

# Organic Ginger (*Zingiber officinale* Rosc.) Development in a Short Temperate Growing Season: Effect of Seedling Transplant Type and Mycorrhiza Application

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## Abstract

Global warming and consumer demand for medicinal plants present an opportunity to introduce ginger growth to the US Delmarva Peninsula. High tunnel and field studies were conducted to assess the development of organic ginger (*Zingiber officinalis*, Rosc) seedling transplants in mycorrhiza-amended soil. Transplant types were tissue culture derived with less than three tillers (TCS1), three or more tillers (TCS2), and nontissue culture derived (NTCS1). Transplants were grown with or without mycorrhiza (2.8 g per plant) in a split plot design with soil amendments as main plot and transplant type as subplot. Data were collected for air temperatures, plant height, tiller number, leaf chlorophyll index (LCI), rhizome fresh weight, plant biomass, rhizome nutrients, and levels of As and Pb. TCS2 transplants produced significantly higher, or trended to higher rhizome yield than transplants with less than three tillers, except for year two field study. The maximum rhizome fresh weight per plant was 648.3 g for TCS2 in high tunnel in year one. Generally, TCS2 had most tillers throughout the growing season ranging from 6.9 to 25.7 tillers per plant over three studies. Mycorrhiza had no effect on ginger height, tiller number, LCI or rhizome yield. Sustained high temperatures above 37°C, plus high light in the field caused dieback and stunted shoot growth in year two. There were no consistent effects of mycorrhiza or transplant type on rhizome nutrient content. Content of total Pb, As and other elements were at safe threshold levels for rhizome consumption. These results suggest that gingers grown from TCS2 transplants with at least three tillers yielded more rhizome than those grown from S1 transplants with fewer tillers. Introduction of ginger to a short season region such as the Delmarva may require consideration of environmental condition such as high temper-

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ature and light to which seedling transplants may be exposed in summer.

## Keywords

Seedling Transplants, Mycorrhiza, Organic Agriculture, Tissue Culture, Medicinal Plants

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## 1. Introduction

Ginger is generally grown in tropical and subtropical climates such as those of Southeast Asia, its origin [1]. As a popular spice crop with medicinal and nutraceutical value [1] [2] [3], consumers demand it, and some small land holders in the USA desire to grow it as a niche crop in the less tropical, short season environment. In fact, outreach and research efforts to grow ginger have been ongoing in several eastern [4] [5] and southern states of the USA [6] [7] [8] by growing the plant in high tunnels or in the field. Ginger grows best under some shading, particularly in the seedling stage, but some production was done in direct sunlight in some countries [1] [9] [10].

As consumers' demands for organic products continue to rise [11], there is the need to adapt ginger production to organic culture. Concomitantly, there is also a problem getting reliable and consistently disease clean transplants from rhizome. The main method of propagation is from rhizome pieces called seed setts. These develop tillers after planting. However, ginger production in short growing season can be a challenge for attaining optimal rhizome production. Ginger can be harvested as young baby ginger after 3 to 4 months of transplanting. Typically, ginger takes 8 - 10 months to produce mature rhizomes after planted from setts [8]. Therefore, there is a need to determine other types of propagation materials and growing medium amendments that can potentially accelerate development of the crop in organic conditions in short growing seasons. One type of propagation material is seedlings with developed shoots/tillers, and this is rarely done or studied for organic culture. The only report found in the literature was one that included two sprout transplants and single sprout transplants in a non-organic system [12]. Since the number of tillers is positively correlated with rhizome production [1], this may be an approach to get a head start on plant development. With respect to growing media amendments, there is evidence that some such as composts [13] and microorganisms like *Azospirillum* and mycorrhiza [14] [15] [16] [17] have an effect on the yield of ginger grown from setts under conventional conditions or organic. Vesicular arbuscular mycorrhiza (VAM) fungi form a symbiotic relationship with host plants and, thus, support soil health and plant nutrition in organic agriculture [18] [19] [20]. They also enhance plant survival and fitness through mechanisms such as increasing water and nutrient uptake by extending the function of their root system and transporting nutrients to plants [21] [22]. In contrast to the many studies on the use of mycorrhiza in conventional ginger development, there is none

for organic that also includes the use of seedling transplants in different stages, whether tissue cultured or nontissue cultured. Tissue culture of apical bud segments has been an established method to produce clean seedling explants in ginger [23] [24] [25]. The objectives of this study were to: 1) Compare the development of organic transplanted tissue cultured and nontissue cultured ginger of different tiller stages in mycorrhiza and non-mycorrhiza amended soil in high tunnel and field conditions, and 2) Assess the rhizome nutrient, lead and arsenic content of the organic ginger.

