Quality Changes in Grafted Pepper (Capsicum annumm L.) Scion Fruit

THESIS

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By

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Abstract

Root systems are known to have profound influences on nearly every aspect of plant development and biology, including vegetative and reproductive capacity. However, the specific impacts of intra-specific combinations of root and shoot systems, when combined in physical hybrids, on important physical, chemical, and sensory properties of *Capsicum annum* pepper fruit are largely unknown. Grafting was used to combine the canopy and root system of two types of *Capsicum* plants (producers and non-producers of capsaicin and small, more elongated versus large, blocky fruit), thereby making it possible to examine the separate and combined roles of variety-specific roots and shoots in shaping key fruit characteristics, among them the concentration of capsaicin. Capsaicin is an ideal metabolite to study root-shoot interaction and roles because early stages of its biosynthetic pathway occur in the roots, with final assembly in the fruit at advanced stages of development. Fruit size, shape, wall thickness, and soluble solids levels were similarly tracked as they and capsaicin influence consumer acceptability and fruit marketability. The overall program involved field studies in 2016 and 2017, a targeted wintertime greenhouse study, and consumer sensory analysis. Overall, it was found that the *Capsicum* variety supplying the root system of the grafted plant had little influence on the variables measured when a sweet pepper was used as a scion. When a hot pepper was used as a scion the root system played a large role in influencing the capsaicinoid profile of the fruit. Implications of this finding include: a) that the variables measured are influenced by more than the root systems used here and b) that it may possible to employ rootstock-scion combinations without concern over rootstock influence on fruit in commercial production.
This thesis is dedicated to my father James Fisk
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Chapter 1: Introduction

The influence of the root system on specific physical and chemical characteristics of fruit are not well characterized in Capsicum. The major functions of roots (e.g., transport, regulation, storage, metabolite production, etc.) make it reasonable to hypothesize that the root system of a variety may influence fruit characteristics such as sugar or acid content, shape, or metabolite levels and that this impact may vary according to the variety of the shoot to which it is attached. Grafting was used as a tool to create physical hybrids by combining the canopy (scion) and the root system (rootstock) of two pepper plants in order to examine whether specific fruit characteristics are impacted by the genotype of the root system. Capsaicin and related alkaloid metabolites are ideal compounds to study the root systems’ impact on the metabolic profile of fruit because precursors of the compound are produced in the roots but the final step which produces capsaicin occurs in the fruit itself. Combining the roots and shoots of Capsicum varieties known to produce fruit differing in size, shape, capsaicin concentration, and other properties could result in a spectrum of possible outcomes. Imbedded on one end was observing little difference in fruit characteristics between non-grafted control plants and plants with grafted root systems and on the other was significant changes in nearly every characteristic. Regardless of outcome, the test was positioned to provide important information to scientists and practicing horticulturalists. Large changes in characteristics as a function of root system origin would require that root-shoot interactions in pepper and the use of grafted pepper plants be examined more carefully. No change may allow for selecting varieties for use in production relying on grafted plants without concern over how specific rootstock-scion combinations may impact fruit acceptability. This program tested the extent to which rootstock-scion combination influenced Capsicum fruit characteristics, using varieties with different fruit traits as experimental stock and
physical (e.g., shape, weight, lobe count, etc.), chemical (e.g., Brix, acids, color, capsaicinoids and capsinoids), and sensory variables as indicators. The overall program involved field studies in 2016 and 2017 followed by fruit quality analysis. The first field study focused on the physical and chemical fruit quality examination of a diverse set of grafts between sweet and hot cultivars. The second field study focused on the sensory quality analysis of the sweet on hot graft combinations using discriminatory and preference testing. The field studies were followed by a greenhouse experiment examining the capsaicinoid profiles in grafts between hot and sweet cultivars.
Chapter 2- Literature Review

Fruit Quality

What is Fruit Quality

Quality as it relates to a horticultural crop differs depending on the role of the person (ie. grower, consumer, etc.). Growers often focus on the size and weight of the fruit due to the fact that their revenue is based on that; meanwhile consumers will focus more on the sensory aspects of the fruit (flavor). Quality to a consumer, other than flavor (taste and aroma) consists of fruit properties which conform with preconceived notions. The attributes of fruit and vegetable quality important to consumers are based on the content of health beneficial metabolites, convenience (where and how quickly the food can be consumed), and the freshness (Schreiner et al., 2013). Overall the quality of fruiting vegetables themselves encapsulates aspects of physical characteristics, flavor, and health-related compound content (Rouphael et al., 2010).

Fruit Quality in Pepper

General fruit quality traits of a bell pepper include fruit weight, length, color and thickness of the flesh (Donas-Ucles et al., 2014). Bell peppers also contain an array of health beneficial compounds such as vitamins C, B, and E, capsaicinoids, capsinoids, phenolics, and carotenoids (De et al., 2003; Carvalho et al., 2015; Jarret et al., 2007). Variability in health-beneficial compound levels across the USDA germplasm collections is extensive (Antonious et al., 2006) and the concentrations of specific compounds in fruits can depend on cultivar and location (Lee et al., 2005), or on the ripening stage of the pepper (Loizzo et al., 2008).
Both the physical and chemical properties of bell pepper fruit listed above are important quality parameters. For instance, studies conducted on processed peppers focus on the content of vitamin C as the main antioxidant and the physical traits of color, appearance, and firmness (Vega-Galvez et al., 2008). In addition to physical and chemical quality, sensory factors, such as texture and flavor are important in determining overall quality from a consumer standpoint (Harker et al., 2003). The flavor of a bell pepper is defined primarily by the taste and the aroma. Important taste quality parameters in pepper are the sweet/sour taste, which can be linked to secondary compounds in the fruit (Eggink et al., 2011; Luning et al., 1994a; Luning et al., 1994b; Govindarajan et al., 1985).

Some secondary metabolites such as carotene, ascorbic acid, and vitamin A have been found to influence the quality of pepper due to their impact on the health beneficial properties of the fruit (Howard et al., 1994). Peppers also contain a unique family of secondary metabolites, capsaicinoids and capsinoids, composed of pungent and non-pungent alkaloid compounds respectively. Sweet peppers contain primarily the non-pungent capsinoids, while hot peppers contain both pungent capsaicinoids and capsinoids.

Hot pepper fruit quality is influenced by fruit size and phytochemical components such as flavonoids and capsaicinoids (Butcher et al., 2012). The presence and level of pungency in peppers is a sensory characteristic that is of particular interest to consumers (Butcher et al., 2012; Eggink et al., 2011; Govindarajan et al., 1985). Pungent secondary products in pepper may influence two key factors of pepper quality: consumer acceptability and health beneficial properties.
Capsaicin and Capsiate

What are they?

The alkaloids capsaicin and dihydrocapsaicin, are the main pungent compounds in peppers (De et al., 2003). Capsaicinoids are most concentrated in the vacuole of the placental tissue cells as well as the blisters on the inner pepper walls, increasing as the fruit matures. They are synthesized directly from two precursors, an acid amide (i.e., vanillylamine) and a nine to 11 chained carbon fatty acid (Johnson et al., 1996; Kim et al., 2009) (Figure 2.1). Bell peppers and other sweet peppers mainly produce, capsiate and dihydrocapsiate, alkaloids that are non-pungent as well as trace amounts of capsaicinoids (Rowland et al., 1983). These secondary metabolites are produced from similar precursors as capsaicin a vanillyl alcohol and a fatty acid. Both alkaloids are dependent on the valine and phenylalanine pathways to supply the starting compounds for the production of precursors (Sutoh et al., 2006; Baas-Espinola et al., 2016). Currently it is unclear which plays a larger role in determining the final concentration of capsaicin (Kobata et al., 2011; Castro-Concha et al., 2016; Thiele et al., 2008; Baas-Espinola et al., 2016; Narasimha et al., 2006; Prasad et al., 2006).

Beneficial Properties

These metabolites, similar to other secondary metabolites have a number of health beneficial properties. Capsaicinoids have been found to have many medical applications such as analgesia, anticancer, anti-inflammatory, antioxidative, anti-obesity, fatigue reduction, dermatological disorders, aid digestive health, prevent ulcers, combat cardiovascular disease, and combat elevated blood sugar (Anandakumar et al., 2012; Baboota et al., 2014; Hsu et al., 2007;
Hsu et al., 2016; Ibrahim et al., 2015; M et al., 2015; Maihofner et al., 2014; Mankowski et al., 2017; Patwardhan et al., 2015; Srinivasan et al., 2016; Sun et al., 2016; Treatment, 2017; Tsi et al., 2003; Yuan et al., 2016). The non-pungent capsinoids have a large number of health beneficial compounds similar to their pungent counterparts such as analgesic, lipid oxidation, antitumor, and antioxidant (Josse et al., 2010; Kawabata et al., 2009; Ohyama et al., 2015; Luo et al., 2011; Rosa et al., 2002; Yoneshiro et al., 2012; Barbero et al., 2016). These metabolites not only aid in improving human health but they also give the pepper plant adaptive advantages.

