FLOWER PRODUCTION AND EFFECT OF FLOWER HARVEST ON BERRY YIELDS WITHIN SIX AMERICAN ELDERBERRY GENOTYPES

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By
Kelly G. McGowan

Andrew L. Thomas, Thesis Supervisor
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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled:

FLOWER PRODUCTION AND EFFECT OF FLOWER HARVEST ON BERRY YIELDS WITHIN SIX AMERICAN ELDERBERRY GENOTYPES

presented by Kelly Gail McGowan,

a candidate for the degree of Master of Science

and hereby certify that, in their opinion, it is worthy of acceptance.

Andrew Thomas

Mark Coggeshall

Michael Gold

I would like to dedicate this to my husband Brent and my daughters, Anna and Lily. Thank you for your love, support and encouragement through this process.

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Abstract

Cultivation of American elderberry (*Sambucus canadensis*) is increasing in North America for its use in dietary supplements. While the flowers of European elderberry (S. *nigra*) are commonly consumed as an anti-viral in Europe, the horticultural production of American elderberry flowers is nascent. A large field experiment with American elderberry was established in 2015 in southwest Missouri, U.S.A to evaluate flower production and to determine the impact of a partial flower harvest on fruit yield and quality in addition to plant morphology. The study concurrently compared four promising new genotypes to two established cultivars. In 2017, 96 randomized plots of six genotypes were assigned treatments of 0, 15, 39, and 100% flower harvest, with four replicated plots per genotype/treatment. Flower and fruit production data, pest and disease incidence, phenology, plant growth response, and fruit quality data were determined. All six genotypes showed differences in flower cyme number, total flower dry matter produced, mean cyme size, bud break, fruit ripening date, fruit yield, berry size, and plant height. Pocahontas and Rogersville showed promise in terms of flower and fruit productivity, and produced taller plants compared with standard cultivars Bob Gordon and Wyldewood. While total fruit yields were correspondingly reduced in plots that received 15 and 39% flower cyme harvests, these differences were not statistically significant. Likewise, mean fruiting cyme weight, berry size, soluble solids in fruit, and plant height were not affected by the various flower cyme harvest treatments. While these results are preliminary, up to 39% harvest of flower cymes did not significantly reduce elderberry fruit yields, but neither did it improve berry size or fruit quality as was expected.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Introduction and Hypothesis

European elderberry (*Sambucus nigra*) is an important agricultural crop in many parts of Europe. Both berries and flowers are utilized, but flower demand continues to increase (Kite et al., 2013). Interest in the health benefits of elderflowers has led to a surge in product development and sales. These products include elderflower infused water, lemonade, energy drinks and health supplements. Culinary uses of elderflower have risen in popularity, as have the development of spirits, liqueurs and beauty products.

While recognizing the popularity of European elderflower markets and with the rise of elderberry production in the U.S., more research is needed on the feasibility of elderflowers as a commercial crop. In addition, increasing insect pest pressure from spotted-wing drosophila (*Drosophila suzukii*), which attack ripening fruit, highlights the need for an alternative crop that was not attractive to this insect (Pinero 2013).

Hypothesis

On mature elderberry plants, harvesting a portion of existing flowers will reduce a plant's fruiting crop load, possible resulting in the remaining fruiting cymes being larger and heavier, with larger individual berries and improved berry quality. Removal of all or part of the elderberry fruit crop (through flower harvest) might result in increased plant growth. Harvest of two high-value marketable commodities (elderberry flowers and fruit) from the same plants may be possible.

Plant Taxonomy

American elderberry belongs to the family Adoxaceae and the genus *Sambucus*. The Adoxaceae family consists of a variety of flowering shrubs and the addition of the American elderberry to this family was a recent development (Applequist 2015). *Sambucus* is derived from the Greek word Sambuke and the Latin word Sambuca which represents a kind of flute or small harp made out of an elderberry twig (Grulova and Salamon 2015).

Previously, American elderberry belonged to the Caprifoliaceae family, commonly referred to as the honeysuckle family. This reclassification occurred due to observed variations in morphology among species and sub-species (Applequist 2015). Genus and species name variations still exist, with American elderberry sometimes listed as a subspecies of black, or European elderberry. *Sambucus canadensis* will be examined for purposes of this study.

Plant Characteristics

American elderberry (*Sambucus canadensis*) is a deciduous shrub or small tree ranging from 3 to 4.5 meters tall and wide. Bark is light gray and smooth when young, becoming furrowed with age. Upright canes arise from the base of the plant. American elderberry displays a shrub-like appearance compared to European elderberry, which develops a more tree-like appearance with age. American elderberry can tolerate a wide range of habitats and growing conditions including roadsides, streambanks, forest edges and fields. It prefers growing on sites with full sun and moist, well-drained soil. (Byers et al. 2014). Ivory to cream- colored flowers bloom in late spring to mid-summer transitioning to a glossy, dark purple berry in late summer. Oppositely arranged compound leaves are 10-30 cm long and pinnate with 5-7 leaflets. The leaflets range from 5-12 cm long and 3-5 cm broad with a serrated margin (Miraj 2016). In addition to heat tolerance, American elderberry is also cold tolerant and found in many areas of the northern United States and Canada.

Elderberry is thought to be both wind and insect pollinated with the best yields resulting from more than one cultivar or genotype adjacent in a planting (Byers 2012). In addition to naturally occurring plants, several genotypes of American elderberry have been selected from native germplasm for superior characteristics. These characteristics include larger berry size, uniform ripening within the fruit cluster and within the plant, disease resistance and ripening time. Two of these selected genotypes, Bob Gordon (Byers and Thomas 2011) and Wyldewood (Byers et al. 2010), were utilized in this study.

