Influence of Incubation Temperature on the Behavior of Triethylamine-Extractable Glyphosate (*N*-Phosphonomethylglycine) in Four Soils

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The behavior of glyphosate, extracted from four soils using aqueous triethylamine, was investigated at two temperatures. For each soil, and at both temperatures, there was a marked loss in the amount of extractable glyphosate immediately after addition of the herbicide to soil. This rapid loss of glyphosate was ascribed to adsorption of the herbicide into a nonextractable form. For three of the four soils used when incubated at 25 °C, the rates of loss of extractable glyphosate were similar to previously measured rates of degradation of this herbicide in these soils. However, loss of extractable glyphosate from the Culgoa clay loam was due not only to substrate degradation but also to slow sorption of glyphosate into the nonextractable form in this soil over the experimental period. For the Rutherglen and Walpeup soils, when incubated at 10 °C, the rates of loss of extractable glyphosate were half of the previously measured rate of degradation of this herbicide in these soils. However, there was no measured loss of extractable glyphosate from the Wimmera clay. As previous work has shown glyphosate to decompose readily in these soils at this temperature, these findings suggest that desorption of glyphosate may occur at a rate greater than degradation at this temperature and, hence, that temperature may play a pivotal role in sorption processes. Investigations with these soils when sterilized by γ-irradiation showed that for the Walpeup, Wimmera, and Rutherglen soils, sorption was complete soon after the addition of the herbicide; however, for the Culgoa soil, further adsorption occurred over the entire experimental period.

Keywords: *Glyphosate; AMPA; soil; triethylamine; sorption; degradation*

INTRODUCTION

Glyphosate, (*N*-phosphonomethyl)glycine, is the most extensively applied herbicide to control annual and perennial weeds. Although a substantial body of literature exists which details the activity of this compound in plants [i.e., *The Herbicide Glyphosate*; Grossbard and Atkinson (1985)], the literature dealing with its behavior in soils is not extensive (Torstensson, 1985; Piccolo et al., 1996). Numerous publications report glyphosate to be readily inactivated in soils (Sprankle et al., 1975a; Rueppel et al., 1977). However, evidence exists regarding the residual bioactivity of this compound in soil (Campbell, 1974; Salazar and Appleby, 1982; Eberbach and Douglas, 1983; Cornish, 1992); hence, its inactivation may not be complete in all circumstances.

Much of our understanding of the mechanism of glyphosate degradation in soil is from studies using ^{14}C-labeled glyphosate (Sprankle et al., 1975b; Nomura and Hilton, 1977; Torstensson, 1982; Eberbach, 1998) and from studies in which levels of glyphosate in soil are estimated using bioassays (Torstensson and Aamisepp, 1977; Hensley et al., 1978; Cornish, 1992). From these studies our general understanding of glyphosate decomposition in soil is that degradation is initially rapid but slows over time to a quasi-steady state. As logarithmic transformation of these degradation curves rarely achieves a linear relationship with respect to time, either degradation of this compound does not obey first-order kinetics or some other process in soil appears to restrict the availability of glyphosate residues for decomposition. Nomura and Hilton (1977) suggested that sorption of glyphosate to soil solids may restrict the availability of the compound for decomposition, and Eberbach (1998) recently showed that decomposition of ^{14}C-labeled glyphosate in soil could be numerically separated into degradation of soluble glyphosate and degradation of the sorbed fraction of glyphosate. However, as glyphosate degrades to CO_2 via intermediary compounds such as aminomethylphosphonic acid (AMPA) (Rueppel et al., 1977), studies using ^{14}C-labeled glyphosate only infer decomposition behavior of the substrate in soil. Similarly, although bioassay species are specific to glyphosate, limitations to their use exist as a response by the test species indicates that only greater than subclinical amounts of the herbicide remain in soil; therefore, their use is only qualitative.

In a search of the literature pertinent to decomposition of glyphosate in soil, few papers were available in which quantitative extraction and analysis of glyphosate and its primary metabolite, AMPA, were used to monitor degradation of glyphosate over an extended period of time. Ragab et al. (1985) reported that glyphosate degraded rapidly in the field and that no traces of the substrate were detected in a sandy loam soil after 122 days. Similarly, Torstensson and Stark (1979) found glyphosate to degrade rapidly in a forest soil; however, traces were still detected after 259 days. The analytical procedure used by Ragab et al. (1985) was the method

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10.1021/jf980785g CCC: $18.00
Published on Web 04/30/1999

recommended by the U.S. EPA (*Pesticide Analytical Manual*, 1977) in which 0.5 M ammonium hydroxide was used as the extractant. Several workers have reported poor and often irreproducible recoveries when using this procedure (Miles and Moye, 1988), and as such, the report of Ragab et al. (1985) may have underestimated the amount of glyphosate extracted and the duration of persistence of the chemical in soil. More recently, aqueous triethylamine (TEA) has been used to extract residues of glyphosate from soils (Lundgren, 1986; Eberbach and Douglas, 1991). In the latter study Eberbach and Douglas (1991) showed that regardless of when extraction occurred after addition of the herbicide, the standard error of the mean of the amount recovered using triplicate samples was within ~10% of the mean and represented a considerable improvement in reproducibility when compared with the ammonium hydroxide extractant.

The purpose of this investigation was to study the behavior of TEA-extractable glyphosate in four soils at 10 and 25 °C, representing winter and spring soil temperatures, respectively. Although many extractants have been shown to extract glyphosate residues from soil (Nomura and Hilton, 1977; Glass, 1983; Lundgren, 1986; Miles and Moye, 1988; Alferness and Iwata, 1994), aqueous TEA has been used in several studies to extract glyphosate residues from soil and is compatible with the procedure for chemical derivatization as in Deyrup et al. (1985) prior to quantification using electron capture gas−liquid chromatography (ECD-GLC). In addition, like many other extractants used [i.e., combinations of NH_4OH and KH_2PO_4 as in Alferness and Iwata (1994)], the amount of glyphosate able to be extracted by aqueous TEA decreases with time (Eberbach and Douglas, 1991). As the loss of extractable glyphosate 13 h after addition of the substrate (as in the above study) occurred at a much greater rate than could be accounted for by substrate decomposition that could occur over this time interval [as per Eberbach (1998)], then it is unlikely that the loss in glyphosate extracted could be accounted for solely by degradation, but instead could be ascribed to sorption of the substrate into a nonextractable form. Therefore, it is reasonable to infer that TEA extracts only readily accessible glyphosate (i.e., water soluble and weakly sorbed), the fraction likely to be bioactive but not strongly sorbed glyphosate (Eberbach and Douglas, 1991).

