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Impacts of *S*-Metolachlor Application Timing on Sweetpotato Growth and Development

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Abstract

S-metolachlor is used to control/suppress yellow nutsedge, annual grasses and several broadleaf weeds in sweetpotato. However, a decline in storage root quality is suspected when excessive rainfall occurs within 24-h after application. A greenhouse study was conducted to determine the effect of *S*-metolachlor application timing on sweetpotato growth and development. *S*-metolachlor treatments (0 and 1 kg·ha^{-1}) were applied over-the-top at 0, 5 and ten days after transplanting (DAT) and a simulated rainfall treatment delivered 25 mm of rain, 51 mm·h^{-1} intensity, immediately after herbicide application. Plants were harvested at 5, 10, 15, 20 and 80 DAT. During the first four harvests, roots were scanned and analyzed with WinRHIZO-Pro image analysis system to estimate root number, length, volume, and surface area along with aboveground growth parameters. At the final harvest, plant growth and biomass components, and quality of storage roots were recorded. Plants treated with *S*-metolachlor on day 0 and 5 DAT were significantly less than those of 10 DAT and untreated control for all measured parameters for the initial 20 days of plant growth. Even though vine length, leaf number, stem biomass, and total storage roots were not different among the treatments at 80 DAT, all other plant components and total biomass production and leaf area development for plants treated at 0 and 5 DAT were significantly ($P < 0.05$) less than from those of 10 DAT and the untreated control. Marketable storage root conversion efficiency declined by 18% and 16% for plants treated at 0 and 5 DAT, respectively, relative to the untreated check. These results indicate that delaying *S*-metolachlor application to 10 DAT will be less damaging to sweetpotato growth and development, particularly marketable storage roots and yield.

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Keywords

Adventitious Root, Development, Growth, Herbicide Phytotoxicity, Injury, Marketable Storage Roots, Sweetpotato

1. Introduction

Effective weed management, particularly within the first six weeks after transplanting, is essential to optimize sweetpotato yield [1] [2] [3]. Growers use a combination of hand-weeding, mechanical, and chemical practices to manage weeds. However, hand-weeding is labor-intensive and time-consuming, and mechanical cultivation is restricted to the initial stages of crop development because of the prostrate growth habit of sweetpotato. As a result, chemical weed control has become necessary to supplement other weed control measures. Nutsedge species are particularly difficult to manage due to extensive rhizomes and tubers that facilitate their persistence and dispersal [4] [5].

S-metolachlor, one of few herbicides registered for use in sweetpotato, has a 24(c) Special Local Needs registration to control or suppress yellow nutsedge, annual grasses, and several small-seeded broadleaf weeds in sweetpotato production systems. *S*-metolachlor is physically and chemically equivalent to metolachlor (a 1:1 mixture of *R*- and *S*-isomers) but requires use rates 35% lower than metolachlor due to increased activity at the site of action in susceptible plants [6]. It is applied pre-emergence (PRE) to weeds and post-transplanting to sweetpotato [7]. The herbicidal action seems to involve conjugation of acetyl coenzyme A and once absorbed, the herbicide is mainly transported acropetally and inhibits biosynthesis of several plant components [6]. The chemical is absorbed into the plant through the roots and shoots but shoot tissues are generally more sorptive and the site of herbicidal activity [6] [8] [9]. Metolachlor also causes loss of root cell integrity through its interference of phospholipid synthesis, an important component of plant cell membranes [10] [11] and has been reported to induce leakage of ^{32}P-labeled orthophosphate from roots of susceptible species [11].

Even though *S*-metolachlor is an effective herbicide, growers are reluctant to use it because misshaped storage roots have been attributed to its use when applications are made soon after transplanting and followed by moderate to heavy rainfall [12]. Monks *et al.* [13] reported that metolachlor at 3.4 kg·ha^{-1} caused storage root injury in sweetpotato. However, information is limited on the effects of *S*-metolachlor application timing on sweetpotato root initiation, growth, and development in a controlled environment.

Transplanted sweetpotato vine tip cuttings (slips) produce adventitious roots, some of which develop into storage roots through the proliferation of cambial cells that form starch-accumulating parenchyma [14] [15] [16]. Storage root formation in sweetpotato is a complex developmental process associated with

the expression of several genes, which are influenced by aerial and soil environmental factors [15] [17] [18]. This developmental process occurs in the first two weeks after transplanting [18] [19] [20]. Stress before and during this stage will detrimentally impact storage root number, quality, and yield. A greater understanding of the influence of rainfall or soil moisture conditions and their interaction with herbicides on storage root formation is needed. Therefore, the objective of this study was to determine the influence of *S*-metolachlor application timing on sweetpotato growth and development including root system development.

