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PHYTOTOXICITY OF SORGOLEONE FOUND IN GRAIN SORGHUM ROOT EXUDATES

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Abstract—Root exudates of *Sorghum bicolor* consist primarily of a dihydroquinone that is quickly oxidized to a *p*-benzoquinone named sorgoleone. The aim of this investigation was to determine the potential activity of sorgoleone as an inhibitor of weed growth. Bioassays showed 125 μM sorgoleone reduced radicle elongation of *Eragrostis tef*. In liquid culture, 50-μM sorgoleone treatments stunted the growth of *Lemna minor*. Over a 10-day treatment period, 10 μM sorgoleone in the nutrient medium reduced the growth of all weed seedlings tested: *Abutilon theophrasti*, *Datura stramonium*, *Amaranthus retroflexus*, *Setaria viridis*, *Digitaria sanguinalis*, and *Echinochloa crus-galli*. These data show sorgoleone has biological activity at extremely low concentrations, suggesting a strong contribution to *Sorghum* allelopathy.

Key Words—Sorgoleone, phytotoxin, allelochemical, allelopathy, root exudate, *Sorghum bicolor*, Sorghums, weed inhibition.

INTRODUCTION

Several *Sorghum* species, including *Sorghum halepense*, *S. vulgare*, *S. sudanense*, and *S. bicolor*, have been shown to cause allelopathic interference (Breazeale, 1924; Abdul-Wahab and Rice, 1967; Hussain and Gadoon, 1981; Putnam and DeFrank, 1983; Putnam et al., 1983; Alsaadawi et al., 1986). Both autotoxicity and interspecific allelopathic effects have been noted in sorghums (Rice, 1984). They produce and release cyanogenic glycosides and an array of

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phenolic acids that can contribute to suppression of plant growth (Martin et al., 1938; Guenzi and McCalla, 1966; Nicollier et al., 1983; Lehle and Putnam, 1983; Rice, 1984; Weston et al., 1989).

Work in Michigan demonstrated that *S. bicolor* (grain sorghum) could be used as an allelopathic cover crop to control weeds in vegetable crops and orchards (Putnam and DeFrank, 1983; Putnam et al., 1983). Weeds were suppressed by either fall-planted–winter-killed or spring-planted–herbicide-desiccated grain sorghum. Forney et al. (1985) found a sorghum–sudangrass hybrid (*S. bicolor* × *S. sudanense*) was effective in suppressing weeds prior to late-summer, no-till planting of alfalfa (*Medicago sativa*). Purvis (1990) reported that wild oat (*Avena sterilis*) germination was suppressed in *S. bicolor* stubble when compared to its germination in the stubble of four other crops. Einhellig and Rasmussen (1989) showed that inhibitory effects of grain sorghum on weeds carried over from one growing season to the next in a field situation in northeastern Nebraska. It is not likely that this carryover is due entirely to inhibitory phenolic acids that are released from the residue, since their activity seems to diminish substantially in a few months (Guenzi et al., 1967).

Netzly and Butler (1986) isolated several hydrophobic *p*-benzoquinones in exudates from grain sorghum roots. Subsequently, these compounds were identified and named sorgoleones (Netzly et al., 1988). The initial exudate consists of related dihydroquinones, which appear to be natural stimulants of witchweed (*Striga asiatica*) germination (Chang et al., 1986; Netzly et al., 1988; Fate et al., 1990). The dihydroquinones readily oxidize to the more stable quinones. Netzly et al. (1988) reported these quinones may have herbicidal activity. They also found that soil containing sorghum roots had methylene chloride-extractable sorgoleone equivalent to 10^{-4} to 10^{-5} mol/liter in the soil (Netzly et al., 1988). In personal communication, Dr. Larry Butler (Purdue University) stated they recovered sorgoleone from the soil in the spring where a sorghum plant was grown the year before. Hence, sorgoleone may be an integral part of the explanation for some of the allelopathic effects of grain sorghum.

Our objectives in these investigations were to determine the relative toxicity of sorgoleone as an allelopathic chemical and to investigate this compound as a possible inhibitor of weed growth under laboratory conditions.

