



# Fruit and Vegetable

## 2020 ANNUAL RESEARCH REPORT







# 2020 Fruit and Vegetable Crops Research Report

Edited by Rachel Rudolph

## AUTHORS:

### Horticulture

#### Faculty

Doug Archbold  
Winston Dunwell  
Rachel Rudolph  
John Snyder  
John Strang

#### Area Extension Associates

Daniel Becker, Princeton, West Kentucky (fruits and vegetables)  
Chris Smigell, Lexington, Central Kentucky (fruits and vegetables)

#### Professional Staff

Ginny Travis  
Dwight Wolfe  
Benjamin Yates

#### Graduate Students

Mohammad Dawood

### Plant Pathology

#### Faculty

Emily Pfeufer

### Dietetics and Human Nutrition

#### Faculty

Bob Perry

#### Extension Associate

Emily DeWitt

### Kentucky State University

College of Agriculture Communities and Environment

#### Faculty

George F. Antonious  
Kirk Pomper

#### Professional Staff

Sheri Crabtree  
Jeremiah Lowe  
Eric Turley

### University of Arkansas

John Clark

**Cover:** Strawberry grown at the University of Kentucky College of Agriculture, Food and Environment's Horticultural Research Farm in Lexington, Kentucky.

Photographer: Steve Patton

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## Important Note to Readers

The majority of research reports in this volume do not include treatments with experimental pesticides. It should be understood that any experimental pesticide must first be labeled for the crop in question before it can be used by growers, regardless of how it might have been used in research trials. The most recent product label is the final authority concerning application rates, precautions, harvest intervals, and other relevant information. Contact your county's Cooperative Extension office if you need assistance in interpreting pesticide labels.

This is a progress report and may not reflect exactly the final outcome of ongoing projects. Please do not reproduce project reports for distribution without permission of the authors.



# The 2020 Fruit and Vegetable Crops Research Program

*Rachel Rudolph, Horticulture*

This year has been especially challenging for all of us in dealing with the global pandemic. Kentucky fruit and vegetable growers faced new obstacles with production and labor while following guidelines to maintain health and safety. Despite these unprecedented disruptions to our normal way of life, Kentucky produce cash receipts increased in 2020 and there was substantial direct market growth. Growers reported record sales at farmers markets and gross sales at produce auctions increased. As fruit and vegetable production continues to show sustained growth in Kentucky, the need remains for applied practical research to support the industry. The 2020 Fruit and Vegetable Crops research report includes results from 12 different projects. Many projects were conducted on research farms while others were conducted on commercial farms with the assistance of grower-cooperators. Research was conducted by University of Kentucky faculty and staff from the Horticulture, Plant Pathology, and Dietetics and Human Nutrition departments as well as faculty and staff of Kentucky State University.

The variety trials in this year's publication include primocane-fruiting blackberries, matted-row strawberries, pie pumpkins, miniature pumpkins, and high tunnel broccoli. Evaluation of varieties is a continuing necessity and allows us to provide current information to growers across the state about the production and performance of various crops. The vegetable variety trial results are the basis for updating the recommendations in our Vegetable Production Guide for Commercial Growers (ID-36). These updates are not based solely on one season's data or location. It is necessary to trial varieties in multiple seasons and if at all possible, multiple locations. We may also collaborate with researchers in surrounding states such as Ohio, Indiana, and Tennessee to discuss results of variety trials they have conducted. The results presented in this publication often reflect a single year of data at a limited number of locations. Although some varieties perform well across Kentucky year after year, others may not. Additional research includes the effect of container and substrate composition on the productivity and growth of 'Duke' highbush blueberry, rootstock effects on apple tree growth and yield, zingiberene content of interspecific hybrid tomatoes grown in the open field, tree tube effects on early-planted paw paw, tomato grafting effects on tomato yield under root-knot nematode pressure, the impact of biochar on soil enzyme activity, and the mobility of sewage sludge trace metals. Below are guidelines for interpreting the results of fruit and vegetable variety trials.

## Our Yields vs. Your Yields

Yields reported in variety trial results are often extrapolated from small plots. Depending on the crop, individual plots range from 1 to 200 plants. Our yields are calculated by multiplying the yields in these small plots by correction factors to estimate per-acre yield. For example, if you can plant 4,200 tomato plants per acre (assuming 18-inch in-row spacing) and our trials only

have 10 plants per plot, we must multiply our average plot yields by a factor of 420 to calculate per-acre yields. Thus, small errors can be greatly amplified. Due to the availability of labor, research plots may be harvested more often than would be economically possible. Keep this in mind when reviewing the research papers in this publication.

## Statistics

Often yield or quality data will be presented in tables followed by a series of letters (a, ab, bc, etc.). These letters indicate whether the yields of the varieties are statistically different. Two varieties may have average yields that are numerically different, but statistically are the same. For example, if tomato variety 1 has an average yield of 2,000 boxes per acre, and tomato variety 2 yields 2,300 boxes per acre, one would assume that variety 2 had a greater yield. However, just because the two varieties had different average yields does not mean that they are statistically or significantly different. In the tomato example, variety 1 may have consisted of four plots with yields of 1,800, 1,900, 2,200, and 2,100 boxes per acre. The average yield would then be 2,000 boxes per acre. Tomato variety 2 may have had four plots with yields of 1,700, 2,500, 2,800, and 2,200 boxes per acre. The four plots together would average 2,300 boxes per acre. The tomato varieties have plots with yield averages that overlap and, therefore, would not be considered statistically different, even though the average per acre yields for the two varieties appear to be quite different. This example also demonstrates variability. Good varieties are those that not only yield well, but also yield consistently. Tomato variety 2 may have had yields similar to variety 1, but it also much greater variation. Therefore, all other things being equal, tomato variety 1 may be a better choice due to less variable yield in the field.

Statistical significance is shown in tables by the letters that follow a given number. For example, when two varieties have yields followed by completely different letters, they are significantly different; however, if they share even one letter, statistically they are no different. Thus, a variety with a yield that is followed by the letters "bcd" would be no different than a variety followed by the letters "cdef" because the letters "c" and "d" are shared by the two varieties. Yield data followed by the letters "abc" would be different from yield data followed by "efg."

When determining statistical significance, we typically use a  $P$  value of 0.05. In this case,  $P$  stands for probability. If two varieties are said to be different at  $P \leq 0.05$ , then at least 95 percent of the time those varieties will be different. If the  $P$  value is 0.01, then 99 percent of the time those varieties will be different. Different  $P$  values can be used, but typically  $P \leq 0.05$  is considered standard practice for agricultural research. This approach may be confusing, but without statistics our results would not be useful. Using statistics ensures that we can make more accurate recommendations for growers.

# Rootstock Effects on Apple Tree Growth and Yield

*Dwight Wolfe, Doug Archbold, Daniel Becker, and Gimmy Travis, Horticulture*

Although apple and peach are the principal tree fruits grown in Kentucky, the hot and humid summers and heavy clay soils make their production more difficult here than in some neighboring tree fruit producing regions and can lead to high disease and insect pressure in Kentucky orchards. Despite these challenges, orchards can offer high per-acre income and are suitable for rolling hills and upland soils.

Identification of improved rootstocks and cultivars is fundamental for advancing the Kentucky tree fruit industry. For this reason, Kentucky cooperates with researchers from 29 other states in the United States, three Canadian provinces, Mexico, and Chile in the Cooperative Regional NC-140 Project entitled, "Improving Economic and Environmental Sustainability in Tree Fruit Production through Changes in Rootstock Use." The NC-140 trials are critical to Kentucky growers, allowing access to and testing of new rootstocks from around the world (Table 1). The detailed and objective evaluations allow growers to select the most appropriate rootstocks for Kentucky.

## Materials and Methods

Grafts of known cultivars on the various rootstocks were produced by nurseries on the West Coast and distributed to cooperators. Kentucky's NC-140 rootstock plantings are located at UK Research and Education Center (UKREC) at Princeton, KY. They are:

**The 2010 apple rootstock trial bitter pit evaluation.** The 2010 apple rootstock trial consisted of thirty-one different rootstocks with 'Aztec Fuji' as the scion cultivar (Table 1). These were compared in a randomized complete block experimental design in four blocks with one to three trees per rootstock per block. The trees were planted in March 2010, on a 6 by 15-foot spacing, and trained to the tall spindle system. This trial was completed in 2018 and details and a final summary were reported previously (Wolfe, 2018; Wolfe et al., 2018).

From this planting, one tree from each replication (where available) was selected from each of the rootstocks listed in Table 2 for a follow-up study to evaluate the influence of rootstock on the incidence of bitter pit (Wolfe et al., 2019). A 50-fruit sample was collected at harvest from each of these trees, evaluated for the presence of bitter pit, and then stored in a cooler for approximately 90 days at about 40°F. The fruit from each sample were then reevaluated for flesh firmness and the presence of bitter pit, and Brix readings were recorded from a subsample of 10 fruits from each 50-fruit sample.

**The 2019 apple rootstock trial.** A new apple rootstock trial was planted 11 Apr. 2019 at the UKREC orchard in Princeton, KY. The trial consists of 'Buckeye Gala' as the scion grafted onto seven different rootstocks. These are: M.9 NAKBT-337,

**Table 1.** Rootstocks in the 2010 apple rootstock trial with 'Aztec Fuji' as the scion cultivar.

Rootstock	Clone status	Breeding Program	Location of Program
B.9	named	Budagovsky	Michurinsk State Agrarian University Michurinsk, Tambov Region, Russia
B.10	named	Budagovsky	
B.7-3-150	not released	Budagovsky	
B.7-20-21	not released	Budagovsky	
B.64-194	not released	Budagovsky	
B.67-5-32	not released	Budagovsky	
B.70-6-8	not released	Budagovsky	
B.70-20-20	not released	Budagovsky	
B.71-7-22	not released	Budagovsky	
G.11	named	Cornell-Geneva	
G.41 N (stool bed produced)	named	Cornell-Geneva	
G.41 TC (tissue culture produced)	named	Cornell-Geneva	
G.202 N (stool bed produced)	named	Cornell-Geneva	
G.202 TC (tissue culture produced)	named	Cornell-Geneva	
G.214 (formerly CG.4214)	named	Cornell-Geneva	
G.814 (formerly CG.4814)	named	Cornell-Geneva	
G.222 (formerly CG.5222)	named	Cornell-Geneva	
G.935 N (stool bed produced)	named	Cornell-Geneva	
G.935 TC (tissue culture produced)	named	Cornell-Geneva	
CG.2034	not released	Cornell-Geneva	
CG.3001	not released	Cornell-Geneva	
CG.4003	not released	Cornell-Geneva	
CG4004	not released	Cornell-Geneva	
CG.4013	not released	Cornell-Geneva	
CG.5087	not released	Cornell-Geneva	
Supp.3	named	Pillnitz	Institut fur Obstforschung Dresden-Pillnitz, Germany
PiAu.9-90	not released	Pillnitz	
PiAu.51-11	not released	Pillnitz	
M.9 NAKBT337	named	NAKB clone of M.9	NAKB, Netherlands CTIFL, France East Mallang Res. Station, Kent, England
M.9 Pajam2	named	CTIFL clone of M.9	
M.26 EMLA	named	E. Mallang clone of M.26	

<sup>1</sup> For more information on Geneva rootstocks, see: <http://www.ctl.cornell.edu/plants/GENEVA-Apple-Rootstocks-Comparison-Chart.pdf>.

M.26 EMLA, G.41, G.814, and G.969, B.10, and one New Zealand rootstock (NZ.2) that is purported to have M.9 vigor, high yield efficiency, and tolerance to aphids and fire blight (possibly immune). Three trees of each rootstock were planted in each row (replication) in a randomized complete block design and trained to the tall spindle system. In order to eliminate the effect of more vigorous stocks competing with the less vigorous ones, only the center tree of each of the three-tree subplots will be evaluated. Thus, the confounding effect due to different rootstock sizes adjacent to one another will be eliminated in this trial. For 2019, only tree height and trunk circumference 20 cm above the graft union were measured. Trunk cross-sectional area was calculated from the trunk circumference. All

data was analyzed using SAS v.9.4 (SAS Institute).

### Results and Discussion

**The 2010 apple rootstock trial bitter pit evaluation.** Neither flesh firmness nor Brix of the apple samples 90 days after cold storage (measured January 2020) were significantly different among rootstocks (Table 2). Variation in bitter pit was very high in both the 2019 and 2020 samples such that statistically significant differences in the percentage of fruit with bitter pit among rootstocks either at harvest or after being in cold storage for 90 days could not be detected.

Bitter pit has been shown to be related to calcium levels in the fruit, and calcium levels in fruit are influenced by rootstock (Autio et al., 1991). Caution in prematurely interpreting these results is warranted as there was only one tree available for sampling on CG.2034. Data from the samples from the 2020 apple harvest now in cold storage will be collected in mid-January 2021. At that time, this study will be completed and a more complete analysis and final summary reported.

**The 2019 apple rootstock trial.** For 2020, trunk cross-sectional area at 30 cm above the graft union (TCSA), yield, and yield efficiency were both statistically different among the seven rootstocks (Table 3). G.814, G.969, and NZ.2, were the largest trees in terms of TCSA and were significantly different from B.10, M.9 NAKBT337, and G.41, the smallest trees in terms of TCSA. M.26 EMLA was not significantly different from either the largest or smallest trees but fell in about the middle of the range of tree size for the rootstocks in this trial.

The average weight per fruit, number of flower clusters per tree, and the number of root suckers per tree did not vary significantly among the five rootstocks. Trees on G.41 produced significantly more fruit than those on G.814, but not more than for any of the other rootstocks. G.41 was significantly more yield efficient than any of the other rootstocks in this trial. Yield efficiency is a measure of the amount of fruit that a tree produces relative to the amount of vegetative growth it has. This was the first year that these trees were fruited, and the plan is for this trial to be evaluated for at least several more years.

**Table 2.** 2019-2020 results for the 2010 NC-140 apple rootstock trial bitter pit evaluation, Princeton, KY.

Rootstock <sup>1</sup>	Initial Number of Trees	2020 TCSA (sq.in.)	Flesh Firmness (lbs.) <sup>3</sup>	Brix (%) <sup>3</sup>	Bitter Pit at 2019 Harvest (%)	Bitter Pit after Storage <sup>3</sup> Jan 2020 (%)	Bitter Pit at 2020 Harvest (%)
M.26 EMLA	4	22.7	12.1	15.9	0.50	8.50	0.50
G.222 (G.5222)	4	16.2	12.3	15.1	0.00	8.00	1.00
M.9 Pajam2	2	14.8	12.2	15.7	0.00	3.00	1.00
CG.3001	3	15.9	12.7	15.2	2.00	9.33	1.33
G.202 N	4	15.6	13.1	15.1	1.00	7.50	1.00
G.935 N	4	15.4	12.6	15.8	0.50	4.50	0.00
G.814 (G.4814)	4	14.2	13.4	15.8	2.50	9.00	0.50
CG.4004	4	13.0	12.9	15.6	0.00	7.50	0.50
G.11	4	12.4	14.0	15.0	1.00	7.00	1.50
CG.5087	2	12.4	11.8	15.2	0.00	7.00	1.00
G.214 (G.4214)	3	12.2	12.8	15.5	0.00	2.67	1.33
M.9 NAKBT337	3	12.2	12.3	15.9	0.67	4.00	0.00
B.10	4	10.4	12.5	15.7	0.50	3.50	0.50
G.41 N	3	9.2	12.5	15.3	1.33	5.33	0.67
CG.4003	4	8.0	12.7	15.3	1.50	4.00	0.00
CG.2034	1	6.2	12.9	15.3	6.00	16.00	6.00
B.9	4	4.1	13.7	14.9	1.50	2.00	0.50
Means	NA	12.9	12.8	15.44	0.96	6.07	0.77
LSD (5%) <sup>2</sup>	NA	4.8	ns	ns	ns	ns	ns

<sup>1</sup> Arranged in descending order of the fall trunk cross-sectional area (TCSA) for each rootstock.

<sup>2</sup> Least significant difference (LSD) at  $P \leq 5\%$ . Differences between two means within a column that are less than the LSD value are not significantly different. "ns" indicates variable was not significant in the analysis of variance at  $P \leq 5\%$ .

<sup>3</sup> From fruit stored for 90 days after harvest in a cooler at 40°F.

**Table 3.** 2020 results for the 2019 NC-140 apple rootstock trial, Princeton, KY.

Rootstock <sup>1</sup>	Number of Data Trees	TCSA (sq.in.)	Yield (lbs. of fruit per tree)	Fruit wt. g / fruit	Bloom (clusters per tree)	Number of Root suckers	Yield Efficiency (lb. per sq. in. of TCSA)
G.814	5	2.02	6.6	152	31.8	2.2	3.3
G.969	5	1.98	8.3	142	38.8	0.0	4.8
NZ.2	5	1.83	8.2	165	23.6	0.4	4.5
M.26 EMLA	5	1.73	8.5	153	40.4	0.0	5.3
B.10	5	1.39	8.3	152	46.6	0.0	6.3
M.9 NAKBT337	5	1.39	5.0	154	22.6	0.2	3.7
G.41	5	1.38	13.9	159	55.8	0.0	10.9
Means	NA	1.67	8.4	154	37.1	0.4	5.5
LSD (5%) <sup>2</sup>	NA	0.40	6.0	ns	ns	ns	4.0

<sup>1</sup> Arranged in descending order of the fall trunk cross-sectional area (TCSA) for each rootstock.

<sup>2</sup> Least significant difference (LSD) at  $P \leq 5\%$ . Differences between two means within a column that are less than the LSD value are not significantly different. "ns" indicates variable was not significant in the analysis of variance at  $P \leq 5\%$ .

The results from both the bitter pit study and the 2019 apple rootstock trial are preliminary and future data from this work will be needed to come to any final conclusions.

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## Performance of Three Primocane-fruiting Blackberry Selections Grown Organically at Kentucky State University

*Jeremiah D. Lowe, Sheri B. Crabtree, and Kirk W. Pomper, College of Agriculture, Community, and the Sciences, Kentucky State University; John R. Clark, Department of Horticulture, University of Arkansas; John G. Strang, Department of Horticulture, University of Kentucky*

In Kentucky, more than 776 farms grow berry crops, including 487 farms that grow blackberries, which are valued at over \$2,620,000 annually (Census of Agriculture, 2017). Blackberries are native to Kentucky, and Kentucky's climate is well-suited for blackberry production. Two cane types exist within brambles: primocanes (or first-year canes), which are usually vegetative, and floricanes, which are the same canes that flower and produce fruit the next growing season. Primocane-fruiting blackberries, also known as fall-fruiting and ever-bearing blackberries, have the potential to produce two crops per year: a normal summer crop on the floricanes and a later crop on the current season's primocanes. Primocanes flower and fruit from mid-summer until frost, depending on temperature, plant health, and the location in which they are grown. Growers can reduce pruning costs by mowing canes in late winter/early spring to obtain a primocane crop only; this also provides control for anthracnose, cane blight, and red-necked cane borer without pesticides. Relying only on a primocane crop also avoids potential winter injury of floricanes. However, late-ripening blackberries are more prone to spotted wing *Drosophila* infestations, so growers who are marketing the berries will need to maintain a pest-control program.

The first commercially available primocane-fruiting blackberry varieties, 'Prime-Jim'® and 'Prime-Jan'®, were released by the University of Arkansas in 2004 (Clark et al., 2005). 'Black Magic'™ is a thorny, primocane-fruiting selection suited for home growers and on-farm sales (Clark et al., 2014). 'Prime-Ark'®45, released in 2009 for commercial use, has improved heat tolerance and shipping traits compared to previous selections (Clark and Perkins-Veazie, 2011). 'Prime-Ark'® Freedom was the first thornless primocane-fruiting blackberry and produces large fruit, but displays inferior shipping traits compared to 'Prime-Ark'® 45 (Clark, 2014). 'Prime-Ark'® Traveler, also a thornless primocane-fruiting selection, has improved storage and shipping characteristics compared to 'Prime-Ark'® Freedom and is recommended for commercial production (Clark and Salgado, 2016). In the fall of 2017, APF-205T was released as 'Stark'® Black Gem'. APF-268 is an advanced selection from the University of Arkansas breeding program. It is a primocane-fruiting blackberry that is not thornless, but has a reduced number of thorns compared to other thorny primocane-fruiting cultivars.

Summer temperatures above 85°F can greatly reduce fruit set, size, and quality on primocanes, which results in substantial reductions in yield and fruit quality (Clark et al., 2005; Stanton et al., 2007). The objective of this study was to determine if 'Prime-Ark'® Traveler is superior to 'Stark'® Black Gem'® and the advanced selection APF-268 in terms of yield and fruit quality under Kentucky growing conditions. Here we report results from the variety trial in its third and fourth years of fruit production.

### Materials and Methods

In May 2016, a primocane-bearing blackberry variety trial was planted at the KSU Research and Demonstration Farm on certified organic land. The planting contained the selections 'Prime-Ark'® Traveler, 'Stark'® Black Gem'®, and APF-268, which are all primocane-fruiting selections from the University of Arkansas. Plants were arranged in a completely randomized design, with four replicate plots each containing five plants of 'Prime-Ark'® Traveler, 'Stark'® Black Gem'®, or APF-268 (total of 20 plants of each selection) in 10-foot plots with a plant spacing of 2 feet. This trial was managed using organic practices following the National Organic Program standards. A combination of cultivation, hand weeding, and straw mulch was used for weed control. Drip irrigation was used as needed. Plots were fertilized with NatureSafe 10-2-8 fertilizer (Griffin Industries LLC, Cold Spring, KY) at 100 lb/acre of N. Primocanes were tipped on all selections at one meter, beginning in early June to promote lateral branching and flowering. Ripe fruit were harvested twice per week from early July through mid-October. Analysis of variance and least significant difference means separation were performed using CoStat Statistical Software (CoHort Software, Monterey, CA).

### Results and Discussion

Fruit were harvested from early July until mid-October. The results presented in this report are for floricanes and primocane crops combined for 2019 and 2020. Growing conditions in 2019 and 2020 were hot; daily high temperatures were above 85°F for 83 out of 122 days from June through September in 2019 and 55 out of 122 days in 2020. The average high for July was 87.4°F in 2019 and 88.0°F in 2020. July, August, and September all had average highs of above 85°F in 2019 (Kentucky



Mesonet, 2020). The high temperatures likely reduced fruit set, size, and quality on primocanes, especially in 2019.

