Oxabetrinil Reversal of Metolachlor and Acid Soil Stress*

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Metolachlor and excess Mn^{2+} (acid soil stress) induce alterations in gibberellin precursor biosynthesis that can explain the morphological responses to these physiological stresses. Oxabetrinil protects sorghum [*Sorghum bicolor* (L.) Moench] from the influence of metolachlor and excess Mn^{2+}. Sorghum cultivar variations in response to excess Mn^{2+} are explicable as differential rates of *ent*-kaurene biosynthesis between acid soil sensitive and tolerant cultivars. Concentrations of Mn^{2+} present in vegetative leaves and reproductive stem tissues were not different. Therefore, cultivar differences in *ent*-kaurene biosynthesis explain the acid soil tolerance differences rather than differential Mn^{2+} absorption, translocation, and/or compartmentation. Metolachlor and safener responses are found in cellular compartments and tissues that do not match a decreased herbicide concentration through absorption, transport, or degradation as a sole mode of action for safeners.

Introduction

Metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] is a preemergence herbicide used in sorghum [*Sorghum bicolor* (L.) Moench] production which can be highly deleterious to the crop [1]. Seed applied oxabetrinil [α-(1,3-dioxolan-2-yl-methoxy)-iminobenzene acetonitrile] serves as a safener to metolachlor in sorghum [1]. The mechanisms of action for these morphological responses have been reported to be an inhibition of gibberellic acid (GA) precursor biosynthesis by metolachlor which is reversed by oxabetrinil [2].

Acid soils are characterized by high concentrations of H^+, Mn^{2+}, and aluminum in the soil solution that can be phytotoxic [3, 4]. Excess H^+-induced seedling shoot growth inhibitions that were explicable as an influence on GA transport from the plumule to the endosperm [5]. This response was reversed by the addition of exogenous GA_3 [5]. Manganese or magnesium are required for GA precursor biosynthesis [6]; but the enzyme activity is highly Mn^{2+} concentration-dependent [6, 7] and high Mn^{2+} concentration resulted in a massive decrease in *ent*-kaurene synthesis [7]. Presumably, the increased shoot growth inhibition of sorghum seedlings produced under high Mn^{2+} and low pH conditions was due to an influence of Mn^{2+} on GA precursor biosynthesis and a failure of transport of the GA that was produced under adverse conditions. Since sorghum (*cv.* DeKalb "BR 64") dry weight production was relatively tolerant to excess Mn^{2+} [8], the possibility existed that if the major mechanisms of action of metolachlor and acid soil stress are on GA synthesis, oxabetrinil would reverse some of the acid soil stress responses in sorghum as well as the herbicidal effects of metolachlor.

Consequently, seed of several sorghum cultivars were planted in soils of differing pH and oxabetrinil was tested for activity in reversing both metolachlor and acid soil stress. Finally, *in vitro* enzyme assays on GA precursor biosynthesis in several sorghum cultivars were conducted where Mn^{2+} concentration was the variable.

Additionally, since sorghum appears to show different responses to acid soil stress at the seedling, vegetative, and reproductive stages of growth [9], there was a possibility that the Mn^{2+} concentrations in the plant tissue might vary and be the basis for the differences in growth responses between the vegetative and reproductive stages. Therefore, field-grown leaf and stem tissues were harvested for Mn^{2+} content analyses.

Materials and Methods

Growth studies

Field studies were conducted on a Cecil sandy clay loam (Typic Hapludult) adjusted to pH 4.2

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and >6.0 by the addition of sulphur or $CaCO_3$. Sorghum cultivars tested are shown in Table I along with their relative acid soil tolerance. Seed were treated with 0 or 1.25 g a.i. oxabetrinil/kg seed. Metolachlor (2.24 kg a.i./ha) was applied to the soil after seed planting. Germination was insured by sprinkler irrigation [1, 9] within 24 h after planting. Seed were planted at 7.5 cm^{-1}. Stand counts were taken at 7 and 30 days after irrigation. Four replications were planted in a randomized complete block design. Seed germination of oxabetrinil-treated seed was determined in a dark germinator at 28 °C after 72 h, in standard germination paper.

Table I. Sorghum [*Sorghum bicolor* (L.) Moench] cultivars utilized and their relative tolerance to acid soil stress.