2. Materials and Methods

2.1. Experimental Facilities, Plant Material, and Treatments

A greenhouse study was conducted at the Rodney Foil Plant Science Center, Mississippi State University, Mississippi (lat. 33˚28'N, long. 88˚47'W) to determine the influence of *S*-metolachlor (Dual Magnum®, Syngenta Crop Protection Inc., Greensboro, NC, USA) application timing on "Beauregard" sweetpotato, which is the major cultivar grown in Mississippi, USA. White polyvinyl chloride pots (20 cm diameter × 30 cm deep) with detachable blue polyethylene (plug) bottoms containing a 2 mm drainage hole were filled with 600 g of coarse gravel then sandy loam soil (71% sand, 23% clay, 5% silt and 1% OM) obtained by mixing sand and topsoil in a 3:1 v/v ratio. On 21 June 2013, pots were irrigated to soil field capacity, and a single four node Beauregard slip was transplanted into each pot with two nodes below the soil surface and two nodes above the soil surface. Nodes above the soil surface each contained one recently fully expanded leaf.

Treatments were a factorial of three *S*-metolachlor application timings [0, 5 and 10 d after transplanting (DAT)] by two rates (0 and 1 kg ai ha^{-1}) by five harvest timings (5, 10, 15, 20 and 80 DAT). *S*-metolachlor was applied with a tractor-mounted compressed-air spraying system fitted with Teejet 8002 XR flat fan nozzles (Teejet Spraying Systems Co., Wheaton, IL) and calibrated to deliver 140 $L{\cdot}ha^{-1}$ at 166 kPa. After the application, all pots from the same application timing received 25 mm of simulated rainfall at an intensity of 5.1 $m{\cdot}h^{-1}$. The rainfall simulator was modeled after one described by Meyer and Harmon [21] with droplet size, fall velocity, and kinetic characteristics similar to natural rainstorms. It delivered droplets at 2.4 m height [22] and rain gauges were used to measure the actual amount of rainfall at the plant height level. No other pesticides or insecticides were applied during the experimental period.

In the greenhouse, pots were arranged in a split-split plot design with application timing as the main-plot factor, application rate as the sub-plot factor and harvest timing as the sub-sub-plot factor. Each treatment was replicated five times.

All plants received Hoagland's nutrient solution in irrigation water at 8:00,

12:00 and 16:00 h each day, to ensure optimum nutrient [23] and water conditions for plant growth through an automated drip irrigation system. Air temperature and relative humidity (RH) at the plant canopy level were measured daily (WatchDog Model 3621 WD, Spectrum Technologies, Inc., Aurora, IL). Day and night air temperatures ranged from 24˚C to 35˚C and 23˚C to 30˚C, respectively. Day and night RH ranged from 60% to 95% and 79% to 95%, respectively. Soil temperature was monitored using a soil thermometer (Veksler Engineering, New Delhi, India) and day/night vapor pressure deficit (VPD) determined with RH was 0.42/0.18 kPa. The photosynthetically active radiation (PAR), measured with a line quantum sensor (LI-191; LI-COR, Inc., Lincoln, NE), was greater than 1300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on clear days at 12:00 h from 21 June to 11 September 2013.

2.2. Data Recorded

2.2.1. Photosynthesis and Fluorescence

Net photosynthetic rate, stomatal conductance, and intercellular CO_2 concentration of the uppermost recently fully expanded main-stem leaves were measured between 10:00 and 12:00 h using an open gas exchange system (LI-6400, LiCOR Inc., Lincoln, NE, USA) at 20 DAT. While measuring photosynthesis, PAR, provided by a 6400-02 LED light source, was set to 1500-$\mu mol \cdot m^{-2} \cdot s^{-1}$, temperature inside the leaf cuvette was set to 30˚C (average growing temperatures in the greenhouse during the experimental period), RH was adjusted to near ambient level (50%), and leaf chamber CO_2 concentration was set to 400-$\mu mol \cdot mol^{-1}$. Fluorescence was measured with the built-in leaf chamber fluorometer, which uses two red LEDs, center wavelength about 630 nm and a detector. The software in the instrument provides data on the fluorescence parameters and calculates parameters such as PSII reaction centers under light (Fv'/Fm') (LI-6400 Photosynthesis system, LI-COR, Inc.).

