Developing Physical Property-Based Rules Which Govern the Greenhouse Activity of Herbicidal 4-Aryl-1,2,4-triazole-5(1*H*)-thiones

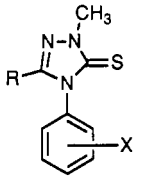
Kirk A. Simmons,* John A. Dixson, Blaik P. Halling, Ernest L. Plummer, Marjorie J. Plummer, John M. Tymonko, Robert J. Schmidt,† Mike J. Wyle, Charles A. Webster, William A. Baver, Debra A. Witkowski, George R. Peters, and William D. Gravelle

Agricultural Chemical Group, FMC Corporation, P.O. Box 8, Princeton, New Jersey 08543

The understanding of physical properties which significantly influence greenhouse efficacy of a series of soil-applied pre-emergent herbicides is discussed. The compounds, 4-aryl-1,2,4-triazole-5(1*H*)-thiones, display broad spectrum control of grass weeds. Eighty-five percent control is achieved for several species at greenhouse rates as low as 33 g/ha. Favorable selectivity toward corn, wheat, and rice was observed in greenhouse testing. The greenhouse and field efficacy and weed spectrum are reviewed.

INTRODUCTION

During the course of optimization of herbicidal activity of a series of 4-aryl-1,2,4-triazole-5(1*H*)-thiones (I) a 25 000-



I

fold improvement in laboratory potency was realized (Simmons et al., 1992). The concentrations (I_{50}) required to inhibit the growth of hydroponic cucumber ranged from ca. 100 μM to 3 nM for a series of analogues. Selected analogues were tested for their herbicidal activity in pre-emergent and post-emergent greenhouse evaluations. Over the course of this project the greenhouse efficacy of this series improved at least 250-fold. Some of the early analogues required application rates greater than 8 kg/ha for 85% control of grass weeds pre-emergent, while later analogues exhibited 85% control levels at rates as low as 33 g/ha. During this process it became apparent that only about 1% of the improvement in cucumber potency was being expressed in greenhouse evaluations. Since this chemistry exhibited favorable selectivity toward most of the major crops, we were interested in understanding the physical properties that were impacting the translation of laboratory potency into greenhouse efficacy.

MATERIALS AND METHODS

Biological Evaluations. *Hydroponic Cucumber Assay.* This assay was adapted from a published procedure (Ross, 1974). Cucumbers (*Cucumis sativus* cv. Wisconsin) were grown for 10 days in the required medium treated with 3-fold dilutions of the experimental compound starting at 100 μM. A pI_{50} for each compound was calculated from the relative growth inhibition measured as the weight gain after treatment relative to that of untreated controls. The fit of the experimental data to a line was evaluated using regression analysis plotting percent inhibition against the logarithm of the dose.

Laboratory Green Foxtail Assay. Four grams of sifted (14 mesh) soil was placed into an 8-dram vial. Fifty milligrams of washed green foxtail seed was placed into the center of the soil and covered with another 4 g of sifted soil. The herbicide treatments were made by diluting a 50 mM acetone solution of the experimental into distilled water to achieve a 100 μM concentration. These herbicide solutions, serially diluted 3-fold, were each applied to the vials by pipetting two 1.0-mL aliquots down the sides of the vial. The vials were closed with a no. 25 Teflon membrane cap and placed into an illuminated incubation chamber under a 16 h/8 h light/dark photoperiod with a light intensity of 150 μE m^{-2} s^{-1}. The temperature was maintained at 25 °C during the light and at 20 °C during the dark photoperiod. The shoot height was measured to the nearest 5 mm 5 days after treatment. A pI_{50} was calculated from the average growth inhibition relative to that of the untreated controls as in the hydroponic cucumber assay. The fit of the experimental data to a line was evaluated using regression analysis plotting percent inhibition against the logarithm of the dose.

Greenhouse Herbicide Assay. Whole plant efficacy of these herbicides was determined in greenhouse herbicide screens on several crop and weed species. These tests were completed by filling pressed fiber pots (10 in. × 6 in. × 3 in.) with a sandy loam soil, pH 6.7, 1.9% organic matter, and planting the test species in rows, in two pots. The test species included cotton (*Gossypium hirsutum* cv. DPL61), soybean (*Glycine max* cv. Williams 82), wheat (*Triticum aestivum* cv. Wheaton), rice (*Oryza sativa* cv. Labelle), field corn (*Zea mays* cv. PN3733), ivyleaf morningglory [*Ipomoea hederacea* (L.) Jacq.], wild mustard (*Sinapsis arvensis* L.), velvetleaf (*Abutilon theophrasti* Medik.), barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.], green foxtail [*Setaria viridis* (L.) Beauv.]. broadleaf signalgrass (*Brachiaria platyphylla*), goosegrass (*Eleusine indica*), texas panicum (*Panicum texanum*), and seedling johnsongrass [*Sorghum halepense* (L.) Pers.]. Candidate herbicides were dissolved in a 50/50 (v/v) acetone/water solution containing 0.5% (v) Tween 20 surfactant. The herbicide solutions, serially diluted 2-fold, were applied to the pots using an overhead spray system with 8003 flat fan nozzles set to deliver 1000 L/ha at a spray pressure of 15 psi. Following application of the herbicides the pots were placed in the greenhouse and grown under a 14-h day-length regime with temperatures of 30 °C during the day and 25 °C at night. Herbicidal activity was determined visually, 14 days after treatment, as an estimate (0–100%) of biomass reduction compared to that of untreated controls.