In 2019, APF-268 and 'Stark® Black Gem®' had significantly larger fruit sizes (3.14 g and 2.87 g) compared to 'Prime-Ark® Traveler' (2.15 g; Table 1). There was no significant difference in berry size in 2020, with all selections having berry sizes over 3.5 g. There was no significant difference in yield in 2019 or 2020, but there was a trend for APF-268 to have a higher yield both years. Yields in 2020 were much higher and berry sizes larger than they were in 2019, likely due to the extreme temperatures present in the summer of 2019.

The University of Arkansas Blackberry Breeding Program recommends that commercial producers plant 'Prime-Ark® Traveler' due to its superior shipping and storage qualities. Due to softer fruit, 'Stark® Black Gem®' is recommended for pick-your-own (also called U-pick) and on-farm sales as well as for home gardens. Year-to-year yield characteristics will need to be evaluated further; however, the data to date suggest that 'Stark® Black Gem®' has large fruit, yields well in Kentucky, and should be considered by growers interested in producing primocane-fruiting blackberries for markets with little to no shipping.

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Clark, J.R. and P. Perkins-Veazie. 2011. 'APF-45' primocane-fruiting blackberry. *HortScience*. 46:670-673.

**Table 1.** 2019-20 yields and berry weights for 'Prime-Ark® Traveler', 'Stark® Black Gem®', and APF-268 at the Kentucky State University Harold R. Benson Research and Demonstration Farm, Frankfort, KY.

Selection	2019		2020	
	Fruit Weight (g)	Yield (lb/acre)	Fruit Weight (g)	Yield (lb/acre)
'Stark® Black Gem®'	2.87 <sup>1</sup> a	279 a	4.61 a	1235 a
'Prime-Ark® Traveler'	2.15 b	206 a	3.55 a	967 a
APF-268	3.14 a	475 a	4.49 a	2187 a

<sup>1</sup> Numbers in a column followed by the same letter are not significantly different (least significant difference  $P = 0.05$ )

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# Performance of 'Duke' Highbush Blueberry Grown in Two Container and Three Soilless Substrate Combinations

Daniel Becker, Dwight Wolfe, Winston Dunwell, and Ginny Travis, Horticulture

The highbush blueberry (*Vaccinium corymbosum* L.) is popular with consumers for its taste and reported health benefits. Excellent sales potential exists at local markets across the state, but exacting soil requirements limit suitable production sites. Blueberries require well-drained, acidic soils with a pH between 4.5–5.2 and high organic matter (Strang, et. al., 2003). When grown in elevated pH and poorly drained soils, blueberries experience weakened growth and iron deficiency. Phytophthora root rot, a devastating fungal disease is common and can destroy plantings. Without extensive soil amendments and building raised beds, costs which can exceed \$7,000/acre during site preparation, few sites in Kentucky will meet the conditions necessary for sustained productivity and long-term profitability (Ernst, 2019).

Often, acceptable sites are located some distance from desirable markets, reducing the viability of on-farm direct-to-customer sales and increasing transportation costs. Grown on sites with good market potential, but poor suitability, blueberries will fail to thrive, leading to either abandonment or further expenses of upkeep without a reasonable increase in returns. By growing blueberries in containers, potential exists for growers without an optimum site to successfully diversify their operations. The ob-

jective of this study is to determine the adaptability of highbush blueberries to perennial container production in Kentucky.

## Materials and Methods

One-year-old bare-root 'Duke' blueberry plants were grown in 7-gal pots filled with pine bark fines substrate in 2017. Irrigation was set automatically and delivered with a staked emitter. All flowers were removed to eliminate cropping the first two years (2017 and 2018). Plants were top-dressed with 156 g per container of a slow-release fertilizer in May. Using the 7-gal pots the first year conserved substrate and reduced the possibility of overwatering. Substrate without widespread root colonization is slow to dry once wet and encourages root rot pathogens.

In April 2018, we selected 54 (out of 70) of the largest plants for transplanting into the 25-gal container and soilless substrate treatment combinations. Conventional blow-molded black plastic pots or flexible artificial fabric SmartPots (High Caliper Growing System, Oklahoma City, OK) were used as containers. Substrates consisted of 3/8 inch average particle size pine bark fines, sphagnum moss (Premier Tech Horticulture, Quakertown, PA) or a 1 pine bark : 1 sphagnum moss (by volume)

**Table 1.** 2020 containerized blueberry trial results, including 2019 yield at UKREC, Princeton, KY.

Container	Substrate	Mean yield/bush (oz) <sup>z</sup>		Wt. 50 berries (oz) <sup>y</sup>	Percent Yield of Harvest			Canopy Volume (cu ft) <sup>x</sup>		
		2019	2020		1st wk.	2nd wk.	3rd wk.			
Fabric	1 pine bark : 1 sphagnum moss (by volume)	77.0	bc	12.5	a	2.5	31.9	44.7	23.3	26.2
Fabric	Pine bark	89.2	ab	9.8	a	2.6	52.7	34.2	13.0	31.3
Fabric	Peat moss	73.9	c	13.3	a	2.7	41.8	38.9	19.1	30.7
Plastic	1 pine bark : 1 sphagnum moss (by volume)	84.7	bc	13.2	a	2.7	39.5	44.4	16.0	26.2
Plastic	Pine bark (control)	99.5	ab	12.2	a	2.6	42.3	36.6	20.9	38.6
Plastic	Peat moss	112.1	a	8.8	a	2.6	35.3	42.2	22.6	28.7

<sup>z</sup> Means within columns followed by the same letter are not significantly different (Duncan's multiple range test LSD,  $P \leq 0.05$ ).

<sup>y</sup> Weight of 50 berry subsample collected during the second harvest week.

<sup>x</sup> Canopy volume calculated as the volume of a cylinder ( $V = \pi r^2 h$ ).

mixture. Limestone gravel contaminant was removed from the pine bark and bales of sphagnum moss uncompressed by hand before use as substrates. Half (27) of the plants were assigned to the black plastic and the other half to fabric containers. Plants were further subdivided into three groups of nine each and assigned a substrate. While transplanting, containers were filled until the root ball of each plant and the substrate was nearly level with the edge of the fabric pots and up to the equivalent volume level in the plastic pots. Water was applied immediately to settle the substrate, with more added as needed to cover the root ball. The containers were placed on a gravel bed 4 ft apart in three rows spaced 13 ft apart. Plots consisted of three plants of each container and substrate treatment combination, with each row being a replication of six plots and 18 plants in a randomized complete block design. The black plastic and pine bark substrate treatment is considered the control.

Plants were fertilized with 267 g per container of Osmocote Plus 15-9-12 (12-14 months at 70°F) split into three, 89 g applications at six-week intervals in mid-April, late May, and early July. Automatic irrigation, programmed to run twice per day at 10:00 a.m. and 2:00 p.m. for one minute durations supplied 0.65 gal of water per container through two 9.8 gal per hour 6-inch staked emitters (Murray Irrigation Limited, Deniliquin NSW, Australia) inserted into the substrate on opposite sides of each plant. Additional 10 minute per week irrigations prevented underwatering when no supplemental rainfall occurred. The irrigation system was turned on in March about budbreak and turned off and winterized in October at the beginning of dormancy. In December, the containers were moved together as close as possible. Frost covers were draped along the outside perimeter of the containers and the entire canopy and containers covered with three layers of 3 oz/sq. yd. winter blankets to provide freeze protection.

Data collected to assess adaptability included yield, canopy volume, and ongoing plant mortality. Harvest passes were performed weekly in 2020 on 6, 18, and 24 June. Ripe fruit from each plant was weighed at each pass, including a 50-berry subsample used to determine average berry weight during the second harvest. The percent yield of each harvest passes was determined by dividing the weight of fruit collected by the sum total yield per plant from all harvests. Plant height and width were measured in mid-Sept. to ascertain canopy volume, plant mortality was recorded at the same time. The data was statistically analyzed using SAS v.9.4 (SAS Institute, Cary, NC), sub-

jecting it to analysis of variance (ANOVA) and means separation using Duncan's multiple range test LSD ( $P \leq 0.05$ ).

## Results and Discussion

The 2020 season was wetter than normal, over 52 inches of rain fell from January until October, compared to 41 inches expected for the same period in Princeton, KY (Kentucky Climate Center, 2020; Kentucky Mesonet, 2020). Polar vortex-related lows of 8.2 and 7.2 °F on 12 and 13 Nov. did not cause noticeable winter injury as the bushes were covered before, then uncovered once temperatures were above freezing. Overall plant health was good coming out of winter. Temperatures in 2020 were normal, except for Apr. and May when they were above average. A 25.8 °F freeze on 15 Apr. thinned some blooms and reduced, but did not eliminate, the crop as experienced by blueberry growers in other parts of the state. The bushes were just at the beginning of the late pink bud stage at the time.

Pruning had a far greater impact on yield in 2020. Bushes were intensively pruned to limit their crop load in anticipation of having restricted access to harvest labor. Intensive pruning, combined with an estimated 10% bloom loss from the April freeze, high winds in early June that damaged fruiting shoots, and earlier and more intense bird pressure increased fruit losses, greatly reducing yield compared to 2019 (Table 1). Berry weight was also unaffected by treatment. While not significant, the percentage of yield collected during each pass was as expected and is similar to 2019 with around 80% of fruit harvested in the first two weeks. Intensive pruning also contributed to the elimination of treatment differences in canopy volume. Two bushes had died as of Sept. 2020, one each in the fabric-sphagnum moss and plastic-1 pine bark : 1 sphagnum moss treatments, resulting in an 11% mortality rate.

In response to high pour-through water pH tested on 18 Sept. 2019, an injector was installed in July 2020 to lower the water pH to the desired 4.5–5.2 range. A 1.5% solution derived from 93% tech grade sulfuric acid was injected at a 1:256 gallon-to-gallon ratio. The base pH of the municipal water source is 7.1–7.3 and its electrical conductivity (EC), tested at the same time is 210–230. Prior to installing the injector, the average (derived from 18 subsamples, one from each plot) pour-through water pH was 6.2 compared to 5.2 when tested on 16 Sept. 2020. The EC was 385 (2019) vs. 1,312 (2020)  $\mu\text{S}/\text{m}$ , indi-

cating that the acidified irrigation water is dissolving the coating of the slow-release fertilizer more rapidly than the untreated water, making more nutrients available for plant uptake. Increased fertilizer availability along with a more suitable pH for blueberry growth are undoubtedly factors contributing to the enlarged canopy volume compared to 2019 (Becker, 2019).

While treatment effects were not evident in 2020, the non-significance does show that pruning, fertility and water-substrate pH management are powerful tools at growers' disposal. Depending on circumstances, growers can prune more extensively to reduce crop and harvest requirements while monitoring, and if necessary, increasing fertility and lowering pH to promote a growth response. One year's crop is sacrificed, but future plant health is encouraged.

### Acknowledgements

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## Tree Tubes Improve Early Field-Planted Pawpaw (*Asimina triloba*) Growth and Survival

Sheri B. Crabtree, Kirk W. Pomper, and Jeremiah D. Lowe, Kentucky State University Land Grant Program

The North American pawpaw (*Asimina triloba*) is a tree fruit native to the understory of hardwood forests in eastern North America and is increasing in small-scale commercial production across the United States and internationally (Pomper and Layne, 2005). Pawpaws are generally planted at a smaller size than other tree fruits; transplanting larger trees can be difficult due to the taproot. First-year survival of young pawpaw trees may be low due to weed pressure, lack of irrigation, and animal damage. Pawpaws are prone to sunscald and also have a tendency to branch lower on the trunk than is desirable with a bushy growth habit, as opposed to a single-trunked central leader tree, which is easier to maintain and harvest from in an orchard setting. Tree tubes can help young trees grow taller, straighter, and faster and improve establishment (Evans and Potter, 1985; Frearson and Weiss, 1987; Potter, 1988). Tree tubes also protect from sunscald, animal browsing and rubbing, and lawnmower or weedeater damage (Pomper et al., 2010). Therefore, tree tubes may be of great benefit in pawpaw orchards. However, solid tree tubes can create a greenhouse effect (Bergez and Dupraz, 1997), causing heat damage to young pawpaw plants, so tree tubes with some ventilation are required. The objective of this study was to determine if tree tubes would enhance growth and survival of field-planted pawpaw trees.

### Materials and Methods

A randomized complete block design was planted in June 2019 at the Kentucky State University H.R. Benson Research and Demonstration Farm in Frankfort, Kentucky, on certified organic land, consisting of the cultivars KSU-Atwood, KSU-Chappell, Shenandoah, Sunflower, and seedlings. Treatments were no shelter (control), open mesh tree tube, and a solid tree tube with ventilation slits (Rigid Seedling Protector Tubes and Tubex® Combitube Tree Shelters, respectively; Forestry Suppliers, Inc. Jackson, MS). Tree tubes, 46 cm in height, were installed upon planting in Spring 2019 and were removed in November 2019 to avoid issues with winter injury, then replaced with 122 cm tree tubes in Spring 2020. The orchard was managed using National Organic Program (NOP) standards, with regular irrigation and weed control via wood chip mulch and hand cultivation. Trunk diameters were measured at planting at 10 cm above ground level, and survival data and trunk diameters were collected in April 2020. Data were analyzed using CoStat Statistical software (CoHort Software, Monterey, CA) and subjected to Analysis of Variance and Least Significant Difference (LSD) means separation. Treatment means were separated based on a significance level of  $P \leq 0.05$ .



## Results and Discussion

Pawpaw trees covered with tree tubes had significantly higher survival than control trees. Trees covered with solid ventilated tubes had 85 percent survival, trees covered with open mesh tubes had 60 percent survival, and trees with no shelter had significantly lower survival, 20 percent after the first year (Table 1). Significantly higher growth was also seen in pawpaws covered with tree tubes. Trees covered with open mesh tree tubes had an average increase in diameter of 1.09 mm in their first year; trees with solid vented tubes had an increase of 0.94 mm diameter, while control trees with no tree tube grew only an average of 0.23 mm in diameter in their first year. This is in contrast to previous studies by Famiani et al. (2007) and Kjelgren and Rupp (1997) which showed lower diameter in trees grown in tree shelters. This may be due to pawpaws' affinity to shade at an early age and therefore increased growth in the shade provided by tree tubes. No differences in survival or growth were seen among the cultivars (Table 2). The shading and protection provided by the tubes likely protected the small trees that are sensitive to UV light and led to increased growth and improved survival.

## Conclusion

The use of tree tubes is recommended for young pawpaw transplants to enhance survival and early growth. Both survival and increase in trunk diameter were higher in young pawpaws covered with tree tubes. The amount of ventilation in the tubes did not affect diameter or survival in Year 1, but some ventilation is needed to avoid overheating. Tubes were replaced with taller tubes to determine their effect on survival and growth in the second year and beyond, and data will continue to be collected.

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**Table 1.** Trunk diameter, growth, and survival of pawpaw trees grown with two types of tree tubes and no tubes at the Kentucky State University Research and Demonstration Farm, Frankfort, KY.

Tree tube	Diameter at planting (mm)	Diameter after one year (mm)	Increase in diameter (mm)	Survival
no tube	4.05	4.61	0.23 b <sup>1</sup>	20% b
open mesh	4.37	6.37	1.09 a	60% a
solid vented	4.81	6.00	0.94 a	85% a
significance	NS	NS	***	***

<sup>1</sup> Numbers followed by the same letter are not significantly different (Least Significant Difference  $P \leq 0.05$ ).

**Table 2.** Trunk diameter, growth, and survival of four cultivars and seedling pawpaw trees grown with two types of tree tubes and no tubes at the Kentucky State University Research and Demonstration Farm, Frankfort, KY.

Cultivar	Diameter at planting (mm)	Diameter after one year (mm)	Increase in diameter (mm)	Survival
KSU-Atwood	4.32 bc <sup>1</sup>	5.58	0.89	58%
KSU-Chappell	5.27 a	6.67	0.99	67%
Shenandoah	4.73 ab	6.15	0.92	75%
Sunflower	3.71 c	5.35	0.82	33%
seedling	4.02 bc	5.41	0.77	42%
significance	**	NS	NS	NS

<sup>1</sup> Numbers followed by the same letter are not significantly different (Least Significant Difference  $P \leq 0.05$ ).

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# Evaluation of Strawberry Varieties as Matted Rows, 2020

John Strang, Chris Smigell, and John Snyder, Horticulture

Strawberries are one of the first fruit crops to ripen in the spring and are very popular with Kentucky consumers. Since the fruit do not develop higher sugar levels after harvest, leaving the berries on the plants longer allows them to develop their full flavor for local sales. There are approximately 200 acres of strawberries grown in Kentucky, and about 130 of these are grown using the matted row system while the rest are grown using the annual plasticulture system.

This study evaluated newer strawberry varieties planted in the matted row system at the University of Kentucky Horticultural Research Farm in Lexington. This report covers the third growing season, or second fruiting year of this study and incorporates data from previous years.

## Materials and Methods

Thirteen dormant, bare-rooted strawberry varieties were planted on 13 Apr. 2018, in a Maury Silt Loam soil after 50 lb/acre of nitrogen (19-19-19) was incorporated. 'Allstar,' 'Chan-

dlar, 'Earliglow', and 'Jewel' were included as standards. All plants were dipped into Viterra® Agri-gel™ (Nepera Chemical Company, Inc.) prior to planting to enhance water retention and plant survival. Each plot was a 10 ft long single row and consisted of six plants set 2 ft apart in the row with 4 ft between rows. Plots were replicated four times in a randomized block design.

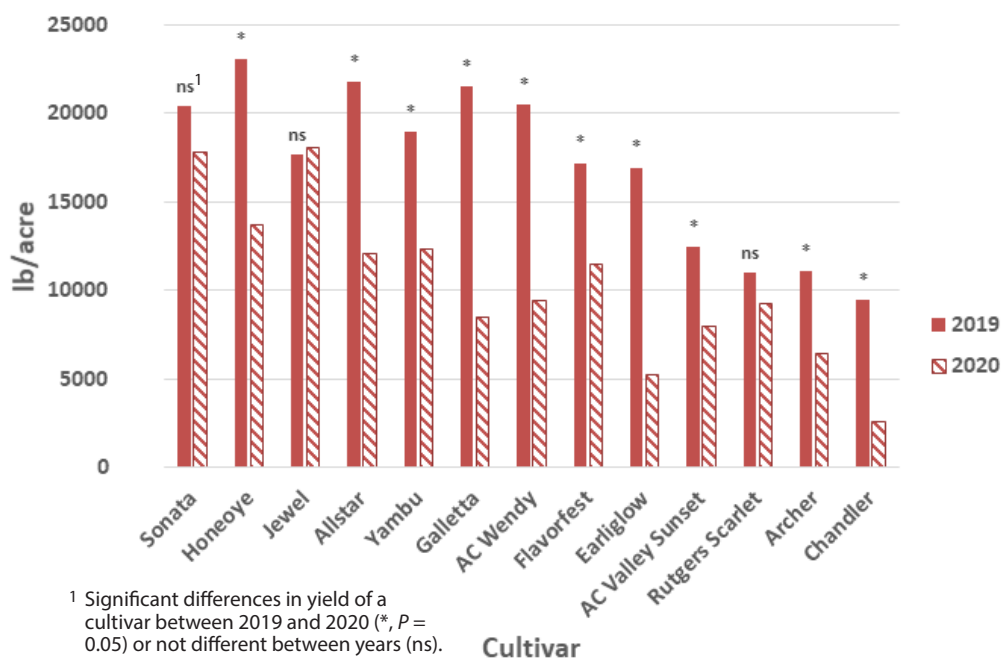
Insect, disease, and weed control were conducted in accordance with the Midwest Fruit Pest Management Guide (ID-232) (Beckerman et al., 2019). No fungicides were applied in 2019, while captan and pyraclostrobin + boscalid (Pristine) were applied two times each during bloom in 2019 and captan was applied once and pyraclostrobin + boscalid three times during bloom in 2020. No insecticide applications were made.

Flumioxazin (Chateau) pre-emergence herbicide was applied over the top of the dormant plants five days after transplanting and napropamide (Devrinol) was applied 28 Aug. during the 2018 season for pre-emergence weed control. At renovation, 1 July 2019, plants were mowed off just above the crowns and 290 lb/acre of sulfur was applied to lower the soil pH. The planting was rototilled to incorporate sulfur between the rows, to narrow plant rows to a width of 14 inches and to control weeds. Fifty pounds of N per acre as ammonium sulfate was broadcast over the planting and terbacil (Sinbar) herbicide was applied for pre-emergent weed control. Rows were again narrowed to a width of 14 inches on 2 Sept. 2018 and 31 Aug. 2019, to keep plants separated. Strawberry plants that rooted in row middles after this were manually removed. Chateau was applied 28 Feb. 2019 and 12 Mar. 2020. Glyphosate (Roundup WeatherMAX) was spot applied between rows, particularly for perennial weed control several times during all three seasons.

Plants were drip irrigated as needed. The field was mulched with wheat straw on 5 Dec. 2018, and 11 Nov. 2019, for winter protection.

Ten-foot sections in each plot were harvested in the spring of 2019 and 2020. Yield, fruit size, flavor, and appearance data were collected. Plant vigor was rated on 23 Nov. 2018, 16 May 2019, and 27 Apr. 2020.

Data are shown for the 2019 (Strang et al., 2019) and 2020 harvest seasons. All marketable fruit were weighed at each harvest and a randomly selected sample of 20 berries were weighed at each harvest to determine average berry weight. Berry flavor was assessed by two individuals four different times and fruit firmness and attractiveness were assessed twice for each variety and replication.



**Figure 1.** Average strawberry yield per acre for 2019 and 2020 seasons, Lexington, KY.

On 9 and 18 May 2019 and 12 June 2020, replicates were assessed for leaf spot (*Mycosphaerella fragariae*) disease. Eight entire (trifoliate) leaves were randomly sampled from each replicate. The number of leaf spot lesions on each leaf were counted and the percent of leaf area showing disease symptoms surrounding the lesions was estimated. On 18 June 2019 and 12 June 2020, replicates were assessed for leaf blight (*Phomopsis obscurans*) and on 12 June 2020, replicates were evaluated for leaf scorch (*Diplocarpon earliana*), following the same protocol used in the 9 May 2019 assessment.