**Plant Benefits and Environmental Production Impacts**

The production of these precursors are influenced by both the environment and genotype of the plant. Pungency has arisen in pepper cultivars in order to give pepper plants an evolutionary advantage when challenged with certain biotic stresses. For instance pungency is beneficial in high moisture environments due to the metabolites ability to combat fungal disease, however under low water conditions pungency leads to a decrease in seed count (Hakk et al., 2012). Stress from low soil moisture can also increase the amount of capsaicin in a pepper with the increase depending on the original pungency level in the pepper (Phimchan et al., 2012; Ruiz-Lau et al., 2011). Temperature has a variable effect on capsaicin production, which, depends on the genotype of the plant (Gonzalez-Zamora et al., 2013). The environment influences the level of secondary metabolites, however, the genotype of the plant plays a larger role in determining the amount of metabolites produced (Meckelmann et al., 2015; Zewdie et al., 2000).
Genetic Influences

Cultivars develop differently as the fruit matures; 40-60 days after formation some cultivars decrease capsaicinoid content while others maintain capsaicinoid levels (Rosa et al., 2002). Ten genes (Phenylalanine ammonia-lyase (PAL), cinnamatic 4-hydroxylase (C4H), 4-Coumarate: Coenzyme A Ligase (4CL), hydroxycinnamoyl transferase (HCT), coumarate-3-hydroxylase (C3H), caffeoyl-CoA 3-O-methyltransferase (CCoAOMT), putative aminotransferase (p-AMT), acyl-transferase (AT), Kas, and FAO13 are known to regulate capsaicin synthesis, another 20 candidate genes have been identified to be related to synthesis (Han et al., 2013). Of the ten genes the genes of Pun1, p-AMT, KAS, and BCAT are strongly upregulated in placental tissue, the area in which capsaicin is produced (Tanaka et al., 2017). The vanillylamine precursor is synthesized from the phenylpropanoid pathway which starts with phenylalanine and involves the enzymes PAL, C4H, 4CL, HCT, C3H, COMT, HCHL, and p-AMT (Figure 2.2). The branched fatty acid is synthesized from the valine pathway which starts with valine and involves the enzymes BCAT, KAS, ACL, FAT, ACS (Aza-Gonzalez et al., 2011). Of the genes located in the placental tissue Pun1 and p-AMT are most important genes due to their roles in producing the final product and in determining the amount of precursor produced.

Pun1 and p-AMT

Pun1 controls the synthesis of both capsaicinoids and capsinoids (Kobata et al., 2013; Han et al., 2013; Ogawa et al., 2015). The Pun1 locus in the pepper genome has two allelic forms that determines which secondary metabolite is produced. Sweet peppers have a series of recessive alleles at this locus and hot peppers a dominant one (Stewart et al., 2007). A recessive
copy of this gene has a deletion in the promoter and first exon region leading to a loss of pungency (Stewart et al., 2005). Several recessive alleles of Pun1 have been identified all are mutations, such as a frameshift mutation, which results in a loss of pungency (Kirii et al., 2017). The AT3 gene which encodes for an acyl transferase is located within the Pun1 locus and, it is now know that the Pun1 gene the CS gene, and the AT3 gene are the same and function in the final production of capsaicin (Stewart et al., 2005 ; Aza-Gonzalez et al., 2011). The expression of the gene AT3 follows a similar pattern to the accumulation of capsaicin and, when it is silenced, the capsaicinoid content decreases, likely due to a decrease of fatty acid synthesis (Arce-Rodriguez et al., 2015).

The gene p-AMT is responsible for producing the enzyme which produces vanillylamine and other acid amide precursors used to produce capsaicinoids and capsinoids (Gururaj et al., 2012). p-AMT is associated with the production of capsinoids and non-pungency (Tanaka et al., 2010 ; Tanaka et al., 2015). The p-AMT gene is also associated with pungency, low pungency genotype is correlated with this gene and mutations of this gene increases pungency (Park et al., 2015 ; Tanaka et al., 2015 ; Peng et al., 2012). Pungency may be linked to the nucleic acid sequence of both the Pun1 and p-AMT genes, however, the expression level of these genes also plays a large role (M et al., 2016).

**Why Root system Changes Matter**

The genetic transcripts, enzymes, and precursors to both capsaicinoids and capsinoids can be found in various parts of the plant, ranging from the whole plant system to a specific location. The genes which encode the enzymes involved in the synthesis of the capsaicinoid precursors
show a high level of transcription in the fruit. Genes for the fatty acid synthesis can be found in the root, stem, leaves, flowers, seed (Figure 2.3). The direct acid amide precursor to both capsaicin or capsiate along with the $p$-AMT gene are found in the fruit, however, vanillin, the precursor to vanillylamine or vanillyl alcohol, is produced in the roots of plants (Suresh et al., 2005). Peppers vary in pungency levels with the genotype of the plant this stems from the level of transcription of the related pungency genes. Any changes to the genotype and therefore the transcription levels may change the capsaicinoid and capsinoid profile.

Root systems were shown to modify the canopy of the plant in a plethora of ways such as the mitigating abiotic and biotic stresses and modifying the metabolic and genetic profile of the canopy. (Dorji et al., 2005; Howell, 2003; Mora-Romero et al., 2015; Signs and Flores, 1990; David et al., 1984). In order to better understand these impacts, grafting was used as a tool to further study the impacts of the roots by combining two genotypes of plants (Holbrook et al., 2002). Through the use of grafting it was found that root systems play a role in mineral uptake, combating water or heat stress, preventing soil borne diseases, influencing the amounts and locations of metabolites, as well as influencing quality parameters of the canopy (Pulgar et al., 2000; King et al., 2008; Schwarz et al., 2010; Yetisir et al., 2003; Faiss et al., 1997; Flachowsky et al., 2012). It was found that while not all unique traits of the root system were imbued into the canopy the plants produced were genetic hybrids of the two cultivars with impacted traits showing a combined phenotype (Guri et al., 1988).

Since the discovery that physical hybrids with new root systems caused changes in the canopy by which the system was attached studies were conducted to identify a mechanism. Mobile RNA, mRNA and some proteins have been found to be sent to the canopy from the root system via phloem sap. (Goldschmidt, 2014). Other types of RNA such as small interfering RNA
(siRNA) and microRNA were also found to have the ability to travel from the root to the shoot and generate changes in the canopy such as fluctuations in leaf morphology (Zhang et al., 2014; Bhogale et al., 2014). These modifications occurred in a manner similar to systemic resistance and was found to be stability inherited due to changes in canopy DNA methylation patterns (Palauqui et al., 1997; Goldschmidt, 2014). There were also cases where the genetic changes were influenced by changes in expression levels of associated genes (Albert et al., 2017). There is still some debate into if the genetic changes are one dimensional meaning the changes were strictly from the root system to the canopy or bi-dimensional meaning the root system and canopy may both alter one another genetically (Palauqui et al., 1997; Goldschmidt, 2014).
Grafting

What is Grafting?

Grafting is a horticultural technique that allows two genotypes to be combined and studied. Grafting combines two seedlings into a single plant by physically joining one (the scion or aerial portion) to the other (the rootstock or lower portion) and then allowing the graft union to heal. Successful formation of a graft union lies in the differentiation of callus cells into vascular cells, this healing process is largely influenced by temperature and humidity, optimally occurring at 20-30°C with 95% humidity (Kubota et al., 2008a; Oda et al., 2002a; Lee et al., 2007). Within 1-2 weeks the xylem and phloem of the scion and rootstock should be exchanging materials (Oda et al., 2002b). In some instances this connection is not optimal, which may result in the vascular bundles reconnecting in a nonlinear fashion. This subpar vascular connection slows the transport of materials and can impact canopy growth, fruit growth, as well as secondary growth of the stem tissue. Grafting of two plants causes proteins and mRNA to travel through the phloem of the rootstock and scion to aid in different processes in the plant such as such as the growth of leaf and meristematic tissue. (Davis et al., 2008). Although grafting has been used since antiquity to create fruit tree combinations, vegetable grafting was implemented for fruiting vegetables in the 1920’s.

Benefits of Grafting

The vegetable industry commercially adopted grafting during the 1950’s as a means to control soil borne diseases, with commercial grafted seedling production beginning in the United States in 2008 (Davis et al., 2008; King et al., 2008; Kubota et al., 2008a; Kubota et al., 2008b.
Afterwards, the development of commercially-available graft combinations has increased vegetable production opportunities over a wider range of environments. Resistance to diseases such as fusarium, phytophthora, and verticillium through grafting have been reported for cucurbit and solanaceous crops. (King et al., 2008; Rivard et al., 2008; King et al., 2010). Grafting is also used to improve growth, enhance nutrient uptake, facilitate heat or cold resistance, and increase yields (Lee et al., 2007; Oda et al., 2002). Grafting imparts benefits on the crop by altering root architecture and function, resulting in a reduction of water and fertilizer inputs (Colla et al., 2010a; Colla et al., 2010b; Rouphael et al., 2008). Grafting can be used as an alternative to chemical treatment of crops to prevent soil borne diseases (Gilardi et al., 2014; Morra et al., 2006; Oka et al., 2004). Bell pepper production can suffer from a number of abiotic and biotic stress factors, which grafting may address. Tolerant hybrids and germplasm, if used as rootstocks, may offer the grower opportunities to produce peppers in areas where Capsicum culture is historically limited by disease pressures or unfavorable environments while simultaneously capitalizing on market-driven demands for fruits with specific quality characteristics. However, the grafted efforts should focus on scion-rootstock combinations of Capsicum due to the incompatibility with other solanaceous crops.

