COMMUNICATIONS

Dinitroaniline Herbicides Adsorb to Glass

Constant concentrations of dinitroaniline herbicides in aqueous solutions are difficult to maintain because these compounds adsorb to glass. In 1% ethanol in water solutions, only oryzalin and nitralin maintained constant concentrations near 5 μM. This concentration of oryzalin is 2 times the concentration required to inhibit cell division in *Chlamydomonas* by 90% (LC_{90}), whereas this concentration of nitralin is less than its LC_{90} value. It is recommended that oryzalin be used as the model dinitroaniline compound for in vitro herbicide studies and that the solubility of any dinitroaniline compound be determined before quantitative in vitro analyses are attempted.

Dinitroaniline herbicides disrupt mitosis in meristematic cells of seedling plants by inhibiting the formation of microtubules (Bartels and Hilton, 1973; Jackson and Stetler, 1973; Hess and Bayer, 1974). For further understanding of the biochemical mechanism of action of these compounds, in vitro analyses must be employed. Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) is often considered as the model for dinitroaniline herbicide studies (Parka and Soper, 1977). However, constant concentrations of trifluralin in aqueous solution are difficult to maintain because trifluralin binds to glass. Exact trifluralin concentrations must be known before researchers can accurately determine concentrations responsible for in vitro physiological and biochemical plant responses. The purposes of this study were (1) characterize trifluralin sorption to glass and (2) find a substrate to which trifluralin does not bind or find a dinitroaniline herbicide that can be maintained at a constant concentration in aqueous solution.

MATERIALS AND METHODS

Lilly Research Laboratories (Greenfield, IN) supplied technical-grade and ^{14}C-labeled trifluralin, isopropalin (2,6-dinitro-*N*,*N*-dipropylcumidine), and oryzalin (3,5-dinitro-N^4,N^4-dipropylsulfanilamide). Shell Development Co. (Modesto, CA) supplied technical-grade and ^{14}C-labeled nitralin [4-(methylsulfonyl)-2,6-dinitro-*N*,*N*-dipropylaniline], and American Cyanamid Chemical Co. (Princeton, NJ) supplied technical-grade and ^{14}C-labeled pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine]. Stock solutions of ^{14}C-labeled herbicides were prepared in either 95% ethanol or 10% ethanol in water. Ten-microliter samples of the stock solutions were added to 990 μL of water in 5-mL siliconized (Sigmacote, Sigma Chemical Co., St. Louis, MO) glass shell vials. The ethanol content of these aqueous solutions was either 1% or 0.1% (v/v). The aqueous solutions were mixed for 1 min and then were not disturbed during the remainder of the experiment. Two-hundred-microliter samples were removed from the aqueous solutions at 3, 10, 30, and 120 min after addition of the herbicide. The aliquots were diluted with Bray's aqueous scintillation fluid (Bray, 1960) and radioassayed for ^{14}C in a Beckman LS 7500 liquid scintillation spectrometer. Herbicide standards were prepared in 200 μL of water and diluted with Bray's scintillation fluid. All data reported are averages of four analyses, and in all cases, greater than 90% of the radioactivity was recovered.

Effective concentrations for herbicidal activity of the dinitroaniline compounds were determined with a *Chlamydomonas* algal bioassay (Hess, 1980). Various concentrations of technical-grade herbicide in Me_2SO (dimethyl sulfoxide) were added to 50-mL Erlenmeyer flasks containing 20 mL of nutrient solution plus algal cells (10^5 cells/mL). The final concentration of Me_2SO in the nutrient solution was 1%. The algal cell suspensions were incubated for 48 h, and cell populations were then measured with a coulter counter (Model Fn, Coulter Electronics Inc., Hialeah, FL). All data reported are averages of at least four analyses.

