Phytotoxicity and Distribution of Sorgoleone in Grain Sorghum Germplasm

Chandrashekhar I. Nimbal,*,† Jeffery F. Pedersen,‡ Carla N. Yerkes,§ Leslie A. Weston,† and Stephen C. Weller§

Department of Horticulture and Landscape Architecture, University of Kentucky, Lexington, Kentucky 40546, Agricultural Research Service, U.S. Department of Agriculture, University of Nebraska, Lincoln, Nebraska 68583, and Department of Horticulture, Purdue University, West Lafayette, Indiana 47907

The relative phytotoxicity of sorgoleone as measured by seed germination and seedling growth of selected crop and weed species and inhibition of photosynthetic oxygen evolution in atrazine-resistant and -susceptible cell cultures of potato (*Solanum tuberosum* L.) and common groundsel (*Senecio vulgaris* L.) were investigated. Relatively little or no effect of sorgoleone was observed on radicle elongation at concentrations less than 500 μM in Petri dish bioassays. Sorgoleone was very phytotoxic to large crabgrass (*Digitatia sanguinalis*), with a GR_{50} of 10 μM for shoot growth in a hydroponic culture bioassay. Inhibition of shoot and root growth of velvetleaf (*Abutilon theophrasti*) and barnyardgrass (*Echinocloa crus-galli*) was also observed at higher concentrations ranging from 10 to 200 μM, but ivyleaf morningglory (*Ipomea hederacea*) was tolerant. Sorgoleone inhibited photosynthetic oxygen evolution in both susceptible and resistant cell cultures of potato and common groundsel, and the effect was similar to that of diuron, a strong inhibitor of PS II electron transport. Chlorophyll fluorescence response to sorgoleone in both resistant and susceptible cell cultures was nearly the same. Grain sorghum (*Sorghum bicolor* L. Moench) genotypes varied considerably in the amount of sorgoleone produced. Root exudates generally contained 85−90% pure sorgoleone on the basis of HPLC analysis. These data indicate that sorgoleone is phytotoxic at micromolar concentrations, exhibits marked selectivity, and inhibits photosynthetic electron transport similar to diuron.

Keywords: *Sorgoleone; bioassay; hydroponics; inhibition; phytotoxicity; photosynthetic oxygen evolution; electron transport; root exudate*

INTRODUCTION

Sorghum species are currently used as cover crops and green manures and for the production of grain and molasses. In the southern United States, they are often grown as summer annual cover crops because of their rapid growth and ability to suppress weeds (Forney et al., 1985). Numerous studies have shown that grain sorghum residues can suppress weeds for at least 8 weeks after cover crop kill and, when turned under, have inhibited weed growth throughout the following season (Putnam et al., 1983; Einhellig and Rasmusssen, 1989). Several *Sorghum* species have shown strong allelopathic interference (Rice, 1984; Panasiuk et al., 1986; Geneve and Weston, 1988; Einhellig and Souza, 1992). Sorghum shoots release cyanogenic glucosides, and a number of phenolic breakdown products of these glucosides contribute to short-term plant growth suppression (Einhellig and Rasmussen, 1989; Putnam and DeFrank, 1983; Guenzi and McCalla, 1966; Weston et al., 1989).

Recently, a more phytotoxic inhibitor was discovered within the root exudates of grain sorghum (*Sorghum bicolor* L. Moench). Netzley and Butler (1986) isolated sorgoleone {2-hydroxy-5-methoxy-3-[(8′*Z*,11′*Z*)-8′,11′,14′-pentadecatriene]-*p*-benzoquinone} from hydrophobic root exudates of sorghum. Sorgoleone, the major *p*-benzoquinone, and three other structurally related minor *p*-benzoquinones together constitute 90% or more of the root exudates. These *p*-benzoquinones are also contact allergens with effects similar to those of poison ivy (Netzly et al., 1988). Sorgoleone has been shown to be a potent inhibitor of chlorophyll formation in *Lemna minor* L. and also inhibits the growth of some broadleaf and grass weeds (Einhellig and Souza, 1992). Sorgoleone appears to act by blocking mitochondrial electron transport as evidenced by inhibition of state III and state IV respiration in mitochondria isolated from etiolated seedlings of soybean [*Glycine max* (L.) Merr.] and corn (*Zea mays* L.) (Rasmussen et al., 1992). Recent studies using soybean leaf disks and isolated pea chloroplasts have indicated that sorgoleone is an effective inhibitor of CO_2-dependent oxygen evolution (Einhellig et al., 1993). However, the site at which sorgoleone acts to inhibit photosynthetic oxygen evolution is not known precisely. An important method of measuring interference with photosynthetic electron transport by a herbicide or other inhibitor involves measuring chlorophyll fluorescence (Devine et al., 1993). If a herbicide compound inhibits transfer of electrons from the primary electron acceptor (Q^-_A) to the secondary electron acceptor (Q_B), the excitation energy builds up in photosystem II and leads to increased fluorescence emmission, which can be conveniently measured with a fluorometer.

