted 1 hr after equilibration, i.e., time 0. The simazine data were subjected to ANOVA, and means were separated at the 5%

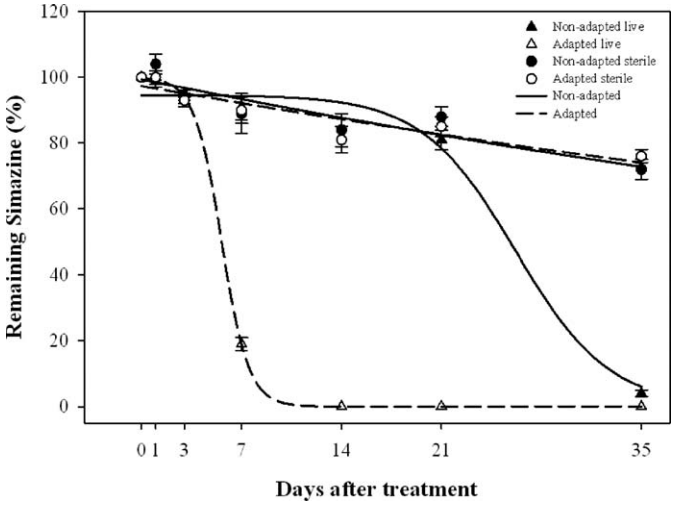

Figure 1. Simazine dissipation in adapted and nonadapted soils in the laboratory as affected by sterilization. Dissipation-logistic equation (Equation 2) for nonadapted live soil: $y = 94.6/\{1 + \exp[-(t - 26.18)/-3.3)]\}$; for adapted live soil: $y = 100.1/\{1 + \exp[-(t - 5.61)/-0.96]\}$. The dissipation first-order kinetics equation (Equation 1) for nonadapted sterile soil: $y = 99.4 \times \exp(-0.0089 \times t)$, and for adapted sterile soil is: $y = 97.3 \times \exp(-0.0078 \times t)$. Error bars indicate the standard errors of the means.

level of significance using Fisher's Protected LSD test. Dissipation data for sterile soils were described using a first-order kinetics model and Sigma Plot (Systat Software Inc., Richmond, CA):

$$Y = Ae^{-kt} \quad [1]$$

where Y is the response variable, A is the concentration of simazine (as a percentage) in the soil at time 0, t is the time variable (in days), and k is the rate constant (per day). Simazine dissipation in live soil was described using the three-parameter, sigmoidal-logistic function:

$$Y = a \Big/ \left[1 + (t/t_0)^b\right] \quad [2]$$

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. Half-life values for simazine were subjected to ANOVA, followed by Tukey's test.

Mineralization Assay. The mineralization data were analyzed as a split-plot experiment, where soil was the whole plot (adapted or nonadapted), and sampling time was the subplot (0, 2, 7, 11, 14, 18, 22, 25, 28, 32, and 35 d). ANOVA and mean separation were performed using PROC MIXED (SAS version 9.1; SAS Institute Inc., Cary, NC). All results were considered significantly different at $P < 0.05$. 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 used is as follows:

$$Y = \langle a \times \exp\{-\exp[-(t - t_0)]\}\rangle / k \quad [3]$$

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

Field Experiment. Simazine concentration in field soil was corrected for soil moisture at each time point and expressed as a percentage of the extractable simazine at day 0. The relationship between simazine concentration and days after treatment was described using the three-parameter, sigmoidal-logistic model, as described previously. Weed count data in the field experiment were expressed as a percentage of the untreated plots at each location to allow comparison among the sites. Means were separated using Fisher's Protected LSD test at the 5% significance level.

