Brief Report

Effect of Glyphosate and Carbaryl Applications on Okra (Abelmoschus esculentus) Biomass and Arbuscular Mycorrhizal Fungi (AMF) Root Colonization in Organic Soil

Ariel Freidenreich 1,2,*, Saoli Chanda 1, Sanku Dattamudi 1 and Krishnaswamy Jayachandran 1,*

1 Department of Earth and Environment, Florida International University, Miami, FL 33199, USA; afreidenreich@ufl.edu (A.F.); schanda@fiu.edu (S.C.); sdattamu@fiu.edu (S.D.)
2 Department of Soil, Water, and Ecosystem Sciences, University of Florida, Gainesville, FL 32611, USA
* Correspondence: jayachan@fiu.edu; Tel.: +1-(305)-348-6553

Abstract: Pesticide application in horticultural crops has recently multiplied to increase crop yields and boost economic return. Consequently, the effects of pesticides on soil organisms and plant symbionts is an evolving subject of research. In this short-term study, we evaluated the effects of glyphosate (herbicide) and carbaryl (insecticide) on okra biomass and AMF root colonization in both shade house and field settings. An additional treatment, the combination of glyphosate and carbaryl, was applied in the field trial. Soil and root samples were collected three times during the experiment: 30 days after planting (before first spray, or T0), 45 days after planting (before second spray, or T1), and at full maturity (at 66 days after planting, or T2). Our results indicate that glyphosate and combined treatments were most effective in controlling weeds and produced almost 40% higher okra biomass than the control. There was a ~40% increase in AMF root colonization in glyphosate-treated plots from T0 to T1. This result was likely due to high initial soil P content, high soil temperature, and low rainfall, which aided in the rapid degradation of glyphosate in the soil. However, at T2 (second spray), high rainfall and the presence of excess glyphosate resulted in a 15% reduction in AMF root colonization when compared to T1. We found carbaryl had little to negligible effect on AMF root colonization.

Keywords: herbicide; horticultural crop; insecticide; rhizosphere; soil health

1. Introduction

It is estimated that about 3.5 million tons of pesticides are applied annually to agricultural crops worldwide, approximately 25% of which is used in the United States alone [1]. However, the amount of substance that comes into contact or is consumed by pests is a small percentage of the amount applied. Studies have shown that approximately 0.3–1.0% of the pesticide applied reaches the target pest [2,3]. Overapplication of pesticides often results in the exposure of non-target organisms to chemicals. This can cause unintended harm to organisms and a subsequent decline in overall environmental health [4,5]. Crop production is highly dependent on soil microbial biodiversity, as microbes are crucial for organic matter decomposition and assist with converting nutrients to plant-available forms [6]. Pesticides have major impacts on soil microbial communities, specifically by altering response with shifts in microbial biomass, ultimately having negative effects on soil health and fertility [7–9].

Glyphosate (N-phosphonomethylglycine; C3H8NO5P) and carbaryl (1-naphthyl methylcarbamate; C12H11NO2) are common broad-spectrum pesticides that are widely used in agricultural soils in the US. Glyphosate kills weeds through the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is also present in fungi and bacteria [10]. Glyphosate is applied to 298 million acres of agricultural cropland annually and is the most used herbicide in the U.S. and in Florida [11,12]. Glyphosate became
popular through providing successful broad-spectrum weed control and is easily available to farmers, with an application cost ranging from USD 1 to USD 13 per acre [11]. Since okra (Abelmoschus esculentus) is grown during warmer months in Florida, USA, warm-season weeds (nutsedges, amaranth, goosegrass, pusley, and more) can cause major problems during production. Not many herbicide options are available for okra in the US, and so far, glyphosate has been shown to work effectively as a pre-emergence herbicide for okra.

Furthermore, more than 700,000 lbs (350 tons) of carbaryl is applied to over 650,000 acres of agricultural land annually in the U.S. [12]. Carbaryl is primarily used as an insecticide to treat apples, pecans, and soybeans. While carbaryl is applied prominently to these specific crops, in some states in the US, carbaryl is crucially important for pest control. For example, 91% of asparagus in Michigan is treated with carbaryl, making it a vital aspect of production [13]. Historically, carbaryl was primarily used in the production of grapefruit, sweet corn, peanuts, and potato in Florida, USA [13].

