

## Interactions of Calcium Ions with Weakly Acidic Active Ingredients Slow Cuticular Penetration: A Case Study with Glyphosate

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Potassium and calcium salts of glyphosate were obtained by titrating glyphosate acid with the respective bases to pH 4.0, and rates of penetration of these salts across isolated astomatous cuticular membranes (CMs) were measured at 20 °C and 70, 80, 90, and 100% humidity. K-glyphosate exhibited first-order penetration kinetics, and rate constants ( $k$ ) increased with increasing humidity. Ca-glyphosate penetrated only when the humidity above the salt residue was 100%. At 90% humidity and below, Ca-glyphosate formed a solid residue on the CMs and penetration was not measurable. With Ca-glyphosate, the  $k$  value at 100% humidity decreased with time and the initial rates were lower than for K-glyphosate by a factor of 3.68. After equimolar concentrations of ammonium oxalate were added to Ca-glyphosate, high penetration rates close to those measured with K-glyphosate were measured at all humidities. Adding ammonium sulfate or potassium carbonate also increased rates between 70 and 100% humidity, but they were not as high as with ammonium oxalate. The data indicate that at pH 4.0 one  $\text{Ca}^{2+}$  ion is bound to two glyphosate anions. This salt has its deliquescence point near 100% humidity. Therefore, it is a solid at lower humidity and does not penetrate. Its molecular weight is 1.82 times larger than that of K-glyphosate, and this greatly slows down rates of penetration, even at 100% humidity. The additives tested have low solubility products and form insoluble precipitates with  $\text{Ca}^{2+}$  ions, but only ammonium oxalate binds  $\text{Ca}^{2+}$  quantitatively. The resulting ammonium salt of glyphosate penetrates at 70–100% humidity and at rates comparable to K-glyphosate. The results contribute to a better understanding of the hard water antagonism observed with glyphosate. It is argued that other pesticides and hormones with carboxyl functions are likely to respond to  $\text{Ca}^{2+}$  ions in a similar fashion. In all of these cases, ammonium oxalate is expected to overcome hard water antagonism.

**KEYWORDS:** Adjuvants; auxins; abscisic acid; foliar uptake; gibberellins; permeability; prohexadione calcium

### INTRODUCTION

Glyphosate is an ionic herbicide (1) with three ionizable groups; their  $\text{p}K_{\text{a}}$  values are 2.27, 5.57, and 10.25, respectively. Glyphosate acid has a low water solubility, and in commercial formulations, monovalent cations such as isopropylammonium, trimesium, ammonium, sodium, potassium, and ethanolamine are used to neutralize the carboxyl group that dissociates between pH 2 and pH 4 (2). These salts have a high water solubility, but in the presence of divalent cations such as  $\text{Ca}^{2+}$ , sparingly soluble salts and/or complexes are formed (3–5) and this reduces the rates of penetration and efficacy of glyphosate. This calcium antagonism has been studied extensively, and adding ammonium sulfate has been shown to overcome it

(6–12). However, the reasons for calcium antagonism are still not entirely clear as complex mixtures containing commercial formulations, various surfactants, and salts were employed in the studies of efficacy and penetration, and humidity was generally not considered an important variable. Recently, humidity and wetting have been shown to be the most important factors in the penetration of glyphosate salts (13). Rates of cuticular penetration increased by factors of 5–10 (depending on glyphosate salt) when the humidity increased from 70 to 100%. Metal ions form complexes with polyoxyethylene chains (14, 15). This has been overlooked in previous studies, even though these generally employed ethoxylated nonionic surfactants.

Smith and Raymond (4) prepared a crystalline glyphosate compound with the formula  $\text{Ca}(\text{O}_3\text{PCH}_2\text{NH}_2\text{CH}_2\text{CO}_2)(\text{H}_2\text{O})_2$  (formula weight: 243.96) and determined the solid state