Field Herbicide Assay. Field evaluation was conducted in 1990 at Champaign, IL, on a silt loam soil with 1.8% organic matter content. Soil pH at this site was 6.4, with the soil composition consisting of 21% clay content, 27% sand, and 52% silt, with an overall cation-exchange capacity (CEC) of 9.8. Herbicide applications were made using a CO_2 bicycle sprayer with 8002 flat fan nozzles, set to deliver a spray volume of 200 L/ha at a pressure of 275 kPa. Test plots were 10 × 3.6 m, with the test designed as a split plot, the main plots being herbicide treatments (rates) and the subplots being application method

† Present address: Bristol-Myers Squibb Co., Princeton, NJ.

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Table I. Physical Properties of Compounds I

compd	mp, °C	molecular formula	anal. (C, H, N) calcd/found	^{1}H NMR ($CDCl_3$) δ, multiplicity,[a] (area)
10	96–98	$C_{13}H_{16}F_3N_5S$	47.13/47.07, 4.83/5.13, 21.15/20.92	1.22 t (6 H), 3.55 q (4 H), 3.90 s (3 H), 6.55 d (1 H), 7.35 dd (1 H), 8.05 d (1 H)
14	161–163	$C_{13}H_{14}F_3N_3O_3S$	44.70/44.53, 4.01/4.03, 12.03/12.03	3.80 s (6 H), 3.85 s (3 H), 3.90 s (3 H), 6.65 s (2 H)
16	77–78	$C_{13}H_{12}F_5N_3OS$	MS M^+ 353.0621/353.0621	1.40 d (6 H), 3.85 s (3 H), 4.60 m, (1 H), 6.90–7.00 m (2 H)
17	83–85	$C_{13}H_{13}F_5N_4S$	44.32/44.53, 3.72/3.41, 15.90/15.64	1.15 t (3 H), 2.95 s (3 H), 3.20 q (2 H), 3.85 s (3 H), 6.85 d (2 H)
18	oil	$C_{10}H_5ClF_5N_3S$	36.43/36.36, 1.53/1.41, 12.75/12.71	3.90 s (3 H), 7.25 dd (1 H), 7.45 dd (1 H)
19	73–76	$C_{12}H_9F_7N_4S$	38.51/38.80, 2.42/2.43, 14.97/15.06	3.05 s (6 H), 3.90 s (3 H)
20	118–121	$C_{12}H_{10}Cl_2F_3N_3OS$	42.21/42.32, 3.02/2.82, 10.55/10.35	1.50 t (3 H), 3.90 s (3 H), 4.15 q (2 H), 7.07 s (1 H), 7.35 s (1 H)
22	111–114	$C_{12}H_8Cl_2F_3N_4OS$	38.92/39.14, 2.16/2.06, 11.35/11.29	3.90 s (3 H), 4.78 dd (1 H), 5.03 d (1 H), 6.60 dd (1 H), 7.25 s (1 H), 7.40 s (1 H)
23	165–168	$C_{14}H_{10}Cl_2F_3N_3O_2S$	40.78/40.56, 2.43/2.49, 10.19/10.05	1.60 s (6 H), 3.90 s (3 H), 7.60 s (1 H)
24	140–143	$C_{13}H_{12}Cl_2F_3N_3OS$	40.41/40.17, 3.11/2.87, 10.88/10.69	1.10 t (3 H), 1.95 m (2 H), 3.90 s (3 H), 4.05 q (2 H), 7.10 s (1 H), 7.38 s (1 H)
26	37–40	$C_{14}H_{13}F_7N_4S$	41.80/41.97, 3.26/3.18, 13.93/13.73	1.20 t (6 H), 3.40 q (4 H), 3.90 s (3 H)
31	123–126	$C_{11}H_8Cl_2F_3N_3S$	MS M – Cl^+ 306.0080/306.0084	2.45 s (3 H), 3.90 s (3 H), 7.35 s (1 H), 7.50 s (1 H)
35	56–60	$C_{14}H_{12}Cl_2F_3N_3OS$	42.21/42.09, 3.02/2.74, 10.55/10.40	1.60 s (6 H), 3.15 d (1 H), 3.22 d (1 H), 3.90 s (3 H), 7.15 s (1 H)
38	150–152	$C_{15}H_{11}ClF_5N_3O_3S$	40.59/40.29, 2.50/2.53, 9.47/9.18	1.60 s (6 H), 3.93 s (3 H), 6.75 t (1 H), 7.45 s (1 H)
39	oil	$C_{15}H_{13}ClF_5N_3O_2S$	41.91/41.67, 3.05/3.09, 9.78/9.65	1.60 s (6 H), 3.12 d (1 H), 3.20 d (1 H), 3.86 s (3 H), 6.62 t (1 H), 7.00 s (1 H)
41	132–133	$C_{13}H_{10}Cl_2F_3N_3OS$	MS M^+ 382.9868/382.9853	0.92 m (4 H), 3.83 m (1 H), 3.90 s (3 H), 7.35 s (1 H), 7.50 s (1 H)