This research was conducted with an objective to investigate the effects of sorgoleone on seed germination, seedling growth in selected crop and weed species,

* Author to whom correspondence should be addressed.
† University of Kentucky.
‡ University of Nebraska.
§ Purdue University.

S0021-8561(95)00561-9 CCC: $12.00

and inhibition of photosynthetic electron transport in resistant and susceptible cell cultures of potato (*Solanum tuberosum* L.) and common groundsel (*Senecio vulgaris* L.). A second objective was to evaluate sorgoleone production within a diverse collection of grain sorghum germplasm.

MATERIALS AND METHODS

Extraction of Sorgoleone. Sorgoleone was extracted according to a procedure described by Netzly and Butler (1986) and Netzly et al. (1988) with some modification. Seeds of sorghum (cv. Pioneer 8333) were germinated in a seed germination chamber at 29 °C for 5 days in the dark after 25 surface sterilized seeds were placed in each of the 100 Petri dishes lined with moistened filter paper. Seedling roots were excised and dipped (20 s) in methylene chloride containing 1% glacial acetic acid to extract sorgoleone. The crude extract was filtered and then evaporated to dryness with a stream of N_2 gas. The dried extract was dissolved in acetonitrile and analyzed for sorgoleone content by HPLC (Waters, Model 486) using a reversed phase Nova-Pak C_{18} column (3.9 × 150 mm, 4 μm). The mobile phase was 75% acetonitrile/25% acidified water. Water was acidified with glacial acetic acid (97.5:2.5 v/v). Sorgoleone was detected at 280 nm using a Waters tunable absorbance detector after 20 μL of the acetonitrile-solubilized crude extract sample was injected. The column flow rate was 2 mL/min with a 10 min total run time.

Weed and Crop Germination Bioassays. Sorgoleone activity was bioassayed using selected weed and vegetable crops. Growth inhibition as measured by radicle elongation was evaluated in velvetleaf (*Abutilon theophrasti* Medic.), foxtail millet [*Setaria italica* (L.) Beauv.], curly cress (*Lepidium sativum* L.), barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], lettuce (*Lactuca sativa* L.), and tomato (*Lycopersicon esculentum* L.). Twenty seeds of each weed/crop were germinated in 4.5 cm Petri dishs containing appropriate sorgoleone test solution. Sorgoleone was formulated at concentrations of 0, 125, 250, 500, and 1000 μM by initially dissolving in methanol and then mixing with water. Methanol concentration in the test solution did not exceed 4%. A 1000 μL aliquot of the appropriate test solution was added to individual Petri dishes lined with Whatman No.1 filter paper. Control dishes received 1000 μL of methanol−water solution. After seeds were placed on moistened filter paper, petri dishes were transferred to a container and incubated in a seed incubator at 29 °C for 3 days in the dark. At the end of the incubation period, radicle length was measured. Treatments were replicated four times, and the experiment was repeated.