**Grafting Fruit Quality Changes**

Besides peppers two other crops watermelon and tomato are often grafted, however, in some cases these crops have fruit quality characteristic changes from this process. Grafting was shown to have an impact on the fruit quality of watermelon (Qin et al., 2016; Proietti et al., 2008; Cushman et al., 2008). In tomato grafting, however, the overall fruit quality with the does not
change dramatically based on the rootstock (Rouphael et al., 2010). Grafted peppers have been shown to have a positive alteration in physical quality parameters such as fruit length and weight (Donas-Ucles et al., 2014). Not only do the physical qualities (i.e., length, width, shape, and thickness) change but the chemical qualities (i.e., vitamin C, soluble solids, and acidity) change across scion and rootstock combinations of Capsicum as well (Soltan, Unpublished). Grafted pepper also display changes in nutrition and the production of bioactive compounds. Grafting increases the amount of antioxidant compounds in the peppers (Chavez-Mendoza et al., 2015). Changes in nutritional composition of bell peppers depend on the genotype of the combined cultivars (Sanchez-Torres et al., 2016). There is antidotal evidence that grafting may alter the production of capsaicinoids in the scion fruit as well (Kleinhenz et al., 2011; Yagishita et al., 1985). The mechanism behind these grafting changes was not fully understood but it was found that mRNA fragments were able to travel through the phloem from the rootstock to the scion, affecting genetic and metabolic changes in the latter (Harada et al., 2010; Kudo et al., 2007). These mRNA fragments were not accompanied with a genome change but a difference in levels of transcription correlating with the genes of the impacted qualities (Tsaballa et al., 2013).
Conclusions

With the act of grafting peppers known to change some aspects of quality, such as fruit shape and metabolite profile it is important to examine the changes across the *Capsicum* genus and quantify the degree to which these changes impact consumer acceptability. Capsaicinoids and capsinoids are health beneficial metabolites unique to *Capsicum* that can serve to examine the metabolic changes, due to the way they are synthesized in the plant, as well as the chemical and sensory acceptability of the fruit, when the root system is changed. Sensory, chemical, and physical fruit quality was examined to illustrate if grafted *Capsicum* follows the trend of tomato grafting, any rootstock may be used with minimal fruit quality changes, or the trend of cucurbit grafting, rootstock must be specifically selected in order to maximize benefits of the root system while simultaneously reducing or eliminating the likely consequences of the root system.
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Figure 2.1: Synthetic Pathway of Capsaicin: This figure highlights the key intermediates involved in the synthesis of capsaicin across the two pathways. Adapted from Kcharov - Own work on common.wikimedia.org.
Figure 2.2: Enzymatic Pathway of Capsaicin: This figure highlights the key enzymes involved in the synthesis of capsaicin. Circles indicate the gene is involved in a cyclic reaction.
Figure 2.3: Location of Enzymes Involved in Capsaicin Synthesis: This figure highlights the locations within the plant the various pathway enzymes involved in the synthesis of capsaicin are located.
Chapter 3: Grafted Scion Fruit Analysis

Introduction:

Quality to a consumer encapsulates aspects of physical characteristics, flavor (taste and aroma), and health-related compound content which conform with previously held notions of the product (Rouphael et al., 2010). General fruit quality traits of a bell pepper include fruit weight, length, color and thickness of the flesh (Donas-Ucles et al., 2014). Bell peppers also contain an array of health beneficial compounds such as vitamins C, B, and E, capsaicoids, phenolics, and carotenoids (Capsicum, 2003 ; Carvalho et al., 2015 ; Jarret et al., 2007). Both the physical and chemical properties of bell pepper fruit listed above are important quality parameters. In addition to physical and chemical quality, sensory factors, such as texture and flavor are important in determining overall quality from a consumer standpoint (Harker et al., 2003). Important taste quality parameters in pepper are the sweet/sour taste, which can be correlated to primary compounds in the fruit (Eggink et al., 2012 ; Luning et al., 1994a ; Luning et al., 1994b ; Govindarajan et al., 1985). Some secondary metabolites such as carotene, ascorbic acid, and vitamin A have been found to influence the quality of pepper due to their impact on the health beneficial properties of the fruit (Howard et al., 1994). Peppers all contain a unique family of secondary metabolites, capsaicoids and capsinoids, composed of pungent and non-pungent alkaloid compounds, respectively. Sweet peppers contain primarily the non-pungent capsinoids, while hot peppers contain both pungent capsaicoids and capsinoids. The presence and level of pungency in peppers is a sensory characteristic that is of interest to consumers (Butcher et al., 2012; Eggink et al., 2012; Govindarajan et al., 1985). Pungent secondary products in pepper may
influence two key factors of pepper quality: consumer acceptability and health beneficial properties.

Capsaicinoids have been found to have many medical applications such as analgesia, anticancer, anti-inflammatory, antioxidative, anti-obesity, fatigue reduction, dermatological disorders, aid digestive health, prevent ulcers, combat cardiovascular disease, and combat elevated blood sugar (Anandakumar et al., 2012; Baboota et al., 2014; Hsu et al., 2007; Ibrahim et al., 2015; M et al., 2015; Maihofner et al., 2014; Mankowski et al., 2017; Patwardhan et al., 2015; Hsu et al., 2016; Srinivasan et al., 2016; Sun et al., 2016; Treatment, 2017; Tsi et al., 2003; Yuan et al., 2016). The non-pungent capsinoids have a large number of health beneficial uses similar to their pungent counterparts such as analgesic, energy expenditure, antitumor, and antioxidant (Josse et al., 2010; Kawabata et al., 2009; Ohyama et al., 2015; Yoneshiro et al., 2012; Rosa et al., 2002; Barbero et al., 2016; Luo et al., 2011).

These compounds are produced from two pathways the phenylpropanoid pathway and the branched fatty acid pathway. The direct acid amide precursors to both capsaicin or capsiate are found in the fruit. However, vanillin, the precursor to vanillylamine or vanillyl alcohol, is made in the roots of plants (Suresh et al., 2005). Peppers vary in pungency levels with the genotype of the plant therefore any changes to the genotype and therefore the precursor levels may change the capsaicinoid and capsinoid profile.

Grafting is a horticultural technique that allows two genotypes to be combined and studied. Grafting has also been shown to have an impact on the fruit quality of cucurbit crops, such as watermelon and no impact on tomato (Qin et al., 2016; Proietti et al., 2008; Cushman et al., 2008; Rouphael et al., 2010). Grafted peppers have been shown to have a positive alteration
in physical quality parameters such as fruit length and weight (Donas-Ucles et al., 2014). Not only do the physical qualities (i.e., length, width, shape, and thickness) change but the chemical qualities (i.e., vitamin C, soluble solids, and acidity) change across scion and rootstock combinations of *Capsicum* as well (Soltan, Personal Communication). Grafted pepper also display changes in nutrition and the production of bioactive compounds. Grafting increases the amount of antioxidant compounds in the peppers (Chavez-Mendoza et al., 2015). Changes in nutritional composition of bell peppers depend on the genotype of the combined cultivars (Sanchez-Torres et al., 2016). There is anecdotal evidence that grafting may alter the production of capsaicinoids in the scion fruit as well (Kleinhenz et al., 2011; Yagishita et al., 1985).

Grafting was used a tool because it was known to change some aspects of physical and chemical quality, it is important to utilize cultivars across the whole *Capsicum* genus to quantify the degree to which fruit quality may change and how that may impact consumer acceptability. The objectives are as follows: to evaluate general physical fruit quality, to judge general chemical fruit quality, to examine secondary products, and to determine sensory quality differences through consumer taste panel analysis using hot and sweet rootstocks.
Materials and Methods

Materials, Solvents, and Reagents

Acetone, water, phosphoric acid, methanol, and ethyl acetate solvents (all HPLC grade), chromatographic vials and septum caps, 0.45 um filters, and 3mL syringes were purchased from Fisher Scientific. Gallic acid was purchased from Sigma Aldrich.

Year 1 Field Study

This field study was centered on the physical and chemical fruit quality analysis as it produced the fruit that went towards the physical fruit quality measurements, the soluble solids measurements, the titratable acidity measurements, and the HPLC analysis. One of the widely grown bell pepper cultivars, Aristotle, was grafted as a scion onto four cultivars used as rootstocks. The cultivars, Black Pearl, Ghost Pepper, Charleston Hot, and Carolina Wonder, were selected in order to encompass some of the diversity of the Capsicum genus and based on their fruit shape, fruit size, and chemical components such as capsaicin. These peppers were grown in the greenhouse with the following settings 20-23C during the night, 25-28C during the day, with supplemental lighting activating when the lighting was below 250-350 watts/square meter from 9am to 5pm. The cultivars were seeded into a 96 cell tray filled with Promix BX media and then placed on a capillary fabric with plastic underneath. The trays were watered with a fine mist every one to two days and the capillary fabric was watered as needed in order to maintain proper moisture for germination. After germination the trays were watered through the capillary fabric with a hose. When the seedlings reached the four true leaf stage they were clef grafted to make the following combinations Aristotle on Black Pearl, Aristotle on Ghost Pepper,
Aristotle on Charleston Hot, Aristotle on Carolina Wonder, and Aristotle on Aristotle. Some Aristotle seedlings were not grafted in order to maintain a nongrafted control and were left in the tray on the capillary fabric. The grafted plants were placed in a healing chamber constructed by placing PVC pipe around an automatic misting system on a greenhouse bench then adding a layer of plastic secured with binder clips and topped with black mesh shade cloth. The healing chamber was maintained at 20-30°C by keeping the sides secured and when the temperature was too high one side of the plastic was opened for five to ten minutes to allow the temperature to decrease. The humidity in the chamber was stabilized at 95% through the use of the automatic misting apparatus which misted the grafted plants for fifteen seconds every hour. After two weeks the grafted plants were healed, had their grafting clips carefully removed, and were placed with the nongrafted controls back on a greenhouse bench with capillary fabric and allowed to acclimate to the greenhouse setting for one additional week. After the one week period the plants were transplanted into a field in Wooster and watered with a balanced fertilizer solution. The plants were arranged in a randomized complete block design (RCBD) with five blocks, each treatment in the block contained five plant sets. The guard plants used consisted of the leftover seedlings of Black Pearl, Charleston Hot, Carolina Wonder, and Ghost Pepper. As the guard plants grew it was apparent that the cultivar Charleston Hot was not Charleston Hot but another cultivar and as a result, that the treatment was removed from analysis. The fruit of the middle three treatment plant sets in each block were harvested twice, once 10 weeks after planting and again at 13 weeks at the mature green stage with any red fruit or fruit which started to turn red removed from analysis. The plants were analyzed for general horticultural traits such as plant volume and the fruit was analyzed for fruit quality characteristics such as fruit shape, fruit
weight, color, titratable acidity, total soluble solids, lobe count, wall thickness, and alkaloid levels.