Risks of glyphosate and carbaryl toxicity to non-target organisms in soil have been increasingly studied. This is mainly because the mobility and toxicity of these pesticides are highly dependent on various soil management and agroclimatic conditions [14]. For instance, phosphate concentrations may directly determine the quantity of glyphosate adsorption and, therefore, the addition of phosphate fertilizers in the soil can increase glyphosate availability as P competes for the adsorption sites [15]. Therefore, understanding the complexity and fate of these chemicals in soil ecosystems is of growing interest among environmental scientists. Plant symbionts (microbes that live in a symbiotic relationship with plants) are an integral part of the soil biomes, and the long-term consequences of pesticide applications on plant symbionts are an imperative area of concern. Arbuscular mycorrhizal fungi (AMF), an important plant symbiont, can easily be found in a wide range of soil ecosystems [16]. AMF are reported to improve the growth of major agricultural crops, assist plants in nutrient uptake, increase water availability, reduce the impacts of soil salinity, and help to protect plants against pathogens [16–19]. Mycorrhizal hyphae extending through the soil can also help to facilitate rhizosphere activity via enhanced microorganism interactions [20].

The effect of glyphosate on AMF function and activity is well documented [18,21–23]. However, the effect of carbaryl or carbamate group of insecticides on the performance of AMF is limited [24]. To the best of our knowledge, no research study on carbaryl and AMF in the subtropical U.S. is available. We recognize that carbaryl application in horticultural crops is increasing, specifically because of the quick degradation of carbamate insecticides in soil [25]. Thus, we planned this study to evaluate the individual and combined effects of these pesticides on AMF colonization in a subtropical climate. We hypothesized that both these pesticides, individually and in combination, will impact AMF population and subsequently influence soil and plant parameters. The specific goals of this experiment were to evaluate the individual effects of glyphosate and carbaryl on AMF root colonization, plant growth parameters, and nutrient contents in a controlled environment and in field conditions during okra production. Additionally, we intended to analyze the combination effects (synergistic vs. masking effects) of these two pesticides on AMF growth in field conditions.

2. Materials and Methods
2.1. Shade House and Field Experiments

This 10-week research project followed an integrated approach where the experiments were conducted both in the shade house settings (controlled environment) and in an agricultural field. The shade house experiment was conducted in the Organic Garden (25.7540° N, 80.3801° W) at Florida International University (FIU). Three-gallon (11.40 L) plastic pots were used for growing okra (Abelmoschus esculentus; Var: Gold Coast). Soils were collected from the Organic Garden to fill the pots. The soil was loamy sand with more than 15% organic matter content and was slightly alkaline, with a pH of 7.5. Additional information about the Organic Garden soil can be found via Gaffar et al. 2021 [26]. Okra
seeds were treated with 15 mL of commercially available mycorrhizae inoculant (Nature's Solution Mycorrhizae) to ensure robust and diverse presence of fungi and root colonization. The solution was a mix of fungal species, including five types of ectomycorrhizal fungi (Pisolithus tinctorius, Rhizopogan villosullus, Rhizopogan amylopogon, Rhizopogan fulvigate, and Rhizopogan luteolus) and six types of endomycorrhizal fungi (Glomus mosseae, Glomus intraradices, Glomus dussi, Glomus clarum, Glomus deserticola, and Glomus migroaggregatum). Principles and applications of seed inoculation were similar to Garcia et al., 2019 (research conducted in the same agroclimatic region) [19]. Seeds were started in starter trays to ensure an equal amount of inoculant was provided to each seed. Seedlings were transplanted into the pots one week after germination. Treatments used in the greenhouse study include (a) control, (b) glyphosate (1.2 mL substance per plant), and (c) carbaryl (1.6 mL substance per plant). Pots were organized in a randomized complete block design (RCBD), with five replications for each treatment. Composted poultry manure (PM) was applied in the pots with a similar dose to the one used in the field study (4.7 L PM for 1050 L of soil volume).

The field experiment was conducted in Homestead, Florida (June to August 2015). Plots were prepared by removing overlying grass, followed by soil tillage with a rototiller (Figure 1). Soils used in the field study were sandy clay loam in texture, with a pH of 5.4 ± 0.26 and high organic matter (OM) content (41 ± 0.3%) (Table 1). We created rectangular (6.1 m × 9.1 m × 0.2 m) raised beds using cinderblocks filled with the field soil (Figure 1).