## Results and Discussion

The spring seasons of 2019 and 2020 were cool and rainy. The 2019 season was frost-free so both yield and berry size were generally good, but berry flavor was reduced. In 2020, damaging freezes occurred on 15 and 16 Apr. and 9 May. Plants were not covered for the 15 and 16 Apr. freezes and early blooming varieties sustained flower losses. Plants were covered with two floating row covers for the 9 May freeze and did not sustain additional injury. A minimal fungicide spray program was used to provide a means to evaluate plant and fruit disease susceptibility.

Figure 1 shows yields for the 2019 and 2020 seasons ranked for average yields. Yields were significantly greater in 2019 than in 2020 for all but 'Sonata,' 'Jewel,' and 'Rutgers Scarlet.' This is primarily because of greater freeze losses in 2020 on early and mid-season blooming and maturing varieties, compared to late varieties. Consequently, 'Earliglow,' 'Chandler,' 'Galletta,' and 'AC Wendy' were particularly poor performers in 2020 and had at least a 50 percent lower yield in 2020 in comparison to 2019.

'Sonata' and 'Honeoye' had the highest average yields with 19,132 and 18,384 lb/acre, respectively, over both harvest

seasons (Table 1). 'Archer' and 'AC Valley Sunset' had the largest fruit sizes in the trial in 2019, while 'AC Valley Sunset' tended to have the largest fruit in 2020, but fruit size was severely affected by the freezes in 2020. 'Flavorfest', 'Yambu', and 'AC Wendy' also had very large fruit.

'Galletta', 'AC Valley Sunset', 'Sonata', 'Honeoye', 'Yambu', 'Flavorfest' and 'Earliglow' were rated as having some of the more attractive fruit over the two years of the trial. 'Allstar', 'Jewel', 'Flavorfest', 'Galletta', and 'Archer' tended to have firmer fruit.

'Earliglow' topped the flavor rating for both years followed by 'Allstar', 'AC Valley Sunset', 'Rutgers Scarlet', and 'Flavorfest'. 'Earliglow' was the earliest variety to be harvested in 2019 followed by 'Galletta' and 'AC Wendy', while 'AC Valley Sunset' was the latest variety to be harvested. Harvest mid-point date data or the date at which half the berries of a variety were harvested before and half were harvested afterward for 2020 are not shown because the harvest period was drastically skewed by the freezes. Plant vigor ratings over three seasons were particularly high for 'Galletta', 'Honeoye', 'Earliglow', 'Allstar', 'Flavorfest', and 'Sonata'. However, 'Archer', 'Chandler', and 'AC Valley Sunset' had some of the lowest plant vigor ratings and yields were reduced substantially.

Plant evaluations for leaf spot incidence and severity for 2019 and 2020 showed that most varieties had very low incidence of this disease, while 'Yambu', 'Rutgers Scarlet', 'Honeoye', and 'AC Wendy' had higher ratings (Table 2). Phomopsis leaf blight evaluations did not show any statistical differences in disease incidence among varieties. However, 'AC Valley Sunset' had a slightly higher severity rating. Leaf scorch incidence ratings were very low for 'Earliglow', 'Flavorfest', 'Sonata', 'Galletta', 'AC Wendy', and 'Rutgers Scarlet', while 'Archer', 'Yambu', 'Chandler', and 'AC Valley Sunset' had significantly higher incidences. Examination of leaf scorch severity ratings shows that ratings were significantly lower for 'Earliglow', 'Flavorfest', 'Sonata', 'Galletta', and 'AC Wendy'. 'Chandler', 'Archer', 'Jewel', and 'Honeoye' displayed higher leaf scorch severity levels. Angular leaf spot is

**Table 1.** Two-year averages for yield, fruit characteristics, plant vigor, and 2019 harvest mid-point.

Variety	Yield 2019-20 (lb/acre) <sup>1</sup>	Berry wt. 20 berries (lb) <sup>2</sup>		Attractiveness 2019-20 (1-5) <sup>3</sup>	Firmness 2019-20 (1-5) <sup>4</sup>	Flavor 2019-20 (1-5) <sup>5</sup>	Harvest mid-point <sup>6</sup> 2019 (date)	Plant vigor 2018-20 (1-5) <sup>7</sup>
		2019	2020					
Sonata	19,132	a	.41 .33	4.3	3.5	3.9	31 May	4.3
Honeoye	18,384	a	.39 .36	4.3	3.6	3.8	27 May	4.8
Jewel	17,880	ab	.49 .42	4.2	3.9	3.9	31 May	4.0
Allstar	16,914	abc	.46 .41	4.2	4.3	4.2	29 May	4.5
Yambu	15,654	bc	.57 .45	4.3	3.6	3.9	26 May	4.1
Galletta	15,015	c	.45 .37	4.5	3.8	3.9	25 May	4.9
AC Wendy	14,987	c	.52 .34	4.1	3.5	4.0	25 May	4.2
Flavorfest	14,368	c	.58 .42	4.3	3.9	4.1	30 May	4.4
Earliglow	11,094	d	.35 .27	4.3	3.6	4.5	23 May	4.8
AC Valley Sunset	10,230	d	.68 .58	4.4	3.7	4.2	08 Jun	3.4
Rutgers Scarlet	10,148	d	.50 .37	4.1	3.9	4.2	31 May	4.0
Archer	8,794	d	.71 .42	3.8	3.8	4.0	29 May	2.6
Chandler	6,037	e	.40 .31	3.9	3.6	3.8	01 Jun	3.3
LSD (5%) <sup>8</sup>	2,618			0.28	0.16	0.19		1.98

<sup>1</sup> Numbers followed by the same letter are not significantly different ( $P \leq 0.05$ ).

<sup>2</sup> Based on 20 berries at each harvest.

<sup>3</sup> Attractiveness: 1 = poor; 5 = excellent.

<sup>4</sup> Firmness: 1 = soft; 5 = very firm.

<sup>5</sup> Flavor based on four evaluations by two individuals each year: 1 = poor; 5 = excellent.

<sup>6</sup> Date on which half of the berries were harvested, based on total yield weight.

<sup>7</sup> Plant vigor rated on 23 Nov. 2018, 16 May 2019, and 27 Apr. 2020; 1 = poor vigor, 5 = excellent.

<sup>8</sup> Least significant difference (LSD)  $P \leq 0.05$  probability level. Differences between two numbers within a column that are less than or equal to the LSD value are not significantly different.

**Table 2.** Average estimated incidence and severity of strawberry plant leaf spot and phomopsis for 2019 and 2020 and leaf scorch for 2020.

Variety	Leaf spot <sup>1</sup>		Phomopsis leaf blight <sup>2</sup>		Leaf scorch <sup>3</sup>							
	Incidence <sup>4,5</sup>	Severity <sup>6</sup>	Incidence	Severity	Incidence	Severity						
Sonata	2.7	c	0.2	c	0.00	a	0.00	b	3.9	de	0.8	cde
Honeoye	14.9	b	2.1	cb	0.03	a	0.09	b	9.8	bcd	2.7	cde
Jewel	0.9	c	0.3	c	0.00	a	0.00	b	12.1	bc	3.4	cd
Allstar	0.2	c	0.0	c	0.03	a	0.06	b	10.2	bcd	2.1	cde
Yambu	31.8	a	4.4	ab	0.00	a	0.00	b	17.3	ab	6.4	ab
Galletta	0.2	c	0.0	c	0.00	a	0.00	b	7.2	cde	1.9	cde
AC Wendy	7.0	bc	6.1	a	0.00	a	0.00	b	8.0	cde	1.5	cde
Flavorfest	0.2	c	0.0	c	0.00	a	0.00	b	3.4	de	0.4	de
Earliglow	0.5	c	0.1	c	0.00	a	0.00	b	1.7	e	0.2	e
AC Valley Sunset	0.5	c	0.0	c	0.03	a	0.63	a	12.3	bc	2.4	cde
Rutgers Scarlet	26.3	a	6.1	a	0.03	a	0.06	b	8.6	cde	2.0	cde
Archer	0.3	c	0.0	c	0.00	a	0.00	b	20.4	a	3.8	bc
Chandler	0.5	c	0.0	c	0.00	a	0.00	b	17.1	ab	7.0	a

<sup>1</sup> Leaf spot caused by *Mycosphaerella fragariae* on 9 May and 18 June 2019 and 12 June 2020.

<sup>2</sup> Phomopsis leaf blight caused by *Phomopsis obscurans* on 18 June 2019 and 12 June 2020.

<sup>3</sup> Leaf scorch caused by *Diplocarpon earliana* on 12 June 2020.

<sup>4</sup> Number of leaf lesions on a trifoliate leaf, averaged from eight leaves per replicate.

<sup>5</sup> Means within same column followed by the same letter are not significantly different ( $P \leq 0.05$ ).

<sup>6</sup> Percent of leaf area showing infection symptoms on the same leaves used to determine disease incidence.

a bacterial disease and all varieties showed some symptoms in 2019, but no symptoms were observed in 2020.

The best performing early maturing varieties in this trial were 'Galletta' and 'AC Wendy'. 'Galletta' had a very good yield in comparison with the other early maturing varieties and its fruit were rated as being the most attractive in the trial. Fruit were relatively firm, but flavor was not quite as good as many of the other varieties. It ranked at the top in plant vigor and had some of the lowest leaf spot and leaf scorch ratings. 'AC Wendy' had a similar yield to 'Galletta'. Its fruit were slightly less attractive and firm, but had a higher flavor rating in both 2019 and 2020 and higher average fruit size in 2019 than 'Gal-



letta.' Plant vigor was good, and it did not differ significantly from 'Galletta' in leaf spot incidence, but it had a higher leaf spot severity rating than 'Galletta.' 'AC Wendy' also had a very low leaf scorch incidence and severity rating.

'Sonata,' 'Honeoye,' 'Jewel,' 'Allstar' and 'Flavorfest' were the best performing mid-season varieties. 'Sonata,' 'Honeoye,' 'Jewel' and 'Allstar' had similar yields. 'Flavorfest' tended to have the largest average fruit size and 'Honeoye' the smallest. All four varieties had similar fruit attractiveness ratings, while 'Allstar,' 'Jewel,' and 'Flavorfest' had firmer fruit and 'Allstar' and 'Flavorfest' had higher fruit flavor ratings. Four of these varieties had very good plant vigor ratings, while the rating for 'Jewel' was slightly lower, but still very good. 'Flavorfest' and 'Sonata' had very low leaf spot and leaf scorch ratings, while 'Allstar' and 'Jewel' had the fourth and fifth highest leaf scorch ratings when compared with other varieties. 'Honeoye' had the third highest leaf spot and fourth highest leaf scorch ratings.

'AC Valley Sunset' was the best performing late maturing variety. It had a lower yield than eight of the varieties in the trial, very attractive, moderately firm large fruit with excellent flavor. Plant vigor and leaf spot incidence and severity were very low, but its leaf scorch rating was slightly higher than many of the other varieties in the trial. This variety will require a more exacting fungicide program, particularly in wet seasons.

'Chandler,' which is one of the primary varieties used in plasticulture production in Kentucky performed poorly in this

study. Chandler leaves were slightly chlorotic throughout the season and a plot soil pH measurement following harvest in 2019 indicated a pH of 7.4 suggesting an iron deficiency. Sulfur was applied to the plot at renovation to lower the pH, but 'Chandler's' leaves were again chlorotic throughout the 2020 season. It appears that 'Chandler' may be less suitable for production on higher pH soils compared to many other varieties.

## Acknowledgments

The authors would like to thank Steve Diver, Dave Lowry, Joseph Tucker, and Jackson "Cade" Laumas for their help and assistance in the successful completion of this trial.

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# Evaluation Of Broccoli Cultivars for Winter High Tunnel Production

Rachel Rudolph and Benjamin Yates, Horticulture

Broccoli (*Brassica oleracea*) is an herbaceous biennial crop grown as an annual. It is part of the Brassicaceae family which includes cauliflower, cabbage, mustard greens, and Brussels sprouts. Broccoli grows as a single stalk, which supports large branching flower heads. Typically grown in the spring and fall, the objective of this trial is to evaluate the cold tolerance of different cultivars. Uncommonly grown in high tunnels, their relatively large space requirements and low economic return per square foot pale when compared to other cool weather high-value crops such as lettuce. However, the extra protection afforded by the high tunnel, coupled with the use of row cover, allowed for a much later harvest window than otherwise possible in Kentucky's climate, thus allowing small-scale growers the opportunity to further diversify their wintertime crop production. Additionally, broccoli is a favorite among school lunch programs which may be another market opportunity for high tunnel growers.

## Materials and Methods

Six cultivars of broccoli (Blue Wind, Imperial, Diplomat, Emerald Crown, Eastern Magic, Arcadia; Table 1) were seeded on 24 Aug. 2019, into 50-cell trays (Landmark Plastic Corporation, Akron, OH) using PRO-MIX BX Mycorrhizae (Premier Tech Horticulture, Quakertown, PA) as the potting media. Broccoli seedlings were transplanted on 4 Oct. 2019 inside a 30 x 96 ft high tunnel with an air-filled, 6 mil double polyethylene layer located at University of Kentucky Horticulture Research Farm in Lexington, KY. The Maury silt loam soil had been tilled and shaped into four raised beds and covered by black landscape fabric from a previous ground cherry study in the spring. Each bed consisted of a single row of burned holes spaced one foot apart and one layer of drip tape laid in the middle of each bed. The trial was arranged as a randomized complete block design with four replications of the six cultivars. Treatment plots consisted of 10 plants with an in-row spacing of 12 inches.

Plants were fertilized three times for a rate of 100 lb/acre of actual nitrogen using calcium nitrate (15.5N-0P-0K). Additionally, boron was also applied to the broccoli crop (1 gal; Cell Force Max, 6N-0P-0K) to achieve the recommended boron (0.23 lb), and another fertilization through the drip irrigation lines for the necessary sulfur (0.2 lb). No pesticide applications were made. When necessary, row cover (Agribon®, Berry Plastics, Evansville, IN) was placed over the plants for cold protection. Supported by metal hoops and held down by rock bags while the plants were still small, the broccoli outgrew the hoops and the row cover was draped over the plants. Initially, a single layer of Agribon 30 (0.9 oz/yd<sup>2</sup>) was used, but when the nighttime lows dropped into the low 20s and below, an additional layer Agribon 19 (0.55 oz/yd<sup>2</sup>) was placed on top of the Agribon 30.

Each broccoli plant was harvested once. On 3 Jan. 2020, the majority of heads from all cultivars, except 'Imperial', had reached maturity, at which time they were harvested, and their marketability evaluated. Crowns were harvested by cutting 3

inches below the base of the crown. Marketable and unmarketable crowns were sorted based on USDA grading recommendations. Crowns were considered marketable based on head size, floret tightness, lack of cold damage, and absence of disease, specifically *Botrytis*. 'Imperial' was harvested on Jan. 16 as well as the remaining heads from other cultivars that had yet to mature by the first harvest.

## Results and Discussion

No disease was observed on any broccoli cultivar during the trial. Overall, 'Imperial' performed the best. It was the highest yielding of all of the cultivars and had significantly higher yield than 'Diplomat' and 'Arcadia' (Table 2). 'Imperial' also had a significantly heavier mean head or crown weight than all other cultivars. However, 'Imperial' was the slowest cultivar to mature which meant harvest was delayed by almost two weeks. The second best performing cultivar was 'Blue Wind' It had 78 percent of the average marketable yield of 'Imperial', but had a higher mean marketable head count than 'Imperial' (Table 2). Nearly all of the crowns of 'Blue Wind' were harvested on the first harvest date, 3 Jan.

The worst performing cultivars were 'Diplomat' and 'Arcadia' which were not significantly different from one another with respect to mean marketable yield, mean head weight, and mean marketable head count (Table 2). Both of these cultivars had the smallest mean marketable yield and head weight. These two cultivars produced small heads that were not very uniform.

Even though the month the broccoli cultivars were transplanted, October 2019, was warm with average high and low temperatures of 69.9°F and 49°F (Kentucky Mesonet, 2019), respectively, none of the broccoli cultivars was harvested within their reported days to maturity. Calendar days to maturity can be unreliable because it does not take the fluctuations of temperature into account. Growing degree days (GDD), also called heat units, is the accumulation of both temperature and time. Each crop requires a certain amount of heat to develop and mature. GDDs are the units used to calculate the amount of heat accumulated over time. Utilizing GDDs to predict when a crop will be harvested will be more accurate and reliable for growers. The lowest temperature in October recorded at the Lexington weather station was 33.1°F on 31 Oct. (Kentucky Mesonet, 2019). November temperatures got as low as 13.1°F on 13 Nov. (Kentucky Mesonet, 2019). These low temperatures can greatly affect broccoli growth and development. Optimum air temperature for growth for broccoli is 60°-70°F (Maynard and Hochmuth, 2007), but can tolerate temperatures below freezing depending on the cultivar. Temperatures in December were higher than normal. The average high temperature was 49.1°F and the average low was 34.3°F. The monthly climatological normal temperatures in Lexington in December are 43.9°F (high) and 28.0°F (low; National Weather Service, 2020).

Although, not the highest yielding, 'Blue Wind' performed the best with respect to head uniformity, both with size of the

**Table 1.** Broccoli cultivar characteristics.

Cultivar	Days to Maturity	Description
Blue Wind	49	Blue-green, medium-size heads with small beads. Does not hold in field
Emerald Crown	59	Best for crown cut, tolerance for purpling in cold
Diplomat	68	Uniform, medium-large heads with small bead. Best in northeast and northwest
Eastern Magic	69	Blue-green heads, high stress tolerance
Arcadia	63	Large, dark green domed heads, high cold tolerance
Imperial	71	Dark green beads, grows slowly in cold

heads and the quality. The 'Blue Wind' plant is low-growing and more open compared to some of the other cultivars. 'Emerald Crown' was also low-growing, but denser and less open compared to 'Blue Wind.' 'Diplomat' had upright growth and did not spread out. Overall, there was few signs of cold damage, and none on the broccoli heads themselves, despite the low temperatures reached in November and December. Growers who are interested in a lower maintenance winter crop with market potential for school cafeterias may want to consider broccoli. With a 12-inch in-row spacing and six or seven beds in a 30 x 96 ft high tunnel, a grower could potentially have 510 to 595 broccoli plants. This kind of dense plant spacing would not be recommended in the warmer months, but would be possible in the coldest months of the year with little to no pest and disease issues. Although not part of the evaluation in this trial, multiple harvests are possible if plants are left to grow after the initial harvest of the main head. Multiple harvests may make a broccoli crop more attractive and lucrative for growers.

**Table 2.** Marketable yield and head weight and count of broccoli grown in a high tunnel from October to January in Lexington, KY.

Cultivar	Mean marketable yield (lb) <sup>z</sup>	Mean head weight (lb)	Mean marketable head count
Blue Wind	4.90 ab <sup>y</sup>	0.57 b	8.75
Emerald Crown	4.95 ab	0.53 bc	9.50
Diplomat	2.95 b	0.34 cd	7.00
Eastern Magic	4.30 ab	0.45 bcd	9.50
Arcadia	2.65 b	0.31 d	8.50
Imperial	6.23 a	0.79 a	7.75

<sup>z</sup> Mean marketable yield is based on the harvest of four replications of each cultivar with 10 broccoli plants in each treatment replicate.

<sup>y</sup> Values within the same column followed by the same letter(s) are not significantly different at  $P \leq 0.05$ .

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## Miniature Pumpkin Cultivar Trial

Daniel Becker, Dwight Wolfe, and Ginny Travis, Horticulture

Miniature pumpkins (*Cucurbita pepo*), like the pie and larger carving Jack-o-Lantern types, are valued as seasonal decoration. Since conducting the last trial in 1998 which included miniature pumpkins, many newer cultivars have become available. Cultivars were evaluated in a replicated trial to determine their performance in Western Kentucky.

### Materials and Methods

Twelve cultivars were seeded on 29 May into 50-cell plug trays filled with BM2 Seed Germination and Propagation Mix (Berger, Saint-Modeste, QC, Canada) at the University of Kentucky Research and Education Center (UKREC) greenhouse in Princeton, KY. On 18 June, seedlings were transplanted into white-on-black plastic drip irrigated beds in a single row, 1 ft apart and 6 ft center-to-center between beds (7,260 per acre plant population). Cultivars were arranged in plots consisting of 10 plants and 10 ft of bed length in a randomized complete block design with five plot replications. The trial field is a Sadler silt loam soil fertilized prior to planting according to soil test recommendations. Irrigation was provided as needed

depending on soil moisture content. Fertigation procedures followed the recommendations of ID-36 (Rudolph et al., 2020) for cucurbit crops; eight applications at about 10-day intervals supplied 5 lb/acre of actual nitrogen and a total of 40 lb for the season.

Acetamiprid (Assail 30 SG, IRAC group 4A) was applied to control squash vine borer using a hand sprayer the day after planting (19 June, 1,224 GDD base 50°F with 1 Jan. biofix), spraying for complete coverage. Pheromone trapping to monitor moth flight was not performed as emergence occurs around 1,000 GDD and their presence in the field was expected based on past experience. Two weeks later (3 July, 1,585 GDD), acetamiprid was rotated with permethrin (Pounce 3.2 EC, IRAC group 3A). A final rotation occurred on 15 July (1,923 GDD). Thereafter, insecticides were applied to control squash bugs and cucumber beetles only when necessary, as determined by integrated pest management scouting.

Foliar fungicide applications started on 15 July and continued every 10–14 days with mancozeb (Manzate ProStik, FRAC group M), pyraclostrobin (Cabrio, FRAC group 11),



and quinoxifen (Quintec, FRAC group 13) in tank mix and rotation, as necessary. Cucurbit downy mildew was confirmed in Pulaski County, KY on 30 July and at the trial field in Caldwell County, KY on 10 Aug. through diagnostic laboratory analysis. Thereafter, the spray interval occurred weekly, including mono- and dipotassium salts of phosphorous acid (Rampart, FRAC group 33), chlorothalonil (Bravo Weatherstik, FRAC group M), and Elumin (Ethanboxam, FRAC group 22) as additional control options for downy mildew. A total of seven fungicide applications were made with the final on 14 Sept., seven days before harvest.

