

Evaluating sorgoleone herbicidal activity on weed species and wheat (*Triticum aestivum*)

Manish K. Bansal¹, Thierry E. Besançon² , Estefania G. Polli¹ ,
James D. Burton³ and Wesley J. Everman⁴ 

Research Article

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Corresponding author:

Wesley J. Everman;
Email: wjeverman@iastate.edu

¹Graduate Research Assistant, Department of Crop Science, North Carolina State University, Raleigh, NC, USA; ²Assistant Professor, Department of Plant Biology, Rutgers University, New Brunswick, NJ, USA; ³Professor Emeritus, Department of Horticultural Science, North Carolina State University, Raleigh, NC, USA and ⁴Associate Professor, North Carolina State University, Raleigh, NC, USA

Abstract

Sorgoleone, an allelochemical exuded from root hairs of grain sorghum [*Sorghum bicolor* (L.) Moench], has proven herbicidal activity on several weeds and crops. Due to its hydrophobic nature and affinity for organic matter, sorgoleone can persist in the soil for a long period. Although sorghum residues are beneficial for weed suppression, growers in the southeastern United States have raised concerns that these residues may negatively affect wheat (*Triticum aestivum* L.) emergence in double-crop systems. Two independent laboratory studies were conducted to evaluate sorgoleone activity: (1) weed species/wheat variety study, which included four weed species, Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], sicklepod [*Senna obtusifolia* (L.) Irwin & Barneby], and velvetleaf [*Abutilon theophrasti* Medik.], and two wheat varieties, ‘USG3251’ and ‘Shirley’; and (2) wheat variety and group study, which included a total of 20 varieties distributed in four wheat groups. In both studies, sorgoleone was applied at 0.025, 0.05, 0.10, 0.15, 0.20, and 0.30 g L⁻¹ to seeds grown in petri dishes under laboratory conditions. In Study 1, sorgoleone activity had a stronger effect on grass weeds than on broadleaf weeds. *Lolium perenne* ssp. *multiflorum* was the most susceptible weed, showing 77% growth reduction at 0.3 g L⁻¹ of sorgoleone. At the same concentration, *D. sanguinalis* showed the second-highest response, with a 32% growth reduction, followed by *A. theophrasti* and *S. obtusifolia*, with reductions of 23% and 20%, respectively. No effect of sorgoleone was observed on wheat varieties. In Study 2, growth reduction was only observed for six wheat varieties at the highest sorgoleone concentrations. Furthermore, in between group comparisons, Hard Red wheat presented higher response to sorgoleone. These findings demonstrate strong herbicidal activity of sorgoleone on grass weeds, particularly *L. perenne* ssp. *multiflorum*, while having minimal effects on most wheat varieties.

Introduction

Allelopathy is defined as the production and release of chemical substances by one species that inhibit the growth of another species (Inderjit and Duke 2003; Weston and Duke 2003). Allelochemicals are typically secondary metabolites, such as phenolics, terpenoids, alkaloids, coumarins, tannins, steroids, and quinones (Weir et al. 2004; Xuan et al. 2005). These compounds are commonly produced or released into the neighboring environment via root exudation, leaching from leaves and other plant parts, or decomposition of plant material.

Allelochemicals have been identified in several plant species, including black walnut [*Juglans nigra* L.], wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and sorghum [*Sorghum bicolor* (L.) Moench] (Bertin et al. 2003; Duke et al. 2005; Inderjit and Duke 2003). As naturally occurring compounds, they are generally more environmentally friendly (Macías et al. 2007), because they tend to degrade more rapidly than many synthetic herbicides, thereby reducing persistence and potentially minimizing environmental impact. Furthermore, crop species with allelopathic properties can serve as cover crops or can be included in intercropping systems to help suppress weeds.

Sorghum is an important cereal grain crop grown worldwide. This species is known to have an allelopathic effect on several crops and weed species. These allelopathic properties were first observed in crops grown in rotation with sorghum (Breazeale 1924) and later confirmed in several studies (Einhellig and Rasmussen 1989; Forney et al. 1985; Panasiuk et al. 1986; Putnam et al. 1983). Sorghum species have been reported to produce several phytotoxins, which are mainly exuded from the root systems or leached from leaves and other plant parts (Hussain et al., 2021).