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structure of the chelate by X-ray diffraction. The structure is polymeric. Each calcium ion bonds with four glyphosate molecules, and each glyphosate bonds with four calcium ions. A network of hydrogen bonds connects each glyphosate molecule to another glyphosate molecule and each water molecule to a glyphosate molecule. The nitrogen atom is protonated and, therefore, does not bind to the calcium (4). The solubility product ( $K_{sp}$ )

$$K_{sp} = [\text{Ca}^{2+}] \times [\text{Hgly}^{2-}] \quad (1)$$

of calcium glyphosate ( $\text{CaHgly}$ ) in saturated solutions and in the presence of  $\text{KNO}_3$  was determined at a pH ranging from 7 to 8. A  $\log K_{sp}$  value of  $-5.32$  was obtained. The data are consistent with a 1:1 calcium glyphosate complex. No evidence for a 1:2 calcium glyphosate species was found. Gauvrit et al. (16) used a commercial glyphosate formulation (Roundup) and measured biological effectiveness in the presence of various concentrations of  $\text{CaCl}_2$ . The efficacy decreased with increasing  $\text{CaCl}_2$  concentrations, and they interpreted their data as evidence for the formation of a 1:1 complex of calcium with glyphosate. Rates of penetration of glyphosate in the presence of various  $\text{CaCl}_2$  concentrations indicated that the calcium complex did not penetrate into barley leaves.

In plant cuticles, penetration of ionic species is confined to aqueous pores (13, 18) and size selectivity of these pores has been measured using calcium salts (19) with anhydrous molecular weights between 111 and 755  $\text{g mol}^{-1}$ . All salts penetrated the cuticle, but permeability decreased with increasing molecular weight, and half times of penetration increased from 1.6 to 23 h. From these data, it would appear that a  $\text{CaHgly}$  complex should readily penetrate. As already pointed out, in previous studies of efficacy or penetration rates of glyphosate in the presence of calcium ions, various nonionic surfactants and commercial formulations were used, and pH and humidity were rarely controlled and were not considered important in cuticular permeability. Various cations and anions were present in sprays and treatment solutions. Their contribution to the calcium antagonism is not known.

In an attempt to overcome these limitations, cuticular penetration of Ca-glyphosate was studied at constant pH while the humidity was controlled and varied. No ions other than Ca-glyphosate were present, and ethoxylated surfactants and other salts were not used. The size of the penetrating glyphosate species was estimated by comparing rates of penetration with those of other calcium salts varying in molecular weights.

## MATERIALS AND METHODS

**Plant Material.** Experiments were conducted using astomatous adaxial cuticular membranes (CMs) of mature leaves from gray poplar [*Populus x canescens* (Aiton) Sm.]. They were isolated enzymatically (20), dried, and stored at 8 °C until used. Poplar CMs had been used previously as model CMs to study glyphosate penetration as affected by cations, humidity, and surfactants (13).

**Chemicals.** Donor solutions were prepared by dissolving glyphosate acid (Bayer, Leverkusen, Germany; 99% purity) in deionized water and titrating the solutions to pH 4.0 with KOH or  $\text{Ca}(\text{OH})_2$ . Stock solutions with a concentration of 0.04  $\text{mol L}^{-1}$  were prepared. Titration was stopped at pH 4.0 because at higher pH a precipitate was formed [ $\text{Ca}(\text{OH})_2$ ]. [ $^{14}\text{C}$ ]Glyphosate, labeled in the methyl position (American Radiolabeled Chemicals, St. Louis, MO; specific activity, 6.66 GBq  $\text{mol}^{-1}$ ; radiochemical purity, 99%), was added as a tracer (approximately 200 Bq  $\mu\text{L}^{-1}$ ). The penetration of  $\text{CaCl}_2$ , Ca-gluconate, and Ca-glyphosate was also measured using  $^{45}\text{CaCl}_2$  (NEN, Boston, MA; specific activity, 44.7 GBq  $\text{mmol}^{-1}$ ; radiochemical purity, 99.9%) as the tracer (approximately 200 Bq  $\mu\text{L}^{-1}$ ). The concentration of the