![Figure 1](image-url). Pictures showing raised bed preparations (A), okra (Abelmoschus esculentus) production (B), okra roots (C), and microscopic images of infected roots (D).
Beds were created in particular locations of the farm to avoid shade effects (received ambient sunlight and rainfall) from large canopy fruit trees. Each bed was divided into four equal segments (sub-plot), which received the following treatments: (a) control (no herbicide was applied), (b) glyphosate (60 mL of liquid per bed), (c) carbaryl (75 mL of liquid per bed), and (d) a combination of both glyphosate and carbaryl. Please note that we introduced the combination treatment (both glyphosate (60 mL of liquid per bed) and carbaryl (75 mL of liquid per bed)) in the field experiment to see the synergistic or masking effect of those pesticides on AMF in the soil. We installed thick plastic barriers around each segment of the raised beds to ensure no spray drift or contamination between treatments. Similar to the shade house experiment, treatments were organized in RCBD with three replications for each treatment (12 total sup-plots). Each sub-plot received about 4.7 L of USDA organic chicken manure (total C and N contents were 33.76 ± 0.29% and 5.53 ± 0.13%, respectively) and thoroughly homogenized with a shovel. Okra seeds were inoculated with mycorrhizae solution (details mentioned earlier) and sowed (10 seeds per m² area; [27]) directly into the beds and lightly covered with soil. The beds were equally watered daily depending on the rainfall during our experiment.

After germination, plants grew for 30 days prior to spray application to allow for sufficient root development [18]. Glyphosate and carbaryl were uniformly sprayed on each bed at 30 and 45 days after okra germination, respectively. Plants were sampled for root colonization at ~15 days after spray, a method modified from Druille et al. [18]. No manual or mechanical weeding was carried out during this experiment. Control pots were left as is without any weed control measures applied. Similarly, no weeding was carried out for control beds in the field.

2.2. Sample Collection for Both Experiments

Rhizosphere soil (soil immediately attached to plant roots) and root samples from the pots were collected three times during the experiment: 30 days after planting (before first spray, T0), 45 days after planting (before second spray, T1), and at full maturity (at 66 days after planting or during harvesting (T2). Okra is a short-duration vegetable crop and generally harvested 60 to 70 days after planting [27]. Root, shoot, and leaf samples were collected from three randomly selected plants from each treatment during each sampling date (a total of 5 plants per treatment were sampled in the potted study and 9 in the field study). The entire plant (including roots) was collected using a shovel for plant parameter analysis. Throughout plant growth, weekly ambient measurements were taken to determine light intensity, soil moisture, soil pH, and soil temperature using a digital meter (Digital 4-Way Soil Meter, Sunleaves, Bloomington, IN, USA). At full maturity, all okra fruits were harvested and weighed to determine total crop yield per plot. Similar sample collection time, principles, and methods were followed for the field experiment.

2.3. Laboratory Analyses

Each plant was thoroughly washed to remove soil particles and insects left on roots, stems, and leaves. Each sample (i.e., roots, stems, and leaves) was separated and allowed to dry at 70 °C for 72 h for dry weight determination (±0.0001 g).
Dried and ground soil and leaf samples were analyzed for total carbon and total nitrogen via dry combustion with a LECO CN Analyzer (St. Joseph, MI, USA). Total P in the plant tissue samples was analyzed following the USEPA [28] semi-automated colorimetry method using the SEAL Analytical AQ2 Discrete Auto Analyzer (Mequon, WI, USA).

The degree of mycorrhizal colonization in the root samples was identified following a modified method by McGonigle et al. [29]. Roots of each plant sample were carefully washed in a 2 mm sieve to remove all remaining soil particles. Twenty-five 1.5 cm long root fragments were removed for processing from each sample. Each set of 25 roots was placed in a micro centrifuge tube and submerged in 1.5 mL of 10% KOH. Samples were placed in the oven at 70 °C for 2 h. Once out of the oven, each sample was rinsed twice in deionized water. The roots were stained by adding a 0.5% Trypan blue/lactoglycerol solution and then placed in the oven at 70 °C for 30 min. After removal from the oven, the samples were thoroughly washed to remove excess blue stain and lactoglycerol solution was added to each set. Twenty-five roots from each sample set were randomly selected and placed on a slide for examination. The roots were examined individually under a stereo microscope (Olympus SZX7, Waltham, MA, USA) and recorded as colonized or not colonized. Qualification for colonization was the visual presence of any three structures: hyphae, vesicles, or arbuscules. Percent colonization was then calculated by dividing the number of colonized roots by 25 and multiplying by 100.

