

DISSERTATION

AN EVALUATION OF YIELD AND QUALITY CHARACTERISTICS FOR VEGETABLE
CROPS GROWN UNDER ORGANIC MANAGEMENT IN FORT COLLINS, COLORADO

Submitted by

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ABSTRACT

AN EVALUATION OF YIELD AND QUALITY CHARACTERISTICS FOR VEGETABLE CROPS GROWN UNDER ORGANIC MANAGEMENT IN FORT COLLINS, COLORADO

Colorado is known for having a strong natural foods industry. It also ranks ninth in the country for organic food purchases, totaling \$155 million annually. Within that, the national average sales of organic fruits and vegetables accounts for 40% of total organic food sales. In Colorado, this would equate to \$72 million in organic fruit and vegetable sales. Yet, the actual value of certified organic vegetable sales has only reached the level of \$40 million annually. This difference represents an economic opportunity for Colorado's organic vegetable producers to meet the demand for organic foods. It is also important to note that more than 50% of consumers between the ages of 18-29 years include organic foods in their diets, whereas only 33% of those that are 65 years and older consume organic foods, which indicates a generational difference in preference. This also suggests that the organic foods market will continue to grow with the millennial generation as they predominate the market and likely beyond.

The mission of the Specialty Crops Program (SCP) at Colorado State University (CSU) is to empower growers and producers throughout Colorado by supplying them with science based information to inspire innovation, competitiveness and success. In order to help vegetable producers address the demand for organic foods, system input studies are needed to help growers optimize the performance of their organic systems; cultivar selection (Chapter 1) is an important consideration to any production system. Over three years (2016-2018), we observed significant yield and quality characteristics differences between cultivars of tomato (*Solanum lycopersicum*), sweet corn (*Zea mays* var. *saccharata*), cabbage (*Brassica oleracea* var.

capitata), bell and roasting peppers (*Capsicum annuum*), and winter squash (*Cucurbita pepo*) in an organic production system located at the Agricultural Research, Development and Education Center South (ARDEC S.) in Fort Collins, CO (lat. 40°36'N., long. 104°59'W.) elevation 5,003 feet. The top performers of the cultivar yield trial, by crop, were 'Mountain Merit' (tomato), 'Nirvana' (sweet corn), 'Lennox' (cabbage), 'Aristotle' (pepper), and 'Bush Delicata' (winter squash), respectively. However, there were many other top tier performers based on yield and quality characteristics that would also make for a valuable input into an organic system. Flavor was another one of the important quality characteristics assessed in this cultivar trial.

We conducted consumer sensory panels at the Larimer County Farmers Market to assess what direct-market shoppers thought of the flavor of the eight tomato and fourteen sweet pepper cultivars included in the trial. This afforded us the opportunity to characterize an important quality attribute. However, it was a time-consuming and laborious way to collect what is known to be relatively subjective data on flavor. Postharvest quality characteristics, such as taste, nutrition, and bioactive compound content can also be evaluated using mass spectrometry platforms coupled with chemometric analysis (Chapter 2). Gas chromatography-mass spectrometry (GC-MS) coupled with solid phase micro extraction (SPME) has been used to profile aroma-contributing metabolites in food crops. However, sample preparation, long acquisition times, and the need for technical lab expertise presents barriers preventing routine application of this type of technology. Ambient mass spectrometry (AMS), which detects metabolites in minimally processed samples under ambient conditions, has emerged as a promising analytical platform for assessing quality characteristics in plant products.

Here, we evaluated SPME-GC-MS and two AMS platforms, direct analysis in real time (DART) mass spectrometry and rapid evaporative ionization-mass spectrometry (REIMS)

coupled with chemometric analysis, to assess quality characteristics in 40 pepper (*Capsicum annuum* L.) cultivars. SPME-GC-MS was able to detect metabolite differences between red and green bell peppers based on volatile aroma-contributing metabolites. DART-MS exhibited the ability to discriminate between pod colors based on metabolite fingerprints, while REIMS could distinguish pepper market class (e.g. bell, lunchbox, and popper). Furthermore, DART-MS analysis resulted in the detection of important bioactive compounds in human diet such as vitamin C, *p*-coumaric acid, and capsaicin. The results of this study demonstrate the potential for these approaches as accessible and reliable tools for high throughput screening of pepper quality. AMS platforms could be employed to evaluate genotypes in a cultivar trial or to evaluate the impact of a crop input on the metabolome.

In addition to the cultivar trial and metabolomics platform evaluation studies, the SCP and I evaluated the impact of crop inputs on yield and quality characteristics of three organic vegetable crops (sweet corn, onions, and peppers). Our research team quantified the impact of applying an Organic Materials Review Institute (OMRI) approved rock-powder based soil amendment (Azomite) on roasting pepper yield (Chapter 3). Azomite (0-0-0.2) supplies trace minerals from mined sources including: calcium (1.8%), magnesium (0.5%), chlorine (0.1%), sodium (0.1%). Two grades of Azomite (granulated and ultrafine) were assessed separately: granulated Azomite was banded in the field and ultrafine Azomite was applied via drip system and/or foliar application in a high tunnel. Interestingly, particle size and the application method were statistically significant predictors of pepper yield. Granulated Azomite that was banded in the field did not increase pepper yield. However, ultrafine Azomite that was applied as a foliar spray significantly increased red pepper yield compared to the water foliar control in ‘Early Perfect Italian’ peppers. In addition, the number of marketable pods significantly increased following an

Azomite foliar application in ‘Stocky Red Roaster’ compared to the no foliar spray control. This study has implications for pepper growers wanting to increase their yields by applying rock powder based soil amendments that contain K and trace minerals.

The last input evaluated for organic vegetable production systems was caprylic/capric acid (CA) (Chapter 4). It is a promising next-generation herbicide for organic specialty crop production; similar to acetic acid (AA, 20% volume/volume), it functions as a post-emergent, non-selective herbicide that damages any green plant tissue it contacts. It was evaluated in a conventional onion system in a commercial onion farmer’s field in Adams County, Colorado, and it was further evaluated in an organic sweet corn system at ARDEC S. Importantly, we observed that application volumes, which are typical of herbicides applied in conventional systems (e.g. 20 gallons per acre) are insufficient carrier volumes for herbicides approved for organic systems. At 50 gallons per acre, CA and AA were equally effective at desiccating small weeds that were less than two inches in height. Each of these herbicides achieved about 50 percent weed control one day after treatment (DAT). However, CA was significantly more effective than AA at controlling weeds that were between six and nine inches in height one day after a high volume (200 gallons per acre) treatment. This is the first documentation of the superior weed control ability of CA. CA is labelled for use in and around all food crops, including organic, which could make this next generation herbicide a valuable tool for Colorado and other Intermountain West organic specialty crop producers. Taken all together, the cultivar trial results, the knowledge we gained of Azomite grades and application method, and our increased understanding of carrier volumes using next generation herbicides has positive implications for growers managing small and large acreage operations. Input system management studies, such as these, are important because they provide useful information to

growers, which helps them identify the management practices and inputs that align with their production preferences and financial situation. In addition, our high throughput method for postharvest quality assessments using AMS could be useful to cultivar trial managers or research horticulturists that are interested in assessing the impact of cultivar, production system inputs, or environment on the metabolome of peppers.

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CHAPTER 1: Organic Vegetable Cultivar Trial: Evaluating Yield and Quality Characteristics

Introduction and Literature Review

Boulder County, centered in Colorado, is known for having a strong natural foods industry. Colorado ranked ninth in the country in 2015 for organic food purchases, which totaled \$155 million. In 2016, the amount of organic foods sold in Colorado grew to \$181 million (USDA, 2016). The national average sales of organic fruits and vegetables accounted for 40% of total organic sales in 2016 (Greene et al., 2017). If the national average held true for Colorado, this would equate to \$72 million in organic fruit and vegetable sales. However, the actual value of certified organic vegetable sales in Colorado was \$40 million (USDA, 2016). The difference between the national average for organic vegetable sales and the actual value produced in Colorado represents an economic opportunity for the state's organic vegetable producers.

Consumer demand for certified organic foods has grown by double-digits annually since the National Organic Program was established in 2000 (Greene et al., 2017). In addition, results from a 1,000 person online survey on household economic activity and motivations pertaining to Colorado agriculture indicate consumer support for Colorado food systems has risen from 79% in 2011 to 85% in 2016 (CDA, 2016). Factors influencing these Colorado organic market trends are listed in Figure 1.1. It is notable that more than 50% of consumers between the ages of 18-29 include organic foods in their diets, whereas only 33% of those that are 65 and older consume organic foods indicating a generational difference in preferences (Greene et al., 2017). In addition, this suggests that the organic foods market will continue to grow with the millennial generation, those born 1981-1996, and likely beyond.

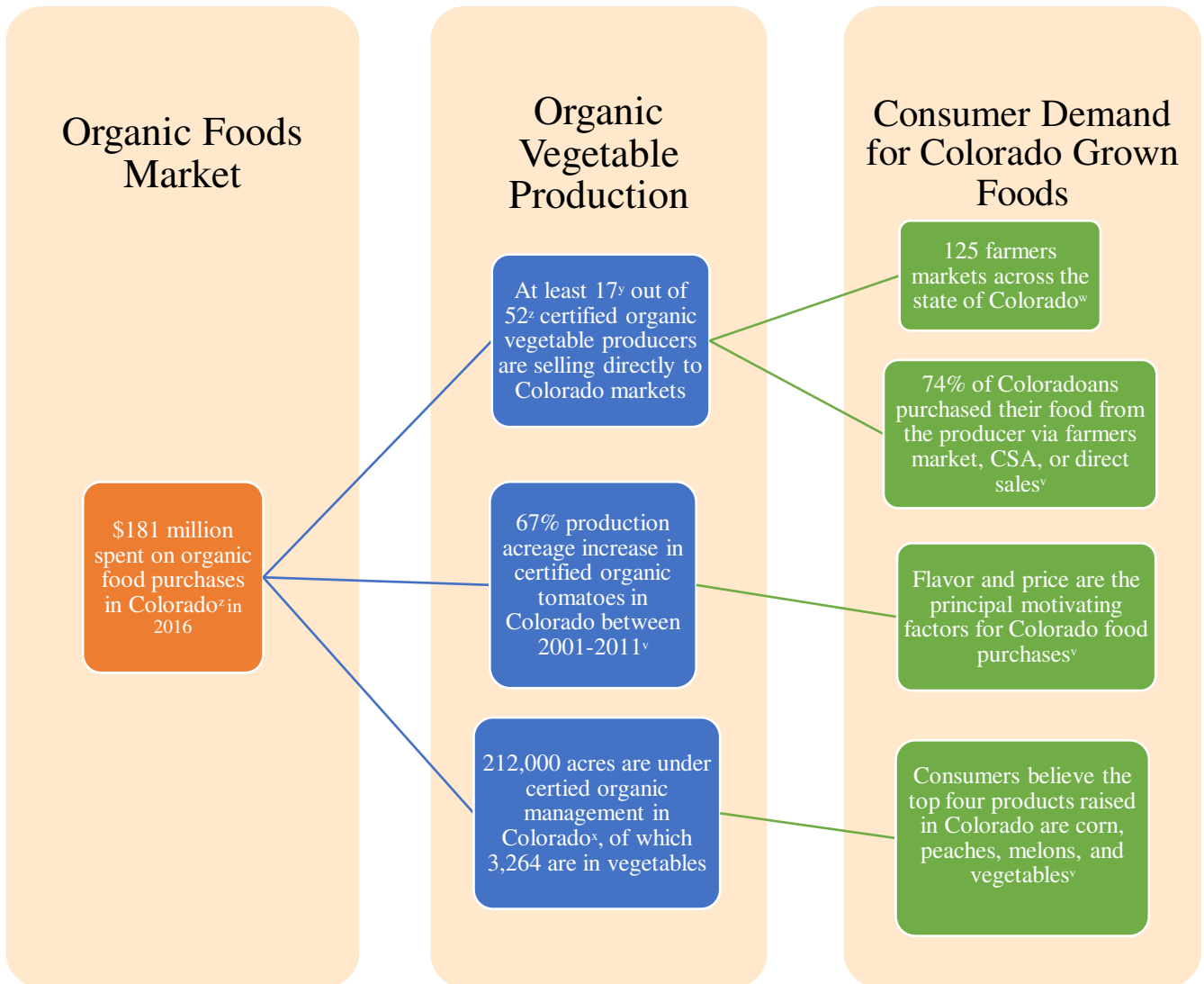


Figure 1.1. Consumer demand and organic vegetable production factors that help shape the organic foods market in Colorado.

^z USDA. 2016. Certified organic survey – Colorado.

<https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/OrganicProduction/2016StatePublications/CO.pdf>.

^y Colorado Department of Agriculture. 2019. Colorado farm fresh directory.

<https://www.colorado.gov/pacific/sites/default/files/Colorado%20Farm%20Fresh%20Directory_1.pdf>.

^x Driscoll, L. and N. Ichikawa. 2017. Growing organic, state by state: A review of state-level support for organic agriculture. Berkeley Food Institute. <https://food.berkeley.edu/wp-content/uploads/2017/10/BFI_Organics_Report_2017_WEB.pdf>.

^w Colorado Department of Agriculture. 2019. Colorado farmers’ market locations.

<<https://www.colorado.gov/pacific/sites/default/files/Colorado%20Farmers%27%20Markets.pdf>>.

^v Colorado Department of Agriculture. 2016. Public attitudes about agriculture in Colorado.

<<https://www.colorado.gov/pacific/sites/default/files/2016%20Public%20Attitudes%20Report%20Final.pdf>>.

In order to help producers keep pace with the demand for organic foods, system input studies are needed to help growers optimize the performance of their organic systems. For example, Williams and Roberts (2002) state that cultivar choice is one of the most important production input decisions made in a year. A cultivar's time to maturity, pest and disease resistance, total marketable yield, quality characteristics, and consumer acceptability are all characteristics of interest to vegetable growers (Stonaker, 2009). In addition, organic growers would benefit from cultivar trials that report on other plant traits that are advantageous to organic systems such as crop canopy architecture and root abundance. Crop canopies that are quick to close help shade out weeds, and a robust root system is better able to tolerate mechanical weeding. Cultivar trials that report on these traits empower organic growers to select a genotype that suits their production needs and their consumers' expectations (Davies and Lennartsson, 2005).

Not only do replicated cultivar trials at universities or in farmers' fields offer recommendations to growers, they also provide a forum to share feedback regarding genotypic performance to plant breeders. Plant breeders with modest resources can use detailed cultivar trial reports to guide their selection practices to further genetic gains. The Northern Organic Vegetable Improvement Collaborative (NOVIC) uses a participatory plant breeding approach that incorporates input from vegetable breeders, researchers, farmers, and consumers in order to advance experimental breeding lines for organic systems. It originally focused on organic hot spots, but has since expanded to less developed regions such as the Intermountain West. Now researchers and Extension educators at Oregon State University, University of Wisconsin-Madison, Cornell University, Colorado State University, Washington State University, the Organic Seed Alliance, and the USDA are working together to identify top performing cultivars

for organic systems (eOrganic, 2019). In addition, NOVIC has the goal of developing open-pollinated cabbage, tomato, winter squash, pepper, and sweet corn cultivars that are specifically adapted to address the needs of organic growers in northern climate or high elevation locations, in the case of Colorado (5,003 feet).

It is estimated that approximately 95% of all the cultivars grown in organic systems were bred for high-input, conventional systems (Lammerts van Bueren et al., 2011). However, the inputs (synthetic fertilizers, pesticides, and seeds) that go into conventional systems are different from organic systems. There is evidence that cultivar performance can be dependent on the system in which it was evaluated. Genotype x production system interaction studies have focused on agronomic crop performance as opposed to vegetable crop performance, which is why it has been summarized here. For example, Murphy et al. (2007) demonstrated a significant genotype x production system interaction when the soft white winter wheat cultivars that were top performers under conventional management were not the same as those under organic management. Further, Lammerts van Bueren et al. (2011) propose that breeding selection practices that occur in organic systems are more likely to lead to the expression of crop traits that are better suited to organic systems. Beneficial to organic system traits include high nutrient-use efficiency, rhizosphere competence for disease suppression, competition against weeds, mechanical weeding resilience, and biotic and abiotic stress resistance. However, maintaining separate breeding pipelines for organic and conventional vegetable systems is expensive in terms of resources and time; it can take up to 10 years to release a new vegetable cultivar. Therefore, there is some value in growing the top performing cultivars in an organic system, even though selections were made under conventional management. However, organic growers should consider cultivar trial results conducted under organic system where available (Wortman et al.,

2017). This emphasizes the need to evaluate genotypic performance in a certified organic production system for vegetable crops. However, there are very few studies reported in the past eight years; summarized here.

During spring and fall 2007-2008, a leafy green cultivar trial was conducted in Lexington, Kentucky under organic management. It included 38 cultivars of leafy greens from Brassicaceae including multiple species of mustard, kale, collards, turnip, Swiss chard, and arugula. Coolong et al. (2013) aimed to provide cultivar recommendations within leafy green genera to market growers managing small-acreage operations that sell directly to consumers. The authors concluded that 'Florida Broadleaf', 'Siberian', 'Georgia Southern', 'Alamo', 'Fordhook Giant', and 'Astro' were top performing cultivars for mustard, kale, collards, turnip, swiss chard and arugula greens, respectively for the midsouth growing region.

Between 2010 and 2011, a winter squash trial was conducted at multiple locations across the state of Pennsylvania under conventional and organic management (Sánchez et al., 2012). The parameters of interest were fruit number per plant, fruit weight per plant, and individual fruit weight. The authors made cultivar recommendations of 'JWS 6823', 'Betternut 401', and 'Metro' for butternut squash produced under conventional management based on yield. For acorn squash under conventional management, 'Autumn Delight', 'Harlequin', 'Table Star', 'Tip Top', 'Jet', 'Table Treat', 'Celebration', and 'Black Bellota' were all top performers. For buttercup cultivars under conventional management, 'Red Kuri', 'Sweet Mama', and 'Sun Spot' were all recommended. The top performing acorn squash cultivars for organic systems differed from those for conventional systems; 'Celebration' and 'Table Queen' were recommended for organic systems. This reinforces the need to make cultivar recommendations for organic systems based on cultivar trials conducted under organic growing conditions and by growing region. In

addition, the ornamental qualities of the squash cultivars were described in the report, which reminds that a majority of market growers selling directly to the consumer are interested in quality components that entice customers, as much as if not more than the total marketable yield.

Another cultivar trial was conducted in Athens, Georgia between 2011 and 2012 on determinate, indeterminate, and semi-determinate tomatoes. Boyhan and colleagues (2014) compared open-pollinated to F1 hybrid beefsteak-type tomato cultivars grown in a transitioning to organic management system. The parameters of interest for the 19 cultivars in the trial were early and total graded yield. The authors reported that the F1 hybrids generally outperformed the open-pollinated cultivars. ‘HSX 8115H’ produced the highest yield of total early fruit. Whereas ‘Celebrity’ had a significantly greater total graded yield compared to the other tomato entries. The top five productive tomatoes in the trial were ‘Celebrity’, ‘Mountain Fresh Plus’, ‘HSX 8115H’, ‘Fletcher 0377’, and ‘Costoluto Fiorentino’ based on total yield. The authors referenced the interesting visual and flavor components associated with open-pollinated cultivars, but did not report on specific sensory attributes at the cultivar level in the trial.

Boyhan et al. (2019) recently published bell pepper cultivar recommendations for organic growers in the Southeast U.S. based on trials conducted in Athens, Georgia. The authors evaluated the genotypic performance of 13 sweet pepper cultivars; the selections were based on grower input and disease resistance descriptions from seed catalogs. The parameters of interest were early graded yield, fancy (>3 in diameter, 3.5 in length) graded yield and total graded yield. Because of a significant interaction, the graded yield data was analyzed by year. The authors state that in 2016, ‘Sweet Chocolate’ had the highest number of total graded fruit, but the fruits were the smallest among cultivar entries. ‘Aristotle X3R[®]’ produced the highest number of fancy fruit in 2016. The yields in 2017 were much higher than in 2016. In 2017, the authors report that

‘Aristotle X3R[®]’ had the greatest total yield in terms of boxes per acre, however it was not significantly more than ‘Gridiron’, ‘King Arthur’, ‘Flavorburst’, ‘Blitz’, ‘Touchdown’, or ‘PS 099793325 X10[®]’. In addition, ‘Blitz’ had the highest number of fancy fruit. ‘Flavorburst’ on the other hand, had the most No. 1 fruit (2.5” diameter and length), although it was not significantly different from ‘Aristotle X3R[®]’. In addition to reporting on the total yield of graded fruit for each cultivar, the authors conclude that in a terminal market the peppers would sell for approximately \$17.58/box (28 pounds/box). This demonstrates that the cultivar recommendations are likely focused on pepper growers managing a large-acreage operation, and those that sell to terminal markets. Direct to market sales would more than likely receive a price premium per box compared to terminal markets (Davies and Lennartsson, 2005). Other than the color of the pepper pod, there was no mention of a quality characteristic assessment in this trial.

Under organic management sixteen watermelon cultivars including open-pollinated and F1 hybrids were evaluated in Watkinsville, Georgia between 2016 and 2017 (Boyhan et al., 2019). In addition to total marketable yield and total fruit count, data were collected on fruit quality characteristics including rind thickness, fruit color, and soluble solids content. Based on the literature, this is the first reporting of fruit quality characteristics included as part of a cultivar trial. Results of the trial are targeted at providing recommendation to producers managing large-acreage operations and market growers selling directly to consumers. The authors conclude ‘Georgia Rattlesnake’ was the highest yielding cultivar based on a pounds per acre basis; it was included in the top five productive cultivars along with ‘Nunhems 800’, ‘Nunhems 860’, ‘Orangelo’, and ‘SSX 8585’. In regards to desirable fruit characteristics, the authors report ‘Nunhems Premium’ and ‘Orangelo’ had bright colored flesh, which is appealing to market shoppers. ‘Sangria’ had the great soluble solids content among watermelon cultivar entries, and

‘Nunhems 790 hq’ was the firmest watermelon, which suggests it is better able to withstand transportation without splitting.

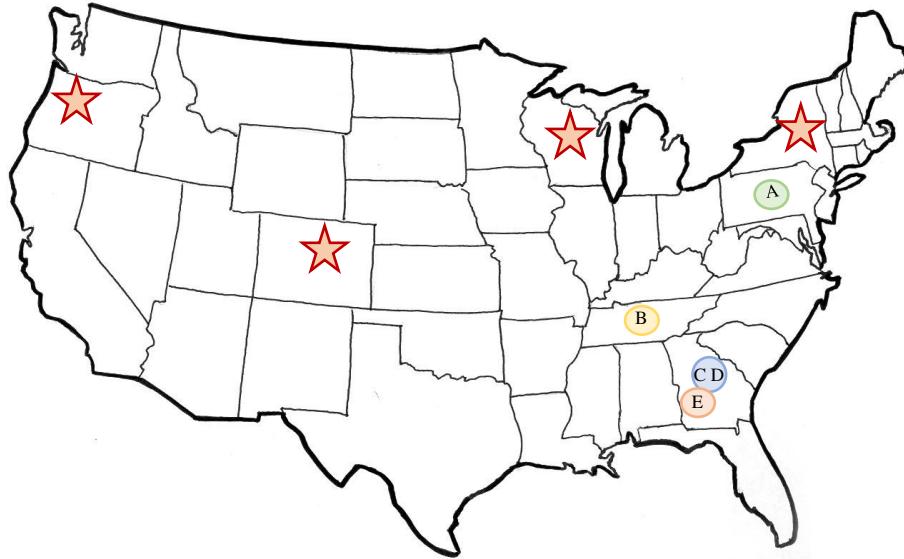


Figure 1.2. Vegetable cultivar trial locations conducted under organic management in the U.S. over the past 8 years (2012-2020).

A: Sánchez et al. (2012); winter squash across Pennsylvania

B: Coolong et al. (2013); leafy greens in Lexington, KY

C: Boyhan et al. (2014); tomatoes in Athens, GA

D: Boyhan et al. (2019); bell peppers in Athens, GA

E: Boyhan et al. (2019); watermelon in Watkinsville, GA

★ = NOVIC III participating sites

United States map. (2019); printable maps. < https://akphotostudio.com/wear_rm.php>

It is worth noting that growers are also interested in planting cultivars with modern productivity traits such as disease resistance, ease of harvesting, earliness, and storability. These factors impact the amount of labor needed, price premiums received, and the length of time one has to sell the crop. Growers consider several other factors when selecting which cultivars to plant. They consider whether the seed was produced under organic or conventional management, and whether the seed has intellectual plant property rights attached to it or is an open seed source. Purchasing from an open seed source affords growers the opportunity to plant, preserve, save, and create new crosses from seed.

Growers also must decide whether to plant F1 hybrids or open-pollinated cultivars because some of their customers may have a preference. These decisions are based on the financial inputs a grower wants to invest in his/her crop, the cost/benefit relationship of planting an F1 hybrid with unique crop traits such as improved quality or disease resistance, and the intellectual plant property protections that may be associated with the certain genetics. The aim of this trial is to identify top performing vegetable cultivars that were bred in and for organic systems and are open source. This trial is also unique because it evaluated cultivar performance for market growers from a vegetable quality and yield perspective. To our knowledge, it is the first organic cultivar trial in the Intermountain West, and the first to evaluate five vegetable species at one site using open source seed and breeding lines from public and vegetable industry breeding programs (e.g. Johnny's Selected Seeds).

Materials and Methods

Commercially available cultivars and unreleased breeding lines of tomato (*Solanum lycopersicum*), sweet corn (*Zea mays* var. *saccharata*), cabbage (*Brassica oleracea* var. *capitata*), bell and roasting peppers (*Capsicum annuum*), and winter squash (*Cucurbita pepo*) were grown on certified organic land at the Agricultural Research, Development and Education Center (ARDEC) South in Fort Collins, CO (lat. 40°36'N., long. 104°59'W.) elevation 5,003 feet (Table 1.1). The experimental lines were a product of breeding efforts at Cornell University, the University of Wisconsin-Madison, Oregon State University, and Colorado State University to improve yield and quality characteristics for vegetable crops grown under organic management.

A soil analysis determined the crops were grown in a sandy clay loam with a pH of 7.9, an organic matter content of 2.1%, and parts per million (PPM) of nitrate, phosphorous, and potassium at 6.0, 20.2, and 444 PPM, respectively. Zinc, iron, manganese, and copper were at the

levels of 2.5, 2.2, 1.8, and 4.3, respectively. Macronutrient needs were met with monthly applications of Drammatic “One” fertilizer 4-4-0.5 (Dramm Corporation, Manitowoc, WI). In addition, a half teaspoon each of blood meal (12-0-0) and bone meal (3-15-0) from Down to Earth Distributors, Inc. was placed in the planting hole at the time of transplanting.

Sweet pepper, tomato, and cabbage seeds were sown on 24 March, 3 April, and 10 April 2017 respectively. A soilless media composed of approximately 40 L Sunshine mix #4, 10 L of worm castings, 250 ml of blood meal, and 250 ml of bone meal was used to germinate and grow the transplants while they were in the greenhouse. The average daily temperature was 25°C and the relative humidity was 50% in the greenhouse (CSU Horticulture Center). The transplants were hardened by placing them outside under an insect-netting covered high tunnel with the east and west end walls removed for 7-10 days prior to transplanting.

Table 1.1. Commercially available cultivars and unreleased breeding lines included in the trial; 2016-2018.

Crop	Commercially available cultivars (90)	Breeding lines (12)	Total
Cabbage	(19) ‘Alessandro’, ‘Bartolo’, ‘Cantasa’, ‘Deadon’, ‘Green Mariner’, ‘Integro’, ‘January King’, ‘Kilmaro’, ‘Lennox’, ‘Marabel’, ‘Paressa’, ‘Reaction’, ‘Red Granite’, ‘Ruby Perfection’, ‘Sieron’, ‘Stanton’, ‘Turkis’, ‘Typhoon’, and ‘Wirosa’	(0)	19
Sweet corn	(18) ‘Allure’, ‘Anthem XR’, ‘AP426’, ‘Double Standard’, ‘Festivity’, ‘Luscious’, ‘Mirai 131Y’, ‘Mirai 308BC’, ‘My Fair Lady’, ‘New Mama’, ‘Nirvana’, ‘Temptation’, ‘Who Gets Kissed’, ‘Xtratender 2171’, ‘Xtratender 274A’, ‘XTH20173’, ‘Zanadoo’, and ‘2472XR’	(1) ‘Jared Synthetic 21’	19
Tomato	(8) ‘Crimson Sprinter’, ‘Damsel’, ‘Iron Lady’, ‘Mountain Merit’, ‘Plum Perfect’, ‘Plum Regal’, ‘Stupice’, and ‘Stellar’	(4) ‘NC12TMV007 x 141233-62’, ‘LB8-3-1-1-1’, ‘LB8-7-1-1-1’, and ‘S200-1-1’	12

Winter squash	(18) 'Acorn #1', 'Bush Delicata', 'Candystick', 'Carnival', 'Celebration', 'Delicata JS', 'Festival', 'Gill's Golden Pippin', 'Honey Bear', 'Jester', 'JWS 6823 PMR', 'Mardi Gras', 'Sugar Bush', 'Sugar Dumpling', 'Sweet Reba Bush', 'Thelma', 'Tuffy', and 'Zeppelin'	(3) 'CU 1', 'CU 2', and 'CU 3'	21
Sweet peppers	(24) 'Ace', 'Aristotle X3R [®] ', 'Belcanto', 'Bella Italia', 'Bridge to Paris', 'Carmen', 'Classic Italian', 'Cornito Rosso', 'Corno di Toro', 'Crest Yellow', 'Early Perfect Italian', 'Early Red Sweet', 'Escamillo', 'Flavorburst', 'Gypsy Queen', 'Karma', 'King Krimson', 'King of the North', 'Laerte', 'Lively Yellow', 'Red Knight X3R [®] ', 'Revolution', 'SBGO 10408', 'Stocky Red Roaster', and 'Whitney'	(3) 'CU 1', 'CU 2', and 'CU 3'	27
Pungent peppers	(2) 'CSU Mosco' and 'CSU Pueblo Popper'	(2) 'CSU 256' and 'CSU 321'	6

All five crops were planted as a randomized complete block design with three blocks in 2016, 2017, and 2018. The planting configuration, number of plants per cultivar/plot combination, number of border plants between cultivars, the in-row spacing, and the between row spacing used for the trial are listed in Table 1.2. A mini-layer 2400 (Rain-Flo Irrigation, East Earl, Pennsylvania) was used to shape the beds. Black plastic drip tape was placed approximately 2 in below the soil surface combined with 1.5 ml thick black plastic weed barrier (Rain-Flo Irrigation, East Earl, Pennsylvania). Holes were punched with a #26 super wheel with the appropriate number of adjustable C-spikes (Rain-Flo Irrigation, East Earl, Pennsylvania) to accommodate the within row plant spacing (Table 1.2).

The tomato, pepper, and cabbage transplants were planted in the field on 31 May, 6 June, and 7 June 2017, respectively. The tomatoes were planted in a high tunnel. The sweet corn and winter squash seeds were direct-sown on 8 June and 22 June 2017, respectively. The sweet corn seeds were sown in four-row plots.

Table 1.2. Planting location, configuration, number of plants per cultivar/block combination, and row spacing for indicated crops.

Crop	Planting configuration	# of Plants/ cultivar	Border plants between cultivars	In-row spacing (in)	Between row spacing (ft)
Cabbage	Double row	10	-	12	6
Peppers	Single row	10	2	18	6
Sweet corn	Double row	20	2	8	6
Tomato	Single row	6	-	24	2.5
Winter squash	Single row	12	2	24	6

Data were collected on the inner two rows; the outer rows served as a border rows, and they helped to pollinate the silks. The winter squash seeds were sown in two row plots. ‘Harelquin’ was sown as a border plant because it has powdery-mildew resistance; monitoring powdery-mildew disease incidence was a parameter of interest in the trial. Weeds were controlled in all vegetable crops with a wheeled stirrup hoe used every 2-3 weeks, as needed. In addition, weeds were hand pulled from the holes where the respective crops were growing.

At the beginning of the season, weeds were hand pulled from the high tunnel. Plant-based compost (A-1 Organics, Eaton CO) was applied at the rate of approximately 2 yards³/525 feet². The compost was incorporated when the soil was rototilled to a depth of 0.15 to 0.30 m using a Harvester 722 (BCS, Portland, OR). Woven weed fabric was laid to help suppress weed growth from Canada thistle, field bindweed, and other weeds. Black plastic drip tape emitting water at a rate of 500L/h/100m with emitters spaced 20cm apart was used to irrigate the crop. Using an irrigation controller, we provided 15-30 minutes of irrigation once or twice daily.

Insect netting was used to cover the high tunnel to mitigate potential damage to the crop from hail storms, which are common in Colorado during the growing season. In addition, a trellising system was created for the tomatoes by running six metal overhead cables (east to west) above the plants. Trellising spools containing white twine and hooks were used to connect the tomato

plants to the cable wires. The tomatoes were attached to the twine with trellising clips spaced approximately every 18 in. Furthermore, lateral vegetative growth on the tomatoes was pruned to maintain a central vertical leader on the indeterminate cultivars. The crops were scouted in the greenhouse and the field for pests on a weekly basis. A single application of *Bacillus thuringiensis* (Thuricide® BT Caterpillar Control, Southern Agriculture Insecticides, Inc., Palmetto, Florida) was made to the cabbage crop for *Trichoplusia ni* (cabbage loopers) in 2017 according to national organic program (NOP) practices. In addition, pyrethrin applications (PyGanic® Crop Protection EC 1.4. II (MGK Insect Control Solutions, Minneapolis, Minnesota) were used to control leaf-feeding insects such as *Myzus persicae* (green peach aphids) on pepper transplants in the greenhouse in 2018.

Cabbage

Data for cabbage was collected on yield of marketable heads (pounds/head), storability, head height and width (cm), and core length using a 1-3 scale. A (1) indicated the entire head contained 1/3 or less of ridged core material that is usually discarded as indicated in Figure 3; a (2) indicated approximately half of the head contained core material; a (3) indicated 2/3 or more of the head contained core material as pictured in Figure 4. Head uniformity was evaluated using



Figure 1.3. Cabbage heads exhibiting a short core length (score of 1 = short).

a 1-9 scale where a (1) indicated the heads are highly variable across several traits; a (3) indicated the heads were variable across several to a few traits; a (5) indicated the heads were moderately variable across a few traits; a (7) indicated the heads were uniform for most traits; a (9) indicated the heads were highly uniform for all or nearly all traits. The number of days (after transplanting) to maturity was also recorded for each cultivar (Table 1.2).



Figure 1.4. Cabbage heads exhibiting a long core length (score of 3 = long).

Sweet Corn

Data for sweet corn were collected on marketable ear number, average ear weight (pounds/ear), and final stand count at each harvest, based on the protocol described by Silva and Bruce (2016). Sweet corn was harvested when it reached the kernel blister stage. Harvests occurred on 30 Aug, 6 Sept., 8 Sept., and 13 Sept 2017. The final stand count was used to normalize the harvest data for each plot to an average per plant basis. Quality characteristics including husk appearance, husk protection, row configuration, tip fill, ear shape, kernel color, flavor, and tenderness for each cultivar were also evaluated based on the protocol described in Silva and Bruce (2016). The sweet corn scoring guide for quality parameters is listed in Appendix Table A.3. In addition, we recorded the date at which 50% or more of a plot had visible silks. The silk date is an indicator that is used to distinguish early maturing from later

maturing cultivars. We also measured the height to the tassel and height to first mature ear at harvest for each cultivar/block combination.

Peppers

For the roasting and bell pepper crops, the stand count, marketable count, and marketable weight (pounds/plant) were recorded at each harvest. The peppers were harvested when 70% of their respective mature color was visible. Weekly harvests occurred on 30 Aug., 8 Sept., 13 Sept., 20 Sept., 27 Sept., and 4 Oct. 2017. Soluble solids content was determined for each cultivar/block combination by combining together 10 mature pods, freezing the samples in a -80 °C walk in freezer, thawing the samples at room temperature for 2-3 days, and expressing the pepper liquid over a digital Milwaukee (MA871) refractometer (Milwaukee Instruments, Rocky Mount, North Carolina).

Sensory evaluation

A sensory evaluation study for flavor was conducted at the Larimer County Farmers' Market (LCFM) on 23 Sept. 2017 for sweet peppers only using a consumer sensory panel. No personal identifying information such as age, gender, or occupation was collected from the consumer sensory panelists. A convenience sample of 66 market shoppers served as our sample size. We identified the LCFM as a viable location to conduct the sensory evaluation because the market shoppers are the demographic to whom many market growers sell their produce. Consumer sensory panelists were asked to indicate what they thought of the sweet pepper flavor using a 9-point Hedonic scale, based on the method described in Silva and Bruce (2016). We also conducted a sensory evaluation for appearance, flavor, sugar-acid balance, texture, and skin thickness for the tomato entries at the LCFM on 16 Sept 2017. Panelists were asked to indicate their preference for a tomato entry using a 5-point Hedonic scale; a (1) indicated that an entry

was ‘least desirable’ for that quality parameter, whereas a (5) indicated an entry was ‘most desirable’ for that parameter. Our consumer sensory panel sample size for tomatoes also included 66 farmers’ market shoppers.

Tomatoes

Total fruit count and weight of all marketable fruit and the stand count was recorded at each harvest for tomatoes, based on protocols described in Silva and Bruce (2016). Tomato harvests occurred on 6 Sept., 15 Sept., 20 Sept., and 27 Sept 2017. Tomatoes were harvested at the breaker stage, half of the fruit was green and the other half was pinkish-red. Reasons why a tomato was considered unmarketable were recorded for each entry. Days to maturity was recorded for each cultivar by subtracting the transplant date, 31 May, from the date of the first ripe fruit. The tomato growth habit was recorded for each cultivar/block combination. In addition, we evaluated leaf curl and leaf cover severity during vegetative growth using a 1-5 scale based on the protocol described in Silva and Bruce (2016). Leaf curl is an indicator related to a crop’s physiological stress tolerance. At peak maturity, we measured the width and height of a group of 10 representative fruits, and we recorded the soluble solids content using the same method as the sweet peppers.

Winter Squash

Data for winter squash were collected on marketable fruit count and weight at harvest. All of the cultivars were harvested and the final stand count was recorded for each cultivar/block combination on 6 Oct. 2017. The reasons why fruits were determined to be unmarketable were recorded for each cultivar. The date at which 50% or more of the plants in a plot had a female flower was recorded as the date of peak pollination. This served as a relative earliness indicator for the cultivars. In addition, pest pressure from striped cucumber beetle and powdery mildew on

the leaves and petioles was monitored using the winter squash scoring guide (Appendix Table A.4). Further, we evaluated fruit storability by monitoring the deterioration of 10 mature squash fruits every month for four months. The squash were stored in a 7°C walk-in cooler. Rotten fruits were removed each month and a percent deterioration over time was calculated. A sensory evaluation was conducted at the Cheyenne Botanic Gardens in Cheyenne, Wyoming on 14 Nov. 2017 after a brief presentation about the project's objectives. Untrained sensory panelists were asked to rate the texture, sweetness, and flavor of the winter squash cultivars using a 9-point Hedonic scale (Table 1.5) (Silva and Bruce, 2016).

Statistical Analysis

Analysis was conducted using R statistical software version 3.6.2 (released 12 Dec. 2019) and the emmeans package (Lenth, 2019). The response variables were marketable yield of produce and °Brix; the predictor variable was cultivar. Included in the mixed linear model were block and year, which were treated as random and fixed effects, respectively. An analysis of variance (ANOVA) was performed. Tukey adjusted pairwise comparisons ($\alpha=0.05$) were used for mean separation. We separated bell peppers from roasting style peppers and acorn squash from delicata squash to account for differences between market classes. A log transformation was performed when the assumption of equal variance was in question. This was the case for the average number of marketable pods per plant for bell and roasting peppers, the average number and yield of marketable fruits for acorn squash, and the average number of marketable ears and yield of sweet corn. The results were back transformed and means are presented.

Results and Discussion

Cabbage

All of the cabbage entries were ready for harvest on 135 days after transplanting except for 'Paresa', which was ready after 152 days. 'Sireon' and 'Lennox' had the highest yields among all cultivars at 3.1 and 3.2 pounds/head, respectively (Figure 1.5). These cultivars yielded significantly heavier heads than those of 'Paresa', 'Cantasa', 'Deadon', 'Marabel', 'Alessandro', 'Wirosa', and 'January King'. According to Kaiser and Ernst (2017), the average planting density for cabbage is 14,000 plants per acre, and an acceptable yield for fresh market cabbage is 40,000 pounds per acre. Yields from the top five cultivars in our trial were on par with this. Cabbage is harvested fresh, but may be stored for several months before it is sold to consumers, therefore storability is an important cultivar characteristic.

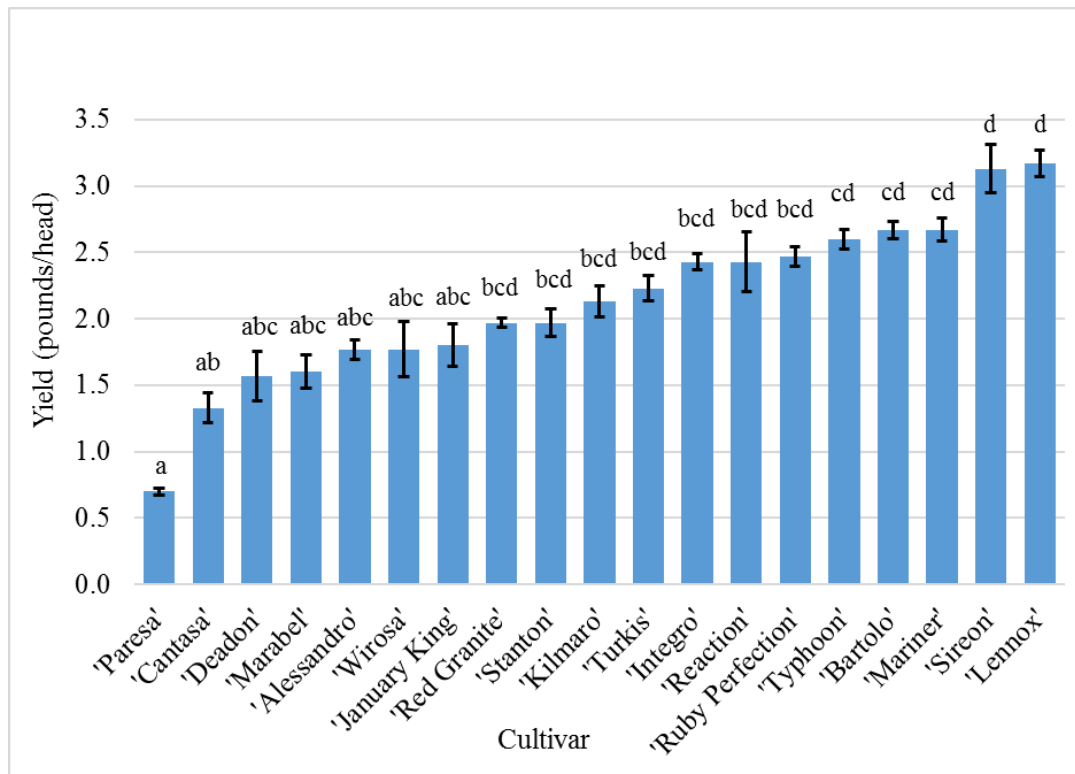


Figure 1.5. A total yield comparison of vegetable cultivar trial cabbage entries on certified organic agricultural land in Fort Collins, Colorado; 2017.; Bars with different letters indicate significant differences ($\alpha = 0.05$) according to Tukey's multiple means comparison.

Cultivar was a significant predictor for the amount of time a cabbage head could be stored in a produce cooler. After three months in storage, 100 percent of the cabbage heads from ‘Alessandro’, ‘Bartolo’, ‘Integro’, ‘Lennox’, ‘Green Mariner’, ‘Reaction’, and ‘Sireon’ were still marketable. These cultivars stored significantly longer than ‘Cantasa’, ‘Deadon’, ‘January King’, ‘Marabel’, ‘Paresa’, ‘Stanton’, and ‘Wirosa’, which had no marketable heads after three months in cold storage (Table 1.10). A cabbage crop is not typically recognized for its ornamental value, however market growers might be interested to know that ‘Deadon’ forms an attractive looking head with purple-red color wrapper leaves covering green and white interior leaves (Figure 1.6). ‘Deadon’ was a top performer because it was a handsome looking savoy cabbage with minimal core material.