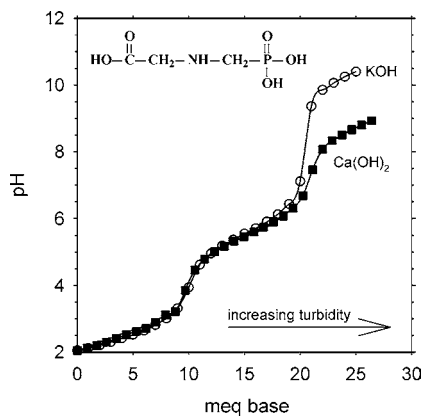
treatment solutions was 0.02  $\text{mol L}^{-1}$ . The nonionic wetting agent C8/10-alkylpolyglucoside Glucocon 215 CSUP (Fluka, Neu-Ulm, Germany) was added to donor solutions at a concentration of 0.25  $\text{g L}^{-1}$ . A citric acid buffer (2  $\text{g L}^{-1}$ ) was used as receiver solution, and its pH was adjusted to 6.0 using KOH. Receiver solutions contained  $10^{-3}$   $\text{mol L}^{-1}$  sodium azide to prevent the growth of microorganisms during experimentation. Donor solutions were freshly prepared immediately before use, and sodium azide was not added to avoid contamination of the donor with  $\text{Na}^+$ . Chemicals were obtained from Fluka, and with the exception of the surfactant, they were of purum or purissima grade.

**Penetration Experiments.** Rates of cuticular penetration were measured using SOFU (simulation of foliar uptake) as described in detail elsewhere (13, 21). The method allows studying the time course of cuticular penetration for each CM separately, and CMs can be used repeatedly. CMs were mounted between the lid and the bottom of desorption chambers using silicon grease (Baysilon, Bayer). After it was mounted, each CM was tested for leaks and imperfections (15), and the chambers were filled with receiver solution and placed into the wells of a thermostated aluminum block, which was rocked at 90 rpm for mixing of receiver solutions. After an equilibration period of about 24 h, the receiver solutions were withdrawn and a 5  $\mu\text{L}$  drop of donor solution was applied to the center of each CM. In a previous study using proline as an ionic model solute, no evidence for droplet size effects on rates of cuticular penetration had been observed (22), and for this reason, relatively large drops were used, which covered a large part of the area of the CM exposed inside the lid. The water evaporated within 60 min when a stream of ambient air was blown over the droplets. The chambers were again filled with receiver solution and returned to the aluminum block. During penetration, air of constant humidity was blown over the salt residue on the CMs to ensure a constant level of hydration of the salt residues on the CMs. The air flow rate was 2.5  $\text{mL s}^{-1}$ , and with an air volume of 0.25  $\text{mL}$  over the CM, this equated to 10 air changes per second. The humidity of the air was adjusted using the dew point method (KF-24 Kältefalle, Heinz Walz GmbH, Effeltrich, Germany). In some instances, when penetration was measured at constant 100% humidity, the donor compartment was closed with a transparent adhesive film (Tesafilem, Beiersdorf, Hamburg) after droplet drying. Receiver solutions were quantitatively withdrawn for scintillation counting and replaced by fresh ones in intervals ranging from 2 to 10 h. After the last sample had been taken, the CMs were washed to remove the salt residues that had not penetrated. For the first wash, 0.3  $\text{mL}$  of 0.1 N HCl was used. After 15 min, three additional washes with deionized water (300  $\mu\text{L}$ ) followed and the combined washes were assayed for radioactivity. In this way, the same set of CMs could be used for all experiments. The amount applied ( $M_0$ ) was obtained by summing up amounts penetrated plus the amount left on the CM for each individual CM. Recoveries ranged from 95 to 100%. The radioactivity of samples was determined at constant quench using a Wallac 1409 liquid scintillation counter (Wallac, Turku, Finland) set to a  $2\sigma$  error of 2%.

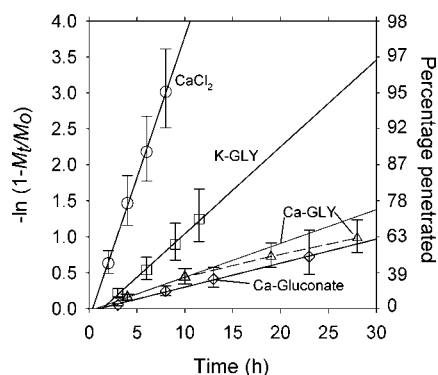
Data were plotted as  $-\ln(1 - M_t/M_0)$  vs time ( $t$ ) where  $M_t$  is the amount of  $^{14}\text{C}$ -glyphosate (or  $^{45}\text{Ca}$ -salt) recovered from the desorption medium at time  $t$ . When the natural logarithm of the fraction left on the CM is plotted against time, the slope of the line is the first-order rate constant ( $k$ ). Variability among individual CM can be large (23), and for this reason, 40 CMs were used as replicates, and arithmetic means and 95% confidence intervals were calculated from log-transformed data.  $-\ln(1 - M_t/M_0)$  was calculated for each CM separately, and arithmetic means with 95% confidence intervals are presented below.