Harvesting began on 21 Sept., 96-days post-transplanting, when most of the fruits were mature. We considered fruits mature when they had hard rinds, characteristic coloration, and firm dark green stems. Fruit was sorted into marketable and unmarketable categories based on appearance (absence or presence of defects), counted, and weighed separately to determine the yield collected from each 10-plant plot. The mean weight of marketable fruits was determined by dividing the yield by the number of fruits collected. Five randomly selected marketable fruits were measured for width across the base and rind thickness, not including stems, to the nearest quarter inch. The canopy area infected with downy mildew was visually assessed on the same day, immediately after the initial harvest using a 1–5 rating scale: 1 = 1–20%; 2 = 21–40%; 3 = 41–60%; 4 = 61–80%; 5 = 81–100%. The following week, five randomly selected fruits of each cultivar, absent of defects, were arranged on tables in the UKREC lobby as typical examples of marketable fruits. During the week, 30 participants voted for their two favorite cultivars based on personal preference. A total of 60 votes were cast. Two further harvest passes followed on 2 and 15 Oct., 106- and 121-days post-transplant, respectively. The data were statistically analyzed using SAS v.9.4 (SAS Institute, Cary, NC), subjecting it to analysis of variance (ANOVA) and means separation using Duncan's multiple range test LSD ( $P \leq 0.05$ ).

**Table 1.** Miniature pumpkin cultivar powdery mildew tolerance, downy mildew infection rating, and plant growth habit.

Cultivar	Powdery Mildew <sup>z</sup>	Downy Mildew (1-5) <sup>y</sup>	Growth Habit	Comments
Crunchkin	UN	4.6	Small bush	Shortened length, moderate vine diameter; slow, well-contained growth that does not spread widely
Flame	UN	4.0		
Orangita	IR	3.4		
Lil Orange Mon	UN	2.4	Large bush	Thick and well-anchored vines; robust, spreading, and mounded but orderly growth habit; large leaves
Gold Dust	HR	3.8	Small vine	Thin and sometimes frail vines that are easy to damage if moved; growth slows once fruiting starts
Gold Speck	IR	3.8		
WeeeeeOne	IR	4.8		
Bumpkin	IR	3.0	Large vine	Thin to moderate vine diameter, average to spreading growth that continues with fruit production
Jack-B-Quik	UN	4.0		
Munchkin <sup>x</sup>	UN	4.0		
Jill-Be-Little	R	2.0	Very large vine	Moderate vine diameter; rampant, very wide-spreading growth; fruit production continues apace
Spark	HR	2.6		

<sup>z</sup> Resistance descriptors obtained from seed source: HR = highly resistant; R = resistant; IR = intermediate resistance; UN = unknown or not specified.

<sup>y</sup> A rating of the area of leaf canopy infected with downy mildew during the initial harvest (21 Sept.). Rating scale: 1 = 1–20%; 2 = 21–40%; 3 = 41–60%; 4 = 61–80%; 5 = 81–100%.

<sup>x</sup> Noted by seed source as being more susceptible to mosaic viruses.

**Table 2.** Marketable and unmarketable yield of miniature pumpkin cultivars.<sup>z</sup>

Cultivar	Marketable					Unmarketable <sup>v</sup>	
	Yield/plot (lb)	Count/plot	Fruit wt. (oz) <sup>x</sup>	Fruit thickness (in) <sup>w</sup>	Fruit width (in) <sup>w</sup>	Yield/plot (lb)	Count/plot
Lil Orange Mon	57.0 a <sup>y</sup>	55.0	16.4	2.4	4.2	14.1 a	13.8
Bumpkin	36.0 b	65.0	8.7	2.3	3.3	10.5 ab	19.0
Spark	34.2 bc	96.0	5.7	1.7	2.8	6.5 bc	19.8
Orangita	31.8 bcd	42.8	11.8	2.6	3.5	8.1 bc	11.4
Jill-Be-Little	29.9 bcde	83.0	5.7	1.8	3.0	6.6 bc	22.4
Flame	27.1 cdef	44.2	9.8	2.3	3.3	6.4 bc	7.2
Gold Dust	23.8 cdef	67.4	5.6	1.9	2.8	4.3 c	14.6
Munchkin	20.9 def	70.0	4.8	1.7	2.7	4.0 c	11.0
Crunchkin	20.4 def	47.0	6.9	1.9	2.9	4.1 c	15.4
WeeeeeOne	19.2 ef	80.8	3.8	2.3	2.6	5.2 bc	14.8
Jack-B-Quik	18.9 ef	63.6	4.7	1.7	2.6	4.9 bc	17.0
Gold Speck	16.0 f	74.0	3.5	1.7	2.5	4.6 c	20.0

<sup>z</sup> Yield and fruit count means are for 10 plant plots with a 60 ft<sup>2</sup> area (multiply by 726 for yield or count/A). Each plot was replicated five times, and results are derived from a sample size of 50 plants/cultivar at the beginning of the trial.

<sup>y</sup> Means within columns followed by the same letter are not significantly different (Duncan's multiple range test LSD,  $P \leq 0.05$ ).

<sup>x</sup> Calculated by dividing the marketable fruit weight by the total number of fruits collected from each plot.

<sup>w</sup> Measurements were collected from five fruits/plot only during the initial harvest on 21 Sept. Fruit thickness does not include the stem (handle) and width is the measure across the base (bottom).

<sup>v</sup> Primary reasons for culling include warts caused by edema located on the fruit's base or side with soil contact, cracking due to excess moisture, rots, animal damage, discoloration, and lopsided or misshapen fruits.

## Results and Discussion

The 2020 season between June planting and final harvest in October was wetter than normal, over 25 inches of rain fell compared to 19 inches considered average for the five-month period in Princeton, KY (Kentucky Climate Center, 2020; Kentucky Mesonet, 2020). Downy mildew arrived in early August at the trial site, transported by persistent southerly winds and heavy rainfall in July. Frequent precipitation and high humidity assisted disease spread until the incidence of infection was severe, despite frequent application of systemic fungicides. Most cultivars had substantial areas of the canopy infected when

**Table 3.** Miniature pumpkin cultivar fruit characteristics and consumer preference.

Cultivar	Seed Source <sup>z</sup>	Days to Maturity <sup>y</sup>	Comments	Consumer Preference <sup>x</sup>
Lil Orange Mon	HR	100	Dark orange, mottled coloration, pebbled and shallowly ribbed surface, flattened and sometimes triangular in shape, stems very long, well-attached	5
Bumpkin	HR	85	Orange, smooth, striated to shallowly ribbed surface, variable sizes and shapes (some flat-rounded or tall-rounded), stems are long and well-attached	3
Spark	SW	90	Variegated cream and orange coloration, smooth, moderately ribbed surface, consistently flattened shape and size, stems moderate in length and secure	16
Orangita	SW	90	Dark orange, smooth surface, distinctive deeply ribbed and rounded shape (looks like a miniature pumpkin), stems moderate in length and secure	12
Jill-Be-Little	SW	100	Consistently light orange, some green flecked on surface, smooth, moderately ribbed, flattened to slightly rounded shape, stems moderate and secure	0
Flame	SW	90	Variegated cream and orange coloration, smooth and slightly pebbled texture, deeply ribbed surface, tall blocky (acorn) shape, thick and sturdy stems	7
Gold Dust	RU	95	Light orange color, some are flecked green on surface, moderately to deeply ribbed, tall flattened to rounded shape, short stems, some soft, less secure	0
Munchkin	HR	100	Dark orange, smooth, moderately ribbed surface, distinctive flattened shape (top rarely slopes, "puck-like" overall), moderate length stems, secure	10
Crunchkin	SW	100	Dark orange to slightly tan, pebbled, deeply ribbed surface, consistently tall flattened to rounded shape, short thick stems, sometimes difficult to cut	0
WeeeeeOne	RU	95	Light orange to orange, striated to wrinkled surface, rounded shape, variable sizes (small to very small), mixed seed lot, stems small, sometimes weak	7
Jack-B-Quik	RU	95	Light orange to orange, smooth surface, moderate ribbing, flattened to slightly rounded shape, some vary in size, stems secure and moderately long	0
Gold Speck	RU	95	Light orange to orange, some green flecks, smooth, shallow to moderately ribbed surface, most flat to slightly rounded, stems short to moderate length	0

<sup>z</sup> See Appendix A for seed companies and addresses.

<sup>y</sup> Average number of days from seeding to harvest according to seed source.

<sup>x</sup> Compiled from votes cast by 30 participants acting as potential consumers at the UKREC in Princeton, KY. Each participant was allowed two votes to designate their two favorite cultivars from a display containing five representative fruits from each cultivar. A total of 60 votes were collected. Cultivars with more votes indicate greater preference.

rated in late September (Table 1). Only for 'Jill-Be-Little,' 'Lil Orange Mon,' and 'Spark' was the severity not great enough to diminish leaf area, all three cultivars maintained green leaves until the final harvest and planting removal. In contrast, 'WeeeeeOne' and 'Crunchkin,' notably among others, were the first to show symptoms and were mostly defoliated after the second harvest. The earliest infected cultivars generally had the highest incidence while affected leaf area was less for those cultivars that showed noticeable symptoms later. Lower incidence may imply some tolerance, but it certainly does not imply resistance; downy mildew was present on all trial cultivars and all are considered susceptible.

The early and widespread presence of downy mildew in the field undoubtedly influenced the yield capacity of some cultivars compared to others. Severe downy mildew will diminish photosynthetic capacity, slowing or halting growth and further fruit set. Cultivars with the most incidence generally had lower yields (Table 2). In contrast, some of the best performers in terms of yield, namely 'Lil Orange Mon,' 'Bumpkin,' 'Spark,' 'Orangita,' and 'Jill-B-Little,' had moderate incidence of downy mildew. 'Lil Orange Mon' had the highest marketable yield, but was among the lowest, along with 'Orangita,' for marketable count. Both cultivars compensated for lower count numbers with heavier and larger fruit dimensions (thickness and width). 'Spark' had a higher mean marketable count than all other cultivars with the exception of 'Jill-Be-Little' and 'WeeeeeOne.' That 'WeeeeeOne' did not achieve greater yields is mainly due to low fruit weight, of which it is similar to 'Gold Speck.' The mean unmarketable yield per plot for 'Lil Orange Mon' was

significantly more than any other cultivar except for 'Bumpkin.' A majority of unmarketable fruit for both cultivars came during the final harvest when all were collected ahead of field renovation, most of these were underripe and poorly colored. The same is true for 'Jill-Be-Little' and most other cultivars, except 'Flame' which had few fruits left after the second harvest. It is probable that fewer fruits for all other cultivars would have been culled had planting occurred earlier, in the first week of June, rather than the middle of the month.

Beyond classing cultivars according to growth habit (Table 1) it is possible to categorize their fruit based on appearance (Table 3). 'Lil Orange Mon' is a "large" miniature that is dark mottled orange with squat disk-shaped fruits and long handles, somewhat similar to 'Bumpkin' which has a more traditional solid orange coloration. 'Spark' and 'Flame' are variegated cream and orange colored, with the former being flattened and the latter being "acorn"-shaped. Of the traditional orange flattened "mini-pumpkin" types there is 'Jill-Be-Little,' 'Munchkin,' 'Gold Dust,' 'Jack-B-Quik,' and 'Gold Speck.' Ones with thicker and more rounded, instead of flat fruits include 'Orangita,' 'Crunchkin,' and 'WeeeeeOne.' Voting participants acting as potential consumers preferred 'Spark' most, followed by 'Orangita' and 'Munchkin.' Appearance matters in addition to novelty; likely, the distinctive features of each and their uniformity drew favor. Yield is not the only factor to consider during cultivar selection, others include disease tolerance, growth habit, fruit size, appearance, and perhaps most important, market potential.

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SAS Institute Inc., Cary, NC, USA.

# Pie Pumpkin Cultivar Evaluation

*Chris Smigell, John Strang, and John Snyder, Horticulture; Emily Pfeufer, Plant Pathology; Bob Perry and Emily DeWitt, Dietetics and Human Nutrition*

The University of Kentucky Vegetable Production Guide for Commercial Growers (ID-36) lists only one recommended pie pumpkin cultivar. Thus, 14 cultivars were evaluated in a replicated trial to determine their performance under Central Kentucky growing conditions. Pie pumpkins are often purchased as seasonal decorations, so these were also evaluated for visual attributes. Culinary evaluations of roasted pumpkin slices and pies were also conducted.

## Materials and Methods

Cultivars were seeded on 26 May 2020 into 72-cell plastic plug trays filled with ProMix BX multipurpose media (Premier Horticulture, Inc.) at the University of Kentucky Horticulture Research Farm in Lexington. Plants were set into black plastic-mulched, raised beds using a waterwheel setter on 17 June. Plots were 15 ft long, containing seven plants of one cultivar set 30 inches apart within the row. Rows were 8 ft apart. Each plot was replicated four times in a randomized complete block design.

Fifty pounds per acre of nitrogen, phosphorus and potassium were applied as 19N-19P-19K prior to planting, and incorporated into the soil using a roto-tiller. Approximately one cup per plant of starter solution (3 lb, Miller Sol-U-Gro 12N-48P-8K in 50 gal of water) was applied at transplanting. The plot was drip-irrigated and fertigated weekly with 0.6 lb/acre of nitrogen (calcium nitrate) from 16 July through 27 Aug. for a total of six fertigations and 15 lb/acre of nitrogen. Teff grass (*Eragrostis tef*) was seeded at a rate of 36 lb/acre and lightly tilled in to suppress weed growth.

Eleven weekly fungicide sprays were applied, 24 June through 1 Sept. Fungicides included chlorothalonil (Initiate 270; 4 applications), thiophanate methyl (Topsin M; 2 applications), mancozeb (Gavel; 2 applications), penthiopyrad (Fontelis; 2 applications), pyraclostrobin (Cabrio; 3 applications), cyazofamid (Ranman; 2 applications), and propamocarb HCl (Previcur Flex; 2 applications). Scanner surfactant was mixed with fungicides on 12 and 19 Aug. Insecticides were applied weekly from 24 June through 22 July, and on 5 and 19 Aug. These included one application of esfenvalerate (Asana), and

three applications each of acetamiprid (Assail) and zeta-cypermethrin (Mustang Maxx). Clethodim (Select) herbicide was used on 5 Aug. to kill the teff grass, which had grown approximately 15 inches tall, hiding the plant runners and pumpkins growing in the row middles. All pesticide application rates were based on recommendations in the 2020-21 University of Kentucky Vegetable Production Guide for Commercial Growers (ID-36).

All pumpkins were harvested, counted, and weighed from 14 to 19 Sept., regardless of the published number of days to harvest. All pumpkins of a cultivar were then gathered and rated in the field for shape, uniformity, and attractiveness. A week later, they were stored in an unheated building until remaining evaluations began on 8 Oct. One representative pumpkin of each cultivar from each of the four replications was evaluated for size (height and width), exterior color, flesh thickness, fruit shape, stem color, diameter, and attractiveness by two horticulture department personnel. Juice was expressed from skinless pumpkin slices using a multi-purpose food processor (Omega Inc, Harrisburg, PA). Sugar content of the juice was measured as °Brix using a handheld refractometer (American Optical model 10431, Deerfield, IL). To gauge pumpkin size variability among all pumpkins of one cultivar, the coefficient of variability (CV) for pumpkin weight was calculated by dividing the sample standard deviation of the pumpkin weight by average pumpkin weight, and expressing the result as a percentage.

On 25 Aug. foliage of the cultivars was evaluated for severities of the fungal diseases downy mildew (*Pseudoperonospora cubensis*) and powdery mildew (*Sphaerotheca fuliginea*). Seven leaves per plot were evaluated for both diseases by estimating the percentages of the tops and bottoms of leaves covered by each disease separately.

All cultivars were evaluated for eating qualities. The heirloom squash 'North Georgia Candy Roaster' was also included, as it is known for making high-quality pumpkin pies. The evaluation was conducted in the kitchen of the University of Kentucky Dietetics and Human Nutrition Program. The evaluators included the authors and three students. All pumpkins/squash were cut top-to-bottom, cleaned of seeds, and cut into



**Table 1.** Pumpkin yields, weight, and fruit dimensions.

Cultivar	Seed Source	Days To Harvest <sup>1</sup>	Total Marketable Yield (lb/acre)	Pumpkins (no./acre)	Average Fruit Weight (lb)	Fruit Weight Variability (CV) <sup>2</sup>	Cull (% weight) <sup>3</sup>	Fruit Height (in) <sup>4</sup>	Fruit Width (in) <sup>4</sup>	Flesh Thickness (in) <sup>4</sup>			
Bisbee Gold	RU	90	33,500	a <sup>5</sup>	7,160	bcde	4.7	c	22	0.0	6.5	6.9	1.1
Speckled Hound (squash)	SW	95	33,400	a	6,920	bcde	4.8	bc	33	2.8	4.8	7.7	1.5
Baby Wrinkles	CL	105	28,400	b	5,210	f	5.4	b	28	2.2	4.8	7.0	1.1
Darling	RU	90	28,300	b	6,770	cde	4.2	cd	25	8.3	4.8	5.7	1.1
Lumina (squash)	SW	90	27,500	bc	2,860	g	9.6	a	35	0.0	4.8	8.8	1.4
Fall Splendor Plus	CL	105	25,000	bcd	6,850	bcde	3.7	def	27	0.0	4.8	6.2	1.1
New England Pie	JO	105	24,300	bcd	8,320	ab	3.0	fgh	33	0.0	4.8	5.9	0.9
Jack Sprat	SW	100	22,900	cde	9,800	a	2.3	hi	25	1.6	4.8	5.4	0.8
Mystic Plus	CL	105	22,800	cde	5,830	ef	3.9	de	20	1.2	4.8	6.8	1.1
Spookie	HO	90	21,800	def	6,770	cde	3.3	ef	39	0.4	5.8	6.3	1.2
Small Sugar New England	HO	100	20,400	def	6,770	cde	3.1	fg	32	1.7	4.8	6.1	1.1
Little Giant	SW	105	18,900	ef	8,010	bc	2.4	ghi	37	4.4	4.8	5.2	0.8
Baby Pam	RU	100	17,100	fg	7,550	bcd	2.2	i	31	1.8	4.8	5.2	1.0
Cinnamon Girl	JO	85	13,800	g	6,380	def	2.2	i	25	8.1	4.8	5.6	0.8
Naked Bear	SW	105	13,400	g	6,300	def	2.2	i	30	0.8	4.8	5.4	0.7

<sup>1</sup> Days to harvest from seed catalogs.

<sup>2</sup> CV = coefficient of variability; a smaller CV means that there is less of a spread of harvested pumpkin weights, compared to a cultivar with a higher CV.

<sup>3</sup> Weight of culled pumpkins divided by sum of marketable + immature+ culled pumpkins X 100.

<sup>4</sup> Values are the average of four pumpkins, one sampled from each replicate.

<sup>5</sup> Means in column followed by the same letter are not significantly different (Waller-Duncan Multiple Range Test LSD, P < 0.05).

one-inch thick half-circles. All samples were coated with a light film of canola oil, sprinkled with kosher salt, and placed on parchment paper covered aluminum trays. Samples were baked in a convection oven at 400°F for about 20 minutes until done. As soon as samples cooled, they were evaluated for color (light yellow to orange), intensity of aroma, sweetness and flavor, firmness while chewing, texture (soft/creamy to stringy/grainy), and overall appeal.

Five cultivars and the 'North Georgia Candy Roaster' were also used to make pies. Cultivars were chosen based on the "good for pies" description in seed catalogs and prior experience with the 'North Georgia Candy Roaster.' Fillings consisted of 15 oz of roasted pumpkin, 14 oz of sweetened, condensed milk, two large eggs, two teaspoons of pumpkin pie spice, and a half-teaspoon of kosher salt. The ingredients were mixed well and poured into commercially-produced, frozen pie crusts. Pies were baked in a preheated oven at 425°F for 15 minutes, and another 35 minutes at 350°F. Pies were at room temperature when evaluated by authors and students. Valerie Powell of Trike Bake in Paris, Kentucky, also baked several of the pumpkin cultivars and her comments are found in Table 4.

## Results and Discussion

Throughout the growing season, the weather was cool with only one day reaching 90°F between the planting date and harvest. During this period 14.7 inches of rain fell. The 5.4 inches in July were 0.6 inches above the local monthly average, and the 3.5 inches in August were 0.2 inches below average for August. Although the cultivars' advertised days to harvest ranged from 85 to 105 days, all cultivars were harvested at about 90 days after planting. Very few immature pumpkins remained in the field by then, and several cultivars had dried stems and dead vines, indicating they were overripe.

Teff grass, when planted early, can effectively restrict weed growth in row middles. However, tilling the teff seed in delayed

emergence, and weed growth was not sufficiently inhibited. The herbicide (Select) was slow to kill the teff at the late stage of growth at which the herbicide was applied. Although the teff was dead and dry by harvest, it still hampered tracing vines and pumpkins back to their source plants for positive identification. Commercial producers are advised to broadcast teff seed, without tilling, and prior to a rain for better germination, and better weed control. If using an herbicide to manage teff, it should be killed with a graminicide when it is approximately 8 inches in height. The other option is to apply a preemergent herbicide at planting for weed control.