Health Benefits

Elderberry is one of the oldest medicinal plants used by man. Native Americans used a concoction of elder plant roots and bark for stomach issues and elderberry tea for rheumatism (Banks 2004). Hippocrates, a Greek physician (460-370 BC), called elderberry his 'medicine chest' because of the wide range of ailments it was thought to treat.

The healing and preventative health effects of elderberry are linked to the presence of phyto-chemicals, including flavonoids, phenolic acids, organic acids, major and trace elements, and vitamins (Senica et al. 2016). Anti-viral properties have also been found in elderberry and have been shown to be a useful treatment for influenza (Gregorio-Jauregui et al. 2014). Flavonoids derived from elderberry bind to H1N1 virus particles, which blocks the ability of the virus to infect host cells (Roschek et al. 2007). In berry to flower comparisons, freeze-dried elderflower powder samples have shown to provide an almost complete extraction of flavonoids and phenolic acids (Christensen et al. 2007). In addition, substances found in elderberry were shown to work as a defense compound against viruses, bacteria and fungi (Kite et al. 2013).

Polyphenol extracts of elderberry have been shown to reduce arterial pressure resulting in a decrease of systolic and diastolic blood pressure (Ciocoiu et al.2016). Polyphenols are also shown to significantly lower glycosylated hemoglobin in diabetic test groups (Ciocoiu et al. 2009). Elderberry is also a good source of antioxidants. These antioxidants have been shown to protect colon cells in lab studies (Olejnik et al. 2015), and can help the human body protect organs and slow down other aging processes that can lead to disease. Elderberry has also been shown to be an immune-boosting component part of the human diet. Nutritional supplements containing elderberry extracts are widely available and used for their role in fighting and reducing the symptoms of cold and flu. One study favorably compared elderberry supplements to the common drug, Tamiflu^{tm} which is widely used to treat the flu (Tiralongo et al. 2016). Although scientists have been aware of the presence of these compounds, recent research has determined their quantity and location (Mikulic-Petkovsed et al. 2015). It is thought that the fruit, flowers, bark, rhizomes and leaves all contain these beneficial components.

In vitro studies have shown that formulations containing *Sambucus nigra* can fulfill requirements for developing commercial sunscreen products, due to the high photo stability properties of the polyphenols they contain (Jarzycka et al. 2013). Elderberry supplements also possess anti-inflammatory properties. Inflammation in the human body can lead to diseases such as arthritis, asthma, allergies, multiple sclerosis, cardiovascular disease, Alzheimer's, Parkinson's, diabetes and some types of cancer (Olejnik et al. 2016). Extracts from elderflower have also been shown beneficial in breast cancer prevention and treatments in early, preliminary studies (Schroder et al. 2016).

Commercial Production of Elderflower and Berries

The health benefits of elderberry have led to a rise in demand. Since both berries and flowers contain healthful compounds, commercial elderberry production in Europe has been a substantial and profitable agricultural product for many years, with flowers often the more valuable commodity.

In 2002, three companies in England and Scotland processed the majority of elderflowers, with approximately £5 million paid per company for the product. One hundred tonnes a year is the average harvest with about 70% from wild sources (Kite et al. 2013). Members of the public desiring to earn extra income collect wild elderflowers from May-June at a rate of £1.10 to 2.60 per kg (Sanderson and Prendergast 2002). In recent years, trespassing and over-harvesting have become a problem and companies are now moving toward sourcing from commercial elderflower producers and private landowners.

In the U.S., commercial elderberry production is common, but commercial flower production is only now beginning to gain attention for its potential as an income-producing crop. There is currently a small amount of elderflower production in the U.S., reflecting its infancy as a commercial crop. In 2017, fresh and dried elderflowers retailed for $14 - $18 per pound (Reneker 2017). Both were popular in the restaurant industry for use in salads, baked goods and desserts.

Elderberry juice in the U.S. is used in products such as energy drinks, health supplements, syrup, colorant, juice, wine, jelly and baked products. Elderflowers are used in wine, spirits, tea, energy drinks, enhanced water and beauty products. Commercial elderberry production in the United States is growing. However, the limiting factor in production is harvest and processing. Elderberries must be hand harvested and the highly perishable fruit must be promptly removed from the field and placed in refrigeration.

Elderberry foliage and stems contain low levels of cyanide, so individual berries must be removed from the clusters (Ulbricht et al. 2014). This can be done by freezing or by vigorous, but careful shaking. Harvested and destemmed berries are either dried or processed into juice. For commercial production in the United States, GAP, or good agricultural practices, must be followed during growing and harvesting and follow-up processing must take place in certified processing facilities. Elderflower is listed on the U.S. Food and Drug Administration (FDA) Generally Recognized as Safe List (GRAS) (www.fda.gov).

Insect and Disease Considerations for Elderberry

Elderberries have a number of disease and insect issues. Spotted wing drosophila (*Drosophila suzukii*), or SWD, is a small fruit fly that can seriously impact yields and fruit quality. Female SWD can penetrate and lay eggs inside of unripe or ripening soft-skinned fruit, such as elderberry. Larvae feed within the fruit and are undetectable until visible fruit damage occurs. The primary season for SWD damage in the United States is mid to late summer, which is the peak of elderberry ripening and harvest. Control measures can be taken but require additional time and expense (Pinero 2013).

Eriophyid mites are microscopic pests that cause disfiguration of leaves. They overwinter on plant debris and care should be taken for proper pruning and sanitation at the end of the growing season to prevent re-infestation the following year (Warmund and Armrine 2015). Other insect pests include Japanese beetle (*Popilla japonica*), elderberry longhorn beetle (*Desmocerus palliates)* and elderberry sawfly (*Tenthrado grandis)* (Byers et al. 2012).

