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# Vinasse and Biochar Effects on Germination and Growth of Palmer Amaranth (*Amaranthus palmeri*), Sicklepod (*Senna obtusifolia*), and Southern Crabgrass (*Digitaria ciliaris*)

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Vinasse and biochar are by-products of biofuel production that can be used as sources of nutrients to crops or soil amendments to improve soil quality. Despite the recent interest in biochar and vinasse effects on soil properties, little is known about their effect on weed communities. We hypothesized that the addition of biochar and vinasse to the soil could affect weed seed germination and growth, and that different weed species would show different responses to these soil amendments. Therefore, the objectives of this study were to determine the effects of vinasse and biochar on the germination and growth of Palmer amaranth, sicklepod, and southern crabgrass. The study was conducted under laboratory and growth chamber conditions. Treatments consisted of four levels of vinasse (0, 10, 20, and 40 L $m^{-2}$) and biochar (0, 0.5, 2.5, and 12.5 kg $m^{-2}$) applied to a sandy loam soil. Biochar at 0.5 and 2.5 kg $m^{-2}$ increased germination of Palmer amaranth but had no effect on sicklepod and southern crabgrass. Vinasse reduced germination of all species. However, sicklepod germination was less affected by vinasse at 10 and 20 L $m^{-2}$ than the other two species. Vinasse at 40 L $m^{-2}$ decreased Palmer amaranth, southern crabgrass and sicklepod germination 57, 26 and 87%, respectively. Biochar had no consistent effect on the vegetative growth of the species studied. Vinasse at 10 L $m^{-2}$ stimulated growth of sicklepod and southern crabgrass compared to the nontreated control. Our results suggested that vinasse used as a soil amendment could affect weed community structure by decreasing germination of susceptible species, but plants and weed species that can get established in vinasse amended soils might show higher growth rates.

**Nomenclature**: Biochar; vinasse; Palmer amaranth, *Amaranthus palmeri* S. Wats, AMAPA; sicklepod, *Senna obtusifolia* (L.) H.S. Irwin & Barneby CASOB; southern crabgrass, *Digitaria ciliaris* (Retz) Koel DIGSP.

**Keywords**: Charcoal, plant growth, seed viability, soil amendments, stillage.

La vinaza y el biochar son subproductos de la producción de biocombustibles que pueden ser utilizados como fuentes de nutrientes para cultivos o como enmiendas para mejorar la calidad del suelo. A pesar del reciente interés en los efectos del biochar y la vinaza sobre las propiedades del suelo, es poca la información disponible sobre su efecto en las comunidades de malezas. Planteamos la hipótesis de que la adición de biochar o vinaza al suelo podría afectar la germinación y el crecimiento de malezas, y que la respuesta a estas enmiendas puede ser distinta dependiendo de la especie de malezas. Por lo tanto, los objetivos de este estudio fueron determinar los efectos de la vinaza y el biochar en la germinación y crecimiento de *Amaranthus palmeri, Senna obtusifolia* y *Digitaria ciliaris*. El estudio fue realizado en condiciones de laboratorio y cámara de crecimiento. Los tratamientos consistieron en cuatro niveles de vinaza (0, 10, 20, and 40 L $m^{-2}$) y biochar (0, 0.5, 2.5, and 12.5 kg $m^{-2}$) aplicados a un suelo franco arenoso. Biochar a 0.5 y 2.5 kg $m^{-2}$ incrementó la germinación de *A. palmeri*, pero no tuvo efecto en *S. obtusifolia* ni en *D. ciliaris*. La vinaza redujo la germinación de todas las especies. Sin embargo, la germinación de *S. obtusifolia* fue menos afectada que las otras dos especies cuando se usó vinaza a 10 y 20 L $m^{-2}$. Vinaza a 40 L $m^{-2}$ redujo la germinación de *A. palmeri*, *D. ciliaris* and *S. obtusifolia* en 57, 26 and 87%, respectivamente. Biochar no tuvo un efecto consistente sobre el crecimiento vegetativo de las especies estudiadas. Vinaza a 10 L $m^{-2}$ estimuló el crecimiento de *S. obtusifolia* and *D. ciliaris* al comparase con el testigo no-tratado. Nuestros resultados sugieren que el uso de vinaza como enmienda para el suelo podría afectar la estructura de las comunidades de malezas al disminuir la germinación de especies

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susceptibles de malezas. Sin embargo, las malezas que logren establecerse en suelo enmendado con vinaza podrían mostrar mayores tasas de crecimiento.

The US Energy Independence and Security Act of 2007 set a goal of producing 136 billion liters of renewable biofuels by 2022 (U.S. DOE 2009). Plants represents one of the largest sources of renewable energy in the United States (Mohan et al. 2006), and pyrolysis and fermentation are currently preferred processes to convert plant biomass to biofuel. Pyrolysis is a process in which the thermal decomposition of organic material occurs with limited oxygen at elevated temperatures (Lehmann and Joseph 2009). During pyrolysis, feedstock material is converted into solid (biochar), gas (syngas) and liquid (bio-oil) fractions (Mohan et al. 2006). Syngas and bio-oil are used as fuels, while biochar is considered a by-product. Biochar has been used as a soil amendment because of its stability and high nutrient and water holding capacity compared to other organic amendments (Lehmann 2007).