Cultivar	Relative acid soil tolerance [%]
Milo Blanco	90
SC283	90
SC574	90
SC689	90
SC599	60
SC326 × SC103	50 (or greater)
GP140	50
SC214	25
RTx430	5
TAM428	5

Manganese determination

Leaf and unemerged stem tissues were collected for Mn analyses at 75 days after planting. Samples were inserted into tared test tubes, weighed, dried, weighed, wet ashed with nitric perchloric acid and analyzed for Mn content by atomic absorption spectrophotometry. Data were converted to mmol Mn/g dry weight, and nmol Mn/g H_2O.

Gibberellin precursor biosynthesis

Procedures followed those published previously [7, 10] except for use of [4-^{14}C]isopentenyl pyrophosphate (0.1 μCi/analysis) and Mn^{2+} concentrations (.1, .5, 1, 3, 5, 7, 10, 15, or 20 μM).

All data were subjected to analysis of variance on a randomized split plot design and means were separated by least significant differences.

Results and Discussion

Influence of oxabetrinil on sorghum seed germination

Oxabetrinil (1.25 g a.i/10 ml water/kg seed) increased germination for SC214, caused no change in germination for SC689, SC599, and SC326 × SC103, and decreased seed germination for Milo Blanco, SC283, SC574, GP140, RTx430, and TAM428 (Table II). These variations in germination of sorghum seed corroborate previous reports [1].

Influence of oxabetrinil on the growth of sorghum planted in acid stress soils treated with metolachlor

The influence of metolachlor is developed in seedlings within seven days in plants grown on soils at pH >6.0. Plants which survive this early herbicide stress grow vegetatively at equal to or better rates than untreated plants [1]. When grown at pH >6.0, oxabetrinil induced an increased 7-day plant stand in the presence of metolachlor in nine of ten cultivars (Table III). Thus, the early (7-day) plant stands demonstrate metolachlor, oxabetrinil, and acid soil stress responses. Late (30-day) plant stand was also influenced by metolachlor, oxabetrinil, and soil pH (data not shown).

Data in Table IV show that in nine of ten cultivars, oxabetrinil treatment improved percent survival at 7 days under pH >6.0 growth conditions. Additionally, oxabetrinil increased survival in all

Table II. Influence of oxabetrinil on germination of sorghum [*Sorghum bicolor* (L.) Moench] cultivars.

Cultivar	Oxabetrinil Treated A	Oxabetrinil Untreated B	Ratio of A/B
	Percent germination [%]		
SC214	50.8 g h i[a]	34.8 j k	↑
SC689	85.8 a b c	87.5 a b	–
SC599	68.5 f	76.8 c–f	–
SC326 × SC103	55.2 g h	57.0 g	–
Milo Blanco	41.8 i j	78.8 b–e	↓
SC283	69.8 e f	83.8 a–d	↓
SC574	75.0 d e f	88.2 a	↓
GP140	16.5 L	27.2 k	↓
RTx430	38.0 j	47.5 h i	↓
TAM428	42.2 i j	69.5 f	↓

[a] Values followed by the same letter are not significantly different at the 5% level.

Table III. Plant stand (7 day) of oxabetrinil-treated and -untreated sorghum [*Sorghum bicolor* (L.) Moench] cultivars planted in soils at pH 4.2 and >6.0 treated with metolachlor.

Cultivar	pH >6.0 Oxabetrinil +	pH >6.0 Oxabetrinil −	pH 4.2 Oxabetrinil +	pH 4.2 Oxabetrinil −
	Plant stand (number of plants)			
Milo Blanco	61 b c d[a*b]	30 i j k$^{+,++c,d}$	30 k–n*	7 p–s
SC283	52 d e f*	30 i j k$^{+,++}$	12 o–s	2 rs
SC574	64 a b c*	39 g h i^{++}	64 a b c*	11 o–s
SC689	74 a*	58 b c d^{++}	67 a b*	22 k–o
SC599	55 c d e*	33 g–k^{++}	43 e f g*	7 p–s
SC326 × SC103	43 f g*	17 L–p^{++}	42 f g h*	5 q r s
GP140	28 i–L	29 i j k$^{+,++}$	15 m–q	12 o–s
SC214	30 h–k*	12 o–s^{+}	14 n–r*	1 s
RTx430	51 d e f*	25 k l m$^{+,++}$	27 j k L*	5 q r s
TAM428	53 c–f*	38 g–i$^{+,++}$	39 g h i*	8 p–s

[a] Values followed by the same letter are not significantly different at the 5% level.
[b*] = Significant differences between antidote–no antidote.
[c+] = Significant differences between antidote at high and low pH.
[d++] = Significant differences between no antidote at high and low pH.