Results and Discussion

Dissipation Assay. When vineyard soil was sterilized via autoclaving, only 20% of the applied simazine disappeared during the 35-d incubation period (Figure 1). The similarity in simazine dissipation between sterilized-adapted and -nonadapted soils suggests that simazine degradation in these vineyard soils is principally governed by microbial activity and not by abiotic factors. In both live soils, simazine concentration after 35 d was less than 5% of the amount applied (Figure 1). Calculated half-life for simazine in the live-nonadapted soil (27.1 d) was within the broad range reported in the literature, i.e., 16 to 186 d (Nearpass et al. 1978; Senseman 2007; Walker 1976; Wauchope 1992). However, the half-life estimate for the live-adapted soil was only 5.7 d, which is nearly five times shorter than that of the nonadapted soil (Table 1) and considerably shorter than the values reported in the literature. These results are similar to previous research with *s*-triazines that indicated that atrazine persistence in adapted soils can be 10 times shorter than in adjacent, nonadapted soils (Krutz et al. 2008; Shaner and Henry 2007; Shaner et al. 2007). Interestingly, after an initial lag phase, the

Table 1. Three-parameter, sigmoidal-logistic equation (Equation 2) and first-order kinetics equation (Equation 1) values for simazine degradation in live and sterile, adapted and nonadapted, California vineyard soils during a 35-d laboratory time course experiment (parameter estimate ± 95% CI).

	t_0, 50% dissipation[a]	b, Slope	
Soil	d	95% CI	P value
Nonadapted live[b]	27.1 a	−2.9 ± −1.3	< 0.0001
Adapted live[b]	5.7 b	−1.1 ± 0.2	< 0.0001
	k, rate constant d^{-1}		
Nonadapted sterile[c]	0.009 ± 0.004		< 0.0001
Adapted sterile[c]	0.007 ± 0.004		< 0.0001

[a] Values followed by the same letter within the column are not significantly different based on Tukey's test at $P \leq 0.05$.
[b] Fit to a three-parameter sigmoidal logistic model: $y = \{a/[1 + (t/t_0)^b]\}$.
[c] Fit to a first-order kinetics model: $y = Ae^{-kt}$.

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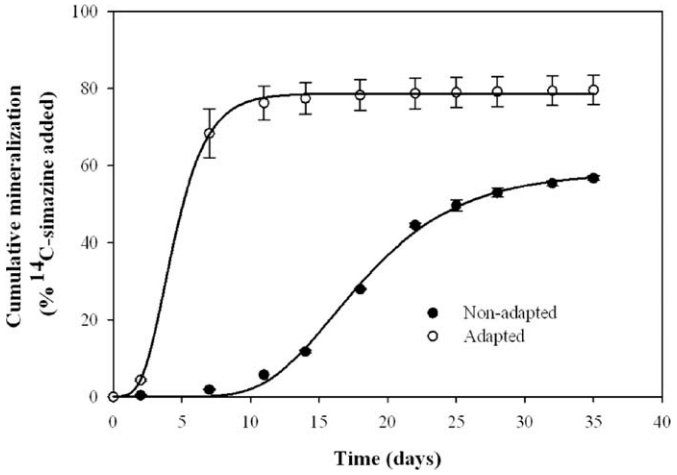

Figure 2. Cumulative mineralization of ^{14}C-simazine in adapted and nonadapted soils. The simazine mineralization Gompertz equation (Equation 3) for nonadapted soil: $y = 58.3 \times \exp[-\exp(-t - 16.11)/5.02)]$ and for adapted soil: $y = 78.7 \times \exp[-\exp(t - 3.77)/1.68)]$. Error bars indicate the standard errors of the means.

nonadapted soil degraded simazine rapidly, indicating that it likely has a small population of the adapted microbial population that was able to respond to the selective agent.

Mineralization Study. Cumulative mineralization of ^{14}C ring-labeled *s*-triazine herbicides can be used to confirm enhanced degradation. For example, Krutz et al. (2009) proposed that a soil can be considered adapted when cumulative mineralization exceeds 50% by 30 d after application under optimum temperature and moisture conditions. Cumulative simazine mineralization in these vineyard soils exceeded this threshold 5 d after application indicating, therefore, that this site does harbor a microbial community able to rapidly degrade *s*-triazine herbicides (Figure 2). The mineralization kinetics from the site with no recent simazine application history was depressed relative to the adapted location (Table 2). However, after an extended lag phase, simazine mineralization at the nonadapted site accelerated and cumulative mineralization approached 50% by 30 d after application. Gunasekara et al. (2007) and Krutz et al. (2009) suggested that once a soil is adapted to *s*-triazine herbicides, the *s*-triazine degrader population is likely to persist over time. In the absence of continued *s*-triazine use, the population declines to a low, but static, value but can quickly rebound following a subsequent application of an *s*-triazine herbicide. Similar to related work in Central Valley, CA, citrus orchards (Abit et al. 2012), these results suggest that the nonadapted vineyard site may have received *s*-triazine applications in the past and retains a population of the *s*-triazine–degrading microbial community.