**Second Year Field Study**

The main focus of this field season was to acquire fresh fruit for sensory analysis. This field study was conducted a year later and consisted of two scions Aristotle and Carolina Wonder both grafted onto the two rootstocks of Jalapeno and Anaheim. The additional sweet cultivar Carolina Wonder was included as a scion in order to ensure legitimacy of results due to the fact that Aristotle is known to be resistant to graft changes (Kokalis-Burelle et al., 2009). The plants were seeded, maintained, grafted, and in the same fashion described above for the first field experiment. This experiment was then transplanted at the North Central Agricultural Research Station located in Fremont OH with a small portion planted at a Wooster field location. Each location was planted in a randomized block design with four plant sets per treatment in each block. The plants were again transplanted and watered with a starter fertilizer solution. The plants located in Fremont were harvested twice based on the scion. The Aristotle scions were harvested first with all the fruit at the mature green stage being picked followed by the Carolina Wonder scions at the mature green stage being harvested a week later.

**Greenhouse Study**

This experiment was designed to illuminate if metabolic changes occurred due to grafting and if there were changes, were they limited to a specific cultivar. Fruit from this experiment was used to study the alkaloid content on the HPLC. For this experiment a total of four scion varieties and four rootstock varieties were selected. Two of the scions were the sweet varieties of Aristotle
and Carolina Wonder, while the other two were the hot cultivars of Jalapeno and Ghost Pepper. The graft combinations produced for this experiment included Aristotle on Jalapeno, Carolina Wonder on Jalapeno, Aristotle on Ghost Pepper, Carolina Wonder on Ghost Pepper, Jalapeno on Aristotle, Jalapeno on Carolina Wonder, Ghost Pepper on Aristotle, Ghost Pepper on Carolina Wonder, along with the self-grafted and nongrafted controls of each cultivar. The seeds were sown, grown, cared for, grafted, healed, and reacclimated in a similar manner to the field experiments above. After the one week acclimation period in the greenhouse the plants were transplanted into two gallon pots filled with Promix BX media and placed in a completely randomized design across three greenhouse benches. Plants were hand watered every one to two days as needed and fertilized with 425 ppm 20-20-20 fertilizer solution once per week. After flowering occurred the plants had drip irrigation installed and were watered three times a day for two minutes where six of the weekly waterings consisted of the fertilizer. Plants were staked and twined when fruit set started in order to prevent plant toppling and graft union destruction. The fruit was harvested at the mature red stage twice for the sweet cultivars, 10 and 13 weeks after transplant, and three times, 13, 15, and 17 weeks after transplant for the hot cultivars.

General Horticultural Qualities (Year 1)

Measurements of horticultural performance included the plant growth rate, the fruit weight, and the fruit shape. As the plants grew in the field they were assessed for their horticultural aptitude. The growth rate was assessed using a method developed by Scheerens et al. (1999) by taking the plant height, widest width, and the width 90° to the widest width of each plant of the middle three plants of each treatment per block on a weekly basis. The fruit was
harvested then examined for physical fruit quality measures including, fruit weight, fruit shape, marketability, lobe count, wall thickness, and color readings which were used to ensure that all fruit were at the same maturity stage. The fruit was assessed for marketability by examining the imperfections and if it had more than two it was removed from analysis. The ratio of marketable fruit to total fruit was used to get a marketability percent. Fruit was weighed to the nearest 0.1 g using a standard laboratory scale.

**General Fruit Quality (Year 1)**

The general fruit quality measurements were the fruit shape, color, lobe count, the carpel wall thickness, the total soluble solids content, and the acid levels of the fruit. After the fruit was weighted it was measured in mm using calipers. The length (height) and diameter of the fruit was taken. The diameter was taken both horizontally and vertically and averaged. The average diameter and the length was used to calculate the shape index by dividing the diameter by the length. Color readings were taken using a Minolta Chromameter reporting the data in International Commission on Illumination (CIE) color space values. Lobe count was taken by simply turning the bell pepper upside down and counting the number of fully formed lobes. If a lobe had a division for a new lobe, but was not fully formed it was counted as one lobe not two. After the non-destructive measures listed above were taken the destructive fruit quality measures were taken. First the wall thickness was calculated by cutting the bell pepper vertically and then the middle of both walls was measured then averaged to get one wall thickness rating per wall. The pepper halves were then put through a dicing chopper to create 4mm³ cubes which were then frozen at -20°C until further analysis was conducted. The pepper cubes were used in the total
soluble solids (Brix) and titratable acidity (TA) measurements. The Brix measurements were taken by expressing the liquid from one tablespoon of chopped fruit using a garlic press had the soluble solids measured on an ATAGO pocket refractometer (PAL-1). The refractometer was blanked before and after each sample using HPLC grade water. The TA analysis was conducted on a slurry of the fruit made by combining a 2:1 mixture of HPLC grade water and chopped fruit in a Kinematica polytron (CH-6010 Kriens-LU) with a PT-DA 12/2 EC-A 154 blade attachment. Five grams of the slurry was weighed into a 50 mL falcon tube accompanied by 45 mL of HPLC grade water. The tubes were allowed to sit at 4°C for at least three hours before they were vacuum filtered through a Buchner funnel with a No1 whatman filter paper into a 125 mL side arm flask. The filtered extract was then partitioned into 20 mL aliquots and frozen. One aliquot was thawed in a beaker with a stir bar. A few drops of phenolthlrien indicator was added then 50 uL sequential aliquots of 0.1N sodium hydroxide was transferred using a pipet into the beaker until the indicator changed color at a pH of 8.2.

Secondary Metabolite Composition (Year 1 and Greenhouse)

Capsaicin and capsiate analysis was performed with a container of cubed peppers lyophilized using a Labconco FreeZone® 12 Liter Freeze Dry System equipped with a Stopping Tray Dryer. When the cubes were dried they were ground in a KRUPS Coffee and Spice Grinder (F203). The fine powder was then weighted into 1.5 g aliquots for non-pungent tissue, placed into a 50 mL polypropylene tubes, and frozen at -20°C for future analysis. The powder was then extracted using a modified protocol established by Bae et al (Bae et al., 2013). Briefly the powder had 40 mL of HPLC grade acetone added to along with 0.5mL of a 0.25
mg/mL internal standard of gallic acid. The mixture was vortexed and allowed to sit for a total of 30 minutes with agitation occurring every five minutes. After the time elapsed the samples were placed into a Thermo Scientific Sorvall® Legend™ T/RT Centrifuge and spun for 15 minutes at 7800 g. The supernaunt was then filtered through a Buchner funnel lined with Whatman No1 filter paper into a 125mL side arm flask. This process was repeated two more times to give a total of 120 mL of extract. From this point the 120 mL was placed into a 500 mL round bottom flask and spun on a BUCHI RII Rotovaporator System equipped with a V-700 vacuum pump and a water bath temperature of 35°C, also equipped with a Brinkmann cooling unit. The solution was allowed to evaporate until it was around 20 mL at which point it was then placed into a 50 mL round glass tube. The round bottom flask was then washed twice with 10 mL of acetone which was added to the round glass tube as well. The tubes were then dried by applying nitrogen gas at 35°C to the liquid surface with an OA-SYS Nitrogen evaporator system. Once the sample was completely dry, it was dissolved in 1 mL of HPLC grade methanol. The dissolved sample was then filtered using 3 ml disposable luer-lock syringe attached to a disposable 0.45 µm nylon filter. The filtered samples were then transferred to an amber chromatographic sample vial with a septum lid. A reversed-phase HPLC System Gold 406A liquid chromatograph (Beckman Coulter, Inc., Fullerton, CA) equipped with an autosampler (model 508) and a diode array detector (model 168) interfaced to an IBM computer with Beckman Coulter, Inc. 32 Karat V.8.0 software. The machine was equipped with a Gemini C18 column held at 30C was used to analyze each sample. The mobile phase for the analysis consisted of solvent A (0.03M phosphoric acid in HPLC grade water) and solvent B (100% HPLC grade methanol). The injection volume was 40 uL with a flow rate of 0.7 mL per minute. The 46 minute program consisted of a solvent gradient which started at 0% B for five minutes followed by a linear
increase to 50% B over seven minutes and held at those conditions for 10 minutes, followed by a linear increase to 100% B over seven minutes and held at those conditions for ten minutes, followed by a return to initial conditions over five minutes. The effluent was analyzed on a diode photo array spectrophotometer set to scan from 168-282 nm. This method was developed and successfully used to quantify capsaicin previously in previous lab work (Appendix A).