## Field Trial Results

Figure 1 displays all tested cultivars. Cultivars are ranked in Tables 1 and 2 by the total marketable yields. While pounds per acre and fruit counts are important, size, color, and shape uniformity as well as stem attractiveness are also important for producers selling decorative pumpkins. 'Speckled Hound' and 'Lumina' are not pumpkins, but rather squashes. Both were deeply ribbed and quite variable in size, compared to the pumpkins. Most pumpkin cultivars had shallow ribbing, smooth skin with no warts, and, except for 'Darling', were round to slightly taller than wide. Based on attributes in these tables, the top-performing cultivars were 'Bisbee Gold', 'Baby Wrinkles', 'Darling', 'Fall Splendor Plus', 'Jack Sprat', 'Little Giant' and 'Cinnamon Girl'. 'Bisbee Gold' (Figure 2) stood out as one of the most attractive and highest-yielding pumpkins, with a very consistent shape, dark orange color with thick, green to dark green stems. It also had the second-least variability in pumpkin weight (CV), and was one of the largest pumpkins in the trial. 'Baby Wrinkles' was the heaviest pumpkin; the yield weight was high, but the number of pumpkins per acre was low. It was also dark orange, with dark green stems of varying thickness, and had a high sugar content. 'Darling' (Figure 3) was the only tall, oblong pumpkin. It was also dark orange, with dark green prominent buttressed stems,



**Figure 1.** Pumpkins: 1. Baby Pam, 2. Small Sugar New England, 3. Cinnamon Girl, 4. New England Pie, 5. Naked Bear, 6. Baby Wrinkles, 7. Fall Splendor Plus, 8. Little Giant, 9. Speckled Hound, 10. Darling, 11. Jack Sprat, 12. Mystic Plus, 13. Lumina, 14. Bisbee Gold, 15. Spookie.

that rated highest for stem attractiveness among all cultivars. ‘Darling’ pumpkins were uniform in shape and weight. Its sugar content was 5.3 °Brix, and it had a high percentage of culls due to four off-type fruit. ‘Fall Splendor Plus’ (Figure 4) produced a high number of pumpkins per acre having similar weights. The fruit of this cultivar were attractive, a little wider at the bottom than the top, and medium orange with long, green stems. It had a high sugar content. ‘Jack Sprat’ (Figure 5) produced the most pumpkins per acre of all cultivars tested, and they had low weight variability. It was very attractive, medium orange with dark green stems, and had a high sugar content. ‘Little Giant’ (Figure 6) and ‘Cinnamon Girl’ were among the smallest pumpkins, dark orange with very shallow ribbing and very attractive stems. Both had high sugar content, with ‘Little Giant’ having the highest mean sugar content of any cultivar in the trial. ‘Little Giant’ also yielded the third-highest number of pumpkins per acre in the trial. ‘Naked Bear’ (Figure 7) is unusual in that its seeds do not have seed coats, hence the name. The seeds can be roasted and sold as shell-free seeds.

**Table 2.** Cultivar mean<sup>1</sup> evaluation ratings and comments.

Cultivar	External Appearance (1-5) <sup>2</sup>	Shape Uniformity (1-5) <sup>2</sup>	Fruit Shape (1-4) <sup>3</sup>	Smoothness (1-5) <sup>4</sup>	Ribbing (1-5) <sup>5</sup>	Stem Diameter (in)	Stem Appearance (1-5) <sup>2</sup>	Sugar Content (°Brix) <sup>6</sup>	Comments and Disease Resistance <sup>7</sup>
Bisbee Gold	4.5	4.0	3.0	2.3	2.8	1.4	3.6	8.6	Most fruit near plant base; thick, straight, dark green stems; uniform size and dark orange color; attractive; good pie size
Speckled Hound (squash)	3.5	3.0	1.0	4.0	4.3	0.8	2.5	7.2	A squash; most fruit near plant base; short, tan stems; variable sizes, most good for one pie; varies from orange with few green spots to green with few orange spots; corky warts; IR: zucchini yellow mosaic virus
Baby Wrinkles	4.1	4.0	2-4	2.1	2.7	1.1	3.9	7.8	Long vines, scattered fruit; most fruit dark orange; straight, attractive stems vary in thickness; IR: PM
Darling	4.5	4.4	3.0	3.5	2.1	0.9	4.8	5.3	Most fruit near plant base; nice, short, buttressed stems; some darker skin freckles; nice size for a pie; a tall pumpkin; IR: PM
Lumina (squash)	2.0	2.0	2.0	4.3	4.7	0.7	2.0	8.0	A squash; very long vines, scattered fruit; stubby weak stems; variable fruit size; white to bluish gray with white streaks; turns gray if left in the field too long after maturity; corky warts
Fall Splendor Plus	4.1	3.6	2.0	2.5	2.9	1.0	4.5	8.1	Most fruit near plant base; long, dark green stems; fruit size varies; most fruit wider at bottom; IR: PM
New England Pie	3.5	4.0	2.3	3.0	2.3	0.8	3.0	7.5	Long, thin vines, scattered fruit; variable stem thicknesses; most fruit light orange
Jack Sprat	4.6	4.1	2.5	3.0	2.1	1.0	4.5	8.3	Moderately long vines; medium length stems; uniform color, attractive; IR: PM
Mystic Plus	4.1	4.3	2.0	2.2	2.6	1.2	3.4	7.3	Moderately long vines; long, thick, straight stems; mostly uniform color among fruit; good size for one pie; IR: PM
Spookie	3.9	3.0	2.8	2.9	2.8	0.8	3.0	5.9	Long vines, scattered fruit; good, long, thin stem; variable fruit shape, size & color
Small Sugar New England	3.3	3.3	2.0	3.1	2.5	1.0	2.8	5.6	An old standard; most fruit near plant base; long, thin stems; variable fruit sizes
Little Giant	4.6	4.1	3.5	3.8	1.9	1.0	4.6	8.9	Most fruit near plant base; strong, dark, buttressed, attractive stems; uniform fruit shape & color; attractive; IR: PM
Baby Pam	3.7	4.4	2.5	4.1	2.0	0.8	2.6	6.7	Long vines, scattered fruit; long, thin stems with varying thicknesses
Cinnamon Girl	4.3	4.2	2.0	3.0	1.9	1.1	4.3	8.0	Most fruit near plant base; long, dark green stems fading to tan; straight & curved stems; several rot culls; IR: PM
Naked Bear	2.8	4.3	2.0	4.1	2.5	1.2	2.3	7.9	Most fruit near plant base; fruit are close to yellow; decent-sized seeds with no shells; IR: PM

<sup>1</sup> Values are the average of 4 pumpkins, one sampled from each replicate.

<sup>2</sup> 1 = poor; 5 = excellent.

<sup>3</sup> 1 = flattened, 2 = oval, 3 = blocky, 4 = round, 5 = highly variable.

<sup>4</sup> 1 = smooth, 5 = rough and warty.

<sup>5</sup> 1 = no ribbing, 5 = deep ribbing.

<sup>6</sup> Refractometer measurement of soluble solids (primarily sugars) in pumpkin juice sample.

<sup>7</sup> Disease resistances from seed catalogs: IR = intermediate resistance; PM = powdery mildew.

### Disease Ratings

All cultivars had both powdery and downy mildew by the time the disease ratings occurred. Powdery mildew was severe, with the percent of lower leaf surface covered by this fungus ranging from 44% to 80% for different cultivars (Table 3). Powdery mildew on upper leaf surfaces ranged from 4% to 20%. The two squash, ‘Speckled Hound’ and ‘Lumina,’ had the lowest powdery mildew severity ratings on upper leaf surfaces and among the lowest for lower leaf surfaces. Cultivars with advertised powdery mildew resistance did not, as a group, tend to have lower severity ratings. ‘Baby Pam,’ ‘Baby Wrinkles,’ ‘Bisbee Gold’ and ‘Jack Sprat’ had some of the lower powdery mildew severity ratings. Downy mildew was less severe, with the percent lower leaf area affected with downy mildew ranging from 5% to 23% for different cultivars. Upper leaf surface affected by downy mildew ranged from 1% to 9%. Again, ‘Baby Pam,’ ‘Baby Wrinkles,’ ‘Bisbee Gold’ and ‘Jack Sprat,’ plus ‘Little Giant,’ ‘Naked Bear’ and ‘Cinnamon Girl’ had some of the lower downy mildew severity ratings.

### Culinary Evaluation Results

Based on the evaluation of roasted pumpkin samples (Table 4), ‘Speckled Hound,’ ‘Lumina,’ ‘Cinnamon Girl,’ ‘Little Giant’ and ‘Jack Sprat’ rated the highest. ‘Speckled Hound,’ ‘Lumina’ and ‘North Georgia Candy Roaster’ are not pumpkins, but squash types. They have a smooth texture, with no stringiness or granular structure found in nearly all the pumpkins in this evaluation. All of these top performers rated highly for chewing softness, smooth texture, aroma and sweetness, but not necessarily for strong flavor. There was not much variability

**Table 3.** Powdery and downy mildew severity ratings conducted on 25 Aug. 2020.

Cultivar <sup>1,2</sup>	Powdery mildew		Downy mildew	
	Leaf under sides <sup>3</sup>	Leaf top sides <sup>3</sup>	Leaf under sides <sup>3</sup>	Leaf top sides <sup>3</sup>
Speckled Hound	44.1	7.3	16.8	4.1
Lumina	55.5	3.8	7.6	1.7
Baby Pam	56.8	6.8	4.6	1.3
Baby Wrinkles (pm)	58.1	9.8	8.8	4.1
Jack Sprat (pm)	60.4	13.0	6.4	1.3
Bisbee Gold	61.1	7.1	7.1	2.3
Mystic Plus (pm)	71.3	15.0	12.7	4.3
New England Pie	73.1	12.3	22.6	9.3
Spookie	73.6	10.5	21.2	5.5
Little Giant (pm)	75.0	16.7	5.0	1.0
Fall Splendor Plus (pm)	75.5	19.5	9.5	3.3
Darling (pm)	76.4	15.2	10.0	3.0
Naked Bear (pm)	76.9	19.8	8.3	4.3
Small Sugar New England	80.2	12.6	11.2	3.6
Cinnamon Girl (pm)	80.2	14.8	6.4	2.4

<sup>1</sup> Ranked in increasing percent coverage by powdery mildew on the underside of leaves.

<sup>2</sup> (pm) indicates that powdery mildew resistance was advertised in seed catalogs.

<sup>3</sup> Averages of seven sampled leaves.

of flavor and aroma ratings among the cultivars, but the flavor and aroma ratings of the three squash cultivars were significantly higher than most of the pumpkins. Five pumpkin cultivars and the ‘North Georgia Candy Roaster’ were made into pies. All the pies rated similarly for color and texture (Table 5). The ‘North Georgia Candy Roaster’ pie rated highest for overall appeal, aroma, sweetness, softness, and smooth texture. ‘Fall



**Figure 2.** Bisbee Gold.



**Figure 3.** Darling.



**Figure 4.** Fall Splendor Plus.



**Figure 5.** Jack Sprat.



**Figure 6.** Little Giant.



**Figure 7.** Naked Bear.

Photos by Steve Patton



**Table 4.** Roasted pumpkin evaluation data and comments.

Cultivar	Color (1-5) <sup>1</sup>	Aroma (1-5) <sup>2</sup>	Sweetness (1-5) <sup>2</sup>	Flavor (1-5) <sup>2</sup>	Firmness (1-5) <sup>3</sup>	Texture (1-5) <sup>4</sup>	Overall Appeal (1-5) <sup>2</sup>	Comments
Speckled Hound (squash)	3.9	3.3 ab <sup>5</sup>	3.9 a	4.1 a	4.7 a	4.9 a	3.9 a	Fruity flavor, strong flavor, very smooth texture, slightly bitter, dark orange
Lumina (squash)	2.4	3.4 a	3.4 ab	3.7 ab	4.6 a	3.9 bc	3.6 ab	Slight fishy aroma, distinct flavor, smooth texture, slight green color
Cinnamon Girl	2.7	2.3 bcd	2.7 bcd	2.7 bcd	3.6 bcd	3.4 cdef	3.4 abc	Good mouthfeel, light sweet flavor and delicate texture, flesh a little dry
Little Giant	2.2	2.1 cd	3.1 abc	2.9 bcd	3.9 ab	3.5 cde	3.3 abc	Tender and easy to cut up, very sweet, full-flavored and rich, flesh slightly dry and fibrous
Jack Sprat	2.3	2.1 cd	2.4 cdef	3.0 bcd	3.7 bc	3.8 bcd	3.2 abc	Delicious, unique savory flavor
North Georgia Candy Roaster (squash)	1.7	2.6 abc	2.6 bcde	3.1 bcd	4.6 a	4.6 ab	3.1 abcd	Delicious
Bisbee Gold	2.7	2.1 cd	1.9 def	2.6 cd	2.9 cde	2.1 g	2.9 bcde	Good sweet buttery mild flavor
Spookie	3.5	2.6 abc	2.1 def	3.3 abc	3.4 bcd	2.6 efg	2.9 bcde	Distinct savory flavor
Fall Splendor Plus	2.2	2.1 cd	1.9 def	2.6 cd	3.1 bcd	2.4 fg	2.7 bcde	Good, mild flavor, not sweet, looks stringy, but has good mouthfeel
Baby Wrinkles	2.5	2.0 cd	1.9 def	2.1 d	2.1 ef	2.6 efg	2.6 cdef	Mild flavor, neutral flavor, faint aroma
Baby Pam	2.6	1.6 cd	1.9 def	3.0 bcd	1.4 f	3.1 cdefg	2.4 cdef	Strong savory flavor, not stringy
New England Pie	2.9	1.8 cd	1.9 def	2.5 cd	2.7 de	3.1 cdefg	2.4 cdef	Neutral flavor, slightly bitter, smooth texture
Small Sugar New England	3.6	2.4 bcd	1.4 f	2.7 bcd	3.4 bcd	2.8 defg	2.4 cdef	Delicious
Naked Bear	2.3	2.3 bcd	2.2 cdef	3.0 bcd	4.0 ab	3.1 cdefg	2.2 def	Unique flavor, slightly bitter, smooth
Mystic Plus	2.4	1.5 d	1.9 def	3.0 bcd	2.1 ef	2.2 g	2.1 ef	Savory flavor, bitter, bad mouthfeel
Darling	2.9	2.3 bcd	1.6 ef	2.4 cd	1.7 f	2.3 fg	1.6 f	Bland, watery, fibrous

<sup>1</sup> 1 = lightest, 5 = darkest.

<sup>2</sup> 1 = least, 5 = most; a high flavor rating indicates flavor intensity, not necessarily good flavor.

<sup>3</sup> 1 = firmest to chew, 5 = softest.

<sup>4</sup> 1 = fibrous or grainy texture, 5 = smoothest texture.

<sup>5</sup> Means in column followed by the same letter are not significantly different (Waller-Duncan Multiple Range Test LSD P < 0.05).

Splendor Plus' was the highest-rated pumpkin overall. All evaluators considered 'Baby Pam' inedible because of its bitterness. The catalog advertises it as excellent for pies, so the bitterness may have been due to a problem with the particular pumpkin chosen for the pie.

## Summary

Considering yield, fruit attractiveness, roasted pumpkin evaluations, and powdery- and downy mildew resistance ratings, 'Bisbee Gold', 'Baby Wrinkles', 'Fall Splendor Plus' and 'Jack

Sprat' were the best pumpkins in this trial. 'Speckled Hound' was the better-performing squash. Although few cultivars were made into pies and evaluated, cultivars with high-quality roasted flesh tended to make high-quality pies.

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**Table 5.** Pie evaluation data and comments.

Cultivar	Color (1-5) <sup>1</sup>	Aroma (1-5) <sup>2</sup>	Sweetness (1-5) <sup>2</sup>	Flavor (1-5) <sup>2</sup>	Firmness (1-5) <sup>3</sup>	Texture (1-5) <sup>4</sup>	Overall Appeal (1-5) <sup>2</sup>	Comments
North Georgia Candy Roaster (squash)	2.7	3.0	3.3	3.3	4.7	4.6	4.4	Smooth mouth feel, slightly sweet
Fall Splendor Plus	2.5	1.8	2.8	2.8	3.5	3.4	3.7	Good balance, not too sweet, visually appealing, strong flavor
New England Pie	3.2	2.7	3.2	3.3	3.8	2.6	3.3	Pasty, chunky, strong flavor, more texture, no aroma, bland
Small Sugar New England	2.7	1.7	2.4	2.9	3.7	3.2	3.1	Some bitterness, chunky, strong flavor, pleasant taste
Mystic Plus	2.7	2.2	2.8	3.1	4.2	3.5	3.1	Distinct savory flavor
Baby Pam	3.2	2.0	1.0	4.2	4.0	3.6	1.0	Bitter, salty

<sup>1</sup> 1 = lightest, 5 = darkest.

<sup>2</sup> 1 = least, 5 = most; a high flavor rating indicates flavor intensity, not necessarily good flavor.

<sup>3</sup> 1 = firmest to chew, 5 = softest.

<sup>4</sup> 1 = fibrous or grainy texture, 5 = smoothest texture.

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## Yield and Zingiberene Content of Two Interspecific Hybrid Tomato Lines Grown in the Open Field

Mohammad H. Dawood, Department of Horticulture, College of Agriculture, Food and Environment, University of Kentucky, and Department of Horticulture and Landscape, College of Agriculture, University of Kufa, Iraq  
John C Snyder, Department of Horticulture, College of Agriculture, Food and Environment, University of Kentucky

Worldwide, tomato is the second most significant vegetable crop, next to potato. Current world production amounts to approximately 200 million tons on 12 million acres (FAOSTAT, 2017). In modern breeding programs, genetic variation available in wild tomato relatives has often been the source of characteristics used to breed for enhanced yield, fruit quality, disease and insect resistance (Rick and Chetelat, 1995). Yield is a genetically complex character and genetic selection for yield requires tremendous attention by the breeder. An increase in yield and quality of self-pollinated crops such as tomato is usually accomplished by choosing those genotypes that have the desired combination of phenotypic characters (de Souza et al., 2012). It is extremely important to understand the extent of genetic diversity available to improve the yield of tomatoes (Bhattarai et al., 2016).

The sesquiterpene hydrocarbon, 7-epizingiberene, is a semi-volatile compound naturally synthesized by plants of *Solanum habrochaites*, a wild relative of cultivated tomato. 7-epizingiberene is one of the main anti-insect chemicals present in its leaf trichomes (Antonious and Kochhar, 2003; Antonious and Snyder, 2006; Snyder et al., 1993). Tomato breeders around the world are attempting to introgress high levels of 7-epizingiberene from wild tomatoes into cultivated types. Their intent in doing so is to improve insect resistance of tomato because 7-epizingiberene has been associated with resistance to arthropods such as spider mites, aphids, and whiteflies (Aragão et al., 2000; Bleeker et al., 2011; Freitas et al., 2002; Gonçalves et al., 2006; Maluf et al., 2001; Weston and Snyder, 1990). Since 7-epizingiberene is an oily compound, the tomato plant expends a great deal of energy to synthesize it, and because of this, there may be a negative association between yield and production of 7-epizingiberene. Also, yield in interspecific hybrids may be reduced due to genic incompatibilities, often referred to as Bateson-Dobzhansky-Muller interactions. This research is the second report of yield for interspecific hybrid tomatoes having high concentrations of 7-epizingiberene. In 2019, we reported yield for 13 interspecific hybrid breeding lines representing two distinct families, D90 and F22 (Dawood and Snyder, 2019). Yield was compared to those of two modern tomato cultivars, 'BHN589' and 'Red Deuce'. The breeding lines produced 15 to 18 lb per plant, compared to 23-25 lb per plant for the commercial cultivars, an expected result because the recurrent parent of the interspecific hybrids was a very early variety. Unfortunately, plants of the recurrent parent

of the interspecific hybrids, 'Zaofen 2' were not available for the 2019 trial, so the yields of the breeding lines could not be directly compared to the yield of 'Zaofen 2', the most relevant comparison. Consequently, in 2020 we conducted a limited tomato yield trial that allowed comparison of the yields of two interspecific hybrid lines, one from each of the previously tested families, to the yields of the recurrent parent and standard tomato cultivars.

### Materials and Methods

The experiment took place in 2020 at the University of Kentucky Horticulture Research Farm, Lexington, KY. Each experimental plot consisted of four tomato plants spaced 2 ft apart within the row, and rows were set on 7-ft centers in raised beds with trickle irrigation and black plastic mulch. The statistical design was a randomized complete block design that included two interspecific hybrid breeding lines, the donor parent and three commonly grown, F1 hybrid tomato cultivars in each of four blocks. The cultivars evaluated were 'Mountain Fresh Plus,' 'BHN589' and 'Red Deuce'. The two breeding lines were BC3F7 generation lines obtained from crossing between a wild tomato relative, *S. habrochaites* (LA2329) and the donor parent, 'Zaofen 2', an early, pink-fruited, determinate variety released in 1962. The BC3F7 lines had been selected for high yield and for high zingiberene production and one was chosen from the D90 family (line SG87) and one from the F22 family (line SH13). On 10 Apr., seeds were soaked in 50% sodium hypochlorite for 30 minutes, rinsed in tap water and were then directly sown into 72-cell flats containing compost-based potting soil (Fort Light, Vermont Compost Co., Montpelier, VT). Transplanting occurred on 26 May. Transplant and field production cultural methods were followed in accordance with UK ID-36 (<http://www2.ca.uky.edu/agcomm/pubs/id/id36/id36.pdf>), except that plants were not pruned.

### Harvest

Harvest began on 25 July and plants were harvested weekly for four weeks. Harvested tomatoes were weighed and counted.

### Determination of 7-epizingiberene in Leaves of Plants

The center third portion of one leaflet from the third or fourth leaf positions on each of the four plants in a plot was placed into a 20 mL vial and then 2 mL of *n*-hexane containing 20 µL/L of *n*-tetradecane as internal standard was added. Vi-

als were vortexed for 30 seconds. Subsequently, the 7-epizingiberene content of the extract was determined by gas chromatography and area of the extracted leaflets was determined by image analysis. Results were expressed as  $\mu\text{g}$  of 7-epizingiberene/ $\text{cm}^2$  of leaflet.

### Statistical Analysis

All data were analyzed using the GLM procedure of SAS version 9.4 statistical software (SAS Institute, Inc., Cary, NC; Der and Everitt, 2015). Means were compared using Duncan's Multiple Range Test.

## Results and Discussion

Analysis of variance revealed significant differences among lines for yield, number of fruits per plant, and average fruit weight. Fruit number per plant was significantly higher in the recurrent parent and interspecific lines compared to the three tomato cultivars (Table 1). For the interspecific hybrid lines and recurrent parent, average number of fruits per plant ranged between 53 and 58, contrasted to 32 to 38 fruit per plant for the three cultivars. For the cultivars, the range for average total fruit weight per plant was narrow, ranging from 21.6 to 22.8 lb/plant (Table 1). The range for total weight for the breeding lines and recurrent parent was similarly narrow, but lower, 16.9 to 17.8 lb/plant. Fruit from the interspecific hybrids and recurrent parent were smaller than those of the cultivated lines; fruit size for the former ranged between 9.1 and 11.3 oz/fruit and for the latter, the range was 4.5 to 5.5 oz/fruit (Table 1).

As expected, 7-epizingiberene was present only on leaves of the two interspecific hybrid and was not detected on leaves of the parental line or on the cultivars (Table 1). Quantities of 7-epizingiberene on the interspecific hybrids were very similar to those present on these hybrids when grown in 2019 (Dawood and Snyder, 2019).