In commercial elderberry production, birds can potentially be a nuisance. Netting can be used on smaller plantings in addition to scare tactics. Timely harvest can keep damage to a minimum. Other wildlife such as deer, rabbits, raccoons and opossums can also browse plants.

Elderberry has few other insect pest issues and are even thought to repel certain insect pests. Experiments have demonstrated that utilizing proteins from elderberry bark and inserting them into commercial tobacco plants (*Nicotiana tabacum* L. *cv Samsun-NN*) will convey resistance to insect pests (Shahidi-Noghabi et al. 2008).

Elderberry is a relatively disease-resistant plant. Instances of bacterial leaf spot (*Pseudomonas viridiflava*), elderberry rust (*Puccinia bolleyana*) and a selection of viruses have been found in North American elderberry (Byers et al. 2012).

Weed management can also be a challenge. Plants are multi-stemmed with new shoots arising from the crown. Herbicide use must be done with care, using carefully selected products in addition to timely applications. Inadequate weed control can lead to a decrease in yields and plant vigor. Weeds such as sedges (*Cyperaceae* spp.) can serve as an alternate host for *Puccinia bolleyana* rust diseases (Figure 1.) (Warmund 2017).

Figure 1. *Puccinia bolleyana* Rust on Elderberry Leaf

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CHAPTER 2

FLOWER PRODUCTION AND EFFECT OF FLOWER HARVEST ON BERRY YIELDS WITHIN SIX AMERICAN ELDERBERRY GENOTYPES

Introduction

Preparation for the study began in 2015. Two sites were selected to provide replication and for data comparison. The first site was at the University of Missouri Southwest Research Center located at 37.073635, -93.878839 near Mt. Vernon, Missouri (aerial photo, Appendix C) with an elevation of 351 meters (https://earth.google.com). The second site was at the University of Missouri Horticulture and Agroforestry Research Center (HARC) located at 39.015292, -92.751515 in New Franklin, Missouri. The elevation of this location is 201 meters (https://earth.google.com). Both sites are within the USDA Hardiness Growing Zone of 6a (www.planthardiness.ars.usda.gov). Following a several month period, it was determined that the HARC site could not be included in the 2017 data collection due to late establishment of plants.

Genotypes and Cultivars of Interest

Six genotypes of American elderberry were used in this study. All six were wild selections derived from native germplasm. Three of the genotypes, Bob Gordon, Ozark and Wyldewood have been used in previous elderberry research and replicated trials resulting in Bob Gordon (Byers and Thomas 2011) and Wyldewood (Byers et al. 2010) having been officially released as cultivars. ‘Pocahontas’, ‘Rogersville’ and ‘Kelly 7-14’ are more recent additions and were included as part of this study.

Bob Gordon

Bob Gordon is a benchmark American elderberry cultivar. An orchardist named Bob Gordon near Osceola, Missouri U.S.A introduced this selection in September 1999. Rooted cuttings from the original plant displayed superior characteristics in field trials, leading to the plant being named after Mr. Gordon.

Bob Gordon was officially released in 2011 (Byers and Thomas 2011). It is the most widely grown cultivar in the Midwestern United States. Due to many years of previous research and trials, much is known about berry yields and plant phenology (Byers and Thomas 2011). Bob Gordon is preferred for its large, decumbent flower and berry cymes. The large flower size is a good choice for flower harvest considerations. Bob Gordon is a medium size shrub up to 220-225 cm wide, with a spreading to upright growth habit. Blooming occurs in late May through mid-June. Blooms transition to large berry cymes, which can yield up to 2.3 kg per plant (Byers 2014). The dark purple berries of Bob Gordon are preferred for their uniform ripening, resistance to shattering and good quality fruit and juice.

Kelly 7-14

The original plant came from the property of Mr. Michael Kelly in Columbia, Missouri U.S.A. The plant was purchased as a seedling through the Missouri Department of Conservation, likely derived from native plants and showed great promise. The Kelly 7-14 genotype has previously been included in observation plots, with this study being the first replicated trial (Byers and Thomas 2017).

Ozark

The Ozark genotype was collected by Patrick Byers near Deer, Arkansas U.S.A. and first used in field trials in 2008. Based on many years of data, its release as a cultivar is anticipated. Previous research included a cultivar evaluation, in addition to flowering comparisons against other types of elderberry (Thomas et al. 2015). Characteristics of Ozark include high berry yields that are high in antioxidants (Thomas et al. 2015). Ozark is generally available in the nursery trade.

Pocahontas

Pocahontas originated in Pocahontas, Arkansas U.S.A. by collector Mr. Rocky Starns. Although it blooms slightly later than other elderberry genotypes, it yields large flower and berry cymes. Pocahontas has previously been included in observation plots with this study being the first replicated trial (Byers and Thomas 2017).

Rogersville

Rogersville originated from a wild, roadside plant in Rogersville, Missouri U.S.A. collected by Michele and Patrick Byers. The plant rebounded well from annual mowing and had large flower and fruit cymes. Rogersville has previously been included in observation plots with this study being the first replicated trial (Byers and Thomas 2017).

Wyldewood

Wyldewood was originally collected from the wild by Mr. Jack Millican near Eufaula, Oklahoma U.S.A in 1995. Field trial results reported yields averaging 2.1 kg per plant (Byers 2014). Wyldewood is a tall shrub reaching upwards of 225 cm in height with a spreading to upright growth habit. Blooming occurs in June transitioning to a reliable and prolific fruit set in late July. Unpruned plants ripen over a four week period. Secondary cymes often form from axils below the main cyme, a characteristic that increases bearing potential of each shoot. Dark purple berries ripen uniformly within cymes and are resistant to shattering. Wyldewood was officially released as a cultivar in 2010 (Byers et al. 2010).