Samples from pungent cultivars were also processed following a modified version of this method. 0.5g aliquots of powder was placed into a polypropylene tube and extracted with 15 mL of HPLC grade acetone. After filtration the 45 mL total was then transferred to a round bottom glass tube and dried down utilizing nitrogen gas. The dried samples were then dissolved in 2 mL of HPLC grade methanol and filtered. 1 mL was placed into the chromatographic vial for analysis while the remaining portion of sample was placed in a separate vial and stored at -20C. The sample was run on the same HPLC system, with the sample program listed above with a modified injection volume of 20 uL.

Sensory Quality Analysis (Year 2)

Sensory analysis was conducted in accordance with The Ohio State University, Institutional Review Board Protocol # 2015B0276, on the scion-rootstock combinations from the second year field study. This study included the scion varieties from two sweet peppers, Aristotle and Carolina Wonder, grafted onto two hot rootstocks, Anaheim and Jalapeno for a total of four graft combinations and two nongrafted controls. The Aristotle scion and Carolina Wonder scions were harvested one week apart at 10 and 11 weeks after transplant respectively, each harvest consisted of the nongrafted control, the scion of Jalapeno, and the scion on Anaheim. The fruit
was harvested at the mature green stage in Fremont OH, placed into a cooler with ice and transported back to Wooster, OH where it was stored in food grade cooler at 4C with 80% humidity for three to four days. The fruit was then taken into a food grade processing lab and chopped using clean food grade equipment into diced bell peppers. One tablespoon of the diced fruit was then placed into a four ounce plastic cup with a clear plastic lid. Each cup was labeled with a three digit number indicative of if it was a nongrafted control, grafted onto Jalapeno, or grafted onto Anaheim. The cups were stored overnight in the food grade cooler and then transported to Columbus OH for consumer sensory analysis.

The analysis consisted of three tests, two triangle test, and one preference test (Meilgaard et al., 2016). The purpose of the triangle tests was to determine if sensory property differences between fruit from nongrafted plants and those harvested from plants grafted to either Jalapeno or Anahiem could be distinguished by consumers. The preference test attempted to distinguish if the consumer favored from one root system over another. The triangle tests consisted of three plastic cups where two of the cups contained the same treatment and one cup contained a different treatment (i.e., Three cups with one outlier). Each triangle test focused on examining the nongrafted control vs one of the rootstocks: one triangle test consisted of samples of nongrafted Aristotle and Aristotle grafted onto Jalapeno while the other consisted of the nongrafted Aristotle and Aristotle grafted onto Anaheim. The preference test consisted of one sample each of nongrafted Aristotle, Aristotle grafted onto Jalapeno and Aristotle grafted onto Anaheim (Figure 3.1). Each sensory analysis took place on one day in the lobby of Kottman hall on The Ohio State University main campus with the consumer panelists consisting of students and staff at the university. Each panelist signed a consent form then was given one test at a time and had to return to the testing table for each set of tests. The first triangle tests came on a tray
which consisted of the three treatment samples, a napkin, a pen, a ballot, two packages of crackers and a cup of water. The second tray consisted of the second triangle test with an additional package of crackers, and the final tray was the preference test. The panelists were given miniature candy bars for their time after they handed in their ballots. Ballots were numbered and stored as they were handed in.

**Data Analysis**

The collected data from the field trials was analyzed in SAS 9.2 software. An ANOVA was conducted along with an LSD to detect differences among the treatments, which was the graft combination. The sensory quality analysis was analyzed using both qualitative and quantitative measures. The analytical method used was a chi square test to examine if the number of correct guesses produced from the sensory analysis exceeded the threshold of significance. The numerical values representing preference test values was also analyzed using an ANOVA in the SAS 9.2 software.
Results and Discussion

General Horticultural Behavior (Year 1)

Grafted plants had a lower change in volume than the non grafted controls (Figure 3.2). The average growth rate for Aristotle grafted onto the two hot rootstocks of Black Pearl and Ghost Pepper were the lowest at 12,833 and 13,008 cubic centimeters respectively. The two controls of self-grafted and nongrafted Aristotle had the highest growth rate at 14,686 and 14,675 respectively. In Harvest 1 the fruit weight, lobe count, and carpel wall thickness were not significantly different based on the rootstock (Table 3.1). The first harvest had significant differences in the shape index measurements and marketability. The shape index has a standard rating of 1 for bell peppers with results less than one indicating a longer fruit and a result larger than 1 indicating a wider diameter fruit. The shape of Aristotle fruit grafted onto Carolina Wonder did not significantly differ from the nongrafted or self-grafted Aristotle treatments, however, they did differ from the Aristotle fruit from the hot rootstocks of Ghost Pepper and Black Pearl. The marketability of the Aristotle grafted onto Carolina Wonder was significantly higher at 95% marketable compared to the self grafted control at 77% marketable. In Harvest 2 the fruit weight, fruit shape, wall thickness, and marketability were not significantly impacted by the root system (Table 3.2). The second harvest did show a significant impact in lobe count with the ghost pepper and self grafted control producing a higher number of lobes, 3.5 and 3.6, respectively compared to the Aristotle on Carolina Wonder graft of 3.2.

The relative differences in growth rate associated with grafting could stem from improper healing at the graft union. It is possible that while the plants survived the grafting process the vascular bundles did not reconnect properly which would limit the amount of water and minerals
transported to the canopy and the amount of solutes transported to the roots. Both Black Pearl and Ghost pepper had small seedlings before grafting. This lead to a stem diameter difference between the rootstock and scion (Aristotle) which impaired the healing and the subsequent growth of the plants. The changes in lobe count were likely due to natural fruit to fruit variability within the cultivar. Aristotle is known to produce 3-4 lobed peppers and while the results were significant the results did not produce a lobe count outside of this natural range. It is known that grafting causes a significant fruit shape change that is inherited stably through backcross breeding (Yagishita et al., 1985). Grafting an elongated scion onto a blocky rootstock produced a wider scion fruit shape (Taller et al., 1999) and the reciprocal graft produced a longer scion fruit (Tsaballa et al., 2013). The fruit shape change presented in the first harvest, however, is likely related to the maturity of the fruit selected since the changes were slight and the literature-documented rootstock changes were dramatic. Further evidence that the rootstock was not the driving force behind the fruit shape change was that the shape change persist into the second harvest. These fruit shape changes while significant were likely lower than a consumer may detect. The changes in marketability that were observed stemmed from the unique way these plants were grown. As flowering started the plants were twined in order to prevent damage to the graft union from the plants falling over. This event however, caused a change in the plant architecture where the first fruit was set between more branched and the twine itself. This also may have played a role in the fruit shape change that was observed in the first harvest and not the second because at the second harvest fruit were not as densely packed as they were at the time of the first harvest.
General Fruit Quality (Year 1)

In both Harvest 1 and Harvest 2 the acidity levels were not impacted by the rootstock used (Figure 3.3). In Harvest 1 the total soluble solid (Figure 3.4) of the fruit were not significantly impacted by the rootstock while in Harvest 2 the total soluble solids content was impacted with the Carolina wonder graft producing a sweeter fruit at 5.4 compared to the graft of Ghost Pepper and the self-grafted control which produced a fruit with a lower sugar content of 4.95 and 4.87 respectively.

Previous studies indicate that both total soluble solid and titratable acidity levels in grafted pepper scion fruit were not significantly different based on the rootstock (Colla et al., 2008). The likely reason total soluble solid content was increased in Harvest 2 compared to Harvest 1 stemmed from an increased maturity stage that was closer to red maturity than green maturity which correlates with an increase in the soluble solid content. The fruit from the second harvest contained a much higher incidence of red and red-tinged fruit (not harvested) than harvest one likely due to the cooler night temperatures. The color readings for this harvest indicated that the L value was not significantly different but the chroma and hue values were. This indicates that the fruit were closer slightly less green and had a darker pigmentation. The significant increase in the total soluble solid content also may have stemmed from the improper healing at the graft union site, however, this is less likely due to the fact that Black Pearl and Ghost Pepper did not have higher soluble solid content. The changes in Brix levels that a human can detect has been studied in the past in strawberry samples (Scheerens, Unpublished). It was found in that study that a Brix unit difference of at least 2 was needed for a human panelist to detect a difference. While the changes in soluble solids reported here were significant they were still much lower than a human could may have been able to detect.
Secondary Metabolite Composition (Year 1 and Greenhouse)

The grafted Aristotle bell peppers from both harvests of produced levels of the non-pungent alkaloid compound capsiate, however the levels of capsiate found were at the lower limits of the chromatographic detection capabilities. Both harvest 1 and harvest 2 (Table 3.3) did not have significant differences in capsiate levels based on the different root systems. In harvest 1 the highest level was 0.5668 Mega Pixels (Mp) while the lowest level was 0.3313 Mp and in harvest 2 the highest level was 1.7534 Mp while the lowest was 0.7753 Mp. In the greenhouse experiment the graft combinations of Aristotle on Jalapeno and Carolina Wonder on Jalapeno did not produce detectable levels of capsiate. The grafted Jalapeno fruit showed a significant difference in the capsaicin levels based on the root system the plant. Jalapeno grafted onto Carolina Wonder produced the most capsaicin with a level of 683 mg/g and the nongrafted control produced the least amount of capsaicin with a level of 60 mg/g (Table 3.4). There was also an unidentified putative capsaicinoid present in the jalapeno fruit. This alkaloid presented the same spectra (Figure 3.5) as capsaicin and also presented the same influence due to root system. The levels of this putative capsaicinoid were significantly higher in the jalapenos from the Jalapeno on Carolina Wonder and Jalapeno on Aristotle grafts with 6.754 and 5.815 Mp respectively compared to the self grafted and nongrafted controls which had 1.948 Mp and 1.010 Mp respectively.

