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Morphological and Physiological Development of Organic Greenhouse Grown Ginger (*Zingiber officinalis*, Rosc) in a Temperate Climate as Influenced by Container and Transplant Origin

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Abstract

Ginger (*Zingiber officinale* Rosc) is a spice produced from underground rhizomes. This makes it necessary to consider the size of its growing area. There is limited information on the phenological development of the plant in containerized greenhouse conditions in temperate regions where natural day-length decreases as the growing season advances. This study determined the effects of container and rhizome sources on ginger shoot growth, chlorophyll concentration, leaf chlorophyll index, transpiration rate, and rhizome yield. Ginger, from non-tissue culture (O1) and tissue culture (O2) origins, were transplanted in a greenhouse in June 2019, 2020 and 2021, and monitored in five container types of different sizes. These were (C1) plastic Supertub (113.2 L), (C2) large Sterilite box (55.3 L), (C3) small Sterilite box (36.7 L), (C4) Husky heavy duty contractor plastic clean up bags (26.3 L) and (C5) Root Trapper Grounder Squat bag (27.9 L). The results did not show consistent trends for the effects of the respective size and origin combinations on most of the morphological characteristics, and all the physiological characteristics evaluated. Increasing container size increased the shoot biomass in all studies and increased fresh rhizome yield in two of three studies in the greenhouse. The effect of transplant origin was inconclusive, with a tissue culture advantage one year and no effect the other year. During the first 5 months after transplanting, the morphological development of tillers and height increased. Leaf chlorophyll index, chlorophyll concentration and stomatal conductance varied across sampling months, and within container and rhizome origin at individual sampling dates. The development of the plants in a greenhouse with decreasing natural day length posed a challenge as some plants senesced within 5 months after transplanting. Further opportunities to arrest senescence and extend growth should be introduced as another approach to extend

growth and increase rhizome yield.

Keywords

Container, Transplant Origin, Organic Agriculture

1. Introduction

Ginger, *Zingiber officinale* is an economically important plant grown for a variety of uses, especially for its medicinal and flavoring potentials [1] [2]. The active ingredients in its rhizomes include phytochemicals with reported digestive, antioxidant, and anti-inflammatory properties [1] [2] [3]. The plant is indigenous to Southeastern Asia which has warm tropical climates with long growing periods. The USA is the biggest importer of ginger rhizomes valued at around US$ 105 million yearly, taking 14% of the global market share according to 2022 data [4]. However, there is consumer interest in growing organic ginger locally to meet the demand of the growing organic industry.

In tropical climates, the plant is generally propagated by rhizome pieces grown in the field. Some research has also been done to test conventionally produced rhizome potential in protected structures like greenhouses and rain shelters to produce disease clean ginger [5] [6]. There is limited information on ginger production in subtropical and temperate areas in USA. It is hardy only in USDA Zones 8 - 12 [7]. Minimum temperature for its growth is 13˚C [8]. From one subtropical region, a report [9] on growing edible ginger as a greenhouse crop, indicated that the plants grown under natural short days showed considerable yellowing and initiation of dormancy by harvest in January. In colder locations below USDA zone 8, where the season is too short for the rhizomes to mature, ginger can be grown in containers and moved indoors before frost [7]. In a short season temperate environment with decreasing natural day length, such as the Delmarva Peninsula of the USA, organic ginger can be grown in the high tunnel [10] [11], but limited information is available on its phenological development in different containerized growing structures in protected environment. Growing ginger in pot culture can enable the production of clean disease-free edible ginger [6]. Additionally, containers in protected structures such as greenhouses and high tunnels can serve as alternate growing spaces when open land area is limited, or there is the need to use an extended growing season.

Ginger can also be produced from seedling transplants. These may be of tissue culture origin or non-tissue cultured. Tissue culture of apical bud segments has been an established method to produce clean seedling explants in ginger [12] [13] [14]. Tissue culture and non-tissue cultured ginger when grown in pots in nonorganic management did not differ in yield [15]. A similar trend was reported for organic and non-organic ginger produced from transplants with less than three tillers and grown directly in the soil in high tunnel [10]. However, there are no reports on how tissue culture derived and non-tissue culture trans-

plants perform in organic management when grown in containers in protected structures in temperate climate.