EXPERIMENTAL PROCEDURES

Instrumentation. A 439 Packard gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) was used for all measurements. A 2.2. m × 4 mm i.d. silanized glass column was packed with 1.5% OV-17 + 1.95% QF1 Chromosorb WHP 80/100 mesh (S.G.E., Ringwood, VIC, Australia). Carrier gas flow (N_2) was maintained at 15 mL min^{-1} while the make up gas flow rate was maintained at 30 mL min^{-1}. Column temperature was 160 °C while the injector and detector were maintained at 260 and 280 °C, respectively.

Glassware and Reagents. Analytical grade glyphosate (free acid form) was obtained from Monsanto Australia Ltd. AMPA, trifluoroacetic anhydride (TFAA), and trifluoroethanol were purchased from Sigma Chemical Co. TEA (laboratory grade) was obtained from Unilab Chemicals and was glass distilled in the laboratory before use. Dimethyldichlorosilane (2% v/v in dichloroethane) was obtained from Ajax Chemicals. All other chemicals used were obtained from Mallinckrodt Chemicals and were of pesticide grade quality or better. All glassware was chromic acid washed, solvent washed (methyl alcohol followed by hexane), and salinized in dimethyldichlorosilane before use. Cation-exchange resin (Ag 50W-X8, 100−200 mesh, H^+ form) and anion-exchange resin (Ag 1-X8, 100−200 mesh, Cl^- form) were obtained from Bio-Rad Laboratories Pty. Ltd., Australia. Derivatizations were performed in 15 mm × 125 mm unsalinized borosilicate glass culture tubes fitted with Teflon-lined screw caps.

Table 1. Soil Chemical Properties

soil[a]	pH[b]	g kg^{-1} silt	clay	org C	Ceq charge kg^{-1} CEC[c]	Ca	Mg	K	Na	Fe (ppm)
Walpeup LS	7.3	11	64	6.7	4.5	4.2	0.3	0.5	0.1	25.5
Wimmera C	8.4	145	466	11.3	45.8	41.8	5.8	1.7	0.4	1.1
Culgoa SiCL	8.4	26	168	15.6	25.7	27.9	3.3	1.2	0.1	1.9
Rutherglen L	5.3	118	214	13.1	5.9	4.6	1.2	0.4	0.1	26.0

[a] Letters following soil names denote soil texture class: C, clay; L, loam; LS, loamy sand; SiCL, silty clay loam. [b] 1:5 water. [c] CEC, cation exchange capacity (centimoles of negative charge per kilogram of soil).

Soils. Soils used in this study were surface (0−5 cm) samples from Victoria, Australia, selected for their wide range of chemical and physical properties and for their importance as cereal and pulse cropping. The soils selected for study were Walpeup loamy sand, Wimmera gray clay, Culgoa silty clay loam, and Rutherglen loam and are classified as Typic Halpoxerid, Calcic Haploxerert, Calcic Palexeralf, Typic Haploxeralf, respectively (Soil Survey Staff, 1988). Some physicochemical properties of the surface layer (0−5 cm) of these soils have been described in previous publications (Eberbach and Douglas, 1991; Eberbach, 1998) (Table 1).

Soil Preparation. After collection from the field, soils were immediately refrigerated (4 °C) and kept in this state until use. Except for studies requiring the use of sterile soil, soils stored for >2 weeks after collection were discarded.

Pretreatment of Nonsterile Soils. When required for use, soils were laid out thinly on a plastic sheet and air-dried overnight at room temperature. After air-drying, a subsample was taken and oven-dried to determine air-dry moisture content. Air-dried soil was then mixed and sieved gently to pass through a 2 mm sieve prior to being weighed into incubation vessels. Microbial respiration by these soils pre- and post-air-drying and following wetting to field capacity was not determined, as it was assumed that passive air-drying was unlikely to have much effect on overall microbial activity. Evidence in support of this assertion has recently been shown by Degens et al. (1995).

Pretreatment of Sterile Soils. Prior to sterilization, soils were air-dried in the same manner as the nonsterilized soils, then sealed in glass screw-top containers, and irradiated with 5 Mrad of γ radiation.

Preparation of Glyphosate. Glyphosate was dissolved in sufficient Milli-Q deionized water to achieve a final solution concentration of 59.2 mmol L^{-1}. Once in solution, the glyphosate solution was sterilized by passing through a Millipore 0.45 μm filter. Samples of glyphosate were taken pre- and post-filtering and confirmed that no appreciable loss of glyphosate occurred during the filtration process.

Behavior of Glyphosate in Nonsterile Soil. Metabolic studies were undertaken by weighing out 3.5 g of soil into a silanized (Coatasil) borosilicate glass incubation vessel (four replicates per soil). Flasks were sealed and the contents allowed to equilibrate at their intended temperature overnight. After equilibrating for temperature, the flasks were opened and 1 mL of a glyphosate solution (59.2 mM) was added to the soil, bringing the final concentration of glyphosate to 2.86 mg of glyphosate (g of soil)$^{-1}$. When necessary, additional deionized water was added at this stage to the soil to bring its moisture content to ~75% of its field capacity, calculated on a volumetric basis. The containers were then stoppered and placed into incubators set at either 25 or 10 °C.

The soils were sampled at 0, 1, 3, 7, 14, 21, and 28 days after addition of the glyphosate solution. At sampling, the soils were completely washed from the containers into a silanized

plastic centrifuge tube with 3 × 10 mL of 0.1 M aqueous TEA and the samples extracted and analyzed for glyphosate and AMPA.

Behavior of Glyphosate in Sterile Soil. Sterile soils (3.5 g) were aseptically weighed into previously sterilized, silanized glass incubation vessels in a sterile air cabinet (four replicates per soil). The vessels were sealed and the contents allowed to equilibrate at their intended temperature overnight.