The effect of sorgaab, water extract of mature sorghum plants, on weed control has been evaluated in various crops, including wheat, corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.),

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and mungbean [*Vigna radiata* (L.) R. Wilczek]. Studies have reported a reduction in weed density for several weed species, such as common lambsquarters (*Chenopodium album* L.), littleseed canarygrass (*Phalaris minor* Retz.), wild oat (*Avena fatua* L.), field bindweed (*Convolvulus arvensis* L.), and toothed dock (*Rumex dentatus* L.), when subjected to foliar application of sorgaab (Cheema et al. 2000; Cheema et al. 2004). Furthermore, Cheema et al. (2000) observed a reduction of 48% in weed biomass when sorghum stalks were incorporated into the soil at 4 mg ha⁻¹. In a laboratory study conducted by Guenzi et al. (1967), wheat root and shoot growth was reduced by 77% and 69%, respectively, when seedlings were soaked in water extracted from sorghum roots. In the same study, exposure to water extract from sorghum stems resulted in 81% reduction in wheat seedling growth. Netzly and Butler (1986) reported that six compounds belonging to the quinone class of organic compounds from droplets exuded by sorghum root hairs resulted in 85% inhibition of root elongation in lettuce (*Lactuca sativa* L.), while no effect was observed in corn.

Several studies have identified sorgoleone (2-hydroxy-5-methoxy-3-[(Z,Z)-8',11',14'-pentadecatriene]-p-benzoquinone) as the main allelopathic component of exudate produced in the root hairs of sorghum species that is responsible for weed-inhibiting properties of this species (Czarnota et al. 2001; Dayan et al. 2007, 2010; Erickson et al. 2001; Rimando et al. 2003). Sorgoleone production starts in the root hairs shortly after radicle emergence (Czarnota et al. 2003), and it is exuded dynamically as oily droplets at the tip of the root hairs and released directly in the soil. If root hairs are functional, sorgoleone production is a continuous process (Dayan et al. 2009). Laboratory studies have confirmed several modes of action to explain herbicidal properties of sorgoleone.

Nimbal et al. (1996) reported that sorgoleone acts similar to photosystem II (PSII) inhibitors, such as atrazine, by binding to the QB-binding niche on the D1 protein and inhibiting PSII. However, Dayan et al. (2009) did not find any cross-resistance when testing sorgoleone response of wild and atrazine-resistant types of redroot pigweed (*Amaranthus retroflexus* L.), suggesting that atrazine and sorgoleone belong to two different families of PSII inhibitors. Hejl and Koster (2004) observed a significant decrease of plasma membrane H⁺-ATPase with increasing sorgoleone concentration, leading to disturbance in root metabolism. Sorgoleone has also been found to inhibit 4-hydroxyphenylpyruvate dioxygenase (4-HPPD), causing reduction of the plastoquinone and, consequently, carotenoid pools, which resulted in foliage bleaching of mouse-ear cress [*Arabidopsis thaliana* (L.) Heynh.] (Meazza et al. 2002). Uddin et al. (2009, 2012) observed that broadleaf weed species were more susceptible than grass weed species when treated with sorghum exudate.

Lolium perenne ssp. *multiflorum* is one of the most troublesome weeds in wheat systems across the southern United States (Grey et al. 2012). Its germination and emergence typically coincide with winter wheat (Hoveland et al., 1976). As a winter annual, it establishes in the fall and persists through the spring (Scarpato De Sanctis 2024), often outcompeting wheat due to its higher growth rate and nutrient uptake capacity (Liebl and Worsham 1987). *Lolium perenne* ssp. *multiflorum* is also a prolific seed producer, with density as high as 212 plants m² which can reduce wheat yield up to 38% (Appleby and Brewster 1992). In North Carolina, Liebl and Worsham (1987) reported an average wheat yield reduction of 5% for every 10 *Lolium perenne* ssp. *multiflorum* plants m². If left uncontrolled, yield losses may exceed 50% (Brewster et al. 1977).

Moreover, seed production potential has been estimated at up to 45,000 seeds plant⁻¹ (Bararpour et al. 2017), contributing to rapid spread and persistence in infested fields. Compounding these issues, herbicide-resistant *L. perenne* ssp. *multiflorum* populations are now widespread, presenting a persistent management challenge for growers. In a recent study, Scarpato De Sanctis (2024) found that 97% of 38 ryegrass populations sampled across North Carolina exhibited resistance to one or more herbicides, including glufosinate, clethodim, glyphosate, nicosulfuron, and paraquat, highlighting the urgent need for effective for alternative, nonchemical management strategies such as allelopathy.

While previous studies have evaluated the allelopathic effects of sorgoleone on weed and crop species, limited information is available in the literature regarding sorgoleone's impact on certain troublesome weed species and wheat. Therefore, the objective of this study was to evaluate the effect of sorgoleone on shoot length of *L. perenne* ssp. *multiflorum* and other troublesome grass and broadleaf weed species, as well as in wheat varieties and groups.

Materials and Methods

Two separate laboratory studies were conducted to evaluate the effect of sorgoleone on shoot length. Study 1 focused on weed species and wheat varieties, while Study 2 examined different wheat groups. Both studies shared similar materials and methods, explained in the next three sections, followed by study-specific methods. The following methodology used for root and sorgoleone production and quantification is based on Besançon et al. (2020).