Procedures and Methodology

Study Area

Site preparation began in August 2015 based on a soil test analysis. Test results (Appendix A) showed adequate levels of potassium (K), calcium (Ca), Magnesium (Mg) and organic matter percentage. Soil pH was determined to be at 6.6, which is acceptable for elderberry growth and development (Byers et al. 2012). Phosphorus (P) levels, determined by a Bray 1 extraction test, were shown to be low and triple super phosphate with a 0-46-0 analysis at a rate of 16.78 kg per acre was recommended and applied. The study site receives full sun and has a soil type of

Hoberg silt loam with a 2-5 percent slope (NRCS, 2017). The site received about 40 inches (101.60 cm) of accumulated precipitation during the 2017 study period (www.agebb.missouri.edu/weather/stations/lawrence/framemtv.htm) which falls within the mean annual precipitation in Missouri (www.climate.missouri.edu/climate.php). Missouri climate is considered continental, with frequent changes in temperature and strong seasonal changes (www.climate.missouri.edu/climate.php).

Nursery Stock and Propagation

Both hardwood and softwood cuttings, 3-4" (7.62 cm – 10.16 cm) in height, were rooted by dipping the proximal end in a butyric acid rooting hormone and potting in 4" (10.16 cm) plastic pots with commercial grade potting media. Cuttings were placed in a greenhouse to root under an automatic mist system. Cuttings developed adequate root systems within 4-5 weeks and were then moved to an on-site outdoor nursery to undergo the hardening off process for a period of two weeks. Final planting stock consisted of rooted cuttings 6-8" (15.24 – 20.32 cm) in height with 6-8 sets of true leaves.

Site Preparation and Transplanting

Ninety-six plots were prepared at the study site and included 8-foot (2.43 m) spacing in-row between plots and 10-foot (3.04 m) spacing between rows. Three transplants were established in each linear plot at a spacing of 3.5 feet (1.06 m) between plants. The planting site was located in a turf grass area and a glyphosate herbicide application was used to eliminate existing grass.

Drip irrigation was installed within rows. Lines remained above ground within plots but were buried between plots for mower accessibility. Transplants were established from September 25 to October 8, 2015. A total of 288 transplants were planted in the study site (96 plots x 3 plants per plot). After planting, wood chip mulch was applied to a depth of six inches (15.24 cm). Rainfall was monitored and supplemental irrigation added via drip irrigation as needed during dry periods at a rate of one inch (2.5 cm) per week. Fertilization was done at the time of transplant using a 13-13-13 nitrogen fertilizer at a rate of 0.445 pounds (0.20 kg) per plot.

Experimental Design and Treatments

Four treatments were used in this study. These include: no flowers harvested within a plot, 15% of flowers harvested within a plot, 39% of flowers harvested within a plot, and 100% of flowers harvested within a plot.

The six elderberry genotypes received each of the flower harvest treatments randomly repeated four times across the site (a total of 96 plots utilizing a split-plot design; Appendix B).

Sampling and Data Collection

For the treatment plots that received 15% and 39% flower harvests, cyme counting took place shortly after development in May and June of 2017. Cymes on each individual plot were counted and quantities noted as harvest took place. The number of cymes to be harvested were determined by taking the total number on each three plant plot. Whenever possible, flowers were harvested on the lower 10% of the plant because fruit cymes near the base of the plant typically produce lower quality berries.

Flower harvest was done after all of the individual flowers within a cyme were open. Hand pruners were used with cuts designed to leave as much residual stem on the plant as possible. Harvested flowers from each plot were placed in brown paper bags (labeled with the plot number, date and flower quantity) and dried at approximately 100° F for 24 hours in a commercial grade dryer. Fresh weight in grams was taken before drying and dry weight in grams taken after. After drying, de-stemming was done. Individual flowers were removed from the cyme by rubbing on a .25 inch (0.635 cm) mesh screen (Figure 2) and post de-stemming weight in grams were recorded.

Ripe berries were also harvested for data collection purposes from late July through August 2017 as ripening occurred. Following harvest, fresh weight and fruit quality characteristics were noted. Juice was extracted using a hand press and a brix (percent soluble solids) reading taken from each plot using a Palm Abbe® digital refractometer (Misco Company, Cleveland, Ohio). Berry size data was taken by counting and weighing 50 random berries from each harvested plot.

Cultivar evaluation data was taken as part of the study. Phenology information including peak flowering date (when 50% of the flowers within a plot were in full bloom), verasion date (color change indicating the onset of ripening) and full ripeness date. Plant characteristics including maximum height, average height and number of stems per plant were also recorded.

Insect and disease data were taken twice during the 2017 study period. A severity scale ranging from 1-5, with one representing no damage and five representing severe damage was used. Insects including Japanese beetles (*Popillia japonica*), sawfly (*Tenthrado grandis*), eriophyid mites (*Phyllocoptes wisconsinensis*) damage levels were noted. Disease data was collected on bacterial leaf spot (*Pseudomonas sp.*) (Byers et al. 2012).

Spot treatment for Japanese beetle damage was applied as needed using carbaryl insecticide, while spotted winged drosophila was controlled with Mustang Max™ at a rate of 4 oz per acre.

Figure 2. Dried Elderflowers on Destemming Screen

Results and Discussion

Results

Data was analyzed using the software program SAS version 9.4 (www.sas.com) from SAS Institute Inc. Cary, NC. The SAS program determines results based on Fishers Least Significant Difference (LSD) test using the general linear model procedure. LSD computes the smallest significant difference between means being compared. The general linear model procedure combines statistical procedures to draw conclusions on given data sets.