## 2. Materials and Methods

### 2.1. Experimental Sites

All high tunnels and field experiments were conducted at the University of Maryland Eastern Shore Agriculture Experiment Station located in Princess Anne, MD., USA (38°12'N 75°42'W), following the National Organic Program guidelines [26]. The certified organic field sites had a sandy loam soil and phosphorus soil levels were high, 300 - 400 ppm, due to previous extensive poultry manure application from the local chicken industry over many years.

### 2.2. Year 1 High Tunnel Study

Yellow ginger rhizomes were purchased from a local grocery store and used for both the tissue culture (TC) and the nontissue cultured (NTC) studies. The axillary buds of rhizomes were used to aseptically regenerate clean tissue plantlets as follows. Buds were cultured in Murashige and Skoog medium supplemented with indole acetic acid (0.5 mg/L), benzyl amino purine (0.1 mg/L), Gamborg's B5 vitamin (1 mL/L), and sucrose (30 g/L) (Phytotechnology Laboratories, Inc., Overland Park, Kans., USA). In spring 2011 plantlets were acclimated in a chamber at 28°C, then transferred to organic growing medium (Sungari Sunshine Mix #1 Organic Planting Mix, Sungro Horticulture, Agawan, Mass., USA) in 11.4-cm plastic pots in a greenhouse to develop tillers. The NTC seedlings were established directly from Yellow ginger rhizome using the following procedure. Rhizomes were rinsed and surface sterilized with 10% bleach before being placed in 27.9 × 54.6 cm black plastic trays/flats (Hummert International, Earth City, MO) where they were covered and kept in a growth chamber at 28°C. Once buds formed, rhizomes were cut into 5 cm- size- pieces containing one bud each and planted into 11.4 cm (11.4 L × 11.4 W × 8.3 H) plastic pots containing Sunshine organic mix, similar to the ones for tissue culture (TC). Transplants were kept in the greenhouse until transplanted in the high tunnel. The TC and nontissue culture derived seedlings (NTC) were grouped according to their development as S1 (fewer than three tillers) or S2 (three or more tillers) resulting in three separate sets of seedlings: NTCS1, TCS1, and TCS2. Transplants were kept in greenhouse until transplanted to raised beds in a high tunnel on certified organic site at the University of Maryland Eastern Shore Experiment Station (Princess Anne, Md., USA) on June 17, 2011. The transplants were planted 25 cm deep in two row-beds with rows spaced 1.25 m apart and plants were spaced 0.3 m plants

apart. The experimental design was a split plot with mycorrhiza and non-mycorrhiza treatments as main plot and the three seedling types as the sub-plot, and three replications. Vesicular-arbuscular mycorrhiza (VAM) inoculant was applied to the soil around each plant at a rate of 2.8 g per plant. The VAM, (*Glomus etunicatum*) inoculum (100 spores and propagules per gram of inoculum) was provided by Becker Underwood, Inc. (Ames, Iowa, USA). Beds were drip-irrigated twice weekly throughout the growing season. Plants were fertilized with Organic Materials Review Institute (OMRI)-certified fertilizers (Phy-ta-Grow Big Red Blood Meal 13-0-0) (California Organic Fertilizers, Inc., Fresno, Calif., USA) at a rate of 50.48 g/m<sup>2</sup> and Sulfate of Potash Ultrafine Diamond K Fertilizer (Great Salt Lake Minerals Co., Odgen, Utah., USA) at 17.87 g/m<sup>2</sup> three times during growth.