Previous research on the metabolism of ^{14}C ring-labeled *s*-triazine herbicides revealed nominal or no release of $^{14}CO_2$ from the soil (Dao et al.1979; Skipper et al.1967). At that time, researchers concluded that ring cleavage of the *s*-triazine contributed little to dissipation of the herbicide. This interpretation was also supported by field work indicating that atrazine and other *s*-triazines have very long residual activities (Buchanan and Hiltbold 1973; Frank and Siron 1985; Kells et al.1980; Roeth et al.1969).

The hydrolytic degradation pathway discussed by Wackett et al. (2002) is most likely still true for soils that do not have a history of *s*-triazine use or where *s*-triazine is rarely used. In this study, in the nonadapted soils, which had no history of simazine use for at least 15 yr, the estimated half-life was approximately 27 d, compared with 5 d in adapted soils. The pattern of metabolism in the nonadapted soils was similar to that reported by earlier researchers, thus, in nonadapted soils, the widely accepted dogma on how *s*-triazines dissipate in soil is probably correct. However, with the selection of soil microbes that can rapidly degrade simazine, chemical hydrolysis may not fully explain soil behavior of the herbicide.

Table 2. Gompertz equation (Equation 3)[a] fitted values for simazine mineralization in adapted and nonadapted California vineyard soils during a 35-d laboratory time-course experiment (parameter estimate ± 95% CI).

	a, Max evolved	*b*, Lag phase	*k*, Rate constant	
Soil	% ^{14}C applied	d	d	P value
Adapted	78.7 ± 23.3	3.8 ± 0.4	1.7 ± 0.3	< 0.0001
Nonadapted	58.3 ± 2.9	16.1 ± 0.6	5.0 ± 0.9	< 0.0001

[a] Gompertz model: $y = a \times \exp\{-\exp[-(t - t_0)/k]\}$.

Field Experiment. The field dissipation of simazine was adequately described by the three-parameter logistic model similar to the live soils in the laboratory-dissipation assay. However, there were significant differences among the two growing seasons; therefore, data are presented separately by year. Simazine half-lives were 21 and 56 d for the adapted and 157 and 87 d for the nonadapted soils in 2008 and 2009, respectively (Figure 3a). In 2008, the simazine dissipation curve from the adapted soil indicated a rapid initial loss during the first 7 to 14 d, followed by slower rates of loss. The initial rates of loss and the half-life suggest that these changes may not have been due solely to degradation in the soil. Similar rapid loss of atrazine were reported by Walker and Zimdahl (1981) and Rocha and Walker (1995) who suggested that volatilization, photochemical degradation, or even wind erosion could also be contributing factors, although the significance of these processes are difficult to assess. The differences between 2008 and 2009 may have been influenced by environmental conditions, such as higher soil temperatures during spring 2009 compared with 2008 (Anonymous 2012).

The predominant weed species in these vineyards were horseweed, hairy fleabane, spotted spurge [*Chamaesyce maculata* (L.) Small], annual sowthistle (*Sonchus oleraceus* L.), and yellow nutsedge (*Cyperus esculentus* L.). A late-winter application of 4.5 kg ai ha^{-1} simazine (the high label rate) should have provided excellent, season-long control of the broadleaf weed community; however, residual weed control varied among site-year locations (Figure 3b). Simazine provided excellent, residual weed control at the nonadapted site in 2008 and reduced the total weed population by 90% or more up to 176 DAT. Conversely, residual weed control at the adapted site was below 60% by 112 DAT at the adapted site. In 2009, there were no differences in residual weed control between the adapted and nonadapted sites most likely due to the relatively faster rate of simazine dissipation in the second year (Figure 3a). Although there was a dramatic weed-control difference among years at the adapted site, duration of weed control was associated with simazine persistence. For example, at the nonadapted site in 2008, simazine half-life was about 158 d, and weed control was 90% or greater through the summer. Simazine dissipated more quickly in the