Figure 1.6. The attractive pigmented wrapper leaves and white/green interior characteristic of the cabbage cultivar ‘Deadon’.

Tomatoes

The interaction between cultivar and year was statistically significant ($p < 0.0001$), so separate comparisons were made between cultivars in 2016 and 2017. Cultivar had a significant

impact ($p < 0.0001$) on the average marketable tomato yield in terms of pounds/plant in 2016 and 2017. In 2016, ‘S200-1-1’ yielded significantly more pounds/plant than ‘Plum Regal’ at 2.5 and 0.6 pounds, respectively. The other entries in 2016 were intermediary performers compared to ‘S200-1-1’ and ‘Plum Regal’, however no other statistical differences were observed (Table 1.3).

Table 1.3. A total yield comparison of vegetable cultivar trial entries for tomatoes on certified organic agricultural land in Fort Collins, Colorado; 2016-2017.

Cultivar	Yield (pounds/plant) ^z			
	2016		2017	
	Mean	SE	Mean	SE
'Crimson Sprinter'	2.2 ab	0.50	4.0 ab	0.41
'Damsel'	- ^y	-	3.6 ab	0.41
'Iron Lady'	1.0 ab	0.41	-	-
'LB8-3-1-1-1'	2.2 ab	0.41	-	-
'LB8-7-1-1-1'	1.5 ab	0.41	4.7 bc	0.41
'Mountain Merit'	1.3 ab	0.41	6.6 d	0.41
'NC12TMV007 x 141233-62'	2.1 ab	0.41	-	-
'Plum Perfect'	0.8 ab	0.41	2.7 a	0.41
'Plum Regal'	0.6 a	0.41	6.3 cd	0.41
'S200-1-1'	2.5 b	0.41	4.9 bcd	0.41
'Stellar'	0.9 ab	0.41	6.1 cd	0.41
'Stupice'	0.8 ab	0.41	-	-

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey’s honestly significant difference test.

^y Cultivars not included in the statistical analysis because they were not grown in that year.

‘S200-1-1’ was also among the top performers in 2017, averaging 4.93 pounds/plant. It was not significantly different from the 2017 trial winner, ‘Mountain Merit’ at 6.6 pounds/plant. ‘Mountain Merit’ yielded significantly more pounds per plant than ‘Plum Perfect’ at 2.7 pounds. The cultivars ‘Stellar’, ‘Plum Regal’, and ‘LB8-7-1-1-1’ were also identified as top performers because their yields were significantly different from ‘Mountain Merit’. ‘Damsel’ and ‘Crimson Sprinter’, which were intermediary performers. No other statistical differences were observed.

There was a significant interaction ($p < 0.0001$) between year and cultivar for the number of tomatoes produced on a per plant basis. Therefore, cultivar comparisons were made separately for 2016 and 2017. There were no significant differences between cultivars in 2016 (Table 1.4). Therefore, the average number of tomatoes per plant was calculated from all cultivar entries, and it was determined that 7.6 tomatoes per plant was the average for the season (data not shown).

Table 1.4. Number of tomatoes produced per plant comparison of vegetable cultivar trial entries for tomatoes on certified organic agricultural land in Fort Collins, Colorado; 2016-2017.

Cultivar	Yield (number of tomatoes/plant) ^z			
	2016		2017	
	Mean	SE	Mean	SE
'Crimson Sprinter'	7.5 a	2.9	13.4 ab	2.4
'Damsel'	- ^y	-	12.4 a	2.4
'Iron Lady'	6.1 a	2.4	-	-
'LB8-3-1-1-1'	10.7 a	2.4	-	-
'LB8-7-1-1-1'	9.9 a	2.4	37.6 c	2.4
'Mountain Merit'	4.0 a	2.4	18.1 ab	2.4
'NC12TMV007 x 141233-62'	9.7 a	2.4	-	-
'Plum Perfect'	4.1 a	2.4	11.7 a	2.4
'Plum Regal'	4.0 a	2.4	40.6 c	2.4
'S200-1-1'	13.7 a	2.4	23.4 b	2.4
'Stellar'	4.1 a	2.4	22.4 ab	2.4
'Stupice'	10.4 a	2.4	-	-

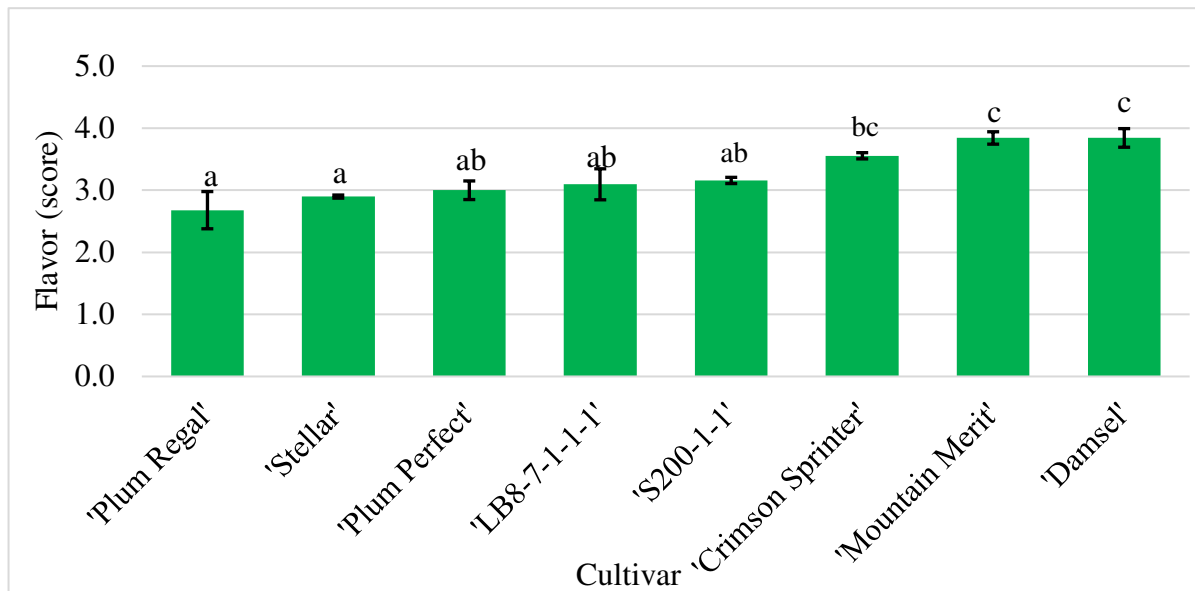
^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

^y Cultivars not included in the statistical analysis because they were not grown in that year.

Unlike 2016, there were significant differences between cultivars for the total number of tomatoes produced per plant in 2017. 'Plum Regal' and 'LB8-7-1-1-1' yielded significantly more tomatoes/plant than all other cultivar entries at 40.6 and 37.6 tomatoes, respectively in 2017. In addition, 'OS00-1-1' at 23.4 tomatoes/plant yielded significantly more than 'Plum Perfect' and 'Damsel', at 11.7 and 12.4 tomatoes, respectively. 'Stellar', 'Mountain Merit', and 'Crimson Sprinter' were intermediary performers; however, no other statistical differences were observed.

It is also worth noting that ‘Plum Regal’ took the most time (89 days after transplanting) to develop the first ripe fruit of the season. This was significantly longer than the time it took ‘Damsel’ (77 days) to produce its first ripe fruit. The days to maturity, growth habit, market class, and breeding system for each cultivar are listed in Appendix Table A.14.

The average tomato fruit widths, heights, leaf curl scores, and leaf cover score are listed for each cultivar in Appendix Table A.15. We recorded this information to evaluate if leaf curl or leaf cover were correlated with yield. However, there was no observable trend (data not shown). Regarding tomato fresh-eating quality parameters, ‘Damsel’, ‘Crimson Sprinter’, and ‘Mountain Merit’ were found to have significantly better flavor than ‘Plum Regal’ and ‘Stellar’ (Figure 1.7).



^z Means sharing the same letters are not statistically significantly different ($\alpha = 0.05$) according to Tukey’s honestly significant difference test.

Figure 1.7. A flavor score comparison of vegetable cultivar tomato entries ^z; 2017 (1 = least desirable flavor, 5 = most desirable flavor, n = 66 tasters).

‘Damsel’ also had a significantly higher soluble solids content than ‘Plum Regal’ and ‘Stellar’. In addition, ‘Damsel’ and ‘Mountain Merit’ were among the most preferred tomato cultivars for their texture, sugar/acid balance, and skin thickness at the LCFM sensory evaluation (Appendix Table A.10). ‘Mountain Merit’ appears to be a top performer in the tomato trial considering the

consumer tasting panel's high regard for its appearance and taste, as well as its ability to produce a significantly higher yield compared to other tomato entries.

Overall, total yields from the top performing cultivars in our tomato trial were below the average yield (30,000-50,000 pounds/acre) for the southeastern U.S. (Ivors, 2010). Assuming a planting density of 4,840 plants/acre, 'Mountain Merit' and 'S200-1-1' yielded 19,206 and 18,139 pounds/acre, respectively. The total yields for each of the top two performers in the organic tomato cultivar trial in Georgia were 33,574 and 28,064 pounds/acre from 'Celebrity' and 'Mountain Fresh Plus', respectively. The lower yields from our trial may be related to the fact that the priority for the Oregon State University tomato breeding program, from which some of the experimental lines were derived is late blight resistance, not necessarily high yield (Lammerts van Bueren et al. 2011). Late blight is more of an issue for the maritime climate of the Pacific Northwest than it is for the semi-arid climate of Colorado. It is possible that the NOVIC tomato cultivar entries may not be the most productive cultivars for the Front Range. Another probable factor contributing to the lower yields in our trial could be lower fertility levels.

To further illustrate this point, Mitchell and Uchanski (2019) carried out an organic tomato cluster pruning experiment at the same time, under similar management practices, and less than 100 yards away from where this cultivar trial took place. Averaging over year and treatment, 'Jet Star' was the highest yielding tomato with a total marketable yield of 22,409 pounds/acre. If 'Jet Star' had been included in the NOVIC tomato cultivar trial, it would have been the top performer. Still, this yield is lower than those reported in the Georgia cultivar trial. This could be related to the fact the Athens, Georgia has more heat units in a year and a longer growing season with an average of 225 frost-free days (McLaurin and Granberry, 2017) than Fort Collins,

Colorado with an average of 151 frost-free days (Colorado State University Extension, 2020). In addition, the average °Brix for the cultivars reported by Mitchell and Uchanski (2019) was around 5.0. This is comparatively higher than the 4.0 average from the entries in the NOVIC tomato cultivar trial.

Tomato breeders that are interested in improving upon these cultivars or the unreleased breeding lines might consider addressing the reasons that lead to transplant death and reasons that left the fruits unmarketable (Table 1.5). For example, ‘Crimson Sprinter’ did not yield significantly different from ‘Mountain Merit’ over the length of the trial, but in 2016 we lost significantly more ($p < 0.0001$) transplants compared to the other entries to a viral problem that resembled Tomato Spotted Wilt Virus (Figure 1.8). In 2017, we also recorded and removed the diseased-looking transplants, however there were not significant differences observed.

Table 1.5. Physiological reasons that lead to unmarketable tomato fruits for each entry in the vegetable cultivar trial on certified organic agricultural land in Fort Collins, Colorado; 2016-2017.

Reasons unmarketable	Cultivars
Zippering, viral issues	'Crimson Sprinter'
Proximal end cracking	'Damsel', 'LB8-7-1-1-1', and 'Stupice'
Proximal end cracking	'Plum Perfect' and 'Plum Regal'
Proximal end cracking, blossom end rot, viral issues	'Iron Lady' and 'LB8-3-1-1-1'
Proximal end cracking, zippering	'Mountain Merit'
Proximal end cracking, scaring	'NC12TMV007 x 141233-62'
Proximal end cracking, zippering	'S200-1-1' and 'Stellar'



Figure 8. A visual representation of the physiological reasons leading to unmarketable tomato fruits. Clockwise from top left: proximal end cracking, *Tomato spotted wilt virus* (Mariman, 2011), blossom end rot (Teasley, 2019), and zippering.

Bell Peppers

Bell peppers grown in years 2016, 2017, and 2018 were analyzed separately due to a significant ($p < 0.037$) cultivar by year interaction. In 2016, there were no significant differences between cultivars, so the average per plant pepper yield was calculated; it was 0.6 pounds/plant. In 2017, ‘Aristotle X3R®’ yielded significantly more than ‘CU 1’, at 3.8 and 2.4 pounds/plant, respectively (Table 1.6). ‘Ace’ and ‘CU 2’ were intermediary performers, but no other statistical differences were observed. In 2018, ‘Flavorburst’ and ‘SBGO 10408’ at 2.4 and 2.0 pounds/plant yielded significantly more than ‘Early Red Sweet’ and ‘Whitney’ at 0.7 pounds/plant each. ‘Ace’ and ‘Aristotle X3R®’, ‘CU 3’, ‘CU 4’, and ‘King of the North’ were intermediary performers, but no other statistical differences were observed.

Table 1.6. A total yield comparison of vegetable cultivar trial bell pepper entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018.

Cultivar	Total yield (pounds/plant) ^z					
	2016		2017		2018	
	Mean	SE	Mean	SE	Mean	SE
'Ace'	0.4 a	0.3	2.7 ab	0.3	1.4 ab	0.3
'Aristotle X3R®'	0.4 a	0.3	3.8 b	0.3	1.9 ab	0.3
'CU 1'	- ^y	-	2.4 a	0.3	-	-
'CU 2'	-	-	2.8 ab	0.3	-	-
'CU 3'	-	-	-	-	0.9 ab	0.3
'CU 4'	-	-	-	-	1.2 ab	0.4
'Early Red Sweet'	-	-	-	-	0.7 a	0.3
'Flavorburst'	-	-	-	-	2.4 b	0.3
'Gypsy Queen'	0.9 a	0.3	-	-	-	-
'King Krimson'	0.6 a	0.4	-	-	-	-
'King of the North'	0.5 a	0.3	-	-	0.8 ab	0.4
'Red Knight X3R®'	0.5 a	0.3	-	-	-	-
'Revolution'	0.6 a	0.3	-	-	-	-
'SBGO 10408'	-	-	-	-	2.0 b	0.3
'Whitney'	-	-	-	-	0.7 a	0.3

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

^y Cultivars not included in the statistical analysis because they were not grown in that year.

If the marketable pod count/plant is of interest to market growers, then they might consider growing 'Gypsey Queen', 'Ace' or 'SBGO 10408'. These were the top performers for pod count per plant in 2016, 2017, and 2018, respectively (Table 1.7). They each yielded more pods/plant than 'Aristotle X3R®' in their respective years. 'Ace', 'King Krimson', 'King of the North', 'Revolution', and 'Red Knight' were intermediary performers in 2016, however no other statistical differences were observed. In 2017, 'CU 1' and 'CU 2' were intermediary performers with 8.4 and 10.7 pods/plant, respectively. No other statistical differences were observed in that year. In 2018, 'King of the North', 'CU 4', 'Early Red Sweet', 'CU 3', 'Ace', 'Whitney', and 'Flavorburst' were intermediary performers, but no other statistical differences were observed.

Table 1.7. Number of pepper pods produced per plant comparison of vegetable cultivar trial bell pepper entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018.

Cultivar	Yield (pod count/plant) ^z					
	2016		2017		2018	
	Mean	SE	Mean	SE	Mean	SE
'Ace'	2.5 ab	0.9	11.9 b	0.9	6.7 ab	0.9
'Aristotle X3R®'	1.0 a	0.9	7.2 a	0.9	4.8 a	0.9
'CU 1'	- ^y	-	8.4 ab	0.9	-	-
'CU 2'	-	-	10.7 ab	0.9	-	-
'CU 3'	-	-	-	-	5.9 ab	1.0
'CU 4'	-	-	-	-	5.5 ab	1.4
'Early Red Sweet'	-	-	-	-	5.8 ab	0.9
'Flavorburst'	-	-	-	-	-	-
'Gypsy Queen'	5.5 b	0.9	-	-	-	-
'King Krimson'	2.7 ab	1.4	-	-	-	-
'King of the North'	2.8 ab	0.9	-	-	3.5 ab	1.4
'Red Knight X3R®'	3.8 ab	0.9	-	-	-	-
'Revolution'	3.6 ab	0.9	-	-	-	-
'SBGO 10408'	-	-	-	-	9.2 b	0.9
'Whitney'	-	-	-	-	6.8 ab	0.9
'Flavorburst'	-	-	-	-	7.3 ab	1.0

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

^y Cultivars not included in the statistical analysis because they were not grown in that year.

'Aristotle X3R®' was identified as a top performer over the length of the trial due to its large pod size and impressive pepper yields in 2017 and 2018. However, it did not have enough time to mature to red on the plant, so we harvested the pods as they were turning from green to red. 'Revolution' had a similar issue, which may explain why the °Brix is significantly lower compared to the other cultivars. While not all of the 'Aristotle X3R®' pods had time to mature to their ripe red colored on the plant, the ones that did had significantly better flavor than 'Whitney'. The results of the bell pepper sensory evaluation for flavor are indicated in Table 1.8.

The USDA Economic Research Service (2013) indicates the national average yield for bell peppers was 33,513 pounds/acre in 2013. This equates to a per plant yield of 1.92 pounds/acre. Therefore, the top five highest yielding cultivars in our trial in 2017 and 2018, which were

'Aristotle X3R®', 'Ace', 'CU 1', 'CU 2', and 'Flavorburst', yielded as well as, if not better than the national U.S. average. The average bell pepper yield reported in Boyhan et al. (2019) was 34,412 pounds/plant in 2016 and 49,112 in 2017. 'Aristotle X3R®', 'Flavorburst', and 'Red Knight X3R®' were top performing bell pepper cultivars in Georgia in 2017, as reported in Boyhan et al. (2019). With the exception of 'Red Knight X3R®', these cultivars were also among the top five performers for yield in our trial. 'Red Knight X3R®', however, was among the bottom three in our trial. This demonstrates that some cultivars perform well across multiple growing regions while others have narrow adaptability.

Roasting Peppers

Results from the LCFM sensory evaluation indicated there no significant differences in sweet pepper flavor for the roasting-style pepper cultivars (data not shown). In addition, the average marketable yield (pounds/plant) for roasting-style peppers was not significantly different among the cultivar entries in 2017 (Table 1.8). The average roasting pepper yield in 2017 was 0.5 pounds per plant. In 2018, 'CSU 321' yielded significantly more pounds per plant, at 4.9 pounds, than 'CSU Mosco', 'CSU Pueblo Popper', 'Crest Yellow', 'Stocky Red Roaster', and 'Bridge to Paris'. 'Escamillo', 'CSU 256' and 'Carmen' were intermediary performers at 3.5, 3.7, and 3.9 pounds per plant, respectively. However, no other statistical differences were observed. In 2018, the top performer, 'Early Perfect Italian' yielded significantly more than 'CSU 256'. No other statistical differences were observed.

The U.S. national average for chile pepper production, assuming a planting density of 17,424 plants/acre, is 25,000 pounds/acre or 1.43 pounds/plant (Ag Marketing Resource Center, 2019). The top ten performers in our pepper trial were at or above the national average. The top five cultivars in 2017 outperformed the national average. However, yields in 2016 and 2018 were

below the national average, which indicates that production year is a significant ($p < 0.0001$) predictor of chile pepper yields. There were significant differences in the number of marketable pods produced on a per plant basis in 2017 and 2018 (Table 1.9). There were no significant differences between cultivars in 2016. The average number of pods produced on a per plant basis in 2016 was 3.6. In 2017, 'CSU 256' yielded significantly more pods per plant, at 44.1, than 'Escamillo', 'Crest Yellow', 'Carmen', and 'Bridge to Paris' at 12.7, 12.8, 16.1, and 18.4 pods, respectively.

In 2018, 'CSU Pueblo Popper' at 41.1 pods per plant, yielded significantly more than all of the other cultivar entries in that year except for 'Stocky Red Roaster'. It is worth noting that these are pungent popper-sized peppers that were significantly shorter than the other cultivar entries (Appendix Table A.15). We also recorded that 'CSU 321', a green pungent pepper had a significantly lower °Brix at 6.9° compared to 'Bridge to Paris', 'Carmen', and 'Escamillo' at 9.4°, 9.5°, and 9.2°, respectively. International Ag Labs, INC, (2019) a consultancy company, proposed a °Brix scale for bell pepper quality; poor, average, good, and excellent quality peppers have °Brix values of 4, 6, 8, and 12, respectively. Based on this scale, the top three performers in our roasting pepper trial exhibited °Brix values that were better than what the International Ag Labs, INC refers to as good quality peppers.

Table 1.8. Total yield comparison of vegetable cultivar trial roasting pepper entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018.

Cultivar	Yield (pounds/plant) ^z					
	2016		2017		2018	
	Mean	SE	Mean	SE	Mean	SE
'Belcanto'	0.6 a	0.3	-	-	-	-
'Bella Italia'	0.5 a	0.3	-	-	-	-
'Bridge to Paris'	0.7 a	0.3	3.2 a	0.31	1.3 ab	0.31
'Carmen'	0.7 a	0.3	3.9 ab	0.31	1.2 ab	0.31
'Classic Italian'	0.4 a	0.3	-	-	-	-
'Cornito Rosso'	0.3 a	0.3	-	-	-	-
'Corno di Toro'	0.6 a	0.3	-	-	-	-
'Crest Yellow'	- ^y	-	3.2 a	0.31	-	-
'CSU 256'	-	-	3.7 ab	0.31	0.3 a	0.31
'CSU 321'	-	-	4.9 b	0.31	0.7 ab	0.31
'CSU Mosco'	-	-	2.6 a	0.31	0.6 ab	0.31
'CSU Pueblo Popper'	-	-	2.8 a	0.31	1.3 ab	0.31
'Early Perfect Italian'	0.6 a	0.3	-	-	1.9 b	0.31
'Escamillo'	-	-	3.5 ab	0.31	1.1 ab	0.31
'Karma'	-	-	-	-	1.9 ab	0.31
'Laerte'	0.4 a	0.3	-	-	-	-
'Lively Yellow'	-	-	-	-	1.1 ab	0.31
'Stocky Red Roaster'	1.3 a	0.3	2.9 a	0.31	0.7 ab	0.54

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

^y Cultivars not included in the statistical analysis because they were not grown in that year.

Table 1.9. Total number of pods per plant comparison of vegetable cultivar trial roasting pepper entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018.

Cultivar	Yield (number of pods/plant)					
	2016		2017		2018	
	Mean	SE	Mean	SE	Mean	SE
'Belcanto'	4.4 a	4.8	-	-	-	-
'Bella Italia'	4.3 a	4.8	-	-	-	-
'Bridge to Paris'	5.4 a	4.8	18.4 a	4.8	10.1 a	4.8
'Carmen'	4.7 a	4.8	16.1 a	4.8	6.9 a	4.8
'Classic Italian'	2.3 a	4.8	-	-	-	-
'Cornito Rosso'	1.3 a	4.8	-	-	-	-
'Corno di Toro'	1.9 a	4.8	-	-	-	-
'Crest Yellow'	^y	-	12.8 a	4.8	-	-
'CSU 256'	-	-	44.1 b	4.8	6.2 a	4.8
'CSU 321'	-	-	35.0 ab	4.8	5.2 a	4.8
'CSU Mosco'	-	-	19.6 ab	4.8	9.4 a	4.8
'CSU Pueblo Popper'	-	-	24.1 ab	4.8	41.1 b	4.8
'Early Perfect Italian'	1.9 a	4.8	-	-	13.8 a	4.8
'Escamillo'	-	-	12.7 a	4.8	6.1 a	4.8
'Karma'	-	-	-	-	13.6 a	4.8
'Laerte'	0.8 a	4.8	-	-	-	-
'Lively Yellow'	-	-	-	-	7.7 a	4.8
'Stocky Red Roaster'	9.0 a	4.8	21.7 ab	4.8	8.0 ab	8.6

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

^y Cultivars not included in the statistical analysis because they were not grown in that year.

Sweet Corn

In regards to sweet corn, a significant ($p < 0.019$) cultivar by year interaction restricted cultivar comparisons for weight of the average ear to within year. In 2016 and 2017, no significant differences between cultivars were observed (Table 1.10). The average ear weighed 0.3 and 0.6 pounds in 2016 and 2017, respectively. In 2018, 'Nirvana' at 0.6 pounds yielded a significantly heavier average ear weight than 'Double Standard', 'Zanadoo', 'Temptation', 'My Fair Lady', 'Allure', and 'Who Gets Kissed'. 'Luscious', 'Xtratender 2171', 'New Mama', and 'Festivity' were intermediary performers, but not other statistical differences were observed. The

national range of marketable sweet corn weight on a per ear basis is 0.4 - 0.7 pounds/ear (Harper and Orzolek, 2005), which indicates that 2016 was a below average year. Whereas, 2017 was an average year as far as yields go. In addition, the top performer of the trial in 2018, ‘Nirvana’, yielded on the high side of average. This is based on the assumption of a national yield of 9,807.69 pounds/acre and a planting density between 14,000 and 24,000 plants per acre (Harper and Orzolek, 2005).

In addition, there were no significant differences between cultivars for the number of ears produced on a per plant basis (Figure 1.9), the average ear width, or the average ear length (Appendix Table A.12). Whereas, the heights to the first ear (cm) for ‘Anthem XR’, ‘Festivity’, ‘Mirai 131Y’, ‘Mirai 308BC’, and ‘Zanadoo’ were significantly taller than ‘Jared Synthetic 21’ and ‘My Fair Lady’. Height to the first ear is important to keep in mind if one has a vertebrate pest such as a fox from whom growers are trying to protect their sweet corn crop. Further, height to the tassel is a measure of vigor. ‘Allure’, ‘AP426’, and ‘Luscious’ were significantly taller than ‘Xtratender 2171’ and ‘Zanadoo’.

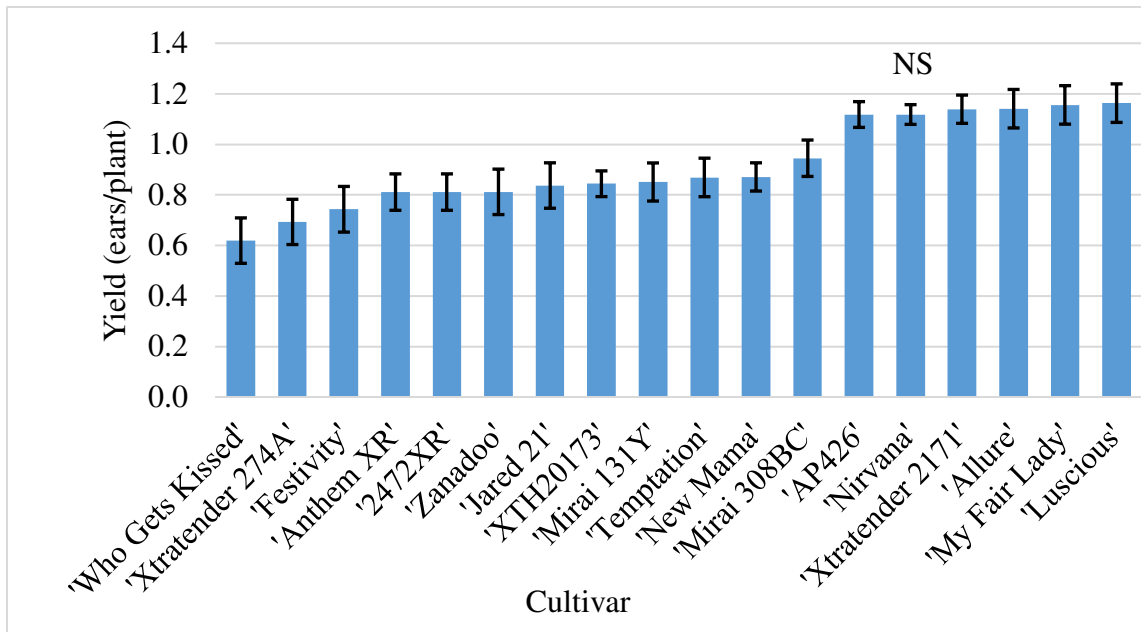
When it comes to quality characteristics, sweet corn flavor often dominates. ‘Nirvana’ was found to have superior flavor compared to ‘New Mama’ and ‘Mirai 308BC’ (Figure 1.10). Those that evaluated ‘Nirvana’ for consumer acceptability agreed the cultivar was appropriately named. Because consumers also make purchasing decisions based on appearance, it is important to report that the kernels of ‘Nirvana’ and ‘XTH20173’ looked significantly more “buttery” than ‘Double Standard’.

Table 1.10. Average ear weight comparison of vegetable cultivar trial sweet corn entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018.

Cultivar	Yield (ear weight in pounds) ^z					
	2016		2017		2018	
	mean	se	mean	se	mean	se
'Allure'	– ^y	-	-	-	0.4 a	0.0
'Anthem XR'	-	-	0.6 a	0.0	-	-
'AP426'	0.2 a	0.0	0.5 a	0.0	-	-
'Double Standard'	-	-	-	-	0.3 a	0.0
'Festivity'	-	-	-	-	0.5 ab	0.1
'Jared 21'	-	-	-	-	0.4 ab	0.1
'Luscious'	-	-	-	-	0.4 ab	0.0
'Mirai 131Y'	0.4 a	0.0	0.6 a	0.0	-	-
'Mirai 308BC'	-	-	-	-	-	-
'My Fair Lady'	-	-	-	-	0.3 a	0.0
'New Mama'	-	-	0.6 a	0.1	0.4 ab	0.0
'Nirvana'	0.3 a	0.0	0.5 a	0.0	0.6 b	0.0
'Temptation'	-	-	-	-	0.3 a	0.0
'Who Gets Kissed'	-	-	-	-	0.4 a	0.1
'XTH20173'	0.2 a	0.0	0.5 a	0.0	-	-
'Xtratender 2171'	-	-	0.5 a	0.0	0.4 ab	0.0
'Xtratender 274A'	0.3 a	0.1	-	-	-	-
'Zanadoo'	-	-	-	-	0.3 a	0.1
'2472XR'	-	-	0.6 a	0.0	-	-

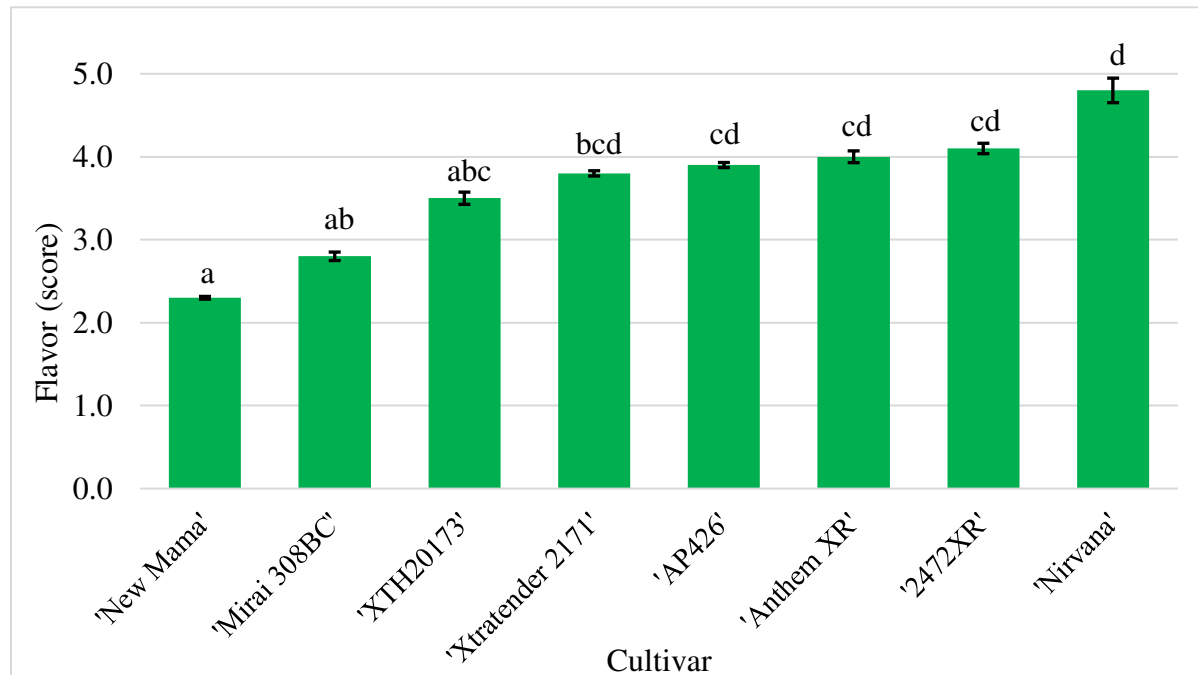
^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test; ^y Cultivars not included in the statistical analysis because they were not grown in that year.

In addition, the husk appearances of 'AP426' and 'Mirai 308BC' looked visually more appealing than 'My Fair Lady' and 'Temptation' as determined by the length of the flag leaves and the color of the husk. Significantly better husk protection from corn borer pests was provided by 'Allure', 'Festivity', and 'Luscious' as compared to 'Double Standard'. Considering the importance of sweet corn flavor and yield to a market grower, 'Nirvana' appears to be a top performer among cultivar entries.



^{NS} The cultivars means are not significantly difference from one another ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Figure 9. Number of ear per plant comparison of vegetable cultivar trial sweet corn entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018.

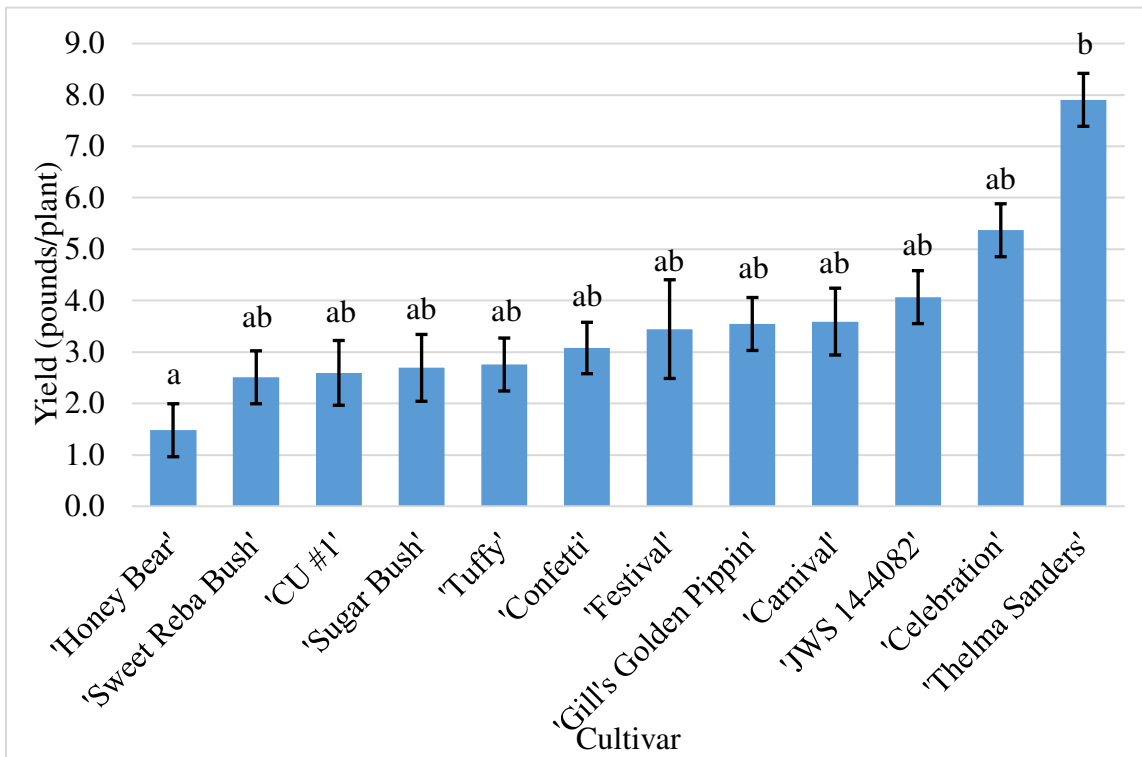


^z Bars sharing the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Figure 1.10. A comparison of sweet corn flavor among participating entries; 2016-2018 combined (1 = objectionable, 5 = excellent sweet corn flavor, n = 10 tasters).

Acorn Squash

'Thelma Sanders' yielded the most at 7.9 pounds/plant. It was significantly more than 'Honey Bear' (Figure 1.10). 'Thelma Sanders' also produced significantly more fruits/plant than 'Honey Bear' (Table 1.17). No other statistical differences were observed. In addition, there were significant differences between cultivars in terms of °Brix. 'Honey Bear' and 'Sugar Bush' yielded fruits with the highest °Brix at 15.9 and 16.2, respectively. They both produced fruits that were significantly higher in °Brix than 'Thelma Sanders' (5.8) at harvest. Squash fruits at or above 14 °Brix are considered excellent quality, based on the scale 6, 8, 12, and 14 for poor, good, average, and excellent quality sweet corn ears (International Ag Labs, 2019).



^z Bars sharing the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Figure 1.11. A total yield comparison of vegetable cultivar trial winter squash entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018 combined.

After four months in storage, ‘Honey Bear’ had °Brix that were significantly higher than ‘Sweet Reba Bush’. There were no significant differences between cultivars in terms of marketability after four months in cold storage. Therefore, the means were combined and the average percent marketable after four months in cold storage was 68 among cultivar entries (Appendix Table A.18). The cultivar ‘Cornell University 1’ (‘CU 1’) yielded the most fruits/plant (4.3), and had the heaviest yield at 4.7 pounds/plant. However, it was not significantly more than the other cultivar entries in the trial. Still, it was among the top five performers in the trial; these cultivar all yielded more than the national average of 3.4 pounds/plant (University of Massachusetts Amherst, 2020).

Delicata Squash

Unlike the acorn and butternut squash results, there were no significant differences between delicata squash cultivars for the number of fruits produced per plant, yield (pounds/plant), and °Brix (Appendix Table A.19). The average total yield and count of fruits per plant for delicata squash entries in the trial were 4.0 pounds/plant and 3.3 fruits/plant, respectively (data not shown). In addition, there were no significant differences in fresh-eating quality parameters for delicata squash entries related to texture, sweetness, or flavor in the consumer sensory panel. Because there were no significant differences, means were combined and the average °Brix reading for fresh delicata squash was 14.5.

After four months in cold storage, the °Brix for ‘Jester’ was significantly higher than ‘Cornell University 2’ (‘CU 2’), ‘Sugar Dumpling’, and ‘Zeppelin’. In addition, there were differences between cultivars in powdery mildew severity on the leaves. ‘Jester’ had a higher incidence of powdery mildew than ‘Candystick’ and ‘CU 1’.

In light of the fact that there are no significant difference in yield or quality parameters, one might choose to grow bush-type cultivars such as ‘Bush Delicata’, ‘CU 1’, ‘CU 2’, ‘CU 3’, or ‘Mardi Gras’ because the fruits are held closely to the plant as in Figure 1.12, which makes harvesting faster.



Figure 1.12. ‘Cornell University 1’ (‘CU 1’) is an example of bush-type delicata squash.

Conclusion

The top performers from each of the five vegetable species included in the trial are listed in Table 1.11. This summary of performance focuses on vegetable characteristics that are important to market growers such as fresh eating quality and total marketable yield. To our knowledge, this is the first cultivar trial to evaluate five vegetable species grown under organic management in the Intermountain West. Our objective was to provide cultivar recommendations for tomato, cabbage, bell and roasting peppers, sweet corn, and winter squash to market growers. As NOVIC continues to evaluate and develop open-pollinated cultivars that are bred in and for organic systems, more regional reports will be released.

The eOrganic (2020) website has over 100 regional organic vegetable crop cultivar trial reports available to interested growers. These are similar to the bulletins made available to regional growers through the Midwest Vegetable Variety Trial Reports webpage via Purdue University (2019). Regional cultivar performance results provide guidance to growers in that region. However, it is important to remember that some cultivars have broad adaptability spanning several or more growing regions. As such, it would be helpful to develop a national organic vegetable trialing program in order to identify top performing cultivars with broad adaptability. The All-America Selection (2019) program is one example of a national database of garden cultivar performance under conventional management that spans multiple growing regions.

Table 1.11. The top performers for each crop based on results from the cultivar trial on certified organic agricultural land in Fort Collins, Colorado; 2016-2018.

Crop	Cultivar	Comments
Cabbage	'Deadon'	Very attractive crop; Green savoy cabbage with outer purple leaves; Moderate yield; Does not store well
	'Lennox'	Green cabbage; High yield, Stores well for 3 months in cold storage
	'Sireon'	Green cabbage; High yield, Stores well for 3 months in cold storage
	'Integro'	Purple cabbage, High yield; Minimal inner core material; Stores well for 3 months in cold storage
	'Ruby Perfection'	Purple cabbage, High yield; Stores well for 3 months in cold storage
Tomato	'Damsel'	Indeterminate slicing tomato; Reaches maturity 77 days after transplanting, Moderate yield; Heirloom appearance; Delicious taste
	'Mountain Merit'	Determinate slicing tomato; High yield; Delicious taste; Nice sugar/acid balance for flavor
	'Plum Regal'	Determinate plum tomato; Lots of small-medium sized fruit; Great leaf protection
Roasting Pepper	'Carmen'	Non-pungent, red pepper; High yield; Delicious taste
	'Crest Yellow'	Non-pungent, yellow pepper with green shoulders; High yield; Pleasant taste
	'CSU 321'	Pungent, green pepper; High yield; Averages 20 marketable pods/plant; Mild chile taste
	'Pueblo Popper'	Pungent, red pepper, Moderate yield; Averages 32 marketable pods/plant; Sweet and spicy taste
Bell Pepper	'Aristotle X3R®'	Red; Moderate yield; Averages four large pods/plant; Excellent red pepper taste; Not enough heat units for all pods to turn red
	'CU 2'	Red; High yield; Averages 10 pods/plant
	'Flavorburst'	Yellow; High yield
Sweet Corn	'Anthem XR'	Sweet corn with good flavor and tenderness; Good husk protection from borer insects; Attractive husk appearance; Large ears
	'Nirvana'	Sweet corn with exceptional flavor and tenderness; Kernels look buttery; Large ears
	'2472 XR'	Sweet corn with good flavor and tenderness; Good husk protection from borer insects; Attractive husk appearance; Largest ears
Winter Squash	'Thelma Sanders'	Acorn squash; High yield; Averages four fruits/plant
	'Bush Delicata'	Delicata squash; Moderate yield; Nice sweet taste; Shrubby growth habit makes harvesting easy
	'Candystick'	Delicata squash; Moderate yield; Over 90% of fruit still marketable after three months in cold storage; Nice sweet taste

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CHAPTER 2: Metabolomics for Horticulturists: Employing Ambient Mass Spectrometry to Assess Inherent Postharvest Pepper Quality Characteristics

Introduction and Literature Review

Inherent postharvest quality characteristics, such as containing an abundance of human-health related compounds (HC) or having a pleasing aroma profile are important to vegetable crops, and horticulturists are interested in evaluating the impact of production system inputs and farming practices on postharvest plant products such as flowers, fruits, and seeds. In addition, breeders are making strides to advance experimental lines that have exceptional flavor (Tieman et al., 2017) and/or high nutritional content (Rembialkowska et al., 2007). Furthermore, high quality vegetable crops could serve to provide a competitive advantage for market growers selling directly to the end-user such as a restaurant or shopper at a farmers' market. In fact, conducting on-farm sensory evaluations is a novel way to better understand consumer expectations for vegetable quality attributes such as appearance, texture, and flavor, which are parameters that are difficult to directly quantify. The problem with conducting consumer sensory panels is that they are expensive in terms of both time and other resources, and the information that is collected is relatively subjective (Kemp, 2008; Birwal and Yadav, 2015). These factors limit the use of sensory evaluations by cultivar trial managers to assess vegetable quality.