[a] s, singlet; d, doublet; t, triplet; q, quartet; br, broadened; dd, doublet of doublets; dt, doublet of triplets; m, multiplet.

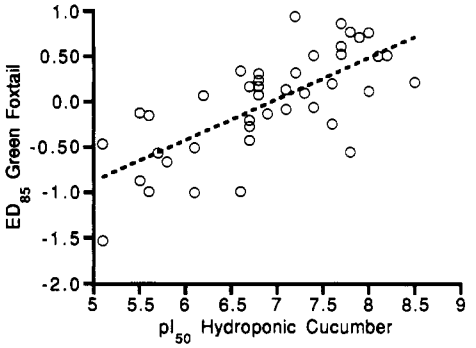


Figure 1. ED_{85} green foxtail vs pI_{50} hydroponic cucumber.

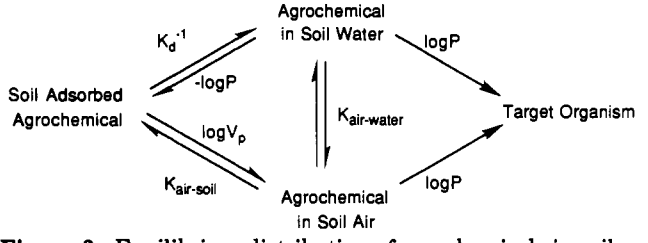


Figure 2. Equilibrium distribution of agrochemicals in soil.

(pre-emergence or preplant incorporated), with all treatments replicated four times. Experimentals, formulated as 50 g/L emulsifiable concentrates, were applied at four rates from 1 to 0.125 kg/ha. Species tested in this field trial included sorghum (*Sorghum vulgare*), field corn, redroot pigweed (*Amaranthus retroflexus* L.), Eastern black nightshade (*Solanum ptycanthum* Dun.), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], barnyardgrass, proso millet (*Panicum miliaceum* L.), giant foxtail (*Setaria faberi* Herrm.), green foxtail, shattercane [*Sorghum bicolor* (L.) Moench], and seedling johnsongrass. The relative growth inhibition was visually determined 41 days after treatment. An ED_{85} was calculated by fitting the experimental data to a line using regression analysis plotting percent inhibition against the logarithm of the dose. In those cases where greater than 85% control was observed at the lowest rate (125 g/ha) tested, ED_{85} values were estimated by extrapolation.

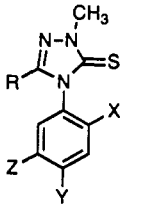
Chemical Methods. The general synthetic route for the 4-aryl-1,2,4-triazole-5(1*H*)-thiones has been described (Simmons et al., 1992). Compounds disclosed here have been prepared according to similar synthetic procedures. The structures are consistent with the elemental analyses, ^{1}H NMR, and melting point data reported in Table I. Vapor pressures (V_p) were determined by a gas chromatographic technique (Kim et al., 1984; Hamilton, 1980) in which the retention time of the experimental is related to the retention time for dibutyl phthalate, a standard of known vapor pressure. The correction for the compound's melting point was based upon the empirical relationship in eq 1 (Yalkowsky, 1979). All vapor pressures, determined using an

$$\ln (V_p)_{solid} - \ln (V_p)_{liquid} = 6.79[1 - (mp/298\ K)] \quad (1)$$

SE-30 microbore column, are reported as log V_p in units of millimeters of Hg at 25 °C. Partition coefficients between 1-octanol and water were determined according to the shake-flask method (Dearden and Bresnen, 1988) at 25 °C for four analogues. The log P values for the other analogues were estimated using the Medchem ClogP program (version 3.33 from A. Leo and D. Weiniger, Pomona College). These four measured values were used to estimate the missing fragment for the heterocycle in this program.