Data were recorded on plant height, number of tillers per plant, leaf chlorophyll index (LCI), and air temperature throughout the plant growth period. The LCI was measured with a SPAD 502 chlorophyll meter manufactured by Spectrum Technologies, Incorporated, Aurora, IL. Measurements were taken from five fully expanded healthy green leaves per treatment combination per replication. Matured rhizomes were harvested on December 14, the day of the first frost (**Figure 1**) in tunnel, and data were collected on rhizome weights, and rhizome nutrient content. For nutrient analysis, the rhizome samples were dried at 75°C to constant weight, then ground in a Wiley Mill, (Wiley, and Philadelphia, PA) to pass through a 1-mm screen. Samples were submitted to A & L Eastern Laboratories, Richmond, VA, for the analysis of 13 macro- and micro-elements: N, P, K, S, Ca, Mg, Na, Fe, Al, Mn, Cu, Zn, and B.

### 2.3. Year 2 High Tunnel

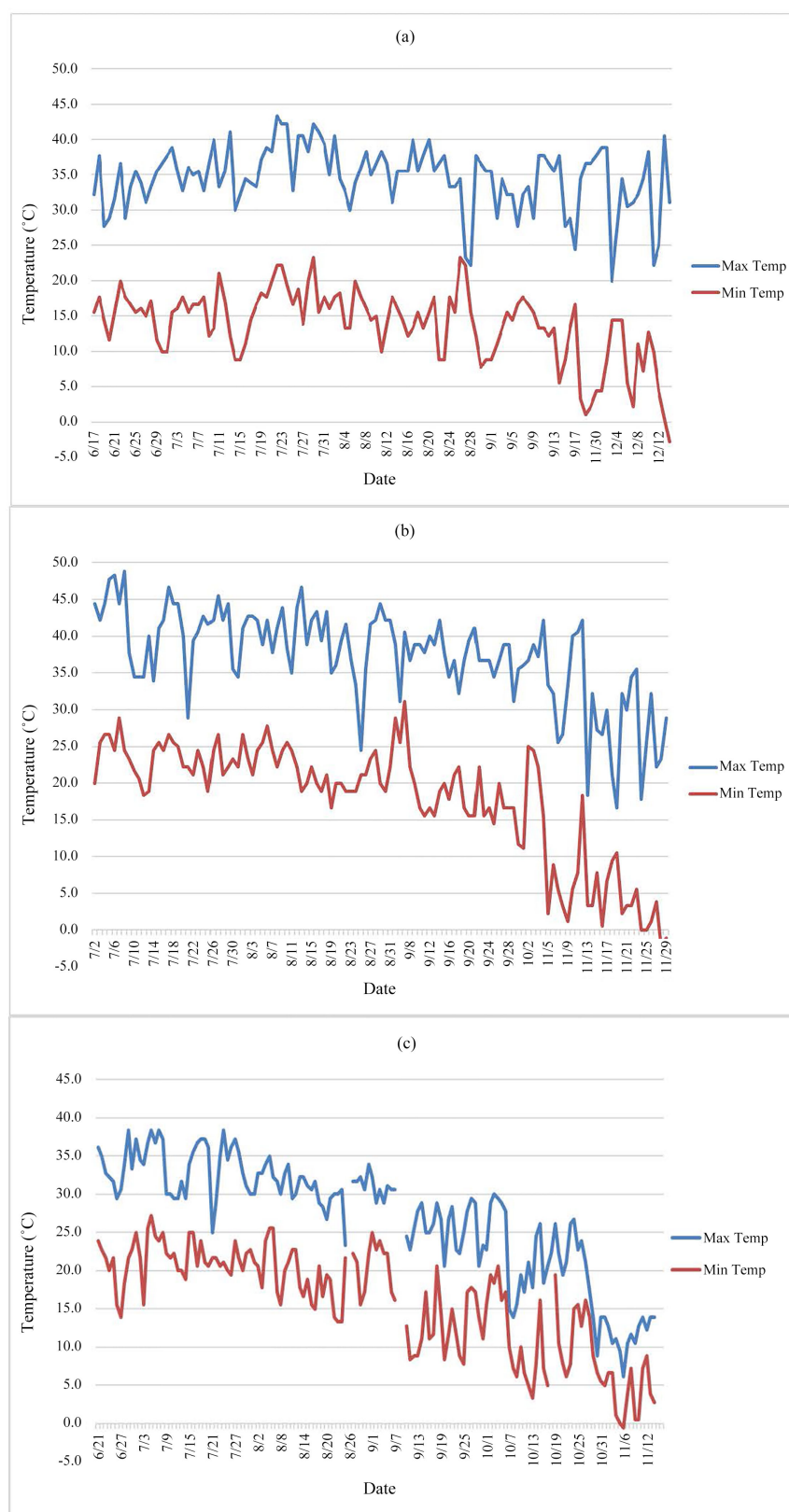
The Year 1 high tunnel study was repeated in 2012 where ginger seedlings were transplanted to the organic high tunnel on June 26, 2012. Data on tiller number and plant height were collected for both the high tunnel and a field study done that year. Under the moderating temperature influence of the high tunnel with warm temperatures, the rhizomes were harvested late in fall on November 29. In addition to the rhizome nutrient analyses for 13 elements, total Pb and total As contents were also determined.