2.4. Statistical Analyses

All data were analyzed with SAS 9.4 software (SAS, Cary, NC, USA). A one-way analysis of variance (ANOVA) test was performed on the data set using the Proc Mixed procedure. Post hoc mean comparison was performed using Tukey–Kramer to find significance between treatments.

3. Results and Discussion

3.1. Okra Biomass Production and Plant Nutrient Status

Okra biomass (root, shoot, and total) treated with glyphosate and carbaryl in both shade house and field settings is presented in Figure 2. We combined root and shoot (stem and leaves) biomass together to obtain total biomass for each treatment.

In field trials, the glyphosate treatment produced the highest total biomass (93.58 g), similar (89.26 g) to the combination application of carbaryl and glyphosate (Figure 2) and more than 40% higher than control plots. Glyphosate, a commonly used herbicide for okra production in Florida [30], is capable of reducing 40 to 70% of the weed populations in okra [31] and subsequently may lead to an increase in crop biomass. In this study, we found that plots treated with glyphosate had 70–80% lower weed presence than control plots. Furthermore, a study conducted in Abeokuta, Nigeria, showed that integrated weed management, including application of herbicides and hoe weeding, generated maximum okra yield and profitability [32]. This trend of biomass production impacted by pesticide treatments was different in the shade house experiment. During the T1 sampling time (after the first pesticide application), glyphosate-treated plots showed a 35% and 30% decrease in total biomass production than control and carbaryl, respectively. However, during harvesting (T2 sampling time), glyphosate plots resulted in 25% and 17% higher biomass production than control and carbaryl, respectively. The decrease in biomass production during the T1 sampling time was likely due to a glyphosate drift problem in the shade house. Although okra pots were spaced about 3 ft apart from each other there was still a drift problem that influenced the outcome of the experiment. Drifting of herbicide can decrease herbicide effectivity [33], which can subsequently reduce biomass yield and introduce herbicide resistance in weeds [34].
decrease herbicide effectivity [33], which can subsequently reduce biomass yield and introduce herbicide resistance in weeds [34].

Shade house Field trials

Figure 2. Line diagrams represent the trend of total biomass in okra as impacted by different pesticide application treatments (control = no pesticide applied, glyphosate, carbaryl, and combine = glyphosate + carbaryl applied) over time (T0 = 30 DAP, before pesticides spray; T1 = 45 DAP, after first spray and before second spray; T2 = 66 DAP, at maturity after second spray) in shade house and field settings.

To avoid glyphosate drift issues in the field setting, we followed preventive measures. We applied thick plastic dividers to separate treatments, and pesticides were applied on days with minimum wind effects. Okra biomasses in carbaryl-treated plots and control plots were not significantly different. Typically, vegetable production in South Florida often experiences yield loss from insect pest infestations [35]. However, this was not the case in our experiment, where few or no insects were visible in control plots, likely due to high air temperature (average temperature was more than 35 °C) and low relative humidity (average RH was 85%) during the experiment. Therefore, the effect of carbaryl as an insecticide was negligible in our field experiment and thus no significant difference in biomass production between control and carbaryl plots was observed. Additionally, soil with low pH and high organic matter content was reported to have higher carbaryl adsorption and subsequently low efficacy in controlling pests [36]. Since the soil in the experimental site had low pH and high organic matter content, the recommended dose of carbaryl treatment did not produce any significant effect on crop parameters compared to control.

While comparing the difference in crop biomasses over time, we observed that the effect of treatment was more prominent during the T2 sampling time (at full maturity or 66 DAP). The magnitude of difference in root biomass for glyphosate and control is at least
24% higher than in shoot biomass (Figure 2). It is also a well-known fact that glyphosate sorption depends on the presence of micronutrients in the soil [37]. This is mainly because glyphosate acts as a chelating agent and binds with Fe, Mn, and Zn micronutrients and becomes unavailable in soil. The soil used in our study site was also acidic and expected to have higher micronutrient contents, which explains the possibility that glyphosate was less available in the soil to affect AMF root colonization. The significance is discussed later (Section 3.2) in this manuscript.