All data were analyzed for variance. Listed variances are ±one standard deviation from the mean. Where appropriate, means were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Trifluralin. The initial purpose of the research was to find a surface to which trifluralin in an aqueous solution would not bind. Preliminary studies with 1 μM solutions of [^{14}C]trifluralin in 1% ethanol were conducted with plastic (Nalgene), Teflon, stainless steel, glass, and siliconized glass containers, but the herbicide was not quantitatively recovered from any of these containers. A 1 μM trifluralin concentration is less than the maximum trifluralin concentration soluble in 1% ethanol in water, so the herbicide should not have precipitated from the aqueous solution. The reported solubility of trifluralin varies from a low value of <1 ppm ("Herbicide Handbook of the Weed Science Society of America", 1979) to a high value of 24 ppm (Windholz, 1976). We have chosen 1 ppm (3.0 μM) as the maximum concentration of trifluralin soluble in pure water. Siliconized glass was used in all of the following studies because it provided the best recovery of trifluralin in the preliminary studies, it was the easiest material to use, and adsorption studies could be done directly in 5-mL siliconized glass shell vials. These shell vials could also be used as liquid scintillation vials, thus avoiding loss during transfer.

At all concentrations tested, the amount of trifluralin in solution decreased with time, suggesting the compound either precipitates out of solution or binds to the walls of the glass container (Figure 1, lines A–C). The herbicide did not volatilize from the aqueous solutions because >90% of the radioactivity could be recovered in the aqueous samples. Sorption to the glass is the favored explanation because trifluralin, at an initial concentration of 0.5 μM in 1% ethanol, did not remain in solution (Figure 1, line C). This concentration is less than the maximum solubility of trifluralin in water, so the herbicide should not have precipitated from the aqueous solution.

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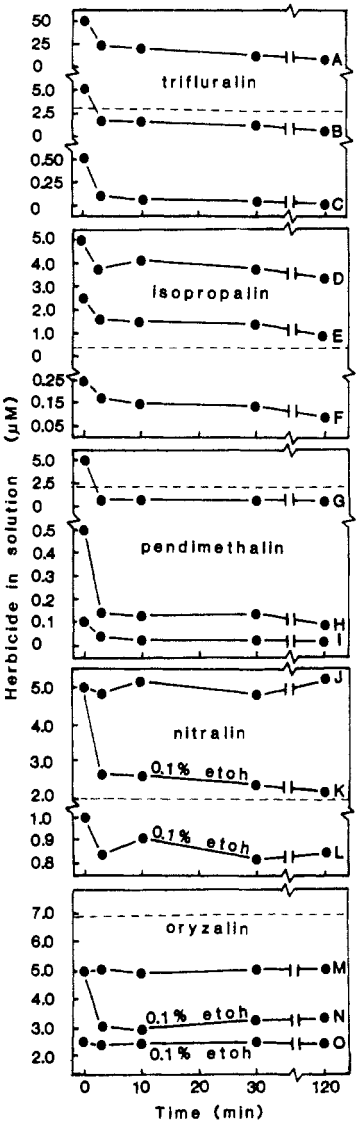

Figure 1. Amount of dinitroaniline herbicide remaining in aqueous solution as a function of time. All solutions are 1% ethanol in water (v/v) unless otherwise indicated. The dashed lines represent the maximum solubilities of these compounds in water as published in the "Herbicide Handbook of the Weed Science Society of America" (1979).

We attempted to saturate the sorption sites of the glass by pretreating siliconized glass vials with nonradioactive trifluralin. Two pretreatment procedures were tested. In the first procedure, 5 μM solutions of trifluralin in 1% ethanol in water were placed in the vials for 2 h before the aqueous solutions were discarded. In the second procedure, the glass walls were coated with 200 μL of a 100 μM solution of trifluralin in acetone until all of the acetone evaporated. The solubility of [^{14}C]trifluralin was then measured in these treated vials. Both sets of vials previously treated with nonradioactive trifluralin exhibited the same reduced recovery of radioactive trifluralin as the vials not pretreated with trifluralin (Figure 1, line B), suggesting that trifluralin binds reversibly to glass or the sorption sites on the glass surfaces cannot be saturated with herbicide at these concentrations.