The by-product of the fermentation process during ethanol production is vinasse, also known as stillage, distillery wastewater or thin stillage (Wilkie et al. 2000). Vinasse chemical composition can vary substantially depending on feedstock and conversion technology, but vinasse typically contains organic compounds and mineral elements. Thus, because of the mineral content and relatively high biological and chemical oxygen demand (BOD and COD), improper disposal of vinasse can lead to pollution (Wilkie et al. 2000), especially for aquatic ecosystems and local water sources. An option for disposal is addressed by using vinasse as a crop fertilizer (Sheehan and Greenfield 1980). A consequence of increased biofuel production via these conversion processes will be the generation of large amounts of by-products such as vinasse and biochar.

Despite potentially beneficial effects on soil chemical and physical properties (Lehmann 2007), research has shown that biochar can transiently decrease plant growth through numerous mechanisms, including the release of toxic compounds from hydrocarbons, presence of toxic heavy metal elements, and nitrogen immobilization because of high C:N (Deenik et al. 2010). For example, Solaiman et al. (2011) reported biochar from wheat straw applied at 100 Mg $ha^{-1}$ reduced the germination of subterranean clover (*Trifolium subterraneum* L.) by 46% and mung bean [*Vigna radiata* (L.) R. Wilczak] by 10% compared to nontreated controls. Additionally, mung bean shoot and root biomass decreased by 20% and 50%, respectively, when biochar was applied at 100 Mg $ha^{-1}$. However, both shoot and root biomass increased compared to the non-treated control when biochar was applied at 10 Mg $ha^{-1}$. In the case of subterranean clover, shoot and root biomass were negatively related to biochar rate, and reductions were observed even with the 10 Mg $ha^{-1}$ rate. These findings indicate that biochar effects on plant growth are likely to be species-dependent.

Murillo et al. (1993) reported that sugar beet (*Beta vulgaris* L.) vinasse applied at 3 Mg $ha^{-1}$ at sowing increased ryegrass (*Lolium multiflorum* Lam cv Barwoltra) fresh weight 43%, but root length decreased 56% compared to the nontreated control. In addition, low beet vinasse concentrations (2.5% v/v) increased pea (*Pisum sativum* L.) and sunflower (*Helianthus annuus* L.) shoot, root and leaf dry weight, whereas higher concentrations (i.e. 10, 25 and 50%) decreased the dry weight accumulation for both species (Algur and Kadioglu 1992). Therefore, vinasse potential positive effects on growth depend on application rate.

The increasing use of vinasse and biochar as soil amendments in cropping systems could be beneficial for soil structure and fertility with minimal adverse environmental consequences (Booth and Lightfoot 1990; Lehmann and Joseph 2009). However, their impact on crop yield and weed growth must be assessed in order to determine if weed-crop interactions might also be affected. Currently there is no information about the effect of vinasse and biochar on Palmer amaranth, southern crabgrass and sicklepod. Therefore, the objective of this research was to determine the effects of the application of different rates of vinasse and biochar on the germination and growth of Palmer amaranth, sicklepod and southern crabgrass, which are three economically important weed species in the southeastern United States.



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Table 1. Characteristics of biochar used in the study. Biochar was produced from pine wood chips by pyrolysis at 800 C.

| Characteristic | Value |
|---|---|
| pH | 9.2 |
| EC ($\mu S/cm^{-1}$) | 1775 |
| Nitrogen (%) | 0.2 |
| Carbon (%) | 62.5 |

## Materials and Methods

Palmer amaranth seeds were obtained from Azlin Seed Service (Leland, MS), southern crabgrass seeds from Estel Farm and Seeds (Thomas, OK), and sicklepod seed was collected from natural populations at the West Florida Research and Education Center (WFREC) in Jay, FL. Sicklepod seeds were scarified with sand paper for 15 s. The soil used was a Dothan sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiudult) with a pH of 6.3. The soil was steamed using the sheet steaming method (Runia 2000) to kill weed seeds already present in the soil. After air-drying, soil was sieved through a 1.18 mm diameter sieve.

