Allelopathic effect of stressing sorghum on weed growth

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Allelopathic effect of stressing sorghum on weed growth

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Abstract: The effect of stressing sorghum by leaf stripping on the emergence and growth of *Amaranthus hybridus* was investigated in 2018 at Henderson Research Station in Mazowe, Zimbabwe. The experiment was set up as a randomised complete block design with three treatments replicated 6 times. A control with *A. hybridus* only, and another with *A. hybridus* sprayed with atrazine were maintained for comparison. Stripping 2 leaves significantly (P < 0.001) inhibited *A. hybridus* height, leaf area, and dry weight compared to no stripping. Increasing leaf stripping intensity from 2 to 4 leaves caused an increase in *A. hybridus* height, leaf area, and dry weight, possibly because intense leaf stripping reduced the number of sorghum leaves available to capture photosynthetically active radiation required for photosynthesis, subsequently reducing the volume of photoassimilates channelled to sorghum roots for allelochemical production. Leaf excision may have created an additional sink that modified assimilate allocation at the expense of roots.

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1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important staple food crop in the semi-arid tropics (ICRISAT, 2004). It is most widely grown in areas where water availability is limited and frequently...
subjected to drought (Deb, Bantilan, Roy, & Rao, 2004). Much of the crop is grown by small-scale farmers at the subsistence level (0.20–4.0 ha per household) (Akintayo & Sedgo, 2001). Apart from being an important food and industrial crop, sorghum produces a number of bioherbicidal phyto-toxins, or allelochemicals that can inhibit the growth of small-seeded weed species.

Allelochemicals are produced as tools for survival when neighbouring plants compete for resources (Maqbool, Wahid, Farooq, Cheema, & Siddique, 2013), and therefore act as a first line of defence against abiotic stresses (Asao et al., 2004; Jimenez et al., 2003). Various stress conditions substantially modulate the levels and types of allelochemicals biosynthesis (Maqbool et al., 2013). Temperature, UV radiation, water, and nutrient availability and competition stress have been identified as some of the key environmental factors that influence allelopathy (Croteau, Kutchan, & Lewis, 2000; Meiners, Kong, Ladwig, Pisula, & Lang, 2012). Ozone, heavy metals, and herbicides are also known to enhance allelochemical levels in plants (Rice, 1984). Changes in temperature modulate the allelochemicals production and their effectiveness. Dayan (2006) found that optimum root growth and sorgoleone production were at 30°C and decreased rapidly at temperatures below 25°C and above 35°C. Sorghum seeds treated with half strength Hoagland solution and 5 μg ml\(^{-1}\) of IBA significantly increase root growth and sorgoleone content in grain sorghum roots (Uddin, Park, Kim, Park, & Pyon, 2010). In grain sorghum, cyanogenic glycoside level was significantly increased under drought (Maqbool et al., 2013). Applying mechanical pressure over developing seedlings stimulated root formation and not the biosynthesis of sorgoleone (Dayan, 2006). Competition stress can be a key factor in triggering the production of phytotoxins. The presence of other plants surrounding sorghum can exacerbate the impact of the environmental stress on the production of sorgoleone (Dayan, 2006). Rice seedlings cultivated with *Echinochloa crus-galli* L. Beauv. (barnyard grass) showed a concentration of momilactone B almost seven times higher than that in rice seedlings grown alone (Kato-Noguchi, 2011).