## RESULTS

When glyphosate acid (10  $\text{mmol L}^{-1}$ ) was titrated with KOH, the three ionizable groups could be clearly identified (Figure 1). Ten milliequivalents of KOH was needed to neutralize the carboxyl group of glyphosate and reach pH 4.0. Up to pH 4.0, the titration curves obtained with  $\text{Ca}(\text{OH})_2$  and KOH were superimposed on each other and the solution remained clear. At higher pH values, the solution became increasingly turbid



**Figure 1.** Titration of 10 mmol of glyphosate acid dissolved in 1 L of deionized water at 20 °C with KOH or  $\text{Ca}(\text{OH})_2$ . The chemical structure of glyphosate is shown as an inset.

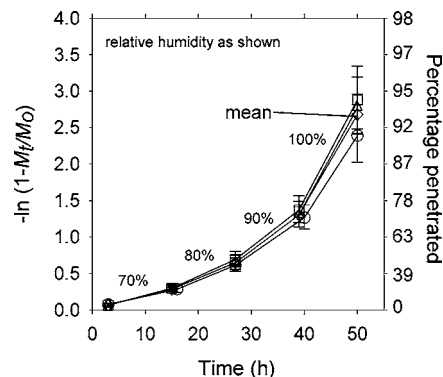


**Figure 2.** Time course of cuticular penetration of  $\text{CaCl}_2$ , Ca-gluconate, K-glyphosate, and Ca-glyphosate at 100% humidity and 20 °C. The same set of CMs was used for all four salts. Means of 40 CMs and 95% confidence intervals are shown.

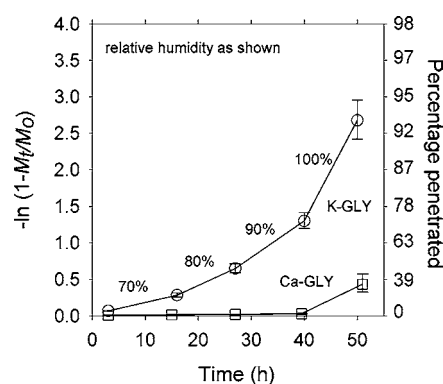
when  $\text{Ca}(\text{OH})_2$  was used and a precipitate formed, even though the solution was well-stirred and the base was added very slowly. Above pH 4.0, the two titration curves began to diverge, and near pH 6, the titration curve obtained with  $\text{Ca}(\text{OH})_2$  was clearly below that obtained with KOH. For penetration experiments, stock solutions ( $0.04 \text{ mol L}^{-1}$ ) were prepared by titration with either KOH or  $\text{Ca}(\text{OH})_2$  up to a pH of 4.0. These solutions were clear and stable over weeks when stored at 4 °C. The glyphosate salts used in the experiments will be abbreviated as K-glyphosate and Ca-glyphosate, respectively.

Plotting  $-\ln(1 - M_t/M_0)$  vs time resulted in linear plots with  $\text{CaCl}_2$ , K-glyphosate, and Ca-gluconate (**Figure 2**). Slopes of these lines are the first-order rate constants ( $k$ ), and they amounted to 0.393 ( $\text{CaCl}_2$ ), 0.121 (K-glyphosate), and 0.033 (Ca-gluconate)  $\text{h}^{-1}$ , respectively. With Ca-glyphosate, a non-linear plot with decreasing rate constants was obtained. The slope for the 4–10 h interval was  $0.047 \text{ h}^{-1}$ .