Information on the morphological and physiological characteristics of organic produced ginger under containerized conditions is lacking. According to a meta-analysis of the effects of rooting volume on plant growth, on average, a doubling of the pot size increased biomass production by 43% [16]; however, the analysis did not include ginger. Under long-day conditions in a greenhouse, large containers, (16-gallon) worked best for non-organic ginger production compared to 2-gallon pots [15]. While it is not known if increased pot size will accelerate morphological development of the plant, one report [17] indicates that early foliage development and high yield capacity to channel dry matter into the rhizomes are included in the physiological attributes for high yield in ginger varieties. Ginger grows slowly; the growth period takes more than 135 to 150 days from sprouting [18]. Moreover, there has been no progress in developing ginger that can grow rapidly to produce rhizomes. The plant does not produce seeds [1]. Recent allele research may provide the future opportunity for genome editing in ginger to improve this trait [19]. However, gene editing technology cannot be applied to prompt the growth of organic ginger because genetic engineering is prohibited in plants used for certified organic production [20].

When growing ginger in containers in decreasing day length environment, factors such as temperature and photoperiod can impact its growth. The favorable range for its growth is 19˚C - 28˚C [8]. It is a quantitative short-day plant for flowering and rhizome swelling whereas its vegetative growth is promoted by a longer light period up to a certain limit [21] [22]. Ginger plants grown at 12-hour photoperiod did not become dormant, and produced the highest fresh rhizome yield in a study that included 10-hour and 14-hour photoperiods [22]. Therefore, the objective was to determine the effects of container size and plant origin on morphological and physiological characteristics of greenhouse grown ginger in protected structures in a temperate location with decreasing day length. The hypothesis was that the size of the container and the rhizome origin would affect the morphological and physiological characteristics of the ginger plant as it developed in the containers in the greenhouse.

2. Materials and Methods

2.1. Experimental Sites

All greenhouse experiments were conducted at the University of Maryland Eastern Shore Agriculture Experiment Station located in Princess Anne, MD., U.S.A. (38˚12'N 75˚42'W; (USA climatic zone 7) [23], following National Organic Program guidelines [20].

2.2. Year 1 Greenhouse Study

Ginger seedling transplants of two origins; non-tissue culture (O1) and tissue culture (O2)/7th generation rhizomes harvested from tissue cultured plants were

planted in the University of Maryland Eastern Shore polycarbonate greenhouse on June 3, 2019. Four container sizes of plastic tubs, boxes and heavy-duty bags were used (Table 1). Containers of different volumes were (C1) black plastic super tub (113.2 L), (C2) Large Clear Sterilite Box (55.3 L, (C3) Small Clear Sterilite Box (36.7 L, and (C4) Husky heavy duty contractor plastic clean up bags (26.3 L and 50.8 μm thick). Eight, seven and five holes were bored equidistantly in each bottom of the tubs, large plastic and small plastic boxes, respectively. Due to the thinness of the bags, to maintain their holding capacity no holes were bored in them. The growing media was (Sungro Sunshine Mix #1 Organic Planting Mix, Sungro Horticulture, Agawan, Mass., U.S.A. Seedlings were at the one to two leaf stage and 15 - 25 cm tall and spaced 15 cm apart to ensure plant rhizomes having enough room to expand. Commercial transplant stage is 20 cm in height for ginger [24]. Developmentally, this seedling stage precedes the three-fork tiller stage, and is the first phase of the grand growth phase of ginger as defined by Xizhen *et al.* [18]. The experimental design was a split plot with container as main plot and transplant origin as subplot and with three replications.

Plants were fertilized with Organic Materials Review Institute (OMRI)-certified fertilizers Nature Safe (13-0-0) (Darling Ingredients Inc., Nature Safe, Cold Spring, KY, USA), Ultra Fines Sulfate of Potash (0-0-50) (Diamond K Gypsum, Richfield, UT, USA) and Phyta-Grow Bone Meal (4-14-0) (California Organic Fertilizers, Inc., Hanford, CA, USA) at rates of 80 lbs. nitrogen/acre, 160 lbs. potassium/acre, and 40 lbs./acre phosphorus, respectively on 6/20/19, and 8/23/19 during the growing period. Growth and physiological characteristics were monitored monthly from 1 - 6 months after transplanting. Data were collected on shoot height (from the base of the pseudo stem to the top of the stem) and tiller number (number of aerial shoots arising around each plant), chlorophyll concentration, leaf chlorophyll index (LCI), stomatal conductance, and rhizome yield. Chlorophyll concentration, leaf chlorophyll index, and stomatal conductance were measured with MC-100 chlorophyll meter, manufactured by Apogee Instruments, Inc., Logan, UT, USA; Spectrum Technologies, Inc. SPAD 502 chlorophyll meter manufactured by Spectrum Technologies, Incorporated, Aurora, IL, and SC-1

Table 1. Container descriptions for ginger growth.