RESULTS AND DISCUSSION

QSAR Development. The quantitative structure–activity relationship (QSAR) model (eq 2) describes the relationship between structure and growth inhibition of

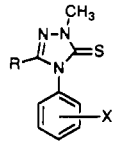


$$pI_{50}\ (M) =$$
$$0.90\ (\pm0.18)\pi_y - 0.40\ (\pm0.09)\pi^2_y - 0.86\ (\pm0.15)\sigma_y + 0.93\ (\pm0.14)B1_x - 1.07\ (\pm0.19)I_0 + 1.09\ (\pm0.17)\pi_z + 3.48\ (\pm0.46)B1_R - 0.88\ (\pm0.17)L_R + 0.05 \quad (2)$$

$$n = 79 \qquad r^2 = 0.84 \qquad s = 0.45 \qquad F = 46.3$$
$$\pi_{y(optimum)} = 1.1$$

hydroponic cucumber (Simmons et al. 1992). In this equation n is the number of analogues, s the standard

Table II. Compounds Used To Develop Translation Models



						green foxtail		
							greenhouse	
compd	R	X	log P	log V_p	cucumber pI_{50}[a]	lab ED_{50}[b]	ED_{85}[c]	ED_{85}[d]
1	CF_3	2,4-Cl_2	4.28	−4.07	5.8	4.6	−0.66	−0.78
2	CF_3	4-NMe_2	3.05	−6.24	5.5	4.4	−0.87	−0.99
3	CF_3	4-*i*Pr	4.43	−4.12	5.6	4.7	−0.99	−0.65
4	CF_3	2-CH_3,4-F	3.64	−3.74	5.1	4.2	−1.53	−1.31
5	CF_3	3,4,5-Cl_3	4.99	−5.79	6.7	5.1	−0.42	0.04
6	CF_3	2,4,5-Cl_3	4.99	−5.51	6.7	4.7	−0.27	−0.32
7	CF_3	2-CH_3,4-CCH	3.77	−5.49	6.1	5.0	−0.50	−0.38
8	CF_3	4-$N(C_2H_5)_2$	3.79	−6.32	6.7	5.2	−0.20	−0.14
9	CF_3	2-Cl,4-$N(C_2H_5)_2$	4.83	−6.62	7.8	5.2	−0.55	0.15
10	CF_3	4-$N(C_2H_5)_2$,(3-pyridyl)	3.14	−6.01	6.6	4.7	−0.99	−0.75
11	CF_3	3,5-Cl_2,4-NEt_2	5.63	−6.49	6.8	5.4	0.31	0.54
12	CF_3	4-$N(CH_3)CH(CH_3)_2$	3.72	−6.07	6.1	4.7	−1.00	−0.59
13	CF_3	3,5-Cl_2,4-NMe_2	4.73	−5.68	6.6	5.4	0.34	0.20
14	CF_3	3,4,5-$(OCH_3)_3$	2.04	−6.48	5.1	4.9	−0.46	−0.83
15	CF_3	3,5-Cl_2,4-$OCH(CH_3)_2$	4.68	−4.96	6.8	5.2	0.08	−0.07
16	CF_3	3,5-F_2,4-$OCH(CH_3)_2$	4.10	−4.50	5.5	5.4	−0.12	−0.13
17	CF_3	3,5-F_2,4-$N(CH_3)(C_2H_5)$	4.04	−4.83	5.6	5.4	−0.15	−0.10
18	CF_3	2,5-F_2,4-Cl	3.85	−3.44	5.7	4.8	−0.56	−0.81
19	CF_3	2,3,5,6-F_4,4-$N(CH_3)_2$	3.94	−4.31	7.1	5.7	−0.08	0.04
20	CF_3	2,5-Cl_2,4-OC_2H_5	4.70	−6.10	7.7	5.9	0.52	0.64
21	CF_3	2,5-Cl_2,4-NHC_2H_5	4.79	−6.81	7.8	5.7	0.77	0.60
22	CF_3	2,5-Cl_2,4-$OCH{=}CH_2$	4.69	−5.68	7.7	5.8	0.86	0.50
23	CF_3	2,5-Cl_2-3,4-$COC(Me)_2O$	4.61	−7.14	7.9	6.1	0.71	0.91
24	CF_3	2,5-Cl_2,4-$OCH_2CH_2CH_3$	5.20	−6.69	7.3	4.6	0.10	−0.19
25	CF_3	2,5-Cl_2,4-NEt_2	5.63	−5.51	8.0	5.2	0.16	0.26
26	CF_3	2,3,5,6-F_4,4-NEt_2	4.84	−4.18	7.6	5.3	−0.24	−0.05
27	CF_3	2,5-Cl_2,4-$OCH(CH_3)_2$	4.96	−5.87	7.2	5.9	0.94	0.68
28	CF_3	2,5-Cl_2,4-OCH_3	4.13	−6.13	6.7	5.4	0.17	0.09
29	CF_3	2-F,5-Cl,4-OCH_3	3.56	−5.46	6.8	5.7	0.17	0.09
30	CF_3	2-F,5-Cl,4-$OCH(CH_3)_2$	4.39	−5.18	7.4	5.9	0.51	0.44
31	CF_3	2,5-Cl_2,4-CH_3	4.90	−5.50	6.9	5.2	−0.13	0.06
32	CF_3	2,5-Cl_2,4-$OC(CH_3)_3$	5.38	−5.87	7.2	5.1	0.32	0.16
33	CF_3	2-SCH_3,4-$OCH(CH_3)_2$,5-Cl	4.84	−6.75	7.4	5.1	−0.06	0.12
34	CF_3	2,5-Cl_2,4-OcC_4H_7	5.05	−6.90	8.1	5.4	0.50	0.44
35	CF_3	2,5-Cl_2-3,4-$CH_2C(Me_2)O$	5.59	−5.84	8.0	5.7	0.76	0.69
36	CF_2Cl	2,5-Cl_2,4-O*i*Pr	5.20	−6.64	6.8	4.8	0.24	−0.03
37	CF_2CF_3	2,5-Cl_2,4-O*i*Pr	5.30	−5.70	6.2	4.5	0.07	−0.37
38	CF_3	2-Cl,5-OCF_2H-3,4-$COCMe_2O$	4.40	−6.62	7.1	5.7	0.14	0.47
39	CF_3	2-Cl,5-OCF_2H-3,4-CH_2CMe_2O	5.33	−5.14	8.2	5.8	0.51	0.61
40	CF_3	2,5-Cl_2,4-$N(CH_3)_2$	4.74	−4.96	8.5	5.6	0.22	0.26
41	CF_3	2,5-Cl_2,4-OcC_3H_5	4.49	−6.60	7.7	5.6	0.61	0.41