It is possible that the capsiate levels between harvests differed in level due to the maturity level of the fruit. The green fruit from the second harvest were much closer to the red maturity stage than the first harvest. This is supported by the color data which indicated the pigmentation in this fruit was darker and not as green. These alkaloid compounds start to accumulate as the fruit grows and is at a maximum at red maturity (Rosa et al., 2013). Of the two harvests Harvest
The synthesis of capsaicin is a complicated process due to the fact that it involves two pathways to produce the needed intermediates. Because of this there are a number of factors that are known to impact the synthesis of capsaicin and capsaicinoids including plant hormones, plant wounding, outside temperatures and light levels (Arce-Rodriguez et al., 2017; Kim et al. 2009). This may explain how the act of grafting itself increased the levels of capsaicin. The importance of the root system to the capsaicin biosynthetic pathway relies on the roots ability to send up genetic information. It was found that the root system can genetically influence the canopy it is grafted to (Goldschmidt, 2014). Other quality parameters in Capsicum such as fruit shape changed based on the root system in which they were attached. Fruit shape was known to be influenced by two genes, which when the root system changed caused stably inherited changes in the canopy (Tsaballa et al., 2011; Tsaballa et al., 2013; Vilarinho et al., 2015). The mechanism behind the fruit shape changes lies in the fact that the fruit shape genes has single nucleotide polymorphisms and expression level changes which can be associated to the root system. It is likely that a similar scenario is occurring in the capsaicin biosynthesis, where the root system sends up genetic material that causes genetic and expression level changes in the fruit. It has also been shown that if root systems have access to a higher amount of building blocks to these precursors then a higher level of the precursors can be produced (Suresh et al., 2003). Therefore it may have been possible for the grafted root system to better acquire nutrients and building blocks for capsaicin. It has also been documented that the act of grafting a pungent pepper onto a non-pungent rootstock the capsaicin levels decrease (Yagishita et al., 1985). This result is likely because the hot cultivar used has been shown to vary in the capsaicin levels based on the environment the plant was grown in. This alludes to a certain level of plasticity in the production of capsaicin in this cultivar that other common pungent
peppers used commercially today do not have. This is a likely reason to why a capsaicin level decrease was observed in this Japanese study.

Peppers in particular were shown to be susceptible to changes originating from the root system, therefore it is likely that genetic mechanisms will play a role in changing important fruit quality aspects of pepper (Goldschmidt, 2014). In the past some quality parameters in *Capsicum* were changed based on the root system in which they were attached. These factors included fruit shape, total soluble solids content, and the metabolic profile, specifically capsaicin profile, of the canopy. For instance fruit shape is known to be influenced by two genes, which when changed by the root system cause stably inherited changes in the canopy (Tsaballa et al., 2011; Tsaballa et al., 2013; Vilarinho et al., 2015). The mechanism behind the fruit shape changes lies in the fact that the fruit shape genes has single nucleotide polymorphisms and expression level changes which can be associated to the root system. One the other hand soluble solids content, which is also genetically controlled, has conflicting reports on if changes occur due to the root system (Vilarinho et al., 2015; Colla et al., 2008; Soltan, Unpublished Data). This study has shown that an increase in capsaicin content occurred when creating hybrids between a hot pepper canopy and a sweet pepper root system. The mechanism at play for this study was likely similar to the known mechanism for fruit shape change, which increases expression levels while modifying the associated genes. This modification was also likely due to RNA sent from the root system which induced both the expression and gene changes. This phenomena and the exact genetic controls behind the changes, however, warrant more study.
Sensory Quality Analysis (Year 2)

Both the Anaheim and jalapeno rootstocks had no impact on the sensory quality of either the Aristotle or the Carolina Wonder scion fruit (Table 3.5). In the triangle tests the participants selected the correct sample for the Carolina wonder scion and Aristotle scion about 33% of the time. This showed that the participants selected the different treatment at a rate similar to random chance. The chi squared values which were 0.29 for the Carolina Wonder scion and 0.23 for the Aristotle scions were compared to a chi squared table which showed that a value higher than 43.77 was needed for these results to be significant. Hedonic analysis showed there were no significant differences between the nongrafted control, the Anaheim rootstock, and the Jalapeno rootstock. On a scale of 1-9 the Carolina wonder scions scored 6.4, 6.5, and 6.6 respectively while the Aristotle scions scored a 6.8, 6.6, and a 6.5 respectively which correlates to “moderately like” for the test.

The sensory quality in grafted vegetables has been studied in the past. Since the flavor of vegetables is composed of a sense reaction to acid, sugar, and volatile compounds early studies focused mainly on the sugar components of grafted vegetables (Rouphael et al., 2010). Some studies however, also examined the sensory quality with the sugar levels and found that when the sugar level changed perceptively so did the overall sensory quality of the grafted product. Additional studies have been conducted on grafted cucurbits and tomatoes that supported the link between sugar content and sensory quality. In grafted cucurbits it was found that an elevated sugar level, a lower sugar level, and an unchanged sugar level all corresponded with an elevated, lower, and unchanged sensory score, respectively (Verzera et al., 2014; Villocino and Quevedo, 2015; Guan et al., 2014). Sensory quality and its relation to chemical compounds has also been studied in grafted tomatoes where it was observed that no change in sugar levels corresponded
with no change in sensory quality (Barrett et al., 2012). While few studies to date have focused on grafted pepper quality the consensus of the literature and the results above, which indicated a non-distinguishable change in sugar content correlates with no distinguishable sensory change, together show that sensory quality is correlated with other chemical changes such as sugar, acids, and volatile compounds. The consumer sensory analysis showed no change due to the fact that the levels of the chemical components of flavor such as sugar and acid levels did not change depending on the root system of the plant.


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Scheerens, Joseph Unpublished Data

Soltan, Mahmoud. Personal Communication.


<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Fruit Weight (g)</th>
<th>Lobe Count</th>
<th>Carpel Wall Thickness (mm)</th>
<th>Shape Index</th>
<th>Marketability (%)</th>
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<td>Aristotle</td>
<td>243.25</td>
<td>3.51</td>
<td>7.13</td>
<td>1.02 ab</td>
<td>77.42 c</td>
</tr>
<tr>
<td>Control</td>
<td>241.60</td>
<td>3.62</td>
<td>6.98</td>
<td>1.01 ab</td>
<td>92.78 ab</td>
</tr>
</tbody>
</table>

**Table 3.1 Physical Measurements of Harvest 1 from the 2016 Field Study:** The table above indicates the impact of the rootstock on the fruit weight, lobe count, wall thickness, shape index, and marketability measured in the first harvest of the 2016 field season. Measures were taken on fruit at green maturity 10 weeks after planting grown in a field setting on a raised bed. Shape index was calculated by dividing the average diameter by the length of the fruit and marketability is the percent of fruit with less than two imperfections. Numbers with different letters indicate a statistically different average.
<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Fruit Weight (g)</th>
<th>Lobe Count</th>
<th>Carpel Wall Thickness (mm)</th>
<th>Shape Index</th>
<th>Marketability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Pearl</td>
<td>238.29</td>
<td>3.33 ab</td>
<td>8.49</td>
<td>1.03</td>
<td>90</td>
</tr>
<tr>
<td>Ghost Pepper</td>
<td>283.62</td>
<td>3.53 a</td>
<td>8.28</td>
<td>0.98</td>
<td>86</td>
</tr>
<tr>
<td>Carolina Wonder</td>
<td>270.50</td>
<td>3.25 b</td>
<td>8.98</td>
<td>0.98</td>
<td>72</td>
</tr>
<tr>
<td>Aristotle</td>
<td>272.02</td>
<td>3.58 a</td>
<td>8.93</td>
<td>1.03</td>
<td>89</td>
</tr>
<tr>
<td>Control</td>
<td>253.91</td>
<td>3.37 ab</td>
<td>8.62</td>
<td>1.03</td>
<td>85</td>
</tr>
</tbody>
</table>

Table 3.2 Physical Measurements of Harvest 2 from the 2016 Field Study: The table above indicates the impact of the rootstock on the fruit weight, lobe count, wall thickness, shape index, and marketability measured in the second harvest of the 2016 field season. Measures were taken on fruit at green maturity 14 weeks after planting grown in a field setting on a raised bed. Shape index was calculated by dividing the average diameter by the length of the fruit and marketability is the percent of fruit with less than two imperfections. Numbers with different letters indicate a statistically different average.
<table>
<thead>
<tr>
<th>Root System</th>
<th>Harvest 1 (Mp)</th>
<th>Harvest 2 (Mp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Pearl</td>
<td>0.3313</td>
<td>1.7534</td>
</tr>
<tr>
<td>Ghost Pepper</td>
<td>0.5668</td>
<td>0.9589</td>
</tr>
<tr>
<td>Carolina Wonder</td>
<td>0.5444</td>
<td>0.9831</td>
</tr>
<tr>
<td>Aristotle (Self)</td>
<td>0.4875</td>
<td>1.4743</td>
</tr>
<tr>
<td>Nongrafted</td>
<td>0.4129</td>
<td>0.7753</td>
</tr>
</tbody>
</table>