Containers	Description	Dimensions (cm)			Volume (L)
		L	W/Diameter	H	
C1	Black Supertub	91.4[Z]	61.0	20.3	113.2
C2	Large Clear Sterilite Box	65.4	46.7	18.1	55.3
C3	Small Clear Sterilite Box	58.4	41.3	15.2	36.7
C4	Husky heavy duty contractor clean up bags—(cylinder)		40.6[y]	20.3	26.3
C5	Trapper Grounder Squat bag with knit fabric base—(cylinder)		35.6	27.9	27.8

[Z]Dimensions based on manufacturers measurements except for Husky heavy duty bag; [y]Measurements in column for width represent diameter for Husky heavy duty bag and trapper grounder bag.

Leaf Porometer manufactured by Decagon Devices, Inc., Pullman, WA, USA, respectively. Chlorophyll content, LCI measurements and stomatal conductance were taken from fully expanded healthy green leaves. All growth and physiological measurements were collected from five samples per treatment combination per replication.

2.3. Year 2 and 3 Greenhouse Study

In Years 2 and 3, follow up studies were conducted on June 30, 2020, and June 17, 2021, respectively. In Year 2, a fifth container, a 27.9 L Root Trapper Grounder Squat bag from Backyard RootMaker® Pro, Huntsville, AL 35815 was added (Table 1) and O2 was the 8th generation derived rhizomes from the previous year's study. The design was similar to that of Year 1 and with three replications. In Year 3, the heavy duty bags were omitted due to their thinness and low rhizome yield, and the tissue culture derived material was no longer available from the previous year. The reason was that the rhizomes were disposed of because cadmium levels were above the permissible 0.3 mg/kg due to exposure to a contaminated water source. For Year 3 the design was a completely random design with containers as treatment and with three replications.

2.4. Ambient Temperature Monitoring

Air temperatures during the growing season were monitored and recorded using a Spectrum 1000 Series Watchdog Micro Station (Spectrum Technologies, Inc., Aurora, IL, USA). In 2019 maximum and minimum temperatures ranged from 40.6˚C (7/21) to 19.5˚C (10/20) and 28.3˚C (7/21) to 7.3˚C (11/4), respectively. In 2021 maximum and minimum temperatures were from 40.3˚C (8/13) to 25.8˚C (10/17), and 27.6˚C (7/13) to 19.2˚C (10/26), respectively. Natural day length (14.4 to 14.5 hours) at planting decreased to below 10 hours, (9.4 - 9.5 hours) at harvest at the end of the study.

2.5. Experimental Design and Statistical Analysis

Two-way and one-way analyses of variance (ANOVA) were used to analyze the data using the SAS program (version 9.1, SAS Institute Inc., Cary, N.C., U.S.A.). When analyses over sampling dates were significant, data were analyzed by sampling dates. 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 Greenhouse Study

In general, shoot height increased up to M4, then decreased at M5 as plant senesced, and some tillers died off (Table 2 and Table 3). However, there were no consistent trends in the effects of container size and rhizome origin. Generally, the tissue cultured plants were taller than non-tissue culture plants at M1 for all

Table 2. Effects of container and rhizome origin on plant height and chlorophyll index from 1 - 5 months (M) after planting in June 2019.