[a] pI_{50} is −log (concentration) in mol/L which provides 50% reduction in weight gain relative to untreated controls for hydroponically grown cucumber. [b] ED_{50} is −log (concentration) in mol/L which provides 50% reduction in plant height relative to untreated controls in the laboratory green foxtail assay. [c] ED_{85} is −log (applied dose) in mol/ha which provides 85% control of growth of green foxtail in the greenhouse evaluations. [d] Predicted using eq 9.

error for the model, r^2 the explained variance, and F the value for the F statistic. The r^2 value is adjusted for the number of variables in the equation. The values in parentheses are the standard error for the coefficients. All terms are significant at least at the 99% level based upon the Student t-test. The values for the physiochemical parameters π, σ, L, $B1$, and $B4$ are taken from the literature (Hansch and Leo, 1978; Hansch et al., 1991). π is the Hansch hydrophobicity index (Hansch and Fujita, 1964); σ is the Hammett sigma constant (Hammett, 1940; Hansch and Fujita, 1964; Hansch and Leo, 1978); L, $B1$, and $B4$ are the sterimol length, minimum radius, and maximum radius parameters (Verloop et al., 1976); and I_0 is an indicator variable (Kubinyi, 1976, 1988).

In the aromatic ring an optimal lipophilicity (π_y) was associated with the para substituent which should also be electron donating. Activity improved as the minimum radius of the ortho substituent ($B1_x$) increased, suggesting that coplanarity of the two rings was unfavorable for activity. Increasing lipophilicity at the 5-position (π_z) of the aromatic ring improved activity. The indicator variable (I_0) assumed the value of 1 when the aromatic ring was only substituted in the ortho positions (i.e., 2- or 2,6-substituted or unsubstituted). Its high level of statistical significance (t-test = −5.63, p-tail = 0.00) and large negative coefficient suggested that the aromatic ring was likely susceptible to metabolism if unsubstituted in the para position (Testa and Jenner, 1976).

Compounds were evaluated in greenhouse tests when their intrinsic potencies (as measured by the hydroponic cucumber assay) were sufficiently high (pI_{50} > 5.0) so as to expect that an ED_{85} could be obtained at doses of 8 kg/ha or less. Analysis of the observed greenhouse activity (ED_{85}, −log [mol/ha]) for control of green foxtail vs the potency (pI_{50}, −log [mol/L]) for growth inhibition of hydroponic cucumber afforded eq 3 (see Figure 1). While