Table IV. Percent survival at 7 days of sorghum [*Sorghum bicolor* (L.) Moench] cultivars planted in metolachlor-treated soils at pH 4.2 and >6.0.

Cultivar	pH >6.0 Oxabetrinil +	pH >6.0 Oxabetrinil −	pH 4.2 Oxabetrinil +	pH 4.2 Oxabetrinil −
	Percent survival[a] [%]			
Milo Blanco	175 ± 36*[b]	31 ± 10$^{+,++c,d}$	81 ± 53*	6 ± 5
SC283	78 ± 12*	32 ± 10$^{+,++}$	12 ± 2*	2 ± 1
SC574	81 ± 12*	50 ± 14^{++}	88 ± 13*	13 ± 5
SC689	84 ± 4	72 ± 9^{++}	82 ± 5*	32 ± 2
SC599	84 ± 4*	44 ± 12$^{+,++}$	64 ± 15*	6 ± 2
SC326 × SC103	77 ± 4*	38 ± 11^{++}	74 ± 5*	7 ± 4
GP140	179 ± 24*	94 ± 6$^{+,++}$	88 ± 25*	36 ± 15
SC214	50 ± 5*	31 ± 9$^{+,++}$	24 ± 8*	4 ± 3
RTx430	135 ± 12*	59 ± 12$^{+,++}$	72 ± 25*	8 ± 4
TAM428	133 ± 6*	51 ± 8$^{+,++}$	74 ± 24*	7 ± 3

[a] $\text{Survival} = \dfrac{\#\ \text{Plants} \times 100}{\text{Germinated} - \text{without antidote}}$
[b*] = Significant difference at a pH between antidote treatments.
[c+] = Significant difference between pH in antidote treated.
[d++] = Significant difference between pH in non-antidote treated.

cultivars grown on pH 4.2 soils. Differences between plants grown at different pH's without oxabetrinil treatment were present in all cultivars while three cultivars did not show significant differences between pH conditions when the seed were treated with oxabetrinil. Oxabetrinil induced an increase in 30-day survival in all cultivars at pH >6.0 and in seven of ten cultivars at pH 4.2 (data not shown). Again, these data show the influence of metolachlor, oxabetrinil, and acid soil

stress on seedling development in the various sorghum cultivars. These responses could be due to: a) combined influence on GA biosynthesis and metabolism, or b) reactivities in different enzymatic processes. Metolachlor and acid soil stress influence GA biosynthesis and transport [2, 5], while oxabetrinil is known to reverse the inhibition of metolachlor on GA precursor biosynthesis [2]. These data corroborate the concept that these responses are involved with a common physiological system.

Using Colby's method [11] for evaluation of synergism or antagonism, the interaction between metolachlor and acid soil stress was shown to be additive indicating that these stresses were reactive at the same site of action.

Manganese content of sorghum cultivars grown in acid stress soils

Concentrations of Mn^{2+} present in the dry weight do not correlate with acid soil resistance in either the bud or leaf tissue at either soil pH (Table V). Thus, Mn^{2+} absorption on a dry weight basis was uniform across cultivars but was influenced by the total Mn^{2+} available for absorption in the soil solution [12].

Total manganese concentration in the water within each tissue shows acid soil responses which were highest in the respective tissues from plants grown at pH 4.2 (Table VI). There were not any significant differences that correlated with acid soil susceptibility. Thus, these Mn^{2+} concentrations were more explicable on the basis of Mn^{2+} availa-

Table V. Manganese content of reproductive bud and leaf tissue taken from sorghum [*Sorghum bicolor* (L.) Moench] cultivars grown on soils of pH >6.0 and 4.2.