Bioassay of Weed Growth in Hydroponics. Sorgoleone activity was bioassayed in hydroponic culture with four weed species. Seeds of velvetleaf (*A. theophrasti* Medic.), ivyleaf morninglory [*Ipomea hederacea* (L.) Jacq.], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and barnyardgrass [*E. crus-galli* (L.) Beauv.] were grown in the greenhouse in plastic containers with vermiculite as growth medium. When seedlings were approximately 3 weeks old, they were washed and transferred to beakers containing half-strength Hoagland's solution (Hoagland and Arnon, 1950). After 3 days of acclimation, uniformly grown seedlings were placed in flasks containing fresh nutrient solution and various concentrations of sorgoleone including 0, 10, 50, 100, and 200 μM. Because of the difficulty of getting sorgoleone into aqueous solution due to its hydrophobicity, a concentrated stock solution was prepared in acetone. This stock was added to nutrient medium to obtain appropriate concentrations of sorgoleone. Acetone concentration in the medium was below 2.5%, and controls were formulated with and without acetone. Each treatment was replicated two or three times with five seedlings per replicate. The nutrient solution was aerated, and plants were maintained under fluorescent lighting for 10 days. Shoot and root dry weights of plants were recorded after drying for approximately 120 h at 47 °C. Mean dry weights were calculated, and GR_{50} values were estimated for roots and shoots of each weed by regression analysis of sorgoleone concentration versus root or shoot dry weights.

Table 1. Inhibition of Growth As Measured by Radicle Length of Selected Plant Species in the Presence of Various Concentrations of Sorgoleone

	radicle length[a] (mm)					
sorgoleone concn (μM)	velvet-leaf	foxtail millet	curly cress	barnyard-grass	lettuce	tomato
0	19.2a	18.3a	10.0a	21.7a	14.9a	7.9a
125	18.5a	19.8a	10.1a	24.5b	13.1a	8.5a
250	20.2a	20.5a	7.8ab	21.0a	14.9a	7.9a
500	16.1a	20.2a	9.2ab	16.8c	15.3a	8.6a
1000	16.6a	17.8a	7.3b	16.5c	16.2a	8.0a

[a] Means followed by the same letter within a column are not different at the 5% level of probability (Fisher's protected lsd).

Screening of Sorghum Germplasm for Sorgoleone Production. A diverse collection of grain sorghum germplasm (Table 3) was screened for sorgoleone production with 5-day-old seedling roots. Germination percentage and root fresh weight were recorded for each germplasm, and total quantity of sorgoleone produced was determined. The extraction procedure for sorgoleone is as outlined above. The purity of the sorgoleone extract was also assessed using reversed phase HPLC as described above.

Cell Cultures. An atrazine-susceptible genotype of potato (PO-WT) was established in liquid suspension culture, made photoautotropic (LaRosa et al., 1984), and used for the selection of an atrazine-resistant (PO-VAR) cell line (Smeda et al., 1993). For additional comparison, atrazine-susceptible (CG-WT) and resistant (CG-VAR) common groundsel plants were used to establish photoautotrophic liquid suspension cultures (Yerkes and Weller, 1993). Both WT and VAR photoautotrophic potato and common groundsel cell suspension cultures were maintained on carbohydrate-free media under conditions of 2% CO_2 and 200 μmol m^{-2} s^{-1} photosynthetic photon flux density. All experiments were conducted with cells in mid-exponential phase of growth and suspended in their respective growth media (Smeda et al., 1993; Yerkes and Weller, 1993).

Measuring Photosynthetic Electron Transport. Photosynthetic electron transport was measured with a Clark-type oxygen electrode (Hansatech D2/2 system) in the absence and presence of sorgoleone at concentrations ranging from 0.01 to 100 μM. For each measurement, 1 mL of cell suspension (30 mg mL^{-1}) was added to the sample cuvette. Temperature of the samples was maintained at 29 °C with a circulating water bath. After a steady rate of respiration was attained, sorgoleone was added under dim light and allowed to absorb for 3 min. A light intensity of 225 μmol m^{-2} s^{-1} photosynthetic photon flux density was used to drive electron transport, and oxygen evolution was measured polarographically.

Chlorophyll Fluorescence. Cell suspensions (30 mg mL^{-1}) of WT and VAR potato and common groundsel were incubated with sorgoleone at concentrations ranging from 0 to 100 μM for 30 min and harvested by vacuum filtration on Whatman No. 4 filter paper. Two hundred milligrams of cells was packed into a 35 μL cuvette and dark-adapted for 15 min before chlorophyll fluorescence measurement. A portable fluorescence meter (Morgan CF-1000) was used for measurement of chlorophyll fluorescence. At the end of dark adaptation, cells were exposed for 10 s to an actinic light of 200 μmol m^{-2} s^{-1} photosynthetic photon flux density. F_v/F_m (ratio that reflects photochemical efficiency), $T_{1/2}$ (half-time to fluorescence maximum), and quenching capacity were measured and expressed as percent of control.