What humans perceive as overall flavor is influenced by interactions between taste, aroma, mouthfeel, sight, and sound (Eggink, 2013). The chemical composition of non-volatile metabolomic compounds (metabolites) contributes to pepper (*Capsicum annuum* L.) taste, whereas volatile metabolites characterize aroma (Luning et al., 1994). Aromas that have been characterized in peppers are listed in Table 2.1. Together, all of these metabolites, which are < 1kDa in mass, comprise the metabolome, much of which contributes to what people recognize as

flavor. Therefore, analysis platforms that collect qualitative and/or quantitative chemical data offer a way to objectively characterize vegetable quality attributes (Moreno et al., 2012).

Table 2.1. An overview of important aroma contributing volatile compounds identified in *C. annuum*, *C. frutescens*, and *C. chinense*, their associated aroma descriptors, and the authors that first identified them; 1969-2012.

Compound	Aroma descriptors	Pepper species	Authors
2-Isobutyl-3-methoxypyrazine	Green bell pepper	<i>C. annuum</i>	^z
2,3-Butanedione	Caramel	<i>C. annuum</i>	^y
1-Penten-3-one	Chemical/pungent, spicy	<i>C. annuum</i>	^y
2,3-Penten-3-one	Fruity, sweet, caramel	<i>C. annuum</i>	^y
Hexenal	Grassy	<i>C. annuum</i>	^y
3-Carene	Red bell pepper, rubbery	<i>C. annuum</i>	^y
(<i>Z</i>)- β -Ocimene	Rancid, sweaty	<i>C. annuum</i>	^y
Octanal	Fruity	<i>C. annuum</i>	^y
(<i>E</i>)-2-Hexenal	Fruity, sweet	<i>C. annuum</i>	^y
(<i>E</i>)-2-Hexenol	Almond, fruity, spicy	<i>C. annuum</i>	^y
(<i>Z</i>)-3-Hexenal	Grassy, green bell pepper, fruity	<i>C. annuum</i>	^y
(<i>Z</i>)-3-Hexenol	Grassy, lettuce, cucumber	<i>C. annuum</i>	^y
o-Xylene	Geranium, rubbery, spicy	<i>C. annuum</i>	^y
1-Hexanol	Fruity, green bell pepper, herbal	<i>C. annuum</i>	^y
Nonal	Mushroom, herbal	<i>C. annuum</i>	^y
(<i>E</i>)-2-Octenal	Almond, sweet, herbal	<i>C. annuum</i>	^y
1,2,4,5-Tetramethylbenzene	Rancid, sweet	<i>C. annuum</i>	^y
2-Sec-butyl-3-methoxypyrazine	Carrot, lettuce, grassy	<i>C. annuum</i>	^y
Linalool	Floral, green bell pepper	<i>C. annuum</i>	^y
Ethyl 2,3-methylbutanoate	Fruity, sweet	<i>C. annuum</i>	^x
Ethyl 3-methylpentanoate	Fruit, exotic	<i>C. annuum</i>	^x
Ethyl 4-methylpentanoate	Fruit, exotic	<i>C. annuum</i>	^x
Hexyl 2,3-methylbutanoate	Fruity	<i>C. annuum</i>	^x
α -Pinene	Pine wood	<i>C. annuum</i>	^x
1,8-Cineole	Eucalyptus	<i>C. annuum</i>	^x
2-Heptanethiol	Paprika, green, kerosene-like	<i>C. annuum</i>	^x
3-Isobutyl-2-methoxypyrazine	Paprika, green, earthy	<i>C. annuum</i>	^x
Methyl salicylate	Green, sweet, phenolic	<i>C. annuum</i>	^x
β -Ionone	Fruity, floral	<i>C. chinense</i>	^x
7,8-Dehydro- β -ionone	Fruity, floral	<i>C. annuum</i>	^x
(<i>Z</i>)-2-Nonenal	Green, cucumber	<i>C. annuum</i>	^x
(<i>E,Z</i>)-2,6-Nonenal	Cucumber	<i>C. annuum</i>	^x
(<i>E</i>)-2-Nonenal	Cucumber, musty	<i>C. annuum</i>	^x
Ectocarpene	Green, sweet	<i>C. frutescens</i>	^x
Sotolone	Soup seasoning, spicy	<i>C. annuum</i>	^x
Unidentified, RI 1020	Sweet, alcoholic	<i>C. chinense</i>	^x
Unidentified, RI 1078	Passion fruit	<i>C. chinense</i>	^x
p-Menth-1-en-9-al	Spicy, herbal	<i>C. annuum</i>	^w
(<i>E</i>)- β -Ocimene	Sweet, herbal	<i>C. annuum</i>	^w
(<i>Z</i>)-2-Penten-1-ol	Rubber, plastic, green	<i>C. annuum</i>	^w
(<i>E</i>)-Geranylacetone	Floral, fruity, pear	<i>C. annuum</i>	^w

^z Buttery et al., 1994; ^y Luning et al. 1994; ^x Rodríguez-Burruezo et al. 2010; ^w Eggink et al. 2012

The literature indicates gas chromatography mass spectrometry (GC-MS) coupled with solid phase micro extraction (SPME) is an analytical platform that pepper breeders have used to profile the volatile metabolites contributing to aroma (Rodríguez-Burruezo et al., 2010). GC-MS has been used to screen for pepper aroma-contributing metabolites (ACM) for over 50 years (Buttery, 1969). Recently, Aranha et al. (2017) characterized Brazilian pepper accessions from the Embrapa Clima Temperado active germplasm collection using GC-MS (Aranha et al., 2017). Three pepper accessions were reported to have separated from the others based on principal component analysis (PCA), which the authors indicated was due to a difference in fructose levels. However, the analyses of metabolic profiles were unable to differentiate among species based on PCA. Interestingly, the metabolic profiles of 32 pepper accessions from *C. annuum*, *C. chinense*, *C. frutescens*, and *C. baccatum* clustered according to differences in pungency, not species (Wahyuni et al., 2013). On the other hand, PCA was able to reveal differences between species based on the semi-polar metabolite profiles assessed using liquid chromatography mass spectrometry (LC-MS).

Analyses of samples by SPME-GC-MS involves some form of sample homogenization, as in Figure 2.1, and extraction to enable sample uniformity (Linxing et al., 2019).



Figure 2.1. Homogenized pepper samples ready for SPME-GC-MS analysis.

However, the steps for sample preparation such as freezing the peppers, grinding them into fruit powder, and mixing the solvents for chemical extraction are time consuming and expensive. In addition, once processed, it can take as long as 30 minutes to acquire the chemical profile for one sample. These barriers, as well as the cost per sample, might keep a horticulturist or cultivar trial manager from using this technology to screen genotypes for ACM or other HCs such as *p*-coumaric acid, for example. *p*-Coumaric acid is an antioxidant known for its ability to reduce the risk of type 2 diabetes and cardiovascular diseases (Wahyuni, 2013). Therefore, mass spectrometry platforms that can objectively screen for vegetable quality attributes using minimally processed samples and under ambient conditions would be a useful tool for horticulturists.

Ambient mass spectrometry (AMS) has emerged as a promising analytical tool that can quickly generate chemical profiles based on the metabolites that are present in the sample (Linxing et al., 2019). AMS platforms operate under ambient conditions and require minimal sample preparation, thereby enabling a high-throughput method for quality analysis. One example of an AMS platform is rapid evaporative ionization mass spectrometry (REIMS). It has been used in the biomedical industry to screen for cancerous tissue in real time during operations (Weston, 2010) and to distinguish between fish species, which the authors indicate could be useful for helping to prevent fish fraud in the seafood (Black et al., 2017). It has also been used to assess porcine meat quality (Verplanken et al., 2017) and to classify quality attributes such as grade and muscle tenderness in beef (Gredell et al., 2019). To our knowledge, there are no reported studies using REIMS to assess vegetable quality.

Another type of AMS platform is direct analysis in real time (DART) mass spectrometry, which has been used in natural product discovery applications in plants since at least 2008

(Banerjee, 2008). In another study, DART-MS was able to detect monoterpenes, sesquiterpenes, flavonoids, and organic compounds in the leaf and stem tissue between four *Eucalyptus* species (Maleknia, 2009). A recent review article indicated DART-MS was also able to detect arecaidine, arecoline, and guvacoline, which are human health-related compounds in plant tissues (Yew, 2019). These articles demonstrate the utility of DART-MS to quickly detect aroma-contributing metabolites as well as HCs in plant tissues without a separate extraction step. Novotná et al. (2012) analyzed extracted tomato and pepper samples from crops grown under organic and conventional management over two years using DART-MS coupled with time of flight (TOF). Interestingly, the metabolite fingerprint was better able to predict growing season than production system (Novotná et al., 2012).

The goal of this study was to develop a high-throughput, objective method that would allow a horticulturist or cultivar trial manager, for example, to easily and quickly characterize quality indicators for different peppers cultivars. To this end, we evaluated SPME-GC-MS as well as two AMS platforms: REIMS and DART-MS. We hypothesized that the chemical fingerprints generated by these analytical techniques coupled with computer-based predictive modelling would enable characterization of quality differences between market classes within the same species. To test this hypothesis, we compared the chemical fingerprints of 13 pepper phenotypes (grown in the 2018 field season) using SPME-GC-MS, red peppers from three phenotypes using REIMS, and 40 different pepper phenotypes (grown in the 2019 field season) using DART-MS. To our knowledge, this is the first study to evaluate a large number of pepper phenotypes using multiple technologies.

Materials and Methods:

SPME-GC-MS

Plant material for SPME-GC-MS

Pepper (*C. annuum*) seeds (Table 2.1) were sown on 24 March 2018 into plastic plug trays (50 cell, Harris Seeds, Rochester, NY) containing a mixture of 40L of soilless media (Sunshine mix #4, Sun Gro Horticulture, Agawam, MA), 10L of worm castings, 250ml of blood meal, and 250ml of bone meal. The average daily temperature in the greenhouse was 25°C and the relative humidity was 50%. The transplants were hardened by placing them outside under an insect-netting covered high tunnel with the east and west end walls removed for 7 days prior to transplanting. Pepper plants were grown between 5 June and 4 Oct. 2018 in a field at the Agricultural Research, Development and Education Center (ARDEC) South in Fort Collins, CO (lat. 40°36'N., long. 104°59'W.) elevation 1,524m.

Table 2.2. Pod types, colors, and cultivar names for peppers evaluated using solid phase micro extraction gas chromatography mass spectrometry (SPME-GC-MS); 2018.

Cultivar	Pod Type	Color
'Ace'	Bell	Red
'Aristotle'	Bell	Green
'Cornell University'	Bell	Red
'Early Red Sweet'	Bell	Red
'Flavorburst'	Bell	Yellow
'SBGO 10408'	Bell	Orange
'Carmen'	Italian-style	Red
'Early Perfect'	Italian-style	Red
'Escamillo'	Italian-style	Yellow
'Karma'	Italian-style	Red
'Lively Yellow'	Italian-style	Yellow
'Stocky Red'	Italian-style	Red
'Bridge to Paris'	Italian-style	Red

The pepper cultivars were planted 0.5m apart in black plastic mulched beds, which were spaced 1.8m apart. Macronutrient needs (57kg/acre nitrogen) were met with monthly applications of Drammatic "One" (4-4-0.5) fertilizer (Dramm Corporation, Manitowoc, WI) through the drip

irrigation system. Black plastic drip tape emitting water at a rate of 500L/h/100m with emitters spaced 20cm apart was used to irrigate the crop. Using an irrigation controller, we provided 15-30 minutes of irrigation once or twice daily. The crop was scouted for pests in the field on a weekly basis.

SPME-GC-MS detection of volatile metabolites

Mature pepper pods were harvested on 13, 20, and 27 Sept. and on 4 Oct. 2018.

Representative pods from each cultivar were stored in plastic bags and held in a -80°C freezer until additional sample preparation could be made for SPME-GC-MS chemical analysis. An effort was made to keep the pepper pods frozen throughout sample preparation. The shoulders and tips of the frozen pods were discarded, and the middle was chopped into 2cm pieces. These pieces were frozen in liquid nitrogen and ground into a fine fruit powder using mortar and pestle. A total of 39 experimental units were produced from 3 replicates of 13 cultivars. Each replicate was derived from two representative pepper pods. Frozen fruit powder (2g) was weighed in a 20-ml screw top vial (Waters Corporation, Milford, MA). A 7.5 pH solution of EDTA-NaOH was prepared by adding NaOH to 100mM EDTA, which produced a final EDTA concentration of 50mM. Then 2ml of the EDTA-NaOH solution was added to the 20ml vial containing the fruit powder. Finally, solid CaCl₂ was immediately added to create a 5M final concentration. The vials were placed in an Allegra X-12R centrifuge (Beckman Coulter, Brea, CA) for 4 minutes at 2000 revolutions/min prior to data acquisition.

The volatile metabolites were profiled using as previously described (Tikunov et al., 2005). Briefly, samples in headspace vials were prepared and submitted 1 hour before data acquisition. Frozen samples were first incubated at 50°C for 5 min, and then the headspace volatiles were extracted by a DVB/PDMS/CAR fiber that was 50/30 µm, Stableflex, (Sigma-Aldrich, St. Louis,

MO) for 20 min, and injected into a DB-WAXUI column (30 m x 0.25 mm x 0.25 μ m, Agilent) in a Trace1310 GC (Thermo Fisher Scientific, Waltham, MA) coupled to an ISQ-LT MS (Thermo Fisher Scientific, Waltham, MA). The SPME fiber desorbed at injection port (250°C) for 5 min, and then at fiber conditioning port (260°C) for 10 min. GC inlet was operated under splitless mode during fiber desorption. The oven program started at 45°C for 3 min, ramped to 220°C at a rate of 8°C/min, and a final hold at 220°C for 0.5 min. Data were acquired under electron impact mode, with full scan 35-400 amu and a scan rate 5 scans/sec Transfer line and source temperatures were 250°C. Quality control samples, containing 1g of each cultivar/replicate combination, were acquired after every fifth sample.

SPME-GC-MS data processing and statistical analysis

Data analysis was performed as previously reported (Linxing et al., 2019). Briefly, mass spectra from the SPME-GC-MS analysis were converted to .cdf file format, and peak detection, grouping, retention time alignment, and peak filling were performed using XCMS algorithms in the R statistical environment (version 3.6.2) (R Project, 2020). The molecular features were deconvoluted into spectral clusters using the RAMclustR package in R. The outputs of this data processing workflow resulted in a data set consisting of metabolites defined by (i) mass spectra, (ii) retention time, and (iii) relative abundance and normalized to the total ion current. Metabolites were annotated by spectral matching in NIST MS Search software to an in-house database of ~1,500 compounds and to external and theoretical databases including NIST v14 (<http://www.nist.gov>) and the Human Metabolome Database (2020). The effects of pod color, market class, and cultivar on the metabolite profile were evaluated using analysis of variance (ANOVA) with the aov function in R. Using the p.adjust function in R, we used a Benjamini-Hochberg false discovery rate adjustment to identify metabolites of statistical significance at the α

= 0.05 level. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (O2PLS-DA) of the metabolites was performed using SIMCA (version 15) (Umetrics, Umea, Sweden) on unit variance scaled data. Fold variation between red and green bell pepper pods was assessed by dividing red by green using GraphPad (version 8.1.0) (GraphPad Software, San Diego, CA).

Plant material for DART-MS

Pepper plants from 40 different cultivars (Table 2.3) were seeded and transplanted following the same timeline and protocol in 2018. Pepper plants were grown between 5 June and 3 Oct. 2019 in a field at the Agricultural Research, Development and Education Center (ARDEC) South in Fort Collins, CO (lat. 40°36’N., long. 104°59’W.) elevation 1,524m. The same production practices used in 2018 were followed in 2019 including fertility levels, irrigation amounts, the use of black plastic mulch, drip tape, as well as in-row and between row plant spacing. Fully mature pepper pods were harvested on 9 and 23 Sept. as well as on 3 Oct. 2019. The pods were stored in 7°C walk-in cooler until quality characteristics could be evaluated via DART-MS. Fresh pods were taken to the lab where they were stored in a 7°C refrigerator until they could be rinsed with deionized water and dried with disposable paper towels. Pepper pungency was determined by the cultivar description in grower seed catalogs or by speaking with the pepper breeder.

Table 2.3. The pod types, pod colors, and pungency for cultivars evaluated using direct analysis in real time (DART) mass spectrometry; 2019.

Cultivar	Pod type	Pod color	Pungency
‘Abay’	Bell	Yellow	Sweet
‘Ace’	Bell	Red	Sweet
‘Aristotle’	Bell	Green	Sweet
‘Aristotle’	Bell	Red	Sweet
‘Bianca’	Bell	White	Sweet
‘Early Red Sweet’	Bell	Red	Sweet
‘Flavorburst’	Bell	Yellow	Sweet
‘Jupiter’	Bell	Red	Sweet

'Karisma'	Bell	Red	Sweet
'King of the North'	Bell	Red	Sweet
'Orange Marmalade'	Bell	Orange	Sweet
'Peacework'	Bell	Red	Sweet
'Procraft'	Bell	Red	Sweet
'Sunrise'	Bell	Yellow	Sweet
'Whitney'	Bell	White	Sweet
'Wisconsin Lakes'	Bell	Red	Sweet
'Yankee Bell'	Bell	Red	Sweet
'Sweet Chocolate'	Bell	Chocolate	Sweet
'Anaheim College 64'	Anaheim	Green	Pungent
'Anaheim TMR #9457'	Anaheim	Green	Pungent
'Bridge to Paris'	Italian-	Red	Sweet
'Cañoncita Field 7 Landrace'	Anaheim	Red	Pungent
'Carmen'	Italian-	Red	Sweet
'Colorado State University 321'	Anaheim	Green	Pungent
'Colorado State University 384'	Anaheim	Green	Pungent
'Colorado State University 390'	Anaheim	Green	Pungent
'Colorado State University Mosco'	Anaheim	Red	Pungent
'Early Perfect Italian'	Italian-	Red	Sweet
'Escamillo'	Italian-	Yellow	Sweet
'Highlander'	Anaheim	Red	Pungent
'Joe E. Parker'	Italian-	Green	Pungent
'Karma'	Italian-	Red	Sweet
'Liebesapfel'	Anaheim	Green	Pungent
'Melrose'	Italian-	Red	Sweet
'Stocky Red Roaster'	Italian-	Red	Sweet
'Sweet Delilah'	Italian-	Red	Sweet
'Yellow Bardo'	Italian-	Yellow	Sweet
'Paradicsom Alaku Sarga Szentes'	Pimento	Yellow	Sweet
'Red Ruffled Pimento'	Pimento	Red	Sweet
'Colorado State University Pueblo Popper'	Popper	Red	Pungent

DART-MS detection of metabolites

The experiment was run as a randomized complete block design with eight replicates spanning eight days. One representative pepper pod served as the replicate. After every 8th sample, a quality control (QC) sample was analyzed. The QCs (6/day) consisted of a sub-sample from the same pod belonging to 'Ace'. A disposable utility razor blade was used to cut a 7cm long by 2cm wide slice of pepper longitudinally down the center of the pod just prior to metabolite analysis. From this

piece, 2mm thick cross-sectional cuts were made to expose the exocarp, mesocarp, and endocarp tissue (Figure 2). The sliced pepper samples were laid sideways on the tablet carrier adapter for the sample introduction rail system (IonSense, Inc., Saugus, MA).

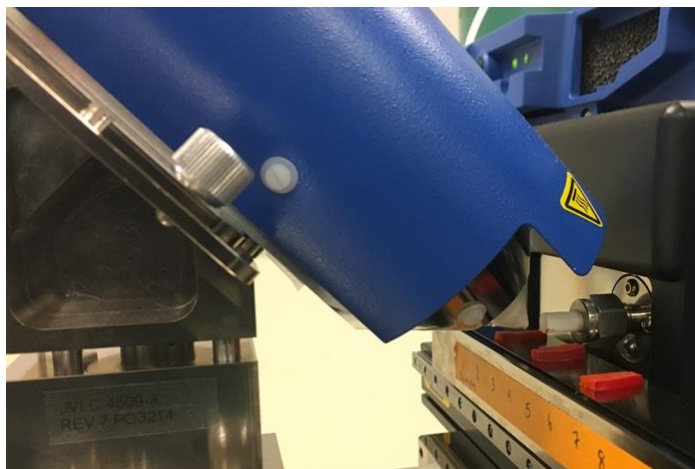


Figure 2.2. Intact pepper samples ready for DART analysis.

The DART-MS analysis was conducted using the DART-Standardized Voltage and Pressure (DART-SVP) model ion source (IonSense, Inc., Saugus, MA). It was coupled to a single quadrupole mass spectrometer (ACQUITY QDa; Waters Corporation, Manchester, UK) via a Vapour interface (IonSense, Inc., Saugus, MA). The DART-SVP was equipped with a motorized linear rail where the tablet carrier was mounted. The helium flow rate for the ion source was set to ~3L/min heated to 350°C. The cone voltage was set to 20V. Spectra were acquired in negative ionization mode over the mass range of 50-500 m/z. The speed of the motorized linear rail system holding the tablet carrier adapter was set to 1.0mm/sec. The standby temperature was held at 245°C. The samples were arranged on the 10 Tablet™ module (metal rail) such that three pepper sub-samples from the same pod were placed in every other tablet location. This allowed the signal from each sample to return to the baseline before the next acquisition could be started.

Authentic analytical standards for *p*-coumaric acid, *trans-p*-coumaric acid, capsaicin, L-ascorbic acid, and 2-methoxy-3-(1-methylpropyl) pyrazine were analyzed using the same instrument conditions with the exception of the sample introduction ($\geq 98\%$ purity) (Sigma-Aldrich, St. Louis, MO). A 10mg/mL solution was made for L-ascorbic acid using HPLC grade water. Solutions (10mg/mL) were made for *p*-coumaric acid, *trans-p*-coumaric acid, and capsaicin using 100% ethanol. The standard 2-methoxy-3-(1-methylpropyl) pyrazine was analyzed directly since it came in liquid form. Twelve replicates of each sample were run through the DART using the DIP-IT™ method. This involved dipping individual 10 μ L glass capillary rods into each solution and placing the rods into the DIP IT™ module holder, which allowed the analytical standards to pass directly in front of the ionizing source.

Compounds were putatively identified and annotated by cross-referencing the *m/z* values observed in the DART-MS spectra against all compounds that have been previously detected in red bell peppers as defined in the food database (FoodB, 2020) (version 1).

DART-MS data processing and statistical analysis

Preprocessing was conducted using a beta version of WRC Abstract Model Builder (Waters Corporation, Manchester, UK). Peaks corresponding to the three pepper sample spectra were selected so that a model could be constructed. The model construction options of “one spectrum per sample” and “apply normalization” were selected. The resulting data is a sum of the three sub-samples normalized to the total ion current for each sample. Peak binning was conducted at an interval of 0.5 *m/z*, which resulted in 900 total bins.

The effects of pod color, market class, cultivar, and pungency on the metabolite profile were evaluated using analysis of variance (ANOVA) with the *aov* function in the R statistical environment (version 3.6.2). Using the *p.adjust* function in R, we used a Benjamini-Hochberg

false discovery rate adjustment to identify metabolites of statistical significance at the $\alpha = 0.05$ level. O2PLS-DA of the metabolites was performed using SIMCA (version 15) on unit variance scaled and LOG_{10} transformed data. Fold variation between red and green bell pepper pods was assessed by dividing red by green using GraphPad (version 8.1.0). The heat map, which is based on z-scores for each pepper pod color and metabolite, was generated using the *pheatmap* function in R. Z-scores were calculated by comparing the average relative abundance value for a metabolite to the population mean and population standard deviation for that metabolite.

REIMS

REIMS detection of metabolites

Approximately 24 pepper samples were purchased for each of the two market classes, bell and popper (Table 2.4, Appendix Figure A.2) from Whole Foods Market (Fort Collins, CO) on 12 Feb. 2018. On the same day, 5 bags of ~24 tri-color (red, yellow, orange) sweet mini lunchbox pepper phenotypes were purchased from King Soopers (Kroger supermarket, Fort Collins, CO).

Table 2.4. The market classes and colors for pepper phenotypes evaluated using rapid evaporative ionization mass spectrometry (REIMS); 2018.

Cultivar/Phenotype	Market Class	Color
'Aloha'	Bell	Red with yellow stripes ^z
Red sweet mini	Lunchbox	Red
Yellow sweet mini	Lunchbox	Yellow
Red popper	Popper	Red

^z An image of the bell pepper cultivar 'Aloha' is available in the Appendix Figure A.2.

Metabolite data on the pepper phenotypes was collected as a randomized complete block design with 16 replicates. The individual pepper phenotypes were cut into thirds. The exocarp of the pod was placed flat on the conduction pad so that the mesocarp was exposed. The “iKnife”

was held perpendicular to the mesocarp tissue so the vacuum component could draw in the smoke generated by the ionizing source.

The chemical fingerprint was detected using the protocol described [18]. Briefly, the samples were analyzed using a Synapt G2 Si Q-ToF, fitted with a REIMS ionization source attached to a monopolar electrosurgical hand piece called an “iKnife” (Waters Corporation, Manchester, UK). It was powered with an Erbotom ICC 300 electrosurgical generator (Erbe Elektromedizin GmbH, Turbingen, Germany) using the “liquid coagulation” mode at a power of 40W. A solution of 2 ng/mL leucine-enkephalin at a continual flow rate of 200 μ L/min was directed to the REIMS source during sampling. The heater bias was set to 80V and the cone voltage was set to 20V. At least 3 “burns” were collected from each sample within a 3 cm X 3 cm square from the center of the pod. Each burn lasted approximately 3 sec. Spectra were collected from 50 – 1200 m/z using positive ionization mode.

REIMS data processing and statistical analysis

Preprocessing was performed using a beta version of Waters Abstract Model Builder. At least three peaks corresponding to three “burns” were selected. The model construction options of “one spectrum per sample” and “apply normalization” were selected as described above. Peak binning was conducted at an interval of 0.5 m/z, which resulted in 900 total bins using an m/z range from 50-550 m/z.

The effects of market class on the metabolite profile were evaluated using analysis of variance (ANOVA) with the *aov* function in the R statistical environment. Using the *p.adjust* function in R, we used a Benjamini-Hochberg false discovery rate adjustment to identify metabolites of statistical significance at the $\alpha = 0.05$ level. PCA of the metabolites was performed using SIMCA (version 15) on unit variance scaled and LOG₁₀ transformed data. The heat map illustrating differences in

metabolite profiles for each market class was generated using z-scores and the *pheatmap* function in R. Compounds were putatively annotated using a similar method as to what was described above for DART-MS.

Results and Discussion

SPME-GC-MS

PCA provides an unbiased overview of the SPME-GC-MS metabolite profiles and indicated that 34.9% of the variability in the model could be explained by pod color (Figure 3a).

Orthogonal projection to latent structure discriminant analysis (O2PLS-DA) was used to generate predictive models for pod color. Using just two colors (red versus green) an overall model fit of

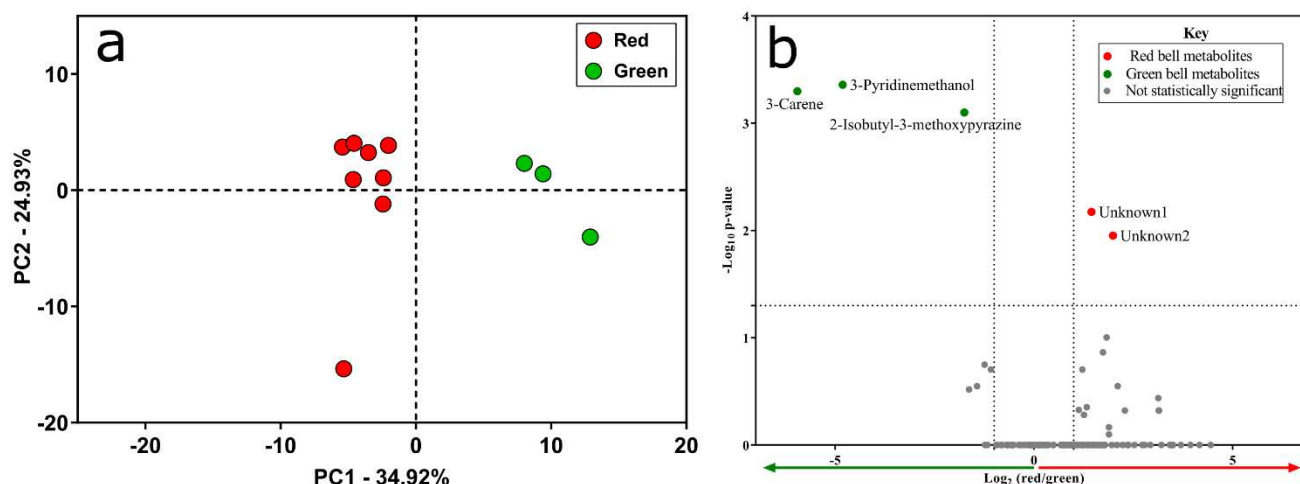


Figure 2.3. SPME-GC-MS metabolite profiles (a) PCA scores plot demonstrating separation between the first (34.9%) and second (24.9%) principal components; (b) volcano plot showing the statistically significant differences between metabolites detected in red and green bell peppers using the Benjamini-Hochberg false-discovery rate adjustment and $\alpha = 0.05$.

0.99 (R^2) and a cross-validated predictive accuracy of 96% (Q^2) were obtained. Including all three pepper colors (red, yellow, and green) yielded a model with an overall fit of 0.87 (R^2) and a cross-validated predictive accuracy of 82% (Q^2). Univariate statistical analysis revealed that the metabolites significantly enriched in green pods included 3-carene, 3-pyridinemethanol, and 2-isobutyl-3-methoxypyrazine (Figure 3b). We were unable to annotate the compounds

significantly enriched in red bell peppers. However, our results demonstrate that there are significant differences in the metabolite profiles between red and green pepper cultivars of the same species that can be detected by SPME-GC-MS.

The observed significant increase of 3-carene and 2-isobutyl-3-methoxypyrazine in green bell pepper pods as compared to red (Appendix Figure A.3) aligns with previous results in the literature (Luning et al. 1994; Pino et al., 2004; Junior et al., 2012). These compounds are both known volatile aroma compounds with descriptors including “green bell pepper, rubbery, and lettuce” (Luning et al., 1994). Previous studies (Liu et al., 2010) also report statistically significant differences in the volatile metabolome between red and green bell peppers. Thus, our results support that metabolite profiles generated by SPME-GC-MS are reflective of pod color and that specific aroma compounds important for consumer sensory can be detected.

DART-MS

O2PLS-DA modeling (red vs green) of the metabolite profiles generated by DART-MS demonstrate that 39.2% of the variation in the data can be explained by pod color (Figure 2a). The O2PLS-DA model exhibited an overall model fit of 0.89 (R^2) and a cross-validated predictive accuracy of 75% (Q^2). Overall, 92 metabolites were determined to be significantly different (Benjamini-Hochberg false-discovery rate adjustment and $\alpha = 0.05$; Figure 2b) and of these, 25 metabolites were putatively identified based on a combination of mass, previous detection in peppers, and comparison to DART-MS analysis of authentic standards (Table 2.5, Appendix Figures A.4, A.5, A.6, and A.7). Additional information for the unknown, but significant metabolites is presented in Appendix Table A.18.

Table 2.5. Putative metabolite annotations from DART-MS analysis of pepper samples.

M/Z^z	Molecular weight of putative compound (g/mol)	Mass error (Da)	Putative identification	Chemical class
269.26	269.27	-0.01	capsiamide	acetamides
121.26	121.14	0.12	p-aminobenzaldehyde	aldehydes
240.26	240.48	-0.22	N-heptadecane	alkanes
254.26	254.30	-0.04	4-methylheptadecane	
268.26	268.50	-0.24	nonadecane	
160.76	161.16	-0.40	aminoadipic acid	amino acids
104.26	104.06	0.20	malonic acid	dicarboxylic acids
216.26	216.32	-0.06	alpha hydroxylauric acid	fatty acids
282.26	282.47	-0.21	oleic acid	
286.26	286.24	0.02	luteolin	flavonoids
164.26	164.04	0.22	<i>p</i> -coumaric acid ^y	hydroxycinnamic acids
313.26	313.20	0.06	N-cis-feruloyltyramine	
313.76	313.30	0.46	moupinamide	methoxyphenols
305.26	305.41	-0.15	capsaicin ^y	
319.26	319.43	-0.17	homocapsaicin	
321.26	321.46	-0.20	homodyhydrocapsaicin	
291.26	291.39	-0.13	norcapsaicin	
277.26	277.36	-0.10	dinorcapsaicin	
120.26	120.10	0.16	tetrose	monosaccharides
136.26	136.24	0.02	limonene	monoterpenes
174.26	174.11	0.15	dehydroascorbic acid	nutrients
176.26	176.12	0.13	ascorbic acid (vitamin C) ^y	
219.26	219.23	0.03	pantothenic acid	
166.26	166.22	0.04	2-methoxy-3-(1-methylpropyl) pyrazine ^y	pyrazines

^z Statistically significance m/z for putatively identified compounds using the Benjamini-Hochberg false-discovery rate adjustment and $\alpha = 0.05$.

^y Compound annotations validated by authentic standards.

The compound putatively identified as 2-isopropyl-3-methoxypyrazine, was observed to be significantly ($p = 0.002$) enriched in green pepper pods, a result that agrees with previous studies using SPME-GC-MS (Liu et al., 2010; Howard et al., 1994; Troconis-Torres et al., 2012). Interestingly, two of the compounds detected in red pepper samples were putatively identified as *p*-coumaric acid and ascorbic acid (vitamin C) (Figure 4). Vitamin C has been previously reported to accumulate in mature red peppers (Thuphairo et al., 2019). Previous studies have also

reported detection of *p*-coumaric acid in red peppers (Masek et al., 2016). Two additional compounds were putatively annotated as capsaicin and 2-methoxy-3-(1-methylpropyl) pyrazine, both of which have been previously detected in peppers and contribute to pepper sensory and flavor quality (Birwal and Yadav, 2015; Buttery et al., 1969). To increase confidence in these compound annotations, authentic standards were analyzed by DART-MS under the same experimental conditions (Appendix Figure A.7).

Using the putatively annotated metabolites only, a comparison of the six different bell

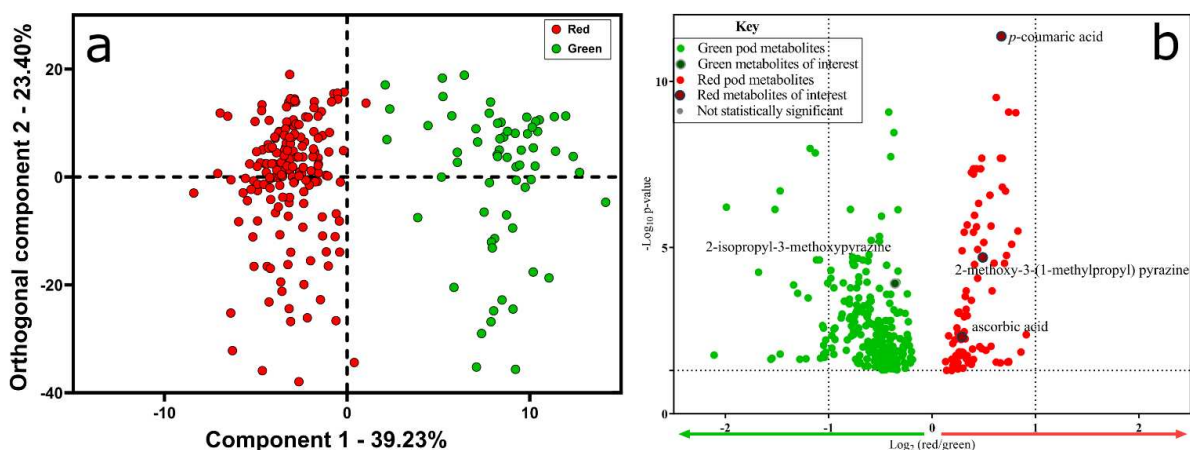


Figure 2.4. DART-MS metabolite profiles for red and green peppers (a) O2PLS-DA scores plot demonstrating separation between the first component (39.2%) and orthogonal components (23.4%) (b) volcano plot showing the statistically significant differences between metabolites associated with red and green bell peppers using the Benjamini-Hochberg false-discovery rate adjustment and $\alpha = 0.05$.

peppers analyzed by DART-MS indicates subgrouping (based on hierarchical clustering) of red and yellow and orange and chocolate (Figure 2.5). Green followed by white bell peppers were the most distant among all phenotypes. Interestingly, chocolate colored bell peppers contained the most *p*-coumaric acid in their metabolite profiles. Yellow bell peppers contained the highest abundance of limone, a terpene compound that has a “citrus” aroma (Good Scents Company, 2020). Red bell peppers contained the highest abundance of 2-methoxy-3-(1-methylpropyl) pyrazine, which is described to have a “musty, earthy, peppery” aroma (Junior et al., 2012).

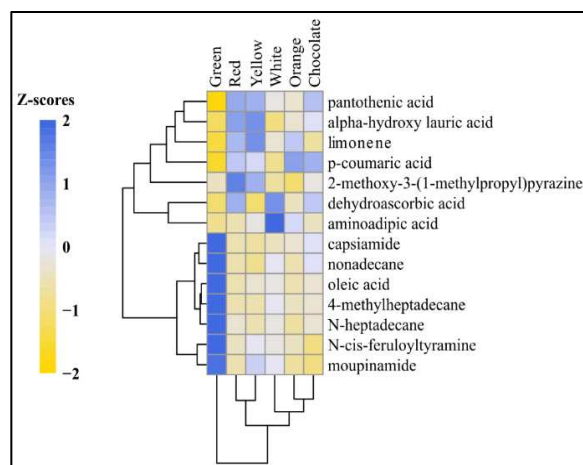


Figure 2.5. Heat map showing the differences in putatively identified compounds in the metabolite profiles of green, white, red, yellow, chocolate, and orange bell pepper phenotypes.

The trend of more N-heptadecane in green peppers has been reported previously in the literature (Thuphairo et al., 2019). Our results indicate significantly higher abundances in nonadecane and oleic acid in green peppers compared to red peppers, a result that diverges from what has been reported in the previous studies (Pino et al., 2004; Lin et al., 2008). Nonadecane appears to be a metabolite that serves as a maturity indicator. It has been reported to be absent in the volatile fraction of green bells, whereas it has the highest levels at the ripening stage and lower levels at maturity. Thus, our observation of higher abundance of nonadecane in green peppers compared to mature red peppers, suggests that some of the green pods likely were developing a metabolite fingerprint that was beginning to resemble ripening. Given that peppers were harvested for up to three weeks prior to analysis, it is possible that some of the green pods continued to mature postharvest. The observed trends for N-heptadecane and alpha-hydroxylauric acid, align with the literature (Novotná et al., 2012) for bell pepper pod color comparisons. DART-MS was also able to detect qualitative metabolite differences between sweet and pungent peppers. The O2PLS-DA model exhibited an overall model fit of 0.79 (R²)

and a cross validated predictive accuracy of 60% (Q^2). Figure 2.6 illustrates that the distinction between sweet and pungent peppers (component 1) explained 23.6% of the variability in the data, and the orthogonal component was able to explain 26.2% of the variability. Using the Benjamini-Hochberg false-discovery rate adjustment, the putatively annotated compounds

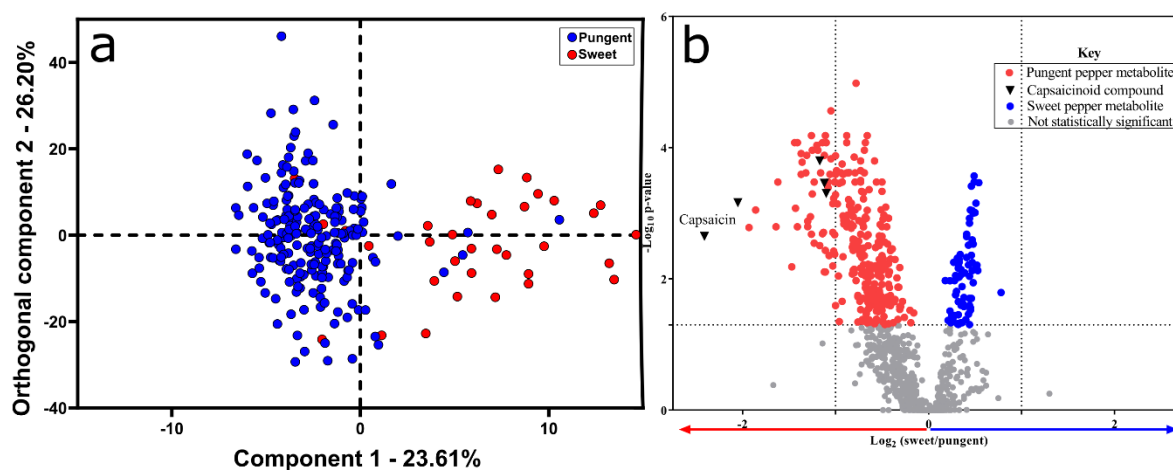


Figure 2.6. DART-MS metabolite profiles for pungent and sweet peppers (a) O2PLSDA scores plot demonstrating separation between the first (23.6%) and orthogonal (26.2%) components (b) volcano plot showing the statistically significant differences between metabolites associated with pungent and sweet peppers using the Benjamini-Hochberg false-discovery rate adjustment and $\alpha = 0.05$.

capsaicin ($p = 0.002$), homocapsaicin ($p = 0.0003$), homodihydrocapsaicin ($p = 0.001$), norcapsaicin ($p = 0.004$), and dinorcapsaicin ($p = 0.0001$) were significantly enriched in the pungent pepper metabolite profiles.

REIMS

O2PLS-DA of the metabolites generated by REIMS demonstrates that 49% of the variation in the data can be explained by market class (Figure 7a). The O2PLS-DA model (market class) exhibited an overall model fit of 0.85 (R^2) and a cross-validated predictive accuracy of 74% (Q^2). Overall, 201 metabolites were determined to be statistically significant (Benjamini-Hochberg false-discovery rate adjustment and $\alpha = 0.05$; Figure 2.7a) and of these, 37 metabolites were putatively identified based on a combination of mass and previous detection in peppers.

Additional information for the unknown but significant metabolites is presented in Appendix Table A.19. Representative mass spectra generated by REIMS are indicated in Appendix Figures A.8, A.9, and A.10. Previous studies have demonstrated that metabolite profiles generated by GC-MS grouped together according to pungency rather than species [12]. The metabolite fingerprints for the three market classes are presented as a heat map (Figure 2.7b) and the chemical classes are listed (Table 2.6)

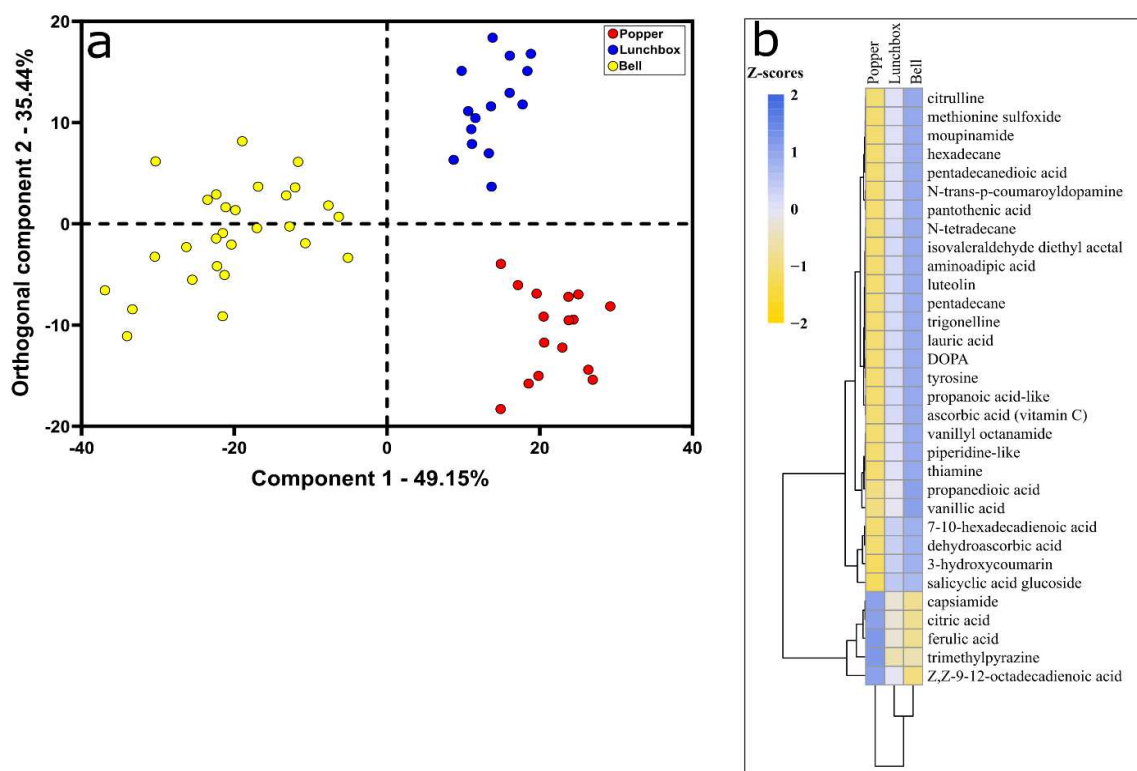


Figure 2.7. The impact of market class on the metabolome of peppers using rapid evaporative ionization mass spectrometry (REIMS): (a) O2PLS-DA scores plot demonstrating separation between component 1 (49.1%) and the orthogonal component (35.4%); (b) heat map showing the differences in metabolite profiles for popper, lunchbox, bell peppers using the Benjamini-Hochberg false-discovery rate adjustment and $\alpha = 0.05$.