A commercial biochar (Table 1) derived from pyrolysis of pine wood chips was used (AGCARB, Standard Purification company, Dunnellon, FL). Vinasse from the lignocellulosic fermentation of sugarcane bagasse to ethanol (Table 2) was obtained from the University of Florida Stan Mayfield Biorefinery Pilot Plant in Perry, FL. Biochar and vinasse application rates were based on the biomass that can be produced and processed for sweet sorghum (i.e. 16.5 Mg $ha^{-1}$ DW) (Erickson et al. 2011). Conversion efficiency per Mg of sweet sorghum biomass was assumed to be 6,060 L for vinasse and 303 kg of biochar. Rates were calculated assuming vinasse and biochar incorporation in the top 10 cm of soil. Vinasse was stirred to keep solids in suspension and then mixed with soil at levels of 0, 10, 20, and 40 L $m^{-2}$, which were equivalent to 0X, 1X, 2X and 4X, respectively. Biochar was applied and mixed with soil at rates of 0, 0.5, 2.5, and 12.5 kg $m^{-2}$, which were equivalent to 0X, 1X, 5X, and 25X, respectively. Soil and amendments were mixed and homogenized in containers.

**Germination Experiment.** Approximately 70 g of treated and control soils were placed in petri dishes (8.0 cm diameter, 2 cm height) and fifty seeds of each weed species were planted per petri dish at 1 mm depth. Each petri dish was watered with 25 mL of deionized water. Dishes were then placed in a germinator chamber (CONVIRON A1000, Controlled Environments Inc., Pembina, ND) in dark conditions with a temperature regime of 12 h at 28 C and 12 h at 25 C. These temperatures were in the range of optimal germination for the Palmer amaranth, southern crabgrass and sicklepod (Chauan and Johnson 2008; Creel et al. 1968; Steckel et al. 2004). Daily germination was evaluated during 14 d. Seeds were considered germinated when the radicle was 2 mm long. A crush test was conducted at the end of the experiment to determine viability of nongerminated seeds (Sawma and Mohler 2002).

Table 2. Characteristics of vinasse used in the study. Vinasse was produced from lignocellulosic fermentation of sugarcane bagasse to ethanol.

| Characteristic | Value |
|---|---|
| Color | dark brown |
| pH | 5.7 |
| EC ($\mu S\ cm^{-1}$) | 13750 |
| Total N ($mg\ L^{-1}$) | 2298 |
| $P_2O_5$ ($mg\ L^{-1}$) | 387 |
| $K_2O$ ($mg\ L^{-1}$) | 44 |
| Mg ($mg\ L^{-1}$) | 39 |
| Na ($mg\ L^{-1}$) | 90 |
| Ca ($mg\ L^{-1}$) | 49 |
| Biological oxygen demand ($mg\ L^{-1}$) | 170 |

**Soil and Vinasse Sterilization Experiment.** Preliminary results indicated that vinasse adversely affected weed germination but also promoted soil microbial activity. Thus, an experiment was conducted to determine if germination reduction was a direct effect of vinasse chemical characteristics or an indirect effect because of proliferation of soil microbial activity. For this study, four treatments were evaluated: soil without vinasse plus autoclaving (CA), soil with vinasse at 40 L $m^{-2}$ plus autoclaving (VA), nonautoclaved soil without vinasse (C) and nonautoclaved soil with vinasse at 40 L $m^{-2}$ (V). Weed species, petri dishes, germinator conditions and seed number were the same as described for the germination experiment. Soil mixtures were autoclaved at 121 C for 1 h (Trevors 1996). Experiment preparation was done in a laminar flow hood to avoid microbial contamination from external sources.



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**Vegetative Growth Experiment.** An experiment was conducted to evaluate the effect of vinasse and biochar on early vegetative weed growth. The same rates of vinasse and biochar rates were used in germination and growth studies. Soil mixtures were prepared, placed in pots (0.94 L, 11.5 cm height and 15 cm diam), and watered 7 to 14 d prior to transplanting. Seedlings were grown in trays using control soil, and four seedlings were transplanted to the pots at the four-leaf stage. Transplanting was used to avoid potential effects of vinasse and biochar addition on seed germination and seedling establishment. Pots were placed in a growth chamber (CONVIRON SH10, Controlled Environments Inc., Pembina, ND) with a temperature regime of 28 C, 325 μmol $m^{-2}s^{-1}$ of photosynthetically active radiation (PAR), a photoperiod of 14 h, and relative humidity of 80% for 4 weeks. The pots were watered daily to maintain field capacity. Plants were thinned seven days after transplanting, leaving one plant per pot. Plant mortality associated to the amendment treatments was recorded during the first 7 d of the experiment. Plant height, number of tillers (for southern crabgrass), and number of leaves were measured weekly at the end of the growth period. After 30 days, the shoots were harvested. Leaf area of functional leaves was measured with a LI-3100 (LI-COR Inc, Lincoln, NE) leaf area meter. Shoots were dried at 60 C for 7 d and then dry weight (DW) was determined.