Container (C)	Rhizome Origin (O)	Height					Leaf Chlorophyll Index (LCI)		
		M1	M2	M3	M4	M5	M3	M4	M5
				cm				LCI	
C1	**O1**	22.3bcd	34.2c	55.4	94.4a	86.2a	52.7a	42.5d	44.7ab
C1	**O2**	25.7abc	32.8c	51.7	73.7c	58.0c	53.0a	51.0ab	42.5ab
C2	**O1**	19.7cd	47.0a	55.8	83.4abc	74.6abc	49.6bc	53.0a	42.7ab
C2	**O2**	30.0ab	35.9bc	55.9	89.1ab	82.7ab	49.9bc	48.2bc	45.1ab
C3	**O1**	19.4cd	34.3c	50.6	74.2c	67.0bc	49.5bc	49.3abc	41.9b
C3	**O2**	35.4a	45.3ab	48.1	78.9bc	73.3abc	49.1c	46.2cd	45.9a
C4	**O1**	15.8d	21.8d	47.5	70.8c	62.9c	50.5b	45.6cd	45.7a
C4	**O2**	32.3a	39.5abc	56.8	90.0ab	85.6a	49.4bc	51.9ab	41.6b
SE		3.2	3.6	4.7	4.3	5.8	0.38	1.47	1.20
C		NS	*	NS	NS	NS	*	NS	NS
O		**	NS	NS	NS	NS	NS	NS	NS
C X O		NS	**	NS	**	**	NS	***	**

Means with different lower case letters are significantly different for the given month after transplanting (M) according to T adjusted multiple comparison tests. O1 = non-tissue culture, O2 = tissue culture origins, C1, 2, 3, 4, and 5 = 113.2, 55.3, 36.7, 26.3 L and 27.8 L volumes, respectively. NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, and 0.001, respectively.

Table 3. Effects of container and rhizome origin on tiller number, chlorophyll concentration and stomatal conductance of greenhouse ginger from 1 - 5 months (M) after planting in June 2019 and on rhizome yield and biomass.

Variable[z]	Tiller Number (TN)/Plant					Chlorophyll concentration			Stomatal conductance			Rhizome wt.	Fresh Biomass	Dry Biomass
	M1	M2	M3	M4	M5	M3	M4	M5	M3	M4	M5			
			TN			$\mu mol \cdot m^{-2}$			$mmol \cdot m^{-2} \cdot s^{-1}$			g	g	g
Container size (C)														
C1	2.0	3.1	4.6	6.1	5.0	472.5a	392.6	443.0	163.0a	195.7ab	113.7	187.1	30.6a	16.2
C2	2.1	3.5	4.8	6.0	4.9	451.2ab	441.6	438.6	108.4b	137.0b	96.5	220.4	17.4b	14.8
C3	2.6	3.6	5.0	6.5	4.9	419.9bc	393.3	385.3	164.9a	162.6ab	71.0	192.6	15.5b	13.2
C4	2.2	3.0	5.1	5.4	5.0	411.8c	418.3	429.8	157.7ab	211.9a	102.8	170.1	16.4b	12.5
SE	0.20	0.32	0.49	0.50	0.39	13.06	20.93	20.62	18.0	24.30	19.7	20.1	3.3	1.5
Rhizome origin (O)														
O1	1.8b	3.2	4.9	6.1	5.1	452.0	414.6	429.4	178.9a	183.9	105.2	156.2b	16.9	11.8b
O2	2.7a	3.4	4.8	5.9	4.8	425.8	408.3	419.0	118.0b	169.7	86.8	228.9a	23.0	16.6a
SE	0.14	0.22	0.34	0.36	0.27	9.2	14.8	14.58	12.7	17.18	13.93	14.2	2.4	1.1
C	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	*	NS
O	***	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	**	NS	**
C X O	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Within Container size and Rhizome origin, the values in each column followed by a different letter are significantly different according to Fisher LSD at $P \leq 0.05$ O1 = non-tissue culture, O2 = tissue culture origins, C1, 2, 3, 4, and 5 = 113.2, 55.3, 36.7, 26.3 L and 27.8 L volumes , respectively. M = Months after Transplanting. NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, and 0.001, respectively.

container sizes; this was also true for all sampling dates for the smallest container, C4. At M2, plants of both origins in C1 had a similar height while those within the other container types differed by origin. At M4, the non-tissue culture plants in the largest containers were the tallest, and the tissue cultured was among the shortest; however, tissue cultured ones in the smallest container, C4, were the tallest while the non-tissue cultured were among the shortest.

Tiller number had no significant interactions (**Table 3**). Container size had no effect, and rhizome origin had limited effect on tiller number except that those tillers of tissue cultured rhizomes at M1 were greater than non-tissue culture derived ones (**Table 3**). The number of tillers increased up to 4 months after planting (10/10). Thereafter, they decreased as the leaves turned yellow, stems senesced, lodged, and died off. Similar results in ginger leaf yellowing have been reported under the natural short day [9]. At M4, day length was decreasing from 11 to 10 hours, and based on previous work, this shortening day length decreases vegetative growth such as tillering while promoting rhizome swelling [21] [22].