2.2.2. Leaf Pigments

At 20 DAT, five 39-mm^2 discs, one each from five recently fully expanded main-stem leaves, were cut from every plant using a cork borer. The discs were placed into a vial containing four mL dimethyl sulfoxide and held at room temperature overnight in the dark. Absorbance of the extract at 470, 648 and 664 nm was recorded using a Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA) and chlorophyll a and b and carotenoid concentrations were computed following the formula of Chappelle *et al.* [24] and expressed on a leaf area basis, $\mu g \cdot cm^{-2}$.

2.2.3. Shoot and Root Development and Biomass

At each harvest timing, total vine length was measured and leaf number counted for each plant. Plant components (vines, leaves, roots) were separated, and leaf area measured (Li-COR 3100 Leaf Area Meter, LiCOR Inc.). All plant parts were bagged separately, oven-dried at 80˚C for 72 h and weighed. At harvests 5, 10,

15, and 20 DAT, roots were gently washed with water on a 3-mm mesh hardware cloth to remove soil. Roots were then placed into transparent acrylic trays (30 cm wide × 40 cm long × 2 depth) containing ~1 cm of water and scanned to acquire digital images using a flatbed scanner optimized for root analysis (Epson Expression 11000XL, Regent Instruments, Montreal, QC, Canada). Images were acquired at a resolution of 800 dpi then analyzed with root analysis system software (WinRHIZO Pro, Version 2012b, Regent Instruments, Montreal, QC, Canada) for root volume, length, and surface area. At the final harvest (80 DAT), storage roots were separated into marketable and non-marketable, counted, weighed, then oven-dried as described previously. Marketable storage roots were those longer than 7.6 cm, greater than 2.5 cm diameter, firm, smooth, and well-shaped without any disease [25].

2.2.4. Anatomical Features of Storage Roots

At 20 DAT, washed storage roots ≥ 10 mm long were immediately removed and fixed in formalin-acetic acid-alcohol. The samples were dehydrated in a graded tertiary butyl alcohol series and embedded in paraplast. Blocks were sectioned at 8 microns with a rotary microtome (AmericanOptical Corp., Scientific Instrument Div., Buffalo, NY, USA), and sections stained with toluidine blue. Digital micrographs were taken with a Motic AE2000 microscope equipped with a Canon EOS Rebel T3i/600D 18.0-megapixel camera (MartinMicroscope Co., Easley, SC, USA).

2.2.5. Data Analysis

All data were subjected to analysis of variance using the General Linear Model procedure of the Statistical Analysis System [26] to determine the main factor effects. Means were separated by Fisher's protected LSD test at the 0.05 level of probability. Data on plant variables were regressed and graphical analysis conducted with SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA). Best-fit models between *S*-metolachlor rates and measured parameters were determined with a coefficient of determination and root mean square error.

3. Results and Discussion

3.1. Photosynthesis and Fluorescence

Leaf photosynthetic rate of the untreated check was 31.7 μmol CO_2 $m^{-2}{\cdot}s^{-1}$ and decreased 21, 19% and 12% when *S*-metolachlor was applied at 0, 5 and 10 DAT, respectively (**Table 1**). *S*-metolachlor application timing, however, did not affect stomatal conductance (0.85 to 0.92 mol H_2O $m^{-2}{\cdot}s^{-1}$), transpiration rate (11.4 to 11.9 μmol H_2O $m^{-2}{\cdot}s^{-1}$), internal CO_2 concentration (313 to 319 μmol CO_2 mol^{-1}), electron transport rate (173 to 200 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$), and chlorophyll fluorescence (0.559 to 0.573) (data not shown). Chlorophyll fluorescence, a measure of the efficiency of PSII photochemistry, can be used to estimate the rate of linear electron transport. Ebert (1980) found no inhibition of the electron transport

Table 1. Effects of *S*-metolachlor application timing on leaf photosynthesis, leaf chlorophyll a, leaf chlorophyll b, leaf chlorophyll a and b, and carotenoids of greenhouse-grown Beauregard sweetpotato at 20 days after planting.