After equilibrating for temperature, the flasks were opened and 1 mL of sterilized glyphosate solution (59.2 mM) was added. When necessary, sufficient sterile deionized water was added to bring each soil to 75% of its field capacity. The flasks were then sealed and incubated at 25 °C. The incubation vessels were sampled at the same intervals as in the previous experiment.

Extraction and Analysis for Glyphosate and AMPA. The instrumentation, procedure, and chemicals used in this study for the extraction, cleanup, derivatization, and analysis for glyphosate and AMPA by GLC have previously been described in full in Eberbach and Douglas (1991).

Extraction. In brief, the procedure was as follows: Soil samples (3.5 g) were extracted by shaking mechanically for 15 min in 30 mL of aqueous 0.1 M TEA (repeated twice). The supernatant from each extraction was pooled and then centrifuged at 2300*g* for 10 min. After centrifugation, the supernatant was cleaned in 5 mL of cation-exchange resin for 5 min, following which time the supernatant was transferred into a flask containing the 9 mL of anion-exchange resin. The contents of the flask were shaken for 10 min, after which time the supernatant was disposed. The contents of the flask were then rinsed in three washes of deionized water, after which the phosphonic acids were liberated from the exchange resin with 3 × 10 mL washes with 0.1 M HCl as in Lundgren (1986). The acid eluant was concentrated at 32 °C under reduced pressure to dryness. The sample was resuspended in 1 mL of deionized water and reduced again to dryness. The sample was then resuspended in 1 mL of deionized water in preparation for derivatization.

Derivatization. The derivatization procedure employed here has previously been described by Deyrup et al. (1985). In brief, the method employed was as follows: A 10 μL sample of extracted glyphosate in deionized water was added to a derivatization tube and evaporated to dryness at 100 °C under a stream of dry nitrogen. After the evaporation was complete and the tube had cooled, 120 μL of TFAA and 60 μL of trifluoroethanol were added, and the tube was capped and heated at 100 °C for 1 h. After derivatization, excess reagents were removed by flushing with dry nitrogen at room temperature and the glyphosate and AMPA derivatives resuspended in 200 μL of nanograde ethyl acetate; 0.5 μL samples were injected into the gas chromatograph.

Data Handling and Statistical Analysis. All data prior to and after transformation were tested for homogeneity of variance using Bartlett's test (Sokal and Rohlf, 1969) and proved to be homogeneous. Data were logarithmically transformed for the purpose of linearization. The transformed decomposition data were analyzed by linear regression, and from the regression coordinates pertinent kinetic parameters were calculated.

RESULTS AND DISCUSSION

The amounts of glyphosate and AMPA extracted from each of the four soils at both temperatures over the incubation period are shown in Figures 1 and 2, respectively.

Consistent with previous observations (Eberbach and Douglas, 1991), <50% of the amount of glyphosate applied was recovered from all soils 24 h after the addition of glyphosate except for the Culgoa soil when incubated at 10 °C (66%). In a previous paper, it was shown that <10% of the glyphosate initially added was metabolized and liberated as CO_2 in the initial 24 h

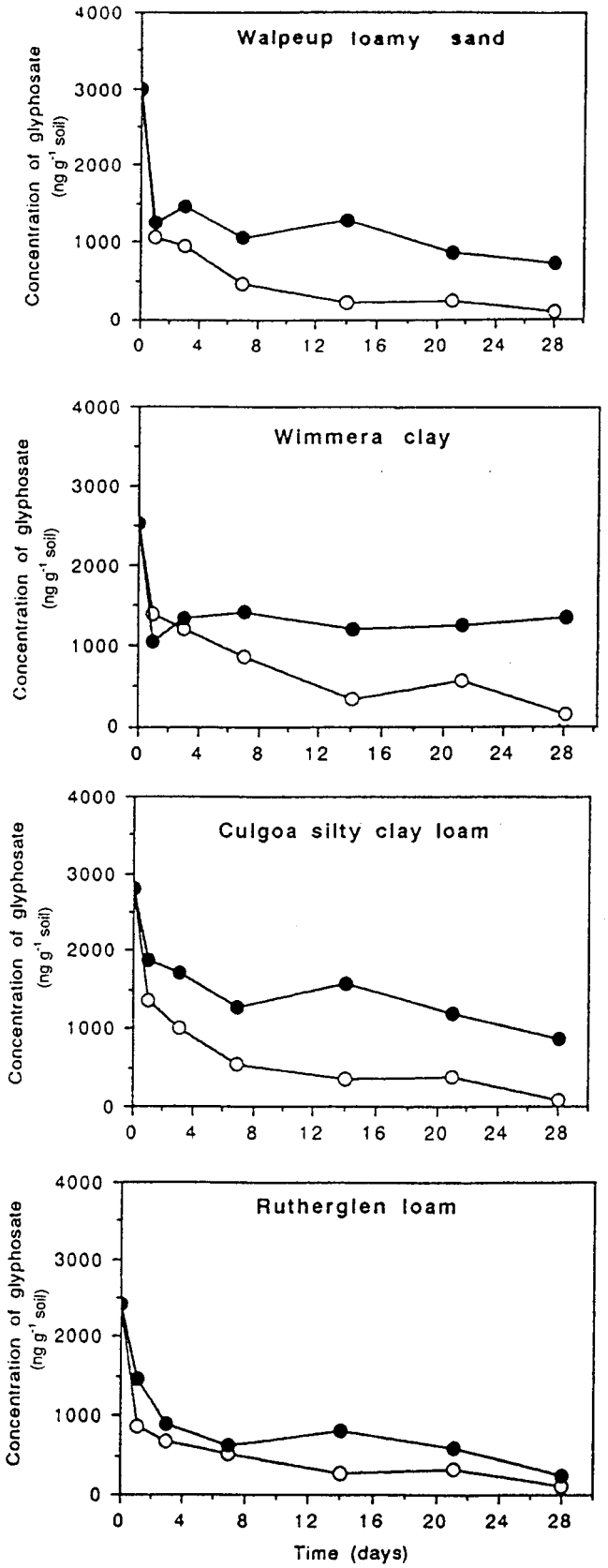

Figure 1. Recovery of TEA-extractable glyphosate in four soils at two temperatures: (○) 25 °C; (●) 10 °C.

period (Eberbach, 1998), and, as in the present study, little AMPA could be detected in soil during this period (Figure 2), it was assumed that the initial loss of extractable glyphosate was not due to metabolism but mainly to rapid sorption of the herbicide by soil solids.