METHODS AND MATERIALS

Exudate Collection. Grain sorghum [*Sorghum bicolor* (L.) Moench; Dekalb Hybrid DK28] root exudate was collected, as needed, by germinating seeds in 9-cm-diameter Petri dishes on Whatman No. 1 filter paper moistened with deionized water. Ten seeds were placed in each of 40 Petri dishes and germinated for five days under room light (day: 10 $\mu E/m^2/sec$) and temperature (23

± 2°C) conditions. Seedling roots were dipped in 20 ml of methylene chloride containing 50 μl of concentrated acetic acid (Netzly et al., 1988). The methylene chloride solution was evaporated to dryness and the residue recovered. Fate et al. (1990) indicated that the methylene chloride-soluble hydrophobic exudate collected in this way contains only sorgoleone and its dihydroquinone (Figure 1). The exudate collected will be referred to as sorgoleone, since the dihydroquinone readily oxidizes to the quinone (Netzly et al., 1988). Analysis of the exudate components by high-pressure liquid chromatography (HPLC: Spectra Physics, model SP8800) using a Spherisorb 5 μM ODS2 column (4.6 × 250 mm) showed approximately 90% of the exudate corresponded to the sorgoleone peak. HPLC methods followed procedures of Netzly and Butler (1986) and Netzly et al. (1988) with Dr. Larry Butler (Purdue University) providing the initial sample of sorgoleone for comparison. In the numerous repetitions of exudate collection, the quantity of sorgoleone obtained ranged from 1.3 to 1.8 mg/100 roots dipped. Sorgoleone was stored at −4°C until used.

Growth Bioassays. Initial investigations of the effects of sorgoleone on plant growth employed a bioassay with *Eragrostis tef* (Zucc) Trotter, commonly known as teff. *E. tef* is a small-seeded cereal crop common to northern Africa that is being tested in South Dakota and other areas of the northern Great Plains as a forage crop (Boe et al., 1986). This bioassay was developed in our laboratory to test the biological activity of a natural product when only a small quantity of test compound is available. *E. tef* germination approaches 100%, radicle elongation is rapid, and many seeds can be germinated in a small volume of test solution.

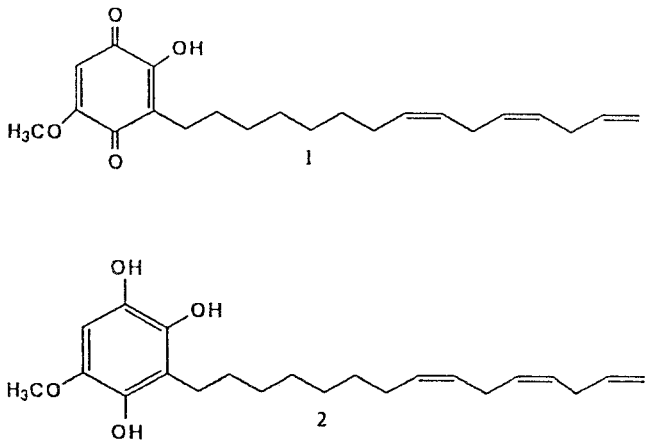

FIG. 1. Structures of (1) sorgoleone and (2) dihydroquinone of sorgoleone (Chang et al., 1986; Netzly et al., 1988; Fate et al., 1990).

Seed germination and radicle elongation studies were conducted in 4.5-cm-diameter Petri dishes with three replicates per treatment. Twenty-five seeds were germinated in a Petri dish on Whatman No. 1 paper moistened with 700 μl of test solution. Sorgoleone was first added to each Petri dish in 50 μl of ethanol, the ethanol evaporated by air drying, and deionized water was then added. The same protocol was used for controls, except they received no sorgoleone. Tests were conducted with 0, 125, and 250 μM sorgoleone. All references to sorgoleone concentrations used in these treatments and subsequent work assume that the compound dissolved in the aqueous test solutions. This is probably an overestimation of true concentration due to the relative hydrophobicity of sorgoleone. Petri dishes were placed in the dark at 25 ± 1°C and percentage germination and radicle lengths were obtained after 48 hr.

A *Lemna minor* L. bioassay was used to determine the effects of sorgoleone on whole plant growth and chlorophyll content (Einhellig et al., 1985). *L. minor* was grown in 24-well tissue culture cluster dishes. The culture media was E medium (Cleland and Briggs, 1967) modified to contain no sucrose or tartaric acid. Each culture dish well contained 1.5 ml of sterile medium and sorgoleone was added in 5 μl of methylene chloride; the control group also received 5 μl of methylene chloride. Treatment solutions were 0, 10, 50, and 100 μM sorgoleone. Efforts to enhance the solubility of sorgoleone by utilizing Tween 20 or Triton X were abandoned because *L. minor* was very sensitive to these surfactants. Each well was inoculated with three fronds, and six replications were used per sorgoleone treatment. *L. minor* was grown under continuous light (200 $\mu E/m^2/sec$) and 28°C. Frond condition, number, and dry weight (24 hr, 80°C) were obtained after seven days. In other experiments with the same design, the chlorophyll content of fronds was determined at the end of the treatment. Fronds were soaked in ethanol for two 24-hr periods, and the absorbance of the chlorophyll extract was read at 665 and 649 nm for quantification using standard equations (Wintermans and DeMots, 1965; Ramirez-Toro et al., 1988; Einhellig and Rasmussen, 1979).