RESULTS AND DISCUSSION

Since sorgoleone is relatively hydrophobic and is less soluble in very polar solvents, biological assays with this compound can be difficult. In Petri dish assays with germinating seeds, the problem was overcome by adding sorgoleone dissolved in methanol. The response of weed and crop species to inhibition by sorgoleone varied (Table 1). Radicle length of barnyardgrass was inhib-

Table 2. Effect of Sorgoleone on the Growth of 15-Day-Old Weed Seedlings under Hydroponic Culture

	shoot dry wt (g)				root dry wt (g)			
sorgoleone concn (μM)	large crabgrass	barnyardgrass	velvetleaf	ivyleaf morningglory	large crabgrass	barnyardgrass	velvetleaf	ivyleaf morningglory
0	0.11	0.33	0.13	0.27	0.20	0.17	0.06	0.07
10	0.06	0.31	0.15	0.25	0.20	0.13	0.05	0.08
50	0.04	0.27	0.09	0.28	0.20	0.09	0.04	0.09
100	0.04	0.27	0.08	0.25	0.20	0.08	0.04	0.09
200	0.02	0.18	0.06	0.26	0.20	0.08	0.03	0.10
GR_{50}[a]	~10	~200	~200	>200	>200	~100	~200	>200

[a] GR_{50} is the concentration of sorgoleone required for 50% growth inhibition. The GR_{50} values were estimated by linear regression analysis.

ited by increasing sorgoleone concentration (>250 μM), with nearly 25% inhibition occurring at the highest concentration of 1000 μM compared to control. Conversely, radicle elongation of velvetleaf, foxtail millet, lettuce, and tomato was not significantly inhibited by sorgoleone. Inhibition did not occur in curly cress over a concentration range of 125−500 μM, but at 1000 μM radicle length was inhibited by 27% compared to control. Overall, sorgoleone had no effect on seed germination and had limited effect on the radicle elongation of weed and crop species. Our results do not agree with those of Einhellig and Souza (1992), who reported that sorgoleone caused complete inhibition of radicle length at 250 μM in *Eragrostis tef* after 48 h of treatment. It is possible that *Eragrostis* may be comparatively more susceptible than other species evaluated. Alternatively, in our studies, part of the applied sorgoleone may be bound to filter paper, causing reduced availability to the germinating seed.

The bioassay in hydroponic culture showed that sorgoleone could remain in solution over a 10 day period when dissolved in a suitable solvent and sonicated before and after addition to the nutrient medium. Sorgoleone inhibited the growth of large crabgrass, velvetleaf, and barnyardgrass, and the inhibition was concentration dependent (Table 2). Among the species tested, large crabgrass was most sensitive, with a GR_{50} of 10 μM for shoot growth. However, there was no apparent effect of sorgoleone on root growth of large crabgrass. Studies by Einhellig and Souza (1992) have demonstrated similar response of large crabgrass shoot growth to sorgoleone in aqueous culture, with a 10 μM concentration reducing more than 50% of shoot growth. Reduction in root growth compared to control was observed in their studies, but no significant differences were found between 10, 50, and 100 μM. Differential tolerance to sorgoleone was observed among weed species, with ivyleaf morningglory showing the most tolerance as evidenced by little effect of sorgoleone on either shoot or root growth over the range of conentrations tested (Table 2). Sorgoleone caused chlorosis in sensitive tissues, with chlorotic shoot tissue observed particularly in large crabgrass and barnyardgrass. The effect of sorgoleone on tissue chlorosis is an indication that sorgoleone may inhibit chlorophyll biosynthesis. Research on its mode of action has indicated that sorgoleone is an inhibitor of CO_2-dependent O_2 evolution (Einhellig et al., 1993). In our experiments, living root systems were often discolored by the presence of sorgoleone, but root weights were generally less affected by sorgoleone presence than shoot weights. Compared to other natural products with herbicidal activity, sorgoleone is very active at concentrations of 10−200 μM, similar to many synthetic herbicides.