Application timing	Photosynthesis	Chl a	Chl b	Chl a and b	Carotenoids
	μmol CO_2 $m^{-2}·s^{-1}$	————μg·cm^{-2}———			
Untreated check	31.7A	29.61A	17.29A	46.91A	8.76 A
0 DAT	25.0B	26.81C	15.00B	41.81C	7.81B
5 DAT	25.6B	27.84B	15.62B	43.45B	8.32B
10 DAT	27.8AB	29.49A	17.13A	46.62A	8.71A

Means within columns followed by different letters are significantly different based on Fisher's least significant difference mean separation test ($P < 0.05$).

system in isolated pea chloroplast at metolachlor concentrations of up to 50 ppm. These results indicate that non-stomatal and non-photochemical processes are the causative factors limiting photosynthesis under *S*-metolachlor application. Similar to our results, Obando [8] found no differences in stomatal conductance of sacred lotus (*Nelumbonucifera* Gartn.) seedlings when *S*-metolachlor treated plants were compared to the untreated check.

3.2. Leaf Pigments

Leaf chlorophyll (Chl) a and b, total chlorophyll, and carotenoid concentrations decreased 9% and 6%, 12% and 9%, 11% and 7%, and 11% and 5% compared to the untreated check in plants treated 0 and 5 DAT, respectively (**Table 1**). Chlorophyll and carotenoid concentrations of plants treated with *S*-metolachlor 10 DAT did not differ from the untreated check. Liu *et al.* [27] reported decreased leaf Chl a, Chl b, and total chlorophyll content 96 h after an application of 3.1 μM *S*-metolachlor to hydroponically grown rice (*Oryza sativa* L.) seedlings. Similarly, a 20% to 35% reduction in chlorophyll content and 50% reduction in carotenoid content in green algae (*Scenedesmus acutus* Meyen. and *Bumilleriopsisfiliformis* Vischer.) have been observed 24 to 48 h after an application of 50 μM metazachlor (a chloroacetamide). The researchers attributed the chlorophyll and carotenoid reduction to the disruption of their production, and not pigment degradation [28].

3.3. Shoot and Root Development and Biomass

At 20 DAT, there was an interaction between *S*-metolachlor application timing and harvest timing for vine length, leaf number, leaf area and total biomass ($p < 0.05$) (**Table 2**; **Figure 1**). While leaf number displayed a linear response (**Figure 1(B)**), the response of vine length (**Figure 1(A)**), leaf area (**Figure 1(C)**) and total biomass (**Figure 1(D)**) to harvest timing were quadratic with R^2 values of at least 97%. At 20 DAT, vines of plants treated at 0 and 5 DAT were 69.5 cm longvines of plants treated at 10 DAT and the untreated control were 71.5 cm

long (Figure 1(A)). The effect of *S*-metolachlor application timing was transient because vine lengths did not differ among the *S*-metolachlor application timings for plants harvested at 80 DAT (714.5 to 808.3 cm·plant^{-1}). Internode length per plant did not differ among all the application and harvest timings (7.2 to 9.2 cm) (Data not shown).

Table 2. Analysis of variance and significance levels on the effect of *S*-metolachlor application timing (AT) and harvest (H) on greenhouse-grown Beauregard sweetpotato leaf number (LN), leaf area (LA), vine length (VL), internode length (IL), total biomass (Bio), root number (RN), root length (RL), root volume (RV), and root surface area (RSA) harvested at 5, 10, 15 and 20 days after transplanting.

Source of variation	Measured parameters								
	LN	LA	VL	IL	Bio	RN	RL	RV	RSA
AT	***	***	***	*	***	***	***	***	***
H	***	***	***	***	***	***	***	***	***
H x AT	*	***	***	ns	***	ns	***	***	***

ns, *, **, *** Non-significant and significant at $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$, respectively.