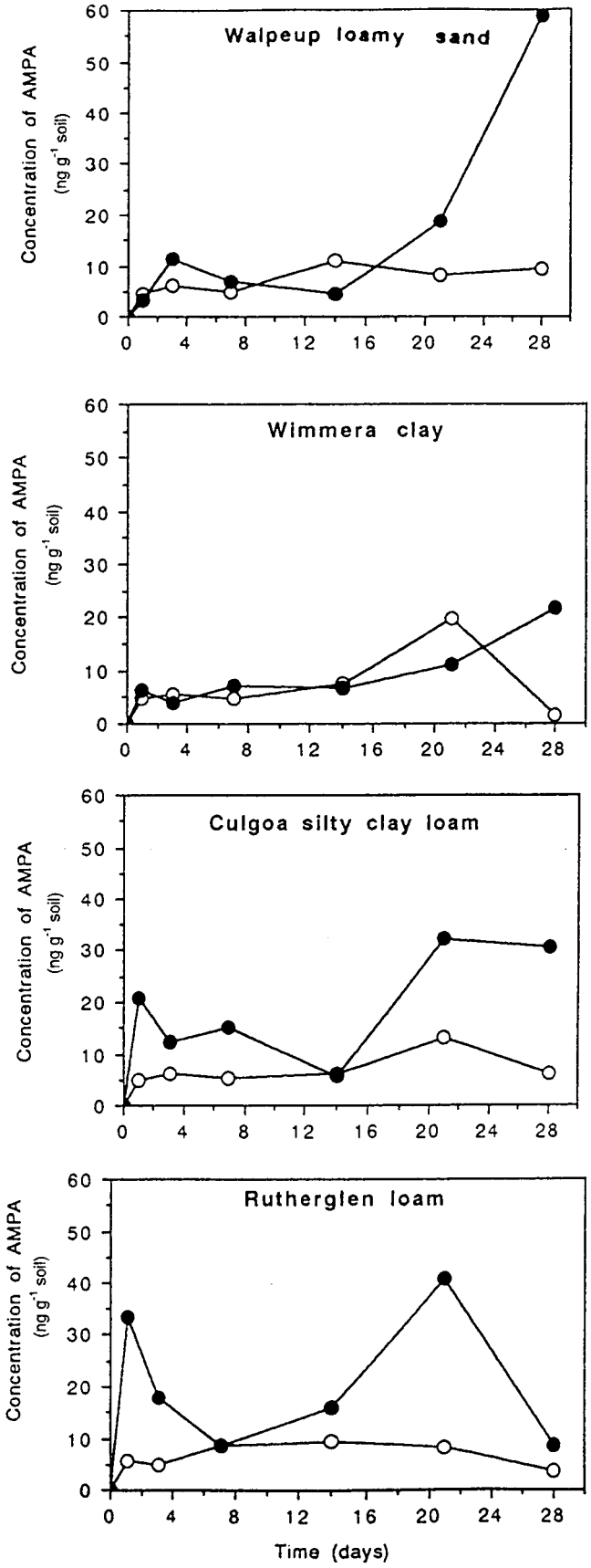

Figure 2. Production of TEA-extractable AMPA from four glyphosate-treated soils at two temperatures: (○) 25 °C; (●) 10 °C.

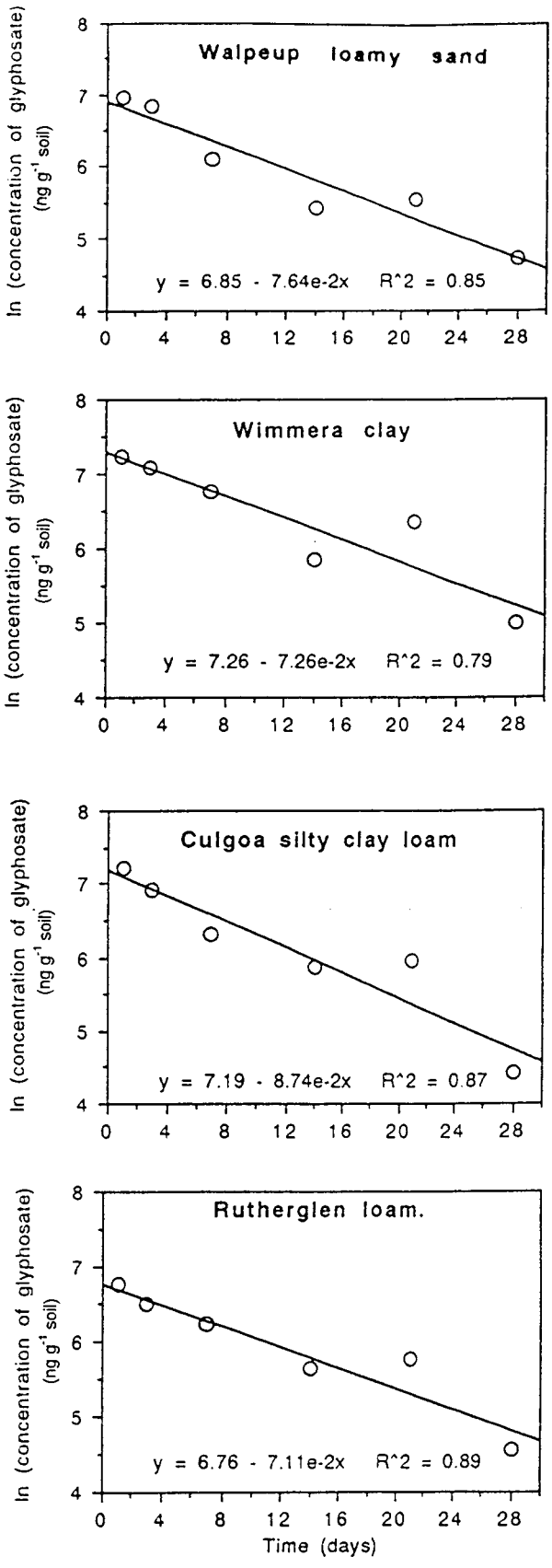

Figure 3. Natural logarithmic transformation of TEA-extractable glyphosate recovered from four soils incubated at 25 °C. The regression line applicable for each soil was calculated with time zero data excluded.