Screening of sorghum germplasm for sorgoleone production indicated that considerable variability exists among genotypes with regard to the amount of sorgoleone produced (Table 3). RTx433 produced as little as 0.67 mg/g of root fresh weight, whereas B Redlan and IS 1318C produced 17.8 and 14.2 mg of sorgoleone, respectively. Most other genotypes ranged betweeen 1.5 and 10 mg/g of root fresh weight. In a different collection of germplasm, Hess et al. (1992) observed that all genotypes of *Sorghum* produced equal amounts of sorgoleone regardless of whether they were resistant or susceptible to striga (*Striga asiatica*). Furthermore, they indicated that sorgoleone production is quite sensitive to environmental conditions, particularly to the amount of moisture on the filter paper. It appears, from our studies performed under controlled environmental conditions, that production and secretion of sorgoleone may also be dependent on inherent genetic differences among *Sorghum* genotypes, apart from environmental factors. On average, sorgoleone accounted for 85−90% of the root exudate composition of the germplasm tested, although the actual amount ranged between 76 and 99%. It is interesting to observe that *Sorghum* species vary not only in the amount of sorgoleone produced but also in its purity, which may suggest differential allelopathic interference among genotypes.

Table 3. Sorgoleone Production by Various Sorghum Genotypes[a]

sorghum genotype	root fresh wt (RFW) (g)	amt of sorgoleone (mg)	sorgoleone per unit RFW (mg/g)	% purity
RTx433	0.15 ± 0.03	0.10 ± 0.00	0.67	91.8
R NB9040	0.13 ± 0.01	1.00 ± 0.25	7.70	89.5
IS 3723C	0.29 ± 0.01	1.23 ± 0.18	4.24	85.7
IS 8266C	0.22 ± 0.01	1.10 ± 0.10	5.00	87.2
B N122	0.35 ± 0.01	2.00 ± 0.10	5.71	75.9
RTx7078	0.15 ± 0.01	1.80 ± 0.21	12.00	98.7
B Martin	0.32 ± 0.03	1.73 ± 0.13	5.40	78.2
IS 8160C	0.22 ± 0.03	2.00 ± 0.15	9.10	83.8
RTx415	0.20 ± 0.02	1.87 ± 0.03	9.35	92.4
RTx430	0.08 ± 0.04	0.30 ± 0.10	3.75	94.9
IS 1318C	0.17 ± 0.03	2.43 ± 0.47	14.20	87.0
RTx7000	0.25 ± 0.02	2.30 ± 0.32	9.20	78.9
Greenleaf	0.06 ± 0.01	0.73 ± 0.47	11.40	99.1
IS 7333C	0.27 ± 0.01	1.63 ± 0.27	6.00	83.4
EH-Sart	0.30 ± 0.04	1.60 ± 0.21	5.33	87.1
IS 5893C	0.19 ± 0.02	0.33 ± 0.07	1.74	82.4
BTx3042	0.19 ± 0.02	0.30 ± 0.06	1.58	77.7
B Redlan	0.15 ± 0.03	2.67 ± 1.31	17.80	80.2
R N97	0.25 ± 0.00	1.70 ± 1.15	6.80	81.6
Piper	0.17 ± 0.01	0.17 ± 0.06	1.00	98.6
IS 1269C	0.20 ± 0.03	0.77 ± 0.15	1.10	83.2
IS 7041C	0.37 ± 0.02	0.60 ± 0.31	1.62	87.9
IS 1098C	0.08 ± 0.01	0.20 ± 0.10	2.50	88.2
IS 12611C	0.27 ± 0.02	0.67 ± 0.03	2.48	84.1
B Wheatland	0.33 ± 0.06	0.83 ± 0.23	2.50	78.3

[a] The data are means (and SD) of three replicates of 25 seedlings each.

Oxygen evolution, a measure of whole chain photosynthetic electron transport, decreased in both atrazine-

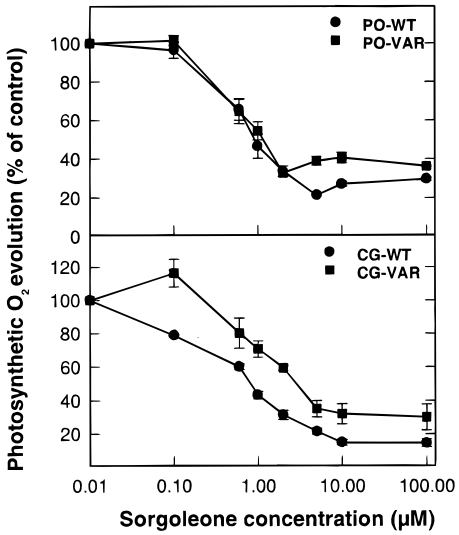

Figure 1. Photosynthetic oxygen evolution expressed as percent of control in susceptible (WT) and resistant (VAR) cell cultures of potato (PO) and common groundsel (CG) treated with different concentrations of sorgoleone. Oxygen evolution was measured in micromoles of O_2 per milligram of Chl per hour, and the data are means of three experiments.