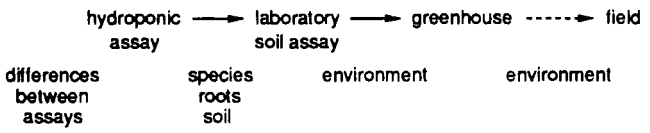
$$ED_{85}\ \text{green foxtail} = 0.45\ (\pm 0.07)pI_{50}\ \text{cucumber} - 3.14 \quad (3)$$

$$n = 41 \qquad r^2 = 0.50 \qquad s = 0.41$$
$$F = 40.5\ (p\text{-tail} = 0.00)$$

there appears to be a significant relationship between greenhouse efficacy and growth inhibition of hydroponic cucumber, compounds with very similar levels of potency against cucumber display greenhouse activities that differ by about 50-fold. Some of this variability could be attributed to interspecies differences (cucumber vs green foxtail) and to day-to-day variation in the tests used to quantify this activity, but we would not have expected this to be 50-fold. The relatively poor explained variance in eq 3 suggested that other factors were responsible for the poor greenhouse efficacy of some of the more potent analogues and the remarkable efficacy of some of the less potent analogues.

Development of Translation Models. Numerous papers have addressed the factors, namely lipophilicity (log P) and volatility (log V_p), which are known to affect the performance of soil-applied agrochemicals (Briggs, 1984; Graham-Bryce, 1981, 1984). Changes in these properties affect soil bioavailability of an agrochemical through alteration of its equilibrium distribution between soil organic matter, water, and air (Figure 2).

We were interested in understanding how these properties impact the translation of laboratory potency into greenhouse efficacy for this chemistry. To facilitate this understanding, a soil-based laboratory assay was developed which allowed for the determination of herbicidal efficacy for small quantities of experimental materials. Additionally, this assay could be performed under more controlled environmental conditions than typically found in a greenhouse. Green foxtail was chosen for this assay as it was especially sensitive to I. Except for the species, the only significant differences between these two laboratory assays (hydroponic cucumber vs soil-based laboratory assay) were the presence or absence of soil in the test system and the presence or absence of roots. Our



approach was to develop physical property-based rules that would facilitate understanding of the differences in observed activities between the hydroponic cucumber assay, the soil-based laboratory assay, and the greenhouse for the 4-aryl-1,2,4-triazole-5(1*H*)-thiones. To develop these translation rules, a set of compounds (Table II), which had been tested earlier in both the greenhouse and hydroponic cucumber assay, was tested in the laboratory soil-based green foxtail assay.

Analysis of the relationship between activities in the hydroponic cucumber assay (pI_{50}, -log [mol/L]) and the soil-based laboratory assay (ED_{50}, -log [mol/L]) for the series of analogues of I afforded eq 4 (Figure 3, broken

$$ED_{50}\ \text{green foxtail} = 0.35\ (\pm 0.06)pI_{50}\ \text{cucumber} + 2.83 \quad (4)$$

$$n = 41 \qquad r^2 = 0.43 \qquad s = 0.36$$
$$F = 30.6\ (p\text{-tail} = 0.00)$$

line). As had been seen earlier, there appears to be a

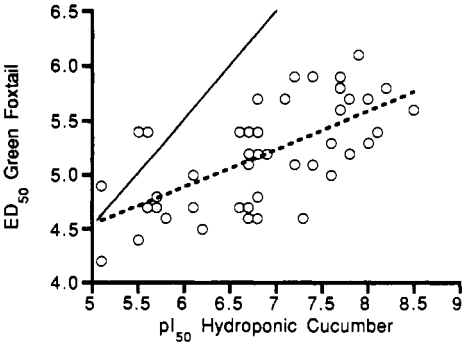


Figure 3. ED_{50} laboratory soil-based green foxtail vs pI_{50} hydroponic cucumber.

Table III. Site of Uptake Studies for 8 in Sorghum

	greenhouse % control	
rate, kg/ha	root	shoot
1.00	0	1
2.00	0	99

Table IV. Root vs Shoot Uptake in Hydroponic Cucumber

compd	$C\log P$	$\log V_p$	-roots	+roots	Δ
1	4.28	-4.07	5.8	4.7	1.1
7	3.77	-5.49	6.1	4.9	1.2
15	4.68	-4.96	6.9	~3.8	3.1
25	5.63	-5.51	8.0	<4.0	>4

Table V. Root Uptake—Systemic Translocation Studies

		concn, μM	
compd	$C\log P$	xylem	phloem
14	2.04	4.3	2.5
25	5.63	<0.1	<0.1

significant relationship between observed activity (ED_{50}) and potency (pI_{50}), and compounds with essentially identical potencies against hydroponic cucumber displayed widely disparate soil-based efficacies (at least 35-fold). Reasoning that potency against a target site is necessary, but not sufficient, for observing greenhouse efficacy, we developed rules in which potency, bioavailability, and stability (in the target organism or environment) together define the overall activity.

efficacy =

potency × bioavailability (log P, log V_p) × stability

This was accomplished using statistical analysis to analyze the difference in activity between assays vs the physical properties of the compounds. In this way an understanding of the sequential effect of roots, soil, and differential environments on translation would be developed. Specifically, analysis of the difference in potency for each compound between assays allows understanding of the origin of the residuals relative to the one-to-one correspondence line (Figure 3, solid line). This one-to-one line represents the ideal case, whereby improvements in cucumber potency are fully expressed in the green foxtail assay. Understanding the impact of physical properties on these deviations from the one-to-one correspondence line facilitated understanding why less-than-ideal translation occurred between the laboratory soil-based green foxtail and hydroponic cucumber assays.