Root Production

Germination frames (28 by 43 cm) were built using four Bailey sash sections (C.R. Laurence, Los Angeles, CA, USA) cut to the proper length and assembled. A piece of aluminum window screen (New York Wire, Mt Wolf, PA, USA) was cut to fit and secured in place with a spline inserted into the spline channel. For root collection, approximately 200 g of sorghum seeds were surface sterilized to prevent fungal growth during the root production phase by soaking in a 20% sodium hypochlorite solution for 15 min, following the methodology detailed by Czarnota et al. (2003). The seeds were then removed from the solution and rinsed under a continuous flow of distilled cold water for 10 min. A double layer of cheesecloth was placed on the screen (28 by 43 cm) and humidified under a flow of cold tap water before seeding. The screen was then oriented vertically for 5 min to drain excess water. Sorghum seeds were uniformly spread on the cheesecloth, and a second double layer of previously wetted cheesecloth was placed on top to form a sandwich enclosing the seeds. The screens were then placed in a 10-L nursery vented tray (Kadon, Dayton, OH, USA) containing a moistened layer of Vattex-P capillary mat (Hummert International, Earth City, MO, USA) placed at the bottom. A layer of Weed-X® landscape fabric (Dallen Products, Knoxville, TN, USA) was placed between the screen and the capillary mat. The tray containing the germination screen was then placed in a 14-L nursery solid tray (Kadon) filled with tap water. The water level was adjusted to reach the bottom of the 10-L tray, so that the capillary mat remained moist. The trays were then covered with a 122 by 51 cm black seedling heat mat (Hydrofarm, Petaluma, CA, USA) to keep seedlings under darkness and at a temperature of 27 °C. The water within the 14-L tray was monitored daily and adjusted to maintain its initial level.

Table 1. Wheat (*Triticum aestivum*) varieties within groups.

Group	Variety
Soft White	Jasper
	Ovation
	Xerpha
	Otto
Soft Red	Puma
	Dynagro 9811
	Syngenta 547
	Agrimaxx 415
	Progeny turbo
Hard Red	Seedway 64
	Zenda
	Joe
	Larry
Hard White	Everest
	Wb4458
	Monument
	Danby
	Clara
	Thunder cl
	Antero

Sorgoleone Production

The collection of the oily droplets exuded from the root hairs of sorghum, hereafter referred to as “root exudate,” was adapted from a procedure developed by Czarnota et al. (2003). Seeds were allowed to germinate and grow on the capillary mat system for 7 d. During this period, the root system developed through the mesh of the screen and completely covered its underside. Seven days after sowing, seedling roots were shaved from the screen using a single-edge razor blade and placed in a 1-L beaker. Next, 500 ml of methylene chloride acidified with 0.25% acetic acid was added to the beaker to extract root exudate. The roots remained in methylene chloride for approximately 2 min to allow sufficient time for sorgoleone extraction to occur (LA Weston, personal communication). The roots were then removed, allowed to dry for 5 min at room temperature, and weighed to determine their fresh weight. The remaining methylene chloride crude extract was decanted through a fluted glass funnel lined with Whatman no. 42 filter paper (Tisch Scientific, Cleves, OH, US) to remove root debris. The filtrate mixture was transferred to a 1-L round-bottom flask and evaporated to dryness using a Büchi® rotary evaporator model R-215 (Büchi Labortechnik AG, Flawil, Switzerland) with a water bath at 40 C. The residual dry extract appeared as a golden oily substance left inside the flask after complete evaporation of the methylene chloride. The residual dry extract was resuspended with 2 ml of methylene chloride and transferred with a glass pipette to a pre-weighed 40 ml amber glass vial. This procedure was repeated three times to remove any remaining dry extract from the round-bottom flask. The contents of the amber glass vial were concentrated under nitrogen (N₂) flow to complete dryness. The vial was then flooded with nitrogen and tightly sealed with a PTFE-lined cap. The dry weight of the methylene chloride extract was determined for each replicate sample by weighing the vial containing the dry extract on a precision scale (Model AE160, Mettler-Toledo, Columbus, OH, USA) and subtracting the weight of the empty vial. The dry extract was finally stored at –20 C until experiment was conducted.

Sorgoleone Quantification

To quantify sorgoleone in the sorghum root exudate, dried extracts were dissolved in 100% acetonitrile acidified with 2.5% glacial

acetic acid to obtain a stock solution (1 mg ml^{–1}), which was further diluted to 100 µg ml^{–1}. Samples were then analyzed using an Agilent 1260 Infinity HPLC (high-performance liquid chromatography) system with a diode-array detector (Agilent, Santa Clara, CA, US) and a 100 × 4.6 mm biphenyl reversed-phase column (Phenomenex). The mobile phase consisted of 65% acetonitrile and 35% water (both acidified with 0.1% glacial acetic acid), delivered at 1 ml min^{–1}. The injection volume was 5 µl, with a run time of 10 min. Sorgoleone was detected at 280 nm and quantified using an external calibration curve prepared with purified sorgoleone (provided by FE Dayan, USDA-ARS). Standard concentrations ranged from 1 to 200 µg L^{–1}, and correlation coefficients (R²) were ≥0.999 for all calibration runs. A bulk stock solution (1,000 µg L^{–1}) was used to prepare fresh standard dilutions before each set of analyses. Peak areas of sorgoleone were integrated, and concentrations were calculated using linear regression generated by Agilent OpenLAB CDS ChemStation software.