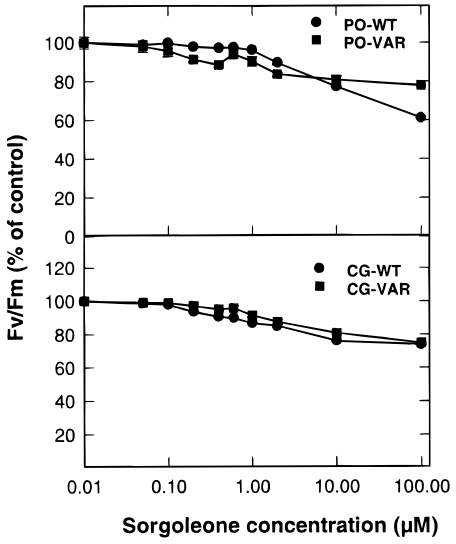

Figure 2. Chlorophyll fluorescence response measured as F_v/F_m (ratio that reflects photochemical efficiency) in susceptible (WT) and resistant (VAR) photoautotrophic cell cultures of potato (PO) and common groundsel (CG) treated with different concentrations of sorgoleone. Data are means of two experiments.

resistant (VAR) and -susceptible (WT) cell cultures of potato and common groundsel with increasing concentrations of sorgoleone (Figure 1). In potato cell cultures, 1 μM sorgoleone effectively reduced oxygen evolution by 50% compared to the untreated control. Furthermore, responses of both WT and VAR cells were identical over the range of sorgoleone concentrations investigated. In contrast, WT common groundsel was more sensitive to inhibition by sorgoleone as compared to VAR, although the pattern of oxygen evolution followed a similar decreasing trend in both as a function of concentration. Less than 1 μM sorgoleone reduced oxygen evolution by 50% in WT common groundsel cells compared to nearly 2 μM sorgoleone required by the VAR cells to obtain a similar level of inhibition. Interestingly, the response of sorgoleone is very similar to the observed response for diuron in inhibiting oxygen evolution of WT and VAR common groundsel cells (Yerkes and Weller, 1995). Also, in studies with spinach thylakoids, sorgoleone strongly inhibited oxygen evolution, and the pattern of inhibition was strikingly similar to that of diuron (Nimbal et al., unpublished data). Evidence for the inhibition of photosynthetic oxygen evolution by sorgoleone has also been reported in *Glycine max* leaf disks and intact chloroplasts of *Pisum sativum* (Einhellig et al., 1993). Atrazine, another well-known inhibitor of photosynthethesis, in contrast, exhibited differential response by inhibiting oxygen evolution in WT, but not in the VAR common groundsel cells (Yerkes and Weller, 1995). These studies suggest that both sorgoleone and diuron may act at the same site to inhibit photosynthetic electron transport. Atrazine resistance in potato VAR cells is due to a serine to threonine substitution at position 264 of the photosystem II reaction center polypeptide, D1 (Smeda et al., 1993). On the other hand, in common groundsel VAR cells and most other triazine-resistant higher plants characterized, resistance is due to a serine to glycine substitution at the same position of the D1 polypeptide.

Chlorophyll fluorescence has been regarded as an important technique in determining inhibition of photosynthetic electron transport. Dark-adapted, photoautotrophic cells, when exposed to photosynthesis-