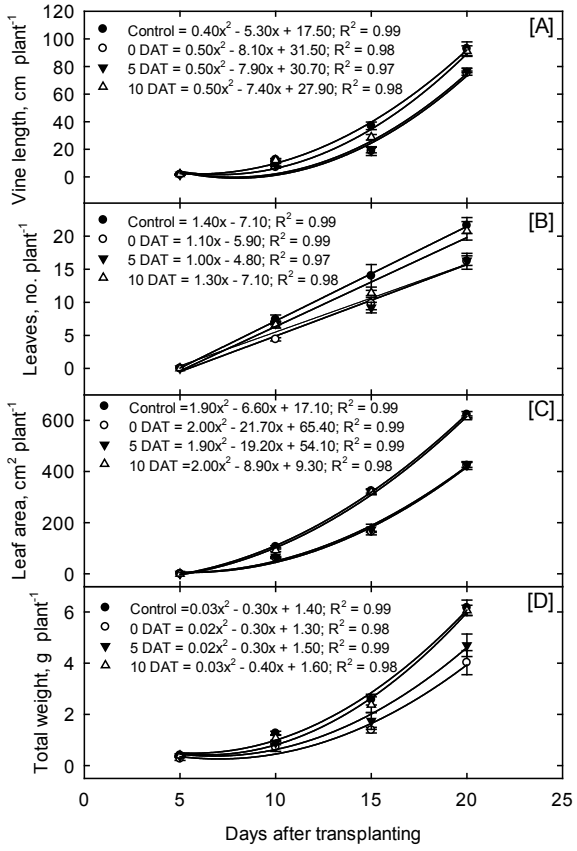

Figure 1. *S*-metolachlor application timing effects on (A) vine length, (B) leaf number, (C) leaf area and, (D) total biomass of greenhouse-grown Beauregard sweetpotato within the first 20 days of growth. Values represent the mean of five plants,and the error bars are ±SE of the mean.

During the first 20 days of plant growth, leaf addition per plant displayed a linear response to harvest timing at all application timings (**Figure 1(B)**). Plants treated at 0 and 5 DAT had a low leaf addition rate (1.1 and 1.0 leaves plant^{-1}, respectively) compared to plants treated at 10 DAT and the untreated check (1.3 and 1.4 leaves plant^{-1}, respectively). Similar to vine length, the effect of *S*-metolachlor application timing was transient, and leaf number did not differ by application timing at 80 DAT (80 to 104 leaves plant^{-1}).

Leaf area per plant displayed a quadratic response to harvest timing within the first 20 DAT (**Figure 1(C)**). Leaf area of plants treated at 10 DAT and the untreated check increased from 32 to 645 cm^2 from 5 to 20 DAT compared to plants treated at 0 and 5 DAT (7 to 431 cm^2) (**Figure 1(C)**). The herbicide effect on photosynthesis, chlorophyll and carotenoid concentrations appeared to have impacted leaf expansion over the entire 80 d duration of this study. Leaf area of the untreated check and *S*-metolachlor at 10 DAT was similar and greater than *S*-metolachlor at 0 (32% reduction) and 5 DAT (32% reduction). At 80 DAT, leaf area was reduced 27, 16% and 9% for plants treated at 0, 5, and 10 DAT, respectively, compared to the untreated check (**Table 3**). The decline in leaf area could be attributed to the inhibited biosynthesis of several plant components such as fatty acids, lipids, proteins, isoprenoids and flavonoids by *S*-metolachlor [6] and this may have interfered with leaf cell division and development. Bollman and Spraque [29] observed a 23% reduction in sugar beet leaf area with *S*-metolachlor PRE. Reduced sweetpotato leaf area may limit storage root yield due to a low canopy photosynthate supply, weak storage root sink and poor translocation of photosynthates to the storage roots [30] [31] [32] [33]. Total plant dry weight increased from 5 to 20 DAT; plants treated at 10 DAT and the untreated check increased from about 0.65 to 7.4 g·plant^{-1} while those treated at 0 and 5 DAT had a minimal increase from 0.3 to 3 g·plant^{-1} (**Figure 1(D)**).

Table 3. *S*-metolachlor application timing effects on shoot and root growth parameters including storage roots (SR), and marketable storage root conversion efficiency (MSRCE) of greenhouse grown Beauregard sweetpotato harvested 80 days after transplanting in 2013.

Application timing	Leaf area m^2·plant^{-1}	Biomass					MSRCE
		Leaf	Stem	Fibrous	SR	Total	
		g·plant^{-1}					%
Untreated check	0.9A	42.5A	69.5A	6.8B	268.8A	390.7A	86.6A
0 DAT	0.63C	33.9B	62.74A	12.3A	176.1B	288.0B	68.5B
5 DAT	0.72BC	37.0AB	64.02A	7.4B	190.7B	302.0B	70.4B
10 DAT	0.78AB	41.8A	64.22A	7.1B	267.2A	383.A	86.6A

Means within rows followed by different letters are significantly different based on Fisher's least significant difference mean separation test ($P < 0.05$).