Phenology data were collected during the study period. Comparisons were done amongst genotype and flower harvest treatment levels (Table 1.). For mean bud break (initiation of growth from a bud in late winter) comparisons between genotypes, Wyldewood was the first to break bud followed by Pocahontas, Ozark and Bob Gordon all breaking bud two days later. Rogersville and Kelly 7-14 were the last to break bud nine days after Wyldewood. Mean bud break between flower harvest treatments was not evaluated because the flower harvest treatments had not been imposed by that time.

Flowering date data across genotypes had little variance, with Kelly 7-14 and Pocahontas blooming the earliest, Bob Gordon, Ozark and Rogersville flowering one day later and Wyldewood the last to flower. Flowering dates within treatments also had little variance as expected, because treatments should not have directly influenced flowering date.

Fruit ripening date had some variance across genotypes. Kelly 7-14 was the earliest to ripen, followed by Bob Gordon four days later and Pocahontas six days later. Ozark, Wyldewood and

Rogersville all ripened last. Fruit ripening dates were not initially affected by flower harvest treatments.

Plant growth within plots was taken at the beginning and end of the 2017 study period and a mean calculated (Table 1). Within maximum height data recorded, Rogersville was tallest at 208 cm, followed by Pocahontas at 193 cm, Bob Gordon at 178 cm, Wyldewood at 168 cm, and Kelly 7-14 at 150 cm. Ozark was the shortest at 137 cm.

Within average height data, Rogersville was again the tallest and Ozark the shortest. Pocahontas had the second tallest average at 171 cm, followed by Wyldewood at 157 cm, Bob Gordon at 150 cm and Kelly 7-14 at 140 cm. Neither maximum nor average height were affected by flower harvest treatments, as might have been expected.

Table 1. Phenology and plant height means found in six American elderberry genotypes within four flower harvest treatments at Mt. Vernon, MO (Per 3 plant plot; 2017).

Factor	Mean Bud Break	Flowering Date**	Fruit Ripening Date	Max Height (cm)	Ave. Height (cm)
Genotype					
Bob Gordon	49 b*	166 ab	220 bc	178 bc	150 cd
Kelly 7-14	56 a	165 b	216 c	150 de	140 d
Ozark	49 b	166 ab	222 ab	137 e	124 e
Pocahontas	49 bc	165 ab	221 ab	193 ab	171 ab
Rogersville	56 a	166 ab	225 a	208 a	173 a
Wyldewood	47 c	167 a	224 ab	168 cd	157 bc
P value	0.0001	0.2046	0.0021	0.0001	0.0001
Flower Harvest Treatments					
0%	NA	165 a	221 a	173 a	152 a
15%	NA	165 a	221 a	174 a	155 a
39%	NA	165 a	223 a	175 a	153 a
100%	NA	166 a	NA	175 a	154 a
P value	NS	NS	NS	NS	NS

*Means within the sub-columns with the same letters are not significantly different according to the least significat difference test ($P \leq 0.05$)

**Dates given in 'day of year after January 1st' value

NS - non-significant

NA - not applicable

cm - centimeters

One of the purposes of this study was to determine potential flower production within six American elderberry genotypes (Table 2). Rogersville had the largest number of flowers harvested with 144 cymes within 3-plant plots. Pocahontas was the next comparable genotype with a 78-cyme average. Wyldewood had a good production value with a 68-flower cyme average, although the cymes were small, diseased and low quality (Appendix D and E).

Wyldewood cyme production was statistically comparable with Kelly 7-14 and Ozark. Bob Gordon had the lowest mean cyme harvest, but the flower cymes were very large compared to the other genotypes in the study. The high flower cyme production of Rogersville and Pocahontas showed great promise within the study period.

Total cyme fresh weight per 3-plant plot data were taken. Pocahontas had the highest fresh weight value and was comparable to Rogersville. Kelly 7-14, Wyldewood, Ozark were all statistically comparable with Bob Gordon, which had the lowest cyme fresh weight in this study.

Pocahontas had the highest individual cyme fresh weight. Kelly 7-14 and Rogersville were comparable with each other while Ozark, Wyldewood and Bob Gordon had the lowest individual fresh weight recordings.

Data on percent water removed from fresh cymes to produce a dried product determined that Ozark had the highest percentage and was comparable with the others in the study. Bob Gordon had the lowest percentage of water in flower cymes at 77%.

Rogersville had the highest total cyme dry weight per 3-plant plot at 267 g. At 241 g, Pocahontas was comparable with Rogersville. Kelly 7-14 had an average of 109 g. Wyldewood, Bob Gordon and Ozark produced the lowest dry weight with Ozark the least productive at 45 g.

Pocahontas had the highest individual average cyme dry weight at 2.9 g. Kelly 7-14 and Rogersville had the same weight average at 1.9 grams. Rogersville, Bob Gordon and Wyldewood had the lowest weights. Wyldewood was the lightest at 0.9 grams.

Destemmed flower dry weight per 3-plant plot was recorded following removal of individual dried flowers from stems within a cyme. The destemmed flowers are the useable final product and determining this weight can be important to production. Rogersville had the highest weight per 3-plant plot at 0.14 g with Pocahontas comparable at 0.136 g. The other genotypes in the study were statistically comparable with Ozark having the lowest weight.

Rogersville and Pocahontas had superior performance in flower harvest comparisons with other genotypes in this study. Calculations to compare cyme fresh weight (beginning product) to de-stemmed flower dry weight (end product) determined that only 9% of the beginning weight was present in the end product. The exception to this was Ozark, with only 4% of beginning weight present in the end product.

Table 2. Flower harvest comparisons of six American elderberry genotypes at Mt. Vernon, MO (Per 3 Plant Plot, 2017).