Interestingly, the bell pepper market class contained the highest abundance of vitamin C as well as its precursor compound dehydroascorbic acid. Bell peppers also contained the most luteolin, which is a flavonoid with antioxidant and anti-inflammatory activity (Troconis-Torres et

al., 2012). The metabolite fingerprint for the popper market class was the most different from the bell market class, while the lunchbox peppers were most similar to the bell pepper market class (Figure 2.5a). The highest abundance of the compounds putatively annotated as capsiamide, citric acid, 3-beta-lanostenol, fumaric acid, and octadecadienoic acid was observed in the popper market class. Poppers also contained the highest relative abundance of the compounds putatively annotated as coumaric acid and ferulic acid, which belong to a class known as coumaric acids. Coumaric acids are important antioxidants with the ability to scavenge free radicals (Thuphairo et al., 2019). In addition, poppers also contained the highest relative abundances of the compound putatively annotated as trimethylpyrazine, which is known to have a “nutty” aroma and “musty” flavor (Good Scents Company, 2020).

Table 2.6. Putative metabolite annotations from REIMS analysis of pepper samples.

M/Z⁺	Molecular weight of putative compound (g/mol)	Mass error (Da)	Putative identification	Chemical class
160.24	160.25	-0.01	isovaleraldehyde diethyl acetal	acetals
269.74	269.27	0.47	capsiamide	acetamides
299.24	299.32	-0.08	n-trans-p-coumaroyloctopamine	
137.24	137.14	0.10	trigonelline	
226.24	226.24	0.00	hexadecane	alkanes
198.24	198.39	-0.15	n-tetradecane	
212.24	212.42	-0.18	pentadecane	
179.24	179.17	0.07	glucosamine	amines
161.24	161.16	0.08	aminoadipic acid	amino acids
175.24	175.20	0.04	citrulline	
197.24	197.19	0.05	DOPA	
165.24	165.21	0.03	methionine sulfoxide	
181.24	181.19	0.05	tyrosine	
165.24	165.19	0.05	phenylalanine	
192.74	192.12	0.62	citric acid	carboxylic acids
74.24	74.08	0.16	propanoic acid	
104.24	104.06	0.18	propanedioic acid	
168.24	168.14	0.10	vanillic acid	
296.74	296.23	0.51	coumaric acid	coumaric acids
194.74	194.18	0.56	ferulic acid	
250.24	250.38	-0.14	4,7,10,13-hexadecatetraenoic acid	fatty acids
252.24	252.39	-0.15	7,10-hexadecadienoic acid like	

329.24	329.24	0.00	7Z,10Z,13Z-docosapentaenoic acid	
200.24	200.32	-0.08	lauric acid	
272.24	272.38	-0.14	pentadecanedioic acid	
280.74	280.40	0.34	(Z,Z)-9,12-octadecadienoic acid like	
286.24	286.24	0.00	luteolin	flavonoids
300.24	300.26	-0.02	salicylic acid glucoside	glucosides
174.24	174.11	0.13	dehydroascorbic acid	
176.24	176.12	0.12	ascorbic acid (vitamin C)	
265.24	265.36	-0.12	thiamine	
162.24	162.14	0.10	hydroxycoumarin	hydroxycoumarins
219.24	219.23	0.01	pantothenic acid	nutrients
85.24	85.15	0.09	piperidine like	piperidine
151.24	151.19	0.05	4-ethyl-2-methoxyphenol like	phenols
313.24	313.30	-0.06	moupinamide	
279.24	279.37	-0.13	vanillyl octanamide	
162.24	162.14	0.10	3-hydroxycoumarin	pyrans
122.24	122.17	0.07	trimethylpyrazine	pyrazines
213.24	213.16	0.08	pyridine like	pyridines
217.24	217.27	-0.03	pyrimidine like	pyrimidines

^z The m/z of putatively identified compounds of statistical significance using the Benjamini-Hochberg false-discovery rate adjustment and $\alpha = 0.05$.

Conclusion

The analytical platforms evaluated in this study differed in their abilities to detect pepper quality characteristics. SPME-GC-MS was able to detect metabolite differences between red and green bell peppers based on volatile aroma-contributing metabolites. DART-MS was also able to detect metabolites known to contribute to aroma between red and green peppers (e.g. 2-methoxy-3-(1-methylpropyl) pyrazine) as well as metabolites associated with fresh-eating quality characteristics (e.g. pungency) and bioactive compounds (e.g. *p*-coumaric acid). REIMS was able to detect human-health related and quality related metabolite differences between red pepper phenotypes. For example, luteolin, a metabolite with antioxidant and anti-inflammatory activity (Lin et al., 2008) had the highest abundance in red bell peppers. In addition, REIMS also detected differences in vanillic acid, a metabolite known to be associated with a “smooth, vanilla” type aroma (Howard, et al., 1994). Taken together, the results of this study demonstrate

that all three platforms (SPME-GC-MS, DART-MS, and REIMS) were able to detect volatile and non-volatile compounds important in the characterization of inherent quality attributes. The AMS platforms have the advantage of throughput and minimal sample preparation and thus represent technologies that could be feasibly developed for screening applications in agricultural studies.

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CHAPTER 3: Evaluating a Trace Mineral Product as a Crop Input for Organic Vegetable Production

Introduction and Literature Review

Chile pepper consumption in the U.S. increased 8% between 2015 and 2017, with the average person consuming about 7.7 pounds per year in 2017 (Economic Research Service, 2018). Approximately 4.7 million pounds of chiles were produced on 18,900 acres in the U.S. in 2018 (National Agriculture Statistics Service, 2018), which equates to an economic value of \$143 million. During this time, California contributed the highest percentage (64%) of chiles to the U.S. fresh market. Chile peppers are an economically important crop to other Intermountain West growers in Arizona, New Mexico, and Texas. Colorado is not included in the top four list of chile pepper production by state, but the chile is a historically and culturally important crop to Colorado fresh market consumers (Haverluk, 2002). In fact, there is a friendly rivalry that exists between consumers who prefer Hatch (NM) to those that prefer Pueblo (CO) grown green chiles, which are two regions (about 500 miles apart) in the U.S. that are known for producing high quality chile peppers (Arellano, 2018). Regardless of growing region though, farmers, including organic growers, are interested in ways to increase their pepper yields. Some even consider adding crop inputs such as rock powder based soil amendments, which supply trace minerals and potassium (K) from mined sources.

The impact of rock powder based soil amendments on food crops has been investigated since the 1700s (Encyclopedia Britannica, 2020). However, more recent research since the 1980s has generated a body of knowledge that researchers, Extension professionals, and farmers rely on today. The literature indicates that rock powders are attractive as soil amendments because they

are relatively less expensive than soluble commercial fertilizers, and they release other mineral nutrients and K slowly over time (Weerasuriya et al., 1993). Plants require K in order to maintain their health and vigor. Potassium is the soil cation that plants need in the largest amount; it plays a role in vital plant functions such as osmoregulation, enzyme activation, water relations, and photosynthate translocation to the fruits (Mikkelsen, 2007).

Feldspars and micas are the most common mineral sources of K, but other sources include: ballast, biotite, granite, and greensand. Hydrated sodium calcium aluminosilicate is another type of rock powder soil amendment that is mined and sold under the trade name AZOMITE (Azomite), which is an acronym for “A to Z of minerals including trace elements” (Azomite Mineral Products, Inc., 2018). The Organic Materials Review Institute (OMRI) approved the use of granulated and ultrafine grades of Azomite for organic production in 2010 and 2018, respectively (Organic Materials Review Institute, 2020). Azomite (0-0-0.2) supplies K (0.2%), Ca (1.8%), Mg (0.5%), chlorine (0.1%), sodium (0.1%) and other trace minerals. The aluminosilicate mineral, which composes Azomite, formed when a volcanic eruption encountered a seabed an estimated 30 million years ago in what is now Utah. Rock types from Sri Lanka such as pink granite, granulitic gneiss, and migmatitic gneiss have also demonstrated the ability to serve as potassium sources (Niwas et al., 1987). Mined K sources are considered non-renewable, but plentiful, and have the expectation that they were not processed or altered from their original form.

In addition to serving as a mineral supplying soil amendment, some rock powders have demonstrated the ability to improve the physical properties of soil. One study demonstrated that the addition of volcanic ash significantly improved the cation exchange capacity (C.E.C.) of the soil (Blum et al., 1989). A recent publication indicated that greensand, a glauconite-based

mineral product containing micro-pores that is native to parts of New Jersey, Delaware, Maryland, and Virginia in the U.S., helps improve soil C.E.C. and the water-holding capacity (Heckman and Tedrow, 2004). Not only does glauconite improve the physical aspects of soil, but it also contains ~8% potassium oxide, small amounts of calcium (Ca), phosphorus (P), and other trace minerals. Interestingly, greensand helps to improve the exchange of micronutrients and macronutrients in soil such as K, Ca, and magnesium (Mg). Still, improved soil nutrient exchange did not lead to an increased nutritional content in a leaf tissue analysis of potato or a higher tuber yield, as reported by Heckman and Tedrow (2004). In another study, glauconite-based soil amendments yielded a comparable plant biomass to a potassium chloride-based fertilizer in grass species (Franzosi et al., 2014), which demonstrates its usefulness as a K fertilizer.

The literature also indicates that rock powder based soil amendments contain K, but the release rate is dependent on weathering conditions, mineral composition, pH, the biological activity of the soil, and particle size (Ramos et al., 2014). Mikkelsen (2007) noted that as the particle size of the rock powder increased, the surface area, reactivity, and the weathering rate decreased. Therefore, large particle size is a limitation to using rock powder, but it is not the only one. Examples of other limitations include: the slow release of nutrients over a long period (months to years), insolubility issues with the potassium, and the need to apply large quantities in order to achieve a positive response (Bolland and Baker, 2000). There is only one study that demonstrated significant yield increases from rock powder soil amendments, and it involved the application of acidulated pegmatic phlopite mica to rice at a rate of 200 kg per hectare (Weerasuriya et al., 1993).

The literature regarding the impact of Azomite on plant growth and marketable yield is mixed. In one study, Azomite did not increase fresh or dry shoot and root biomass in *Chrysanthemum* cuttings, *Malus* seedlings, or *Citrus* seedlings, when it was mixed 1:1 with soilless media in container grown plants (Ely and Hubbard, 1998). However, another study reported that Azomite significantly increased stem length, fresh and dry shoot biomass, as well as chlorophyll, nitrogen, phosphorus, and potassium content in the leaves of two drought stressed tomato cultivars (Azad et al., 2016). However, differences in tomato yields were not reported. The focus of this study was to investigate the yield impact of applying Azomite to Italian-style roasting peppers under organic management over two growing seasons. The hypothesis of the study could be defined as, trace minerals and rock powder based K delivered by Azomite would significantly increase the total marketable yields of two Italian-style roasting pepper cultivars. In addition, our team hypothesized both grades of Azomite would lead to significant increases in yield, whether it be the granulated grade incorporated through banding in the row (2017-2018) or the ultrafine grade applied as foliar and/or fertilizer irrigation (fertigation) treatments (2018 only).

Material and Methods

Seeds from two non-pungent, Italian-style roasting pepper (*Capsicum annuum* L.) cultivars ‘Stocky Red Roaster’ and ‘Early Perfect Italian’ were sown into 20 row seed furrow trays (Grower’s Solution, Cookeville, TN) containing soilless media on 25 April 2017 and again on 27 April 2018. Trays were placed on heated (75-80°F) propagation mats (Redi-Heat HD Mats, Danville, IL). A soilless media composed of approximately 40 L Sunshine® mix #4 (Sun Gro Horticulture, Agawam, MA), 10 L of worm castings, 250 ml of blood meal (12-0-0) (Down to Earth Fertilizers, Eugene, OR), and 250 ml of bone meal (3-15-0) (Down to Earth Fertilizers, Eugene, OR) was used to germinate and grow the transplants while they were in a climate-

controlled greenhouse. Pepper seedlings were transplanted into 50 cell plastic trays 18 days later (Denver Wholesale Florist, Denver, CO).

Design and Materials for Granulated Experiment

The experiment was setup as a split-plot design with Azomite treatment as the whole plot factor and pepper cultivar as the sub-plot factor. This study was conducted during the 2017 field season, at the Agricultural Research, Development and Education Center (ARDEC) South in Fort Collins, CO (lat. 40°36’N., long. 104°59’W.) elevation 1,524 m, and it was repeated again in 2018. Four replicates of three treatments rates (0, 100, and 200 #/acre) and two cultivars (Table 3.1) produced 24 experimental units. Each experimental unit contained 11 plants with two plants, one on each end, serving as border plants. Data were not collected from the border plants. Three rates of granulated Azomite (Azomite Mineral Products, Inc., Nephi, Utah) were banded into the row prior to transplanting on 8 June 2017 and on 7 June 2018, respectively. Granulated Azomite has a granule size of 1.0 – 2.0 mm.

Table 3.1. Pepper cultivars and treatments included in the granulated Azomite field experiment occurring on certified organic agricultural land in Fort Collins, Colorado; 2017-2018.

Treatments	Pepper cultivars
0 #/ac granulated Azomite	‘Early Perfect Italian’
100 #/ac granulated Azomite	‘Stocky Red Roaster’
200 #/ac granulated Azomite	

The pepper plants were transplanted into 1.5 ml black plastic (Rain-Flo Irrigation, East Earl, PA) mulched beds on certified organic land. Planting holes were punched with a #26 super wheel with the appropriate number of adjustable C-spikes (Rain-Flo Irrigation, East Earl, Pennsylvania) to accommodate the within row (18 in) plant spacing. Black plastic drip tape emitting water at a rate of 132 gal/h/328 ft with emitters spaced 8 in apart was used to irrigate the crop. Using an irrigation controller, we provided 15-30 min of irrigation once or twice daily. Macronutrient

needs were met with monthly applications (June, July, and Aug.) of Drammatic “One” fertilizer (4-4-0.5) (Dramm Corporation, Manitowoc, WI).

Plant height, yield of marketable peppers, days to first open flower, flower bud and pod count, and °Brix were measured to assess the impact of granulated Azomite on two pepper cultivars. A soil sample was collected from a depth of 4-6 in prior to the application of Azomite. Soil samples were also collected 26 and 134 days after treatment (DAT). A leaf tissue test for macro and micronutrients (including silicon) was taken 61 and 117 DAT (data presented in the Appendix). The first fully-ripe red pepper harvest occurred on 17 Sept. 2017 and 10 Sept. 2018. The remaining marketable pods were harvested on 4 Oct. 2017 and 24 Sept. 2018. A fruit was defined as fully-ripe if it exhibited two-thirds or more of its mature red color. A marketable fruit was defined as two-thirds its average mature size and free from blemishes attributed to physiological disorders (e.g. sunscald) or plant diseases (e.g. blossom end rot). Soluble solids content (°Brix) was measured with a digital refractometer (MA871, Milwaukee Instruments, Rocky Mount, NC) after the pods were frozen, thawed and the pepper liquid was filtered through cheesecloth.

Economic Analysis

An economic analysis was conducted using the total marketable yields for each cultivar, based on the granulated field experiment. In addition, a separate analysis was also conducted for both cultivars based on the ultrafine Azomite foliar treatments. The gross revenues for each cultivar are based on the average total marketable yield on a per plant basis, a planting density of 17,424 plants/acre (Boyhan et al., 2019), and a per pound price of \$3.20 (Colorado State University Extension, 2020). Variable expenses, estimated at \$7,682 per acre, include the costs of materials such as seed, soil, plastic trays, Azomite, and irrigation drip tape. The variable

expenses also include the labor associated with tillage, planting bed construction, transplanting, weeding with a stir-up hoe, and harvesting. Labor costs were estimated at \$12 an hour. The cost of banding granular Azomite in the field was estimated at \$20 per acre (Marty Campfield, Azomite Mineral Products, Inc., personal correspondence, 10 Nov. 2018), which is priced for a pepper grower managing a large-acreage operation. Granulated Azomite was priced at \$0.40 a pound, whereas ultrafine Azomite was priced at \$1.50 a pound (Marty Campfield, Azomite Mineral Products, Inc., personal correspondence, 10 Nov. 2018). Fixed expenses, estimated at \$6,819, were based on Farm Service Agency loan applicant data from 2012; the expenses were consumer price indexed to 2017 dollars (Martha Sullins, Colorado State University Extension, personal correspondence, 22 Apr. 2020).

Design and Materials for Ultrafine Azomite Experiment

The ultrafine Azomite experiment took place in an uncovered (20 ft x 45 ft) high tunnel on certified organic land at (ARDEC) South in Fort Collins, CO during the 2018 field season. The experiment was repeated that same year in a second high tunnel that was covered with insect netting (40 mesh, Greenhouse Megastore, Danville, IL) in order to protect the crop from hail damage. A split-plot design with three replicates per high tunnel was used to conduct the experiment. Fertigation treatment was the whole plot factor and foliar treatment was the sub-plot factor. Thirty-six experimental units were derived from six treatments (Table 3.2), two pepper cultivars, and three replicates. Each experimental unit contained seven plants with two plants, one on each end, serving as border plants. Data was not collected from the border plants.

Table 3.2. Fertigation treatments, foliar treatments, pounds per acre of Azomite applied, and the treatment identifiers for the ultrafine Azomite high tunnel experiment occurring on certified organic agricultural land in Fort Collins, Colorado; 2018.

Treatment key	Fertigation treatments	Foliar treatments	Pounds/acre of Azomite (season)
AZO-AT	Ultrafine Azomite (AZO)	Ultrafine Azomite foliar treatment (AT)	50 #/ac
AZO-WT	Ultrafine Azomite (AZO)	Water foliar treatment (WT)	25 #/ac
AZO-NT	Ultrafine Azomite (AZO)	No foliar treatment (NT)	25 #/ac
Water-AT	Water irrigation only (Water)	Ultrafine Azomite foliar treatment (AT)	25 #/ac
Water-WT	Water irrigation only (Water)	Water foliar treatment (WT)	0 #/ac
Water-NT	Water irrigation only (Water)	No foliar treatment (NT)	0 #ac

The pepper plants were transplanted into 24 in bed tops covered with black woven weed fabric (994GC, Lumite, Alto, GA). The high tunnels were transplanted on 15 June 2018 with an in-row spacing of 12 in. Plants were irrigated using the same volume and frequency as described for the granulated experiment. Macronutrient needs were met with monthly applications of Drammatic “One” fertilizer (4-4-0.5) (Dramm Corporation, Manitowoc, WI). The ultrafine Azomite fertigation treatments were delivered using a fertilizer injector (Dosatron model D14MZ2, 1:50, 14 GPM) and drip tape emitting 132 gal/h/328 ft with emitters spaced 8 in apart and 5 pounds/acre Azomite was suspended in 3,000 gallons/acre of water. This was possible because 90% of Azomite ultrafine can pass through a 450-mesh screen (Azomite Mineral Products, Inc., 2018). Five Azomite fertigation treatments occurred over a ten-week period (17 to 73 days after transplant) with 5 pounds/ac delivered at each application. Water irrigation only (water) treatments also received an additional 3,000 gallons/acre water every other week for 10 weeks. The fertigation treatments were in addition to the normally scheduled watering events. Ultrafine Azomite foliar treatments were delivered using a ½ gal handheld sprayer (GroundWork, Tractor Supply Co., Brentwood, TN) and the appropriate spray volume and Azomite weight so as to mimic the coverage that would be required to achieve a 40 gallon per acre spray volume and 5 pounds/ac ultrafine Azomite. The 25 pounds/ac foliar Azomite treatment was delivered in 5 pounds/ac increments over five separate applications spanning 10 weeks (17 to 73 DAT). A blanket served as a physical barrier protecting one foliar treatment plot from the others during the application process.

Plant heights, yield of marketable red and green peppers, count of marketable red and green peppers pods, days to first open flower, flower bud and pod counts, and °Brix were measured to assess the impact of Azomite on two roasting pepper cultivar. A soil sample was taken prior to

the application of Azomite, 55 DAT, and 126 DAT. A tissue test for macro and micronutrients (including silicon) was conducted on 53 and 109 DAT (results are in the Appendix). A fully-ripe red pepper harvest occurred 17 and 22 Sept. 2018. The remaining marketable pods were harvested 1 Oct. 2018. Pods were defined as fully-ripe and marketable using the same conditions as those defined in the granulated experiment.

Statistical Analysis

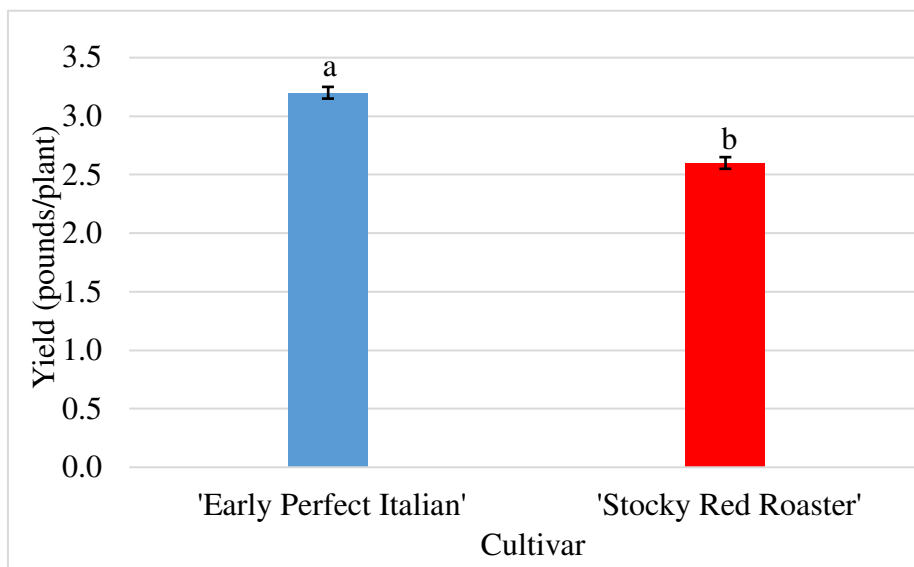
A check of the diagnostic plot, Pearson residual versus fitted values, indicated equal scatter so the analysis of variance (ANOVA) assumption of equal variance was met. ANOVA was performed using R Statistical Software (version 3.6.2) (R Statistical Software, 2019) and the emmeans package (Lenth, 2019). Tukey adjusted pairwise comparisons ($\alpha = 0.05$) were used for mean separation if statistical significance was observed in the ANOVA test. The predictor variables for the granulated Azomite experiments were cultivar ('Early Perfect Italian' and 'Stocky Red Roaster') and treatment (0, 100 and 200 pounds/ac Azomite). The predictor variables for the ultrafine Azomite experiments were fertigation treatment (AZO and Water) and foliar treatment (AA, WA, and NA). Year was included as a random effect in the granulated Azomite model, while high tunnel was included as a random effect in the ultrafine Azomite model. The response variables were plant height, days to first open flower, flower bud and pod count, °Brix, total marketable pounds per plant, total marketable pods per plant, total marketable pounds per plant of ripe red peppers, and total marketable pods per plant of ripe red peppers.

Results and Discussion

Granulated Azomite Experiment

There were no statistically significant differences between granulated Azomite treatments at 0, 100, and 200 pounds/ac for yield parameters, days to flower, flower bud count, °Brix, and plant height (Appendix Tables A.20 - A.22). Therefore, granulated Azomite treatment means

were averaged to explore the effect of cultivar on yield and °Brix. ‘Early Perfect Italian’ yielded significantly ($p = 0.0001$) more pounds per plant of marketable pods than ‘Stocky Red Roaster’, at 3.2 and 2.6 pounds, respectively (Figure 3.1).

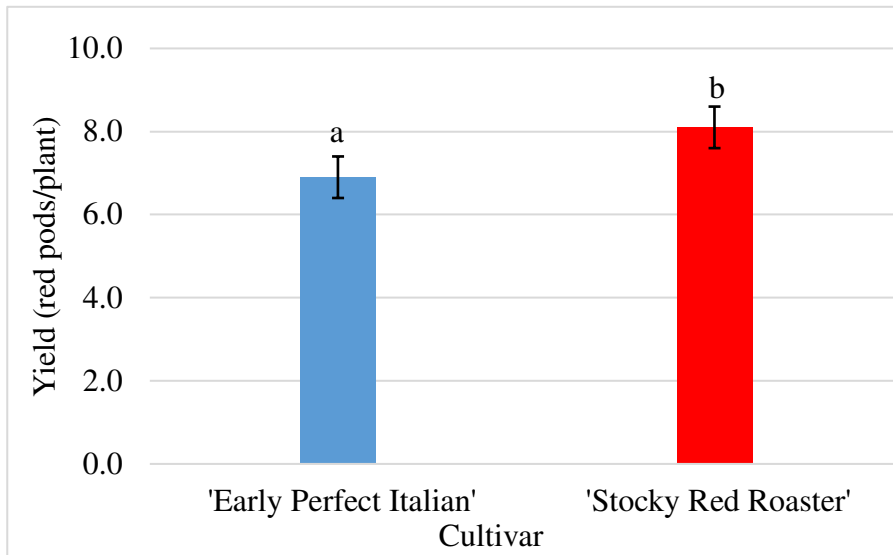


^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test. The error bar indicates mean \pm standard error ($n=4$).

Figure 3.1. Total marketable pounds per plant comparison between two Italian-style roasting pepper cultivars on certified organic agricultural land in Fort Collins, Colorado; 2017-2018 combined ^z.

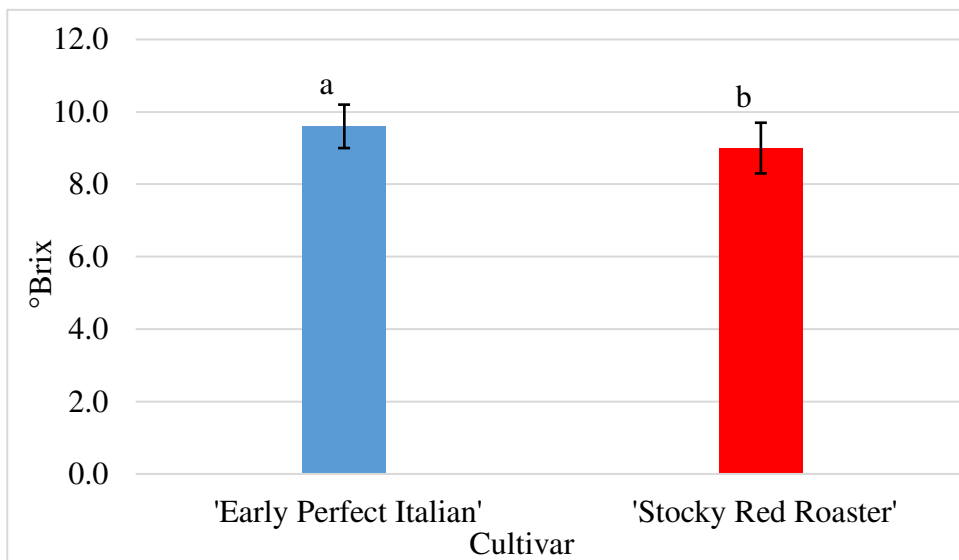
‘Stocky Red Roaster’, however, produced significantly more ($p = 0.006$) red pods per plant than ‘Early Perfect Italian’ at 8.1 and 6.9 pods, respectively (Figure 3.2). It is worth noting that ‘Stocky Red Roaster’ pods (14.3 cm) were shorter, although not significantly, than ‘Early Perfect Italian’ pods (15.5 cm). In addition to yield, the cultivars differed in soluble solids content. Figure 3.3 indicates the ripe pods of ‘Early Perfect Italian’ (9.6 °Brix), had a significantly higher ($p = 0.001$) °Brix value than ‘Stocky Red Roaster’ (9.0 °Brix). Results from the leaf tissue analysis and soil sample analysis (1 replicate) did not indicate that Azomite appreciably

impacted the nutritional content in pepper leaves (Appendix Tables A.23 - A.26) or in the soil profile (Appendix Tables A.27 – A.29).



^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test. The error bar indicates mean \pm standard error (n=4).

Figure 3.2. Total number of marketable red pods per plant comparison between two Italian-style roasting pepper cultivars on certified organic agricultural land in Fort Collins, Colorado; 2017-2018 combined ^z.



^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test. The error bar indicates mean \pm standard error (n=4).

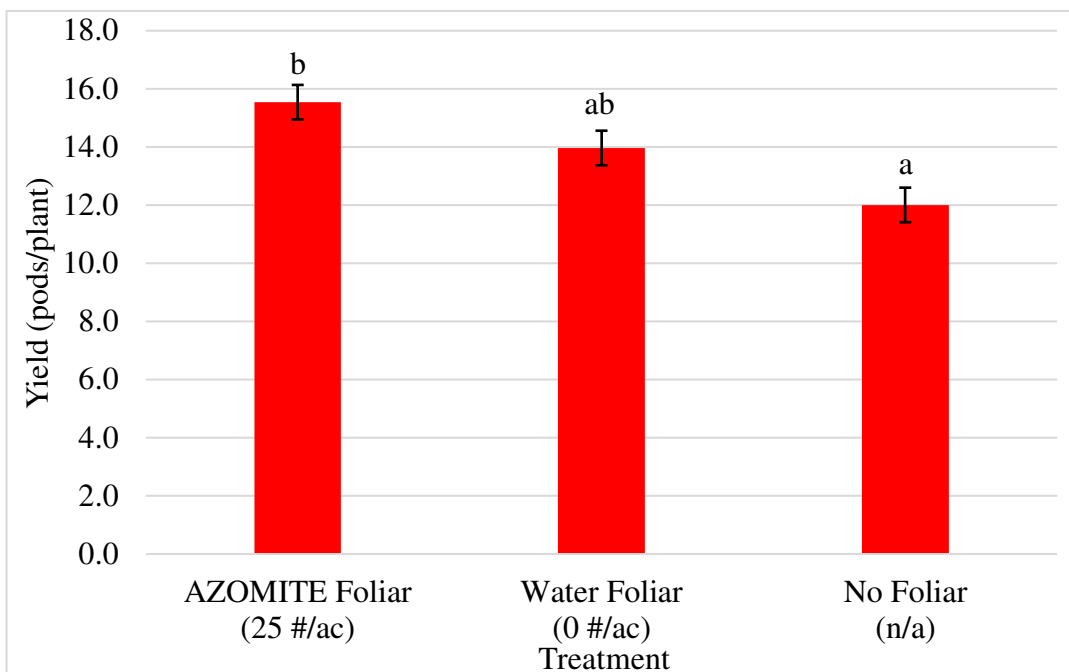
Figure 3.3. Comparison of °Brix for Italian-style roasting pepper cultivars on certified organic agricultural land in Fort Collins, Colorado; 2017-2018 combined ^z.

After recording the total marketable yield for each granulated Azomite treatment, an economic analysis was developed for each cultivar/treatment combination (Appendix Table A.30). Averaging over treatments, ‘Early Perfect Italian’ had a net revenue ranging from \$149,000 – \$175,018 per acre, while ‘Stocky Red Roaster’ had a net revenue ranging from \$119,263 - \$145,281 per acre. The economic analysis provides additional evidence that cultivar choice can significantly impact pepper yields and subsequent financial returns.

Ultrafine Azomite Experiment

Unlike the granulated Azomite experiment, we observed statistical differences in the total number of marketable pepper pods produced between ultrafine foliar Azomite treatments. In ‘Stocky Red Roaster’, there were no statistical differences between fertigation treatments for the total number of marketable pods produced (Figure 3.4). Therefore, we averaged over fertigation treatments to explore the main effect of foliar treatment. The foliar treatment of 25 pounds/ac of ultrafine Azomite produced significantly ($p = 0.002$) more marketable ‘Stocky Red Roaster’ pods per plant than the no foliar treatment. The water foliar treatment was intermediate, but no other statistical differences were observed. To our knowledge, this is the first reporting of significant differences in a yield parameter after Azomite was applied as a foliar treatment.

Given there are no reported studies of Azomite impacts to total marketable yield, comparisons were made to aluminosilicate foliar applications to vegetable crops. It is worth noting that hydrated sodium calcium aluminosilicate is the mineral composing Azomite. A review article indicates that foliar applications of kaolin, which is also an aluminum silicate derived from a kaolinite mineral, (Encyclopedia Britannica, 2020) helped reduce the incidence of fruit splitting in muskmelon (*Cucumis melo* L.).



^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test. The error bar indicates mean \pm standard error (n=3).

Figure 3.4. Total pepper pod count per plant comparison between three foliar ultrafine treatments of Azomite for 'Stocky Red Roaster' on certified organic agricultural land in Fort Collins, Colorado; 2018 combined high tunnels ^z.

The foliar applications of kaolin clay act as particle film technology, which helps the crop mitigate stress related to excessive solar radiation due to its reflective properties (Fernández-Trujillo et al., 2013). Another article reported that foliar applications of kaolin to drought stressed tomatoes resulted in significantly higher yields and reduced incidence of sunscald as well as blossom end rot on tomatoes. Interestingly, there was no effect on yield when kaolin was applied under non-limiting water conditions (AbdAllah, 2019).

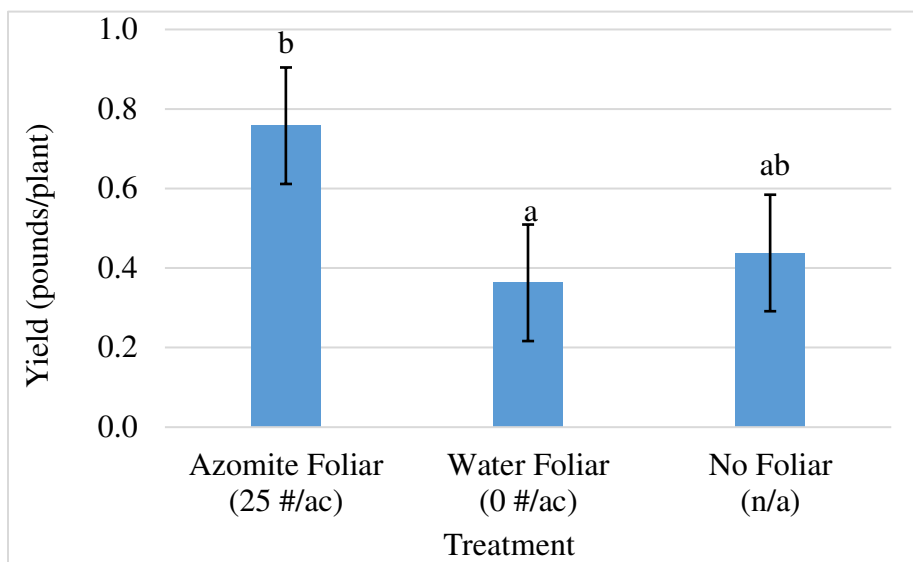
There are also studies demonstrating that conventional input foliar applications of salt-based K sources increase °Brix, K content in leaf tissues, as well as ascorbic acid and β -carotene content in the fruits of muskmelon (Jifon and Lester, 2009). This demonstrates that plants take in K via conventional input foliar applications, but it is unclear if plants are able to absorb K

through the leaves in combination with aluminosilicate foliar applications. When we compared the water foliar treated and untreated pepper yields (Figure 3.4), we see evidence in support of foliar applications of water mitigating against excessive solar radiation and leading to numerically higher yields, although not significantly higher.

There were also statistical differences in the total weight of marketable pods produced per plant between ultrafine Azomite foliar treatments in ‘Early Perfect Italian’. There were no statistical differences between fertigation treatments for the total weight of marketable pods per plant. Therefore, we averaged over fertigation treatments to explore the main effect of foliar treatment. The foliar treatment of 25 pounds/ac of ultrafine Azomite produced a significantly ($p = 0.01$) heavier total weight of marketable pods per plant compared to the water foliar application. The no foliar treatment was intermediate, but no other statistical differences were observed (Figure 3.5). Interestingly, the water foliar application did not lead to higher yields compared to the no foliar application in ‘Early Perfect Italian’, as it did in ‘Stocky Red Roaster’. This suggests the impact of foliar applications of water on total marketable yield is cultivar dependent.

There were also significant treatment differences between °Brix values in ‘Early Perfect Italian’ as indicated in Figure 3.6. The ultrafine Azomite fertigation plus water foliar treatment produced pods that had significantly ($p = 0.003$) higher °Brix values than the pods produced from the water irrigation plus water foliar treatment, at 9.0 °Brix and 8.1 °Brix, respectively. All of the other treatments were intermediates, but no other statistical differences were observed. As a comparison point, conventional input foliar applications of salt-based K sources have led to significant increases in °Brix content in the fruits of muskmelon (Jifon and Lester, 2009).

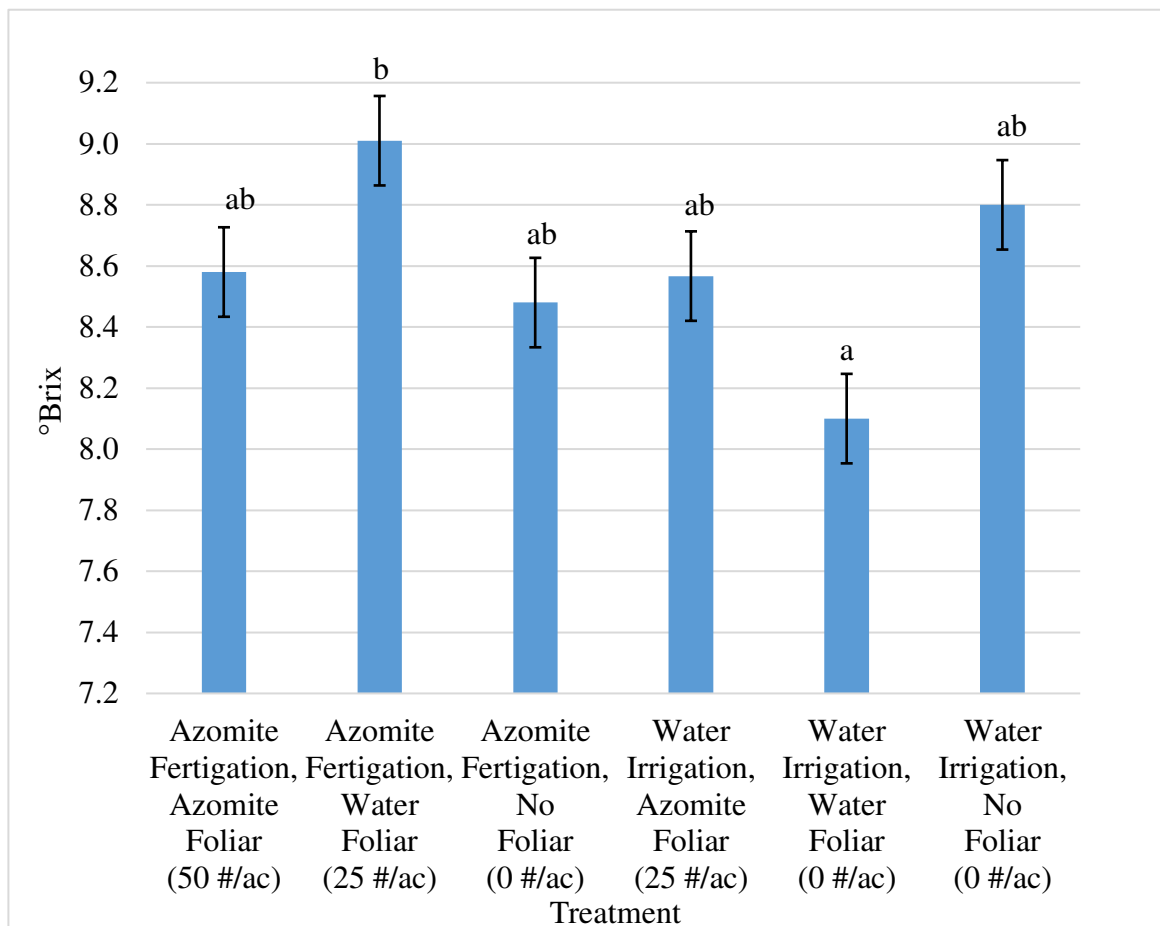
However, this is the first reporting of significant differences in quality components in the pods of an organic roasting pepper crop, under organic management.



^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test. The error bar indicates mean \pm standard error (n=3).

Figure 3.5. Yield comparison of marketable red pepper pods between three ultrafine foliar application treatments of Azomite for the cultivar 'Early Perfect Italian' on certified organic agricultural land in Fort Collins, Colorado; 2018 combined high tunnels ^z.

The economic analysis for applying ultrafine Azomite as a foliar treatment to a pepper crop is indicated for each cultivar (Table 3.3). In 'Stocky Red Roaster', the net revenues increased with increasing costs of inputs. The no foliar treatment, water foliar treatment, and ultrafine Azomite foliar treatment had net revenues of approximately \$80, \$92, and \$99 thousand, respectively. Net revenues for 'Early Perfect Italian' also increased with increasing cost of inputs. The no foliar treatment, water foliar treatment, and ultrafine Azomite foliar treatment had net revenues of approximately \$110, \$113, and \$132 thousand, respectively. It is important to point out that these revenues are derived from the economic analysis model assumptions (Appendix Tables A.47-A.48), and they are not predictive of future pepper returns



^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test. The error bar indicates mean \pm standard error ($n = 3$).

Figure 3.6. Comparison of °Brix between six foliar/fertigation treatments of ultrafine Azomite for 'Early Perfect Italian' pepper pods on certified organic agricultural land in Fort Collins, Colorado; 2018 combined high tunnels.

Table 3.3. Economic comparison of net revenue based on total marketable yields for ultrafine Azomite foliar treatments for 'Stocky Red Roaster' and 'Early Perfect Italian' pepper cultivars on certified organic agricultural land in Fort Collins, Colorado; 2018 combined high tunnels.

		Azomite treatment	Water treatment	No treatment
'Stocky Red Roaster'	Mean	\$98,718	\$92,390	\$79,678
	SE	\$14,241	\$15,070	\$17,107
'Early Perfect Italian'	Mean	\$132,094	\$113,267	\$110,360
	SE	\$19,472	\$27,344	\$17,972

Conclusion

Taken all together, these results indicate that granulated Azomite applications that were banded in the field did not significantly impact roasting pepper yields in ‘Early Perfect Italian’ or ‘Stocky Red Roaster’. Cultivar selection, however, did significantly impact the total marketable yield in field grown peppers under organic management. ‘Early Perfect Italian’ yielded significantly more pounds per plant, and exhibited a significantly higher °Brix content compared to ‘Stocky Red Roaster’. However, ‘Stocky Red Roaster’ produced significantly more, smaller red pods per plant compared to ‘Early Perfect Italian’. Interestingly, foliar applications of ultrafine Azomite significantly increased the number of marketable pods per plant in ‘Stocky Red Roaster’ compared to the no foliar application control. Foliar applications of Azomite also significantly increased the total marketable yield of ‘Early Perfect Italian’ peppers compared to water foliar applications only.