Sorgoleone Inhibition Studies

Two laboratory experiments were conducted separately to investigate the allelopathic effect of sorgoleone. In the first study, four weed species—*L. perenne* ssp. *multiflorum*, *D. sanguinalis*, *S. obtusifolia*, *A. theophrasti*—along with two winter wheat varieties, ‘Shirley’ and ‘USG325’, commonly grown in North Carolina were evaluated. The second study focused on five wheat varieties from four different groups: Hard Red, Hard White, Soft Red, and Soft White (Table 1). The following methods were identical for both studies. All work was conducted in a sterile environment under a laminar flow hood. Equipment used in the studies was sterilized using 70% ethanol solution before use. Before germination, seeds were surface sterilized with 5% sodium hypochlorite solution for 1 min. Seeds were then rinsed twice with sterilized water to remove the excess sodium hypochlorite. Germination paper was cut to fit 205-cm² square culture-growth plates (Corning, Corning, NY, USA) and moistened with deionized water. Next, 15 g of seeds were spread uniformly on germination paper and covered with lid and sealed with 6.5 cm PM-992 Parafilm® (Bemis, Oshkosh, WI, USA). Plates were put under laminar flow hood at room temperature. Seeded plates were inspected for germination. At the onset of germination, 80 g L^{–1} (w/v) of agar molecular genetic powder (Fisher Scientific, Hampton, NH, USA) was added to seven volumetric flasks with the required amount of deionized water to make a final volume of 450 ml (Table 2). Flasks were then covered with aluminum foil and placed into autoclave water-bath trays and subjected to an autoclave cycle at 123 C for 30 min at 103 to 130 kPa. After the agar cooled to 45 C, sorgoleone stock solutions were prepared at concentrations of 0.025, 0.05, 0.10, 0.15, 0.20, and 0.30 g L^{–1} by dissolving approximately 0.25, 0.50, 1.0, 1.5, 2.0, and 3.0 mg of sorgoleone extract, respectively, in 10 ml of methanol in individual flasks. A non-treated control was added for comparison. All solutions were prepared on the same day as the agar. Flasks were gently swirled to mix, and 10 ml of solution was transferred to 20 by 100 mm petri dishes (Corning) using a 5-ml pipette. Each concentration was individually replicated in three petri dishes and two experimental runs. Once the agar solution solidified (~30 min), 20 pre-germinated seeds exhibiting visible coleoptile or radicle lengths <1 mm were removed from the germination plates using precision forceps and carefully placed onto the hardened agar. Petri dishes were closed, sealed with Parafilm®, labeled, and arranged in a completely randomized block design in a growth chamber at 18/24 C (night/day). Finally, 10 d after treatment, petri dishes were opened, and coleoptile or shoot length was measured

Table 2. List of compounds used for treatment preparation.

Sorgoleone concentration g L ⁻¹	Sorgoleone 4.6% WP ^a g	Stock solution ^b ml	Agar g	Water ml	Final volume ml
0.000	0.000	10	3.60	436.40	450
0.025	0.245	10	3.53	436.22	450
0.050	0.489	10	3.47	436.04	450
0.100	0.978	10	3.34	435.68	450
0.150	1.467	10	3.21	435.33	450
0.200	1.957	10	3.08	434.97	450
0.300	2.935	10	2.81	434.25	450

^aWP, wettable powder.^bStock solutions were prepared by dissolving sorgoleone extract (WP) in 10 ml of methanol.

for each seed. Data were converted into percentage of coleoptile/shoot length compared with the non-treated control by using following formula:

$$\% \text{ Shoot length} = \frac{\text{Observed shoot length}}{\text{Non - treated control shoot length}} \times 100 \quad [1]$$

where values below 100% indicate a reduction in shoot length growth and values above 100% indicate an increase.