Weeds that emerge with or before the crop are the most competitive and result in the greatest yield loss (Swanton, Nkoa, & Blackshaw, 2015). Threshold levels of redroot pigweed (*Amaranthus retroflexus* L.), barnyard grass (*Echinochloa crus-galli* L.), common ragweed (*Ambrosia artemisiifolia* L.), and velvetleaf (*Abutilon theophrasti* Medic.) in corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and dry bean (*Phaseolus vulgaris* L.) may be 2–10 times higher for weeds emerging 3–4 weeks after the crop compared with those that emerge with the crop (Cardina, Regnier, & Sparrow, 1995; Dieleman, Hamill, Fox, & Swanton, 1996; Knezevic, Weise, & Swanton, 1994). Weeds that emerge later than the crop are much less competitive in terms of crop yield loss but still may be considered problematic if they influence crop harvestability or reduce crop quality (Swanton et al., 2015). Pre-emergence herbicides are usually applied against weeds that emerge with or before the crop. They remove much of the early season weed competition pressure on a crop and can protect yield better than post-emergent herbicides (Grains Research and Development Corporation [GRDC], 2015). However, concerns over environmental problems caused by herbicides and the growing number of herbicide-resistant weed biotypes suggest that alternative methods of weed management be explored.

*Amaranthus hybridus* (L.) has been reported to substantially reduce yields of maize, soyabean, cotton, sugarbeet, peas and sorghum (Holm, Plucknett, Pancho, & Herberger, 1977; Weaver & McWilliams, 1980). Additionally, herbicide resistance of *Amaranthus hybridus* has been reported for decades in different herbicide groups such as photosystem II inhibitors (Bhowmik, 2010; Hirschberg & McIntosh, 1983) and ALS inhibitors (Maertens, Sprague, Tranel, & Hines, 2004; Riar, Tehranchian, Norsworthy, & Nandula, 2015; Whaley, Wilson, & Westwood, 2006). In a manner similar to the application of pre-emergence herbicides against weeds, weeds that have a propensity to emerge with or before the crop may also be managed by the use of allelopathic crops that produce and exude highly phytotoxic compounds in their seedling stages of growth. Sorghum produces a high concentration of sorgoleone (2-hydroxy-5-methoxy-3-[8Z,11Z]-8,11,14-pentadecatriene]-p-benzoquinone), a highly potent bioherbicide, during the seedling stage through root exudation. Uddin et al. (2010) found that a high concentration of sorgoleone
Sorgoleone was produced in 5- to 10-day old sorghum seedlings. Sorgoleone interferes with several molecular target sites, including inhibition of photosynthesis in germinating seedlings (Dayan et al., 2010), and therefore has the potential to inhibit the growth of weeds that germinate with the crop.

The open-pollinated sorghum variety Shirikure produced high content of sorgoleone (185.6 μg mg⁻¹ of root fresh weight) when sorghum varieties that were reported by farmers to be allelopathic against weeds were assayed for sorgoleone content (Tibugari et al., unpublished data). The objective of the study was to examine the effects of leaf stripping IS9456 on emergence and early growth of *Amaranthus hybridus*.

2. Materials and methods

2.1. Study site

A pot experiment was conducted in a glasshouse at the Weed Research Unit at Henderson Research Station (17°34’0”S and 30°58’0”E) in Mazowe, Zimbabwe, 25 km from Harare. The average temperature in the glasshouse was 28°C. No artificial lighting was provided.

2.2. Experimental procedure

The treatments comprised *A. hybridus* + sorghum that received no stripping, *A. hybridus* + sorghum whose two bottom leaves were stripped and *A. hybridus* + sorghum whose four bottom leaves were stripped. Controls included *A. hybridus* that had no sorghum, and *A. hybridus* that was sprayed with atrazine. Leaf stripping was conducted 12 days after sorghum emergence. Leaf stripping was accomplished by cutting the required number of leaves at the junction between the leaf sheath and the stem (Mashingaidze et al., 2012) using a scalpel blade. Treatments were replicated 6 times.

Certified sorghum seed was purchased from K2 Seeds. The *A. hybridus* seed was obtained from the Weed Research Unit of Henderson Research Station. Pots measuring 10 cm top diameter and 15 cm height were used. Each pot was filled with 0.5 kg of sandy loam soil which was sterilized by autoclaving for 24 h at 100°C before the experiment. After filling the pots with soil, 2–3 seeds of sorghum were dibbled at the centre of the pot. Then, 20 seeds of *A. hybridus* were sown in a circular pattern around the sorghum seeds. This was meant to allow radial and even spread of lateral roots of sorghum. Prior to the commencement of the experiment, a germination test was conducted to assess the germinability of both the sorghum and *A. hybridus* seeds. Percent germination of both Shirikure and *A. hybridus* was 99%.