This experiment, in addition to the information published in 2019 (Dawood and Snyder, 2019) provides a snapshot of field performance of an inbred population, an F7 resulting from seven successive self-pollinations of a relatively advanced interspecific backcross generation, a BC3. Yields of the interspecific hybrids were similar in 2020 to those observed in 2019, and yield of the interspecific hybrids were statistically indistinguishable from the yield of the recurrent parent. 7-Epizingiberene was present on the two interspecific hybrids, and its concentration on SG87 was the same as that routinely observed on the donor parent, *Solanum habrochaites* accession LA2329. Of the two interspecific hybrids tested in 2020, SG87 had numerically higher 7-epizingiberene concentration and numerically lower yield compared to SH13. In 2019, we reported a negative correlation between yield and 7-epizingiberene concentration for the interspecific hybrids, supporting the idea that there may be a yield penalty associated with high zingiberene production. Results obtained in 2020 with regard to relationship between yield and 7-epizingiberene concen-

**Table 1.** Yield and total numbers of fruit per plant, average fruit size and foliar 7-epizingiberene concentration for two breeding lines and their recurrent parent, and three standard F1 hybrid tomato cultivars grown in 2020.

Variety or line name	Type	Yield (lb/plant) <sup>1</sup>	Fruit number (no/plant)	Fruit size (oz/fruit)	7-Epizingiberene concentration ( $\mu\text{g}/\text{cm}^2$ )
Zaofen 2	Recurrent Parent	17.8 b	53 a	5.5 c	0 c
SG87	Breeding line	15.9 b	58 a	4.5 d	40 a
SH13	Breeding line	17.6 b	54 a	5.3 c	24 b
Mountain Fresh Plus	Cultivar	21.8 a	38 b	9.1 b	0 c
Red Deuce	Cultivar	22.8 a	32 b	11.3 a	0 c
BHN589	Cultivar	21.6 a	32 b	10.9 a	0 c

<sup>1</sup> Means within a column followed by the same letter are not significantly different as determined by Duncan's Multiple Range Test,  $P = 0.05$ .

tration are consistent with a negative relationship. However, whether this relationship is causal needs additional research.

## Conclusion

The current study revealed that the fruit number per plant in interspecific hybrid families was higher than that in the tested tomato cultivars while total yield per plant was higher in cultivars than that in interspecific hybrids. Yields for the interspecific hybrids was similar to that of the recurrent parent and in the case of one of these hybrids 7-epizingiberene content was similar to that of the wild donor parent. All in all, we believe we have made good progress in the introgression of 7-epizingiberene into cultivated tomato. Despite the breeding and selection challenges that can occur in interspecific hybrid development, we were successful in breeding interspecific hybrids having high yield similar to their recurrent parent and 7-epizingiberene production similar that in the wild donor parent. These results suggest that it may be possible to breed tomatoes with both high yield and sufficient 7-epizingiberene contents in their leaves, which could lead to genetic improvement in tomato plant pest resistance and yield production and could also lead to reduction or elimination of insecticide use.

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## Evaluation of Tomato Grafting for Improved Production in High Tunnels

Rachel Rudolph, Horticulture

High tunnels are passively heated and cooled temporary structures used to extend the growing season for high value crops. They provide protection from the weather and serve as a moderately controlled environment. High tunnels have the potential to provide the grower with the ability to extend the growing season and moderate the environment with respect to precipitation and temperature. This can translate to increased income from increased marketable yields and improved produce quality. Nearly 1,200 high tunnels have been installed in the state of Kentucky (KY) since the inception of the High Tunnel System Initiative within the Natural Resource Conservation Service Environmental Quality Incentives Program (NRCS EQIP) in 2012 (NRCS, 2020). High tunnels play an important role in the local food system of Kentucky.

High tunnel production is nuanced and growers face unique production challenges from pests and diseases. One of those particular challenges are plant-parasitic nematodes, specifically the root knot nematode (RKN; *Meloidogyne* spp.). RKN invade plant roots and cause root gall. This impairs root function, which causes reduced water and nutrient uptake. RKN have a wide host range, including nearly all vegetable crops. Tomatoes are the most high-value crop grown in high tunnels in KY and the most common high tunnel crop (Carey et al., 2009; Chase and Naeve, 2013). Infection of RKN into plant roots also opens up wounds in the roots that increase the likelihood of infections by soilborne pathogens, such as *Verticillium* or *Phytophthora*. This can be devastating for a tomato crop. Several KY high tunnel growers have already observed issues with RKN. In 2018, 11 different vegetable samples from

nine different counties were submitted to the University of Kentucky Plant Disease Diagnostic Lab all were exhibiting symptoms of RKN infection. Ten of the nine samples were from crops grown in a high tunnel (Julie Beale, personal communication).

There are fumigants and nematicides labeled for use on tomatoes. However, they are either not permitted for use in high tunnels, are extremely costly for growers, or are only moderately effective against plant-parasitic nematodes. There is a need for another management technique that is both affordable, sustainable, and effective. Grafting is a management method that growers could incorporate into their production system with relative ease. Grafting involves combining the desirable fruit characteristics of one plant (called the scion) with the desirable root characteristics of another plant (called the rootstock). Desirable root characteristics include plant-parasitic nematode and disease resistance, stress tolerance (such as drought or heat stress), and vigorous root systems. Grafting is used both in the United States and around the world to improve plant development, vigor, quality, and yield. However, very few growers in KY are familiar with grafting and even fewer growers actually utilize grafting in their production system.

The right rootstock for KY high tunnel production needs to be tested and evaluated as not all rootstock is resistant to RKN and not all rootstock will be suitable for KY high tunnel production because of the high temperatures that can be reached during the summer months, this includes air and soil temperatures. Additionally, there are two different species of RKN present in Kentucky. The resistant rootstocks are only re-

**Table 1.** Yield of non-grafted and grafted tomato plants grown in a high tunnel in Knox County, Kentucky, in 2019.

Treatment	Mean marketable yield (lb)	Total marketable yield <sup>z</sup> (lb)	Estimated mean yield per plant (lb)
Non-grafted Primo Red	142.74 <sup>by</sup>	713.70	15.86
Primo Red + Arnold	193.04 <sup>a</sup>	965.20	21.45
Primo Red + Estamino	213.10 <sup>a</sup>	1,065.50	23.68
Primo Red + Maxifort	190.64 <sup>a</sup>	953.20	21.18

<sup>z</sup> Yields are from 45 plants per treatment (9 plants per treatment plot x 5 replications).

<sup>y</sup> Values within the same column followed by the same letter(s) are not significantly different at  $P \leq 0.05$ .

**Table 2.** Root knot nematode (RKN) juvenile population densities in roots and soil of non-grafted and grafted plants grown in a high tunnel in Knox County, Kentucky, in 2019.

Treatment	Mean RKN/100 cc of soil	Mean RKN/g of root
Non-grafted Primo Red	2,044 <sup>az</sup>	1
Primo Red + Arnold	20 <sup>bc</sup>	1
Primo Red + Estamino	0 <sup>c</sup>	0
Primo Red + Maxifort	174 <sup>b</sup>	11

<sup>z</sup> Values within the same column followed by the same letter(s) are not significantly different at  $P \leq 0.05$ .

sistant to *Meloidogyne incognita*, not *M. hapla*. The objective of this study was to evaluate grafting as a non-chemical method for management of RKN in Kentucky high tunnel systems. Grafted plants were evaluated based on their resistance to RKN (population densities in both plant roots and soil), plant vigor, stem diameter, and marketable fruit yield.

## Materials and Methods

A trial was conducted in 2019 in a commercial high tunnel in Knox County, Kentucky. A soil sample collected in the fall of 2018, approximately six months before the start of the experiment, revealed there were approximately 710 RKN/100 cc of soil. This is considered a high population density and management action is required in order to continue future (University of Clemson Nematode Assay Lab). The species of RKN has been confirmed as *M. incognita* by DNA sequencing (North Carolina Department of Agriculture, Agronomic Division, Nematode Assay Section). The experiment comprised of 20 rows each containing nine tomato plants. The in-row spacing was 12 inches and the between row spacing was 3 ft. A soil sample was collected prior to planting. The soil characteristics in this high tunnel were as follows: 736 lb/acre P, 1517 lb/acre K, 210 lb/acre Mg, 11013 lb/acre Ca and soil pH 7.6. The grower applied 50 lb/acre of N (calcium nitrate) prior to transplanting.

Experimental treatments were arranged in a completely randomized block design with five replicates. One replicate consisted of one row of nine plants. The treatments included 'Primo Red' (the scion) grafted onto four different rootstocks. The RKN-resistant rootstocks were 'Arnold', 'Estamino', and 'Maxifort'. Non-grafted 'Primo Red' served as the control.

Plants were grafted on 5 Mar. 2019 at the University of Kentucky Horticulture Research Farm, Lexington, KY, using the splice grafting method. Newly grafted plants were maintained in a dark growth chamber at 26°C and 85 percent humidity for three days and then gradually reintroduced to light. All plants, grafted and non-grafted, were transplanted into the tunnel on 20 Mar. 2019, making sure to keep the graft union above the soil. Once transplanted, the plants were maintained, managed, and harvested by the grower for the entire growing season according to University of Kentucky recommendations (Rudolph et al., 2019). Stem diameter measurements were collected on 15 Apr. and 23 May. The first harvest occurred on 19 June. Harvesting continued

every five to seven days until the last harvest on 29 July.

Destructive sampling occurred on 3 Aug. 2019 and included pulling the three middle plants out of the soil and cutting the roots off. Plants, excluding the roots, were placed in paper bags and oven dried for seven days at 180°F. Soil samples were collected from each treatment replicate. The roots of three plants and the soil samples from each treatment replicate were placed in sealed plastic bags and mailed to Clemson University Nematode Assay Lab, Pendleton, South Carolina for RKN extraction.

## Results and Discussion

Tomato fruit were harvested by the grower over the course of seven weeks. Non-grafted 'Primo Red' plants yielded significantly less compared to all three 'Primo Red' grafted rootstock combinations (Table 1). The total yield of non-grafted 'Primo Red' for the entire season was 713 lb. The highest yielding grafted rootstock Primo Red + Estamino produced over 1,000 lb, almost 33 percent more than the non-grafted control. The three grafted rootstock treatments were numerically different, but not significantly different from one another. Primo Red + Maxifort produced the lowest mean yield (Table 1), but was three pounds less than the next highest yielding, Primo Red + Arnold.

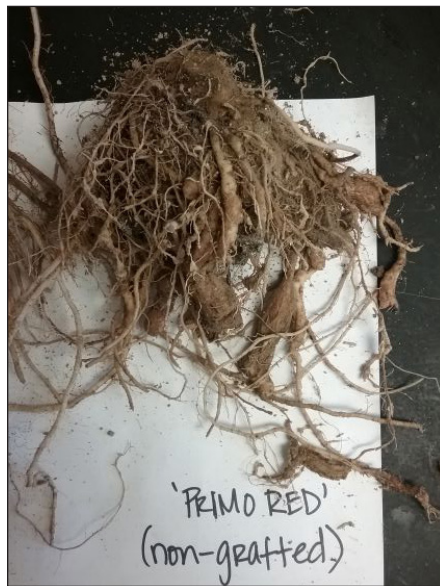
Primo Red + Estamino also had significantly more plant biomass compared to the non-grafted control. It was not significantly different, however, than the other grafted rootstocks. The other grafted rootstocks were also not significantly different from the non-grafted control (data not shown). Rootstocks are generally known for growing vigorously. However, a high RKN population density can weaken plants and slow growth. This may have happened at this site.

The non-grafted control had significantly higher RKN population densities in the soil around plant roots compared to the three grafted rootstock treatments (Table 2). Primo Red + Maxifort had a significantly higher mean RKN population density in soil compared to Primo Red + Estamino, but was not significantly different than Primo Red + Arnold. The mean RKN population densities of Primo Red + Estamino and Primo Red + Arnold were not significantly different from one another. The RKN root population densities across all treatments were low, even in the non-grafted control (Table 2). This means that very few RKN were extracted from roots. This was unexpected as the non-grafted control is not resistant to RKN and the roots were extremely galled (Figure 1). The grafted plant roots either exhibited no signs of galling or very little in comparison to the non-grafted control (Figure 2). RKN enters plant roots

and feeds off plant proteins in the roots. One explanation for the low density of RKN found in non-grafted roots is that non-grafted roots were so damaged by RKN throughout the season that RKN was no longer able to inhabit and feed off of those roots. Another explanation is the extraction and identification method utilized by the Clemson University Nematode Assay Lab. The lab extracted and quantified juvenile RKN, but juveniles often leave the root once they have hatched from their eggs (Mitkowski and Abawi, 2011). Extracting and quantifying eggs, not juveniles, may have provided a more accurate picture of RKN infection in roots.

Yield was significantly greater in grafted plants compared to non-grafted. This demonstrates the potential benefit of the use of grafted plants in the presence of the plant-parasite, RKN. Crop rotation is also recommended, but given that *M. incognita* has an extremely wide host range of over 3,000 species (Castagnone-Sereno, 2000), crop rotation may be difficult for specialty crop growers. Growers should consider an integrated approach to managing RKN. This could include plant resistance, such as grafting with resistant rootstock, rotation with non-host crops, and chemical control, such as a nematicide.

Future research should include more frequent sampling of RKN from both soil and roots to get a better perspective on any population density changes throughout the season. Extraction of eggs from roots should also be considered after destructive sampling of plants. Other resistant rootstocks also need to be evaluated. An economic evaluation would also be useful as rootstock seed is more expensive than regular seed. Additionally, grafting takes time which would affect labor costs. Growers can construct their own healing chamber for newly grafted plants, but maintaining the right temperatures and humidity is crucial and is labor and expense to the normal transplant production process. Growers will need to determine their RKN population in order to determine whether or not to utilize grafted plants.



**Figure 1.** Non-grafted 'Primo Red' tomato roots after being grown in a high tunnel with root knot nematode for over four months.

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**Figure 2.** Galled tomato roots on the non-grafted 'Primo Red' and less or not galled roots on 'Arnold,' 'Maxifort,' and 'Estamino' rootstocks after being grown in a high tunnel with root-knot nematode for over four months.



# Duality of Biochar Impact on Soil Enzymes Activity

George F. Antonious and Eric T. Turley, Division of Environmental Studies, College of Agriculture, Community and the Sciences, Kentucky State University; Mohammad H. Dawood, Department of Horticulture and Landscape, College of Agriculture, University of Kufa, Iraq

The production of municipal solid waste (MSW) is continuously increasing worldwide due to global urbanization of society and the increase in wastewater treatment coverage. Soil amendments, such as MSW and animal manures, such as chicken manure, horse manure, and vermicompost (worm casting) are contributors of soil fertility due to their microbial content. Soil quality is significantly dependent on soil biology in which microorganisms play energetic parts in soil fertility and crop production through enzymatic activity, organic matter decomposition, and nutrient cycling. Recycling animal manures for use as fertilizers would reduce dependence on synthetic inorganic fertilizers and provide amendments useful for improving soil structure and nutrient status at low-cost to limited resource farmers. Biochar is a product of incinerating wood by a process known as pyrolysis. Studies have indicated that biochar used as a soil amendment could increase plant nutrients, soil cation exchange capacity (CEC), soil organic matter, and nutrients availability (Haipeng et al., 2017). Investigators reported that biochar application to agricultural soils has a potential for climate change mitigation, soil, and water retention, and positive influences on soil microbial communities and crop yield (Ferreira et al., 2017).

Accordingly, monitoring soil enzymes as bioindicators of soil health and potential impact of animal manures have been recommended (Antonious 2018; 2016). Studies of enzyme activities that provide information on the biochemical processes occurring in the soil profile are needed. The rhizosphere is a zone of increased microbial and enzyme activity where soil and root make contact. Soil organisms in the rhizosphere of growing plants secrete extracellular enzymes. Secreted enzymes decompose complex organic resources into accessible nutrient elements, such as C-, N-, and P- produced due to soil invertase, urease, and phosphatase activity, respectively. Soil urease that hydrolyzes urea plays a significant role in the N-cycle yielding ammonia, and CO<sub>2</sub> is important in regulating the efficiency of urea as a nitrogen fertilizer. Invertase catalyzes the hydrolysis of sucrose to glucose and fructose due to β-fructofuranosides, predominantly available in microorganisms, animals, and plants (Alef and Nannipieri, 1995). Phosphatase is a hydrolytic enzyme involved in the P-cycle capable of hydrolyzing organic phosphate esters of phosphoric acid to inorganic phosphorus, which can then be absorbed by plants.

The use of soil amendments in agricultural production systems is an affordable way to improve crop yield and soil quality. Antonious et al. (2008) found that sewage sludge mixed with yard waste compost provided the greatest marketable yield and greatest number of eggplant fruit compared to the control treatments. Azarmi et al. (2008) reported that the addition of vermicompost to agricultural soil increased tomato yield and elemental content of tomato fruit compared to the control treatment. Laczi et al. (2016) found that the best yield of the

Chinese cabbage was obtained when horse manure was used as a soil amendment. The use of soil amendments is popular in agricultural fields because of the value of this waste as a low-cost alternative to inorganic fertilizers. The main objective of this investigation was to assess the impact of various soil amendments on soil urease, invertase, acid, and alkaline phosphatase activity.

## Materials and Methods

A field experiment at the University of Kentucky Horticulture Research Farm in Lexington, KY, USA, was established in a randomized complete block design (RCBD). The native soil in the experimental plots is a Bluegrass-Maury Silty Loam (2.2% organic matter, pH 6.2). The soil has an average of 56% silt, 38% clay, and 6% sand. Each plot was 4 × 10 ft<sup>2</sup> (1.23 × 3.05 cm<sup>2</sup>) and the entire study area contained 42 plots (3 replicates × 14 treatments). The soil treatments were: control (NM soil), sewage sludge (SS), horse manure (HM), chicken manure (CM), vermicompost (Vermi), organic fertilizer (Org; Nature Safe 10N-2P-8K), synthetic inorganic fertilizer (Inorg; Southern State 19N-19P-19K), and biochar added to NM soil, SS, HM, CM, Vermi, Organic, and Inorg fertilizers. The soil in the seven treatments was mixed with 10% (w/w) biochar (Wakefield Agricultural Carbon, Columbia, MO). Properties of the biochar used in this investigation were: surface area 366 m<sup>2</sup> g<sup>-1</sup> dry, bulk density 480.6 kg m<sup>-3</sup>, total organic C 88%, moisture 54%, temperature 200 °C, total inorganic C 0.34%, particle size (< 0.5 mm), and pH 7.4. All soil amendments were applied at 5% nitrogen (N) on dry weight basis to eliminate variations among soil treatments due to N content. SS was purchased from the Metropolitan Sewer District (Louisville, Kentucky), and CM was obtained from the Department of Animal and Food Sciences, University of Kentucky (Lexington, Kentucky). HM was obtained from the Kentucky horse park (Lexington, Kentucky). Vermicompost (worm castings) was obtained from Worm Power (Montpelier, Vermont, USA) and organic and inorganic commercial fertilizers were obtained from the Southern States Cooperative Stores (Lexington, Kentucky). Soil amendments were added to native topsoil, mixed, and rototilled to a depth of 15 cm of topsoil. Sixty-day-old seedlings of eggplant, *Solanum melongena* cv. Epic were planted in a freshly tilled soil at 18 inches (45 cm) in-row spacing and drip irrigated as needed. Weeding and other agricultural operations were carried out regularly as needed. The plants were sprayed with the insecticide esfenvalerate (Asana XL) three times during the growing season at seven days intervals for insect control (Rudolph et al., 2020).

Soil samples (n = 3) were collected from the rhizosphere of growing plants of each treatment 4 months after planting to a depth of 15 cm. Samples were collected using a core sampler (Clements Associates, Newton, IA) equipped with a plastic

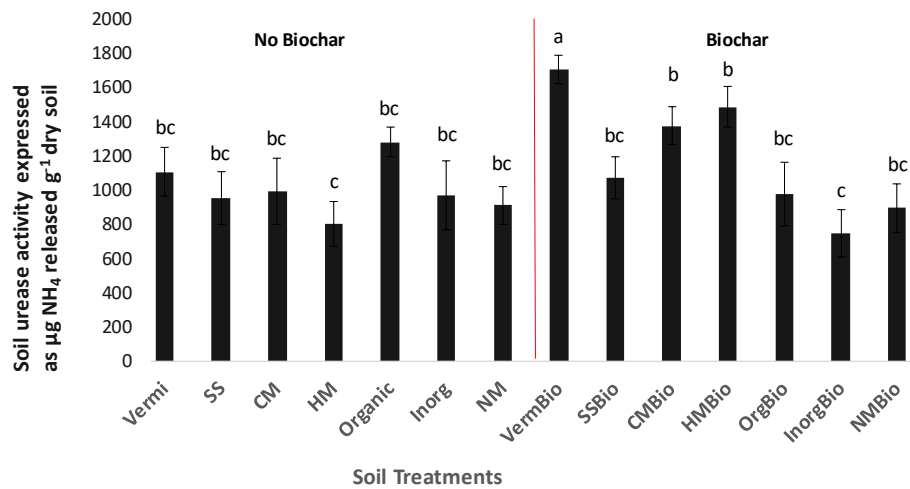
liner tubes of 2.5 cm i.d. for maintenance of sample integrity. Soil samples were air-dried at room temperature, passed through a 2 mm non-metallic sieve, and kept at 4 °C up to 24 hours before use.

For determination of soil urease activity, the procedure was completed as described by Tabatabai and Bremner (1972). Urease activity was expressed as  $\mu\text{g NH}_4\text{-N released g}^{-1}$  dried soil. Invertase activity in soil was estimated by the method described by Balasubramanian et al. (1970). Acid and alkaline phosphatase activities in soil were determined by the method developed by Tabatabai and Bremner (1969), which determines p-nitrophenol released when soil is incubated with buffered sodium p-nitrophenol phosphate solution (pH 6.7 for acid phosphatase assay and pH 11 for alkaline phosphatase assay). Data containing soil urease, invertase, acid and alkaline phosphatase activity were statistically analyzed using analysis of variance (ANOVA) and the means were compared using Duncan's multiple range test (SAS Institute, 2016).