**Experimental Design and Statistical Analysis.** The germination experiments were conducted as completely randomized designs. The vegetative growth experiment was a randomized complete block design with growth chamber as the block factor. All experiments had four replications and were conducted twice. Results from all experiments were analyzed using the GLIMMIX procedure in the Statistical Analysis Software (SAS 9.3, SAS Institute Inc., Cary NC). For all experiments, data from both experimental repetitions were combined ($n=8$) and analyzed using experiment as a random effect. Germination experiments were analyzed using an ordinal logistic regression model and Tukey-Kramer Honestly Significant Difference ($\alpha=0.05$) was used for mean separation. Vegetative growth experiment results from surviving plants (completed the growth period) were analyzed using analysis of variance (ANOVA). In addition, linear and nonlinear regression analyses for growth variables were conducted with SigmaPlot (version 12.5, Systat Software, San Jose, CA). From the same experiment, plant mortality for each treatment was analyzed using a logistic regression. Tukey-Kramer Honestly Significant Difference was used for mean separation ($\alpha=0.05$) in both cases.

## Results and Discussion

**Germination.** Biochar application at the levels tested in this study did not affect germination or viability of southern crabgrass and sicklepod (Figure 1). However, Palmer amaranth seed germination was higher for biochar at 0.5 and 2.5 kg $m^{-2}$ (82 and 86%, respectively) compared to 0 and 12.5 kg $m^{-2}$ rates (66 and 72%, respectively). In addition, Palmer amaranth seed viability from non germinated seeds was lower in biochar treatments compared to the nontreated control. Solaiman et al. (2011) reported different responses to biochar depending on crop species. Wheat (*Triticum aestivum* L.) seed germination was stimulated by biochar at 1 kg $m^{-2}$, but at the same rate germination was reduced for mung bean and subterranean clover. Biochar from paper mill waste at 1 kg $m^{-2}$ increased wheat germination, but no effect on radish and soybean was observed (Van Zwieten et al. 2010). Corn (*Zea mays* L.) seed germination was not affected after exposure to six different aqueous extracts of biochars applied at 2.6 mL of liquid $g^{-1}$ paper (Rogovska et al. 2012). However, three of those biochar extracts reduced shoot length of corn seedlings, suggesting the presence of phytotoxic compounds. Therefore, as reported for crop species biochar effect on germination appears to depend on weed species and biochar properties.

In contrast, vinasse reduced germination for the three weed species (Figure 2). This reduction was the consequence of increased seed mortality, which was reflected in a higher number of nonviable seeds at the end of the experiment for all species, especially at the highest vinasse rate. Palmer amaranth exhibited the greatest decrease in seed germination and viability among the three weed species on vinasse treatments. At 40 L $m^{-2}$ of vinasse, Palmer amaranth germination was 9%, which was lower than the germination observed for the treatments with 10 and 20 L $m^{-2}$ of vinasse (19 and 22%, respectively). Southern crabgrass germination was lower on vinasse treatments compared to



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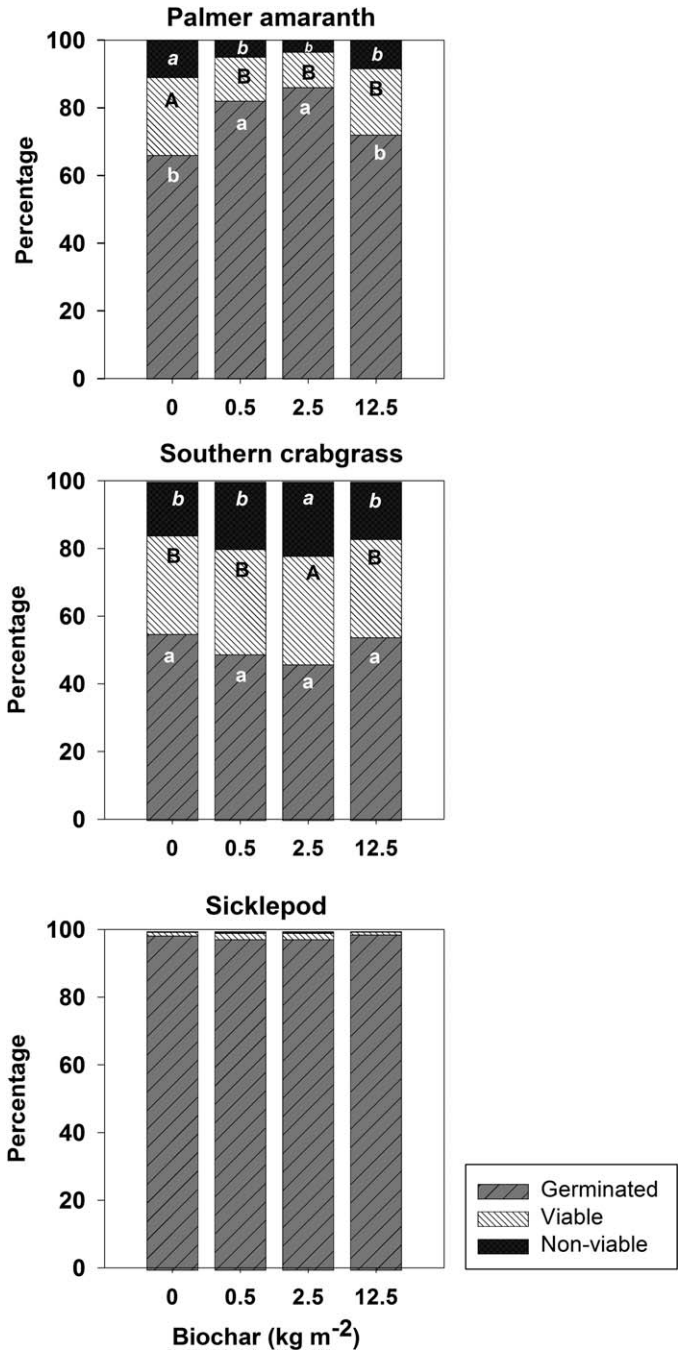