At 20 DAT, untreated control plants had four and five adventitious and storage roots, respectively (**Figure 2**, **Figure 3(A)**). Root surface area, root volume, and root length fit quadratic curves for all application timing with R^2 values of at least 92% (**Figure 3**). Between 5 to 20 DAT root surface area increased from 0.028 to 0.2 $m^2 \cdot plant^{-1}$ for the untreated control and plants treated 10 DAT, but when plants were treated at 0 and 5 DAT, it increased from 0.02 to 0.08 m^2 $plant^{-1}$ (**Figure 3(B)**). Root volume of plants treated 0 and 5 DAT increased from 4.7 to 8.4 $cm^3 \cdot plant^{-1}$ between 5 and 20 DAT, but for the untreated check and plants treated 10 DAT, it increased from about 5.6 to 14 $cm \cdot plant^{-1}$ (**Figure 3(C)**). Total lateral root length between 5 and 20 DAT, increased from 6 to 93 $cm \cdot plant^{-1}$ for the untreated control and plants treated at 10 DAT, but when plants were treated 0 or 5 DAT, it increased from 5 to 60 $cm \cdot plant^{-1}$ (**Figure 3(D)**). Similar to our results, Liu *et al.* [34] reported a reduction in lateral root number and main and lateral root lengths of rice and maize (*Zea mays* L.) seedlings. Wu *et al.* [35] also reported *S*-metolachlor inhibition of root growth of rice, maize, and sorghum [*Sorghum bicolor* (L) Moench] seedlings.

Sweetpotato marketable storage root conversion efficiency (MSRCE), defined as the percentage of marketable storage roots to total numbers of roots produced, did not differ between 10 DAT and the untreated check (87%) (**Table 3**). However, when *S*-metolachlor was applied at zero and five DAT, MSRCE declined to 68% and 70%, respectively, showing that the conversion rate is time and herbicide dependent. Storage root number at 80 DAT did not differ among application timings, however, storage root fresh weight per plant was reduced by 35% for plants treated at 0 and 5 DAT (**Figure 4**), indicating that the plants can compensate for a delay in storage root development, but the delay will ultimately result in reduced storage root yield. These additional roots might have developed from the callus tissue on the distal end of the slip instead of the nodes. Grichar and Dotray [36], in one of two years, reported stunting of "Runner" and "Virginia" peanut with 1.6 $kg \cdot ha^{-1}$ *S*-metolachlor applied 7 d after cracking but not when applied at 14, 21, and 28 d after cracking.

Figure 2. Pictorial representation of greenhouse-grown Beauregard sweetpotato root system with *S*-metolachlor application timing effects harvested 20 days after transplanting (DAT).

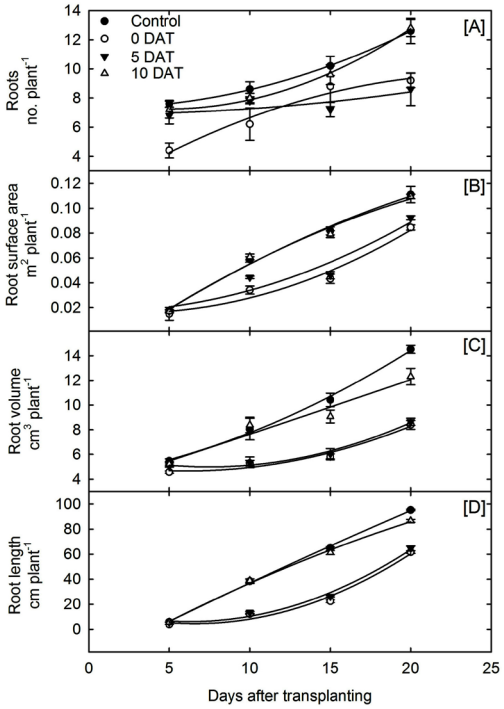

Figure 3. *S*-metolachlor application timing effects on growth and development of (A) root numbers, (B) root surface area, (C) root volume, and (D) root length of greenhouse-grown Beauregard sweetpotato within the first 20 days of plant growth. Values represent the mean of five plants, and the error bars are ±SE of the mean.