Sorgoleone was tested against six commonly occurring field weeds (three broadleaf and three grass weed species) using an aqueous growth system. Seedling growth of velvetleaf (*Abutilon theophrasti* Medic.), jimsonweed (*Datura stramonium* L.), redroot pigweed (*Amaranthus retroflexus* L.), green foxtail [*Setaria viridis* (L.) Beauv.], crabgrass [*Digitaria sanguinalis* (L.) Scop.], and barnyard grass [*Echinocholoa crus-galli* (L.) Beauv.] was studied. Seeds were germinated in vermiculite in the greenhouse for seven to 10 days; then seedlings were individually transplanted into opaque vials containing 70 ml of one-half strength Hoagland's solution that contained two and one-half times the normal iron (Hoagland and Arnon, 1950). After two days acclimatization, seedlings were selected for uniformity and transferred to fresh nutrient solution, which contained either 0, 10, 50, or 100 μM sorgoleone. Eight plants were utilized

for each treatment and the control. Since sorgoleone was not fully soluble in the growth media, each treatment concentration was prepared by direct addition of sorgoleone rather than by a dilution process from the most concentrated solution. Seedlings were grown in the greenhouse for 10 days and harvested. Shoot and root dry weights were obtained after drying for 48 hr at 105°C. Leaf area and leaf weight were also obtained from the broadleaf weeds with the exception that dried and dead leaves in pigweed treatments did not allow for collection of leaf area measurements. Shoot–root ratios and the specific leaf weight (SLW = leaf weight/leaf area) were computed for *A. theophrasti* and *D. stramonium*.

Data Analyses. Comparisons among treatments in an experiment were analyzed using analysis of variance (ANOVA) and Duncan's multiple-range test.

RESULTS

The small volume of solution required for a test fostered the use of *E. tef* as the first bioassay for potential inhibitory effects of sorgoleone. In repeated trials, *E. tef* seed had a germination rate above 95%, with the radicle first appearing by 24 hr. No sorgoleone concentration tested inhibited or delayed this initial evidence of germination. However, 48-hr radicle elongation was almost completely stopped by 250 μM sorgoleone, and radicle lengths of seeds germinated in 125 μM sorgoleone were about half those of controls (Table 1).

Tests with *L. minor* showed that sorgoleone was inhibitory to the total growth of this plant. After seven days of treatment, *L. minor* grown with a 100-μM concentration of sorgoleone had only 73% of the frond count and 78% of the dry weight of controls (Table 2). *L. minor* fronds growing in this treatment also exhibited less total chlorophyll, but there was no difference from control plants in the ratio of chlorophyll a to b (data not shown). The treatment of 50 μM sorgoleone significantly inhibited frond number. Although not statistically significant, the 10-μM sorgoleone treatment resulted in a slight increase in frond number and dry weight.

TABLE 1. EFFECTS OF SORGOLEONE ON RADICLE ELONGATION OF *Eragrostis tef*[a]

	Sorgoleone treatment (μM)		
	0	125	250
Radicle Length after 48 hr (mm)	11.4 a	6.1 b	0.6 c

[a] Each value is the mean of 75 seedlings. Those not followed by the same letter are significantly different, $P < 0.05$, ANOVA with Duncan's multiple-range test.

TABLE 2. EFFECTS OF SORGOLEONE ON *Lemna minor* GROWTH AND CHLOROPHYLL CONTENT[a]

Plant parameter	Sorgoleone treatment (μM)			
	0	10	50	100
Frond number	45.5 a	52.2 a	41.7 b	33.0 c
Dry weight (mg)	5.0 a	5.3 a	4.9 a	3.9 b
Total chlorophyll (mg chl/frond)	2.07 a	2.09 a	2.06 a	1.87 b

[a] Values ($N = 6$) in a row not followed by the same letter are significantly different, $P < 0.05$, ANOVA with Duncan's multiple-range test.