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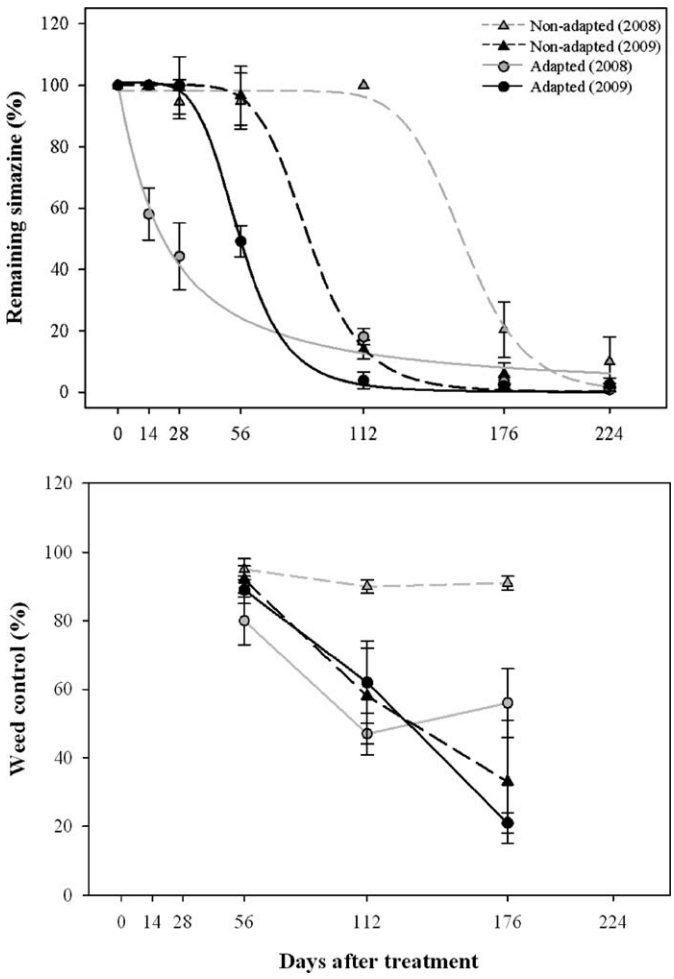

Figure 3. (A) Field dissipation of simazine in adapted and nonadapted vineyard soils near Parlier, CA, in 2008 and 2009. Dissipation-logistic equation (Equation 2) for nonadapted soil (2008): $y = 98.2/\{1 + \exp[-(t - 157.4)/-11.3]\}$; for nonadapted (2009): $y = 100.2/\{1 + \exp[-(t - 87.1)/-7.1]\}$; for adapted (2008): $y = 99.5/\{1 + \exp[-(t - 20.8)/-1.1]\}$; and for adapted (2009): $y = 100.8/\{1 + \exp[-(t - 55.6)/-5.3]\}$. Error bars indicate standard errors of the means. (B) Weed control relative to the untreated control in simazine-adapted and -nonadapted vineyard soils near Parlier, CA, in 2008 and 2009. Error bars indicate standard errors of the means.

adapted site in 2009 (half-life of 86 d) and in the nonadapted site in both years (half-life of 21 to 56 d); consequently, residual weed control was reduced. These results are similar to the reported performance of other *s*-triazine herbicides when applied in soils with continuous use of these herbicides (Krutz et al. 2007).

Although simazine must be applied in orchards and vineyards before weeds emerge for greatest efficacy, excessively early applications could lead to reduced residual weed control because of microbial or chemical degradation, photolysis, or leaching through the target zone (Armstrong et al. 1967; Evgenidou and Fytianos 2002; Mandelbaum et al. 1995; Morgante et al. 2012). To reduce early winter losses, vineyard 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.

These experiments indicated that simazine persistence can vary among vineyards with different herbicide use histories because of rapid degradation of the herbicide. Simazine dissipated in soil with a history of simazine use four times faster in the laboratory and 1.5 to 8 times faster in the field compared with soil from vineyards with no recent simazine history. Laboratory mineralization assays verified that the dissipation was due to microbial degradation. Moreover, residual weed control is correlated with the persistence of simazine in these soils. California growers and pest-control consultants should be aware that simazine can perform differently under seemingly similar vineyard conditions and should monitor and adjust weed control strategies accordingly.

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