### 2.4. Field Study

In Year 2, a follow up field study was conducted where ginger seedlings were transplanted to the site on June 21, 2012. Ginger rhizomes were harvested from the field site on November 14, after the first frost. Similar data were collected in the field as those for the high tunnel that year.

### 2.5. Ambient Temperature Monitoring

Air temperature during the growing season was periodically monitored and recorded (**Figure 1**) using a hygrothermograph, (NovaLynx, Grass Valley, CA).



**Figure 1.** Maximum and minimum air temperatures for 2011 high tunnel (a), 2012 high tunnel (b) and 2012 organic field (c) during growth of ginger from transplants to rhizome harvest.

## 2.6. Statistical Analysis

Two-way analysis of variance (ANOVA) was used to analyze the data using the SAS program (version 9.1, SAS Institute Inc., Cary, NC, USA). When interactions for variables were not significant, the data were reanalyzed to compare the main effects. The means were compared using LSD at ( $P \leq 0.05$ ).

## 3. Results and Discussion

### 3.1. Year 1 High Tunnel Study

Plant height and tiller number steadily increased (**Table 1**) during the growing period up to November 4, 2011, four and a half months after transplanting. Thereafter, the plants began to senesce, and growth ceased as the temperature decreased (**Figure 1**). For the first 2 months after transplanting the maximum air temperatures ranged from 27.8°C to 38.9°C, except for two days in the first month and 10 days in the second month which were in the low 40 s. For the remainder of the growing period, maximum temperatures were generally between 20.6°C and 38.9°C. Minimum air temperatures ranged from 23.3°C to -2.8°C and did not dip below freezing until December 14 due to the moderating effect of the tunnel. Mycorrhiza did not significantly promote ginger development and had no effect on plant height, tiller number or LCI within any of the sampling dates (**Table 1**). The available reports of the effects of mycorrhiza on ginger are for nonorganic culture and have been mixed. The research of Santos *et al.* [16] showed better post vitro acclimation of micro propagated ginger micro plantlets with mycorrhiza than non-mycorrhiza plants grown in greenhouse. However, the work of Silva *et al.* [27] with micro-propagated ginger reported mixed results when using different isolates of arbuscular mycorrhizal fungi to compare ginger shoot biomass and height against non-mycorrhiza controls. In strawberry cultivars, AM fungi applied to high phosphorus soil, fertility (Mehlich-3 extractible P = 498 mg·kg<sup>-1</sup>) produced mixed results, including increased daughter plants in early growth [28].

Seedling type did not affect plant height (**Table 1**). The more advanced TCS2 transplants consistently produced most tillers at each sampling date. When tiller production ceased, the 25.7 for TCS2 was approximately double that of each of the TCS1 and NTCS1 plants.

Leaf chlorophyll index analyses were done for each of the four separate sampling dates, 7/22, 8/23, 9/8 and 11/4 (M1, M2, M3 and M4.5) because sampling date was significant (**Table 1**). SPAD meter measures leaf chlorophyll index (LCI) which serves as relative measure of chlorophyll content [29]. The latter is often correlated with leaf N status and photosynthetic activity [30] [31]. Mycorrhiza did not have any effects on leaf chlorophyll index in this study (**Table 1**). However, under water stressed conditions, unlike our optimized watered study, it has been reported to increase the chlorophyll content of ginger plants over that of non-mycorrhizal plants [32]. Leaf chlorophyll index at two and three months after transplanting was highest in TCS2, perhaps indirectly due to higher photosynthesis in this active growth period (**Table 1**).

**Table 1.** Effects of soil additive and seedling transplant type on plant height, tiller number and leaf chlorophyll index (LCI), dry shoot biomass per plant and fresh rhizome weight per plant for high tunnel organic ginger development by month (M) after transplanting in 2011.