The growth of each of the broadleaf weeds tested was markedly inhibited by 10 μM and higher concentrations of sorgoleone in the nutrient medium (Table 3). Inhibition was concentration-dependent, with the most severe effects occurring from 100 μM sorgoleone. In the case of *A. theophrasti* and *A. retroflexus*, plants grown with 100 μM sorgoleone appeared slightly smaller than controls at the end of three days of treatment. Differences in growth between the treated plants and controls became more apparent as the experiment progressed, and by the time of harvest several 100-μM treated *A. retroflexus* seedlings had wilted or dried leaves and appeared dead. The visible effects of sorgoleone on *Datura stramonium* seedlings were not as severe as for the other two species.

Leaf area and shoot, root, and leaf weights of all weed species were inhibited by sorgoleone even at 10-μM treatments (Table 3). However, the shoots of *A. theophrasti* and *D. stramonium* seedlings were more affected than the roots, as shown by the lower shoot–root ratios among treated plants. The reduction in shoot–root ratio was greatest in plants grown with 100 μM sorgoleone. A similar reduction in the shoot–root ratio occurred in sorgoleone-treated *A. retroflexus* plants except at the highest treatment level. This exception was probably due to early cessation of growth and/or death of *A. retroflexus* seedlings grown in 100-μM sorgoleone solutions. Calculations of SLW (specific leaf weight) for *A. theophrasti* and *D. stramonium* did not show any clear trends across treatment levels.

Similar to effects found with broadleaf weeds, growth of the three grass weeds evaluated was suppressed by each of the sorgoleone concentrations tested, and the extent of the inhibition was concentration-dependent (Table 4). *D. sanguinalis* seedlings showed the greatest reductions in plant weight with the 10-, 50-, and 100-μM sorgoleone treatments having 34%, 23%, and 22%, respectively, of the weight of untreated plants at harvest. Most of the 100-μM treated *D. sanguinalis* seedlings were dead by the time of harvest. Because of the strong

TABLE 3. EFFECTS OF SORGOLEONE ON GROWTH OF BROADLEAF WEEDS[a]

	Sorgoleone treatment (μM)			
	0	10	50	100
Abutilon theophrasti				
Total plant wt (mg)	79.0 a	48.6 b	38.4 b	22.4 c
Shoot wt[b] (mg)	66.4 a	39.9 b	30.5 b	16.9 c
Root wt (mg)	12.6 a	8.7 b	7.9 b	5.5 c
Shoot–root ratio	5.4 a	4.6 ab	3.9 bc	3.3 c
Leaf wt (mg)	48.6 a	27.8 b	20.3 b	10.9 c
Leaf area (cm^2)	20.9 a	12.6 b	7.9 c	4.1 d
SLW[c] (mg/cm^2)	2.3 a	2.2 a	2.6 a	2.7 a
Datura stramonium				
Total plant wt (mg)	220.5 a	131.6 b	116.3 bc	99.3 c
Shoot wt (mg)	191.5 a	109.3 b	96.6 bc	80.4 c
Root wt (mg)	29.0 a	22.3 ab	19.7 b	19.0 b
Shoot–root ratio	7.5 a	5.4 ab	5.7 ab	4.7 b
Leaf wt (mg)	140.4 a	76.4 b	66.6 b	57.2 b
Leaf area (cm^2)	23.9 a	15.1 b	12.0 c	10.9 c
SLW (mg/cm^2)	5.9 a	5.1 b	5.6 ab	5.2 ab
Amaranthus retroflexus				
Total plant wt (mg)	73.1 a	39.8 b	24.2 b	5.7 c
Shoot wt (mg)	65.2 a	35.4 b	20.7 bc	5.2 c
Root wt (mg)	7.9 a	4.4 b	3.5 b	0.5 c
Shoot–root ratio	8.3 b	7.9 bc	6.1 c	11.0 a
Leaf wt (mg)	49.0 a	26.4 b	14.6 c	2.9 d

[a] Each value is the mean of eight plants. Row values not followed by the same letters are significantly different, $P < 0.05$, ANOVA with Duncan's multiple-range test.
[b] Shoot weight included leaf and stem weights.
[c] SLW (specific leaf weight) = leaf weight/leaf area.

sorgoleone effects found in other species, *E. crus-galli* seedlings were not tested at the 100-μM level. In the experiments with each of the grass weeds, shoots and roots were about equally inhibited and no significant differences in the shoot–root ratio were evident.