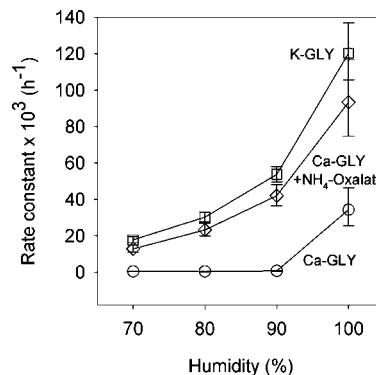
CMs were robust, and results were repeatable, as seen in **Figure 3**, where penetration of K-glyphosate was studied (with the same set of CMs) in weekly intervals and humidity over the residue was varied. The experiment was started at a humidity of 70%. After 10–12 h, the receiver sample was drawn, new receiver was added, and the humidity was increased to 80%. This procedure was repeated with 90 and 100% humidity. The new dew point was established almost instantaneously as the Peltier cold trap responded within 30 s. A similar experiment was conducted with Ca-glyphosate. With this compound, penetration took place only at 100% humidity (**Figure 4**).



**Figure 3.** Effect of relative humidity above the donor on cuticular penetration of K-glyphosate. The experiment was started at 70% humidity, and the humidity was increased after withdrawing the receiver and replacing it with fresh solution. The experiment was repeated three times in weekly intervals with the same set of CMs. Means and 95% confidence intervals are shown.

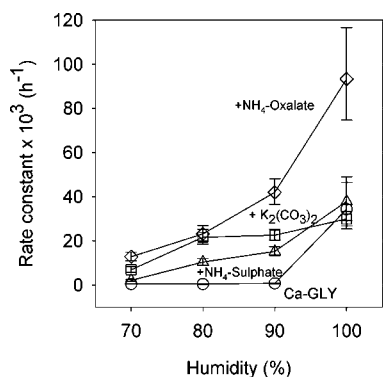


**Figure 4.** Effect of relative humidity above the donor on cuticular penetration of K-glyphosate (the mean of three experiments is shown in **Figure 3**) or Ca-glyphosate. The experiment was started at 70% humidity, and the humidity was increased after withdrawing the receiver and replacing it with fresh solution. The same set of CMs was used for both salts. Means and 95% confidence intervals are shown.



**Figure 5.** Rate constants of cuticular penetration of K-glyphosate, Ca-glyphosate, or an equimolar mixture of Ca-glyphosate with ammonium oxalate as affected by humidity above the donor. The same set of CMs was used for all salts. Means and 95% confidence intervals are shown.

Mixing  $20 \text{ mmol L}^{-1}$  Ca-glyphosate with  $20 \text{ mmol L}^{-1}$  ammonium oxalate immediately resulted in the formation of a whitish precipitate. If this mixture was used as donor, rate constants of penetration were much higher than with Ca-glyphosate alone and almost as high as with K-glyphosate, especially at low humidity (**Figure 5**). At the end of the experiment, crystalline precipitates were visible on the CMs under the binocular in the Ca-glyphosate and the Ca-glyphosate



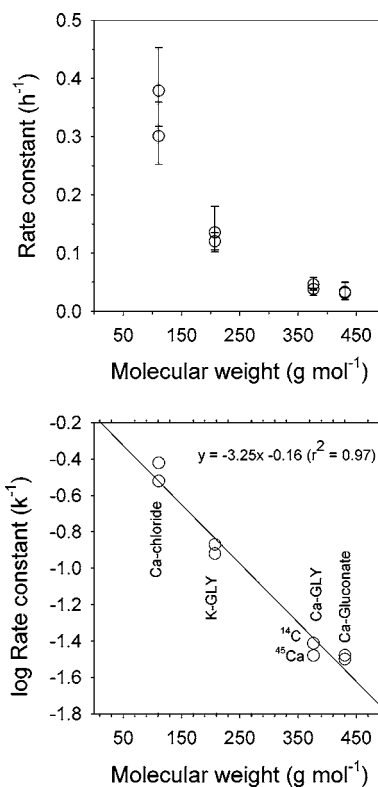
**Figure 6.** Rate constants of cuticular penetration of Ca-glyphosate or equimolar mixtures of Ca-glyphosate with ammonium oxalate, potassium carbonate, or ammonium sulfate as affected by humidity above the donor. The same set of CMs was used for all salts. Means and 95% confidence intervals are shown.

+ ammonium oxalate treatments but not with K-glyphosate. The precipitates were soluble in 0.1 N HCl used for washing the CMs at the end of the experiment.