Cultivar	Bud pH >6.0	Bud pH 4.2	Leaf pH >6.0	Leaf pH 4.2
		μmol Mn/g DW		
SC 574	0.502 ± 0.164[a*]	1.068 ± 0.129[b,c]	0.434 ± 0.092*	0.998 ± 0.166
SC 599	0.796 ± 0.022*	1.490 ± 0.191[+,++]	0.482 ± 0.050*	0.807 ± 0.197
SC 326 × SC 103	0.606 ± 0.041*	37.5 ± 33.0	0.648 ± 0.086*	2.587 ± 1.297
SC 214	0.880 ± 0.108	1.151 ± 0.194[+,++]	0.618 ± 0.143	0.809 ± 0.068
RTx 430	1.074 ± 0.067	0.783 ± 0.087	0.783 ± 0.136	0.858 ± 0.146
TAM 428	0.439 ± 0.114*	1.296 ± 0.151	0.374 ± 0.083*	1.001 ± 0.206

[a*] = Significant difference between pH >6.0 and pH 4.2.
[b+] = Significant difference between bud and leaf tissue at pH >6.0.
[c++] = Significant difference between bud and leaf tissue at pH 4.2.

Table VI. Manganese concentration in the water of reproductive bud and leaf tissue taken from sorghum [*Sorghum bicolor* (L.) Moench] cultivars grown on soils of pH >6.0 and 4.2.

Cultivar	Bud pH >6.0	Bud pH 4.2	Leaf pH >6.0	Leaf pH 4.2
		mmol Mn/g water		
SC 574	0.087 ± 0.025*[a]	0.149 ±0.018[++b]	0.062 ± 0.013*	0.229 ± 0.034
SC 599	0.104 ± 0.003*	0.200 ± 0.025[+c]	0.072 ± 0.007*	0.155 ± 0.033
SC 283 × SC 103	0.072 ± 0.005*	0.4 ± 0.301[+,++]	0.057 ± 0.007*	0.227 ± 0.117
SC 214	0.103 ± 0.012*	0.149 ± 0.025	0.077 ± 0.017*	0.148 ± 0.012
RTx 430	0.105 ± 0.007*	0.140 ± 0.011[+,++]	0.082 ± 0.013*	0.195 ± 0.026
TAM 428	0.065 ± 0.015*	0.203 ± 0.022	0.055 ± 0.009*	0.220 ± 0.043

[a*] = Significant difference between pH >6.0 and pH 4.2.
[b++] = Significant difference between bud and leaf tissue at pH 4.2.
[c+] = Significant difference between bud and leaf tissue at pH >6.0.

bility in the soil solution and a relatively uniform Mn^{2+} absorption capacity than on the basis of cultivar differential capacity to absorb Mn^{2+}. Thus, if Mn^{2+} concentration is a major factor in the response of plants to acid soil stress, there must be a sorghum cultivar differential in response to Mn^{2+} concentration in the capacity to produce GA.

Influence of manganese on GA precursor biosynthesis

Manganese concentration is known to influence gibberellin precursor biosynthesis [6, 7]. Small concentrations are highly beneficial in the synthesis of *ent*-kaurene but excess concentrations are highly deleterious [6, 7]. The sorghum cultivar utilized for studies of the metolachlor influence on gibberellin precursor biosynthesis was Funk G 522 DR which is highly sensitive to acid soil stress. Fig. 1a shows Funk G 522 DR to have a typical Mn^{2+} concentration response curve with a stimulation of *ent*-kaurene synthesis up to 5 µM Mn^{2+} concentrations and a 50% decrease in *ent*-kaurene biosynthesis at a concentration of 15 µM Mn^{2+}. The concentration of Mn^{2+} required for