2.3. Data collection and analysis

Data on percent emergence, plant height, leaf area, and dry weight were measured. Percentage emergence was calculated by dividing the number of seeds that emerged by the total number of seeds that were planted and then multiplying by 100. Height was measured using a string and a ruler. Leaf area was estimated destructively by tracing detached leaves on graph paper and subsequently counting the number of squares covered by the outline. At the end of the experiment, the shoot of *A. hybridus* shoot was cut at the soil surface and dried in an oven for 72 h before weighing with a digital scale. The collected data were subject to analysis of variance (ANOVA) using GenStat (version 14). Treatment means were separated at 95% level of significance.

3. Results

All *A. hybridus* seeds that were sown and exposed to an atrazine spray failed to emerge (Figure 1).

Inhibition of emergence by intact sorghum, sorghum with two leaves stripped and sorghum with four leaves stripped was significantly similar (Figure 2). Intact sorghum and sorghum with two leaves stripped significantly reduced the height of *A. hybridus* (Figure 2).
In contrast, *A. hybridus* growing together with sorghum that had four leaves stripped grew significantly taller than *A. hybridus* growing together with sorghum that had two leaves stripped (Figures 3 and 4).

Stripping two leaves significantly (*P* < 0.001) reduced leaf area of *A. hybridus* by 84.3% compared to stripping four leaves. Leaf area of *A. hybridus* increased in sorghum that had four leaves stripped (Figure 4). Stripping sorghum of two leaves significantly (*P* < 0.001) reduced *A. hybridus* dry weight by 56.4% compared to no stripping (Figure 5).

Increasing stripping intensity of sorghum to four leaves increased the dry weight of *A. hybridus* by 58.6%.
4. Discussion

Percent emergence was not affected by leaf stripping intensity. This result suggests that it is the mere presence of sorghum, and the production of allelochemicals, that caused poor emergence across treatments. An unpublished study by Tibugari et al. in which sorghum varieties that were reported by farmers to be allelopathic were assayed for sorgoleone content, showed that Shirikure...
produces high sorgoleone (185.6 μg mg⁻¹ of root fresh weight) levels. The result that there was poor A. hybridus emergence in pots that had sorghum and the weed is not surprising. Dayan and Duke (2009) report that certain species of Sorghum (e.g. Sudan grass, S. sudanese) can easily be grown as weed-free monocultures without additional herbicide application (Dayan & Duke, 2009). It is therefore likely that inhibition of emergence and growth that was observed in the current study might have been caused by sorgoleone. Additionally, Dayan (2006) found that sorgoleone biosynthesis appears to be stimulated by the presence of root exudates of other plants, suggesting that the allelopathic potential of sorghum may be enhanced in the presence of competitors (Dayan, 2006). Panasiuk, Bills, and Leather (1986) investigated the allelopathic interaction between sorghum and 10 grass and broadleaf weed species under laboratory and greenhouse conditions. They found that germination of weeds incubated in closed petri dishes with germinating sorghum was slightly inhibited or stimulated. In the current study, poor emergence of A. hybridus in pots that had intact sorghum and sorghum with 2 and 4 leaves stripped was comparable to A. hybridus response to atrazine. The sparse emergence of A. hybridus that was observed suggests that allelochemicals produced by seedlings of Shirikure, possibly sorgoleone, work in a manner more or less similar to pre-emergence herbicides.