To test the reversibility of sorption, we pretreated siliconized vials with [^{14}C]trifluralin and repeated the solubility studies with 5 μM solutions of nonradioactive trifluralin in 1% ethanol in water. No radioactivity was detected in any of the nonradioactive trifluralin samples taken from vials previously treated with 5 μM solutions of [^{14}C]trifluralin in 1% ethanol in water. However, the radioactivity of the samples taken from vials previously treated with 100 μM solutions of [^{14}C]trifluralin in acetone increased as the incubation time increased. These data indicate the sorption of trifluralin to glass is irreversible until all of the sorption sites are filled; then sorption becomes a reversible process. A constant concentration of trifluralin in an aqueous solution would therefore be difficult to maintain because of the dynamic equilibrium between trifluralin in solution and trifluralin bound to glass. This equilibrium would be dependent upon the type of glass, the volume to surface area ratio of the liquid and glass, and the initial concentration of trifluralin.

The sorbed trifluralin can be recovered from the glass walls by rinsing the vials with acetone. Samples of the [^{14}C]trifluralin adsorbed to glass and in aqueous solution were spotted on silica gel plates (Eastman silica gel chromatography plates, 0.25 mm) and chromatographed with benzene, chloroform, acetone, or methanol. The plates were cut into 10-mm sections, and the radioactivity was determined by liquid scintillation spectrometry. Only one spot was found on each of the plates. This spot had the same R_f value as the [^{14}C]trifluralin standard for each solvent system, indicating that no chemical decomposition of trifluralin had occurred.

Trifluralin might bind to glass as a result of the highly electronegative fluorine atoms of the trifluralin molecule electrostatically interacting with cations on the glass surface. However, Martell and Calvin (1952) report that fluorine cannot interact with cations when this halogen is covalently bonded to carbon. Also, isopropalin, pendimethalin, nitralin, and oryzalin do not contain fluorine and yet bind to glass (Figure 1). Therefore, some other portion of the dinitroaniline molecule must interact with the surface of the glass.

Other Dinitroaniline Compounds. The solubilities of isopropalin, pendimethalin, 5 μM nitralin in 0.1% ethanol in water, 2.5 μM nitralin in 0.1% ethanol in water, and 5 μM oryzalin in 0.1% ethanol in water also decreased with time (Figure 1, lines D–I, K, L, and N). At least one concentration of each of these herbicides was less than the maximum concentration reported to be soluble in water, indicating these compounds also bind to glass. If a 5 μM solution of each of these dinitroaniline herbicides is prepared with 1% ethanol in water (Figure 1, lines A, D, G, J, and M), then only nitralin and oryzalin maintain constant concentrations. All dinitroaniline compounds bind to glass, but the sulfone group and the sulfonamide group of nitralin and oryzalin, respectively, hydrogen bond to the water molecules and increase the solubility of both compounds. An initial nitralin concentration of 5.0 μM in 1% ethanol maintains a constant concentration of 5.01 ± 0.13 μM in aqueous solution (Figure 1, line J). Initial oryzalin concentrations of 5.0 μM in 1% ethanol and 2.5 μM in 0.1% ethanol maintain a constant concentration of 4.99 ± 0.07 and 2.49 ± 0.07 μM in aqueous solutions, respectively (Figure 1, lines M and O).

It is possible dinitroaniline herbicides bind to glass by chelating a cation on the glass surface. The substituted amino group and the two nitro groups in ortho positions cause several centers of high electron density (the O of the NO_2 groups and the lone pair of electrons on the amino nitrogen) to be close to each other. Because of their electron-donating properties, nitrogen and oxygen form strong complexes with a variety of cations (Martell and Calvin, 1952), which, in the dinitroaniline molecule, could form a stable six-membered ring with cations. To study this possibility, we evaluated several cations for their

Table I. Amount of [^{14}C]Trifluralin Removed from Solution, Two Hours after Preparation, as a Function of Na^+, K^+, Mg^{2+}, or Ca^{2+} Ion Concentrations

cation concn,[a] μM	[^{14}C]trifluralin removed from solution, μM[b]			
	Na^+	K^+	Mg^{2+}	Ca^{2+}
5	4.19 BC	4.13 AB	4.28 D	4.36 E
50	4.19 BC	4.09 A	4.24 CD	4.42 E
500	4.37 E	4.13 AB	4.21 BCD	4.39 E