It is interesting that application method and particle size significantly impacted Italian-roasting pepper yields. Granulated Azomite banded in the row did not impact pepper yield, whereas the ultrafine Azomite product significantly improved pepper yield when it was applied as foliar spray. It is unclear whether Azomite ultrafine foliar applications act as particle film technology helping to dissipate excessive solar radiation, or whether the pepper leaves are able to absorb the potassium and other trace minerals supplied by the rock powder based crop input. This could serve as the starting point for future research studies to investigate Azomite’s mode of action. Pepper growers in the high solar incidence of the Intermountain West would benefit from knowing the yield impact of applying foliar applications of ultrafine Azomite to their crops, but they should also be aware that the yield response is impacted by cultivar choice.

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CHAPTER 4: Next Generation Herbicides for Organic Specialty Crop Production

Introduction and Literature Review

Weed control is a major issue for organic production systems; weeds compete with the crop for essential resources such as water, nutrients and light. They often cause yield reductions and can negatively impact financial returns. Heavy weed pressure in a sweet corn crop is depicted by Figure 4.1.



Figure 4.1. Uncontrolled weeds in sweet corn crop.

Weeds can reduce vegetable crop yields by 45-95% on average, depending on the level of weed pressure and a specialty crop's ability to compete with weeds (Mennan et al., 2020). Specialty crops vary widely in their ability to compete with weeds. For example, yields were reduced by 25-30% in peas and by 67% in onions (Singh et al., 2019). Onion crops are known for being particularly vulnerable to pressure from weeds because they do not form a crop canopy until late in the season. Crop canopies help prevent weeds from getting sunlight, which leads to photosynthate reduction and reduced vigor.

Vegetable growers make weed management decisions based on the amount of time to crop canopy closure, the crop's growth habit, and the type of annual or perennial weeds present. In addition, growers also consider the suite of tools that are available to them based on the

management of the crop. Inputs for organic systems are different from conventional systems, and they must be Organic Materials Review Institute (OMRI) or Washington State Department of Agriculture (WSDA) approved. Some inputs, including herbicides, are allowable with restrictions. This means that an herbicide may be used “only if the requirements of 205.206(e) are met, which requires the use of preventive, mechanical, physical, and other weed management practices” and these steps must be documented (OMRI, 2019). It is also important to note that an herbicide must be listed on the organic system plan, and be approved by the certifier before use.

Input selection is also tied to grower management practices. For example, herbicide applications are common on agricultural land under conventional management (Benbrook, 2016). It is estimated that herbicide-tolerant (HT) seed technology was implemented on 94% of acre planted in soybeans and on 95% of the acres planted in corn in the U.S. in 2014 (Benbrook, 2016). By comparison, herbicide adoption among organic producers is low. A survey of weed management practices among Midwest organic vegetable, grain, and diversified operations revealed that only 3% of organic producers considered making an herbicide application as part of their weed management plan (DeDecker et al., 2014). The list of top seven most commonly adopted weed management practices among organic growers includes crop rotation (86%), between-row cultivation (78%), tillage (76%), cover cropping (66%), delayed planting (65%), green manure (63%), and hand-weeding (57%). This list and a previous survey (Walz, 1999) indicate that organic operations manage weeds predominately using cultural and mechanical controls.

There are a variety of reasons that could explain why herbicide adoption among organic farmers remains low, such as not having spray equipment, a lack of understanding of product efficacy, having a concern for promoting HT weeds, having a minimal input farming ideology,

or having an environmentally-conscious customer base or a combination of factors. Part of the issue may be related to the fact that herbicides that were approved for organic systems in the early 2000s had the reputation for being only somewhat effective. For example, acetic acid (AA) has long been the standard in post-emergent, non-selective herbicides because it has the ability to desiccate young annual broadleaf weeds that are four inches in height or shorter (Evans and Bellinder, 2009). This creates a narrow time window for a grower to make an effective application. In addition, AA is expensive to use at its most effective spray volume (100 gallons per acre) and concentration (20% volume/volume) (Webber et al., 2018).



Figure 4.2. Field bindweed encroaching on the crop growing in plastic mulch.

These factors may have deterred an organic producer from considering the use of herbicides to manage weedy “trouble spots” such as field edges, perennial weed patches, and the plastic mulch-soil interface common to vegetables grown in plasticulture as in Figure 4.2, which involves the use of plastic mulch and drip irrigation. Herbicide adoption by organic farmers is not likely to reach the same levels as producers managing land conventionally both by design

and practical reality, but the organic farmers that are open to using herbicides need accurate and current information regarding the efficacy of new active ingredients and product formulations. A survey by the Organic Farming Research Foundation (Jerkins and Ory, 2016) revealed that 67% of farmers indicate weed control is their most difficult management issue. The survey also indicated that small and medium acreage farmers are interested in learning more about cost effective weed control measures, including herbicides for organic systems.

The first herbicide to be evaluated was derived from a mixture of caprylic acid and capric acid (CA) at 47 and 32% v/v, respectively). Caprylic acid is a plant extract derived from coconut oil and palm seed kernel (Penner et al., 2011). It is currently sold under the trade names Suppress® and Homeplate® Herbicide EC. CA demonstrated the ability to control weeds $\leq 9''$ in height when applied between rows in a strawberry crop (Daugovish et al., 2015). CA also reduced woolly distaff thistle as much as 88% on rangeland (DiTomaso et al., 2017). These results encouraged us to evaluate CA in an organic specialty crop system.

We also chose to evaluate another herbicide derived from the active ingredient eugenol (EU) (Weed Slayer®), or otherwise known as clove oil. Research in the early 2000s demonstrated that eugenol was among the most effective essential oils with herbicidal activity (Tworkoski, 2002). A study in 2015 revealed that eugenol caused weed injury as high as the 75% level in some grass species (*P. minor*, *S. halepense*, and *L. chinensis*), which are known by their common names as bunchgrass, Johnson grass, and false wheatgrass, respectively. It also caused injury as high as the 89% level in some broadleaf weed species (*C. benghalensis*, *B. pilosa*, and *A. conyzoides*), which are known by their common names as spiderwort, common aster, and hardy ageratum (Ahuja et al., 2015). Weed Slayer® is unique from other eugenol-based herbicides because it also contains a proprietary biosurfactant, which is marketed as a way to improve herbicide “translocation” to

the roots of weeds (Anonymous, 2017). The results of this study would be useful to growers that are interested in using herbicides to control weeds on their farms.

The objective of this study was to evaluate two new herbicides derived from CA and EU, respectively for organic specialty crop production. We aimed to quantify the yield impact and the percent weed injury caused by the application of post-emergent, non-selective herbicides in sweet corn and onion crops. We hypothesized that CA and EU-based herbicides would provide adequate weed control without negatively impacting crop yields.

Materials and Methods

Sweet Corn Weed Management Experiment

In 2018 a post-emergent, non-selective herbicide study was designed on certified organic land at the Agricultural Research, Development and Education Center (ARDEC) South in Fort Collins, CO (lat. 40°36'N., long. 104°59'W.) elevation 5,003 feet (or about 1524 m). The field was prepared by rototilling to a depth of 6 - 12 inches using a Harvester 722 (BCS, Portland, OR) twice before planting. 'Nirvana' sweet corn (*Zea mays* L. var. *saccharata*) seeds were sown ½ inch deep into certified organic agricultural land on 26 June 2018. 'Nirvana' was a top performer for yield and flavor based on previous cultivar trials (Chapter 1).

Three rows of sweet corn (180 feet in length) were planted on 30-inch centers. A randomized complete block design with three replicates of five weeding treatments served as the experimental design. The five weeding treatments (Table 4.1) consisted of an untreated weedy control, an acetic acid herbicide treatment, a caprylic/capric herbicide treatment, a hand weeded once treatment, and a hand weeded weed free control. Fifteen experimental units were derived from five treatments and three replicates. Each treatment plot was a 75 ft² block of sweet corn. A three-foot wide buffer strip separated the treatment plots; no data was collected from the buffer zones. The research plots were under moderate weed pressure from Palmer amaranth, field

bindweed, common purslane, Eastern black nightshade, prickly lettuce, common lambsquarters, Venetian mallow, and green foxtail among others.

Table 4.1. Comparison of weeding method, concentration, application volume, and timing for five weeding treatments on certified organic agricultural land; 2018.

Treatment	Method	Concentration	Application volume	Timing
Untreated (weedy control)	N/A	N/A	N/A	Not weeded
Hand weeded (weed free control) ^z	Stirrup hoe	N/A	N/A	Weekly
Hand weeded (once at V4-V5) ^y	Stirrup hoe	N/A	N/A	at V4/V5 ^v
Acetic acid herbicide ^x	Sprayer	20% v/v (undiluted)	210 gallons·ac ⁻¹	at V4/V5
Caprylic/capric acid herbicide ^w	Sprayer	9% v/v	210 gallons·ac ⁻¹	at V4/V5

^zHand weeded (weed free control) treatment involved weekly cultivation events with a stirrup hoe for 10 weeks from 6-22-18 to 8-31-18.

^yHand weeded (once at V4/V5) treatment involved two mechanical cultivation events with a stirrup hoe from 6-22-18 to 8-31-18.

^xAcetic acid treatment involved one mechanical cultivation then one herbicide application at the 4-leaf collar/5-leaf collar (V4/V5) stage from 6-22-18 to 8-31-18.

^wCaprylic/capric acid treatment involved one mechanical cultivation then one herbicide application at V4/V5 from 6-22-18 to 8-31-18.

^vThe V4/V5 timing refers to the sweet corn crop development stage when 50% of the crop has reached the 5-leaf collar stage and the other 50% of the crop is at the 4-leaf collar stage.

Before the treatments were made, all of the weeds in the sweet corn study were cultivated with a stir-up hoe on 16 July 2018. Two weeks later, a 1 x 1 m² quadrat was placed in a semi-random spot that was also representative of the weed cover, crop cover, and exposed soil for each of the treatment plots. This facilitated the quantification of percent weed cover, crop cover, and exposed soil 1 day before treatment. The treatments were made when 50 percent of the crop was at the 5-leaf collar stage (V5), which was on 31 July 2018. The application volume was 210 gallons·ac⁻¹ or approximately 5 gallons·1,000 square feet⁻¹. No adjuvants were used in this study. A backpack sprayer (model #475, Solo, Newport News, VA) with a flat spray jet nozzle (model TeeJet 8005-VP, Spraying Systems Company, Wheatland, IL) and a walking speed of approximately 2.2 mph were used. In addition, the nozzle was held at a 45° angle to the crop and approximately 8 in away; it was positioned 10 in off the ground to simulate a shielded spray. The hand weeded treatments were made with a stirrup hoe.

The percent weed cover by species was assessed 1 day after treatment (DAT), 14 DAT, and 28 DAT. The percent weed cover reduction 1, 14, and 28 DAT was calculated as [(original % weed cover - % weed cover after application) / original % weed cover] x 100, using Excel

(Microsoft Office Professional Plus 2016, Denver, Colorado). The weeding treatments ended when all of the weeds were hand pulled on 31 August 2018. The sweet corn ears were harvested by hand when their kernels reached the milk stage between 17 Sept. and 26 Sept. 2018. Data was collected on percent crop damage from herbicide contact (phytotoxicity), marketable yield, average ear weight, kernel soluble sugars and fresh eating quality characteristics (flavor and tenderness).

Economic Analysis

A time study was conducted by recording the time it took to hand weed a 180 ft² plot with a stirrup hoe for all treatments except the untreated (weedy control). The time (28.25 minutes) it took to hand weed was extrapolated to 1,000 ft². Labor costs were estimated at \$12 per hour (2018), which brought the cost of the first hand weeding event to \$31 per 1,000 ft². The time it took to hand weed the weed-free check (24.75 minutes) was also recorded. This brought the cost per maintenance weeding event to \$28 per 1,000 ft². The AA material cost was calculated based on \$22 per gallon; CA was based on \$72 per gallon, which were the market prices in 2018. Both AA and CA applications included 1.5 hours for labor (\$18). These season long costs were based on information available in summer 2018. Fennimore (2019) provides a point of comparison for our economic analysis; the estimated cost to hand weed an acre of onions was \$294. The material cost for a 50 gallons per acre herbicide application of eugenol, caprylic/capric acid, and acetic acid was estimated at \$300, \$450, and \$619, based on information available in 2018.

Fresh Eating Quality

A sensory evaluation for flavor and tenderness was conducted using parameters from the Northern Organic Vegetable Improvement Collaborative (NOVIC) protocols (Silva and Bruce,

2016). The scale for flavor and tenderness ranged from 5-1, with (5) representing “excellent, sweet and good corn flavor” and (1) representing “objectionable”.

Statistical Analysis

Differences between treatment means for percent broadleaf weed control, marketable yield, average ear weight, kernel soluble sugars ($^{\circ}$ Brix), flavor, and tenderness were analyzed using an analysis of variance (ANOVA) statistical approach in R statistical software, $\alpha=0.05$, with a Tukey adjustment for multiple comparisons.

Storage Onion Experiment

An assessment of post-emergent, non-selective herbicides (Table 4.2) applied to weeds in an onion crop occurred on land under conventional management at Sakata Farms ($40^{\circ} 0'58.75''$ N latitude and $104^{\circ}51'6.70''$ W longitude) located in Adams County, Colorado during the 2019 growing season. The elevation was 5,005 feet or 1524 meters. Onion seeds from two Spanish cultivars, ‘Delgado’ and ‘Joaquin’, were sown $\frac{1}{2}$ inch deep on 10 April 2019. The onion seeds were sown in rows of three across a 30-inch bed top as in Figure 4.3. The within row spacing was four inches. The experiment was setup a randomized complete block design with four replicates and 13 weeding management treatments, which produced a total of 52 experimental units. The individual treatment plot size was 12 feet long and 30 inches wide, with a 1 foot buffer zone between treatment plots. No data was collected from the buffer zone.



Figure 4.3. Post-emergent herbicide research plots (bottom right) depicting weed pressure within a well-managed commercial onion operation (background) in Brighton, CO (Thaddeus Gourd, Colorado State University Extension).

The post-emergent herbicide applications were broadcasted over the bed top using a backpack sprayer (Model JR, Bellspray, Inc., Opelousas, LA) equipped with a four-row spray boom, a carbon dioxide cylinder (30 PSI), and disposable plastic 2-liter bottles. In order to minimize cross contamination, the lines of the sprayer and the spray boom were triple rinsed with water before the next application was made.



Figure 4.4. Onions at the two-leaf stage growing in conventionally managed agricultural land.

In addition, care was taken to apply the treatments in the order of least concentrated herbicide solution to most. For example, caprylic/capric acid at 3% was applied first; it was followed by the 6% solution, and then by the 9% solution volume/volume. Because the herbicides made contact with both the weeds and the onion crop, percent phytotoxicity of the cash crop was recorded. Furthermore, the percent weed injury, the total weed biomass, percent weed cover from a ½ meter² quadrat and the dry weights (grams) were recorded.

The first herbicide application occurred 50 days after planting on 30 May 2019 between 10:00 and 11:00 a.m. The treatment applications were made to their respective plots when the onions were at the two-leaf growth stage as in Figure 4.4. Oxyfluorfen, acetic acid, and eugenol were applied at the 0.2, 20, and 4.0% concentrations, respectively. Caprylic/capric acid was applied to three different plots at three different rates: 3.1, 6.3, and 9.4%. A walking speed of 2.8 mph, a sprayer tank pressure of 30 PSI, and an application volume of 20 gallons per acre were used to make the first herbicide application.

A second herbicide application was also made at the two-leaf stage on 10 June 2019 between 7:00 and 8:00 a.m. to the same treatment plots as the first application. Oxyfluorfen, acetic acid, and eugenol were applied at the 0.2, 20, and 4.0% concentrations, respectively. Caprylic/capric acid was also applied at 3.1, 6.3, and 9.4% concentration to separate treatment plots. On 21 June 2019 (11 DAT) the weeds were cut with a knife at ground level so as to collect total weed biomass by species for each treatment plot. The weeds were placed in an industrial dryer for three days at 158°F.

Table 4.2. Comparison of onion growth stage, allowance in organic production, application method, volume of applications, and the spray concentrations for 13 weeding management treatments on conventional agricultural land; 2019.

Growth stage	Weeding treatments	Organic production	Application method	Volume 1, ^{zy} volume 2 (gallons/acre)	Spray Concentration
Season	Untreated (weedy control)	Yes	n/a	n/a	n/a
Season	Hand weeded (weed free control)	Yes	Stirrup hoe	n/a	n/a
2-leaf	Oxyfluorfen (grower check)	No	Sprayer	20 gpa, 40 gpa ^z	0.2%
2-leaf	Acetic acid	Yes	Sprayer	20 gpa, 40 gpa	20.0%
2-leaf	Eugenol	Yes	Sprayer	20 gpa, 40 gpa	4.0%
2-leaf	Caprylic/capric acid	Yes	Sprayer	20 gpa, 40 gpa	3.1%
2-leaf	Caprylic/capric acid	Yes	Sprayer	20 gpa, 40 gpa	6.3%
2-leaf	Caprylic/capric acid	Yes	Sprayer	20 gpa, 40 gpa	9.4%
3-leaf	Acetic acid	Yes	Sprayer	50 gpa ^y	20.0%
3-leaf	Eugenol	Yes	Sprayer	50 gpa	4.0%
3-leaf	Caprylic/capric acid	Yes	Sprayer	50 gpa	3.1%
3-leaf	Caprylic/capric acid	Yes	Sprayer	50 gpa	6.3%
3-leaf	Caprylic/capric acid	Yes	Sprayer	50 gpa	9.4%

^z A spray application volume of 20 gallons per acre was made to the treatment plot on 30 May 2019, and a separate application volume of 40 gallons per acre was made to the same treatment plot on 10 June 2019.

^y A single spray application volume of 50 gallons per acre was made to separate treatment plots on 17 June 2019.

A third application occurred on 17 June 2019 between 7:00 and 8:00 am when the onions were at the three-leaf stage. The percent concentrations of active ingredients applied for oxyfluorfen, acetic acid, and eugenol were 0.2, 20, and 4.0%, respectively. Caprylic/capric acid was applied at 3.1, 6.3, and 9.4% concentrations to separate plots. Weeds were again removed using the protocol described above on 28 June 2019 (11 DAT). After biomass data was collected 11 DAT, respectively for the two onion growth stages, all of the remaining weeds in the study were hand pulled. No other weeding treatments occurred throughout the rest of the season.

The onion crop was harvested by hand on 19 Sept. 2019. All of the onion leaves were removed using shears, so as to leave a 1/3 inch long onion neck. Onions from the entire 30 feet² of the treatment plots were harvested, and placed in labelled 50-pound burlap onion sacks until sorting and grading could occur on 20 Sept. 2019. A semi-automatic potato sorter was modified to sort and grade the onions. Onions that did not form a $\geq 2 \frac{1}{4}$ inch in diameter bulb were not considered marketable. The other grading sizes are indicated in Table 4.3. The count and weight of each onion size were recorded using USDA 2014 ‘Bermuda’-‘Granex’-‘Grano’ standards. Finally, each plot yield was extrapolated to per acre basis.

Table 4.3. Yield parameters assessed using five onion grades based on bulb diameters; 2019.

Grade ^z	Bulb diameter (inches)
Unmarketable	Bulbs < 2.25
Small	Bulbs = 2.25
Medium	Bulbs $2.25 \leq 3$
Jumbo	Bulbs $3 \leq 4$
Colossal	Bulbs ≥ 4

^z Grades were assessed using USDA ‘Bermuda’-‘Granex’-‘Grano’ grades, 2014.

Results and Discussion

Sweet Corn Experiment

AA provided significantly better control of broadleaf weed species such as field bindweed, Palmer amaranth, and Eastern black nightshade (*Convolvulus arvensis*, *Amarathus palmeri*, and *Solanum ptychanthum*) compared to the untreated control (Table 4.4). CA provided significantly better control of broadleaf weed species, at 86%, compared to the untreated and AA treatments at 0 and 41%, respectively (Figure 4.6). The hand weeded at V4/V5 and the hand weeded weekly treatments were not significantly different from the CA treatment. It is worth noting that CA provided less control of grassy weed species such as green foxtail at 20%. The level of green foxtail control was observationally lower, although formal statistical comparisons were not possible. We also observed that CA demonstrated significantly better control than AA on broadleaf weeds at 65 and 26%, respectively 14 DAT. These results indicate the CA is a more effective post-emergent herbicide than AA on weeds that are $\geq 4''$ in height. There were no significant differences in broadleaf control between the hand weeded at V4/V5 treatment, the CA treatment, and the hand weeded weekly treatment at 14 DAT.

Table 4.4. Comparison of percent injury to broadleaf weeds 1, 14, and 28 days after treatment, number of ears per plant, and the average ear weight for five weed control treatments for organic sweet corn production on certified organic agricultural land; 2018.

Treatment	Percent injury to broadleaf weeds ^z (days after treatment)						Average ear weight	
	<u>1 DAT</u>		<u>14 DAT</u>		<u>28 DAT</u>		Mean	SE
	Mean	SE	Mean	SE	Mean	SE		
Untreated (weedy control)	0.0 a	4.6	0.0 a	7.9	0.0 a	4.6	0.4 a	0.0
Acetic acid (210 gpa)	41.0 b	4.6	26.0 a	7.9	11.0 a	4.6	0.5 ab	0.0
Hand weeded (once at V4-V5)	89.0 c	4.6	33.0 ab	7.9	16.0 ab	4.6	0.5 ab	0.0
Caprylic/capric acid (210 gpa)	86.0 c	4.6	65.0 b	7.9	34.0 b	4.6	0.5 b	0.0
Hand weeded (weed free control)	100.0 c	4.6	66.0 b	7.9	100.0 c	4.6	0.5 ab	0.0

^zMeans within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.



Figure 4.5. Acetic acid (20%) application showing 41% initial burn down 1 DAT.



Figure 4.6. Caprylic/capric acid application showing 86% initial burn down 1 DAT. Sweet corn at the ≤ 3 -leaf collar stage died after lodging due to herbicide contact.

Our results regarding the efficacy of AA align with what has been previously been reported in the literature. Evans and Bellinder (2009) observed that weed injury increased as weed height decreased. They reported that the percent weed injury from an acetic acid application in redroot pigweed (*Amaranthus retroflexus*) at the cotyledon stage was 90% whereas the percent weed injury in common chickweed (*Stellaria media*) at the 8-10 leaf stage was only 55%. Rhoades (2007) also reported similar results, noting that that AA was much less effective on weeds that were taller than 2” in height.

Crop Injury

It is important to note that crop injury occurred after the herbicide treatments. An average of 4% overall injury to a sweet corn plant was attributed to an AA application. This was significantly more injury to the crop compared the untreated, hand weeded at V4/V5, and hand weeded weekly treatments. A CA application led to 7% overall injury to a sweet corn plant (1 DAT), which was significantly more injury compared to the AA treatment. CA lead to statistically significantly more injury than AA, however the injury caused by both herbicides was

below the limit of acceptable percent crop injury for sweet corn at 24% 3 DAT (Evans and Bellinder, 2009). The authors reported no statistically significant differences in total yield between an OMRI-approved post-emergent herbicide application, which caused 24% visual crop injury and the hand weeded control, which caused 0% crop injury (Evans and Bellinder, 2009). Acceptable crop injury is relative to the herbicide applied, the volume of application, the percent concentration, and the crop growth stage.

As a point of comparison, paraquat is a post-emergent, non-selective herbicide that is used to control weeds in a sweet corn crop under conventional management. It is understood that post-emergent applications of paraquat are known to cause injury to corn. Sperry et al. (2019) demonstrated that corn injury was directly related to the stage at which paraquat was applied. For example, injury to corn decreased from 65% to 29% (3 DAT) as the crop matured from the 3-leaf to the 9-leaf growth stage. However, paraquat applied as a post-emergent herbicide lead to reduced corn yields at all growth stages. This demonstrates one of several key differences between herbicides used in conventional systems and those used in organic systems. It also reminds that crop injury is expected after post-emergent, non-selective herbicide applications, however impacts to yield are dependent on the crop growth stage at application and the type of herbicide applied.

Unlike the yield results reported for paraquat, applying CA and AA did not significantly reduce the number of sweet corn ears produced (Table 4.5). In addition, the plots treated with CA had an average ear weight (0.49 pounds) that was significantly higher than the untreated control (0.39 pounds). It is not surprising, but it is still important to note, that an herbicide application of CA or AA did not affect the sweet corn flavor or kernel soluble sugar content (Table 4.6).

Table 4.5. Comparison of yield in terms of the number of ears per plant, crop phytotoxicity, Brix, flavor scores, and tenderness scores for five weeding treatments in an organic sweet corn system; 2018 (Flavor and tenderness scales: 1 = objectionable, 5 = excellent sweet corn flavor, n = 10 tasters).

Treatment	Number of ^z ears/plant		Injury		Brix		Flavor	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Untreated (weedy control)	0.9 a	0.1	0.0 a	0.5	13.2 a	0.3	4.1 a	0.3
Hand weeded (weed free control)	1.0 a	0.1	0.0 a	0.5	13.2 a	1.0	4.2 a	0.2
Hand weeded (once at V4-V5)	1.0 a	0.0	0.0 a	0.5	13.3 a	0.2	3.7 a	0.3
Acetic acid (200 gpa)	0.9 a	0.1	4.3 b	0.5	14.6 a	0.2	4.4 a	0.2
Caprylic/capric acid (200 gpa)	0.9 a	0.1	7.3 c	0.5	14.3 a	0.3	4.4 a	0.2

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

In the sweet corn crop, an assessment of 210 gallons per acre post-emergent herbicide application revealed caprylic/capric acid caused significantly more broadleaf weed injury 1, 14, and 28 DAT compared to the acetic acid treatment. Results regarding the efficacy of acetic acid in this trial align with the literature. High spray volume applications of acetic acid (20% v/v) at 100 gallons per acre lead to injury at the 91% level to weeds that have six leaves or less (Evans and Bellinder, 2009). Injury was reduced to 76% when the weeds had more than six leaves. Rhoads (2007) reported ineffective weed control with AA when the weeds were taller than two inches. For the first time, this study demonstrates that CA provides significantly better weed control than acetic acid on weeds that were six inches in height and taller. Our results and the literature demonstrate the importance of the relationship between the weed growth stage, the timing of application, and the active ingredient of the herbicide applied.

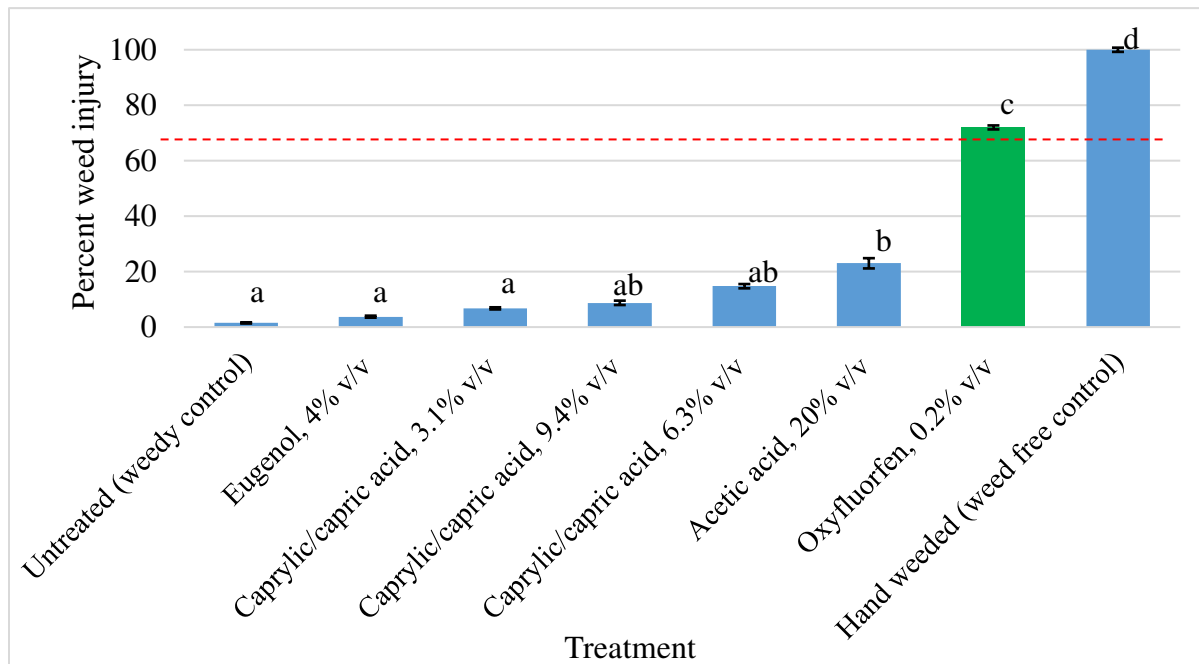
Economic Analysis

The economic analysis demonstrated that CA (\$52/1,000 feet²) is about half the cost of AA (\$128/1,000 feet²), while providing superior weed control. A single hand weeding at V4/V5 costs \$59/1,000 feet² and weekly hand weeding through the season costs \$279/1,000 feet². This demonstrates some post-emergent herbicides are more economical than others.

Storage Onion Experiment

Low spray volume applications

There were significant ($p < 0.001$) differences observed 7 DAT in the level of weed injury sustained among the weeding treatments at a spray volume of 20 gallons per acre. The hand weeded treatment (weed free control) exhibited a significantly higher percent weed injury compared to all other treatments (Figure 4.7). The application of the conventional herbicide, oxyfluorfen, led to a significantly higher percent weed injury, at 72%, compared to all other treatments, except the hand weeded control. AA exhibited a significantly higher percent weed injury at 23% compared to the untreated (weedy control) at 2% injury. However, AA (20% concentration) did not provide satisfactory weed control; satisfactory weed control is defined as $\geq 50\%$ weed injury (Patton et al., 2019). No other statistical differences were observed.



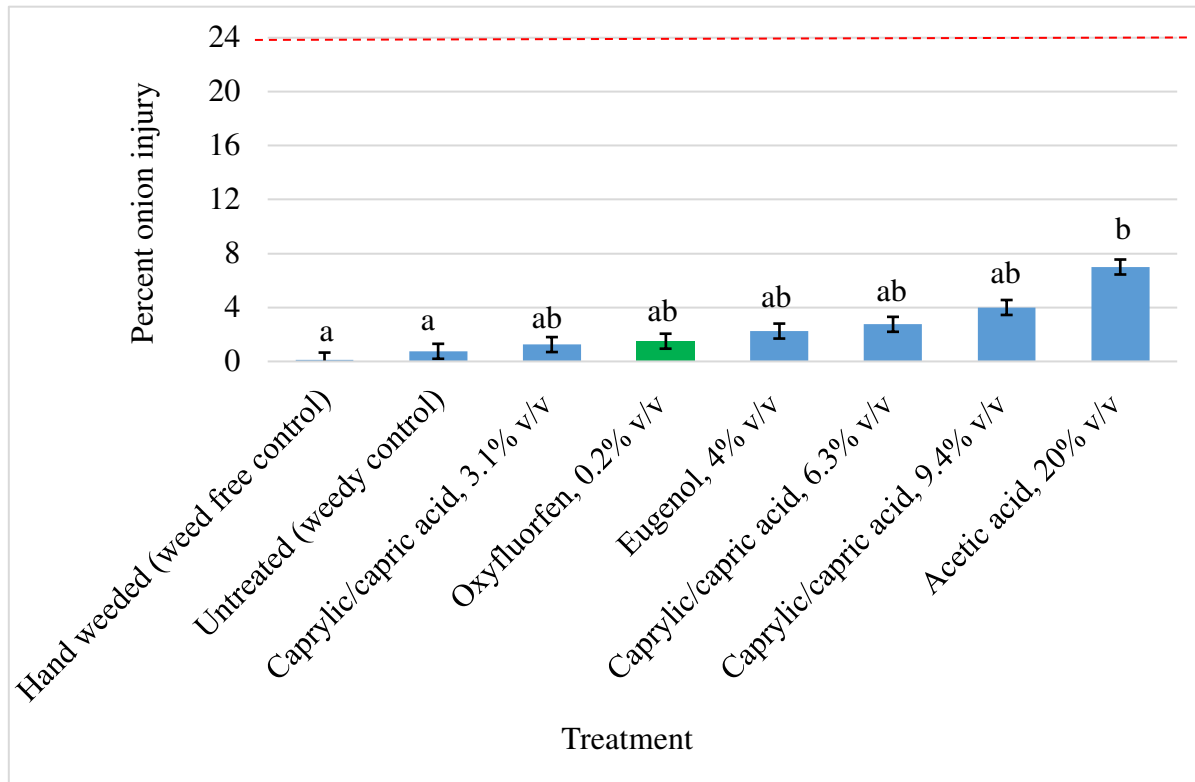
^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test; The error bar indicates mean \pm standard error ($n = 4$); The horizontal red line indicates acceptable ($\geq 50\%$) weed control; the green bar indicates the herbicide is not approved for organic systems.

Figure 4.7. Percent weed injury comparison of seven weeding treatments for organic systems (blue) and one weeding treatment for conventional systems (green) at an application volume of 20 gallons per acre in a storage onion crop at the two-leaf stage 7 DAT; 2019.



Figure 4.8. Visual weed (center) and onion crop (right) injury following a post-emergent herbicide application.

Weeding treatment was also a significant ($p < 0.012$) predictor for the percent injury to the onion crop (phytotoxicity). An application of AA led to a significantly higher percent onion injury compared to the untreated control and the hand weeded control (Figure 4.9). Caprylic/capric acid at 3.1, 6.3, and 9.4% volume/volume (v/v), eugenol, and oxyfluorfen were intermediaries, but not other statistical differences were observed. The results demonstrated that low spray volume application rates, such as 20 gallons/acre, which are typical of herbicides used in conventional systems, did not lead to adequate weed injury among the herbicides evaluated that are OMRI or WSDA approved for organic systems. At this rate, the injury to the crop was minimal, and it was well-below the acceptable threshold of 24% visual injury (Herrmann et al., 2017).

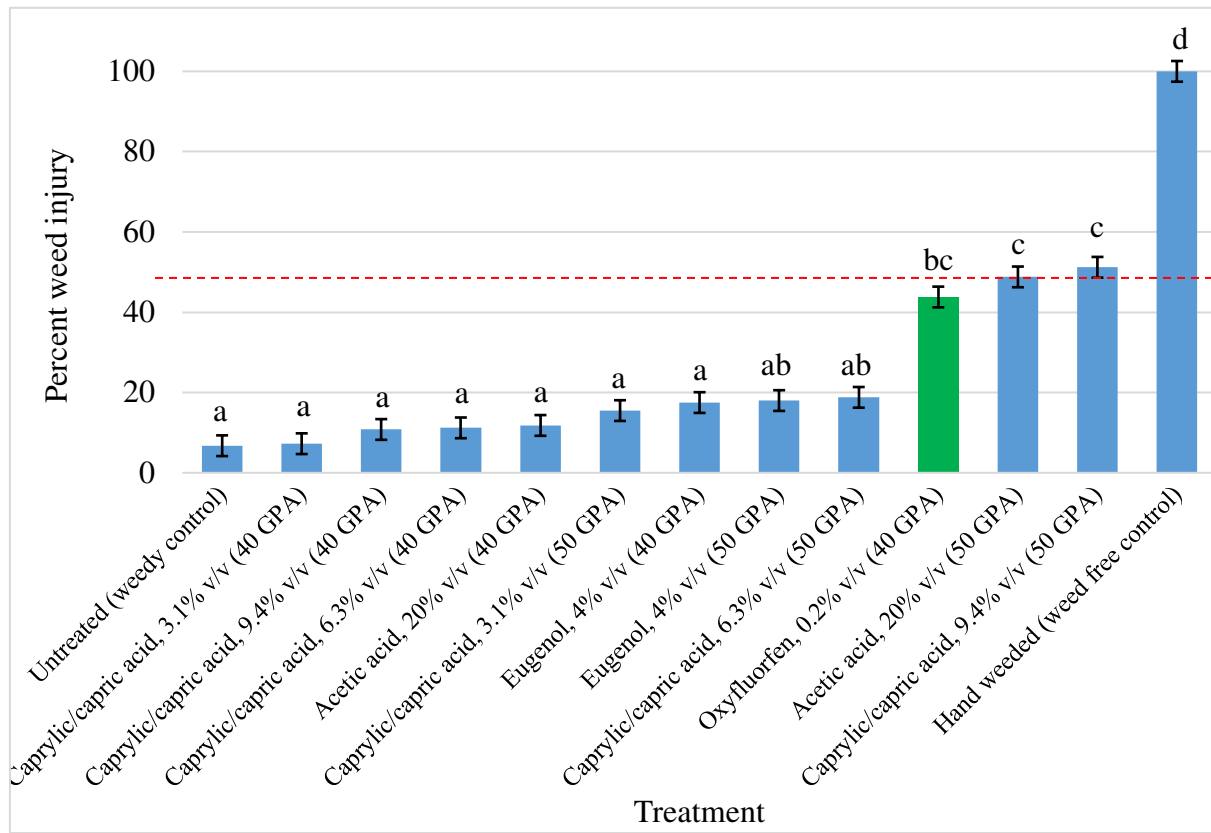


^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test; the error bar indicates mean \pm standard error ($n = 4$); the horizontal red dotted line indicates the threshold (<24%) for acceptable crop phytotoxicity.

Figure 4.9. Percent onion crop injury comparison of seven weeding treatments for organic systems (blue) and one weeding treatment for conventional systems (green) at an application volume of 20 gallons per acre in a storage onion crop at the two-leaf stage 4 DAT; 2019.

Medium spray volume applications

There were also significant ($p < 0.001$) differences observed 4 DAT in the percent weed injury among the weeding treatments at the spray volumes of 40 and 50 gallons per acre (Figure 4.10). The hand weeded (weed free control) led to a significantly higher percent weed injury, at



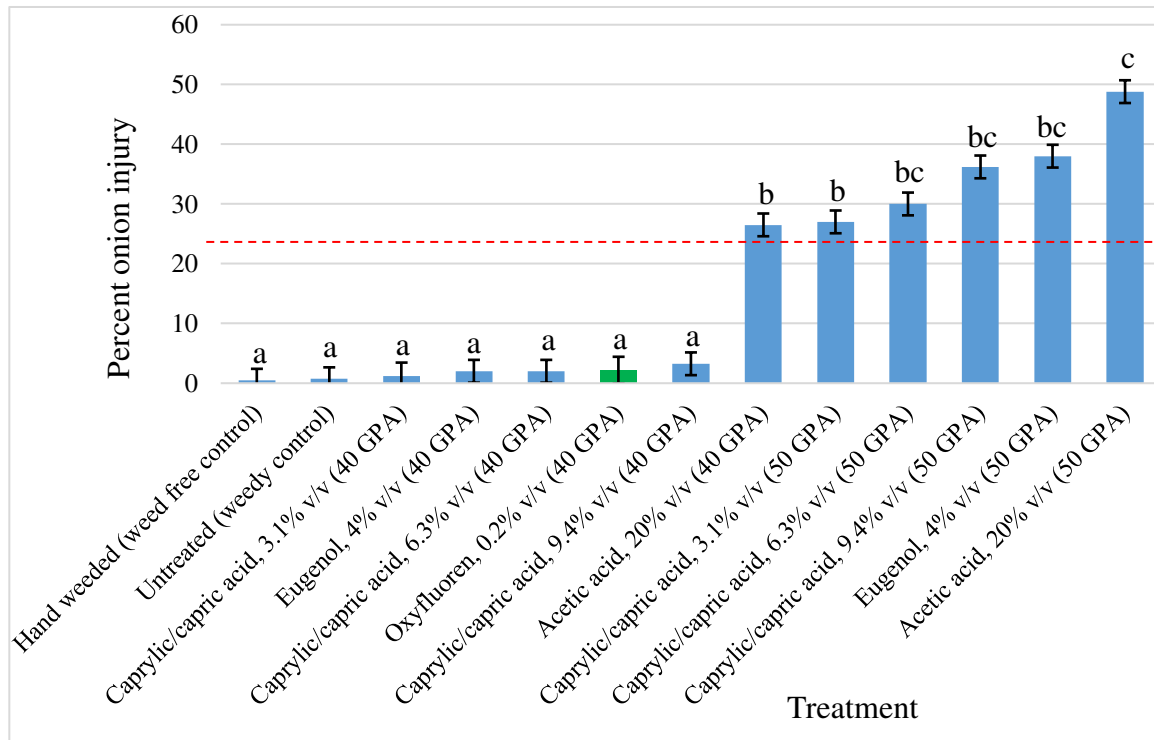
^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test; the error bar indicates mean \pm standard error ($n = 4$); the horizontal red dotted line indicates the threshold ($\geq 50\%$) for acceptable weed injury.

Figure 4.10. Percent weed injury comparison of twelve weeding treatments for organic systems (blue) and one weeding treatment for conventional systems (green) at the indicated application volume of 40 or 50 gallons per acre in a storage onion crop at the three-leaf stage 4 DAT; 2019 ^z.

100%, compared to all other treatments. Applications of oxyfluorfen, AA (50 GPA), and CA 9.4% v/v (50 GPA) caused a significantly higher percent weed injury compared to the untreated (weedy control), CA 3.1% (40 GPA), CA 9.4% v/v (40 GPA), CA 6.3% v/v (40 GPA), AA (40 GPA), CA 3.1% v/v (50 GPA), and EU (40 GPA). EU (50 GPA) and CA 6.3% v/v (50 GPA)

were intermediaries, but not other statistical differences were observed. In short, the percent weed injury associated with the application of an herbicide commonly used in conventional systems, oxyfluorfen, applied at a spray volume of 40 gallons per acre was comparable to the injury associated with two herbicides (CA and AA) that are allowable in organic systems.

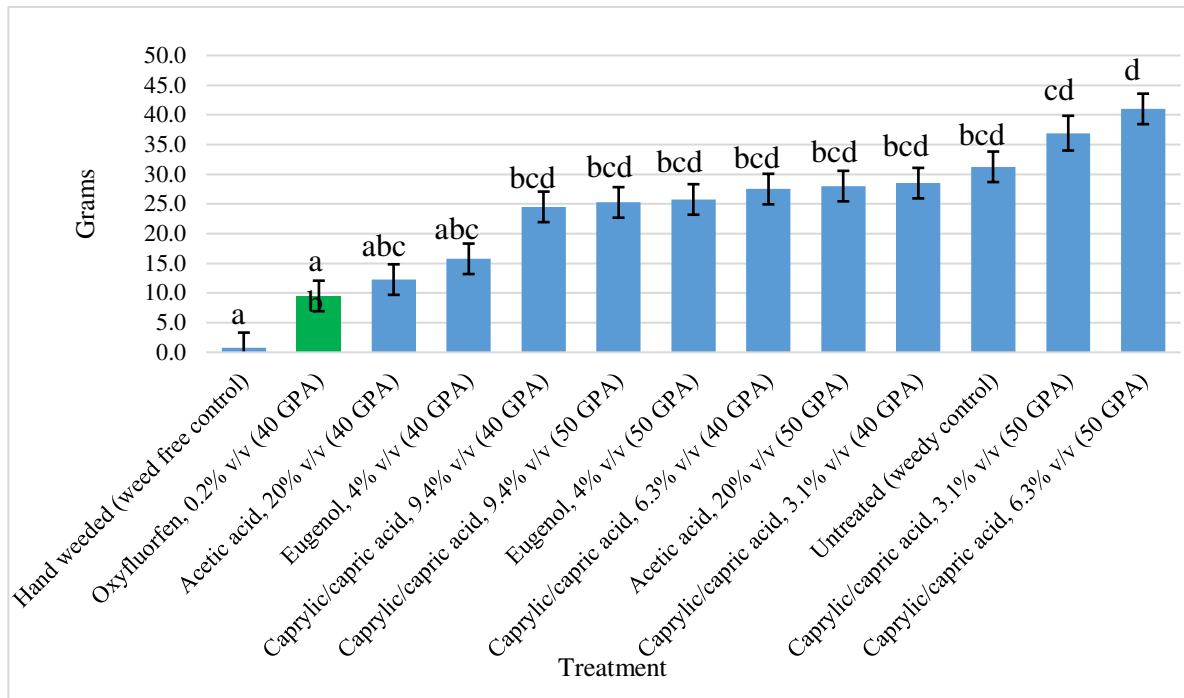
It was unexpected that oxyfluorfen applied at 50 gallons per acre to 3-leaf stage onions lead to 44% weed injury, while 20 gallons per acre applied to 2-leaf stage onions lead to 72% weed injury. Of note, 17 days passed between these two application events. This demonstrates that herbicide efficacy is related to the size of the weeds at application. There were also significant differences between weeding treatments for the percent onion crop injury four DAT at the spray volumes of 40 and 50 gallons per acre (Figure 4.11). AA (50 GPA) caused a significantly higher percent onion injury, at 49%, compared to CA 3.1% (50 GPA), AA (40 GPA), all other herbicide treatments at 40 gallons per acre, the untreated (weedy control), and the hand weeded (weed free control). In addition, the percent onion injury sustained after an application of acetic acid (40 GPA), which was 27%, was comparable to the level of injury sustained from applications of CA 3.1% v/v (50 GPA), CA 6.3% v/v (50 GPA), CA 9.4% v/v (50 GPA), and EU (50 GPA) at 27, 30, 36, and 38%, respectively. Total weed biomass collected 18 DAT also revealed significant differences in the weed control abilities of 13 weeding management treatments (Figure 4.12).