Figure 1. Effect of biochar rates on seed germination and viability of non germinated seeds for Palmer amaranth, southern crabgrass and sicklepod. Means with the same letter within seed categories and species are not significantly different based on Tukey-Kramer Honestly Significant Difference ($\alpha = 0.05$).

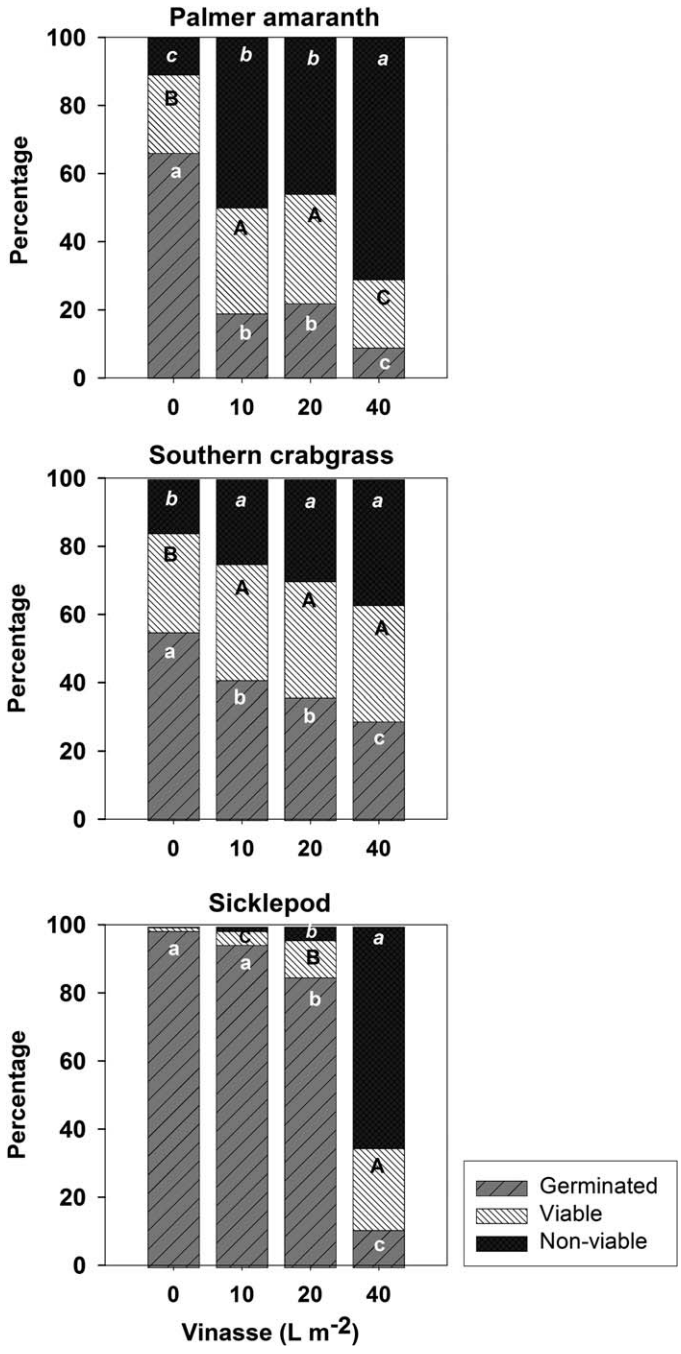


Figure 2. Effect of vinasse rates on seed germination and viability of non germinated seeds for Palmer amaranth, southern crabgrass and sicklepod. Means with the same letter within seed categories and species are not significantly different based on Tukey-Kramer Honestly Significant Difference ($\alpha = 0.05$).

the nontreated control. The germination for this species under the highest vinasse rate was almost half of the non-treated control. Previous research has shown similar results in other species. For example, when incubated in 0.15 and 0.50% v/v vinasse concentration during 96 h, ryegrass seed germination was 81 and 35% respectively, compared with 100% for the non-treated control (Murillo et al. 1993). Additionally, Ramos et al. (2008) found a negative effect of vinasse on