The activity difference between the hydroponic cucumber (pI_{50}) and the laboratory soil-based green foxtail (ED_{50}) assays was analyzed. The model (eq 5) related this difference to lipophilicity (log P) and volatility (log V_p), where n is the number of analogues, s is the standard error for the model, r^2 is the explained variance and is adjusted

Table VI. Weed Spectrum for Compound 27

AMARE	BRAPP	DIGSA	ECHCG	ELEIN	PANMI	PANTE	SETFA	SETVI	SORBI	SORSE
Greenhouse ED_{85} (g/ha)										
nd	91 ± 30	38 ± 14	143 ± 77	33 ± 16	133 ± 75	145 ± 62	55 ± 20	44 ± 13	210 ± 92	300 ± 126
Field ED_{85} (g/ha)										
104 ± 51	nd	104 ± 51	242 ± 115	nd	110 ± 61	nd	~62[a]	~62[a]	171 ± 80	115 ± 70

[a] Estimated since replicates averaged 95–96% control at 125 g/ha for these species.

$$ED_{50}\ \text{foxtail} - pI_{50}\ \text{cucumber} = -0.55\ (\pm 0.10)\ \log P + 0.21\ (\pm 0.08)\ \log V_p + 1.98 \quad (5)$$

$$n = 41 \qquad r^2 = 0.47 \qquad s = 0.50$$
$$F = 19.0\ (p\text{-tail} = 0.00)$$

for the number of variables in the equation, and F is the value of the F statistic. The values in parentheses are the standard error for the coefficients. All terms are significant at least at the 98% level based upon the Student t-test.

Compounds that displayed the smallest deviation from the ideal one-to-one line were those with low lipophilicity ($\log P$) and high volatility ($\log V_p$). By referring to Figure 2, this relationship was clarified. Since the predominant difference between these two assays was the presence or absence of soil (although root and species differences also exist), one conclusion supported by eq 5 was that the organic component of soil represented a significant sink for these compounds. Decreasing lipophilicity and increasing volatility improved their bioavailability in the presence of soil by shifting their equilibrium toward dissolution in soil water and soil air. Since this analysis could only explain about half of the variance of the residuals from the ideal line, other factors, some perhaps specific to this class of chemistry, may be operative and as yet are undiscovered.

The second analysis involved the relationship between the greenhouse efficacy (ED_{85}) and the laboratory efficacy (ED_{50}) for green foxtail. The final model (eq 9) and its development are presented (eqs 6–8). All terms were

$$ED_{85}\ \text{foxtail (greenhouse)} = \mathbf{a} \times ED_{50}\ \text{foxtail (lab)} + \mathbf{b} \times \log P + \mathbf{c} \times \log Vp + \mathbf{d}$$

a	b	c	d	r^2	s	F	eq
0.97 ± 0.12			−5.08	0.63	0.35	69.4	6
0.90 ± 0.11		−0.16 ± 0.06	−5.61	0.69	0.32	44.9	7
0.84 ± 0.10	0.29 ± 0.06		−5.73	0.76	0.28	65.0	8
0.80 ± 0.09	0.27 ± 0.05	−0.13 ± 0.04	−6.14	0.80	0.25	55.8	9

significant at least at the 99% level based upon the Student t-test, and the r^2 values are adjusted for the number of parameters in each equation. The inclusion of each additional term was justified using the sequential F-test and adjusted r^2, s, and F values (Draper and Smith, 1966). At first glance eq 9 appears to contradict eq 5, suggesting that *increasing* lipophilicity and *decreasing* volatility improve the translation from the laboratory soil assay to the greenhouse. To clarify these results, several additional experiments were conducted which elaborated on the mobility in and the site of uptake for these compounds in plant systems. In a greenhouse test compound 8 ($\log P = 3.79$ and $\log V_p = -6.32$) was selectively applied to the roots and shoots of sorghum by imposing an activated carbon barrier in the growing pot between these two regions. For the root uptake study the sorghum seed was placed above the carbon barrier, while the portion of soil below the barrier was treated with the experimental. Conversely, for the shoot uptake experiment the seed was placed below the carbon barrier and the soil above this barrier was treated with the experimental. The percent growth inhibition of sorghum (Table III) via these two modes of uptake suggested that the plant's shoot was the predominant site of effective uptake.