Leaf chlorophyll index (LCI) is used as relative measure of chlorophyll concentration [25], which is often correlated with leaf N status and photosynthetic activity [26] [27]. Leaf chlorophyll index fluctuated within month sampling, showing differences among some container x origin combination, but no set pattern (**Table 2**). One exception was at M3 where it was significantly higher for the plants in the largest container compared to those in the two smaller containers. Throughout the sampling period, LCI values ranged from 41.6 to 53.0 and were within the range of acceptable plant health for ginger suggested minimum of 40 for good ginger growth as cited by Li *et al.* [28].

Leaf chlorophyll concentration had no significant interactions. Within each sampling month, for the three to five-month period following transplanting the content was generally similar, except for the plants in the largest container trending or having significantly higher values (472.5 μmol·m^{-2}) at M3 than those in the smaller containers (411.8 μmol·m^{-2}) (**Table 3**). Leaf chlorophyll concentration ranged from 385.3 for C3 at M5 to 472.5 μmol·m^{-2} for C1 at M3.

Stomatal conductance (mmol·m^{-2}·s^{-1}) measured by a porometer is the rate of CO_2 entering, or water vapor exiting through stomata. Stomatal conductance within sampling date was generally not affected by container and rhizome origin, except that non-tissue culture plants at M3 had higher rates than the tissue cultured ones, the C1 and C3 plants had greater rates than C2 at M3; and C4 was higher (211.9 mmol·m^{-2}·s^{-1}) than C2 (137.0 mmol·m^{-2}·s^{-1}) at M4 (**Table 3**). Conductance varied across sampling dates with a decline at M5. At this date there were no significant responses to container or origin, and conductance ranged from 113.7 for C1 to 71.0 mmol·m^{-2}·s^{-1} for C3. The range of stomatal conductance rates in our study seems comparable to values obtained for other ginger studies. Control ginger plants grown under adequate conditions in one study had just above 200 mmol·m^{-2}·s^{-1} while abscisic acid treated leaves had values that ranged from less than 50 to 150 mmol·m^{-2}·s^{-1} [29].

Tissue cultured plants produced higher rhizome fresh weight per plant and shoot dry biomass/plant than the non-tissue culture plants in 2019. Per plant fresh shoot biomass was highest in the largest container (Table 3). Contrary to expectations, the rhizome weight in the largest containers was not the highest and not statistically different from the other sizes.

Overall, for Year 1, tiller and height increased up to M4 after transplanting, the physiological data of chlorophyll concentration, leaf chlorophyll concentration and stomatal conductance varied across sampling dates and there were no consistent effects of container and origin at each sampling date, except that tissue culture plants were taller and had more tillers at M1.

3.2. Year 2 Greenhouse Study

In 2020 study, height rapidly increased from M1 to M2, thereafter the plants grew slowly up to M4, then decreased (Table 4). As in the 2019 study, where the height decreased at M5 (Table 3), some heights were also decreased. Container X origin were significant for plant height at most of the sampling months (Table 4). For M2 to M4, the plants in the largest container C1, and the non-tissue culture plants C2O1 and the tissue culture plants C5O2 in the root maker container, had the tallest plants. Plants in the plastic bags, C4 and in C3, the smallest rectangular container, had lowest heights.

Table 4. Effects of container and rhizome origin on plant height and tiller number of greenhouse ginger from 1 - 5 months (M) after planting in June 2020 (PD June 30, 2020).