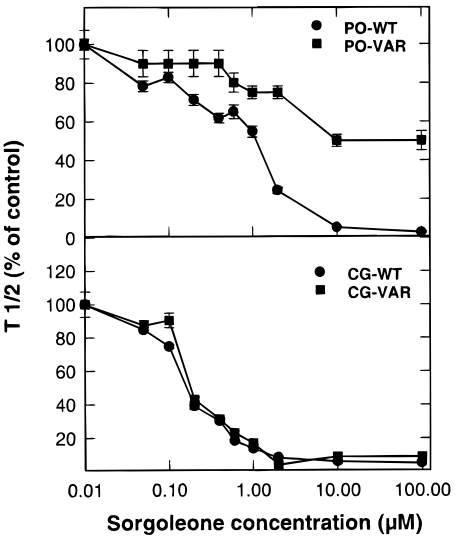

Figure 3. Half-time to fluorescence maximum ($T_{1/2}$) in sorgoleone-treated susceptible (WT) and resistant (VAR) photoautotrophic cell cultures of potato (PO) and common groundsel (CG). Data are means of two experiments.

saturating light, exhibit a typical rise in fluorescence followed by a rapid exponential decay in the absence of any PS II inhibiting herbicide or inhibitor. The Presence of such an inhibitor/herbicide can markedly slow the fluorescence decay, indicating that the reoxidation of Q^-_A by Q_B is inhibited. When we measured the flash-induced chlorophyll fluorescence in the presence of sorgoleone, the F_v/F_m was found to be similar for WT and VAR cell types of both potato and common groundsel (Figure 2). This indicates that sorgoleone caused fluorescence in the VAR cells almost to the same extent as in WT cells. This is not unexpected because photosynthetic electron transport, as measured by oxygen evolution, was affected similarly in both WT and VAR cell types. Increasing sorgoleone concentrations progressively reduced the elapsed time to reach fluorescence maximum (Figure 3). $T_{1/2}$ was reduced similarly in WT and VAR cells of common groundsel, but the effect was less for VAR potato cells compared to WT

potato cells. The reason for this differential response is not clear, although VAR potato cells exhibited inherently low $T_{1/2}$ time compared to WT potato cells.

Sorgoleone is a natural plant product that has herbicidal activity at very low concentrations. The biological activity of this compound is much greater than those of many phenolics, flavonoids, coumarins, and sesquiterpene lactones (Einhellig et al., 1970; Einhellig, 1986; Einhellig and Rasmussen, 1989; Scholes, 1987; Harr, 1990) and can be compared to synthetic herbicides such as diuron, atrazine, and metribuzin that inhibit photosynthesis. Although all grain sorghum genotypes evaluated exude sorgoleone from their roots, our studies suggest that the amount of sorgoleone produced is not the same in all of the genotypes tested. It is also noteworthy that sorgoleone exhibits selectivity among plant species. At the present time, investigations are underway to elucidate the precise mode of action of sorgoleone and the inheritance of sorgoleone production in sorghum. With an ability to cause phytotoxicity at micromolar concentrations potentially by inhibiting photosynthetic oxygen evolution, sorgoleone is a potent allelochemical.

ABBREVIATIONS USED

WT, atrazine-susceptible; VAR, atrazine-resistant; PO, potato; CG, common groundsel; F_v/F_m, ratio that reflects photochemical efficiency; $T_{1/2}$, half-time to fluorescence maximum.

LITERATURE CITED

Devine, M. D.; Duke, S. O.; Fedtke, C. In *Physiology of Herbicide Action*; P T R Prentice Hall: Englewood Cliffs, NJ, 1993; pp 113−136.

Einhellig, F. A. Mechanisms and modes of action of allelochemicals. In *The Science of Allelopathy*; A. R. Putnam and C. S. Tang, Eds.; Wiley: New York, 1986; pp 171−188.

Einhellig, F. A.; Rasmussen, J. A. Prior cropping with grain sorghum inhibits weeds. *J. Chem. Ecol.* **1989**, *15*, 951−960.

Einhellig, F. A.; Souza, I. F. Phytotoxicity of sorgoleone found in grain sorghum root exudates. *J. Chem. Ecol.* **1992**, *18*, 1−11.

Einhellig, F. A.; Rice, E. L.; Risser, P. G.; and Wender, S. H. Effects of scopoletin on growth, CO_2 exchange rates, and concentration of scopoletin, scopolin and chlorogenic acids in tobacco, sunflower, and pigweed. *Bull. Torrey Bot. Club* **1970**, *97*, 22−23.

Einhellig, F. A.; Rasmussen, J. A.; Hejl, A. M.; Souza, I. F. Effects of root exudate sorgoleone on photosynthesis. *J. Chem. Ecol.* **1993**, *19*, 369−375.