For each soil at both temperatures, depletion of the extractable glyphosate was not linear with respect to time (Figure 1), as confirmed by linear regression analysis. Transforming logarithmically the amount of

Table 2. Linear Regression and Regression Statistics of the Loss of TEA-Extractable Glyphosate from Four Victoria Soils at 25 and 10 °C

soil type[a]	incubation temp (°C)	regression eq[b]	R^2	F test	SE[c] of y	SE of b
Walpeup LS[c]	25	$\ln y = 6.85 - 0.076x$	0.85	111.9***[d]	0.32	0.007
	10	$\ln y = 7.25 - 0.022x$	0.55	25.5***	0.2	0.004
Wimmera C	25	$\ln y = 7.26 - 0.073x$	0.79	79.7***	0.39	0.008
	10	$\ln y = 7.09 + 0.003x$	0.02	0.42	0.23	0.005
Culgoa SiCL	25	$\ln y = 7.19 - 0.087x$	0.87	128.1***	0.36	0.008
	10	$\ln y = 7.49 - 0.023x$	0.55	24.8***	0.21	0.005
Rutherglen L	25	$\ln y = 6.76 - 0.071x$	0.89	162.1***	0.25	0.006
	10	$\ln y = 7.11 - 0.048x$	0.76	43.6***	0.32	0.007

[a] LS, loamy sand; C, clay; SiCL, silty clay loam; L, loam. [b] Equation in the form of $\ln y = a + bx$, where y = the loss of glyphosate from that particular phase, a = intercept (or pool size), b = gradient of the regression (rate constant), and x = time (days). [c] Standard error. [d] *** indicates significant at a probability of <0.1%.

Table 3. Rate Constants Describing the Loss of TEA-Extractable Glyphosate, Half-Life, and Calculated Pool Size of Extractable Glyphosate from Four Victoria Soils at 25 and 10 °C

soil type[a]	incubation temp (°C)	rate constant (ng day^{-1})	half-life (days)	pool size[b] (% of applied)
Walpeup LS	25	-7.64×10^{-2}	9.1	41.1
	10	-2.20×10^{-2}	31.5	61.1
Wimmera C	25	-7.26×10^{-2}	9.6	61.9
	10	3.26×10^{-3}	NA[c]	52.4
Culgoa SiCL	25	-8.74×10^{-2}	7.9	58.1
	10	-2.3×10^{-2}	30.0	78.3
Rutherglen L	25	-7.11×10^{-2}	9.8	37.5
	10	-4.84×10^{-2}	14.6	52.4

[a] LS, loamy sand; C, clay; SiCL, silty clay loam; L, loam. [b] Pool size calculated from the time zero (T_0) intercept from the regression equation and presented as a proportion of the amount of glyphosate initially added. [c] As the rate constant was positive, a prediction of the half-life pertaining to the loss of extractable glyphosate was unattainable.

glyphosate recovered from each soil slightly improved the regression relationship (data not shown); however, in each instance, rapid initial sorption still biased the regression. Hence, for each soil and temperature, regressions were performed in which time zero (T_0) data were excluded (Figure 3).

Exclusion of T_0 markedly improved R^2 values, decreased standard errors for the y and b coefficients, and allowed for the kinetics of TEA-extractable glyphosate to be more fully explored minus the effect of the initial sorption. Similarly, T_0 data have been excluded in previous studies [i.e., Thirunarayanan et al. (1985)] to more adequately explain the behavior of chlorsulfuron in soil.

Loss of Extractable Glyphosate from Nonsterile Soil at 25 °C. At each time interval, less glyphosate was extracted from soils incubated at 25 °C than at 10 °C (Figure 1). The general pattern depicting loss of extractable glyphosate at 25 °C was a curvilinear decomposition curve (Figure 1). Log-transformation of extractable glyphosate from each soil over the incubation period improved the linearity of the decomposition curve (Figure 3) (significant at $P < 0.001$, Table 2) and enabled the kinetics pertaining to loss of extractable glyphosate in these soils to be determined (Table 3).

For all soils except the Culgoa silty clay loam, the rate constants for loss of extractable glyphosate as measured in the present study were similar (Table 3) and concur, albeit slightly lower, with rate constants for the decomposition of ^{14}C-glyphosate from the same soils as estimated in a previous study (Eberbach, 1998). This numeric similarity between loss of extractable glyphosate as shown in the present study and decomposition of ^{14}C-glyphosate measured using evolution of $^{14}CO_2$ (Eberbach, 1998) suggests that at 25 °C, aqueous TEA extracts the readily decomposable fraction of glyphosate associated with the soluble phase. However, the slightly lower rate of loss of extractable glyphosate as measured here for the Walpeup, Wimmera, and Rutherglen soils suggests that while the pool of extractable glyphosate is being progressively depleted of glyphosate due to degradation, it is slowly replenished with glyphosate desorbed from the sorbed phase, but at a rate lower than the rate of decomposition. Hence, the amount of TEA-extractable glyphosate remaining in these soils decreases until the amount remaining reflects the rate of desorption of glyphosate from the sorbed phase as shown in Eberbach (1998).

Furthermore, the similarity between the rates of disappearance of extractable glyphosate and the decomposition of soluble glyphosate as measured in a previous study (Eberbach, 1998) suggests that once glyphosate undergoes decomposition, its metabolism is rapid and complete. Given that the analytical procedure employed in this paper has high sensitivity to soil residues of AMPA (Eberbach and Douglas, 1991), the lack of accumulation of AMPA as shown here (Figure 2) lends support to this assertion. It could therefore be speculated that AMPA is only a short-lived decomposition product and that the fraction of glyphosate that can be extracted using aqueous TEA readily undergoes cometabolism to CO_2.

The rates of loss of extractable glyphosate from the Culgoa soil was greater than previously reported rates of decomposition of the substrate from this soil (Eberbach, 1998). Slow adsorption of glyphosate into the nonextractable form may be occurring over time, which could account for this observation.