Treatment <sup>z</sup>	Height				Tiller Number (TN)/Plant				Leaf Chlorophyll Index (LCI)				Biomass Yield	Rhizome Yield
	M0	M1	M2	M4.5	M0	M1	M2	M4.5	M1	M2	M3	M4.5		
	cm				TN				LCI				g	g
Soil additive (SA)														
VAM	20.9	24.6	31.2	48.3	2.5	4.4	8.3	16.5	31.6	39.8	43.4	33.5	34.1	500.2
Control	21.0	24.7	29.8	48.0	2.9	5.1	9.0	17.7	30.8	38.5	43.3	32.4	30.0	481.7
SA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Seedling Type (S)														
NTCS1	21.4	26.1	33.6	45.3	1.4b <sup>y</sup>	3.3b	6.0b	11.8b	32.0	38.8ab	41.1b	31.6	20.6b	375.8b
TCS1	19.0	22.1	31.1	47.7	2.1b	3.5b	6.3b	13.8b	30.3	36.7b	44.4a	31.3	26.4ab	448.6b
TCS2	22.5	25.7	26.8	51.4	4.4a	7.6a	13.5a	25.7a	31.4	41.8a	44.6a	35.9	49.2a	648.3a
S	NS	NS	NS	NS	***	***	***	***	NS	*	**	NS	*	**

<sup>z</sup>Treatments VAM = vesicular arbuscular mycorrhiza, NTC = non tissue culture, TC = tissue culture, S1 = seedling with <3 tillers; S2 = seedling with ≥3 tillers. <sup>y</sup>Within soil additive and seedling type, the values in each column followed by a different letter are significantly different according to LSD at  $P \leq 0.05$ . NS, \*, \*\*, \*\*\*Nonsignificant or significant at  $P < 0.05$ , 0.01, and 0.001, respectively.

Year-one high tunnel study showed that seedling transplant type affected the fresh rhizome yield produced after 5 months. Regardless of origin, the transplant type, TCS2, with three or more tillers, had higher yield than the two S1 types (Table 1). Dry biomass of TCS2 was also higher than that of NTCS1. The superior performance of the TCS2 derived transplants over the S1, regardless of whether tissue culture or not, is as expected. TCS2 plants at transplanting were already in the three-fork stage, the first phase of the grand growth phase as defined by Xizhen *et al.* [9]. The TCS1 and NTCS1 transplants were still in the seedling stage.

### 3.2. Year 2 High Tunnel Study

One day after transplanting, high tunnel plants were visually observed as healthy. A week later, a few plants in the high tunnel showed a symptom of sun burn, under the high temperatures (Figure 1). The maximum temperatures were mainly above 40°C, reaching as high as 48.3°C during the first month after transplanting. Two weeks after transplanting, the plants appearance looked better following fertilizer application. However, few weeks later and up to a month and half after transplanting (August 16), several plants from all the treatment combinations had some shoots die. During this period, and up to three months after transplanting they continued to periodically experience high maximum temperature above 40°C. These responses are consistent with the reports of Bhosale and Shinde, and Kandiannan, *et al.* [10] [32] that ginger grows best at temperatures around 28°C - 32°C, and very high temperatures can be desiccated.



ing and result in sunburn and plant death. Both high temperature and high light intensity, particularly during the seedling phase, may cause photo inhibition and result in decreased photosynthesis efficiency and yield [9]. The base temperature requirement is 13°C [10], and 1200 to 1300 accumulated temperature is required in the growing period [9]. Minimum temperature during our study ranged from -2°C - 2°C at harvest to 28.9°C during the growing period.

Due to the inconsistency in finding healthy developed leaves for sampling, no LCI data could be sampled for high tunnel or field plants, this year. There was little to no growth in shoot height during this period due to plant dieback and regrowth of new shoots under the high temperatures in the high tunnel (**Table 2, Figure 1**). Consequently, after plant regrowth, the plant heights for month three were generally less than at the time of transplanting. Mycorrhiza did not affect shoot height or number of tillers, and seedling type did not impact shoot height after transplanting. This lack of response to mycorrhiza application is similar to the results of year one (**Table 1**) of our study. Although the tillers in the high tunnel grew slowly under the temperature stress in year 2 (**Table 2**), transplant type still had a significant effect on the number, as in year one (**Table 1**). The TCS2 consistently produced highest number of tillers. While not significant, rhizome yield per plant trended higher for TCS2 than TCS1 and NTCS1.