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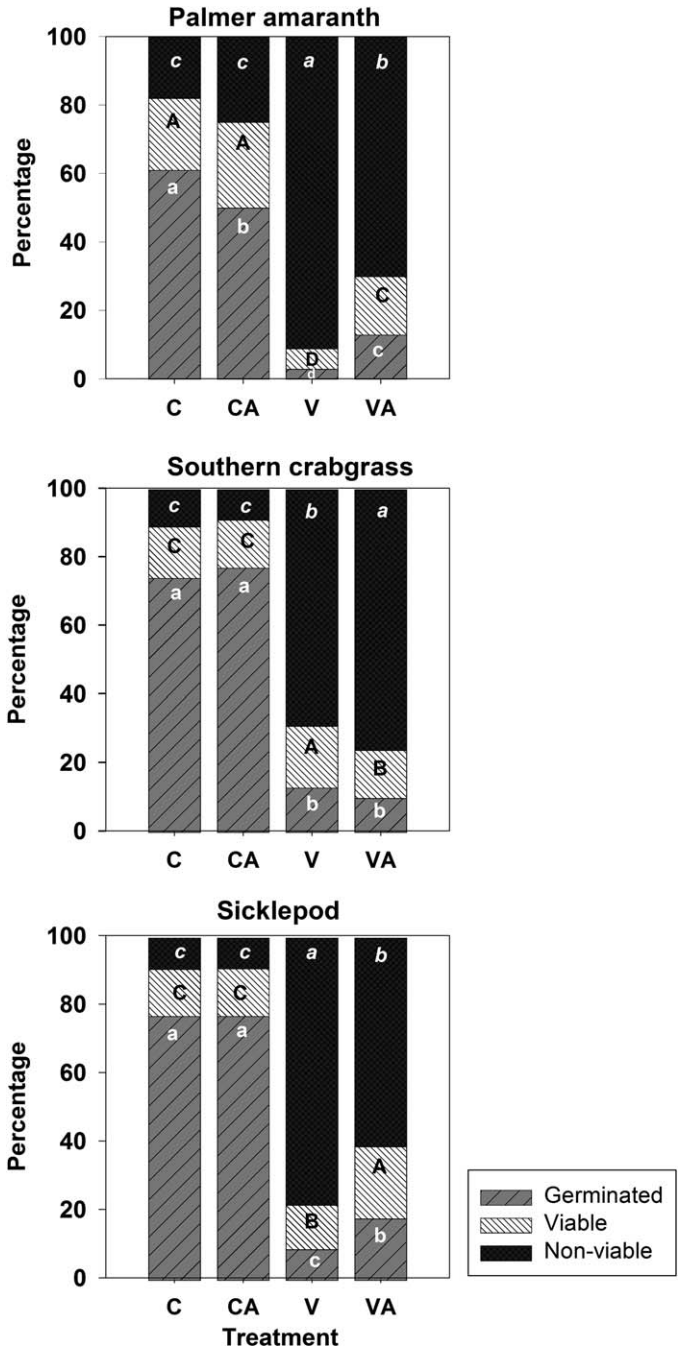


Figure 3. Seed germination and viability of non germinated seeds for Palmer amaranth, southern crabgrass and sicklepod seed after incubation in non-treated soil (C), non-treated autoclaved soil (CA), soil with vinasse at 40 L $m^{-2}$ (V) and autoclaved soil with vinasse at 40 L $m^{-2}$ (VA). Means with the same letter within seed categories and species are not significantly different based on Tukey-Kramer Honestly Significant Difference ($\alpha = 0.05$).

sunflower and peanut emergence. Vinasse at 15 L $m^{-2}$ reduced peanut emergence 56% and sunflower emergence only 5% compared to the non-treated control. In this case, the authors attributed vinasse interference with seed germination to increased osmotic potential reducing seed water uptake (Ramos et al. 2009). In the present study, reduction in germination and increase in nonviable seeds in response to vinasse occurred at 10 and 20 L $m^{-2}$ for Palmer amaranth and southern crabgrass. Electrical conductivity values were similar among vinasse and biochar treatments. Vinasse at 40 L $m^{-2}$ was the exception with an electrical conductivity three times higher than biochar at 12.5 kg $m^{-2}$ (data not shown). Therefore, the vinasse negative effect on germination could not be attributed mainly to a high osmotic potential as has been proposed in other studies (Ramos et al. 2009). Without further chemical and physiological analysis we cannot rule out the possibility that vinasse contained chemicals that caused seed mortality through a different physiological mechanism.

Sicklepod was less affected by vinasse than the other two weed species. For example, sicklepod seed germination and viability were reduced less than 15% by vinasse at 10 and 20 L $m^{-2}$, while Palmer amaranth and southern crabgrass reductions at the same rates ranged between 25% and 40%. However, vinasse at the highest rate (40 L $m^{-2}$) reduced sicklepod seed germination by 87% and increased nonviable seeds up to 65%. Sicklepod tolerance to the lower vinasse rates could be because of its impermeable hard seed coat (Creel et al. 1968) and bigger seed size compared with Palmer amaranth and southern crabgrass.