Loss of Extractable Glyphosate from Nonsterile Soil at 10 °C. Similar to that at 25 °C, the general pattern depicting loss of extractable glyphosate at 10 °C was a curvilinear decomposition curve (Figure 1). Log-transformation of extractable glyphosate over time markedly improved the linearity of the data for the Walpeup, Culgoa, and Rutherglen soils (significant at $P < 0.001$) but had little effect on the Wimmera soil data (Figure 4; Table 2). Rate constants, half-life, and pool-size calculated from regression equations of the transformed data are presented in Table 3.

The rates of loss of extractable glyphosate as reported here for the Walpeup and Rutherglen soils (2.02×10^{-2} and 4.75×10^{-2} ng day^{-1}, respectively) were approximately half the rates of decomposition of soluble ^{14}C-glyphosate as previously estimated for these soils (6.95×10^{-2} and 9.77×10^{-2} ng day^{-1}, respectively) at

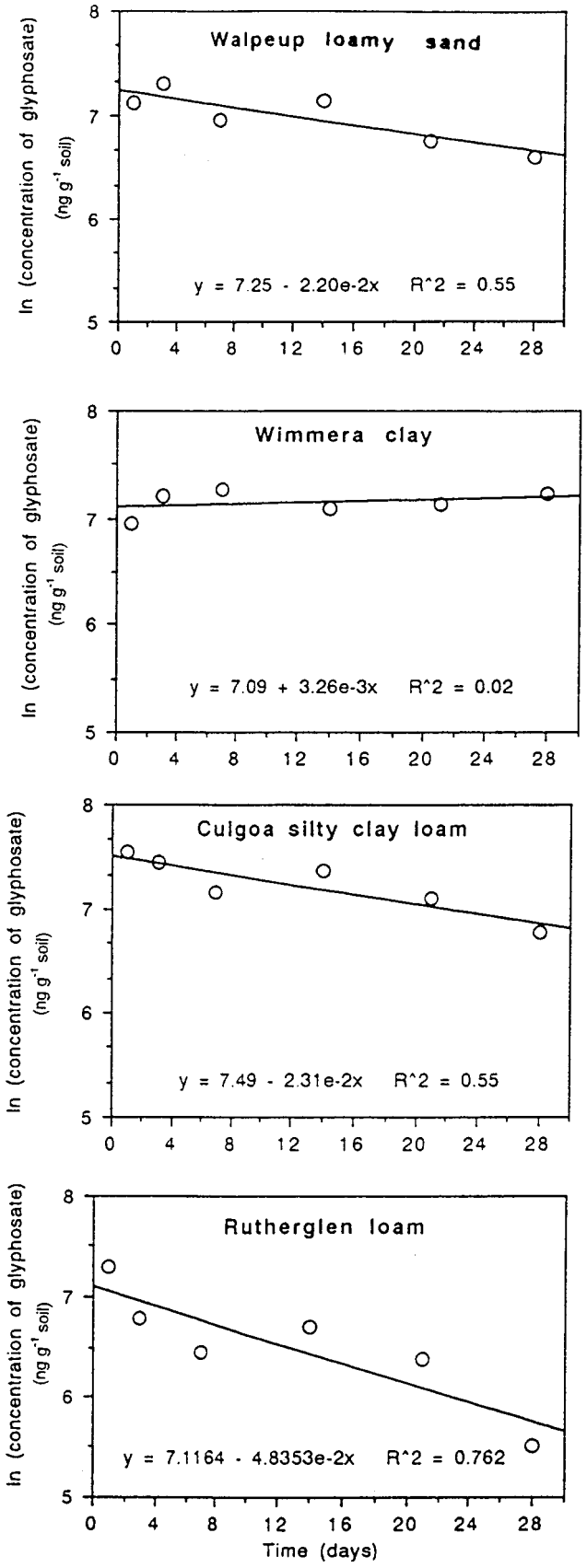

Figure 4. Natural logarithmic transformation of TEA-extractable glyphosate recovered from four soils incubated at 10 °C. The regression line applicable for each soil was calculated with time zero data excluded.

similar temperature (Eberbach, 1998). There are two possible explanations for this observation: incomplete extraction of glyphosate at this temperature relative to 25 °C or replenishment of extractable glyphosate from the sorbed form. Given that extractions were all performed at room temperature (22 °C) irrespective of the imposed incubation temperature, it is difficult to understand why extraction efficacy per se should be greater when samples were incubated at one temperature relative to another. Evidence from the present study and from the previous study (Eberbach, 1998) suggests that desorption of sorbed glyphosate may be an appreciable source of extractable glyphosate at lower temperatures.

No loss of extractable glyphosate occurred from the Wimmera soil over the 28 day incubation period (Figure 4; Table 2). As ^{14}C-glyphosate has been previously shown to decompose in this soil at this temperature (Eberbach, 1998) and as AMPA was detected in this soil at this temperature (Figure 2), we assume that desorption of sorbed glyphosate occurred at a rate similar to the rate of cometabolism of this herbicide in this soil at this temperature. Furthermore, as the regression for loss of extractable glyphosate in this soil was positive, desorption of glyphosate may have occurred at a greater rate than decomposition, increasing the concentration of soluble glyphosate in this soil at this temperature. This finding supports previous observations for this soil at 10 °C, when apparent desorption occurred at a faster rate than decomposition (Eberbach, 1998).

Although previous work has suggested that the decompositions of labile glyphosate are similar in the Wimmera and Culgoa soils at this temperature (Eberbach, 1998), data presented here suggest that the rate of loss of extractable glyphosate from the Culgoa soil is quite different from that in the Wimmera soil (Figure 4; Tables 2 and 3). The rate of loss of extractable glyphosate from this soil in the present study (2.3×10^{-2} ng day^{-1}) was an order of magnitude greater than the measured rate of decomposition (2.84×10^{-3} ng day^{-1}) from Eberbach (1998). Although the reason for this discrepancy is not completely apparent, similar to incubation at 25 °C, it is likely that further adsorption into the sorbed form may occur in this soil over time.