Genotype	Total Number of Flower Cymes	Total Cyme FW (g)*	Average Cyme FW (g)	Percent Water
Bob Gordon	34 c	279 b	5.4 c	77 b
Kelly 7-14	51 bc	553 b	11.0 b	83 a
Ozark	44 bc	331 b	6.9 c	86 a
Pocahontas	78 ab	1477 a	20.3 a	85 a
Rogersville	114 a	1239 a	10.7 b	82 a
Wyldewood	68 bc	439 b	5.6 c	84 a
P Value	0.0027	0.0001	0.0001	0.4568

Genotype	Total Cyme DW (g)	Average Cyme DW (g)	De-stemmed Flower DW (g)
Bob Gordon	58 c	1.1 c	33 b
Kelly 7-14	109 bc	1.9 b	52 b
Ozark	45 c	0.9 c	28 b
Pocahontas	241 ab	2.9 a	136 a
Rogersville	267 a	1.9 b	141 a
Wyldewood	70 c	0.9 c	46 b
P Value	0.0022	0.0001	0.0001

* (g) = grams
DW = dry weight
FW = fresh weight
Means within sub-columns with the same letters are not significantly different according to the least significant different test ($P \leq 0.05$)

Berry harvest data were compared within the six genotypes on plots with no flower harvest treatments (Table 3). Mean number of berry cymes harvested determined Rogersville to have the highest amount at 148. Pocahontas was comparable at 103 and closely compared with Kelly 7-14 at 96.

Total fruit fresh weight data were recorded on harvested cymes. Kelly 7-14 and Pocahontas had the highest weights at 13.5 and 13.3 pounds (lb) respectively with Rogersville comparable at 10.1 lb. Bob Gordon and Wyldewood were comparable at 5.6 lb and 2.7 lb respectively and Ozark had the lowest weight at 0.4 lb.

Average berry cyme fresh weight determined that Bob Gordon had the highest weight at 0.169 lbs. and was comparable to Kelly 7-14 at 0.141 lbs and Pocahontas at 0.129 lbs. Single berry weight and brix levels among genotypes was non-significant.

Table 3. Mean berry harvest data and brix readings of six American elderberry genotypes at Mt. Vernon, MO (Per 3 plant plot, 2017) .

Genotype	Total of Berry Cymes Harvested	Total Fruit FW (lb)	Average Berry Cyme FW (lb)
Bob Gordon	41 bc	5.6 abc	0.169 a
Kelly 7-14	96 ab	13.5 a	0.141 a
Ozark	20 c	0.4 c	0.026 b
Pocahontas	103 ab	13.3 a	0.129 a
Rogersville	148 a	10.1 ab	0.062 b
Wyldewood	80 abc	2.7 bc	0.039 b
P value	0.0323	0.0123	0.0001
Genotype	Single Berry Weight (mg)	Brix	
Bob Gordon	86 a	9.9 a	
Kelly 7-14	90 a	11.8 a	
Ozark	69 a	11.3 a	
Pocahontas	73 a	10.1 a	
Rogersville	77 a	9.0 a	
Wyldewood	79 a	7.4 a	
P value	NS	NS	

**Means within the sub-columns with the same letters are not significantly different according to the least significant difference test ($P \leq 0.05$)

NS - non-significant

Berry harvest information was taken and compared across the four flower harvest treatments in the 3-plant plots (Table 4.). The 0% flower harvest level had the highest number of berry cymes, as expected since no flowers were removed. The 0% flower harvest plots correspondingly had the highest total berry fruit weight and average berry cyme weight. The 15% harvest level had the highest single berry weight although differences were not significant in comparison to the other treatments. Total berry fruit fresh weight, average berry cyme weight and single berry weight data were compared and found to be unaffected by flower harvest. Brix levels were also found to be non-significant between treatments. Flower harvest did not have any effect on brix levels within this study.

Table 4. Mean berry harvest data within three flower harvest treatments of American elderberry at Mt. Vernon, MO (Per 3 plant plot, 2017).

Flower Harvest Treatments	Total # berry cymes***	Total Berry Fruit FW (lb)	
0%	86 a	7.9 a	
15%	69 a	6.6 a	
39%	63 a	6.3 a	
P value	NS	NS	
Flower Harvest Treatments	Average Berry Cyme wt (lb)	Single Berry (mg)	Brix
0%	0.096 a	78.9 a	10.0 a
15%	0.090 a	85.4 a	10.7 a
39%	0.091 a	78.9 a	10.5 a
P value	NS	NS	NS

*Means within sub-columns with the same letters are not significantly different according to the least significant difference test ($P \leq 0.05$).
**Dates given in 'day of year after January 1st' value
***Total number of flower cymes harvested
FW = fresh weight, g = grams, lb = pounds, mg = milligrams
NS = non-significant

Hypothesis Evaluation

Hypothesis

On mature elderberry plants, harvesting a portion of existing flowers will reduce a plant's fruiting crop load, possible resulting in the remaining fruiting cymes being larger and heavier, with larger individual berries and improved berry quality. Removal of all or part of the elderberry fruit crop (through flower harvest) might result in increased plant growth. Harvest of two high-value marketable commodities (elderberry flowers and fruit) from the same plants may be possible.

Evaluation

Elderberry flower harvest had no significant effect on plant phenology within the study period. Since genotype responses to flower harvest was unknown prior to this study, these data could be considered baseline information for any future studies.

When comparing flower production across genotypes, Pocahontas and Rogersville had superior harvest potential in number of cymes harvested, cyme dry and fresh weight, and destemmed weight.

Single berry weights and brix levels were compared across genotypes. Although Pocahontas and Rogersville had superior results in flower harvest data, Kelly 7-14 had top numbers in both berry weight and brix. Pocahontas and Rogersville would be good selections for flower production with Kelly 7-14 a good selection for berry production.

While these results should be considered preliminary, up to a 39% harvest of flowering cymes did not significantly reduce berry fruit yields nor did it improve berry size or fruit quality across all genotypes. Pocahontas and Rogersville displayed superior flower harvest potential and would be a good consideration for commercial production.