Container	Rhizome Origin	Height					Tiller Number (TN)/Plant				
		M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
		cm					TN				
C1	O1	46.6bc	77.7ab	79.9a	81.5a	84.1	2.6d	3.7b	4.2b	4.8bc	4.4bc
C1	O2	53.0abc	78.9a	80.6a	81.5a	75.0	3.3bc	4.9a	6.2a	6.7a	6.1a
C2	O1	61.9a	77.7ab	77.9ab	79.0ab	70.4	4.1abc	4.6ab	4.6b	4.7bc	4.4bc
C2	O2	42.5bcd	65.9c	67.4bc	69.9bc	74.5	3.7abc	4.3ab	4.5b	5.0bc	4.7bc
C3	O1	40.9cd	62.0cd	63.1c	64.5c	71.3	3.9ab	4.7ab	4.9ab	5.1bc	4.3bc
C3	O2	32.9d	52.4d	61.7c	63.7c	64.8	3.3bc	4.1ab	4.6b	4.8bc	4.6bc
C4	O1	44.9bcd	65.2c	65.4c	66.7c	69.2	3.6abc	4.6ab	4.9ab	5.2b	5.2b
C4	O2	47.7bc	66.2bc	66.9c	68.7c	67.2	3.6abc	4.2ab	4.2b	4.7bc	4.1c
C5	O1	46.9bc	60.0cd	60.4c	63.6c	65.2	4.2a	4.5ab	4.5b	4.7bc	4.3c
C5	O2	53.9ab	80.1a	80.7a	81.7a	75.0	3.2cd	3.7b	3.7b	4.0c	3.9c
SE		4.6	3.9	3.6	3.2	5.0	0.2	0.4	0.5	0.4	0.30
C		*	***	***	***	NS	**	NS	NS	*	*
O		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C X O		*	***	**	**	NS	**	NS	*	*	**

Means with different lower case letters are significantly different for the given month after transplanting (M) according to T adjusted multiple comparison tests. O1 = non-tissue culture, O2 = tissue culture origins, C1, 2, 3, 4, and 5 = 113.2, 55.3, 36.7, 26.3 L and 27.8 L volumes, respectively. NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, and 0.001, respectively.

Tiller numbers were influenced by sampling dates, particularly the increase between M1 and M2. However, tiller growth across the sampling dates M2 to M5 was generally slow. At M5 the values were the same as M4 or trended lower due to abscission of stems from lodging. This trend in tiller decrease at M5 was also observed in the 2019 study (Table 3), where the plants senesced, and tillers lodged and died. The number for the tissue cultured, C1O2 at M4 was among the highest (6.7) as was plant height (Table 4). Within sampling months, most of the C × O interactions were significant. Generally, the lowest tiller numbers tended to be for C5O2, Rootmaker bags; this is in contrast to the highest shoot heights recorded for this container x origin combination (Table 4).

Leaf chlorophyll concentration varied across sampling dates and ranged from 430.9 μmol·m^{-2} for C4O1 at M3 to 247.6 μmol·m^{-2} for C1O2 at M6 (Table 5). Within the sampling months after transplanting, there was little to no effect of container and origin on chlorophyll concentration, except at M4 where C4O1, (353.8 μmol·m^{-2}) and C5O2 (352.4 μmol·m^{-2}) plants had higher levels than the C2O1 (234.4 μmol·m^{-2}), C4O2 (263.3 μmol·m^{-2}) and the C1 plants, respectively. The C4O1 had the highest values at each sampling month.

Table 5. Effects of container and rhizome origin on chlorophyll concentration and stomatal conductance of greenhouse ginger from 1 - 5 months (M) after planting in June 2020.

Container (C)	Rhizome Origin (O)	Chlorophyll concentration				Stomatal Conductance			
		M3	M4	M5	M6	M3	M4	M5	M6
		μmol·m^{-2}				mmol·m^{-2}·s^{-1}			
C1	O1	326.0b	278.6bc	320.8abc	362.1	270.3a	155.0bc	216.4a	120.4ab
C1	O2	328.4b	278.9bc	312.5bc	247.6	188.7bc	203.1bc	184.4abc	130.2ab
C2	O1	358.1ab	234.4c	276.1c	279.8	130.5cd	187.9bc	155.5bcd	123.9ab
C2	O2	383.3ab	303.6abc	326.6abc	281.0	221.4ab	345.4a	200.4ab	122.9ab
C3	O1	321.6b	291.6abc	338.8abc	294.6	118.1cd	149.1bc	117.1de	169.5a
C3	O2	343.8ab	313.3ab	316.5abc	316.6	133.8cd	213.2bc	183.3abc	182.7a
C4	O1	430.9a	353.8a	405.7a	387.7	114.2d	228.5b	140.6cde	148.7ab
C4	O2	398.7ab	263.3c	290.6c	280.0	141.9cd	161.5bc	86.0e	69.7b
C5	O1	376.4ab	283.3abc	371.6ab	369.9	113.0d	134.6c	140.3cde	135.1ab
C5	O2	368.6ab	352.4a	378.3a	328.6	126.5cd	226.9b	174.9abcd	148.1ab
SE		31.9	25.0	27.7	43.3	23.6	27.9	19.8	31.6
C		NS	NS	NS	NS	***	*	**	NS
O		NS	NS	NS	NS	NS	**	NS	NS
C X O		NS	*	NS	NS	*	**	*	NS