^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test; the error bar indicates mean \pm standard error ($n = 4$); the horizontal red dotted line indicates the threshold (<24%) for acceptable visual crop phytotoxicity.

Figure 4.11. Percent crop injury comparison of twelve weeding treatments for organic systems (blue) and one weeding treatment for conventional systems (green) at the indicated application volume of 40 or 50 gallons per acre in a storage onion crop at the three-leaf stage 4 DAT; 2019 ^z.

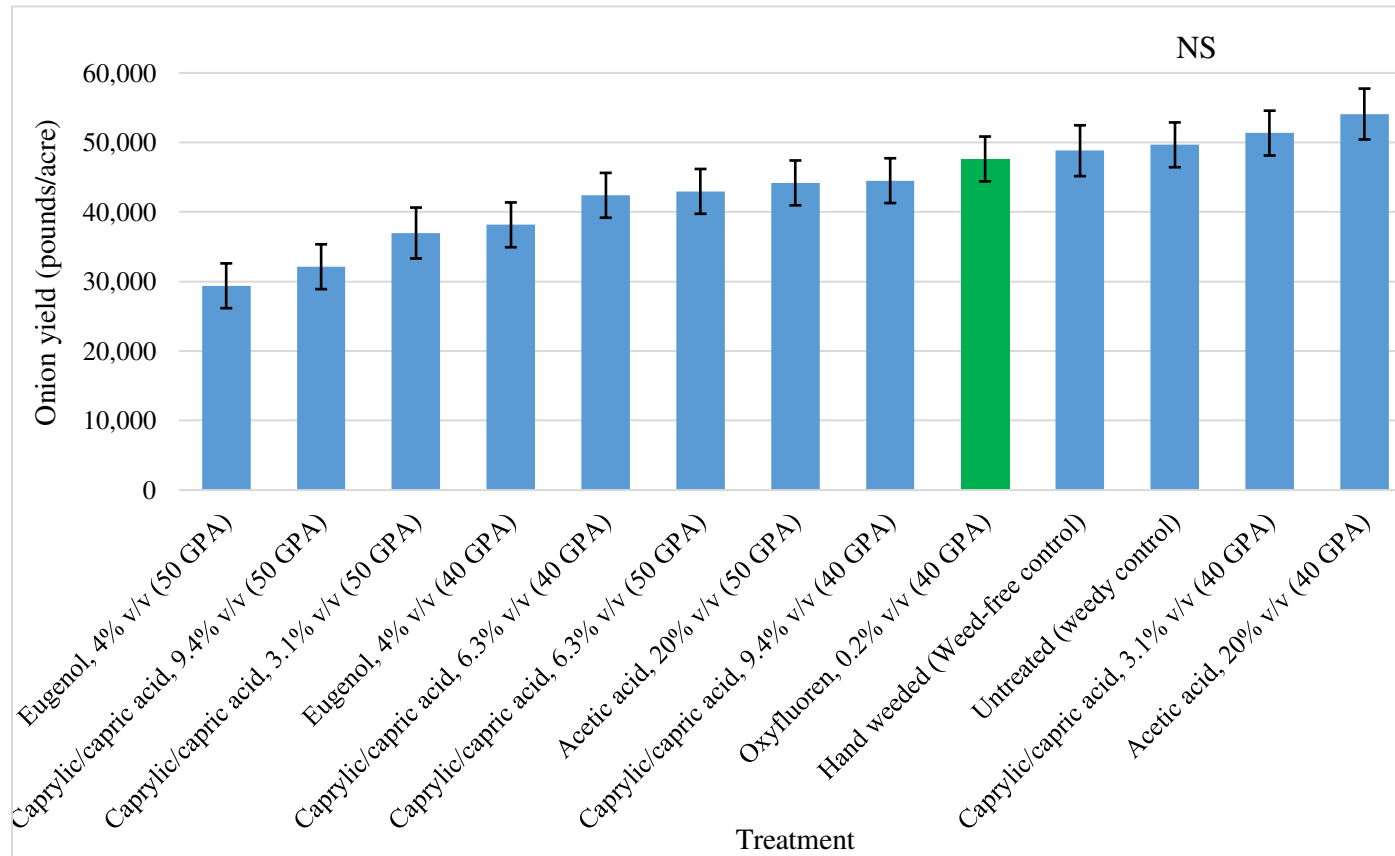
The hand weeded (weed free control) treatment, at 0.8 grams, had a significantly lower weed biomass compared to CA 9.4% v/v at 40 or 50 gallons per acre, EU (50 GPA), CA 6.3% v/v (40 GPA), AA 20% v/v (50 GPA), CA 3.1% v/v (40 GPA), untreated, CA 3.1% v/v (50 GPA), and CA 6.3% v/v (50 GPA). It is also worth noting that oxyfluorene, significantly reduced the total weed biomass compared to caprylic/capric acid 3.1% (50 GPA) and caprylic/capric acid 6.3% (50 GPA).



^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test. The error indicates mean \pm standard error ($n = 4$).

Figure 4.12. Total broadleaf weed biomass (grams) of a half square meter quadrat comparison of twelve weeding treatments for organic systems and one weeding treatment for conventional systems at the indicated application volume of 40 or 50 gallons per acre in a storage onion crop at the two-leaf or three-leaf stage, respectively 18 DAT; 2019^z.

There were significant differences in the weed control abilities of the 13 treatments. However, this did not lead to significant differences in total marketable yield assessed on a pounds per acre basis (Figure 4.13) or the total number of marketable onions (Table 4.6).



The error indicates mean \pm standard error (n = 4).

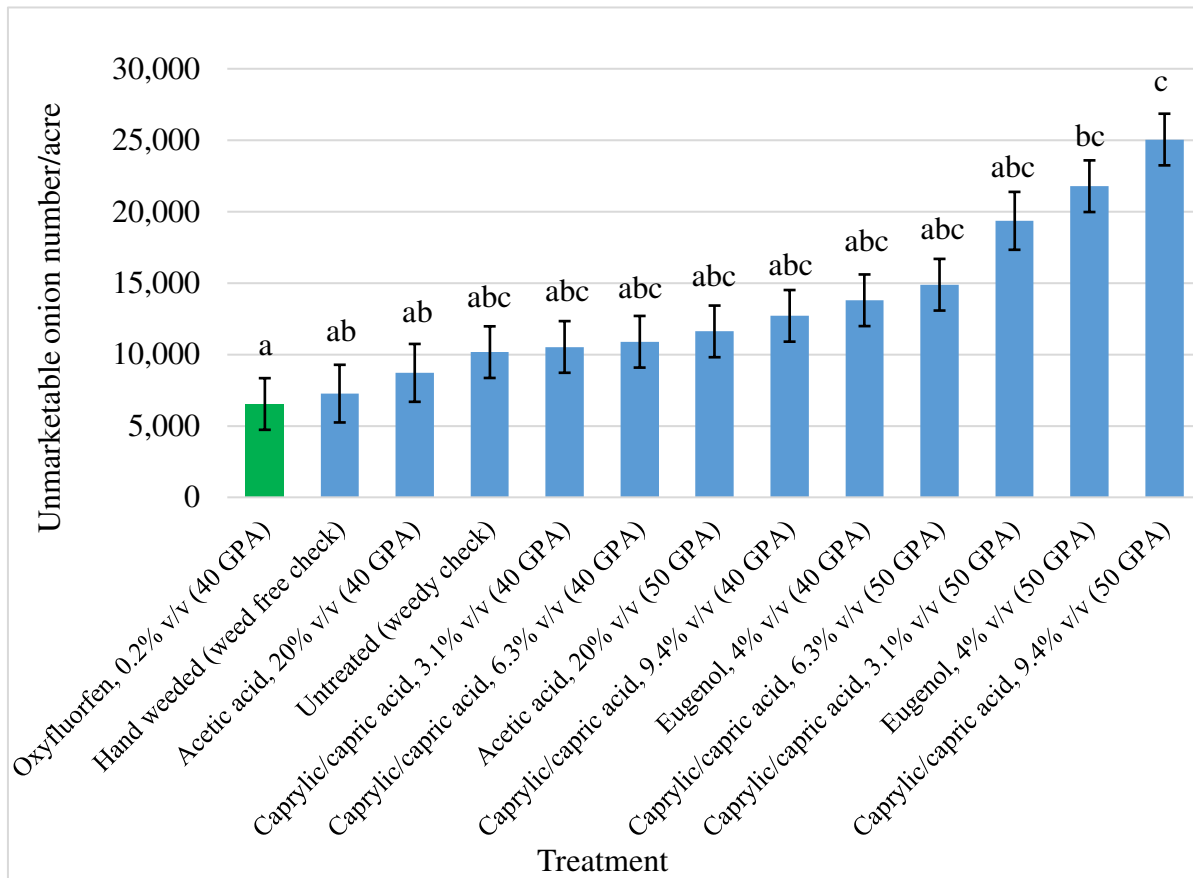
Figure 4.13. Total yield (pounds/acre) comparison of twelve weeding treatments for organic systems (blue) and one weeding treatment for conventional systems (green) at the indicated application volume of 40 or 50 gallons per acre in a storage onion crop 101 DAT; 2019.

Table 4.6. Total number of marketable onions on a per acre basis and a distribution by grade comparison between twelve weed management treatments for organic systems and one weeding treatment for conventional systems at the indicated application volume of 40 or 50 gallons per acre in a storage onion crop 101 DAT; 2019.

Treatment	Total		Small		Medium		Jumbo		Colossal	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Eugenol, 4% v/v (50 GPA)	97,284	7,855	19,602	5,140	60,258	3,615	17,424	6,607	0	857
Caprylic/capric acid, 3.1% v/v (50 GPA)	97,284	8,930	13,068	6,026	50,820	4,051	31,944	7,739	1,452	989
Caprylic/capric acid, 9.4% v/v (50 GPA)	99,462	7,855	19,239	5,140	57,717	3,615	21,780	6,607	726	857
Caprylic/capric acid, 6.3% v/v (50 GPA)	104,181	7,855	11,616	5,140	51,909	3,615	40,656	6,607	0	857
Untreated check	107,085	7,855	9,801	5,140	48,279	3,615	46,827	6,607	2,178	857
Caprylic/capric acid, 3.1% v/v (40 GPA)	107,811	7,855	9,075	5,140	43,560	3,615	52,272	6,607	2,904	857
Caprylic/capric acid, 9.4% v/v (40 GPA)	108,174	7,855	12,705	5,140	49,368	3,615	45,375	6,607	726	857
Acetic acid, 20% v/v (50 GPA)	109,989	7,855	14,883	5,140	48,279	3,615	46,827	6,607	0	857
Weed-free check	110,831	8,930	11,131	6,026	52,272	4,037	45,491	7,739	1,931	989
Oxyfluorfen, 0.16% v/v (40 GPA)	112,530	7,855	11,616	5,140	55,176	3,615	43,560	6,607	2,178	857
Eugenol, 4% v/v (40 GPA)	113,982	7,855	19,602	5,140	67,518	3,615	26,499	6,607	363	857
Acetic acid, 20% v/v (40 GPA)	119,064	8,930	14,999	6,026	55,176	4,051	47,422	7,739	1,452	989
Caprylic/capric acid, 6.3% v/v (40 GPA)	122,331	7,855	26,136	5,140	63,525	3,615	30,855	6,607	1,815	857

The error indicates mean \pm standard error (n = 4); no statistically significant difference were observed in the distribution of onion sizes for weed management treatments based on an $\alpha = 0.05$ and a Tukey's honestly significant difference test.

Post-emergent, non-selective herbicides that are allowable in organic systems, which were broadcasted over the top of a storage onion crop did not lead to statistically significant differences in yield from the oxyfluorfen treatment. However, applications of EU (50 GPA) and CA 9% v/v (50 GPA) lead to a significantly higher number of unmarketable onions compared to oxyfluorfen (40 GPA) (Figure 4.14). In addition, AA (40 GPA) had significantly fewer unmarketable onions compared to caprylic/capric acid 9% (50 GPA).



^z Means with different letters indicate statistically significant differences ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Figure 4.14. Number of unmarketable onions on a per acre basis comparison between twelve weeding treatments for organic systems (blue) and one weeding treatment for conventional systems (green) at the indicated application volume of 40 or 50 gallons per acre in a storage onion crop 101 DAT; 2019^z.

Research evaluating post-emergent herbicides in conventionally managed horticultural and agronomic crops has been thorough, and wide spread adoption is common. As a comparison point, many reduced-tillage conventional producers rely on the judicious use of herbicides as a critical component to their integrated weed management plan. Studies evaluating post-emergent herbicides in organic systems, on the other hand, have not lead to wide scale adoption. This could be due to the fact that studies have concluded that low volume spray applications (< 50 gallons per acre) of AA (20% v/v) do not provide long-term weed control (Webber et al., 2018), and that acetic acid is most effective on weeds that are less than four inches in height (Evans and Bellinder, 2009).

This study is unique from what has been reported on in the literature because it compared two new product formulations (one derived from caprylic/capric acid and the other from eugenol) to acetic acid, which has been considered the organic industry standard for a number of years. It also evaluates low spray volume applications of 20, 40, and 50 gallons per acre. We have demonstrated that caprylic/capric acid is superior to acetic acid-based herbicides in terms of weed injury on weeds that were taller than four inches. Furthermore, our results indicate that low volume spray applications of 20-30 gallons per acre, which are typical of herbicide volumes used in conventional systems are not adequate for herbicides approved in organic systems. In addition, broadcasting a post-emergent herbicide over the top of an onion crop did not lead to a statistically significant yield reduction. This has implications for onion crop producers wanting to add organic to their production portfolio, but whom may not have available labor. As the organic foods market continues to expand, so must the options for weed control. In addition to providing practical guidance on post-emergent herbicides for organic systems, this study is a first step in understanding the relationship between weed growth stage, crop growth stage, and

product efficacy. A next step would be to evaluate the addition of an adjuvant on product efficacy. Additionally, it would be worthwhile to explore application volumes ranging from 50 to 100 gallons per acre.

Extension and Outreach

Grower outreach was a critical component to this project. We received financial support in the form of a Colorado Department of Agriculture Specialty Crop Block Grant (\$24,999; 2018-2019) to teach growers about these new herbicide formulations and their potential uses. Having designed curriculum for adult learners and having a background in delivering Extension programs encouraged us to explore modern teaching methodologies. Research has shown that Extension outreach methods that strictly focus on content, such as explaining the aspects of an integrated weed management have led to poor adoption rates (Wilson et al., 2009). This is not surprising given that content-focused learning, which is traditionally determined, organized, delivered, and evaluated by the teacher, does not take adult learning principles (Figure 15) or adult learner experiences into account.

In practice, providing research-based results to growers is essential, but their adoption rates are also influenced by prior beliefs, previous experience, and intrinsic values. Therefore, it seems likely that the issue of technology adoption may be related to a breakdown in learning strategy, where researchers and Extension professionals may be applying pedagogy principles to an adult learning setting. Instead, Extension educators are likely to be more successful if they emphasize the learning process. This would involve setting up an environment that respected adult learner tendencies and their experiences, and focused on including the learner in the process and evaluation of their learning.

Learning Characteristics	Description
Independent self-concept	• An adult's self-concept grows increasingly independent as they age.
Experience as a resource	• Adults accumulate a wealth of experience, which is the foundation for future learning.
Readiness to learn	• Adults' readiness to learn is influenced by their professional and social roles.
Immediate application	• As a person matures, their interest in immediate application grows.
Internally motivated	• Adults are more internally, rather than externally motivated.
Rationale	• Adults need to understand the reason for learning something.

Figure 4.15. Hallmark characteristics (Merriam and Bierema, 2014) for adult learning (andragogy).

It has been demonstrated that discovery learning, (i.e. knowledge gained through past failures and successes) influences grower decisions (Eckert and Bell, 2005). This phenomenon is observable when solutions that have worked in the past are not effective for current problems. This forces a producer to re-assess their mental model. Furthermore, it is important to note that some producers have a significant preference for attaining information through their personal experiences or through the experiences of their peers (DeDeck et al., 2014).

In short, farmers are more willing to adopt a new practice if someone they know is already having success. Therefore, grower involvement in the learning process and peer-to-peer teaching are critical aspects to communicating research results and facilitating grower adoption. Our outreach objective was to develop a learning process based Extension-type program that focused on using discovery learning opportunities to empower growers across the spectrum of production systems (organic to conventional) to adopt appropriate cultural or chemical controls for weed

management. We evaluated the impact of our Extension program using surveys. Table 7 reports the aspects that growers, researchers, and Extension professionals will remember from the workshop. It was interesting that many of the attendees mentioned a willingness to consider alternative methods of weed control (e.g. herbicide applications and flame weeding). Table 8 summarizes the concepts that our workshop attendees would like to learn more about. Some expressed an interest in learning more about pre-emergent herbicides, additional organic herbicide options, and the impact of herbicides on the soil microbiome. These aspects serve as possible starting points for future workshops and research ideas.

Table 4.7. Self-identified participant professions and their survey responses to an open-ended question for the integrated weed management workshop (4 Sept. 2018) post-assessment.

Respondent profession	I will remember that
Farmer/plant research	“Herbicides with similar ingredients can not be automatically used the same. Also, steam may be feasible on some scales.”
Extension	“Get weeds early! Some interesting organic herbicides that might be economical in some crops.”
Plant researcher	“Weed slayer has no REI!”
Plant researcher/Extension	“Flame weeding can be effective/economical.”
Extension	“Flame control weed method.”
Farmer	“Use Suppress.”
Farmer	“Suppress not that expensive.”

Table 4.8. Self-identified participant professions and their survey responses to an open-ended question for the integrated weed management workshop (4 Sept. 2018) post-assessment.

Respondent profession	I would like to know more about
Farmer/plant researcher	“How damaging the herbicides are/are not to other vegetable crops.”
Extension	“Organic herbicides.”
Plant researcher	“Microbe impacts from organic herbicides.”
Plant researcher/Extension	“Cost/efficiency control comparison w/ Thad’s products vs. Suppress.”
Extension	“Continue learning about organic weed methods.”
Extension	“Pre-emergent.”
Farmer	“Pre-emergent.”
Farmer	“High tech cultivating in row.”

We also recorded workshop attendees' likelihood of technology adoption through Likert-scale statements such as "I could see myself making an herbicide application to weeds on my farm" (Figure 16), and "I learned something new about weed management today" (Figure 17). Interestingly, 33% and 50% of the attendees indicated that they agreed or strongly agreed, respectively with the statement, "I could see myself making herbicide applications to weeds on my farm." It is also notable that 88% of the workshop attendees indicated that they learned something new and 88% said they would consider sharing what they learned with a peer. Considering that growers learned new information about post-emergent, non-selective herbicides, and they indicated they were willing to share some of what they learned with a peer, we think the Extension workshop and the delivery method were successful.

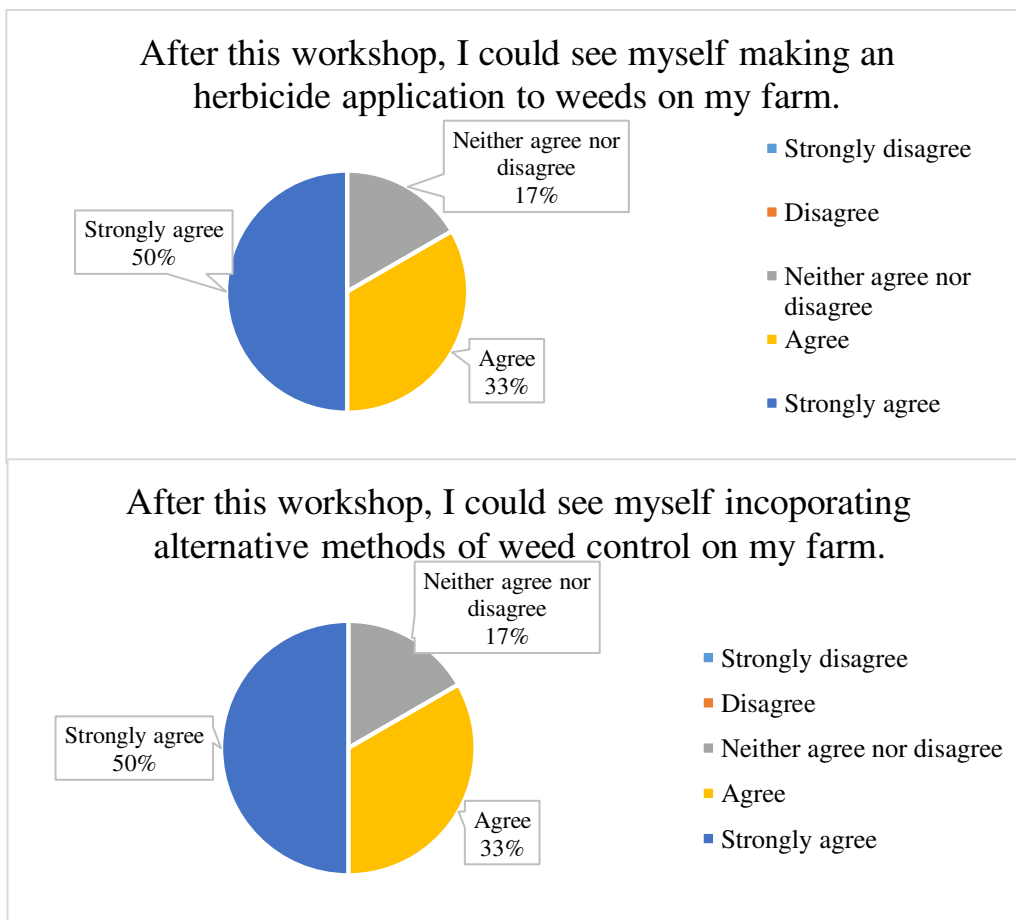


Figure 4.16. Summarized survey responses to two Likert-scale statements for the integrated weed management workshop (4 Sept. 2018) post-

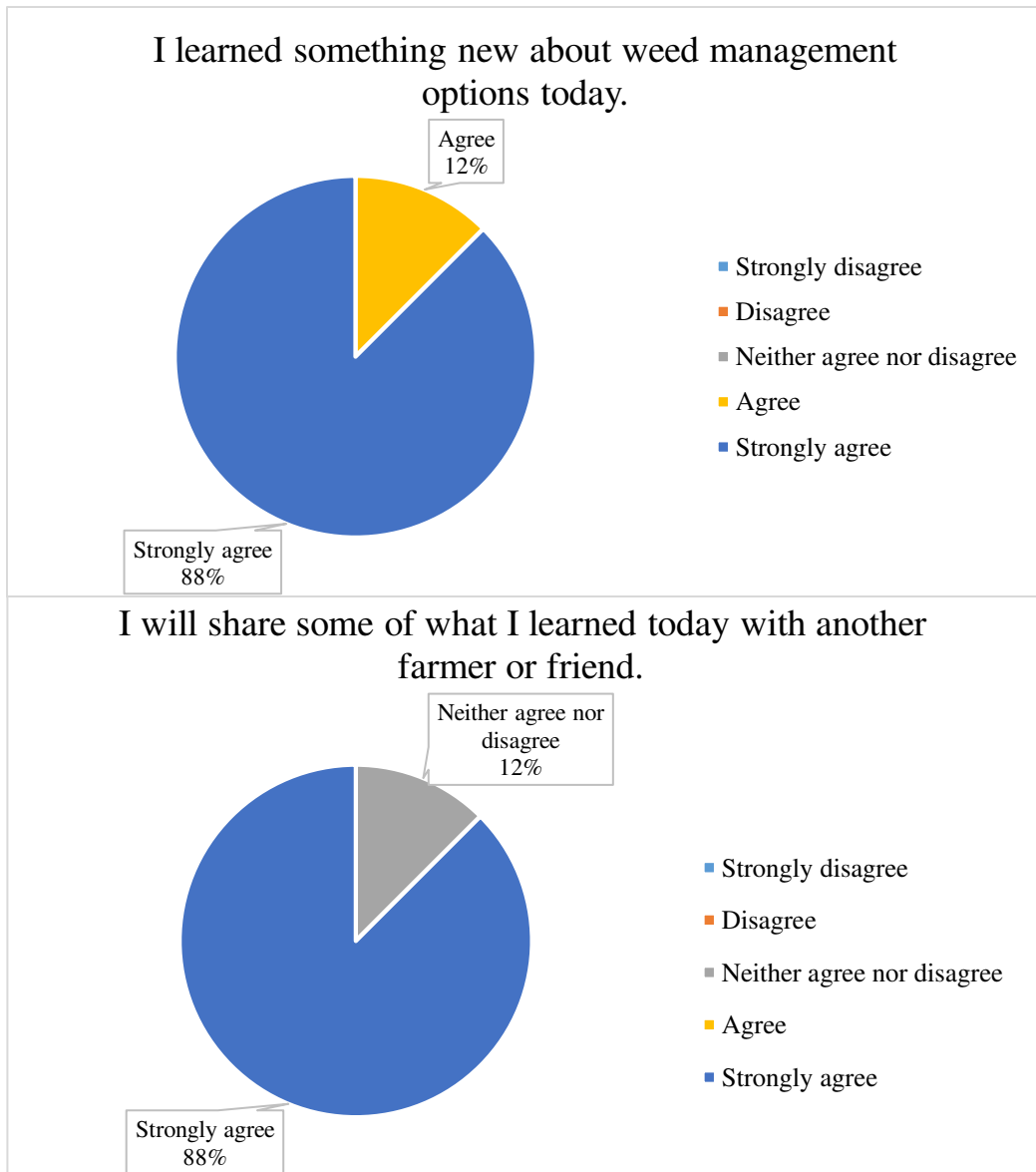


Figure 4.17. Summarized survey responses to two Likert-scale statements for the integrated weed management workshop (4 Sept. 2018) post-assessment.

Conclusion

Weed management is an important issue for specialty crop producers including organic vegetable producers. Growers need a variety of tools to control weeds at different growth stages. This study demonstrates that post-emergent, non-selective herbicides derived from eugenol, acetic acid (20%), and caprylic/capric acid are effective at controlling annual weeds and injuring perennial weeds that are four inches in height or less. When the weeds exceed four inches,

caprylic/capric acid demonstrates significantly better control than acetic acid at 86% and 41% initial burn down 1 day after treatment. Fourteen DAT, caprylic/capric acid achieved 65% control of broadleaf weed species, whereas acetic acid only achieved 26% control. This demonstrates that caprylic/capric acid provides better weed control than acetic acid. Interestingly, two additional caprylic/capric acid based herbicides (Homeplate® and FireWorxx®) have been OMRI-approved since the start of this project. Price is an important consideration in selecting a weed management strategy. The product costs for a 50 GPA herbicide application of eugenol, caprylic/capric acid, or acetic acid (20%) were \$300, \$450, and \$619, respectively.

A caprylic/capric acid herbicide application (50 gallons per acre, 9% v/v) costs approximately \$450 per acre. This relatively high input cost is more easily recovered if one is growing a high-value crop, such as organic tomatoes or leafy greens. Another approach to managing input costs is to reduce the amount of product applied. This could be achieved by spraying only the weediest “trouble spots” in a field, or by reducing the gallons of active ingredient applied. However, growers need to be aware that low-volume spray application rates (20-30 GPA), which are typical of conventional herbicides are not effective for herbicides that are approved for organic systems. This study demonstrates that 40 gallons per acre of acetic acid (20%) and 50 gallons per acre of caprylic/capric acid is needed to achieve adequate (greater than 50%) weed injury.

Future uses, which require additional research, might include using caprylic/capric acid to control weeds in an organic dry bean crop, such as pinto beans, or to help terminate a cover crop prior to planting. It would also be interesting to evaluate the number of herbicide applications it would take to manage weeds in a reduced-till system for several (2-3) years. Additionally, it would be worthwhile to evaluate the weed control ability of caprylic/capric acid with the

addition of an adjuvant. All in all, projects that focus on providing adequate weed control at an affordable cost would be beneficial for organic producers who are wanting to control weeds with an herbicide approved for organic systems. It is important to note, that not all producers are interested in applying post-emergent herbicides for practical or philosophical reasons. However, those that are interested in making herbicide applications need specific recommendations regarding product efficacy at various spray volumes and application rates. This study serves as an important starting point to provide recommendations to organic vegetable growers.

Furthermore, it provides evidence that organic systems and the management inputs that support those systems are not the same as conventional systems. Growers need to be aware that managing an organic system takes more than an input substitution strategy to achieve adequate weed control. In addition, Extension educators would benefit from employing adult educational principles and practices such as discovery learning opportunities to improve adoption of recommended research-based practices identified from input system management studies.

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APPENDIX

Table A.1. Scoring guide for pest pressure parameters including striped cucumber beetle, powdery mildew on the leaves, and powdery mildew on the petioles for vegetable cultivar trial winter squash entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018.

Trait	Description	Score
Striped cucumber beetle damage	No visible damage on any of the plants	9
	25% defoliation with plants growing and fruiting normally	7
	50% defoliation with some compromise in fruiting/flowering and minimal fruit scarring	5
	75% defoliation with much compromise in fruiting/flowering and much fruit scarring	3
	All plants dead from defoliation due to feeding	1
Powdery mildew rating (leaves)	No visible mildew on tops of leaves	9
	25% leaf canopy covered with powdery mildew	7
	50% leaf canopy covered with powdery mildew	5
	75% leaf canopy covered with powdery mildew	3
	Tops of leaves completely covered with powdery mildew; the plant has died	1
Powdery mildew rating (petioles)	No visible mildew on tops of leaves	9
	25% leaf petioles covered with powdery mildew	7
	50% leaf petioles covered with powdery mildew	5
	75% leaf petioles covered with powdery mildew	3
	Leaf petioles are completely covered with powdery mildew; the plant has died	1

Table A.2. Scoring guide for the sensory evaluation including texture, sweetness, and flavor for vegetable cultivar trial winter squash entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018.

Trait	Description	Score
Texture	Fine grain, smooth	9
	Some fibers	5
	Stringy (like spaghetti squash)	1
Sweetness	Very sweet, sugary	9
	Sweet, but not overpowering	5
	No sweetness	1
Flavor	Noticeably "squashy"	9
	Bland	5
	Off-flavors, bland	1

Table A.3. Scoring guide for sweet corn quality parameters including husk appearance, protection, row configuration, tip fill, ear shape, kernel color, flavor, and tenderness for vegetable cultivar trial entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018.

Trait	Description	Score
Husk appearance	Dark green, flag leaves >15 cm	5
	Green husks, 10-15 cm flags	4
	Green husks, 5-10 cm flags	3
	Pale green 3-5 cm flags	2
	Yellow or brown or no flag leaves	1
Husk protection	V. Long ≥ 7 cm beyond ear tip	5
	Long 5-7 cm	4
	Medium 3-5 cm	3
	Short ≤ 3 cm	2
	Exposed ear tips	1
Row configuration	Absolutely straight rows	5
	Nearly straight one or two breaks	4
	Spiral rowing or occasional breaks	3
	Many broken rows	2
	No definable rows or large gaps between rows	1
Tip fill	One to four kernels at the tip	5
	Less than 2 cm without kernels (blank)	4
	Top 2 cm blank	3
	Top 2-3 cm blank	2
	Top >3 cm blank	1
Ear shape	Perfectly cylindrical	5
	Tip slightly tapered	4
	Gradual taper from the middle of the ear	3
	Strong taper or curve	2
	Pyramidal	1
Kernel color	Buttery, glossy yellow	5
	Darker yellow	4
	Pale yellow	3
	Tan yellow	2
	Mottled, discolored, brown	1
Flavor	Excellent, sweet and good corn flavor	5
	Pleasant	4
	Acceptable	3
	Little sweetness	2
	Objectionable	1

Table A.4. Market class, yield, days to harvest, and storability for vegetable cultivar trial cabbage entries on certified organic agricultural land in Fort Collins, Colorado; 2017.

Cultivar	Market class	Yield ^z		Days to harvest		Percent marketable after time in storage					
		(pounds/head)		Mean	SE	<u>1 Month</u>		<u>2 Months</u>		<u>3 Months</u>	
		Mean	SE			Mean	SE	Mean	SE	Mean	SE
'Alessandro'	Purple	1.8 abc	0.1	132 a	3	100 b	0	100 b	0	100 c	0
'Bartolo'	Green	2.7 cd	0.1	135 a	0	100 b	0	100 b	0	100 c	0
'Cantasa'	Savoy	1.3 ab	0.1	135 a	0	100 b	0	17 a	8	0 a	0
'Deadon'	Savoy	1.6 abc	0.2	135 a	0	100 b	0	67 b	19	0 a	0
'Green Mariner'	Green	2.7 cd	0.1	135 a	0	100 b	0	100 b	0	100 c	0
'Integro'	Purple	2.4 bcd	0.1	135 a	0	100 b	0	100 b	0	100 c	0
'January King'	Savoy	1.8 abc	0.1	135 a	0	100 b	0	67 b	17	0 a	0
'Kilmaro'	Purple	2.1 bcd	0.1	135 a	0	100 b	0	100 b	0	93 c	7
'Lennox'	Green	3.2 d	0.1	135 a	0	100 b	0	100 b	0	100 c	0
'Marabel'	Savoy	1.6 abc	0.1	135 a	0	100 b	0	92 b	8	0 a	0
'Paresa'	Savoy	0.7 a	0.0	152 b	0	0 a	0	0 a	0	0 a	0
'Reaction'	Green	2.4 bcd	0.2	135 a	0	100 b	0	100 b	0	100 c	0
'Red Granite'	Purple	2.0 bcd	0.0	135 a	0	100 b	0	93 b	7	93 c	7
'Ruby Perfection'	Purple	2.5 bcd	0.1	135 a	0	100 b	0	87 b	13	87 bc	13
'Sireon'	Green	3.1 d	0.2	135 a	0	100 b	0	100 b	0	100 c	0
'Stanton'	Savoy	2.0 bcd	0.1	135 a	0	100 b	0	100 b	0	52 b	8
'Turkis'	Green	2.2 bcd	0.1	135 a	0	100 b	0	100 b	0	77 bc	23
'Typhoon'	Green	2.6 cd	0.1	135 a	0	100 b	0	93 b	7	93 c	7
'Wirosa'	Savoy	1.8 abc	0.2	135 a	0	100 b	0	17 a	17	0 a	0

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.5. Cabbage head characteristics including height, width, core material length score, and head uniformity score for vegetable cultivar trial cabbage entries on certified organic agricultural land in Fort Collins, Colorado; 2017.

Cultivar	Height (cm) ^z		Width (cm)		Core length		Head score	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Alessandro'	12.3 bcdefg ^w	0.5	11.1 ab	0.3	1.0 a	0.0	8.3 ab	0.3
'Bartolo'	12.9 cdefg	0.8	13.7 bc	0.4	1.7 ab	0.7	9.0 b	0.0
'Cantasa'	10.4 abcd	1.2	12.3 abc	0.8	1.0 a	0.0	8.0 ab	0.0
'Deadon'	9.5 ab	0.9	13.6 bc	1.0	1.0 a	0.0	6.7 a	0.3
'Green Mariner'	13.0 cdefg	0.5	13.1 abc	0.1	2.7 ab	0.3	7.7 ab	0.3
'Integro'	13.1 cdefg	0.6	12.8 abc	0.3	1.0 a	0.0	9.0 b	0.0
'January King'	10.1 abc	0.6	14.6 bc	1.1	2.0 ab	0.6	7.0 ab	0.6
'Kilmaro'	14.5 efg	0.2	11.4 abc	0.5	1.7 ab	0.3	7.7 ab	0.9
'Lennox'	13.6 defg	0.9	14.7 c	0.4	2.0 ab	0.0	8.7 ab	0.3
'Marabel'	9.1 ab	0.3	13.3 abc	1.4	3.0 b	0.0	6.7 a	0.9
'Paresa'	8.6 a	0.8	9.9 a	0.5	1.0 a	0.0	8.7 ab	0.3
'Reaction'	13.3 cdefg	1.2	12.4 abc	1.0	1.3 ab	0.3	8.3 ab	0.3
'Red Granite'	13.5 cdefg	0.4	11.1 ab	0.1	1.3 ab	0.3	8.7ab	0.3
'Ruby Perfection'	13.7 defg	0.2	13.0 abc	0.3	3.0 b	0.0	8.3 ab	0.3
'Sireon'	15.5 g	0.7	13.7 bc	0.5	1.7 ab	0.3	8.3 ab	0.7
'Stanton'	11.4 abcdef	0.5	12.9 abc	0.7	1.7 ab	0.3	8.0 ab	0.0
'Turkis'	11.9 abcdef	0.3	12.8 abc	0.2	2.0 ab	0.6	8.3 ab	0.3
'Typhoon'	14.7 fg	0.4	12.4 abc	0.2	1.3 ab	0.3	9.0 b	0.0
'Wirosa'	11.2 abcde	0.9	13.2 abc	1.4	2.3 ab	0.7	7.7 ab	0.3

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.6. Average number of marketable ears per plant, average yield per ear, ear width, ear length, height of ear, and height of tassel for vegetable cultivar trial sweet corn entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018 combined.

Cultivar	Number of ^z ears/plant		Ear width (cm)		Ear length (cm)		Height of ear (cm)		Height of tassel (cm)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Allure'	0.9 a	0.0	5.5 a	0.0	24.0 a	0.3	39.8 abcde	0.1	125.6 bc	2.9
'Anthem XR'	0.9 a	0.3	5.0 a	0.1	20.0 a	0.2	49.7 efgh	2.6	136.7 abc	2.3
'AP426'	1.2 a	0.1	4.4 a	0.2	20.9 a	0.2	35.7 abc	1.6	139.1 c	5.7
'Double Standard'	1.0 a	0.0	5.1 a	0.1	19.9 a	0.8	43.1 bcdefg	3.1	118.3 abc	5.7
'Festivity'	0.5 a	0.1	4.7 a	0.5	18.3 a	1.9	56.6 h	1.2	121.5 abc	2.5
'Jared Synthetic 21'	0.6 a	0.1	5.0 a	0.5	17.7 a	2.7	33.0 a	3.2	112.3 abc	3.6
'Luscious'	1.0 a	0.0	5.9 a	0.1	21.1 a	0.6	40.1 abcdef	1.1	125.1 bc	4.5
'Mirai 131Y'	1.0 a	0.0	5.0 a	0.1	21.0 a	0.3	48.3 gh	2.6	125.8 abc	2.6
'Mirai 308BC'	1.0 a	0.1	3.7 a	1.1	17.2 a	4.5	48.0 defgh	1.0	135.3 abc	0.3
'My Fair Lady'	1.0 a	0.2	5.4 a	0.1	21.0 a	1.1	35.7 ab	1.3	112.4 abc	5.1
'New Mama'	0.8 a	0.1	5.3 a	0.1	21.7 a	0.2	43.9 bcdefg	1.7	129.8 abc	6.2
'Nirvana'	1.1 a	0.1	5.2 a	0.2	21.4 a	0.7	38.6 abcd	1.1	121.9 ab	4.8
'Temptation'	0.7 a	0.1	5.4 a	0.2	17.1 a	0.9	45.5 cdefg	0.8	112.7 abc	4.5
'Who Gets Kissed'	0.4 a	0.0	5.2 a	0.2	20.4 a	1.2	48.9 efgh	2.7	112.3 abc	5.5
'Xtratender 2171'	1.1 a	0.1	5.0 a	0.6	20.6 a	2.7	43.4 bcdefg	0.7	121.8 a	5.8
'Xtratender 274A'	0.8 a	0.1	4.5 a	0.3	20.6 a	0.5	29.9 abc	2.4	111.3 ab	2.6
'XTH20173'	0.9 a	0.1	4.6 a	0.1	19.4 a	0.4	39.5 abcdef	1.5	125.5 ab	4.4
'Zanadoo'	0.6 a	0.1	5.0 a	0.2	19.1 a	1.6	49.6 fgh	1.5	106.9 a	3.0
'2472XR'	0.9 a	0.2	4.9 a	0.1	21.8 a	0.6	37.7 abc	1.2	146.3 abc	3.7

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.7. Parameters used to assess quality characteristics including husk appearance, husk protection, tip fill, ear shape, kernel color, flavor, and tenderness for vegetable cultivar trial sweet corn entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018 combined.

Cultivar	Husk ^z appearance		Husk protection		Tip fill		Ear shape		Kernel color		Flavor	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Allure'	3.7 cd	0.7	5.0 b	0.0	4.7 bcd	0.3	3.7 a	0.3	5.0 ab	0.0	-	-
'Anthem XR'	4.7 bcd	0.3	4.0 ab	0.0	4.7 abcd	0.3	3.7 a	0.3	4.3 ab	0.7	4.0 cd	0.3
'AP426'	4.8 d	0.2	4.2 ab	0.3	4.0 abcd	0.4	3.8 a	0.2	3.8 ab	0.6	3.9 cd	0.3
'Double Standard'	2.0 abc	0.0	2.0 a	0.6	3.7 abcd	0.3	3.3 a	0.3	3.0 a	0.0	-	-
'Festivity'	2.0 abc	0.6	4.3 b	0.3	1.7 a	0.3	3.0 a	0.0	4.7 ab	0.3	-	-
'Jared Synthetic 21'	2.0 abc	0.0	3.0 ab	0.6	3.0 abcd	0.6	3.3 a	0.3	4.3 ab	0.7	-	-
'Luscious'	2.7 abcd	0.3	4.3 b	0.3	4.3 bcd	0.3	3.7 a	0.7	5.0 ab	0.0	-	-
'Mirai 131Y'	3.8 abcd	0.7	3.0 ab	0.0	2.7 abcd	0.3	3.0 a	0.0	3.0 ab	0.0	-	-
'Mirai 308BC'	4.7 bcd	0.3	3.7 ab	0.7	4.0 abcd	0.0	4.0 a	0.0	4.0 ab	1.0	2.8 ab	0.4
'My Fair Lady'	1.7 ab	0.3	2.0 a	0.0	2.7 abc	0.7	3.7 a	0.3	5.0 ab	0.0	-	-
'New Mama'	4.2 d	0.3	3.5 ab	0.4	2.8 ab	0.4	3.3 a	0.2	4.0 ab	0.3	2.3 a	0.4
'Nirvana'	3.9 bcd	0.2	3.2 ab	0.1	4.3 cd	0.2	3.7 a	0.2	4.6 b	0.2	4.8 d	0.1
'Temptation'	1.3 a	0.3	3.3 ab	0.9	4.3 bcd	0.3	4.0 a	0.0	5.0 ab	0.0	-	-
'Who Gets Kissed'	2.3 abcd	0.3	4.3 b	0.7	2.7 abc	0.7	3.7 a	0.3	4.7 ab	0.3	-	-
'Xtratender 2171'	3.5 bcd	0.4	2.8 ab	0.3	4.8 d	0.2	3.3 a	0.4	4.7 ab	0.4	3.8 bcd	0.4
'Xtratender 274A'	4.0 abcd	0.8	3.0 ab	0.0	3.5 abcd	0.4	4.0 a	0.0	3.5 ab	0.4	-	-
'XTH20173'	5.0 d	0.0	3.7 ab	0.2	4.2 abcd	0.3	3.3 a	0.2	4.7 b	0.3	3.5 abc	0.3
'Zanadoo'	2.0 abc	0.6	4.7 b	0.3	3.0 abcd	0.6	4.0 a	0.0	5.0 ab	0.0	-	-
'2472XR'	5.0 cd	0.0	4.7 ab	0.3	4.3 abcd	0.3	3.7 a	0.3	5.0 ab	0.0	4.1 cd	0.1

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.8. The breeding system, market class, growth habit, average yield per plant, average number of fruits per plant, and the number of days to the first ripe fruit for vegetable cultivar trial tomato entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2017 combined.