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CHAPTER 3

Future Direction and Research Considerations

The harvest and production of elderflowers has little to no research baseline information in the U.S. In addition to research and data collection from this study, an easier and more efficient way to de-stem dried flowers needs development. Current destemming methods are time consuming and allow for loss of pollen, which could possibly effect flavor and health benefits.

More efficient ways of drying flowers need further refinement, as well. This study revealed that flowers were prone to molding if airflow was restricted during the drying process, such as packing too many flower cymes into a paper bag.

Further studies are needed on healthful compounds and benefits of elderflowers and consumer education of such benefits. Although cyanide is a known component of the entire elderberry plant, more research is needed to determine the exact location, in what concentration and if consuming raw elderflower products could be harmful.

In addition, consuming raw or dried elderflowers could pose food safety risks in the form of contamination if improperly handled by workers or if herbicides and other chemicals were used. Additional research and production standards are needed in this area.

Research in freezing of fresh flowers is needed. Enzyme activity that causes the flowers to brown shortly after thawing, limits the storage capacity of elderflowers.

Insect, mite and disease issues of elderflower could also benefit from additional research. Field observances from this study found that mite damage on flowers caused small and tightly bound clusters. Rust led to deformed flowers. In addition, Japanese beetles, which favor elderberry foliage, did not seem to feed on flowers during this study period.

Additional research on indeterminate and determinate growth characteristics could also affect flower production in American elderberry.

It was noted in this study that a few of the plants in the 100% flower harvest treatments re-bloomed to varying degrees although not a significant amount. Further studies could be done on variances between genotypes and flower harvest treatment levels.

Closing Statements and Implications

Elderberry and elderflower production in the United States is increasing and will continue to do so. As more is discovered about health benefits, consumer demand will drive producers to consider growing this crop. Flower production is likely to become a secondary or niche market crop as both an escape from berry insect pest issues while providing many of the same health benefits as the berries. American consumers continue to demand healthful, sustainably gown food, and elderberry and elderflowers can be a good fit for that market.

Results from this study show that a portion of the flowers can be harvested and have little to no effect on remaining berries. Subsequent studies will likely build upon this and encourage more producers to grow this crop.

APPENDIX A

University Extension
University of Missouri Columbia

Soil Test Report

-------------------**MU Laboratories**--------------

23 Mumford, Columbia, MO, (573) 882-0623 or P.O. Box 160, Portageville, MO 63873, (573) 379-5431

Serial No. H82178H-3	County Lawrence	Region
Submitted 6/23/2016	Processed 6/28/2016	

http://www.soiltest.psu.missouri.edu/

Sample ID: ELDER

Lab No: C1613139

This report is for: ANDREW THOMAS
14548 HIGHWAY H
MT VERNON MO 65712
thomasal@missouri.edu

Last Limed: unknown

Submitted by:
Firm No. Outlet:

SOIL TEST RESULTS		RATING					
		Very low	Low	Medium	High	Very high	Excess
pHs	6.6	********************************					
Phosphorus (P)	37 lbs/a	***************					
Potassium (K)	278 lbs/a	**********************************					
Calcium (Ca)	3796 lbs/a	*************************					
Magnesium (Mg)	307 lbs/a	********************************					
Organic Matter:	3.7 %	Neutr. Acidity: 0.5 meq				CEC: 11.6 meq	

Fertilizer & Limestone Recommendations (lbs/1000 sq ft)

Crop	Nitrogen (N):	Phosphorus(P_2O_5)	Potash (K_2O)	Zinc(Zn)	Sulfur(S)	LIME
9 ELDER perennial bedding plants	0.0	2.0	0.0			0 0 0

Comments: ---Some herbicide labels list restrictions based on soil pH in water. Use the estimated pH in water of 7.1 as a guide to the label. If you wish to have soil pH in water analyzed, contact your dealer or local Extension specialist listed below.
---The soil should be tested every 2 to 3 years to determine the effects of your fertilization practices and to develop a new set of fertilizer and limestone guidelines.
***The soil has adequate calcium and an adequate pH for perennial bedding plants (). Application of lime, wood ashes, or calcium rich fertilizer is not recommended.

Your soil test report suggests that phosphorus may be limiting in your soil. A good source of phosphorus is triple superphosphate, which has the analysis of 0-46-0. Divide the recommended *application rate for phosphorus by 0.46 to arrive at a recommended rate of triple superphosphate per 1000 sq ft. For best results, apply phosphorus in late winter in advance of planting and incorporate into the soil. For fertilization rates and timing, see MU Guide G6135 Home Fruit Production: Strawberry Cultivars and Their Culture for more information. I recommend retesting your garden soil in 2-3 years.*

This soil test is coded as perennial ornamental plants.

Regional Specialist Robert Balek **Phone** 417-358-2158 *Robert Balek*

MP 552 7/96 **Signature**

APPENDIX B; PLOT LAYOUT MAP

Elderberry Flower Production Study

Two sites: Mt. Vernon
Six cultivars: Bob Gordon, Kelly 7-14, Ozark, Pocahontas, Rogersville, Wyldewood
Four treatments: 1 = no flowers harvested, 2 = 15% flowers harvested, 3 = 40% flowers harvested, 4 = 100% flowers harvested
Four reps (plots) of each cultivar × flower harvest treatment = 96 plots
Each plot contains 3 plants
96 plots × 3 plants each = 288 plants per site
Spacing 3.5 feet between plants within plots, 8 feet in-row between plots, 10 feet between rows
Mt. Vernon site planted Sept 25 - Oct. 8, 2015 (just a few missing -- to be planted in spring)