### Result and Discussion

Urease activity in the rhizosphere of eggplant varied due to the different types of soil amendments mixed with NM native soil. Urease activity, that indicates the cycling of N in the soil, was significantly greater ( $P < 0.05$ ) in vermicompost biochar (VermBio) treatments compared to Vermi treatments that had no biochar (Figure 1) indicating the role of biochar in promoting soil urease activity when mixed with vermicompost. On the contrary, biochar added to SS (SSBio), CM (CMBio), organic (OrgBio), inorganic (InorgBio) commercial fertilizers, and NM soil (NMBio) did not increase soil urease activity compared to the no biochar treatments. The loss of organic matter during the biochar preparation process (pyrolysis) contributes to an increase in the concentration of trace metals in biochar. Zn for example inhibited urease activity (Yang et al., 2006). In fact, many investigators are not in complete agreement on the impact of biochar on agricultural soils. Accordingly, the influence of biochar on soil microbial communities have been reported to be either negative, positive or insignificant depending on the source of biochar as well as the type of soil used (Bruun et al., 2012; Galvez et al., 2012; Luo et al., 2013).

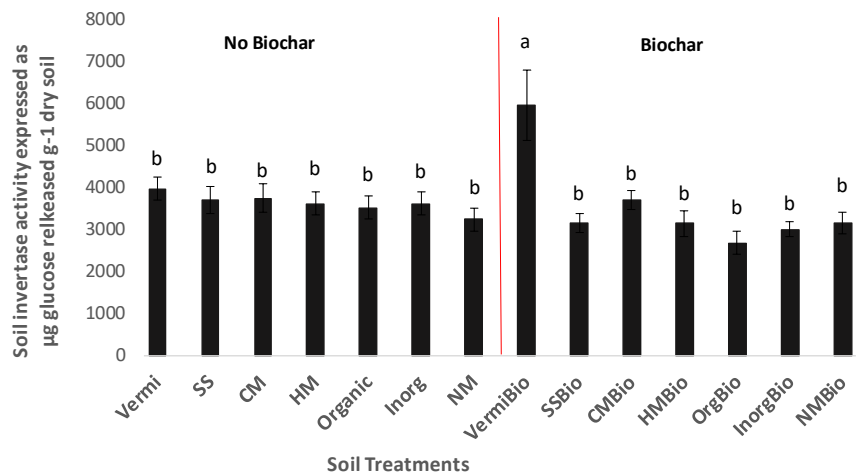
Invertase also plays an important role in increasing soluble nutrients in soil and is often used to monitor and characterize soil



**Figure 1.** Impact of soil amended with Vermi (vermicompost), SS (sewage sludge), CM (chicken manure), HM (horse manure), Organic (organic commercial fertilizer), Inorg (inorganic commercial fertilizer), NM (no-mulch bare soil), Vermi mixed with biochar (VermBio), SS mixed with biochar (SSBio), CM mixed with biochar (CMBio), horse manure mixed with biochar (HMBio), organic commercial fertilizer mixed with biochar (OrgBio), inorganic fertilizer mixed with biochar (InorgBio), and no-mulch soil mixed with biochar (NMBio) on soil urease activity in the rhizosphere of field-grown eggplants. Statistical comparisons were carried out among soil treatments. Bars  $\pm$  standard deviation accompanied by different letter(s) indicate significant differences ( $P \leq 0.05$ ) using Duncan's multiple range test.

fertility. VermBio was superior in increasing soil invertase activity compared to other soil amendments tested in this investigation (Figure 2). VermBio significantly increased soil invertase activity from 3970 to 5,947  $\mu\text{g g}^{-1}$  dry soil (50% increase) compared to Vermi with no biochar addition indicating the role of biochar in promoting invertase activity. Other than VermBio, there were no significant differences among other animal manure treatments on increasing soil invertase activity.

Soil acid phosphatase activity was significantly reduced by 43% due to addition of commercial inorganic fertilizer to NM



**Figure 2.** Impact of soil amended with Vermi (vermicompost), SS (sewage sludge), CM (chicken manure), HM (horse manure), Organic (organic commercial fertilizer), Inorg (inorganic commercial fertilizer), NM (no-mulch bare soil), Vermi mixed with biochar (VermBio), SS mixed with biochar (SSBio), CM mixed with biochar (CMBio), horse manure mixed with biochar (HMBio), organic commercial fertilizer mixed with biochar (OrgBio), inorganic fertilizer mixed with biochar (InorgBio), and no-mulch soil mixed with biochar (NMBio) on soil invertase activity in the rhizosphere of field-grown eggplants. Statistical comparisons were carried out among soil treatments. Bars  $\pm$  standard deviation accompanied by different letter indicate significant differences ( $P \leq 0.05$ ) using Duncan's multiple range test.

native soil whereas, soil amended with commercial organic fertilizer increased acid phosphatase activity by 7% compared to NM soil (Figure 3A). Acid phosphatase activity was 1414 and dropped to 1124  $\mu\text{g g}^{-1}$  dry soil (about 21% reduction) after the addition of biochar to Vermicompost amended soil. Whereas biochar added to SS (SSBio), Org (OrgBio), and NM (NMBio) soil did not impact acid phosphatase activity compared to SS, Org, and NM treatments not amended with biochar (Figure 3A).

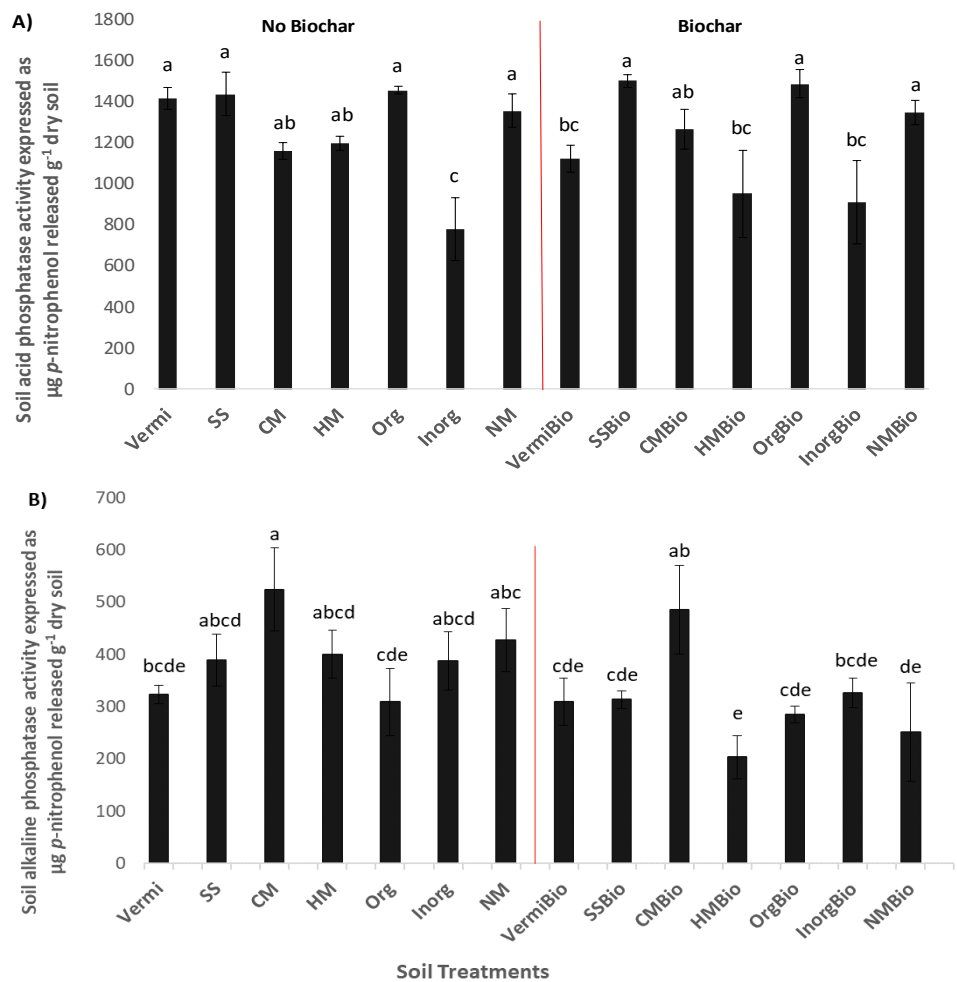
No significant differences were found in soil alkaline phosphatase activity between organic and synthetic inorganic fertilizers amended or not amended with biochar (Figure 3B). Results also revealed that biochar added to CM (CMBio) did not increase alkaline phosphatase activity compared to CM treatments not amended with biochar. Biochar added to no mulch soil (NMBio) reduced soil alkaline phosphatase activity by 41% compared to NM native soil not amended with biochar. One possible reason is that biochar added to soil amendments might contain one or more alkaline phosphatase inhibitors. Many microorganisms multiply and others removed, due to a trace metal contamination, which results in shifts in the quality and functionality of soils. Cd significantly inhibited alkaline phosphatase activity, whereas Zn inhibited urease activity (Yang et al., 2006).

### Conclusions

Utilization of vermicompost amended with biochar, rather than synthetic inorganic fertilizer will be beneficial in large-scale crop cultivation systems for promoting the activities of soil urease and invertase. Soil acid phosphatase activity was reduced by the addition of commercial inorganic fertilizer compared to soil amended with commercial organic fertilizer. No significant differences were found in soil alkaline phosphatase activity between organic and inorganic fertilizers even after amended with biochar.

### Acknowledgments

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**Figure 3.** Impact of soil amended with Vermicompost (Vermi), SS (sewage sludge), CM (chicken manure), HM (horse manure), Organic (organic commercial fertilizer), Inorg (inorganic commercial fertilizer), NM (no-mulch bare soil), Vermicompost mixed with biochar (VermiBio), SS mixed with biochar (SSBio), CM mixed with biochar (CMBio), horse manure mixed with biochar (HMBio), organic commercial fertilizer mixed with biochar (OrgBio), inorganic fertilizer mixed with biochar (InorgBio), and no-mulch soil mixed with biochar (NMBio) on soil acid phosphatase activity (A) and alkaline phosphatase activity (B) in the rhizosphere of field-grown eggplants. Statistical comparisons were carried out among soil treatments. Bars  $\pm$  standard deviation accompanied by different letter(s) indicate significant differences ( $P \leq 0.05$ ) using Duncan's multiple range test.

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## Mobility of Trace Metals from Sewage Sludge Amended Soil into Plants

*George F. Antonious, Division of Environmental Studies, College of Agriculture, Community, and the Sciences, Kentucky State University*

Municipal sewage sludge (SS) also known as biosolids is processed at the Wastewater Treatment Plant (Metropolitan Sewage Facility, Louisville, Kentucky) to eliminate odor, pathogens, and reduce its volume by drying to make it easier to handle and transport to agricultural lands. SS has non-biodegradable trace metals and some of these metals are toxic to humans and animals even at trace concentrations. However, in agricultural production systems soil microorganisms need certain metals for their survivals. Metals are toxic to soil microorganisms when present above certain concentrations (Chakrabarti et al., 2005) as indicated by diminished activities of the enzymes they release. Accordingly, accumulation of trace metals in soil and edible plants grown in municipal SS amended soil requires a continuous monitoring. Risks of soil contamination when waste materials such as SS are used as fertilizer have been a matter of frequent concern (Vidal-Vázquez et al., 2005).

Plants require copper (Cu) as an essential micronutrient for normal growth and development. In plants, Cu exists as Cu<sup>2+</sup> and Cu<sup>+</sup> and acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism, and hormone signaling (Raven et al., 1999). Chromium (Cr) is toxic to plants and does not play any role in plant metabolism (Dixit et al., 2002). However, accumulation of Cr by

plants can reduce growth, induce chlorosis in young leaves, reduce pigment content, reduce enzymatic function, damage root cells, and cause ultrastructural modifications of the chloroplast and cell membrane (Panda et al., 2002). Zinc (Zn) is an essential plant micronutrient involved in a wide variety of physiological processes (Bradley et al., 2007) and is one of the most ubiquitous trace-metals in soil. Industrial and agricultural activities, such as smelter and incinerator emissions, dispersal from mine wastes, excessive applications of Zn-containing fertilizers or pesticides and use of Zn-contaminated SS can increase Zn concentration in the environment and make it dangerous.

Molybdenum (Mo) represents one of the scarcest trace elements in biological systems (Kaiser et al., 2005). The behavior of Mo in soils has been extensively studied because it has a unique position among other micronutrients in that it is least soluble in acidic soils and readily mobile in alkaline soils. This is due to the great affinity of Mo to adsorb to soil organic matter. Liming of acidic soils is a common practice to increase Mo availability to plants. Mo is known to be essential to microorganisms, such as *Rhizobium* bacteria and other N-fixing microorganisms which have an especially large requirement for Mo. Some fungi and nitrogen-fixing bacteria tend to concentrate Mo up to 100 ppm. Since the most important function of Mo in plants is NO<sub>3</sub> reduction, a deficiency of this micronutrient causes symptoms similar to those of N deficiency. In

terms of solubility, metals can reach toxic levels inside plant cells and tissues through the potential of metal uptake by plant root-to-shoot translocation (bioaccumulation). Accordingly, the objectives of this investigation were to: 1) assess the impact of soil incorporated with municipal SS on Cu, Cr, Zn, and Mo concentration at three locations in Kentucky (Adair, Meade, and Franklin counties), 2) determine the bioaccumulation factor (BAF) of each metal in plants grown under this practice, and 3) compare metal concentrations detected in edible plants to their allowable limits.

## Materials and Methods

The field study was conducted at three locations in Kentucky (Adair, Meade, and Franklin counties) in areas where limited resource farmers used municipal SS as an alternative source of fertilizers for commercial production of fresh vegetables. Before planting, the soil was mixed with municipal SS obtained from Metropolitan Sewer District, Louisville, KY, at 37.5 t hectare<sup>-1</sup> on dry weight basis. SS was incorporated into the top soil with a plowing depth of 15 cm. Thirty cm of extra space was allowed between planting rows, and the plants were watered, irrigated, and weeded as needed, but no mineral fertilizer was applied. At harvest, eight plants were collected randomly from the Meade experimental site, four plants were collected from the Adair experimental site, and seven plants were collected from the Franklin experimental site (Table 1). Randomly selected fruit, pods, bulbs, or leaves (n = 3) from each location were harvested at full maturity. In each instance, fruits were harvested from throughout the plants to reduce the effect of fruit, pods, bulbs, and leaves position on the concentration of metals analyzed.

Soil samples (three replicates per location) were collected from the rhizosphere (a zone where soil and plant root make contact) of growing plants to a depth of 15 cm using a soil core sampler equipped with a plastic liner (Clements Associates, Newton, IA, USA) of 2.5 cm i.d. Soil samples were air-dried at room temperature, passed through a 2 mm non-metallic sieve and kept at 4 °C up to 24 h before use (Antonious et al., 2011). Soluble metals concentration in soil that are available to plants were extracted using CaCl<sub>2</sub> (Antonious et al., 2013). For quantification of Cr, Mo, Cu, and Zn in harvested plants, ten fruit, pods, bulbs, or leaves of comparable size were collected at random from each of the three field locations in Adair, Meade, and Franklin counties (three replicates from each plant) at full maturity and dried in an oven at 65 °C for 48 h. The dried samples were ground manually with ceramic mortar and pestle to pass through 2 mm non-metallic sieve. Concentrations of metals were determined using inductively coupled plasma-mass spectrometer (ICP-MS) in standard mode following the U.S. EPA method 6020a (EPA, 1998). Metals root-to-shoot translocation (bioavailability) is the proportion of the soluble metals concentration in soil that is available for incorporation into plant tissues. The bioaccumulation factor (BAF) was calculated by dividing the metal content in plant by the soluble metal content in the soil on dry weight basis (Anton and Mathe, 2005; Antonious et al., 2010).

**Table 1.** Plants grown at three locations (Meade, Adair, and Franklin counties) in Kentucky where farmers used municipal sewage sludge for land farming.

Location	Plant	Latin Name	Cultivated Variety	
Meade Site	Green bean	<i>Phaseolus vulgaris</i>	Blue Lake	
	Green pepper	<i>Capsicum annuum</i>	Aristotle X3R	
	Green squash	<i>Cucurbita pepo</i>	Costata Romanesco	
	Yellow squash	<i>Curcubita pepo</i>	Conqueror III)	
	Onion	<i>Allium cepa</i>	Super Star- F1	
	Tomato	<i>Lycopersicon esculentum</i>	Mountain Spring	
	Okra	<i>Abelmoschus esculentus</i>	Clemson Spineless	
Adair Site	Beets	<i>Beta vulgaris</i>	Red Ace- F1	
	Tobacco	<i>Nicotiana tabacum</i>	Burley	
	Red potato	<i>Ipomoea batatas</i>	Norland Red	
	Onion	<i>Allium cepa</i>	Super Star- F1	
	Sweet potato	<i>Ipomoea batatas</i>	Beauregard	
	Franklin Site	White potato	<i>Ipomoea batatas</i>	Kennebec
		Green pepper	<i>Capsicum annuum</i>	Aristotle X3R
Tomato		<i>Lycopersicon esculentum</i>	Mountain Spring	
Onion		<i>Allium cepa</i>	Super Star- F1	
Broccoli		<i>Brassica oleracea</i>	Packman	
Yellow squash		<i>Curcubita pepo</i>	Conqueror III	
Sweet potato		<i>Ipomoea batatas</i>	Beauregard	

Metal concentrations in soil mixed with SS and plants grown in soil amended with SS at each of the three locations were statistically analyzed using ANOVA procedure (SAS Institute, 2016) and the means were compared using Duncan's multiple range test.

## Results and Discussion

Cr concentrations in plant grown at the Meade site ranged from 0.08 µg g<sup>-1</sup> in been seeds to 1.4 µg g<sup>-1</sup> dry tissue in onion bulbs (Table 2). Cr BAF of onion bulbs was significantly greater than all the other plants tested at this site. As described earlier, BAF is defined as the concentration of a metal in plant tissue divided by metal concentration in soil. Therefore, BAF values > 1 indicates the ability of a plant to tolerate and accumulate a trace metal. Onion bulbs accumulated more Cr than onion leaves, although the surface area of the leaves is much greater compared to the bulb. This could be attributed to the proximity of the onion roots (bulbs) to the soil compared to the leaves. Most soils contain significant amounts of Cr, but its availability to plants is highly limited. A higher Cr content was observed in the roots than in leaves or shoots. Mo concentrations ranged from 0.08 µg g<sup>-1</sup> in onion bulbs to 1 µg g<sup>-1</sup> dry tissue in bean pods and the Mo BAF values were lowest in onion bulbs (0.37) and highest in bean pods (4.5) (Table 2).

Cu concentrations in plants grown in Meade area ranged from 7.7 µg g<sup>-1</sup> in onion bulbs to 26 and 25 µg g<sup>-1</sup> dry weight in tomato and yellow squash fruits, respectively with BAF values ranged from 1.4 in onion bulbs to 4.7 and 4.5 in tomato and yellow squash fruits, respectively (Table 2). The average Cu content in plant tissue is 10 µg g<sup>-1</sup> dry weight (Baker and Senef, 1995). Minnich et al. (1987) observed that the Cu concentration in shoot tissues of snap beans increased linearly with the Cu content of the sludge applied to soil. Zn concentrations ranged from 31.5 µg g<sup>-1</sup> to 83.6 µg g<sup>-1</sup> dry weight in onion

**Table 2.** Concentrations of trace metals expressed as  $\mu\text{g g}^{-1}$  dry tissue and bioaccumulation factor (BAF) of plants grown in soil amended with municipal sewage sludge in Meade County, Kentucky.

Meade Plants	Bean Pods	Bean Seeds	Green Pepper	Green Squash	Yellow Squash	Onion Leaves	Onion Bulbs	Tomato Fruits	Okra	Beets
Cr	0.35 ± 0.05	0.079 ± 0.03	0.10 ± 0.04	0.14 ± 0.07	0.186 ± 0.6	0.436 ± 0.19	1.348 ± 0.07	0.14 ± 0.04	0.155 ± 0.07	0.177 ± 0.05
BAF	1.816 c	0.416 d	0.532 d	0.747 d	0.979 d	2.295 b	7.095 a	0.726 d	0.816 d	0.932 d
Mo	1.02 ± 0.12	0.722 ± 0.07	0.15 ± 0.05	0.28 ± 0.09	0.25 ± 0.11	0.16 ± 0.05	0.082 ± 0.02	0.14 ± 0.06	0.31 ± 0.03	0.137 ± 0.09
BAF	4.53 c	3.27 b	0.683 d	1.244 c	1.118 c	0.701 d	0.371 e	0.647 d	1.398 c	0.620 d
Cu	15.53 ± 5.0	13.74 ± 4.2	19.05 ± 7.2	21 ± 10.1	24.95 ± 7.44	21.04 ± 8.0	7.69 ± 1.4	26.01 ± 9.3	13.08 ± 7.2	16.02 ± 3.5
BAF	2.795 c	2.473 c	3.429 b	3.780 b	4.491 a	3.787 b	1.384 d	4.681 a	2.354 c	3.930 b
Zn	48.77 ± 13	63.05 ± 18	39.66 ± 12	83.57 ± 15	79.85 ± 13	31.49 ± 9.4	35.15 ± 8.8	47.86 ± 6.4	49.19 ± 7.8	51.01 ± 12
BAF	4.424 c	5.720 b	3.598 b	7.581 a	7.244 a	2.857 d	3.189 d	4.342 c	4.462 c	4.628 c

Note: Metal concentration is an average of three replicates ± standard deviation. Bioaccumulation factor (BAF) was calculated by dividing metal content in plant tissue by soluble metal in the soil. Statistical comparisons were carried out among plants. Values accompanied by different letters in the same row indicate significant differences ( $P \leq 0.05$ ) using Duncan's range test.

leaves and green squash fruits, respectively among other vegetables grown at the Meade location. Soluble Zn that occur particularly in municipal SS is very mobile in soils and therefore is easily available to plants (Langerwerff et al., 1978). Roots often contain much more Zn than do plant tops, particularly if the plants are grown in Zn-rich soils. Zn stimulates the resistance of plants to dry and hot weather and to bacterial and fungal diseases (Cabot et al., 2019). Accordingly, Zn-deficient plants are more susceptible to diseases. Table 2 shows that green and yellow squash fruits are two Zn hyperaccumulator plants.