Cultivar	Breeding system	Market class	Growth habit	Days to maturity ^z	
				Mean	SE
'Crimson Sprinter'	Open pollinated	Slicer	Indeterminate	80.7 ab	1.2
'Damsel'	F1 hybrid	Slicer	Indeterminate	77.0 a	0.0
'Iron Lady'	F1 hybrid	Slicer	Determinate	-	-
'LB8-3-1-1-1'	F1 hybrid	Slicer	Determinate	-	-
'LB8-7-1-1-1'	F1 hybrid	Plum	Determinate	82.7 abc	0.5
'Mountain Merit'	F1 hybrid	Slicer	Determinate	82.7 abc	0.5
'NC12TMV007 x 141233-62'	F1 hybrid	Slicer	Determinate	-	-
'Plum Perfect'	F1 hybrid	Plum	Determinate	84.0 bc	0.0
'Plum Regal'	F1 hybrid	Plum	Determinate	88.7 c	1.0
'Stellar'	F1 hybrid	Slicer	Determinate	85.0 bc	1.5
'Stupice'	Open pollinated	Slicer	Determinate	-	-
'S200-1-1'	F1 hybrid	Slicer	Determinate	81.0 ab	1.5

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.9. The width, height, leaf curl rating, and leaf cover rating for vegetable cultivar trial tomato entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018 combined.

Cultivar	Width (cm) ^z		Height (cm)		Leaf curl		Leaf cover	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Crimson Sprinter'	6.9 cd	0.2	6.0 bc	0.1	2.8 ab	0.3	2.8 abc	0.2
'Damsel'	6.9 cd	0.1	5.9 bc	0.1	2.3 ab	0.3	3.7 cd	0.9
'Iron Lady'	4.5 ab	0.3	4.8 ab	0.4	3.3 ab	0.7	2.3 abc	0.3
'LB8-3-1-1-1'	6.0 abcd	0.1	6.1 bcd	0.1	1.7 a	0.3	2.7 abc	0.3
'LB8-7-1-1-1'	4.6 a	0.2	6.6 cd	0.1	1.2 a	0.2	4.3 d	0.2
'Mountain Merit'	7.4 d	0.2	6.5 cd	0.2	2.3 ab	0.5	2.2 ab	0.2
'NC12TMV007 x 141233-62'	5.8 abc	0.2	5.8 bcd	0.3	1.7 a	0.7	3.7 abcd	0.3
'Plum Perfect'	5.4 ab	0.2	7.0 d	0.1	4.0 b	0.4	2.7 abc	0.3
'Plum Regal'	4.6 a	0.2	6.6 cd	0.4	1.5 a	0.2	3.3 bcd	0.2
'Stellar'	6.2 bc	0.2	6.0 bc	0.1	1.3 a	0.2	2.0 a	0.4
'Stupice'	4.4 a	0.4	4.1 a	0.2	2.3 ab	0.3	2.0 abc	0.0
'S200-1-1'	6.0 bc	0.3	5.4 ab	0.1	2.0 a	0.5	2.0 a	0.4

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.10. Parameters used to assess quality characteristics including: °Brix, appearance, flavor, texture, sugar/acid balance, and skin thickness for vegetable cultivar trial tomato entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2017 combined.

Cultivar	°Brix ^z		Appearance		Flavor		Texture		Sugar/acid balance		Skin thickness	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Crimson Sprinter'	4.0 abc	0.3	4.1 bcd	0.3	3.6 bc	0.5	3.9 de	0.4	3.6 bcd	0.4	3.9 bc	0.4
'Damsel'	5.1 d	0.1	3.3 a	0.5	3.8 c	0.6	4.1 e	0.5	3.7 cd	0.6	3.9 c	0.5
'Iron Lady'	3.4 abc	0.3	-	-	-	-	-	-	-	-	-	-
'LB8-3-1-1-1'	3.8 abcd	0.2	-	-	-	-	-	-	-	-	-	-
'LB8-7-1-1-1'	4.3 cd	0.1	4.3 cd	0.4	3.1 ab	0.5	2.9 a	0.5	3.3 abcd	0.4	3.3 a	0.4
'Mountain Merit'	4.4 cd	0.1	4.5 d	0.3	3.8 c	0.4	3.8 cde	0.4	3.8 d	0.4	3.8 abc	0.4
'NC12TMV007 x 141233-62'	3.9 abcd	0.2	-	-	-	-	-	-	-	-	-	-
'Plum Perfect'	4.0 abc	0.1	4.1 bcd	0.4	3.0 ab	0.5	3.5 bcde	0.5	3.1 abc	0.5	3.3 a	0.4
'Plum Regal'	3.2 a	0.2	4.1 bc	0.4	2.7 a	0.4	3.3 abc	0.4	3.1 a	0.4	3.5 abc	0.4
'Stellar'	3.5 ab	0.1	3.9 bc	0.4	2.9 a	0.5	3.2 ab	0.5	3.2 ab	0.4	3.4 abc	0.4
'Stupice'	4.1 abcd	0.1	-	-	-	-	-	-	-	-	-	-
'S200-1-1'	4.1 bcd	0.2	3.8 ab	0.4	3.2 ab	0.5	3.4 abcd	0.4	3.3 abcd	0.4	3.4 ab	0.4

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.11. Market class, average number of marketable fruits per plant, average yield per plant, °Brix for fresh samples, and °Brix for stored samples (4 months in cold storage) for vegetable cultivar trial winter squash entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018 combined.

Cultivar	Market class	Yield ^z (pounds/plant)		Number of fruits/plant		°Brix (fresh)		°Brix (after storage)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Acorn #1'	Acorn	2.6 ab ^w	0.4	2.2 a	0.4	9.2 abc	1.1	-	-
'Carnival'	Acorn	3.6 ab	0.6	2.3 a	0.3	9.7 abc	1.8	-	-
'Celebration'	Acorn	5.4 ab	1.8	4.4 a	1.6	9.9 abc	0.6	-	-
'Confetti'	Acorn	3.1 ab	0.8	3.6 a	1.2	11.8 abc	0.9	-	-
'Festival'	Acorn	3.4 ab	-	2.6 a	-	11.7 abc	-	-	-
'Gill's Golden Pippin'	Acorn	3.5 ab	0.2	6.2 a	0.8	8.4 abc	0.8	-	-
'Honey Bear'	Acorn	1.5 a	0.1	1.5 a	0.2	15.9 c	0.3	13.9 ab	1.7
'Sugar Bush'	Acorn	2.7 ab	0.3	1.8 a	0.2	16.2 c	1.6	17.4 b	1.1
'Sweet Reba Bush'	Acorn	2.5 ab	0.7	1.9 a	0.3	10.5 ab	0.5	7.7 a	0.1
'Thelma Sanders'	Acorn	7.9 b	2.3	4.8 a	0.9	5.8 a	1.0	-	-
'Tuffy'	Acorn	2.8 ab	0.3	2.6 a	0.3	12.1 abc	0.3	15.5 ab	2.8
'JWS 6823 PMR'	Butternut	4.1 ab	0.5	3.0 a	0.4	8.9 abc	0.3	-	-

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.12. Market class, powdery mildew severity on the leaves and petioles, and percent marketable fruits after time in cold storage for vegetable cultivar trial winter squash entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018 combined.

Cultivar	Market class	Percent marketable after time in cold storage ^z							
		<u>1 Month</u>		<u>2 Months</u>		<u>3 Months</u>		<u>4 Months</u>	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Acorn #1'	Acorn	-	-	-	-	-	-	-	-
'Carnival'	Acorn	-	-	-	-	-	-	-	-
'Celebration'	Acorn	-	-	-	-	-	-	-	-
'Confetti'	Acorn	-	-	-	-	-	-	-	-
'Festival'	Acorn	-	-	-	-	-	-	-	-
'Gil's Golden Pippin'	Acorn	-	-	-	-	-	-	-	-
'Honey Bear'	Acorn	93.3 a	6.7	76.7 a	8.8	76.7 a	8.8	76.7 a	0.9
'JWS 6823 PMR'	Butternut	-	-	-	-	-	-	-	-
'Sugar Bush'	Acorn	100.0 a	0.0	85.0 a	5.0	85.0 a	5.0	85.0 a	0.5
'Sweet Reba Bush'	Acorn	90.0 a	5.8	70.0 a	15.3	70.0 a	15.3	70.0 a	1.5
'Thelma Sanders'	Acorn	-	-	-	-	-	-	-	-
'Tuffy'	Acorn	100.0 a	0.0	40.0 a	10.0	40.0 a	10.0	40.0 a	10.0

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.13. Market class, average number of marketable squash per plant, average yield per plant, °Brix for fresh samples, and °Brix for stored samples (4 months in cold storage) for vegetable cultivar trial winter squash entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018 combined.

Cultivar	Market Class	Yield ^z (pounds/plant)		Number of fruits/plant		°Brix (fresh)		°Brix (after storage)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Bush Delicata'	Delicata	3.7 a ^w	0.7	3.1 a	0.6	13.7 a	0.2	13.9 ab	0.6
'Candystick'	Delicata	3.9 a	0.5	3.1 a	0.3	14.9 a	0.3	14.5 ab	0.7
'CU 1'	Delicata	4.7 a	0.6	4.3 a	0.4	14.6 a	0.5	12.6 ab	0.8
'CU 2'	Delicata	4.2 a	0.3	3.9 a	0.2	14.1 a	0.5	12.0 a	0.5
'CU 3'	Delicata	4.5 a	0.3	3.9 a	0.4	15.6 a	0.3	14.2 ab	0.3
'Delicata JS'	Delicata	4.4 a	0.9	3.7 a	0.6	16.0 a	0.4	14.5 ab	0.7
'Jester'	Delicata	4.1 a	0.6	2.6 a	0.3	16.1 a	0.9	16.3 b	0.6
'Mardi Gras'	Delicata	3.2 a	0.3	3.8 a	0.5	12.0 a	1.4	-	-
'Sugar Dumpling'	Delicata	2.7 a	0.2	2.2 a	0.2	14.0 a	1.8	12.0 a	0.5
'Zeppelin'	Delicata	4.1 a	0.6	3.5 a	0.5	14.4 a	0.5	13.4 a	0.3

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.14. Market class, texture, sweetness, and flavor for vegetable cultivar trial winter squash entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018 combined.

Cultivar	Market Class	Texture		Sweetness		Flavor	
		Mean	SE	Mean	SE	Mean	SE
'Bush Delicata'	Delicata	4.2 a	0.8	3.5 a	0.6	4.7 a	0.7
'Candystick'	Delicata	6.0 a	0.6	4.8 a	0.6	6.2 a	0.7
'CU 1'	Delicata	5.3 a	0.6	4.1 a	0.6	5.1 a	0.7
'CU 2'	Delicata	5.5 a	0.6	4.6 a	0.5	5.1 a	0.7
'CU 3'	Delicata	3.9 a	0.7	3.2 a	0.5	5.1 a	0.8
'Delicata JS'	Delicata	6.0 a	0.8	5.5 a	0.6	6.6 a	0.8
'Jester'	Delicata	6.3 a	0.7	4.8	0.6	5.8 a	0.6
'Mardi Gras'	Delicata	-	-	-	-	-	-
'Sugar Dumpling'	Delicata	-	-	-	-	-	-
'Zeppelin'	Delicata	5.0 a	0.6	4.3	0.6	5.8 a	0.6

Table A.15. Powdery mildew severity on the leaves and petioles, and percent marketable after time in cold storage for vegetable cultivar trial winter squash (delicata) entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018 combined.

Cultivar	Powdery mildew ^z severity				Striped cucumber beetle severity		Percent marketable after time in cold storage							
	Leaves		Petioles		Mean	SE	1 Month		2 Months		3 Months		4 Months	
	Mean	SE	Mean	SE			Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Bush Delicata'	3.7 ab ^w	0.2	6.0 ab	0.8	9.0 a	0.0	93.3 a	6.7	81.7 a	12.5	80.0 a	12.1	80.0 a	1.2
'Candystick'	6.0 bc	0.4	7.0 ab	0.4	9.0 a	0.0	100.0 a	0.0	95.0 a	3.4	95.0 a	3.4	93.3 a	0.3
'CU 1'	7.0 c	0.6	8.3 b	0.3	9.0 a	0.0	100.0 a	0.0	100.0 a	0.0	90.0 a	10.0	86.7 a	1.3
'CU 2'	4.7 abc	0.7	8.0 b	0.0	9.0 a	0.0	100.0 a	0.0	100.0 a	0.0	96.7 a	3.3	86.7 a	1.3
'CU 3'	5.0 abc	0.6	7.7 b	0.3	9.0 a	0.0	100.0 a	0.0	96.7 a	3.3	93.3 a	3.3	90.0 a	0.6
'Delicata JS'	4.3 ab	0.2	5.0 a	0.4	9.0 a	0.0	100.0 a	0.0	88.3 a	6.0	73.3 a	9.4	63.3 a	1.0
'Jester'	3.3 a	0.2	6.7 ab	0.2	9.0 a	0.0	96.7 a	1.7	85.0 a	4.6	70.0 a	5.6	50.0 a	1.2
'Mardi Gras'	-	-	-	-	-	-	-	-	-	-	-	-	-	-
'Sugar Dumpling'	-	-	-	-	-	-	100.0 a	0.0	75.0 a	5.0	75.0 a	5.0	75.0 a	0.5
'Zeppelin'	5.0 abc	0.4	7.7 b	0.3	9.0 a	0.0	98.0 a	2.0	84.0 a	10.3	78.0 a	7.7	74.0 a	0.8

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.

Table A.16. The average yield per plant, number of marketable pods per plant, °Brix, average pod length, average pod width, and flavor results for vegetable cultivar trial roasting-style pepper entries on certified organic agricultural land in Fort Collins, Colorado; 2016-2018 combined.

Cultivar	Pod color	Pungency	°Brix ^z		Pod length (cm)		Pod width (cm)		Flavor score	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Belcanto'	Red	Non-pungent	7.7 abc	0.6	15.0 a-d	0.2	5.0 a	0.1	- ^y	-
'Bella Italia'	Red	Non-pungent	7.4 abc	0.2	16.2 bd	0.6	5.3 a	0.0	-	-
'Bridge to Paris'	Red	Non-pungent	9.4 c	0.4	17.2 d	0.3	4.2 a	0.1	5.7 a	0.6
'Carmen'	Red	Non-pungent	9.5 c	0.3	18.1 cd	0.8	5.6 a	0.3	5.5 a	0.7
'Classic Italian'	Red	Non-pungent	8.0 abc	0.6	13.3 a-d	0.9	5.2 a	0.1	-	-
'Cornito Rosso'	Red	Non-pungent	9.0 abc	0.2	12.9 a-d	0.4	4.7 a	0.5	-	-
'Corno di Toro'	Red	Non-pungent	7.9 abc	0.5	9.9 abc	3.6	6.2 a	2.7	-	-
'Crest Yellow'	Yellow	Non-pungent	8.7 abc	0.1	15.7 cd	0.0	5.9 a	0.3	5.3 a	1.3
'CSU 256'	Red	Pungent	7.1 ab	0.6	15.2 cd	0.2	3.7 a	0.1	-	-
'CSU 321'	Green	Pungent	6.9 a	0.9	16.2 cd	0.1	4.2 a	0.1	-	-
'CSU Mosco'	Red	Pungent	7.9 abc	0.6	15.5 cd	0.7	4.4 a	0.1	-	-
'CSU Pueblo Popper'	Red	Pungent	8.1 abc	0.2	5.7 ab	0.1	5.4 a	0.1	-	-
'Early Perfect Italian'	Red	Non-pungent	8.8 abc	0.5	15.4 a-d	0.8	5.0 a	0.5	5.2 a	0.8
'Escamillo'	Yellow	Non-pungent	9.2 bc	0.1	20.1 cd	0.1	6.6 a	0.1	5.4 a	0.9
'Karma'	Red	Non-pungent	9.4 abc	0.9	14.3 a-d	0.5	5.0 a	0.1	-	-
'Laerte'	Red	Non-pungent	6.0 abc	0.0	15.6 a-d	0.2	5.8 a	0.2	-	-
'Lively Yellow'	Yellow	Non-pungent	9.7 abc	0.5	-	-	-	-	-	-
'Stocky Red Roaster'	Red	Non-pungent	8.5 abc	0.4	15.4 cd	0.7	4.9 a	0.2	5.5 a	0.9

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test; ^y Cultivars not included in the statistical analysis because they were not grown in that year.

Table A.17. The average yield, number of marketable pods, °Brix, average pod length, average pod width and flavor results for vegetable cultivar trial bell pepper on certified organic agricultural land in Fort Collins, Colorado; 2016-2017 combined.

Cultivar	Pod color	Pungency	°Brix		Pod length (cm)		Pod width (cm)		Flavor score	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
'Ace'	Red	Non-pungent	8.2 bc	0.3	10.0 a	0.5	6.8 a	0.2	5.6 bc	0.7
'Aristotle X3R®'	Green	Non-pungent	7.5 bc	0.3	10.0 a	0.6	9.3 a	0.3	5.8 c	0.7
'CU 1'	Red	Non-pungent	8.2 bc	0.1	8.2 a	0.2	7.7 a	0.4	4.3 ab	1.2
'CU 2'	Red	Non-pungent	8.5 bc	0.1	9.1 a	0.4	7.4 a	0.2	4.8 bc	1.3
'CU 3'	Red	Non-pungent	9.7 c	0.7	-	-	-	-	-	-
'CU 4'	Red	Non-pungent	6.8 abc	-	-	-	-	-	-	-
'Early Red Sweet'	Red	Non-pungent	8.5 bc	0.1	7.3 a	0.1	6.4 a	0.3	4.4 bc	1.0
'Flavorburst'	Yellow	Non-pungent	8.1 bc	0.8	9.9 a	0.4	7.6 a	0.2	4.5 bc	1.3
'Gypsy Queen'	White	Non-pungent	5.9 abc	0.2	5.2 a	0.1	5.2 a	0.1	-	-
'King Krimson'	Red	Non-pungent	6.4 abc	-	7.0	-	6.5	-	-	-
'King of the North'	Red	Non-pungent	6.7 bc	0.3	8.4 a	3.0	8.5 a	2.9	-	-
'Red Knight X3R®'	Red	Non-pungent	7.5 bc	0.3	9.4 a	0.1	7.8 a	0.1	-	-
'Revolution'	Green	Non-pungent	3.2 a	2.6	10.2	-	11.4	-	-	-
'SBGO 10408'	Orange	Non-pungent	8.8 bc	0.1	-	-	-	-	5.2 bc	1.3
'Whitney'	White	Non-pungent	6.0 ab	0.2	11.4 a	0.2	7.2 a	0.2	2.5 a	1.1

^z Means within a column followed by the same letter are not statistically significantly different ($\alpha = 0.05$) according to Tukey's honestly significant difference test.



Figure A.1. Image ‘Sweet Chocolate’ bell pepper, which was evaluated using direct application real time (DART) mass spectrometry; 2019.



Figure A.2. Image ‘Aloha’ bell pepper cultivar, which was evaluated using rapid evaporative ionization mass spectrometry (REIMS); 2018.

Table A.18. The m/z values for statistically significant metabolites detected, but unidentified (negative mode) using direct application real time (DART) mass spectrometry for different colors of bell pepper.

M/Z ^z (1-100)	M/Z (101-140)	M/Z (141-170)	M/Z (171-198)	M/Z (199-216)	M/Z (217-243)	M/Z (244-281)	M/Z (282- 500)
10.75	102.25	142.25	170.25	199.25	217.25	244.75	282.25
59.75	103.75	144.25	170.75	202.25	217.75	245.25	284.75
60.25	104.25	151.75	171.25	208.75	226.75	246.25	350.25
65.75	117.75	152.25	171.75	209.25	227.25	252.75	356.75
82.75	118.25	153.75	172.25	309.75	227.75	253.75	359.25
83.75	119.25	156.25	183.25	310.25	228.25	256.75	360.25
85.75	120.25	157.75	188.25	325.75	232.25	257.25	368.25
94.25	127.75	158.25	188.75	211.75	239.75	269.75	462.75
97.75	128.25	160.25	189.25	213.75	240.25	270.25	
98.25	134.75	164.25	189.75	214.75	241.25	270.75	
98.75	136.75	167.75	190.25	216.25	241.75	280.75	
99.75	138.25	168.75	198.75	216.75	242.25	281.75	

^z The m/z values for unidentified compounds of statistical significance using the Benjamini-Hochberg false-discovery rate adjustment and $\alpha = 0.05$.

Table A.19. The m/z values for statistically significant metabolites detected, but unidentified (positive mode) using rapid evaporative ionization mass spectrometry (REIMS) for market classes of pepper.

M/Z ^z (1-185)	M/Z (186-202)	M/Z (203-214)	M/Z (215-238)	M/Z (239-254)	M/Z (255-275)	M/Z (276-308)	M/Z (309-500)
110.25	186.25	203.25	216.25	240.25	256.25	276.25	310.25
114.75	187.25	204.25	222.25	242.25	258.25	280.75	312.25
136.25	188.25	206.25	223.25	244.25	261.25	282.75	316.25
160.25	188.75	206.75	224.75	245.25	262.25	284.25	318.25
164.25	190.25	207.75	226.25	246.25	262.75	285.25	320.75
168.25	190.75	208.25	226.75	248.25	264.25	286.25	
172.25	192.25	208.75	228.25	248.75	264.75	288.25	
172.75	192.75	209.75	228.75	249.25	266.75	290.25	
173.25	194.25	210.25	230.25	250.25	267.75	290.75	
174.25	194.75	210.75	232.25	250.75	268.25	292.25	
174.75	196.25	211.75	233.25	252.25	268.75	296.25	
182.75	196.75	212.25	234.25	252.75	271.25	304.25	
183.25	197.25	212.75	236.25	254.25	272.25	306.75	
185.25	202.25	214.75	238.25	254.75	275.25	308.75	

^z The m/z values for unidentified compounds of statistical significance using the Benjamini-Hochberg false-discovery rate adjustment and $\alpha = 0.05$.

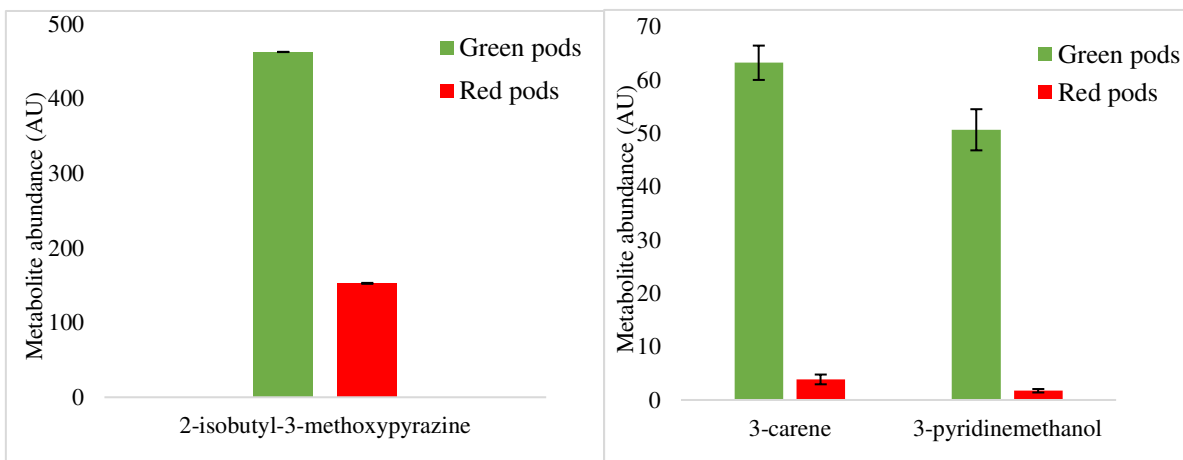


Figure A.3. Relative abundance (AU) of volatile metabolites detected in red and green peppers using solid phase micro extraction gas chromatography mass spectrometry (SPME-GC-MS).

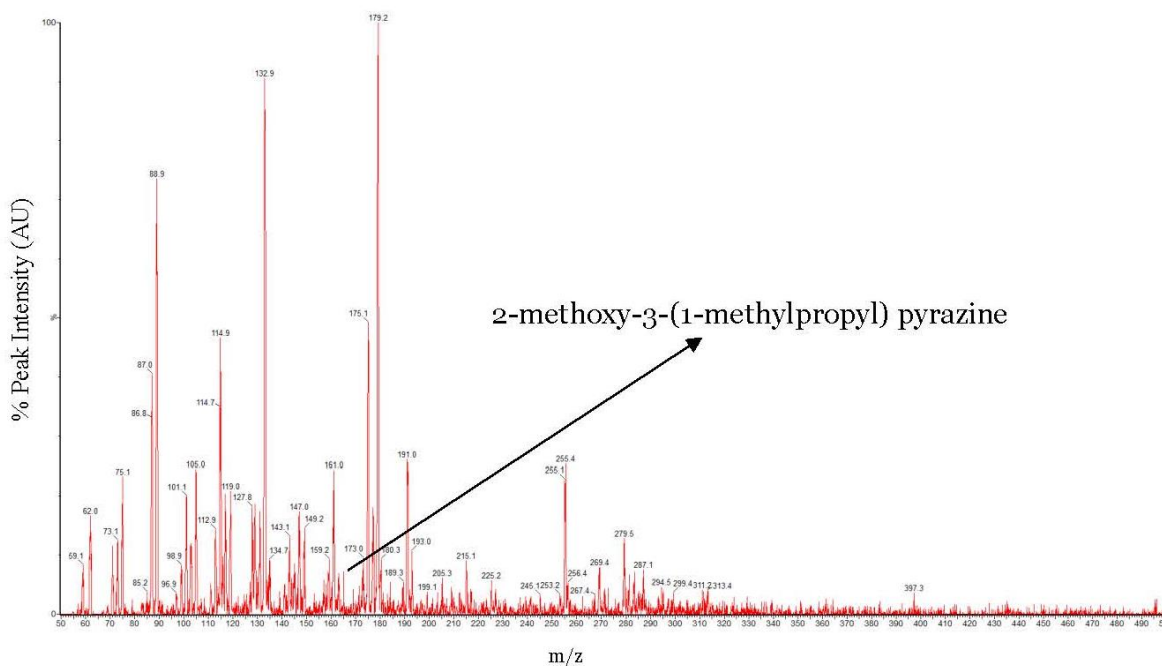


Figure A.4. Representative mass spectrum for the 'Sweet Chocolate' pepper cultivar; DART-MS.

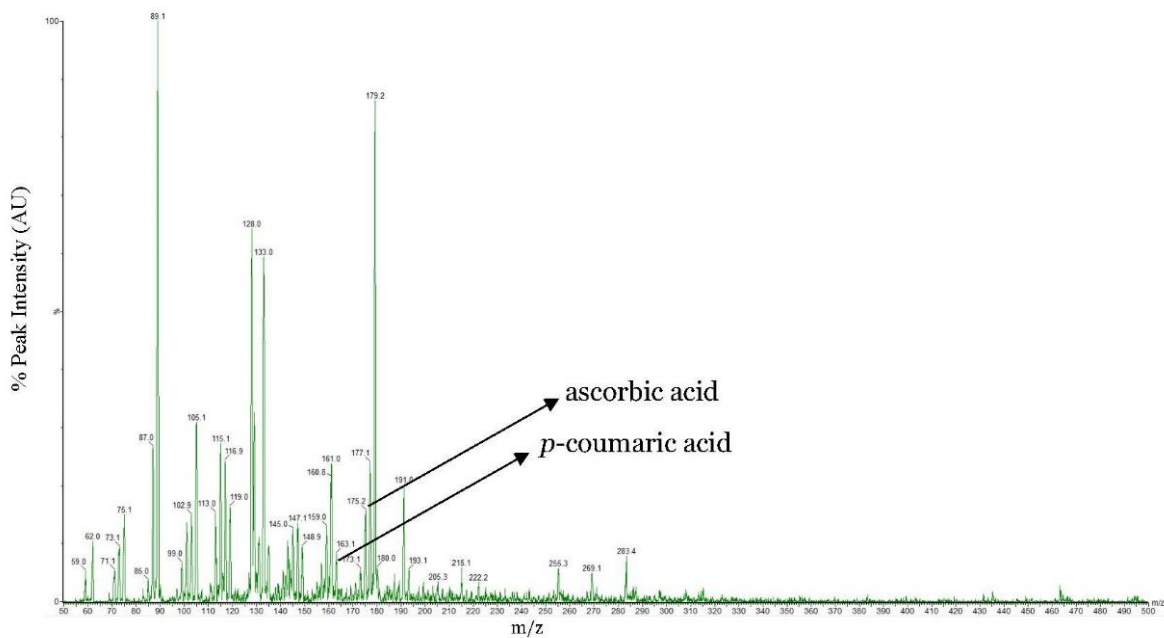


Figure A.5. Representative mass spectrum for the 'Early Perfect Italian' pepper cultivar; DART-MS.

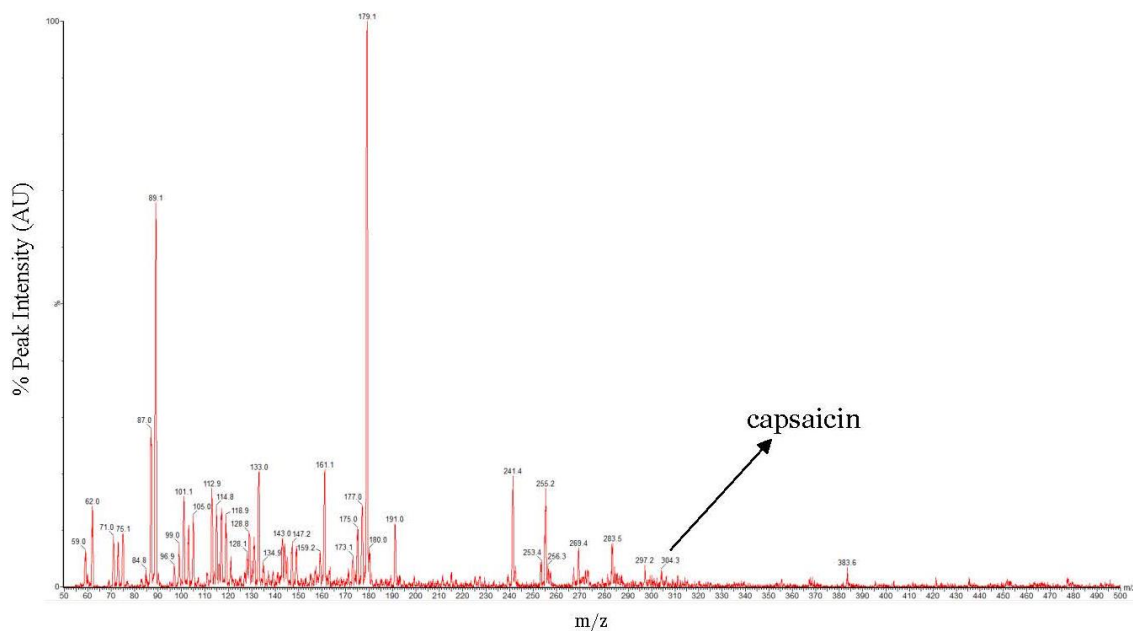


Figure A.6. Representative mass spectrum for the 'Highlander' pepper cultivar; DART-MS.

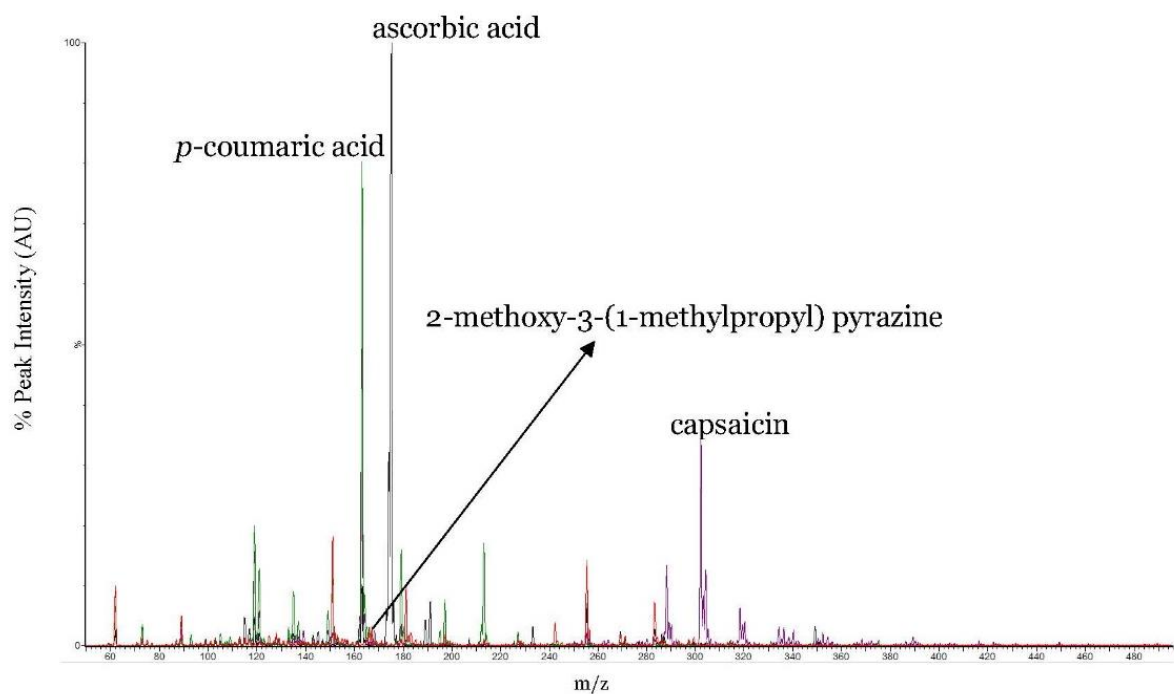


Figure A.7. Mass spectrum for four authentic standards (*p*-coumaric acid, ascorbic acid, 2-methoxy-3-(1-methylpropyl) pyrazine, and capsaicin; DART-MS).

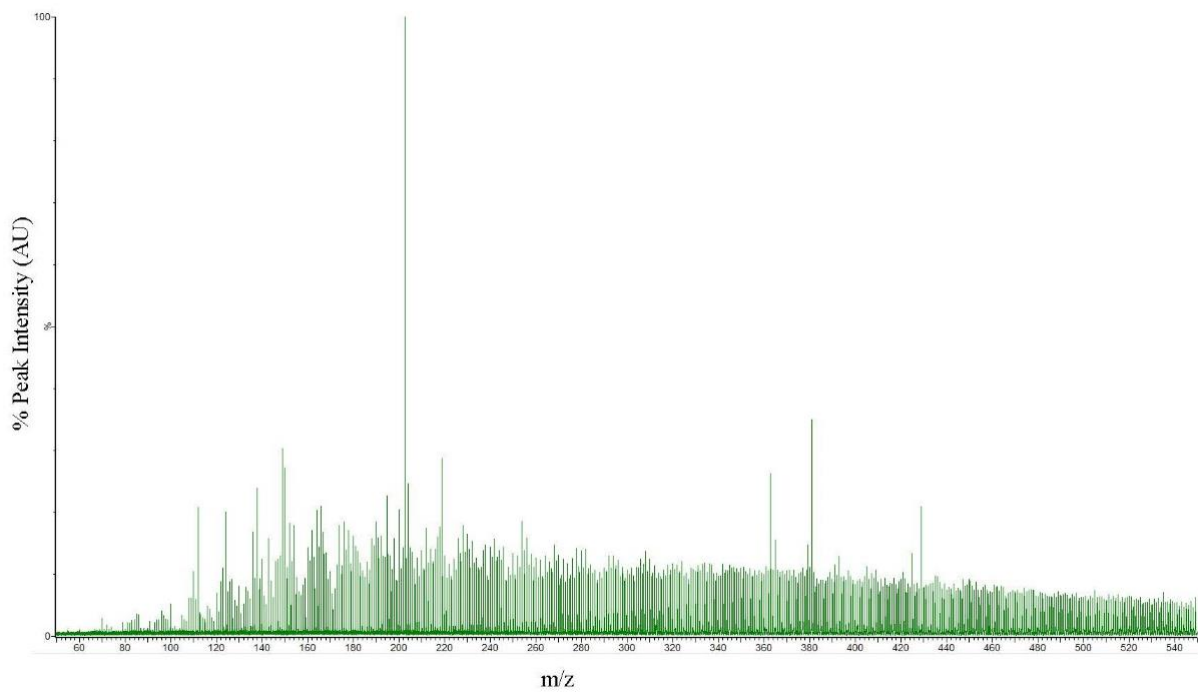


Figure A.8. Representative mass spectrum for the 'Aloha' pepper phenotype: REIMS.

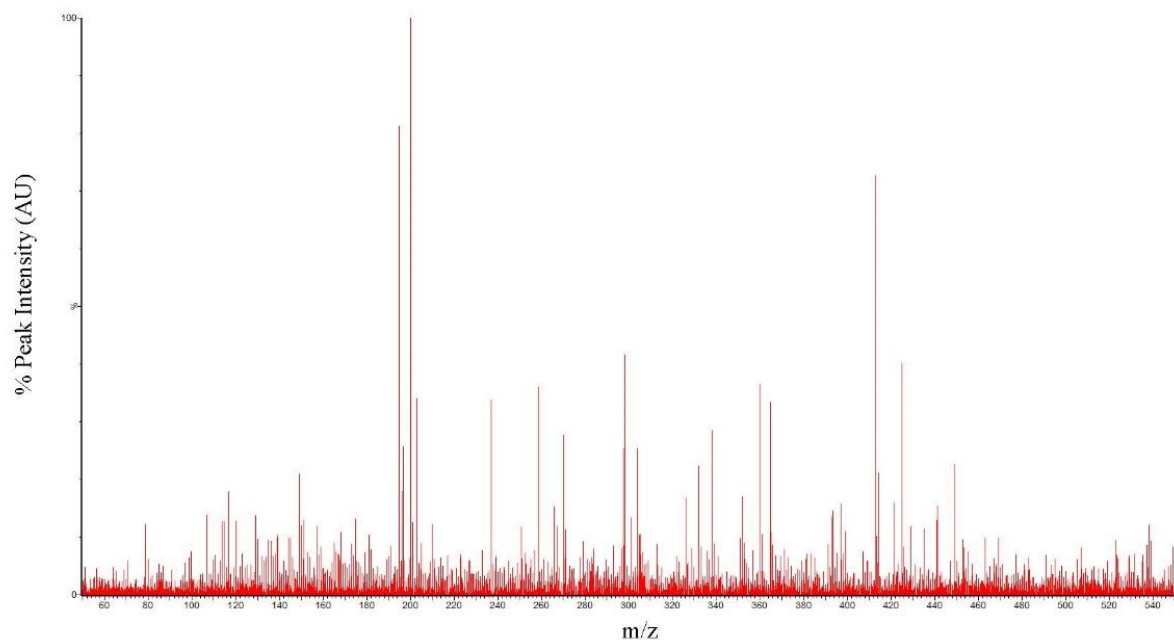


Figure A.9. Representative mass spectrum for the lunchbox pepper phenotype: REIMS.

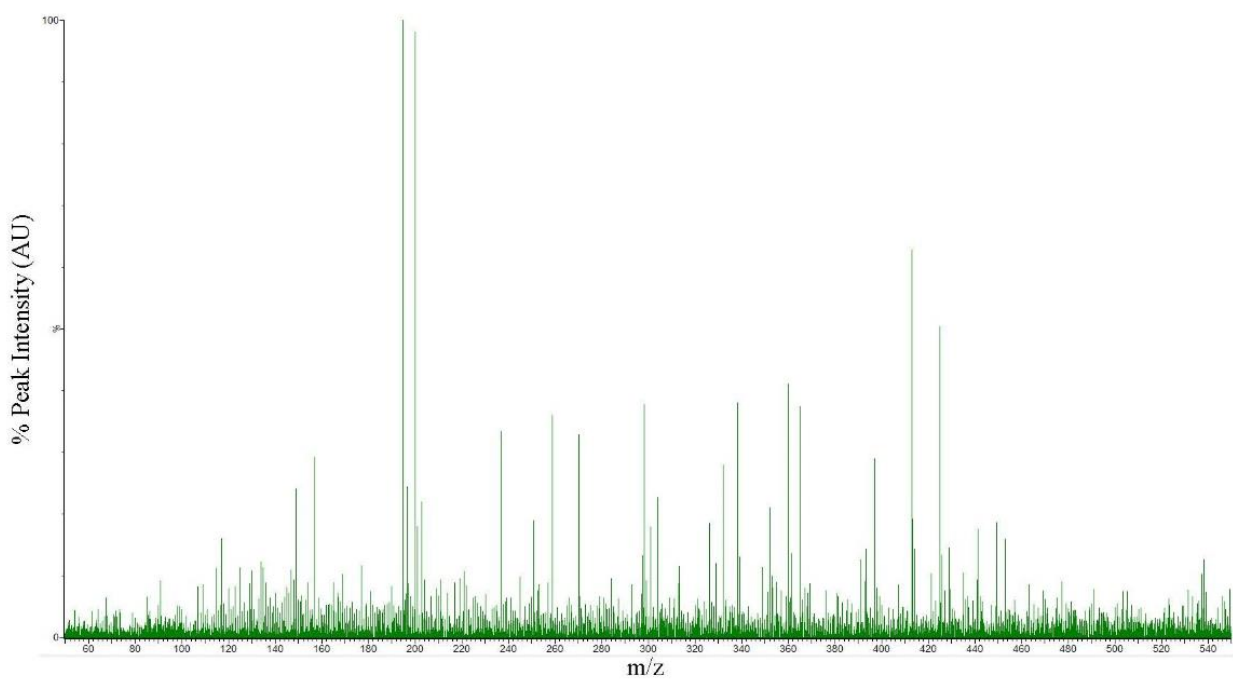


Figure A.10. Representative mass spectrum for the red popper phenotype: REIMS.

Table A.20. Comparison of granulated Azomite treatments on total yield, red pod yield, total number of marketable pods, and number of red pods produced on a per plant basis for roasting pepper cultivars on certified organic agricultural land in Fort Collins, Colorado; 2017-2018.

	Total yield^{ns} (pounds/plant)				Red pod yield^{ns} (pounds/plant)				Total number of^{ns} pods/plant				Number of red^{ns} pods/plant			
	'EPI' ^z		'SRR' ^y		'EPI'		'SRR'		'EPI'		'SRR'		'EPI'		'SRR'	
	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
0 #/ac AZOMITE	3.1	0.2	2.5	0.2	1.4	0.2	1.3	0.2	17.8	1.6	18.6	0.8	6.5	1.1	8.1	1.4
100 #/ac AZOMITE	3.2	0.3	2.6	0.3	1.5	0.1	1.3	0.2	20.0	1.3	20.8	1.4	7.7	0.6	8.3	1.1
200 #/ac AZOMITE	3.2	0.2	2.8	0.2	1.3	0.2	1.3	0.2	18.5	0.7	21	1.1	6.4	1.2	7.9	1.1

^{ns} There are no statistically significant differences between treatments within cultivar according to Tukey's multiple means comparison at $\alpha = 0.05$.

^z 'EPI' is an abbreviation for the roasting pepper cultivar 'Early Perfect Italian'.

^y 'SRR' is an abbreviation for the roasting pepper cultivar 'Stocky Red Roaster'.

Table A.21. Comparison of granulated Azomite treatments on number of days to flowering and °Brix for roasting pepper cultivars on certified organic agricultural land in Fort Collins, Colorado; 2017-2018.

	Number of DAT^{ns} to flowering				Flower bud^{ns} count 85 DAT^x				°Brix^{ns}			
	'EPI' ^z		'SRR' ^y		'EPI'		'SRR'		'EPI'		'SRR'	
	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
0 #/acre AZOMITE	42.1	0.6	42.1	0.6	20.8	2.3	19.3	1.8	9.8	0.3	9.1	0.2
100 #/acre AZOMITE	41.9	0.6	41.8	0.4	18.0	1.7	17.6	1.1	9.6	0.1	9.0	0.1
200 #/acre AZOMITE	42.4	0.9	41.5	0.3	18.8	1.9	22.1	1.6	9.4	0.2	8.9	0.2

^{ns} There are no statistically significant differences between treatments within cultivar according to Tukey's multiple means comparison at $\alpha = 0.05$.