Statistical Analysis

Percentage of shoot length (% non-treated control) data were subjected to ANOVA using the PROC GLIMMIX in SAS v. 9.4 (SAS Institute, Cary, NC, USA). Experimental run, weed species, wheat varieties, wheat group, and sorgoleone concentration were treated as fixed effects, while replication was treated as a random effect. Mean comparisons were performed using Fisher's protected LSD test when *F*-values were statistically significant ($P \leq 0.05$). Concentration-response curves for weed species data were generated using a three-parameter log-logistic model in R software utilizing the base packages plus the DRC: ANALYSIS OF DOSE-RESPONSE CURVES package (Ritz et al. 2015; Seefeldt et al. 1995):

$$y = \frac{d}{1 + \exp[b(\log x - \log e)]} \quad [2]$$

where *y* represents the percentage of shoot length, *d* is the upper limit, *b* is the slope of each curve, *e* is the sorgoleone concentration required for 50% shoot length reduction (GR₅₀ values), and *x* is the sorgoleone concentration.

Furthermore, when the log-logistic model presented lack of fit, linear regression was used instead:

$$y = a + bx \quad [3]$$

where *y* represents the percentage of shoot length, *a* is the intercept, *b* is the slope of each line, and *x* is the concentration of sorgoleone (g L⁻¹).

Results and Discussion

Weed Species/Wheat Varieties

The main effect of experimental run was not significant at $\alpha = 0.05$ (Table 3). Thus, data were pooled across experimental runs. The main effects of weed species/wheat variety and sorgoleone

Table 3. Two-way ANOVA results for the weed species/wheat variety study (Study 1).

Effect ^a	df	<i>F</i> ratio	Prob > <i>F</i> ^b
spp	5	304.26	<0.0001
concentration	6	74.18	<0.0001
spp*concentration	30	21.97	<0.0001
run	1	0.00	0.9494
spp*run	5	3.94	0.0019
concentration*run	6	1.13	0.3430
spp*concentration*run	30	1.25	0.1817

^aspp, species.^bValues ≤ 0.05 are statistically significant.**Table 4.** One-way ANOVA results for the weed species/wheat variety study (Study 1).

Effect ^a	df	<i>F</i> ratio	Prob > <i>F</i> ^b
spp	5	282.86	<0.0001
concentration	6	68.96	<0.0001
spp*concentration	30	20.42	<0.0001

^aspp, species.^bValues ≤ 0.05 are statistically significant.**Table 5.** Estimates of regression parameters for the weed species/wheat variety study (Study 1).

Species ^a	Regression parameters ^b		
	<i>d</i>	<i>b</i>	GR ₅₀
IR	102.09 (2.54)	1.15 (0.08)	0.08
LC	99.59 (2.69)	0.65 (0.15)	1.08
SP	100.46 (3.33)	0.50 (0.21)	2.50
VL	99.94 (3.52)	0.30 (0.08)	8.10
	<i>a</i>	<i>b</i>	
USG3251	100.15 (2.35)	0.82 (1.53)	
Shirley	98.45 (0.66)	-1.15 (0.43)	

^aAbbreviations: IR, Italian ryegrass (*Lolium perenne* ssp. *multiflorum*); LC, large crabgrass (*Digitaria sanguinalis*); SP, sicklepod (*Senna obtusifolia*); VL, velvetleaf (*Abutilon theophrasti*). Wheat varieties (*Triticum aestivum*): 'USG3251' and 'Shirley'.

^bWeed species were modeled with a three-parameter log-logistic regression, while wheat varieties were modeled with a linear regression due to the lack of fit to log-logistic model. Three-parameter log-logistic regression equation: $y = \{d / [1 + \exp(b(\log x - \log e))]\}$, where *y* represents the shoot length (% of non-treated control), *d* is the upper limit, *b* is the slope of each curve, and *e* is the sorgoleone concentration needed for 50% response. GR₅₀ is the effective concentration (g L⁻¹) of sorgoleone for 50% shoot length or growth reduction. Linear regression equation: $y = a + bx$, where *y* represents the shoot length (% of non-treated control), *a* is the intercept, *b* is the slope of each line, and *x* is the concentration of sorgoleone (g L⁻¹).

concentration, as well as their interaction, significantly influenced shoot length (Table 4). Sorgoleone reduced the shoot growth of weed species, whereas the wheat varieties demonstrated no concentration-dependent response, as indicated by the lack of fit of the log-logistic model (Table 5). Similarly, Uddin et al. (2014) reported that crops, such as barley (*Hordeum vulgare* L.), wheat, corn, soybean [*Glycine max* (L.) Merr.], tomato (*Solanum lycopersicum* L.), and Chinese cabbage [*Brassica rapa* L. (Pekinensis group)], were less susceptible to sorgoleone than weed species. Furthermore, in the present study, weed species exhibited varying levels of response to sorgoleone. Grass species, *L. perenne* ssp. *multiflorum* and *D. sanguinalis*, were more susceptible to sorgoleone than broadleaf weeds, *S. obtusifolia* and *A. theophrasti*. Broadleaf weed species required a higher concentration of sorgoleone (2.50 to 8.10 g L⁻¹) to reach GR₅₀, compared with the lower range observed in grass species (0.08 to 1.08 g L⁻¹). Response ranking to sorgoleone concentration from highest to lowest was as follows: *L. perenne* ssp. *multiflorum* > *D. sanguinalis*