Glyphosate-treated pots in the shade house experiment showed 2% and 9% less P uptake by plant tissue as compared to control and carbaryl, respectively (Table 2). A similar trend was observed in the field experiment. Glyphosate and combined treatments showed about 8% less P uptake than control and carbaryl treatments in the field. It is well known that both P and glyphosate compete for exchangeable sites in the soil [15,38] and, therefore, P availability and uptake inversely affect glyphosate contents in the soil. As a result, in both the shade house and field experiments, we observed glyphosate-application-reduced P uptake by okra plants. However, no significant difference of total C and N contents in plant tissue was found for varying treatments both for shade house and field experiment. Glyphosate produced the lowest fruit yield (204.49 kg ha$^{-1}$), while control treatment produced the highest fruit yield (408.31 kg ha$^{-1}$) in the field experiment. Though glyphosate increased biomass yield in the field experiment, it reduced fruit yield. This could be a result of adverse effects in fruit production and retention of fruits within the plant. Studies mentioned that glyphosate increases the production of ethylene, which results in fruit abscission and drop when applied in citrus fields [39].

Table 2. Nutritional parameters of okra leaf and fruit yield of okra for both shade house and field settings.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Nitrogen (mg g$^{-1}$ dw)</th>
<th>Total Carbon (mg g$^{-1}$ dw)</th>
<th>Total Phosphorous (mg g$^{-1}$ dw)</th>
<th>Fruit Yield *§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shade house experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>31.29 ± 9.22</td>
<td>413 ± 10.34</td>
<td>4.11 ± 0.83</td>
<td>-</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>33.77 ± 4.20</td>
<td>418 ± 3.97</td>
<td>4.03 ± 0.74</td>
<td>-</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>28.71 ± 4.10</td>
<td>419 ± 14.58</td>
<td>4.41 ± 1.01</td>
<td>-</td>
</tr>
<tr>
<td>Field experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>37.98 ± 2.90</td>
<td>430 ± 9.31</td>
<td>5.10 ± 1.10</td>
<td>408 ± 132</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>39.58 ± 4.58</td>
<td>430 ± 9.21</td>
<td>4.70 ± 0.91</td>
<td>204 ± 132</td>
</tr>
<tr>
<td>Combine</td>
<td>38.76 ± 4.54</td>
<td>433.35 ± 9.53</td>
<td>4.71 ± 0.87</td>
<td>295 ± 90</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>39.19 ± 4.14</td>
<td>431.89 ± 11.93</td>
<td>5.10 ± 0.92</td>
<td>386 ± 154</td>
</tr>
</tbody>
</table>

* Fruit yield in the field trial is expressed as kg ha$^{-1}$. § Fruit yield of okra was not obtained in the shade house because of drift issues.

3.2. Arbuscular Mycorrhizal Root Colonization

Root colonization by AMF is the most crucial step of plant symbiosis, with a higher number (%) of colonized roots indicating a greater symbiotic relationship between AMF and the plant. In this experiment, we evaluated root colonization by AMF in both glyphosate- and carbaryl-treated plots (Figure 3). In the shade house, a steady decreasing trend in root colonization was found within glyphosate treated plants. Conversely, our results from the field study indicate that initially at T1 sampling time, there was 40% and 44% increase in AMF root colonization in glyphosate-treated plots and control plots, respectively, as compared to T0 time. After T1 sampling time (45 DAP), a decreasing trend in AMF colonization was observed for both control and glyphosate-treated plots. At the T2 sampling time, glyphosate-treated plots had a 15% and control plots had a 29% reduction of AMF root colonization as compared to the T1 sampling time. No distinct change in AMF colonization was found in carbaryl-treated plots for both shade house and field trials. Plots with combination treatments of carbaryl and glyphosate showed an initial 35%
reduction in AMF colonization during sampling time T1 as compared to sampling time T0. Later, both T1 and T2 sampling times showed similar AMF colonization. In our field study, glyphosate was sprayed directly on weeds. In the shade house, glyphosate was mostly sprayed on soil surface due to less weed population than in the field condition. Druille et al. [21] reported that glyphosate application had a greater negative effect on spore viability and root colonization when applied directly to soil as opposed to plant foliage in field conditions. Weeds present in all treatment plots consisted primarily of purple nutsedge (Cyperus rotundus) [40]. Purple nutsedge can be colonized by AMF but has been shown to possess a non-functional relationship with endomycorrhizal fungi [41]. Koske et al. [42] found that roots of the purple nutsedge contained vesicles and hyphal structures but lacked arbuscules. Therefore, it is possible that a higher presence of weeds in the field may have impacted the root colonization results, whereas this was not a factor in the shade house.