**Table 3.3 Capsiate Levels of Harvest 1 and 2 from the 2016 Field Study**: The table above shows the average capsiate metabolite levels from the green mature fruit harvested from the field at 10 and 14 weeks. The capsiate was extracted from a mixture of lyophilized fruit tissue using acetone. The extract was measured on the HPLC using a solvent gradient of phosphoric acid water and methanol on a C18 reverse phase column for 46 minutes. Three rootstocks along with the self-grafted, and non-grafted control were analyzed. Numbers with different letters indicate a statistically different average.
<table>
<thead>
<tr>
<th>Root system</th>
<th>Capsaicin (mg/g)</th>
<th>Putative Capsaicinoid (mg/g cap.eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aristotle</td>
<td>1.99 ab</td>
<td>2.13 a</td>
</tr>
<tr>
<td>Carolina Wonder</td>
<td>2.54 a</td>
<td>2.47 a</td>
</tr>
<tr>
<td>Jalapeno (Self)</td>
<td>1.03 bc</td>
<td>0.71 b</td>
</tr>
<tr>
<td>Nongrafted</td>
<td>0.22 c</td>
<td>0.37 b</td>
</tr>
</tbody>
</table>

**Table 3.4 Capsaicinoid Levels in Jalapeno Tissue from the Greenhouse Study:** The table above shows the average capsaicin and putative capsaicinoid metabolite levels from the red mature jalapeno fruit harvested from the greenhouse study. The plants were grown in the greenhouse setting until fruit reached the red maturity stage and were harvested. The alkaloids were extracted from a mixture of lyophilized fruit tissue using acetone. The extract was measured on the HPLC using a solvent gradient of phosphoric acid water and methanol on a C18 reverse phase column for 46 minutes. Two rootstocks along with the self-grafted, and non-grafted control were analyzed. Numbers with different letters indicate a statistically different average.
Table 3.5 Consumer Sensory Analysis on the Second Year Field Study: The table above shows the results of the consumer triangle tests. The triangle tests were used to determine if a consumer can determine which of three samples is different from the others. Triangle Test 1 consists of the nongrafted scion cultivar compared against the scion grafted onto Jalapeno whereas Triangle Test 2 is the nongrafted scion cultivar versus the scion grafted onto Anaheim. The triangle tests were given in sequence at a consumer taste panel, which took place in the lobby of Kottman Hall on The Ohio State University Campus. Both Aristotle triangle tests were conducted on one day while the two Carolina Wonder tests were conducted a week later. Correct panelists indicate how many of the total panelists were able to determine which of the three samples was different from the other two.

<table>
<thead>
<tr>
<th>Sensory Test</th>
<th>Correct Panelists</th>
<th>Total Panelists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aristotle Triangle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 1</td>
<td>36</td>
<td>111</td>
</tr>
<tr>
<td>Aristotle Triangle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 2</td>
<td>40</td>
<td>111</td>
</tr>
<tr>
<td>Carolina Wonder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triangle Test 1</td>
<td>23</td>
<td>66</td>
</tr>
<tr>
<td>Carolina Wonder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triangle Test 2</td>
<td>20</td>
<td>66</td>
</tr>
</tbody>
</table>

Table 3.5 Consumer Sensory Analysis on the Second Year Field Study: The table above shows the results of the consumer triangle tests. The triangle tests were used to determine if a consumer can determine which of three samples is different from the others. Triangle Test 1 consists of the nongrafted scion cultivar compared against the scion grafted onto Jalapeno whereas Triangle Test 2 is the nongrafted scion cultivar versus the scion grafted onto Anaheim. The triangle tests were given in sequence at a consumer taste panel, which took place in the lobby of Kottman Hall on The Ohio State University Campus. Both Aristotle triangle tests were conducted on one day while the two Carolina Wonder tests were conducted a week later. Correct panelists indicate how many of the total panelists were able to determine which of the three samples was different from the other two.
Bell Pepper Quality Evaluation

We would like you to assist us in determining quality differences among various samples of bell pepper. There are three parts to this test.

Part 1:

You will be given two sets of three bell pepper samples each that have been coded with three-digit numbers. Two of the samples in each set are identical and the third is different. Please attempt to determine which of the samples is the “different” one in each set, and then indicate it by circling or marking its code number on the ballot for Part 1 below. As you evaluate each sample within the set, you may wish to consider a variety of quality factors including: color, sweetness, tartness, flavor, crunchiness, etc. If necessary, please cleanse your palate between samples with the water and/or crackers provided with them.

<table>
<thead>
<tr>
<th>Set 1 Sample Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>116</td>
</tr>
<tr>
<td>287</td>
</tr>
<tr>
<td>349</td>
</tr>
</tbody>
</table>

Once you are finished with the first set of samples, please signal one of the research assistants that you are ready for the second set of samples. Evaluate these as you did the first set.

<table>
<thead>
<tr>
<th>Set 2 Sample Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>459</td>
</tr>
<tr>
<td>501</td>
</tr>
<tr>
<td>653</td>
</tr>
</tbody>
</table>

Once you are finished with the second set of samples, please signal one of the research assistants that you are ready for the third set of samples.
Part 2:

You will be given another set of three bell pepper samples each that have been coded with three-digit numbers. The samples in this third set are all different from each other. Please indicate how you feel about each sample individually using the “like-dislike” scale below. After determining the most accurate description of a given sample, place an X or a ☐ in the box corresponding to that description in the tables provided. If you perceive two or more samples to be similar, you may rate them the same. If necessary, please cleanse your palate between samples with the water and/or crackers provided with them.

<table>
<thead>
<tr>
<th>Acceptability Statements</th>
<th>Set 3 Sample Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>722</td>
</tr>
<tr>
<td>Like extremely</td>
<td></td>
</tr>
<tr>
<td>Like very much</td>
<td></td>
</tr>
<tr>
<td>Like moderately</td>
<td></td>
</tr>
<tr>
<td>Like slightly</td>
<td></td>
</tr>
<tr>
<td>Neither like nor dislike</td>
<td></td>
</tr>
<tr>
<td>Dislike slightly</td>
<td></td>
</tr>
<tr>
<td>Dislike moderately</td>
<td></td>
</tr>
<tr>
<td>Dislike very much</td>
<td></td>
</tr>
<tr>
<td>Dislike extremely</td>
<td></td>
</tr>
</tbody>
</table>

Part 3:

Using the same sample set in Part 2, please indicate which sample you prefer.

<table>
<thead>
<tr>
<th>Set 3 Sample Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>722</td>
</tr>
</tbody>
</table>

For Part 3, please indicate why you prefer one sample over the other two by placing an X or a ☐ in boxes next to quality characteristics you consider to be superior in the preferred sample. Check all boxes that are relevant to your decision.
## Quality Characteristics

<table>
<thead>
<tr>
<th>General appearance</th>
<th>Overall aroma</th>
<th>Lack of bitterness or sourness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shininess</td>
<td>Overall flavor</td>
<td>Crispness/crunchiness</td>
</tr>
<tr>
<td>Color</td>
<td>Sweetness</td>
<td>Other (please explain)</td>
</tr>
</tbody>
</table>

**Comments Welcome!**

**Figure 3.1: Sensory Analysis Ballot:** The above ballot shows the layout for the tests utilized in the consumer sensory analysis. Analysis took place on three days the first and third of which were in the lobby of Kottman Hall and the second of which was in the lobby of the Agricultural administration building on The Ohio State University campus. The three tests conducted were two triangle tests which determine if a consumer can detect a difference between the three samples and a discriminatory test which determines if a consumer has a preference between the three samples. Aristotle grafted onto Jalapeno is represented by numbers 116, 349, 232, and 839. Aristotle grafted onto Anaheim is represented by numbers 459, 653, 549, and 915. Nongrafted Aristotle is represented by numbers 287, 147, 375, 501, 423, 609, and 722. The same layout but with different numbers was used for the Carolina Wonder scion.
**Figure 3.2: Change in Canopy Volume of Bell Pepper Plants from the 2016 Field Study:**
The above graph shows the average weekly canopy volume of the plants in the 2016 field season. Canopy volume was measured by taking the plant height, horizontal width, and vertical width and then using those measurements to calculate the volume of a rectangular prism. Measurements were taken weekly starting the day before transplant into the fields and ending two weeks before the first harvest.
Figure 3.3: Acidity Levels From Both Harvest from the 2016 Field Study: The above chart displays the total potential acidity in the mature green fruit collected across both harvests of the 2016 field season. The fruit was grown in a field setting until green maturity at which point it was harvested and chopped into composite samples per treatment. A slurry of chopped fruit and water was created and used to measure the potential acid levels. Phenolthanien indicator and sodium hydroxide were used to determine the acid levels. Bars with different letters indicate a statistically different average.
Figure 3.4: Soluble Solids Content of Both Harvests from the 2016 Field Study: The above chart displays the total soluble solids content in the green mature fruit collected across both harvests of the 2016 field season. Plants were grown in a field setting and harvested once the fruit reached green maturity. The fruit was then chopped and mixed into composite samples per treatment. Two tablespoons of the mixed chopped fruit was then placed into a garlic press lined with cheese cloth. The liquid was pressed from the fruit and read on a pocket refractometer. Bars with different letters indicate a statistically different average.
**Figure 3.5: Absorption Spectra of Capsaicin Standard and Unknown Capsaicinoid:** The above spectra are of the capsaicinoid capsaicin and a putative capsaicinoid sample detected in samples from the greenhouse study in Jalapeno tissue. The plants were grown in a greenhouse setting until the fruit reached red maturity. The harvested fruit was then chopped into composite samples and lyophilized and ground into a powder. The powder was then extracted using acetone and run on an HPLC with a C18 reverse phase column under a solvent gradient of phosphoric acid and methanol. The fact that these spectra match but the peaks are at different times may indicate that the compounds are both capsaicinoids. However, it is also possible that the putative capsaicinoid compound is a precursor or similarly structured metabolite and the identity needs to be confirmed using another analytical method such as NMR or LC/MS before it is possible to confirm that it is a capsaicinoid.
Appendix A: Capsaicinoid Extraction and Identification Method Optimization