Means with different lower case letters are significantly different for the given month after transplanting (M) according to T adjusted multiple comparison tests. O1 = non-tissue culture, O2 = tissue culture origins, C1, 2, 3, 4, and 5 = 113.2, 55.3, 36.7, 26.3 L and 27.8 L volumes, respectively. NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, and 0.001, respectively.

Stomatal conductance had significant container × origin interactions at each sampling month, except M6 (Table 5). At M3, C1O1 was highest (270.3 mmol $m^{-2}·s^{-1}$) and this was significantly greater than the values for plants in the smaller containers, C3, C4, and C5. At M4, the C2O2 was highest and different from all the other container x origin combinations. At M5, highest conductance (216.41 mmol $m^{-2}·s^{-1}$) was for C1O1, and lowest (86.0 mmol $m^{-2}·s^{-1}$) was for C4O2. At M6 all combinations were similar, except that C4O2 plants had significantly lower (69.7 mmol $m^{-2}·s^{-1}$) rates than those of C3O1 (169.5 mmol $m^{-2}·s^{-1}$) and C3O2 (182.7 mmol $m^{-2}·s^{-1}$).

Leaf chlorophyll index was generally higher for the first 3 months after transplanting than the latter three months (Table 6). For M1, M2 and M3 most of the LCI values were above 40, then trended down to a low of 29.5 for Origin 2 in M6. At that time many of the plants were senescing. Throughout the period, M1-M5, container had a significant effect on the LCI values. While there were differences in the LCI at each sampling date, there was no consistent trend in the pattern of container differences at each month. Leaf chlorophyll index was generally not affected by transplant origin, except at M2 and M3 when the tissue cultured ones were of higher value than the non-tissue culture ones.

Table 6. Effects of container and rhizome origin on leaf chlorophyll index, fresh rhizome yield, and dry shoot biomass per plant of greenhouse ginger after planting in June 2020.

Variable [z]	Leaf Chlorophyll Index (LCI)						Rhizome wt.	Dry Biomass
	M1	M2	M3	M4	M5	M6		
	LCI						g	g
Container size (C)								
C1	43.5a	42.5ab	45.6a	34.5a	31.1c	30.3	176.0a	14.0a
C2	44.3a	40.7ab	45.6a	26.8b	33.0bc	29.9	110.3b	8.8b
C3	44.4a	40.0bc	38.6d	36.0a	37.0abc	30.3	72.2c	4.0c
C4	37.7b	44.3a	40.7c	32.5a	37.7ab	29.4	86.4bc	6.5bc
C5	44.8a	36.8c	43.6b	35.7a	40.0a	35.8	103.7b	5.4bc
SE	1.5	1.3	0.5	1.3	2.0	3.9	10.1	1.2
Origin (O)								
O1	41.7	39.3b	42.2b	33.7	36.2	32.8	114.7	7.7
O2	44.2	42.4a	43.4a	32.4	35.3	29.5	104.7	7.9
SE	0.9	0.8	0.3	0.8	1.3	2.4	6.4	0.8
C	*	**	***	***	*	NS	***	***
O	NS	*	*	NS	NS	NS	NS	NS
C × O	NS	NS	NS	NS	NS	NS	*	***

[z]Within Container size and Rhizome origin, the values in each column followed by a different letter are significantly different according to Fishers LSD at P.05. O1 = non-tissue culture, O2 = tissue culture origins, C1, 2, 3, 4, and 5 = 113.2, 55.3, 36.7, 26.3 L and 27.8 L volumes, respectively. M = Months after Transplanting. NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, and 0.001, respectively.

Table 7. Effect of Container Size on Growth characteristics of greenhouse ginger in 2021 greenhouse study.