Figure 3. Line diagrams represent the trend of AMF root colonization in okra impacted by different pesticide application treatments (control = no pesticide; glyphosate = only glyphosate applied; combine = both glyphosate and carbaryl applied, and carbaryl = only carbaryl applied) over time (T0 = 30 DAP before pesticides spray; T1 = 45 DAP after first spray and before second spray; T2 = 66 DAP at maturity after second spray) in the shade house and field experiments.

The experimental site also had organic soil with a high P content. A high phosphate content, or the addition of phosphate to soil, also helps in the breakdown of glyphosate [43]. Therefore, the excess P in soil (please see Table 1) could have assisted in the initial breakdown of glyphosate. After the second spray, the adverse effect of glyphosate on AMF root colonization was more prominent. Our results indicate that glyphosate reduced P uptake from the soil by okra plants (Table 2), mainly because it competes with P for soil-
exchangeable sites. Furthermore, AMF helps to solubilize phosphates in soil and release more P in the soil solution [44]. Thus, those freed exchangeable sites in the soil become available to glyphosate for adsorption. Soil temperature [45] and aeration [46] play important roles in glyphosate mineralization. From the first spray (T0) to the T1 sampling time, 56% of the experimental days received rainfall (total rainfall was 5.30 cm). From the time of the second spray (T1) to the final sampling time (T2), about 79% of the experimental days received rainfall (total rainfall was 17.80 cm). Therefore, lower rainfall (aerobic soil condition) and high soil temperature (more than 30 °C) during the T1 sampling period played a role in the quick degradation of glyphosate in the soil. This is reflected in the initial increase in AMF root colonization in glyphosate-treated sites within the field experiment. We did not measure glyphosate toxicity in the soil; however, it is not unlikely that after the second spray (at T2), an excess amount of glyphosate was accumulated in the soil, which adversely affected AMF root colonization.

We understand that species identification and quantification of various microorganism species in the soil would help us to better evaluate the effects of these pesticides on AMF colonization. However, it was beyond the scope of this short-term preliminary study. Arbuscular mycorrhizal fungi, a common symbiont, was indeed affected by the applications of glyphosate and carbaryl in this study. We observed varying magnitudes of AMF colonization, which has been reported in previous studies [18,22,23], possibly because our experiments were conducted in organic-rich soils. A higher amount of food source (SOM were 16% and 40% for shade house and field, respectively) has a positive influence on AMF population, an indication of the symbiotic biodiversity of the soil used in this study. Additionally, high SOM induces higher enzyme activity in the soil and subsequently results in more breakdown of glyphosate.

4. Conclusions

In this study, glyphosate promoted okra biomass production by controlling the weed population. Yet, due to higher fruit drop, the yield of okra was the lowest in glyphosate-treated plots compared with other treatments in the field trial. Not much insect damage was visually observed in the field research. Therefore, carbaryl has negligible effect on plant biomass production and produced a similar okra biomass to that of control. Arbuscular mycorrhizal fungal root infection was higher in the field experimental site during T1 sampling (after first glyphosate spray) and was likely due to high soil temperature, low rainfall, and higher soil P content. Later, at the T2 sampling time (after second spray), the AMF root colonization was reduced by 15% because of high rainfall and excess glyphosate concentration in the soil. Carbaryl did not show any effect on AMF root colonization throughout the experiment in the field study. Within the shade house study, it was clear that glyphosate had the most negative effect on AMF root colonization, followed by carbaryl, when compared to the control at T1 and T2. This is likely the result of glyphosate application directly to the soil surface, which may have had more detrimental effects on non-target organisms in a more contained and controlled setting.

Overall, this preliminary study has revealed that treating okra with glyphosate can be positive for biomass production yet detrimental for fruit production. In the controlled shade house experiment (no influence of weeds or other confounding factors), AMF was negatively impacted by glyphosate application. Since glyphosate is heavily used in the sub-tropics for weed control, this study can have important implications for the use of glyphosate in warm humid climates as it relates to okra production and soil microbial communities. We recommend more in-depth studies on this topic to get a true grasp of the interaction of these chemical pesticides in the sub-tropics as it relates to soil biota and crop production.

and K.J.; funding acquisition: K.J. All authors have read and agreed to the published version of the manuscript.

**Funding:** The research was funded through FIU’s Agroecology Program via the U.S. Department of Agriculture-National Institute of Food and Agriculture, National Needs Fellowship (2013-38420-20499).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**