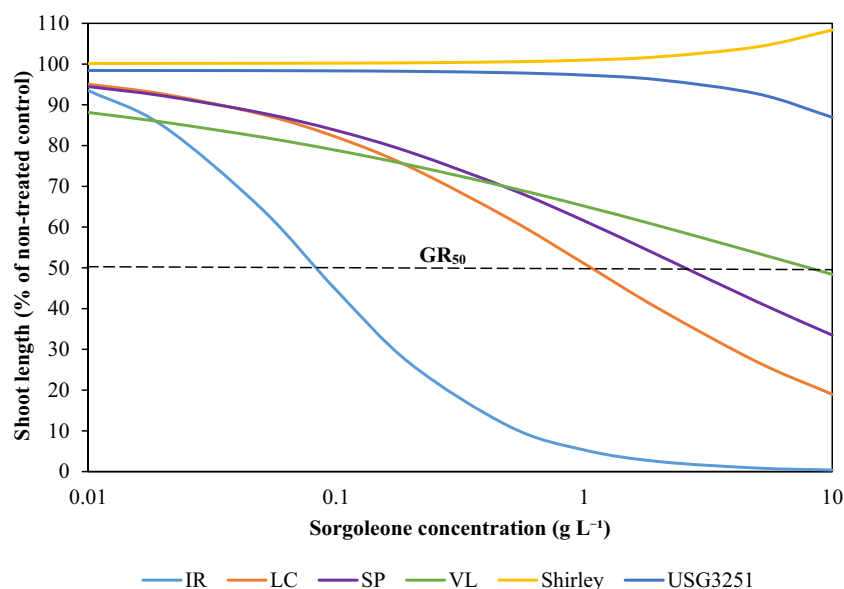


Figure 1. Dose–response curves representing the shoot length (% of nontreated) weed species: IR, Italian ryegrass (*Lolium perenne* ssp. *multiflorum*), LC, large crabgrass (*Digitaria sanguinalis*), SP, sicklepod (*Senna obtusifolia*), and VL, velvetleaf (*Abutilon theophrasti*) at 10 d after treatment. Predicted response can be described as $y = \{d/1 + \exp [b (\log x - \log e)]\}$, where y represents the shoot length (% of nontreated control), d is the upper limit, b is the slope of each curve, e is the sorgoleone concentration (g L^{-1}) needed for 50% response (GR50 values), and x is the sorgoleone concentration. Due to the lack of fit of the log-logistic model for wheat varieties ('Shirley' and 'USG3251'), data were fit to a linear regression, $y = a + bx$, where y represents the shoot length (% of nontreated control), a is the intercept, b is the slope of each line, and x is the sorgoleone concentration (g L^{-1}).

Table 6. Two-way ANOVA results for the wheat variety and group study (Study 2).

Effect ^a	df	F ratio	Prob > F ^b
var	19	6.29	<0.0001
concentration	6	6.93	<0.0001
var*concentration	114	1.92	<0.0001
run	1	2.03	0.1548
var*run	19	1.60	0.0508
concentration*run	6	0.23	0.9686
var*concentration*run	114	0.28	1.0000

^avar, wheat varieties.

^bValues ≤ 0.05 are statistically significant.

> *S. obtusifolia* > *A. theophrasti*. Shoot length in *L. perenne* ssp. *multiflorum* and *D. sanguinalis* decreased exponentially with increasing concentrations of sorgoleone, reaching ~77% and 32%, respectively, at the highest concentration of 0.3 g L^{-1} (Figure 1). Likewise, at the same sorgoleone concentration, shoot length of *S. obtusifolia* and *A. theophrasti* declined gradually as sorgoleone concentration increased, reaching a reduction of ~20% and 23%, respectively. Previous research has investigated the herbicidal activity of sorgoleone on different weed species (Czarnota et al. 2001; Dayan et al. 2009; Nimbal et al. 1996; Uddin et al. 2009, 2012, 2014). In hydroponic assays conducted by Nimbal et al. (1996) to evaluate effect of sorgoleone in weed species, sorgoleone was phytotoxic to both broadleaf and grass weed species at concentrations as low as 10 μM . Similarly, in the present study, reduction in shoot length was observed in weed species even at the lowest concentration of sorgoleone (0.025 g L^{-1}). Furthermore, in the same studies, the authors observed a greater reduction in barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] radicle length in response to sorgoleone compared with *A. theophrasti*, further supporting the results of the present study, in which grass weeds were generally more susceptible to sorgoleone than broadleaf weeds. This difference in response to sorgoleone may be attributed

Table 7. Two-way ANOVA results for the wheat variety and group study (Study 2).