DISCUSSION

This research clearly shows that sorgoleone is a potent inhibitor of plant growth that may likely be involved in allelopathic interference from *Sorghum* species. Sorgoleone addition at low concentrations resulted in suppressions of radicle elongation in the *E. tef* bioassay and reduction in total plant growth in

TABLE 4. EFFECTS OF SORGOLEONE ON GROWTH OF GRASS WEEDS[a]

	Sorgoleone treatment (μM)			
	0	10	50	100
Setaria viridis				
Total plant wt (mg)	42.9 a	29.4 b	15.8 c	13.4 c
Shoot wt (mg)	32.8 a	22.0 b	11.8 c	10.5 c
Root wt (mg)	10.1 a	7.4 b	4.0 c	2.9 c
Shoot–root ratio	3.3 a	3.2 a	3.6 a	3.6 a
Digitaria sanguinalis				
Total plant wt (mg)	29.1 a	9.9 b	6.8 b	6.5 b
Shoot wt (mg)	24.3 a	7.9 b	5.7 b	4.8 b
Root wt (mg)	4.8 a	2.0 b	1.1 b	1.7 b
Shoot–root ratio	5.2 a	7.3 a	5.9 a	3.2 a
Echinochloa crus-galli				
Total plant wt (mg)	134.8 a	87.4 b	40.6 c	NT[b]
Shoot wt (mg)	105.0 a	65.7 b	29.9 b	NT
Root wt (mg)	29.8 a	21.7 a	10.7 b	NT
Shoot–root ratio	3.5 a	3.1 a	2.9 a	

[a] Each value is the mean of eight plants. Row values not followed by the same letter are significantly different, $P < 0.05$, ANOVA with Duncan's multiple-range test.
[b] NT = no test.

L. minor. Growth suppression was also pronounced in the six weed species tested. Even the 10-μM treatments of sorgoleone significantly reduced growth of each of the weed seedlings over the 10-day treatment. In general, growth reductions were nonselective, as sorgoleone affected both broadleaf and grass weeds. Tests of the effects of sorgoleone on a greater diversity of weed species are needed to confirm this lack of selectivity. However, it appears that the action of sorgoleone alone cannot explain the findings of Einhellig and Rasmussen (1989) that broadleaf weed abundance and biomass were more reduced after a crop of grain sorghum than were grass weeds.

It is important to recognize that the treatment concentrations that reduced seedling growth were very low in comparison to many allelopathic chemicals that have been tested in similar experimental designs. Inhibition of seedling growth by 10 μM sorgoleone in the nutrient medium is one to two orders of magnitude lower than the concentration required for growth reduction by many phenolic acids, which is often in the 100–1000 μM range (Einhellig, 1986, 1989). Allelopathic coumarins, flavonoids, and sesquiterpene lactones are typically inhibitory in a concentration range similar to phenolic acids (Einhellig et al., 1970; Scholes, 1987; Haar, 1990). We did not test sorgoleone concentrations below 10 μM, but the data suggest the threshold for growth inhibition of

some seedlings, such as *D. sanguinalis*, may be below this level. Inhibition by sorgoleone at micromolar concentrations is even more surprising when it is recognized that sorgoleone was not completely soluble in the aqueous nutrient medium. Hence, the treatment concentrations cited for the tests must be considered somewhat higher than what was available in the treatment solutions.

Relatively few quinones have been implicated in allelopathy, and even fewer as allelochemicals from higher plants (Rice, 1984). Juglone (5-hydroxy-1,4-naphthoquinone) is well known as the principle chemical responsible for walnut (*Juglans nigra*) allelopathy. Its potency is similar to the effects of sorgoleone. Micromolar levels of juglone have been reported to suppress the growth of several herbaceous species even though no visible signs of injury occurred (Rietveld, 1983).

We speculate that sorgoleone interferes with membrane functions. Investigations are now in progress to elucidate its possible physiological effects. Regardless of the mechanism(s) of sorgoleone action, it is a powerful inhibitor of plant growth. The occurrence of sorgoleone as a relatively pure compound in root exudates indicates it must be considered a part of the explanation for the allelopathic activity of the *Sorghum* crops in conjunction with the action of phenolic acids and other allelopathic compounds known to be released from *Sorghum* species (Guenzi and McCalla, 1966; Nicollier et al., 1983; Lehle and Putnam, 1983; Panasiuk et al., 1986; Weston et al., 1989). In summary, sorgoleone is a novel allelochemical that has biological activity at extremely low concentrations.

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