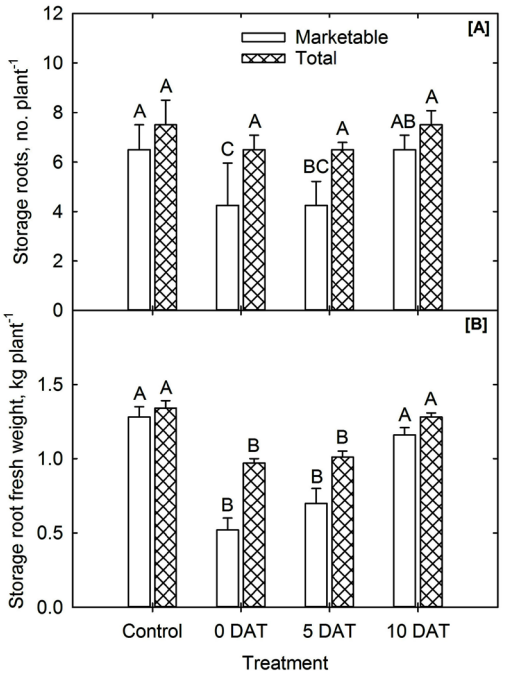

Figure 4. *S*-metolachlor application timing effect on greenhouse-grown Beauregard sweetpotato (A) total and marketable and total storage root numbers, and (B) storage root fresh weight harvested at 80 days after transplanting (DAT). Values represent the mean of four plants, and the error bars are ±SE of the mean. The values on the on the top bars with different letter are significantly different among treatments at $P < 0.05$.

Additionally, peanut (*Arachis hypogaea* L.) grade and yield were not affected, probably because peanut has an indeterminate growth habit, which allows for compensation from early season stress like herbicide injury if given good growing conditions and sufficient recovery time. Also, Cardina and Swann [37] reported that suppression of early peanut growth with metolachlor did not reflect in final yield except at 6.7 kg·ha^{-1}, which is well above the labeled rate. Ritter and Menbere [38] reported that, in one of three years, wheat plants outgrew early season stunting resulting in *S*-metolachlor not affect grain yield.

Fresh storage root weight per plant, declined by 78% and 15% at 20 DAT, and 28% and 25% at 80 DAT for plants treated at 0 and 5 DAT, respectively, when compared to the untreated check (**Figure 4** and **Figure 5**). Marketable storage root weight at 80 DAT also declined by 59% and 45% for plants treated at 0 and 5 DAT, respectively, when compared to the untreated check (**Figure 4**). However, no differences were detected between the untreated check and *S*-metolachlor at 10 DAT, with regards to marketable storage root weight at 80 DAT, and fresh storage root weight at 20 and 80 DAT. Similarly, Meyers *et al.* [12] [39] reported that *S*-metolachlor applied immediately after transplanting reduced US no. 1 and total marketable sweetpotato yields compared to the untreated check and plants treated at 14 DAT. Sweetpotato root systems in this study were qualitatively and quantitatively affected by *S*-metolachlor application timing. These root systems harvested at 80 DAT are pictorially represented in **Figure 5**.

At 80 DAT, stem dry biomass per plant ranged from 63 to 69 g and did not differ among application timings (**Table 3**). Fibrous root dry biomass for plants treated at 0 DAT was greater than that of the untreated check and those treated 5 or 10 DAT. At 80 DAT, reduced total plant dry biomass was reduced by 26% and 23%, for plants treated 0 and 5 DAT, respectively, compared to the untreated check. In a greenhouse study, Fleming *et al.* [40] reported that a metolachlor application reduced maize dry biomass 35% to 49%, relative to the untreated check. Similarly, Wu *et al.* [35] concluded that *S*-metolachlor is a strong inhibitor of shoot growth of rice, maize, and sorghum seedlings. *S*-metolachlor PRE under field conditions decreased black bean (*Phaseolus vulgaris* L) and sugar beet (*Beta vulgaris* L.) biomass 16% and 36%, respectively [29] [41]. However, when *S*-metolachlor application was delayed until 10 DAT, plant biomass was not different from the untreated check for plants harvested at 20 and 80 DAT.

Plant biomass partitioned to leaves, stems, fibrous roots, storage roots and total roots at 80 DAT are presented in **Table 4**. While no differences were observed in the proportion of total plant biomass partitioned to leaves, significant differences occurred in the stem, fibrous root, storage root, and total root biomass partitioning. Plants treated at 0 or 5 DAT partitioned a greater proportion of their biomass to stems and less to storage roots. However, biomass partitioning in plants treated at 10 DAT did not differ from the untreated check. Plants from the untreated check and those treated at 10 DAT, partitioned at least 70% of total plant biomass to storage roots. Belehu [42] reported as much as 73% of

total plant dry matter partitioned to sweetpotato storage roots in one of three cultivars.