Adding ammonium sulfate or potassium carbonate (20 mmol L<sup>-1</sup>) to the Ca-glyphosate donor (20 mmol L<sup>-1</sup>) resulted in significant penetration rates even at 70–90% humidity (**Figure 6**). However, rate constants of penetration were lower than with ammonium oxalate, especially at 90 and 100% humidity. When ammonium sulfate or potassium carbonate was added to Ca-glyphosate, the solutions remained clear for some time, but precipitates formed after several hours. Crystalline residues could be seen under the binocular at the end of the penetration experiment, but they dissolved in 0.1 N HCl.

## DISCUSSION

When the carboxyl group ( $pK_a = 2.27$ ) was titrated with KOH, the end point of titration was reached at about pH 4.0, and for 1 mol of COOH groups, 1 mequiv of KOH was consumed. With Ca(OH)<sub>2</sub> at pH 4.0, one Ca<sup>2+</sup> ion neutralized two carboxyl groups; that is, 2 mol of glyphosate reacted with 1 mol of Ca<sup>2+</sup>. Hence, the molar ratio of calcium to glyphosate was 1:2, which is clearly in conflict with the conclusion drawn by Smith and Raymond (4), Sundaram and Sundaram (5), Gauvrit et al. (16), and de Ruiter et al. (17), who reported a 1:1 relationship. The molecular weight of the Ca-glyphosate species that penetrated the cuticles can be estimated as the size selectivity of poplar CMs is known (19). CaCl<sub>2</sub> and Ca-gluconate were included in the present study to serve as molecular weight reference points. Data from **Figure 2** and from an additional experiment are plotted in **Figure 7**. Measurements were made at 100% humidity. Rate constants of penetration decreased with increasing molecular weight (**Figure 7**). A good linear relationship was obtained when log  $k$  was plotted against the anhydrous molecular weights. The slope of the plot ( $-3.25$ ) is a quantitative measure of size selectivity. The plot indicates that the molecular weight of Ca-glyphosate is close to 360 g mol<sup>-1</sup>, which is similar to the molecular weight of Ca(H<sub>2</sub>Gly)<sub>2</sub> (376.3 g mol<sup>-1</sup>). This clearly demonstrates that during initial penetration (**Figure 2**) each Ca<sup>2+</sup> ion was accompanied by two glyphosate anions. Identical rate constants for <sup>45</sup>Ca-glyphosate and Ca-<sup>14</sup>C-glyphosate were obtained (**Figure 7**). This shows that electroneutrality was maintained during penetration and two glyphosate anions accompanied each Ca<sup>2+</sup> ion. Clearly, counterion exchange with K<sup>+</sup> or Na<sup>+</sup> from the donor did not occur. Our data are not compatible with the penetration of very large



**Figure 7.** Effect of anhydrous molecular weight on rate constants of penetration of <sup>45</sup>CaCl<sub>2</sub>, <sup>45</sup>Ca-gluconate, <sup>45</sup>Ca-glyphosate, Ca-<sup>14</sup>C-glyphosate, and K-<sup>14</sup>C-glyphosate. Results of duplicate determinations using the same set of CMs are shown. Means and 95% confidence intervals of 40 CMs are shown.

complexes present in the solid state or with the complex CaHGly characterized by Smith and Raymond (4).

Rate constants of penetration of Ca-glyphosate decreased with time, while with the other compounds this was not the case (**Figure 2**). Penetration was studied at 100% humidity, and the concentration of the residue on the CMs is not known. The donor had an initial concentration of 0.02 mol L<sup>-1</sup>, but during droplet drying, most water evaporated. However, the salt residue became rehydrated when air saturated with water vapor was blown over the residue. Likewise, when the donor compartment was closed with Tesafilm, water penetrated from the receiver solution across the CMs and the air over the salt residue quickly reached 100%. The solubility of Ca-glyphosate was not determined, but the concentration of the stock solution was 0.04 mol L<sup>-1</sup>, and Ca-glyphosate did not precipitate at 4 °C. The product of the molar concentrations of Ca<sup>2+</sup> (0.02) and glyphosate (0.04) in the stock solutions is  $8 \times 10^{-4}$ . Even though the solution was not saturated, this is much higher than the solubility product of  $4.8 \times 10^{-6}$  measured by Smith and Raymond (4). Again, this is good evidence that the Ca salt of glyphosate formed by titrating glyphosate acid with Ca(OH)<sub>2</sub> up to pH 4.0 differed in structure from the complex formed at pH 7–8 when the phosphonate groups are also ionized and interact with Ca<sup>2+</sup> ions (4, 5, 16, 17). These complexes clearly have a lower solubility as seen from the fact that precipitates formed when titration was continued beyond pH 4.0 (**Figure 1**). During droplet drying, concentrations much higher than 0.04 mol L<sup>-1</sup> were reached and crystals were observed on the CMs when the experiment was terminated. Thus, the decrease with time of rate constants (**Figure 2**) might have been due to a decrease in driving force (concentration) with time by crystallization from a supersaturated solution. Other processes such as the formation of