In the current study, stripping two leaves caused a significant reduction in height, leaf area and dry weight of A. hybridus compared to intact sorghum and sorghum with four leaves stripped. Leaf stripping might have induced allelochemical production in sorghum. Research has shown that plants exposed to biotic stress respond with similar defensive mechanisms by the allocation of carbon skeletons from plant productivity to producing in higher concentrations and continuous synthesis of molecules of the defensive mode of action (Gawronska & Golisz, 2006). Tissue injury, pathogen attack, herbivory, and infection by microsymbionts such as rhizobium can cause synthesis and release of phenolics (Dakora, 1994; Lawson, Rolfe, & Djordjevic, 1996). When Hovary (2011) induced benzoxazinone allelochemical production in 21-day old rye (Secale cereale) plants by wounding or applying methyl jasmonate, benzoxazinone levels significantly increased in both mechanically and chemically induced tissue. In another study, Kruidhof et al. (2014) investigated whether mechanical damaging in spring could induce the production of allelochemicals in late summer sown lucerne, winter rye, and winter oilseed rape. Their work showed that although mechanical wounding enhanced the allelopathic activity per unit biomass of all the three cover crop species, the effect was only minor and often just sufficient to compensate for the loss in biomass resulting from wounding.

The reduced growth in terms of height, leaf area and dry weight in intact sorghum and sorghum with two leaves stripped that was observed in the current study may have been caused by sorgoleone. Sorgoleone has multiple potential modes of action (Dayan & Duke, 2009). Sorgoleone inhibits photosynthesis in germinating seedlings and plants (Dayan et al., 2010). It inhibits plant growth by strongly inhibiting PSII in vitro (Einhellig, Rasmussen, Hejl, & Souza, 1993; Gonzalez, Kazimir, Nimbal, Weston, & Cheniae, 1997; Rimando, Dayan, Czarnota, Weston, & Duke, 1998), inhibiting mitochondrial functions (Rasmussen, Hejl, Einhellig, & Thomas, 1992), inhibiting p-hydroxyphenylpyruvate dioxygenase (HPPD) (Meazza et al., 2002) and by impairing essential plant processes such as solute and water uptake (Hejl & Koster, 2004).

Production of sorgoleone is dependent on the presence of root hairs (Yang, Owens, Scheffler, & Weston, 2004) and is mostly constitutive and proportional to the root biomass (Dayan, 2006). In the current study, increasing leaf stripping intensity in sorghum from 2 to 4 leaves caused an increase in plant height, leaf area, and dry weight. Leaf excision wounds may have created an additional sink that modified assimilate allocation at the expense of roots. Immune response through callose deposition to wound areas may have redirected resources meant for allelochemical production. Intense leaf stripping reduced the number of leaves available to capture photosynthetically active radiation required for photosynthesis, subsequently reducing the volume of photoassimilates channelled to growing sorghum roots. This might have reduced the amount of
sorgoleone available to inhibit A. hybridus growth. It has been reported that low concentrations of allelochemicals can stimulate plant growth (Belz, 2007).

Seeds of A. hybridus are small and lenticellular in shape; with each seed averaging 1–1.5 mm in diameter (Akubugwo, Obasi, Chinyere, & Ugbofu, 2007). Response of A. hybridus to the presence of sorghum and to leaf stripping confirms reports by other scientists that the herbicidal activity of sorgoleone is strongest on small-seeded weeds (Einhellig & Souza, 1992; Netzy & Butler, 1986; Nimbali, Yerkes, Weston, & Weller, 1996; Rimando et al., 1998) just like many soil-applied commercial herbicides (Dayan et al., 2010).

5. Conclusion
Stressing sorghum by removing two bottom leaves during early growth possibly triggers an increase in the production and exudation of allelochemicals that can inhibit the emergence and growth of A. hybridus. Increasing leaf-stripping intensity of sorghum seedlings to four leaves reduces the total leaf surface area that can intercept light for maximum photosynthesis to occur, and this possibly reduces the volume of roots produced by the sorghum plant, subsequently leading to a reduction in sorgoleone production by the roots. Further studies that confirm levels of sorgoleone and other allelopathic compounds prior to and after leaf stripping are necessary.

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