Influence of Temperature on Amount of Extractable Glyphosate and Its Half-Life. Previous work showed that lowering the incubation temperature acts to reduce the rate of decomposition of soluble glyphosate in soil, as well as to increase the apparent rate of desorption from the sorbed phase, particularly in neutral-alkaline soils (Eberbach, 1998). These observations suggest that the net affect of lowering temperature is likely to be an increase in the amount of soluble (extractable) glyphosate present in soil and is consistent with findings of the present study (Table 3). However, lowering the temperature also substantially increased the half-life of extractable glyphosate in three of the four soils examined. These findings therefore imply that when applied at lower temperatures such as those experienced during winter in the major cereal cropping regions of Australia, more glyphosate may reside in soil in the soluble form for longer time periods after application. Work is currently underway in our laboratory to elucidate the effect of temperature on the dynamic relationship between degradation and sorption of glyphosate.

Behavior of Glyphosate in Sterile Soil Incubated at 25 °C. Consistent with observations made using nonsterile soil, considerable loss of extractable glypho-

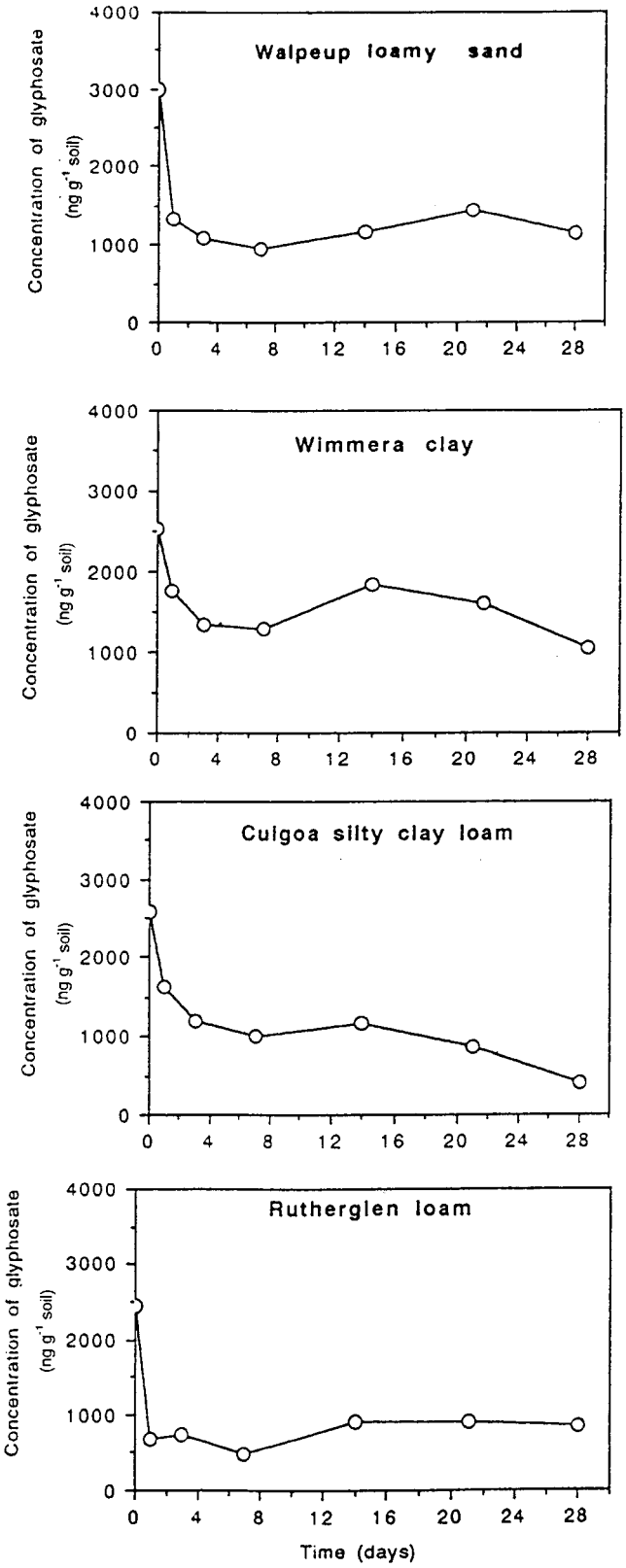

Figure 5. Recovery of TEA-extractable glyphosate from four sterile soils incubated at 25 °C.

sate occurred immediately after the addition of herbicide into soil (Figure 5).

Logarithmically transforming the amount of glyphosate extracted when T_0 data were excluded improved linearity of the data set with respect to time (Figure 6). For each soil and at each time period, no AMPA was detected in the extract; hence, it was assumed that any