### 3.3. Field Study

The data of the field-grown ginger showed no significant differences between soil additives or seedling types for the shoot development and rhizome yield, even though there were differences when they were transplanted (**Table 3**). The shock due to intense light exposure from direct sunshine and high temperature (**Figure 1**) quickly wiped out any advantage they had started with in their tiller number or height. One day after transplanting, the leaves of some had sun burn. Three weeks after transplanting (7/5/2012), leaf burn was still observed on some plants. During the first month after transplanting, the maximum temperatures ranged from 25.0°C to 38.3°C (**Figure 1**). By month two after transplanting, there were no new leaf burns observed. Maximum temperatures in months two and three after transplanting ranged from 20.6°C - 38.3°C. For the remainder of the study the maximum range was 6.1°C - 30.0°C (**Figure 1**); growth remained slow (**Table 3**), and there were no differences in any of the characteristics measured to test the effects of mycorrhiza or seedling transplant type. Minimum temperature during study ranged from -0.6°C at harvest to 25.6°C during the growing period. The study was terminated a week before month five of the growing season due to frost.

The fresh rhizome yield per plant was not significantly affected by mycorrhiza or propagation type (**Table 3**). Across these three studies, the maximum rhizome yield (648 g per plant) five months after transplanting was for the TCS2 derived transplants in year one high tunnel experiment (**Table 1**). Longer duration studies such as the 10-month field study of Rafie *et al.* [8] have produced more rhizomes, nearly 5 pounds /plant in the field in Florida, USA.



**Table 2.** Effects of soil additive and seedling transplant type on plant height, tiller number and fresh rhizome weight per plant for high tunnel organic ginger development by month (M) after transplanting in 2012.

Treatment <sup>z</sup>	Height			Tiller Number (TN)			Yield/Plant
	M0	M3	M4.5	M0	M3	M4.5	
	cm			TN			
Soil additive (SA)							
VAM	20.7	15.8	23.8	2.3a	4.0	6.7	104.7
Control	19.4	15.6	22.6	2.1b	3.6	5.1	79.9
SA	NS	NS	NS	NS	NS	NS	NS
Seedling Type (S)							
NTCS1	24.8a <sup>y</sup>	13.9ab	20.0a	1.6b	3.3b	5.0ab	72.3
TCS1	19.8b	13.7b	21.6a	1.8b	3.3b	4.8b	84.3
TCS2	15.6c	19.6a	28.0a	3.2a	4.9a	7.8a	120.2
S	***	NS	NS	***	*	*	NS

<sup>z</sup>Treatments VAM = vesicular arbuscular mycorrhiza, NTC = non tissue culture, TC = tissue culture, S1 = seedling with <3 tillers; S2 = seedling with ≥3 tillers. <sup>y</sup>Within soil additive and seedling type, the values in each column followed by a different letter are significantly different according to LSD at  $P \leq 0.05$ . NS, \*, \*\*\*Nonsignificant or significant at  $P < 0.05$ , and 0.001, respectively.

**Table 3.** Effects of soil additive and seedling transplant type on plant height, tiller number and fresh rhizome weight per plant for ginger development by month (M) after transplanting to organic field in 2012.

Treatment <sup>z</sup>	Height			Tiller Number (TN)			Yield/Plant
	M0	M3	M4.5	M0	M3	M 4.5	
		cm			TN		g
Soil additive (SA)							
VAM	13.0	18.0	18.8	1.8	5.4	6.4	94.9
Control	11.9	17.5	19.2	2.0	4.7	5.7	98.4
SA	NS	NS	NS	NS	NS	NS	NS
Seedling Type (S)							
NTCS1	13.1ab <sup>y</sup>	17.6	21.1	1.2b	4.4	5.3	98.9
TCS1	15.7a	17.3	17.8	1.5b	5.0	6.1	100.6
TCS2	9.1b	18.4	18.6	3.1a	5.8	6.9	90.4
S	***	NS	NS	***	NS	NS	NS

<sup>z</sup>Treatments VAM = vesicular arbuscular mycorrhiza, NTC = non tissue culture, TC = tissue culture, S1 = seedling with <3 tillers; S2 = seedling with ≥3 tillers. <sup>y</sup>Within soil additive and seedling type, the values in each column followed by a different letter are significantly different according to LSD at  $P \leq 0.05$ . NS, \*\*\*Nonsignificant or significant at  $P < 0.001$ , respectively.