[a] All cation solutions were prepared from the chloride salts. [b] The initial [^{14}C]trifluralin concentration was 5 μM in 1% ethanol in water (1 mL). All values are the average of four replicates. Means followed by the same letter are not significantly different at the 5% level. The amount of [^{14}C]trifluralin in 1% ethanol in pure water removed from solution 2 h after preparation is 4.37 μM (Duncan's multiple range test for the control represents E).

chelating properties with trifluralin (Table I). If trifluralin interacts with a cation on the glass surface, then addition of the appropriate cation should eliminate the adsorption of trifluralin. None of the Ca^{2+} concentrations tested reduced trifluralin adsorption. The 5 and 50 μM concentrations of Na^+ decreased sorption 4%. Mg^{2+} decreased sorption by an average of 3% and K^+ by an average of 6%. These data suggest that dinitroaniline herbicides have some chelation potential; however, the decreased glass binding achieved is inconsequential in maintaining these compounds in aqueous solution.

The only other functional group common to all of the dinitroaniline compounds tested is the aromatic ring. The aromatic ring may form π complexes with transition metals on the surface of the glass which would cause these molecules to adsorb to glass (Cotton and Wilkinson, 1972). Surface analysis techniques could be employed to test this mechanism of adsorption. The electronic and electrostatic interactions of the aromatic ring, when coupled with the hydrophobic characteristics of the dinitroaniline compounds, may explain why dinitroaniline compounds in aqueous solutions preferentially bind to diverse materials such as plastic, Teflon, glass, and stainless steel.

The sorption of dinitroaniline herbicides to glass surfaces is governed by physical phenomena and not by chemical reaction mechanisms. Trifluralin, isopropalin, pendimethalin, and nitralin bind to glass in what appears to be a diffusion-controlled process (Figure 1, lines A–I and K). In a more detailed series of experiments, the concentration of trifluralin in 1% ethanol in water decreased from an inital 5 to 0.63 μM after 120 min when the vial contents were left undisturbed. If the vial contents were continuously shaken during the 120-min incubation, the concentration decreased from the initial 5 to 0.39 μM. If the vial contents were incubated undisturbed for 60 min and then shaken for 1 min, there was an immediate drop from 0.85 μM before to 0.71 μM after the 1-min agitation period. No mathematical model can yet be presented to predict dinitroaniline sorption to glass surfaces.

Biological Activity. To correlate biological activity with obtainable dinitroaniline concentrations, we determined the concentrations necessary to affect cell division in the green alga *Chlamydomonas*. Ethanol concentrations ≤ 1% (v/v) do not affect cell division in this bioassay (Hess, 1980). Initial concentrations necessary to reduce cell division by 50% (LC_{50}) were 0.23 μM for trifluralin, 0.04 μM for pendimethalin, 0.7 μM for isopropalin, 2.8 μM for nitralin, and 1.25 μM for oryzalin. Dose–response analysis for trifluralin, pendimethalin, and isopropalin may be inaccurate because these compounds do not maintain a constant concentration in aqueous solutions at concentrations needed to reduce cell division. This demonstrates the potential difficulty of working with dinitroaniline compounds in vitro.

The lethal dosage rate (LC_{90}) of nitralin in the *Chlamydomonas* assay cannot be accurately measured because the LC_{90} obtained for nitralin (8.8 μM) is greater than the maximum constant solubility of nitralin in 1% ethanol in water. Oryzalin is the preferred compound for quantitative in vitro assays because the constant solubility of oryzalin in 1% ethanol in water (5.0 μM) is approximately 2 times the LC_{90} concentration (2.2 μM) for *Chlamydomonas*. It is therefore recommended that oryzalin be considered as the model dinitroaniline herbicide for in vitro studies, because it has the same mode of action as trifluralin (Upadhyaya and Noodén, 1978; Parka and Soper, 1977; Struckmeyer et al., 1976; Bartels and Hilton, 1973; Lignowski and Scott, 1971) and because the concentration of oryzalin remains constant in aqueous solutions. If any dinitroaniline molecule is chosen for quantitative in vitro analyses, then the solubility of the compound in aqueous solution should be tested before in vitro experiments are begun.

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