Forney, D. R.; Foy, C. L.; Wolf, D. D. Weed suppression in no-till alfalfa (*Medicago sativa*) by prior cropping of summer-annual forage grasses. *Weed Sci.* **1985**, *33*, 490−497.

Geneve, R. L.; Weston, L. A. Growth reduction of eastern redbud (*Cercis canadensis* L.) seedlings caused by interaction with a sorghum-sudangrass hybrid (sudex). *J. Environ. Hortic.* **1988**, *6*, 24−26.

Guenzi, W. D.; McCalla, I. N. Phenolic acids in oats, wheat, sorghum and corn residues and their phytotoxicity. *Agron. J.* **1966**, *58*, 303−304.

Harr, M. J. Allelopathic effects of several sesquiterpene lactones. M.A. thesis, University of South Dakota, Vermillon, SD, 1990; 73 pp.

Hess, D. E.; Ejeta, G.; Buttler, L. G. Selection of sorghum genotypes expressing a quantitative biosynthetic trait that confers resistance to Striga. *Phytochemistry* **1992**, *31*, 493−497.

Hoagland, D. R.; Arnon, D. I. The water culture method for growing plants without soil. *Calif. Agric. Exp. Stn. Circ.* **1950**, *No. 347*.

LaRosa, P. C.; Hasegawa, P. M.; Bressan, R. A. Photoautotrophic potato cells: transition from heterotrophic to autotrophic growth. *Physiol. Plant.* **1984**, *61*, 279−286.

Netzly, D. H.; Butler, L. G. Roots of sorghum exude hydrophobic droplets containing biologically acitve components. *Crop Sci.* **1986**, *26*, 775−778.

Netzly, D.; Riopel, J. L.; Ejeta, G.; Butler, L. G. Germination stimulants of witchweed (*Striga asiatica*) from hydrophobic root exudates of sorghum (*Sorghum bicolor*). *Weed Sci.* **1988**, *36*, 441−446.

Panasiuk, O.; Bills, D. D.; Leather, G. R. Allelopathic influence of *Sorghum bicolor* on weeds during germination and early development of seedlings. *J. Chem. Ecol.* **1986**, *12*, 1533−1543.

Putnam, A. R.; DeFrank, J. Use of phytotoxic plant residues for selective weed control. *Crop Prot.* **1983**, *2*, 173−181.

Putnam, A. R.; DeFrank, J.; Barnes, J. P. Exploitation of allelopathy for weed control in annual and perennial cropping systems. *J. Chem. Ecol.* **1983**, *8*, 1001−1010.

Rasmussen, J. A.; Hejl, A. M.; Einhellig, F. A.; Thomas, J. A. Sorgoleone from root exudate inhibits mitchondrial functions. *J. Chem. Ecol.* **1992**, *18*, 197−207.

Rice, E. L. In *Allelopathy*, 2nd ed.; Academic Press: Orlando, FL, 1984, 422 pp.

Scholes, K. A. Effects of six classes of allelochemicals on growth, photosynthesis, and chlorophyll content in *Lemna minor* L. M.A. thesis, University of South Dakota, Vermillon, SD, 1987; 81 pp.

Smeda, R. J.; Hasegawa, P. M.; Goldsbrough, P. B.; Singh, N. K.; Weller, S. C. A serine-to-threonine substitution in the triazine herbicide-binding protein in potato cells results in atrazine resistance without impairing productivity. *Plant Physiol.* **1993**, *103*, 911−917.

Weston, L. A.; Harmon, R.; Mueller, S. Allelopathic potential of sorghum-sudangrass hybrid (sudex). *J. Chem. Ecol.* **1989**, *15*, 1855−1865.

Yerkes, C. N.; Weller, S. C. Photoautotrophic plant cell suspension cultures and their use in photosynthesis-inhibitor studies. *Proc. North Cent. Weed Sci. Soc.* **1993**, *48*, 69−70.

Yerkes, C. N.; Weller, S. C. Response of atrazine-susceptible and -resistant photoautotrophic cells to photosynthesis-inhibiting herbicides. *Weed Sci. Soc. Am. Abstr.* **1995**, *35*, 88.

Received for review August 18, 1995. Revised manuscript received February 28, 1996. Accepted March 18, 1996.[⊗]

JF950561N

[⊗] Abstract published in *Advance ACS Abstracts*, April 15, 1996.