For the three weed species, vinasse treatments at 20 and 40 L $m^{-2}$ developed high fungal activity after 2 d of incubation. *Penicillium* spp. and *Aspergillus* spp. mycelia were identified. Azania et al. (2003) reported fungal growth on arrowleaf sida (*Sida rhombifolia* (L.) and *Brachiaria decumbens* (Stapf) seeds after 48 h of incubation with different concentrations of vinasse. Miyamoto et al. (2012) suggested that vinasse composition contributed to increased number of bacteria and fungi, but the vinasse itself did not contain these microorganisms.

**Soil and Vinasse Sterilization.** Autoclaved and nonautoclaved vinasse treatments (40 L $m^{-2}$) decreased germination at similar levels in all three species (Figure 3). Germination in nonautoclaved vinasse treatments was reduced by 97, 87, and 91% for Palmer amaranth, southern crabgrass and sicklepod, respectively. Although seed mortality was slightly higher in the nonautoclaved vinasse



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Table 3. Plant mortality as a result of the addition of biochar and vinasse rates on southern crabgrass, Palmer amaranth and sicklepod growing in pots in a growth chamber.

| Treatment | Mortality (%) |
|---|---|
| Non-treated control | 4[a] b |
| Biochar 0.5 kg $m^{-2}$ | 0 b |
| Biochar 2.5 kg $m^{-2}$ | 4 b |
| Biochar 12.5 kg $m^{-2}$ | 33 a |
| Vinasse 10 L $m^{-2}$ | 13 b |
| Vinasse 20 L $m^{-2}$ | 54 a |
| Vinasse 40 L $m^{-2}$ | 67 a |
| P-value | 0.001 |

[a] There is no species by treatment interaction, thus treatment effect was averaged across species. Means with the same letter within columns and species are not significantly different based on Tukey-Kramer Honestly Significant Difference ($\alpha = 0.05$).

than in the autoclaved vinasse treatment for Palmer amaranth and sicklepod, most of the reduction in germination in comparison with the treatments without vinasse was observed in the absence of visible fungal growth (i.e. autoclaved vinasse). Therefore, these results suggest that vinasse effects on seed germination and viability were not mainly because of increased activity of microbial communities present in the soil.

**Vegetative Growth.** Although this experiment was not designed to accurately quantify plant mortality, seedling establishment for the three species was affected when the rate of biochar and vinasse increased. Treatments such as biochar at 12.5 kg $m^{-2}$ and vinasse at 20 and 40 L $m^{-2}$ showed mortality significantly higher than the nontreated control (Table 3). Overall, seedling mortality was two-fold higher in vinasse treatments at 40 L $m^{-2}$ compared to biochar at 12.5 kg $m^{-2}$ indicating the high rate of vinasse was more injurious than biochar not only reducing seed germination but also seedling establishment.

For seedlings that survived the establishment phase, linear and nonlinear regression results showed no clear trend ($R^2 < 0.28$ and $P > 0.05$) between biochar and vinasse rates and growth variables. However, ANOVA results indicated that vinasse added at 10 L $m^{-2}$ stimulated plant response among species (Table 4). In addition, biochar treatments had no effect on plant growth compared to the non-treated control. Southern crabgrass was the exception; when biochar was applied at 12.5 kg $m^{-2}$ leaf area decreased 39% compared to the nontreated control (Table 5). Adams et al. (2013) reported that the addition of biochar increased growth parameters for big bluestem (*Andropogon*

Table 4. Effect of vinasse rates on plant height, leaf number, leaf area and shoot biomass of Palmer amaranth, southern crabgrass and sicklepod, after growing in pots for 30 d in a growth chamber.

| Species | Rate (L $m^{-2}$) | Height (cm) | Number of leaves | Leaf area ($cm^2$) | Number of tillers | Biomass DW[a] per plant (g) |
|---|---|---|---|---|---|---|
| Palmer amaranth[b] | 0 | 35 | 17[c] b | 71 a | NA | 1.26 |
| | 10 | 19 | 48 ab | 154 a | NA | 1.34 |
| | 20 | 21 | 66 a | 202 a | NA | 1.99 |
| | 40 | —[d] | — | — | NA | — |
| | P-value | 0.35 | 0.02 | 0.04 | | 0.23 |
| Southern crabgrass[b] | 0 | 46 | 28 b | 142 b | 5 b | 2.38 c |
| | 10 | 56 | 91 a | 474 a | 20 a | 7.72 a |
| | 20 | 57 | 83 a | 401 a | 19 a | 5.43 b |
| | 40 | 53 | 79 a | 434 a | 19 a | 5.10 b |
| | P-value | 0.11 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Sicklepod[b] | 0 | 11 b | 5 | 62 b | NA | 0.44 b |
| | 10 | 15 a | 7 | 184 a | NA | 1.23 a |
| | 20 | 9 b | 6 | 55 b | NA | 0.47 b |
| | 40 | 9 b | 5 | 70 b | NA | 0.56 b |
| | P-value | 0.0004 | 0.08 | 0.0001 | | 0.0002 |

[a] Abbreviation: DW, dry weight; NA, not applicable.