Effect ^a	df	F ratio	Prob > F ^b
var	19	7.03	<0.0001
concentration	6	7.80	<0.0001
var*concentration	114	2.14	<0.0001

^avar, wheat varieties.

^bValues ≤ 0.05 are statistically significant.

to seed size. Grass weeds typically produce smaller seeds, which provide limited resources and makes seedlings more vulnerable to growth inhibition compared with large-seeded broadleaf weeds. However, Uddin et al. (2012) observed higher response of broadleaf weeds Japanese dock (*Rumex japonicus* Houtt.), false cleavers [*Galium aparine* L.; syn.: *Galium spurium* L.], *A. retroflexus*, eclipta [*Eclipta alba* (L.) L.] and Indian jointvetch [*Aeschynomene indica* L.] subjected to sorgoleone treatment compared with grass weeds (*E. crus-galli* and *D. sanguinalis*). For example, at 0.2 g ml^{-1} , growth of broadleaf species was reduced from 80% to 100%, compared with a reduction of 62% to 83% in grass weeds. Thus, the sorgoleone effect may be species-specific and depending on other factors, including physiological traits (e.g., seed coat permeability and capacity for metabolic detoxification), environmental conditions (e.g., soil type, organic matter content, moisture availability, and microbial activity), as well as methodological differences in extract concentration and exposure duration.

Wheat Variety and Group

For this second experiment, the main effect of experimental run was not significant at $\alpha = 0.05$ (Table 6). Thus, data were pooled across experimental runs. The main effects of wheat varieties and sorgoleone concentration, as well as their interaction, significantly influenced shoot length (Table 7). At the 0.0 g L^{-1} baseline, all varieties were normalized to 100%. As sorgoleone concentration

Table 8. Interaction of sorgoleone concentration and wheat (*Triticum aestivum*) variety on shoot length (% of non-treated control) 10 d after treatment.

Wheat variety	Sorgoleone concentration —g L ⁻¹ —													
	0		0.025		0.05		0.1		0.15		0.2		0.3	
	Shoot length ^a													
	% non-treated control													
Agrimaxx 415	100	d-q	100.8	c-p	94.9	g-x	95.1	g-x	104.4	b-k	96.1	g-w	98.6	d-t
Antero	100	d-q	97.1	f-u	101	c-p	93.5	k-x	100.4	c-p	91.8	m-y	101	c-p
Clara	100	d-q	98.8	d-s	98.3	d-u	100.5	c-p	95.1	g-x	97.7	e-u	81.9	y
Danby	100	d-q	102.3	b-n	96.6	f-u	101.4	c-o	96.9	f-u	97.6	e-u	79.1	y-z
Dynagro 9811	100	d-q	92.4	l-y	94.5	i-x	96.2	g-w	108.3	a-e	94.8	h-x	95	g-x
Everest	100	d-q	102.3	b-n	96.3	g-w	104.8	b-i	87.7	u-y	85.7	v-y	85.7	v-y
Jasper	100	d-q	99.8	d-q	99.5	d-r	99.1	d-s	111	a-c	91.6	m-y	94.4	i-x
Joe	100	d-q	88.7	r-y	94.8	h-x	95.4	g-x	84.6	x-y	92.3	l-y	93.5	k-x
Larry	100	d-q	92.6	l-y	101	c-p	95.1	g-x	92	m-y	88.6	s-y	84.6	x-y
Monument	100	d-q	97.4	f-u	99.7	d-q	96.2	g-w	98.9	d-s	99.3	d-s	87.4	u-y
Otto	100	d-q	105.5	b-h	100.4	c-p	105.9	b-g	102.4	b-l	103.1	b-l	103.7	b-l
Ovation	100	d-q	93.4	l-x	89.4	q-y	104.4	b-k	103.1	b-l	97.6	e-u	99	d-s
Progeny turbo	100	d-q	96	g-w	94.5	i-x	93.4	l-x	103.2	b-l	99.7	d-q	91.2	o-y
Puma	100	d-q	98.2	d-u	90.3	p-y	96.5	f-v	87.9	t-y	85.6	w-y	95.4	g-x
Seedway sw64sr	100	d-q	93.7	j-x	101.9	b-o	101.5	c-o	105.6	b-h	98.7	d-t	98.7	d-t
Syngenta sy547	100	d-q	100.7	c-p	101	c-p	91.3	m-y	94.9	g-x	99.5	d-r	92.2	l-y
Thunder cl	100	d-q	107.4	b-f	108.4	a-e	102.5	b-l	104.5	b-j	103.6	b-l	112.7	a-b
WB4458	100	d-q	105	b-i	102.4	b-m	98	d-u	106.3	b-g	92.6	l-y	88.5	s-y
Xerpha	100	d-q	94.3	i-x	104.3	b-l	95.9	g-w	106.5	b-g	108.8	a-d	118.7	a
Zenda	100	d-q	95	g-x	99	d-s	91.4	m-y	89.3	q-v	88.5	s-y	81.4	v

^aMeans followed by the same letter in the columns and rows do not differ according to Fisher's LSD at $\alpha = 0.05$. Means in bold differ from baseline treatment (0 g L⁻¹) according to Fisher's LSD at $\alpha = 0.05$.