associates with higher molecular weight as observed by Thelen et al. (8) may also have contributed to the decrease in rate constants.

Ca-glyphosate did not penetrate the CMs at humidity below 100%, while K-glyphosate penetrated, albeit at lower rates (Figure 3). Dissolution of a salt is the prerequisite for penetration, and this depends on humidity and the point of deliquescence (POD) (21). Data in Figure 4 clearly show that POD of Ca-glyphosate is above 90% and probably near 100%, while POD for K-glyphosate is below 70%. Thus, efficacy of glyphosate in the presence of  $\text{Ca}^{2+}$  ions greatly depends on humidity, and 100% humidity is required for penetration to occur.

Our data show that two factors are involved in calcium antagonism: (i) Ca-glyphosate has a much higher molecular weight than K-glyphosate, and for this reason, the rate constant of penetration ( $k$ ) of Ca-glyphosate is much lower (Figure 2). From the data of Figures 2 and 4, half times of penetration ( $t_{1/2} = \ln 0.5/k^{-1}$ ) at 100% humidity of 5.7 and 21 h are obtained for K- and Ca-glyphosate, respectively. The molecular weight of Ca-glyphosate is larger by a factor of 1.82, and this increased the half time of penetration by a factor of 3.68. (ii) For penetration of Ca-glyphosate to occur, humidity must be 100%, while K-glyphosate penetrates at considerably lower humidity. The dependence on humidity of rate constants observed with monovalent glyphosate salts has been attributed to swelling of CMs (13, 21). This crucial role of humidity on efficacy of glyphosate and on calcium antagonism has been overlooked in the past.

Mixing equimolar quantities of Ca-glyphosate with ammonium oxalate results in an insoluble Ca-oxalate precipitate. The glyphosate salt is in the ammonium form, which is soluble and has a POD below 70%, and the dependence on humidity of its penetration rates was similar to that found with K-glyphosate (Figure 5). This is in agreement with results obtained in a previous study, which also demonstrated that rate constants of penetration of K-glyphosate were identical at pH 4.0, 7.7, and 9.5 (13).  $\text{Ca}^{2+}$  ions react with  $\text{SO}_4^{2-}$  and form  $\text{CaSO}_4 \times 2\text{H}_2\text{O}$ , but water solubility ( $8.8 \text{ g L}^{-1}$ ) (24) and the  $K_{\text{sp}}$  ( $7.1 \times 10^{-5}$ ) (24) of this salt are relatively high as compared with Ca-oxalate  $\times \text{H}_2\text{O}$ , for which  $K_{\text{sp}}$  is  $2.34 \times 10^{-9}$  (24). The solubility product for  $\text{CaCO}_3$  is similar to that for Ca-oxalate ( $4.96 \times 10^{-9}$ ) (24), but in equilibrium with  $\text{CO}_2$  in air,  $\text{Ca}(\text{HCO}_3)_2$  is formed (24) and its solubility amounts to  $0.85 \text{ g L}^{-1}$ . It follows from these physicochemical data that after mixing Ca-glyphosate with equimolar concentrations of  $(\text{NH}_4)_2\text{SO}_4$  or  $\text{K}_2\text{CO}_3$  significant concentrations of  $\text{Ca}^{2+}$  ions occur in donor solutions and a fraction of the glyphosate is still present as Ca-glyphosate in addition to K- or  $\text{NH}_4$ -glyphosate, as was suggested before (7). In the experiment depicted in Figure 6, rate constants were measured for equimolar mixtures of Ca-glyphosate, ammonium oxalate, or potassium carbonate. Donor solutions were freshly prepared, and rate constants were measured with increasing humidity, starting at 70%. With ammonium oxalate, the only glyphosate salt present was ammonium-glyphosate and it penetrated at rates similar to K-glyphosate (Figures 5 and 6). With the other two additives, a mixture of glyphosate salts occurred in the donor and the glyphosate in the form of a monovalent salt penetrated faster, by virtue of the lower molecular weight and the lower POD, as compared with Ca-glyphosate. Hence, the ammonium glyphosate and K-glyphosate gradually disappeared from the donor and after 100 h only Ca-glyphosate was left, and it penetrated at rates comparable to those measured with pure Ca-glyphosate (Figure 6).