In Adair plants, leaves of tobacco grown in soil amended with SS contained the greatest Cr concentration ( $0.25 \mu\text{g g}^{-1}$  dry tissue) compared to 0.06, 0.08, and  $0.06 \mu\text{g g}^{-1}$  dry tissue in red potato, onion bulbs, and sweet potato, respectively (Table 3). This could be due to the large surface area of tobacco leaves  $\text{g}^{-1}$  tissue compared to edible tissues of plants tested at this site. Mo BAF values were < 1.0 in red potato, onion bulbs, and sweet potato. Whereas Cu concentrations in plants grown in Adair site ranged from  $8.9 \mu\text{g g}^{-1}$  -  $12.2 \mu\text{g g}^{-1}$  dry tissue and Zn concentrations ranged from  $14.8$  to  $37.8 \mu\text{g g}^{-1}$  dry tissue in sweet potato and tobacco leaves, respectively. Accordingly, all Cu and Zn BAF values were > 1.0.

Concentrations of Cr in plants grown at the Franklin site ranged from  $0.04 \mu\text{g g}^{-1}$  dry tissue in sweet potato to  $0.72 \mu\text{g g}^{-1}$  dry tissue in potato tubers. Whereas Mo concentrations ranged from <  $0.01 \mu\text{g g}^{-1}$  dry tissue in tomato fruits to  $0.05 \mu\text{g g}^{-1}$  dry tissue in pepper fruits. Mo BAF values were > 1.0 in pepper fruits, onion bulbs, and sweet potato (Table 4). Cu concentration in Franklin plants ranged from  $6.6 \mu\text{g g}^{-1}$  -  $30.0 \mu\text{g g}^{-1}$  dry tissue with a maximum BAF value of 3.3 in onion bulbs. Zn concentrations were high and ranged from  $4.2 \mu\text{g g}^{-1}$  dry tissue in sweet potato to  $28.2 \mu\text{g g}^{-1}$  dry tissue in broccoli heads. However, Zn BAF values in all plants tested in the Franklin location were all either below or around 1.0.

**Table 3.** Concentrations of trace metals expressed as  $\mu\text{g g}^{-1}$  dry tissue and bioaccumulation factor (BAF) of plants grown in soil amended with municipal sewage sludge in Adair County, Kentucky.

Adair Plants	Tobacco Leaves	Red Potato	Onion Bulbs	Sweet Potato
Cr	0.251 ± 0.04	0.06 ± 0.02	0.075 ± 0.01	0.059 ± 0.02
BAF	0.258 a	0.061 b	0.077 b	0.061 b
Mo	0.709 ± 0.08	0.56 ± 0.04	0.105 ± 0.03	0.078 ± 0.01
BAF	1.196 a	0.944 b	0.177 c	0.132 c
Cu	12.23 ± 1.25	10.0 ± 0.91	9.454 ± 0.6	8.940 ± 0.9
BAF	4.395 a	3.616 b	3.398 b	3.214 b
Zn	37.83 ± 9.12	24.6 ± 5.22	26.334 ± 7.46	14.851 ± 3.55
BAF	7.792 a	5.066 b	5.424 b	3.059 c

Note: Metal concentration is an average of three replicates ± standard deviation. Bioaccumulation factor (BAF) was calculated by dividing metal content in plant tissue by soluble metal in the soil. Statistical comparisons were carried out among plants. Values accompanied by different letters in the same row indicate significant differences ( $P \leq 0.05$ ) using Duncan's range test.

Regarding the permissible limits for trace metals in soil and edible plants, the world health organization (WHO, 1996) reported that the desirable levels of Cr, Cu, and Zn in unpolluted soil should not exceed 100, 36, and  $50 \mu\text{g g}^{-1}$  dry soil, respectively. Whereas these values should not exceed 1.3, 10, and  $0.6 \mu\text{g g}^{-1}$  dry edible plant tissue, respectively. Regarding Mo, plants vary widely in their requirements for Mo and their ability to extract it from soils. Sims (1981) reported that the Mo level in Kentucky plants was found at  $0.3 \mu\text{g g}^{-1}$  dry tissue and generally detected at a high level of 1.6 in leguminous crops and  $0.1$ - $0.5 \mu\text{g g}^{-1}$  in non-leguminous crops. In the present investigation Cr in plants grown at the Meade, Adair,

**Table 4.** Concentrations of trace metals expressed as  $\mu\text{g g}^{-1}$  dry tissue and bioaccumulation factor (BAF) of plants grown in soil amended with municipal sewage sludge in Franklin County, Kentucky.

Franklin Plants	Potato	Pepper Fruits	Onion Bulbs	Tomato Fruits	Broccoli	Yellow Squash	Sweet Potato
Cr	0.716 ± 0.11	0.461 ± 0.06	0.314 ± 0.05	0.235 ± 0.09	0.397 ± 0.07	0.259 ± 0.03	0.043 ± 0.01
BAF	0.122 a	0.079 b	0.054 c	0.040 c	0.068 b	0.044 c	0.007 d
Mo	0.009 ± 0.002	0.05 ± 0.006	0.039 ± 0.004	0.007 ± 0.003	0.026 ± 0.002	0.017 ± 0.005	0.039 ± 0.003
BAF	0.317 d	1.667 a	1.283 b	0.233 d	0.783 c	0.567 c	1.280 b
Cu	11.13 ± 1.8	8.15 ± 1.5	29.96 ± 2.3	11.34 ± 1.5	6.55 ± 0.99	8.31 ± 1.6	7.04 ± 2.1
BAF	1.227 b	0.897 c	3.303 a	1.251 b	0.722 c	0.916 c	0.776 c
Zn	17.36 ± 1.3	14.43 ± 2.4	15.55 ± 1.4	22.46 ± 0.9	28.21 ± 2.1	24.46 ± 1.3	4.21 ± 1.6
BAF	0.754 c	0.626 c	0.675 c	0.975 b	1.224 a	1.062 a	0.183 d

Note: Metal concentration is an average of three replicates ± standard deviation. Bioaccumulation factor (BAF) was calculated by dividing metal content in plant tissue by soluble metal in the soil. Statistical comparisons were carried out among plants. Values accompanied by different letters in the same row indicate significant differences ( $P \leq 0.05$ ) using Duncan's range test.



and Franklin sites was below the permitted level of  $1.3 \mu\text{g g}^{-1}$  tissue. Similarly, Mo concentrations were below the level in leguminous crops and non-leguminous crops at the three sites tested. Other than onion bulbs, Cu concentrations were above the permissible level of  $10 \mu\text{g g}^{-1}$  tissue in all plants grown at the Meade site. At the Adair site, Cu was above the limit only in tobacco leaves. Whereas at Franklin site, Cu was above the limit in potato tubers, onion bulbs, and tomato fruits. The Zn permissible level in plants is  $0.6 \mu\text{g g}^{-1}$  and this level was exceeded in the plants tested at the three sites.

## Conclusions

Crop species differed in their trace metal uptake from agricultural soils amended with sewage sludge (biosolids) and accumulation in their tissues. This process of elemental flow from nonliving (soil particles) to the living compartments (plants) determines metals toxicity. Selecting plants that have low bioaccumulation factor (BAF) reduces metal concentration in edible tissues and consequently in the food chain. Plants with high trace metal uptake (bioaccumulation) have been recommended for phytoremediation which is a widely accepted cost-effective environmental restoration technology that uses plants to remediate excess levels of toxic contaminants, such as trace metals in soil. Results revealed that low metal accumulating plants might be appropriate selections for growing in Cr, Mo, Cu, and Zn contaminated soils. Growing onion bulbs, potato tubers, sweet potatoes, and other below ground crops in soil amended with biosolids is not recommended due to their direct root contact with the soil and potential accumulation of trace metals in their below ground edible tissues compared to plant tops.

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## Appendix A

# Sources of Vegetable Seeds

The abbreviations used in this appendix correspond to those listed after the variety names in tables of individual trial reports.

AAS.....	All America Selection Trials, 1311 Butterfield Road, Suite 310, Downers Grove, IL 60515 <a href="https://all-americaselections.org">https://all-americaselections.org</a>	HN.....	HungNong Seed America Inc., 3065 Pacheco Pass Hwy., Gilroy, CA 95020
AC.....	Abbott and Cobb Inc., Box 307, Feasterville, PA 19047 <a href="http://www.abbottcobb.com">www.abbottcobb.com</a>	HO.....	Holmes Seed Co., 2125-46th St., N.W., Canton, OH 44709 <a href="http://www.holmesseed.com">www.holmesseed.com</a>
AT.....	American Takii Inc., 301 Natividad Road, Salinas, CA 93906 <a href="http://www.takii.com">www.takii.com</a>	HR.....	Harris Seeds, 60 Saginaw Dr., P.O. Box 22960, Rochester, NY 14692 <a href="http://www.harrisseed.com">www.harrisseed.com</a>
BHN.....	BHN Seed, Division of Gargiulo Inc., 16750 Bonita Beach Rd., Bonita Springs, FL 34135 <a href="http://www.bhnseed.com">www.bhnseed.com</a>	HS.....	Heirloom Seeds, P O Box 245, W. Elizabeth PA 15088-0245 412-384-0852
BBS.....	Baer's Best Seed, 154 Green St., Reading, MA 01867 <a href="http://www.baersbest.com">www.baersbest.com</a>	HZ.....	Hazera Seed, Ltd., P.O.B. 1565, Haifa, Israel <a href="https://www.hazera.com">https://www.hazera.com</a>
BC.....	Baker Creek Heirloom Seeds, 2278 Baker Creek Rd., Mansfield, OH 65704 <a href="http://www.rareseeds.com">www.rareseeds.com</a>	JU.....	J. W. Jung Seed Co., 335 High St., Randolph, WI 53957 <a href="http://www.jungseed.com">www.jungseed.com</a>
BK.....	Bakker Brothers of Idaho Inc., P.O. Box 1964, Twin Falls, ID 83303 <a href="http://www.bakkerbrothers.nl">www.bakkerbrothers.nl</a>	JS/JSS.....	Johnny's Selected Seeds, Foss Hill Road, Albion, MA 04910-9731 <a href="http://www.johnnyseeds.com">www.johnnyseeds.com</a>
BL.....	Burrell Seed Growers, P.O. Box 150, Rocky Ford, CO 81067 <a href="https://burrellseeds.us">https://burrellseeds.us</a>	KS.....	Krummrey & Sons Inc., P.O. 158, Stockbridge, MI 49285 517-851-7550
BU.....	W. Atlee Burpee & Co., P.O. Box 6929, Philadelphia, PA 19132 <a href="http://www.burpee.com">www.burpee.com</a>	KU.....	Known-You Seed Co., Ltd. <a href="https://knownyouseed.com/">https://knownyouseed.com/</a>
BZ.....	Bejo Zaden B.V., 1722 ZG Noordscharwoude, P.O. Box 9, The Netherlands <a href="https://www.bejo.com">https://www.bejo.com</a>	KTS.....	Kitazawa Seed Co., PO Box 13220 Oakland, CA 94661-3220 <a href="http://www.kitazawaseed.com">www.kitazawaseed.com</a>
CA.....	Castle VegTech Inc., 190 Mast St., Morgan Hill, CA 95037 <a href="https://seedquest.com">https://seedquest.com</a>	LI.....	Liberty Seed, P.O. Box 806, New Philadelphia, OH 44663 <a href="https://libertyseeds.com">https://libertyseeds.com</a>
CF.....	Cliftons Seed Co., 2586 NC 43 West, Faison, NC 28341 <a href="https://www.cliftonseed.com">https://www.cliftonseed.com</a>	MB.....	Malmborg's Inc., 5120 N. Lilac Dr., Brooklyn Center, MN 55429 <a href="http://www.malmborgsinc.com">www.malmborgsinc.com</a>
CH.....	Alf Christianson, P.O. Box 98, Mt. Vernon, WA 98273 (360) 336-9727	MKS.....	Mikado Seed Growers Co. Ltd. <a href="http://en.mikadokyowa.com/about-us-en/">en.mikadokyowa.com/about-us-en/</a>
CL.....	Clause Home Garden, 100 Breen Road, San Juan Bautista, CA 95045 <a href="http://www.clausehomegarden.com">www.clausehomegarden.com</a>	MR.....	Martin Rispins & Son Inc., 3332 Ridge Rd., P.O. Box 5, Lansing, IL 60438 <a href="http://rispensseeds.com">rispensseeds.com</a>
CR.....	Crookham Co., P.O. Box 520, Caldwell, ID 83605 <a href="https://www.crookham.com">https://www.crookham.com</a>	MWS.....	Midwestern Seed Growers, 10559 Lackman Road, Lenexa, KS 66219 <a href="http://www.midwesternbioag.com">www.midwesternbioag.com</a>
D.....	Daehnfeltd Inc., P.O. Box 947, Albany, OR 97321 <a href="https://seedquest.com">https://seedquest.com</a>	NE.....	Neuman Seed Co., 202 E. Main St., P.O. Box 1530, El Centro, CA 92244 619-337-3100
DR.....	DeRuiter Seeds Inc., P.O. Box 20228, Columbus, OH 43320 <a href="http://www.deruiterseeds.com">www.deruiterseeds.com</a>	NU/NH.....	Nunhems (see Cannery Seed Corp.) <a href="http://nunhems.us/">http://nunhems.us/</a>
EV.....	Evergreen Seeds, P.O. Box 2036 Sunnyvale, CA 94087 <a href="http://evergreenseeds.com/">http://evergreenseeds.com/</a>	NS.....	New England Seed Co., 3580 Main St., Hartford, CT 06120 <a href="https://www.neseed.com">https://www.neseed.com</a>
EX.....	Express Seed, 300 Artino Drive, Oberlin, OH 44074 <a href="https://www.expressseed.com">https://www.expressseed.com</a>	NZ.....	Nickerson-Zwaan, P.O. Box 19, 2990 AA Barendrecht, The Netherlands <a href="http://www.rijkszwaan.com">www.rijkszwaan.com</a>
EZ.....	ENZA Zaden, P.O. Box 7, 1600 AA, Enkhuisen, The Netherlands 02280-15844 <a href="https://www.enzazaden.com/us/">https://www.enzazaden.com/us/</a>	ON.....	Osbourne Seed Co., 2428 Old Hwy 99 South Rd Mt Vernon, WA 98273 <a href="http://www.osborneseed.com">www.osborneseed.com</a>
FED.....	Fedco Seed Co., P.P. Box 520 Waterville, ME, 04903 <a href="http://www.fedcoseeds.com">www.fedcoseeds.com</a>	OUT.....	Outstanding Seed Co., 354 Center Grange Rd, Monaca PA 15061 <a href="https://outstandingseed.com">https://outstandingseed.com</a>
FM.....	Ferry-Morse Seed Co., P.O. Box 4938, Modesto, CA 95352 <a href="https://ferrymorse.com">https://ferrymorse.com</a>	OLS.....	L.L. Olds Seed Co., P.O. Box 7790, Madison, WI 53707 <a href="http://www.oldsgardenseed.com">www.oldsgardenseed.com</a>
GB.....	Green Barn Seed, 18855 Park Ave., Deephaven, MN 55391 <a href="http://greenbarngardens.com/">http://greenbarngardens.com/</a>	OR.....	Orsetti Seed Co., P.O. Box 2350, Hollister, CA 95024 <a href="http://www.orsettiseeds.com">www.orsettiseeds.com</a>
GO.....	Goldsmith Seeds Inc., 2280 Hecker Pass Highway, P.O. Box 1349, Gilroy, CA 95020 <a href="http://www.voeksinc.com/goldsmith-seeds/">www.voeksinc.com/goldsmith-seeds/</a>	P.....	Pacific Seed Production Co., P.O. Box 947, Albany, OR 97321 <a href="http://www.pacificseed.com">www.pacificseed.com</a>
GU.....	Gurney's Seed and Nursery Co., P.O. Box 4178, Greendale, IN 47025 <a href="http://www.gurneys.com">www.gurneys.com</a>	PA/PK.....	Park Seed Co., 1 Parkton Ave., Greenwood, SC 29647-0002 <a href="http://www.ParkSeed.com">www.ParkSeed.com</a>
HI.....	High Mark Seeds, 5313 Woodrow Ln, Hahira, GA 31632 <a href="http://www.highmarkseed.com">www.highmarkseed.com</a>	PARA.....	Paragon Seed Inc., P.O. Box 1906, Salinas CA, 93091 831-753-2100
HL/HOL.....	Hollar & Co. Inc., P.O. Box 106, Rocky Ford, CO 81067 <a href="http://www.hollarseeds.com">www.hollarseeds.com</a>	PG.....	The Pepper Gal, P.O. Box 23006, Ft. Lauderdale, FL 33307-3006 <a href="http://www.peppergal.com">www.peppergal.com</a>
H/HR.....	Harris Moran Seed Co., 3670 Buffalo Rd., Rochester, NY 14624, Ph: (716) 442-0424 <a href="https://www.hmclause.com">https://www.hmclause.com</a>	PL.....	Pure Line Seeds Inc., Box 8866, Moscow, ID <a href="https://purelineseed.com">https://purelineseed.com</a>
HMS.....	High Mowing Organic Seeds, 76 Quarry Rd., Walcott, VT 05680 <a href="http://www.highmowingseeds.com">www.highmowingseeds.com</a>	PAN.....	Pan American Seed Company, P.O. Box 438, West Chicago, IL 60185 <a href="http://www.panamseed.com">www.panamseed.com</a>
		PT.....	Pinetree Garden Seeds, P.O. Box 300, New Gloucester, ME 04260 <a href="http://www.superseeds.com">www.superseeds.com</a>
		RM.....	Reimer Seed Co., PO Box 236, Mt. Holly, NC 28120 <a href="http://www.reimerseeds.com">www.reimerseeds.com</a>

RG.....	Rogers Seed Co., P.O. Box 4727, Boise, ID 83711-4727 928 783 7204	SW .....	Seedway Inc., 1225 Zeager Rd., Elizabethtown, PA 17022 <a href="http://www.seedway.com">www.seedway.com</a>
RI/RSP.....	Rispens Seeds Inc., 3332 Ridge Rd., P.O. Box 5, Lansing, IL 60438 <a href="http://rispensseeds.com">rispensseeds.com</a>	SY.....	Syngenta AG, 600 N Armstrong Place (83704), Box 4188, Boise, ID 83711 <a href="http://www.syngenta.com">www.syngenta.com</a>
RS.....	Royal Sluis, 1293 Harkins Road, Salinas, CA 93901 <a href="http://www.sluisgarden.com">www.sluisgarden.com</a>	TR.....	Territorial Seed Company, Box 158, Cottage Grove, OR 97424 <a href="http://www.territorialseed.com">www.territorialseed.com</a>
RU.....	Rupp Seeds Inc., 17919 Co. Rd. B, Wauseon, OH 43567 <a href="http://www.ruppseeds.com">www.ruppseeds.com</a>	TGS.....	Tomato Growers Supply Co., P.O. Box 2237, Ft. Myers, FL 33902 <a href="http://www.tomatogrowers.com">www.tomatogrowers.com</a>
SM .....	Seminis Inc., 2700 Camino del Sol, Oxnard, CA 93030- 7967 <a href="http://www.seminis.com">www.seminis.com</a>	TT.....	Totally Tomatoes, P.O. Box 1626, Augusta, GA 30903 <a href="http://www.totallytomato.com">www.totallytomato.com</a>
SE.....	Southern Exposure Seed Exchange, P.O. Box 460 Mineral, VA 23117 <a href="http://www.southernexposure.com">www.southernexposure.com</a>	TW .....	Twilley Seeds Co. Inc., P.O. Box 65, Trevese, PA 19047 <a href="http://www.twilleysseed.com">www.twilleysseed.com</a>
SHUM .....	Shumway Seed Co., 334 W. Stroud St. Randolph, WI 53956 <a href="http://www.rhshumway.com">www.rhshumway.com</a>	UA.....	US Agriseeds, San Luis Obispo, CA 93401 <a href="http://www.voloagri.com">www.voloagri.com</a>
SI/SG.....	Siegers Seed Co., 8265 Felch St., Zeeland, MI 49464- 9503 <a href="http://www.siegers.com">www.siegers.com</a>	UG .....	United Genetics, 8000 Fairview Road, Hollister, CA 95023 <a href="http://unitedgenetics.com">unitedgenetics.com</a>
SK.....	Sakata Seed America Inc., P.O. Box 880, Morgan Hill, CA 95038 <a href="http://www.sakata.com">www.sakata.com</a>	US .....	US Seedless, 12812 Westbrook Dr., Fairfax, VA 22030 <a href="http://usseedless.com">usseedless.com</a>
SN.....	Snow Seed Co., 21855 Rosehart Way, Salinas, CA 93980 <a href="http://dev.snowseedcompany.com">dev.snowseedcompany.com</a>	VE .....	Vesey's Seed Limited, York, Prince Edward Island, Canada <a href="http://www.veseys.com">www.veseys.com</a>
SOC.....	Seeds of Change, Santa Fe, NM <a href="http://www.seedsofchange.com">www.seedsofchange.com</a>	VL.....	Vilmorin Inc., 6104 Yorkshire Ter., Bethesda, MD 20814 <a href="http://www.shamrockseed.com">www.shamrockseed.com</a>
SST .....	Southern States, 6606 W. Broad St., Richmond, VA 23230 <a href="http://www.southernstates.com/farm-store/">www.southernstates.com/farm-store/</a>	VS .....	Vaughans Seed Co., 5300 Katrine Ave., Downers Grove, IL 60515-4095 <a href="http://www.vaughans.com/">www.vaughans.com/</a>
ST.....	Stokes Seeds Inc., 737 Main St., Box 548, Buffalo, NY 14240 <a href="http://www.stokeseeds.com">www.stokeseeds.com</a>	WI .....	Willhite Seed Co., P.O. Box 23, Poolville, TX 76076 <a href="http://www.willhiteseed.com">www.willhiteseed.com</a>
SU/SS.....	Sunseeds, 18640 Sutter Blvd., P.O. Box 2078, Morgan Hill, CA 95038 <a href="https://vitakraftsunseed.com">https://vitakraftsunseed.com</a>	WP .....	Wood Prairie Farms, 49 Kinney Road, Bridgewater, ME 04735 <a href="http://www.woodprairie.com">www.woodprairie.com</a>
SV .....	Seed Savers Exchange, 3094 North Winn Rd., Decorah, IA 52101 <a href="http://www.seed savers.org">www.seed savers.org</a>	ZR .....	Zeraim Seed Growers Co. Ltd., P.O. Box 103, Gedera 70 700, Israel <a href="http://www.zeraim.com/en">www.zeraim.com/en</a>





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