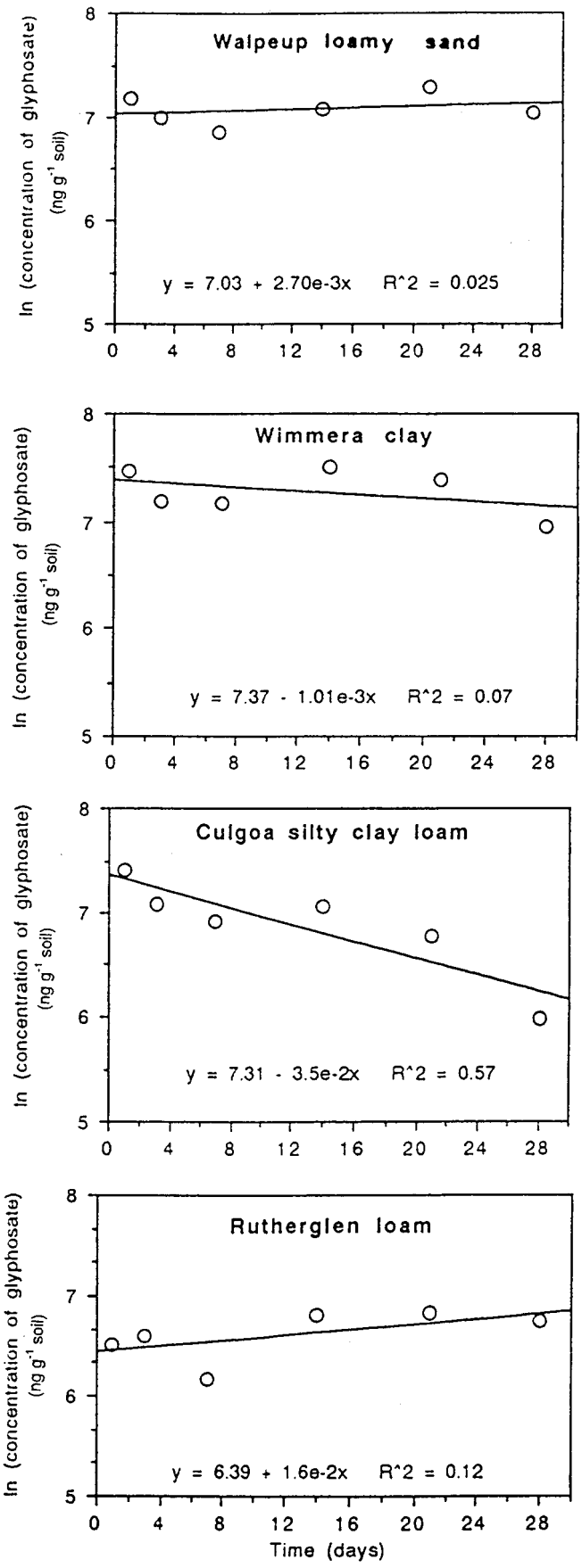

Figure 6. Natural logarithmic transformation of TEA-extractable glyphosate recovered from four γ-irradiated soils incubated at 25 °C. The regression line applicable for each soil was calculated with time zero data excluded.

Table 4. Linear Regression and Regression Statistics of the Loss of TEA-Extractable Glyphosate from Four γ-Irradiated Soils at 25 °C

soil type[a]	regression eq[b]	R^2	F test	SE[c] of y	SE of b
Walpeup LS	$\ln y = 7.03 + 0.0027x$	0.025	0.44	0.52	0.004
Wimmera C	$\ln y = 7.37 - 0.001x$	0.07	2.6	0.29	0.006
Culgoa SiCL	$\ln y = 7.31 - 0.035x$	0.57	28.6***[d]	0.27	0.007
Rutherglen L	$\ln y = 6.39 - 0.016x$	0.12	3.8	0.34	0.008

[a] LS, loamy sand; C, clay; SiCL, silty clay loam; L, loam. [b] Equation in the form of $\ln y = a + bx$, where y = the loss of glyphosate from that particular phase, a = intercept (or pool size), b = gradient of the regression (rate constant), and x = time (days). [c] Standard error. [d] *** indicates significant at a probability of <0.1%.

loss of extractable glyphosate was due to adsorption of the substrate into a nonextractable form and not due to decomposition.

For the Walpeup, Wimmera, and Rutherglen soils, only minor changes occurred in the status of sorption of glyphosate between the extractable and the strongly adsorbed form over the 28 day incubation period. Regression analysis of these functions was not significant (Table 4), which confirmed these observations. Hence, for these three soils we postulate that adsorption of glyphosate by the soil is complete within the first 24 h after addition of the substrate. In addition, further equilibration shifts between the extractable and nonextractable forms in the absence of external factors such as substrate degradation are minor. Similarly, equilibrium in the sorption of glyphosate from the soluble into the adsorbed phase was shown to have been achieved within 6 h in soils and clays when an aqueous solvent was used (Miles and Moye, 1988).

Unlike the other three soils, the amount of glyphosate extracted from the Culgoa soil decreased significantly ($P < 0.001$) over the incubation period (Figures 5 and 6; Table 4). Consistent with observations made in the studies using nonsterile soils, sorption equilibrium did not appear to be reached in this soil over the incubation period. Although poor sterilization may offer some explanation for the apparent loss of extractable glyphosate observed in this soil, no AMPA was measured in any of the extracts, which leads us to the inference that slow adsorption of glyphosate into the nonextractable form occurred over the 28 day incubation period. Other studies have shown that the pH of the soil/solvent system could influence the degree of adsorption of glyphosate (Glass, 1987) and the rate of onset of sorption equilibrium (Miles and Moye, 1988). In this study, Miles and Moye (1988) showed that when glyphosate was added to soil in an alkaline solvent, sorption equilibrium was not achieved after 120 h. Given that glyphosate is thought to bind to clays through the phosphonic acid moiety (Hance, 1976; Sprankle et al., 1975a), it is interesting to consider that slow adsorption of phosphorus also occurs in soil (Barrow, 1979; Barrow and Shaw, 1974; Madrid and de Arambaus, 1985; Munns and Fox, 1976). An explanation of this phenomenon is that molecular rearrangement may occur at the adsorption site, allowing the newly arranged crystalline surface to adsorb more substrate (Munns and Fox, 1976; Chen et al., 1973). A process similar to this was thought to influence the adsorption of glyphosate into the sorbed form in an alkaline soil (Miles and Moye, 1988). This too may account for the observations reported here for the Culgoa soil; however, given that the Wimmera soil also was alkaline, the apparent difference regarding rate to reach sorption equilibrium between these two soils is not at this stage understood.

Conclusion. Although insufficient data are available in the present study to enable prescriptive statements to be made regarding the influence of temperature on the dynamics of glyphosate in soils, evidence presented here does suggest that lower incubation temperatures increase the residence time of extractable glyphosate in soil. We postulate for the Walpeup, Wimmera, and Rutherglen soils that this is the net effect of a reduction in the rate of glyphosate cometabolism at 10 °C and an increase in the rate of desorption of glyphosate into the TEA-extractable form. However, for the Culgoa soil, a decrease in the amount of glyphosate extractable over time was due to cometabolism of the substrate and incomplete adsorption. Although further experimentation is required before an association can be confidently made between TEA-extractable glyphosate and bioactive residues of glyphosate in soil, it is likely that the forms of glyphosate extracted from soil by TEA include glyphosate in the bioactive form. This being the case, then the implications of these results and those from previous work (Eberbach, 1998) suggest that at lower temperatures, such as those experienced over winter in cool climate cropping zones, typical of much of southern Australia, more bioactive glyphosate may be present in some soils to affect susceptible plants. We are currently undertaking further work to investigate the influence of temperature on sorptive processes in relation to soil properties and the bioavailability of soil residues of glyphosate.

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Received for review July 20, 1998. Revised manuscript received February 26, 1999. Accepted March 5, 1999.

JF980785G