HPLC optimization experiments were conducted throughout May 2015 to July 2017 to assess the methodology for optimal recovery of both capsaicinoids and capsinoids. Variables examined in these experiments included organic extraction solvents, and solvent gradients.

Materials and Methods

Extraction Solvents

The extraction solvents used were Acetone Extraction Solvent (AES), acetone, methanol, and ethyl acetate all HPLC grade. The AES procedure was adapted from Ozgen et al. (2008). Briefly the fruit was cut into chunks and lyophilized using a Labconco FreeZone® 12 Liter Freeze Dry System equipped with a Stoppering Tray Dryer. When the pieces were dried they were ground in a KRUPS Coffee and Spice Grinder (F203). The powder was then extracted with 30 ml of Acetone Extraction Solvent (AES) mixture (Acetone 70%, Water 29.5%, Acetic Acid 0.5%) for 30 minutes, the samples were agitated every five minutes. After the time elapsed the samples were centrifuged for 15 minutes at 7800g using a Thermo Scientific Sorvall® Legend™ T/RT Centrifuge. The supernaunt was then filtered through a Buchner funnel lined with Whatman No1 filter paper into a 125 mL side arm flask. This process was repeated two more times to give a total of 90 mL. From this point the 90 mL was placed into a 500 mL round bottom flask and the volume reduced to approximately 20 mL using a BUCHI RII Rotovaporator System equipped with a V-700 vacuum pump and a water bath temperature of 35°C, also equipped with a Brinkmann cooling unit. The solution was allowed to evaporate until it was around 20 mL. The round bottom flask was then scrubbed of residual solids and the solution was
brought up to 25ml. Twenty ml were then transferred to a 50 mL polyurethane tube, with 10 ml of 0.4 M sodium acetate, and an additional 20 mL of ethyl acetate was added and mixed until separation. The ethyl acetate layer (top) was then removed and placed in a 50 ml round glass tube. This step was repeated with a second 20 mL extraction and a third 10 ml extraction. The tubes were then dried by applying nitrogen gas at 35°C to the liquid surface with an OA-SYS Nitrogen evaporator system. Once the sample was completely dry it was dissolved in 1 mL of HPLC grade methanol. The dissolved sample was then filtered using 3 ml disposable luer-lock syringe attached to a disposable 0.45 µm nylon filter. The filtered samples were then transferred to an amber chromatographic sample vial with a septum lid. A reversed-phase HPLC System Gold 406A liquid chromatograph (Beckman Coulter, Inc., Fullerton, CA) equipped with an autosampler (model 508) and a diode array detector (model 168) interfaced to an IBM computer with Beckman Coulter, Inc. 32 Karat V.8.0 software. The machine was equipped with a Gemini C18 column held at 30C was used to analyze each sample. The mobile phase for the analysis consisted of solvent A (0.03M Phosphoric acid in HPLC grade water) and solvent B (100% HPLC grade methanol). The injection volume was 30 uL with a flow rate of 1 mL per minute. The 28 minute program consisted of a solvent grade which started at 0% B for five minutes followed by a linear increase to 100% B over seven minutes and held at those conditions for 10 minutes, followed a linear decrease to 0% B over five minutes. The effluent was analyzed on a photo diode array spectrophotometer set at 168-282 nm.

A similar protocol was followed for the other extraction solvents. 1.5g of powder was extracted with 30 mL of each solvent. The solvent was added and allowed to extract for 30 minutes, agitation occurred every five minutes. The 90 mL filtered supernatant was rotary evaporated down to 20 mL and placed into a 50 mL round bottom glass tube. The round bottom
was washed twice with 10 mL of the solvent used in the extraction and added to the round bottom tube. The samples were dried, dissolved, and analyzed in the same manner listed above.

Solvent Gradient

Five solvent gradients were also analyzed on the HPLC system listed above. The extractions were carried out using the AES protocol outlined above and run through five different program gradients. The first gradient was outlined above. The second gradient was a 46 minute program consisted of a solvent grade which started at 0% B for five minutes followed by a linear increase to 50% B over seven minutes and held at those conditions for 10 minutes, followed by a linear increase to 100% B over seven minutes and held at those conditions for ten minutes, then decreased linearly to 0% B over five minutes. The third, fourth, and fifth programs consisted of an isocratic runs lasting for 40 minutes each with the solvent ratios of 30% A, 70% B, 20% A, 80% B, and 10% A and 90% B, respectively. The effluent was analyzed on a photo diode array spectrophotometer set at 168-282 nm.
Results and Discussion

Extraction Solvents

Of all the solvents utilized acetone had the highest overall extraction of all compounds with an average of 21.89 Mp, 14.26 Mp, 1.97 Mp, and 0.55 Mp for capsaicin, dihydrocapsaicin, capsiate, and dihydrocapsiate respectively. This solvent extracted levels of capsaicin, dihydrocapsaicin, and capsiate that were significantly higher compared to two other tested solvents which were ethyl acetate and methanol. It was higher than the third solvent, an acetone extraction mixture, but not significantly. The solvent method that did the worst is methanol which had an average of 17.49 Mp, 10.415 Mp, 0.105 Mp, and 0.22 Mp in capsaicin, dihydrocapsaicin, capsiate, and dihydrocapsiate respectively. This solvent was statistically lower than all the other tested solvents for the compounds capsaicin, dihydrocapsaicin, and capsiate.

Solvent Gradients

All five programs produced chromatograms which could be used to quantify level of capsaicinoids. The three isocratic programs produced the most clustered chromatograms which made identification of different capsaicinoids difficult, therefore they were excluded. The program which has two gradients of B (Methanol) produced staggered peaks which moved some of the unwanted peaks before the peaks of interest. Due to its ability to separate out peaks of interest the most efficiently the program consisting of two gradients of B was selected.
Summary

The solvent of acetone extracted the highest average of all the compounds examined. This methodology is also much easier compared to the next method of AES due to the fact that there was no partitioning with another solvent and use of a solvent mixture. This solvent also produced spectra with the most separated peaks. The other solvent systems also had their own set of issues, such as complexity of extraction methodology with the AES, methanol leaving an oily residue that could not be dried down, and the ethyl acetate requiring additional protection measures. Due to the overall ease and high extraction levels the methodology using only acetone was selected. The solvent gradients tested all produced usable chromatograms. The one that was able to best separate out the peaks of interest consisted of two solvent B gradients, which led its selection.
Citations

### Table A.1: Metabolite Concentration Across Solvent Systems

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Capsaicin</th>
<th>Dihydrocapsaicin</th>
<th>Capsiate</th>
<th>Dihydrocapsiate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>21.89 a</td>
<td>14.26 a</td>
<td>1.67 a</td>
<td>0.55</td>
</tr>
<tr>
<td>AES</td>
<td>20.62 ab</td>
<td>13.92 ab</td>
<td>1.66 a</td>
<td>0.39</td>
</tr>
<tr>
<td>Methanol</td>
<td>17.49 c</td>
<td>10.42 c</td>
<td>0.11 c</td>
<td>0.22</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>19.62 b</td>
<td>12.40 b</td>
<td>1.06 b</td>
<td>0.39</td>
</tr>
</tbody>
</table>

The table above shows the average metabolite levels measured on the HPLC using different solvent methodologies. Extracts from composite fruit samples were created by mixing the solvent with lyophilized composite samples of commercial hot and sweet peppers. The alkaloids were analyzed on an HPLC equipped with a C18 reverse phase column run with a solvent gradient of phosphoric acid water and methanol over a 46 minute time period. Numbers with different letters indicate a statistically different average.
Complete Citations


Kleinhenz, Matt, and Jenny Moyseenko. “Grafting to Improve Pepper Production, Especially under Stressful Conditions,” 2011.


Ogawa, Kana, Katsunori Murota, Hanako Shimura, Misaki Furuya, Yasuko Togawa, Takeshi Matsumura, and Chikara Masuta. “Evidence of Capsaicin Synthase Activity of the Pun1-
Encoded Protein and Its Role as a Determinant of Capsaicinoid Accumulation in Pepper.”


Qin, Y. Effects of Dual/Threefold Rootstock Grafting on the Plant Growth, Yield and Quality of Watermelon - ProQuest


Scheerens, Joseph Unpublished Data