Variable[z]	Chlorophyll concentration		Leaf Chlorophyll Index (LCI)		Tiller Number (TN)/Plant		Ht		Stomatal conductance		Fresh Biomass Wt./plant	Dry Biomass Wt./plant	Rhizome wt./plt
	μmol·m^2		LCI		TN		cm		mmol·m^{-2}·s^{-1}		g	g	g
	M4	M5	M4	M5	M4	M5	M4	M5	M4	M5	M5	M5	M5
C1	308.9a	297.7a	39.2a	39.2a	9.3a	7.3a	97.7a	86.5a	189.5	127.7	54.0a	35.5a	349.7a
C2	302.7a	254.3ab	33.6b	33.9ab	7.2b	6.7ab	89.2b	88.1a	163.6	114.6	23.2b	20.8b	251.5b
C3	276.5ab	208.9b	32.7b	24.5c	6.bc	6.0bc	78.0c	63.7b	158.7	97.8	17.7c	16.0c	169.6c
C5	258.2b	206.8b	33.0b	32.2b	5.7c	5.0c	65.8d	51.5b	133.5	117.2	8.7d	8.7d	113.2d
SE	16.0	16.0	1.4	1.4	0.4	0.4	4.0	4.0	20.3	20.3	2.7	1.4	12.8

[z]Means in each column followed by a different letter are significantly different according to Fisher LSD at P ≤ 0.05. O1 = non-tissue culture, O2 = tissue culture origins, C1, 2, 3, 4, and 5 = 113.2, 55.3, 36.7, 26.3L and 27.8L volumes, respectively. M = months after transplanting. NS, *, **, *** Nonsignificant or significant at P < 0.05, 0.01, and 0.001, respectively.

Rhizome weight and shoot biomass had significant C X O interaction. These weights were highest in the largest container, C1 and significantly greater than C2. While the small rectangular C3 containers produced lowest rhizome fresh weight and biomass, those weights did not differ among the C3, C4 and C5 for biomass, or the C3 and C4 for rhizome weight. Transplant origin did not affect rhizome weight or shoot biomass.

3.3. Year 3 Greenhouse Study

In 2021 greenhouse ginger, the growth and physiological characteristics measured, except for stomatal conductance, trended or were higher in the two largest containers than in the small pan and the Rootmaker container (Table 7). All measurements, declined by M5, or were similar to the previous month after transplanting. By M5 some leaves were turning yellow and some tillers were lodging or abscising. The ranges in the characteristics measured from M4 to M5 were as follows; chlorophyll concentration from 308.9 for C1 to 206.8 μmol·m^{-2} for C5, LCI from 39.2 for C1 to 24.5 for C3, tiller number from to 9.3 for C1 to 5.0 for C5, height from 97.7 cm for C1 to 51.5 cm for C5, and stomatal conductance from 189.5 for C1 to 97.8 mmol·m^{-2}·s^{-1} for C3.

Biomass and rhizome weight significantly increased as container size increased. This trend is in agreement with previous meta-analysis that increasing pot size of plants increased biomass [16].

4. Conclusion

In general, the results did not show consistent trends for the effects of the respective size and origin combinations on most of the morphological characteristics, and all the physiological measurements. For the rhizome yield and biomass characteristics, these findings partially support our hypothesis that increased volume will increase the yield and biomass of organic ginger in the greenhouse.

While biomass consistently increased in the larger containers, rhizome fresh yield was unaffected in year 1 and consistently increased with pot size in years 2 and 3. However, the effect of the tissue culture origin versus the non-tissue culture origin is inconclusive. Tissue cultured plants produced higher rhizome fresh weight and shoot dry biomass than the non-tissue culture plants in 2019, but did not have an effect in year 2. The tissue cultured materials were several generations removed from the first culture and this may have affected their inconsistent yield performance. During the first 5 months after transplanting, the morphological development of tillers and height increased; the physiological parameters of leaf chlorophyll index, chlorophyll concentration and stomatal conductance varied across sampling months, and within container and rhizome origin at individual sampling dates. The continued development of the plants in a greenhouse with decreasing day length during the active growth posed a challenge as plants senesced within 5 months after transplanting. Further opportunities to arrest senescence and extend growth are another approach to increase the rhizome yield. One recent report [30] suggests that interrupting the dark period with light can prolong ginger growth. Therefore, for future studies on growing ginger in temperate areas in a greenhouse with decreasing natural day length, consideration may be given to the inclusion of light to interrupt the short days.

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Conflicts of Interest

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

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