### 3.4. Rhizomes Nutrient Content

In the 2011 high tunnel study, the following 13 macroelements and trace elements were analyzed for the rhizomes; N, S, P, K, Mg, Ca, Na, B, Zn, Mg, Fe, Cu, and Al. The mycorrhiza had no effect on the rhizome content for macroelements and trace elements (Table 4). Except for boron and zinc, transplant seedling type did not affect nutrient content. The NTC transplant derived rhizomes had the highest boron (8.3 mg·kg<sup>-1</sup>) and zinc (36.8 mg·kg<sup>-1</sup>). In Year 2 high tunnel

experiment, calcium level in the control ( $3.0 \text{ g}\cdot\text{kg}^{-1}$ ) was higher than in the mycorrhiza treatments, ( $2.1 \text{ g}\cdot\text{kg}^{-1}$ ) (**Table 5**). In the field study, the boron content of the controls was higher ( $11.4 \text{ mg}\cdot\text{kg}^{-1}$ ) than that of the mycorrhizae treatments ( $9.4 \text{ mg}\cdot\text{kg}^{-1}$ ) (**Table 6**). This nutrient content was also the highest in NTCSI in the 2011 high tunnel study (**Table 4**). In the field the NTCSI transplant types had highest copper and boron levels (**Table 6**). Lead ( $0.5 - 1.1 \text{ mg}\cdot\text{kg}^{-1}$ ) and arsenic ( $0.14 - 1.1 \text{ mg}\cdot\text{kg}^{-1}$ ) (**Table 5** and **Table 6**) analyzed were within acceptable levels of  $10 \text{ mg}\cdot\text{kg}^{-1}$  for Pb and  $3.0 \text{ mg}\cdot\text{kg}^{-1}$  for As, respectively, for consumption per USA FDA guideline, from Gupta *et al.* [33]. Macro and trace element content for nutrient levels, were comparable to those from other ginger studies [34].

**Table 4.** Effects of soil additive and seedling transplant type on nutrient content of organic ginger rhizomes grown in high tunnel in 2011.

Treatment <sup>a</sup>	N	S	P	K	Mg	Ca	Na	B	Zn	Mn	Fe	Cu	Al
	$\text{g}\cdot\text{kg}^{-1}$							$\text{mg}\cdot\text{kg}^{-1}$					
Soil additive (SA)													
VAM	20.6	1.8	3.6	25.6	4.0	2.1	5.0	6.7	29.0	97.2	117.1	8.9	42.9
Control	21.7	1.9	3.6	25.6	4.1	2.2	5.7	7.4	26.7	104.7	118.3	10.0	51.6
SA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Seedling Type (S)													
NTCSI	22.0	1.9	3.8	25.6	4.1	2.3	5.5	8.3a <sup>y</sup>	36.8a	132.3	124.0	9.7	45.5
TCSI	21.5	1.9	3.6	24.5	4.1	2.1	5.1	6.5b	24.3b	92.0	111.0	9.2	44.3
TCS2	20.0	1.8	3.4	26.6	4.2	2.1	5.3	6.3b	22.3b	78.5	118.2	9.5	51.8
S	NS	NS	NS	NS	NS	NS	NS	*	***	NS	NS	NS	NS

<sup>a</sup>Treatments VAM = vesicular arbuscular mycorrhiza, NTC = non tissue culture, TC = tissue culture, S1 = seedling with <3 tillers; S2 = seedling with  $\geq 3$  tillers. <sup>y</sup>Within soil additive and seedling type, the values in each column followed by a different letter are significantly different according to LSD at  $P \leq 0.05$ . NS, \*, \*\*\*Nonsignificant or significant at  $P < 0.05$ , and  $0.001$ , respectively.

**Table 5.** Effects of soil additive and seedling transplant type on nutrient content of organic ginger rhizomes grown in high tunnel in 2012.