^z 'EPI' is an abbreviation for the roasting pepper cultivar 'Early Perfect Italian'.

^y 'SRR' is an abbreviation for the roasting pepper cultivar 'Stocky Red Roaster'.

^x DAT is an abbreviation for days after transplant.

Table A.22. Comparison of three granulated Azomite treatments on plant height for roasting pepper cultivars on certified organic agricultural land in Fort Collins, Colorado; 2017-2018.

	Plant height ^{ns} 28 DAT (cm)				Plant height ^{ns} 49 DAT ^x (cm)				Plant height ^{ns} 63 DAT (cm)			
	'EPI' ^z		'SRR' ^y		'EPI'		'SRR'		'EPI'		'SRR'	
	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
0 #/ac AZOMITE	21.8	2.3	21.3	0.5	39.3	2.7	41.3	1.2	51.3	1.1	49.8	0.5
100 #/ac AZOMITE	23.0	2.1	22.3	1.7	42.1	1.8	41.5	1.5	51.3	2.1	50.1	1.2
200 #/ac AZOMITE	24.5	1.4	22.5	0.6	40.1	2.5	41.7	1.1	52.5	0.3	48	0.8

^{ns} There are no statistically significant differences between treatments within cultivar according to Tukey's multiple means comparison at $\alpha = 0.05$.

^z 'EPI' is an abbreviation for the roasting pepper cultivar 'Early Perfect Italian'.

^y 'SRR' is an abbreviation for the roasting pepper cultivar 'Stocky Red Roaster'.

^x DAT is an abbreviation for days after transplant.

Table A.23. Leaf tissue test results for three granulated Azomite treatments in roasting pepper cultivars on certified organic agricultural land in Fort Collins, Colorado 54 DAT ^y; 2017.

Element	Unit	'Stocky Red Roaster'				'Early Perfect Italian'			
		0 #/ac	100 #/ac	200 #/ac	% change ^z	0 #/ac	100 #/ac	200 #/ac	% change
Nitrogen	%	5.25	5.63	5.44	3.6	5.84	2.86	5.26	-9.9
Phosphorus	%	0.336	0.398	0.407	21.1	0.463	0.369	0.368	-20.5
Potassium	%	5.14	5.44	5.68	10.5	6.06	5.75	5.38	-11.2
Calcium	%	1.73	1.69	1.94	12.1	2.42	2.13	2.02	-16.5
Magnesium	%	0.599	0.624	0.678	13.2	0.857	0.678	0.651	-24.0
Sulfur	%	0.491	0.512	0.531	8.1	0.598	0.586	0.515	-13.9
Silicon	%	0.057	0.068	0.058	1.8	0.065	0.075	0.059	-9.2
Boron	ppm	24.9	27.5	28.6	14.6	27.4	27.7	24.9	-9.1
Iron	ppm	119	120	125	5.0	147	142	125	-14.9
Manganese	ppm	50.3	52.9	60.2	19.7	67.2	59	55.4	-17.6
Copper	ppm	8.1	9.7	9.3	14.8	11.2	8.6	8.4	-25.0
Zinc	ppm	34.2	39.2	41.5	21.3	47.7	39	36.8	-22.9
Aluminum	ppm	125	103	114	-8.8	129	141	116	-10.1

^z Percent change refers to the difference between the 0 #/ac and 200 #/ac Azomite treatments.

^y DAT is an abbreviation for days after transplant.

Table A.24. Leaf tissue test results for three granulated Azomite treatments in roasting pepper cultivars on certified organic agricultural land in Fort Collins, Colorado 116 DAT; 2017.

Element	Unit	‘Stocky Red Roaster’				‘Early Perfect Italian’			
		0 #/ac	100 #/ac	200 #/ac	% change ^z	0 #/ac	100 #/ac	200 #/ac	% change
Nitrogen	%	4.07	3.82	4.02	-1.2	3.91	3.71	3.95	1.0
Phosphorus	%	0.089	0.22	0.238	167.4	0.27	0.25	0.132	-51.1
Potassium	%	4.05	3.85	4.18	3.2	4.02	4.09	4.34	8.0
Calcium	%	0.04	2.89	3.18	7850	3.58	3.96	0.08	-97.8
Magnesium	%	0.344	0.727	0.774	125	0.976	0.974	0.542	-44.5
Sulfur	%	0.434	0.419	0.42	-3.2	0.545	0.566	0.58	6.4
Silicon	%	0.056	0.072	0.07	25	0.081	0.081	0.083	2.5
Boron	ppm	24.4	24.2	27.2	11.5	27.3	30.1	31.4	15.0
Iron	ppm	4.2	80.5	74.9	1683.3	90.9	108	4.9	-94.6
Manganese	ppm	10.9	45.9	49.7	355.9	60.6	63.8	15.8	-73.9
Copper	ppm	2.2	7.4	7.9	259.1	11.3	9.6	4.1	-63.7
Zinc	ppm	4.8	24.7	25.4	429.2	38.1	34.6	6.2	-83.7
Aluminum	ppm	2.2	85	72.4	3190.9	88.9	125	5.3	-94.0

^zPercent change refers to the difference between the 0 #/ac and 200 #/ac Azomite treatments.

Table A.25. Leaf tissue test results for three granulated Azomite treatments in roasting pepper cultivars on certified organic agricultural land in Fort Collins, Colorado 61 DAT; 2018.

Element	Unit	‘Stocky Red Roaster’				‘Early Perfect Italian’			
		0 #/ac	100 #/ac	200 #/ac	% change ^z	0 #/ac	100 #/ac	200 #/ac	% change
Nitrogen	%	4.52	4.12	4.35	-3.8	4.16	4.16	4.4	5.8
Phosphorus	%	0.318	0.303	0.315	-0.9	0.289	0.274	0.276	-4.5
Potassium	%	4.41	4.03	4.31	-2.3	4.57	4.4	4.44	-2.8
Calcium	%	2.05	1.96	2.05	0.0	2.29	2.31	2.47	7.9
Magnesium	%	0.623	0.576	0.613	-1.6	0.608	0.618	0.622	2.3
Sulfur	%	0.484	0.447	0.462	-4.5	0.602	0.579	0.642	6.6
Silicon	%	0.219	0.22	0.217	-0.9	0.262	0.221	0.233	-11.1
Boron	ppm	34.3	31	30.8	-10.2	32.5	32	32.6	0.3
Iron	ppm	135	134	164	21.5	145	139	167	15.2
Manganese	ppm	51	46.8	52.7	3.3	49.1	46.7	46.9	-4.5
Copper	ppm	9.1	8.3	8.8	-3.3	8.3	8.3	7.8	-6.0
Zinc	ppm	33.1	30.1	34.7	4.8	32.3	35.1	32.5	0.6
Aluminum	ppm	159	168	201	26.4	171	175	207	21.1

^z Percent change refers to the difference between the 0 #/ac and 200 #/ac Azomite treatments.

Table A.26. Leaf tissue test results for three granulated Azomite treatments in roasting pepper cultivars on certified organic agricultural land in Fort Collins, Colorado 117 DAT; 2018.

Element	Unit	‘Stocky Red Roaster’				‘Early Perfect Italian’			
		0 #/ac	100 #/ac	200 #/ac	% change ^z	0 #/ac	100 #/ac	200 #/ac	% change
Nitrogen	%	4.1	4.1	4.11	0.2	3.73	3.83	3.93	5.4
Phosphorus	%	0.207	.209	0.196	-5.3	0.175	0.207	0.184	5.1
Potassium	%	4.38	4.42	4.18	-4.6	4.04	4.42	4.07	0.7
Calcium	%	3.73	3.61	3.63	-2.7	3.81	4.04	4.18	9.7
Magnesium	%	0.976	0.925	0.872	-10.7	0.889	0.999	0.869	-2.2
Sulfur	%	0.479	0.523	0.493	2.9	0.63	0.728	0.65	3.2
Silicon	%	0.059	0.061	0.060	1.6	0.071	0.059	0.0545	-22.7
Boron	ppm	48.7	47.9	47.7	-2.1	46.6	47.1	46	-1.3
Iron	ppm	104	88.5	116	11.5	80.6	92.8	113	40.2
Manganese	ppm	58	54.3	52.1	-10.2	52.1	57.9	51	-2.1
Copper	ppm	7.2	6.7	6.3	-12.5	5.9	7.6	7	18.6
Zinc	ppm	24.1	23.8	22.4	-7.1	20.4	24.6	20.7	1.5
Aluminum	ppm	114	87.7	127	11.4	80.9	101	134	65.6

^z Percent change refers to the difference between the 0 #/ac and 200 #/ac Azomite treatments.

Table A.27. Soil test results before, 14 days after, and 134 days after granulated Azomite treatment applications were made at 0, 100 and 200 #/ac on certified organic agricultural land in Fort Collins, Colorado; 2017.

Element	Unit		Before Application				After Application (14 DAT)				After Application (134 DAT)			
			Clean Field				Combined Cultivars				Combined Cultivars			
			0 #/ac	100 #/ac	200 #/ac	% change ^x	0 #/ac	100 #/ac	200 #/ac	% change	0 #/ac	100 #/ac	200 #/ac	% change
Nitrogen ^z	lb/ac	120.4	69	70	66	-4.3	68	68	65	-4.4				
Phosphorus	lb/ac	62	518	518	531	2.5	586	573	568	-3.1				
Potassium	lb/ac	948	1228	1180	1172	-4.6	1408	1230	1276	-9.4				
Calcium	lb/ac	-	9696	9414	6258	-35.5	12972	12358	11916	-8.1				
Sodium	lb/ac	-	132	120	108	-18.2	126	124	108	-14.3				
Magnesium	lb/ac	-	1544	1518	1522	-1.4	1818	1670	1648	-9.4				
Sulfur	ppm	-	32	26	26	-18.8	29	26	28	-3.4				
Silicon	ppm	-	137.3	136.8	133.1	-3.1	125.5	138.7	138.2	10.1				
Boron	ppm	-	1.59	1.69	1.58	-0.6	1.59	1.32	1.44	-9.4				
Iron	ppm	22.6	41	48	43	4.9	54	56	52	-3.7				
Manganese	ppm	18.2	116	120	123	6.0	133	131	127	-4.5				
Copper	ppm	7.6	3.51	3.53	3.42	-2.6	4.06	3.88	3.71	-8.6				
Zinc	ppm	6.2	4.79	4.42	4.49	-6.3	5.08	5	4.61	-9.3				
Aluminum	ppm	-	177	186	170	-4.0	266	272	261	-1.9				
Organic Matter	%	3	2.43	2.5	2.32	-4.5	2.42	2.42	2.26	-6.6				
pH		8.4	8.2	8.1	8.2	0.0	8	8.2	8	0.0				
T.E.C. ^y		-	33.61	32.71	32.26	-4.0	43.57	41.01	39.88	-8.5				

^z Estimated nitrogen release.

^y Total exchange capacity takes soil pH into account.

^x Percent change refers to the difference between the 0 #/ac and 200 #/ac Azomite treatments.

Table A.28. Soil test results before, 26 days after, and 134 days after granulated Azomite treatment applications were made at 0, 100 and 200 #/ac on certified organic agricultural land in Fort Collins, Colorado 117 DAT; 2018.

Element	Unit	Before Application	After Application (26 DAT)				After Application (134 DAT)			
		Clean Field	'Stocky Red Roaster'				'Stocky Red Roaster'			
			0 #/ac	100 #/ac	200 #/ac	% change ^x	0 #/ac	100 #/ac	200 #/ac	% change
Nitrogen ^z	lb/ac	70	70	69	72	-4.3	84	77	78	-7.1
Phosphorus	lb/ac	490	550	545	682	24.0	485	518	518	6.8
Potassium	lb/ac	948	1358	1312	1354	-0.3	930	1194	1094	17.6
Calcium	lb/ac	11788	13444	13916	13466	0.2	9120	10438	9558	4.0
Sodium	lb/ac	206	142	156	160	12.7	126	140	130	3.2
Magnesium	lb/ac	1446	1720	1750	1732	0.7	1272	1534	1382	8.6
Sulfur	ppm	-	36	41	38	5.6	32	32	40	25.0
Silicon	ppm	-	163.47	163.42	162.07	-0.9	137.96	139.89	136.28	-1.2
Boron	ppm	1.55	1.87	1.92	3.12	66.8	1.07	1.23	1.17	9.3
Iron	ppm	30	45	47	54	20.0	27	27	26	-3.7
Manganese	ppm	86	117	120	120	2.6	84	94	86	2.4
Copper	ppm	2.49	3.47	3.64	3.66	5.5	2.48	2.94	2.6	4.8
Zinc	ppm	3.26	4.41	4.57	5.55	25.9	5.37	5.67	5.74	6.9
Aluminum	ppm	175	296	308	304	2.7	193	213	190	-1.6
Organic Matter	%	2.48	2.52	2.47	2.58	2.4	3.43	2.87	2.88	-16.0
pH		8	8.3	8.2	8.3	0.0	8.4	8.2	8.2	-2.4
T.E.C. ^y		38.98	44.2	45.56	44.32	0.3	30.66	35.46	32.37	5.6

^zEstimated nitrogen release.

^yTotal exchange capacity takes soil pH into account.

^xPercent change refers to the difference between the 0 #/ac and 200 #/ac Azomite treatments.

Table A.29. Soil test results before, 26 days after, and 134 days after granulated Azomite treatment applications were made at 0, 100 and 200 #/acre on certified organic agricultural land in Fort Collins, Colorado; 2018.

Element	Unit	Before	After Application (26 DAT)				After Application (134 DAT)			
		Application	'Early Perfect Italian'				'Early Perfect Italian'			
		Clean Field	0	100	200	%	0	100	200	%
		#/ac	#/ac	#/ac	change ^x	#/ac	#/ac	#/ac	change	
Nitrogen ^z	lb/ac	70	71	71	68	-4.2	79	77	75	-5.1
Phosphorus	lb/ac	490	582	550	531	-8.8	522	485	495	-5.2
Potassium	lb/ac	948	1200	1320	1252	4.3	1052	1092	1228	16.7
Calcium	lb/ac	11788	12928	13632	13352	3.3	10194	9564	10058	-1.3
Sodium	lb/ac	206	144	142	138	-4.2	140	128	138	-1.4
Magnesium	lb/ac	1446	1586	1716	1598	0.8	1410	1386	1476	4.7
Sulfur	ppm	-	35	39	35	0.0	34	38	38	11.8
Silicon	ppm	-	163.96	168.84	157.66	-3.8	139.90	140.63	139.51	-0.3
Boron	ppm	1.55	1.59	1.96	2.49	56.6	1.15	1.16	1.27	10.40
Iron	ppm	30	46	45	49	6.5	34	27	26	-23.5
Manganese	ppm	86	112	120	123	9.8	93	89	92	-1.1
Copper	ppm	2.49	3.49	3.39	3.46	-0.9	3.02	2.79	2.84	-6.0
Zinc	ppm	3.26	4.85	4.41	4.33	-10.7	5.73	5.54	5.45	-4.9
Aluminum	ppm	175	284	305	312	9.9	210	200	207	-1.4
Organic Matter	%	2.48	2.56	2.53	2.39	-6.6	2.94	2.86	2.73	-7.1
pH		8	8.2	8.2	8.1	-1.2	8.3	8.1	8.1	-2.4
T.E.C. ^y		38.98	42.13	44.66	43.37	2.9	34.07	32.43	34.3	0.7

^z Estimated nitrogen release.

^y Total exchange capacity takes soil pH into account.

^x Percent change refers to the difference between the 0 #/ac and 200 #/ac Azomite treatments.

Table A.30. Comparison of total variable costs, average fixed costs, total costs, gross revenue, and net revenue range associated with granulated Azomite treatments at 0, 100 and 200 #/ac; 2017-2018 combined.

	0 #/ac		100 #/ac		200 #/ac	
	'SRR' ^v	'EPI' ^u	'SRR'	'EPI'	'SRR'	'EPI'
Seeds and soil	\$467	\$467	\$467	\$467	\$467	\$467
Plastic trays (1006)	\$62	\$62	\$62	\$62	\$62	\$62
Plastic mulch (black)	\$730	\$730	\$730	\$730	\$730	\$730
AZOMITE material	\$0	\$0	\$40	\$40	\$80	\$80
Sowing seeds and transplanting	\$578	\$578	\$578	\$578	\$578	\$578
Irrigation drip tape	\$297	\$297	\$297	\$297	\$297	\$297
Tillage and bed construction	\$507.80	\$507.80	\$507.80	\$507.80	\$507.80	\$507.80
Planting to field	\$1,733	\$1,733	\$1,733	\$1,733	\$1,733	\$1,733
AZOMITE application	\$0	\$0	\$20	\$20	\$20	\$20
Cultivation by hand ^z	\$862	\$862	\$862	\$862	\$862	\$862
Weeding and harvesting	\$2,328	\$2,328	\$2,328	\$2,328	\$2,328	\$2,328
Irrigation water (cost)	\$79	\$79	\$79	\$79	\$79	\$79
Irrigation set-up and labor	\$37	\$37	\$37	\$37	\$37	\$37
Total variable costs	\$7,682	\$7,682	\$7,742	\$7,742	\$7,782	\$7,782
Average fixed costs ^y	\$6,819	\$6,819	\$6,819	\$6,819	\$6,819	\$6,819
Total costs	\$14,501	\$14,501	\$14,561	\$14,561	\$14,601	\$14,601
Gross revenue of pepper sales ^x	\$139,392	\$172,846	\$144,968	\$178,422	\$156,119	\$178,422
Net revenue of pepper sales ^w within 1 standard error	\$124,891 ± 11,151	\$158,345 ± 11,151	\$130,407 ± 16,727	\$163,861 ± 16,727	\$141,518 ± 11,151	\$163,821 ± 11,151

^z \$12/hour for labor, hand-weeding and stirrup cultivation.

^y Average fixed costs from 2012 in 2018 dollars.

^x \$3.20/lb. for organic roasting peppers for northern Colorado farmers' markets

^w Based on one standard error about the mean.

^v SRR is an abbreviation for the pepper cultivar 'Stocky Red Roaster.

^u EPI is an abbreviation for the pepper cultivar 'Early Perfect Italian'.

Table A.31. Fertigation treatments, foliar treatments, pounds per acre of Azomite applied, and the treatment key for the ultrafine AZOMITE high tunnel experiment occurring on certified organic agricultural land in Fort Collins, Colorado; 2018.

Treatment key	Fertigation treatments	Foliar treatments	Pounds/acre of AZOMITE
AZO-AT	Ultrafine AZOMITE (AZO)	Ultrafine AZOMITE foliar treatment (AT)	50#/acre
AZO-WT	Ultrafine AZOMITE (AZO)	Water foliar treatment (WT)	25#/acre
AZO-NT	Ultrafine AZOMITE (AZO)	No foliar treatment (NT)	25#/acre
Water-AT	Water irrigation only (Water)	Ultrafine AZOMITE foliar treatment (AT)	25#/acre
Water-WT	Water irrigation only (Water)	Water foliar treatment (WT)	0#/acre
Water-NT	Water irrigation only (Water)	No foliar treatment (NT)	0#/acre

Table A.32. Comparison of ultrafine Azomtie treatments on total yield, red pod yield, total number of marketable pods, and number of red pods produced on a per plant basis as well as number of days to flowering, flower bud count 85 days after transplanting, and °Brix for ‘Early Perfect Italian’ peppers on certified organic agricultural land in Fort Collins, Colorado; 2018 combined high tunnels.

		AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Red pod yield (pounds/plant) ^{ns}	mean	0.6	0.2	0.8	0.3	0.5	0.7
	se	0.2	0.1	0.2	0.1	0.2	0.3
Total number of pods/plant ^{ns}	mean	16.2	14.7	16.6	13.5	14.9	15.3
	se	1.4	1.8	1.4	1.6	3.0	1.9
Number of red pods/plant ^{ns}	mean	4.8	2.4	5.5	3.2	4.6	4.9
	se	0.8	1.0	1.3	1.6	1.8	2.3
Number of DAT to flowering ^{ns}	mean	37.7	39.8	39.2	41.5	40.8	38.7
	se	2.2	3.7	3.9	2.4	2.6	3.4
Flower bud count 85 DAT ^{ns}	mean	15.4	14.1	16.8	12.7	12.1	15.6
	se	2.3	1.4	3.7	2.1	2.1	2.1

^{ns} Indicates that the means are not statistically significantly different ($\alpha = 0.05$) according to Tukey’s honestly significant difference test. Standard errors about the mean are presented (n = 3).

Table A.33. Leaf tissue test results for ‘Early Perfect Italian’ for six ultrafine Azomite treatments from in covered high tunnel 53 DAT; 2018.

		AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen	%	3.56	3.16	3.50	2.86	2.82	3.06
Phosphorus	%	0.25	0.24	0.27	0.27	0.27	0.25
Potassium	%	3.78	3.70	4.05	3.68	4.13	3.90
Calcium	%	1.46	1.57	1.80	1.33	1.72	1.84
Magnesium	%	0.36	0.37	0.43	0.34	0.41	0.41
Sulfur	%	0.43	0.42	0.46	0.31	0.41	0.40
Silicon	%	0.18	0.17	0.19	0.19	0.19	0.22
Boron	ppm	19.70	21.10	22.30	20.30	22.00	21.00
Iron	ppm	86.20	91.40	102.00	101.00	108.00	111.00
Manganese	ppm	31.00	32.50	35.80	33.80	34.70	36.40
Copper	ppm	7.20	6.60	7.10	5.60	6.20	6.30
Zinc	ppm	47.10	53.20	56.50	53.60	61.40	58.00
Aluminum	ppm	58.70	68.50	86.90	85.90	92.80	108.00

Table A.34. Leaf tissue test results for ‘Early Perfect Italian’ for six ultrafine Azomite treatments in an uncovered high tunnel, 53 DAT; 2018.

		AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen	%	4.31	4.18	4.16	4.68	4.24	4.26
Phosphorus	%	0.29	0.31	0.28	0.33	0.35	0.31
Potassium	%	3.88	4.18	4.14	4.65	4.41	4.55
Calcium	%	1.72	1.68	1.72	1.84	1.82	2.10
Magnesium	%	0.52	0.51	0.51	0.59	0.55	0.56
Sulfur	%	0.43	0.45	0.47	0.47	0.46	0.54
Silicon	%	0.29	0.23	0.21	0.30	0.31	0.27
Boron	ppm	21.50	25.80	24.90	25.70	29.20	28.40
Iron	ppm	84.70	88.40	84.90	87.80	96.80	101.00
Manganese	ppm	37.90	41.20	41.80	51.40	47.10	49.90
Copper	ppm	7.00	7.70	7.50	8.80	9.10	8.20
Zinc	ppm	42.40	48.70	50.80	55.20	59.90	50.90
Aluminum	ppm	52.30	59.40	60.00	54.60	68.30	81.80

Table A.35. Leaf tissue test results for ‘Stocky Red Roaster’ for six ultrafine Azomite treatments in a covered high tunnel 53 DAT; 2018.

		AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen	%	3.53	-	3.99	2.81	3.19	3.14
Phosphorus	%	0.30	-	0.27	0.28	0.28	0.26
Potassium	%	3.94	-	3.68	3.97	3.59	3.52
Calcium	%	1.35	-	1.28	1.75	1.38	1.32
Magnesium	%	0.38	-	0.38	0.42	0.35	0.36
Sulfur	%	0.37	-	0.33	0.41	0.32	0.33
Silicon	%	0.17	-	0.22	0.19	0.22	0.20
Boron	ppm	19.90	-	18.30	23.60	19.40	18.20
Iron	ppm	94.90	-	89.80	116.00	99.60	93.40
Manganese	ppm	36.00	-	32.70	35.80	33.40	34.00
Copper	ppm	7.70	-	7.20	6.40	6.80	6.60
Zinc	ppm	51.60	-	45.20	63.90	55.10	53.20
Aluminum	ppm	75.90	-	71.10	102.00	82.70	80.80

Table A.36. Leaf tissue test results for ‘Stocky Red Roaster’ for six ultrafine Azomite treatments in an uncovered high tunnel, 53 DAT; 2018.

		AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen	%	4.52	4.43	4.46	4.47	4.84	4.29
Phosphorus	%	0.34	0.31	0.32	0.34	0.38	0.32
Potassium	%	4.26	3.80	3.84	4.44	4.27	4.37
Calcium	%	1.62	1.56	1.53	1.79	1.68	1.72
Magnesium	%	0.52	0.47	0.50	0.63	0.55	0.54
Sulfur	%	0.41	0.37	0.38	0.41	0.44	0.41
Silicon	%	0.30	0.22	0.23	0.21	0.27	0.21
Boron	ppm	23.90	22.80	21.40	25.30	26.60	26.50
Iron	ppm	97.90	84.10	84.90	82.20	89.00	86.40
Manganese	ppm	41.80	37.40	44.00	54.80	51.00	48.10
Copper	ppm	9.60	7.60	8.70	8.70	10.10	8.60
Zinc	ppm	53.50	47.80	51.50	58.20	53.50	55.70
Aluminum	ppm	57.80	53.30	60.60	64.00	53.10	57.00

Table A.37. Leaf tissue test results for ‘Stocky Red Roaster’ for six ultrafine Azomite treatments in a covered high tunnel 109 DAT; 2018.

		AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen	%	2.63	2.62	2.61	2.36	2.45	2.41
Phosphorus	%	0.27	0.34	0.26	0.47	0.37	0.31
Potassium	%	3.88	4.14	4.07	3.73	3.94	3.92
Calcium	%	3.44	3.66	3.66	3.44	3.67	3.75
Magnesium	%	0.62	0.59	0.63	0.59	0.62	0.66
Sulfur	%	0.55	0.49	0.55	0.47	0.53	0.61
Silicon	%	0.07	0.07	0.06	0.07	0.06	0.06
Boron	ppm	65.00	68.00	59.80	64.00	67.00	67.40
Iron	ppm	150.00	160.00	177.00	181.00	242.00	175.00
Manganese	ppm	51.00	54.00	52.80	51.80	56.80	53.80
Copper	ppm	8.40	8.70	9.90	8.70	9.70	9.10
Zinc	ppm	55.00	64.80	57.70	75.10	70.40	63.30
Aluminum	ppm	167.00	167.00	203.00	184.00	272.00	201.00

Table A.38. Leaf tissue test results for ‘Stocky Red Roaster’ for six ultrafine Azomite treatments in an uncovered high tunnel 109 DAT; 2018.

		AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen	%	3.80	3.90	4.05	4.02	4.11	3.93
Phosphorus	%	0.20	0.19	0.17	0.19	0.22	0.19
Potassium	%	4.36	4.37	4.26	4.35	4.66	4.49
Calcium	%	3.61	3.17	3.08	3.39	3.34	3.23
Magnesium	%	0.76	0.69	0.80	0.75	0.82	0.73
Sulfur	%	0.46	0.41	0.43	0.40	0.44	0.45
Silicon	%	0.06	0.06	0.06	0.05	0.05	0.04
Boron	ppm	46.50	37.80	33.20	35.90	46.60	35.60
Iron	ppm	123.00	99.80	93.00	101.00	106.00	92.90
Manganese	ppm	45.10	41.20	47.30	43.70	47.30	43.50
Copper	ppm	5.10	5.20	5.10	5.60	6.10	4.90
Zinc	ppm	34.10	34.10	30.50	28.70	33.50	26.50
Aluminum	ppm	117.00	90.20	83.10	97.00	101.00	87.50

Table A.39. Soil test results for ‘Early Perfect Italian’ for six ultrafine Azomite treatments in an uncovered high tunnel 55 DAT; 2018.

		Clean Field	AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen ^z	lb/ac	83	87	92	90	89	93	85
Phosphorus	lb/ac	1214	820	884	921	788	751	714
Potassium	lb/ac	2522	1302	1376	1194	1180	1116	956
Calcium	lb/ac	10996	10778	11174	10450	10606	11014	10906
Sodium	lb/ac	260	278	250	224	234	206	184
Magnesium	lb/ac	1378	1248	1260	1132	1134	1140	1170
Sulfur	ppm	87	37	28	24	28	23	22
Silicon	ppm	-	127	123	127	125	126	132
Boron	ppm	1	1	1	1	1	1	1
Iron	ppm	75	61	66	57	52	54	54
Manganese	ppm	69	81	73	74	80	78	78
Copper	ppm	4	4	4	4	4	4	4
Zinc	ppm	12	9	9	9	8	8	8
Aluminum	ppm	101	148	187	90	121	192	200
Organic Matter	%	3	4	4	4	4	4	3
pH		8	8	8	8	8	8	8
T.E.C. ^y		38	36	37	34	34	35	35

^zEstimated nitrogen release.

^yTotal exchange capacity takes soil pH into account.

Table A.40. Comparison of ultrafine Azomite treatments on total yield, red pod yield, and number of red pods produced on a per plant basis as well as number of days to flowering, flower bud count 85 days after transplanting, and °Brix for ‘Stocky Red Roaster’ peppers on certified organic agricultural land in Fort Collins, Colorado; 2018 combined high tunnels.

		AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Total yield (pounds/plant) ^{ns}	mean	1.7	1.9	2.2	1.7	1.9	1.9
	se	0.3	0.2	0.3	0.4	0.3	0.2
Red pod yield (pounds/plant) ^{ns}	mean	0.6	0.6	0.6	0.5	0.6	0.6
	se	0.3	0.3	0.3	0.2	0.3	0.2
Number of red pods/plant ^{ns}	mean	5.2	5.5	6.2	4.7	4.9	6.1
	se	1.5	1.5	1.8	0.6	1.7	1.1
Number of DAT to flowering ^{ns}	mean	37.0	36.5	36.8	35.3	38.3	36.8
	se	2.8	1.9	1.8	1.3	2.5	1.8
Flower bud count 85 DAT ^{ns}	mean	14.5	14.6	14.4	10.5	14.4	11.2
	se	2.4	2.6	2.7	1.5	1.4	2.4
°Brix ^{ns}	mean	8.2	8.1	8.3	8.3	7.8	8.3
	se	0.3	0.1	0.2	0.2	0.3	0.2

^{ns} Indicates that the means are not statistically significantly different ($\alpha = 0.05$) according to Tukey’s honestly significant difference test. Standard errors about the mean are presented (n = 3).

Table A.41. Soil test results for ‘Stocky Red Roaster’ for six ultrafine Azomite treatments in a covered high tunnel 55 DAT; 2018.

		Clean Field	AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen ^z	lb/ac	96	84	85	82	89	87	90
Phosphorus	lb/ac	1040	1012	1195	971	1090	994	1200
Potassium	lb/ac	1496	1748	2130	1596	2104	1952	2334
Calcium	lb/ac	76	12210	11138	10846	10792	10336	11582
Sodium	lb/ac	254	218	196	174	218	186	268
Magnesium	lb/ac	1218	1402	1426	1350	1376	1300	1532
Sulfur	ppm	37	28	28	24	28	25	32
Silicon	ppm	-	130	132	133	129	132	135
Boron	ppm	1	1	1	1	1	1	2
Iron	ppm	58	86	94	68	74	75	65
Manganese	ppm	70	86	80	82	79	78	82
Copper	ppm	3	5	5	4	4	4	4
Zinc	ppm	11	11	12	9	9	8	10
Aluminum	ppm	118	225	225	205	169	130	193
Organic Matter	%	5	3	4	3	4	4	4
pH		8	8	8	8	8	8	8
T.E.C. ^y		37	40	38	36	37	35	40

^z Estimated nitrogen release.

^y Total exchange capacity takes soil pH into account.

Table A.42. Soil test results for ‘Stocky Red Roaster’ for six ultrafine Azomite treatments in an uncovered high tunnel 55 DAT; 2018.

		Clean Field	AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen ^z	lb/ac	83	88	85	90	88	90	86
Phosphorus	lb/ac	1214	811	788	907	765	788	756
Potassium	lb/ac	2522	1290	1228	1488	1304	1226	1208
Calcium	lb/ac	10996	11118	10526	11174	11166	10698	77
Sodium	lb/ac	260	260	224	286	250	226	236
Magnesium	lb/ac	1378	1298	1156	1280	1216	1196	1336
Sulfur	ppm	87	31	27	32	28	27	30
Silicon	ppm	-	126	123	123	126	129	130
Boron	ppm	1	1	1	1	1	1	1
Iron	ppm	75	56	61	54	50	53	51
Manganese	ppm	69	80	75	76	90	79	85
Copper	ppm	4	4	4	4	4	4	4
Zinc	ppm	12	9	8	9	9	8	9
Aluminum	ppm	101	195	123	181	193	138	204
Organic Matter	%	3	4	4	4	4	4	4
pH		8	8	8	8	8	8	8
T.E.C. ^y		38	37	34	37	36	35	38

^zEstimated nitrogen release.^yTotal exchange capacity takes soil pH into account.

Table A.43. Soil test results for ‘Early Perfect Italian’ for six ultrafine Azomite treatments in a covered high tunnel 126 DAT; 2018.

		AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen ^z	lb/ac	83	93	88	88	85	86
Phosphorus	lb/ac	724	719	870	852	687	751
Potassium	lb/ac	896	986	1062	1136	808	924
Calcium	lb/ac	9398	8986	9492	9928	9392	10168
Sodium	lb/ac	162	164	182	188	164	172
Magnesium	lb/ac	1066	1002	1094	1088	1010	1136
Sulfur	ppm	31	32	33	32	27	31
Silicon	ppm	115	113	115	115	115	116
Boron	ppm	1	1	1	1	1	1
Iron	ppm	50	50	46	46	40	42
Manganese	ppm	69	62	69	70	68	79
Copper	ppm	3	3	4	3	3	4
Zinc	ppm	9	9	11	11	10	10
Aluminum	ppm	167	139	154	158	162	180
Organic Matter	%	3	4	4	4	4	4
pH		8	8	8	8	8	8
T.E.C. ^y		30	29	31	32	30	33

^zEstimated nitrogen release.

^yTotal exchange capacity takes soil pH into account.

Table A.44. Soil test results for ‘Early Perfect Italian’ for six ultrafine Azomite treatments in an uncovered high tunnel 126 DAT; 2018.

		Clean Field	AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen ^z	lb/ac	83	85	83	87	85	80	85
Phosphorus	lb/ac	1214	838	1365	1113	998	834	1227
Potassium	lb/ac	2522	1456	1532	1698	1854	1668	1812
Calcium	lb/ac	10996	9252	8950	9556	9062	8994	9088
Sodium	lb/ac	260	162	150	258	184	180	178
Magnesium	lb/ac	1378	1272	1168	1290	1194	1218	1222
Sulfur	ppm	87	40	37	47	36	37	41
Silicon	ppm		120	120	121	117	121	122
Boron	ppm	1	1	1	1	1	1	1
Iron	ppm	75	51	59	67	62	47	52
Manganese	ppm	69	64	62	68	65	71	71
Copper	ppm	4	4	3	4	3	3	3
Zinc	ppm	12	10	12	11	10	9	9
Aluminum	ppm	101	164	150	160	152	155	152
Organic Matter	%	3	4	3	4	3	3	4
pH		8	8	8	8	8	8	8
T.E.C. ^y		38	32	31	33	31	31	32

^zEstimated nitrogen release.

^yTotal exchange capacity takes soil pH into account.

Table A.45. Soil test results for ‘Stocky Red Roaster’ for six ultrafine Azomite treatments in a covered high tunnel 126 DAT; 2018.

		AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen ^z	lb/ac	87	88	89	89	89	84
Phosphorus	lb/ac	769	737	751	710	710	696
Potassium	lb/ac	1002	914	1064	1102	882	812
Calcium	lb/ac	9552	9246	9622	9748	9600	9016
Sodium	lb/ac	176	178	170	164	160	156
Magnesium	lb/ac	1066	980	1054	1088	1014	1002
Sulfur	ppm	32	32	31	32	31	32
Silicon	ppm	118	114	111	114	111	115
Boron	ppm	1	1	1	1	1	1
Iron	ppm	43	49	42	38	38	39
Manganese	ppm	69	64	68	71	71	68
Copper	ppm	3	3	3	3	3	3
Zinc	ppm	10	10	9	10	9	10
Aluminum	ppm	157	153	155	155	164	158
Organic Matter	%	4	4	4	4	4	3
pH		8	8	8	8	8	8
T.E.C. ^y		31	30	31	32	31	29

^z Estimated nitrogen release.

^y Total exchange capacity takes soil pH into account.

Table A.46. Soil test results for ‘Stocky Red Roaster’ for six ultrafine Azomite treatments in an uncovered high tunnel 126 DAT; 2018.

		Clean Field	AZO-NT	AZO-WT	AZO-AT	Water-NT	Water-WT	Water-AT
Nitrogen ^z	lb/ac	83	88	85	90	88	90	86
Phosphorus	lb/ac	1214	811	788	907	765	788	756
Potassium	lb/ac	2522	1290	1228	1488	1304	1226	1208
Calcium	lb/ac	10996	11118	10526	11174	11166	10698	77
Sodium	lb/ac	260	260	224	286	250	226	236
Magnesium	lb/ac	1378	1298	1156	1280	1216	1196	1336
Sulfur	ppm	87	31	27	32	28	27	30
Silicon	ppm	-	126	123	123	126	129	130
Boron	ppm	1	1	1	1	1	1	1
Iron	ppm	75	56	61	54	50	53	51
Manganese	ppm	69	80	75	76	90	79	85
Copper	ppm	4	4	4	4	4	4	4
Zinc	ppm	12	9	8	9	9	8	9
Aluminum	ppm	101	195	123	181	193	138	204
Organic Matter	%	3	4	4	4	4	4	4
pH		8	8	8	8	8	8	8
T.E.C. ^y		38	37	34	37	36	35	38

^zEstimated nitrogen release.^yTotal exchange capacity takes soil pH into account.

Table A.47. Comparison of total variable costs, average fixed costs, total costs, gross revenue, and net revenue range associated with six ultrafine Azomite treatments on ‘Early Perfect Italian’ from both high tunnels; 2018.

	AZO-AS	AZO-WS	AZO-NS	Water-AS	Water-WS	Water-NS
Seeds and soil	\$467.46	\$467.46	\$467.46	\$467.46	\$467.46	\$467.46
Plastic trays (1006)	\$62.01	\$62.01	\$62.01	\$62.01	\$62.01	\$62.01
Plastic mulch (black)	\$729.64	\$729.64	\$729.64	\$729.64	\$729.64	\$729.64
Azomite fertigation (materials)	\$37.50	\$37.50	\$37.50	\$0.00	\$0.00	\$0.00
Azomite foliar (materials)	\$37.50	\$0.00	\$0.00	\$37.50	\$0.00	\$0.00
Sowing seeds and transplanting	\$577.81	\$577.81	\$577.81	\$577.81	\$577.81	\$577.81
Irrigation drip tape	\$296.85	\$296.85	\$296.85	\$296.85	\$296.85	\$296.85
Tillage and bed construction	\$507.80	\$507.80	\$507.80	\$507.80	\$507.80	\$507.80
Planting to field	\$1,733.42	\$1,733.42	\$1,733.42	\$1,733.42	\$1,733.42	\$1,733.42
Azomite application (injector)	\$61.60	\$61.60	\$61.60	\$0.00	\$0.00	\$0.00
Azomite application (foliar spray)	\$20.00	\$20.00	\$0.00	\$20.00	\$20.00	\$0.00
Cultivation by hand ^z	\$862.40	\$862.40	\$862.40	\$862.40	\$862.40	\$862.40
Weeding and harvesting by hand	\$2,328.48	\$2,328.48	\$2,328.48	\$2,328.48	\$2,328.48	\$2,328.48
Irrigation water (cost)	\$78.90	\$78.90	\$78.90	\$78.90	\$78.90	\$78.90
Additional water for foliar treatments	\$0.01	\$0.01	\$0.00	\$0.01	\$0.01	\$0.00
Irrigation set-up and labor	\$36.96	\$36.96	\$36.96	\$36.96	\$36.96	\$36.96
Total variable costs	\$7,730.55	\$7,693.05	\$7,673.05	\$7,631.45	\$7,593.95	\$7,573.95
Average fixed costs ^y	\$6,819.28	\$6,819.28	\$6,819.28	\$6,819.28	\$6,819.28	\$6,819.28
Total costs	\$14,549.83	\$14,512.33	\$14,492.33	\$14,450.73	\$14,413.23	\$14,393.23
Gross revenue of pepper sales ^x	\$153,609.95	\$129,820.40	\$142,086.89	\$139,577.84	\$125,638.64	\$107,517.68
Net revenue of all pepper sales ^w	\$139,060 ± 17,299	\$115,308 ± 23,474	\$127,595 ± 21,184	\$125,127 ± 21,644	\$111,225 ± 31,214	\$93,124 ± 14,759

^z \$12/hour for labor, hand-weeding and stirrup cultivation.

^y Average fixed costs from 2012 in 2018 dollars.

^x \$3.20/lb. for organic roasting peppers for northern Colorado farmers’ markets.

^w One standard error about the mean.

Table A.48. Comparison of total variable costs, average fixed costs, total costs, gross revenue, and net revenue range associated with six ultrafine Azomite treatments on ‘Stocky Red Roaster’ from both high tunnels; 2018.

	AZO-AS	AZO-WS	AZO-NS	Water-AS	Water-WS	Water-NS
Seeds and soil	\$467.46	\$467.46	\$467.46	\$467.46	\$467.46	\$467.46
Plastic trays (1006)	\$62.01	\$62.01	\$62.01	\$62.01	\$62.01	\$62.01
Plastic mulch (black)	\$729.64	\$729.64	\$729.64	\$729.64	\$729.64	\$729.64
Azomite fertigation (materials)	\$37.50	\$37.50	\$37.50	\$0.00	\$0.00	\$0.00
Azomite foliar(materials)	\$37.50	\$0.00	\$0.00	\$37.50	\$0.00	\$0.00
Sowing seeds and transplanting	\$577.81	\$577.81	\$577.81	\$577.81	\$577.81	\$577.81
Irrigation drip tape	\$296.85	\$296.85	\$296.85	\$296.85	\$296.85	\$296.85
Tillage and bed construction	\$507.80	\$507.80	\$507.80	\$507.80	\$507.80	\$507.80
Planting to field	\$1,733.42	\$1,733.42	\$1,733.42	\$1,733.42	\$1,733.42	\$1,733.42
Azomite application (injector)	\$61.60	\$61.60	\$61.60	\$0.00	\$0.00	\$0.00
Azomite application (foliar)	\$20.00	\$20.00	\$0.00	\$20.00	\$20.00	\$0.00
Cultivation by hand ^z	\$862.40	\$862.40	\$862.40	\$862.40	\$862.40	\$862.40
Weeding and harvesting by hand	\$2,328.48	\$2,328.48	\$2,328.48	\$2,328.48	\$2,328.48	\$2,328.48
Irrigation water (cost)	\$78.90	\$78.90	\$78.90	\$78.90	\$78.90	\$78.90
Additional water for foliar treatments	\$0.01	\$0.01	\$0.00	\$0.01	\$0.01	\$0.00
Irrigation set-up and labor	\$36.96	\$36.96	\$36.96	\$36.96	\$36.96	\$36.96
Total variable costs	\$7,838.34	\$7,800.84	\$7,780.83	\$7,739.24	\$7,701.74	\$7,681.73
Average fixed costs ^y	\$6,819.28	\$6,819.28	\$6,819.28	\$6,819.28	\$6,819.28	\$6,819.28
Total costs	\$14,657.62	\$14,620.12	\$14,600.11	\$14,558.52	\$14,521.02	\$14,501.01
Gross revenue of pepper sales ^x	\$120,899.31	\$107,703.57	\$93,485.59	\$105,752.08	\$106,216.70	\$94,972.40
Net revenue from all pepper sales ^w	\$106,242	\$93,083	\$78,885	\$91,194	\$91,696	\$80,471
	± \$17,664	± \$13,026	± \$14,692	± \$10,817	± \$17,113	± \$19,521

^z \$12/hour for labor, hand-weeding and stirrup cultivation.

^y Average fixed costs from 2012 in 2018 dollars.

^x \$3.20/lb. for organic roasting peppers for northern Colorado farmers’ markets.

^w One standard error about the mean.