[b] Number of plants (*n*) evaluated for growth parameters was between 4 and 8 per species and treatment combination.

[c] Means with the same letter within columns and species are not significantly different based on Tukey-Kramer Honestly Significant Difference ($\alpha = 0.05$).

[d] Data is not available due the high plant mortality at 40 L $m^{-2}$ of vinasse for Palmer amaranth.



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Table 5. Effect of biochar rates on plant height, leaf number, leaf area and shoot biomass of Palmer amaranth, southern crabgrass and sicklepod, after growing in pots for 30 d in a growth chamber.

| Species | Rate (kg $m^{-2}$) | Height (cm) | Number of leaves | Leaf area ($cm^2$) | Number of tillers | Biomass DW[a] per plant (g) |
|---|---|---|---|---|---|---|
| Palmer amaranth[b] | 0 | 34 | 19[c] ab | 80 | NA | 1.24 |
| | 0.5 | 38 | 23 a | 73 | NA | 1.60 |
| | 2.5 | 30 | 18 ab | 77 | NA | 1.90 |
| | 12.5 | 40 | 8 b | 39 | NA | 0.98 |
| | P-value | 0.82 | 0.01 | 0.18 | | 0.16 |
| Southern crabgrass[b] | 0 | 46 | 28 | 142 a | 5 ab | 2.38 |
| | 0.5 | 41 | 31 | 138 ab | 7 a | 2.78 |
| | 2.5 | 36 | 27 | 119 ab | 6 ab | 2.56 |
| | 12.5 | 38 | 25 | 86 b | 4 b | 2.00 |
| | P-value | 0.24 | 0.59 | 0.03 | 0.02 | 0.23 |
| Sicklepod[b] | 0 | 11 ab | 5 | 62 | NA | 0.44 a |
| | 0.5 | 15 a | 5 | 93 | NA | 0.70 a |
| | 2.5 | 13 a | 5 | 86 | NA | 0.66 a |
| | 12.5 | 7 b | 6 | 53 | NA | 0.35 a |
| | P-value | 0.01 | 0.60 | 0.09 | | 0.04 |

[a] Abbreviation: DW, dry weight; NA, not applicable.

[b] Number of plants (n) evaluated for growth parameters was between 4 and 8 per species and treatment combination.

[c] Means with the same letter within columns and species are not significantly different based on Tukey-Kramer Honestly Significant Difference ($\alpha = 0.05$).

*gerardii* Vitman), whereas sericea [*Lespedeza cuneata* (Dumont) G. Don] had no response to biochar. Thus, similarly to germination responses, growth responses to biochar are species dependent.

Species dependent growth effects were also observed in response to vinasse (Table 4). Palmer amaranth increased number of leaves 3.8 times at 20 L $m^{-2}$ compared to the nontreated control but had no response for the other growth variables. Vinasse at 10 L $m^{-2}$ increased southern crabgrass leaf number, leaf area, and number of tillers compared to the nontreated control. Southern crabgrass biomass increased by 56 and 53% at 20 and 40 L $m^{-2}$, respectively, compared to the nontreated control. In addition, biomass at 10 L $m^{-2}$ was higher than the other vinasse treatments and the nontreated control. Sicklepod growth under the lowest rate of vinasse (10 L $m^{-2}$) increased height, number of leaves, leaf area and biomass compared to the non-treated control and other vinasse rates. Research has shown that addition of vinasse to the soil at 2.5 L $m^{-2}$ activated enzymes related to growth (e.g. cellulose and pectin methyl), increased chlorophyll and protein content on sunflower and pea, while rates over 10 L $m^{-2}$ decreased the production of those factors and reduced growth (Kadioglu and Algur 1990). Our results indicated different growth responses among species. Southern crabgrass and sicklepod increased plant growth when vinasse was added at 10 L $m^{-2}$ while Palmer amaranth was not affected.

Christoffoleti and Bacchi (1985) showed changes in weed community structure in response to vinasse applications. For example, Jamaican crabgrass (*Digitaria horizontalis* Willd.) and lilac tasselflower (*Emilia sonchifolia* L.) densities increased when vinasse rates increased from 0 to 15 L $m^{-2}$. Conversely, purple nutsedge (*Cyperus rotundus* L.), common purslane (*Portulaca oleracea* L.) and arrowleaf sida (*Sida rhombifolia* L.) had lower plant densities when vinasse rates increased from 0 to 15 L $m^{-2}$. Overall, the results of the present study suggested that the use of vinasse as a soil amendment could result in weed community shifts through decreases in weed seed germination. However, weed species that can get established in soil treated with biochar and vinasse might be able to compensate the negative effects on germination by exhibiting increased growth.

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