Treatment <sup>a</sup>	N	P	K	S	Ca	Mg	Na	Fe	Al	Mn	Cu	Zn	B	As	Pb
	$\text{g}\cdot\text{kg}^{-1}$							$\text{mg}\cdot\text{kg}^{-1}$							
Soil additive (SA)															
VAM	18.5	3.0	25.5	2.5	2.1b <sup>y</sup>	3.5	5.5	109.9	43.6	71.3	6.6	25.5	7.9	0.16	0.95
Control	21.4	3.8	31.8	2.9	3.0a	4.2	7.3	115.6	42.5	97.7	7.4	28.8	9.3	0.26	1.1
SA	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Seedling Type (S)															
NTCSI	20.0	3.4	27.0	2.7	2.4	3.7	5.6	114.3	49.0	102.3	7.3	28.3	8.9	0.14	1.07
TCSI	19.8	3.3	30.6	2.6	2.4	3.7	5.9	104.4	37.8	92.1	6.8	26.7	7.9	0.24	0.92
TCS2	20.2	3.5	28.3	2.8	2.7	4.1	7.6	119.5	44.7	59.0	7.0	26.5	9.1	0.24	1.03
S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

<sup>a</sup>Treatments VAM = vesicular arbuscular mycorrhiza, NTC = non tissue culture, TC = tissue culture, S1 = seedling with <3 tillers; S2 = seedling with  $\geq 3$  tillers. <sup>y</sup>Within soil additive and seedling type, the values in each column followed by a different letter are significantly different according to LSD at  $P \leq 0.05$ . NS, \*Nonsignificant or significant at  $P < 0.05$ , respectively.

**Table 6.** Effects of soil additive and seedling transplant type on nutrient content of organic ginger rhizomes grown in field in 2012.

Treatment <sup>z</sup>	N	P	K	S	Ca	Mg	Na	Fe	Al	Mn	Cu	Zn	B	As	Pb
	g·kg <sup>-1</sup>							mg·kg <sup>-1</sup>							
Soil additive (SA)															
VAM	18.0	4.4	33.3	2.3	2.4	5.4	3.2	124.3	38.7	127.7	7.6	37.2	9.4b <sup>z</sup>	0.9	0.6
Control	19.3	5.3	43.7	2.4	2.7	5.4	5.5	115.4	24.1	139.6	7.5	41.5	11.4a	1.1	0.6
SA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS
Seedling Type (S)															
NTCS1	21.0	5.2	38.9	2.5	3.0	5.7	5.3	126.4	42.3	170.5	10.4a	44.0	12.6a	0.9	0.6
TCS1	18.1	4.8	40.2	2.2	2.3	5.1	3.8	113.9	23.3	127.6	6.3b	35.3	9.3b	0.9	0.5
TCS2	17.0	4.6	36.8	2.3	2.5	5.2	4.0	119.4	28.7	102.7	6.0b	38.7	9.3b	1.1	0.7
S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	**	NS	NS

<sup>a</sup>Treatments VAM = vesicular arbuscular mycorrhiza, NTC = non tissue culture, TC = tissue culture, S1 = seedling with <3 tillers; S2 = seedling with ≥3 tillers. <sup>b</sup>Within soil additive and seedling type, the values in each column followed by a different letter are significantly different according to LSD at P ≤ 0.05. NS, \*, \*\*Nonsignificant or significant at P < 0.05, and 0.01, respectively.

## 4. Conclusion

This study shows that using ginger transplant seedlings in more advanced stage with more tillers, such as TCS2, provides an advantage in supporting more tiller growth over types at lower developmental stages like the TCS1 and NTCS1. However, the use of established soil biological amendments such as mycorrhiza was not effective in enhancing ginger growth in the short season. Introducing ginger production in temperate region, such as the Delmarva Peninsula of the USA, has some limitations where the short growing season, high light intensity outdoors, particularly at transplanting, and periodic summer heat waves may occur. While dieback and regrowth will occur, after exposure to such conditions, the resulting stunting of plant will decrease the potential to produce more rhizomes as indicated in this study. This distress may potentially be averted through shading, a practice used in many ginger producing areas; transplanting earlier, by May 15 when warm temperatures occur and are typically not as high as summer; and or using other practices that will ameliorate temperature build-up in the growing environment. The rhizomes produced from our study had nutrient contents that fell within acceptable levels for them to be safely consumed.

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## Conflicts of Interest

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

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