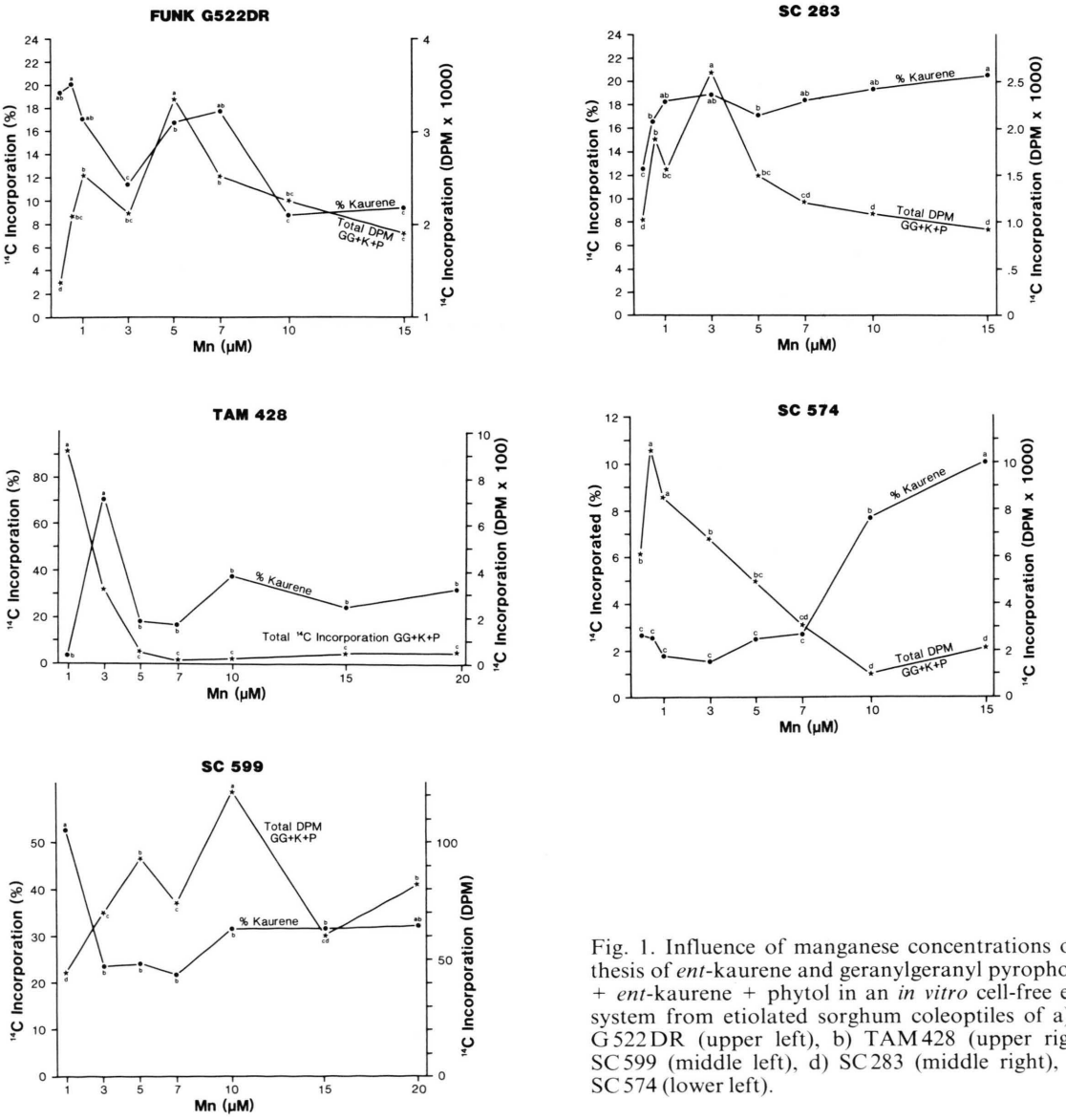

Fig. 1. Influence of manganese concentrations on synthesis of *ent*-kaurene and geranylgeranyl pyrophosphate + *ent*-kaurene + phytol in an *in vitro* cell-free enzyme system from etiolated sorghum coleoptiles of a) Funk G 522 DR (upper left), b) TAM 428 (upper right), c) SC 599 (middle left), d) SC 283 (middle right), and e) SC 574 (lower left).

maximum *ent*-kaurene biosynthesis in Funk G 522 DR is somewhat higher than that found in wheat [7], but the general trends are the same.

Manganese concentration required for maximum *ent*-kaurene biosynthesis in TAM 428 (acid soil stress sensitive) was 3 μM Mn^{2+}; *ent*-kaurene biosynthesis was decreased at higher Mn^{2+} concentrations (Fig. 1 b). *ent*-Kaurene biosynthesis in SC 599 (acid soil stress intermediate) was a maximum at 1 μM Mn^{2+}; *ent*-kaurene biosynthesis was decreased at higher Mn^{2+} concentrations (Fig. 1 c). But, total geranylgeranyl (GG) + products were a maximum at 10 μM Mn^{2+} in SC 599, while the acid soil stress sensitive cultivars were generally more sensitive to excess Mn^{2+} in GG + product biosynthesis (Fig. 1 a–1 c). Finally, the acid soil tolerant cultivars (SC 283, SC 574) were sensitive to excess Mn^{2+} in total GG + product biosynthesis (Fig. 1 d, 1 e) but *ent*-kaurene biosynthesis was not influenced by excess Mn^{2+} (15 μmol Mn) (Fig. 1 d, 1 e). Thus, the influence of metolachlor and acid soil stress (excess Mn^{2+} concentration) in these sorghum cultivars is explicable as an influence on gibberellin precursor biosynthesis. Additionally, the influence of excess H^+ concentration on the growth of sorghum seedling shoots is totally explicable as a major decrease in GA transport from plumule to aleurone where the various cultivars show the same relative percentage of sorghum shoot growth inhibition as culture pH decreased and this inhibition is identical to the partitioning of GA_3 between water and ethyl acetate as pH decreased [5]. Thus, growth and morphological responses of sorghum cultivars to metolachlor, oxabetrinil, and acid soil stress are identical and can be explained by common biosynthetic processes and reactivities.