Table 9. One-way ANOVA results for wheat group study (Study 2).

Effect	df	F ratio	Prob > F ^b
group	3	10.84	<0.0001
concentration	6	5.11	<0.0001
group*concentration	18	3.13	<0.0001

^aValues ≤ 0.05 are statistically significant.

increased, shoot length remained similar for most varieties, especially at sorgoleone concentrations ≤ 2 g L⁻¹ (Table 8). Eleven of the 20 wheat varieties (e.g., 'Agrimaxx 415', 'Antero', 'Otto', 'Ovation') maintained shoot length values close to 100% across concentrations, indicating tolerance. However, at the highest sorgoleone concentration (0.3 g L⁻¹), some varieties ('Clara', 'Danby', 'Larry', 'Monument', 'WB4458', and 'Zenda') exhibited reduced shoot length, with reductions ranging from 11% to 21%. Similarly, Uddin et al. (2014) reported that preemergence treatment of sorgoleone at 0.2 g L⁻¹ and 0.4 g L⁻¹ reduced wheat shoot biomass by 2.2% and 8.5%, respectively. Moreover, Guenzi et al. (1967) observed 77% and 69% reduction in wheat seedlings growth at 72 h after seed soaking in water extract derived from sorghum roots. In contrast, 'Jasper' at 0.15 L⁻¹ and 'Thunder cl' and 'Xerpha' at 0.3 g L⁻¹, presented increased shoot length (11% to 13%). This increase may be attributed to hormesis, a phenomenon in which phytotoxic compounds promote a stimulatory effect at low concentrations (Dayan and Duke 2009). Because the varieties negatively affected by sorgoleone belong either to the Hard Red or Hard White wheat group, a second analysis was conducted to evaluate the influence of wheat group on shoot length. The main effects of wheat group and sorgoleone concentration, as well as their interaction, were significant at $\alpha = 0.05$ (Table 9). In the Soft White group, shoot length remained unaffected across all

sorgoleone concentrations (Table 10). In the Hard White and Soft Red groups, only the highest concentration of sorgoleone reduced shoot length. However, in the Hard Red group, sorgoleone at 0.15, 0.2, and 0.3 g L⁻¹ caused a reduction in shoot length. These results suggest that Hard Red group is more susceptible to sorgoleone than the Soft White, Hard White, and Soft Red groups. In a study examining the effects of straw-water extracts from nine wheat varieties on wheat seedling germination and growth, Guenzi et al. (1967) reported significant differences among varieties, indicating that varietal traits may influence their response to allelopathic compounds.

This study confirmed the herbicidal activity of sorgoleone on weed species, particularly on grass species such as *L. perenne* ssp. *multiflorum* and *D. sanguinalis*. In contrast, most wheat varieties tested exhibited little to no response to this compound. These findings indicate that sorgoleone has the potential to be incorporated into weed management strategies for wheat crop systems. Among the evaluated weed species, *L. perenne* ssp. *multiflorum* poses the greatest challenge due to its overlapping germination window with wheat and widespread herbicide resistance. Sorgoleone may help suppress *L. perenne* ssp. *multiflorum* without affecting wheat growth, potentially enhancing the viability of sorghum in double-crop wheat crop systems. However, further greenhouse and field studies are necessary to validate these findings and assess the impact on wheat yield. Notably, the Hard Red group exhibited greater response to sorgoleone than other wheat groups, emphasizing the need for additional research to understand this variation and optimize management strategies.

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Table 10. Interaction of wheat group and sorgoleone concentration on shoot length (% non-treated control) 10 d after treatment.

Wheat group	Sorgoleone concentration — g L ⁻¹ —						
	0	0.025	0.05	0.1	0.15	0.2	0.3
	Shoot length ^a % non-treated control						
Soft White	100 a-e	98.2 b-e	96.2 c-g	100.4 a-d	102.1 ab	97.3 b-f	102.2 ab
Hard White	100 a-e	100.6 a-c	100.8 a-c	98.8 a-e	99.2 a-e	98.0 b-e	92.4 f-h
Soft Red	100 a-e	96.7 c-g	97.4 b-f	95.5 d-g	103.7 a	97.8 b-e	95.1 f-g
Hard Red	100 a-e	96.7 c-g	98.7 a-e	96.9 c-g	92.0 fg	89.5 hi	86.7 i

^aMeans followed by the same letter in the columns and rows do not differ according to Fisher's LSD at $\alpha = 0.05$.

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