3.4. Anatomical Features of Storage Roots

Micrographs of transverse sectioned storage roots 20 DAT are illustrated in Figure 6. Pigmented adventitious roots of the non-treated control and those treated 10 DAT are anatomically similar (Figure 6). The roots of these treatments contained a continuous regular vascular cambial ring, metaxylem, protoxylem, and secondary meristematic activity. These anatomical features are all indicators that an adventitious root has the potential to become a storage root [14] [16] [43]. However, roots of plants receiving *S*-metolachlor at 0 or 5 DAT, lacked these features of initiated storage roots and displayed a general lignification of the stele, thus resulting in decreased MSRCE. Metolachlor has previously been shown to cause loss of root cell membrane integrity resulting in leakage of exudates or previously absorbed ^{32}P labeled orthophosphate [10] [11] [44], likely a result of its effects on major root cell membrane components such as proteins and phospholipids [6] [11] [44].

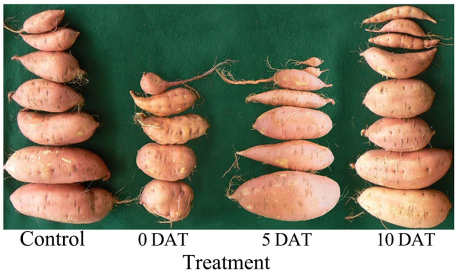

Figure 5. Pictorial representation of greenhouse-grown Beauregard sweetpotato storage roots with *S*-metolachlor application timing effects harvested 80 days after transplanting (DAT).

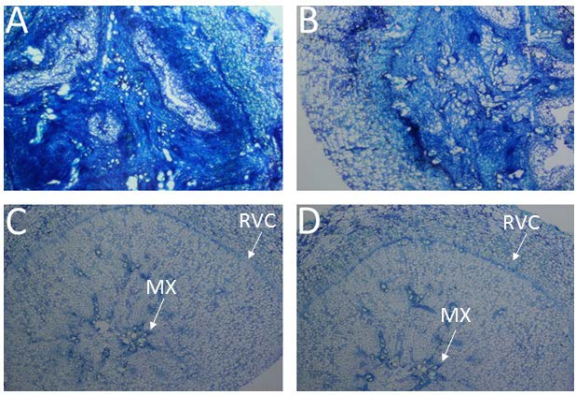

Figure 6. Micrograph of a transverse section of storage roots (400X) of 20 days grown plants treated with *S*-metolachlor at (A) 0, (B) 5, (C) 10 days after transplanting (DAT) and (D) untreated check in 2013. Roots of the untreated check and those treated 10 DAT contain continuous regular vascular cambial rings (RVC) and metaxylem surrounded by secondary meristematic activity (MX). Roots of plants treated 0 and 5 DAT lack RVC, MX, and display a general lignification of the stele (agnification = 400X).

Table 4. *S*-metolachlor application timing effects on biomass partitioning of greenhouse grown Beauregard sweetpotato harvested 80 days after transplanting (DAT) in 2013.

Application timing	Leaf	Stem	Fibrous roots	Storage roots	Total roots
	---------- % biomass ----------				
Untreated check	11 A	15 B	2 B	72 A	74 A
0 DAT	12 A	22 A	4 A	61 B	66 B
5 DAT	12 A	21 A	2 B	64 B	67 B
10 DAT	11 A	17 B	2 B	70 A	72 A

Means within columns followed by different letters are significantly different based on Fisher's least significant difference mean separation test ($P < 0.05$).

4. Conclusion

There were no meaningful differences between plants treated at 10 DAT and those of the untreated check. However, sweetpotatoes receiving *S*-metolachlor 0 or 5 DAT had reduced Chl a and b, total Chl, and carotenoid concentrations as well as reduced leaf area, root surface area, root volume, total lateral root length, fresh storage root, and marketable root weight, and total plant dry biomass. Findings from this study suggest that *S*-metolachlor applications should be delayed until 10 DAT to limit the herbicide's potential impacts on sweetpotato growth, development, and yield.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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