In addition to this greenhouse assessment, several compounds were evaluated in a special hydroponic cucumber assay. In this experiment four compounds, whose properties represented factorially arranged combinations of both high and low values in $\log P$ and $\log V_p$, were evaluated to determine their efficacy at growth inhibition of hydroponic cucumber plants that had their roots excised or in which the root system was intact. The data, summarized in Table IV, support the conclusion from the greenhouse test that these compounds were less effective at plant growth inhibition when taken up via the root system. In all cases the compounds were less effective at inhibiting cucumber growth when intact roots were present. However, it should be noted that the two lower $\log P$ compounds (**1** and **7**) were proportionately more effective than the two higher $\log P$ analogues. This behavior is consistent with similar findings in barley; that is, while more lipophilic compounds adsorb readily into roots, they do not subsequently translocate from them very well (Briggs et al., 1982, 1983).

In addition to these tests, two analogues (**14**, $\log P = 2.0$, and **25**, $\log P = 5.6$) were evaluated (Table V) for their movement in castor bean using established methods (Bromilow et al., 1987). Castor bean was allowed to take up the experimentals from a 20 μM solution for 4 h and the xylem and phloem saps analyzed by HPLC for the concentration of the experimental compounds. Compound **14** was found to be taken up by the castor bean and translocated throughout the plant; the more lipophilic analogue could not be detected.

All of the data confirm that these experimental herbicides were not readily translocated throughout plants when taken up by roots except for lower $\log P$ analogues. This is a generally recognized phenomena for xenobiotics (Briggs et al., 1982, 1983). Therefore, the predominant site of uptake is the shoot of emerging plants, requiring the compounds to remain in the vicinity of the emerging shoot for maximum soil efficacy. Being strongly adsorbed to the soil organic phase, more lipophilic analogues would remain near the soil surface. Given the high $\log P$ of these compounds, movement through the soil would occur predominantly through the vapor phase; thus, some degree of volatility would be required for movement from the soil to the plant with subsequent expression of activity. This clarifies the impact of $\log P$ and $\log V_p$ on the soil efficacy of these experimental herbicides.

Knowledge Application. In the final phase of this project the goal became to balance the physiochemical requirements for high potency (eq 2) with the property requirements for good soil availability (eq 9). Up to this point the most potent compounds against hydroponic cucumber (Table II, **25**, **35**, **36**, **40**, **41**; pI_{50} values in the range 8.0–8.5) displayed 85% control of green foxtail at average rates of 0.43 ± 0.24 mol/ha in the greenhouse.

Alternative substituents in I were explored whose combined properties were anticipated to afford analogues with improved bioavailability. This endeavor ultimately led to compound **27**, which exhibited improved green foxtail efficacy (85% control at 0.11 ± 0.03 mol/ha) in spite of the fact that its cucumber potency was reduced (pI_{50} = 7.2) nearly 10-fold! The lipophilicity and volatility of **27** may well represent an optimal balance between reduced potency and greater bioavailability. The weed spectrum of **27** is shown in Table VI. The rates required for 85% control (ED_{85}) were averaged across several greenhouse tests (n = 3–9 depending upon species) or across the replicates (n = 4) of the field evaluation. By optimally balancing physical properties against potency for these compounds, a good translation between greenhouse and field use rates has been achieved for compound **27**; i.e., ED_{85} values between tests overlap at the 95% confidence intervals. Interestingly, one of the earliest field candidates (**8**) displayed a very poor translation between the greenhouse and the field (field ED_{85} = 16-fold higher than greenhouse ED_{85})! The spectrum of weed control in the field for compound **27** is very similar to that seen for the chloroacetanilides, such as alachlor, which are currently registered for pre-emergence weed control in corn. Given the similar weed spectrum and lower use rate, this class of chemistry may well serve as a lead for developing a pre-emergence corn herbicide.

CONCLUSION

This work indicates that while potency is necessary, it alone is not sufficient for high levels of greenhouse or field efficacy. Factors, such as lipophilicity and volatility, which impact the bioavailability of an agrochemical, must also be considered in the overall design process. Although species differences that exist between the assays and methods used prevent definitive conclusions from being drawn, our findings have strong implications for the way in which compounds are selected for field evaluation. The influence of physical properties may well differ between the greenhouse and the field. As a result, the most efficacious analogue in a series in field testing may not correspond to the most efficacious in greenhouse testing. Therefore, one needs to evaluate in the field a set of compounds which span not only levels of greenhouse potency but also those properties which can affect bioavailability as well. We feel this work serves as a model for further exploration of this possibility.

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