Conclusion

Safener mode of action has been suggested to be due to: a) decreased herbicide absorption, b) decreased herbicide transport to the site of activity, or c) increased herbicide metabolism until a physiologically active concentration no longer exists at the site of activity [13]. At least two additional alternatives exist. They are: (1) a true biochemical interaction of the two exogenously applied compounds at the site(s) of activity, and (2) concomitant reactivity between the herbicide at its site of activity and a product of the activity of the safener. Examples of true competition between exogenously applied compounds at the herbicide site of activity are seen between norflurazon and some norflurazon analogs [14]. Examples of the reactivities of products of herbicide activity are seen in the reversal of phytoene synthetase by EPTC or CDAA [15, 16].

There is no doubt that decreased absorption, transport, or increased metabolism could result in decreased herbicidal activity. But plant herbicide and safener biochemical responses are located in mitochondria, cytosol, microsomes, and chloroplasts. The possibility that a single degradative mechanism is involved in all of these cellular compartments is difficult to postulate because the absorption of these herbicides into the seedling tissues is directly into the coleoptile and transport from roots to plumule is at an absolute minimum. Thus, degradative mechanisms in the coleoptile must be present in all of the cellular compartments, and that has not been shown.

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[1] R. E. Wilkinson, Pestic. Biochem. Physiol. **32,** 25 (1988).
[2] R. E. Wilkinson, Pestic. Biochem. Physiol. **16,** 199 (1981).
[3] C. D. Foy, Soil Acidity and Liming, in: American Society of Agronomy Monograph No. 12, 2nd Ed. (F. Adams, ed.), pp. 57–97, American Society of Agronomy, Madison, Wis. 1984.
[4] R. B. Clark, in: Breeding Plants for Less Favorable Environments (M. N. Christiansen and S. F. Lewis, eds.), pp. 71–142, John Wiley and Sons, New York 1982.
[5] R. E. Wilkinson and R. R. Duncan, J. Plant Nutrit. **12,** 1395 (1989).
[6] J. Knotz, R. C. Coolbaugh, and C. A. West, Plant Physiol. **60,** 81 (1977).
[7] R. E. Wilkinson and K. Ohki, Plant Physiol. **87,** 841 (1988).
[8] K. Ohki, Agron. J. **67,** 30 (1975).
[9] R. R. Duncan and R. E. Wilkinson, in: Plant Nutrition – Physiology and Application (M. L. van Beusichem, ed.), p. 429, Kluwer Acad. Publ., New York 1990.
[10] R. E. Wilkinson, J. Plant Growth Regul. **1,** 85 (1982).
[11] S. R. Colby, Weeds **15,** 20 (1967).
[12] R. E. Wilkinson, E. L. Ramseur, R. R. Duncan, and L. M. Shuman, in: Genetic Aspects of Plant Mineral Nutrition (N. El Bassam *et al.*, eds.), p. 263, Kluwer Acad. Publ., New York 1990.
[13] K. K. Hatzios, in: Crop Safeners for Herbicides: Development, Uses, and Mechanism of Action (K. K. Hatzios and R. E. Hoagland, eds.), p. 365, Academic Press, Inc., San Diego 1989.
[14] R. E. Wilkinson, Pestic. Biochem. Physiol. **28,** 381 (1987).
[15] R. E. Wilkinson, Pestic. Biochem. Physiol. **33,** 257 (1989).
[16] R. E. Wilkinson, Pestic. Biochem. Physiol. **29,** 146 (1987).