Data shown in Figure 6 clearly demonstrate that the current practice of adding ammonium sulfate to overcome calcium antagonism is less than optimum. Potassium carbonate performed somewhat better, but both chemicals resulted in glyphosate penetration rates that were much lower than those obtained with ammonium oxalate. The amounts needed depend on the hardness of water, which usually ranges from 1 to 4 mmol  $\text{L}^{-1}$ . However, in some areas of Germany, up to 30 mmol  $\text{L}^{-1}$  has been found (25). With a glyphosate dose of 200  $\text{g ha}^{-1}$  applied in a spray solution volume of 200 L, the glyphosate concentration in the tank would be about 6 mmol  $\text{L}^{-1}$ . If hard water with a  $\text{Ca}^{2+}$  concentration of 3 mmol  $\text{L}^{-1}$  is used, the entire glyphosate would be in calcium form and it would penetrate only at 100% humidity. Adding 85.2 g of ammonium oxalate would quantitatively precipitate the calcium ions, and penetration of ammonium oxalate would take place at much higher rates, because the molecular weight is much lower. Penetration at humidity below 100% would be possible because the POD of ammonium oxalate is below 70% humidity.

In a previous communication (13), it was shown that rates of penetration of monovalent glyphosate salts were increased about 4-fold by adding 0.2 g  $\text{L}^{-1}$  Glucopon 215 CSUP. This is an alkylpolyglycoside surfactant (APG), and at 0.2 g  $\text{L}^{-1}$ , the static surface tension is around 30 mN  $\text{m}^{-1}$ . This ensures good wetting and access of ions to the aqueous pores in cuticles. In commercial or experimental formulations of glyphosate, tallow amine or alcohol ethoxylates have been used, usually at much higher concentrations. However, as long as good wetting was ensured with 0.2 g  $\text{L}^{-1}$  Glucopon 215 CSUP, adding other adjuvants did not further increase rates of penetration (18). Therefore, an optimum formulation consists of glyphosate with the carboxyl group neutralized by a monovalent cation, a small amount of an APG surfactant, and some ammonium oxalate to prevent hard water antagonism caused by  $\text{Ca}^{2+}$  ions. However, we have not tested if concentrations of ammonium oxalate in excess of those necessary to balance water hardness might be phytotoxic, as they also penetrate into leaves, and they might upset calcium homeostasis. Alternatively, ammonium oxalate could scavenge  $\text{Ca}^{2+}$  in the cell wall thus increasing glyphosate mobility. This needs clarification.

Many pesticides, growth regulators, and hormones are weak electrolytes with carboxyl groups (i.e., auxins, gibberellins, and abscisic acid). Prohexadione-calcium is even marketed as a sparingly soluble calcium salt. All of these react with  $\text{Ca}^{2+}$  ions in hard water, and the calcium salts have a molecular weight almost twice as high as their  $\text{K}^+$  salts. PODs are probably very high, and for penetration, a very high humidity would be needed. These effects will slow penetration and may prevent it altogether at low humidity. Adding a small amount of ammonium oxalate or some other effective scavenger of  $\text{Ca}^{2+}$  ions could overcome these problems.

#### ABBREVIATIONS USED

CM, cuticular membrane;  $k$ , rate constant of penetration;  $t_{1/2}$ , half time of penetration;  $M_0$ , amount applied;  $M_t$ , amount penetrated at time  $t$ .

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