Mt. Vernon Plot Randomization and Map

↑ NORTH ↑ ↑ NORTH ↑

ROW A	PLOT	1	2	3	4	5	6	7	8	9	10	11
	CULT	O	B	B	K	R	P	B	R	O	R	K
	TRT	3	3	4	2	3	4	1	2	3	4	3
ROW B	PLOT	1	2	3	4	5	6	7	8	9	10	11
	CULT	R	W	K	P	B	R	P	K	W	W	B
	TRT	4	2	1	1	2	3	2	4	3	2	2
ROW C	PLOT	1	2	3	4	5	6	7	8	9	10	11
	CULT	O	B	P	K	O	K	K	R	P	O	B
	TRT	1	1	4	2	1	4	4	2	3	2	2
ROW D	PLOT	1	2	3	4	5	6	7	8	9	10	11
	CULT	B	W	R	K	O	P	W	R	W	K	O
	TRT	2	1	1	3	4	2	4	1	3	4	1
ROW E	PLOT	1	2	3	4	5	6	7	8	9	10	11
	CULT	W	O	B	W	P	B	W	O	B	O	K
	TRT	3	1	1	4	3	4	3	4	3	4	1
ROW F	PLOT	1	2	3	4	5	6	7	8	9	10	11
	CULT	K	O	P	O	B	R	R	B	W	R	O
	TRT	3	2	2	2	1	4	3	4	1	1	2
ROW G	PLOT	1	2	3	4	5	6	7	8	9	10	11
	CULT	P	R	R	W	P	O	W	R	W	R	K
	TRT	3	4	2	1	4	4	4	3	1	2	3
ROW H	PLOT	1	2	3	4	5	6	7	8	9	10	11
	CULT	P	O	K	R	B	P	B	O	P	B	K
	TRT	1	3	2	1	3	2	3	3	1	4	2
ROW I	PLOT	1	2	3	4	5	6	7	8			
	CULT	W	P	K	P	W	P	W	K			
	TRT	4	1	1	3	2	4	2	1			

APPENDIX C-ARIEL PHOTO OF STUDY SITE

Image retrieved from www.googleearth.com
November 2017; Study site located within yellow box.

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

Distance Diagnostics through Digital Imaging
Sample: 39075

Sample Number:	39075	Submission Date:	09/14/2017
County:	Out of State	Submitted by:	Keiddy Urrea
Client:	Kelly McGowan University of Missouri Extension 2400 S. Scenic Ave. Springfield Mo. 65807 Springfield, MISSOURI 417-881-8909 mcgowank@missouri.edu		
County Agent:	Out of State		
Date Collected:	09/12/2017	Commodity:	Tree/Shrub
Plant Name:	Elderberry		
Plant Variety:			
Age of Plant:			
Host Parts Affected:	leaves/needles,petiole,twigs/branches		
Locations:			
Symptoms:	blight,leaf spot,spot		
First Noticed:			
Previous Crop:			
Percent Affected:			
Damage Pattern:			
Damage Occurence:			
Soil Checked for:			

Diagnosed:	09/14/2017 at 12:00 AM
Pest Name:	Bacterial leaf spot, Pseudomonas sp./spp. (Confirmed)
Host Name:	Elderberry, Sambucus sp./spp.
Diagnosis:	
Recommendation:	Clean up all fallen leaves if possible. I would use a copper fungicide or lime sulfur during the dormant period.
Diagnosed by:	Sherrie Smith 2601 N. Young Ave. Fayetteville, ARKANSAS 72704 479-575-2727

APPENDIX E

UNIVERSITY OF MISSOURI Extension

DIAGNOSTIC REPORT

Sample#	201700301
Field ID	
Host	Elderberry
Received Date	8/25/2017
County	Lawrence
State	MO

Submitter:

Kelly McGowan
UM Extension
2400 S Scenic Ave

Springfield MO 65807

Phone **417-874-2965** Fax Email **mcgowank@missouri.edu**

Diagnosis and Recommendations

Host/Habitat	Elderberry (Sambucus sp./spp.); Cultivar: Wyldewood
List of Diagnosis/ID(s)	
fungal leaf spot	

Final Report

The causal agent of the leaf spotting is fungal, diagnosed by the mycelium growing in the spots. The fungus was not identified as it did not make any spores during incubation. However, powdery mildew can be ruled out.
At this time there are several plant pathogens that cause leaf spots on elderberry being researched. Due to the plant clinic closing we are unable to culture tissues to diagnose the causal agent.

From the specialist on fungicides:

Any of these can be used- Pristine, Abound, Manzate, Propimax, Quadris, Quash, Neem. I haven't tried to control leaf spot with any of these but they are labeled for Colletotrichum. Pristine is a good fungicide on many fruit crops, but won't available for homeowners.

University of Missouri Plant Diagnostic Clinic 28 Mumford Hall Columbia MO 65211 Telephone : 573-882-3019 Fax : 573-884-4288	Diagnosed By : Patricia Hosack (hosackp@MISSOURI.EDU) Completed Date: 8/31/2017

Sample# 201700301

AUTHOR VITA

Kelly McGowan resides in Springfield, Missouri U.S.A. with her husband and two daughters. She is employed as a Horticulture Educator for the University of Missouri Extension office in Greene County, located at the Springfield Botanical Gardens in Springfield, Missouri.

She has a Bachelor of Science degree in Horticulture from Missouri State University's Darr College of Agriculture in Springfield, Missouri. In addition, she has a Master of Science degree in Natural Resources with an emphasis in Agroforestry from the University of Missouri in Columbia, Missouri.

In addition to a background and interest in *Sambucus canadensis* research, she has an interest in native lepidoptera, sustainable gardening and farming practices, and growing her own food.

In her free time, she enjoys spending time outdoors and traveling.